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DETERMINATION OF SELECTED ACIDIC PHARMACEUTICAL COMPOUNDS IN WASTEWATER TREATMENT PLANTS



Lawrence Mzukisi Madikizela

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

November 2016

DEDICATION

To My Late Father

Mongezi Morris Madikizela

1964 - 2001

DECLARATION

I declare that this thesis is my own work, unaided work. It is being submitted for the Degree of Doctor of Philosophy to the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination to any other university.

.....

Mr. Lawrence Mzukisi Madikizela

School of Chemistry, University of the Witwatersrand, Johannesburg. 2016

This thesis is submitted after examination and approval by the following supervisor

.....

Prof. Luke Chimuka

School of chemistry, University of the Witwatersrand, Johannesburg, 2016

ABSTRACT

This research was directed towards the environmental monitoring and assessment of the most used non-steroidal anti-inflammatory drugs (NSAIDs) which are naproxen, ibuprofen and diclofenac. The work involved the development and application of sensitive techniques for the quantification of naproxen, ibuprofen and diclofenac in the South African aquatic environment. Based on this information, a multi-templates molecularly imprinted polymer (MIP) was synthesized and applied alongside the commercial available sorbent (Oasis MAX) in the solid-phase extraction (SPE) of target compounds from water samples. The extracted compounds were then quantified using high performance liquid chromatography (HPLC).

MIP was synthesized by applying a bulk polymerization approach at 70 °C where all target compounds were used as multi-templates. Other reagents used in synthesis were 2-vinyl pyridine, 1,1'-azobis-(cyclohexanecarbonitrile), ethylene glycol dimethacrylate and toluene as functional monomer, initiator, cross-linker and porogenic solvent, respectively. Synthesis of a non-imprinted polymer (NIP) under similar reaction conditions as MIP was carried out with the omission of templates.

Techniques employed in characterization of MIP and NIP were Fourier transform infrared spectroscopy (FTIR), Brunauer, Emmett and Teller (BET) method, CHNS analyzer, zeta potential, cross-polarization/magic angle spinning nuclear magnetic resonance spectroscopy, thermogravimetric analysis, differential scanning calorimetry and x-ray diffraction. Monomer-template interactions were investigated using molecular dynamics.

The performance of the MIP was evaluated based on its ability to selectively extract target compounds in organic (acetonitrile, acetone, chloroform and toluene) and aqueous media. The extraction capacity of the MIP in organic solvents for naproxen, ibuprofen and diclofenac increased from high polarity to low polarity solvents. In a low polarity solvent (toluene), the extraction capacity achieved for naproxen, ibuprofen and diclofenac were 14.4, 11.0 and 14.0 mg/g, respectively. In this case, the selectivity of the MIP where gemfibrozil was employed as the

competing species was evident. Selectivity of the MIP collapsed during the adsorption of naproxen, ibuprofen and diclofenac from water using gemfibrozil and fenoprofen as competitors. This resulted in high extraction efficiencies for target compounds and competitors, however, both gemfibrozil and fenoprofen were easily desorbed from the MIP using weak organic solvent due to lack of molecular recognition.

During the binding sites characterization, the best fit of pseudo-second-order implied a chemisorption of all target compounds onto MIP sorbent. The data also fitted well in Langmuir isotherm which meant that the adsorption of target pharmaceuticals occurred on the homogeneous binding sites of the MIP.

Optimized adsorption conditions in water such as MIP amount of 50 mg, extraction time of 10 min, sample pH of 2.5 and sample volume of 10 mL were applied for the selective adsorption of naproxen, ibuprofen and diclofenac in contaminated wastewater and river water. In WWTP influent, naproxen recovery was 38%, whereas ibuprofen and diclofenac were 69% and 87%, respectively.

MIP was further used as a selective adsorbent in solid-phase extraction (SPE) of three drugs from environmental samples. The selectivity of the MIP in environmental samples was compared to that of the commercially available Oasis MAX sorbent. The application of molecularly imprinted solid-phase extraction (MISPE) reduced matrix effects and improved the sensitivity of the analytical method. In this case, the detection limits for naproxen, ibuprofen and diclofenac were 0.2, 1 and 0.6 µg/L, respectively. When deionized water was spiked with 5 and 50 µg/L of target compounds, recoveries greater than 80% were obtained.

Thereafter, the developed MISPE was applied for selected acidic drugs from environmental samples. Environmental samples were collected from urban (Durban) and semi-urban/rural areas (Ladysmith) of KwaZulu-Natal Province in South Africa. The most abundant compound in the environment was ibuprofen. In river water samples from Durban, the maximum concentrations found for naproxen, ibuprofen and diclofenac were 6.8, 19 and 9.7 µg/L, respectively. The maximum amounts found for the same drugs in Ladysmith river samples were generally lower

with naproxen, ibuprofen and diclofenac detected at 2.8, 6.7 and 2.6 µg/L, respectively. The same trend was observed in wastewater.

Further work on the monitoring of acidic compounds in wastewater was conducted using Oasis MAX as the SPE sorbent prior to HPLC analysis. All target compounds were detected in Kingsburg and Umbilo WWTPs located in Durban surroundings. The influent and effluent concentrations detected were in the ranges of 6.4 to 69 µg/L and 0.6 to 4.2 µg/L, respectively. Further to this, the removal efficiency of the target compounds during the WWTP process in Kingsburg and Umbilo was in the range of 69 to 97%.

The extent of pollution in the environment was further assessed by the monitoring of ketoprofen and triclosan in wastewater and river water using SPE with Oasis HLB sorbent and HPLC. Traces of both compounds ranging from 1.2 to 9.0 µg/L were detected in wastewater. The maximum concentrations found in river water were 2.0 and 0.9 µg/L for ketprofen and triclosan, respectively.

Overall, the analytical methods implemented in this work were highly accurate, precise and sensitive. The synthesized MIP was highly selective and its application in environmental studies led to the development of a less expensive analytical method. This work also gives the overview of the extent of water pollution caused by acidic pharmaceuticals in various water matrices.

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Jesus once said "I am the light of the world. Whoever follows me will never walk in darkness, but will have the light of life" John 8:12.

Hence, I decided to follow him.

Indeed with God all things are possible.

Ndiyabulela!!!

PUBLICATIONS AND MANUSCRIPTS

This thesis is based on the following papers:

1. **Status of pharmaceuticals in African water bodies: Occurrence, removal and analytical methods**

   Lawrence Mzukisi Madikizela, Nikita Tawanda Tavengwa, Luke Chimuka

   Manuscript. Under Revision in Journal of Environmental Management

2. **Experimental and theoretical study of molecular interactions between 2-vinyl pyridine and acidic pharmaceuticals used as multi-template molecules in molecularly imprinted polymer**

   Lawrence Mzukisi Madikizela, Phumlane Selby Mdluli, Luke Chimuka

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3. **Synthesis, adsorption and selectivity studies of a polymer imprinted with naproxen, ibuprofen and diclofenac**

   Lawrence Mzukisi Madikizela, Luke Chimuka

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4. **Determination of ibuprofen, naproxen and diclofenac in aqueous samples using a multi-template molecularly imprinted polymer as selective adsorbent for solid-phase extraction**

   Lawrence Mzukisi Madikizela, Luke Chimuka

   Published: Journal of Pharmaceutical and Biomedical Analysis, 128 (2016) 210-215.

5. **Occurrence of naproxen, ibuprofen and diclofenac residues in wastewater and river water of KwaZulu-Natal Province in South Africa**

   Lawrence Mzukisi Madikizela, Luke Chimuka

   Manuscript. Under Revision in Journal of Environmental Monitoring and Assessment.

6. **Molecularly imprinted solid-phase extraction of naproxen, ibuprofen and diclofenac from Ladysmith water resources in South Africa: An initial assessment**

   Lawrence Mzukisi Madikizela, Phumlane Selby Mdluli, Luke Chimuka

   Manuscript. Submitted.

7. **Simultaneous determination of naproxen, ibuprofen and diclofenac in wastewater using solid-phase extraction with high performance liquid chromatography**

   Lawrence Mzukisi Madikizela, Luke Chimuka

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8. **Determination of triclosan and ketoprofen in river water and wastewater by solid phase extraction and high performance liquid chromatography**

   Lawrence M. Madikizela, Sindisiwe F. Muthwa, Luke Chimuka

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LIST OF ABBREVIATIONS

BET	Brunauer, Emmett and Teller
COX	Cyclooxygenase
DAD	Diode array detector
DWTPs	Drinking water treatment plants
FLD	Fluorescence detection
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
HF-LPME	Hollow fiber-liquid phase microextraction
HLB	Hydrophilic lipophilic balance
HPLC	High performance liquid chromatography
LLE	Liquid-liquid extraction
LPME	Liquid-phase microextraction
MIP	Molecularly imprinted polymer
MISPE	Molercularly imprinted solid-phase extraction
MS	Mass spectrometry
NIP	Non imprinted polymer
NSAIDs	Non-steroidal anti-inflammatory drugs
SBSE	Stir-bar sorptive extraction
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
WWTP	Wastewater treatment plant

Chapter 1 - INTRODUCTION

This chapter gives the general information about the target compounds which includes their sources in the environment, toxicity as well as the problem statement and motivation.

1 Introduction

1.1 General Introduction

Clean water is an important resource on earth as it supports and maintains human health and sustainable ecosystem development. The growth of population, urbanization, and industrialization, and consumption patterns, change over time, which has generated the ever-increasing demands for freshwater resources worldwide (Sun et al., 2016). Therefore, the continuous monitoring of the quality status of water resources is important for ensuring the good well-being of the aquatic environment. The evaluation of the quality status of aquatic ecosystems requires the estimation of water pollution and possible consequences on the aquatic organisms (Arditsoglou and Voutsa, 2008). The discharge of treated wastewater into rivers is one of the major sources of water pollution. For example, the presence of high quantities of pharmaceuticals in surface water has been reported worldwide in which the primary source has been reported to be the wastewater treatment plant effluents (Paiga et al., 2016). In this regard, large quantities of such pollutants originate from industrial and domestic sources. Currently, pharmaceuticals especially non-steroidal anti-inflammatory drugs (NSAIDs) are regarded as the most detected pollutants in the water bodies.

Water is regarded as a good transport medium for organics with polar groups in their molecules (Bialk-Bielinska et al., 2016). Therefore, acidic pharmaceuticals which contain polar groups in their molecular structures (Table 1) are easily transported from one water matrix to the other. For example, acidic pharmaceuticals can be transported from wastewater into river water, then dam water to drinking water which could result in the occurrence of pharmaceuticals in all water resources. As a result, in many countries the sources of pharmaceuticals in surface water cannot be traced.

1.2 Acidic pharmaceutical compounds

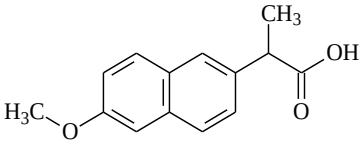
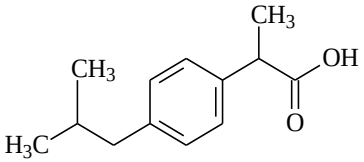
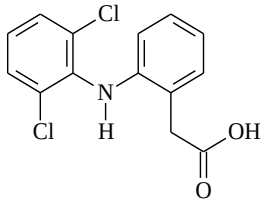
The primary focus of this study was on the detection and quantification of widely used NSAIDs such as naproxen, ibuprofen and diclofenac in South African

water resources. These acidic compounds are used in human medical care as analgesics (Al-Hadithi et al., 2011). NSAIDs are the most frequently used pharmaceuticals in the treatment of pains. Recently in South Africa, the data on consumption of pharmaceuticals indicated that medication with antipyretic activity is the most consumed (Matongo et al. 2015[a]). Based on this information, both diclofenac and ibuprofen were among the top five widely used NSAIDs in South Africa (Matongo et al. 2015[a]). The compounds in the NSAID group are available through medical prescription and over the counter via self-medication (Manrique-Moreno et al., 2016).

Naproxen, ibuprofen and diclofenac are weak organic acids (Table 1) with acid dissociation constants (p*K*a) ranging from 4.15 to 4.91 (Lindqvist et al., 2005), whereas, their octanol-water partition coefficients (K_{ow}) range between 0.7 and 3.97 (Behera et al., 2011). The properties of these compounds include high water solubility at neutral pH and polar nature which could lead to difficulty in their removal efficiency in the sewage treatment procedure (Koutsouba et al., 2003; Larsson et al., 2009; Dahane et al., 2013).

Table 1

The molecular structures of the selected pharmaceutical compounds and their physico-chemical properties.

Compound	Molecular structure	p*K*a	Log K_{ow}	Water solubility (mg/L)
Naproxen		4.15	3.18	44
Ibuprofen		4.91	3.97	58
Diclofenac		4.15	0.7	10

1.3 Mode of action and side effects caused by naproxen, ibuprofen and diclofenac on humans

Naproxen and ibuprofen are usually employed for the medical treatment of rheumatoid arthritis, however their irritant side effects on the gastro-enteric mucous membranes limits their application (Mahkam and Poorgholy, 2011). When ingested, NSAIDs inhibit the cyclooxygenase pathway by blocking the production of prostaglandins. Two of three existing cyclooxygenase (COX) enzymes are related to the biological activity of NSAIDs. In this case, COX-1 enzyme plays a role in synthesizing prostaglandins that act as the stomach lining and intestine protector. COX-2 enzyme is related to the production of prostaglandins that are associated with inflammation. COX-2 is therefore induced by cytokines, mitogens and endotoxins. Therefore NSAIDs act by inhibition of COX-2 enzyme (Manrique-Moreno et al., 2016).

As in the case of several pharmaceutical drugs, the use of naproxen, ibuprofen and diclofenac for healthcare is usually accompanied by several side effects. In general, the side effects of NSAIDs which are related to the COX-1 inhibition are gastrointestinal injury, kidney and liver damage (Manrique-Moreno et al., 2016). In the case of naproxen alone, the known side effects are headaches, dizziness, abdominal pain, nausea, and shortness of breath. Naproxen is labelled as a compound with a potential of increasing risk of suffering heart attack on humans and malignant effect on mucosal hydrophobicity. For ibuprofen, side effects such as cardiovascular, renal and hepatic damage are known (Manrique-Moreno et al., 2009). The latter may be associated with the long-time treatments using drugs with ibuprofen as the active ingredient. On the other hand, the use of diclofenac can lead to hepatotoxicity (Manrique-Moreno et al., 2009).

1.4 Toxicity of naproxen, ibuprofen and diclofenac

Very little information is known about health effects that are associated with the consumption of acidic pharmaceuticals by animals and aquatic species at low levels. Regarding humans, there has been no evidence on the acute toxicity

caused by naproxen, ibuprofen and diclofenac. Regardless of low or no toxicity associated with the presence of naproxen, ibuprofen and diclofenac in water bodies, it is wise to keep water resources free of such pollutants in order to minimise the risks that may be caused by long term exposure.

Negative health effects that are linked with the presence of diclofenac in the aquatic environment have been reported (Taggart et al., 2007; Oaks et al., 2012; Cuklev et al., 2012). For instance, diclofenac has been associated with the decline on the number of vultures in Asia (Taggart et al., 2007; Oaks et al., 2012). Also, diclofenac is known as a compound that affects organ histology and gene expression in fish at a concentration of 1 µg/L (Cuklev et al., 2012).

For naproxen, it has been reported that the quantity of mRNA in three genes was transformed significantly in the intestines of adult zebrafish after two weeks of exposure to naproxen, whereas, in the case of humans, it has been reported that the adverse side effects of NSAIDs occur in the gastrointestinal tract (Stancova et al., 2015).

The toxicity of ibuprofen against *Selenastrum capricornium* (a microalga) has been reported where the toxicity of ibuprofen showed an increase with increasing concentration of the drug (Quero-Pastor et al., 2014). It has also been reported that the ibuprofen degradation products formed after the application of ozone which is used interchangeable with clrorine in WWTPs were more toxic than the ibuprofen itself (Quero-Pastor et al., 2014).

1.5 Sources of pharmaceutical compounds in the aquatic environment

The most common sources of acidic pharmaceuticals in the environment include households, wastewater treatment plants (WWTPs), hospitals, industrial units as well as intensive animal-breeding farms (Kostopuolou and Nikolaou, 2008). However, there are other possible sources which are related to human activities that cannot be ignored. Such sources are the direct discharge of untreated wastewaters to the environment through the leakage of septic tanks, landfill leachates and the use of manure or sludge from WWTP to promote plant growth in agricultural fields (Paiga et al., 2016). In most cases, pharmaceuticals are consumed by humans and thereafter, they are subjected to metabolism, then excreted as

metabolites or unaltered parent compounds. It has been indicated that the consumed ibuprofen, naproxen, and diclofenac are usually eliminated from human body with 10, 70 and 10% of unchanged drugs, respectively (Kermia et al., 2016). Excreted pharmaceuticals usually enter the sewer system through toilets, thereafter, they are transported to WWTPs. Degradation of naproxen, ibuprofen and diclofenac in WWTPs depends on the biological treatment efficiency, and in some cases, evidence of poor removal of pharmaceuticals during wastewater treatment processes has been reported (Kermia et al., 2016). This indicates that transformation of pharmaceuticals in WWTPs is possible. However, some compounds resist biodegradation and are therefore discharged with wastewater effluent into the nearest rivers (Parrilla Vazquez et al., 2013).

Various concentrations of pharmaceuticals arrive in WWTPs depending on several factors which include:

- Frequency of use for each pharmaceutical drug;
- Excretion of un-metabolized pharmaceutical drugs; and
- Resistance of pharmaceutical drugs to biodegradation (Kostopuolou and Nikolaou, 2008).

Since 1994, the permission to discard unused pharmaceutical drugs with household waste was granted by European Union (Kummerer, 2009). In the case of pharmaceutical waste disposal down the domestic drains, the pharmaceutical compounds are transported with domestic wastewater into WWTPs. However, in the case of solid waste disposal, the compounds can end up on landfill sites, where they can be released with landfill effluent and contaminate surface water or drinking water (Kummerer, 2009).

1.6 Problem statement and motivation

South Africa is lagging behind in the evaluation of the status of pharmaceuticals in water resources such as WWTPs, drinking water treatment plants (DWTPs), rivers, dams, swimming pools and lakes. The extent of water pollution in South African water resources caused by pharmaceutical drugs is not

well understood. The challenge behind this problem may be caused by the lack of suitable analytical procedures. In this context, analytical methodologies that are required for selective quantitative monitoring of pharmaceutical compounds in South African water resources are not yet fully developed and verified. In developed countries such as in Europe, the evaluation of pharmaceutical compounds in the aquatic environment is usually conducted using hyphenated techniques like gas and high performance liquid chromatography (HPLC) coupled to a mass spectrometry detector. Such techniques are advantageous as they offer low detection and quantification limits. These techniques also allow for the use of the instrument built-in library for the identification of a pollutant in a complex environmental sample. However, due to high operating and maintenance costs of such techniques, several African laboratories cannot afford them. Such laboratories tend to use the low cost detection methods such as photo diode array detection for the assessment of organic pollutants in aquatic environment. The challenges associated with the use of low cost detectors are limited sensitivity and selectivity which could hinder their application in the trace analysis of pharmaceuticals in the environment. Due to these challenges, the major part of this study was spent in developing suitable sample preparation techniques that can be used for the extraction and pre-concentration of acidic drugs from wastewater treatment plants and river samples.

Furthermore, the capability of WWTPs in South Africa to remove pharmaceutical compounds during the treatment process is not known. The structural changes of naproxen, ibuprofen and diclofenac that might occur during their release into the environment in the context of South African climate are not understood. South Africa is also known for the lack of infrastructure and resources which could be linked to poor sanitation especially in rural communities. This could lead to direct disposal of pharmaceutical products or human waste into surface water. Therefore, this demands the continuous monitoring of pharmaceutical pollutants in the water bodies.

Currently, it is well known that pharmaceutical products are used daily by humans for medical purposes. Therefore, routine monitoring for the occurrence of their active ingredients in the aquatic environment that includes wastewater and

river water is required. This study was based on the development of sensitive and selective methods for the quantification of widely used pharmaceutical drugs in South African water resources. The main focus was on the assessment of WWTPs, however the study was further extended to rivers and DWTPs.

Chapter 2 - LITERATURE REVIEW

This chapter presents the review of analytical methods developed for the quantification of naproxen, ibuprofen and diclofenac in water resources with more focus on sample preparation. Also, the occurrence and removal of naproxen, ibuprofen and diclofenac in water bodies have been reviewed.

2 Literature Review

2.1 Sample preparation techniques

The analysis of complex environmental samples like wastewater and the detection or quantification of organic pollutants such as naproxen, ibuprofen and diclofenac at trace levels are currently the two main analytical problems (Payan et al., 2011). Therefore, the preparation of liquid samples which is employed for the purpose of extracting and pre-concentrating the compounds of interest, as well as the removal of matrix interferences is a crucial step in environmental analysis. Sample preparation leads to better sensitivity and selectivity of the analytical method. There are several sample handling techniques that have been developed for the isolation and pre-concentration of naproxen, ibuprofen and diclofenac in environmental samples. Such sample preparation techniques include solid-phase extraction (SPE), liquid-liquid extraction (LLE), solid-phase microextraction (SPME), liquid phase microextraction (LPME) and stir bar sorptive extraction (SBSE) among others.

2.1.1 Solid-phase extraction

SPE is used extensively for the extraction of organics from aqueous samples. SPE permits the minimization of sample volumes and lowers the risks associated with the handling of toxic organic solvents (Kostopoulou and Nikolaou, 2008). In this extraction technique, the SPE cartridge is mounted on a vacuum manifold which can usually accommodate up to 12 or 24 cartridges. This allows for simultaneous preparation of samples while saving time during sample handling. During the SPE, the liquid sample is percolated into a pre-conditioned cartridge containing a sorbent which is capable of retaining the target compounds. The conditioning of the SPE cartridge is performed by passing small volumes of organic solvents or water through the sorbent with the intention of increasing the effective surface area while reducing the interferences. After the loading of a sample onto the cartridge, the undesired components that get adsorbed on the SPE cartridge are washed off with a suitable solvent. Thereafter, the sorbent is usually allowed to dry

preceding the elution of adsorbed compounds. Thereafter, the desorption of the extracted compounds of interest is carried out by applying small volumes of organic solvents with the intention of disrupting the interactions that occur between the target compounds and the SPE sorbent (Andrade-Eiroa et al., 2016).

To date, a number of effective SPE sorbents are known. Of which, many of these sorbents are commercial available nowadays. Most of the SPE sorbents that include molecularly imprinted polymers (MIPs), hydrophilic lipophilic balance (HLB), mixed-mode strong cation exchanger and reversed-phase (C_{18}) have been reported in the literature for the removal of acidic pollutants particularly naproxen, ibuprofen and diclofenac from environmental samples (Azzouz et al., 2010; Laven et al., 2009; Migowska et al., 2012; Martinez-Sena et al., 2016). These SPE sorbents are discussed in section 2.2.

2.1.2 Liquid-liquid extraction

LLE is the traditional sample preparation system that has been used extensively for the extraction and pre-concentration of organic compounds from aqueous samples (Mahugo-Santana et al., 2011). LLE is based on the removal of target compounds from an aqueous phase into an organic solvent that is immiscible with water. In recent years, the application of the technique has been decreasing which might be due to several disadvantages that include the extensive usage of organic solvents of which some are harmful and costly, as well as the introduction of the modern sample preparation techniques like SPE and SPME. Therefore, due to high usage of organic solvents, the application of LLE technique in sample preparation can be a dangerous and expensive exercise. After all, LLE is time consuming, environmental unfriendly and its application can lead to loss of target compounds due to multi-stage operations that cannot be neglected (Payan et al., 2009; Mahugo-Santana et al., 2011). In order to advance the LLE technique, liquid-phase microextraction was implemented (Mahugo-Santana et al., 2011).

2.1.3 Liquid phase microextraction

To date, different approaches to LPME have been developed. These approaches are single-drop microextraction, hollow-fiber LPME (HF-LPME), dispersive liquid-liquid microextraction and solidified floating organic drop microextraction (Mahugo-Santana et al., 2011). During the identification and quantification of pharmaceuticals in environmental samples, HF-LPME is the most used technique when compared to single-drop microextraction, dispersive liquid-liquid microextraction and solidified floating organic drop microextraction (Mahugo-Santana et al., 2011).

In HF-LPME, the compounds of interest are extracted from aqueous samples through a thin layer of organic solvent immobilized within the pores of a porous hollow fiber and into the acceptor solution that is kept inside the lumen of the hollow fiber (Payan et al., 2010). In the case of HF-LPME, the organic phase is protected by the fiber which in turn decelerates the process of organic solvent dissolution into the bulk solution (Payan et al., 2010). HF-LPME can be operated in two modes that are based on two phase and three phase systems. In the mode that is based on two phases, the acceptor solution is the organic solvent that is retained within the pores of the hollow fiber, whereas in the three-phase mode, the acceptor phase is an acidic or a basic aqueous solution (Payan et al., 2010; Sharifi et al., 2016).

HF-LPME is a relatively new sample preparation technique that offers high sensitivity and selectivity to an analytical method (Quintana et al., 2004). The technique has been applied to the isolation and enrichment of ibuprofen, naproxen and diclofenac from wastewater (Quintana et al., 2004; Payan et al., 2010). In such studies, the extracts obtained using HF-LPME were quantitatively analyzed using liquid chromatography with mass spectrometry detection that yielded low detection limits at ng/L levels (Quintana et al., 2004; Payan et al., 2010). Superior sensitivity could be partly attributed to the greater extraction efficiency and pre-concentration factors that resulted from the efficient sample preparation technique (Quintana et al., 2004; Payan et al., 2010). The popularity of the HF-LPME technique has been increasing which could be due to its low organic solvent consumption (which is line

with green chemistry concepts), low cost, the extraction process is rapid and compounds with wide pH range can be tolerated (Quintana et al., 2004; Payan et al., 2010; Mahugo-Santana et al., 2011).

2.1.4 Solid-phase microextraction

Solid-phase microextraction (SPME) is a fast, cost effective and solvent free technique when used for analyte extraction and pre-concentration prior to gas chromatographic separation and quantification (Bagheri et al., 2012; Nawala et al., 2016; Piri-Moghadam et al., 2016). However, in the case of HPLC applications, a few milliliters of organic solvent are required for desorption of extracted compounds prior to analytical separation (Terzopoulou et al., 2016). In the case of gas chromatographic analysis, target compounds are desorbed thermally in the heated injector port. Therefore, SPME is less favored for HPLC quantification as the desorption process tends to prolong the analysis time and increases the consumption of organic solvents.

In SPME, target compounds are separated between the sample matrix and a stationary phase deposited on a fused silica fiber (Nawala et al., 2016). This is done by exposing a small amount of extracting phase that is dispersed on a solid support to the sample for a well defined period of time (Pawliszyn, 2000). Since naproxen, ibuprofen and diclofenac are not volatile enough for gas chromatography (GC) analysis, SPME-HPLC procedures have been developed (Moraes de Oliveira et al., 2005; Vera-Candioti et al., 2008), where a polydimethylsiloxane-divinylbenzene coated fiber is directly immersed in the sample solution for a pre-determined time during the extraction. Thereafter, the extracted compounds of interest are desorbed into an organic solvent prior to their introduction into a chromatographic column. For HPLC applications, the SPME extractions are usually carried out at room temperature under stirred conditions. In the case of GC applications, a sol-gel technique using poly(ethylene glycol) grafted multi-walled carbon nanotubes coated fiber has been developed for solid-phase microextraction of NSAIDs (Sarafraz-Yazdi et al., 2012). In literature, there are numerous reported applications for the isolation and pre-concentration of naproxen, ibuprofen and diclofenac from

water samples using the SPME technique (Rodriguez et al., 2004; Araujo et al., 2008; Suchara et al., 2008). In these applications, several stationary phase coated fibers that include polyacrylate and polydimethylsiloxane have been explored (Rodriguez et al., 2004; Araujo et al., 2008).

As compared to the most widely used SPE technique for NSAIDs, SPME is an attractive alternative due to a number of advantages as outlined below (Kostopoulou and Nikolaou, 2008).

- SPME leads to faster extraction than SPE. It takes approximately 50 min to process any volume of sample using SPME, whereas SPE extraction time depends largely on sample volume and flow rates.
- SPME generally requires 100 times less organic solvent than SPE.
- SPME requires lower sample volume than SPE such as 25 mL instead of 500 mL.
- SPME utilizes re-usable fibers instead of single-use of most SPE cartridges.

However, in spite of the various applications and advantages reported for SPME, there are several drawbacks that limits the success of the technique which include low thermal and chemical stability of the extracting material, the stripping of coating during extraction or desorption of target compounds and short lifetime of the adsorbent (Sarafraz-Yazdi et al., 2012).

2.1.5 Stir bar sorptive extraction

For stir bar sorptive extraction (SBSE) applications, polydimethylsiloxane is used as a sorbent. For this application, polydimethylsiloxane is coated onto a glass-coated magnetic bar and employed in the extraction of liquid samples. Extraction is executed by introducing the coated magnetic bar directly into the aqueous sample. The bar is allowed to stir the aqueous solution while the target compounds are extracted into the solid-phase. After adsorption, the bar is removed from the sample, rinsed and dried. The extracted compounds are then chemically

desorbed in a liquid or in a gas chromatographic inlet (Camino-Sanchez et al., 2014).

In comparison with SPME, the sorbent amount in SBSE is higher which results in enhanced extraction efficiency, while being robust and very simple to operate (Quintana et al., 2007). For these reasons, SBSE has been applied in environmental, clinical and food analysis, and to a large variety of matrices that include soils, surface water and wastewater, solid and liquid foods, gaseous samples, and biological fluids (Camino-Sanchez et al., 2014). SBSE leads to high pre-concentration factors, broad spectrum of applications and simplicity, therefore, it is becoming one of the widely studied sample extraction techniques for the analysis of organics (Camino-Sanchez et al., 2014). In this context, SBSE has been applied in the extraction and pre-concentration of NSAIDs from environmental samples prior to liquid chromatographic quantification (Silva et al., 2008).

2.2 Solid-phase extraction sorbents

2.2.1 Molecularly imprinted polymers

Molecularly imprinted polymers (MIPs) are synthetic polymeric materials with specific recognition sites complementary in shape, size and functional groups to the molecule of interest in analysis (Batlokwa et al., 2011). MIPs have received significant attention in different research fields that include enzyme mimicking catalysis (Antuna-Jimenez et al., 2014), sensing materials (Zhu et al., 2016), drug delivery systems (Rostamizadeh et al., 2012), chromatographic separations (Haginaka et al., 1999) and solid-phase extraction (Farrington and Regan, 2007).

The application of MIPs as SPE sorbents allows for the isolation and pre-concentration of the compound of interest, as well as the removal of the sample matrix (Caro et al., 2006). MIP sorbents are highly selective with some other added advantages such as physical robustness, resistance to elevated temperatures and pressures, inertness to acids, bases and organic solvents, low production cost and ease of preparation (Li et al., 2014).

In literature, there are three reported ways that are normally followed in the synthesis of MIPs. These are based on covalent, non-covalent and semi-covalent protocols. The covalent approach involves the formation of reversible covalent bonds between the template and monomers, whereas in the semi-covalent method, the template is covalently attached to a functional monomer, however the rebinding is based on non-covalent interactions (Mahkam and Poorgholy, 2011). In the non-covalent approach, the formation of weak non-covalent interactions such as hydrogen bonding, electrostatic interactions, hydrophobic interactions, Van der Waals forces and dipole-dipole interactions occur between the template and functional monomers prior to polymerization (Mahkam and Poorgholy, 2011).

MIPs have been widely used as SPE sorbents in the environmental monitoring of acidic pharmaceuticals. In this context, most studies have focused on the development of MIPs that are suitable for the extraction of single compounds. For example, different studies have investigated the synthesis and application of MIPs for selective recognition of either naproxen, ibuprofen or diclofenac from aqueous media and biological samples (Caro et al., 2004; Farrington and Regan, 2007; Sun et al., 2008). Naproxen, ibuprofen and diclofenac have been simultaneously detected in environmental samples (Carmona et al. 2014; Kermia et al., 2016; Sarafraz-Yazdi et al., 2012), therefore a need for the development of multi-template MIPs to target such compounds concurrently arose. In this case, few studies have been reported for the investigation of MIPs that can be used to selectively extract a group of acidic pharmaceuticals from aqueous media (Dai et al., 2012; Duan et al., 2013). Due to widespread of NSAIDs in the aquatic environment, MIPs are now commercially available for NSAID group and they have been investigated for their suitability in SPE (Gilart et al., 2013; Martinez-Sena et al., 2016). To date, there are very few applications of such commercial sorbents in environmental samples. In some cases, MIPs show limited selectivity to certain NSAIDs and they are mostly tested in less contaminated sample matrices such as river water (Martinez-Sena et al., 2016).

2.2.2 Hydrophilic lipophilic balance

HLB sorbents are widely used for the extraction of pharmaceuticals with a wide range of polarities and pH values (Yu and Wu, 2011). This is because of high surface area and polar functionalities in the structure of hydrophilic lipophilic polymeric sorbents (Gilart et al., 2014). For example, in the case of Oasis HLB that is produced by Waters (Milford, MA, USA), the HLB material is based on a macro porous poly(N-vinylpyrrolidone-divinylbenzene) copolymer and has been one of the most used sorbents in the solid-phase extraction of environmental organic pollutants (Gilart et al., 2014). Oasis HLB is a universal sorbent that contains both hydrophilic N-vinylpyrrolidone and lipophilic divinylbenzene (Fig. 1 (a)) as monomers which help in the extraction of acidic, neutral and basic organic compounds from environmental samples (Kasprzyk-Hordern., 2008; Arsenault, 2012). Although Oasis HLB has got high capacity for the retention of organic compounds with different polarities (K'oreje et al., 2012), these advantages are accompanied by lack of selectivity as the material has got the ability to extract many interfering species and untargeted compounds from the sample matrix together with target compounds (Gilart et al., 2014).

Nonetheless, Oasis HLB is still widely used in the isolation and enrichment of acidic pharmaceuticals from water samples (Vergeynst et al., 2015; K'oreje et al., 2016; Agunbiade and Moodley, 2016; Petrie et al., 2016). Its application in sample preparation is usually coupled with quantitative determination of pharmaceuticals using chromatographic-based techniques (Agunbiade and Moodley, 2014; K'oreje et al., 2016). Regardless of the lack of selectivity, these methods are usually very sensitive, as the target compounds are pre-concentrated during the SPE process. Depending on the detection system, the reported limits of detection in the analysis of naproxen, ibuprofen and diclofenac in environmental samples using Oasis HLB as SPE sorbent varies from low ng/L to µg/L levels (Santos et al., 2005; Zhao et al., 2009). In the case where a photo diode array is employed for HPLC detection, the detection limits reported in literature for naproxen, ibuprofen and diclofenac were 0.02, 0.96 and 0.28 µg/L, respectively (Santos et al., 2005). The detection limits reported for the same compounds using mass spectrometry detection ranged from 0.7 to 1.3 ng/L (Zhao et al., 2009). This

means that SPE is able to enhance the detection of target compounds. However, the sensitivity of the analytical method also rests on the nature of the detection system and the volumes used in sample extraction. The latter occur when large sample volume is extracted using SPE and the retained analytes are eluted with small volume of organic solvent.

2.2.3 Mixed-mode strong cation exchanger

An example of a mixed-mode strong cation exchanger is the Oasis MCX (Fig. 1 (b)) that is produced by Waters (Milford, MA, USA). Oasis MCX is derived from Oasis HLB copolymer and provides both ion exchange and reversed-phase retention properties (Arsenault, 2012). Oasis MCX sorbent can adsorb polar, non-polar, neutral and cationic compounds concurrently from aqueous samples (Yu and Wu, 2011). The presence of sulfonic groups in Oasis MCX promotes cation-exchange interactions (Kasprzyk-Hordern., 2008). Although the MCX sorbent is originally intended for the extraction of neutral and basic compounds (Kasprzyk-Hordern., 2008), the successful application of the sorbent in the SPE of acidic pharmaceuticals from water samples has been reported (Kasprzyk-Hordern., 2008). This was evident when high recoveries of naproxen, ibuprofen and diclofenac were reported in different studies. For instance, in one application of Oasis MCX sorbent the absolute recoveries of naproxen, ibuprofen and diclofenac reported for ground water, surface water, wastewater influent and effluent were in the ranges of 64 to 96% (Lindqvist et al., 2005).

A study that was based on the selection of suitable SPE sorbent for the extraction of a number of compounds that included ibuprofen and diclofenac has been reported (Gracia-Lor et al., 2012). Oasis MCX was evaluated for its performance against the Oasis HLB. The results indicated that Oasis MCX provided higher recoveries (over 90%) for both ibuprofen and diclofenac, however other compounds such as those that are used as UV filters were not strongly retained by this sorbent. Such study (Gracia-Lor et al., 2012), demonstrated that some compounds that are usually extracted with Oasis HLB can also be extracted from water samples using Oasis MCX sorbent.

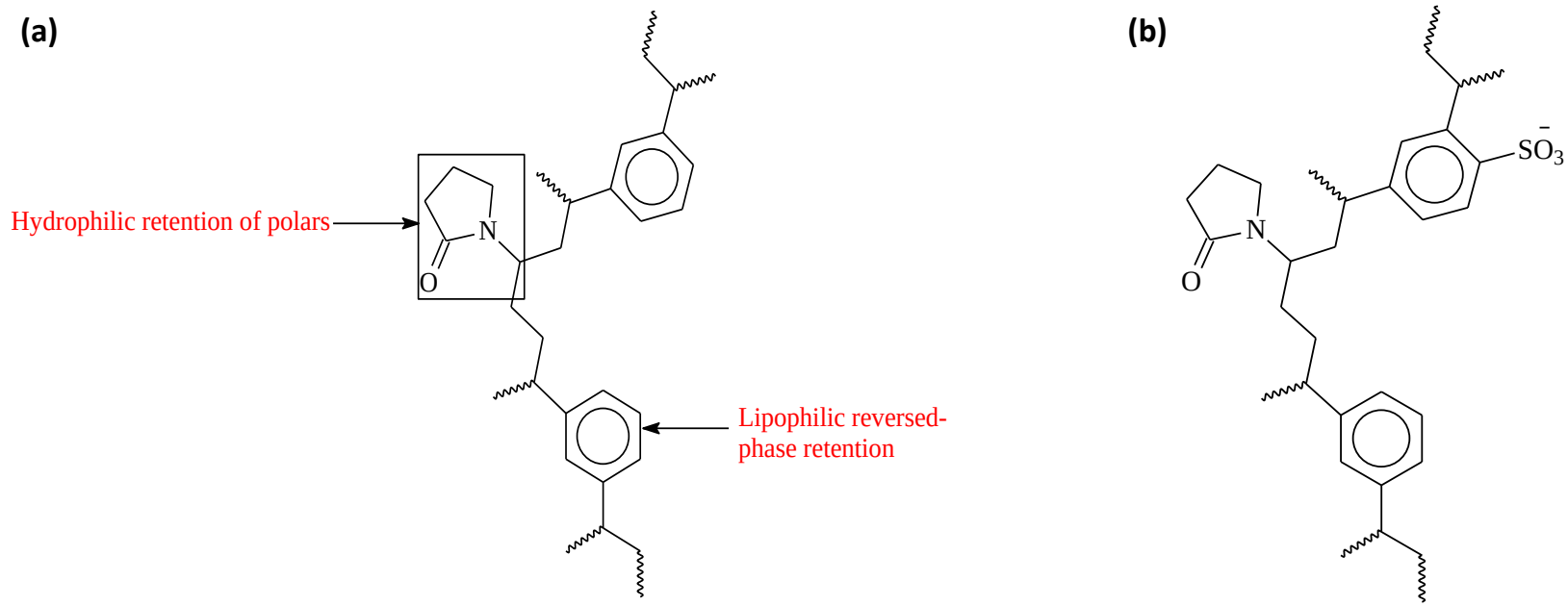


Fig. 1. Structures of Oasis HLB (a) and Oasis MCX (b) (Arsenault, 2012).

2.2.4 Reversed-phase sorbents (C_{18})

Most of the sorbents such as Oasis MCX can be operated in the reverse extraction mode for certain compounds. However, the traditional reversed-phase sorbent such as C_{18} material is commercially available in the form of SPE cartridges. When C_{18} is employed as the SPE sorbent of choice, target compounds may require extreme acidic or alkaline conditions for maximum retention onto C_{18} material. For the purpose of acidic pharmaceutical extraction, water solutions need to be acidified which results in protonation of compounds such as naproxen, ibuprofen and diclofenac, and thus maximizes the retention on the reverse-phase sorbent while reducing the uptake of basic compounds (Bones et al., 2006). Due to the ability of extracting a wide range of acidic compounds, C_{18} sorbents usually lack selectivity.

In the past years, the application of C_{18} as SPE sorbent in the extraction of naproxen, ibuprofen and diclofenac from water samples has been reported. It has been demonstrated that the maximum extraction of naproxen, ibuprofen and diclofenac occurs under acidic conditions and recoveries generally decrease as the pH of water solution increases (Bones et al., 2006). When extracted at pH 6.3, the recovery for diclofenac was 81% in WWTP effluent (Stulten et al., 2008).

2.3 Chromatographic separation

After the extraction and pre-concentration steps, the identification and quantification of naproxen, ibuprofen and diclofenac from the environmental samples is usually carried out with chromatographic based instrumentation. However, recent work has also demonstrated the application of capillary electrophoresis coupled with contactless conductivity detection for the quantitative determination of pharmaceutical pollutants in surface water and wastewater samples using solid-phase extraction for analyte enrichment and matrix interference exclusion (Le et al., 2016).

2.3.1 Gas chromatography

In gas chromatography, the sample is vaporized in the heated injector port, and then transported by carrier gas into the stationary phase for chromatographic separation and detection upon elution from the column. The compounds are detected in the gas phase with various detection systems. In the case of naproxen, ibuprofen and diclofenac studies, mass spectrometry detector is widely used due to better sensitivity and ability to confirm the occurrence of a compound in environmental samples using the built-in library (Koutsouba et al., 2003; Quintana et al., 2007; Araujo et al., 2008; Azzouz et al., 2010). However, the use of electron capture detector has been reported occasionally (Migowska et al., 2012).

Due to poor volatility of naproxen, ibuprofen and diclofenac, such compounds are derivatized prior to gas chromatographic injection. Silylating reagents such as N-methyl-N-(trimethylsilyl)-trifluoroacetamide and N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide are commonly used in the derivatization of acidic pharmaceuticals prior to GC–MS identification and quantification (Azzouz et al., 2010). The derivatives that are formed by these reagents are highly volatile and thermally stable. Generally, the derivatization process increases the analysis time, hence, high performance liquid chromatography (HPLC) is highly favored in the identification and quantification of naproxen, ibuprofen and diclofenac in water samples.

2.3.2 High performance liquid chromatography

In HPLC, the sample components are separated in the chromatographic stationary phase while in liquid form. The detection systems for HPLC that have been reported for identification and quantification of naproxen, ibuprofen and diclofenac in water samples are mass spectrometry (Agunbiade and Moodley, 2016), diode array (DAD) (Payan et al., 2011) and fluorescence (FLD) (Payan et al., 2011). The problems associated with detection systems like DAD are lack of sensitivity and selectivity. Therefore, various sample preparation techniques are

used to enrich the compounds of interest and eliminate matrix interferences, thus improving sensitivity and selectivity.

2.4 Occurrence of selected pharmaceuticals in water bodies

The analytical methods highlighted earlier are widely used worldwide in order to measure the status of water pollution caused by pharmaceuticals. The most popular methods are those that involve the use of Oasis HLB sorbent for SPE and liquid chromatography equipped with mass spectrometry detector for quantification. Also, the method adopted by United States Environmental Protection Agency uses liquid chromatography coupled to mass spectrometry for detection of pharmaceuticals after SPE (USEPA method 1694). The method detection limits for naproxen and ibuprofen when using such method are 0.004 and 0.006 μg/L (USEPA method 1694). Some of the methods that have been applied for the identification and quantification of naproxen, ibuprofen and diclofenac in environmental water samples are highlighted in Table 2.

The studies that are centered around the occurrence of naproxen, ibuprofen and diclofenac in water bodies have been ongoing for a long time in certain countries of Europe (Ollers et al., 2001; Koutsouba et al., 2003; Santos et al., 2005; Araujo et al., 2008; Larsson et al., 2009; Azzouz et al., 2010; Jelic et al., 2011; Gilart et al., 2013; Le et al., 2016). However, in South Africa the existence of such pharmaceuticals in environmental waters have been reported recently in few scientific documents (Agunbiade and Moodley, 2014; Amdany et al., 2014; Amdany et al., 2015; Matongo et al., 2015[a]; Matongo et al., 2015[b]; Agunbiade and Moodley, 2016). In addition, there are other relevant studies on environmental analysis of pharmaceuticals conducted by various South African organizations that have not yet been published. Most of the scientific documents published by South African researchers have been based on the identification and quantification of pharmaceuticals in water samples collected from the KwaZulu-Natal and Gauteng Provinces. As shown in Table 3, the latest projections on the intake of pharmaceuticals in South Africa ranked KwaZulu-Natal and Gauteng Provinces in the 3rd and 1st position, respectively (Matongo et al., 2015). Hence, South African

studies on environmental monitoring of naproxen, ibuprofen and diclofenac focused more on the status of these two Provinces.

Table 2

The selected analytical methods for the quantification of naproxen, ibuprofen and diclofenac in water.

Sample preparation	Analytical technique	Matrix	Detection limits (µg/L)			Reference
			Naproxen	Ibuprofen	Diclofenac	
SPE - Oasis HLB	GC-MS	Wastewater	0.02	0.0005	0.003	Kermia et al., 2016
SPME	GC-MS	Deionized water	0.003	0.0003	-	Araujo et al., 2008
SBSE	GC-MS	Ultrapure water	0.02	0.02	0.02	Quintana et al., 2007
HF-LPME	HPLC/MS-MS	Wastewater	-	0.3	0.1	Payan et al., 2010
SPE – Oasis HLB	HPLC-DAD	Wastewater	0.02	1	0.3	Santos et al., 2005
SPE – Oasis HLB	HPLC-FLD	Wastewater	0.2	0.7		Amdany et al., 2014
SPE - Oasis HLB	HPLC-MS	Surface water	-	0.001	0.01	Agunbiade and Moodley, 2016

Table 3

Predicted pharmaceutical consumption in South African Provinces (Matongo et al., 2015[b]).

Province	Rank	Estimated consumption per Province / %
		100
Gauteng	1	37
Western Cape	2	18
KwaZulu-Natal	3	15
Eastern Cape	4	8
Mpumalanga	5	6
Free State	6	5
North West	7	5
Limpopo	8	3
Northern Cape	9	2

In South Africa, naproxen, ibuprofen and diclofenac have only been monitored in wastewater and surface water in the form of river and dam water. Unlike in several foreign countries such as Europe where such compounds have been monitored in many water matrices such as in wastewater (Gilart et al., 2013), rivers (Al-Hadithi et al., 2011), dams (Felix-Canedo et al., 2013), lakes (Ollers., 2001), swimming pools (Azzouz et al., 2010) and drinking water (Caban et al., 2015). In general, there is not enough data that is based on the environmental monitoring of naproxen, ibuprofen and diclofenac in Africa as a whole with very few studies reported in countries like Kenya (K'oreje et al., 2012; K'oreje et al., 2016), Nigeria (Olarinmoye et al., 2016) and Algeria (Kermia et al., 2016).

The data extracted from few reports indicate that the concentrations of naproxen, ibuprofen and diclofenac reported in South African WWTPs influent, effluent and surface water are generally higher that those reported in other African countries and the rest of the World (Tables 4-6).

In order to elaborate, in a recent study conducted in Algeria which is an African country, the concentrations of naproxen in wastewater influent and effluent were 9.59 and 0.33 µg/L, respectively (Kermia et al., 2016). In comparison with concentrations detected in Europe (Table 4), the pharmaceuticals reported for influents and effluents of WWTPs located in Spain, Finland and Switzerland were in the range of 1.6 to 6.4 µg/L (Ollers et al., 2001; Lindqvist et al., 2005; Gilart et al., 2013). In South Africa, high concentrations of up to 55 and 20 µg/L have been reported in WWTP influent and effluent, respectively (Amdany et al., 2014).

Table 4

Maximum concentrations of naproxen detected in South African samples compared to the rest of the world.

Maximum Concentration (µg/L)				
Influent	Effluent	Surface water	Country	Reference
55	20		South Africa**	Amdany et al., 2014
6.4	1.6		Spain	Gilart et al., 2013
4.9	1.9	0.06	Finland	Lindqvist et al., 2005
	3.5	0.4	Switzerland	Ollers et al., 2001
9.6	0.3		Algeria**	Kermia et al., 2016

** indicate African countries.

The mean concentrations reported for ibuprofen in South African WWTP influent and effluent are in the ranges of 63 to 120 µg/L and 25 to 59 µg/L, respectively (Table 5) (Amdany et al., 2014; Matongo et al., 2015[a]). In one South African study, the highest concentration of ibuprofen found in surface water was 85 µg/L (Matongo et al., 2015[a]). The mean concentrations reported for wastewater influent and effluent of Algeria were 8.6 and 0.4 µg/L, respectively (Kermia et al., 2016). In surface water, mean concentrations were 0.06 and 0.08 µg/L in Spain and Switzerland, respectively (Ollers et al., 2001; Gilart et al., 2013). The concentration

range of 3.9 to 20 µg/L has been observed in Finland and Spain (Lindqvist et al., 2005; Gilart et al., 2013).

Table 5

Maximum concentrations of ibuprofen detected in South African samples compared to the rest of the world.

Maximum Concentration (µg/L)				
Influent	Effluent	Surface water	Country	Reference
120	25		South Africa**	Amdany et al., 2014
63	59	85	South Africa**	Matongo et al., 2015[a]
20	5.5	0.06	Spain	Gilart et al., 2013
13	3.9		Finland	Lindqvist et al., 2005
	1.5	0.08	Switzerland	Ollers et al., 2001
8.6	0.4		Algeria**	Kermia et al., 2016
	30	25	Kenya**	K'oreje et al., 2012

** indicate African countries.

The same trend was observed for diclofenac (Table 6). In trying to explain or justify the variations in distribution patterns of naproxen, ibuprofen and diclofenac in WWTPs and surface water, it has been reported that such variations could be a result of the diversified pharmaceutical usages in various countries (Behera et al., 2011; Yuan et al., 2014). Also, high concentrations of pharmaceuticals are usually detected in urban WWTPs as oppose to rural WWTPs which is likely to be linked to the high number of residents in urban areas (Matongo et al., 2015[a]). In addition, lower concentrations in rural environments may be due to limited access to these pharmaceuticals.

