Development of a novel passive sampler based on a combination of membrane assisted solvent extraction and molecularly imprinted polymer for monitoring of selected pharmaceuticals in surface waters

Sinegugu Khulu

A thesis submitted to the Faculty of Science, University of the Witwatersrand in fulfilment of the requirements for the degree of Doctor of Philosophy

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DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any university

Sinegugu Khulu

26th day of July 2022 at Johannesburg

PUBLICATIONS AND MANUSCRIPTS

This thesis is based on the following papers in which the candidate was the principal author:

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Sinegugu Khulu, Somandla Ncube, Tebogo Kgame, Elizabeth Mavhunga, Luke Chimuka

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Multivariate optimization of a two-way technique for extraction of pharmaceuticals in surface water using a combination of membrane assisted solvent extraction and a molecularly imprinted polymer

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Development and application of a membrane assisted solvent extraction-molecularly imprinted polymer based passive sampler for monitoring of selected pharmaceuticals in surface water

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Sinegugu Khulu, Somandla Ncube, Tebogo Kgame, Elizabeth Mavhunga, Luke Chimuka

Candidate contribution: Principal author, involved in idea conceptualization, performed the experiments and wrote the manuscript. Tebogo Kgame was an honours student who assisted with experimentation. Other co-authors helped with analysis of results and manuscript editing.

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Candidate contribution: Principal author, involved in idea conceptualization, performed the experiments and writing of the manuscript. Co-authors helped with analysis of results and manuscript editing.

CONFERENCE OUTPUTS

Synthesis and application of a selective molecularly imprinted polymer in solid-phase extraction of emerging pollutants from dam water (ChromSA postgraduate student workshop, September 2019).

Multivariate optimization of a two-way technique for extraction of pharmaceuticals in surface water using a combination of membrane assisted solvent extraction and a molecularly imprinted polymer (ChromSA postgraduate student workshop, October 2021) – Awarded 3rd Prize for oral presentation.

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Lawrence Mzukisi Madikizela, Cornelius Rimayi, Sinegugu Khulu, Somandla Ncube, Luke Chimuka**,** Pharmaceuticals and personal care products

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ABSTRACT

Pharmaceuticals are an important group of persistent emerging pollutants due to being continuously detected in the aquatic environment. Pharmaceuticals are compounds whose therapeutic effects enhance the health of individuals. However, these compounds have the potential to enter the environment as part of effluents from wastewater treatment plants while other sources include improper disposal of expired and unused medication and human excreta. Although these pollutants exist in trace levels (μg L^{-1} to ng L^{-1}) in environmental samples, they have gained a lot of attention in the science community owing to the perceived detrimental effects on human health and ecotoxicological effects in aquatic life. As such, determination of pharmaceuticals in the environment has become an essential component in environmental monitoring studies. Various analytical techniques have been reported mainly grab sampling followed by solid phase extraction being the most reported. However, the challenge has been that these pharmaceuticals exist in trace levels which requires extremely sensitive approaches. At the same time, they exist as mixtures which requires the need for analytical methods which allows for multi-residue analysis at a time. One of the obvious choices of sampling is grab sampling that involves instantaneous collection of samples. The main challenge of such is that this requires large amounts of samples as well as potential to miss the episodic events of pollution. In this regard, recent studies now advocate for passive sampler-based approaches where a sorbent is deployed in the environment over a period of time. This allows for estimation of time-weighted average (TWA) concentrations which caters for episodic events of pollution. In this regard, the purpose of the current study was to develop an alternative passive sampling technique based on a combination of a molecularly imprinted polymer (MIP) sorbent and membrane assisted solvent extraction (MASE) for the determination of pharmaceuticals belonging to five different classes in surface water. The approach was to synthesize a smart polymer using molecular imprinting technology, place it inside a semi-permeable polypropylene membrane and finally place it in a protective chamber. The chamber and its content, now referred to as the passive sampler was then deployed

under optimized conditions for monitoring of the five model pharmaceuticals belonging to different groups.

The first part of the work involved synthesizing a smart polymer, the MIP for efficient and selective extraction of pharmaceuticals belonging to different groups. This was done by selecting an appropriate template for molecular imprinting process. Cavity tuning experiments which involved the utilization of all the target pharmaceuticals whether as single or multi-template were conducted. The venlafaxine imprinted polymer was successfully selected based on its cross-selectivity for the selected pharmaceuticals. The synthesized polymer attained maximum matrix-matched adsorption capacities ranging from 206 to 418 ng mg^{-1} for individual pharmaceuticals within 80 min. Batch adsorption and kinetic studies indicated that the binding of the selected pharmaceuticals on the MIP particles resulted in multiple interactions through chemisorption. An analytical method for determination of the target pharmaceuticals was successfully developed using liquid chromatography-mass spectrometry (LC-MS), giving detection limits ranging from 0.03 to 0.31 ng mL^{-1} and quantification limits ranging from 0.12 - 3.81 ng mL^{-1} for individual pharmaceuticals. The venlafaxine imprinted polymer was further applied as a selective sorbent for solid phase extraction of an antiretroviral (nevirapine), an antidepressant (venlafaxine), a muscle relaxant (methocarbamol), an anticonvulsant (carbamazepine) and a cardiac stimulant (etilefrine) in dam water samples, yielding recoveries ranging from 43 - 69%. This preliminary data indicated that MIP cross selectivity can be an essential and attractive approach in the monitoring of organic pollutants belonging to different classes in environmental water bodies. This work, presented as **Paper 1** in the thesis was published in *Polymer Bulletin* Journal.

The synthesized venlafaxine imprinted smart polymer was then used in combination with a membrane assisted solvent extraction technique, referred to as MASE-MIP for the extraction of these compounds in complex environmental water samples. The MASE-MIP combination utilized the cross-selectivity of the synthesized venlafaxine

MIP whilst preventing co-extraction of larger molecules to yield cleaner extracts and increased selectivity. After efficient extraction, the sample extracts were analyzed using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-qTOF/MS). The MASE-MIP was optimized for various significant experimental parameters such as the influence of the sample salt content, the stirring rate, the stirring time and the amount of MIP using a central composite design. Optimum extraction conditions for a sample volume of 18 mL were found to be 5 g of salt content, a stirring rate of 400 rpm, an extraction time of 60 min and 50 mg of MIP, yielding good extraction efficiencies ranging from 38 – 91% for individual pharmaceuticals. The optimized MASE-MIP-LC-qTOF/MS method yielded detection limits in the range of 0.09 to 0.20 ng mL^{-1} and quantification limits ranging from 0.31 to 0.69 ng mL^{-1} for individual pharmaceuticals. Furthermore, the optimized extraction method was applied in environmental monitoring of selected pharmaceuticals in two important rivers in South Africa. All selected model compounds were detected in the water samples at concentrations ranging from 0.19 to 2.48 ng mL^{-1}. This illustrated emphasis of a need to continuously monitor the presence of these compounds in environmental waters. The monitoring could be done through the proposed analytical method which has proven to be precise and accurate. This work, presented as **Paper 2** in this thesis has been published in *Chemosphere* journal.

The developed MASE-MIP technique was further used in combination with the passive sampling technique to form a MASE-MIP based passive sampler for extraction and monitoring of selected pharmaceuticals in environmental water bodies. This technique utilized the cross-selectivity of the synthesized MIP, the size exclusion and protective membrane and allowed for preconcentration of the target pharmaceuticals into the green receiver solvent (ionic liquid). The passive sampler approach was based on allowing the targeted compounds to diffuse selectively in an integrative manner through the polypropylene membrane which housed an ionic liquid as a green receiving solvent and a MIP. Upon successful diffusion, the analytes were selectively adsorbed by a MIP. The technique was optimized for parameters such as effects of biofouling,

deployment time and solvent type for the receiver phase. Furthermore, the passive sampler was calibrated in laboratory-based experiments to obtain sampling rates (Rs) for each target pharmaceutical with the view to attain estimated time weighted average (TWA) concentrations of the targeted pharmaceuticals in environmental waters. The optimum matrix-matched sampling rates obtained ranged from 0.0007 - 0.0018 L d^{-1} for individual pharmaceuticals, whilst the method detection and quantification limits ranged from 2.45 - 3.26 ng L^{-1} and 8.06 - 10.81 ng L^{-1}, respectively. Upon deployment in a dam situated in a highly populated township in South Africa, only etilefrine and methocarbamol were detected and quantified at maximum TWA concentrations of 12.88 and 72.29 ng L^{-1}, respectively. This work is well presented as **Paper 3** in this thesis. Paper 3 is a manuscript under review in *Water Research* journal.

The MASE-MIP based passive sampler showed proven ability to selectively extract targeted pharmaceuticals prior to their determination using LC-qTOF/MS. In this case, the presented experimental procedures allow for detection of trace level environmental concentrations of the targeted pharmaceuticals, making them suitable alternative analytical methods that can be utilized for monitoring of these compounds in environmental water bodies.

DEDICATION

This work is dedicated to my dearest daughter Zibusiso: Thank you for your patience, your love and for all the nights you spent without me. All these kept me going during the most challenging parts of this journey. I hope this accomplishment is enough inspiration and proof that you can do anything you put your mind to.

To my parents, my pillars of strength: Thank you for believing in me without ceasing. Thank you for all your prayers for they have kept me this far. *Ngibonga angiphezi boKhulu KaBayeni!*

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Zondi) and every other person who supported and encouraged me throughout this journey, it really went long a way!

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LIST OF ABBREVIATIONS

DES	Deep eutectic solvent
DVB	Divinylbenzene
HF-LPE	Hollow fiber liquid phase extraction
HF-LPME	Hollow-fibre liquid-phase microextraction
HLB	Hydrophilic lipophilic balance
IL	Ionic liquid
LLE	Liquid-liquid extraction
MASE-MIP polymer	membrane assisted solvent extraction-molecularly imprinted
MIP	Molecularly imprinted polymer
PAH	polycyclic aromatic hydrocarbon
POCIS	polar organic chemical integrative sampler
SA	South Africa
SBSE	Stir-bar sorptive extraction
SPE	Solid phase extraction
SPME	Solid phase microextraction
WWTPs	Wastewater treatment plants

CHAPTER ONE

This chapter gives a general introduction to the study and gives information on the selected model pharmaceuticals which includes their physiochemical properties and health effects.

1. INTRODUCTION

Droughts accompanied with the deteriorating water quality have become the main threats to South Africa's ability to supply sufficient quality water in order to meet its demands and to ensure environmental sustainability [1]. The largest threat to quality water supply is due to water pollution through various ways. The unavoidable wide usages of pharmaceuticals has seen them being frequently detected in the environmental waters and this has raised a lot of attention amongst the science community and the general public at large [2–4]. Subsequently, pharmaceuticals have been identified as part of the emerging water pollutants owing to their negative health effects in biota (especially fish) as well as increased microbial drug resistance in these species [5–8].

Growth in industries and human settlements have been identified as the contributors in the pollution of water in both rural and urban areas in Africa and across the globe [9]. This is due to inaccessibility of modern ablution facilities particularly in most African regions as well as improper disposal of unused or expired medications which in turn contribute to the direct contamination of surface water bodies [10]. Although this is the case, pharmaceuticals are primarily introduced into the environment through treated wastewater treatment plants (WWTPs) effluents [11–14]. WWTPs receive very large quantities of pharmaceuticals via human excreta due to their high excretion rates from the human body [10]. Most pharmaceuticals are highly polar and soluble in water which result in their limited elimination during the wastewater treatments processes and then enter the environment [15–17]. In WWTPs, some pharmaceuticals undergo incomplete degradation whilst some of the degradation products can be reversed to their active biological forms through de-conjugation [4, 18]. Due to this, it becomes important to monitor the amount of pharmaceuticals that are discharged into the aquatic environment.

The selected pharmaceuticals for the study belong to five different therapeutic groups and as a result, they possess different therapeutic and physicochemical properties. The pharmaceuticals were, an antiretroviral (nevirapine), an antidepressant (venlafaxine), a muscle relaxant (methocarbamol), an anticonvulsant (carbamazepine) and a cardiac stimulant (etilefrine).

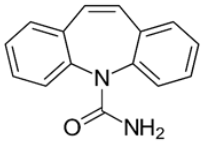
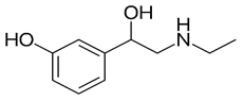
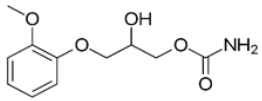
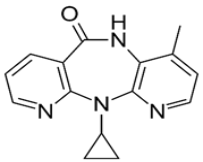
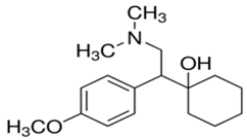
Venlafaxine is a widely used antidepressant whilst methocarbamol is used as a muscle relaxant drug for the treatment of symptoms of musculoskeletal disorders such as painful spasm and dislocations [19, 20]. Nevirapine is commonly used to prevent mother to child transmission of HIV and treating the HIV-1 infection, whilst carbamazepine and etilefrine are widely used as a basic treatment of epilepsy and hypertension, respectively [21, 22]. Physicochemical properties and molecular structures of the studied compounds are summarized in Table 1. As shown in Table 1, these compounds have n-octanol-water partition coefficient logarithms (logKow) ranging from 0.1 – 2.9, dissociation constants (pKa) ranging from 9.1 – 15.96 and high water solubilities which increases their chances of escaping the WWTP process and in turn end up in the environment.

Amongst the investigated pharmaceuticals, venlafaxine and methocarbamol have been linked with neurobehavioral disorders in aquatic animals with reports of neuroendocrine disruption resulting to reduced aggressive nest defense and distracted feeding patterns in exposed fish [20, 23]. In a study conducted in Spain, venlafaxine was found to encourage acute deadly phytotoxicity of *polystichum setiferum* plant [24]. A different target pharmaceutical, carbamazepine has shown severe negative impact on benthic organisms [22]. For example, in a study conducted by Oetken et al, the results obtained revealed that this compound poses a potential threat on the survival of *Chironomus riparius* and other related aquatic insects [25] [21].

The need for sensitive monitoring techniques for monitoring of pharmaceuticals in aqueous medium has led to calls for better methods that can detect them in trace levels where they also exist as mixtures in complex samples. Traditionally, solid phase

extraction of samples that were collected via grab sampling has been the approach of choice. However, this approach is limited for applications that require detection of analytes that exist in parts per trillion, hence the need for advancement of the existing techniques. In this work, a passive sampler based on membrane assisted solvent extraction and a molecularly imprinted polymer combination was developed and applied in the monitoring of the selected model pharmaceuticals. The passive sampler was deployed in the Orlando Dam, a dam situated in the heart of an overly populated Soweto township in South Africa. The idea was to develop a method which can analyze more than one class of pharmaceuticals at a time and determine their environmental TWA concentrations. This was accomplished by synthesizing a MIP with an affinity for all target compounds (Paper 1). The cross-selectivity of the MIP was then used in combination with the semi-permeable polypropylene membrane bag for selective extraction of these compounds in environmental water bodies with high sample matrix (Paper 2). The selective MASE-MIP combination was then used in the development of a passive sampler for monitoring of the selected pharmaceuticals over a period of 14 days. The passive sampler was aimed at pre-concentrating the trace environmental concentrations to quantifiable levels, as well as to understand the extent of environmental pollution by estimation of TWA concentrations for individual compounds (Paper 3). It is the first time that the MASE-MIP combination is applied as a passive sampler in the monitoring of pharmaceutical in the environments. Furthermore, it was the first time that MASE-MIP combination utilized a greener solvent (ionic liquid) in the receiver phase.

Table 1 Physicochemical properties and molecular structures of the selected model pharmaceuticals

Compound	Structure	Therapeutic Class	Molecular weight ($g.mol^{-1}$)	LogKow	pKa		Water Solubility ($mg\ L^{-1}$)
					Acid	Base	
Carbamazepine		Anticonvulsant	236.27	2.45	15.96	-3.8	1.05×10^2
Etilefrine		Cardiac stimulant	181.23	0.1	9.1	9.73	17.7
Methocarbamol		Muscle relaxant	241.24	0.6	13.6	-3.4	267
Nevirapine		Antiretroviral	266.3	2.5	10.37	5.06	1.38×10^4
Venlafaxine		Antidepressant	277.4	2,9	14.42	8.91	7.2×10^3

CHAPTER TWO

This chapter discusses the sources of pharmaceuticals in the environment, the different extraction and sampling techniques for the analysis of the selected pharmaceuticals in the environment, with more emphasis on MIPs as SPE sorbents and their combination with the MASE technique. Occurrence of the selected pharmaceuticals in the environment is also reviewed.

2 LITERATURE REVIEW

2.1 Sources of pharmaceutical compounds in the aquatic environment

Detection of pharmaceuticals in African environmental water bodies and globally is due to various sources, ranging from effluents (WWTP, nursing homes or hospitals, pharmaceutical factories) to improper disposals of medication [4, 11, 16, 26, 27], with the main source of their detection being WWTP [14, 28]. A study conducted in South Africa revealed that WWTP receives variable amounts of pharmaceutical at different times of the day [29]. This could be attributed to the frequency at which the compound is used and the rate at which it is excreted from the body.

A number of studies have been conducted globally to monitor the ability of numerous selected WWTPs for the removal of the selected model pharmaceuticals during the water treatment process [5, 21, 30]. For example, in Kenya, a study aimed at studying the ability of WWTP to remove pharmaceuticals revealed that removal efficacies of nevirapine ranged from 11 to 49% [23]. In a study conducted in South Africa, nevirapine was reported to be resistant to degradation [21]. On a separate South African study, nevirapine also showed removal efficacy of 22.5% during the WWTP process [31], whilst a study in Greece showed negative removal efficacies of carbamazepine in WWTP [30]. All these studies report inefficiencies of various WWTPs to remove these pharmaceuticals in water, which introduces these compounds into surface water bodies.

It was also discovered that although WWTPs are said to be the main source of pharmaceuticals in the environment, medical waste effluents were also found to have a great potential [11]. For example, in most African countries, there is scarcity of proper waste management such as incinerators for medical and pharmaceutical factories [32, 33] which results in their waste being released into the environment untreated. This was observed in studies conducted in Nigeria and Zimbabwe where there was no pre-treatment of medical waste performed, thereby resulting in these wastes being disposed together with municipality wastes into the environment [32, 33].

2.2 Techniques for extraction of pharmaceuticals

Nowadays, high end developed and selective analytical instrumentation such as chromatographic techniques coupled with mass spectrometry are available for determination of pharmaceuticals [34, 35]. The identification of the pharmaceutical compounds through the m/z ions provides selectivity as it detects and identify any co-eluting compounds as separate responses. However, samples cannot be directly injected into the system without any pre-treatment as that could negatively affect the accuracy and precision of results [35] and also damage different components of the techniques e.g. the column. In this regard, sample treatment and clean-up steps are considered as crucial steps prior to chromatographic determination of pharmaceuticals in complex samples such as environmental water bodies [34, 36]. The primary objective of sample treatment is the reduction of matrix effects and preconcentration of analytes (trace enrichment) by separating the analytes from the main sample matrix [35, 37]. There is a variety of existing pre-concentration and extraction techniques applied in the analysis of pharmaceuticals. These include liquid-liquid extraction (LLE), solid phase extraction (SPE), hollow fiber liquid phase extraction (HFLPE), stir-bar sorptive extraction (SBSE), solid-phase microextraction (SPME) etc. [38–41]. LLE as a traditional sample preparation technique has several drawbacks which include consumption of large quantities of organic solvents, not easy to automate as well as being time consuming [34]. The other mentioned alternative sample preparation techniques have an advantage of utilizing less amounts organic solvents compared to LLE. However, they lack selectivity when it comes to analysis of pharmaceuticals in environmental water samples since these coexist with other organic compounds in environmental water bodies.

Although there is a number of SPE sorbents available commercially for environmental applications, such as reversed- phase C_{18}, Oasis hydrophilic lipophilic balance (HLB), anionic-exchange (Oasis MAX), carbon nanotubes and styrene divinylbenzene (DVB) [42]; some of them still lack selectivity towards targeted compounds and are designed for single use [39]. Moreover, for trace analysis, sample preparation may be prolonged

especially with large sample volumes used in trying to preconcentrate the sample into quantifiable levels [43]. In some cases, even after carefully optimizing the SPE extraction method, the analyte signals still get suppressed due to the presence of matrix effects (co-elution of matrix components with analytes) [44]. The SPE technique was further improved by automating the technique and further coupling it to the chromatographic determination system. This did away with the technique being laborious and resulted in improved detection limits. However, this possesses a high potential of analytes passing through the column (mostly polar compounds) where used preconcentration volumes are high [43]. This then raised a need for more selective sorbents for SPE. Over the recent years, researchers have been working hard to develop suitable and selective sorbents for SPE applications. As such, there has been high interest in the application of molecularly imprinted polymers (MIPs) as smart adsorbents for SPE in order to increase selectivity by providing specific interactions between the polymer and the target compound [37, 45, 46].

2.2.1 MIPs as adsorbents for SPE

MIPs are artificial materials which have synthetically generated recognition sites that can specifically bind with target compounds or closely related compounds [44, 47, 48]. These materials are synthesized through an interaction of functional monomers, the target molecule and a cross-linking agent leading to a three-dimensional network polymer [44, 49–51]. Monomers are chosen based on their ability to interact with the functional groups present in the template molecule [44]. Once polymerization has been established, the template molecules are removed with an aid of relevant solvents to create binding sites complementary to target analytes through shape, size and functional groups [44, 47]. MIPs have added advantages of being robust, stable and resistant over a wide range of temperature, pH and solvents (thermal and chemical stability), easy to prepare and can be stored over a long period of time without losing its binding affinity towards targeted compounds [47, 48]. The gained interest into MIPs as adsorbents for SPE is due to their high selectivity towards the targeted compounds [52–54] and their reusability [55, 56]. Other advantages of MIPs over traditional SPE

sorbents are that they are not affected by changes in the matrix composition resulting into better and higher recoveries and improved detection limits [57]. MIPs were mostly introduced to target hydrophilic compounds. There has been advances in the application of MIPs as SPE adsorbents where compounds belonging to different groups are analysed through the phenomenon referred to as cross-selectivity [57, 58]. A few studies have utilized this phenomenon for example, quite recently, Wang et al. analyzed 20 compounds consisting of sulfonamides, tetracyclines and fluoroquinolones using a single MIP [59], whilst Herrero-Hernández et al. used a single MIP for analysis of two classes (3 phenoxy acid herbicides and 4 phenols) [58]. The advantage of this is that multiple analytes can be analyzed by a single MIP whilst retaining good selectivity towards targeted compounds. In this work also, in **Paper 1** this phenomenon was observed where pharmaceutical compounds belonging to five different classes were extracted through a single MIP.

2.2.2 Membrane based extraction techniques

There has been a growing interest in the utilization of membranes for microextraction of pollutants from aqueous environmental samples [60, 61]. These techniques are based on equilibrium partitioning of analytes from the aqueous sample phase into the receiver phase containing the extraction solvent where the analytes are preferentially enriched [62]. Liquid-phase microextraction (LPME) was first introduced as a technique which created a barrier between the aqueous sample and the receiver solvent to avoid the mixing of the two phases [63, 64]. This technique showed high selectivity and high reduction of matrix effects [60, 65]. This is a three-phase system where the analyte is first extracted into an organic solvent that impregnates the walls of the membrane, and then back extracted into an aqueous acceptor solution adjusted to the adequate pH [65, 66]. To minimize the extraction steps, Quintana et al, utilized hollow-fibre liquid-phase microextraction (HF-LPME) for enrichment and clean up in a single step in the analysis of pharmaceuticals in wastewater samples [67]. HF-LPME used very small volumes of about 10 µL which was very difficult to manipulate. For that reason, a technique based on a microporous polypropylene bag was introduced and combined with a number of

applications with an aid of gas chromatography determination [63, 64, 66, 68]. The membrane bag can house up to 1 mL of organic solvent where analytes are extracted through the membrane bag which also acts as protective barrier which limits coextraction of matrix in the sample [35, 62].

Recent studies have shown that using a microporous membrane to prevent interferences from accessing the MIP is an attractive approach to dealing with matrix effect [35]. This approach is known as membrane assisted solvent extraction-molecularly imprinted polymer (MASE-MIP). The approach has been reported to reduce matrix effects by preventing high molecular weight interfering compounds and particulate matter from clogging the MIP cavities, yield cleaner extracts and provide increased selectivity [34, 35, 37, 69]. Typically, its set-up consists of a polypropylene membrane bag (4 cm long, 6 mm internal diameter, 0.22 mm pore size and 160 mm thickness), filled up to 1 mL organic solvent and up to 100 mg MIP particles in the receiver phase. Target compounds diffuse through the membrane bag into a receiver phase containing the organic solvent and the MIP. There is a number of parameters which influences the movement of analytes from the aqueous donor phase into the organic receiver phase such as sample salt content, stirring rate, temperature, contact time, mass of the MIP etc. [60, 63, 68]. To attain acceptable analyte recoveries and improved selectivity, the extraction method needs to be properly optimized. This technique works quite well in samples where matrix components are high because in such instances, MIPs tend to lose their recognition abilities after a few extractions [35]. This is especially for MIPs where interaction of the target template and the monomer is through hydrogen bonding; these interactions tend to be weak and are prone to interferences [34]. The technique has been successfully applied in the extraction of polycyclic aromatic hydrocarbons (PAHs) in wastewater [69] and in the extraction of triazines from aqueous extracts of cow peas and maize baby corn [70], yielding high enrichment factors, high selectivity and low limits of detection and quantification.

Paper 2 of this work studies the extraction of the five selected model pharmaceuticals from environmental water samples. It was observed that this phenomenon resulted in improved analyte recoveries, improved limits of detection and quantification as a result of improved selectivity and reduced matrix effects.

2.3 Sampling techniques for monitoring pharmaceuticals in water

2.3.1 Grab sampling

Normally, pharmaceuticals are present in very low concentrations in aquatic environment (μg L^{-1} – ng L^{-1} or sometimes pg L^{-1}) [27, 71, 72]. Most of the monitoring programmes utilize grab sampling of specific amounts of water at a specific time. However, this sampling method has drawbacks in monitoring the presence of pollutants as it only provides pollutant concentrations at the time of sampling, which might not be a true reflection of the extent of pollution since pollutant concentrations vary over time [73, 74]. For example, wastewater effluent plants receive varying amounts of pollutants in a day [29], which subsequently end up in the environment at variable concentrations. This then result in episodic events of pollution being missed [74]. Automatic sampling systems which can frequently sample a number of water samples at specific times can be a solution to this, however, this is costly and mostly impractical as it would be laborious [73]. In addition, pre-concentrating environmental pollutants to quantifiable concentration levels can be expensive and tedious as large sample volumes are required which is accompanied by several sample preparation steps [75–77]. Furthermore, grab sampling does not offer data on the bioavailable fraction of the pollutants [78].