Table 6

Maximum concentrations of diclofenac detected in South African samples compared to the rest of the World.

Maximum Concentration (µg/L)				
Influent	Effluent	Surface water	Country	Reference
22	19	12	South Africa**	Agunbiade and Moodley, 2016
<0.5	0.8		Spain	Gilart et al. 2013
0.4		0.04	Finland	Lindqvist et al., 2005
	0.7	0.3	Switzerland	Ollers et al., 2001
2.3	2.7		Algeria**	Kermia et al., 2016

** indicate African countries.

2.5 Removal of naproxen, ibuprofen and diclofenac during the wastewater treatment process

The removal efficiency of compounds during the wastewater treatment process is calculated based on the concentrations of drugs in the treated and untreated wastewater using equation (1).

$$Removal\ efficiency = \frac{C_{infl} - C_{effl}}{C_{infl}} \times 100 \qquad (1)$$

where C_{infl} and C_{effl} are the concentrations obtained in the raw influent and final effluent, respectively (Kermia et al., 2016).

The overall removal efficiency of NSAIDs by a few WWTPs located in South Africa has been evaluated (Amdany et al., 2014; Matongo et al., 2015[b]). A comparison with various WWTPs over the world that have relatively similar wastewater treatment process was conducted as shown in Table 7. The compared WWTPs treat domestic wastewater using mechanical, biological and chemical processes (Behera et al., 2011; Kermia et al., 2016; Lindqvist et al., 2005; Zorita et al., 2009).

The average removal efficiencies reported for naproxen and ibuprofen in Goudkoppies and Northern WWTPs located in Gauteng Province of South Africa were in the ranges of 68-86% and 61-82%, respectively (Amdany et al., 2014). In some cases, the concentrations of the pharmaceutical drugs detected in the wastewater effluent were greater than in the influent which is an indication of poor removal efficiency. This case has been reported in a South African WWTP, where ibuprofen concentrations in the influent and effluent were 5.8 and 12.9 µg/L, respectively (Matongo et al., 2015[b]). The same case for diclofenac has been reported for a WWTPs in Sweden and Algeria, which led to the reported removal efficiencies of -105% and -174%, respectively (Kermia et al., 2016; Zorita et al., 2009). The removal efficiency of diclofenac in South African WWTPs has not been documented.

The data presented in various studies (Table 7) indicate that both ibuprofen and naproxen are usually well removed during the wastewater treatment process. Biodegradation has been identified as the possible reason for high removal efficiency of ibuprofen (Martin et al., 2012). Lower removal efficiency for diclofenac was linked to the combination of degradation in wastewater together with the liberation of additional diclofenac molecules by de-conjugation of glucoronidated or sulfated diclofenac and/or its desorption from particles (Martin et al., 2012). Also, high removal efficiency can be associated with the ability of the compounds to get adsorbed onto solid sludge. However, in the case of diclofenac, it can be difficult for the molecule to adsorb in the sludge due to its low octanol-water partition coefficient of 0.7 (Table 1). There are wide variations reported in literature for the removal rates of such compounds, especially in the case of diclofenac. This could indicate that the performance of WWTPs vary all over the world. As well, the poor removal of such compounds in some cases during the wastewater treatment process has raised serious concerns regarding their status in the environment due to an increasing re-use of wastewater effluent for landscape irrigation and crop irrigation (Yuan et al., 2014). South African WWTPs discharge the treated wastewater into rivers and in few cases directly into the sea. Therefore, incomplete removal of pharmaceuticals of this nature during the sewage treatment process can have a negative impact on aquatic life. Pharmaceutical compounds

exert biological effects on people or animals and may have a chronic toxicity impact to aquatic living organisms (Kermia et al., 2016).

To summarize, it has been documented in literature that the removal efficiency of pharmaceuticals during sewage treatment process depends on several factors (Behera et al., 2011; Kermia et al., 2016). These factors are:-

- The chemical structure of the pharmaceutical compound.
- The physico-chemical properties of the pharmaceutical compound.
- The treatment processes employed by individual WWTPs and/or the wastewater residence time at different WWTPs.

Table 7

The removal efficiencies of naproxen, ibuprofen and diclofenac during wastewater treatment process.

Compound	Removal efficiency / %	Country	Reference
Naproxen	73	Algeria**	Kermia et al., 2016
	55-98	Finland	Lindqvist et al., 2005
	94	Sweden	Zorita et al., 2009
	96	Korea	Behera et al., 2011
Ibuprofen	94	South Africa**	Agunbiade and Moodley, 2014
	95	Algeria**	Kermia et al., 2016
	78-100	Finland	Lindqvist et al., 2005
	87	Spain	Martin et al., 2012
	99	Sweden	Zorita et al., 2009
	98	Korea	Behera et al., 2011
Diclofenac	-174	Algeria**	Kermia et al., 2016
	9-60	Finland	Lindqvist et al., 2005
	14	Spain	Martin et al., 2012
	-105	Sweden	Zorita et al., 2009
	81	Korea	Behera et al., 2011

** indicate African countries.

2.6 Challenges relating to the analysis of pharmaceuticals in water samples

Due to the low levels of pharmaceuticals expected in water resources especially in drinking water, very sensitive and selective methods of analysis are required. Most of the analytical methods developed thus far fulfill this requirement to some extent. One of the challenges in the development of analytical methods for the determination of pharmaceutical residues in water samples is the unavailability of certified reference materials or reference methods (Bialk-Bielinska et al., 2016). This has resulted in relying only on using spiked samples for the validation of accuracy and precision of analytical methods.

Another problem that affects the less developed countries is the lack of infrastructure and research funding. For instance, the most sensitive methods for water analysis involve the use of MS detectors. Such detectors are costly, hence many organizations especially in African countries cannot afford them. Therefore, this might be one of the major reasons for the lack of data on the environmental analysis of pharmaceutical residues in African countries. In many countries, the limited data that is available on the occurrence of pharmaceuticals in drinking water and surface water come from the targeted research projects and investigations, hence they only provide the initial indication of the presence of such pollutants in the environment (World Health Organization, 2011).

Most of the data on environmental monitoring of pharmaceuticals in Africa is based on the grab sampling approach. The laboratory quantification of environmental samples collected using grab sampling protocol provides a snapshot of the contaminants levels at a particular time. This sampling technique requires large sample volumes in order to allow for greater pre-concentration factors. Large sample volumes are usually accompanied by high matrix effects, due to incomplete elimination of some untargeted compounds with the matrix (Bialk-Bielinska et al., 2016). A passive sampling technique using polar organic integrative sampler for the extraction of naproxen, ibuprofen and diclofenac from wastewater is one possible alternative. As an example, the passive sampling technique was used for the extraction of naproxen, ibuprofen and triclosan in South African WWTPs where

an estimation of the pharmaceutical concentrations over a two week period in wastewater influents and effluents was determined (Amdany et al., 2014).

Due to the challenges that are already stated, many countries that include South Africa have no specific regulations that are set for NSAIDs residues in the environment (Amdany, 2013). This group of compounds is still regarded as emerging in the African countries hence many research projects on these contaminants are on-going.

Chapter 3 - RESEARCH OBJECTIVES, APPROACH AND SUMMARY OF THE THESIS

In this chapter, aims and objectives of the study are presented. The materials, analytical methods employed in this work, together with results and their discussion are given in this chapter based on paper format.

3 Research objectives and approach

3.1 Aims of the study

3.1.1 General aim of the study

To determine the concentrations of naproxen, ibuprofen and diclofenac in South African water resources that include wastewater and river water.

3.1.2 Objectives

1. To develop the sensitive and selective analytical methods that can be used routinely in the environmental monitoring of naproxen, ibuprofen and diclofenac.
2. To synthesize a molecularly imprinted polymer that can be used as selective SPE sorbent during detection and quantification of naproxen, ibuprofen and diclofenac in water samples.
3. To monitor the concentrations of naproxen, ibuprofen and diclofenac in South African water resources that includes wastewater and river water.

3.2 Research process

In this research work, multi-templates molecularly imprinted polymer was synthesized, characterized and applied as a sorbent in the solid-phase extraction of naproxen, ibuprofen and diclofenac from water samples. The advantages of this polymer over commercial sorbents include improved selectivity and re-usability. The preparation of this smart polymer requires five major elements normally the radical initiator, cross linking monomer, functional monomer, porogenic solvent and templates. The selection and description of these materials are highlighted in the following sections.

3.2.1 Synthesis of molecularly imprinted polymer

The required 5 key elements in the synthesis of MIP are templates, functional monomer, cross linking monomer, radical initiator and a porogenic solvent. The success of the imprinting technique depends on these elements. In this research, the selection of these elements was based on previous work (Dai et al., 2012; Duan et al., 2013) and computational tools were used to justify the selection in each case as demonstrated in our published work attached as Paper 1 of this document.

1. Multi-templates – All three target compounds were used as templates in the synthesis of MIP. The application of multi-templates approach in polymerization was very important as the properties of the binding sites are defined by the functionalities and properties of these compounds. Templates are usually selected based on their availability, cost and their chemical functionalities which determine their ability for the formation of strong interactions with monomers (Pichon and Chapuis-Hugon, 2008). The requirements of a template in molecular imprinting which also favour the suitability of naproxen, ibuprofen and diclofenac are:-
    - Templates should be chemically inert during the polymerization.
    - They should be stable at moderately elevated temperatures (around 60°C) or upon exposure to ultraviolet light (Cormack and Elorza, 2004).

2. Radical initiator – In this work, radical polymerization was initiated with 1,1'-azobis-(cyclohexanecarbonitrile). As documented in literature (Mijangos et al., 2006; Ramelow and Pingili, 2010), the free radical polymerization is initiated by thermal decomposition or ultraviolet radiation of 1,1'-azobis-(cyclohexanecarbonitrile) which yields a stable tertiary free radical shown in Fig. 2. A tertiary radical that was produced has the ability to form the reactive sites in the cross linking monomer during the polymerization process.

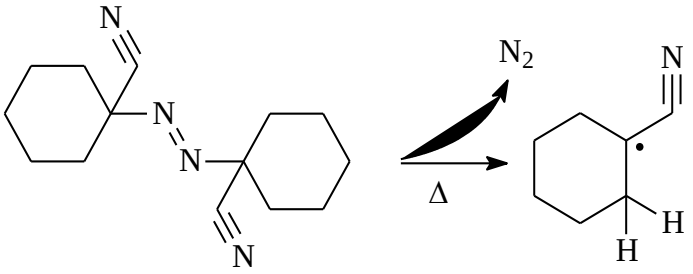


Fig. 2. Thermal decomposition of 1,1'-azobis-(cyclohexanecarbonitrile).

3. Cross linking monomer – Cross linking is a process of covalently joining two or more molecules. Cross linking reagents contain two or more reactive ends that are capable of attaching to specific functional groups (Saad, 2013). Based on this information, in the current project, ethylene glycol dimethacrylate was used as the cross linking monomer. In MIP formation, the cross linkers play several roles that include:-
    - Controlling the morphology of the polymer matrix.
    - They serve to stabilize the imprinted binding site.
    - They impart mechanical stability to the polymer matrix (Cormack and Elorza 2004).

4. Functional monomer – Functional monomer is a compound that is responsible for the binding interactions in the imprinted binding sites (Cormack and Elorza, 2004). In a non-covalent imprinting approach, functional monomer is used in excess relative to moles of a template to favor the formation of template-functional monomer assemblies. In the current study, 2-vinyl pyridine was selected as the functional monomer. 2-vinyl pyridine is a functional monomer that is able form hydrogen bonds with the carboxylic group of target molecules (Farrington and Regan, 2007).

5. Porogenic solvent – The role of porogenic solvents is to bring all components of the polymerization mixture (template, initiator, functional

monomer and cross linker) into one phase. Porogenic solvents are also responsible for the creation of the pores in macro porous polymers (Cormack and Elorza 2004). In the current study, toluene was used as porogenic solvent due to its capability of stabilizing hydrogen bonds between the templates and functional monomer (Cormack and Elorza 2004).

3.2.2 Characterization and application of multi-templates molecularly imprinted polymer

The synthesized MIP was characterized using various physical and chemical methods. These methods apply different laboratory instruments and techniques such as solid-state nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, scanning electron microscopy, thermogravimetric analysis, differential scanning calorimetry, organic elemental analyzer, x-ray diffraction, zeta potential and Brunauer, Emmett and Teller method. Molecular interactions that occur in polymerization and in molecular recognition of naproxen, ibuprofen and diclofenac were examined using molecular dynamics.

Applications investigated in this research involve the adsorption of naproxen, ibuprofen and diclofenac by MIP from organic and aqueous media. As well as the application of MIP as a selective sorbent in solid-phase extraction of naproxen, ibuprofen and diclofenac from wastewater, river water and water destined for human consumption.

3.2.3 Solid-phase extraction of acidic compounds from water samples using commercial sorbents

In this work, SPE cartridges packed with Oasis MAX were purchased and used with HPLC for the identification and quantification of naproxen, ibuprofen and diclofenac from wastewater. SPE method was developed, optimized and applied in wastewater samples. The commercial SPE sorbent was compared with the synthesized multi-templates MIP in terms of selectivity. The work also involved the evaluation of removal efficiency for naproxen, ibuprofen and diclofenac during

the wastewater treatment process at Kingsburg and Umbilo WWTPs located in Durban, KwaZulu-Natal, South Africa.

In a separate study, other organic pollutants such as ketoprofen and triclosan were determined in Amanzimtoti WWTP and Mbokodweni River. The two compounds were quantified using HPLC with photo diode array detection after SPE. In this particular work, the commercial SPE cartridges were packed with Oasis HLB sorbent. This work was very important as it demonstrated that the environmental monitoring should not only focus on the widely used pharmaceuticals. There is a wide range of organic pollutants that might be present in important water resources.

3.3 Summary of the thesis

3.3.1 Introduction

Based on the information presented above, **Paper 1** summarizes the data available in literature for the environmental monitoring of pharmaceutical compounds. In the paper, the work that has been conducted in Africa concerning the identification and quantification of pharmaceutical compounds is reviewed. Further to this, the challenges facing the African continent regarding the lack of information on environmental monitoring of pharmaceuticals are highlighted in the paper together with opportunities and future trends.

Paper 2 was based on the synthesis and characterization of multi-templates molecularly imprinted polymer which was aimed to be used as the selective sorbent in the extraction of naproxen, ibuprofen and diclofenac from water samples. The paper focused more on experimental and theoretical studies of molecular interactions between the functional monomer (2-vinyl pyridine) used in polymerization and target compounds which were naproxen, ibuprofen and diclofenac. Molecular recognition, adsorption capacity and selectivity of the synthesized MIP in polar and non-polar organic solvents were examined. The organic solvents investigated in this study were toluene, chloroform, acetonitrile and methanol. In the light of the scope presented, the paper partially fulfilled the

first and second objectives of this project as the synthesized multi-templates molecularly imprinted polymer was investigated for its ability to extract naproxen, ibuprofen and diclofenac selectively.

In **Paper 3**, the adsorption of naproxen, ibuprofen and diclofenac into MIP from aqueous samples was investigated. The influence of pH, polymer amount, initial concentration and contact time on the adsorption of target pharmaceuticals were investigated. Selectivity of MIP in aqueous solutions was also evaluated using structurally related pharmaceuticals such as gemfibrozil and fenoprofen. Therefore, the second objective of the project was partially achieved in this paper.

Paper 4 focused on applying the synthesized multi-templates MIP as selective sorbent in the solid-phase extraction of naproxen, ibuprofen and diclofenac from wastewater and river water. In this study, SPE procedure was optimized and high performance liquid chromatography equipped with photo diode array detection was used to quantify the extracted compounds. The concentrations of naproxen, ibuprofen and diclofenac in selected WWTPs and Mbokodweni river water were reported. Therefore, the second and third objectives were achieved where the target pharmaceuticals were selectively extracted from wastewater and river water followed by the quantification.

Paper 5 focused on the monitoring of ibuprofen, naproxen and diclofenac in WWTPs and rivers located in eThekwini municipality around the city of Durban. In this study, the methods developed in Paper 4 were applied in order to investigate the occurrence of target acidic pharmaceuticals in aqueous environment. This study was mainly based on environmental monitoring and assessment, hence this part of the work was entirely based on the third objective.

Furthermore, the experimental method developed in Paper 4 was further applied in order to assess the pollution levels in semi-urban areas of Ladysmith. An initial assessment for the occurrence of naproxen, ibuprofen and diclofenac in water samples collected from the surroundings of Ladysmith town in KwaZulu-Natal was presented in **Paper 6.** In this aspect, Ladysmith water resources sampled were river water, drinking water treatment plant and WWTPs. Therefore, Paper 6 addresses the third objective.

Paper 7 reported on the method development for SPE of naproxen, ibuprofen and diclofenac using the commercial cartridges packed with Oasis MAX sorbent. In this study, the developed SPE procedure was applied in high performance liquid chromatographic quantification of acidic pharmaceuticals in Kingsburg and Umbilo WWTPs located in KwaZulu-Natal province, near Durban. Further to this, the removal efficiency for naproxen, ibuprofen and diclofenac were evaluated for the first time in both Kingsburg and Umbilo WWTPs. Therefore, the first and third objectives were achieved.

In order to further evaluate the extent of water pollution, the identification and quantification of other pharmaceuticals and personal care products in wastewater and river water were investigated. Therefore, **Paper 8** is based on the optimization of the SPE method and its application in the quantification of ketoprofen (acidic pharmaceutical) and triclosan (anti-bacterial agent) in wastewater and river water.

In this work, triclosan and ketoprofen were extracted using the optimized standard procedures. During the optimization of SPE using Oasis HLB sorbent, the influence of sample pH, elution solvent, sample volume and ionic strength were investigated. The work was carried out in order to identify other pollutants that are present in wastewater and river water samples. This was very important in order to determine the selectivity of the new methods.

3.3.2 Paper 1

Madikizela L.M., Tavengwa N.T. and Chimuka, L. Status of Pharmaceuticals in African Water Bodies: Occurrence, Removal and Analytical Methods. *Under Revision in Journal of Environmental Management.*

Madikizela – 60% (conducted the research, wrote the first draft);

Tavengwa – 25% (assisted with literature review and edited the first draft);

Chimuka – 15% (supervisor, reviewed the manuscript).

Status of pharmaceuticals in African water bodies: Occurrence, removal and analytical methods

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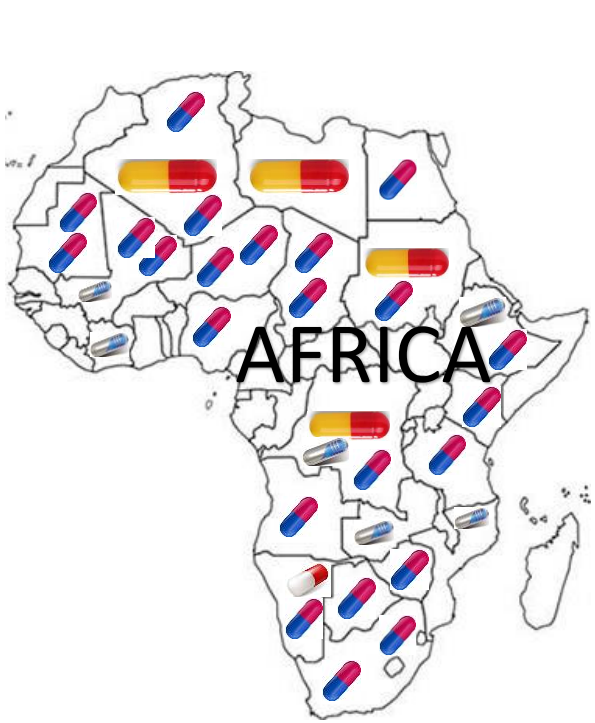
Corresponding Author: LM Madikizela, lawrencem2@dut.ac.za, +2731 373 2315

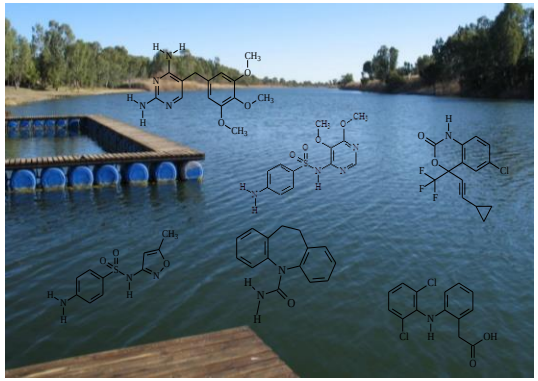
ABSTRACT

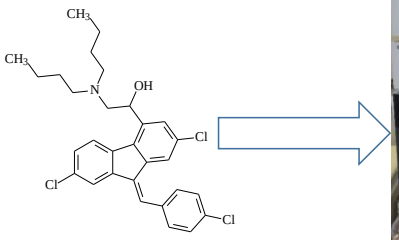
In this review paper, the milestones and challenges that have been achieved and experienced by African Environmental Scientists regarding the assessment of water pollution caused by the presence of pharmaceutical compounds in water bodies are highlighted. The identification and quantification of pharmaceuticals in the African water bodies is important to the general public at large due to the lack of information. The consumption of pharmaceuticals to promote human health is usually followed by excretion of these drugs via urine or fecal matter due to their slight transformation in the human metabolism. Therefore, large amounts of pharmaceuticals are being discharged continuously from wastewater treatment plants into African rivers due to inefficiency of employed sewage treatment processes. Large portions of African communities do not even have proper sanitation systems which results in direct contamination of water resources with human waste that contains pharmaceutical constituents among other pollutants. Therefore, this article provides the overview of the recent studies published, mostly from 2012 to 2016, that have focused on the occurrence of different classes of pharmaceuticals in African aqueous systems. Also, the current analytical methods that are being used in Africa for pharmaceutical quantification in environmental waters are highlighted. African Scientists have started to investigate the materials and remediation processes for the elimination of pharmaceuticals from water.

Keywords: Pharmaceuticals; Africa; environment; aqueous samples; wastewater treatment plants

GRAPHICAL ABSTRACT









1. Introduction

In recent years, many African Scientists embarked on the research based on quantitative analysis of pharmaceuticals in water bodies. Pharmaceuticals are compounds that are designed to prevent, cure, treat disease and improve health (Jelic et al., 2011). After intake of pharmaceutical drugs by the intendent consumer, they are subjected to metabolic reactions, such as hydroxylation, cleavage or glucuronation (Beausse, 2004). However, many pharmaceutical drugs are not completely degraded in the human body, therefore, they are normally excreted after slight transformation or in unchanged form (Debska et al., 2004).

Excreted drugs are transported into wastewater treatments plants (WWTPs) via sewage pipes. Globally, it has been scientifically demonstrated that most WWTPs are unable to remove pharmaceutical drugs completely during the sewage treatment process, which lead to the contamination of surface water (Sun et al., 2014; Gurke et al., 2015; Moreno-Gonzalez et al., 2015; Pereira et al., 2015). However, there are other factors that contribute to pharmaceutical contamination of water resources. For example, in many African communities there are areas whereby there is poor or no sanitation processes (Segura et al., 2015). Such areas do not have the sewage treatment facilities, therefore the human waste is directly disposed on the ground or surface water (Segura et al., 2015). In such areas during the rainy seasons, fecal matter is washed off from the ground into the rivers, thus contaminate the surface water and causes health danger to humans and aquatic species. Other sources of pharmaceuticals in the environment include direct discharge of untreated wastewaters to the environment through the leakage of septic tanks, landfill leachates, animal waste and treatment drugs, and the application of manure or WWTP sludge as fertilizer in agricultural fields (Paiga et al., 2016).

The groups of pharmaceuticals that are being detected in aqueous samples worldwide include non-steroidal anti-inflammatory drugs (NSAIDs), β-blockers, antibiotics, anti-epileptics, anti-retroviral drugs (ARVs), steroid hormones and antipsychotics (K'oreje et al., 2012; Manickum and John, 2014; Matongo et al., 2015a; Matongo et al., 2015b).

The chemical structures of the most detected compounds that belongs to some of these groups are given in Table 1 with their physicochemical properties (Dahane et al., 2013; Fenet et al., 2012; Ngumba et al., 2016; Vymazal et al., 2015; Wood et al., 2015). Such properties indicate that pharmaceuticals could be more detected in water rather than solid matrices such as sediments and aquatic plants. Long-time exposure of some organisms to certain classes of these pharmaceutical groups may result in resistance, which is directly linked to public health (Segura et al., 2015). Hence, there is strong need to monitor the occurrence of pharmaceuticals in the environment.

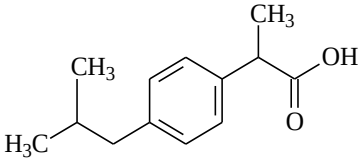
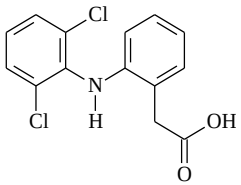
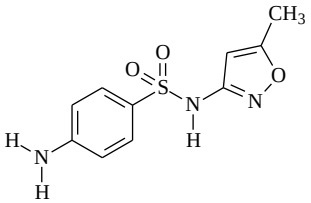
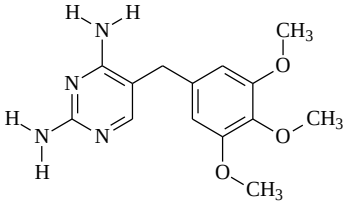
To date, many research papers have indicated the widespread of pharmaceuticals in the environment. However, many of these scientific papers emerge from European based countries, while African countries are still lagging behind in terms of identifying and quantifying pharmaceuticals in environmental samples. Furthermore, the capability of sewage treatment processes for the removal of pharmaceutical constituents in Africa is not fully achievable. This is the area that has been well exploited in Europe over the years (Kasprzyk-Hordern et al., 2009a; Gross et al., 2010; Jelic et al., 2011; Garcia-Lor et al., 2012), whereas the removal efficiency of pharmaceuticals during the wastewater treatment process has been reported recently in few African based studies (Kermia et al., 2016; K'oreje et al., 2016; Zunngu et al., 2016).

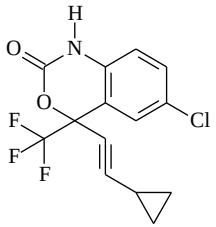
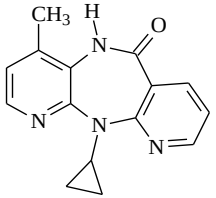
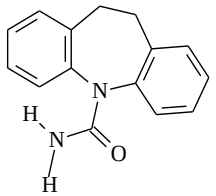
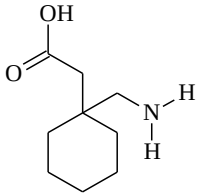
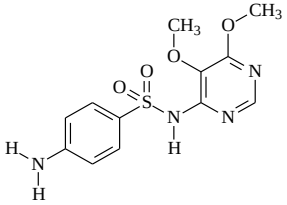
Globally, several review articles that are based on the occurrence of pharmaceuticals in aquatic environment and sediments have been published (Beausse, 2004; Li, 2014; Santos et al., 2010; Savci, 2013). To the best of authors knowledge, none of these published reviews focused on the occurrence of pharmaceutical drugs in African environment. Some of African reviews that have been published focused more on endocrine disrupting chemicals with little information on environmental pollution caused by pharmaceutical drugs (Olujimi et al., 2010; Tijani et al., 2015). Therefore, this is the first review to demonstrate the milestones that have been reached by African Scientific community in the field of environmental analysis of pharmaceuticals. This review also gives an overview of the analytical methods that have been used by African researchers for evaluating pharmaceutical pollutants in the aquatic environment.

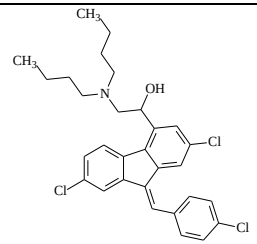
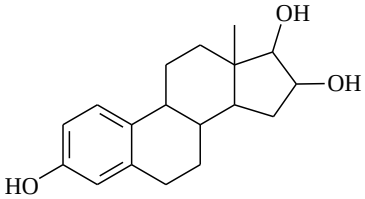
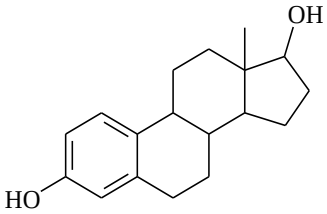
Therefore, in the perspective of the African community, this study attempts to highlight the extent of water pollution on the continent by pharmaceutical compounds, the achievements in the study area by African researchers and the shortcomings with future research possibilities.

Table 1

Chemical structures and physicochemical properties of some commonly used pharmaceutical drugs.

Pharmaceutical class	Compound	Chemical structure	Water solubility (mg L^{-1})	Log K_{OW}
NSAIDs	Ibuprofen		58	3.71
	Diclofenac		10	4.02
Antibiotics	Sulfamethoxazole		610	0.89
	Trimethoprim		400	0.91

ARVs	Efavirenz		10	4.15
	Nevirapine		0.7046	3.89
Anti-epileptics	Carbamazepine		17.7	2.45
	Gabapentin		4490	-1.10
Anti-malarials	Sulfadoxine		296	0.70

	Lumefantrine		Insoluble	8.34
Estrogens	Estriol		3.2–13.3	2.6–2.8
	17-β-estradiol		12.96–13.0	3.1–4.0

2. Data collection for literature review

The scope and the area of this study was Africa. Even though there are limited studies on pharmaceutical compounds in African water bodies, there were enough papers to prepare this review article. Regarding African studies, data from twenty-six articles was reviewed and compared to the global trends. The presented data were obtained after thoroughly searching different scientific journals from mostly three search engines: Web of Science, Scopus, and Google. The expanded keywords were Africa, pharmaceutical, water and each of 53 African countries. In the African context, the cited work was published from 2012 to 2016, however, some sampling for the presented data could have been performed in earlier years. There was not much information obtained relating to the occurrence of pharmaceutical residues in water bodies prior to these years. For simplicity and consistency, in most cases the concentration units reported in literature were converted from ng L^{-1} to µg L^{-1}.

3. Occurrence of pharmaceuticals in African water bodies

3.1. Non-steroidal anti-inflammatory drugs (NSAIDs)

Globally, NSAIDs are widely detected in the environment due to their availability over the counter that do not require any medical prescription which allows for self-medication (Manrique-Moreno et al., 2016). Maximum concentrations of pharmaceuticals detected in African wastewater and surface water are given in Figs S1-S3. As presented in Table 2, naproxen, ibuprofen, diclofenac and ketoprofen are the most common drugs in African aqueous environment. Some of the reported quantities in African wastewater exceed the levels found in WWTPs located in well developed countries such as in Europe, this could be due to poor sanitation in African countries. For example, a maximum concentration of 221 µg L^{-1} for ibuprofen (Fig S1 and Table 2) was reported in a WWTP influent located in South African Province of KwaZulu-Natal (Madikizela and Chimuka, 2016a). Further to this, the mean concentration of ibuprofen detected in the influent of Northern WWTP located in Gauteng Province of

South Africa was 111.9 µg L^{-1} (Amdany et al., 2014). Whereas, the maximum concentrations in influent reported for ibuprofen in several European based studies were 22.8, 1.36 and 20.2 µg L^{-1} (Dahane et al., 2013; Gilart et al., 2013; Larsson et al., 2014). Therefore, ibuprofen is one of the most frequently detected NSAIDs in African wastewater and surface water. In Kenya, among other NSAIDs, ibuprofen had the highest concentration of approximately 30 µg L^{-1} in WWTP effluent (K'oreje et al., 2012). It is speculated that the inefficiency of sewage treatment facilities contributes to the pollution levels of the surface water. As a consequence, traces of ketoprofen, diclofenac, paracetamol, naproxen and ibuprofen have been detected in African rivers that includes Umgeni and Mbokodweni Rivers in South Africa, and Nairobi River basin in Kenya (K'oreje et al., 2012; Agunbiade and Moodley, 2014; Madikizela et al., 2014; Gumbi et al., 2016; Madikizela and Chimuka, 2016a). Generally, variations of concentration levels are observed across different WWTPs which could be due to seasonal effect, different consumption rates and sampling times. Seasonal effects are well documented in literature with little data on the effect of sampling time. For example, in WWTPs located in South Africa, it was found that at certain time of the day the concentration of pharmaceuticals were higher which could be due to high consumption of the drugs at a particular time (Amdany et al., 2015).

Table 2

Mean concentrations / concentration ranges for NSAIDs quantified in water bodies.

Sample	Compound	Mean concentrations or concentration ranges (µg L^{-1})
Influent		
	Naproxen	9.6[a], 1.2[a], 55[b], 52[b], 1.1[c], 2.3[c], 1.22-39.6[h], 2.2[k]
	Ibuprofen	1.6[a], 8.6[a], 39.8[b], 111.9[b], 5.2[c], 7.2[c], 62.8[f], 5.8[g], 6.02-221[h], 6.46-10.6[j], 1.36[k]
	Diclofenac	2.3[a], 0.99[a], 22.3[d], 3.72-104[h], 0.93-1.51[j], 0.85[k]
	Ketoprofen	0.57[a], 3.15[d], 1.7-6.4[e], 0.31[k]
Effluent		
	Naproxen	0.33[a], 13.5[b], 20.4[b], 0.4[c], 0.8[c], <LOQ-5.34[h], 0.098[k]
	Ibuprofen	0.34[a], 0.43[a], 12.6[b], 24.6[b], 1.1[c], 1.6[c], 58.7[f], 12.9[g], 3.87-67.9[h], nd-2.1[j], nd[k]
	Diclofenac	1.6[a], 2.7[a], 19.0[d], <LOQ-20.8[h], 0.03-0.06[j], 0.74[k]
	Ketoprofen	1.0[a], 0.90[d], 1.2-4.3[e], 0.16[k]
Surface water		
	Naproxen	<LOQ-0.68[h], 0.02-0.03[i], nd-<LOQ[k]
	Ibuprofen	0.31[a], 85[f], 0.23-6.2[g], <LOQ-11.4[h], 0.04-8.84[i], nd-17.4[j], nd-<LOQ[k]
	Diclofenac	nd[a], 12.4[d], 0.03-0.27[i], nd- 0.73[j], nd-0.036[k]
	Ketoprofen	0.27[a], 0.38[d], <0.26-2.0[e], nd[k]

Reference/Country/Sampling month and year - [a]Kermia et al., 2016/ Algeria/ November 2014; [b]Amdany et al., 2014/ South Africa/ August and September 2012; [c]Amdany et al., 2015/ South Africa/ August and October 2012; [d]Agunbiade and Moodley, 2016/ South Africa; [e]Madikizela et al., 2014/ South Africa/ August-October 2013; [f]Matongo et al., 2015a/ South Africa/ September 2013; [g]Matongo et al., 2015b/ South Africa/ September 2013; [h]Madikizela and Chimuka, 2016/ South Africa/ January – March 2016; [i]Olarinmoye et al., 2016/ Nigeria/ August – September 2013; [j]K'oreje et al., 2016/ Kenya/ September 2012 and July 2013; [k]Dahane et al., 2013/ Spain.

nd- the compound was not detected; <LOQ – the concentration of the compound was below the quantification limit.

3.2. Antibiotics (anti-infectives)

Nowadays, antibiotics are frequently detected in environmental waters. For example, in four African countries (Ghana, Kenya, Mozambique and South Africa), nineteen antibiotics were quantified in surface waters (Segura et al., 2015). Some of these antibiotics with their detected concentration ranges (ng L^{-1}) were sulfapyridine (0.73-153), sulfamethoxazole (0.11-53828), sulfathiazole (0.32-3971), sulfanerazine (0.23-0.31), salfamethizole (0.11-0.42), sulfadimidine (0.69-330), trimethoprim (0.08-11383), azithromycin (0.6-27), dehydroerythromycin (0.17-2800), clarithromycin (0.11-439), roxithromycin (0.52-11), lincomycin (0.17-241), tetracycline (0.85-465), doxycycline (2.5-50), oxytetracycline (1.4-1793), minocycline (2.9-315) and chlortetracycline (3.9-225) (Segura et al., 2015). Furthermore, the specified antibiotics and compounds such as nalidixic acid were also detected in other African studies at ng L^{-1} to µg L^{-1} levels (Segura et al., 2015; Matongo et al., 2015a, Matongo et al., 2015b; Gumbi et al., 2016; Ngumba et al., 2016). The widespread of such pollutants could be a result of lack of sewage treatment facilities, whereas in some cases such as in Durban (South Africa), the WWTPs serve only the part of the city (Segura et al., 2015). This suggest that the major part of the contamination of surface water could be a result of the untreated sewage. In another study, six antibiotics were detected in water samples collected in Nigeria (Olarinmoye et al., 2016). The highest concentrations (µg L^{-1}) measured for chloramphenicol, erythromycin-A dehydrate, erythromycin, sulfadiazine, sulfamethoxazole and trimethoprim were 0.36, 0.48, 1.00, 0.04, 1.50 and 0.40, respectively (Olarinmoye et al., 2016). Generally, as shown in Fig S1-S3, the concentrations of antibiotics in surface water reported in Africa are relatively lower when compared to NSAIDs which could be due to unavailability of antibiotics over the counter without prescription. However, when compared to China (Table 3), the concentrations for antibiotics detected in African surface water are relatively similar.

For these two regions (Africa and China), it is believed that there are wide variations in terms of antibiotics prescriptions. This was evident as some compounds that are reported in African waters were not detected in China and vice versa.

Table 3

Mean concentrations / concentration ranges for antibiotics quantified in water bodies.

Sample	Compound	Mean concentration and concentration ranges ($\mu g\ L^{-1}$)
Influent		
	Erythromycin	0.61[a], 1.1[b]
	Sulfamethoxazole	34.5[a], 59.3[b], 10.1-54.8[e]
	Trimethoprim	0.13[b], 4.25-72.9[e]
	Chloramphenicol	nd-0.16[e]
	Ciprofloxacin	nd-0.27[e]
	Levofloxacin	nd-0.16[e]
	Metronidazole	0.35-3.0[e]
	Nalidixic acid	nd-2.8[e]
	Sulfadoxin	0.71-3.23[e]
Effluent		
	Erythromycin	0.16[a], 0.24[b]
	Sulfamethazine	1.1[b]
	Trimethoprim	0.16[b], 0.07[c], 0.09-0.16[e]
	Sulfamethoxazole	3.34[c], 2.86-4.09[e]
	Ciprofloxacin	0.07[c]
	Chloramphenicol	nd-0.005[e]
	Metronidazole	0.004-0.02[e]
	Sulfadoxin	0.05-0.52[e]

Surface water		
	Erythromycin	0.03-0.26[b], 0.06-1.00[d], nd-0.12[f], 0.0004-0.007[g]
	Sulfamethoxazole	0.22-6.01[b], <0.008-13.8[c], 0.09-1.50[d], 0.02-38.9[e], nd-0.94[f], 0.0022-0.76[g]
	Sulfamethazine	0.05-1.2[b], nd-0.63[e], nd-0.016[f], 0.02-0.39[g]
	Trimethoprim	0.20-0.87[b], <0.024-2.65[c], 0.12-0.40[d], 0.03-6.95[e]
	Ciprofloxacin	<0.012-0.51[c], nd-0.034[g]
	Chloramphenicol	0.03-0.36[d], nd-0.66[e], nd-0.0039[g]
	Erythromycin-A dihydrate	0.12-0.48[d],
	Sulfadiazine	0.02-0.04[d], 0.0009-0.51[f], 0.0049-0.11[g]
	Levofloxacin	nd-0.04[e]
	Metronidazole	nd-4.02[e]
	Sulfadoxin	nd-1.46[e]

Reference/Country/Sampling month and year - [a]Matongo et al., 2015a/ South Africa/ September 2013; [b]Matongo et al., 2015b/ South Africa/ September 2013; [c]Ngumba et al., 2016/ Kenya/ October 2014; [d]Olarinmoye et al., 2016/ Nigeria/ August – September 2013; [e]K'oreje et al., 2016/ Kenya/ September 2012 and July 2013; [f]Li et al., 2012/ China/ August 2008 and October 2010; [g]Chen and Zhou, 2014/ China/ July 2012.

nd- the compound was not detected.

3.3. Anti-retroviral drugs (ARVs)

ARV treatment comprise a combination of nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors that act to inhibit multiple, viral targets and approved integrase inhibitors used for antiretroviral treatment for patients with viral resistance (Shah et al., 2013). Over the years, this group of pharmaceuticals have been less studied. However most recently, there has been some

progress in the analysis of ARV drugs in aquatic environment. This could emanate from the steady increase in the number of people that receive ARV treatment. In the African continent, more than 7.5 million people were receiving treatment at the end of year 2012 compared to 50 000 people ten years prior to that period (WHO, 2013). As a consequence, most recently there have been some few reports on the occurrence of ARVs in wastewater and in African rivers (Wood et al., 2015; Schoeman et al., 2015; K'oreje et al., 2016; Ngumba et al., 2016). As shown in Table 4, high amounts of ARVs have been found in Kenyan rivers with maximum concentrations of 167 µg L^{-1}, 17 µg L^{-1} and 6 µg L^{-1} for lamivudine, zidovudine and nevirapine, respectively (K'oreje et al., 2016; Ngumba et al., 2016). Lower concentrations in the range of 0.0265 to 0.430 µg L^{-1} in surface water have been reported in South Africa (Wood et al., 2015). In WWTP effluent, maximum concentrations reported in a South African study for nevirapine and efavirenz were 0.350 and 7.100 µg L^{-1}, respectively (Schoeman et al., 2015). For these compounds, African data differs significantly to that obtained from a study reported in China. In China, the ARV drugs reported in wastewater and river water were acyclovir, ganciclovir and ribavirin, whereas the compounds that are being consumed in Africa such as stavudine and zidovudine were not detected (Peng et al., 2014). Such trends may be due to differences in consumption of ARV drug types in different countries.

The high detection frequency for nevirapine in the environment could be due to its wide use for the treatment of HIV and for the prevention of mother to child transmission (Schoeman et al., 2015). Poor removal efficiency for nevirapine during the sewage treatment process could also lead to its frequent detection in surface water and WWTPs effluent.

Table 4

Maximum concentrations for ARV drugs quantified in African water bodies.

Sample	Compound	Maximum concentration (µg L^{-1})
Influent		
	Nevirapine	2.1[a], 3.3[d]
	Efavirenz	17.4[a], 1.02[d]
	Lamivudine	60.7[d]
	Zidovudine	20.1[d]
Effluent		
	Lamivudine	4.0[b], 31.1[d]
	Zidovudine	0.51[b], 0.11[d]
	Nevirapine	0.35[a], 1.4[b], 2.1[d]
	Efavirenz	7.1[a], 0.11[d]
Surface water		
	Lamivudine	5.4[b], 0.24[c], 167[d]
	Zidovudine	7.7[b], 0.97[c], 17.4[d]
	Nevirapine	4.9[b], 1.48[c], 5.62[d]
	Tenofovir	0.24[c]
	Zalcitabine	0.07[c]
	Lopinavir	0.31[c]

Didanosine	0.0541[c]
Stavudine	0.78[c]
Amandatine	0.10[d]
Efavirenz	0.56[d]
Rimantadine	0.02[d]

Reference/Country/Sampling month and year - [a]Schoeman et al., 2015/ South Africa; [b]Ngumba et al., 2016/ Kenya/ October 2014; [c]Wood et al., 2015/ South Africa; [d]K'oreje et al., 2016/ Kenya/ September 2012 and July 2013.

3.4. Anti-epileptics

Carbamazepine is generally present in wastewater and in the environment, therefore it is widely studied globally (Fernandez-Lopez et al., 2016). It is the common anti-epileptic drug that has been detected in the African environment that includes wastewater, drinking water and bio-solids due to its wide use as an anti-seizure medication (Fenet et al., 2012; Odendaal et al., 2015; K'oreje et al., 2016; Matongo et al., 2015a). The highest detected concentrations in wastewater collected from Kenya, Tunisia and South Africa (Table 5) were 0.35, 0.29 and 2.21 µg L^{-1}, respectively (Fenet et al., 2012; Matongo et al., 2015a, K'oreje et al., 2016). The presence of two carbamazepine metabolites (carbamazepine-10, 11-epoxide and 10, 11-dihydroxycarbamazepine) in wastewater have also been reported in concentrations of up to 0.052 ng L^{-1} (Fenet et al., 2012). In comparison with other pharmaceutical groups (Fig S1-S3), it can be noticed that the detection frequency and anti-epileptic drugs quantities in environmental samples is low due to strict dispatching regulations. Overall, the concentrations of carbamazepine reported in Africa are somewhat similar to those reported in USA, Germany and Portugal (Table 5). For instance, the median concentrations of 1.9 and 0.47 µg L^{-1} have been reported in wastewater influent from Germany and Portugal, respectively (Bahlmann et al., 2014). As can be seen in Table 5, these concentrations (1.9 and 0.47 µg L^{-1}) are within the levels of 0.35 to 4.6 µg L^{-1}

reported in South Africa and Kenyan influent. Other anti-epileptic drugs that are not used commonly are gabapentin and cyclobarbital. There are currently no reports on the occurrence of gabapentin and cyclobarbital in the African continent.

Table 5

Mean concentrations / concentration ranges for antibiotics quantified in water bodies.

Sample	Mean concentration and concentration ranges (µg L^{-1})
Influent	2.21[a], 4.6[b],1.9[c], 0.47[c], nd-0.35[e], 0.0475-0.0673[f]
Effluent	0.91[a], 1.5[b], 2.0[c], 0.52[c], 0.11-0.29[d], nd-0.32[e], 0.15-0.22[f]
Surface water	0.17-1.7[b], 0.01-0.11[d], nd-0.43[e]

Reference/Country/Sampling month and year - [a]Matongo et al., 2015a/ South Africa/ September 2013; [b]Matongo et al., 2015b/ South Africa/ September 2013; [c]Bahlmann et al., 2014/ Germany and Portugal; [d]Fenet et al., 2012/ Tunisia/ July and September 2008; [e]K'oreje et al., 2016/ Kenya/ September 2012 and July 2013; Li et al., 2013/ USA/ September and November 2011.

3.5. Anti-malarials

In Africa, malaria is one of the leading causes of morbidity and mortality which have infected millions of Africans (Semakula et al., 2016). In high malaria burdened countries, the disease accounts for up to 40% of public health expenditures; 30% to 50% of inpatient hospital admissions and up to 60% of outpatient health clinic visits, resulting in reduction in economic growth rates by as much as 1.3% (WHO, 2015).

Currently, there is limited information on the occurrence of anti-malarial drugs in water system worldwide. In many studies, anti-malarial drugs have been investigated, but were not detected in water or were found to be below the limit of quantification (0.39 ng L^{-1}) as it was the case in Spain for sulfadoxine (Garcia-Galan et al., 2010; Boix et

al., 2015). In Kenya, a concentration of up to 2 µg L^{-1} for sulfadoxine have been reported in the effluent of Nairobi River Basin (K'oreje et al., 2012). In the same study (K'oreje et al., 2012), concentrations of sulfadoxine in the range of 0.1 to 0.8 µg L^{-1} were detected in river water. Other anti-malarials investigated but not detected in Kenyan waters were quinine, artemether, lumefantrine and pyrimethamine (K'oreje et al., 2012). Lumefantrine is insoluble in water (Table 1), hence it can be easily lost during sample collection using grab and composite sampling approaches. The lack of reports on the occurrence of anti-malarial drugs in African waters may be due to their limited consumption. This may be a result of the introduction of newer anti-malarials which tend to be more expensive than African economies can bear (Foster, 1991). While, the efficacy of readily affordable antimalarial drugs is declining rapidly, with highly efficacious drugs being too expensive (Bloland et al., 2000).

3.6. Steroid hormones

Steroid hormones studied in Nigeria and South Africa include natural estrogens, synthetic estrogens, natural androgen and natural progesterone (Olarinmoye et al., 2016; Manickum and John, 2014). Estrogens that include 16-α-hydroxyesterone, 17-α-ethinylestradiol, estradiol, estriol, estrone and mestranol, were studied in Nigerian wastewater impacted surface water, however none of them were detected at a concentration greater than the detection limit of 0.01 µg L^{-1} (Olarinmoye et al., 2016). Compounds detected in South African WWTP influent, effluent and river water (Table 6) were estrone, 17-β-estradiol, estriol, 17-α–ethinylestradiol, progesterone and testosterone (Manickum and John, 2014). As indicated in Table 6, the detected concentrations were generally in low ng L^{-1} with trace amounts in river water which was largely contaminated due to incomplete removal during wastewater treatment (Manickum and John, 2014). In comparison with a study conducted in United States of America (USA), only estrone, estriol and strone-3-sulfate were detected in wastewater influent at concentrations above their detection limits of 10, 43 and 3.3 ng L^{-1}, respectively (Li et al., 2013). None of the steroid hormones were detected in wastewater

effluent and river water which could be an indication of good removal efficiency of such compounds during the treatment process in USA (Li et al., 2013).

Table 6

Concentrations (ng L^{-1}) reported in literature for steroid hormones in water bodies.

Sample	Compound	African water bodies Manickum and John (2014)			USA water bodies Li et al. (2013)
		Minimum	Mean	Maximum	Maximum
Influent					
	Estrone	13	84	351	16.9
	17-β-estradiol	20	119	199	-
	Estriol	3	5	9	126
	17- α-ethinylestradiol	10	30	95	-
	Progesterone	163	408	904	-
	Testosterone	119	343	635	-
	Estrone-3-sulfate				21.2
Effluent					
	Estrone	3	23	78	-
	17-β-estradiol	4	20	107	-
	Estriol	<1	<1	<1	-
	17- α-ethinylestradiol	1	3	8	-
	Progesterone	0	9	25	-

	Testosterone	0	11	26	-
River					
	Estrone	1	8	32	-
	17-β-estradiol	1	10	66	-
	Estriol	<1	6	2	-
	17- α-ethinylestradiol	0	2	4	-
	Progesterone	0	13	60	-
	Testosterone	3	10	19	-

Reference/Country/Sampling month and year: Manickum and John, 2014/ South Africa/ March 2010-June 2012; Li et al., 2013/ USA/ September and November 2011.