2.3.2 Passive sampling

Passive sampling technique is based on unprompted flow of analytes from the sample phase to the receiver phase of the passive sampler as a result of different analyte concentration between the two phases [73, 79]. This process continues until equilibrium is reached. Passive samplers offer enhanced sensitivity to analytes through pre-concentration over a longer period of time and, reduce the use of organic solvents

in sample preparation [79]. In some cases, passive samplers also offer increased selectivity towards target compounds depending on the trapping material used in the receiver phase [73]. The acceptor phase can be a porous adsorbent, a solvent or a chemical reagent [80]. Passive sampling devices allow for time integrated or time weighted average (TWA) concentrations which are collected *in situ* without having to alter the bulk of sampling medium [81]. This sampling method considers variations in pollutant concentrations and in turn increases the capabilities of the device to detecting and quantifying contaminants at trace levels, which is very difficult with grab sampling [71, 78, 82, 83]. Passive samplers offer more practical approaches to sampling, like ease of operation, less labouring, being very cost effective nonmechanical devices which does not need for energy sources in their operation [73, 76, 82]. An ideal sampler would normally consist of an acceptor phase and a membrane which separates the acceptor phase and the aqueous sample. The acceptor phase is selected based on its ability to accept or extract target compounds from the water body [84].

During the deployment period, the accumulation of analytes onto the sampler sorbent can be described in three regimes; the first one is the linear regime, the second is the curvilinear regime and the third one is the equilibrium regime [85] as presented in Fig. 1. In the time-integrative, the uptake of analytes of the receiver phase is said to be linear meaning that the concentration in the receiver will increase, whilst the concentration of analytes in the sample medium phase decrease. The second one refers to curvilinear, whereas in the third phase the movement of analyte is stationary. This is the phase at which the extraction between the receiver and sample phases is at equilibrium. In this regard, kinetic passive samplers therefore have an advantage of adsorbing contaminants from episodic events where concentrations of the pollutants are variable [73, 79, 81]. There are different types of passive samplers depending on the kind of analytes. There are passive samplers suited for organic compounds such as the polar organic chemical integrative sampler (POCIS), Chemcatcher, etc.) and those suitable for metals such as the one based on diffusive gradients in thin-films technique (DGT) and those based on polymer inclusion membrane (PIM).

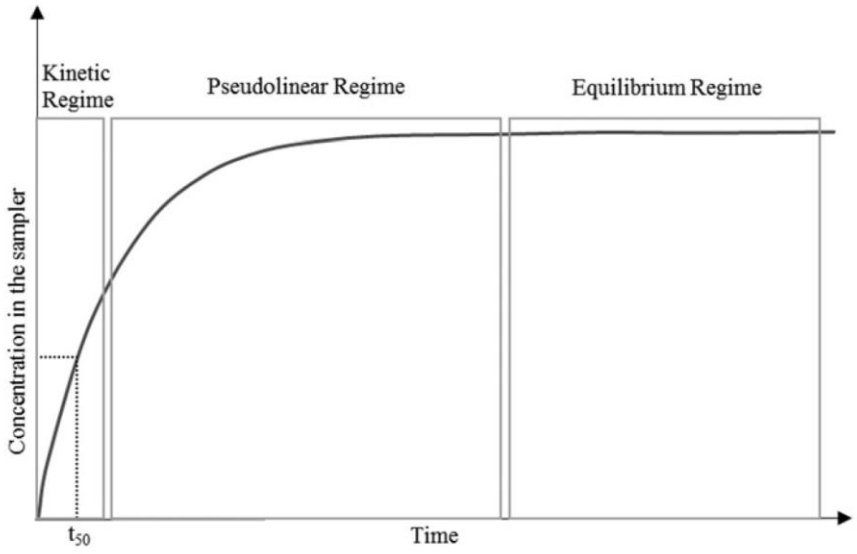


Figure 1. Representation of the three accumulation regimes governed by exposure time of a passive sampler [85]

2.3.2.1 Chemcatcher

The Chemcatcher passive sampler uses a diffusion-limiting membrane and a solid-phase receiving phase. The receiver phase is be placed and sealed in an inert plastic housing. The accumulation and selectivity rates are controlled by the choice of the diffusion-limiting membrane and that of the solid-phase receiving phase [79]. There is a number of different designs available depending on the combination of the sorbent and the diffusion-limiting membrane. One example of a used design is for non-polar organic compounds with logKow values higher than 4. This sampler uses the commercially available C_{18} empore disk as a receiving phase and low-density polyethylene diffusion-limiting membrane [73]. In one study, Vrana et al. calibrated the Chemcatcher sampler for measurements of time-weighted average concentrations of hydrophobic micropollutants, such as polyaromatic hydrocarbons and organochlorine pesticides in water [86].

2.3.2.2 Polar organic chemical integrative sampler

The polar organic chemical integrative sampler (POCIS) is used for monitoring of hydrophilic contaminants, like pesticides, pharmaceutical drugs, etc. At the moment, POCIS is the extensively used and commercially available passive sampler in environmental monitoring of pharmaceuticals [85, 87]. It consists of a sorptive receiver phase normally covered by microporous polyethersulphone membrane and the type of the receiving phase material can be changed to suit the type of targeted analytes [73]. In recent times, it has been observed that the POCIS is very effective in the qualitative analysis of pharmaceuticals but has drawbacks when it comes to quantitative analysis of these compounds. For these reasons, the POCIS has been considered a semi-quantitative tool for monitoring of pharmaceuticals [76, 88, 89]. The drawbacks are caused by its vulnerability to physicochemical parameters like the sample conductivity, dissolved organic matter, pH, biofouling, water flow and temperature [90–92]. For these reasons, the accumulation of pharmaceuticals onto the sorbent material is not clearly understood. Subsequently, the accumulation rates, widely known as sampling rates (Rs) which are obtained from calibrating the sampler in the laboratory may not be applicable for field application with the view to estimate the bioavailable concentration in the field water bodies [93]. There is currently few studies that have resorted to *in situ* calibrations of the POCIS, with the view to minimize the doubts in the estimation of the accumulated concentration [83, 84, 94]. This is an indication that more research is needed in the search for alternative passive samplers for monitoring of the bioavailable concentrations of pharmaceuticals in environmental waters.

It is for these reasons that in the present work, a novel passive sampler based on a combination of membrane assisted solvent extraction and molecularly imprinted polymer (MASE-MIP) was designed and optimized for monitoring of the selected model compounds in aquatic environments. The sampler also investigated the possibilities of using green and stable solvents such as the deep eutectic solvents (DESs) and liquids (ILs) in the receiver phase. In recent years, DESs which share few properties with ILs have gained a lot of interests in the various fields of analytical

chemistry e.g. sample preparation, micro extraction, mass spectrometry etc. [95, 96]. **Paper 3** of this work explains the optimization in detail. This brings the novelty into this approach since DESs and ILs have not been applied as a receiver in the MASE-MIP, even more as a receiver phase component in passive sampling. DESs have mostly been applied in extraction of pharmaceuticals using LLE based extraction techniques, where the extraction solvent would be in contact with the sample medium [97]. The tested IL (aliquat 336) has been applied in a variety of applications in the analysis of pharmaceuticals. This includes its use as functional monomer in the synthesis of a MIP for selective extraction of abacavir from polluted water [98]. Another application was in the synthesis of polymer inclusion membranes for extraction of antibiotics from water samples [99, 100]. In another study, aliquat 336 was used as a carrier for a technique based on emulsion liquid membrane in the extraction of acetaminophen in aqueous solutions [101]. Quite recently, it was utilized in the modification of waste-tyre derived activated carbon for the extraction of non-steroidal drugs in wastewater [102]

2.4 Occurrence of the selected pharmaceuticals in the environment

Occurrence of pharmaceuticals in the environment is still on going as researchers are trying to understand their toxicity and fate. Table 2 summarizes some of the work that has been done in the monitoring of the selected pharmaceuticals around the globe. The data presented on Table 2 projects nevirapine as being the predominant one. Carbamazepine was detected at range of 0.10 – 1.65 ng mL^{-1} in the Umngeni River, South Africa and in the Nairobi Basin, Kenya, whilst it was quantified at 0.42 ng mL^{-1} in ground water in California, United States [23, 103, 104]. In a South African study, methocarbamol was detected at a maximum concentration of 0.096 ng mL^{-1} [105], whilst in a study in the US, its accumulation on the exposed fish was 0.023 ng mL^{-1} [104]. Amongst all the selected pharmaceuticals, etilefrine is the least monitored globally. In a study by Rimayi and co-workers, etilefrine was not detected in all the sampling site [105], whilst in another one also by Rimayi, it was detected in some sites [106]. However, concentrations are not available as that was a screening study. Overall,

it can be noted that these compounds are present in the environment, and this raises a need for their frequent monitoring. Findings on their environmental monitoring for this work are well presented in Paper 2 and Paper 3.

Table 2 Environmental concentrations of the selected pharmaceuticals

Pharmaceutical compound		Matrix	Concentration (ng mL^{-1})	References
Carbamazepine	South Africa	Surface water	0.19 – 1.65	[107]
	Kenya	Surface water	0.10	[23]
	United State	groundwater	0.42	[104]
Etilefrine	South Africa	Surface water	d	[106]
methocarbamol	South Africa	Surface water	Up to 0.096	[105]
	United State	Exposed fish	0.023	[20]
Nevirapine	South Africa	Surface water	0.11 – 0.23	[108]
	South Africa	Surface water	0.13 – 1.48	[109]
	Kenya	Surface water	4.86	[110]
venlafaxine	Soth Africa	Surface water	Up to 0.026	[105]
		Surface water	Up to 0.026	[111]
	Poland	Surface water	Up to 0.250	[112]

d- detected

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CHAPTER 3

In this chapter, aims and objectives of the study are presented. A brief summary of the overall approach is also presented.

3 AIMS AND OBJECTIVES

3.1 Aim of the study

The main aim of the study was to develop a MASE-MIP-based passive sampler for determination of a variety of pharmaceuticals from environmental surface water samples.

3.2 Objectives

- To synthesize and characterize a MIP that can selectively bind to etilefrine, methocarbamol, venlafaxine, carbamazepine and nevirapine.
- To develop and optimize a MASE-MIP technique for effective extraction of targeted pharmaceuticals from environmental water samples
- To design and optimize MASE-MIP based passive sampler for the determination of target analytes
- To apply the developed technique in the analysis of target pharmaceuticals

3.3 Hypothesis

The developed MASE-MIP-based passive will offer an effective and practical approach for monitoring and measuring of the bioavailable concentrations of various pharmaceuticals in surface water.

3.4 Motivation

Pharmaceuticals are key contributors to the pollution of surface water pollution in aquatic environments which includes rivers, dams and lakes to name a few. South Africa is still slacking behind in understanding the extent of water pollution and the assessments of risks associated with pharmaceuticals in its aquatic environments. For this to be achieved, reliable analytical methods for monitoring of pharmaceuticals should be developed and verified. Most of the available methods and techniques for risk assessment of pharmaceuticals in the aquatic environment involve determining the total concentration. Novel techniques that measure the bioavailable fraction are still limited. Passive samplers are an ideal approach for sensitive monitoring and measuring of the bioavailable concentration of pharmaceuticals in environmental surface waters.

As such, in this work a novel MASE-MIP-based passive sampler was successfully designed and investigated as part of the search for effective and practical analytical methods for analysis of the bioavailable fraction of pharmaceuticals in the environment. This encompassed a technical aspect of the designing of the passive sampler followed by laboratory optimization and lastly the application of the sampler in surface waters.

3.5 Overall Approach

The first step in this work was to synthesize a MIP for selective and efficient extraction of model pharmaceuticals belonging to five different therapeutic classes. Extensive MIP cavity tuning experiments were performed for the selection of a polymer with an affinity for all the five model pharmaceuticals. The selected MIP was characterized for stability, parameters related to polymer pores and the functional groups present on the polymer surface. Batch adsorption studies were also conducted with the view to understand the binding of compounds on the MIP cavities. The polymer was further applied to environmental samples using spiked dam water samples. In this case the performance of MIP was investigated. This first part of the research was then presented in **Paper 1** which is already published in *Polymer Bulletin* Journal.

The selected polymer was then used in the development of a two-way technique based on MASE-MIP. A central composite design was applied to optimize several parameters for this technique, and its performance reported as percentage recovery, limit of detection and quantification and relative standard deviation values. The optimized MASE-MIP technique was then applied in the extraction of the model compounds from surface water samples of two of the major South African Rivers. This second part of the research yielded **Paper 2** which is already published in the *Chemosphere* journal.

In the third part of the work a passive sampling device was developed and combined with the MASE-MIP technique to form a MASE-MIP based passive sampler. This sampler was then optimized for extraction of the target pharmaceuticals in environmental water bodies. This was followed by deployment of these samplers in Orlando Dam, a dam situated in the heart of Soweto township (South Africa), for

environmental monitoring of the selected pharmaceutical compounds. This part of the work produced **Paper 3** which is a manuscript under review in *Water Research Journal*.

CHAPTER 4

MANUSCRIPTS AND PUBLICATIONS

This chapter presents two published articles and one manuscript for consideration in the examination of this thesis. The design and reference formatting of the articles and the manuscript were formatted to suit the requirements of this thesis. On all the papers, tables and figures have been placed next to where they appear in the text in order to meet the requirements for the submission of this thesis. Also, the page numbers for published articles and the manuscript have been formatted to cater for submission requirements of this dissertation. Highlights submitted to each journal are not included in this dissertation. Supplementary document for paper 2 is included in this dissertation. **Paper 1 and 2** have been published while **Paper 3** is a manuscript under review.

4 PAPER 1

This Paper is entitled “Synthesis, characterization and application of a molecularly imprinted polymer as an adsorbent for solid phase extraction of selected pharmaceuticals from water samples” has already been published in *Polymer Bulletin*. This article presents extensive MIP-cavity tuning experiments and batch adsorption studies which led to the selection of a venlafaxine imprinted polymer with high affinity for all five model pharmaceuticals belonging to different classes.

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Sinegugu Khulu – Principal Author

Somandla Ncube – Co-Supervisor

Tebogo Kgame – BSc honours student

Elizabeth Mavhunga – Co-author

Luke Chimuka – Main Supervisor

Synthesis, characterization and application of a molecularly imprinted polymer as an adsorbent for solid phase extraction of selected pharmaceuticals from water samples

Sinegugu Khulu [a,b], Somandla Ncube [c], Tebogo Kgame [a], Elizabeth Mavhunga [b], Luke Chimuka [a*]

[a] Molecular Sciences Institute, University of Witwatersrand, Private Bag X3, Johannesburg, 2050, South Africa

[b] School of Education, University of Witwatersrand, Private Bag X3, Johannesburg, 2050, South Africa

[c] Department of Chemistry, University of South Africa, Private X6, Florida, 1710, South Africa

[*] Luke.Chimuka@wits.ac.za

Abstract

Most pollutant compounds exist as mixtures in the environment. In this regard, one of the 12 principles of green analytical chemistry emphasize the need for methods that allow for analysis of multiple compounds versus those that analyze a single analyte at a time. In this work, we present a molecularly imprinted polymer (MIP) synthesized for the selective and efficient extraction of selected pharmaceuticals belonging to five different classes namely: an antiretroviral (nevirapine), an antidepressant (venlafaxine), a muscle relaxant (methocarbamol), an anticonvulsant (carbamazepine) and a cardiac stimulant (etilefrine) from surface water samples. Cavity tuning experiments using the target pharmaceuticals as a single or multi-template were conducted and the venlafaxine imprinted polymer was successfully selected for the study based on its high selectivity towards targeted pharmaceuticals. Batch adsorption and kinetic studies showed that adsorption of the selected pharmaceuticals onto the particles of the polymer followed a Freundlich adsorption isotherm as well as a pseudo second order adsorption model. This indicated heterogeneity of the binding surface

energies on the MIP resulting in multiple interactions through chemisorption. An analytical method for quantification of the compounds using liquid chromatography-mass spectrometry (LC-MS) was successfully developed, with detection limits ranging from 0.03 to 0.31 ng mL^{-1} and quantification limits in the 0.12 - 3.81 ng mL^{-1} range. The imprinted polymer was then evaluated as a selective adsorption sorbent for solid phase extraction (SPE) of the selected pharmaceuticals in dam water samples followed by LC-MS analysis, giving recoveries ranging from 43 - 69%.

Keywords: Molecularly imprinted polymer; pharmaceuticals; nevirapine; carbamazepine; venlafaxine

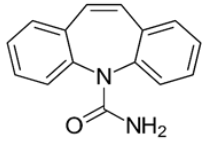
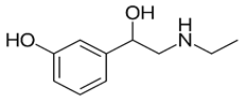
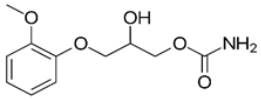
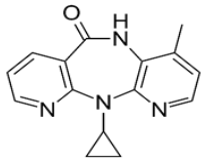
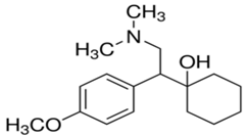
4.1 Introduction

In the modern society, the detection of pharmaceutical drugs and their residues in surface waters has raised concern due to their potential negative impact on the human health if consumed unknowingly from contaminated food and water sources [1, 2]. The widespread usage of pharmaceuticals has seen them primarily entering ground water and surface water through wastewater treatment plant (WWTP) effluents [3–6]. WWTPs receive high quantities of pharmaceuticals via human urinary and faecal excretion, and improper disposal of expired medication from nursing homes, hospitals and domestic effluents as well as untreated effluents from the pharmaceutical industry [5, 7–9]. Literature suggests that the presence of pharmaceuticals in the environmental water is due to them surviving wastewater treatment processes [10]. Pharmaceutical drugs are water soluble and have a high polarity which allows them to escape wastewater treatment processes easily [6], others undergo incomplete degradation while some degradation products could even be returned to their biologically active forms through de-conjugation [3, 10, 11]. Although pharmaceuticals are present in the aquatic environment in relatively small concentrations (µg - ng L^{-1}) [1, 3, 12], their residues can cause ecotoxic effects, hormonal disruption, and drug resistance [13].

Sample preparation prior to chromatographic determination and clean-up steps are of great importance in the analysis of pharmaceuticals from environmental water bodies [14, 15]. There is a number of existing extraction and pre-concentration techniques used in sample preparation including solid-phase extraction, hollow fibre liquid-phase extraction, stir-bar sorptive extraction etc. [16–20]. However, these lack selectivity as pharmaceuticals normally co-exist with other organic compounds in environmental water systems. Over the years there has been a great interest in the application of molecularly imprinted polymers (MIPs) as sorbents for solid phase extraction technique to increase analyte selectivity in the presence of very complex samples [2, 21–23]. The interest in MIPs as adsorbents is due to their great selectivity towards target compounds based on shape, size and functional groups[24–27] and the fact that they can be re-used [5, 28–31].

Most studies in environmental monitoring have mainly focused on synthesizing MIPs that target a single pharmaceutical or a group of pharmaceuticals belonging to the same class. In the environment pharmaceuticals do not exist in isolation but as mixtures of different classes some of which have similar physico-chemical properties. Principle no. 8 of the 12 principles of green analytical chemistry emphasizes the need for methods that allow for analysis of multiple compounds versus those that analyze a single analyte at a time [32]. MIPs have been shown to bear synthetic recognition sites which can specifically bind to a target molecule as well as other closely related molecules [33, 34]. Ncube et al., 2019 observes that this phenomenon, referred to as cross selectivity, can be utilized when there is need to analyze multiple analytes belonging to different classes in complex samples [23]. Studies have been mentioned where a single MIP has been applied in analysis of different classes of environmental pollutants. For example, Herrero-Hernández et al. used a single MIP for analysis of two classes (3 phenoxyacid herbicides and 4 phenols) [35], while recently Wang et al. analyzed 20 compounds consisting of sulphonamides, tetracyclines, and fluoroquinolones using a single MIP [36]. As such, the objective of this present work was to develop a single MIP that can be used to target five different classes of pharmaceuticals from environmental water sources. In this regard, various cavity tuning experiments were done to identify the imprinting template that could produce cavities on a single MIP with a high affinity for all targeted classes of pharmaceuticals. The model compounds included an antiretroviral (nevirapine), an antidepressant (venlafaxine), a muscle relaxant (methocarbamol), an anticonvulsant (carbamazepine), and a cardiac stimulant (etilefrine). Table 1 shows the physicochemical properties and molecular structures of the model pharmaceuticals. The synthesized MIP was applied as an adsorbent for solid phase extraction of the target compounds in dam water samples.

Table 1 Physicochemical properties and molecular structures of the selected pharmaceuticals

Compound	Structure	Class	Molecular weight ($g.mol^{-1}$)	pKa		Water Solubility ($mg\ L^{-1}$)	Biota health side effects
				Acid	Base		
Carbamazepine		Anticonvulsant	236.27	15.96	-3.8	1.05×10^2	Behavioural and neurological effects in juvenile fish
Etilefrine		Cardiac stimulant	181.23	9.1	9.73	17.7	Cardiovascular effects such as increased pulse rate and cardiac output
Methocarbamol		Muscle relaxant	241.24	13.6	-3.4	267	Neurobehavioral disorders in aquatic animals
Nevirapine		Antiretroviral	266.3	10.37	5.06	1.38×10^4	Reduced growth rate on fish, and enlarged livers
Venlafaxine		Antidepressant	277.4	14.42	8.91	7.2×10^3	Neuroendocrine disruption, impacting the stress and feeding responses in fish

4.2 Materials and Methods

4.2.1 Chemicals and reagents

Analytical grade venlafaxine hydrochloride (≥98%), methocarbamol, etilefrine hydrochloride, nevirapine, carbamazepine and p-vinylbenzoic acid (97%) were all obtained in powder form from Sigma-Aldrich, Johannesburg, South Africa, while ethylene glycol dimethacrylate (98%) was in liquid form. LC grade methanol (≥99.9%), dimethyl sulfoxide, acetonitrile (99.8%), toluene (99.7%), formic acid (≥95%), and acetic acid (≥99%) were also purchased from Sigma-Aldrich, Johannesburg, South Africa.

4.2.2 Instrumentation and apparatus

For washing the template out of the MIP cavities, a temperature-controlled WiseCube WIG-105 Precise Shaking incubator by Wisd® Laboratory Instruments (Wertheim, BW, Germany) was used. Centrifugation of samples was done through a Rotofix 32A centrifuge from Hettich® (Frankfurt, Hessen, Germany). Pore size and pore volume were characterized through a Brunauer-Emmett-Teller (BET) Micromeritics TriSar 3000 system by Micromeritics Instrument Corp. (Nocross, Georgia, USA). Thermograms representing stability of MIPs were from a Q500 Thermogrametric analyzer (TGA) by TA Instruments (New Castle, DE, USA. A Thermo Nicolet Antaris FTIR analyzer by Perken Elmer (Waltham, MA, USA) was used for identification of functional groups on the MIP surface.

Deionized water was produced through a Millipore DirectQ 3 UV waters system-2345 from Millipore (Massachusetts, USA). The 6 mL SPE cartridges loaded with the MIP were mounted on an SPE system connected to a VDE 0530 vacuum pump purchased from KNF Neuberger GmbH (Freiburg, BW, Germany). The cartridges were conditioned with 5 mL methanol and then equilibrated with 5 mL of deionized water. This was followed by loading spiked dam water. Washing was done with 5 mL of 5 %v/v methanol in water. Finally, the trapped analytes were eluted from the MIP cavities using 5 mL of methanol. The eluent was evaporated to near dryness using a

slow steady stream of nitrogen gas followed by reconstituting using 1 mL of 0.1 %v/v formic acid in methanol and taken to LC-MS for analysis.

The LC system used for the separation and analysis of compounds was the Thermo Scientific Dionex Ultimate 3000 from ThemoFisher Scientific (Waltham, MA, USA). The instrument consisted of a HPG-3200RS pump and a WPS-3000TRS autosampler. The LC was coupled to compact quadrupole time-of-flight mass spectrometer system from Bruker Daltonics Inc. (Billerica, MA, USA). Bruker Compass DataAnalysis version 4.3 was used to collect and process qualitative data, while Bruker Compass QuantAnalysis version 4.3 was used for collection and processing of quantitative data. Chromatographic separation of analytes was achieved on a Luna Omega C18 column (50 x 4.6 mm, 3 μm) from Phenomenex (Torrance, CA, USA). Separation was through gradient elution using solvent A (0.1% formic acid in deionized water) and solvent B (0.1% formic acid in acetonitrile). The system flowrate was kept constant at 0.3 mL min^{-1}. Initially solvent B was kept at 5% for 1 min. Then it was ramped uniformly until it reached 55% solvent B at 6 min and kept constant for a further 2 min. It was again ramped until it reached 95% solvent B at 10 min and kept constant for a further 2 min. Finally, the gradient was dropped to 5% solvent B over a minute and kept constant for another minute. The total runtime was 14 min.

4.2.3 Synthesis of a molecularly imprinted polymer

In a round bottom flask, 0.05518 mmol (equivalent to 10 - 16 mg) of the pharmaceutical template was dissolved in 3 mL of dimethyl sulfoxide at 40°C for 5 min while stirring at 500 rpm. The p-vinyl benzoic acid functional monomer (0.2207 mmol) was then added into the mixture while heating for a further 10 min. The mixture was then heated to 60°C and ethylene glycol dimethacrylate (167 μL) was added as a cross-linker. Heating and stirring of the mixture continued for a further 10 min. The system was then purged with nitrogen gas for 5 min to remove oxygen from the reaction. A 10 mg mass of an initiator, 1,1´–azobis-(cyclohexanecarbanitrile) was then added and the temperature slowly raised to 80°C. Polymerization was achieved within 2 h. Heating

was stopped, and the mixture allowed to cool to room temperature. The MIP was then transferred into a petri dish and dried in an oven at 70°C overnight. The dried MIP was ground into a fine powder (50 – 100 µm particle size diameter) and transferred into a 50 mL centrifuge vial. A total of six polymers imprinted with the target pharmaceuticals as templates were investigated. Five polymers had one pharmaceutical as an imprint while the sixth polymer comprised of a mixture of all the five pharmaceuticals under investigation as multiple imprints. The MIPs were then washed several times with methanol to remove the imprinting molecules thus creating cavities on the MIP. The washing process was done by placing the vials in a WiseCube shaking incubator, with shaking being done at 150 rpm and temperature maintained at 35°C for 4 h. This process was repeated until the imprinting molecules were no longer detected in the washing solvent. A control polymer referred to as a non-imprinted polymer (NIP) was also synthesized in the absence of the template.