-not studied by the researchers

4. Removal of pharmaceuticals from water – In the context of African conditions

Contamination of water resources with organics is globally well-known (Dahane et al., 2013; Kermia et al., 2016; Larsson et al., 2014; Sun et al., 2014). The presence of pharmaceuticals in WWTPs which lead to the pollution of surface water have been reviewed in this paper (Tables 2 to 6). The excretion of pharmaceuticals in unchanged forms from the human body could lead to high levels in WWTPs. Various metabolic excretion rates for some common drugs in the environment are reported in Table 7. There is no available information on the excretion rates associated with other classes of pharmaceuticals. High excretion rate (100%) reported for gabapentin could be associated with its limited usage (Kasprzyk-Hordern et al., 2009b).

Table 7

Metabolism excretion rates for selected pharmaceuticals.

Pharmaceutical class	Compound	Excretion rate (%)	Reference
NSAID	Ibuprofen	10	Kermia et al., 2016
	Diclofenac	10	Kermia et al., 2016
Antibiotics	Sulfamethoxazole	30	Kasprzyk-Hordern et al., 2009b
	Trimethoprim	80	Kasprzyk-Hordern et al., 2009b
Anti-epileptic	Carbamazepine	10	Kasprzyk-Hordern et al., 2009b
	Gabapentin	100	Kasprzyk-Hordern et al., 2009b

WWTPs were not specifically designed to remove pollutants such as pharmaceuticals during sewage treatment process (Fernandez-Lopez et al., 2016). Various removal efficiencies of pharmaceuticals during the wastewater treatment process have been reported in literature (Table 8). Removal efficiencies differs significantly in different WWTPs as is the case with diclofenac. For example, the removal efficiency for

ketoprofen reported in South Africa was in the range of 88-90% (Zunngu et al., 2016), whereas -83% was observed for the same compound in Algeria (Kermia et al., 2016). In some WWTPs, the concentration of pharmaceuticals reported in the influent is less than in the effluent (Kermia et al., 2016), which lead to removal efficiency of less than 0% and pollution of surface water. This could be the result of differences in the WWTPs designs or desorption of the pharmaceuticals from the particulate matter during the wastewater treatment (Kermia et al., 2016).

Regarding other pharmaceutical groups, poor removal efficiency during the wastewater treatment process have also been reported. To mention just few cases, the removal efficiencies reported for carbamazepine (anti-epileptic drug), lamivudine (ARV drug) and nevirapine (ARV drug) in Kenya were 32%, 24-59% and 11-49%, respectively (K'oreje et al., 2016). Whereas for steroid hormones, the removal efficiencies were 100, 98, 96, 90, 78 and 72% for estriol, progesterone, testosterone, 17-α-ethinylestradiol, 17-β-estradiol and estrone, respectively (Manickum and John, 2014). Perhaps, the high removal effiency for steroid hormones could be the result of the trace amounts detected in effluents and poor or no detection in river water (Manickum and John, 2014; Li et al., 2013).

In the context of African researchers, there has been very few studies that actually focused on phytoremediation for the purpose of pharmaceutical removal from water bodies. In a reported African study, 90% removal of doxycycline (an antibiotic) from pharmaceutical effluents using electro-coagulation coupled electro-flotation process was documented (Zaidi et al., 2015). On the other hand, the adsorption of aspirin and paracetamol from aqueous solutions using Fe/N-CNT/β-cyclodextrin nanocomposites as an adsorbent has been investigated, however the study did not report any environmental application (Mphahlele et al., 2015). Most recently, the application of molecularly imprinted polymer for the removal of naproxen, ibuprofen and diclofenac from contaminated wastewater and river water has been demonstrated (Madikizela and Chimuka, 2016b). In light of the presented scope, the removal potential of these possible phytoremediation materials and procedures in the large scale is not known. In

addition, the reported adsorbents were synthesized for laboratory evaluation therefore it is not yet understood if such materials can be reproduced in a large scale. The applications of such procedures for water treatment in large scale are very important as recent reports in Europe indicated the existence of pharmaceuticals in drinking water (Carmona et al., 2014; Caban et al., 2015).

Table 8

Removal efficiency of pharmaceuticals during the wastewater treatment process.

Pharmaceuticals	Country	Removal efficiency (%)	Reference
Ketoprofen	South Africa	88-90	Zunngu et al., 2016
Ketoprofen	Algeria	-83	Kermia et al., 2016
Naproxen	Algeria	73	Kermia et al., 2016
Ibuprofen	Algeria	79-95	Kermia et al., 2016
Diclofenac	Algeria	-174-30	Kermia et al., 2016
Ketoprofen, diclofenac and ibuprofen	South Africa	64-94	Agunbiade and Moodley, 2014
Naproxen and ibuprofen	South Africa	61-86	Amdany et al., 2014

5. Analytical methods for the determination of pharmaceuticals in water bodies

Traditionally, organic compounds are monitored in environmental samples using high performance liquid chromatography (HPLC) and gas chromatography (GC) for non-volatile and volatile analytes, respectively. In few cases, non-volatile compounds have been derivatized into volatile forms and quantified using GC (Togola and Budzinski, 2007; Kermia et al., 2016). Derivatization is tedious and tends to produce unwanted compounds which compromise the selectivity of the analytical method, hence HPLC is a technique of choice for the analysis of non-volatile compounds. Mostly, the

methods employed in Africa utilize the external calibration approach and standard addition method, where methods validations are performed with spiked solutions. In the context of African studies, pharmaceuticals are usually quantified in environmental samples at ng L^{-1} and µg L^{-1} levels (Tables 2 to 6), therefore sensitive detectors such as mass spectrometry (MS) and fluorescence are highly recommended for chromatographic analysis. However, the low cost and readily available detectors such as UV-visible or photo diode array detectors have been used in Africa where the sensitivity of the analytical method was improved by employing sample pre-concentration step (Agunbiade and Moodley, 2014; Madikizela and Chimuka, 2016a).

In environmental monitoring, a sample preparation step is very important as it enables for analyte pre-concentration and removal of matrix interferences. Consequently, over 80% of analysis time is spent in both sampling and sample preparation (Pavlovic et al., 2007). As shown in Table 9, sample preparation techniques that are being used in Africa for pharmaceutical analysis include solid-phase extraction (SPE) and hollow fiber silicone membrane. Majority of SPE studies have been conducted using hydrophilic lipophilic balance (HLB) sorbents (Agunbiade and Moodley, 2014; Kermia et al., 2016; Matongo et al., 2015b), whereas most recently molecularly imprinted polymers (MIPs) have been developed as selective sorbents for pharmaceuticals (Madikizela and Chimuka, 2016a). Other reported SPE sorbents for pharmaceuticals are Bond Elute sodium sulphate (Schoeman et al., 2015) and ENV+ adsorber material (Olarinmoye et al., 2016).

In order to bypass the critical sample preparation procedure, the application of polar organic integrative sampler (POCIS) have been reported for the determination of NSAIDs in wastewater treatment plants located in Johannesburg, South Africa (Amdany et al., 2014). POCIS application is very important as it combines sampling with sample preparation steps together. Overall, the summary of analytical methods that have been applied for water analysis in Africa is presented in Table 9. Regardless of limited access to sensitive instrumentation in Africa, the methods highlighted in

Table 9 have the detection limits in the range of low ng L^{-1} to µg L^{-1} which are relatively similar to the methods employed in other continents.

Table 9

A summary of analytical methods used for pharmaceutical analysis in Africa.

Pharmaceutical	Sample preparation	Analytical technique	Detection	Matrix	Detection limit (μg L^{-1})	Reference
NSAIDs						
Naproxen	MISPE	LC	Photo diode array	Wastewater	0.15	Madikizela and Chimuka, 2016a
Ibuprofen	SPE	LC	Fluorescence	Wastewater	0.70	Amdany et al., 2014
Diclofenac	SPE	GC	Mass spectrometer	Wastewater	0.0033	Kermia et al., 2016
ARVs						
Nevirapine	SPE	GC	Mass spectrometer	Wastewater	0.0018	Schoeman et al., 2015
Evafirenz	SPE	LC	Mass spectrometer	Surface water	0.090	Wood et al., 2015
Lamivudine	SPE	LC	Mass spectrometer	River water	0.0030	Ngumba et al., 2016
Antibiotics						

Ciprofloxacin	SPE	LC	Mass spectrometer	Surface water and wastewater	0.0012	Agunbiade and Moodley, 2016
Ampicillin	SPE	LC	Photo diode array	River water	0.20	Agunbiade and Moodley, 2014
Trimethoprim	SPE	LC	Mass spectrometer	Wastewater	0.12	Matongo et al., 2015a
Anti-epileptics / Psychiatric						
Carbamazepine	SPE	LC	Mass spectrometer	Wastewater	0.27	Matongo et al., 2015b
Anti-malarials						
Sulfadoxine	SPE	LC	Mass spectrometer	River water	<0.050	K'oreje et al., 2012
Lipid-lowering						
Gemfibrozil	SPE	LC	Mass spectrometer	Wastewater	0.020	Olarinmoye et al., 2016

6. Challenges, opportunities and future trends

Generally, pharmaceuticals end up in big water bodies, thereby diluting them. This leads to reduction of concentration to, at times, trace concentration levels that cannot be detected by traditional detectors. Instruments with low detection limits need to be employed. However, in the African context, where research funds are limited, this becomes a challenge for the identification and quantification of these pharmaceutical compounds. More so, there is need to pre-concentrate these pharmaceutical compounds so that the available instruments such as GCs and HPLCs with high detection limits can still be used. These techniques are widespread and well understood in Europe, however, there is lack of expertise in Africa. For Matongo et al. (2015b), three pharmaceuticals were not detected which could be due to extensive dilution in the environment. Therefore, this is an opportunity for African scientists to have collaborations with researchers outside the continent that have such technological resources. This is very important in order to close the gap that exists, for example, between Africa and Europe in terms of the knowledge regarding the distribution of organic pollutants in the environment.

Variable (episodic) fluctuation of the concentration of pharmaceuticals within a day as observed elsewhere (Amdany et al., 2015) can be rectified by the use of recent sampling techniques such as passive sampling. Furthermore, a wider range of pharmaceuticals should be studied in Africa as there is currently a lack of data on many other classes such as antidiabetics, antiemetics, antifungals, antihistamines, antihelmitics and antiulcers. Therefore, an opportunity to monitor such compounds in African water resources arises. In addition, the occurrence of pharmaceuticals in the environment that belong to antipsychotic and lipid regulator classes have been reported in few studies (Matongo et al., 2015a; Matongo et al., 2015b; Agunbiade and Moodley, 2016). Also, there is currently a lack of data in many African countries such as Zimbabwe, Mali, Lesotho, etc. As observed in this review, the African continent contains 54 countries, but the studies on identification and quantification of pharmaceuticals have been

reported in less than 10 countries which could be due to lack of capacity and financial constraints.

Pharmaceuticals are easily transported from one water matrix to the other because of their properties that include high water solubility (Table 1) which could result in none adsorption on solid matter. More so, they can be transported from urban areas to rural areas and/or vice versa. Most studies on Africa are currently focusing in the assessment of water resources in urban areas such as Durban, Pietermaritzburg, Lagos, Johannesburg, Nairobi and Algiers. However, there is a need to monitor the organics in water from rural areas as some of those areas are characterized as water scarce regions, hence the quality of their limited water is important.

Currently the sewage treatment process in Africa commonly involves screening, aeration, sedimentation and chlorination. Since the current treatment process is unable to remove pollutants completely, an opportunity rises as there is need to cater or redesign the WWTPs that can degrade and exhaustively remove pharmaceuticals from WWTPs.

7. Conclusions

This review shows that a number of pharmaceutical classes including non-steroidal anti-inflammatory, antibiotic, anti-retroviral, anti-epileptic, steroid hormones and anti-malarial drugs have been detected in the water resources of African countries. Furthermore, it was observed that analytical resources have improved in some African countries as most studies have been conducted using the state of the art laboratory equipment such as HPLC equipped with mass spectrometry detection. However, in most African countries there is still lack of laboratory infrastructure, therefore, future international collaborations should be initiated that should help in routine monitoring of pharmaceuticals in African water bodies. Furthermore, an extensive monitoring of pharmaceuticals in different African countries is required where necessary instrumentation is available for trace quantification of drugs in water. In few major

African cities, there is data available on the quantification of pharmaceuticals in water, therefore, future studies in such areas should also focus in monitoring of water resources in rural locations. New information on the status of pharmaceuticals in African cities and rural areas could pave new interest and direction of research on these compounds.

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References

Agunbiade, F.O., Moodley, B., 2014. Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa. Environ Monit Assess 186, 7273-7291.

Agunbiade, F.O., Moodley, B., 2016. Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi River, KwaZulu-Natal, South Africa. Environ. Toxicol. Chem. 35, 36-46.

Amdany, R., Chimuka, L., Cukrowska, E., 2014. Determination of naproxen, ibuprofen and triclosan in wastewater using the polar organic chemical integrative sampler (POCIS): A laboratory calibration and field application. Water SA 40, 407-414.

Amdany, R., Moya, A., Cukrowska, E., Chimuka, L., 2015. Optimization of the temperature for the extraction of pharmaceuticals from wastewater by a hollow fiber silicone membrane. Anal Lett 48, 2343-2356.

Bahlmann, A., Brack, W., Schneider, R.J., Krauss, M., 2014. Carbamazepine and its metabolites in wastewater: Analytical pitfalls and occurrence in Germany and Portugal. Water Res. 57, 104-114.

Beausse, J., 2004. Selected drugs in solid matrices: a review of environmental determination, occurrence and properties of principal substances. Trends Anal. Chem. 23, 753-761.

Bloland, P.B., Ettling, M., Meek, S., 2000. Combination therapy for malaria in Africa: hype or hope? Bull. World Health Organ. 78 (12).

Boix, C., Ibanez, M., Sancho, J.V., Rambla, J., Aranda, J.L., Ballester, S., Hernandez, F., 2015. Fast determination of 40 drugs in water using large volume direct injection liquid chromatography–tandem mass spectrometry. Talanta 131, 719-727.

Caban, M,, Lis, E., Kumirska, J., Stepnowski, P., 2015. Determination of pharmaceutical residues in drinking water in Poland using a new SPE-GC-MS(SIM) method based on Speedisk extraction disks and DIMETRIS derivatization. Sci. Total Environ. 538, 402-411.

Carmona, E., Andreu, V., Pico, Y., 2014. Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: From waste to drinking water. Sci. Total Environ. 484, 53-63.

Chen, K., Zhou, J.L., 2014. Occurrence and behavior of antibiotics in water and sediments from the Huangpu River, Shanghai, China. Chemosphere 95, 604-612.

Dahane, S., Gil Garcia, M.D., Martinez Bueno, M.J., Ucles Moreno, A., Martinez Galera, M., Derdour, A., 2013. Determination of drugs in river and wastewaters using solid-phase extraction by packed multi-walled carbon nanotubes and liquid chromatography-quadrupole-linear ion trap-mass spectrometry. J. Chromatogr. A 1297, 17-28.

Debska, J., Kot-Wasik, A., Namiesnik, J., 2004. Fate and analysis of pharmaceutical residues in the aquatic environment. Crit. Rev. Anal. Chem 34, 51-67.

Fenet, H., Mathieu, O., Mahjoub, O., Li, Z., Hillaire-Buys, D., Casellas, C., Gomez, E., 2012. Carbamazepine, carbamazepine epoxide and dihydroxycarbamazepine sorption to soil and occurrence in a wastewater reuse site in Tunisia. Chemosphere 88, 49-54.

Fernandez-Lopez, C., Guillen-Navarro, J.M., Padilla, J.J., Parsons, J.R., 2016. Comparison of the removal efficiencies of selected pharmaceuticals in wastewater treatment plants in the region of Murcia, Spain. Ecol. Eng. 95, 811-816.

Foster, S.D., 1991. Pricing, distribution, and use of antimalarial drugs. Bull. World Health Organ. 69, 349–363.

Garcia-Galan, M.J., Diaz-Cruz, M.S., Barcelo, D., 2010. Determination of 19 sulfonamides in environmental water samples by automated on-line solid-phase extraction-liquid chromatography–tandem mass spectrometry (SPE-LC–MS/MS). Talanta 81, 355-366.

Garcia-Lor, E., Sancho, J.V., Serrano, R., Hernandez, F., 2012. Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia. Chemosphere 87, 453-462.

Gilart, N., Marce, R.M., Fontanals, N., Borrull, F., 2013. A rapid determination of acidic pharmaceuticals in environmental waters by molecularly imprinted solid-phase extraction coupled to tandem mass spectrometry without chromatography. Talanta. 110, 196-201.

Gros, M., Petrovic, M., Ginebreda, A., Barcelo, D., 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ. Int. 36, 15-26.

Gumbi, B.P., Moodley, B., Birungi, G., Ndungu, P.G., 2016. Detection and quantification of acidic drug residues in South African surface water using gas chromatography-mass spectrometry. Chemosphere, In Press, http://dx.doi.org/10.1016/j.chemosphere.2016.10.105

Gurke, R., Robler, M., Marx, C., Diamond, S., Schubert, S., Oertel, R., Fauler, J., 2015. Occurrence and removal of frequently prescribed pharmaceuticals and corresponding metabolites in wastewater of a sewage treatment plant. Sci. Total Environ. 532, 762-770.

Jelic. A., Gros, M., Ginebreda, A., Cespedes-Sanchez, R., Ventura, F., Petrovic, M., Barcelo, D., 2011. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Res. 45, 1165-1176.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009a. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res. 43, 363-380.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009b. Illicit drugs and pharmaceuticals in the environment – Forensic applications of environmental data. Part 1: Estimation of the usage of drugs in local communities. Environ. Pollut. 157, 1773-1777.

Kermia, A.E.B., Fouial-Djebbar, D., Trari, M., 2016. Occurrence, fate and removal efficiencies of pharmaceuticals in wastewater treatment plants (WWTPs) discharging in the coastal environment of Algiers. Comptes Rendus Chimie 19, 963-970.

K'oreje, K.O., Demeestere, K., De Wispelaere, P., Vergeynst, L., Dewulf, J., Van Langenhove, H., 2012. From multi-residue screening to target analysis of pharmaceuticals in water: Development of a new approach based on magnetic

sector mass spectrometry and application in the Nairobi River basin, Kenya. Sci. Total Environ. 437, 153-164.

K'oreje, K.O,, Vergeynst, L., Ombaka, D., De Wispelaere, P., Okoth, M., Van Langenhove, H., Demeestere, K., 2016. Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. Chemosphere 149, 238-244.

Larsson, E., al-Hamimi, S., Jonsson, J.A., 2014. Behaviour of nonsteroidal anti-inflammatory drugs and eight of their metabolites during wastewater treatment studied by hollow fibre liquid phase microextraction and liquid chromatography mass spectrometry. Sci. Total Environ. 485-486, 300-308.

Li, W.C., 2014. Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil. Environ. Pollut. 187, 193-201.

Li, W., Shi, Y., Gao, L., Liu, J., Cai, Y., 2012. Occurrence of antibiotics in water, sediments, aquatic plants, and animals from Baiyangdian Lake in North China. Chemosphere 89, 1307-1315.

Li, X., Zheng, W., Kelly, W.R., 2013. Occurrence and removal of pharmaceutical and hormone contaminants in rural wastewater treatment lagoons. Sci. Total Environ. 445-446, 22-28.

Madikizela, L.M., Chimuka, L., 2016a. Determination of ibuprofen, naproxen and diclofenac in aqueous samples using a multi-template molecularly imprinted polymer as selective adsorbent for solid-phase extraction. J Pharm Biomed Anal 128, 210-215.

Madikizela, L.M., Chimuka, L., 2016b. Synthesis, adsorption and selectivity studies of a polymer imprinted with naproxen, ibuprofen and diclofenac. J Environ Chem Eng 4, 4029-4037.

Madikizela, L.M., Muthwa, S.F., Chimuka, L., 2014. Determination of triclosan and ketoprofen in river water and wastewater by solid phase extraction and high performance liquid chromatography. S. Afr. J. Chem 67, 143-150.

Manickum, T., John, W., 2014. Occurrence, fate and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa). Sci. Total Environ. 468-469, 584-597.

Manrique-Moreno, M., Heinbockel, L., Suwalsky, M., Garidel, P., Brandenburg, K., 2016. Biophysical study of the non-steroidal anti-inflammatory drugs (NSAID) ibuprofen, naproxen and diclofenac with phosphatidylserine bilayer membranes. Biochim. Biophys. Acta – Biomembranes 1858, 2123-2131.

Matongo, S., Birungi, G., Moodley, B., Ndungu, P., 2015a. Pharmaceutical residues in water and sediment of Msunduzi River, KwaZulu-Natal, South Africa. Chemosphere 134, 133-140.

Matongo, S., Birungi, G., Moodley, B., Ndungu, P., 2015b Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa. Environ Sci Pollut Res 22, 10298-10308.

Moreno-Gonzalez, R., Rodriguez-Mozaz, S., Gros, M., Barcelo, D., Leon, V.M., 2015. Seasonal distribution of pharmaceuticals in marine water and sediment from a mediterranean coastal lagoon (SE Spain). Environ. Res. 138, 326-344.

Mphahlele, K., Onyango, M.S., Mhlanga, S.D., 2015. Adsorption of aspirin and paracetamol from aqueous solution using Fe/N-CNT/β-cyclodextrin nanocomopsites synthesized via a benign microwave assisted method. J Environ Chem Eng 3, 2619-2630.

Ngumba, E., Gachanja, A., Tuhkanen, T., 2016. Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. Sci. Total Environ. 539, 206-213.

Odendaal, C., Seaman, M.T., Kemp, G., Patterton, H.E., Patterton, H.G., 2015. An LC-MS/MS based survey of contaminants of emerging concern in drinking water in South Africa. S. Afr. J. Sci 111, 1-6.

Olarinmoye, O., Bakare, A., Ugwumba, O., Hein, A., 2016. Quantification of pharmaceutical residues in wastewater impacted surface waters and sewage sludge from Lagos, Nigeria. J. Environ. Chem. Ecotoxicol. 8, 14-24.

Olujimi, O.O., Fatoki, O.S., Odendaal, J.P., Okonkwo, J.O., 2010. Endocrine disrupting chemicals (phenol and phthalates) in the South African environment: a need for more monitoring. Water SA 36, 671-682.

Paiga, P., Santos, L.H.M.L.M., Ramos, S., Jorge, S., Gabriel Silva, J., Delerue-Matos, C., 2016. Presence of pharmaceuticals in the Lis river (Portugal): Sources, fate and seasonal variation. Sci. Total Environ. 573, 164-177.

Pavlovic, D.M., Babic, S., Horvat, A.J.M., Kastelan-Macan, M., 2007. Sample preparation in analysis of pharmaceuticals. TrAC, Trends Anal. Chem. 26, 1062-1075.

Peng, X., Wang, C., Zhang, K., Wang, Z., Huang, Q., Yu, Y., Ou, W., 2014. Profile and behavior of antiviral drugs in aquatic environments of the Pearl River Delta, China. Sci. Total Environ.466-467, 755-761.

Pereira, A.M.P.T., Silva, L.J.G., Meisel, L.M., Lino, C.M., Pena, A., 2015. Environmental impact of pharmaceuticals from Portuguese wastewaters: geographical and seasonal occurrence, removal and risk assessment. Environ. Res. 136, 108-119.

Santos, L.H.M.L.M., Araujo, A.N., Fachini, A., Pena, A., Delerue-Matos, C., Montenegro, M.C.B.S.M., 2010, Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J. Hazard. Mater. 175, 45-95.

Savci, S., 2013. A review of occurrence of pharmaceuticals in sediments. Afr J Biotechnol 12, 4539-4541.

Schoeman, C., Mashiane, M., Dlamini, M., Okonkwo, O.J., 2015. Quantification of selected antiretroviral drugs in a wastewater treatment works in South Africa using GC-TOFMS. J Chromatogr Sep Tech 6, 1-7.

Segura, P.A., Takada, H., Correa, J.A., El Saadi, K., Koike, T., Onwona-Agyeman, S., Ofosu-Anim, J., Sabi, E.B., Wasonga, O.V., Mghalu, J.M., dos Santos Junior A.M., Newman, B., Weerts, S., Yargeau, V., 2015. Global occurrence of anti-infectives in contaminated surface waters: Impact of income inequality between countries. Environ. Int. 80, 89-97.

Semakula, H.M., Song, G., Achuu, S.P., Zhang, S., 2016. A Bayesian belief network modelling of household factors influencing the risk of malaria: A study of parasitaemia in children under five years of age in sub-Saharan Africa. Environ Modell Softw. 75, 59-67.

Shah, S.A.B., Mullin, R., Jones, G., Shah, I., Barker, J., Petroczi, A., Naughton, D.P., 2013. Simultaneous analysis of antiretroviral drugs abacavir and tenofovir in human hair by liquid chromatography–tandem mass spectrometry. J Pharm Biomed Anal 74, 308-313.

Sun, Q., Lv, M., Hu, A., Yang, X., Yu, C., 2014. Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in a wastewater treatment plant in Xiamen, China. J. Hazard. Mater. 277, 69-75.

Tijani, J.O., Fatoba, O.O., Babajide, O.O., Petrik, L.F., 2015. Pharmaceuticals, endocrine disruptors, personal care products, nanomaterials and perfluorinated pollutants: a review. Environ Chem Lett 14, 27-49.

Togola, A., Budzinski, H., 2007. Analytical development for analysis of pharmaceuticals in water samples by SPE and GC-MS. Anal Bioanal Chem 388, 627-635.

Vymazal, J., Brezinova, T., Kozeluh, M., 2015. Occurrence and removal of estrogens, progesterone and testosterone in three constructed wetlands treating municipal sewage in the Czech Republic. Sci. Total Environ. 536, 625-631.

WHO, (2013) Global Update on HIV Treatment: Results, Impact and Opportunities.

WHO, (2015) Malaria Factsheet No. 95.

Wood, T.P., Duvenage, C.S.J., Rohwer, E., 2015. The occurrence of anti-retroviral compounds used for HIV treatment in South African surface water. Environ. Pollut. 199, 235-243.

Zaidi, S., Chaabane, T., Sivasankar, V., Darchen, A., Maachi, R., Msagati, T.A.M., 2015. Electro-coagulation coupled electro-flotation process: Feasible choice in doxycycline removal from pharmaceutical effluents. Arabian J. Chem., In Press, http://dx.doi.org/10.1016/j.arabjc.2015.06.009

Zunngu SS, Madikizela LM, Chimuka L, Mdluli PS (2016) Synthesis and application of a molecularly imprinted polymer in the solid-phase extraction of ketoprofen from wastewater. Comptes Rendus Chimie, In Press, http://dx.doi.org/10.1016/j.crci.2016.09.006

3.3.2.1 Paper 1 - Supporting information

Status of pharmaceuticals in African water bodies: Occurrence, removal and analytical methods

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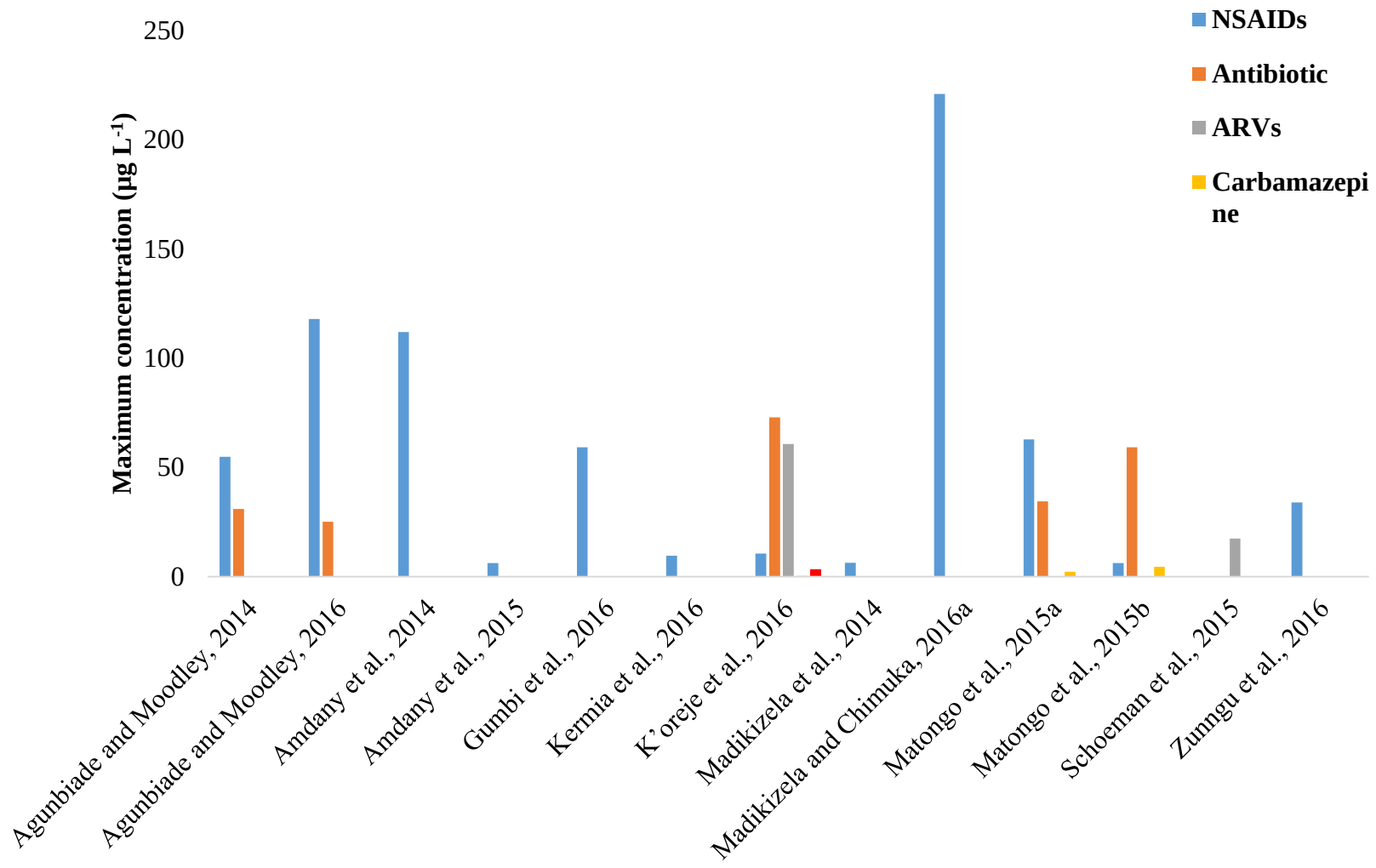


Fig. S1: Maximum concentration levels of pharmaceuticals detected in African wastewater influent.

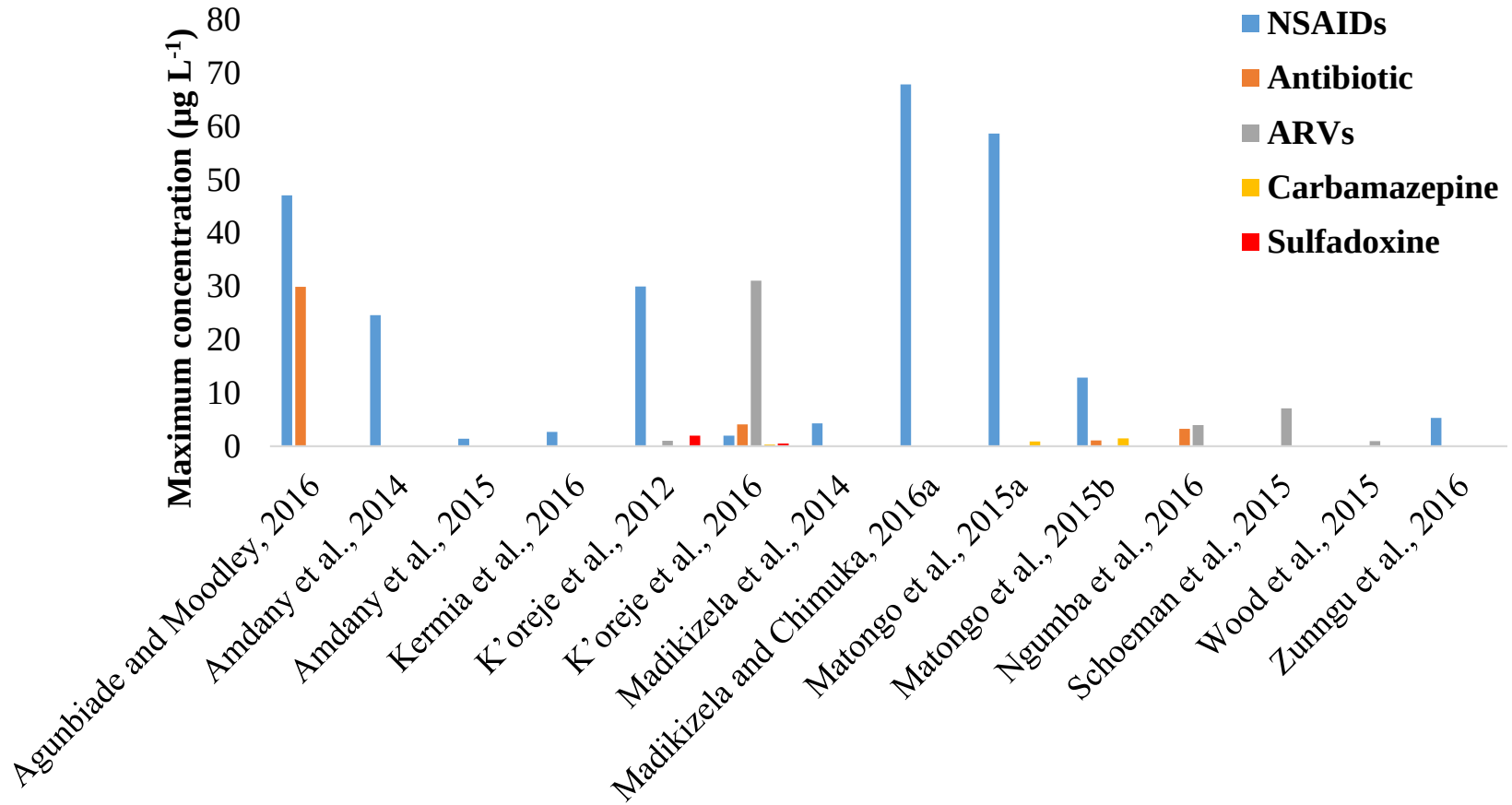


Fig. S2: Maximum concentration levels of pharmaceuticals detected in African wastewater effluent.

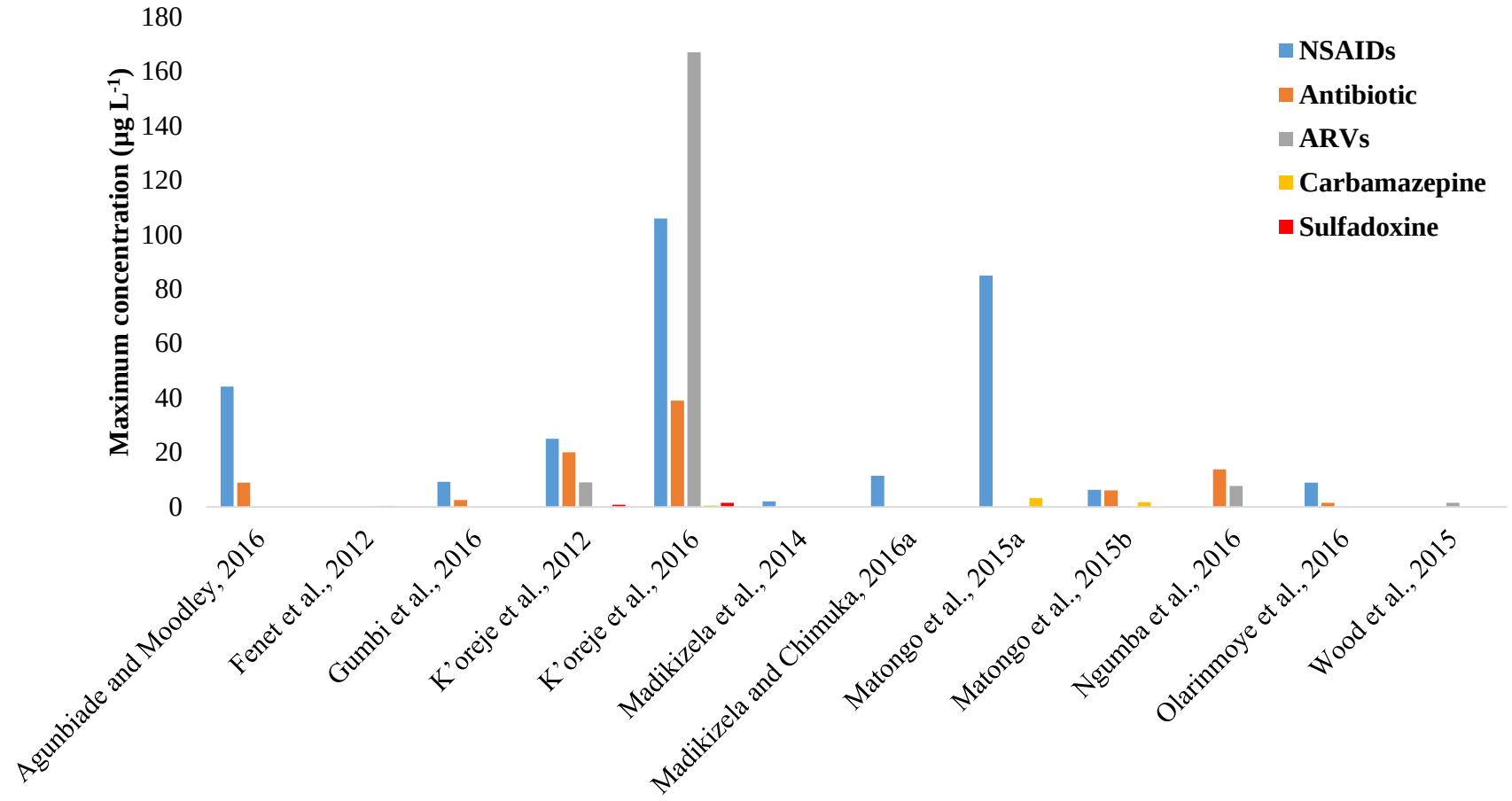


Fig. S3: Maximum concentration levels of pharmaceuticals detected in African surface water.

3.3.3 Paper 2

Madikizela L.M., Mdluli P.S. and Chimuka, L. (2016) Experimental and theoretical study of molecular interactions between 2-vinyl pyridine and acidic pharmaceuticals used as multi-template molecules in molecularly imprinted polymer. *Reactive and Functional Polymers 103, 33-43.* Impact Factor 2.725

Madikizela – 60% (conducted the research, wrote the manuscript);

Mdluli – 20% (assisted with computational modelling and its interpretation);

Chimuka – 20% (supervisor, reviewed the manuscript).

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Experimental and theoretical study of molecular interactions between 2-vinyl pyridine and acidic pharmaceuticals used as multi-template molecules in molecularly imprinted polymer

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ABSTRACT

Molecular interactions between functional monomer and template molecules are regarded as the driving force for the success of a molecularly imprinted polymer. In this study, a multi-template molecularly imprinted polymer (MIP) for ibuprofen, naproxen and diclofenac was synthesized in an oil bath set at 70 °C for 24 hours. 2-vinyl pyridine, ethylene glycol dimethacrylate, toluene and 1,1'-azobis-(cyclohexanecarbonitrile) were used as functional monomer, cross-linker, porogen and radical initiator, respectively. A non-imprinted polymer (NIP) was synthesized using a similar approach with the omission of templates. Monomer-template interactions were examined using Molecular Dynamics and Fourier Transform Infrared Spectroscopy (FTIR). Both molecular dynamics and FT-IR results indicated the formation of the hydrogen bond between the templates and 2-vinyl pyridine. Molecular dynamics further revealed the identity of the hydrogen atoms in the templates involved in interactions with nitrogen atom on the functional monomer in the presence of toluene molecules. Surface area obtained for the MIP using Brunauer, Emmett and Teller method was 282 m^2/g, whereas 232 m^2/g was obtained for the NIP. This indicated that MIP has more binding sites compared to the NIP. Furthermore, batch adsorption and selectivity experiments were carried out in the presence of gemfibrozil as the competitor. When such experiments were carried out in toluene, the adsorption capacities (mg/g) obtained for naproxen, ibuprofen, diclofenac and gemfibrozil were 14.4, 11.0, 14.0 and 7.5, respectively. These results show that the MIP was more selective to the compounds that were used as template molecules.

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1. Introduction

Molecular imprinting is a technique that is used to prepare polymers with highly specific binding sites for small molecules [1]. Molecularly imprinted polymers (MIPs) are prepared using a functional monomer(s), which allows the interactions with the functional group(s) of a molecule to be recognized and they are synthesized with a cross linking monomer(s) in the presence of the target molecule(s). The imprint molecule(s) is removed from the polymer in order to create the molecularly imprinted complementary binding site(s) for the target molecule(s) [2]. Over the last two decades, molecularly imprinted polymers have gained several scientific applications that includes their use as; solid-phase extraction sorbents [3], chromatographic stationary phase [4–6], electrochemical sensor [7], etc. The popularity of MIPs in chemistry applications is attributed to their properties that include high selectivity, mechanical strength, and resistance against acids, bases, organic solvents, high pressures and temperatures [8].

Acidic pharmaceuticals such as ibuprofen, diclofenac and naproxen (organic structures shown in Fig. 1 (a) – (c)) belong to the class of non-steroidal anti-inflammatory drugs. They are among the group of pharmaceutical compounds that is often used to promote human health [9]. Once used by humans, they are excreted during urinary discharges as free drugs or as metabolites. This contributes to the presence of the acidic pharmaceuticals in the influent and effluent of wastewater treatment plants at the low μg/L levels [10–12]. Amdany et al. [12] reported the concentration range of 52 to 128 μg/L for naproxen, ibuprofen and triclosan in wastewater influent, whereas, 11 to 25 μg/L was reported for the same compounds in the effluent. A group of acidic pharmaceuticals have been also detected simultaneously in aquatic environment that includes river water and drinking water at ng/L levels [13,14]. Recent methods that are used for the quantitative determination of acidic compounds in aqueous matrices involves the use of MIPs for the selective extraction and/or pre-concentration of target compounds [15–17]. In this regard, multi-template MIPs are of great importance as they are able to selective extract a group of acidic pharmaceuticals. 2-vinyl pyridine (functional monomer) shown in Fig. 1 (d) and ethylene glycol dimethacrylate (cross-linker) are widely used in the synthesis of MIP that is imprinted with ibuprofen, diclofenac or naproxen [18,19].

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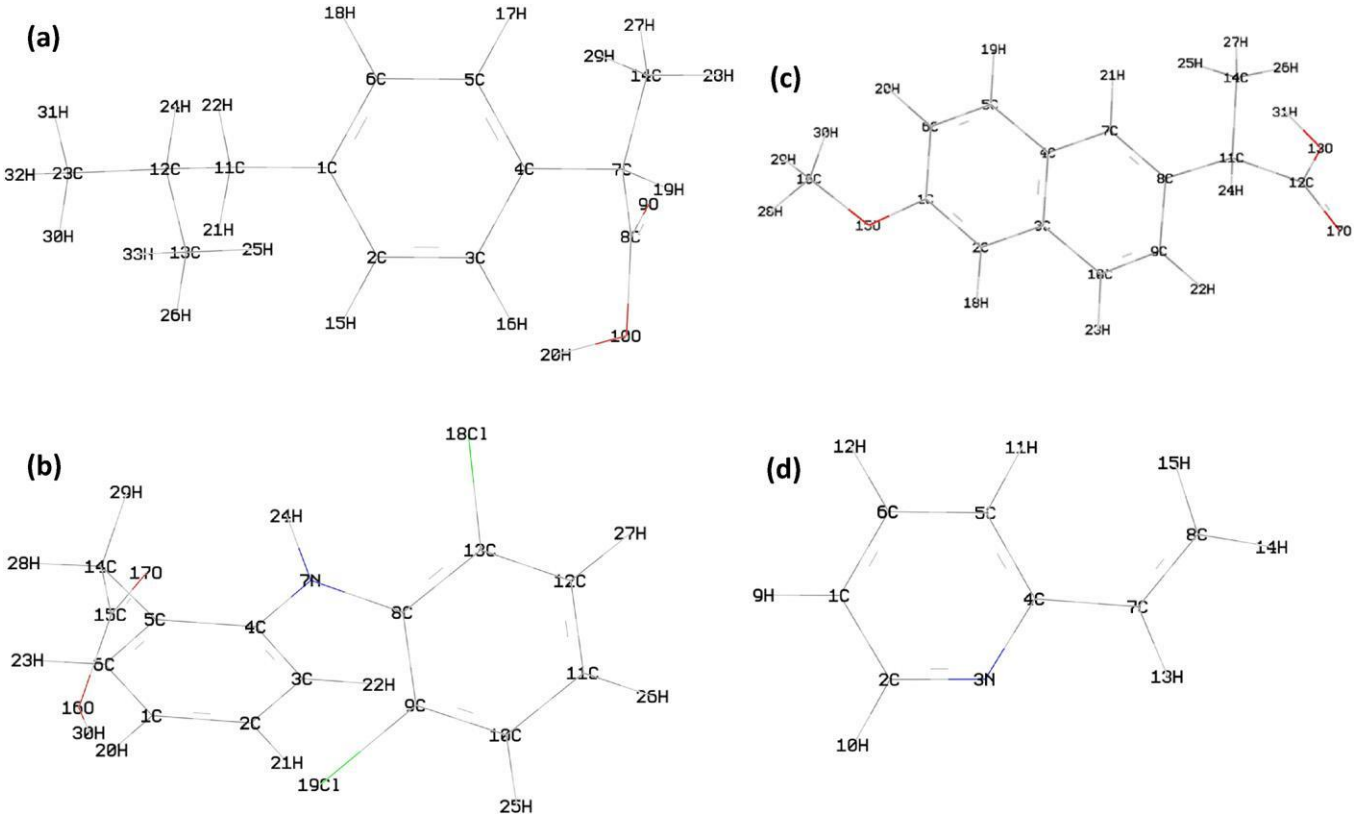


Fig. 1. Computational derived structures of ibuprofen (a), diclofenac (b), naproxen (c), and 2-vinyl pyridine (d).

Functional monomer and ethylene glycol dimethacrylate form polymer matrix around the template thus preserving monomer-template binding sites [1]. Functional monomers are understood to be responsible for the binding interactions in the imprinted binding sites. During the imprinting process, the functionality of the template is matched with that of the functional monomer [20].

Molecular interactions that occur between the functional monomer and template molecules have been previously explained using spectroscopic techniques such as nuclear magnetic resonance (NMR), ultraviolet-visible (UV-Vis) and Fourier transform infrared spectroscopy FT-IR [21,22]. For example, Farrington and Regan [23] have used density functional theory and NMR to demonstrate the interactions that take place between 2-vinyl pyridine and ibuprofen. However, factors that might influence the interactions between 2-vinyl pyridine and acidic pharmaceuticals have not been thoroughly investigated. Despite having a detailed literature for the investigation of monomer-template interactions for the MIP synthesized for acidic pharmaceuticals, the influence of a porogenic solvent during the molecularly imprinting process have not been addressed in details. Insight into monomer-template interactions have been investigated by Lasagabaster-Latorre et al. [24]. In their study, they investigated the interactions that occur between 4-vinyl pyridine (functional monomer) and Bisphenol A (template) using spectroscopic techniques such as UV-Vis, proton NMR and FT-IR.

However, this study investigate the molecular interactions of 2-vinyl pyridine with three acidic pharmaceuticals that have been simultaneously imprinted. FT-IR being the traditional technique that is widely used for functional group characterization is applied in this study along-side the molecular dynamics for gaining insight into molecular interactions that occur between 2-vinyl pyridine and acidic pharmaceuticals. This study further shows that the solvent used during the template re-binding affects the adsorption of target compounds into MIP particles. The objective of this study was to investigate the interactions that occur between 2-vinyl pyridine and acidic pharmaceuticals by employing the spectroscopic techniques in parallel with the molecular dynamics. The selectivity of the polymers synthesized in this study was further studied using a structurally related acidic pharmaceutical as the competitor.

2. Experimental

2.1. Chemicals

Naproxen (98 %), ibuprofen (≥ 98 %), diclofenac sodium salt, 2-vinylpyridine (97 %), 1,1'-azobis-(cyclohexanecarbonitrile) (98 %), ethylene glycol dimethacrylate (98 %), HPLC grade acetone (≥ 99.8 %), HPLC grade chloroform (≥ 99.8 %) and toluene (99.7 %) were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC-grade acetonitrile (≥ 99.9 %) and glacial acetic acid (100 %) were purchased from Merck (Darmstadt, Germany). Formic acid (approx. 98 %) was purchased from Fluka (Steinheim, Germany).

2.2. Synthesis of polymers

A procedure reported by Duan et al (2013) [25], was followed with modification for the synthesis of a MIP in a two-step reaction process. In the first step of reaction, 1.51 mL (7.95 mmol) of ethylene glycol dimethacrylate was mixed with 20 mg of 1,1'-azobis-(cyclohexanecarbonitrile) and 50 mL of toluene in a 250 mL round bot-tom flask. The resulting mixture was purged with nitrogen for 10 minutes to remove oxygen and it was sealed under inert conditions. The mixture was continuously stirred with magnetic stirrer while reacting in an oil bath at 70 °C for 8 hours. The second step of reaction was carried out by mixing separately 76.60 mg (0.333 mmol) naproxen, 68.69 mg (0.333 mmol) ibuprofen, 106.04 mg (0.333 mmol) diclofenac, 0.25 ml (2.37 mmol) 2-vinylpyridine, 3.85 ml ethylene glycol dimethacrylate, 60 mg 1,1'-azobis-(cyclohexanecarbonitrile) and 50 mL mixture of acetonitrile/toluene (50:50, v:v). This second mixture

Table 1
Mulliken charges, (a) ibuprofen, (b) diclofenac, (c) naproxen and (d) 2-vinyl pyridine.

Compound	Number	Atom	Atomic charges
(a) Mulliken charges of ibuprofen			
Ibuprofen	1	C	0.030
	2	C	-0.106
	3	C	-0.085
	4	C	0.024
	5	C	-0.094
	6	C	-0.097
	7	C	-0.124
	8	C	0.548
	9	O	-0.434
	10	O	-0.630
	11	C	-0.228
	12	C	-0.138
	13	C	-0.384
	14	C	-0.395
	15	H	0.100
	16	H	0.077
	17	H	0.098
	18	H	0.097
	19	H	0.130
	20	**H**	**0.277**
	21	H	0.134
	22	H	0.121
	23	C	-0.406
	24	H	0.165
	25	H	0.158
	26	H	0.140
	27	H	0.114
	28	H	0.168
	29	H	0.174
	30	H	0.143
	31	H	0.135
	32	H	0.147
	33	H	0.140
(b) Mulliken charges of diclofenac			
Diclofenac	1	C	-0.085
	2	C	-0.075
	3	C	-0.010
	4	C	0.223
	5	C	0.031
	6	C	-0.086
	7	N	-0.453
	8	C	0.169
	9	C	0.220
	10	C	-0.087
	11	C	-0.150
	12	C	-0.080
	13	C	0.255
	14	C	-0.194
	15	C	0.507
	16	O	-0.593
	17	O	-0.403
	18	Cl	-0.516
	19	Cl	-0.562
	20	H	0.130
	21	H	0.135
	22	H	0.151
	23	H	0.122
	24	**H**	**0.286**
	25	H	0.164
	26	H	0.143
	27	H	0.164
	28	H	0.118
	29	H	0.131
	30	**H**	**0.348**
(c) Mulliken charges of naproxen.			
Naproxen	1	C	0.287
	2	C	-0.096
	3	C	0.017
	4	C	0.042
	5	C	-0.144
	6	C	-0.107
	7	C	-0.097
	8	C	0.030
	9	C	-0.061
	10	C	-0.111
	11	C	-0.131
	12	C	0.561
	13	O	-0.608
	14	C	-0.437
	15	O	-0.526
	16	C	-0.107
	17	O	-0.457
	18	H	0.126
	19	H	0.113
	20	H	0.118
	21	H	0.097
	22	H	0.179
	23	H	0.115
	24	H	0.121
	25	H	0.137
	26	H	0.144
	27	H	0.136
	28	H	0.147
	29	H	0.110
	30	H	0.121
	31	**H**	**0.284**
(d) Mulliken charges of 2-vinyl pyridine			
2-vinyl pyridine	1	C	-0.104
	2	C	0.053
	3	**N**	**-0.317**
	4	C	0.195
	5	C	-0.083
	6	C	-0.106
	7	C	-0.104
	8	C	-0.289
	9	H	0.091
	10	H	0.098
	11	H	0.081
	12	H	0.101
	13	H	0.130
	14	H	0.132
	15	H	0.122

was then transferred into the same round bottom flask (containing the first mixture), this was followed by purging the mixture with nitrogen for 10 minutes. The flask was sealed under nitrogen and stirred in an oil bath at 70 °C for 16 hours. Resulting polymer with synthetic mass yield of 97 % was oven dried at 60 °C to constant mass. Polymer was then milled, sieved and particles ranging from 25 to 50 μm were collected. Non imprinted polymer (NIP) was prepared under the same conditions in the absence of template molecules, where a synthetic mass yield of 98 % was obtained. Templates were eluted from the polymer using a 10 % (v/v) acetic acid in acetonitrile. Elution step was repeated several times until the target compounds were not detected by the HPLC system. Thereafter, 100 % acetonitrile was applied in order to wash off the acetic acid residue.

2.3. Monomer-template interactions: Molecular dynamics simulation

Molecular dynamics simulation in canonical ensemble at constant atom number, volume and temperature (NVT) was utilized to fully understand the interactions of ibuprofen, naproxen and diclofenac with 2-vinyl pyridine. All simulations were executed using the Discover Module of Materials Studio (version 7.0) [26]. The COMPASS force field was used to calculate the intermolecular interaction of ibuprofen, naproxen and diclofenac on 2-vinyl pyridine. All systems were subjected to energy minimization for geometry optimization using minimizer incorporated in the discover module of Materials studio before molecular dynamics simulations were conducted. For minima calculation a maximum iteration of 100000 was used with an ultra-fine convergence level. The molecular dynamic simulation using NVT lasted for 100 ps with a time step of 1 fs.

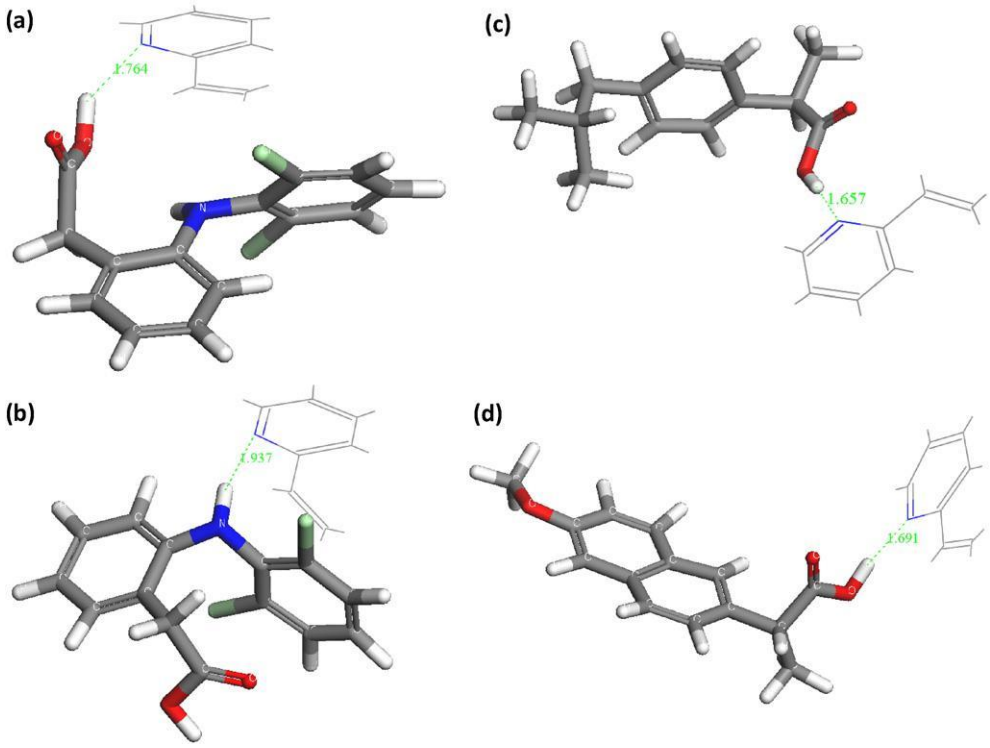


Fig. 2. The interactions that occur between 2-vinyl pyridine and diclofenac (a), (b), ibuprofen (c), and naproxen (d). Hydrogen bonding is indicated with dashed lines and bond distances are given using Angstrom (Å) units.

2.4. Characterization

In order to confirm the molecular dynamics simulation predictions, Fourier-transform infrared spectroscopy equipped with attenuated total reflection from Perkin Elmer (Llantrisant, United Kingdom) was used. Polymers, acidic pharmaceuticals and 2-vinyl pyridine were analyzed without any sample pre-treatment.

Surface area, total pore volume and average pore diameter for the synthesized polymers were determined using the Flow Prep 060 instrument from Micromeritics (Aachen, Germany).

A scanning electron microscopy, JOEL model JSM 6700F (Tokyo, Japan) was used to study the polymer morphology.

Elemental analysis was performed using a Flash 2000 Organic Elemental Analyser obtained from Thermo Scientific (Milan, Italy).

2.5. High performance liquid chromatographic separation

Chromatographic separation using a linear solvent system was per-formed on a high performance liquid chromatography (HPLC) system that consisted of an online mobile phase degasser unit (Model: DGU-20A3), 20 μL sample loop, pump (Model: LC-20AB), and UV/vis detector (Model: SPD-20A), all obtained from Shimadzu Corporation (Kyoto, Japan). The mobile phase used consisted of a mixture of acetonitrile: 0.2 % formic acid in water (70:30, v:v) at a flow rate of 0.8 mL/min.

Table 2
Binding energies, ΔE (kcal/mol), for acidic pharmaceuticals with 2-vinyl pyridine complexes.

Compound	ΔE (kcal/mol)
Ibuprofen	-9.43139
Diclofenac (H_{24})	-10.5409
Diclofenac (H_{30})	-14.7034
Naproxen	-10.8464

Separation was performed on a Kinetex C_{18} HPLC column of 150 x 4.6 mm x 2.6 μm obtained from Phenominex (California, USA). Shimadzu LC solutions software was used for data collection and processing. UV/vis detector was set at 230 nm for naproxen measurement, while ibuprofen and diclofenac were both monitored at 200 nm.

2.6. Batch binding experiments in organic media

5 mg of polymers (NIP and MIP) were incubated overnight at room temperature with 10 ml mixture of naproxen, ibuprofen and diclofenac (10 mg/L mix standard) prepared in appropriate organic solvent. Thereafter, un-adsorbed compounds were injected into the HPLC system after

Table 3
Binding energies, ΔE (kcal/mol), obtained for acidic pharmaceuticals with 2-vinyl pyridine complexes in various organic solvents.

Porogen	$\Delta E_{solvent}$ of template (kcal/mol)	$\Delta E_{solvent}$ of 2-vinyl pyridine (kcal/mol)
(a) Diclofenac		
Toluene	4.97158	11.315987
Chloroform	-2.586086	1.867335
Acetonitrile	-10.44476	-7.688702
Acetone	-23.81805	-7.80626
(b) Ibuprofen		
Toluene	5.445108	11.315987
Chloroform	-2.886558	1.867335
Acetonitrile	-8.931445	-7.688702
Acetone	-19.13455	-7.80626
(c) Naproxen		
Environment	$\Delta E_{solvent}$ of template (kcal/mol)	$\Delta E_{solvent}$ of 2-vinyl pyridine (kcal/mol)
Toluene	0.499213	11.315987
Chloroform	-8.112243	1.867335
Acetonitrile	-8.855985	-7.688702
Acetone	-20.15806	-7.80626

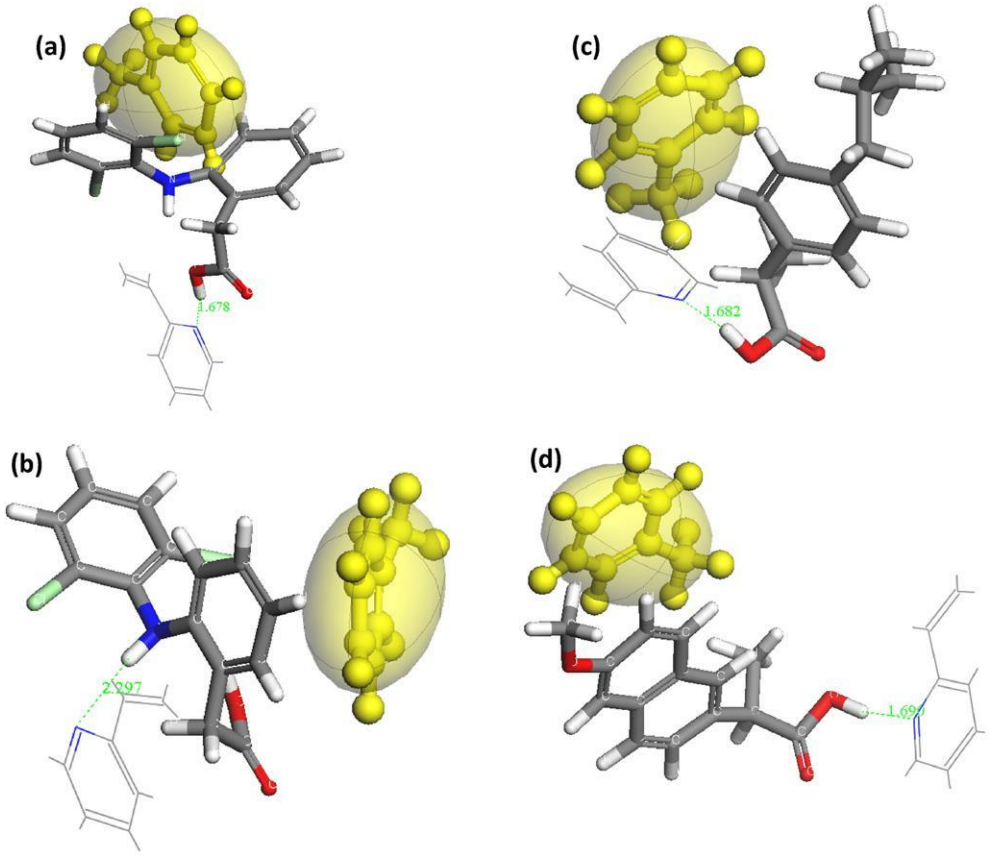


Fig. 3. The interactions that occur between 2-vinyl pyridine and diclofenac (a) and (b), ibuprofen (c) and naproxen (d) in the presence of one toluene molecule.

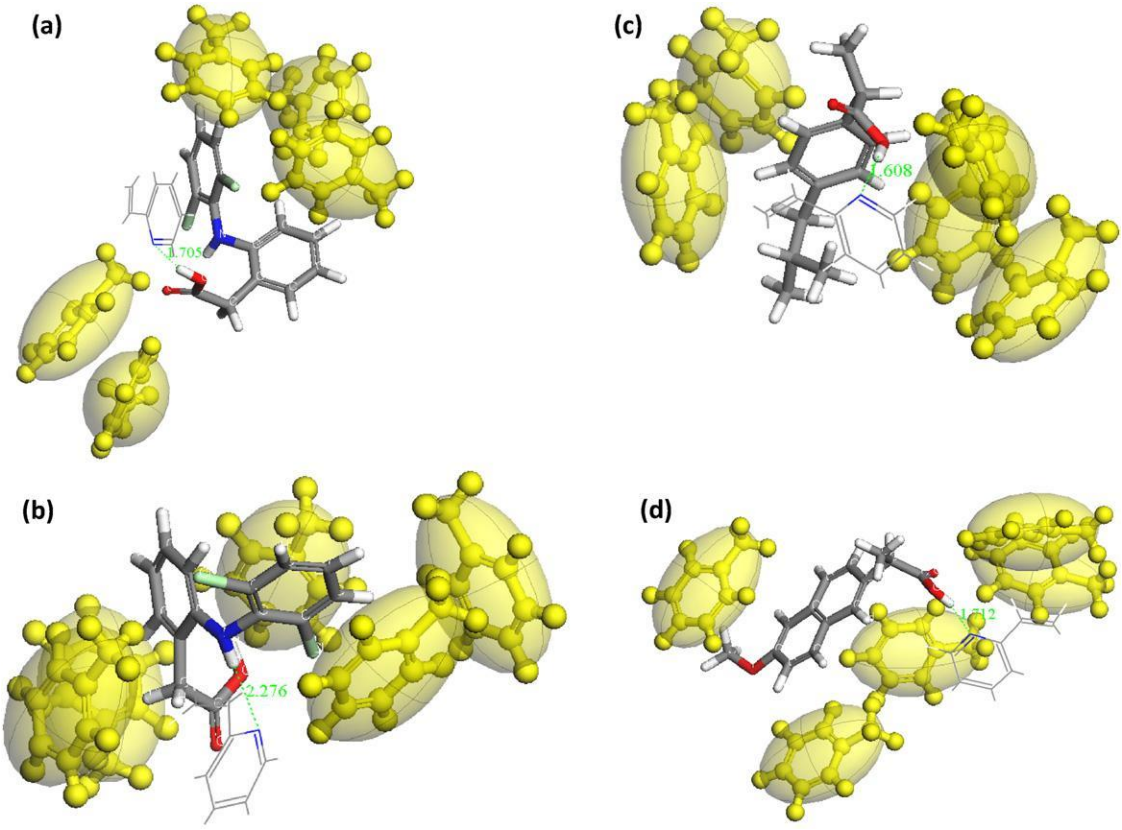


Fig. 4. The interactions that occur between 2-vinyl pyridine and diclofenac (a) and (b), ibuprofen (c) and naproxen (d) in the presence of five toluene molecules.

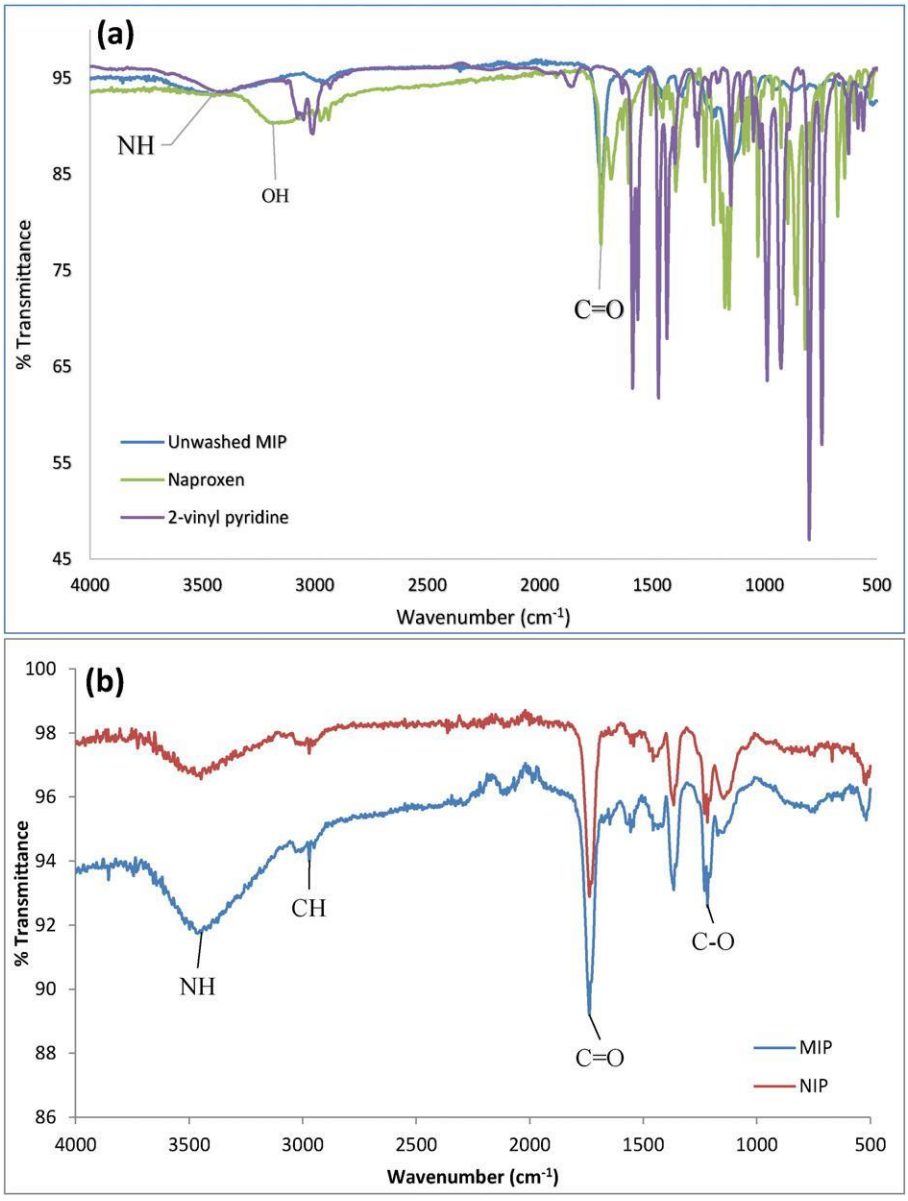


Fig. 5. FT-IR spectra of unwashed MIP, naproxen and 2-vinyl pyridine (a) and washed MIP and NIP (b).

filtration of solutions through a 0.22 μm syringe filter and quantified. The extraction efficiency and adsorption capacity (Q) were calculated using Eqs. (1) and (2), respectively.

$$\text{Extraction efficiency} = \frac{(C_0 - C_e)}{C_0} \times 100 \quad (1)$$

$$Q = \frac{(C_0 - C_e)V}{W} \quad (2)$$

where C_0 (mg/L) and C_e (mg/L) are the initial and final concentration of the incubated solution, respectively. V is the volume of the solution used in litres whereas, W is the mass of the polymer in grams.

Table 4
BET analysis of polymers.

Polymer	Surface Area (m^2/g)	Total pore volume (cm/g)	Average pore diameter (nm)
MIP	282	0.38	5.43
NIP	232	0.20	3.46

2.7. Selectivity experiments

To evaluate the selectivity of the MIP, adsorption studies were per-formed in the presence of the gemfibrozil (competitor). 5 mg of the polymer was stirred overnight at room temperature in glass flask containing 10 mL of naproxen/ibuprofen/diclofenac/gemfibrozil quaternary solution at a concentration of 10 mg/L each. Thereafter, free compounds were injected into the HPLC system after filtration of solutions through a 0.22 μm syringe filter and quantified. Selectivity performance was evaluated by determining distribution coefficient (K_d), selectivity coefficient (k), and relative selectivity coefficient (k') using Eqs. (3), (4) and (5), respectively [27].

$$K_d = \frac{(C_0 - C_e)}{C_e} \times \frac{V}{w} \quad (3)$$

$$k = \frac{K_d(analyte)}{K_d(competitor)} \quad (4)$$

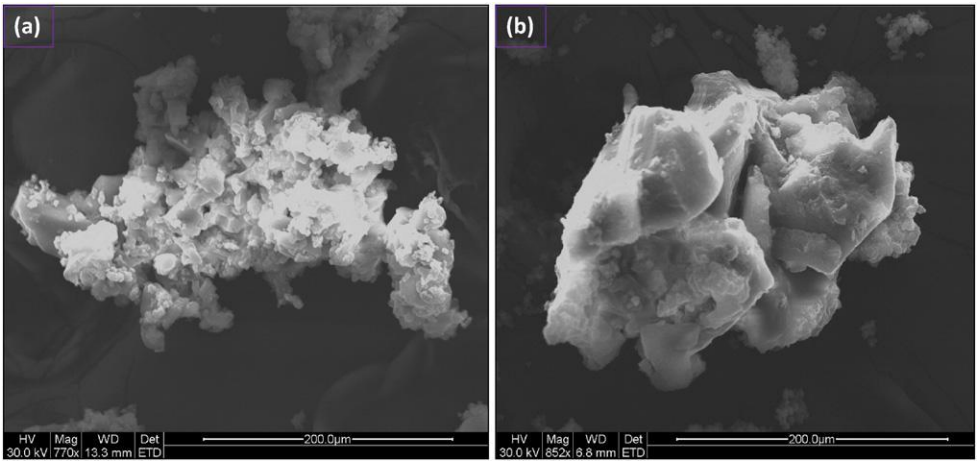


Fig. 6. Scanning electron micrographs of (a) washed MIP and (b) washed NIP.

$$k' = \frac{k_{MIP}}{k_{NIP}} \quad (5)$$

Where C_0 (mg/L), C_e (mg/L), V (litres) and w (grams) are initial concentration, final concentration, volume of the solution and mass of the polymer, respectively.

3.1. Monomer-template interactions - Molecular dynamics simulation

The Mulliken charges of all the atoms present in the functional monomer and all template molecules are recorded in Table 1(a) – (d).

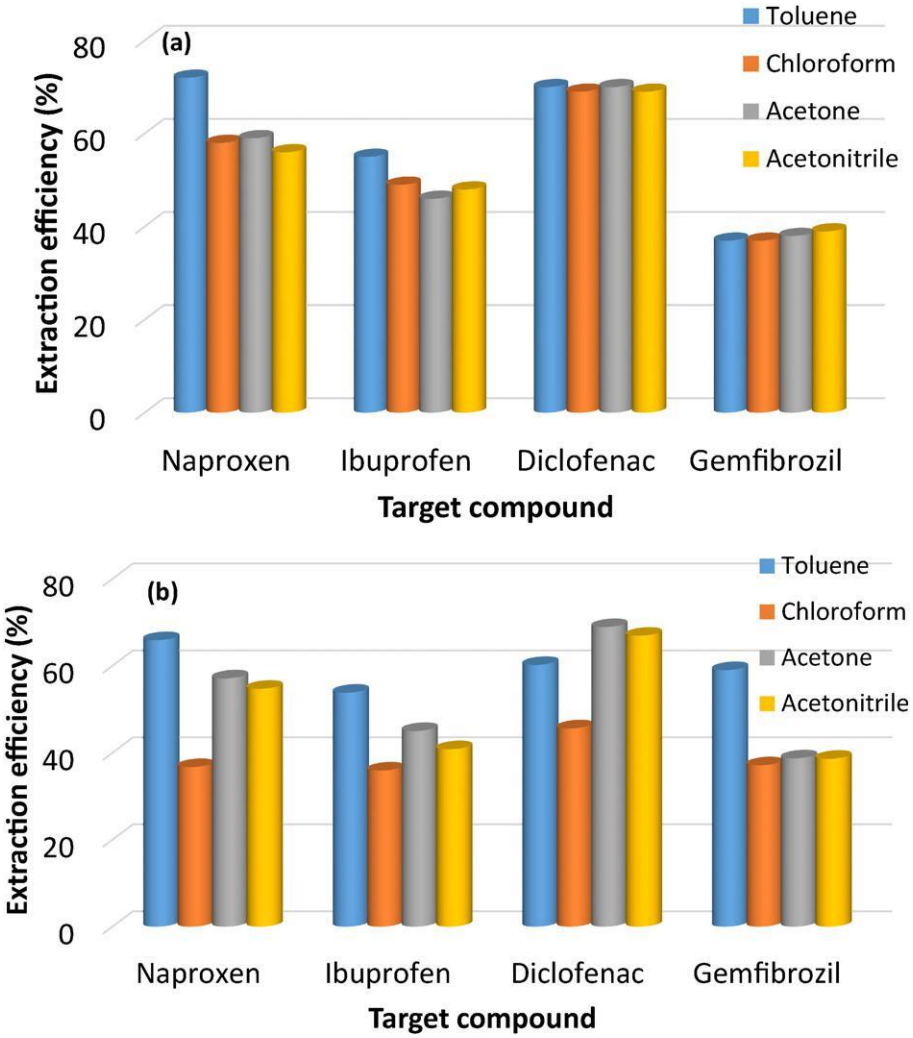


Fig. 7. Extraction efficiencies obtained for all compounds when extracted with MIP (a) and NIP (b).

These charges were used to predict the atoms that are most likely to form hydrogen bonding [28]. Based on these charges, it was found that the possible proton donors for ibuprofen, diclofenac and naproxen were H_{20}, H_{30} and H_{31}, respectively while on the other hand N_3 for 2-vinyl pyridine was the most likely candidate for proton acceptor. It was also noted that H_{24} of diclofenac was available for bonding, however a weak interaction was observed for this hydrogen atom and nitrogen atom of 2-vinyl pyridine. Hydrogen bonding interactions that occur between 2-vinyl pyridine and pharmaceutical compounds as predicted by molecular dynamics simulation are presented in Fig. 2. The conformations of all templates and 2-vinyl pyridine were optimized prior to energy calculations. Then the binding energies, ΔE, for complexes that are formed when each template interacts with 2-vinyl pyridine were calculated using molecular dynamics. Eq. (6) was employed in all cases.

$$\Delta E = E_{(complex)} - E_{(template)} - E_{(2-vinyl\ pyridine)} \quad (6)$$

Binding energy was used to quantitatively measure the strength of the hydrogen bonding. Higher ΔE is an indicative of high binding strength. The obtained energies presented in Table 2 confirmed that the hydrogen bonding interaction that exist between H_{24} of diclofenac and N_3 of 2-vinyl pyridine is weaker than that of H_{30} for diclofenac and N_3 for 2-vinyl pyridine. This phenomenon was further confirmed by using the bond distances, where it was discovered that O-H_{24}…..N_3 bond gave a distance of 1.937 Angstrom whereas in O-H_{30}…..N_3 bond a shorter distance of 1.764 Angstrom (Å) was obtained. It was observed that one of the aromatic rings of diclofenac hinders the hydrogen bonding interaction, and this leads to poor O-H_{24}…..N_3 interaction. The bond distances obtained for ibuprofen and naproxen complexes were 1.657 and 1.691 Å, respectively. These distances are in good agreement with the results given in Table 2.

3.2. Effect of a porogenic solvent in monomer-template interactions

The effect of a porogenic solvent on the interactions between 2-vinyl pyridine and all three acidic pharmaceuticals were computationally investigated. Porogenic solvents investigated in this study are toluene, chloroform, acetonitrile and acetone and these are common organic solvents that are normally used during the synthesis of MIPs [29,30]. The effect of a porogenic solvent was carried out by performing the solvent energy calculations, $\Delta E_{solvent}$ (kcal/mol), using Eq. (7).

$$\Delta E_{solvent} = \Delta E_{(solution)} - \Delta E_{(vacuum)} \quad (7)$$

where E(solution) and E(vacuum) are interaction energies (kcal/mol) in the liquid phase and in gas phase, respectively. Results tabulated in Table 3 (a) – (c) indicates that toluene is unlikely to compete for hydrogen bond formation with all the templates and the functional monomer. These results also demonstrate that high polarity porogenic solvents are likely to disturb the monomer-template interactions. Toluene was therefore selected as the most suitable medium for the polymerization. The interactions that take place between 2-vinyl pyridine and template molecules in the presence of toluene were investigated and the results are given in Figs. 3 and 4. Based on the results presented in Fig. 4, it was observed that the volume of toluene used during the synthesis does not affect the quality of monomer-template interactions. These results clearly show that toluene is not binding to the template molecules, nor to the functional monomer.

3.3. Characterization

3.3.1. FT-IR analysis

The FT-IR spectra for unwashed MIP, washed MIP, washed NIP, naproxen and 2-vinyl pyridine is given in Fig. 5. For spectral clarity, it was decided to omit diclofenac and ibuprofen spectra from Fig. 5. Results obtained show that the OH group of naproxen has disappeared

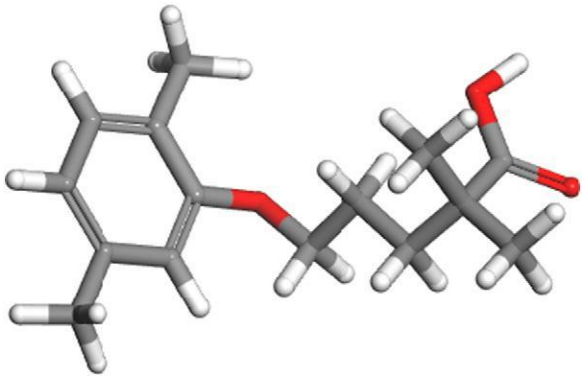


Fig. 8. Chemical structure of gemfibrozil.

in the unwashed MIP, this is caused by its involvement in the hydrogen bonding that leads to the formation of NH group in the MIP. A distinct peak at 1715 cm^{-1} implies the presence of carbonyl groups that originate from the template and cross-linking molecules.

From the spectra in Fig. 5(b), it can be noted that NIP and MIP contains similar functional groups, the shape and position of the bands are identical. This is an indication of the MIP having a similar backbone structure as the NIP and the templates are probably completely removed from the MIP [31]. It was observed that the transmittance of most signals was stronger in the MIP than in the NIP. The strong transmittance of the peak at 3449 cm^{-1} for the MIP in Fig. 5(b) is due to the fact that OH formed hydrogen bond that overlapped with the NH band from the MIP [31]. This peak is slightly flattened in the NIP as it was synthesized without the use of any templates.

3.3.2. Surface characterization

Adsorption of compounds is strongly influenced by the surface area of polymers. According to Brunauer, Emmett and Teller (BET) results presented in Table 4, it was noted that larger surface area, pore volume and pore diameter were obtained for the MIP than the NIP. The same trend has been reported in other study [23]. The imprinting effect for the MIP is attributed to the more binding sites that are distributed in the cavity. Surface areas obtained in this study are higher than those reported in literature by Sanagi et al. [32] for a MIP that was synthesized for organophosphorus pesticides analysis in fruit samples. A greater total pore volume is an indicative of the MIP having a superior sample load capacity as compared to the NIP [23]. Pore diameters obtained for both MIP and NIP are in the range of 2-50 nm, hence the structure for both polymers is mesoporous [33].

3.3.3. Elemental analysis and polymer morphology

Elemental analysis was performed using CHN analyzer to determine the percentage of carbon (C), hydrogen (H) and nitrogen (N) element in each polymer after templates removal. The percentages of C, H and N obtained in MIP were 55.9 %, 6.6 % and 0.5 %, respectively. For NIP, the results were 60.2 %, 6.8 % and 0.7 % for C, H and N, respectively. These results show that the percentages of H and N elements were identical, however a noticeable difference was observed for C element as a result

Table 5
MIP adsorption capacity (mg/g) for each compound in different solvents.

Compound	Toluene	Chloroform	Acetone	Acetonitrile
Naproxen	14.4	11.6	11.8	11.1
Ibuprofen	11.0	9.86	9.00	9.68
Diclofenac	14.0	13.8	14.0	13.8
Gemfibrozil	7.46	7.42	7.74	6.92

of template removal procedure. The sources of nitrogen in both polymers were the cyanide group that was present in the initiator and the functional monomer used in the synthesis.

The morphology of both washed MIP and NIP was studied using scanning electron microscopy. Results presented in Fig. 6 clearly show the differences in the surfaces of the MIP and NIP. It was observed that

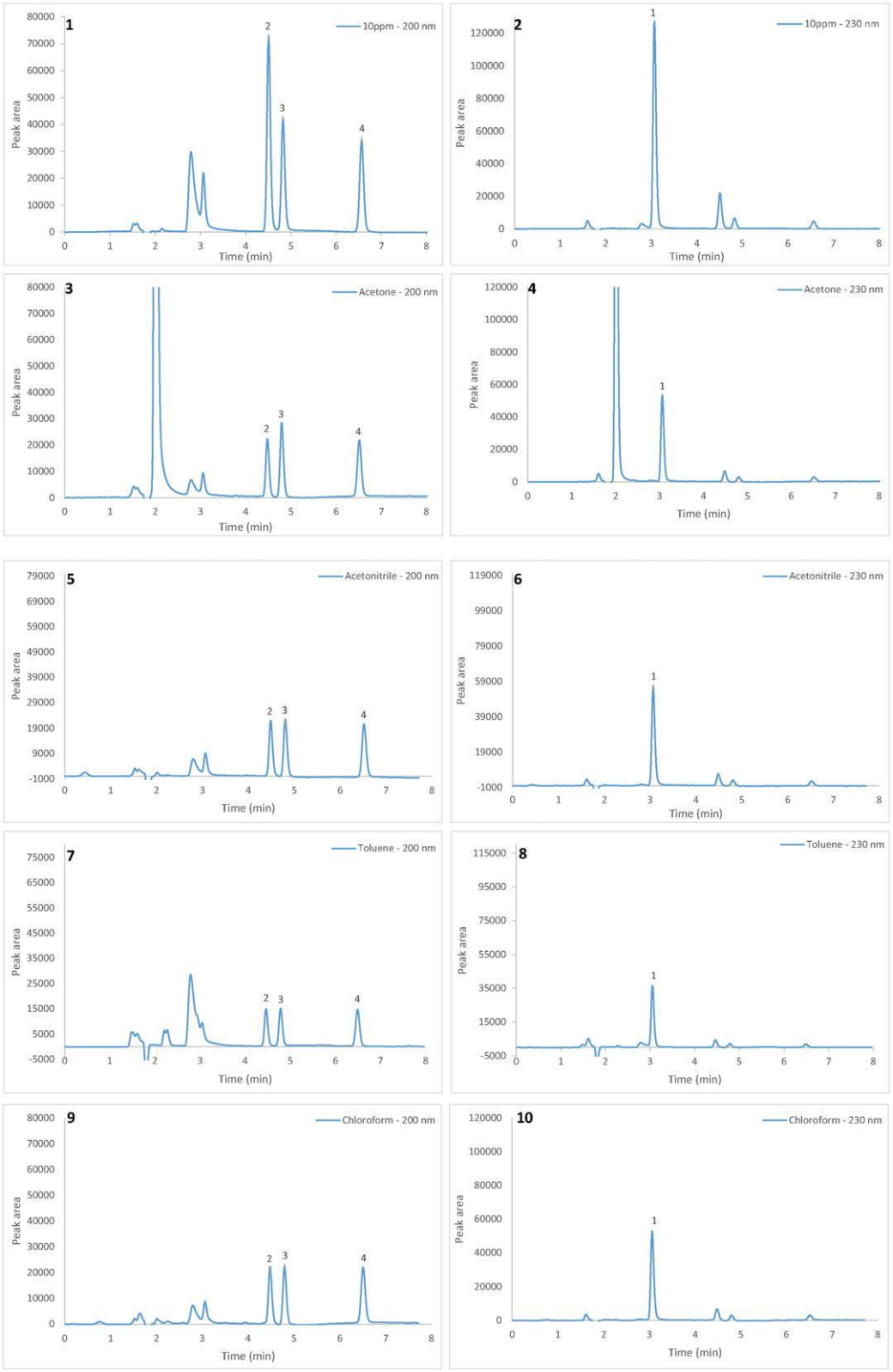


Fig. 9. Chromatograms recorded before incubation at 200 and 230 nm for 10 mg/L acidic pharmaceuticals (1 and 2), after incubation in acetone (3 and 4), after incubation in acetonitrile (5 and 6), after incubation in toluene (7 and 8) and after incubation in chloroform (9 and 10). Peaks are labelled as follows; 1 – naproxen, 2 - diclofenac, 3 – ibuprofen and 4 – gemfibrozil.

Table 6
Imprinting effect parameters on NIP.

Solvent	K_d (L/g)				K
	Naproxen	Ibuprofen	Diclofenac	Gemfibrozil	
Toluene	3.87	2.33	3.03	2.88	1.34
Chloroform	1.16	1.12	1.68	0.93	1.25
Acetone	2.66	1.64	4.43	1.38	1.92
Acetonitrile	2.42	1.38	4.04	1.06	2.28

the structure of the MIP was more rough and irregular on the surface than the NIP.

3.4. Adsorption and selectivity studies

Adsorption experiments were carried out in order to examine the validity of results obtained from molecular dynamics simulation. The extraction efficiency of polymers was evaluated in high polarity (acetonitrile and acetone) and low polarity organic solvents (toluene and chloroform). The results presented in Fig. 7 indicate that the binding affinity of the synthesized MIP for all compounds increases in the order of acetonitrile <acetone <chloroform <toluene. Toluene gave the highest extraction efficiency for all compounds tested; this was expected as toluene was used as a porogenic solvent in this study. High polarity solvents yielded poor extraction efficiencies, this is probable caused by the competition of solvents with target compounds for hydrogen bonding onto the MIP cavities except for diclofenac. High extraction efficiency for diclofenac was observed as this template have two protons that are available for hydrogen bonding and was thus less influenced by the porogen solvent. Extraction efficiency for naproxen was better compared to the one of ibuprofen, this was expected as naproxen-2-vinyl pyridine complex gave a higher binding energy as seen in Table 2. The results in Fig. 7 also indicate that even in a multi template molecularly imprinted polymers, template with higher interaction with the monomer will still dominant such interactions.

The selectivity of the MIP was investigated by performing the batch adsorption experiments in the presence of a gemfibrozil. Gemfibrozil's structural information is shown in Fig. 8. Gemfibrozil is an acidic cholesterol-lowering pharmaceutical compound that is effective in reducing serum cholesterol and triglyceride [34]. Gemfibrozil was selected as the competitor in the selectivity experiments as it contains similar functional groups to the templates and its carboxylic group was expected to form hydrogen bonding with 2-vinyl pyridine. HPLC separation of gemfibrozil from other compounds was achieved by using the conditions explained in Section 2.5 whereas the quantification was performed using a UV-visible detector that was set at 200 nm. The results that are presented in Fig. 7 and Table 5 show that the molecularly imprinted polymer was able to recognize all target compounds in all solvent conditions tested. Table 5 also shows that the MIP can distinguish the three target compounds from their competitor. Lower adsorption capacity (9 – 11 mg/g) was obtained for ibuprofen, this was probable caused by the fact that gemfibrozil's chemical structure resembles that of ibuprofen. This is possible caused by the variations of the three dimensional structures of target compounds and the competitor. The results that are presented in Fig. 9 further shows that target compounds were adsorbed in the presence of both high polarity and low polarity solvents. Fig. 9 also shows the separation of the three templates and the competitor.

Table 7
Imprinting effect parameters on MIP.

Solvent	K_d (L/g)				K	k'
	Naproxen	Ibuprofen	Diclofenac	Gemfibrozil		
Toluene	5.14	2.44	4.69	1.19	4.32	3.22
Chloroform	2.75	1.94	4.41	1.18	2.33	1.87
Acetone	2.90	1.70	4.62	1.26	2.30	1.20
Acetonitrile	2.50	1.88	4.49	1.26	1.99	0.87

Furthermore, parameters related to the selectivity performance for the polymers such as K_d, k and k' are given in Tables 6 and 7. K_d values for three target compounds in MIP were greater than the values for gemfibrozil in all tested solvents which reflected in selectivity coefficients that were greater than the value of 1. The adsorption of target compounds on NIP can be associated to non-specific interactions, however the selectivity of the NIP was lower than the MIP which resulted in poor selectivity coefficients [35]. High relative selectivity coefficient (k' of 3.22) was obtained in toluene, this indicated that the MIP selectivity was 3.22 times greater for target compounds in the presence of gemfibrozil when compared to the NIP. In this case, the selectivity became poor in high polarity solvents such as acetone and acetonitrile. From these results, it was observed that the multi-template MIP possesses good molecular recognition properties as it showed the capability to extract all three target compounds simultaneously, whereas single-template MIP can only extract one compound.

4. Conclusion

A multi-template molecularly imprinted polymer was synthesized and characterized. It was evident from FT-IR characterization that both MIP and NIP have a similar backbone structure. Higher surface area was obtained for the MIP and that translated to the MIP being the polymer that have higher adsorption capacity than the NIP. BET results further indicated that both MIP and NIP have mesoporous structures. SEM images showed that the surface of the MIP was rough and irregular when compared to the NIP. This study also demonstrated the importance of carefully selecting the porogenic solvent as it might affect the monomer-template interactions. Results obtained from molecular dynamics simulation calculations were further confirmed experimentally. High adsorption for diclofenac molecule was observed which is related to high binding energy that was obtained for diclofenac and 2-vinyl pyridine complex in molecular dynamics simulation. Higher adsorption capacity was obtained for the MIP when the porogenic solvent was used as the extraction medium. Based on the batch selectivity studies, MIP was able to selectively remove the target compounds from polar and non-polar organic solvents.

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References

13 J. Saloni, P. Lipkowski, S.S.R. Dasary, Y. Anjaneyulu, H. Yu, G. Hill Jr., Polymer 52 (2011) 1206–1216.
14 J. Haginaka, H. Sanbe, H. Takehira, J. Chromatogr. A 857 (1999) 117–125.
15 Z. Sun, W. Schussler, M. Sengl, R. Niessner, D. Knopp, Anal. Chim. Acta 620 (2008) 73–81.
16 S.A. Piletsky, K. Karim, E.V. Piletska, C.J. Day, K.W. Freebairn, C. Legge, A.P.F. Turner, Analyst 126 (2001) 1826–1830.
17 A. Ellwanger, P.K. Owens, L. Karlsson, S. Bayoudh, P. Cormack, D. Sherrington, B. Sellergren, J. Chromatogr. A 897 (2000) 317–327.
18 R.J. Ansell, J.K.L. Kuah, D. Wang, C.E. Jackson, K.D. Bartle, A.A. Clifford, J. Chromatogr. A 1264 (2012) 117–123.
19 Z. Guo, A. Florea, C. Cristea, F. Bessueille, F. Vocanson, F. Goutaland, A. Zhang, R. Sandulescu, F. Lagarde, N. Jaffrezic-Renault, Sensors Actuators B Chem. 207 (2015) 960–966.
20 B.B. Prasad, G. Rai, Spectrochim. Acta A Mol. Biomol. Spectrosc. 105 (2013) 400–411.
21 S. Dahane, M.D.G. Garcia, M.J. Martinez Bueno, A.U. Moreno, M.M. Galera, A. Derdour, J. Chromatogr. A 1297 (2013) 17–28.
22 J.L. Santos, I. Aparicio, E. Alonso, M. Callejon, Anal. Chim. Acta 550 (2005) 116–122.
23 N. Lindqvist, T. Tuhkanen, L. Kronberg, Water Res. 39 (2005) 2219–2228.
24 R. Amdany, L. Chimuka, E. Cukrowska, Water SA 40 (2014) 407–414.

[13] U. Jux, R.M. Baginski, H. Arnold, M. Kronke, P.N. Seng, Int. J. Hyg. Environ. Health 205 (2002) 393–398.
[14] E. Carmona, V. Andreu, Y. Pico, Sci. Total Environ. 484 (2014) 53–63.
[15] H.A. Panahi, A. Feizbakhsh, S. Khaledi, E. Moniri, Int. J. Pharm. 441 (2013) 776–780.
[16] A. Lagha, N. Adhoum, L. Monser, OCBMJ 4 (2011) 7–13.
[17] N. Gilart, R.M. Marce, N. Fontanals, F. Borrull, Talanta 110 (2013) 196–201.
[18] C. Dai, X. Zhou, Y. Zhang, S. Liu, J. Zhang, J. Hazard. Mater. 198 (2011) 175–181.
[19] C. Dai, J. Zhang, Y. Zhang, X. Zhou, Y. Duan, S. Liu, Chem. Eng. J. 211 (2012) 302–309.
[20] H. Yan, K.H. Row, Int. J. Mol. Sci. 7 (2006) 155–178.
[21] X. Shi, A. Wu, G. Qu, R. Li, D. Zhang, Biomaterials 28 (2007) 3741–3749.
[22] M.C. Cela-Perez, A. Lasagabaster-Latorre, M.J. Abad-Lopez, J.M. Lopez-Vilarino, M.V. Gonzalez-Rodriguez, Vib. Spectrosc. 65 (2013) 74–83.
[23] K. Farrington, F. Regan, Biosens. Bioelectron. 22 (2007) 1138–1146.
[24] A. Lasagabaster-Latorre, M.C. Cela-Perez, S. Fernandez-Fernandez, J.M. Lopez-Vilarino, M.V. Gonzalez-Rodriguez, M.J. Abad, L.F. Barral-Losada, Mater. Chem. Phys. 141 (2013) 461–476.
[25] Y. Duan, C. Dai, Y. Zhang, L. Chen, Anal. Chim. Acta 758 (2013) 93–100.
[26] Accelrys: Materials Studio is a Software Environment for Molecular Modeling (Version 7.0), Accelrys Inc., 2013
[27] R.N. Rao, P.K. Maurya, S. Khalid, Talanta 85 (2011) 950–957.
[28] W. Dong, M. Yan, M. Zhang, Z. Liu, Y. Li, Anal. Chim. Acta 542 (2005) 186–192.
[29] C. Dai, S. Geissen, Y. Zhang, Y. Zhang, X. Zhou, Environ. Pollut. 159 (2011) 1660–1666.
[30] J. Wang, Z. Xu, P. Wu, S. Yin, J. Membr. Sci. 331 (2009) 84–90.
[31] A.M. Chrzanowska, A. Poliwoda, P.P. Wieczorek, Mater. Sci. Eng. C 49 (2015) 793–798.
[32] Z. Wang, X. Cao, Process Biochem. 50 (2015) 1136–1145.
[33] M.M. Sanagi, S. Salleh, W.A.W. Ibrahim, A.A. Naim, D. Hermawan, M. Miskam, I. Hussain, H.Y. Aboul-Enein, J. Food Compos. Anal. 32 (2013) 155–161.
[34] B.A. Roadcap, D.G. Musson, J.D. Rogers, J.J. Zhao, J. Chromatogr. B 791 (2003) 161–170.
[35] K. de Mata, M.Z. Corazza, F.M. de Oliveira, A.L. de Toffoli, C.R.T. Tarley, A.B. Moreira, React. Funct. Polym. 83 (2014) 76–83.

3.3.4 Paper 3

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Synthesis, adsorption and selectivity studies of a polymer imprinted with naproxen, ibuprofen and diclofenac

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ABSTRACT

In this study, selective removal of acidic pharmaceutical from aqueous media was investigated. The purpose of this work was to use the multi template molecularly imprinted polymer (MIP) for the selective extraction of naproxen, ibuprofen and diclofenac from aqueous samples. A multi template MIP was synthesized using a bulk polymerization method. The performance of the MIP in aqueous solutions was evaluated by optimizing several adsorption parameters. The optimized adsorption conditions were 50 mg of MIP, extraction time of 10 min and a sample pH of 4.6. The imprinting factors obtained for naproxen, ibuprofen and diclofenac were 1.25, 1.42, and 2.01, respectively, which corresponded to the selectivity order of diclofenac > ibuprofen > naproxen. Furthermore, the synthesized MIP showed great selectivity to the target compounds in the presence of gemfibrozil and fenoprofen. The data was modelled best by pseudo 2nd order which implied a chemisorption of pharmaceuticals onto MIP particles. Based on R^2 values, it was determined that the adsorption data fitted Langmuir isotherm which meant that the binding occurred on the homogeneous sites. The recovery in wastewater influent for naproxen, ibuprofen and diclofenac was 38%, 69% and 87%, respectively.

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1. Introduction

Naproxen, ibuprofen and diclofenac are acidic pharmaceuticals that belong to the class of non-steroidal anti-inflammatory drugs (NSAIDs) [1]. NSAIDs are analgesics that are used to treat inflammation and fever in humans. NSAIDs can lead to side effects if overdosed [2]. The presence of pharmaceuticals in the environment is a result of direct disposal to aquatic systems and incomplete removal during wastewater treatment. Occurrence of such compounds in the environment have raised serious concerns for the scientific community and general public at large [3]. There are presently no regulatory standards for these pharmaceuticals although they are considered to have potential for adverse human and environmental effects with increased risk potential on exposure [3]. Wastewater treatment plants (WWTPs) receive high concentration of pharmaceuticals through human urinary, fecal excretion and from pharmaceutical manufacturing effluents [4]. WWTPs have been reported to be the major source of pharmaceuticals in the aquatic environment [5–8]. NSAIDs as shown in Table 1 are polar compounds that are soluble in water [9–12], therefore they escape the wastewater treatment process easily. Recently, more work on the determination of NSAIDs in aqueous systems have been reported [13–15]. NSAIDs have been detected globally in wastewater [13], surface water [14] and drinking water [15].