4.2.4 Molecularly imprinted polymer selection

Various experiments were done to select a MIP with an affinity for all the target compounds. In this regard, 10 mL of dam water was spiked with 1 µg mL^{-1} mixture of pharmaceuticals under investigation. A 10 mg mass of the MIP was placed into the aqueous solution and allowed to stand for 3 h to adsorb the pharmaceuticals. The mixture was then centrifuged for 5 min and filtered through 0.45 µm frits and the supernatant was analyzed for pharmaceuticals that were not adsorbed by the MIP. The procedure was repeated with all the synthesized MIPs. A MIP that showed better affinity towards all the target pharmaceuticals was selected as the best MIP and used in successive studies.

4.2.5 Batch adsorption studies

4.2.5.1 Effect of initial concentration

To investigate the effect of initial concentration of pharmaceuticals in solution, 10 mg of the chosen MIP was dispersed into seven different 10 mL spiked solutions with a concentration range of 0.005 - 1 ng mL^{-1} of the five pharmaceuticals. The extraction

was allowed to occur under ambient conditions for an hour. The mixtures were then centrifuged and injected in the LC-MS. These experiments were done in triplicate. The amount of each pharmaceutical adsorbed by a unit mass of the MIP was calculated using Eq. 1. The total adsorption capacity was calculated as the sum of each adsorption capacity at individual spiking concentration using Eq. 2. The adsorption capacity of the NIP was also calculated using Eq. 1. The imprinting factor was then calculated as a ratio of adsorption capacity values of the MIP and the NIP at equilibrium using Eq. 3.

$$q_e = \frac{V(C_i - C_e)}{m} \quad (1)$$

where q_e is the adsorbed amount of each pharmaceutical at equilibrium (ng mg^{-1}) (also referred to as the adsorption capacity), C_i is the spiking concentration of each pharmaceutical (ng mL^{-1}), C_e is the concentration of each pharmaceutical at equilibrium (ng mL^{-1}), V is the volume of spiked solution (mL), and m is the mass of the MIP (mg).

$$Q_e = \Sigma q_e \quad (2)$$

where Q_e is the total adsorption capacity at equilibrium (ng mg^{-1})

$$IF = Q_{MIP}/Q_{NIP} \quad (3)$$

where IF is the imprinting factor, Q_{MIP} is the adsorption capacity for the MIP and Q_{NIP} is the adsorption capacity for the NIP

Calculating the imprinting factor aids in assessing the efficiency of the MIP, whilst calculating the MIP binding capacity assists in determining the amount of MIP that can be used in routine analysis of pharmaceuticals under investigation. The calculated equilibrium adsorption capacities at each concentration were then used in the determination of the extent of binding between the MIP and the pharmaceuticals by plotting graphs of the linearized Langmuir and Freundlich adsorption isotherm models (Eq. 4 & 5 respectively). The plot with a higher coefficient of determination (R^2) was accepted as the model that explains the extent of binding.

$$C_e/q_e = 1/bq_m + C_e/q_m \quad (4)$$

where C_e is the concentration at equilibrium (ng mL^{-1}), q_e is the equilibrium adsorption capacity (ng mg^{-1}), q_m is the maximum adsorption capacity (ng mg^{-1}), and b is the constant related to the energy of binding expressed by (mL mg^{-1})

$$logq_e = logK_f + (1/n)logC_e \quad (5)$$

where K_f is the equilibrium constant (ng $mL^{-1})^{1/n}$ and n is the adsorption intensity

4.2.5.2 Effect of contact time

Investigation of the effect of contact time was done from 5 to 210 min with the view to understand the mechanism of adsorption. A 10 mg mass of the MIP was placed into 30 x 10 mL solutions spiked with 1 µg mL^{-1} of the pharmaceuticals. Three vials were collected at specific times (5, 10, 20, 30, 60, 90, 120, 150, 180 and 210 min) and 5 µL aliquots were then injected into the LC-MS system to determine the amount of pharmaceuticals remaining in solution. The analytical data obtained was then fitted into three commonly used kinetic models; the Lagergren pseudo first order model (Eq. 6), the pseudo second order model (Eq. 7), and the intra-particle diffusion model represented by the Weber–Morris linear model (Eq. 8). Between the pseudo first order and the pseudo second order models, the model with the highest R^2 value was the one used to explain the adsorption mechanism. The intra-particle diffusion model was used to determine the rate controlling step.

$$log\ log\ (q_e - q_t) = logq_e - k_i t/2.303) \quad (6)$$

where q_e and q_t are adsorption capacity parameters (ng mg^{-1}) at equilibrium and at time t (min) respectively. k_i is the Lagergren rate constant for first order adsorption (min^{-1})

$$t/q_e = 1/k_2 q_e{}^2 + t/q_e \quad (7)$$

where k_2 is the Lagergren rate constant for the second order adsorption

$$q_t = K_p \cdot t^{0.5} + I_d \quad (8)$$

where q_t (ng mg^{-1}) is the amount of compound adsorbed at time t (min) and k_p is the initial rate of the intra-particle diffusion (ng mg^{-1} min$^{-0.5}$) and I_d (ng mg^{-1}) is the constant relating to the rate limiting step

4.2.6 Effect of sample pH and elution solvent

The pharmaceuticals under investigation are polar compounds with relatively different physico-chemical properties (Table 1) hence their extent of binding to the functional groups of the MIP cavities is expected to vary. In this regard, experiments were done to find a pH region in which the pharmaceuticals bind to the MIP cavity functional groups effectively as well as a solvent that can effectively elute all the analytes from the cavities. Pharmaceuticals have both basic N atoms and acidic -OH groups whose charges are sensitive to pH changes based on their pKa values given in Table 1. The solvents chosen during elution of analytes from the MIP cavities are based on their ability to disrupt the interactions that occur between the functional groups on the MIP cavities and the analytes. The solvents that were investigated were the two polar solvents viz: methanol and acetonitrile that act by interfering with hydrogen bonding between the MIP and analyte, and one less polar solvent, toluene which mainly targets the π-π interactions.

4.2.7 Application of the MIP in spiked dam water samples

The objective of this step was to demonstrate the applicability of the synthesized MIP in the extraction of trace levels of selected pharmaceuticals from real water samples. Water samples were collected in 1 L bottles from Florida Lake, Florida, South Africa

and transported to the laboratory in cooler boxes for analysis. These were immediately filtered through 0.45 μm. For analysis, the dam water samples were spiked at low analyte concentration levels of 0.5 and 1 ng mL^{-1} which relates to concentrations reported for some of the test compounds in dam water samples such as 0.055 – 1.480 ng mL^{-1} reported for nevirapine [21, 105, 108, 109]. The SPE procedure was done using cartridges packed with 50 mg of the MIP and 60 mL of spiked dam water samples loaded into the cartridges at 10 mL min^{-1} using a vacuum pump. The applicability of the synthesized MIP in the determination of pharmaceuticals at trace levels was calculated as extraction efficiency at each spiking level given as a percentage ratio of the amount spiked versus the amount recovered by the MIP (Eq. 9).

$$Extraction\ efficiency(\%) = \frac{n_R}{n_S} \times 100 \quad (9)$$

where n_R is the amount recovered by the MIP (mg) and n_S is the amount of pharmaceutical spiked into the dam water (mg)

4.3 Results and discussions

4.3.1 Synthesis and characterization of the best MIP

4.3.1.1 Selecting the best imprinted MIP

The performance of the six MIPs imprinted with different compounds is summarized in Fig. 1. Results show that the MIP imprinted with venlafaxine displayed better affinities towards compounds under investigation, with the exception of itself and etilefrine which were better extracted by the MIP imprinted with the mixture of all compounds. It was observed that most analytes did not favour themselves except for etilefrine. It is predicted that etilefrine with its small steric structure creates smaller cavities making it difficult for other larger steric structure compounds. On the other hand, cavities created using larger steric structure imprinting molecules will allow entrance by compounds of smaller or similar steric structure. On average venlafaxine was found to be the best template for the study, which could be mainly attributed to its

functional groups and its size (Table 1). Further experiments were therefore conducted using the venlafaxine imprinted polymer.

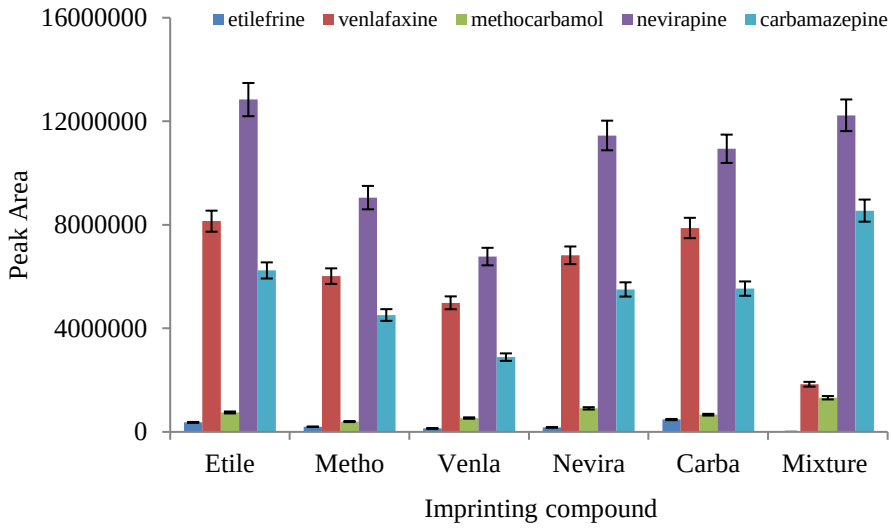


Figure 1 Amount of analytes remaining in solution after extraction with different imprinted polymers. Etile (etilefrine), Metho (methocarbamol), Venla (venlafaxine), Nevira (nevirapine), Carba (carbamazepine)

4.3.1.2 Characterization of the polymer

Based on the BET results, the venlafaxine imprinted MIP had an average pore size of 6.817 nm, pore volume of 0.001034 $cm^3\ g^{-1}$ and a Freundlich surface area of 0.6069 $m^2\ g^{-1}$. The pore size for the NIP was 9.781 nm, pore volume of 0.001041 $cm^3\ g^{-1}$ and a Freundlich surface area of 0.4257 $m^2\ g^{-1}$. These results show that the NIP has a wider pore size while its surface area is smaller compared to that of the MIP. This might be an indication that the cavities of the NIP were not controlled by the imprinting molecule. Fig. 2 shows the FTIR spectra of the venlafaxine imprinted polymer and the

venlafaxine imprinted polymer with extracted target compounds. The spectra for both MIPs exhibited amongst others the presence of important bands at 1155 cm^{-1} (C–O stretch), 1463 cm^{-1} (C=C aromatic stretch), 1725 cm^{-1} (C=O stretch), 2997 cm^{-1} (C–H stretch) and 3552 cm^{-1} (O–H stretch). However, it can be observed that the transmittance of the bands weakens on the spectrum of the polymer with the extracts. This could be attributed to the fact that the monomer functional groups on the cavities are interacting with the functional groups of the adsorbed analytes leading to lower vibrational frequency. In addition, the spectrum for the MIP bound with analytes shows the N-H stretch at a range of 2035 - 2162 cm^{-1} resulting from pseudo NH_3^+ ions due to the interactions of the hydrogen of the monomer and the NH_2 of the analytes. These spectra depict that extraction through the MIP cavities was successful.

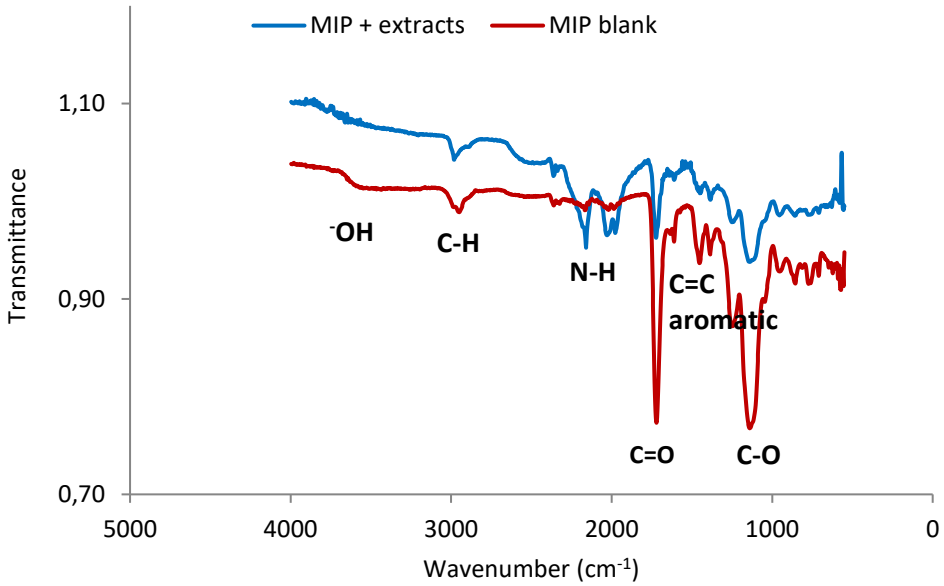


Figure 2 The FTIR spectra of the venlafaxine imprinted polymer, and the polymer with extracted etilefrine, carbamazepine, methocarbamol, nevirapine and venlafaxine

Thermal stability results of the MIP are given in Fig. 3. The results show that the polymer can be applied under extreme environmental conditions without degradation. It can be noted that the initial degradation of the polymer starts happening between 318 and 439°C for the MIP with analytes resulting in 84 %wt loss, and at 449°C for the MIP without analytes resulting in 87 %wt loss of the MIP. The pattern shows that there is further decomposition at a range of 521 and 567°C for the MIP with analytes resulting in 9 %wt loss, and between 524 and 586°C for the MIP without analytes resulting in 6 %wt loss of the polymer.

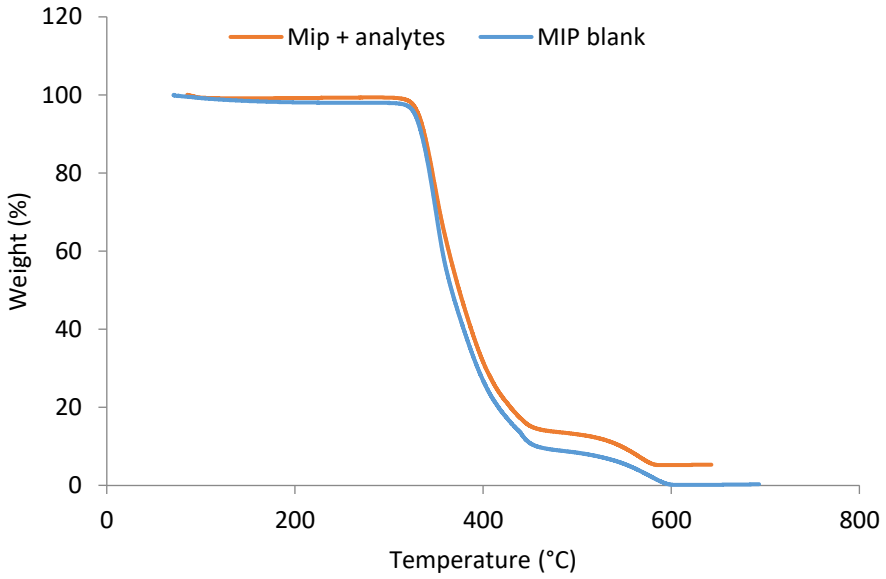


Figure 3 Thermo-gravimetric analysis of the venlafaxine imprinted polymer and the venlafaxine polymer with extracted pharmaceutical analytes

4.3.2 Effect of sample pH

The sample pH may lead to ionization of target compound and in turn affect the analyte binding to MIP cavities. In this work, it was observed that extraction efficiencies of all the target pharmaceutical compounds were greatly affected by the sample pH. Fig. 4 shows that maximum adsorption of target compounds was attained at pH 6. The pKa values of the targeted compounds given in Table 1 indicate that in acidic conditions these compounds get protonated at the basic nitrogen and be in their positively charged form. When the pH of the solution is increasing, these basic nitrogens exist in their neutral state which is favourable for binding with the oxygen of the p-vinyl benzoic acid on the MIP cavities through hydrogen bonding. However, the carboxylic groups of the compounds exist in their neutral form in the acidic region while in the basic region they are negatively charged. In this regard, it was observed that optimum binding occurred at pH 6 for most of the compounds. The observed reduction in binding under extreme acidic conditions might also be attributed to competition with the hydronium ions.

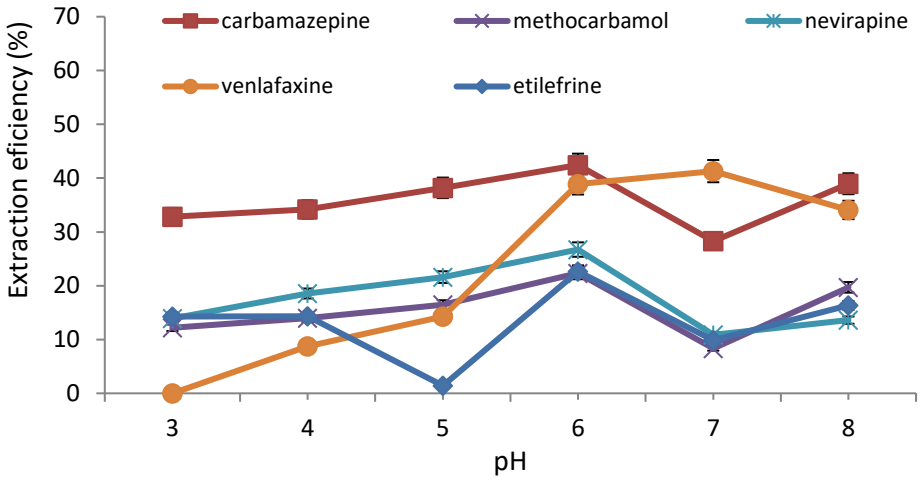


Figure 4 Effect of sample pH on the removal of target pharmaceutical compounds in aqueous medium

4.3.3 Batch adsorption

4.3.3.1 Effect of initial concentration

The adsorption isotherm modelling results depicting the effect of initial concentration are summarized in Table 2. It was observed that the linearized Freundlich model was favored with higher R^2 values ranging from 0.9071 – 0.9989 compared to 0.6452 – 0.9467 for the Langmuir model. This assumes heterogeneity of the binding surface energies of the MIP resulting in multiple interactions between the targeted pharmaceuticals and the MIP. This was expected since all five compounds either consist of an amine group or an alcohol and a benzene ring(s). Interactions expected to be taking place are hydrogen bonding between the hydrogen of the carboxylic group of p-vinylbenzoic acid and nitrogen of the compounds or the oxygen of the hydroxyl group. Once the carboxylic group of the monomer has been occupied, bonding could still take place through the π-π interactions based on the benzene rings.

4.3.3.2 Effect of contact time

The experimental adsorption capacities were obtained at the plateau region of the plots of adsorption capacity versus contact time. The plots were obtained by fitting a polynomial of the 3rd order through the experimental data points. The plot for adsorption of etilefrine is shown in Fig. 5. The experimental binding capacity of the MIP towards the target pharmaceutical compounds ranged from 131 - 418 ng mg^{-1} (Table 3). These matrix-matched binding capacity values were slightly lower than those obtained using spiked de-ionized water which themselves ranged from 199 - 475 ng mg^{-1}. Similar experiments were done with a NIP in order to determine the imprinting factors (IF). The results are shown in Table 3.

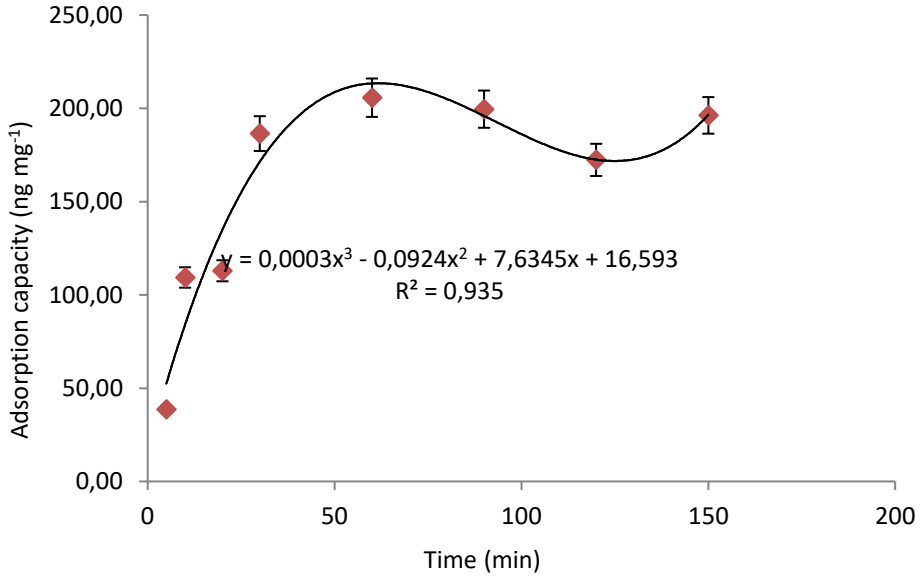


Figure 5 Effect of contact time on adsorption capacity of the MIP towards etilefrine

The parameter values of the kinetic models obtained from the plots of adsorption capacity as a function of contact time are given in Table 3. The R^2 values for the pseudo first order ranged from 0.5748 - 0.9510 while for the pseudo second order the R^2 values ranged from 0.9231 - 0.9968. These results showed that the adsorption process followed the pseudo second order kinetic modelling, indicating that the interaction between the MIP and the targeted pharmaceutical compounds was through chemisorption. This is in agreement with the Freundlich adsorption isotherm which suggested that the adsorption was through multiple interactions. The results for predicted adsorption capacities through the pseudo second order isotherm are also in agreement with the experimental values, as opposed to the ones predicted through the pseudo first order isotherm. Good precision was observed on the experimental results which is indicated by the relative standard deviations ranging between 2.1 and 9.3% for triplicate analysis. Results for the Weber-Morris plot are summarized in Table 3 for all target compounds. The results show the I_d values to be greater than zero which

indicate that the rate limiting step was due to both external mass transfer and intra-particle diffusion. Fig. 6 shows a plot of qe vs $t^{0.5}$ with two linear regions, with the first region being controlled by the external transfer of the target compounds from solution to the surface of the MIP, and the second region being described by the gradual adsorption of the analytes until a plateau is achieved.

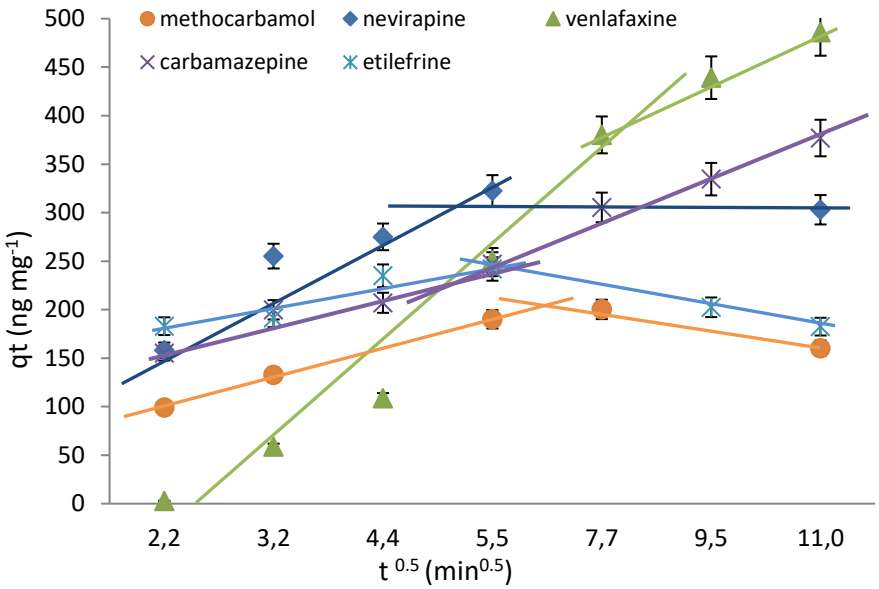


Figure 6 Weber-Morris plot for adsorption of carbamazepine, methocarbamol, nevirapine, venlafaxine and etilefrine on the venlafaxine imprinted polymer

Table 2 Adsorption modelling parameters using venlafaxine-imprinted polymer

	Freundlich isotherm			Langmuir isotherm		
	n	K_f	R^2	q_m	b	R^2
Carbamazepine	0.404	23.9	0.9081	232.6	3.69×10^{-3}	0.9151
Etilefrine	1.095	3.2	0.9171	204.1	4.33×10^{-3}	0.6452
Methocarbamol	0.909	3.6	0.9989	303	1.85×10^{-3}	0.9467
Nevirapine	1.042	2.8	0.9071	188	3.19×10^{-3}	0.8765
Venlafaxine	1.141	1.8	0.9902	471	3.41×10^{-3}	0.7145

n - intensity of the adsorption, K_f - Freundlich isotherm constant, R^2 - the determination co-efficient, q_m - maximum adsorption capacity, b - Langmuir constant

Table 3 Kinetic modelling parameters for matrix matched optimization using venlafaxine-imprinted polymer

	Pseudo first order			Pseudo second order			Experimental values (n = 3, RSD)			Intraparticle diffusion model		
Analyte	q_e (ng mg^{-1})	K_1 (min^{-1})	R^2	q_e (ng $mg^{-1)}$)	K_2	R^2	Q_{MIP} (ng mg^{-1})	Q_{NIP} (ng mg^{-1})	IF	R^2	K_p (ng mg^{-1} $min^{-0.5}$)	I_d (ng mg^{-1})
Carbamazepine	36.4	6.91×10^{-3}	0.6852	250	2.07×10^{-3}	0.9959	418.1 ± 6.9	279.8 ± 9.3	1.5	0.9769	23.09	118.8
Etilefrine	118	2.40×10^{-2}	0.9510	115.5	3.29×10^{-4}	0.9811	205.7 ± 2.1	128.9 ± 9.1	1.6	0.9304	20.30	35.2
Methocarbamol	69.4	9.60×10^{-3}	0.9040	109.9	3.80×10^{-4}	0.9231	131.0 ± 2.8	73.96 ± 4.2	1.8	0.9001	18.40	69.9
Nevirapine	27.9	5.98×10^{-3}	0.5748	147.1	2.83×10^{-3}	0.9968	299.1 ± 7.6	183.4 ± 6.1	1.6	0.8875	45.84	77.5
Venlafaxine	76.6	7.60×10^{-3}	0.9289	208.3	4.84×10^{-4}	0.9781	296.0 ± 5.1	168.8 ± 6.5	1.8	0.9736	63.69	-138.5

q_e is the predicted equilibrium adsorption capacity, K_1 and K_2 are rate constant for pseudo first order and second order adsorption respectively, R^2 is the measure of linearity. Q_{MIP} and Q_{NIP} are the experimental maximum adsorption capacities for the MIP and NIP respectively, IF is the imprinting factor. K_p intial rate of the intra-particle particle diffusion, I_d is the constant relating to the rate limiting step.