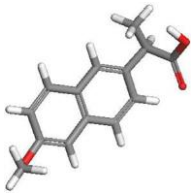
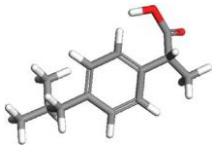
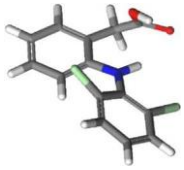
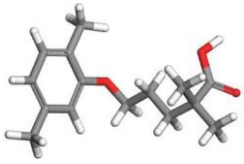
Quantification of NSAIDs is usually carried out with chromatographic techniques after their extraction from aqueous matrices with a suitable sample preparation method. Gas chromatographic analysis employs derivatization procedures for the conversion of NSAIDs into volatile forms [16]. Compounds selected in this study are not volatile, therefore they are best separated in a high performance liquid chromatographic column prior to their detection. Solid-phase extraction (SPE) and solid phase micro-extraction (SPME) are the established techniques that are widely used as sample clean-up steps prior to chromatographic separation [12–15]. Both techniques are based on adsorption of compounds onto packing materials. The adsorbents used in both SPE and SPME techniques provide limited selectivity towards target compound(s). Selectivity is improved in SPE when molecularly imprinted polymer (MIP) is employed as the adsorbent [17].

MIP is a stable synthetic polymer that contains highly specific sites having an affinity for a target molecule [17]. Over the years,

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Table 1
Chemical structures and physicochemical properties of acidic pharmaceuticals [9–12].

Compound	Chemical Structure	Water Solubility (mg L^{-1})	pK_a	Log K_{ow}
Naproxen		44	4.2	3.10
Ibuprofen		58	4.9	3.71
Diclofenac		10	4.2	4.02
Gemfibrozil		8.4	4.7	4.51
Fenoprofen		30	4.5	4.05

Hydrogen, carbon, oxygen, chlorine and nitrogen atoms are represented by white, grey, red, green and blue, respectively.

MIPs have been synthesized in the presence of one target molecule [18–20]. Different classes of pharmaceuticals are being detected in the environmental samples, hence MIPs are being designed for the removal or isolation and pre-concentration of such pollutants. Recent work in this aspect involves the development of MIPs for selective removal of antiviral and antidiabetic drugs in aqueous media [21,22]. Recent work also demonstrate the potential for MIPs in the removal of specific group of pharmaceuticals [21–23]. MIPs are useful adsorbents for acidic pharmaceuticals in water, hence their characterization such as thermal stability is highly required for their correct utilization. The removal of acidic pharmaceuticals from lake water using multi-template MIPs have been reported [23], however, the performance of such polymers in more complicated sample matrix such as wastewater influent and effluent have not been fully evaluated. This is important because although MIPs are selective to particular functional groups, they are not specific to certain molecule(s). The backbone polymer of molecularly imprinted sorbent can adsorb some compounds based on functional groups present especially for aqueous samples. Selective elution in this case is performed.

Owing to the recent studies that have reported the occurrence of pharmaceuticals in South African water bodies that includes wastewater and river water [3,6], there is a strong need to develop and evaluate the performance of selective adsorbents for accurate analysis using HPLC systems with simple detectors such as UV–vis. Besides the gain in selectivity for these materials, they can also be re-used. Smart adsorbents such as MIPs have been well developed and applied in the removal of pharmaceutical pollutants from water resources of well developed countries [17,20–23], however there are no reports on the selective removal of pharmaceuticals from South African aqueous matrix. Therefore, the aim of this work was to give a detailed report on the evaluation of the adsorption, selective washing and elution of naproxen, ibuprofen and diclofenac by MIP sorbent that was synthesized using a multi-template approach from wastewater.

2. Experimental

2.1. Chemicals

Naproxen (98%), ibuprofen (≥98%), diclofenac sodium salt, 2-vinylpyridine (97%), 1,1′-azobis-(cyclohexanecarbonitrile) (98%), ethylene glycol dimethacrylate (98%), HPLC grade methanol (≥99.9%) and toluene (99.7%) were purchased from Sigma-Aldrich

(Steinheim, Germany). HPLC-grade acetonitrile (≥99.9%) and glacial acetic acid (100%) were purchased from Merck (Darmstadt, Germany). Formic acid (approx. 98%) was purchased from Fluka (Steinheim, Germany). Sodium hydroxide pellets were purchased from Associated Chemical Enterprises (Johannesburg, South Africa).

2.2. Chromatographic separation

Separation was performed using liquid chromatography that consisted of an online mobile phase degasser (Model: DGU-20A3), 20 μL sample loop, pump (Model: LC-20AB), and UV/vis detector (Model: SPD-20A), purchased from Shimadzu Corporation (Kyoto, Japan). The mobile phase used consisted of a mixture of acetonitrile: 0.2% formic acid in water (60:40, v:v) at a flow rate of 0.8 mL min^{-1}. Separation was performed on a Kinetex C_{18} HPLC column of 150 4.6 mm 2.6 μm obtained from Phenominex (California, USA). Shimadzu LC solutions software was used for data collection and processing. UV/vis detector settings were 230 nm for naproxen, and 200 nm for ibuprofen and diclofenac.

2.3. Synthesis of polymers

Bulk polymerization of MIP was performed in two steps. In the first step, 20 mg of 1,1′-azobis-(cyclohexanecarbonitrile) was dissolved in toluene (50 mL), this was followed by the addition of ethylene glycol dimethacrylate (1.51 mL). The flask was purged with nitrogen for 10 min and sealed. Then, the reaction was allowed to take place with constant stirring in an oil bath set at 70 °C for 8 h. In the second step, naproxen (76.60 mg), ibuprofen (68.69 mg), diclofenac (106.04 mg) were dissolved in acetonitrile (25 mL), followed by the addition of 0.25 mL 2-vinylpyridine, 3.85 mL ethylene glycol dimethacrylate, 60 mg 1,1′-azobis-(cyclo-hexanecarbonitrile) and 25 mL of toluene. These contents were transferred to the product obtained in the first step of reaction. The mixture was purged with nitrogen for 10 min and sealed. The reaction was carried out in an oil bath set at 70 °C for 16 h. Resulting polymer was oven dried at 60 °C to constant mass. Polymer was then milled, sieved and particles ranging from 25 to 50 μm were collected. Non imprinted polymer (NIP) was prepared under the same conditions in the absence of template molecules. Templates were eluted repeatedly from the polymer using a mixture of 10% (v/v) acetic acid in acetonitrile until the target compounds were not detected by the HPLC system. Thereafter, 100% acetonitrile was applied in order to wash off the acetic acid residue.

2.4. Characterization of polymers

Thermogravimetric analysis (TGA) was performed using a TA instrument, model SDTQ600 (Delaware, Newcastle, USA). TGA curves were recorded at a heating rate of 10 °C min^{-1} from 30 °C to 700 °C under nitrogen purge of 50 mL min^{-1}.

2.5. Optimization of adsorption experiments

Batch adsorption experiments were carried out at room temperature using deionized water that was previously spiked with naproxen, ibuprofen and diclofenac. Various parameters such as sample pH (2.5–11), polymer amount (5–50 mg), initial concentration of target compounds (5–50 mg L^{-1}) and contact time (2–20 min) were optimized. Only one parameter was changed at a time during the optimization. For example, while varying the pH of the sample, the amount of the polymer, concentration of target compounds in spiked water and contact time were kept constant. All experiments were carried out in triplicate. Extraction efficiency and adsorption capacity were determined using Eqs. (1) and (2), respectively.

$$\text{Extraction efficiency (\%)} = \frac{(C_o - C_e)}{C_o} \times 100 \quad (1)$$

$$\text{Adsorption capacity (mg/g)} = \frac{(C_o - C_e)\,V}{W} \quad (2)$$

where C_o represent the initial concentration (mg L^{-1}) before the adsorption and C_e the final concentration (mg L^{-1}) of target compound remaining in solution after adsorption. V is the volume of the solution in liters and W represents the mass of the polymer in grams [24,25].

2.6. Swelling experiments

40 mg of the polymer and 10 mL of water were added into an empty 15 mL centrifuge tube. Swelling was allowed to occur at room temperature. The contents of the tube were then centrifuged at 4000 rpm. Excess solvent was discarded and the mass of the wet polymer was recorded. The swelling capacity was calculated using Eq. (3).

$$\text{Swelling capacity} = \frac{m_w - m_d}{m_d} \times 100 \quad (3)$$

where swelling capacity is expressed as% (m/m), m_w is the mass of the wet (swollen) polymer and m_d is the mass of the dry polymer [26].

2.7. Selectivity experiments

Batch adsorption experiments were carried out at room temperature using deionized water (pH 2.5) that was spiked with 50 μg L^{-1} mixture of naproxen, ibuprofen, diclofenac, gemfibrozil and fenoprofen. 10 mL of the spiked solution was transferred into a glass flask containing 50 mg of the polymer. The mixture was stirred at room temperature for 10 min and transferred into a 3 mL SPE tubes, where the liquid fraction was sent to waste. Frits were employed below and above the polymer to safeguard for sorbent loss. Solid material was rinsed with appropriate solvent. Thereafter, desorption of extracted compounds was performed with 2 mL mixture of acetonitrile: acetic acid (80:20, v:v) and quantified with HPLC.

2.8. Optimization of washing and elution steps

For these steps, spiked deionized water (10 mL, pH 2.5) containing 50 μg L^{-1} of naproxen, ibuprofen, diclofenac, gemfibrozil and fenoprofen was used as the sample. Adsorption was carried out using 50 mg of the polymer. The solution was stirred for 10 min, thereafter, the cartridge was packed with the solution and the liquid was sent to waste. Acetonitrile was investigated as the possible elution solvent with the addition of acetic acid (1–20%) in the elution solvent. For the optimization of the washing solvent, 20% (v:v) acetic acid in acetonitrile was employed as the elution solvent. Prior to elution, the cartridge was washed with water containing methanol (10–20%). Percentage recovery for each compound was determined in each case.

2.9. Extraction of naproxen, ibuprofen and diclofenac from contaminated water

Samples were collected from a local river and wastewater treatment plant located in Durban, South Africa. Sampling sites have been discussed in our previous work [27]. Samples were filtered through a 0.45 μm filter paper and spiked with 50 μg L^{-1} of

each compound. Sample pH was adjusted to 2.5, then 50 mg of the polymer was added to 10 mL of each sample and stirred for 10 min. Samples were transferred into 3 mL SPE tubes, where the liquid fraction was sent to waste. Frits were employed below and above the polymer to safeguard for sorbent loss. Solid material was rinsed with 2 mL of 10% (v:v) methanol in water. Thereafter, desorption of extracted compounds was performed with 2 mL mixture of acetonitrile: acetic acid (80:20, v:v) and quantified with HPLC.

3. Results and discussions

3.1. Synthesis and characterization

Due to the widespread of naproxen, ibuprofen and diclofenac in environmental samples; MIP that is able to adsorb such compounds from water was synthesized. The choice of reagents for synthesis and more details on characterization that includes Fourier-transform infrared spectroscopy, scanning electron mi-croscopy, elemental analysis and Brunauer, Emmett and Teller method have been discussed in our previous work [28].

Thermogravimetric analysis for washed and unwashed MIP, washed NIP and naproxen is presented in Fig. S1 (Support information). 5% of thermal decomposition caused by the degradation of templates on the surface of the unwashed MIP was observed at 155 °C. This was demonstrated when naproxen showed 90% decomposition in a temperature range of 170–285 °C. A weight loss at 250 °C was observed in all polymers, which marks this temperature as the point where the polymer backbone collapses. The only observed difference in the curves obtained for washed NIP and washed MIP, is on 100% thermal decomposition of the MIP at 455 °C while the decomposition for NIP was 97%. The difference may be due to structural variations that might be occurred during templates removal. These results are in agreement with those reported elsewhere [29], where the mass loss was observed at 250 °C for a MIP that was synthesized for the removal of 1,3-diisopropylurea from active pharmaceutical ingredient.

3.2. Adsorption experiments

3.2.1. Effect of sample pH

The effect of sample pH is important in order to achieve a maximum extraction of target compounds. The sample pH was varied over the pH range of 2.5–11 while all other experimental conditions were kept constant. The constant conditions were polymer amount (50 mg), concentration of target compounds (20 mg L^{-1}), sample volume (10 mL) and contact time (10 min). The occurrence of suitable interactions between the adsorbent and target molecules in aqueous media is dependent on the medium's pH [30]. Based on the results (Fig. 1a and Fig. S2), it was observed that the extraction efficiency for all the target compounds was greatly affected by the sample pH. High extraction efficiency (>70%) was achieved when target compounds were protonated. When the sample pH was increased to basic, deprotonation of target molecules occurred that resulted in poor extraction efficiency since hydrogen bonding is the main cause of adsorption. High extraction efficiency for diclofenac (99%) at pH 4.6 was obtained for the MIP while 85 and 89% were obtained for naproxen and ibuprofen, respectively. This was possible due to the fact that diclofenac has got low polarity (log K_{OW} = 4.02) as well as lower water solubility (10 mg L^{-1}) when compared to naproxen and ibuprofen, hence it diffuses easily from water. Therefore, pH 4.6 was selected for subsequent adsorption experiments.

3.2.2. Effect of polymer amount

Amount of polymer used for the batch adsorption was varied from 5 to 50 mg while conditions such as sample pH (4.6),

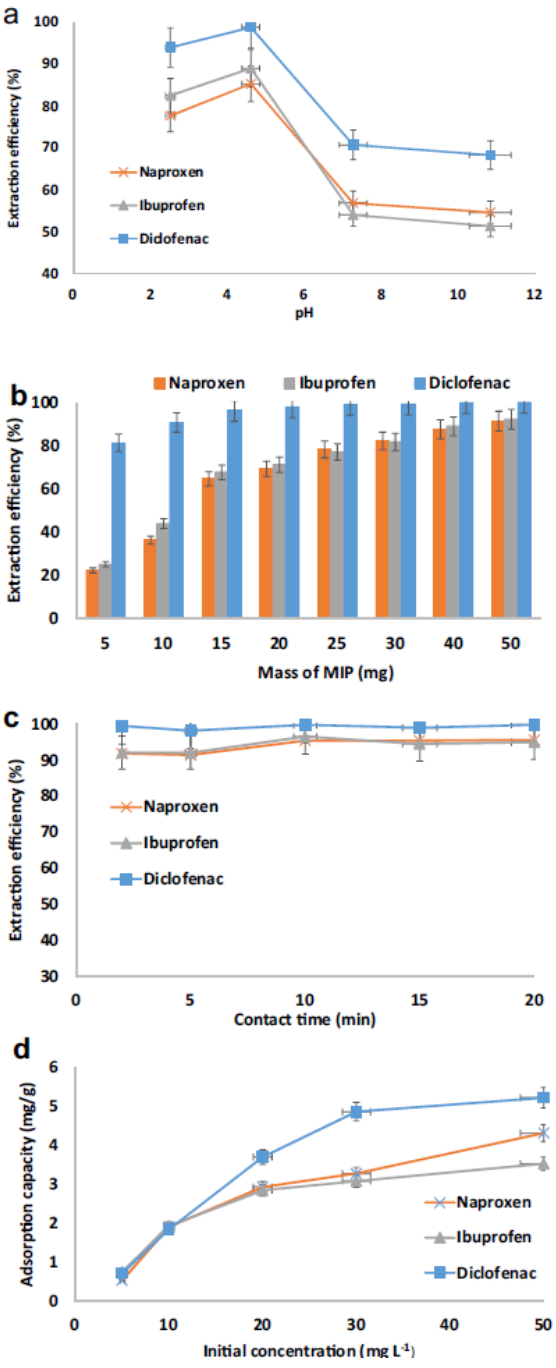


Fig. 1. a. Effect of sample pH on the extraction efficiency of naproxen, ibuprofen and diclofenac using MIP adsorbent. b. Effect of polymer amount on the extraction efficiency of selected NSAIDs using MIP adsorbent. c. Effect of contact time on the extraction efficiency of naproxen, ibuprofen and diclofenac using MIP adsorbent. d. Effect of initial concentration on the adsorption capacity of naproxen, ibuprofen and diclofenac using MIP adsorbent.

concentration of target compounds (20 mg L^{-1}), sample volume (10 mL) and contact time (10 min) were constant. Results (Fig. 1b) indicated that when 50 mg of the MIP was employed, >91% of naproxen and ibuprofen were extracted, and 100% extraction

efficiency was achieved for diclofenac. Higher extraction efficiencies were obtained for the MIP (Fig. 1b) than the NIP (Fig. S3); due to the imprinting effect. Therefore, subsequent adsorption experiments were further carried out using 50 mg of the polymer.

3.2.3. Effect of contact time

The effect of contact time was investigated by determining the adsorption capacity as a function of time. While varying contact time, sample pH (4.6), initial concentration (20 mg L^{-1}) for all compounds, adsorbent mass (50 mg) and sample volume (10 mL) were kept constant. Results in Fig. 1c show that the maximum adsorption was achieved in 2 min, where the extraction efficiency was greater than 90% for all compounds. In order to ensure the maximum uptake of target compounds from aqueous samples, the contact time of 10 min was employed in subsequent experiments. The adsorption capacity obtained for MIP ranged from 1.8 to 2 mg g^{-1}. For NIP, the adsorption capacity for ibuprofen increased slowly from 0.76 to 1.33 mg g^{-1} over 20 min, whereas fast adsorption occurred for naproxen and diclofenac into NIP surface (Fig. S4).

3.2.4. Effect of initial concentration

Results obtained for the adsorption capacity as a function of initial concentration are depicted in Fig. 1d and Fig. S5. During the optimization of initial concentration, the pH of the solution (4.6), polymer amount (50 mg), contact time (10 min) and sample volume (10 mL) were kept constant. The adsorption capacity increased almost linearly as a function of initial concentration, this trend was observed until 30 mg L^{-1}, thereafter the curves slightly flattened towards reaching the equilibrium. The adsorption capacity of 4.32 mg g^{-1} for naproxen at the initial concentration of 50 mg L^{-1} was achieved in this study, whereas Panahi et al. [31] obtained the sorption capacity of 3.26 mg g^{-1} in their study. In these experiments, the concentration was not increased beyond 50 mg L^{-1} as these compounds are expected to be present in water samples at μg L^{-1} levels. In all cases the amount of each compound adsorbed on the NIP was lower than the compounds adsorbed on the MIP; this trend is similar to the literature data [31]. This was caused by the presence of cavities in the MIP which were formed during the templates removal.

3.3. Binding sites characterization

3.3.1. Kinetic modeling

Adsorption process was described by employing Eqs. (4) and (5) for pseudo-first-order and pseudo-second-order kinetic models, respectively.

$$log(Q_e - Q_t) = log\ Q_e - \frac{k_1 t}{2.303} \quad (4)$$

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{1}{Q_e} t \quad (5)$$

where Q_t is the adsorption capacity at any time (mg g^{-1}), Q_e is the adsorption capacity at equilibrium (mg g^{-1}), t is the contact time (min), k_1 and k_2 are pseudo-first-order (min^{-1}) and pseudo-second-order sorption rate constants (g mg^{-1} min^{-1}), respectively [32,33]. The kinetic data is presented in Table 2. Based on the correlation coefficients (R^2) for polymers, pseudo-second-order model was the best fit, which indicates that the adsorption is of chemical nature [34–36]. The adsorption capacities obtained for MIP when employing pseudo-second-order model were 1.93, 2.01 and 2.00 mg g^{-1} for naproxen, ibuprofen and diclofenac, respectively. Whereas, the maximum adsorption capacities obtained from the batch adsorption experiments were 1.91, 1.93 and 2.00 mg g^{-1} for naproxen, ibuprofen and diclofenac, respectively. It has been proposed when the data fits the pseudo-second-order kinetic model, that the target molecule binds to two or more active sites at the adsorbent surface with different binding energies. Moreover, the occupation rate of the adsorption sites is propor-tional to the square of the number of unoccupied sites [34].

Table 2
Calculated parameters for the kinetic models.

Polymer	Compound	Pseudo-first-order	Pseudo-second-order		
		R^2	R^2	k_2 (g mg^{-1} min^{-1})	Q_e (mg g^{-1})
MIP	Naproxen	0.8154	0.9999	3.23	1.93
	Ibuprofen	0.9766	1	11.9	2.01
	Diclofenac	0.102	0.9999	12.1	2.00
NIP	Naproxen	0.943	0.9991	2.86	1.93
	Ibuprofen	0.8947	0.9837	0.073	1.85
	Diclofenac	0.7541	0.9998	3.59	1.99

3.3.2. Adsorption isotherms

The isothermal analysis of the polymers was employed using Eqs. (6) and (7) for the linearized forms of Freundlich and Langmuir isotherms, respectively.

$$\log Q = m \log C_e + \log a \quad (6)$$

$$\frac{C_e}{Q} = \frac{C_e}{Q_{max}} + \frac{1}{Q_{max} K_L} \quad (7)$$

Where Q is the amount of the adsorbed molecule at equilibrium (mg g^{-1}), m is the adsorption intensity or surface heterogeneity, C_e is the equilibrium concentration of the target molecule (mg L^{-1}), a is the adsorption capacity of target molecule (mg g^{-1}), Q_{max} is the maximum adsorption capacity (mg g^{-1}) and K_L is Langmuir adsorption equilibrium constant [35]. Based on the correlation coefficients obtained, the data fitted well with Langmuir isotherm which indicates the homogeneous nature of binding sites [32]. The constants, K_L and Q_{max}, were determined by using the intercepts and slopes of the linear plots of C_e/Q versus C_e and the results are given in Table 3.

Table 3 shows that the maximum adsorption capacities obtained for the MIP were higher than those for NIP; this was expected since MIP contains more binding sites that the NIP. Furthermore, the selectivity of the MIP for the target compounds was evaluated using the imprinting factors. Imprinting factors (α) were calculated from Eq. (8), after determining the partition coefficients (K_D) from Eq. (9).

$$\alpha = \frac{K_D(MIP)}{K_D\,(NIP)} \quad (8)$$

Table 3
Data extracted from the adsorption isotherms.

Polymer	Compound	Langmuir			Freundlich
		R^2	K_L (L mg^{-1})	Q_{max} (mg g^{-1})	R^2
MIP	Naproxen	0.9742	0.428	4.474	0.9696
	Ibuprofen	0.9953	0.846	3.598	0.9955
	Diclofenac	0.9999	1.060	5.453	0.7347
NIP	Naproxen	0.9736	0.270	3.885	0.9657
	Ibuprofen	0.9987	0.790	3.134	0.715
	Diclofenac	0.9995	2.141	4.819	0.7178

$$K_D = \frac{C_p}{C_s} \quad (9)$$

where C_p and C_s are the concentration of target compounds (mg L^{-1}) in polymer and in solution, respectively, at adsorption equilibrium [37]. From the results presented in Table 4, it can be seen from the K_D values that the selectivity of the polymers followed the order of diclofenac > ibuprofen > naproxen. The α value obtained for diclofenac was almost 2-fold of the α value for naproxen and ibuprofen; this might be caused by the strength of monomer-template interactions. It is possible that diclofenac uses two protons, one in the carboxylic group and the other in the amine group, for the formation of hydrogen bonding with 2-vinyl pyridine.

3.4. Swelling studies

The swelling capacity (W) of the polymers was investigated over a one hour period. Fig. 2a shows the rapid swelling of the polymers within 10 min and then the swelling rate decreased when approaching the equilibrium. The swelling is possible caused by the water absorption by the N group of 2-vinyl pyridine used in polymerization. The second-order swelling kinetics was investigated using Eq. (10) as follows:

$$\frac{t}{W} = \frac{1}{K_{is}} + \frac{t}{W_e} \quad (10)$$

where K_{is} and W_e are representing the initial swelling rate (g/ (g min)) and the equilibrium water absorbency (g/g), respectively. The swelling constants were determined by using Fig. 2b. The initial swelling rates (K_{is}) obtained were 2.62 and 5.84 g/(g min) for MIP and NIP, respectively. And the equilibrium water absorbencies (W_e) obtained for MIP and NIP were 11.5 and 18.5 g/g, respectively. These results indicate that water enters the network of the polymer which decreases the osmotic pressure difference between the solution and the polymer, which in turn retards the diffusion rate of water [38]. Additionally, the swelling of the MIP allows the water to diffuse into cavities and results in greater contact with the target compounds. Results in Fig. S6 indicate that the pH of water does not really influence the swelling.

3.5. Selectivity studies

The selectivity of the MIP collapsed in aqueous phase as high extraction efficiencies were obtained for gemfibrozil and feno-profen at pH 2.5. pH was lowered from 4.6 to 2.5 in order to ensure the complete protonation of competitors as well. Gemfibrozil and fenoprofen are used for medical purposes as lipid regulator and as antiphlogistic, respectively [39]. Both competitors contain the carboxylic group in their molecular structures (Table 1). Hydrogen atom of the carboxylic group in competitors is expected to bond with the nitrogen atom of 2-vinylpyridine. Hence, the extraction efficiencies obtained for gemfibrozil and fenoprofen were 75% and 66%, respectively. Also, both gemfibrozil and fenoprofen were easily desorbed from the MIP (Fig. 3(a)), which indicate that both compounds were non-selectively bound to the polymer. The

Table 4
Imprinting factors for the polymers.

Template	K_D (MIP)	K_D (NIP)	A
Naproxen	3,75	3,01	1,25
Ibuprofen	4,60	3,24	1,42
Diclofenac	40,47	20,12	2,01

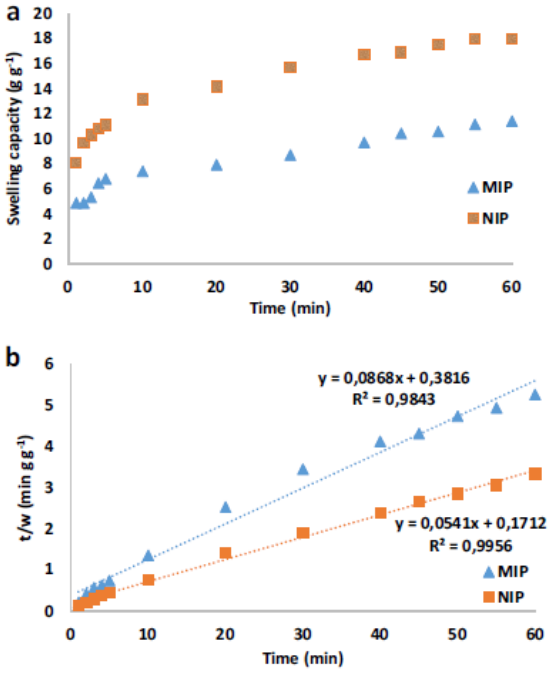


Fig. 2. Swelling capacity as a function of time (a), and second-order kinetic swelling for the polymers (b).

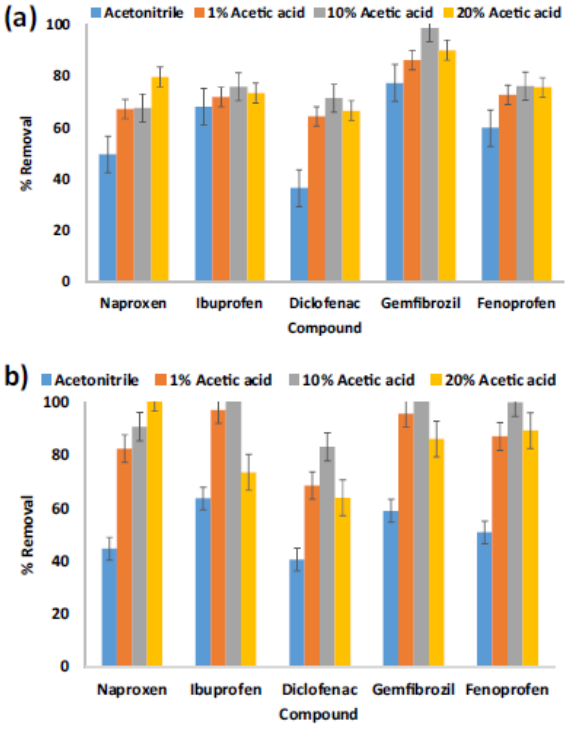


Fig. 3. Desorption of the compounds adsorbed into MIP (a) and NIP (b) by acetic acid in acetonitrile.

percent removal of compounds shown in Fig. 3 was calculated based on the adsorbed amount of each compound. Fig. 3(a) further shows that naproxen, ibuprofen and diclofenac were tightly bound on the surface of the MIP, and hence a strong solvent was required

for their elution. Acetic acid was added into the elution solvent as it has the ability to disrupt the hydrogen bonds that occur between the template molecules and 2-vinylpyridine. Desorption of compounds from the NIP (Fig. 3(b)) did not require a strong solvent as the compounds were adsorbed through non-specific interactions. Mahkam and Poorgholy [40] observed that the pharmaceutical drug release from the NIP was faster when compared to the MIP.

Introduction of methanol in the washing solvent prior to desorption of trapped compounds resulted in the reduction of gemfibrozil and fenoprofen from the MIP surface (Fig. 4(a)). 10% (v:v) methanol in water was selected as the selective washing solvent as higher methanol concentrations tend to desorb the target compounds. A recovery of 48% for naproxen was obtained after washing the MIP with 10% methanol, whereas recoveries obtained for gemfibrozil and fenoprofen were 42% and 34%, respectively. This could be the result of structural similarities and presence of similar functional groups in naproxen, gemfibrozil and fenoprofen. Percent recoveries were reduced significantly during the washing of the NIP (Fig. 4(b)) as the NIP possesses neither cavities nor the recognition sites [40]. In another study [41], the use of 30% (v:v) acetonitrile in water as the washing solvent resulted in a decrease of target compound from the NIP, while the recovery remained unchanged for the MIP. In this study, high portions of methanol (>10%) in the washing solvent resulted in poor recovery for target compounds. Sanagi et al. [41] observed a decrease in the quinalphos recovery when they used >40% acetonitrile in the washing solvent due to the disruption of specific interactions between the target compounds and binding sites.

Overall, this work resulted in pre-concentration factor of 5 for target compounds which could result in the improvement of detection and quantification limits. Hence, the detection limits as defined by the signal to noise ratio of 3 improved to 1.5, 7.5 and 4.7 μg L^{-1} for naproxen, ibuprofen and diclofenac, respectively. Pre-concentration factor is based on the sample volume (10 mL) extracted and volume of extract (2 mL), therefore it can be increased by the extraction of large sample volumes. Another advantage of the proposed work was evident when the synthesized

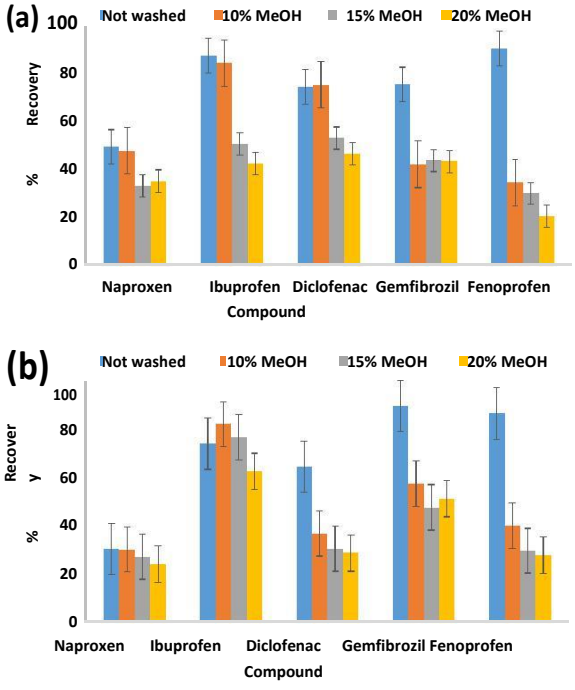


Fig. 4. Selective washing of the MIP (a) and NIP (b).

MIP was recycled by washing with 20% (v:v) acetic acid in acetonitrile, followed by acetonitrile alone. The MIP was re-used in the adsorption of target compounds from spiked de-ionized water at least 5 times without reducing the extraction efficiencies (Fig. S7).

3.6. Removal of selected acidic pharmaceuticals from contaminated water

After successful optimization of adsorption and desorption experiments, spiked river water and wastewater samples were subjected to the optimized conditions. Samples were spiked with 50 μg L^{-1} as target compounds have been detected in the influent and effluent at low μg L^{-1} levels [6,42]. Chromatograms (Fig. 5(a) and (b)) obtained for the compounds extracted using MIP were cleaner when compared to those for the NIP (Fig. 5(c) and (d)). Some degree of selectivity was evident as the NIP chromatograms showed high degree of unwanted peaks due to interfering species. MIP was not really affected by matrix effects as less noise was observed compared to the NIP, hence this is an indication of the MIP being more selective than the NIP. Furthermore, higher recoveries for target compounds were obtained when using the MIP rather than the NIP (Fig. 6). This is a result of strong interactions between the MIP and target compounds. Regardless of the lack of available data on removal of group of pharmaceuticals from difficult matrix such as wastewater influent, the current work was compared to multi-template MIPs used in analytical applications like solid-phase extraction [32,43,44]. For example, in the study reported by Manzo et al. [32] where they used a MIP as the sorptive phase immobilized in a rotating disc extraction device, a recovery of 50% was obtained for diclofenac; whereas in the current study the recovery of diclofenac ranged from 64 to 86%. In a different case, Gilart et al. [43] used a commercial MIP as an SPE sorbent for the determination of acidic pharmaceuticals in environmental waters. In their study [43], the recovery for diclofenac was 56, 62, and 88% for ultrapure water, wastewater effluent, and influent, respectively, whereas higher recoveries were obtained for other acidic pharmaceuticals. It is of interest to note that multi-template MIP can extract a wide range of compounds efficiently. For instance, Farrington and Regan [18] reported a recovery of 83% for ibuprofen when using an ibuprofen MIP, whereas poor recoveries (<35%) were obtained for naproxen and ketoprofen. In this work, ibuprofen percent recovery ranged from 57 to 69%. Recoveries for acidic pharmaceuticals obtained in a study reported by Duan et al. [44] for a multi-template MIP that was used as SPE sorbent were greater than 95% in lake water and wastewater effluent, however wastewater influent was not included in their study which is known as the complicated matrix. Besides the application in SPE, this study have also demonstrated that multi-template MIPs can be applied in the removal of pharmaceuticals in highly contaminated water like influent.

4. Conclusion

A multi-template molecularly imprinted polymer with homogeneous binding sites was successfully synthesized by a simple bulk polymerization method. The extraction efficiencies achieved for naproxen, ibuprofen and diclofenac when using molecularly imprinted polymer were higher than those obtained when non imprinted polymer was used as the adsorbent. The MIP synthesized demonstrated a high selectivity to diclofenac rather that naproxen and ibuprofen. The adsorption kinetics was best fitted with pseudo-second-order, indicating that chemisorption took place and the target molecules bind to two or more active sites at the surface of the polymer. The swelling of the prepared MIP allowed the diffusion of water with polar compounds into the

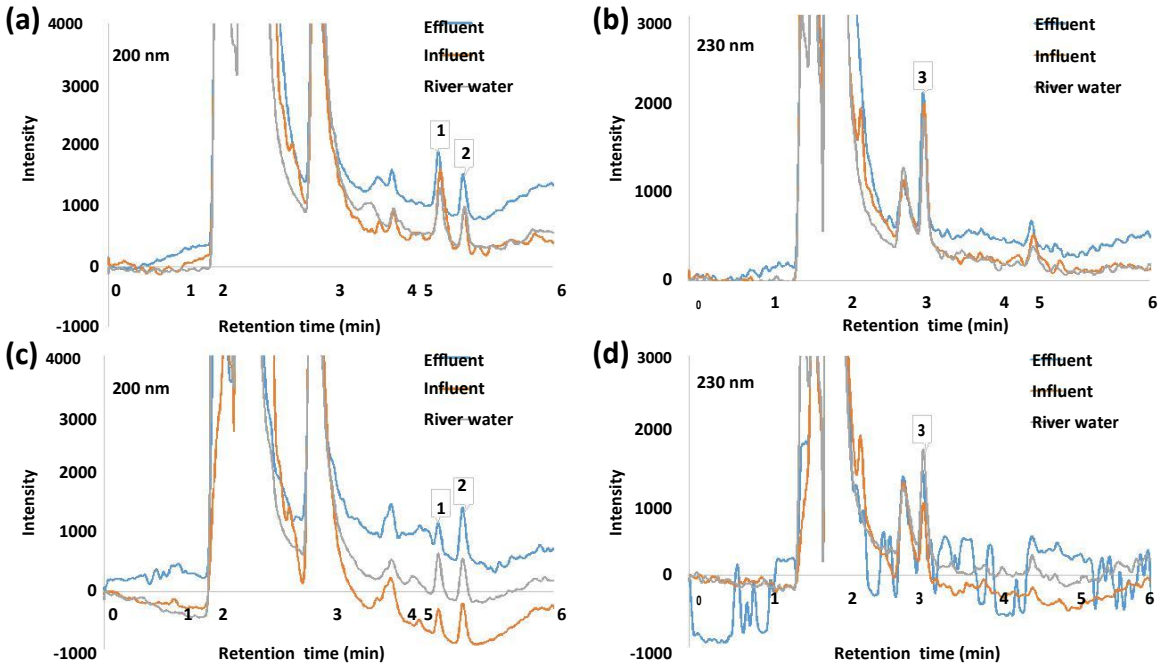


Fig. 5. Chromatograms recorded at 200 nm and 230 nm showing the desorbed compounds. Chromatograms shown in (a) and (b) represent MIP whereas (c) and (d) represent NIP. Compounds elution order was diclofenac (1), ibuprofen (2) and naproxen (3).

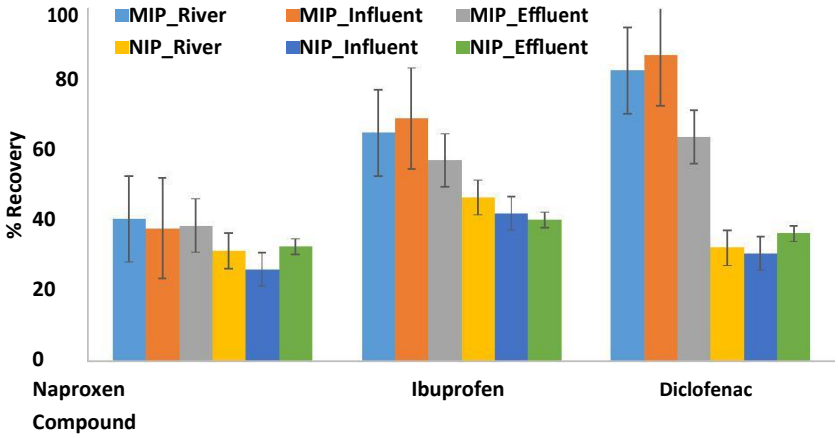


Fig. 6. Recovery of target compounds obtained after extraction of river water, wastewater influent and effluent samples with MIP and NIP.

cavities. Results further demonstrated that the MIP has a potential to remove naproxen, ibuprofen and diclofenac from wastewater treatment plants and river water.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jece.2016.09.012.

References

[1] N. Larsson, E. Petersson, M. Rylander, J.A. Jonsson, Continuous flow hollow fiber liquid-phase microextraction and monitoring of NSAID pharmaceuticals in a sewage treatment plant effluent, Anal. Methods 1 (2009) 59–67.
[2] A. Lagha, N. Adhoum, L. Monser, A molecularly imprinted polymer for the selective solid-phase extraction of ibuprofen from urine samples, TOCBM J. 4 (2011) 7–13.
[3] F.O. Agunbiade, B. Moodley, Pharmaceuticals as emerging organic contaminants in Umgeni River water system KwaZulu-Natal, South Africa, Environ. Monit. Assess. 186 (2014) 7273–7291.
[4] M. Farre, I. Ferrer, A. Ginebreda, M. Figueras, L. Olivella, L. Tirapu, M. Vilanova, D. Barcelo, Determination of drugs in surface water and wastewater samples by liquid chromatography-mass spectrometry: methods and preliminary results including toxicity studies with Vibrio fischeri, J. Chromatogr. A 938 (2001) 187–197.
[5] L. Patrolecco, N. Ademollo, P. Grenni, A. Tolomei, A.B. Caracciolo, S. Capri, Simultaneous determination of human pharmaceuticals in water samples by solid phase extraction and HPLC with UV-fluorescence detection, Microchem. J. 107 (2013) 165–171.
[6] R. Amdany, L. Chimuka, E. Cukrowska, Determination of naproxen, ibuprofen and triclosan in wastewater using the polar organic chemical integrative

sampler (POCIS): a laboratory calibration and field application, Water SA 40 (2014) 407–414.
[7] A.R.M. Silva, F.C.M. Portugal, J.M.F. Nogueira, Advances in stir bar sorptive extraction for the determination of acidic pharmaceuticals in environmental water matrices: comparison between polyurethane and polydimethylsiloxane polymeric phases, J. Chromatogr. A 1209 (2008) 10–16.
[8] G. Ying, A.S. Kookana, D.W. Kolpin, Occurrence and removal of pharmaceutically active compounds in sewage treatment plants with different technologies, J. Environ. Monit. 11 (2009) 1498–1505.
[9] S. Bayen, H. Zhang, M.M. Desai, S.K. Ooi, B.C. Kelly, Occurrence and distribution of pharmaceutically active and endocrine disrupting compounds in Singapore's marine environment: influence of hydrodynamics and physical-chemical properties, Environ. Pollut. 182 (2013) 1–8.
[10] A. Kot-Wasik, J. Debska, A. Wasik, J. Namiesnik, Determination of non-steroidal anti-inflammatory drugs in natural waters using off-line and on-line SPE followed by LC coupled with DAD-MS, Chromatographia 64 (2006) 13–21.
[11] F. Chen, G. Ying, L. Kong, L. Wang, J. Zhao, L. Zhou, L. Zhang, Distribution and accumulation of endocrine-disrupting chemicals and pharmaceuticals in wastewater irrigated soils in Hebei, China, Environ. Pollut. 159 (2011) 1490– 1498.
[12] S. Dahane, M.D. Gil Garcia, M.J. Martinez Bueno, A. Ucles Moreno, M. Martinez Galera, A. Derdour, Determination of drugs in river and wastewaters using solid-phase extraction by packed multi-walled carbon nanotubes and liquid chromatography-quadrupole-linear ion trap-mass spectrometry, J. Chromatogr. A 1297 (2013) 17–28.
[13] V.G. Samaras, N.S. Thomaidis, A.S. Stasinakis, T.D. Lekkas, An analytical method for the simultaneous trace determination of acidic pharmaceuticals and phenolic endocrine disrupting chemicals in wastewater and sewage sludge by gas chromatography-mass spectrometry, Anal. Bioanal. Chem. 399 (2011) 2549–2561.
[14] A. Togola, H. Budzinski, Analytical development for analysis of pharmaceuticals in water samples by SPE and GC–MS, Anal. Bioanal. Chem. 388 (2007) 627–635.
[15] E. Carmona, V. Andreu, Y. Pico, Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: from waste to drinking water, Sci. Total Environ. 484 (2014) 53–63.
[16] L. Guo, H.K. Lee, One step solvent bar microextraction and derivatization followed by gas chromatography–mass spectrometry for the determination of pharmaceutically active compounds in drain water samples, J. Chromatogr. A 1235 (2012) 26–33.
[17] S. Yoon, H. Byun, Molecularly imprinted polymers for selective separation of acetaminophen and aspirin by using supercritical fluid technology, Chem. Eng. J. 226 (2013) 171–180.
[18] K. Farrington, F. Regan, Molecularly imprinted sol gel for ibuprofen: an analytical study of the factors influencing selectivity, Talanta 78 (2009) 653– 659.
[19] J. Haginaka, H. Takehira, K. Hosoya, N. Tanaka, Uniform-sized molecularly imprinted polymer for (S)-naproxen selectively modified with hydrophilic external layer, J. Chromatogr. A 849 (1999) 331–339.
[20] C. Dai, S. Geissen, Y. Zhang, X. Zhou, Selective removal of diclofenac from contaminated water using molecularly imprinted polymer microspheres, Environ. Pollut. 159 (2011) 1660–1666.
[21] Z. Terzopoulou, M. Papageorgiou, G.Z. Kyzas, D.N. Bikiaris, D.A. Lambropoulou, Preparation of molecularly imprinted solid-phase microextraction fiber for the selective removal and extraction of the antiviral drug abacavir in environmental and biological matrices, Anal. Chim. Acta 913 (2016) 63–75.
[22] G.Z. Kyzas, S.G. Nanaki, A. Koltsakidou, M. Papageorgiou, M. Kechagia, D.N. Bikiaris, D.A. Lambropoulou, Effectively designed molecularly imprinted polymers for selective isolation of the antidiabetic drug metformin and its transformation product guanylurea from aqueous media, Anal. Chim. Acta 866 (2015) 27–40.
[23] C. Dai, J. Zhang, Y. Zhang, X. Zhou, Y. Duan, S. Liu, Selective removal of acidic pharmaceuticals from contaminated lake water using multi-templates molecularly imprinted polymer, Chem. Eng. J 211–212 (2012) 302–309.
[24] N.T. Tavengwa, E. Cukrowska, L. Chimuka, Synthesis, adsorption and selectivity studies of N-propyl quaternized magnetic poly(4-vinylpyridine) for hexavalent chromium, Talanta 116 (2013) 670–677.
[25] Z. Li, C. Qin, D. Li, Y. Hou, S. Li, J. Sun, Molecularly imprinted polymer for specific extraction of hypericin from Hypericum perforatum L. herbal extract, J. Pharm. Biomed. Anal. 98 (2014) 210–220.
[26] Y. Lu, C. Li, X. Wang, P. Sun, X. Xing, Influence of polymerization temperature on the molecular recognition of imprinted polymers, J. Chromatogr. B 804 (2004) 53–59.
[27] L.M. Madikizela, S.F. Muthwa, L. Chimuka, Determination of triclosan and ketoprofen in river water and wastewater by solid-phase extraction and high performance liquid chromatography, South Afr. J. Chem. 67 (2014) 143–150.
[28] L.M. Madikizela, P.S. Mdluli, L. Chimuka, Experimental and theoretical study of molecular interactions between 2-vinyl pyridine and acidic pharmaceuticals used as multi-template molecules in molecularly imprinted polymer, React. Funct. Polym. 103 (2016) 33–43.
[29] G. Szekely, J. Bandarra, W. Heggie, F.C. Ferreira, B. Sellergren, Design preparation and characterization of novel molecularly imprinted polymers for removal of potentially genotoxic 1,3-diisopropylurea from API solutions, Sep. Purif. Technol. 86 (2012) 190–198.
[30] M. Tabandeh, S. Ghassamipour, H. Aqababa, M. Tabatabaei, M. Hasheminejad, Computational design and synthesis of molecular imprinted polymers for selective extraction of allopurinol from human plasma, J. Chromatogr. B 898 (2012) 24–31.
[31] H.A. Panahi, A. Feizbakhsh, S. Khaledi, E. Moniri, Fabrication of new drug imprinted polymer beads for selective extraction of naproxen in human urine and pharmaceutical samples, Int. J. Pharm. 441 (2013) 776–780.
[32] V. Manzo, K. Ulisse, I. Rodriguez, E. Pereira, P. Richter, A molecularly imprinted polymer as the sorptive phase immobilized in a rotating disk extraction device for the determination of diclofenac and mefenamic acid in wastewater, Anal. Chim. Acta 889 (2015) 130–137.
[33] A. Ameli, N. Alizadeh, Nanostructured conducting molecularly imprinted polymer for selective uptake/release of naproxen by the electrochemically controlled sorbent, Anal. Biochem. 428 (2012) 99–106.
[34] K.D. Mata, M.Z. Corazza, F.M. de Oliveira, A.L. de Toffoli, C.R.T. Tarley, A.B. Moreira, Synthesis and characterization of cross-linked molecularly imprinted polyacrylamide for the extraction/preconcentration of glyphosate and aminomethylphosphonic acid from water samples, React. Funct. Polym. 83 (2014) 76–83.
[35] Y. Tang, J. Lan, X. Gao, X. Liu, D. Zhang, L. Wei, Z. Gao, J. Li, Determination of clenbuterol in pork and potable water samples by molecularly imprinted polymer through the use of covalent imprinting method, Food Chem. 190 (2016) 952–959.
[36] M.D.M.C. Lopez, M.C.C. Perez, M.S.D. Garcia, J.M.L. Vilarino, M.V.G. Rodriguez, L. F.B. Losada, Preparation, evaluation and characterization of quercetin-molecularly imprinted polymer for preconcentration and clean-up of catechins, Anal. Chim. Acta 721 (2012) 68–78.
[37] W. Dong, M. Yan, M. Zhang, Z. Liu, Y. Li, A computational and experimental investigation of the interaction between the template and the functional monomer used in the molecularly imprinted polymer, Anal. Chim. Acta 542 (2005) 186–192.
[38] T.S. Anirudhan, P.L. Divya, J. Nima, Silylated montmorillonite based molecularly imprinted polymer for the selective binding and controlled release of thiamine hydrochloride, React. Funct. Polym. 73 (2013) 1144–1155.
[39] D. Loffler, T.A. Ternes, Determination of acidic pharmaceuticals, antibiotics and ivermectin in river sediment using liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1021 (2003) 133–144.
[40] M. Mahkam, N. Poorgholy, Imprinted polymers as drug delivery vehicles for anti-inflammatory drugs, Nat. Sci. 9 (2011) 163–168.
[41] M.M. Sanagi, S. Salleh, W.A.W. Ibrahim, A.A. Naim, D. Hermawan, M. Miskam, I. Hussain, H.Y. Aboul-Enein, Molecularly imprinted polymer solid-phase extraction for the analysis of organophosphorus pesticide in fruit samples, J. Food Comp. Anal. 32 (2013) 155–161.
[42] J.L. Santos, I. Aparicio, E. Alonso, M. Callejon, Simultaneous determination of pharmaceutically active compounds in wastewater samples by solid phase extraction and high-performance liquid chromatography with diode array and fluorescence detectors, Anal. Chim. Acta 550 (2005) 116–122.
[43] N. Gilart, R.M. Marce, N. Fontanals, F. Borrull, A rapid determination of acidic pharmaceuticals in environmental waters by molecularly imprinted solid-phase extraction coupled to tandem mass spectrometry without chromatography, Talanta 110 (2013) 196–201.
[44] Y. Duan, C. Dai, Y. Zhang, L. -Chen, Selective trace enrichment of acidic pharmaceuticals in real water and sediment samples based on solid-phase extraction using multi-templates molecularly imprinted polymers, Anal. Chim. Acta 758 (2013) 93–100.

3.3.4.1 Paper 3 - Supplementary data

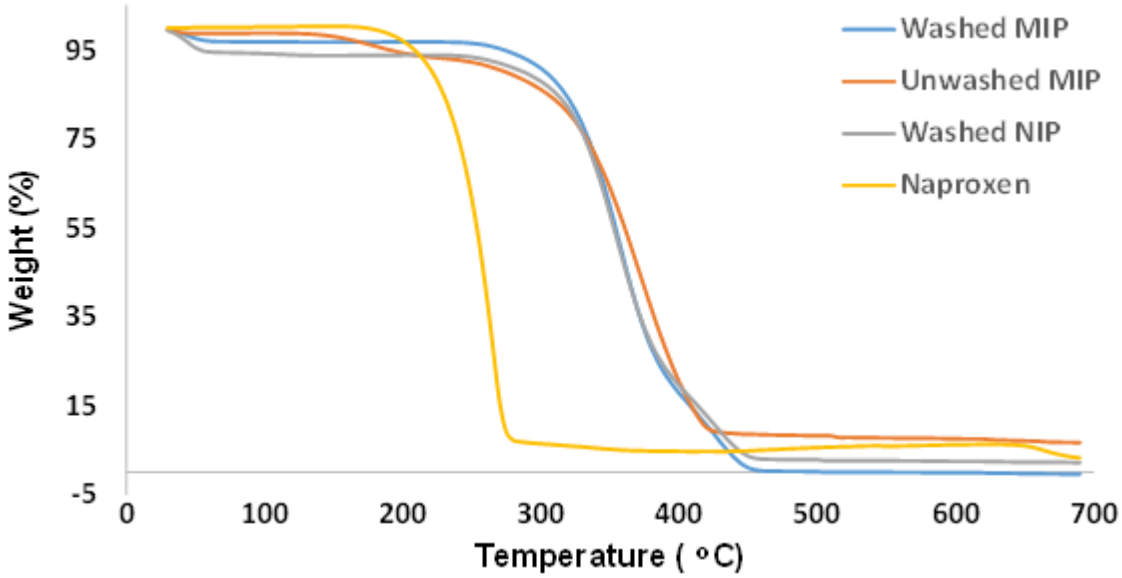


Fig. S1. TGA curves of washed MIP, unwashed MIP, washed NIP and naproxen.

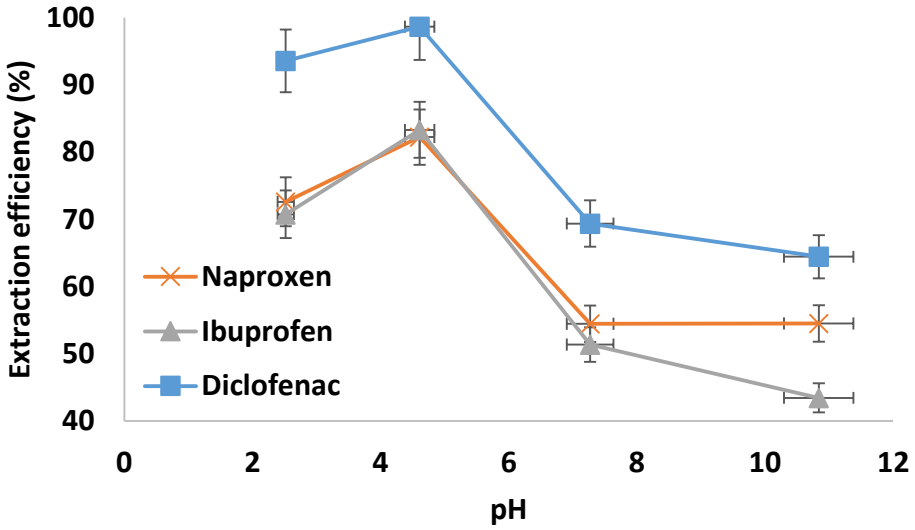


Fig. S2. Effect of sample pH on the extraction efficiency of naproxen, ibuprofen and diclofenac using NIP.

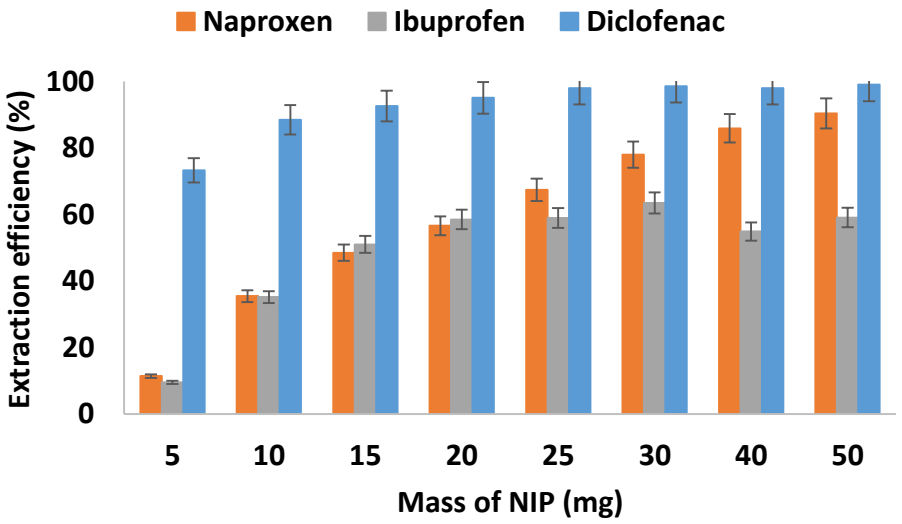


Fig. S3. Effect of polymer amount on the extraction efficiency of naproxen, ibuprofen and diclofenac using NIP.

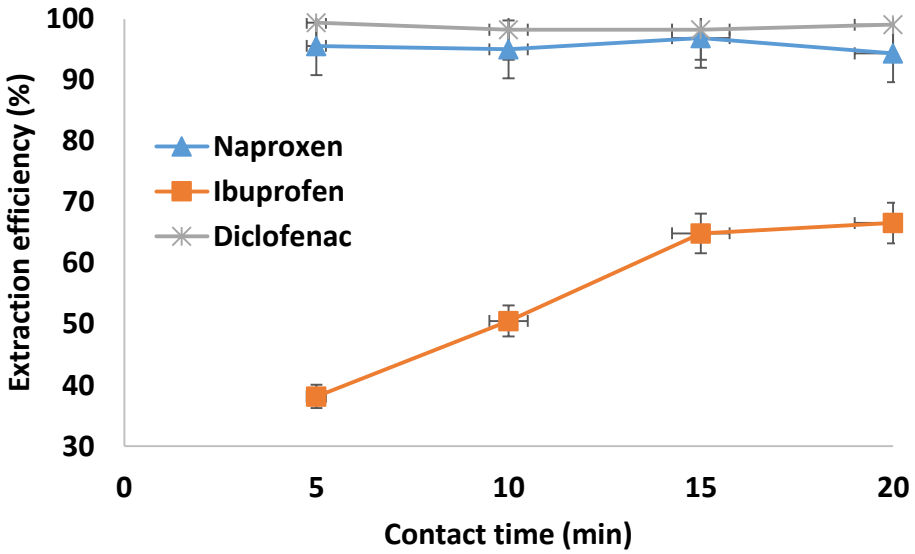


Fig. S4. Effect of contact time on the extraction efficiency of naproxen, ibuprofen and diclofenac using NIP.

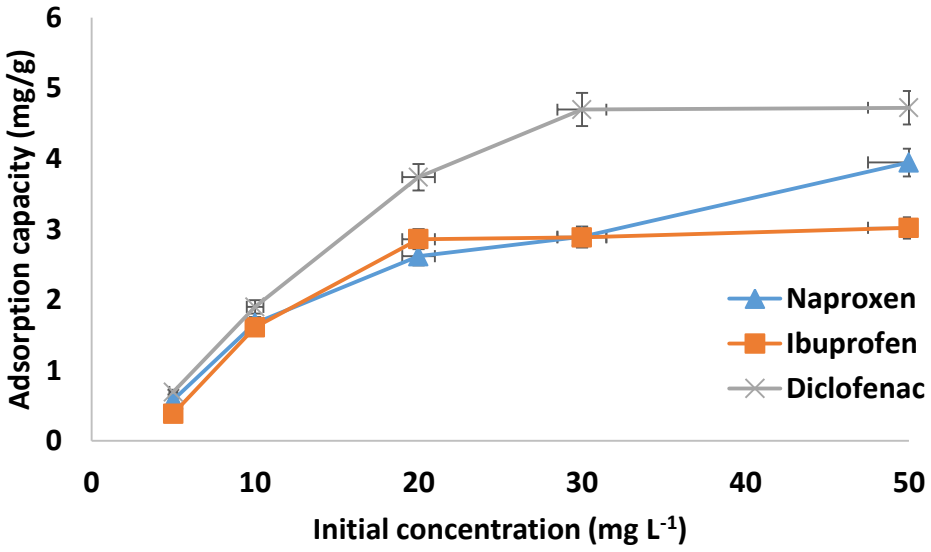


Fig. S5. Effect of initial concentration on the adsorption capacity of naproxen, ibuprofen and diclofenac using NIP.

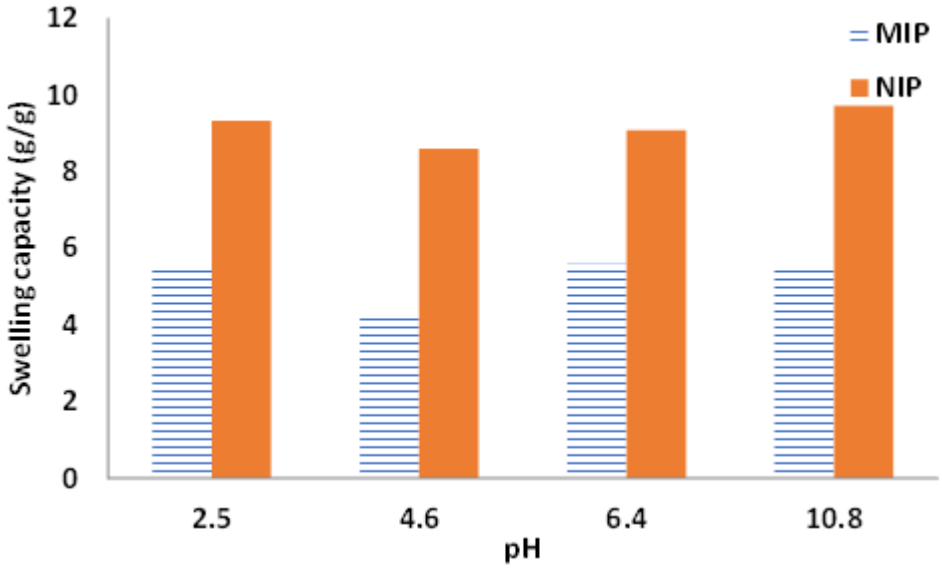


Fig. S6. Swelling capacity as a function of pH.

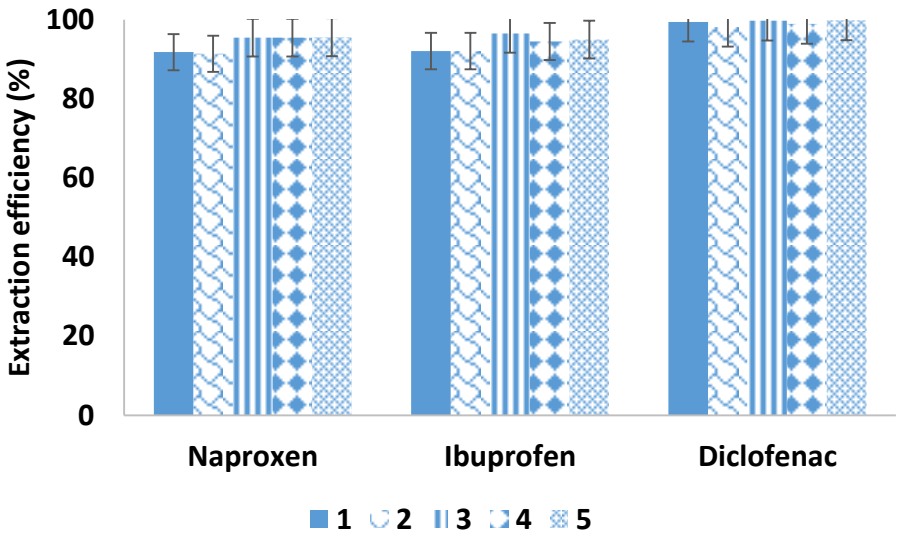


Fig. S7. Re-usability of MIP.

3.3.5 Paper 4

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Determination of ibuprofen, naproxen and diclofenac in aqueous samples using a multi-template molecularly imprinted polymer as selective adsorbent for solid-phase extraction

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ABSTRACT

This study describes the application of multi-template molecularly imprinted polymer (MIP) as selective sorbent in the solid-phase extraction (SPE) of naproxen, ibuprofen and diclofenac from wastewater and river water. MIP was synthesized at 70 °C by employing naproxen, ibuprofen and diclofenac as multi-templates, ethylene glycol dimethacrylate, 2-vinyl pyridine and toluene as cross-linker, functional monomer and porogen, respectively. Wastewater and river water samples (pH 2.5) were percolated through SPE cartridge packed with 50 mg of the MIP. The cartridge was washed with 2 mL of methanol-water 10:90% (v:v) prior to elution with 2 mL of acetic acid-acetonitrile 20:80% (v:v). Quantification of eluted compounds was performed with high performance liquid chromatography equipped with photo diode array detection. The detection limits were 0.15, 1.00 and 0.63 $\mu g L^{-1}$ for naproxen, ibuprofen and diclofenac, respectively. Recoveries for naproxen, ibuprofen and diclofenac in deionized water spiked at 5 and 50 $\mu g L^{-1}$ were greater than 80%. Ibuprofen was the most frequently detected compound with maximum concentrations of 221, 67.9 and 11.4 $\mu g L^{-1}$ in wastewater influent, effluent and river water, respectively.

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1. Introduction

Ibuprofen, naproxen and diclofenac (molecular structures in Table 1) are acidic pharmaceuticals that belong to the class of nonsteroidal anti-inflammatory drugs [1]. These compounds are released into the environment through human wastes [2]. The compounds are polar, therefore, they are not significantly adsorbed in the subsoil and may be transported into the groundwater aquifers from the contaminated surface water [2]. Another important source of acidic pharmaceuticals in the environment is their incomplete removal during the sewage treatment [3]. Occurrence of ibuprofen, naproxen and diclofenac in wastewater and river water is well documented in Europe and some well developed countries [4–8]. However, in some parts of South Africa, these compounds have been reported to be present in wastewater and river water [9–12]. In Spain, these compounds have been detected in drinking water at ng/L levels [13,14].

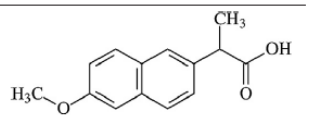
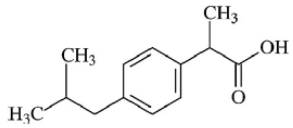
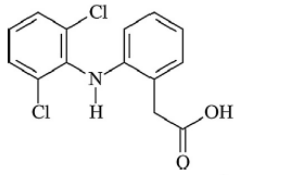
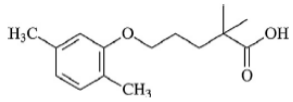
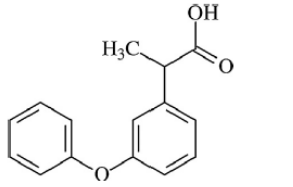
A simple determination of non-volatile pharmaceuticals in the environment requires the use of a high performance liquid chromatographic (HPLC) equipment for separation and quantification. A suitable sample preparation technique for analyte pre-concentration and removal of sample interfering species is employed prior to chromatographic separation. Solid-phase extraction (SPE) is one of the most widely used sample preparation technique for the determination of pharmaceuticals in the environment. Published work involves the use of commercial SPE sorbents such as Oasis HLB, Oasis MCX, Strata X and C_{18} [15–20]. The disadvantages of the mentioned SPE sorbents include poor selectivity and they can only be used once.

Application of molecularly imprinted polymers (MIPs) as SPE sorbents can overcome these disadvantages. MIP is a tailor-made material, which has high affinity and selectivity for its template [21]. MIPs are now widely used as SPE sorbents as they offer high selectivity, easy preparation and regeneration. A great amount of work has been directed towards the use of a MIP for the solid-phase extraction of a single compound from various sample matrices. For instance, a selective extraction of naproxen from urine samples using molecularly imprinted solid-phase extraction (MISPE) have been reported by Caro et al. [22]. In separate studies, a

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Table 1
Molecular structures of the selected compounds.

Compound	Molecular structure
Naproxen	
Ibuprofen	
Diclofenac	
Gemfibrozil	
Fenoprofen	

MIP was developed by Farrington and Regan [23] for the recognition of ibuprofen from the pharmaceutical capsules, whereas, Sun et al. [24] synthesized a MIP for SPE of diclofenac from surface and wastewater samples. Ibuprofen, naproxen and diclofenac are usually detected simultaneously in the environmental samples; hence, nowadays more work is being presented on the use of multi-template MIPs for the extraction of such compounds from aqueous samples [25–27]. Previous works [25,27] have presented the use of multi-template MIPs as SPE sorbents prior to mass spectrometry detection. Due to budget constraints, most laboratories cannot afford the high cost of mass spectrometry detection system. Therefore, in this study, multi-template MIP was used to selectively extract ibuprofen, naproxen and diclofenac from river water and wastewater, as well as to improve the sensitivity of the less expensive HPLC coupled to photo diode array detection method.

In view of the scope presented above, the aim of this work was to imprint a polymer using three template molecules (ibuprofen, naproxen and diclofenac), and to apply the polymer as the selective SPE sorbent for ibuprofen, naproxen and diclofenac in water. Furthermore, the objective of the work was to provide a detailed screening of these drugs in South African wastewater and river water by using a cheap method that is highly accurate, simple, sensitive, selective and rapid.

2. Experimental

2.1. Reagents and materials

Naproxen (98%), ibuprofen (≥98%), diclofenac sodium salt, 2-vinylpyridine (97%), 1,1′-azobis-(cyclohexanecarbonitrile) (98%), ethylene glycol dimethacrylate (98%), HPLC grade methanol (≥99.9%) and toluene (99.7%) were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC-grade acetonitrile (≥99.9%) and glacial acetic acid (100%) were purchased from Merck (Darmstadt, Germany). Formic acid (approx. 98%) was purchased from Fluka (Steinheim, Germany). Sodium chloride (≥99.5%) was purchased from Associated Chemical Enterprises (Johannesburg, South Africa).

Oasis MAX cartridges packed with 150 mg of sorbent and vacuum pump were obtained from Waters Corporation (Milford, Massachusetts, USA) and Millipore (Darmstadt, Germany), respectively.

2.2. Synthesis of multi-template molecularly imprinted polymer

Bulk polymerization of MIP was performed in two steps. The first step was carried out by dissolving 20 mg of 1,1′-azobis-(cyclohexanecarbonitrile) in 50 mL of toluene, followed by the addition of 1.51 mL of ethylene glycol dimethacrylate. The reaction flask was purged with nitrogen for 10 min and sealed. Then, the reaction was allowed to take place with constant stirring in an oil bath set at 70 °C for 8 h. In the second step, naproxen (76.60 mg), ibuprofen (68.69 mg), diclofenac (106.04 mg) were dissolved in 25 mL of acetonitrile, followed by the addition of 0.25 mL 2-vinylpyridine, 3.85 mL ethylene glycol dimethacrylate, 60 mg 1,1′-azobis-(cyclohexanecarbonitrile) and 25 mL of toluene. These contents were transferred to the product obtained in the first step. The mixture was purged with nitrogen gas for 10 min and sealed. The reaction was carried out in an oil bath set at 70 °C for 16 h. The resulting polymer was oven dried at 60 °C to constant mass. The polymer was then milled, sieved and particles ranging from 25 to 50 μm were collected. Non-imprinted polymer (NIP) was prepared under the same conditions in the absence of template molecules. Templates were eluted from the polymer using a mixture of 10% (v/v) acetic acid in acetonitrile. Elution step was repeated several times until the target compounds were not detected by the HPLC system. Thereafter, 100% acetonitrile was applied in order to wash off the acetic acid residue.

2.3. Apparatus

Agilent VNMRS Wide Bore 500 MHz nuclear magnetic resonance (NMR) spectrometer with a 1H frequency of 500 MHz and a Carbon 13 (13C) frequency of 125 MHz was used for characterization. The spectra were acquired utilizing a dual-channel 4 mm Chemagnetics TM T3 HX MAS probe using 4 mm zirconia rotors. The cross-polarization (CP) spectra were recorded at 25 °C with proton decoupling using a recycle delay of 10 s. The CP pulse power parameters were optimized for the Hartmann-Hahn match using a glycine standard sample. The radio frequency fields for the match were γCB1C = γHB1H ≈ 55 kHz. The contact time for cross-polarization was optimized to 2.0 ms. Magic-angle-spinning (MAS) was performed at 10 000 revolutions per second (10 kHz).

Chromatographic separation was performed on a HPLC system that consisted of an online mobile phase degasser unit (Model: DGU-20A3), 20 μL sample loop, pump (Model: LC-20AB), and photo diode array detector (Model: SPD-M20A), all obtained from Shimadzu Corporation (Kyoto, Japan). The mobile phase used consisted of a mixture of acetonitrile: 0.2% formic acid in water (60:40, v:v) at a flow rate of 0.8 mL min^{-1}. Separation was performed on a Kinetex C_{18} HPLC column of 150 × 4.6 mm × 2.6 μm obtained from Phenominex (California, USA). Shimadzu LC solutions software was used for data collection and processing. Photo diode array detector was set at 230 nm for naproxen measurement, while ibuprofen and diclofenac were both monitored at 200 nm.

2.4. Sampling

The samples collected consist of wastewater influent (collected after solid removal from raw waste), effluent (collected after chlorination) and river water samples. Samples were collected monthly from January to March 2016 using pre-cleaned glass bottles. These samples were collected from Northern wastewater treatment plant (WWTP) (GPS: S29.79635^0 E30.99630^0), Amanzimtoti WWTP (GPS: S30.00749^0 E30.91720^0) and Mbokodweni river (GPS: S30.00592^0 E30.92440^0). All sampling sites are located in the province of Kwa-Zulu Natal (South and North of the city of Durban) in South Africa. The collected samples were transported in cooler bags to the laboratory. On arrival in the laboratory, the individual samples were filtered through a 150 mm filter paper with pore size of 10 μm that was obtained from Munktell and Filtrak GmbH (Bernstein, Germany). The samples were kept in the refrigerator at 4 °C after adjusting the pH to 2.5.

2.5. Preparation of molecularly imprinted solid-phase extraction (MISPE) cartridge

A slurry of 50 mg MIP particles was prepared with 3 mL of acetonitrile. The slurry was transferred into an empty 3 mL SPE cartridge. Frits were placed at the bottom and top of the MIP sorbent. For regeneration after each use, the cartridge was washed repeatedly with 20% (v:v) acetic acid in acetonitrile to remove impurities and target compounds, followed by washing with 3 mL of acetonitrile.

2.6. MISPE and oasis MAX SPE procedures

MISPE cartridge was conditioned with 2 mL of acetonitrile, followed by 2 mL of deionized water (pH 2.5). With the aid of the vacuum pump, 50 mL of the acidified sample (pH 2.5) was percolated at 0.3 mL min^{-1}. Thereafter, 2 mL of 10% (v:v) methanol in water was used to wash the cartridge. Elution of retained compounds was performed with 2 mL of 20% (v:v) acetic acid in acetonitrile. Thereafter, 20 μL of eluted extract was injected into the HPLC system.

For comparison in terms of selectivity, the commercial Oasis MAX cartridge was used to percolate the samples using the optimized procedure as follows: The SPE cartridge was conditioned with 5 mL of acetonitrile followed by 5 mL of acidified deionized water (pH 2.5) both loaded at a flow rate of 1 mL min^{-1}. The acidified wastewater sample (pH 2.5, 100 mL) was loaded onto the SPE cartridge at a flow rate of 1 mL min^{-1}. 2 mL of 10% (v:v) methanol in water was used to wash the cartridge prior to elution of retained compounds. Thereafter, the retained compounds were eluted sequentially with 2 mL methanol, 2 mL mixture of methanol-acetic acid 90:10, (v:v) and 2 mL of 2% (v:v) formic acid diluted using methanol-acetic acid 40:60, (v:v). The components of the eluates were combined and evaporated under nitrogen atmosphere to 0.5 mL prior to HPLC analysis.

2.7. Validation of analytical method

A mixture of target compounds (100 mgL^{-1} for each compound) was prepared in acetonitrile. Thereafter, a series of standard solutions were prepared from the stock solution and analyzed using an HPLC system. Instrument detection limits, limits of quantification and linearity were computed.

Accuracy and precision of the analytical method were performed using deionized water that was spiked with target compounds at concentration levels of 5 and 50 μgL^{-1}. The optimized MISPE method was employed for the extraction and pre-concentration of target compounds prior to HPLC quantification.

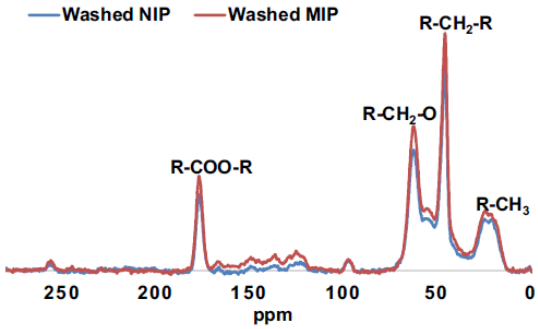


Fig. 1. Solid-state ^{13}C CP/MAS NMR Spectra of the MIP and NIP.

3. Results and discussion

3.1. Characterization of polymers

The solid-state ^{13}C CP/MAS NMR spectra for the MIP and NIP (Fig. 1) were Fourier transformed, baseline corrected and the relevant peaks indicated in accordance to the information obtained from the literature [28,29]. The results showed no differences in the chemical shifts obtained for MIP and NIP, which indicates that both polymers were chemically equivalent. The resonances observed that corresponds to the various methyl groups were represented by the broad peak at 22 ppm. Other groups detected were methylene groups in cross linker at 45 and 60 ppm, and CO_2R group at 175 ppm. All the assigned signals were in agreement with the expected composition of the polymers.

Further characterization was performed with Fourier-transform infrared spectroscopy for functional group analysis, scanning electron microscopy for polymer morphology, organic elemental analyzer for carbon, hydrogen and nitrogen contents of polymers, and Brunauer, Emmett and Teller method for surface area determination in polymers. Details of such tests were presented in our recently published work [30].

3.2. Optimization experiments for MISPE

The success of any SPE experiments relies entirely on optimization experiments. In this work, the effect of sample volume (10–50 mL), salt content (0.0–0.3%), elution volume (2–5 mL) and presence of foreign compounds in sample matrix (selectivity study) were studied. Other parameters such as sorbent mass, sample pH, washing solvent and elution solvent were optimized in our previous work. All the experiments were conducted using deionized water that was spiked with 50 μgL^{-1} of target compounds.

Effect of sample volume was investigated as large sample volumes lead to greater pre-concentration factors. The percent recoveries obtained increased slowly from the sample volume of 10 mL to 30 mL, thereafter they remained unchanged. However, the sample volume of 50 mL was selected for subsequent experiments as it promoted the sensitivity by increasing the pre-concentration factor. The maximum recoveries obtained for naproxen, ibuprofen and diclofenac were 58, 73 and 56%, respectively.

Addition of salt in water increases the ionic strength which tends to affect the retention of target compounds in the SPE sorbent, due to the salting out effect [31]. Furthermore, salt can be added in aqueous samples to improve the extraction of several analytes as the increase in ionic strength usually brings a reduction on the sol-

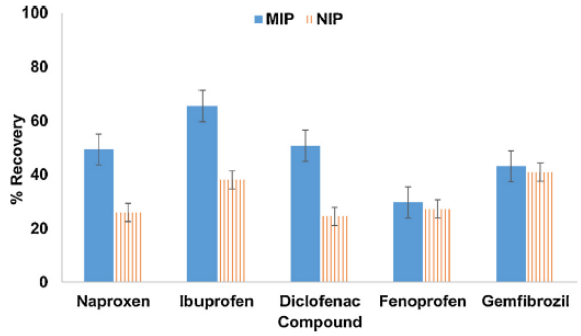


Fig. 2. Selectivity of the polymers.

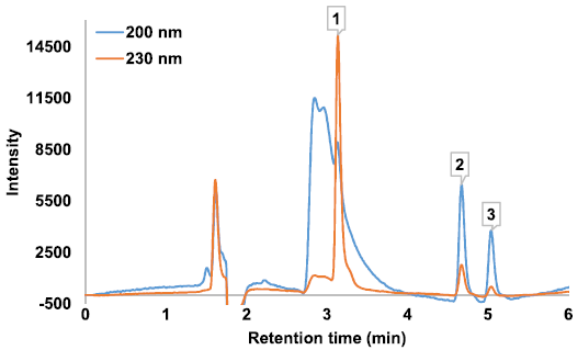


Fig. 3. The chromatograms for 1000 μg/L each of naproxen (1), diclofenac (2) and ibuprofen (3) at 200 nm and 230 nm.

ubility of the hydrophobic analytes in the water solution and forces more of these analytes into the extracting phase [32]. In this work, salt content did not affect the percent recoveries therefore it was not added into the environmental samples.

Flow rates play a significant role in the SPE. The decrease in flow rates from 1 mL min^{-1} to 0.3 mL min^{-1} resulted in the increase of recoveries for target compounds due to the prolonged contact between the adsorbent and target compounds.

The effect of elution volume was investigated in order to ensure the complete removal of target compounds from the MISPE cartridge. The results showed no improvement in the recoveries when the elution volume exceeded 2 mL. Therefore, the elution volume of 2 mL was maintained throughout this work.

Finally, selectivity of polymers was investigated using gemfibrozil and fenoprofen as competitive species. Gemfibrozil and fenoprofen are acidic pharmaceuticals that belong to the therapeutic classes of lipid regulator and anti-inflammatory, respectively [33]. Both competitive species contain similar functional groups as the target compounds (Table 1). The two competitors have been previously detected in environmental water samples that contain the target compounds [33,34]. For selectivity study, deionized water was spiked with five compounds (naproxen, ibuprofen, diclofenac, gemfibrozil and fenoprofen), followed by adjusting pH to 2.5. In order to assess the success of molecular imprinting technology, parallel experiments were carried out using cartridges that were packed with a non-imprinted polymer. Thereafter, 50 mL of the spiked water was percolated into a pre-conditioned cartridge. After loading the sample, the cartridge was washed with 2 mL mixture of methanol-water 10:90% (v:v) and elution was performed with a 2 mL mixture of acetic acid-acetonitrile 20:80% (v:v). Fig. 2 shows the recoveries obtained for five compounds from the MISPE cartridges. The recoveries of MIP were higher than those obtained for the NIP, due to molecular recognition. Also, the recoveries of the competitive species were lower when compared to those of target compounds due to the shape of the molecules. Ibuprofen had the highest recovery (66%), this could be explained by the fact that the structure of ibuprofen as shown in Table 1, contains one aromatic ring, whereas both naproxen and diclofenac have two rings in their respective molecular structures. Fenoprofen contains two aromatic rings, therefore it was expected to compete more strongly with naproxen and diclofenac for adsorption and elution. While gemfibrozil contains one aromatic ring, it was poorly recovered, probable due to long aliphatic chain in its molecule.

3.3. Method validation

The chromatograms obtained for the separation of target compounds are presented in Fig. 3. The target compounds were well separated using a C_{18} HPLC column with resolution values greater than 1.5 for all the compounds. Limit of detection (LOD) and limit of quantification (LOQ) were used to measure the sensitivity of the method. LOD and LOQ were defined as the concentration where signal to noise ratio is 3 and 10, respectively. The results obtained (Table 2) show that the method can be applied to the quantitative analysis of target compounds at low μgL^{-1} levels. These results (Table 2) are in agreement with those reported in literature for the same compounds using HPLC with fluorescence [9] and diode array [10] detection. The solid-phase extraction of target compounds yielded the recoveries that ranged from 82 to 103% which is an indication of a highly accurate method. Standard deviation (SD) was used as a measure of method precision. The experiments were carried out in triplicates and the SD values are reported as ± values in Table 2. Deviations were generally smaller for 5 μgL^{-1} spiked solutions as it may be difficult to notice any errors associated with measurements at such low concentrations. Furthermore, the cartridges were re-used up to at least five times without reducing the percent recoveries.

Table 2
Calculated values of LOD, LOQ, linearity and percent recoveries obtained for deionized water spiked with 5 and 50 μg/L (n = 3) of each target compound.

Compound	LOD (μgL^{-1})	LOQ (μgL^{-1})	Linearity (R^2)	% Recovery ± SD (%)	
				5 μgL^{-1}	50 μgL^{-1}
Naproxen	0.15	0.49	0.999	85 ± 2.8	86 ± 8.9
Ibuprofen	1.00	3.33	0.999	97 ± 9.7	103 ± 8.6
Diclofenac	0.63	2.11	0.998	88 ± 1.1	82 ± 4.9

3.4. Environmental application

The developed MISPE procedure was applied in the environmental monitoring of target compounds in WWTPs and river water that were described elsewhere [35,36]. As shown in Table 3, the target compounds were detected in all influent samples. Naproxen was the least detected compound with maximum concentration of 39.6 μgL^{-1} found in Northern WWTP, whereas, ibuprofen had the highest concentration of 221 μgL^{-1} in the same treatment plant. In some cases, the concentrations of target compounds were higher in the effluent due to malfunctioning of WWTPs at the time of sample collection. Traces of target compounds were detected in river water, which calls for the detailed screening of such compounds in sediments, aquatic plants and animals. The concentrations obtained in this study are in agreement with the data reported for the same compounds in various samples collected in other parts of South Africa [9–12]. Amdany et al. [9] reported the comparable concentration of 12.6 μgL^{-1} for ibuprofen in Goudkoppies WWTP effluent located in Johannesburg. The concentrations of naproxen obtained

Table 3
Range of concentrations ($\mu g L^{-1}$) for naproxen, ibuprofen and diclofenac in contaminated environmental samples.

Sampling site	Compound					
	Naproxen		Ibuprofen		Diclofenac	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Northern WWTP influent	1.84	39.6	6.02	221	10.1	104
Northern WWTP effluent	<LOQ	<LOQ	3.87	13.0	<LOQ	<LOQ
Amanzimtoti WWTP influent	1.22	2.62	14.9	23.6	3.72	13.9
Amanzimtoti WWTP effluent	nd.	5.34	12.0	67.9	<LOQ	20.8
Mbokodweni river	nd.	0.68	nd.	11.4	nd.	<LOQ

Where, nd means the compound was not detected, while <LOQ, means the compound was detected at a concentration that is below the limit of quantification.

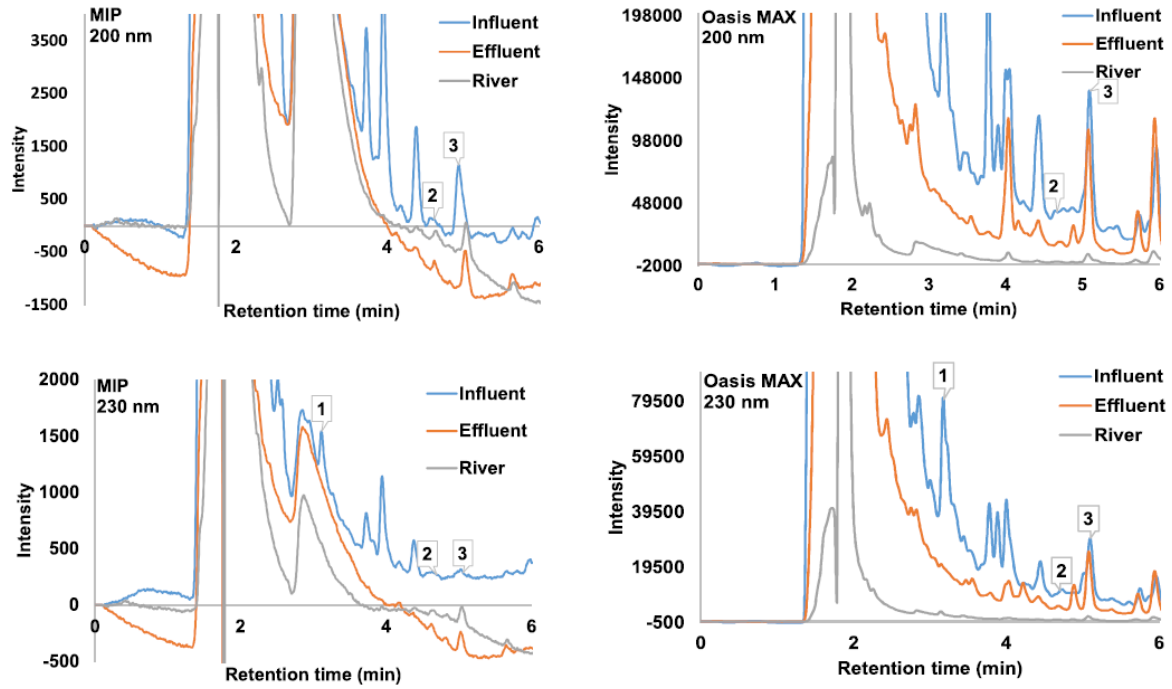


Fig. 4. The chromatograms for river water, Amanzimtoti WWTP influent and effluent. Peaks I, 2 and 3 are for naproxen, diclofenac, and ibuprofen, respectively.

in the current study were lower than those reported by Amdany et al. [9]. Interestingly, the ibuprofen concentration in Northern WWTP effluent of 12.94 $\mu g L^{-1}$ reported by Matongo et al. [11] where they used Oasis HLB sorbent for SPE and HPLC–MS/MS for separation and quantification agreed well with the current results. This indicates that the proposed method is highly reliable. Generally, some concentrations reported in other parts of the World were lower than those observed in the current study [37–39]. This could be probable due to differences in the WWTP design, the pharmaceutical consumptions in different countries and population served by the WWTPs.

River water, Amanzimtoti WWTP influent and effluent were also extracted using Oasis MAX as SPE sorbent. Oasis MAX is made of a mixed-mode polymer sorbent with both reversed-phase and anion-exchange functionalities [37].The chromatograms obtained were compared to those of MIP sorbent. The peaks were more intense in the Oasis MAX chromatograms due to higher preconcentration factor (Fig. 4). Unwanted peaks were reduced when using MIP sorbent and the chromatograms were cleaner due to improved selectivity (Fig. S1 in support information). The re-use of SPE cartridges packed with MIP gave this work an economical advantage.

4. Conclusions

A new method for the rapid determination of naproxen, ibuprofen and diclofenac in water has been optimized. Molecularly imprinted polymer was applied as an alternative SPE sorbent for the selective extraction and pre-concentration of acidic pharmaceuticals in aqueous samples. The primary advantage of using MISPE is the re-use of cartridges without reducing the recovery of the target compounds. It is important to apply this type of study to all water bodies in order to understand the extent of pharmaceuticals in the environment.

All three compounds were detected in the wastewater influent and effluent samples, which indicate the incomplete removal of such compounds from the wastewater treatment process. Among the target compounds, ibuprofen was the most frequently detected pharmaceutical in all samples at higher concentrations. Naproxen concentrations were lower in the environmental samples. The presence of target compounds at trace levels in river water indicates the contamination of Mbokodweni river. To the best of our knowledge, this is the first detailed study of the quantitative determination of naproxen, ibuprofen and diclofenac in aqueous samples obtained from Mbokodweni river and Amanzimtoti WWTP.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2016.05.037.

References

[1] E. Larsson, S. Al-Hamimi, J.A. Jonsson, Behavior of nonsteroidal anti-inflammatory drugs and eight of their metabolites during wastewater treatment studied by hollow fibre liquid phase microextraction and liquid chromatography mass spectrometry, Sci. Total Environ. 485–486 (2014) 300–308.
[2] V. Koutsouba, T. Heberer, B. Fuhrmann, K. Schmidt-Baumler, D. Tsipi, A. Hiskia, Determination of polar pharmaceuticals in sewage water of Greece by gas chromatography-mass spectrometry, Chemosphere 51 (2003) 69–75.
[3] N. Larsson, E. Petersson, M. Rylander, J.A. Jonsson, Continuous flow hollow fiber liquid-phase microextraction and monitoring of NSAID pharmaceuticals in a sewage treatment plant effluent, Anal. Methods. 1 (2009) 59–67.
[4] L. Vergeynst, A. Haeck, P. De Wispelaere, H. Van Langenhove, K. Demeestere, Multi-residue analysis of pharmaceuticals in wastewater by liquid chromatography-magnetic sector mass spectrometry: method quality assessment and application in a Belgium case study, Chemosphere 119 (2015) S2–S8.
[5] J. Zhao, G. Ying, L. Wang, J. Yang, X. Yang, L. Yang, X. Li, Determination of phenolic endocrine disrupting chemicals and acidic pharmaceuticals in surface water of the Pearl Rivers in South China by gas chromatography-negative chemical ionization-mass spectrometry, Sci. Total Environ. 407 (2009) 962–974.
[6] G. Ying, R.S. Kookana, D.W. Kolpin, Occurrence and removal of pharmaceutically active compounds in sewage treatment plants with different technologies, J. Environ. Monit. 11 (2009) 1498–1505.
[7] M.R. Boleda, M.T. Galceran, F. Ventura, Validation and uncertainty estimation of a multiresidue method for pharmaceuticals in surface and treated waters by liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1286 (2013) 146–158.
[8] V.G. Samaras, N.S. Thomaidis, A.S. Stasinakis, T.D. Lekkas, An analytical method for the simultaneous trace determination of acidic pharmaceuticals and phenolic endocrine disrupting chemicals in wastewater and sewage sludge by gas chromatography-mass spectrometry, Anal. Bioanal. Chem. 399 (2011) 2549–2561.
[9] R. Amdany, L. Chimuka, E. Cukrowska, Determination of naproxen, ibuprofen and triclosan in wastewater using the polar organic chemical integrative sampler (POCIS): A laboratory calibration and field application, Water SA 40 (2014) 407–414.
[10] F.O. Agunbiade, B. Moodley, Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa, Environ. Monit. Assess. 186 (2014) 7273–7291.
[11] S. Matongo, G. Birungi, B. Moodley, P. Ndungu, Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa, Environ. Sci. Pollut. Res. 22 (2015) 10298–10308.
[12] S. Matongo, G. Birungi, B. Moodley, P. Ndungu, Pharmaceutical residues in water and sediment of Msunduzi River, KwaZulu-Natal, South Africa, Chemosphere 134 (2015) 133–140.
[13] R. Rodil, J.B. Quintana, E. Concha-Grana, P. Lopez-Mahia, S. Muniategui-Lorenzo, D. Prada-Rodriguez, Emerging pollutants in sewage, surface and drinking water in Galicia (NW Spain), Chemosphere 86 (2012) 1040–1049.
[14] E. Carmona, V. Andreu, Y. Pico, Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: from waste to drinking water, Sci. Total Environ. 484 (2014) 53–63.
[15] J.L. Santos, I. Aparicio, E. Alonso, M. Callejon, Simultaneous determination of pharmaceutically active compounds in wastewater samples by solid phase extraction and high-performance liquid chromatography with diode array and fluorescence detectors, Anal. Chim. Acta 550 (2005) 116–122.
[16] N. Lindqvist, T. Tuhkanen, L. Kronberg, Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters, Water Res. 39 (2005) 2219–2228.
[17] V.G. Samaras, A.S. Stasinakis, D. Mamais, N.S. Thomaidis, T.D. Lekkas, Fate of selected pharmaceuticals and synthetic endocrine disrupting compounds during wastewater treatment and sludge anaerobic digestion, J. Hazard. Mater. 244-245 (2013) 259–267.
[18] A. Togola, H. Budzinski, Analytical development for analysis of pharmaceuticals in water samples by SPE and GC–MS, Anal. Bioanal. Chem. 388 (2007) 627–635.
[19] A. Azzouz, B. Souhail, E. Ballesteros, Continuous solid-phase extraction and gas chromatography-mass spectrometry determination of pharmaceuticals and hormones in water samples, J. Chromatogr. A 1217 (2010) 2956–2963.
[20] T. Kosjek, E. Heath, A. Krbavcic, Determination of non-steroidal anti-inflammatory drug (NSAIDs) residues in water samples, Environ. Int. 31 (2005) 697–785.
[21] Y. Liu, F. Wang, T. Tan, M. Lei, Study of the properties of molecularly imprinted polymers by computational and conformational analysis, Anal. Chim. Acta 581 (2007) 137–146.
[22] E. Caro, R.M. Marce, P.A.G. Cormack, D.C. Sherrington, F. Borrull, A new molecularly imprinted polymer for the selective extraction of naproxen from urine samples by solid-phase extraction, J. Chromatogr. B 813 (2004) 137–143.
[23] K. Farrington, F. Regan, Investigation of the nature of MIP recognition: the development and characterization of a MIP for ibuprofen, Biosens. Bioelectron. 22 (2007) 1138–1146.
[24] Z. Sun, W. Schussler, M. Sengl, R. Niessner, D. Knopp, Selective trace analysis of diclofenac in surface and wastewater samples using solid-phase extraction with a new molecularly imprinted polymer, Anal. Chim. Acta 620 (2008) 73–81.
[25] N. Gilart, R.M. Marce, N. Fontanals, F. Borrull, A rapid determination of acidic pharmaceuticals in environmental waters by molecularly imprinted solid-phase extraction coupled to tandem mass spectrometry without chromatography, Talanta 110 (2013) 196–201.
[26] C. Dai, J. Zhang, Y. Zhang, X. Zhou, Y. Duan, S. Liu, Selective removal of acidic pharmaceuticals from contaminated lake water using multi-templates molecularly imprinted polymer, Chem. Eng. J. 211–212 (2012) 302–309.
[27] Y. Duan, C. Dai, Y. Zhang, L. Chen, Selective trace enrichment of acidic pharmaceuticals in real water and sediment samples based on solid-phase extraction using multi-templates molecularly imprinted polymers, Anal. Chim. Acta 758 (2013) 93–100.
[28] U. Skogsberg, C. Meyer, J. Rehbein, G. Fischer, S. Schauff, N. Welsch, K. Albert, A.J. Hall, B. Sellergren, A solid-state and suspended-state magic angle spinning nuclear magnetic resonance spectroscopic investigation of a 9-ethyladenine molecularly imprinted polymer, Polymer 48 (2007) 229–238.
[29] M. Sobiech, T. Zolek, P. Lulinski, D. Maciejewska, Separation of octopamine racemate on (*R*,*S*)-2-amino-1-phenylethanol imprinted polymer—experimental and computational studies, Talanta 146 (2016) 556–567.
[30] L.M. Madikizela, P.S. Mdluli, L. Chimuka, Experimental and theoretical study of molecular interactions between 2-vinyl pyridine and acidic pharmaceuticals used as multi-template molecules in molecularly imprinted polymer, React. Funct. Polym. 103 (2016) 33–43.
[31] S. Dahane, M.D. Gil Garcia, M.J. Martinez Bueno, A. Ucles Moreno, M. Martine Galera, A. Derdour, Determination of drugs in river and wastewaters using solid-phase extraction by packed multi-walled carbon nanotubes and liquid chromatography-quadrupole-linear ion trap-mass spectrometry, J. Chromatogr. A 1297 (2013) 17–28.
[32] A. Sarafraz-Yazdi, A. Amiri, G. Rounaghi, H. Eshtiagh-Hosseini, Determination of non-steroidal anti-inflammatory drugs in water samples by solid-phase microextraction based sol-gel technique using poly(ethylene glycol) grafted multi-walled carbon nanotubes coated fiber, Anal. Chim. Acta 720 (2012) 134–141.
[33] L. Patrolecco, N. Ademollo, P. Grenni, A. Tolomei, A.B. Caracciolo, S. Capri, Simultaneous determination of human pharmaceuticals in water samples by solid phase extraction and HPLC with UV-fluorescence detection, Microchem. J. 107 (2013) 165–171.
[34] N.H. Tran, J. Hu, S.L. Ong, Simultaneous determination of PPCPs, EDCs, and artificial sweeteners in environmental water samples using a single-step SPE coupled with HPLC-MS/MS and isotope dilution, Talanta 113 (2013) 82–92.
[35] S.B.T. Nzimande, Treated wastewater effluent as potential source of emerging bacterial pathogens in surface water, in: MSc Thesis, University of KwaZulu-Natal, Durban South Africa, 2014.
[36] L.M. Madikizela, S.F. Muthwa, L. Chimuka, Determination of triclosan and ketoprofen in river water and wastewater by solid-phase extraction and high performance liquid chromatography, S. Afr. J. Chem. 67 (2014) 143–150.
[37] H. Lee, T.E. Peart, M.L. Svoboda, Determination of endocrine-disrupting phenols acidic pharmaceuticals, and personal-care products in sewage by solid-phase extraction and gas chromatography-mass spectrometry, J. Chromatogr. A 1094 (2005) 122–129.
[38] J.T. Yu, E.J. Bouwer, M. Coelhan, Occurrence and biodegradability studies of selected pharmaceuticals and personal care products in sewage effluent, Agric. Water. Manag. 86 (2006) 72–80.
[39] Y. Yu, L. Wu, A.C. Chang, Seasonal variation of endocrine disrupting compounds, pharmaceuticals and personal care products in wastewater treatment plants, Sci. Total Environ. 442 (2013) 310–316.