4.3.4 Effect of elution solvent

The results in Fig. 7 show that methanol was the best elution solvent for the study with higher recoveries for targeted pharmaceuticals. This is agreement with most studies where methanol was selected as the MIP elution solvent for nevirapine [41], carbamazepine [42, 43], methocarbamol [44, 45] and venlafaxine [34, 46]. Methanol utilizes its elution strength through competing with the target pharmaceutical compounds for hydrogen bonding at the MIP surface. On the other hand, acetonitrile is aprotic in nature and does not form hydrogen bonds with anionic nucleophiles. All the elution solvents were spiked with 1 %v/v formic acid to enhance the disruption of the hydrogen bonds as well as aid in ionization of the analytes during LC-MS determination. Toluene being a less polar solvent was found to be less effective as an elution solvent for the target compounds.

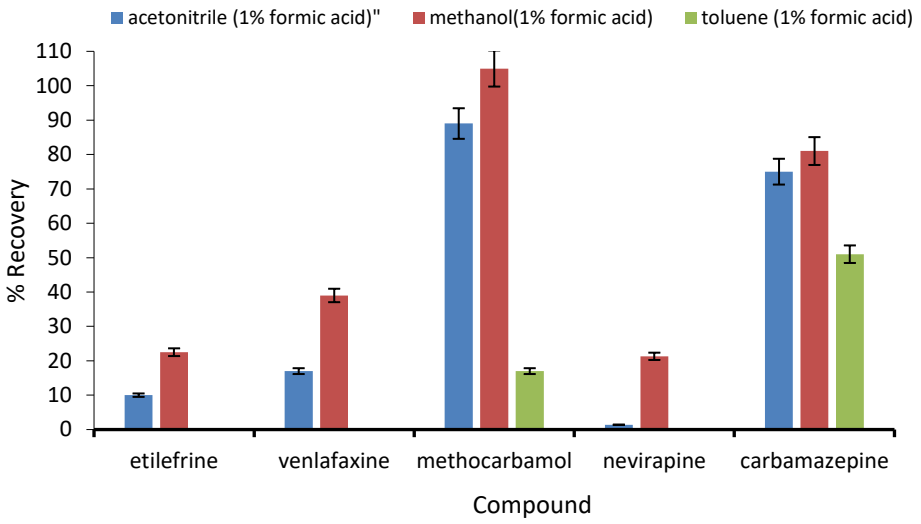


Figure 7 Performance of three selected solvents in the elution of the targeted pharmaceutical drugs from the MIP cavities

4.3.5 Applicability of the synthesized polymer in environmental water samples

Fig. 8 shows mass spectra which reports on the fragment ions present for each targeted compound. It can be observed that the major characteristic fragment ions are: 164 (m/z) for etilefrine, 121 (m/z) for venlafaxine, 118 (m/z) for methocarbamol, 198 and 226 (m/z) for nevirapine and 194 (m/z) for carbamazepine. In order to confirm the presence of target compounds, major fragmentation ions together with the chromatographic retention times reported in Table 4 were used. The use of this technique aided in the selectivity of the LC-MS method for application in dam water samples with relatively high matrix effects.

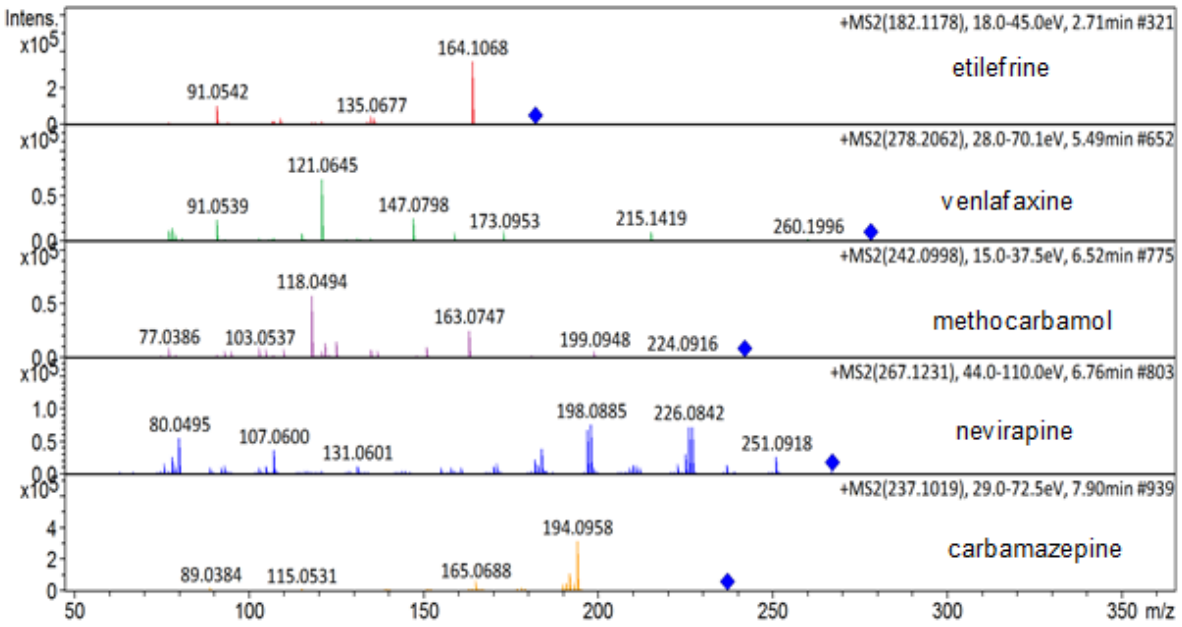


Figure 8 MS spectra of the major fragmentation ions for the selected model pharmaceutical compounds

The parameters used in validating the applicability of the synthesized MIP as an SPE adsorbent in the analysis of targeted pharmaceuticals are summarized in Table 4. The

linearity of the standard calibration curves for the analytes was evaluated over the concentration range of 0.5 - 100 ng mL^{-1} for etilefrine and carbamazepine and 5 - 100 ng mL^{-1} for the other compounds. The R^2 values ranged from 0.9958 - 0.9996, and the recoveries from 43 - 64% for the 0.5 ng mL^{-1} spiked samples and 52 - 69% for 1 ng mL^{-1}. These recoveries are generally lower than those reported for MIPs targeting single analytes used in this study as model compounds. For example, the reported recoveries from MIPs for nevirapine, carbamazepine, methocarbamol and venlafaxine respectively ranged from 89.7 – 98.6% [41], 75 – 101% [42, 43], 92.2 – 96.9% [44, 45] and 84 – 102% [34, 46]. However, the recoveries reported in this study are relatively favourable considering that the focus was on utilizing MIP cross-selectivity using five model compounds belonging to different classes of pharmaceuticals.

The relative standard deviations were found to be less than 9% for triplicate analysis. This was an indication of good precision. The matrix-matched LODs reported in this study (0. 034 - 0.31 ng mL^{-1}) are comparable with other studies reporting analysis of the selected pharmaceutical using an SPE-LC-MS technique including 0.092 ng mL^{-1} for analysis of nevirapine in water samples [38], 3 ng mL^{-1} for venlafaxine [34], 0.04 ng mL^{-1} for methocarbamol [47], 100 ng mL^{-1} for carbamazepine [48].

Table 4 SPE method performance in environmental water samples (n = 3)

		Calibration		Method limits		Recoveries (% ± RSD)	
Compound	t_R min	Range (ng mL^{-1})	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	0.5 ng mL^{-1} spike	1 ng mL^{-1} spike
Carbamazepine	7.90	0.5 - 100	0.9958	0.040	0.13	64 ± 2.9	56 ± 5.1
Etilefrine	2.67	0.5 - 100	0.9981	0.034	0.12	58 ± 3.1	52 ± 6.4
Methocarbamol	6.57	5 - 100	0.9993	0.31	1.03	54 ± 4.2	58 ± 3.1
Nevirapine	6.79	5 - 100	0.9996	0.21	3.81	43 ± 2.9	58 ± 6.9
Venlafaxine	5.51	5 - 100	0.9972	0.30	1	60 ± 1.1	69 ± 8.9

4.4 Conclusions

The study has successfully synthesized a venlafaxine imprinted smart polymer with a high selectivity towards different classes of pharmaceuticals with good extraction efficiencies from surface water samples. Venlafaxine as an imprinting molecule was chosen through cavity tuning experiments in which pharmaceuticals from other classes were also investigated. The target pharmaceuticals included an antiretroviral, an antidepressant, a muscle relaxant, an anticonvulsant and a cardiac stimulant. The synthesis approach is simple, fast and inexpensive. The results obtained show emphasis on utilization of MIP cross selectivity to target multiple analytes from complex aqueous samples. Preliminary studies using spiked dam samples have also indicated that MIP cross selectivity can be an essential approach in the monitoring of organic compounds belonging to different classes in environmental water bodies.

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Conflict of interests

The authors declare that there is no conflict of interests

Availability of data

Not Applicable

Code Availability

Not Applicable

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4 PAPER 2

This paper entitled “Multivariate optimization of a two-way technique for extraction of pharmaceuticals in surface water using a combination of membrane assisted solvent extraction and a molecularly imprinted polymer” has already been published in *Chemosphere* journal. The paper presents a development and multivariate optimization of a two way technique based on membrane assisted solvent extraction-molecularly imprinted polymer (MASE-MIP) for the extraction of five model pharmaceuticals belonging to different therapeutic classes.

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Sinegugu Khulu – Principal Author

Somandla Ncube – Co-Supervisor

Yannick Nuapia - Co-author

Lawrence Mzukisi Madikizela - Co-author

Hlanganani Tutu - Co-author

Heidi Richards - Co-author

Kuria Ndungu - Co-author

Elizabeth Mavhunga – Co-author

Luke Chimuka – Main Supervisor

Multivariate optimization of a two-way technique for extraction of pharmaceuticals in surface water using a combination of membrane assisted solvent extraction and a molecularly imprinted polymer

Sinegugu Khulu [a,b], Somandla Ncube [c], Yannick Nuapia [a,d], Lawrence Mzukisi Madikizela [a,e], Hlanganani Tutu [a], Heidi Richards [a], Kuria Ndungu [f], Elizabeth Mavhunga [b], Luke Chimuka [a*]

[a] Molecular Sciences Institute, University of Witwatersrand, Private Bag X3, Johannesburg, 2050, South Africa

[b] School of Education, University of Witwatersrand, Private Bag X3, Johannesburg, 2050, South Africa

[c] Department of Chemistry, Sefako Makgatho Health Sciences University, P.O Box 60, Medunsa, 0204, South Africa

[d] School of Animal, Plant, & Environmental Science, University of Witwatersrand, Johannesburg, South Africa

[e] Institute for Nanotechnology and Water Sustainability, College of Science, Engineering and Technology, University of South Africa, Florida Science Campus, 1710, South Africa

[f] Norwegian Institute for Water Research-NIVA, Gaustadalleen 21, 0349, Oslo, Norway

[*] Luke.Chimuka@wits.ac.za

Abstract

This work demonstrates development and evaluation of a two-way technique based on the combination of membrane assisted solvent extraction and a molecularly imprinted polymer (MASE-MIP) for selective and efficient extraction of five selected pharmaceuticals belonging to five different therapeutic classes. The pharmaceuticals were extracted from surface water samples followed by liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-qTOF/MS) determination. A central composite design was applied to optimize the influence of the sample salt content, the stirring rate, the stirring time and the amount of MIP on the extraction of an anticonvulsant (carbamazepine), a cardiac stimulant (etilefrine), a muscle relaxant (methocarbamol), an antiretroviral (nevirapine) and an antidepressant (venlafaxine) from surface water. Optimization of the analytical method was performed by spiking water with a mixture of all five pharmaceuticals at 500 ng mL^{-1}. Optimum extraction conditions for a sample volume of 18 mL were found to be 5 g of salt content, a stirring rate of 400 rpm, an extraction time of 60 min and 50 mg of MIP. The MASE-MIP-LC-qTOF/MS method gave detection and quantification limits ranging from 0.09 to 0.20 ng mL^{-1} and 0.31 to 0.69 ng mL^{-1}, respectively. The spiked river water samples yielded recoveries ranging from 38 to 91% for the selected model compounds belonging to the five classes of pharmaceuticals. Upon the application of the developed analytical method in water analysis, all selected pharmaceuticals were detected in South African river water with nevirapine and venlafaxine being more prominent attaining the maximum concentrations of 1.64 and 2.48 ng mL^{-1}, respectively.

Keywords: Molecularly imprinted polymer; membrane assisted solvent extraction; pharmaceuticals; nevirapine; environmental monitoring

4.1 Introduction

The extensive usage of pharmaceuticals has seen them being classified as emerging environmental contaminants owing to the health risks associated with exposure of aquatic life to these compounds [1–4]. Antidepressants and muscle relaxants such as venlafaxine and methocarbamol, respectively may be associated with neurobehavioral disorders in aquatic animals with reports of neuroendocrine disruption resulting to reduced aggressive nest defence and distracted feeding patterns in exposed fish [5, 6]. The unavoidable usage of pharmaceuticals has gained them increasing global consciousness and therefore, regulatory bodies are in the process of establishing maximum permissible concentration levels for these contaminants in the water system [1].

The growth of human settlements and industries has been identified as great contributors towards surface water pollution in rural and urban areas [7]. This is attributed to poor sanitation in most African regions, inaccessibility of modern ablution facilities and improper disposal of expired or unused medications which then contribute to the direct contamination of surface water with pharmaceuticals [8]. However, the primary contributor of pharmaceuticals in the environment has been identified to be the wastewater treatment plants (WWTPs), which receive high quantities of pharmaceuticals through human urinary and faecal excretion [8–13]. Deteriorating water quality is one of the primary threats to South Africa's ability to provide sufficient water of appropriate quality to meet its demands and to ensure environmental sustainability [14]. There has been ongoing research in South Africa for monitoring the water quality where pharmaceuticals have been identified as possible water contaminants [15, 16]. Globally, through advancements of sensitive analytical instrumentation and methods for trace analysis of pharmaceuticals, researchers have been able to do water quality studies especially those related to pollution with these compounds in the environment [12]. Environmental monitoring of pharmaceuticals requires efficient and selective analytical methods which allow

for determination of multiple compounds in a single run [17, 18] since these pollutants exist as mixtures in the environment.

Sample treatment is still considered one of the crucial steps in the analysis of pharmaceuticals from environmental water samples [19]. Its objective is mainly to reduce matrix effects and preconcentrate (trace enrichment) the analyte(s), reduce sample size and the amount of organic solvents used [20, 21]. Solid-phase extraction (SPE), which involves the use of sorbents such C18-bonded silica has been used extensively in sample preparation. However, these sorbents have limited selectivity, are rather expensive and designed for single use [21]. In this regard, for increased selectivity and reusability, molecularly imprinted polymers (MIPs) were introduced [22]. Suitably, MIPs have been broadly applied as sorbents in SPE, referred to as molecularly imprinted solid-phase extraction (MISPE), allowing increased selectivity towards targeted compounds prior to determination [23, 24]. In samples with high matrix effects, MIPs tend to lose their recognition abilities after a few extractions. Recent work shows a use of porous membrane as a protective sleeve to be a simple approach to prevent matrix effects into accessing the sorbent [20]. The combination known as membrane assisted solvent extraction-molecularly imprinted polymer (MASE-MIP) is highly advantageous as it combines separation ability of the membrane (filtration of high molecular weight interfering compounds and particulate matter) with the high selectivity of the MIP [20, 21].

In our previous work, we synthesized a MIP with an affinity for five pharmaceutical compounds belonging to different therapeutic groups. The five groups of pharmaceuticals and their model compounds were a cardiac stimulant (etilefrine), an anticonvulsant (carbamazepine), an antiretroviral (nevirapine), a muscle relaxant (methocarbamol) and an antidepressant (venlafaxine) [25]. Table S1 adapted from our previous work summarizes physiochemical properties and biota health side effects if exposed to the selected pharmaceuticals. The current study was aimed at combining the MIP with the MASE technique, referred to as the MASE-MIP

technique for the extraction of selected pharmaceuticals from complex river water samples impacted by improper dumping of solid waste. The MASE-MIP was optimized for various significant experimental parameters using a multivariate approach with the view of obtaining good extraction efficiencies for all targeted pharmaceuticals. In this regard, the MASE-MIP combination utilized the cross-selectivity of the synthesized venlafaxine MIP whilst preventing co-extraction of larger molecules to yield cleaner extracts and increased selectivity. Furthermore, a comparison between the MASE-MIP and the MASE technique was done, based on their applicability to real surface water samples. Finally, the developed analytical method comprising of MASE-MIP combined with LC-qTOF/MS was applied in environmental monitoring of selected pharmaceuticals in two important South African rivers.

4.2 Materials and Methods

4.2.1 Chemicals and reagents

Analytical grade venlafaxine hydrochloride (≥98%), methocarbamol, etilefrine hydrochloride, nevirapine, and carbamazepine, p-vinylbenzoic acid (97%) were all obtained in powder form from Sigma-Aldrich, Johannesburg, South Africa, while ethylene glycol dimethacrylate (98%) was in liquid form. UHPLC grade methanol (≥99.9%), dimethyl sulfoxide, acetonitrile (99.8%), toluene (99.7%) and formic acid (≥95%) were also purchased from Sigma-Aldrich, Johannesburg, South Africa. Ethyl acetate (99%), chloroform (99%) and cyclohexane (99 %) were purchased from Associated Chemical Enterprises (ACE), Johannesburg, South Africa.

4.2.2 Synthesis of the molecularly imprinted polymer

Synthesis of MIP was adopted from our previous work [25]. This was initiated by dissolving 15 mg of template (venlafaxine) in 3 mL of dimethyl sulfoxide at 40 °C for 5 min while stirring. Functional monomer, p-vinyl benzoic acid (30 mg) was then added into the mixture while heating for a further 10 min. The reaction temperature was then raised to 60 °C and the cross-linker, ethylene glycol dimethacrylate (167 µL),

was added to the reaction mixture while stirring and heating for a further 10 min. A 10 mg mass of an initiator, 1, 1´–azobis-(cyclohexanecarbonitrile), was added to the mixture and the system was purged with nitrogen gas for 5 min. Lastly, the reaction temperature was raised to 80 °C to achieve polymerization. MIP cavities were generated by washing the polymer with methanol for removal of the template. The synthesized MIP is known for its high affinity for all the five model pharmaceuticals with the maximum matrix-matched adsorption capacities for individual pharmaceuticals ranging between 206 and 418 ng mg^{-1} and an extraction efficiency of 43 - 69% for carbamazepine, etilefrine, methocarbamol, nevirapine and venlafaxine. The MIP attains its maximum adsorption capacity within 80 min when it is in direct contact with the pharmaceuticals.

4.2.3 Instrumentation and apparatus

MIP cavities were created by removing the template through excessive washing of the MIP with methanol. Efficient removal of the template from the MIP was attained using a temperature-controlled WiseCube WIG-105 Precise Shaking incubator supplied by Wisd® Laboratory Instruments (Wertheim, BW, Germany). Centrifugation of samples was done through a Rotofix 32A centrifuge from Hettich® (Frankfurt, Hessen, Germany). Deionized water was produced through a Millipore DirectQ 3 UV waters system-2345 from Millipore (Massachusetts, USA). The polypropylene membrane bags (4 cm long, 0.03 mm wall thickness, 6 mm internal diameter) were purchased from Chemetrix Export (Johannesburg, SA) and 1 mL (6 mm internal diameter) empty syringe with 10 µm frits were purchased from ANATECH Instruments (Johannesburg, SA).

The UHPLC system used for the separation and analysis of compounds was the Thermo Scientific Dionex Ultimate 3000 from ThemoFisher Scientific (Waltham, MA, USA). The instrument consisted of a HPG-3200RS pump and a WPS-3000TRS autosampler. The UHPLC was coupled to quadrupole time-of-flight compact mass spectrometer system from Bruker Daltonics Inc. (Billerica, MA, USA). Bruker Compass

DataAnalysis version 4.3 (Build 110.102.1532) was used to collect and process qualitative data, while Bruker Compass QuantAnalysis version 4.3 (Build 110.102.1532) was used for collection and processing of quantitative data. Chromatographic separation of analytes was achieved on a Luna Omega C18 column (50 x 4.6 mm, 3 μm) from Phenomenex (Torrance, CA, USA). Separation was through gradient elution using solvent A (0.1 % formic acid in deionized water) and solvent B (0.1% formic acid in acetonitrile). During the gradient elution, solvent B was initially kept at 5% for 1 min. This was followed by ramping until it reached 55% at 6 min and kept constant for 2 min. It was further ramped until it attained 95% at 10 min and kept constant for another 2 min. Lastly, the gradient was dropped to 5% of solvent B for a minute and kept constant for a further 1 minute [25]. All five pharmaceuticals were separated within 14 min.

4.2.4 MASE preparation

Cyclohexane was used to pre-condition the extraction membranes. This was achieved by shaking the membranes at 150 rpm containing cyclohexane for approximately 3 h. This step was repeated 3 times with fresh portions of cyclohexane. The metal cylinders for the membranes were not removed to ensure that the inside walls were also conditioned. After pre-conditioning, the membranes were dried out and then soaked in an extraction solvent for at least 2 h. The membranes were checked for leakages by filling them with the extraction solvent.

4.2.5 General MASE-MIP extraction procedure

The MASE-MIP system was adapted from our previous work [26]. The set up as shown in Fig. S1, consisted of a 20 mL headspace vial filled with 18 mL deionized water spiked with 0.5 μg mL^{-1} concentration of the targeted pharmaceuticals. The membrane bag was attached to a metal funnel and fixed with a PTFE ring. The membrane bag was then filled with MIP particles and 1mL of an organic acceptor solvent. The membrane bag containing MIP particles mixed with the acceptor phase was placed inside the extraction cell containing 18 mL of aqueous sample and stirred for pre-set times. After

extraction, the acceptor content (MIP and the acceptor solvent) was transferred into a 1 mL empty cartridge with a 10 µm frit at the bottom and mounted onto an SPE unit. The solvent was separated from the MIP particles by opening a valve and allowing it to flow out of the cartridge through gravity. A steady vacuum pump was then applied for 1 min to dry off the MIP particles completely. The targeted pharmaceuticals were then eluted from the MIP cavities using 4 × 1 mL of methanol. Finally, the solvent extract was reduced to 1 mL by purging with a gentle stream of nitrogen prior to injection to the LC-qTOF/MS for identification and quantification of pharmaceuticals.

In the optimized method, extraction was performed by filling the membrane bag with 50 mg of MIP particles, 18 mL saturated with 5 g of NaCl at a stirring rate of 400 rpm for 60 min. Membrane bags were re-used during optimization since extraction was done on pure water samples. The used membrane bags, prior to the conditioning step were first soaked into a solution of 50:50 %v/v acetonitrile and water to remove sodium chloride for about 3 h.

In the MASE procedure, the same steps were followed except that the acceptor phase consisted of acceptor organic solvent only. After extraction, the organic acceptor solvent was reduced to almost dryness and the contents of the sample vial were reconstituted with 0.5 mL methanol prior to injection into the LC-qTOF/MS.

4.2.6 Optimization of extraction parameters

4.2.6.1 Experimental design

Three solvents namely: toluene, ethyl acetate and chloroform were screened as possible acceptor solvents. When the best extraction solvent was chosen, a central composite design approach was then adapted for the optimization of the MASE-MIP technique using MODDE Pro (Sartorius Stedim Biotech, Malmö, Sweden). The experiment design comprised of 27 experimental runs. In this case, a central point of the 27 experiments had three replicates with the same experimental conditions. The screened variables were the sample salt content (0 - 5 g), the stirring rate (400 – 1200 rpm), the stirring time (10 – 90 min) and the amount of MIP (10 - 60 mg). A central point of the

27 experiments had three replicates. Table S2 shows the experimental conditions with the corresponding extracted concentrations of the selected pharmaceutical compounds.

4.2.6.2 Model fitting and predictive efficiency

The predictive efficiency and model fitting between the predicted and the experimental data at optimum extraction conditions were investigated by using the root mean square error (RMSE), mean absolute error (MAE), standard error of prediction (SEP), model predictive error (MPE), chi-square statistic (χ^2), and correlation coefficients (R^2). Table S4 describes the equations corresponding to the calculation of these factors.

4.2.7 Validation and application of the MASE-MIP technique

In this step, the applicability of the optimized MASE-MIP technique for the extraction of the selected pharmaceuticals from surface water samples was investigated. Surface water samples were collected in 1 L bottles from 10 different sites in the KwaZulu-Natal and the Gauteng provinces of South Africa and were transported to the laboratory in cooler boxes. These were immediately filtered through 0.45 μm filters. Prior to LC-qTOF/MS analysis, selected pharmaceuticals were extracted using the two sample preparation techniques (MASE-MIP and MASE) from the blank surface water samples and spiked samples (spiked with a mixture of 10 ng mL^{-1} of each targeted compounds).

The applicability of both the MASE-MIP and MASE methods in the determination of pharmaceuticals was calculated as extraction efficiency given as a percentage ratio of the amount spiked versus the amount recovered. Other validation parameters computed include relative standard deviation and R^2 values of each analyte.