3.3.5.1 Paper 4 - Supplementary data

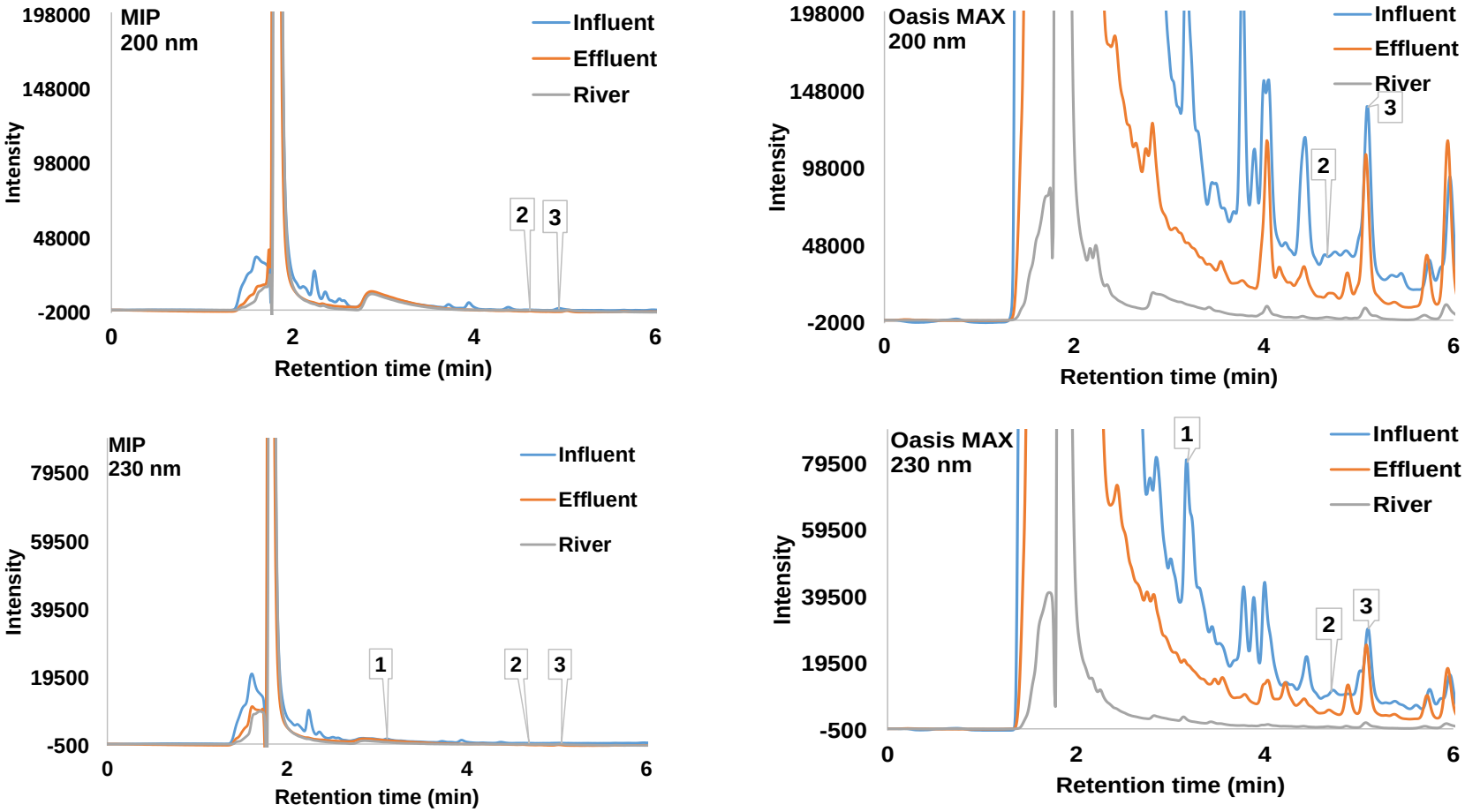


Fig. S1. The chromatograms for river water, Amanzimtoti WWTP influent and effluent. Peaks I, 2 and 3 are for naproxen, diclofenac, and ibuprofen, respectively. Long arrows points where the peaks appear when the chromatograms are zoomed.

3.3.6 Paper 5

Madikizela L.M. and Chimuka, L. Occurrence of naproxen, ibuprofen and diclofenac residues in wastewater and river water of KwaZulu-Natal Province in South Africa. *Under Revision in Environmental Monitoring and Assessment.*

Madikizela – 80% (conducted the research, wrote the manuscript);

Chimuka – 20% (supervisor, reviewed the manuscript).

Occurrence of naproxen, ibuprofen and diclofenac residues in wastewater and river water of KwaZulu-Natal Province in South Africa

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Abstract

The present paper reports a detailed study that is based on the monitoring of naproxen, ibuprofen and diclofenac in Mbokodweni River and wastewater treatment plants (WWTPs) located around the city of Durban in KwaZulu-Natal Province of South Africa. Target compounds were extracted from water samples using a multi-template molecularly imprinted solid-phase extraction prior to separation and quantification on a high performance liquid chromatography equipped with photo diode array detector. The analytical method yielded the detection limits of 0.15, 1.00 and 0.63 µg/L for naproxen, ibuprofen and diclofenac, respectively. Solid-phase extraction method was evaluated for its performance using deionized water samples that were spiked with 5 and 50 µg/L of target compounds. Recoveries were greater than 80% for all target compounds with RSD values in the range of 4.1 to 10%. Target compounds were detected in most wastewater and river water samples with ibuprofen being the most frequent detected pharmaceutical. Maximum concentrations detected in river water for naproxen, ibuprofen and diclofenac were 6.84, 19.2 and 9.69 µg/L, respectively. The concentrations of target compounds found in effluent and river water samples compared well with some studies. The analytical method employed in this work is fast, selective, sensitive and affordable, therefore, it can be used routinely to evaluate the occurrence of acidic pharmaceuticals in South African water resources.

Keywords: molecularly imprinted solid-phase extraction, wastewater, river water, naproxen, ibuprofen, diclofenac

Introduction

Naproxen, ibuprofen and diclofenac are weak organic acids that belong to the group of non-steroidal anti-inflammatory drugs (NSAIDs) (Table 1). Compounds such as ibuprofen and naproxen are widely used by humans for the treatment of rheumatoid arthritis (Mahkam and Poorgholy 2011). These compounds become part of the human waste and they are excreted into the environment as un-metabolized parent compounds and metabolites (Koutsouba et al. 2003). High concentration of pharmaceutical compounds enter wastewater treatment plants (WWTPs) daily through urinary or fecal excretion and from pharmaceutical manufacturing facilities (Farre et al. 2001). Due to the polar nature of naproxen, ibuprofen and diclofenac, they escape the wastewater treatment process easily and contaminate the river water.

Mostly, the environmental monitoring of NSAIDs involves the use of chromatographic tools with solid-phase extraction (SPE) for the reduction of matrix effects and pre-concentration of target compounds. For solid-phase extraction of naproxen, ibuprofen and diclofenac, sorbents such as multi-walled carbon nanotubes (Dahane et al. 2013), single-template molecularly imprinted polymer (Farrington and Regan 2007), multi-template molecularly imprinted polymer (Madikizela and Chimuka 2016; Duan et al. 2013), Oasis HLB (Zhao et al. 2009), Oasis MCX (Lindqvist et al. 2005), C_{18} (Rigobello et al. 2013) have been used. Most of these SPE sorbents except the molecularly imprinted polymers have limited selectivity, and their single usage leads to the production of waste and economic disadvantage. Recent studies are focusing on the application of molecularly imprinted polymer (MIP) for SPE due to its properties that include thermal stability, re-usability, improved selectivity, mechanical strength, etc (Prasad and Rai 2013).

Development of molecularly imprinted solid-phase extraction (MISPE) for NSAIDs is well documented (Caro et al. 2005; Duan et al. 2013; Zorita et al. 2008). However, such technique is not yet fully exploited for the routine

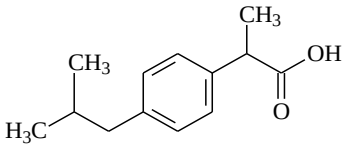
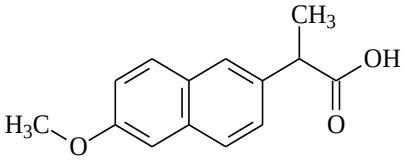
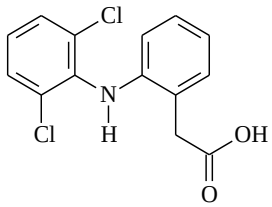
monitoring of selected NSAIDs in wastewater and river water. This is probable due to the slow progress/success in the development of multi-template molecularly imprinted polymers (MIPs) for this purpose. This is important in order to employ a cheap analysis method as there is a growing need for the determination of NSAIDs in aqueous samples.

NSAIDs in aqueous samples such as wastewater, river water and drinking water have been studied extensively in well developed areas such as European and American countries (Carmona et al. 2014; Dahane et al. 2013; Santos et al. 2005; Yu et al. 2006; Yu et al. 2013). However, most African countries including South Africa are lagging behind in this aspect due to limited access to the robust analytical methods and instrumentation. Regardless of these facts, few papers have been published recently that reports on the presence of NSAIDs in South African wastewater and surface water (Agunbiade and Moodley 2014; Agunbiade and Moodley 2016; Amdany et al. 2014; Amdany et al. 2015; Matongo et al. 2015a; Matongo et al. 2015b). These papers demonstrated the occurrence of such compounds in low µg/L levels in wastewater and rivers found in South African major cities that includes Durban and Johannesburg. Moreover, in other African countries such as Kenya and Algeria, all three target compounds have been detected in wastewater, river water and ground water (Kermia et al. 2016; K'oreje et al. 2016). Transportation of pharmaceuticals from wastewater to drinking water has already been reported in Europe (Carmona et al. 2014). This might be due to compounds being hydrophilic and stable in aqueous medium, hence the low removal efficiencies have been documented in some cases. The reported removal efficiencies for naproxen, ibuprofen and diclofenac are in the ranges of 73-100%, 55-100% and 9-98%, respectively (Kermia et al. 2016; Larsson et al. 2014; Lindqivist et al. 2005; Yu et al. 2006).

As a consequence, it is highly important for South African Scientists to develop procedures for the monitoring of NSAIDs in wastewater and rivers as some of

these sites are not restricted from the public use. Therefore, this study is designed to focus on the application of multi-template MIP for the extraction of naproxen, ibuprofen and diclofenac from wastewater and river water. The aim of the study was to determine the concentrations of selected compounds in WWTPs managed by eThekwini Municipality in KwaZulu-Natal Province which is ranked number 3 in terms of pharmaceutical consumption in South Africa (Matongo et al. 2015b). EThekwini Municipality have approximately 28 WWTPs, and there is currently no available data on the simultaneous monitoring of naproxen, ibuprofen and diclofenac in most of these sewage treatment facilities. Therefore, this is the first detailed study based on the occurrence of these acidic pharmaceuticals in the effluent of Amanzimtoti, New Germany and Umhlathuzana WWTPs. This is also the first study that is aimed to monitor the presence of naproxen, ibuprofen and diclofenac in Mbokodweni River.

Table 1 Common name, IUPAC name and Molecular structures of target compounds.

Common name	IUPAC name	Molecular structure
Ibuprofen	α-methyl-4-(2-methylpropyl)benzene acetic acid	
Naproxen	(S)-6-methoxy-α-methyl-2-naphthaleneacetic acid	
Diclofenac	2-[(2,6-dichlorophenyl)amino]benzene acetic acid	

Materials and methods

Analytical reagents

Naproxen (98%), ibuprofen (≥ 98%) and diclofenac sodium salt were purchased from Sigma-Aldrich (Steinheim, Germany) and used as standards and templates in the synthesis of MIP. 2-vinylpyridine (97%), 1,1'-azobis-(cyclohexanecarbonitrile) (98%), ethylene glycol dimethacrylate (98%) and toluene (99.7%) purchased from Sigma-Aldrich (Steinheim, Germany) were used in the synthesis of MIP as functional monomer, radical initiator, cross linking monomer and porogen, respectively. HPLC grade methanol (≥99.9%) from Sigma-Aldrich (Steinheim, Germany), HPLC-grade acetonitrile (≥99.9%)

and glacial acetic acid (100%) purchased from Merck (Darmstadt, Germany) were used as solvents. Formic acid (approx. 98%) was purchased from Fluka (Steinheim, Germany) and used in the chromatographic mobile phase.

Sampling and sample pre-treatment

Influent and effluent samples were collected from WWTPs located around the city of Durban in the Province of KwaZulu-Natal, South Africa (Table 2). Samples were also collected from three points in Mbokodweni River (identified as Mbokoweni River A, B and C in this paper) that is found in South of Durban city. In this case, Mbokodweni River A represent a river sample that was collected approximately 1 km downstream from Amanzimtoti WWTP outfall. Whereas, Mbokodweni River B and C samples were collected from 1 km and 3 km upstream of the WWTP. These samples were collected monthly from January to May in 2016 using glass bottles that were thoroughly cleaned with soap, deionized water and rinsed in sampling sites with real sample. Samples were immediately protected from light and transported to the laboratory where they were filtered twice with filter papers having pore sizes of 10 µm and 0.45 µm purchased from Munktell and Filtrak GmbH (Bernstein, Germany) and Millipore (Darmstadt, Germany), respectively. pH in each sample was adjusted to 2.5, thereafter, samples were stored in the refrigerator at 4 °C until further processing.

Synthesis of multi-template molecularly imprinted polymer

Synthetic procedure was adopted elsewhere (Dai et al. 2012; Duan et al. 2013) and modified in the previous work (Madikizela and Chimuka 2016; Madikizela et al. 2016). Synthesis was performed by dissolving 20 mg of 1,1’-azobis-(cyclohexanecarbonitrile) in 50 mL of toluene, thereafter, 1.51 mL of ethylene glycol dimethacrylate was added. The reaction flask was purged with nitrogen

for 10 minutes and sealed. Then, the reaction was allowed to take place with constant stirring in an oil bath set at 70 °C for 8 hours. Thereafter, naproxen (76.60 mg), ibuprofen (68.69 mg) and diclofenac (106.04 mg) were dissolved in 25 mL of acetonitrile, followed by the addition of 0.25 mL 2-vinylpyridine, 3.85 mL ethylene glycol dimethacrylate, 60 mg 1,1'-azobis-(cyclohexanecarbonitrile) and 25 mL of toluene. These contents were homogenized and transferred to the product obtained in the first step. The resulting mixture was purged with nitrogen gas for 10 minutes and sealed. The reaction was allowed to polymerize in an oil bath set at 70 °C for 16 hours. The obtained polymer dried at 60 °C, milled, sieved and particles ranging from 25 to 50 µm were collected. Naproxen, ibuprofen and diclofenac were removed from the polymer using a mixture of 10% (v/v) acetic acid in acetonitrile.

Multi-template molecularly imprinted solid-phase extraction

Solid-phase extraction cartridge (3 mL) was packed with a slurry that was made with 50 mg of MIP and acetonitrile. Frits were employed below and above the MIP to safeguard against the sorbent loss.

MISPE procedure was adopted from published work (Madikizela and Chimuka 2016). Prior to loading of samples, each MISPE cartridge was conditioned with 2 mL of acetonitrile and equilibrated with 2 mL of acidified deionized water (pH 2.5). With the aid of the vacuum pump, 50 mL of acidified sample (pH 2.5) was percolated at 0.3 mL/min. Thereafter, 2 mL of 10% (v:v) methanol in water was used to wash the cartridge prior to elution of retained compounds with 20% (v:v) acetic acid in acetonitrile (2 mL). The eluted extract (20 µL) was injected into the high performance chromatography (HPLC) system.

Chromatographic separation and quantification

Separation and quantification of target compounds was performed on an HPLC system purchased from Shimadzu Corporation (Kyoto, Japan). HPLC was equipped with an online mobile phase degasser unit (Model: DGU-20A3), 20 µL sample loop, pump (Model: LC-20AB), and photo diode array detector (Model: SPD-M20A). Compounds were separated on a Kinetex C_{18} HPLC column (150 x 4.6 mm x 2.6 µm) purchased from Phenominex (California, USA) using a mixture of acetonitrile: 0.2% formic acid in water (60:40, v:v) as mobile phase at a flow rate of 0.8 mL/min. Naproxen was monitored at 230 nm, whereas, ibuprofen and diclofenac were both studied at 200 nm. The chromatographic system was equipped with Shimadzu LC solutions software for data collection and processing.

Monitoring of physicochemical parameters

Physicochemical properties such as sample pH, conductivity, salinity, dissolved oxygen and total dissolved solids were measured in sampling sites using a calibrated Bante900P multi-parameter water quality meter that was purchased from Bante instruments (Shanghai, China). The calibration of the multi-parameter water quality meter was performed using the pH calibration buffers (pH 4, 7 and 10) and conductivity calibration solutions (84 µS/cm, 1413 µS/cm and 12.88 mS/cm) that were provided by the supplier. Single point calibration for dissolved oxygen was carried out in air.

Table 2 Sampling sites and GPS co-ordinates representing the geographical location of study areas.

Sampling site	GPS Co-ordinate
Amanzimtoti WWTP	S30.00749° E30.91720°
Marrianridge WWTP	S29.87692° E30.88397°
New Germany WWTP	S29.80586° E30.89602°
Northern WWTP	S29.79635° E30.99630°
Shallcross WWTP	S29.87692° E30.88397°
Mbokodweni River A	S30.00592° E30.92440°
Mbokodweni River B	S30.00876° E30.90587°
Mbokodweni River C	S30.00460° E30.90045°

Results and discussion

Performance of analytical method

As shown in Fig. 1, well resolved peaks within six minutes for all three target compounds were obtained using a reverse-phase chromatographic column. In order to evaluate the performance of the analytical method, figures of merit such as limits of detection (LOD), limits of quantification (LOQ), precision, linearity and extraction recoveries were determined for each analyte using deionized water that was spiked with 5 and 50 µg/L mixture of target compounds. LOD and LOQ were defined as the concentration where target compounds gave a signal to noise ratio of 3 and 10, respectively. The detection and quantification limits (Table 3) were similar to those reported for the analysis of the same compounds in wastewater using HPLC with photodiode array detection (Payan et al. 2011; Santos et al. 2005). Calibration curves were plotted for each target compound in the concentration range of 30-1000 µg/L. All calibration curves were linear with correlation coefficients greater than 0.99. As shown in Table 3, the extraction recoveries were greater than 80% with

relative standard deviation (RSD) values ranging from 4.1 to 10%, which is an indication of accepted accuracy and precision.

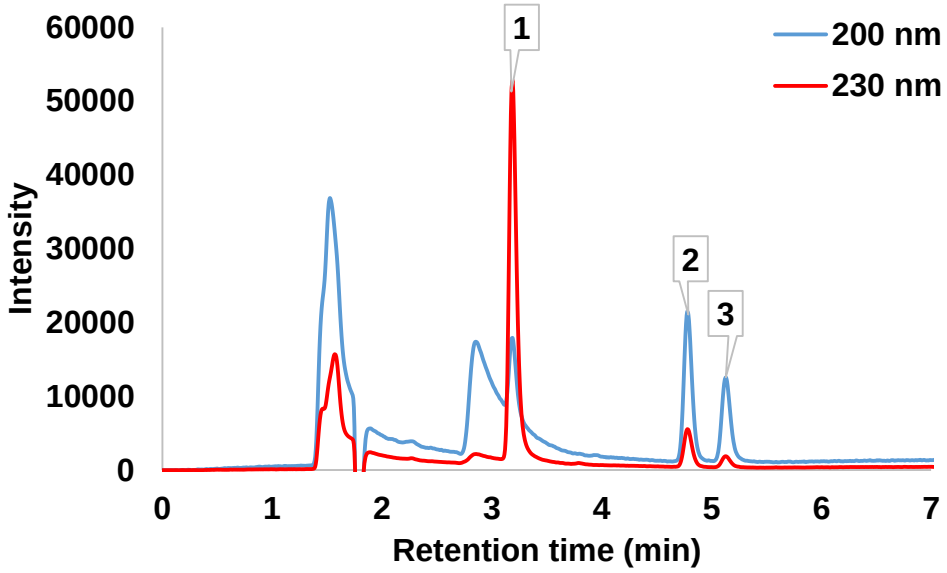


Fig. 1 Chromatograms recorded at 200 and 230 nm for the separation of 1000 µg/L of naproxen (peak 1), diclofenac (peak 2) and ibuprofen (peak 3).

Table 3 Limits of detection, limits of quantification, linearity, relative standard deviation and recovery studies (n=5).

Compound	LOD (µg/L)	LOQ (µg/L)	Linearity (R^2)	Recovery (%) ± RSD (%)	
				5 µg/L	50 µg/L
Naproxen	0.15	0.49	0.9925	83 ± 8.6	84 ± 4.1
Ibuprofen	1.00	3.33	0.9919	101 ± 10	99 ± 8.7
Diclofenac	0.63	2.11	0.9954	81 ± 9.6	86 ± 4.8

Study areas

Wastewater treatment plants sampled in this study have been described in previous studies (Bux and Kasan 1994; Mhlanga et al. 2009; Madikizela et al. 2014; Nzimande 2014). New Germany and Northern WWTPs treat wastewater from the local industries and domestic sources (Bux and Kasan 1994; Nzimande 2014). The treated water is then discharged into the nearest rivers. Shallcross and Mariannridge WWTPs are both located in a site known as Umhlathuzana Works. Their effluent is combined prior to the discharge point. Shallcross receives domestic wastewater only, whereas Mariannridge receives wastewater from industrial (30%) and domestic (70%) sources (Mhlanga et al. 2009). For the purpose of this work, the combined effluent from Shallcross and Mariannridge WWTPs is identified as Umhlathuzana WWTP effluent. Amanzimtoti WWTP receives water from industrial areas and semi-urban areas (Madikizela et al. 2014). Previously, a non-steroidal anti-inflammatory drug known as ketoprofen and triclosan (antibacterial agent) were detected in both influent and effluent of Amanzimtoti WWTP (Madikizela et al. 2014). Mbokodweni River was sampled on the upstream (2 points) and downstream (1 point) of Amanzimtoti WWTP outfall. On the upstream of the River, there is an informal settlement on the river banks with poor sanitation system.

Occurrence of naproxen, ibuprofen and diclofenac in wastewater and river water

Typical chromatograms obtained for environmental analysis are given in Fig. 2. Target compounds were identified in environmental samples based on matching the retention times and photodiode array spectra (Fig. S1) with those of standard solutions. To simplify the presentation of some Figures, the use of acronyms as described in Table 4 was applied.

The results obtained for wastewater and river water concentrations are summarized in Fig. 3 and Table 5. The maximum concentrations in wastewater and river water were compared with the data available in literature (Table 6). As can be seen in Fig. 3, the highest concentration of 221 µg/L was obtained for ibuprofen in Northern WWTP influent with the average concentration of 72 µg/L. The maximum concentration of 128 µg/L with average of 120 µg/L have been reported for Northern WWTP influent located in Johannesburg, South Africa (Amdany et al. 2014). Ibuprofen was also the most frequently detected acidic pharmaceutical in various samples as reported for different matrices in other studies (Bayen et al. 2013; Carmona et al. 2014). This observation was not surprising as ibuprofen has been reported to be the most consumed pharmaceutical in South Africa among the three NSAIDs selected in this study (Matongo et al. 2015a). The average concentration of ibuprofen in Northern WWTP effluent was 10 µg/L, whereas 12 µg/L has been reported previously (Matongo et al. 2015b). Traces of ibuprofen were also detected in river water. At this point, the source of pharmaceuticals in the river could not be traced as the compounds were also detected in the upstream of the river.

The concentrations for naproxen were generally lower in all samples except in New Germany WWTP influent, which could be explained by the lower consumption of this drug in South Africa as per the published script lines (Matongo et al. 2015a). Although the consumption of naproxen is low in South Africa, its presence in the wastewater could not be ignored as it has also been found present in other WWTPs located in another South African Province (Amdany et al. 2014). In most cases, there have been a decrease of pharmaceuticals from the raw influent to the final effluent. This may be due to the adsorption of target compounds on solid sludge. The treatment process in all the investigated WWTPs consists of screening, settling tanks, aeration and chlorination.

Diclofenac was detected in wastewater and river water (Fig 3 and Table 5). The presence of diclofenac in river water was probable due to its poor removal during the wastewater treatment process as reported elsewhere (Rosal et al. 2010; Zorita et al. 2009). The concentration of diclofenac in wastewater and river water is higher than the levels reported for WWTPs and rivers in Europe (Carmona et al. 2014; Gilart et al. 2013; Martin et al. 2012). For instance, the average concentration for diclofenac in wastewater ranges from 3 to 53 µg/L, whereas, a mean concentration of 0.72 µg/L has been reported for the same compound in North WWTP located in Spain (Martin et al. 2012). This could be due to variations in pharmaceutical consumption rates from country to country.

Table 4 Acronyms used for sampling sites

Sampling site	Acronym
Amanzimtoti WWTP influent	AWWTP influent
Amanzimtoti WWTP effluent	AWWTP effluent
New Germany WWTP influent	NGWWTP influent
New Germany WWTP effluent	NGWWTP effluent
Northern WWTP influent	NWWTP influent
Northern WWTP effluent	NWWTP effluent
Shallcross WWTP influent	SWWTP influent
Marrianridge WWTP influent	MWWTP influent
Umhlathuzana WWTP effluent	UWWTP effluent
Mbokodweni river A	River A
Mbokodweni river B	River B
Mbokodweni River C	River C

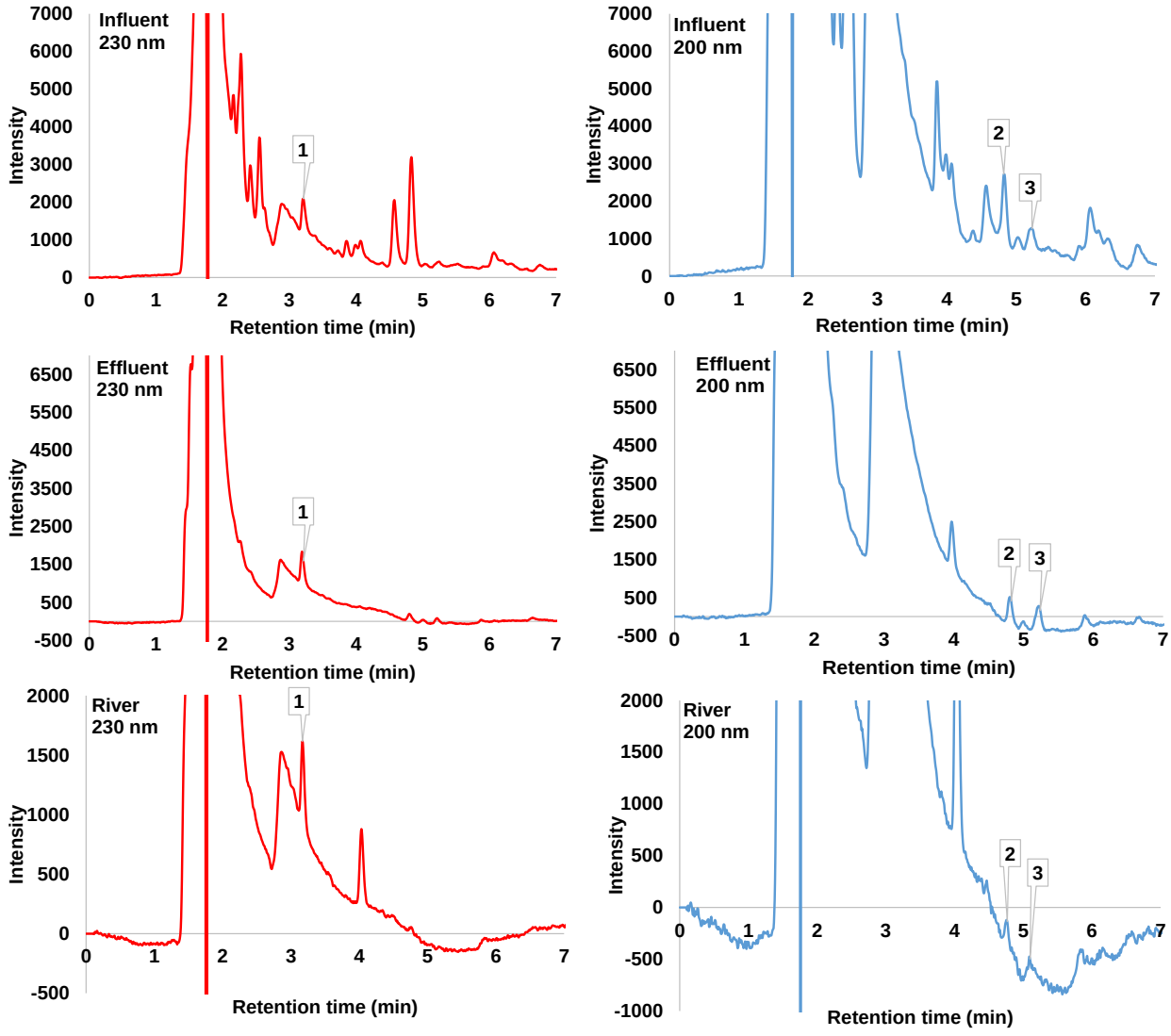


Fig. 2 Chromatograms for Amanzimtoti wastewater influent, effluent and Mbokodweni River A, recorded at 200 and 230 nm. Peaks 1, 2 and 3 are for naproxen, diclofenac and ibuprofen, respectively.

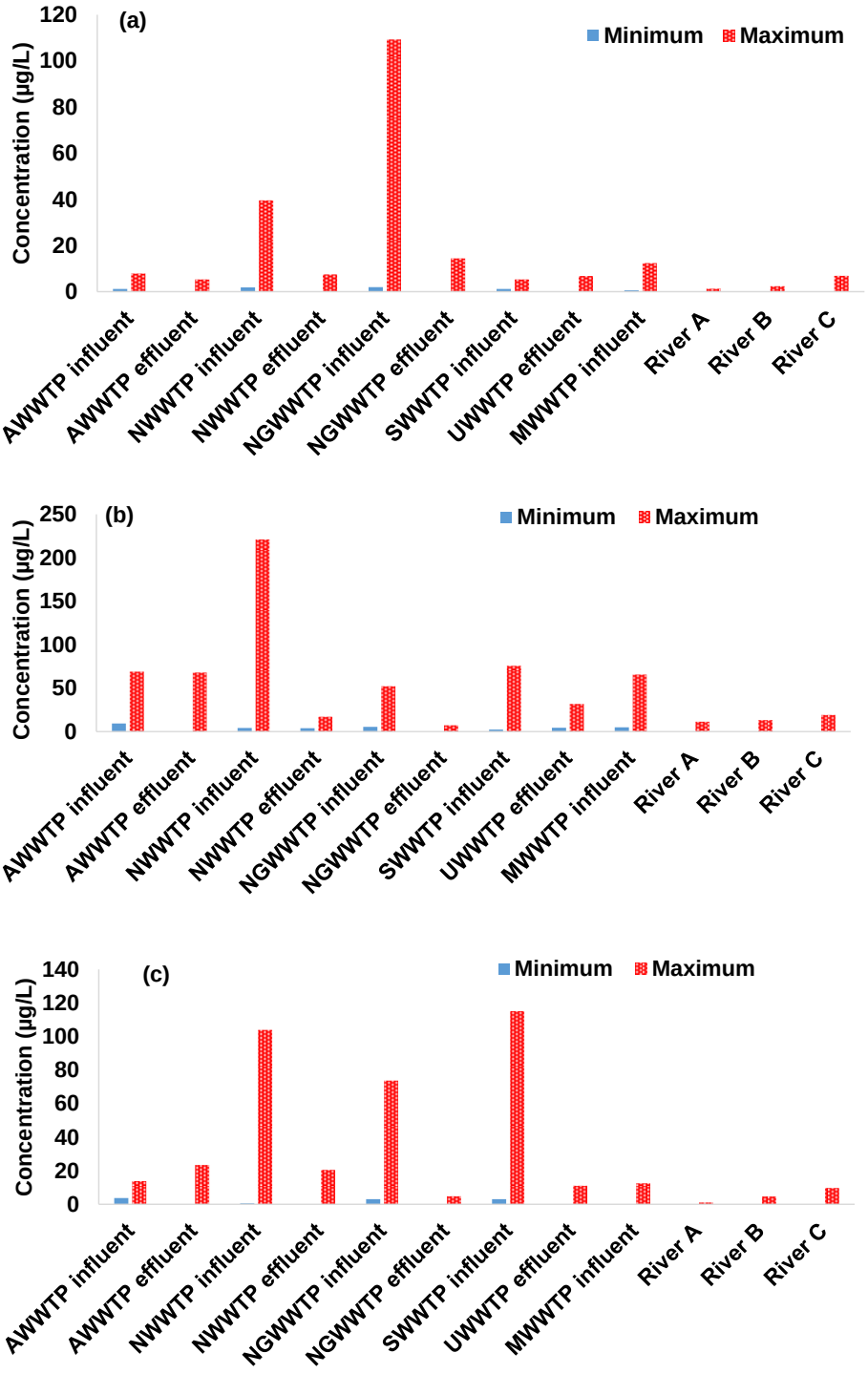


Fig. 3 Minimum and maximum concentrations of naproxen (a), ibuprofen (b) and diclofenac (c) in environmental samples. Acronyms are explained in Table 4.

Table 5 Average concentrations (n = 5) and quantification frequency of naproxen, ibuprofen and diclofenac in water samples.

Sampling site	Naproxen		Ibuprofen		Diclofenac	
	Concentration (µg/L)	Frequency	Concentration (µg/L)	Frequency	Concentration (µg/L)	Frequency
AWWTP influent	3	5	28	5	9	5
AWWTP effluent	3	4	21	5	9	4
NGWWTP influent	37	5	30	5	21	5
NGWWTP effluent	5	4	5	5	3	3
NWWTP influent	11	5	72	5	46	5
NWWTP effluent	4	2	10	5	15	2
SWWTP influent	3	5	34	5	53	5
MWWTP influent	4	5	30	5	6	3
UWWTP effluent	4	2	19	5	4	4
River A	1	2	5	3	0	0
River B	2	3	6	4	4	3
River C	4	2	11	2	5	2

*Average values were calculated considering below detection and quantification limits as zero.

Table 6 Comparison of maximum concentrations (µg/L) found in current study with data in literature.

Compound	Influent	Effluent	River water	Country	Reference
Naproxen	109.3	14.4	6.8	South Africa	Current study
	55.0	13.5	Not studied	South Africa	Amdany et al. 2014
	9.49	0.78	Not studied	Sweden	Larsson et al. 2014
	11.4	3.12	Not studied	Spain	Santos et al. 2005
	52.9	0.83	Not studied	Spain	Santos et al. 2009
Ibuprofen	220.9	67.9	19.2	South Africa	Current study
	128	24.6	Not studied	South Africa	Amdany et al. 2014
	22.8	0.52	Not studied	Sweden	Larsson et al. 2014
	143.0	10.1	Not studied	Spain	Santos et al. 2005
	603	40.2	Not studied	Spain	Santos et al. 2009
	117.5	58.7	8.5	South Africa	Matongo et al. 2015a
	5.8	12.9	62	South Africa	Matongo et al. 2015b
Diclofenac	115.1	23.5	9.7	South Africa	Current study
	2.25	0.36	Not studied	Sweden	Larsson et al. 2014
	<LOD	<LOD	Not studied	Spain	Santos et al. 2005
	<LOD	<LOD	Not studied	Spain	Santos et al. 2009

<LOD: Concentration was less that the method detection limit.

Physicochemical parameters of collected samples

Results for physicochemical analysis are given in Table 7. The pH for all the samples was neutral. Hence the pH of collected solutions was reduced to 2.5 in order to allow for the protonation of target compounds prior to MISPE. Salinity was measured as practical salinity unit (psu) and represents the concentration of the dissolved salts in wastewater. Salinity results indicated that the samples contained small amounts of soluble inorganic salts that were not expected to affect the proposed analytical method. High salinity water is known as water with large quantities of soluble inorganic salts and organic compounds (Zhang et al. 2012). On the other hand, a decrease in TDS between the raw influent and effluent was observed. The results of the current study were much lower than those reported in other study, where Anderson et al (2015) reported a minimum of 981 mg/L for TDS in a wastewater collected from Canada. The conductivity of 703 and 589 µS/cm for wastewater influent and effluent, respectively, has been reported elsewhere (Rosal et al. 2010). In all the investigated WWTPs there was an increase in dissolved oxygen (DO) between the raw influent and final effluent, curtesy of aeration process. In this regard, DO increased from 0.85 to 3.02 mg/L in Amanzimtoti WWTP in which case almost similar concentration of 3.56 mg/L was observed in river water sampled in the downstream of the plant. In comparison to Thesis river in Mpumalanga Province (South Africa) where DO of 1.11 mg/L was recorded (Wanda et al. 2016), Mbokodweni river had much higher value. Based on this data, the WWTPs investigated in this work performs in a similar manner as other treatment works in the world.

Table 7 Physicochemical properties (n = 5) of collected environmental samples. Standard deviations are given as ± values.

Sampling site	Parameter				
	pH	DO (mg/L)	Conductivity (µs/cm)	TDS (mg/L)	Salinity (psu)
Amanzimtoti WWTP influent	7.22 ± 0.19	0.85 ± 0.52	1399 ± 159	699 ± 80	0.70 ± 0.09
Amanzimtoti WWTP effluent	7.61 ± 0.06	3.02 ± 0.52	1110 ± 120	557 ± 60	0.56 ± 0.06
Marrianridge WWTP influent	7.49 ± 0.06	0.74 ± 0.38	1003 ± 57	508 ± 20	0.50 ± 0.03
New Germany WWTP influent	7.02 ± 0.21	0.55 ± 0.28	1206 ± 152	601 ± 72	0.60 ± 0.08
New Germany WWTP effluent	7.17 ± 0.16	3.75 ± 0.52	1000 ± 226	500 ± 115	0.49 ± 0.12
Northern WWTP influent	7.15 ± 0.04	0.64 ± 0.43	959 ± 75	479 ± 36	0.48 ± 0.04
Northern WWTP effluent	7.07 ± 0.17	2.31 ± 0.82	699 ± 19	351 ± 9.3	0.34 ± 0.01
Shallcross WWTP influent	7.25 ± 0.08	0.74 ± 0.42	681 ± 88	341 ± 43	0.33 ± 0.04

Umhlathuzana WWTP effluent	7.25 ± 0.15	3.73 ± 0.90	607 ± 82	302 ± 42	0.29 ± 0.04
River water – sample A	7.32 ± 0.19	3.56 ± 0.73	777 ± 321	388 ± 161	0.38 ± 0.16
River water – sample B	7.17 ± 0.24	3.99 ± 1.63	357 ± 27	178 ± 13	0.17 ± 0.01
River water – sample C	7.17 ± 0.26	4.17 ± 1.61	331 ± 50	165 ± 25	0.16 ± 0.02

4. Conclusion

A rapid analytical method that involves the use of multi-template molecularly imprinted polymer as selective SPE sorbent and HPLC with photodiode array detection for the separation and quantification has been applied for the environmental monitoring of naproxen, ibuprofen and diclofenac. All three pharmaceutical compounds were detected in wastewater and river water. In all samples (influent, effluent and river water), ibuprofen was detected most frequently with higher concentrations. The occurrence of pharmaceuticals in the upstream of Mbokodweni River was observed which could indicate that human activities play a major role in contamination of water resources. The results of this study demonstrated the necessity to conduct more research on the occurrence of acidic pharmaceuticals in all South African water bodies including lakes and dams. Also, the improvement in the wastewater treatment processes is required in order to reduce the pollution of precious resources such as river water.

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REFERENCES

Agunbiade, F. O., Moodley, B. (2014). Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa. *Environmental Monitoring and Assessment, 186,* 7273-7291.

Agunbiade, F. O., Moodley, B. (2016). Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi River, KwaZulu-Natal, South Africa. *Environmental Toxicology and Chemistry, 35(1),* 36-46.

Amdany, R., Chimuka, L., Cukrowska, E. (2014). Determination of naproxen, ibuprofen and triclosan in wastewater using the polar organic chemical integrative sampler (POCIS): A laboratory calibration and field application. *Water South Africa, 40(3),* 407-414.

Amdany, R., Moya, A., Chimuka, L., Cukrowska, E. (2015). Optimization of the temperature for the extraction of pharmaceuticals from wastewater by a hollow fiber silicone membrane. *Analytical Letters, 48,* 2343-2356.

Anderson, J. C., Joudan, S., Shoichet, E., Cuscito, L. D., Alipio, A. E. C., Donaldson, C. S., Khan, S., Goltz, D. M., Rudy, M. D., Frank, R. A., Knapp, C. W., Hanson, M. L., Wong, C. S. (2015). Reducing nutrients, organic micropollutants, antibiotic resistance, and toxicity in rural wastewater effluent with subsurface filtration treatment technology. *Ecological Engineering, 84,* 375-385.

Bayen, S., Zhang, H., Desai, M. M., Ooi, S. K., Kelly, B. C. (2013). Occurrence and distribution of pharmaceutically active and endocrine disrupting compounds in Singapore's marine environment: Influence of hydrodynamics and physical-chemical properties. *Environmental Pollution, 182,* 1-8.

Bux, F., Kasan, H. C. (1994). A microbiological survey of ten activated sludge plants. *Water South Africa, 20*, 61-72.

Carmona, E., Andreu, V., Pico, Y. (2014). Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: From waste to drinking water. *Science of the Total Environment, 484,* 53-63.

Caro, E., Marce, R. M., Cormack, P. A. G., Sherrington, D. C., Borrull, F. (2005). Selective enrichment of anti-inflammatory drugs from river water samples by solid-phase extraction with a molecularly imprinted polymer. *Journal of Separation Science, 28,* 2080-2085.

Dahane, S., Gil Garcia, M. D., Martinez Bueno, M. J., Ucles Moreno, A., Martinez Galera, M., Derdour, A. (2013). Determination of drugs in river and wastewaters using solid-phase extraction by packed multi-walled carbon nanotubes and liquid chromatography-quadrupole-linear ion trap-mass spectrometry. *Journal of Chromatography A, 1297,* 17-28.

Dai, C., Zhang, J., Zhang, Y., Zhou, X., Duan, Y., Liu, S. (2012). Selective removal of acidic pharmaceuticals from contaminated lake water using multi-templates molecularly imprinted polymer. *Chemical Engineering Journal, 211-212,* 302-309.

Duan, Y., Dai, C., Zhang, Y., Ling-Chen. (2013). Selective trace enrichment of acidic pharmaceuticals in real water and sediment samples based on solid-phase extraction using multi-templates molecularly imprinted polymers. *Analytica Chimica Acta, 75*8, 93-100.

Farre, M., Ferrer, I., Ginebreda, A., Figueras, M., Olivella, L., Tirapu, L., Vilanova, M., Barcelo, D. (2001). Determination of drugs in surface water and wastewater samples by liquid chromatography-mass spectrometry: methods and preliminary results including toxicity studies with *Vibrio fischeri. Journal of Chromatography A, 938,* 187-197.

Farrington, K., Regan, F. (2007). Investigation of the nature of MIP recognition: The development and characterisation of a MIP for Ibuprofen. *Biosensors and Bioelectronics, 22(6),* 1138-1146.

Gilart, N., Marce, R. M., Fontanals, N., Borrull, F. (2013). A rapid determination of acidic pharmaceuticals in environmental waters by

molecularly imprinted solid-phase extraction coupled to tandem mass spectrometry without chromatography. *Talanta, 110,* 196-201.

Kermia, A. E. B., Fouial-Djebbar, D., Trari, M. (2016). Occurrence, fate and removal efficiencies of pharmaceuticals in wastewater treatment plants (WWTPs) discharging in the coastal environment of Algiers. *Comptes Rendus Chimie, 19(8),* 963-970.

K'oreje, K. O., Vergeynst, L., Ombaka, D., De Wispelaere, P., Okoth, M., Van Langenhove, H., Demeestere, K. (2016). Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. *Chemosphere, 149,* 238-244.

Koutsouba, V., Heberer, Th., Fuhrmann, B., Schmidt-Baumler, K., Tsipi, D., Hiskia, A. (2003). Determination of polar pharmaceuticals in sewage water of Greece by gas chromatography-mass spectrometry. *Chemosphere, 51,* 69-75.

Larsson, E., al-Hamimi, S., Jonsson, J. A. (2014). Behaviour of nonsteroidal anti-inflammatory drugs and eight of their metabolites during wastewater treatment studied by hollow fibre liquid phase microextraction and liquid chromatography mass spectrometry. *Science of the Total Environment, 485-486,* 300-308.

Lindqvist, N., Tuhkanen, T., Kronberg, L. (2005). Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. *Water Research, 39,* 2219-2228.

Madikizela, L. M., Chimuka, L. (2016). Determination of ibuprofen, naproxen and diclofenac in aqueous samples using a multi-template molecularly imprinted polymer as selective adsorbent for solid-phase extraction. *Journal of Pharmaceutical and Biomedical Analysis, 128,* 210-215.

Madikizela, L. M., Mdluli, P. S., Chimuka, L. (2016). Experimental and theoretical study of molecular interactions between 2-vinyl pyridine

and acidic pharmaceuticals used as multi-template molecules in molecularly imprinted polymer. *Reactive and Functional Polymers, 103,* 33-43.

Madikizela, L. M., Muthwa, S. F., Chimuka, L. (2014). Determination of triclosan and ketoprofen in river water and wastewater by solid-phase extraction and high performance liquid chromatography. *South African Journal of Chemistry, 67,* 143-150.

Mahkam, M., Poorgholy, N. (2011). Imprinted polymers as drug delivery vehicles for anti-inflammatory drugs. *Nature and Science, 9*(5), 163-168.

Martin, J., Camacho-Munoz, D., Santos, J. L., Aparicio, I., Alonso, E. (2012). Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal. *Journal of Hazardous Materials, 239-240,* 40-47.

Matongo, S., Birungi, G., Moodley, B., Ndungu, P. (2015)a. Pharmaceutical residues in water and sediment of Msunduzi River, KwaZulu-Natal, South Africa. *Chemosphere, 134,* 133-140.

Matongo, S., Birungi, G., Moodley, B., Ndungu, P. (2015)b. Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa. *Environmental Science and Pollution Research, 22,* 10298-10308.

Mhlanga, F. T., Brouckaert, C. J., Foxon, K. M., Fennemore, C., Mzulwini, D., Buckley, C. A. (2009). Simulation of a wastewater treatment plant receiving industrial effluents, *Water South Africa, 35*, 447-454.

Nzimande, S. B. T. (2014). Treated wastewater effluent as potential source of emerging bacterial pathogens in surface water, *MSc thesis*, University of KwaZulu-Natal, Durban, South Africa.

Payan, M. R., Lopez, M. A. B., Torres, R. F., Navarro, M. V., Mochon, M. C. (2011). Electromembrane extraction (EME) and HPLC determination of non-steroidal anti-inflammatory drugs (NSAIDs) in wastewater samples. *Talanta, 85,* 394-399.

Prasad, B. B., Rai, G. (2013). Molecular structure, vibrational spectra and quantum chemical MP2/DFT studies toward the rational design of hydroxyurea imprinted polymer. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 105,* 400-411.

Rigobello, E. S., Dantas, A. D. B., Bernardo, L. D., Vieira, E. M. (2013). Removal of diclofenac by conventional drinking water treatment processes and granular activated carbon filtration. *Chemosphere, 92 (2),* 184-191.

Rosal, R., Rodriguez, A., Perdigon-Melon, J. A., Petre, A., Garcia-Calvo, E., Gomez, M. J., Aguera, A., Fernandez-Alba, A. R. (2010). Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. *Water Research, 44,* 578-588.

Santos, J. L., Aparicio, I., Callejon, M., Alonso, E. (2009). Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain). *Journal of Hazardous Materials, 164,* 1509-1516.

Santos, J. L., Aparicio, I., Alonso, E., Callejon, M. (2005). Simultaneous determination of pharmaceutically active compounds in wastewater samples by solid-phase extraction and high-performance liquid chromatography with diode array and fluorescence detectors. *Analytica Chimica Acta, 550,* 116-122.

Wanda, E. M. M., Mamba, B. B., Msagati, T. A. M. (2016). Determination of the water quality index ratings of water in the Mpumalanga and North West provinces, South Africa. *Physics and Chemistry of the Earth, 92,* 70-78.

Yu, J. T., Bouwer, E. J., Coelhan, M. (2006). Occurrence and biodegradability studies of selected pharmaceuticals and personal care products in sewage effluent. *Agricultural Water Management, 86,* 72-80.

Yu, Y., Wu, L., Chang, A. C. (2013). Seasonal variation of endocrine disrupting compounds, pharmaceuticals and personal care products in wastewater treatment plants. *Science of the Total Environment, 442,* 310-316.

Zhang, J., Zhang, Y., Quan, X. (2012). Electricity assisted anaerobic treatment of salinity wastewater and its effects on microbial communities. *Water Research, 46,* 3535-3543.

Zhao, J., Ying, G., Wang, L., Yang, J., Yang, X., Yang, L., Li, X. (2009). Determination of phenolic endocrine disrupting chemicals and acidic pharmaceuticals in surface water of the Pearl Rivers in South China by gas chromatography-negative chemical ionization-mass spectrometry. *Science of the Total Environment, 407,* 962-974.

Zorita, S., Boyd, B., Jonsson, S., Yilmaz, E., Svensson, C., Mathiasson, L., Bergstrom, S. (2008). Selective determination of acidic pharmaceuticals in wastewater using molecularly imprinted solid-phase extraction. *Analytica Chimica Acta, 626,* 147-154.

Zorita, S., Martensson, L., Mathiasson, L. (2009). Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden. *Science of the Total Environment, 407,* 2760-2770.