4.2.8 Sampling and description of study area

In all cases, water samples were grabbed into pre-cleaned plastic containers during the month of October 2020. Two important South African rivers (one in the province of KwaZulu-Natal and the other in Gauteng) were selected as the model study sites. The selected KwaZulu-Natal River is well known as Umdloti River with its Indian Ocean estuary situated approximately 25 km North East of Durban city. Hazelmere Dam

which serves as the recreational site and water storage facility for irrigation and domestic use is situated approximately 20 km upstream of the estuary (about 5 km upstream of the town of Verulam) [14]. The first Umdloti River water sample 1 (S1) was collected in the upstream of the Hazelmere Dam, while sample 2 (S2) was in the downstream of the dam wall. As shown on the Map (Fig. 1), S1 is in a rural location surrounded by lots of vegetation and few houses, while there is more population residing in the vicinity of S2. From Hazelmere Dam, Umdloti River flows through the industrial area (known as Canelands) before it reaches Verulam town. Sample 3 (S3) was collected in the downstream of the Verulam town before the river reaches the Verulam WWTP which discharges its effluent into Umdloti River. Sample 4 (S4) was in the downstream of the Verulam WWTP and finally Sample 5 (S5) was taken from the estuary. As shown in supplementary data (Fig S2 (b)), the river in the S4 is covered with water hyacinth which is a result of eutrophication due to the industrial discharges into the upstream of the river and the contaminated WWTP effluent.

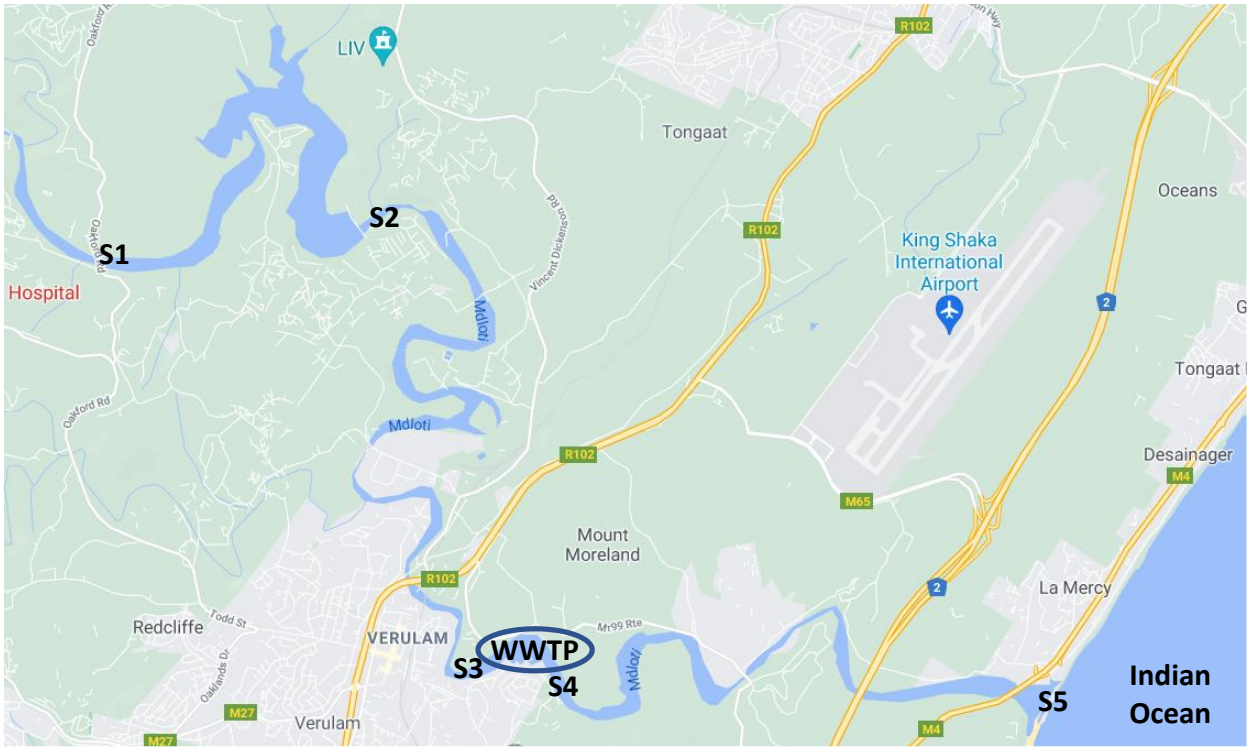


Figure 1 Sampling area – Umdloti River, KwaZulu-Natal Province, South Africa

Five sampling sites were selected along the Hennops River that flows in the Gauteng Province. As shown on the map (Fig. 2), the first sampling site (S1) was in Tembisa Township in the East of Johannesburg, a city that is considered as the economic hub of South Africa. Tembisa Township is a highly populated residential area with a large number of people residing in informal settlements along the Hennops River. Lack of proper ablution facilities and dumpsites for solid materials is evident in the Tembisa Township. As shown in supplementary data (Fig S2 (c) and (d)), Hennops River in Tembisa Township is highly polluted with solid garbage and plastic materials. S2 was collected in Irene (a residential area). S3 was sampled under the bridge of M25 highway next to the Centurion mall towards Pretoria, a capital city of South Africa. Herein, the river flows in between the business area and the residential suburbs. Blackwood Road Bridge was taken as S4, while S5 was collected underneath the Witstinkhout Road. Only the residential suburbs are found in between S3, S4 and S5. But, in all these sampling sites the evidence of illegal dumping of solid waste along the river was present.

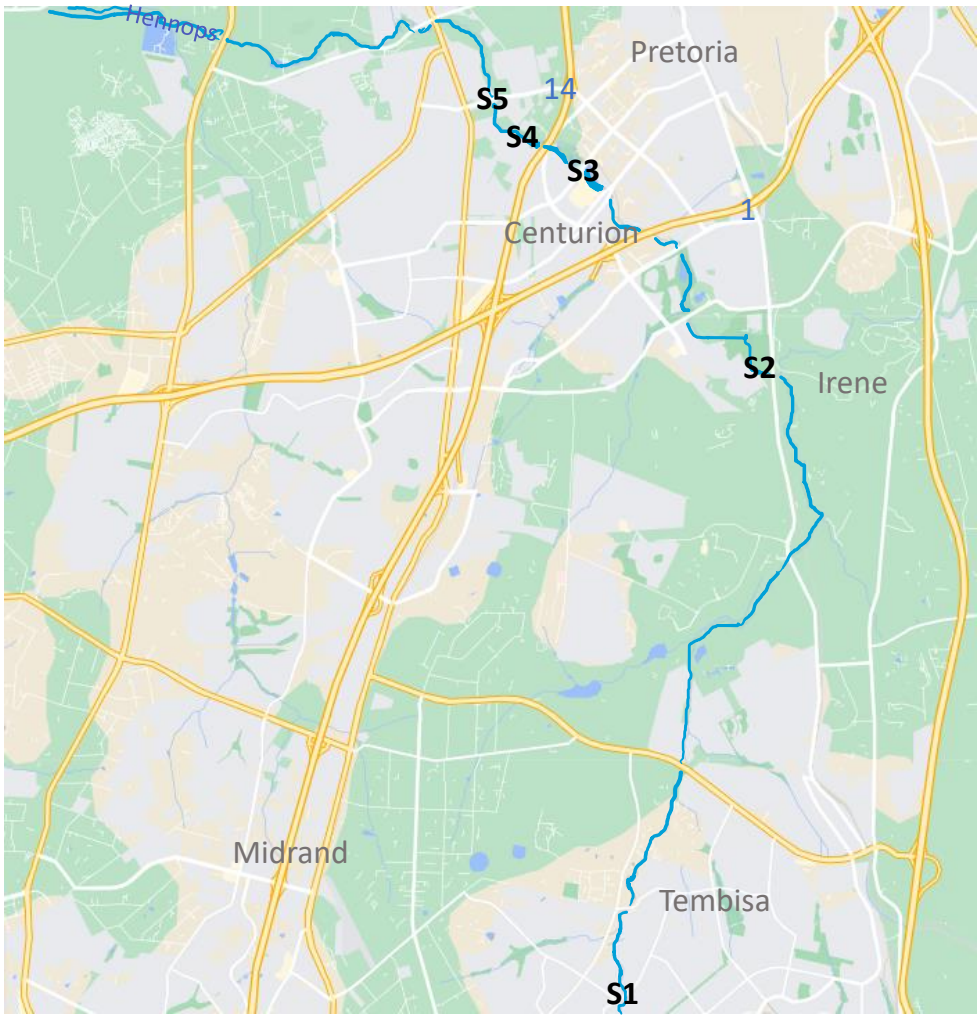


Figure 2 Sampling area- Hennops River, Gauteng Province, South Africa

4.3 Results and discussions

4.3.1 Optimization of extraction conditions

4.3.1.1 *Acceptor phase organic solvent*

A good solvent should yield a high partitioning coefficient of the analytes from the donor sample phase through the membrane bag into the acceptor phase, at the same time not interfere with the binding of the analytes onto the cavities of the MIP particles

[24]. For polar compounds slightly polar solvents should be used for the extraction. However, very polar solvents which are miscible in water like methanol may not be used as extraction solvent as they diffuse through the membrane into the aqueous donor during stirring which results in most of the acceptor phase being lost during extraction [27]. Based on this background, in this study two slightly polar solvents namely: ethyl acetate and chloroform and one not so polar solvent toluene were investigated as possible acceptor phase solvents for the extraction of the polar targeted pharmaceutical compounds. Fig. 3 reports on the amount of the targeted pharmaceuticals eluted from the MIP cavities after being extracted from the donor phase through the different tested solvents. The figure depicts that the target pharmaceuticals were better extracted with chloroform as opposed to the other two solvents. Further experiments were then conducted with chloroform as an acceptor phase solvent

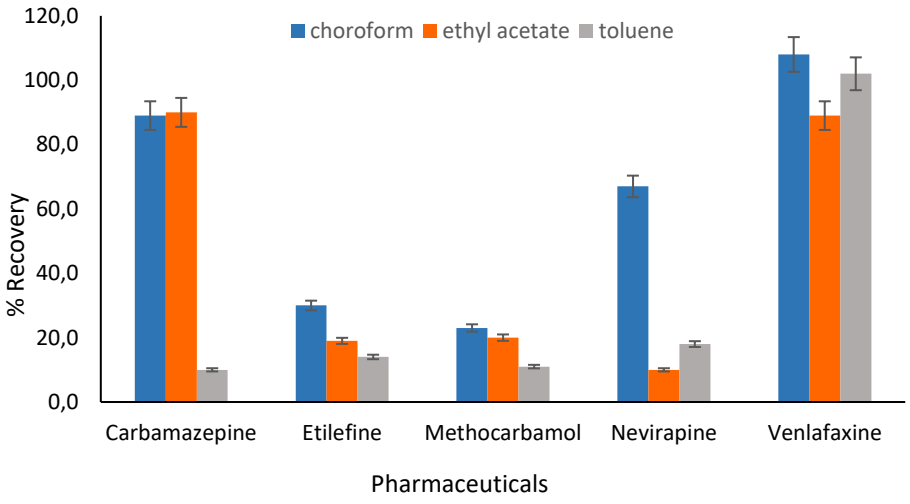


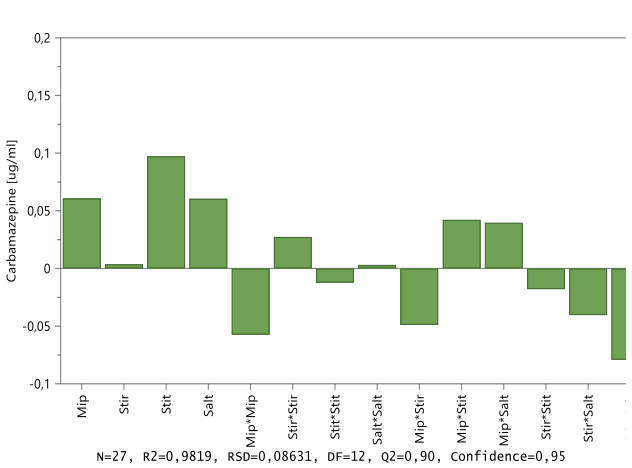
Figure 3 Performance of three selected solvents for extraction of the selected pharmaceuticals from the donor phase

4.3.1.2 Effect of selected experimental parameters on the extraction of the targeted pharmaceutical compounds

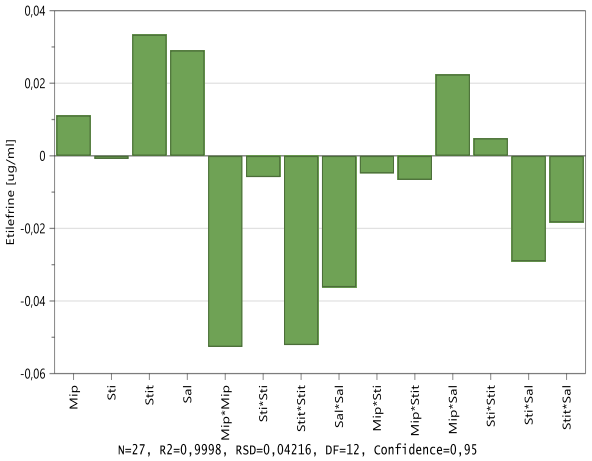
Several parameters were optimized to obtain maximum extracted concentration of the targeted compounds at equilibrium. It has been reported that various factors including surface area, pore size, pore distribution and the number of binding sites in the sorbent control the binding of compounds onto the MIP particles [19]. In most instances including the present study, an increase in the amount of polymer is found to be directly proportional to the uptake of targeted pharmaceuticals. Some of the selected parameters like the sample ionic strength and the solvent acceptor composition tend to influence factors like the movement and the rate at which analytes are transferred from the aqueous donor phase into the organic acceptor phase [28]. The rate at which the sample is stirred reduces the thickness of the membrane walls thus influencing the transitioning of analytes from the donor phase to the acceptor phase through the membrane [27–29]. Lastly the stirring time (extraction time) is important in establishing the time it takes for the partitioning equilibrium of analytes to be reached [29]. Based on this background, all these parameters were optimized in the current study.

The coefficient plots (Fig. 4) show the interaction of the selected parameters on the extraction of the target compounds. Fig. 4a - d show that the MIP amount, the extraction time and the salt content had a significant positive effect on the extraction of four pharmaceutical compounds with P-values lower than 0.05 as shown in Table S5. On the other hand, it can be observed that the stirring rate did not have a significant effect on the extraction of these pharmaceuticals giving P-values between 0.77 and 0.99 (Table S5). This means that the model did not observe an increase in the concentration extracted with an increase in stirring rate i.e. minimum stirring rate was enough to build pressure on the MASE-MIP cell for optimum extraction of these compounds. However, coefficient plot in Fig. 4e reveals that for venlafaxine only one factor (MIP amount) had a significant positive effect in its extraction (P-value 0.042). The other three parameters did not have a significant effect on the extraction of this pharmaceutical as

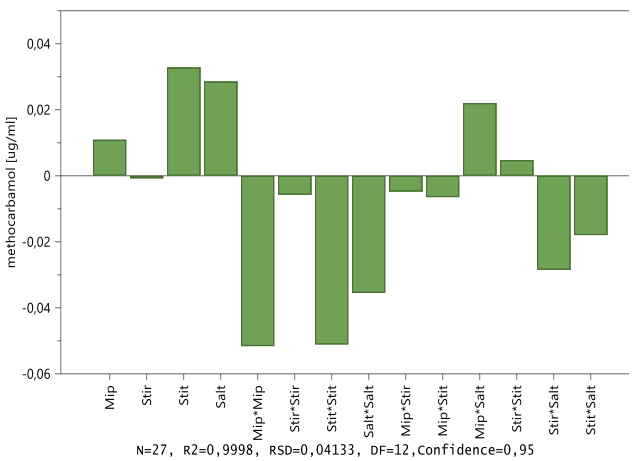
shown by P-values in the range of 0.70 – 0.77 (Table S5). This implies that increasing the amount of MIP will yield a higher recovery of venlafaxine, whilst increasing the other parameters may not have significant influence on the recovery of this compound. This is possibly because the MIP was imprinted with venlafaxine, therefore unlike the other compounds, it does not need as much time to attain its maximum adsorption. The relative standard deviation of the optimization model as presented on the coefficient plots ranged from 4 – 13%, which is an indication of good precision.



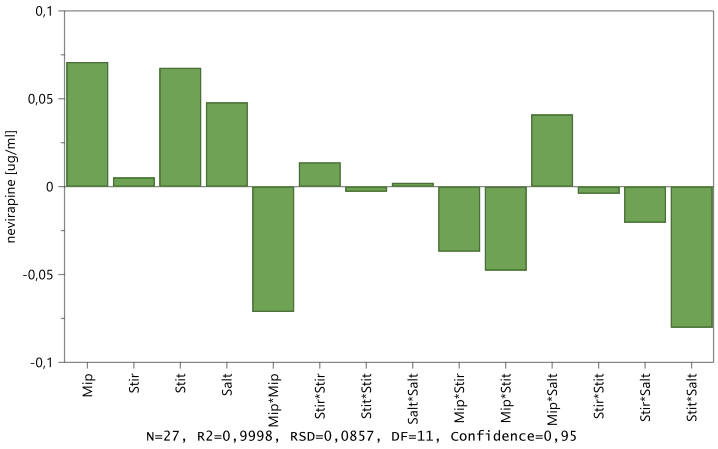
(a)



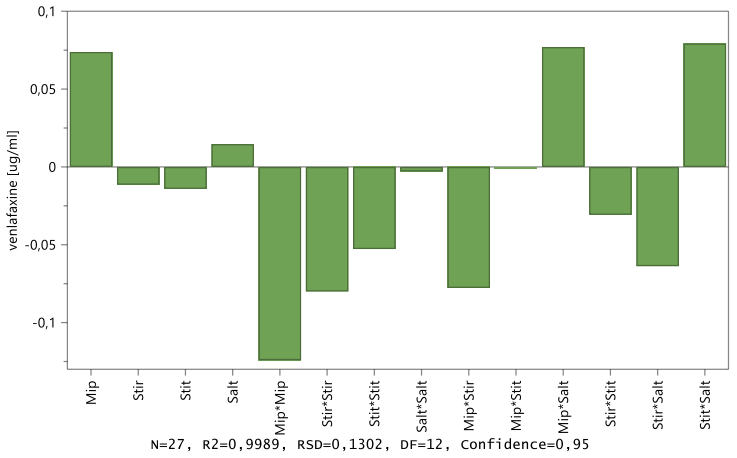
(b)



(c)

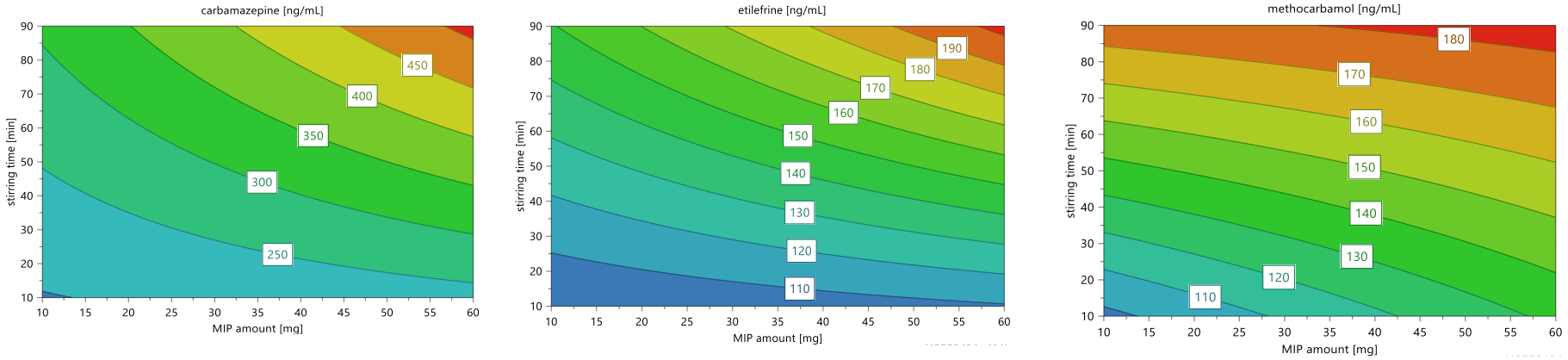


(d)

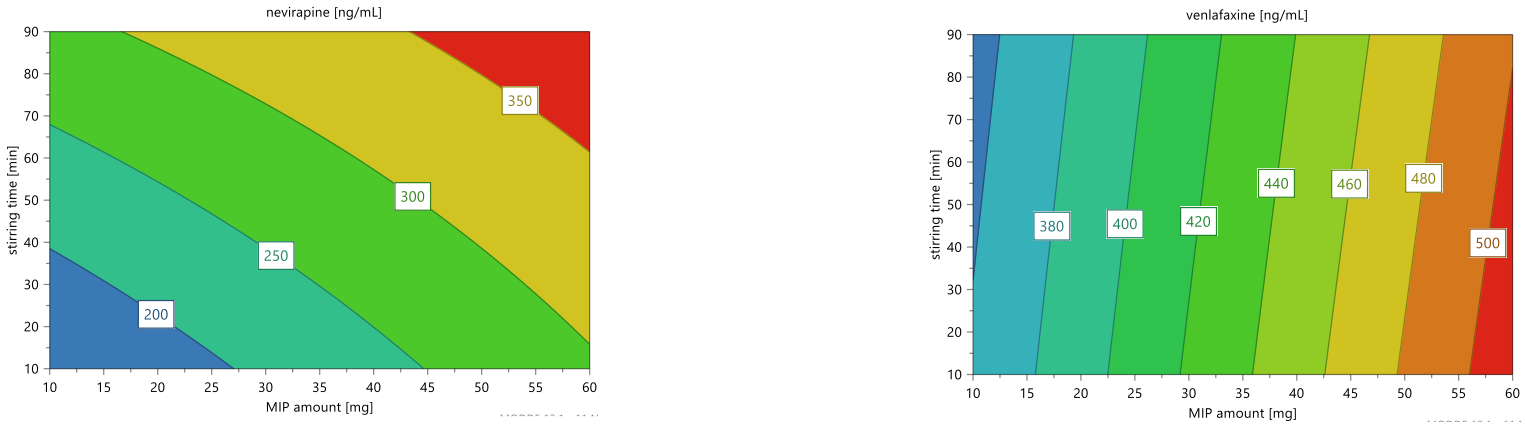


(e)

Figure 4 Coefficient plots of the investigated factors and their influence on the extraction of (a) carbamazepine, (b) etilefrine, (c) methocarbamol, (d) nevirapine and (e) venlafaxine. MIP (MIP amount), Stir (stirring rate), Stit (stirring time), salt (salt content)



(a) (b) (c)



(d) (e)

Figure 5 3D contour plots for the extraction of (a) carbamazepine, (b) etilefrine, (c) methocarbamol, (d) nevirapine and (e) venlafaxine from deionized water using the MASE-MIP technique

Fig. 5 is a representation on an effect of extraction time (s) and the amount of MIP (mg) on the extracted concentrations of the selected pharmaceuticals. The response surface depicted by counter plots reported in Fig. 5 a – e suggests that increasing the extraction time (stirring time) resulted in an increase in the amount of the extracted pharmaceuticals. Venlafaxine also showed this impact on its extraction but did not require as much time as the other target compounds to attain maximum extraction. Extraction of all the pharmaceutical compounds was also influenced positively by an increase in MIP amount. The counter plots (Fig. 5) support the results on the influence of these two parameters on the extraction of target pharmaceuticals as described by the coefficient plots presented in Fig. 4. The predicted optimum extraction conditions were found to be 5 g of salt content, a stirring rate of 400 rpm, an extraction time of 60 min and 50 mg of MIP.

4.3.1.3 Model Fitting and Predictive Efficiency

The model was created and calculated by fitting using partial least square regression. The fitted model showed a described variance range from 98% – 99% (R^2 = 0.9819 – 0.9998), where R^2 indicates the model fit. The linearity of the predicted values vs the corresponding experimental values explained by R^2 values confirmed the validity of the model and its ability to predict the best conditions where optimum extraction of the selected pharmaceuticals is realized. After the optimum conditions were predicted, triplicate analyses were conducted using the optimum conditions. Table 1 presents both the values predicted by the model and the values obtained experimentally. The relative standard deviations for all triplicate analysis were below 6% for all targeted pharmaceutical compounds, which indicates good precision. Furthermore, the model was investigated for its predictive performance by statistical factors including the ADD (%), RMSE, MAE, SEP (%), MPE (%), and chi-square (χ^2) which were evaluated using the equations described in Table S4. Table 1 presents the statistical analysis results obtained. The results depict that the response surface methodology (RSM) has a great ability to investigate the effect of the important parameters in the extraction of the model pharmaceuticals using the proposed MASE-MIP technique. All the statistical

error parameters presented in Table 1 are low, proving that the model has a good fitting.

Table 1 Statistical results of response surface methodology model fitting and predictive efficiency analysis

Pharmaceutical	Predicted values	Experimental values (n=3, % RSD)	ADD (%)	RMSE	MAE	SEP (%)	MPE (%)	X^2	R^2
Carbamazepine	0.554	0.406 ± 4.2	0.0016	0.0702	0.0016	0.8315	0.1611	0.0068	0.9819
Etilefrine	0.225	0.201 ± 4.1	0.0037	0.0847	0.0033	1.9245	0.3367	0.0909	0.9998
Methocarbamol	0.226	0.198 ± 5.8	0.0037	0.0763	0.0033	1.9245	0.3367	0.0909	0.9998
Nevirapine	0.426	0.355 ± 1.6	0.0037	0.1445	0.0034	1.9246	0.3368	0.0909	0.9989
Venlafaxine	0.594	0.536 ± 0.2	0.0037	0.2239	0.0034	1.9246	0.5569	0.0909	0.9989

Predicted and experimental data are expressed in μg mL^{-1}

4.3.2 Method validation and application

4.3.2.1 Validation of the MASE-MIP and the MASE techniques

The developed MASE-MIP and MASE methods for the extraction of selected pharmaceuticals were validated, giving the results summarized in Table 2. The surface water samples were spiked with 10 ng mL^{-1} of each targeted pharmaceutical. For both methods, the relative standard deviations are low i.e. below 8%, which is an indication of good precision. The linearity of the standard calibration curves for the targeted compounds was assessed over the concentration range of 1 – 100 ng mL^{-1} for etilefrine, carbamazepine and nevirapine and 5 – 100 ng mL^{-1} for the other pharmaceuticals. The R^2 values for linear plots ranged from 0.9982 to 0.9999, whilst the matrix-matched LODs were 0.09 – 0.20 ng mL^{-1} for MASE-MIP and ranged from 0.15 – 0.35 for MASE. These results represented highly sensitive analytical methods with acceptable

linearity. The recoveries obtained for all spiked samples ranged from 38 – 91% for MASE-MIP and 23 – 55% for MASE. The recoveries were observed to be higher for MASE-MIP for all analytes in all tested samples. This could be attributed to the fact that in MASE-MIP as selected pharmaceuticals are transferred from donor phase to acceptor phase, they move from acceptor phase to be attached on MIP cavities until equilibrium is reached. In this regard, most interfering compounds are prevented from reaching the MIP surface. On the other hand, in MASE, compounds move from donor phase to acceptor phase until equilibrium is reached. The reason for lower recoveries could be that the acceptor phase become saturated and is not able to accept more analytes, or the compounds could also be diffusing back to the donor phase. For MASE-MIP acceptable recoveries exceeding 70% were achieved for three pharmaceuticals, carbamazepine, nevirapine and venlafaxine. The two exceptions for etilefrine and methocarbamol could be due to their poor recognition by the venlafaxine-MIP in addition to the limited transfer from the aqueous donor phase across the membrane bag. These results therefore imply that the MASE-MIP demonstrated higher sensitivity and selectivity towards the targeted pharmaceuticals as compared to the MASE technique. In this context, the method detection limits attained in this study are better for compounds such as methocarbamol and venlafaxine as compared to other methods, where the LODs were reported to be 0.70 ng mL^{-1} [30] and 0.29 ng mL^{-1} [31], respectively, using SPE-LC-MS. For other compounds, the LODs obtained in our study were comparable with those reported in other analytical methods for an example, carbamazepine found to be 0.10 ng mL^{-1} using MASE-GC-MS [32] and 0.09 ng mL^{-1} for nevirapine [33] using SPE-LC-MS. All samples were analyzed by LC-qTOF/MS and identification of analytes done using major characteristic fragment ions which are: 194 (m/z) for carbamazepine, 164 (m/z) for etilefrine, 198 and 226 (m/z) for nevirapine, 121 (m/z) for venlafaxine and 118 (m/z) for methocarbamol.

Table 2 Performance of the analytical methods in surface water samples (n = 3, RSD).

Target Compound	Calibration			MASE-MIP limits (ng mL^{-1})			MASE limits (ng mL^{-1})		
	t_R min	Range (ng mL^{-1})	R^2	LOD	LOQ	Recovery (%)	LOD	LOQ	Recovery (%)
Carbamazepine	7.90	1 - 100	0.999	0.09	0.31	91 ± 4	0.15	0.49	55 ± 2
Etilefrine	2.67	1 - 100	0.998	0.17	0.56	41 ± 7	0.29	0.97	23 ± 6
Methocarbamol	6.57	5 - 100	0.999	0.20	0.69	38 ± 4	0.35	1.16	24 ± 7
Nevirapine	6.79	1 - 100	0.998	0.12	0.39	72 ± 4	0.24	0.81	41 ± 3
Venlafaxine	5.51	5 - 100	0.999	0.13	0.44	91 ± 5	0.23	0.78	49 ± 3

$t_{R:}$ retention time

4.3.3 Application of the developed analytical methods in monitoring of selected pharmaceuticals in surface water

The main objective of this step was to show the applicability of both the optimized MASE-MIP and MASE techniques, whilst presenting the potential of the MASE-MIP to eliminate matrix effects and increase selectivity towards targeted analytes from surface water samples. It is important to note that for pharmaceuticals that yielded recoveries lower than 70%, the compound concentration on real surface water samples was corrected by the sample recovery to obtain the true value. A T-test was also conducted for all compounds between the application results obtained by MASE and those obtained by the MASE-MIP. The p-values were all > 0.05, indicating that statistically there was no significant difference between the results obtained by both techniques. Results obtained by applying both the MASE, and MASE-MIP techniques are tabulated in Table 3.

It can be observed that the targeted pharmaceuticals were mostly detected from surface water, which is an indication of improper dumping or in the case of site 4 of Umdloti River is an indication that these compounds may also have been injected into the river through the WWTP effluent. In almost all the samples the concentrations of the analytes were in the order venlafaxine > nevirapine > carbamazepine > etilefrine > methocarbamol, apart from S1, S2, and S3 of the Hennops River where carbamazepine had higher concentrations than nevirapine. Etilefrine was detected in all the samples but could only be quantified in S2, S3 and S4 of the Hennops River, in other sites the concentrations were below the quantification limits. On the other hand, methocarbamol could not be quantified in all the sampling sites and in some instances, it was not detected. In all the five sampling sites of the Hennops River, carbamazepine, etilefrile, nevirapine and venlafaxine were detected, although in S1 and S2 etilefrine could not be quantified. In all these five sites illegal dumping of solid waste was evident during the time of sampling, which could be the largest contributor to the presence of these emerging pollutants on these sampling sites. As illustrated in Fig. S2 (c) and (d), the garbage dumping was huge and to some extent even the disposal of raw sewerage was

observed along the Hennops River where it cuts through the overpopulated Tembisa Township. The targeted pharmaceuticals could still be detected and quantified in sites S3 – S5 although these sites were in the residential suburbs and business areas. This means the wetlands in the downstream of Tembisa Township are unable to efficiently remove the pharmaceuticals in the river water. Rimayi and co-workers did a quantitative assessment of emerging pollutants including nevirapine, carbamazepine, methocarbamol and venlafaxine in the Hartbeespoort dam (which receives part of its water from the Hennops River) in 2016 [15]. They found concentrations of these compounds to be in the order of nevirapine > carbamazepine > methocarbamol > venlafaxine [15]. In another study, Rimayi and colleagues performed screening of emerging pollutants in the Juskei and Hennops Rivers with the aid of a Chemcatcher sampling device. In this case, carbamazepine and venlafaxine were detected in the Hennops surface water samples [16].

To the best of our knowledge, this is the first study to monitor the occurrence of pharmaceuticals in Umdloti River. In this river, nevirapine and venlafaxine were quantified in S1 and S2 whilst the other analytes were only detected with concentrations being lower than the limits of quantification. This gave an indication that to some extent the population residing along the two sampling sites (S1 and S2) were involved in inappropriate disposal of these pharmaceuticals. In these sampling sites, the quantified values of venlafaxine and nevirapine were similar. In sampling site 3 (S3) methocarbamol is the only pharmaceutical that could not be detected whilst the other four were present in water samples. This site is located in the downstream of the industrial area and the town of Verulam which indicates the possible improper discharge of pharmaceuticals which make their way into the Umdloti River. In site 4, quantifiable levels of carbamazepine, nevirapine and venlafaxine were observed with their concentrations reaching 0.320, 0.583 and 1.442 ng mL^{-1}, respectively. The concentrations in this site were lower than those found in S3. Interestingly, the concentrations of pharmaceuticals found in Umdloti estuary (S5) were generally higher than the amounts found in the upstream. This may be due to this estuary being

susceptible to closure by sand or sediments, thus in turn resulting to accumulation of these pharmaceuticals on the estuary. Also, Umdloti estuary is a recreational site which is used by residents and tourists for camping, fishing, etc. This presents a likelihood of the direct disposal of medications and human excreta into this site. There are other studies which report the monitoring of these pharmaceuticals all over the world. For an example a study conducted in California, where carbamazepine concentration was found to be 0.42 ng mL^{-1} in groundwater samples [18]. In the Nairobi River basin (Kenya), Ngumba and colleagues quantified 4.86 ng mL^{-1} of nevirapine in 2015 [34]. In another study performed by Shoenfuss and co-workers in the US, the concentrations of methocarbamol and venlafaxine were found to be 0.023 ng mL^{-1} and 0.511 ng mL^{-1}, respectively, after fish exposure to the treated effluent [6]. This means the results of the present study are somewhat similar to related work conducted in other countries.

Table 3 Average concentrations of the selected pharmaceuticals found in different sampling sites of the Hennops and Umdloti Rivers (n = 3, RSD).

sample ID	Carbamazepine (ng mL^{-1})		Etilefrine (ng mL^{-1})		Methocarbamol (ng mL^{-1})		Nevirapine (ng mL^{-1})		Venlafaxine (ng mL^{-1})	
	MASE-MIP	**MASE**	**MASE-MIP**	**MASE**	**MASE-MIP**	**MASE**	**MASE-MIP**	**MASE**	**MASE-MIP**	**MASE**
Hennops River S1	0.62 ± 5.6	0.569 ± 7.2	<LOQ (0.483)	<LOQ (0.420)	<LOQ (0.223)	<LOQ (0.201)	0.499 ± 6.8	0.489 ± 3.3	1.520 ± 9.3	1.314 ± 9
Hennops River S2	0.61 ± 6.2	0.581 ± 6.6	0.557 ± 7.8	<LOQ (0.416)	<LOQ (0.270)	<LOQ (0.250)	1.640 ± 9.6	1.515 ± 3.1	1.592 ± 7.6	1.293 ± 10.2
Hennops River S3	0.74 ± 10.7	0.544 ± 5.4	0.647 ± 10.2	<LOQ (0.457)	ND	ND	0,623 ± 6.6	0.588 ± 5.6	1.784 ± 11.2	1.80 ± 8.2
Hennops River S4	0.36 ± 13.7	<LOQ (0.431)	0.584 ± 11.8	<LOQ (0.470)	<LOQ (0.205)	<LOQ (0.201)	0.580 ± 5.0	0.561 ± 5.7	1.818 ± 2.8	1.569 ± 8.0
Hennops River S5	0.31 ± 8.0	<LOQ (0.274)	<LOQ (0.464)	<LOQ (0.435)	ND	ND	0.792 ± 10.6	0.829 ± 7.4	1.368 ± 4.1	0.957 ± 11.5
Umdloti River S1	<LOQ (0.19)	<LOQ (0.165)	<LOQ (0.407)	<LOQ (0.439)	<LOQ (0.193)	ND	0.810 ± 5.0	0.950 ± 11.9	2.204 ± 2.5	1.046 ± 7.2
Umdloti River S2	<LOQ (0.25)	<LOQ (0.196)	<LOQ (0.413)	<LOQ (0.483)	<LOQ (0.286)	<LOQ (0.201)	0.751 ± 6.7	0.634 ± 2.4	2.481 ± 1.0	1.192 ± 5.8
Umdloti River S3	0.43 ± 9.4	<LOQ (0.381)	<LOQ (0.417)	<LOQ (0.443)	ND	ND	0.781 ± 8.6	0.756 ± 7.0	1.798 ± 3.8	2.143 ± 2.9
Umdloti River S4	0.32 ± 8.2	<LOQ (0.316)	<LOQ (0.454)	<LOQ (0.428)	ND	ND	0.583 ± 9.2	0.524 ± 7.9	1.442 ± 6.1	1.451 ± 2.5
Umdloti River S5	0.51 ± 10.1	<LOQ (0.390)	<LOQ (0.419)	<LOQ (0.448)	<LOQ (0.214)	<LOQ (0.217)	0.917 ± 10.2	0.998 ± 1.3	1.599 ± 3.3	1.375 ± 5.0

4.4 Conclusions

An effective extraction method based on a combination of membrane assisted solvent extraction and a molecularly imprinted polymer was successfully developed and optimized through central composite design for selective extraction of five pharmaceuticals in surface water. This was followed by their chromatographic separation and detection using LC-qTOF/MS. The developed MASE-MIP method was compared to MASE for the extraction of selected pharmaceuticals in river water. In this case, MASE-MIP proved to be a preferred sample preparation technique that yielded lower detection and quantitation limits as well as higher recoveries for all selected pharmaceuticals. Based on recoveries, it was observed that a MIP synthesized with venlafaxine as the template was capable of simultaneously extracting 4 other pharmaceuticals while the membrane prevented the co-extraction of bigger molecules. Although this was the case, recoveries lower than 50 % were obtained for etilefrine and methocarbamol which could have been as a result of poor recognition by the MIP and low transfer rate across the membrane bag. The proposed analytical method was successfully applied for environmental monitoring of selected pharmaceuticals in two major South African rivers. All the investigated pharmaceuticals were found present in river water. This shows a need to conduct extensive environmental monitoring studies in order to determine the sources and spread of pharmaceuticals in South African waters. This could be done by utilizing the analytical method proposed in this study which has been proven to be efficient, precise and accurate for monitoring selected pharmaceuticals in environmental waters.

Conflict of interests

The authors declare that there is no conflict of interests

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and antiretroviral drugs in Nairobi River Basin , Kenya. Sci Total Environ 539:206–213. https://doi.org/10.1016/j.scitotenv.2015.08.139

SUPPLEMENTARY DATA

Multivariate optimization of a two-way technique for extraction of pharmaceuticals in surface water using a combination of membrane assisted solvent extraction and a molecularly imprinted polymer

Sinegugu Khulu [a,b], Somandla Ncube [c], Yannick Nuapia [a,d], Lawrence Mzukisi Madikizela [a,e], Hlanganani Tutu [a], Heidi Richards [a], Kuria Ndungu [f], Elizabeth Mavhunga [b], Luke Chimuka [a*]

[a] Molecular Sciences Institute, University of Witwatersrand, Private Bag X3, Johannesburg, 2050, South Africa

[b] School of Education, University of Witwatersrand, Private Bag X3, Johannesburg, 2050, South Africa

[c] Department of Chemistry, Sefako Makgatho Health Sciences University, P.O Box 60, Medunsa, 0204, South Africa

[d] School of Animal, Plant, & Environmental Science, University of Witwatersrand, Johannesburg, South Africa

[e] Institute for Nanotechnology and Water Sustainability, College of Science, Engineering and Technology, University of South Africa, Florida Science Campus, 1710, South Africa

[f] Norwegian Institute for Water Research-NIVA, Gaustadalleen 21, 0349, Oslo, Norway

[*] Luke.Chimuka@wits.ac.za

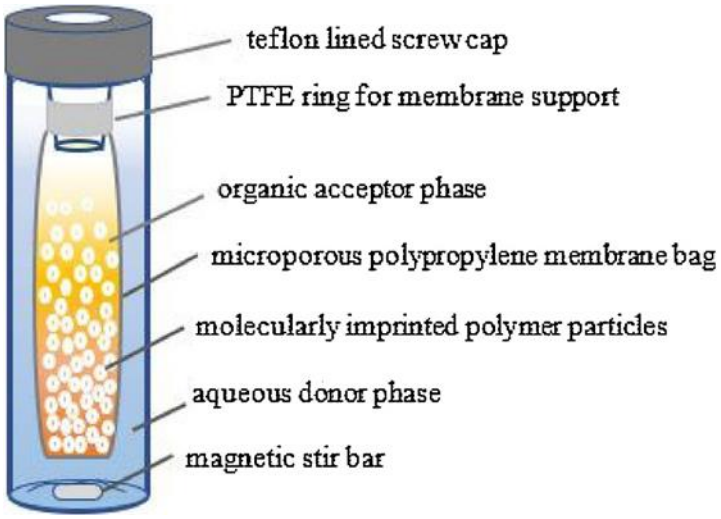


Fig. S1 Schematic representation of a MASE-MIP technique [1]

Fig. S2 Photographs captured in Sampling site 1 (a) and sampling site 4 (b) of Umdloti River, and sampling site 1 (c) & (d) of Hennops River.

Table S1 Molecular structures, physicochemical properties of the selected pharmaceuticals and their health effects on biota [2]

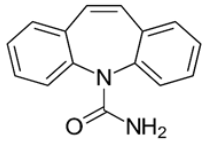
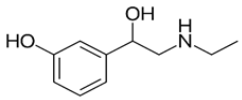
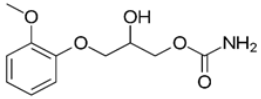
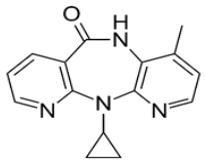
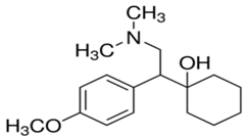
Compound	Structure	Molecular weight ($g.mol^{-1}$)	logKow	pKa		Water Solubility ($mg\ L^{-1}$)	Biota health side effects
				Acid	Base		
Carbamazepine		236.27	2.45	15.96	-3.8	1.05×10^2	Behavioral and neurological effects in juvenile fish
Etilefrine		181.23	0.1	9.1	9.73	17.7	Cardiovascular effects such as increased pulse rate and cardiac output
Methocarbamol		241.24	0.6	13.6	-3.4	267	Neurobehavioral disorders in aquatic animals
Nevirapine		266.3	2.5	10.37	5.06	1.38×10^4	Reduced growth rate on fish, and enlarged livers
Venlafaxine		277.4	2,9	14.42	8.91	7.2×10^3	Neuroendocrine disruption, impacting the stress and feeding responses in fish

Table S2. Experimental conditions with the corresponding extracted amounts of pharmaceuticals (µg mL^{-1})

Run order	MIP amount (mg)	Stirring rate (rpm)	Stirring time (min)	Salt amount (g)	Carbamazepine	Etilefrine	Methocarbamol	Nevirapine	Venlafaxine
1	35	800	50	2.5	0.324	0.214	0.226	0.316	0.547
2	60	800	10	2.5	0.214	0.068	0.078	0.280	0.391
3	35	1200	10	2.5	0.135	0.058	0.073	0.142	0.376
4	35	800	10	0	0.078	0.061	0.070	0.148	0.736
5	35	800	50	2.5	0.332	0.221	0.205	0.328	0.567
6	60	800	90	2.5	0.462	0.159	0.102	0.291	0.378
7	35	400	10	2.5	0.217	0.110	0.121	0.247	0.411
8	10	1200	50	2.5	0.351	0.174	0.180	0.270	0.541
9	60	1200	50	2.5	0.365	0.174	0.189	0.353	0.481
10	10	800	50	0	0.130	0.689	0.078	0.128	0.287
11	35	1200	50	5	0.424	0.167	0.178	0.404	0.331
12	35	400	50	0	0.260	0.068	0.073	0.160	0.358
13	60	400	50	2.5	0.431	0.192	0.205	0.404	0.481
14	10	800	10	2.5	0.187	0.072	0.070	0.1810.	0.249
15	35	1200	90	2.5	0.311	0.152	0.160	0.271	0.261
16	35	800	10	5	0.439	0.155	0.165	0.403	0.447
17	35	400	50	5	0.397	0.187	0.195	0.324	0.586
18	60	800	50	0	0.191	0.071	0.086	0.168	0.340
19	35	800	90	5	0.489	0.192	0.211	0.405	0.586
20	10	400	50	2.5	0.221	0.161	0.177	0.173	0.230
21	35	800	50	2.5	0.335	0.198	0.202	0.252	0.514
22	35	800	90	0	0.445	0.159	0.188	0.471	0.558
23	10	800	50	5	0.155	0.065	0.081	0.116	0.251
24	10	800	90	2.5	0.266	0.117	0.120	0.240	0.239
25	35	400	90	2.5	0.465	0.171	0.189	0.392	0.419
26	35	1200	50	0	0.449	0.169	0.170	0.322	0.358
27	60	800	50	5	0.375	0.178	0.177	0.320	0.612

Table S3 Quadratic equations of the predictive model

Pharmaceuticals	Quadratic equations
Carbamazepine	$Y = 0.061a + 0.004b + 0.097c + 0.061d - 0.057a^2 + 0.027b^2 - 0.012c^2 + 0.003d^2 – 0.049ab + 0.042ac + 0.0397ad – 0.018bc - 0.041bd - 0.079cd + 0.33$
Etilefrine	$Y = - 0.036a + 0.0004b + 0.036c – 0.023d + 0.003a^2 – 0.039b^2 - 0.078c^2 + 0.008d^2 – 0.008ab + 0.012ac + 0.182ad + 0.008bc – 0.030bd – 0.015cd + 0.211$
Methocarbamol	$Y = 0.011a – 0.001b + 0.033c + 0.029d – 0.052a^2 – 0.006b^2 – 0.051c^2 - 0.036d^2 – 0.005ab – 0.006ac + 0.022ad + 0.005bc – 0.029bd – 0.018cd + 0.211$
Nevirapine	$Y = 0.068a + 0.005b + 0.065c + 0.048d – 0.069a^2 + 0.013b^2 – 0.0003c^2 + 0.001d^2 – 0.037ab – 0.039ac + 0.041ad – 0.004bc – 0.021bd – 0.080cd + 0.299$
Venlafaxine	$Y = 0.074a – 0.011b – 0.014c + 0.015d – 0.124a^2 – 0.080b^2 – 0.052c^2 – 0.003d^2 – 0.078ab – 0.001ac + 0.077ad – 0.031bc – 0.064bd + 0.079cd + 0.542$

a = MIP amount, b = stirring rate, c = stirring time, d = salt content

Table S4 Equations of error functions

Error	Equation	
Absolute average deviation (ADD)	$\text{ADD (\%)} = \left[\frac{\sum_{i=1}^{n}\left(\lvert Y_{iexp} - Y_{ical}\rvert / Y_{iexp}\right)}{n}\right]$	(S1)
Root mean square error (RMSE)	$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}\left(Y_{i,e} - Y_{i,p}\right)^2}{n}}$	(S2)
Mean absolute error (MAE)	$\text{MAE} = \frac{i}{n}\sum_{i=1}^{n}\lvert Y_{i,e} - Y_{i,p}\rvert$	(S3)
Standard error of prediction (SEP)	$\text{SEP (\%)} = \frac{RMSE}{Y_{i,e}} \times 100$	(S4)
Model predictive error (MPE)	$\text{MPE (\%)} = \frac{100}{n}\sum_{i=1}^{n}\left\lvert\frac{Y_{i,e} - Y_{i,p}}{Y_{i,p}}\right\rvert$	(S5)
Chi-square (χ^2)	$\chi^2 = \sum_{i=1}^{n}\frac{\left(Y_{i,p} - Y_{i,e}\right)}{Y_{i,p}}$	(S6)

Y_{iexp} and $Y_{i,e}$: experimental value, Y_{ical} and $Y_{i,p}$: predicted value, n : number of replicates

Table S5 Statistical data corresponding to coefficient plots for all targeted compounds

	Carbamazepine			**etilefrine**			**methocarbamol**			**Nevirapine**			**venlafaxine**		
	Coeff. SC	% Std. Err.	P-value	Coeff. SC	% Std. Err.	P-value	Coeff. SC	% Std. Err.	P-value	Coeff. SC	% Std. Err.	P-value	Coeff. SC	% Std. Err.	P-value
Constant	0.33	4.9	2.43E-05	0.21	6.1	0.01	0.211	2.4	1.33E-06	0.30	4.9	8.47E-05	0.54	7.5	1.06E-05
MIP amount	0.06	2.5	0.03	-0.04	3.1	0.26	0.017	1.2	0.38	0.07	2.7	0.02	0.074	3.8	0.04
Stirring rate	0.003	2.5	0.89	0.001	3.1	0.98	-0.001	1.2	0.95	0.01	2.5	0.84	-0.01	3.8	0.77
Stirring time	0.10	2.5	0.002	0.04	3.1	0.27	0.03	1.2	0.02	0.07	2.7	0.03	-0.01	3.8	0.71
Salt amount	0.06	2.5	0.03	-0.02	3.1	0.47	0.03	1.2	0.03	0.05	2.5	0.08	0.02	3.8	0.70
MIP*MIP	-0.06	3.7	0.15	0.002	4.6	0.96	-0.05	1.8	0.018	-0.07	3.9	0.09	-0.12	5.6	0.05
Stir*Stir	0.03	3.7	0.48	-0.04	4.6	0.40	-0.01	1.8	0.75	0.01	3.8	0.72	-0.08	5.6	0.2
Stit*Stit	-0.01	3.7	0.75	-0.08	4.6	0.11	-0.05	1.8	0.01	-0.003	3.9	0.94	-0.05	5.6	0.37
Salt*Salt	0.003	3.7	0.93	0.01	4.6	0.87	-0.04	1.8	0.07	0.002	3.8	0.95	-0.003	5.6	0.96
MIP*Stir	-0.05	3.7	0.28	-0.01	5.3	0.89	-0.01	2.1	0.82	-0.04	4.3	0.41	-0.08	6.5	0.26
MIP*Stit	0.04	4.3	0.35	0.01	5.3	0.83	-0.01	2.1	0.76	-0.05	5.4	0.40	-0.001	6.5	0.99
MIP*Salt	0.04	4.3	0.38	0.18	5.3	0.01	0.02	2.1	0.31	0.041	4.3	0.40	0.077	6.5	0.26
Stir*Stit	-0.02	4.3	0.68	0.01	5.3	0.88	0.01	2.1	0.82	-0.004	4.3	0.93	-0.03	6.5	0.65
Stir*Salt	-0.04	4.3	0.37	-0.03	5.3	0.58	-0,03	2.1	0.19	-0.02	4.3	0.64	-0.06	6.5	0.35
Stit*Salt	-0.08	4.3	0.09	-0.02	5.3	0.78	-0,02	2.1	0.40	-0.08	4.3	0.09	0.08	6.5	0.25

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2. Khulu S, Ncube S, Kgame T, et al (2021) Synthesis , characterization and application of a molecularly imprinted polymer as an adsorbent for solid - phase extraction of selected pharmaceuticals from water samples. Polym Bull. https://doi.org/10.1007/s00289-021-03553-9

4 PAPER 3

This paper entitled “Development and application of a membrane assisted solvent extraction-molecularly imprinted polymer based passive sampler for monitoring of selected pharmaceuticals in surface water” is under review in *Water Research* Journal. The paper presents a development and evaluation of a MASE-MIP based passive sampling device for monitoring of the selected model pharmaceuticals in environmental water bodies.

Under review in Water research: Manuscript number: WR70110 (IF:13.400)

Sinegugu Khulu – Principal Author

Somandla Ncube – Co-Supervisor

Yannick Nuapia - Co-author

Lawrence Mzukisi Madikizela - Co-author

Elizabeth Mavhunga – Co-author

Luke Chimuka – Main Supervisor

Development and application of a membrane assisted solvent extraction-molecularly imprinted polymer based passive sampler for monitoring of selected pharmaceuticals in surface water

Sinegugu Khulu [a,b], Somandla Ncube [c], Yannick Nuapia [a,d], Lawrence Mzukisi Madikizela [a,e], Elizabeth Mavhunga [b], Luke Chimuka [a*]

[a] Molecular Sciences Institute, University of Witwatersrand, Private Bag X3, Johannesburg, 2050, South Africa

[b] School of Education, University of Witwatersrand, Private Bag X3, Johannesburg, 2050, South Africa

[c] Department of Chemistry, Sefako Makgatho Health Sciences University, P.O Box 60, Medunsa, 0204, South Africa

[d] School of Animal, Plant, & Environmental Science, University of Witwatersrand, Johannesburg, South Africa

[e] Institute for Nanotechnology and Water Sustainability, College of Science, Engineering and Technology, University of South Africa, Florida Science Campus, 1710, South Africa

[*] Luke.Chimuka@wits.ac.za

Abstract

Passive sampling has been considered a promising approach to conventional grab sampling for monitoring of pharmaceuticals in the environment. The technique allows for estimation of time weighted average (TWA) concentrations of analytes through unprompted extraction and pre-concentration of analytes over longer periods of time. In this work, development, evaluation and pre-liminary application of a novel passive sampler for monitoring of selected pharmaceuticals in environmental waters is demonstrated. The samplers were calibrated in laboratory-based experiments to obtain sampling rates (Rs) for each target pharmaceutical. Passive sampling was based on the diffusion of analytes (carbamazepine, methocarbamol, etilefrine, venlafaxine and nevirapine) in an integrative manner from surface water through the membrane bag which housed an ionic liquid as a green receiving solvent and a molecularly imprinted polymer for selective adsorption of target pharmaceuticals. Effects of biofouling, deployment time and solvent type for the receiver phase were optimized for selective uptake of analytes in surface water. Notably, there was a decrease in the uptake of selected pharmaceuticals and consequently a decrease in their sampling rates (Rs) in the presence of biofouling. The optimum matrix-matched sampling rates ranged from 0.0007 - 0.0018 L d^{-1} whilst the method detection and quantification limits ranged from 2.45 - 3.26 ng L^{-1} and 8.06 - 10.81 ng L^{-1}, respectively. The optimized passive sampler was finally deployed in a dam situated in the heart of a typical highly populated township in the Gauteng Province of South Africa. Only etilefrine and methocarbamol were detected and quantified at maximum TWA concentrations of 12.88 and 72.29 ng L^{-1}, respectively.

Keywords: Passive sampling; molecularly imprinted polymer; Membrane assisted solvent extraction; pharmaceuticals; aliquat 336

4.1 Introduction

Globally, pharmaceuticals are increasingly being used as medications for both humans and animals. They are now considered an important group of persistent emerging pollutants due to being continuously detected in the aquatic environments [1–3]. As such, they have become a global concern to the scientific community owing to their detrimental health effects and microbial resistance in aquatic life [1, 2, 4, 5]. Although they are typically available at trace levels (μg L^{-1} – ng L^{-1} or even pg L^{-1}) in aquatic environments [6–8], their monitoring is important in the assessment of pollution and identification of pollution sources [9]. Most water monitoring programmes depend on the conventional grab sampling of water at a specific time. However, this sampling method has drawbacks in monitoring the presence of pollutants as it only provides pollutant concentrations at the time of sampling, which in most cases is not a true reflection of the extent of pollution [6, 9–12]. For example, variations in pharmaceutical loads entering the domestic wastewater treatment plants in different times of the day have been recorded in South Africa [13]. Additionally, pre-concentrating environmental pollutants to quantifiable concentration levels can be expensive and tedious as large sample volumes are required which is accompanied by several sample preparation steps [10, 12, 14]. Moreover, grab sampling does not offer data on the bioavailable fraction of the pollutants [15].

Passive sampling technique has displayed qualities of being a promising approach to traditional grab sampling for the monitoring of pharmaceuticals in aquatic environments [16, 17]. The technique makes use of passive sampling devices which permit unprompted *in situ* extraction and pre-concentration of analytes over longer periods of time [16]. This then allows for time integrated or time weighted average (TWA) concentrations of analytes which considers variations in pollutant concentrations and in turn increases the capabilities of the device to detecting and quantifying contaminants at trace levels [6, 15, 18, 19]. Passive samplers are simple less labouring, cost effective, easy to handle and nonmechanical devices which can be operated without power supply [12, 18]. An ideal sampler would normally consist of

an acceptor phase and a membrane which separates the acceptor phase and the aqueous sample. The acceptor phase is selected based on its ability to accept or extract target compounds from the water body [20].

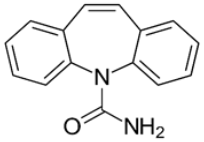
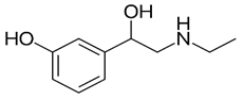
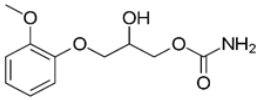
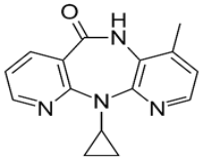
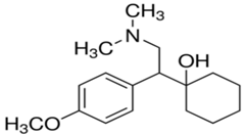
Currently, the widely used and commercially available passive sampler in environmental monitoring of pharmaceuticals is the polar organic chemical integrative sampler (POCIS), consisting of a sorptive receiver phase normally covered by microporous polyethersulphone membrane [21, 22]. However, recent studies have shown that the POCIS is effective for qualitative analysis but has several flaws which makes it a semi-quantitative tool for monitoring of pharmaceuticals [12, 17, 23]. Some of its weaknesses include the misunderstood accumulation mechanism, and its vulnerability to physicochemical parameters like the water pH, conductivity dissolved organic matter, biofouling, water flow and temperature [24–26]. For these reasons, accumulation rates or sampling rates (Rs) obtained from laboratory calibration of the sampler may be biased when applied in the field for the purposes of estimating the bioavailable concentration in the water body [27]. Currently, a few studies have resorted to *in situ* calibrations of the POCIS, with the view to minimize the doubts in the estimation of the accumulated concentration [16, 19, 20]. This is an indication that more research is needed in the search for alternative passive samplers for monitoring of the bioavailable concentrations of pharmaceuticals in environmental waters.

In this work, we designed and optimized a novel alternative passive sampler based on membrane assisted solvent extraction and molecularly imprinted polymer (MASE-MIP) for monitoring of the selected model compounds in aquatic environments. This was achieved by synthesizing a smart polymer and then enclosing it in a membrane bag and finally enclosing them in a polyvinyl chloride (PVC) cage and deployed as a passive sampler. The idea was that the work investigated the possibilities of using greener and stable solvents such as the deep eutectic solvents (DESs) and Ionic liquids (ILs) as an alternative approach to the conventional organic solvents in the receiver phase of a passive sampler. In recent years, DESs which share few properties with ILs

have gained a lot of interests in the various fields of analytical chemistry e.g. sample preparation, micro extraction, mass spectrometry etc. [28, 29]. The two green solvents share unique physicochemical properties such as nonflammability and low volatility. Moreover, DESs have added advantages over ILs which include being eco-friendly, mostly biodegradable, cheaper and easy to be produced (prepared from a hydrogen bond acceptor and a hydrogen bond donor, therefore also good in the dissolution of pharmaceuticals) [28–30]. The developed passive sampler is based on the diffusion of selected pharmaceuticals from surface water through the membrane bag into the green solvent-receiving phase which is followed by the selective adsorption of analytes onto the MIP cavities over a period of 14 days. Upon retrieval of the passive sampler from the surface water, analytes were desorbed from the MIP and analyzed using liquid chromatography equipped with a quadrupole time of flight mass spectrometry detection system (LC-qTOF/MS). Other parameters such as the effect of biofouling and deployment time were optimized for selective uptake of analytes in surface water.

The passive samplers were then calibrated through matrix-matched laboratory-based experiments to obtain sampling rates for each target pharmaceutical for purposes of estimating TWA concentrations for each pharmaceutical. The target pharmaceuticals with their physicochemical properties provided in Table 1 were an antidepressant (venlafaxine), an antiretroviral (nevirapine), a muscle relaxant (methocarbamol), an anticonvulsant (carbamazepine) and a cardiac stimulant (etilefrine). Finally, the MASE-MIP based passive sampler for the selective monitoring of five pharmaceuticals belonging to different therapeutic groups in Orlando Dam situated in the heart of Soweto Township, a biggest South African township. In the regard, this is the first study to investigate the occurrence of pharmaceuticals in Orlando Dam.

Table 1. Molecular structures, physicochemical properties of the selected pharmaceuticals and their health effects on biota [31]

Compound	Structure	Molecular weight ($g.mol^{-1}$)	logKow	pKa		Water Solubility ($mg\ L^{-1}$)	Biota health side effects
				Acid	Base		
Carbamazepine		236.27	2.45	15.96	-3.8	1.05×10^2	Behavioral and neurological effects in juvenile fish
Etilefrine		181.23	0.1	9.1	9.73	17.7	Cardiovascular effects such as increased pulse rate and cardiac output
Methocarbamol		241.24	0.6	13.6	-3.4	267	Neurobehavioral disorders in aquatic animals
Nevirapine		266.3	2.5	10.37	5.06	1.38×10^4	Reduced growth rate on fish, and enlarged livers
Venlafaxine		277.4	2,9	14.42	8.91	7.2×10^3	Neuroendocrine disruption, impacting the stress and feeding responses in fish

4.2 Materials and Methods

4.2.1 Chemicals and reagents

Analytical grade venlafaxine hydrochloride (≥98%), carbamazepine, etilefrine hydrochloride, methocarbamol, nevirapine and p-vinylbenzoic acid (97%) were all obtained in powder form, while ethylene glycol dimethacrylate (98%), 1-Octanol (≥99 %) and trioctylmethylammonium chloride were obtained in in liquid form from Sigma-Aldrich, Johannesburg, South Africa. UHPLC grade methanol (≥99.9%), dimethyl sulfoxide, acetonitrile (99.8%), toluene (99.7%) and formic acid (≥95%) were also purchased from Sigma-Aldrich, Johannesburg, South Africa. Chloroform (99%) and cyclohexane (99 %) and sodium chloride were purchased from Associated Chemical Enterprises (ACE), Johannesburg, South Africa. Nitrogen gas was obtained from Afrox, Johannesburg, South Africa.

4.2.2 Synthesis of a molecularly imprinted polymer

Synthesis of a MIP was adopted from our previous work [31]. To synthesize a polymer, 15 mg of the template (venlafaxine) was dissolved in 3 mL of dimethyl sulfoxide at 40 °C for 5 min while stirring. 30 mg of p-vinyl benzoic acid, functional monomer was added into the mixture while heating for a further 10 min. The reaction mixture temperature was then raised to 60 °C and 167 µL of ethylene glycol dimethacrylate, cross-linker was added to the reaction while stirring and heating for further 10 min. An initiator, 1, 1´–azobis-(cyclohexanecarbonitrile) (10 mg) was added to the mixture and the system was purged with nitrogen gas for 5 min. Finally, the reaction temperature was then raised to 80 °C to achieve polymerization. MIP cavities were generated by washing the polymer excessively with methanol for removal of the template. The synthesized MIP displayed high affinity towards all the five model pharmaceuticals, with maximum matrix-matched adsorption capacities ranging between 206 and 418 ng mg^{-1} for individual pharmaceuticals.

4.2.3 Instrumentation and apparatus

A temperature-controlled WiseCube WIG-105 Precise Shaking incubator supplied by Wisd® Laboratory Instruments (Wertheim, BW, Germany) was used for template removal from the MIP. Samples were centrifuged through a Rotofix 32A centrifuge from Hettich® (Frankfurt, Hessen, Germany). Polypropylene membrane bags (4 cm long, 0.03 mm wall thickness, 6 mm internal diameter) were purchased from Chemetrix Export (Johannesburg, South Africa). Empty SPE cartridges with a 1 mL capacity and 6 mm diameter fitted with 10 µm frits at the bottom were purchased from Anatech Instruments (Johannesburg, South Africa). Deionized water was produced through a Millipore DirectQ 3 UV waters system-2345 from Millipore (Massachusetts, USA). The magnetic stirrer used in deployment experiments was purchased from C.C IMELMANN (Pty) Ltd (Johannesburg, South Africa). The SPE vacuum manifold was purchased from Phenomex (Carlifornia, USA), whilst the vacuum pump was purchased from Gilson (Williers Le Bel, France). The HI98194 multiparamer meter used for in situ measurements of physicochemical parameters was purchased from Hanna Instruments (Johannesburg, South Africa).

The UHPLC system used for separation and analysis of pharmaceuticals was the Thermo Scientific Dionex Ultimate 3000 from ThemoFisher Scientific (Waltham, MA, USA). The instrument consisted of a HPG-3200RS pump and a WPS-3000TRS autosampler. The UHPLC was coupled to a quadrupole time-of-flight compact mass spectrometer system from Bruker Daltonics Inc. (Billerica, MA, USA). For collection and processing of qualitative data, Bruker Compass DataAnalysis version 4.3 (Build 110.102.1532) was used, while Bruker Compass QuantAnalysis version 4.3 (Build 110.102.1532) was utilized for collection and processing of quantitative data. Chromatographic separation of analytes was achieved on a Luna Omega C_{18} column (50 x 4.6 mm, 3 µm) from Phenomenex (Torrance, CA, USA). Separation was through gradient elution using solvent A (0.1 % formic acid in deionized water) and solvent B (0.1% formic acid in acetonitrile). All five pharmaceuticals were separated within 14 min. Initially, solvent B was kept at 5% for 1 min. This was followed by ramping until

it reached 55% at 6 min and kept constant for 2 min. It was further ramped until it reached 95% at 10 min and kept constant for further 2 min. Finally, it was dropped to 5% of solvent B for a minute and kept constant for another minute [31].

4.2.4 Development of a MASE-MIP based passive sampler

4.2.4.1 MASE preparation

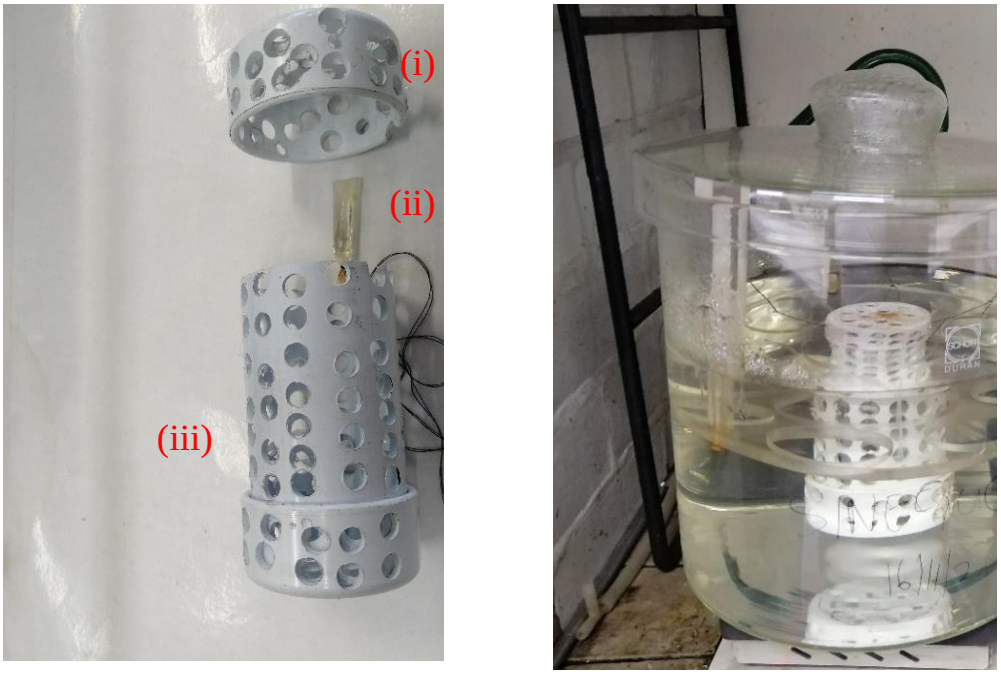
Pre-conditioning of extraction membranes was achieved by immersing the polypropylene membranes into a beaker containing cyclohexane and shaking at 150 rpm for approximately 3 h. This step was repeated 3 times with fresh portions of cyclohexane. The procedure was done with metal cylinders on to ensure that the inside walls of the membranes were also conditioned. After pre-conditioning, the membranes were dried out and then soaked in an appropriate extraction solvent for at least 2 h.

4.2.4.2 Design and preparation of MASE-MIP based passive sampler

The passive sampler device was designed and machined in-house using cost effective PVC pipe purchased from a local hardware supermarket. The PVC pipe consisted of a top and bottom cap; the latter was glued to the bottom of the pipe by PVC glue. The length of the sampler chamber was 10 cm, with internal diameter of 5.5 cm. The sampler chamber was drilled to create multiple holes as shown on Fig. 1a, which became essential for the flow of water.

MIP particles (50 mg) were transferred into the interior of the polyethylene membrane bag. The bag was filled with 1 mL of receiver solution (solvent). The membrane bag containing a mixture of MIP particles and receiver solution was then tied with a cotton string as a means of temporary sealing. The bag was then placed inside the chamber (the chamber can fit 5 to 6 propylene membrane bags). For laboratory deployments, the MASE-MIP based passive samplers were placed in a 5 L glass beaker containing 3 L of either deionized or dam water spiked with 10 ng mL^{-1} of target pharmaceutical compounds as shown in Fig. 1b.

After extraction, the MIP and the acceptor solution mixture was transferred into a 1 mL empty SPE cartridge fitted with a 10 µm frit at the bottom and then mounted onto an SPE vacuum manifold. The receiver solution was allowed to flow through the SPE cartridge and discarded while safeguarding against any MIP particle losses by means of frits. A steady vacuum was then applied by means of external vacuum pump to dry the MIP particles. Then, the adsorbed pharmaceuticals were eluted from the MIP cavities using 4 × 1 mL of methanol. For environmental monitoring, the solvent extracts were reduced to 1 mL by purging with a gentle stream of nitrogen gas prior to injection to the LC-qTOF/MS for identification and quantification of pharmaceuticals.



(a) (b)

Figure 1. (a) components of a MASE-MIP based passive sampler (i) top cap, (ii) propylene membrane bag with MIP particles and acceptor solution, (iii) chamber with bottom cap attached, (b) laboratory scale deployment of the passive sampler.

4.2.5 Optimization of extraction parameters

4.2.5.1 Effect of acceptor phase

In this step, the aim was to adapt conditions from our previous work [32], where chloroform was used as an organic receiver phase. However, owing to chloroform's volatility it was not possible to apply it as an acceptor solvent for long extraction periods. Chloroform volatilized to dryness within two to three days of deployment. This raised a need to explore more stable, low volatility and environmentally friendly solvents. In this regard, a DES, prepared by mixing methyltrioctylammonium chloride (aliquat 336) and 1-octanol in a ratio of 50:50 %v/v and an ionic liquid (aliquat 336) were investigated as possible extraction solvents. DES preparation followed the same method described by Tang and colleagues, where aliquat 336:1-octanol were mixed in a 1:1 ratio in a glass vial and heated at a temperature of 80 °C with constant stirring at 350 rpm for 2 h to form a homogenous mixture [33]. To assess the effect of solvent type, a polypropylene membrane bag was filled with 50 mg MIP particles and 1 mL of either the DES or the IL and then tied with a cotton string. Thereafter, 5 L glass beakers were filled with 3 L of deionized water spiked with 20 ng mL^{-1} of the selected model pharmaceuticals. Thereafter, the prepared passive samplers based on either DES or IL as an acceptor solvent were deployed in the spiked solutions. Each passive sampler chamber consisted of four membrane bags and on day 1, 3, 5 and 7, a membrane bag was removed from each passive sampler chamber and treated using the method explained in 2.4.3 prior to LC-qTOF/MS analysis. These experiments were done in triplicates. Also, on each of those days a 0.5 mL sample of donor phase was sampled and taken to LC-qTOF/MS for analysis.

4.2.5.2 Effect of exposure time and biofouling

Effect of exposure time and biofouling was conducted in order to understand the accumulation of the selected model pharmaceuticals onto the sampling device during the time of deployment. Sampling rates were estimated using Eq. 1.

$$R_S = \frac{M_S}{C_w\, t} \quad (1)$$

where R_s is the sampling rate (L d^{-1}), M_s is the mass of analytes accumulated in the receiving phase (ng), C_w is the mean water concentration (ng L^{-1}), t is the deployment time (days).

Extraction of targeted pharmaceuticals was done in a period of 14 days, where a passive sampler chamber containing six membrane bags with acceptor phase mixture was immersed at about 10 cm deep into a 5 L glass beaker containing 3 L of deionized spiked with 10 ng mL^{-1} of analytes. On day 1, 3, 5, 9, 11 and 14, a single membrane bag was removed from each deployment system, followed by desorption of analytes from MIP and their analysis with LC-qTOF/MS. Additionally, on each of those days a 0.5 mL sample of aqueous sample was sampled and taken to LC-qTOF/MS for analysis. These experiments were done in triplicate.

Furthermore, a second laboratory scale calibration of the sampler was done using environmental surface water sampled from Florida Lake, Gauteng, South Africa (26.1739° S, 27.8971° E). This environmental surface water was initially analyzed for the presence of the target pharmaceuticals using an analytical method that was previously developed by our research group [32]. None of the target pharmaceuticals were found in water samples from Florida Lake. The purpose of performing this calibration with an environmental sample was to investigate the effect of biofouling on the passive sampler sampling rates with the view to understand the behavior of the sampler in the environment. This was achieved by immersing the passive sampler into the glass beaker containing 3 L of the water spiked with 10 ng mL^{-1} of analytes and stirring the system at 100 rpm. The experiments were done in triplicate over a period of 14 days. The pH of the water at the time of sampling was 7.36, and a temperature of 25.1 °C which is equivalent to room temperature. Calculations of the sampling rates were done using Eq. 1.

4.2.6 Study site and field deployment of passive samplers

The passive samplers were deployed at the Orlando Dam (26.2561° S, 27.9222° E) situated in Soweto, a highly populated and biggest South African township located in the Gauteng Province. This dam is situated in the downstream of a big government owned hospital, a private hospital and a shopping mall (Fig 2). Orlando Dam receives water from the Baileyspruit and Diepkloofspruit streams which both cut through the highly populated Soweto township. The dam serves as recreational fishing dam as utilized by the members of the community. During the time of deployment and retrieval of passive sampler, the dam was found to be surrounded by hyacinth and other vegetation, which is a sign of eutrophication. A study by Bengu and colleagues reported a presence of nitrites, nitrates, phosphates and faecal coliform bacteria in the Orlando dam surface water [34]. In the present study, three passive sampling devices were deployed in three parts of the Orlando dam. These three sites were the most accessible and conducive part of the dam for passive sampling. Physicochemical parameters such pH (7.74), conductivity (485 µS cm^{-1}), total dissolved solids (243 mg L^{-1}) and temperature (27.6 °C) were all measured *in situ* at the time of deployment. Water from this dam pours into the Klipspruit stream, a stream which has been documented in reports by the water research commission and the city of Johannesburg as being highly impacted by sewage discharge [34].

The passive samplers were deployed in Orlando Dam for 14 days, the period at which samplers were operating at the integrative regime. For estimation of TWA concentrations of the targeted pharmaceuticals Eq. 2 was used.

$$C_w = \frac{C_s M_s}{R_s\, t} \qquad (2)$$

where C_w is the mean analyte concentration in the water during the deployment period (ng L^{-1}), C_s is the analytes concentration accumulated in the adsorbent phase (ng g^{-1}), M_s is the mass of the adsorbent in the passive sampler (g), R_s is the sampling rate (L d^{-1}) and t is the total deployment time (days).

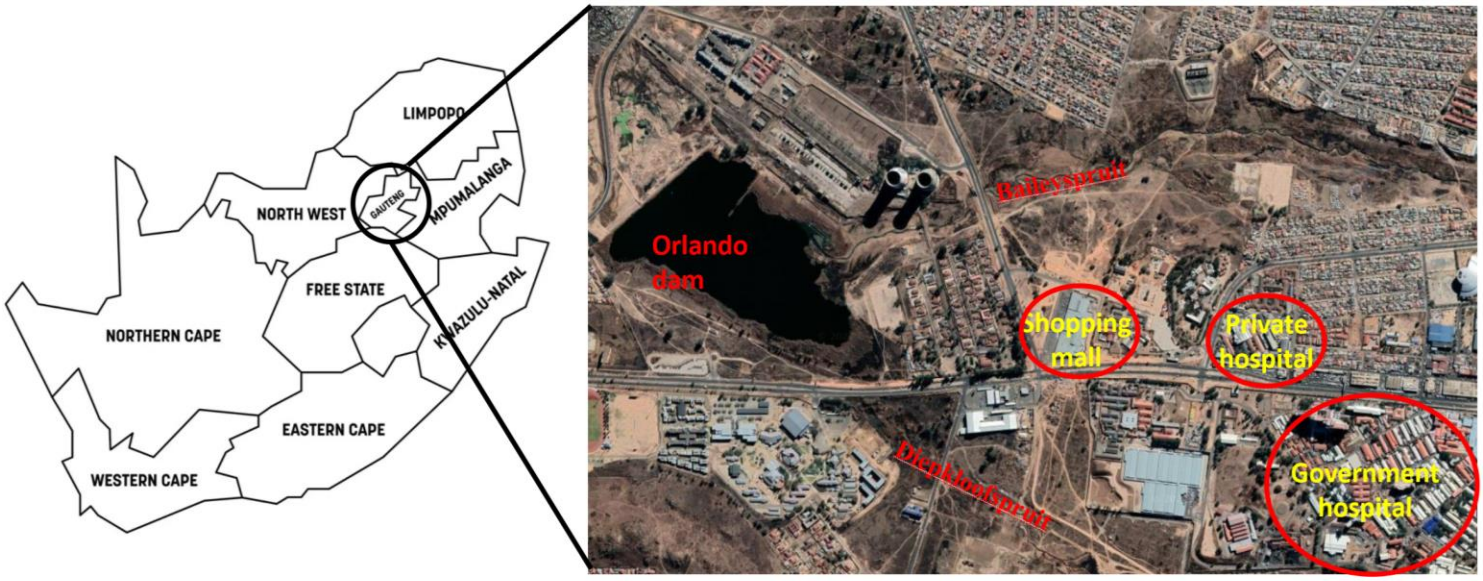


Fig. 2 Deployment site, Orlando Dam, Gauteng Province, South Africa.

4.2.7 MASE-MIP extraction of water samples

At the time of deployment of passive samplers, water samples were grabbed into pre-cleaned plastic bottles from Orlando Dam and transported into the laboratory in a cooler box for further preparation. The samples were filtered through 0.22 µm syringe filters (Merck, Johannesburg, South Africa) followed by pre-treatment with a MASE-MIP technique developed and validated in our previous work [148]. The sample vial consisted of 18 mL water sample saturated in 5 g sodium chloride, and a propylene membrane bag filled with 1 mL chloroform and 50 mg of MIP. The samples were stirred at 400 rpm for a duration of 60 minutes to allow for extraction of targeted compounds. Thereafter, the contents of the membrane bag were transferred into 1 mL empty SPE cartridge with 10 µm frits at the bottom and mounted onto an SPE manifold. Chloroform was allowed to flow through the SPE cartridge utilizing a gravitational form and sent to waste. A steady vacuum was then applied to dry the MIP particles,

followed by elution of analytes with 4 x 1 mL of methanol. After which, the extracts were combined and reduced to 1 mL by gentle purging with nitrogen gas and then finally taken to the LC-qTOF/MS for chromatographic determination and quantification. This step was conducted to check for any variability in the results obtained by MASE-MIP and the passive sampler.

4.3 Results and discussions

4.3.1 Optimization of extraction conditions

4.3.1.1 Acceptor phase

In this step, we investigated the solvent type on the extraction of target compounds. Upon discovering that chloroform could not be utilized as an extraction solvent for long term, a DES (aliquat 336:1-octanol on a 1:1 ratio) and an IL (aliquat 336) were investigated as suitable solvents. These solvents with their chemical structures given in Fig. 3 were chosen based on their stability and their ability to allow for hydrogen bonding (Fig. 3). However, in the MASE-MIP technique, an effective solvent should allow for both the movement of analytes across the polypropylene membrane as well as not interfere with their binding onto the MIP cavities [35]. Fig. 4 demonstrate accumulated amount of each target pharmaceutical on the MIP using the three tested solvents during the 7 days of deployment. From the figure, it was noted that target pharmaceuticals were extracted and transported better using aliquat 336. This is possible due to the interactions taking place between the chloride of aliquat 336 and the hydrogen atom of the amide and/or hydroxyl functional groups present in the target pharmaceuticals (chemical structures provided in Table 1). Also, electrostatic interactions are expected to occur between the nitrogen ion present in aliquat 336 and oxygen of the pharmaceutical compounds. This also means that the movement of analyte from the solvent to the MIP cavities was more pronounced with aliquat 336, than it was with the DES. Even though literature suggests that DESs are good in the dissolution of pharmaceuticals due to their excellent physicochemical properties [28,

36, 37]; it can also be noted that the DESs would have been utilized in direct contact with the aqueous sample (e.g. liquid-liquid extraction). For a example, Tang and co-workers extracted two antibiotics from environmental water through liquid-liquid microextraction with an aid of the same DES. However, it should be noted that several factors like the sample salt content, vortex and sample in contact in separation solvent could have aided on the high recoveries obtained (> 94.8%). In the present study, there is a protective layer offered by the polypropylene membrane bag between the sample and the extraction, also the extraction takes place in two stages. Therefore, based on the results obtained, further experiments were conducted with aliquat 336 as an extraction solvent.

(a) (b)

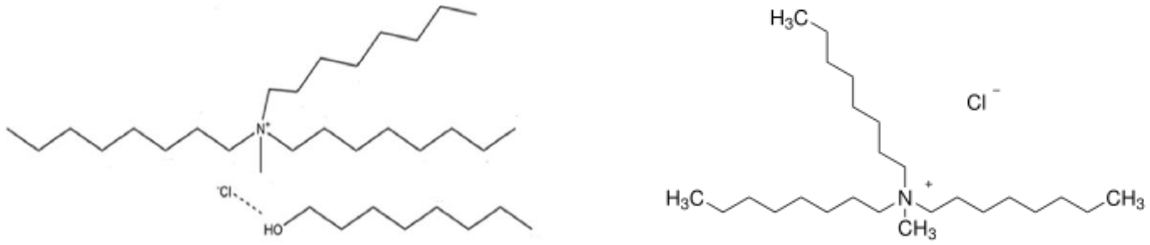
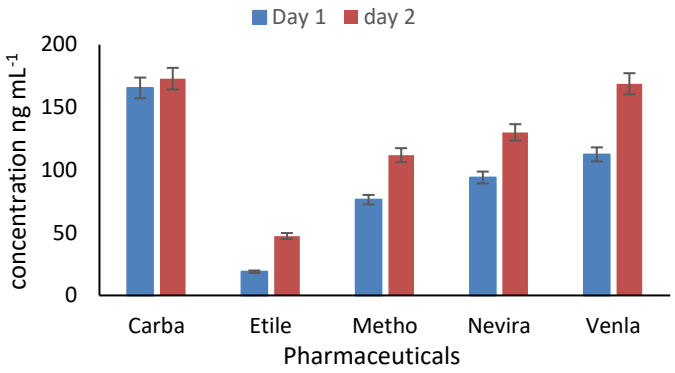
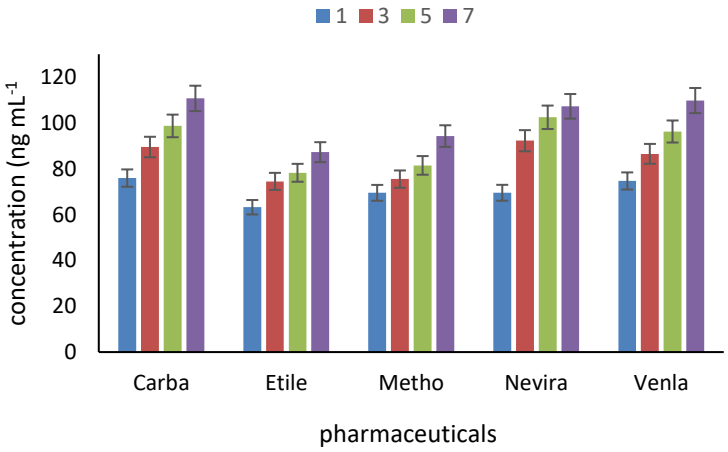


Fig. 3 Chemical structures of (a) DES (aliquat 336:1-octanol) and (b) Ionic liquid (aliquat 336)



(a)



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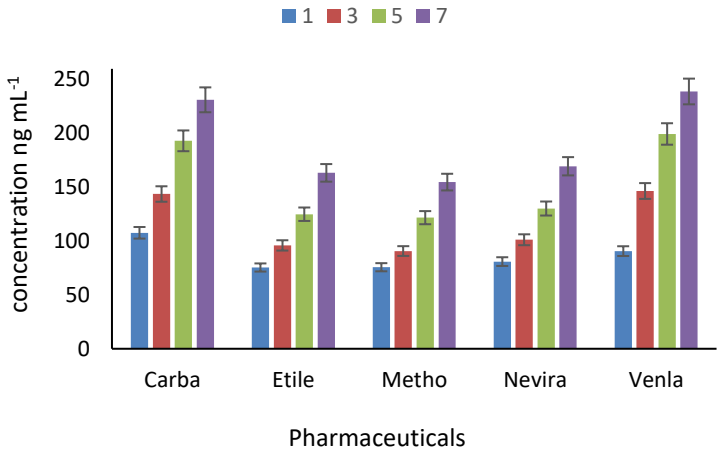

(c)

Fig. 4. Performance of (a) chloroform, (b) 1-octanol:aliquat 336 50:50 %v/v and (c) aliquat 336 on the extraction of Carba (carbamazepine), Etile (etilefrine), Metho (methocarbamol), Nevira (nevirapine) and Venla (venlafaxine) from the aqueous donor phase over a period of 2 and 7 days

4.3.1.2 Effect of exposure time and biofouling

To be able to determine time weighted concentrations of analytes in environmental water, determination of accumulation rates (sampling rates) onto the MIP cavities is paramount. During the deployment period the accumulation of analytes onto the sampler sorbent can be described in three regimes; the first one is the linear regime, the second is the curvilinear regime and the third one is the equilibrium regime. The linear regime is the range at which sampling rates are calculated. This parameter can be affected by a number of factors which can include biofouling, turbulence, temperature, pH and diffusion of analytes through the membrane [19]. In this work, the water pH was adjusted to 7.5 and the system was stirred at 100 rpm with the aim of mimicking environmental water pH and dam water hydrodynamic conditions. In some cases, the stirring rate influences the partitioning of analytes from donor to receiver phase. However, in the case of compounds with logKow < 3 (same as the target compounds, Table 1), not much gain can be obtained by increasing the stirring rate [38]. This phenomenon was proven to be true in the case of the target pharmaceuticals, where increasing the stirring rate from 400 rpm to 1200 rpm did not have a positive effect on their extraction process [32].

The samplers were immersed in a beaker containing 10 ng L^{-1} of each target pharmaceutical and their accumulation on the MIP cavities was measured over a period of 14 days. Good linear regressions were obtained for all compounds (Fig. 5), with R^2 values ranging from 0.9782 to 0.9924. The sampling rates were determined through Eq. 1 using the slope (Ms t^{-1}) of the linear regression plot and the concentration in the water (Cw). The Rs values (L d^{-1}) obtained (Table 2) ranged from 0.0009 to 0.0028 for individual pharmaceuticals. These values assisted in understanding the volume of water that can be extracted by the passive sampler per day. Accepted precision for triplicate results was indicated by relative standard deviation ranging from 5.1 – 7.9%.

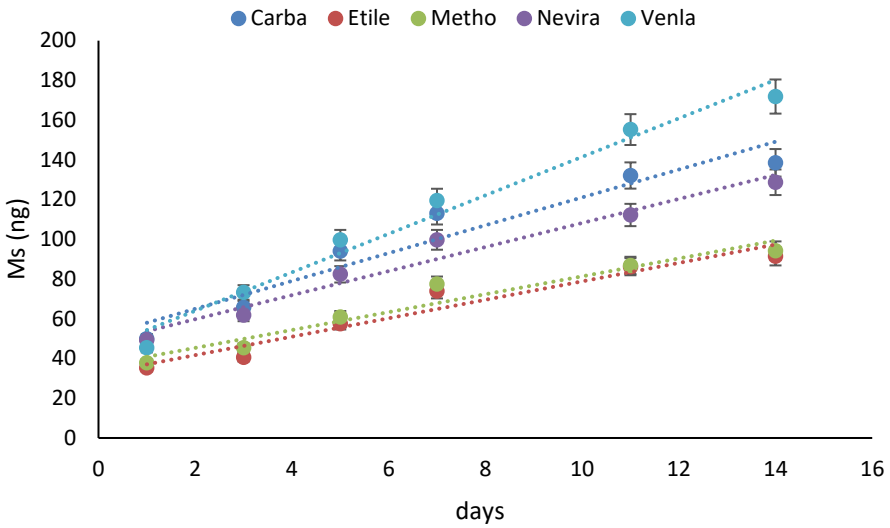

Figure 5: Accumulation of target pharmaceuticals on the Mip cavities over a period of 14 days. Carba (carbamazepine), Etile (etilefrine), Metho (methocarbamol), Nevira (nevirapine), Venla (venlafaxine).

In order to understand and estimate the uptake rates of the passive sampler in environmental water, another laboratory scale matrix-matched calibration was conducted. The purpose of conducting the matrix-matched calibrations was to estimate sampling rates that are closer to field sampling rates, and to understand the effect of biofouling on the uptake of target pharmaceuticals. In this instance, the accumulation of pharmaceuticals on the MIP cavities was measured over a period of 14 days. Good linear regressions (Fig. 6) with good correlation values ranging from 0.9715 to 0.9910 were obtained. The Rs values attained ranged from 0.0007 to 0.0018 (L d^{-1}). Triplicate results yielded relative standard deviation ranging from 7.9 – 13.5%. From Fig. 6, it can be observed that the accumulation of target compounds was affected by biofouling. A drop in the accumulation could have been caused by slowed down diffusion of analytes through the polypropylene membrane bag as biofouling accumulated on the membrane surface over time.

Table 2. Performance of the MASE-MIP based passive sampler, (n=3).

Target compound	t_R (min)	LC-qTOF/MS calibration			D.H_2O sampler calibration		Matrix-matched calibration			
		Range (ng mL^{-1})	R^2	Fragmentation ion (m/z)	Rs (L d^{-1}, ± %RSD)	R^2	Rs (L d^{-1}, ± %RSD)	R^2	LOD (ng L^{-1})	LOQ (ng L^{-1})
Carbamazepine	7.90	1 - 100	0.9995	194	0.0023 ± 5.1	0.9867	0.0012 ± 8.2	0.9720	2.45	8.16
Etilefrine	2.67	1 - 100	0.9998	164	0.0011 ± 6.5	0.9782	0.0008 ± 8.5	0.9715	2.42	8.06
Methocarbamol	6.57	5 - 100	0.9988	118	0.0009 ± 7.9	0.9857	0.0007 ± 13.5	0.9910	3.10	10.33
Nevirapine	6.79	1 - 100	0.9995	198, 226	0.0017 ± 5.6	0.9924	0.0011 ± 8.0	0.9863	2.93	9.76
Venlafaxine	5.51	5 - 100	0.9993	121	0.0028 ± 5.3	0.9896	0.0018 ± 7.9	0.9814	3.26	10.81

t_R : retention time, Rs: sampling rates, D.H2O; deionized water, LOD: limit of detection, LOQ: limit of quantification

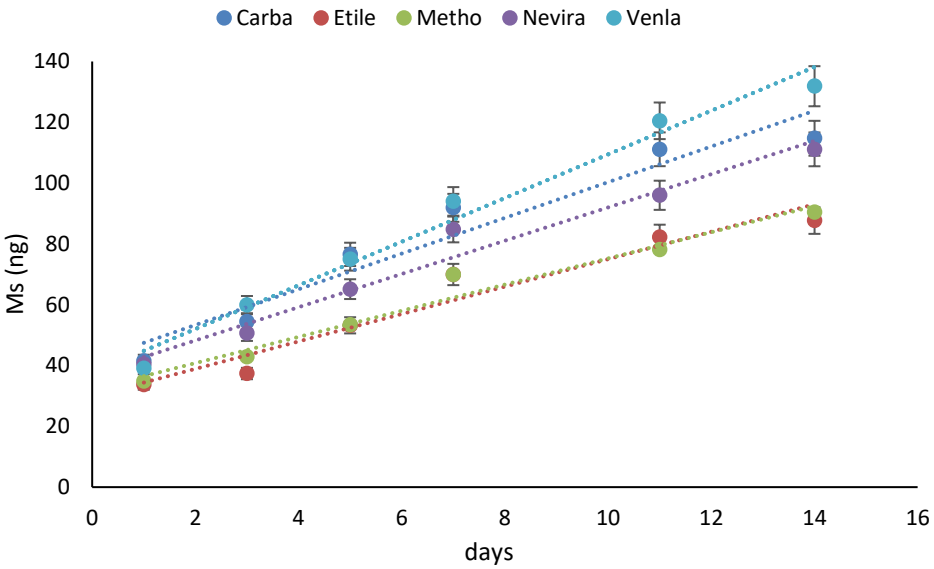

Figure 6 Matrix-matched accumulation of target pharmaceuticals of the MIP cavities over a period of 14 days. Carba (carbamazepine), Etile (etilefrine), Metho (methocarbamol), Nevira (nevirapine), Venla (venlafaxine).

Of all the targeted pharmaceuticals, carbamazepine is the most investigated compound in passive sampling-based studies. Vermeirssen and colleagues estimated the Rs for this compound to be 0.098 and 0.301 L d^{-1} using Chemcatcher and POCIS devices respectively [3]. In another study the estimated Rs was 0.214 L d^{-1} [39], whilst Bayen and coworkers estimated the sampling rates to be 0.600, 0.157 and 0.497 L d^{-1} for the same compound when calibration was carried under different physicochemical conditions [40]. In one study, the Rs for venlafaxine was reported to be 0.23 L d^{-1} [41]. It is well noted that there is quite a variation in the sampling rates for individual compounds which is attributed to the parameters at which calibrations were carried under. This then emphasizes a need for calibration of samplers at conditions closer to the environmental conditions of the chosen deployment site. In the present study, the sampling rates are observed to be lower than those reported in literature which could

be due to several factors. One could be the type of membrane used in the present study, as this influences the permeation of compounds into the receiver phase, another factor could be the density of the applied receiver solvent. Although carrying out the experiments at temperatures higher than the ambient temperature could reduce the viscosity of solvent and in turn improve extraction; it would not be ideal in the optimization of this sampler and in the estimation of the Rs for purposes of estimating TWA concentrations in environmental waters which is hardly ever above a temperature of 30 °C.

4.3.2 Validation and application of a passive sampling device in environmental monitoring

The developed MASE-MIP based passive sampler was validated yielding results presented in Table 2. The linearity of the of the standard calibration curves was evaluated over a range of 5 – 100 ng L^{-1} for venlafaxine and methocarbamol and a range of 1 – 100 ng L^{-1} for the other pharmaceuticals o. The R^2 values ranged from 0.9988 to 0.9998. Matrix-matched method LODs and LOQs ranged from 2.42 to 3.26 ng L^{-1} and 8.06 to 10.81 ng L^{-1}, respectively. Identification of analytes was done on the LC-qTOF/MS using retention times and major characteristic fragment ions summarized in Table 2.

The developed passive sampler was deployed for 14 days at three different sites of the Orlando Dam for the purposes of investigating its application ability in environmental water bodies. Eq. 2 was used in the estimation of the TWA concentrations in the dam with the aid of the Rs obtained in the matrix-matched calibrations of the sampler. Table 3 presents the results obtained. It can be noted that methocarbamol was detected in all deployment sites with TWA concentrations ranging from 16.99 to 72.29 ng L^{-1}, whilst etilefrine was only detected in site 2 and 3 at TWA concentrations of 8.17 and 12.88 ng L^{-1}, respectively. The other three compounds were not detected in all the deployment sites. However, a recent South African based study reported the occurrence of carbamazepine in Klip River with concentrations less than the method detection limits

of 0.094 ng mL^{-1} [42]. In Singapore, carbamazepine had TWA concentrations ranging from 0.56 to 0.96 ng L^{-1} in tropical lakes and 2.8 ng L^{-1} in estuarine water [40]. In a different study, the bioavailable amount of venlafaxine ranged from 34 - 605 ng in the surface waters of the Czech Republic [41].

In addition to the deployed samplers, grab samples were collected at the deployment sites to assess any variation in concentrations when two sample preconcentration techniques are used. The MASE-MIP technique applied was optimized and validated for the extraction of these model pharmaceuticals in surface water in our previous work. However, none of the compounds were detected nor quantified with this method. This was due to the fact that the method LODs for the MASE-MIP for the compounds detected through passive sampling ranged from 0.17 to 0.20 ng mL^{-1}, which is much higher than the concentrations that can be quantified through the sampler (LOQs on Table 2). This then makes the developed passive sampling technique a suitable and attractive technique for the estimations of the average trace level concentration for the targeted pharmaceuticals in environmental water bodies.

Table 3. TWA concentrations of the selected pharmaceuticals found in different sampling sites of the Orlando dam, (n=3, %RSD)

Site ID	Carbamazepine (ng L^{-1})	Etilefrine (ng L^{-1})	Methocarbamol (ng L^{-1})	Nevirapine (ng L^{-1})	Venlafaxine (ng L^{-1})
Orlando Dam Site 1	ND	ND	16.99 ± 13	ND	ND
Orlando Dam Site 2	ND	8.17 ± 11	49.22 ± 14	ND	ND
Orlando Dam Site 2	ND	12.88 ± 13	72.29 ±13	ND	ND

ND: not detected

To the best of our knowledge, this is the first study which investigated the environmentally available concentrations of these target compounds in South African waters through passive sampling technique. This is also the first study which designed, optimized, and applied a MASE-MIP based passive sampler for environmental monitoring of pharmaceuticals. Rimayi and coworkers conducted a study focused on the screening of emerging pollutants in the Hennops and Juskei Rivers of South Africa through a Chemcatcher passive sampler [43]. Carbamazepine, etilefrine, methocarbamol and venlafaxine were detected. However, their study only focused on the screening and not the quantification of these pharmaceuticals.

4.4 Conclusions

A MASE-MIP based passive sampling device was successfully designed, optimized and applied in the monitoring of selected model pharmaceuticals, belonging to five different therapeutic classes. The optimized sampler showed ability of the usage of an ionic liquid, aliquat 336, as a green receiver solvent. Results showed slightly reduced accumulation of target compounds onto the receiver phase, which could be attributed to the viscosity of the aliquat 336. Even though that was the case, the sampler showed ability to be applied in the monitoring of trace environmental concentrations (up ng L^{-1}) in surface water, where methocarbamol and etilefrine trace TWA concentrations were detected. The optimized passive sampling device was proven to be precise, efficient and an attractive alternative to conventional sampling techniques for the monitoring of trace level concentrations of the targeted compounds.

Conflict of interests

The authors declare that there is no conflict of interests

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CHAPTER 5

This chapter highlights the development, validation and environmental application of all the three extraction techniques for the monitoring of the selected pharmaceuticals in surface water.

5 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

A venlafaxine imprinted smart polymer was successfully synthesized for the selective and efficient extraction of selected pharmaceuticals belonging to five therapeutic classes from aqueous solutions. Venlafaxine as an imprinting molecule was selected through various cavity tuning experiments where all the target pharmaceuticals were investigated as either a single or multi-template. The target pharmaceuticals included an antidepressant (venlafaxine), an antiretroviral (nevirapine), a muscle relaxant (methocarbamol), an anticonvulsant (carbamazepine) and a cardiac stimulant (etilefrine). Batch adsorption and kinetic studies displayed that adsorption of the selected pharmaceuticals onto the MIP cavities followed a Freundlich adsorption isotherm as well as a pseudo second order adsorption model. This was an indication of the heterogeneity of the binding surface energies on the MIP resulting in multiple interactions through chemisorption. An analytical method for quantification of the model pharmaceuticals was also successfully developed using liquid chromatography-mass spectrometry (LC-MS), yielding detection and quantification limits ranging from 0.03 to 0.31 ng mL^{-1} and 0.12 - 3.81 ng mL^{-1}, respectively. Furthermore, the venlafaxine imprinted polymer was evaluated as a selective adsorption sorbent for solid phase extraction (SPE) of the selected pharmaceuticals in dam water samples followed by analysis through LC-MS, yielding recoveries ranging from 43 - 69%. The results obtained showed emphasis on the utilization of MIP cross selectivity to target multiple analytes from complex aqueous samples.

A two-way technique based on the combination of membrane assisted solvent extraction and a molecularly imprinted polymer (MASE-MIP) for selective and efficient extraction of five selected pharmaceuticals belonging to five different therapeutic classes was successfully developed and optimized through central composite design. The five model pharmaceuticals were extracted from surface water samples followed by liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-qTOF/MS) analysis, giving detection and quantification limits

ranging from 0.09 to 0.20 ng mL^{-1} and 0.31 to 0.69 ng mL^{-1}, respectively. Spiked environmental water samples yielded recoveries ranging from 38 to 91% for the selected model compounds. Additionally, the developed analytical method was applied in the analysis of water samples collected from two major South African rivers. All the selected model pharmaceuticals were detected in the river water samples with nevirapine and venlafaxine being more prominent reaching maximum concentrations of 1.64 and 2.48 ng mL^{-1}, respectively. This highlighted a necessity to conduct extensive environmental monitoring studies in order to determine the sources and spread of pharmaceuticals in South African waters.

A passive sampler based on a combination of membrane assisted solvent extraction and a molecularly imprinted polymer (MASE-MIP) was successfully designed, optimized and applied in the environmental monitoring of selected model pharmaceuticals, belonging to five different therapeutic classes. The optimized sampler showed to be a promising and innovative sampler which utilizes the MASE-MIP combination as a passive sampling technique. Moreover, the sampler displayed an ability utilize an ionic liquid, aliquat 336, as a green receiver solvent. However, results obtained displayed slightly reduced accumulation of target compounds onto the receiver phase when compared with what is in literature. This could be attributed to the viscosity of the aliquat 336. Even though that was the case, the technique allowed for unprompted extraction and pre-concentration of the target pharmaceuticals over longer periods of time and in turn allowed for estimation of trace time weighted average (TWA) environmental concentrations (up to ng L^{-1}). The optimized MASE-MIP based sampling method attained detection and quantification limits ranging from 2.45 - 3.26 ng L^{-1} and 8.06 - 10.81 ng L^{-1} through liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-qTOF/MS), respectively. Upon deployment at a South African dam, only etilefrine and methocarbamol were detected and quantified at maximum TWA concentrations of 12.88 and 72.29 ng L^{-1}, respectively.

Overall, the developed MASE-MIP passive sampler proved to be an attractive alternative approach to the commercially available passive samplers for monitoring of the selected model pharmaceuticals in surface water. Parameters such as detection limits can still be improved by deploying the sampler for extended deployment periods.

Appendix A

Table A1. MS/MS instrumental parameters for detection of target compounds

Compound name	Precursor ion (m/z)	Fragment ion (m/z)	Collision energy (eV)	Ionization mode	Retention time (min)
Etilefrine	182	164	18 – 45	ESI - positive	2.67
Carbamazepine	237	194	29 – 72.5	ESI - positive	7.90
Methocarbamol	242	118	15 – 37.5	ESI - positive	6.57
Nevirapine	267	298; 226	44 - 110	ESI - positive	6.79
Venlafaxine	278	121	28 – 70.1	ESI - positive	5.51

Table A2. Adsorption modelling parameters using venlafaxine-imprinted polymer

	Freundlich isotherm			Langmuir isotherm		
	n	K_f (ng $mL^{-1})^{1/n}$	R^2	q_m (ng mg^{-1})	b (mL mg^{-1})	R^2
Carbamazepine	0.404	23.9	0.9081	232.6	3.69×10^{-3}	0.9151
Etilefrine	1.095	3.2	0.9171	204.1	4.33×10^{-3}	0.6452
Methocarbamol	0.909	3.6	0.9989	303	1.85×10^{-3}	0.9467
Nevirapine	1.042	2.8	0.9071	188	3.19×10^{-3}	0.8765
Venlafaxine	1.141	1.8	0.9902	471	3.41×10^{-3}	0.7145