3.3.6.1 Paper 5 - Supplementary data

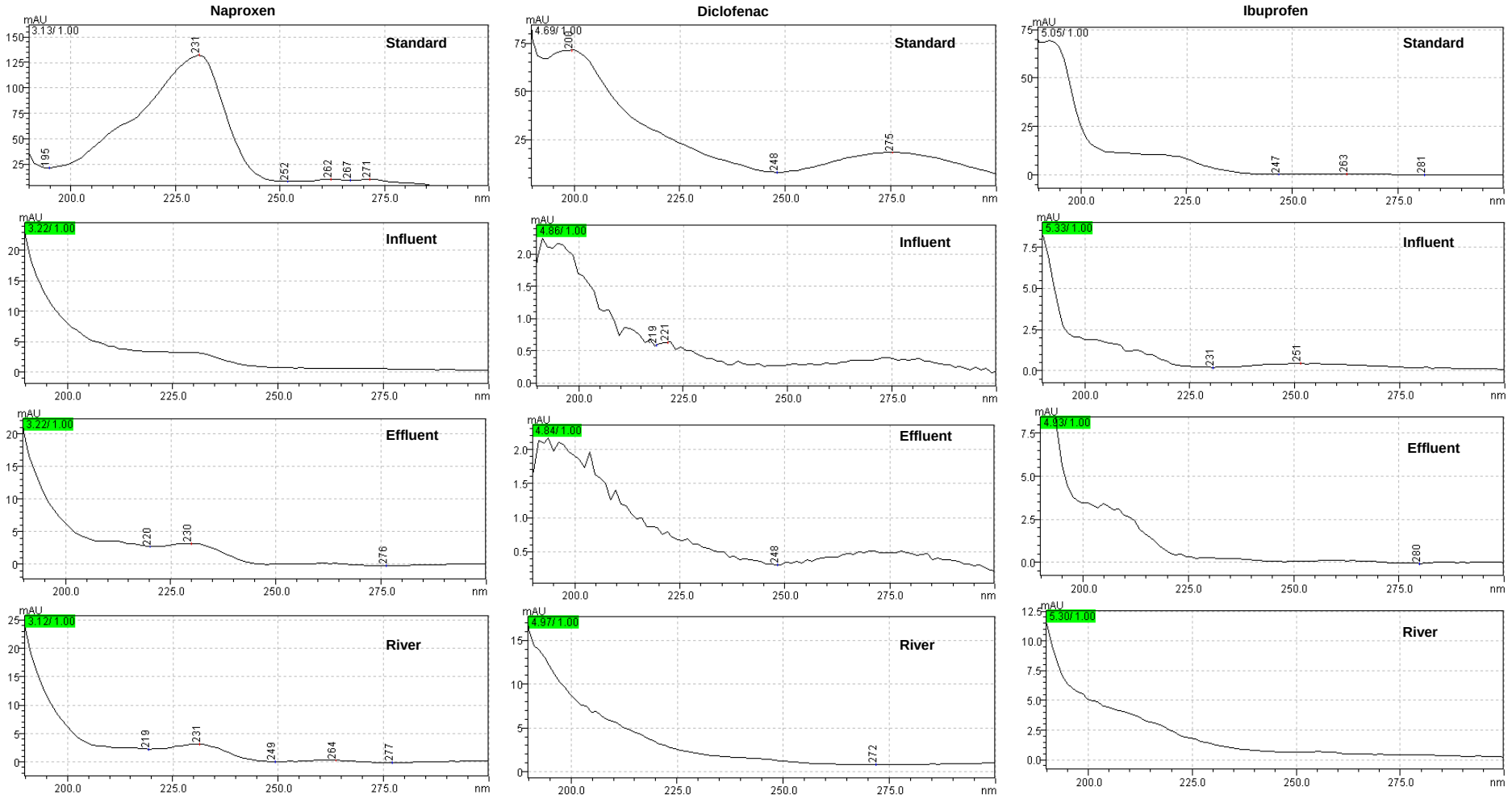


Fig. S1 PDA spectra for wastewater, river water and standard solutions.

3.3.7 Paper 6

Madikizela L.M., Mdluli P.S. and Chimuka, L. Molecularly imprinted solid-phase extraction of naproxen, ibuprofen and diclofenac from Ladysmith water resources in South Africa: An initial assessment. *Submitted.*

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Mdluli – 10% (Assisted in surface characterization, reviewed the manuscript).

Chimuka – 15% (supervisor, reviewed the manuscript).

Molecularly Imprinted Solid-Phase Extraction of Naproxen, Ibuprofen and Diclofenac from Ladysmith Water Resources in South Africa: An Initial Assessment

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ABSTRACT

In this study, the extraction of naproxen, ibuprofen and diclofenac in Ladysmith water resources was conducted by means of a multi-templates molecularly imprinted polymer (MIP) as selective sorbent in solid-phase extraction. Quantification was done using high performance liquid chromatography with photo diode array detection system. Bulk polymerization of MIP was carried out at 70 ℃ for 24 hours and characterized with differential scanning calorimetry, x-ray diffraction and zeta potential. The analytical method detection limits for naproxen, ibuprofen and diclofenac in wastewater treatment plant effluent were 0.23, 1.02 and 0.30 µg L^{-1}, respectively. Recoveries obtained for wastewater, river water, deionized water and drinking water treatment plant (DWTP) samples spiked with 5 µg L^{-1} of target compounds were greater than 80%. All compounds were not detected in DWTP samples, whereas, in river water the concentrations were generally higher in the upstream than the downstream. The maximum concentrations detected in river water for naproxen, ibuprofen and diclofenac were 2.77, 6.72 and 2.58 µg L^{-1}, respectively. Only diclofenac was present in wastewater at concentrations above the limit of quantification. In conclusion, the high levels of naproxen, ibuprofen and diclofenac detected in river water could be attributed to poor sanitation in Ladysmith.

KEYWORDS

Molecularly imprinted polymer, pharmaceuticals, water resources, solid-phase extraction

1. Introduction

Analyte extraction and pre-concentration are very crucial steps in the analysis of environmental pollutants. For instance, in environmental monitoring, both sampling and sample preparation are labour intensive and they constitute more than 80% of the analysis time.[1] To date, several sample preparation techniques including solid-phase extraction (SPE), solid-phase microextraction, hollow fiber-based liquid phase microextraction and stir bar sorptive extraction have been reported for the quantitative analysis of pharmaceuticals in the environment.[2-5] In all these aforementioned procedures, SPE is the most used system where various traditional sorbents that include hydrophilic lipophilic balance (Oasis HLB), Oasis MCX, Strata X and C_{18} are employed.[6-9] Most recently, the application of molecularly imprinted polymers (MIPs) as selective sorbents in solid-phase extraction of pharmaceuticals from aqueous samples have been reported.[10-12] Nowadays, MIPs gain popularity due to high selectivity and thermal stability, re-usability, and stability in aqueous and organic solvents.[13,14]

Once extracted, pharmaceuticals are usually quantified using gas and high performance liquid chromatography (HPLC).[9,15,16] Derivatization is required in gas chromatography (GC) in order to improve the volatility of naproxen, ibuprofen and diclofenac.[15,16] It has been reported that derivatization has a tendency to increase the analysis time and may lead to the formation of unwanted products.[17] Due to derivatization which is one of the cumbersome step in GC, high performance liquid chromatography (HPLC) is a preferred technique for the quantification of naproxen, ibuprofen and diclofenac in environmental samples. Detection of pharmaceuticals using HPLC is usually carried out using photo diode array, fluorescence and mass spectrometry (Martinez-Sena et al., 2016) detectors.[12,18]

Naproxen, ibuprofen and diclofenac are acidic pharmaceuticals that belong to the class of non-steroidal anti-inflammatory drugs (NSAIDs), hence, they are used to treat inflammation and fever in humans.[19] Upon consumption, naproxen, ibuprofen and diclofenac are eliminated with 70, 10 and 10% of unchanged drugs, respectively.[20] The sources of these drugs in the environment are associated with the industries that manufacture medical mixtures, the impact of pharmaceutical

industry on the environment, disposal of expired drugs, hospital wastewater and wastes, and excretion of drugs and their metabolites by animals and humans.[21]

Due to these reasons, naproxen, ibuprofen and diclofenac are widely detected in water samples. In well developed countries such as in Europe, the evidence of environmental monitoring of pharmaceutical compounds exist. In this case, pharmaceuticals have been detected in wastewater influent and effluent, river water, dam water, lake water as well as in drinking water .[9,18,22,23] In the context of African continent, there is currently a lack of database which gives a map out overview of the distribution of pharmaceutical drugs in the environment. This can be due to unavailability of sensitive instrumentation such as liquid chromatography coupled with quadrupole time of flight mass spectrometry detection system as most African research institutions are unable to afford the purchase or/and the running costs associated with this modern technology. To address this, more research is directed towards the development of sample preparation techniques that could enhance the sensitivity of less expensive laboratory instrumentation. In recent years, few papers that are based on the occurrence of naproxen, ibuprofen and diclofenac in South African wastewater and river water have been published.[11,24-27] These published papers focused more on environmental monitoring of these drugs in water samples that were collected from major cities such as Durban and Johannesburg. To date, there are currently no South African reports on the occurrence of naproxen, ibuprofen and diclofenac in water samples collected from rural based areas. Further to this, the assessment of naproxen, ibuprofen and diclofenac in South African studies have only been conducted in wastewater and river water.

To address these problems, this paper was aimed to provide an initial assessment of water quality in rural based Ladysmith by monitoring the occurrence of naproxen, ibuprofen and diclofenac in water resources that includes the wastewater, river water and drinking water. To achieve this aim, the existing method for the synthesis of multi-template molecularly imprinted polymer, SPE application and HPLC quantification was used.[11] The application of selective SPE sorbent was very important as this could enhance the detection of target compounds at low levels.

2. Experimental

2.1. Chemicals, reagents and apparatus

The analytical standards that were used as templates in MIP synthesis were naproxen (98%), ibuprofen (≥ 98%) and diclofenac sodium salt, all purchased from Sigma-Aldrich (Steinheim, Germany). In the synthesis of MIP, 2-vinylpyridine (97%), 1,1'-azobis-(cyclohexanecarbonitrile) (98%), ethylene glycol dimethacrylate (98%) and toluene (99.7%) purchased from Sigma-Aldrich (Steinheim, Germany) were used as functional monomer, radical initiator, cross linking monomer and porogenic solvent, respectively. Solvents used in template removal, solid-phase extraction and chromatographic mobile phase were methanol (≥99.9%, HPLC grade) from Sigma-Aldrich (Steinheim, Germany), acetonitrile (≥99.9%, HPLC grade) from Merck (Darmstadt, Germany), glacial acetic acid (100%) from Merck (Darmstadt, Germany) and formic acid (approx. 98%) from Fluka (Steinheim, Germany). Deionized water was produced from a water purification system purchased from Lasec (Durban, South Africa).

2.2. Synthesis of multi-templates molecularly imprinted polymer

MIP was synthesized based on method reported by Dai *et al.*[13] 2012 and Duan *et al.* [23], and modified for the extraction of naproxen, ibuprofen and diclofenac in our previous work.[11,19,28] In this work, 20 mg of 1,1'-azobis-(cyclohexanecarbonitrile) was dissolved in 50 mL of toluene, thereafter 1.51 mL of ethylene glycol dimethacrylate was added. Nitrogen gas was bubbled for 10 minutes to provide the inert atmosphere. The reaction vessel was sealed and kept in an oil bath set at 70 °C with constant stirring for 8 hours. Thereafter, the following chemicals were added into the reaction mixture; naproxen (76.60 mg), ibuprofen (68.69 mg), diclofenac (106.04 mg), acetonitrile (25 mL), 2-vinylpyridine (0.25 mL), ethylene glycol dimethacrylate (3.85 mL), 1,1'-azobis-(cyclohexanecarbonitrile) (60 mg) and toluene (25 mL). The resulting solution was purged with nitrogen gas for 10 minutes and sealed. Thereafter, the polymerization reaction was refluxed at 70 °C for 16 hours. The produced solid polymer was dried at 60 °C and milled. The polymer was

sieved and the particles ranging from 25 to 50 μm were collected. Non-imprinted polymer (NIP) was synthesized following similar reaction conditions with the exclusion of templates. Polymers were washed repeatedly to ensure complete removal of templates with acetic acid in acetonitrile (10% (v/v)) followed by acetonitrile. Washing solutions from each cycle were analyzed with HPLC for the presence of templates.

2.3. Characterization

Characterization of polymers was done with differential scanning calorimetry (DSC), x-ray diffraction (XRD) and zeta potential. DSC was performed using a thermal analysis instrument (model SDTQ600) from Delaware (Newcastle, USA). Both MIP and NIP were heated from 30 °C to 700 °C using a heating rate of 10 °C/min in DSC under nitrogen purge of 50 mL/min. In XRD analysis, the instrument from Bruker AXS (Karlsruhe, Germany) was equipped with XRD commander for data collection and Eva software for processing. The zeta potentials of polymer particles dispersed in water were determined at 25 °C using a zeta instrument (Model: Nanosight NS 500) obtained from Malvern Instruments Limited (Worcestershire, UK).

2.4. Swelling studies

40 mg of each polymer was transferred into a 15 mL centrifuge tube followed by the addition of 10 mL of appropriate solvent. Solvents investigated were toluene, acetone, acetonitrile and water. The centrifuge tube was sealed and left at room temperature for 48 hours, followed by centrifugation at 4000 rpm for 10 minutes. The excess solvent was discarded and the weight of the wet (swollen) polymer was recorded. Each experiment was done in triplicate. The swelling capacity was calculated using equation (1):

$$\text{Swelling capacity} = \frac{(m_w - m_d)}{m_d} \times 100 \qquad (1)$$

where swelling capacity is expressed as % (m/m), m_w is the weight of the wet MIP/NIP and m_d is the weight of the dry MIP/NIP.[29]

2.5. Sampling and study sites

Water samples were collected from Ladysmith water resources (Table 1 and Fig. 1) using pre-cleaned glass containers. Samples were collected from the following water resources; wastewater treatment plants (WWTPs) (site numbers 3 and 6), river water (site numbers 2, 4, 5 and 7) and drinking water treatment plant (DWTP) (site number 1). Field measurements that includes dissolved oxygen, conductivity, pH, total dissolved solids and salinity were recorded in the sampling sites using a potable Bante900P multi-parameter water quality meter that was purchased from Bante instruments (Shanghai, China). The collected samples were sent to the laboratory, where the suspended solids were immediately removed by filtration through a 0.45 µm membrane filters purchased from Pall Corporation (Michigan, United States). The pH in each sample was adjusted to 2.5, thereafter, samples were kept in the refrigerator at 4 $^{\circ}$C until analysis.

Study sites are located in KwaZulu-Natal province which is positioned on the southeastern seaboard of the Republic of South Africa. The geographic area of the province is 94,361 km^2. This province has a population of just over ten million people with a density of 110 people per/km^2.[30] Ladysmith has one WWTP that receives used water for treatment from the local households in town and surrounding area and small industries, whereas another plant is located in eZakheni Township. EZakheni WWTP only receives wastewater from domestic sources of the township. Water after treatment from both WWTPs is discharged into Klip River. Sampling was done in the upstream and downstream of the Klip River. Within the Ladysmith region, there is a drinking water treatment facility. In this case, the water is withdrawn from the local dam for treatment prior to its release to the consumers. In this study, water prior and post treatment in drinking water treatment facility is referred to as raw water and effluent, respectively.

Table 1 Global Positioning System (GPS) co-ordinates for the sampled water resources.

Sampling site	GPS Co-ordinate	Site number
Ladysmith WWTP	S28.57136°, E29.80273°	3
EZakheni WWTP	S28.63787°, E29.92303°	6
Ladysmith DWTP	S28.56119°, E29.777794°	1
River upstream of Ladysmith WWTP	S28.55212°, E29.74923°	2
River downstream of Ladysmith WWTP	S28.58744°, E29.81491°	4
River upstream of eZakheni WWTP	S28.63435°, E29.91869°	5
River downstream of eZakheni WWTP	S28.63856°, E29.92359°	7

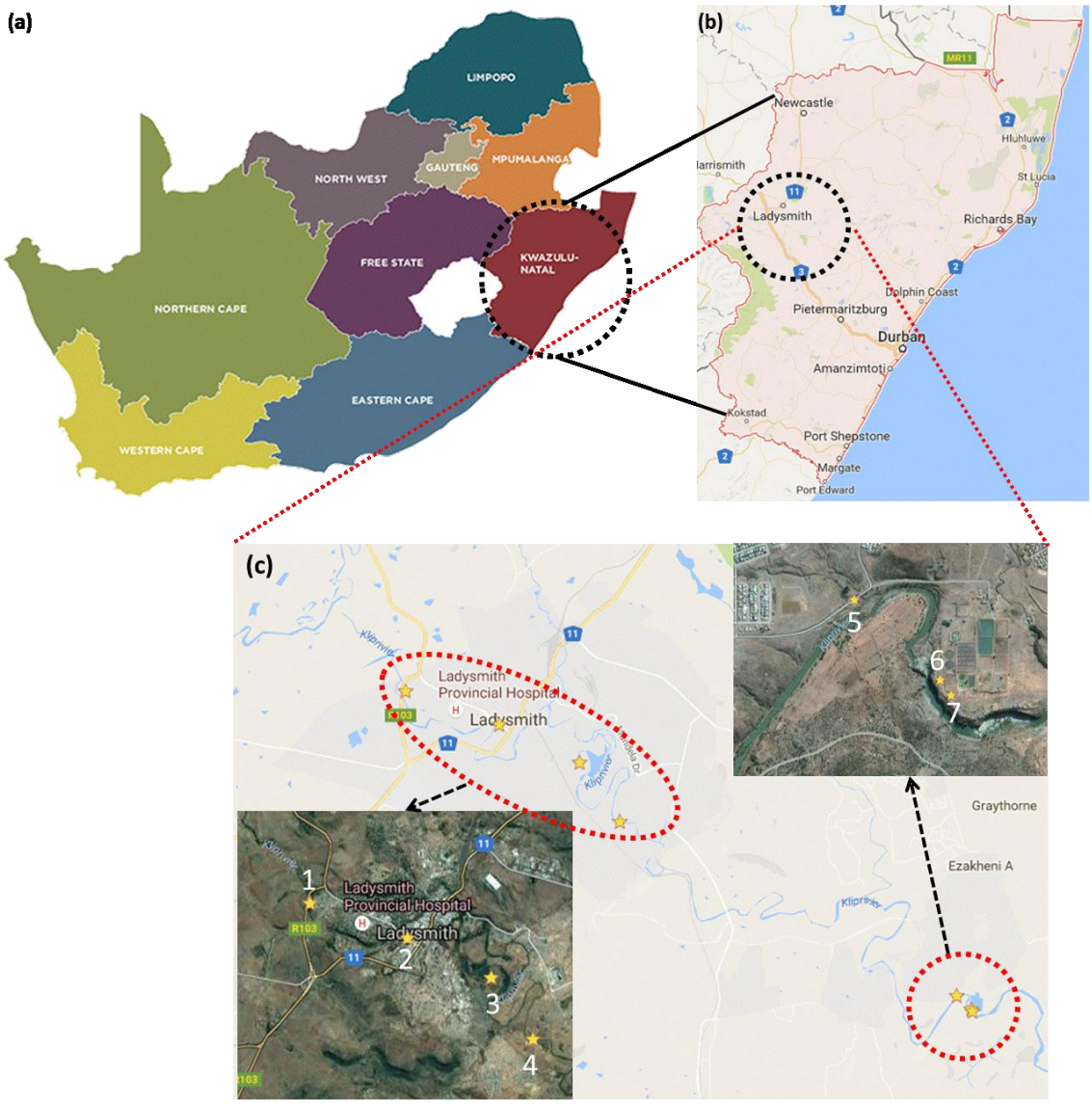


Figure 1 Maps showing the study area, where (a) shows nine South African Provinces, (b) shows the location of Ladysmith in KwaZulu-Natal and sampling sites are shown in (c).

2.6. Molecularly imprinted solid-phase extraction

For sample preparation, the method described previously was adopted and applied.[11] This was done by packing 50 mg of MIP particles in a form of acetonitrile slurry in 3 mL SPE cartridges. Polypropylene frits were fitted at the bottom and top of the prepared MIP sorbent. Molecularly imprinted solid-phase extraction (MISPE) cartridge was conditioned with 2 mL of acetonitrile. Equilibration was done with 2 mL of deionized water at pH 2.5. With the assistance of the vacuum pump, 50 mL of the sample acidified to pH 2.5 was loaded at 0.3 mL/min. Thereafter, washing of the matrix interfering species was done with 2 mL of 10% (v:v) methanol in water. The retained compounds were removed from the sorbent with 2 mL of acetic acid in acetonitrile (20% (v:v)). This was followed by the injection of 20 µL of eluted extract into the HPLC system. For regeneration of MIP after single application, the cartridge was flushed with acetic acid in acetonitrile (20% (v:v)) followed by washing with 3 mL of acetonitrile.

2.7. Chromatographic conditions

Compounds were analyzed using HPLC system that was purchased from Shimadzu Corporation (Kyoto, Japan). HPLC system was equipped with an online mobile phase degasser unit (Model: DGU-20A3), sample loop (20 µL), pump (Model: LC-20AB), photo diode array detector (Model: SPD-M20A) and Shimadzu LC solutions software. The chromatographic separation was performed on a Kinetex C_{18} HPLC column of 150 x 4.6 mm x 2.6 µm obtained from Phenominex (California, USA) using a mixture of acetonitrile: 0.2% formic acid in water (60:40, v:v) as the mobile phase at a flow rate of 0.8 mL min^{-1}. Detector wavelengths were 200 nm for monitoring ibuprofen and diclofenac while naproxen was quantified at 230 nm.

2.8. Quality assurance and analysis

The analytical method was verified using linearity, precision, accuracy, method detection and quantification limits. For analysis, target compounds were observed in the chromatograms of environmental samples based on the retention times

obtained from direct injection of standard solutions and those of real samples after extraction. For quality assurance, the standard solutions and prepared samples were injected in triplicate. The presence of target compounds in environmental samples were confirmed with photo diode array (PDA) spectrum for individual compounds as previously described.[17] This was done by matching the spectrum of the pure compound with the one obtained during the analysis of the environmental sample. External calibration method was used for quantification of naproxen, ibuprofen and diclofenac in water samples.

3. Results and Discussion

3.1. Characterization and swelling analysis

Characterization with DSC resulted in two endothermic peaks for naproxen which were due to the melting transition at 160°C and thermal degradation of the drug at 270°C, as shown in Fig. 2. These two peaks were not observed in both polymers which is an indication of total removal and omission of templates from the MIP and NIP, respectively. DSC thermograms of MIP and NIP were similar with endothermic peak at 360°C which is associated with the thermal decomposition of both polymers.

The X-ray diffractograms (Fig. 3) indicate that the prepared polymers have amorphous nature, due to the lack of peaks. This observation is in agreement with the study which showed the lack of crystallinity for MIP designed for abacavir (an antiviral drug) and the corresponding NIP .[31] Amorphous nature of MIP and NIP was justified due to strong interactions between the template and the monomer used in polymerization.[31]

Zeta potentials for MIP and NIP were -16.5 mV and -18.9 mV, respectively (Fig. 4 (a) and (c)). This means that the hydrodynamic surface charge of both polymers was negative, which also explains high adsorption of templates onto both MIP and NIP surface that was observed previously especially at low pH.[19] It has been reported that the maximum adsorption of target compounds onto the surface of the MIP takes place in acidic conditions where the analytes are protonated.[11,19] MIP that was loaded with all target compounds at pH 2.5 gave a zeta potential of 0 mV

(Fig. 4 (b)). This phenomenon was depicted using a scheme in Fig. 5, which showed the orientation of adsorbed compounds on the surface of MIP molecule. In this figure, a clear picture of the protonated templates which are attached via their carboxyl end is demonstrated. In this case, the pH control played a significant role on the retention of these acidic molecules on the surface of the MIP. This phenomenon implied that the acidic molecules at their ionized forms which can be obtained at high pH would have been less retained by the MIP. However, this can be reversed by lowering the pH of the system which led to high retention of the acids. Further to this, it was previously explained that the hydrogen bonding interactions takes place between the carboxylic groups of target compounds and the nitrogen atom of the functional monomer as shown in previous work.[28]

From swelling analysis, it was discovered that the swelling of the MIP was less when compared to the NIP (Fig. 6). This trend was observed in organic solvents (with different polarities) and water. The swelling trend followed the polarity order of toluene>acetone>acetonitrile>water. It has already been documented that when bulk polymerization was used in the synthesis of MIP, the particles would increase in size (diameter) depending on the type of the solvent used in rebinding and this could cause variations in the binding site cavities.[32] This could ultimately alter the arrangement of functional groups of the MIP, and could lead to a loss of recognition by affecting interactions between the target compounds and the polymer.[32] This was evident in previous study, where the recognition abilities of multi-templates MIP varied in different organic solvents.[28]

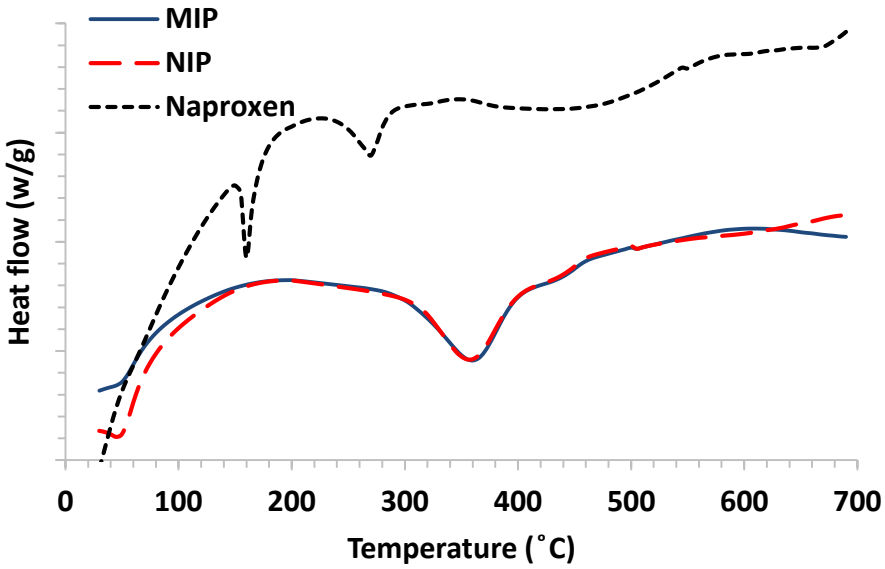


Figure 2 DSC curves for the characterization of MIP, NIP and naproxen.

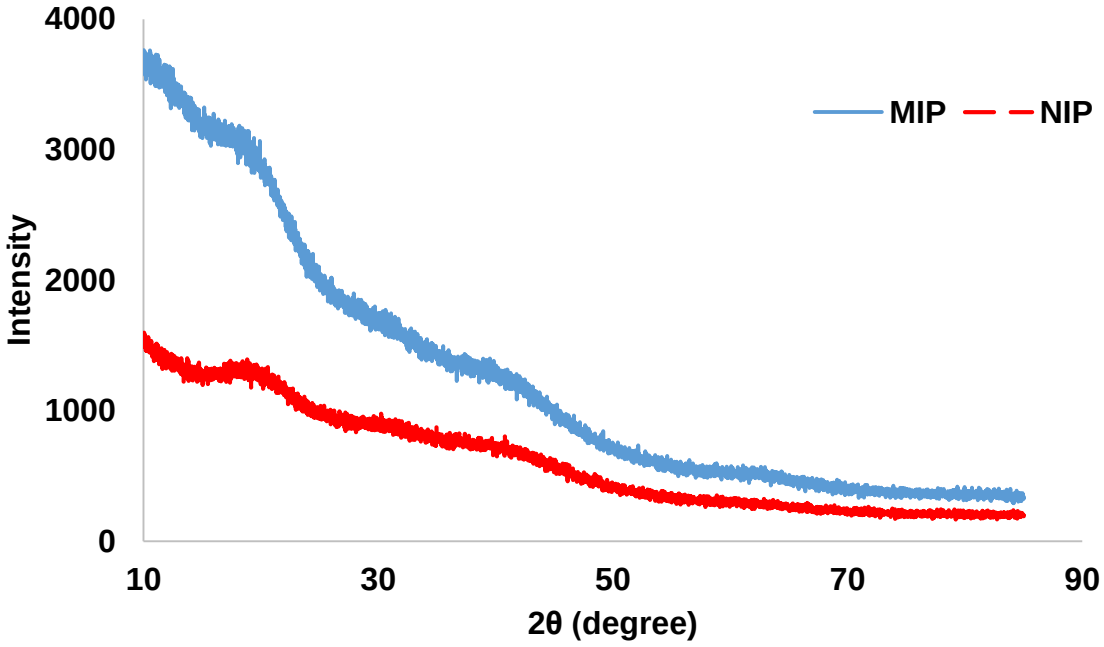


Figure 3 X-ray diffractograms for the characterization of MIP and NIP.

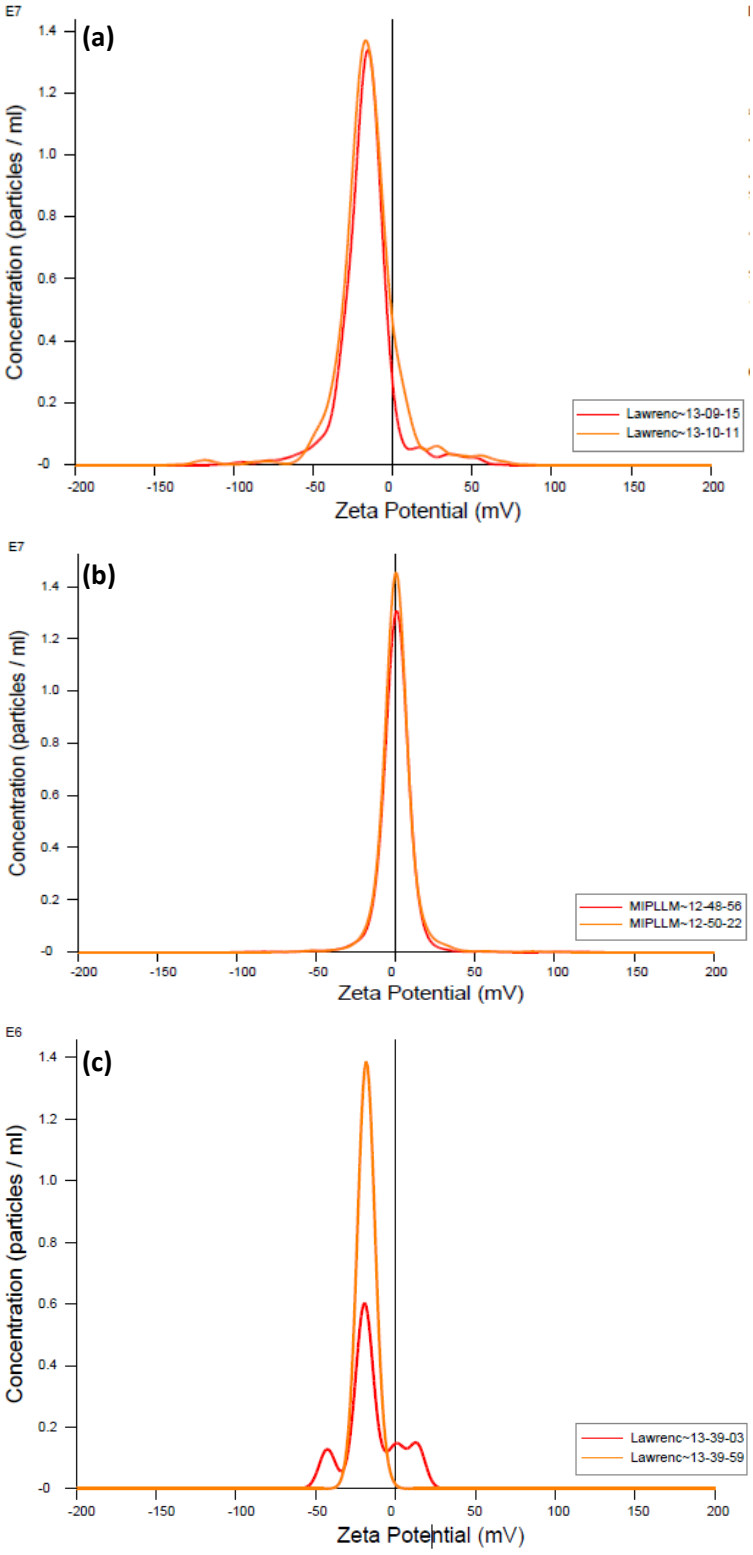


Figure 4 Zeta potential curves for MIP dispersed in water (a), MIP loaded with 1 mg L^{-1} standard solution of naproxen, ibuprofen and diclofenac in water (b), and NIP dispersed in water (c).

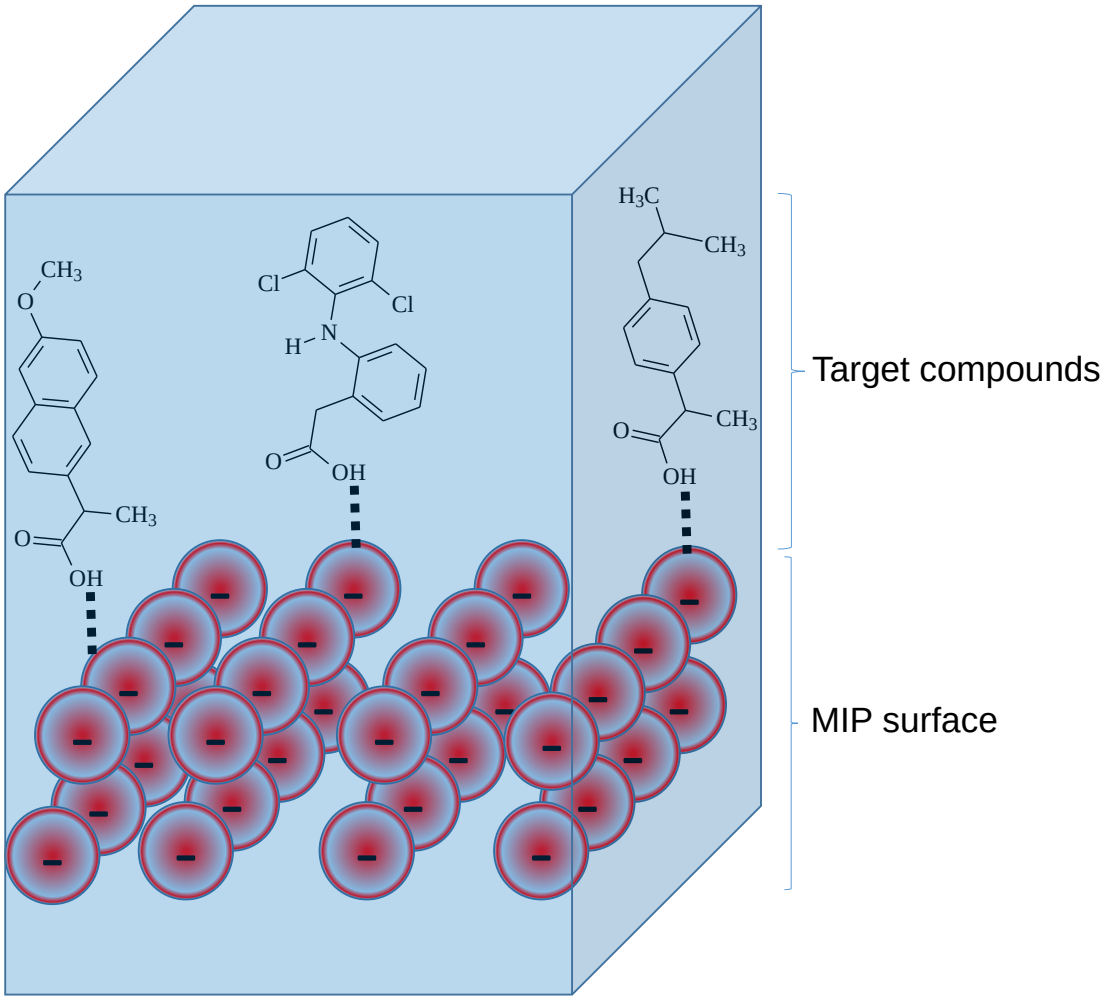


Figure 5 Adsorption mechanism of target compounds onto MIP surface. Hydrogen atom of acidic pharmaceuticals were adsorbed on the negative surface of the polymer. The bond formed between target compounds and the polymer is indicated by dashed lines.

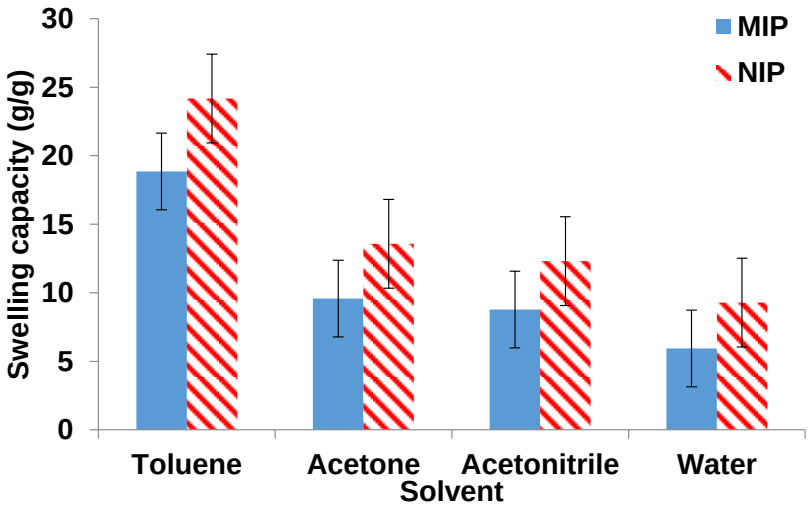


Figure 6 Swelling capacities for polymers in organic solvents and water (n = 3).

3.2. Application

3.2.1. Validation of analytical method

The analytical conditions resulted in well resolved peaks for target compounds and short analysis times (Fig. 7 (a) and (b)). Limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and linearity of the calibration curves were used to assess the validity of the analytical method (Table 2). For this validation, deionized water, raw water from DWTP, WWTP effluent and river water were spiked with all target compounds at a concentration of 5 µg L^{-1}. Chromatograms obtained for spiked and unspiked samples are presented in Fig. 7 (c) to (f). LOD and LOQ were defined as the concentrations which produced the signal to noise ratio of 3 and 10, respectively. Based on LODs and LOQs, the sensitivity of the current method compared well with the results in literature for the HPLC quantification of the same compounds using photo diode array detection.[7,33] The sensitivity of the current method can be improved by increasing sample volume, however, such work can lead to longer analysis time. It was further observed that the analytical method was highly accurate and precise due to high recoveries and lower standard deviations. External calibration curves were linear for all the target compounds with R^2 in the range of 0.994 to 0.999.

Table 2 Detection limits (LOD), quantification limits (LOQ), linearity (R^2), recovery (R) and standard deviation (SD) values (n = 3) obtained for the DWTP, WWTP and river water spiked with target compounds at 5 µg L^{-1}.

Compound	R^2	Drinking water			Deionized water			River water			Wastewater		
		LOD	LOQ	R ± SD	LOD	LOQ	R ± SD	LOD	LOQ	R ± SD	LOD	LOQ	R ± SD
Naproxen	0.995	0.22	0.74	86 ± 11	0.20	0.67	86 ± 5	0.19	0.64	85 ± 8	0.23	0.77	81 ± 7
Ibuprofen	0.999	1.00	3.33	85 ± 8	0.38	1.28	94 ± 13	0.96	3.20	88 ± 11	1.02	3.40	80 ± 9
Diclofenac	0.994	0.25	0.85	88 ± 5	0.48	1.59	73 ± 12	0.24	0.80	80 ± 6	0.30	1.00	84 ± 9

LOD and LOQ are given in µg/L, whereas the recovery is given in % units.

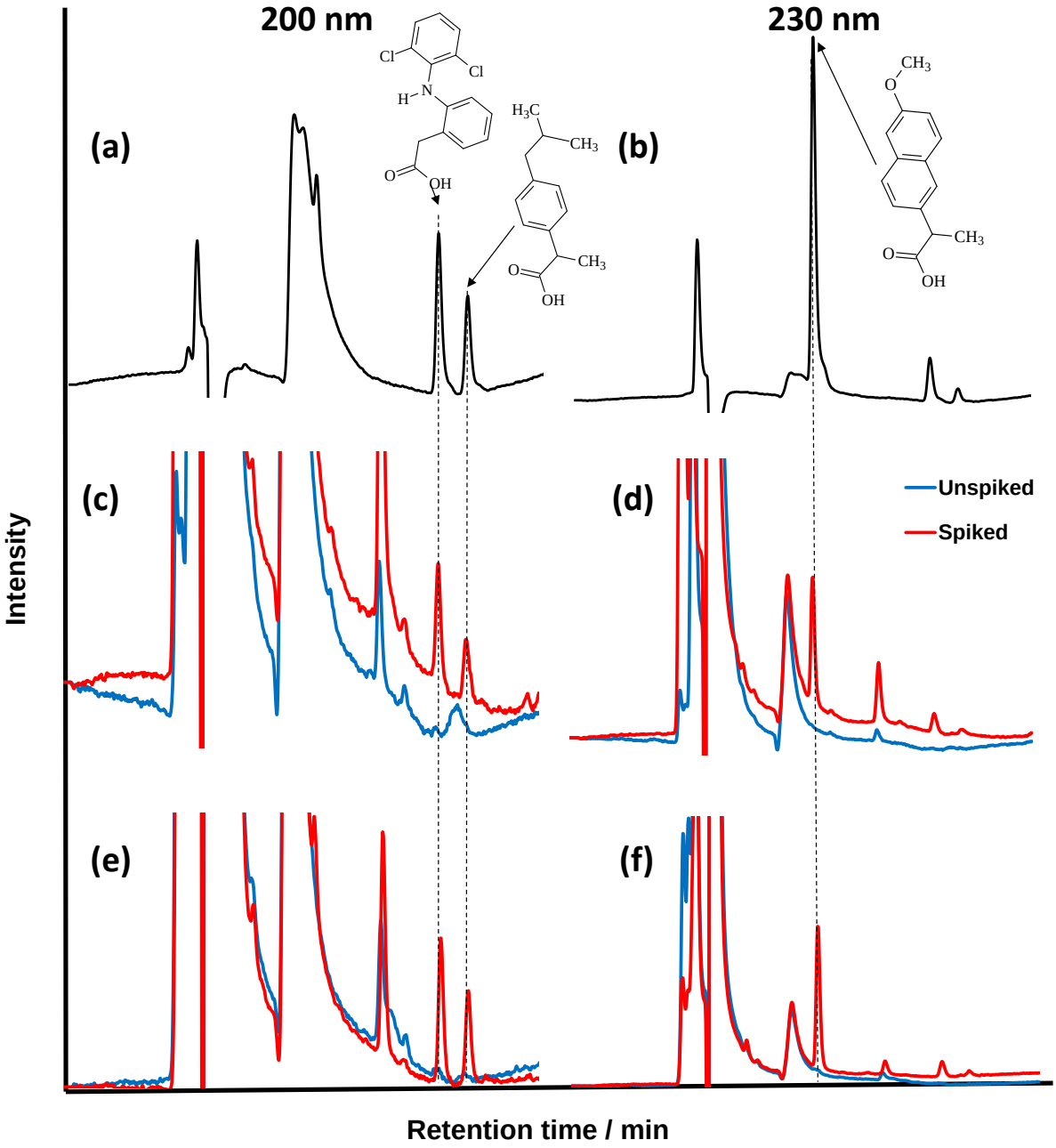


Figure 7 Chromatograms, (a) and (b) recorded at 200 and 230 nm for the separation of 1000 µg L^{-1} of target compounds. The elution in ascending order was naproxen, diclofenac and ibuprofen. Chromatograms, (c) to (f), were recorded at 200 and 230 nm for unspiked and spiked DWTP influent (c and d) and effluent (e and f).

3.2.2. Analysis of naproxen, ibuprofen and diclofenac in water resources

In most cases for WWTPs, the detected concentrations for the three compounds were below the quantification limits (Table 3). The most quantified compound in wastewater samples was diclofenac with the maximum concentration of 1.44 ± 0.29 µg L^{-1} in the effluent. Previously, the relative abundance of ibuprofen and diclofenac in the water system reported in South African study were 0.81 and 2.16 µg L^{-1}, respectively.[24]

In comparison with concentrations reported in various South African WWTPs, the levels of target compounds detected in this study were generally lower.[11,26,34,35] This could be due to the larger population groups served by various WWTPs reported in previous studies. Results can also be influenced by seasonal changes and variations in sample collection times (morning vs afternoon).[27] Further to this, previous studies focused more on the WWTPs that are located in the metropolitan areas which include the well-developed cities of Johannesburg and Durban. In this instance, the concentration ranges reported for diclofenac and ibuprofen in WWTP located in the Msunduzi district in Pietermaritzburg which is a less populated area when compared to Johannesburg and Durban were 12.4 to 22.3 µg L^{-1} and 1.06 to 1.38 µg L^{-1}, respectively.[25] As in the case of the results shown in Table 3, previous work showed that the concentrations for diclofenac in wastewater are generally higher than that of ibuprofen.[25] Poor removal efficiency for diclofenac in Ladysmith WWTP was observed. In literature, there is an evidence of poor removal efficiency of diclofenac during the wastewater treatment process.[9,20] In relation to African studies, diclofenac concentrations were similar to those obtained in wastewater analysis of Algeria.[20] In the same study [20], the concentrations of naproxen and diclofenac did not exceed 10 µg L^{-1} in wastewater samples. To some extent, the concentrations in wastewater were also similar to those obtained in certain European countries.[36] For instance, the median concentrations reported in the influent of a WWTP located in the Spanish Mediterranean area of Valencia for naproxen, ibuprofen and diclofenac were 1.32, 14.6 and 0.53 µg L^{-1}, respectively.[36] Ibuprofen was not detected in effluent, however diclofenac and naproxen were found at median concentrations of 0.34 and 0.13 µg L^{-1}, respectively.[36]

Surprisingly, the concentrations of the three compounds in the river water especially in the upstream were higher than those obtained in wastewater. This is probable due to poor sanitation and lack of WWTPs in this region. The town of Ladysmith is mostly surrounded by rural areas with free grazing of animals such as cattle's and goats along the river. At the time of sampling especially on the upstream of the river, there was a lot of solid materials such as plastics, papers, etc, all these materials were floating on river water which is an indication of direct disposal of solid waste into the river. The reduction of pharmaceuticals in the river downstream might be caused by the dilution effect. Target compounds were previously detected in other South African rivers [11,24, 34] which causes serious concerns to the general public regarding the status of these pollutants. It has already been reported that in few cases the concentration of pharmaceuticals in South African surface water can exceed the one obtained in wastewater.[35] Globally, various concentrations of acidic pharmaceuticals have been reported in river water. In this regard, the maximum concentrations reported for naproxen, ibuprofen and diclofenac in Lis river (Portugal) were 260, 1317 and 38 ng L^{-1}.[37] This actually indicates that there is a serious water pollution caused by pharmaceuticals all over the world. It has been reported that the continuous discharge of these pharmaceutical drugs have contributed to their persistence in the environment.[38] Further to this, the comparison relating the concentrations obtained in this work with previous studies is given in Table 4.

In general, the levels of pharmaceuticals reported in South African water bodies are larger than those reported in other countries. Therefore, the current method was expected to detect the pharmaceuticals present in all samples. However, none of the three compounds were detected in DWTP samples. This is the first South African study that have evaluated the presence of ibuprofen, naproxen and diclofenac in DWTP. Based on European studies, these compounds have been detected in low ng L^{-1} levels in drinking water [9,16] which could indicate that the sensitivity of the current method needs further improvement which could be a separate study all together.

Table 3 Average concentrations (µg L^{-1}) ± standard deviations obtained for target compounds in environmental samples (n = 3). Site numbers are given in brackets.

Compound	Ladysmith WWTP (3)		EZakheni WWTP (6)		Ladysmith DWTP (1)		River water			
	Influent	Effluent	Influent	Effluent	Raw water	Effluent	(2)	(4)	(5)	(7)
Naproxen	<LOQ	<LOQ	nd	<LOQ	nd	nd	2.77 ± 1.66	<LOQ	nd	nd
Ibuprofen	<LOQ	<LOQ	<LOQ	<LOQ	nd	nd	6.72 ± 1.23	<LOQ	5.73 ± 1.16	4.17 ± 2.60
Diclofenac	1.24 ± 0.16	1.44 ± 0.29	1.32 ± 0.64	<LOQ	nd	nd	2.58 ± 1.38	1.82 ± 1.29	nd	nd

Where, nd means the compound was not detected and <LOQ symbolizes the case in which the analyte was detected at a concentration that was below the quantification limit.

Table 4 Maximum concentrations of target compounds detected in various countries.

Compound	Influent /µg L^{-1}	Effluent /µg L^{-1}	Surface water / µg L^{-1}	Drinking water	Country	Reference
Naproxen	3.245	-	0.260	-	Portugal	5
	-	-	0.09	nd	Iran	3
	-	3.176	<LOD	-	Italy	4
	0.24	0.07	<LOD	-	Poland	8
	2.3	0.8	-	-	South Africa	27
	<LOQ	<LOQ	2.77	nd	South Africa	This study
Ibuprofen	19.12	-	1.317		Portugal	5
	-	-	0.12	nd	Iran	3
	-	6.965	<LOD	-	Italy	4
	0.28	0.11	<LOD	-	Poland	8
	5.76	12.94	62	-	South Africa	35
	<LOQ	<LOQ	6.72	nd	South Africa	This study
Diclofenac	0.972	-	0.0038	-	Portugal	5
	-	-	nd	nd	Iran	3
	-	1.171	<LOD	-	Italy	4
	0.46	0.12	<LOD	-	Poland	8
	22.3	19	8.17	-	South Africa	25

	1.32	1.44	2.58	nd	South Africa	This study

<LOD – less than the limit of quantification, nd – not detected.

3.3. Water quality in the studied sites

In general, the water quality of the studied sites was evaluated based on the common physical-chemical parameters. The results (Table 5) obtained compared well with water quality parameters of other African water bodies.[39-41] According to South African National Standards, pH permissible limit is ≥5 to ≤9.7 pH units, while conductivity should not exceed 1700 µS cm^{-1} in drinking water.[41] The increase of conductivity from the raw influent to the effluent could have indicated the poor performance of the WWTPs. This was also evident in river water downstream which could have been caused by the introduction of WWTPs effluents into the surface water. In a previous study,[39] a mean concentration of 8.14 mg L^{-1} for dissolved oxygen (DO) was obtained in river water which corresponded well with the results of the current study. Both low salinity and total dissolved solids (TDS) indicate that the quality of water was fairly good. An increase in TDS from wastewater influent to effluent was observed, this trend has been reported for a WWTP in Kenya (K'oreje et al., 2016).[42]

Table 5 Physicochemical properties measured in the studied sites.

Property	Ladysmith WWTP (3)		EZakheni WWTP (6)		Ladysmith DWTP (1)		River water			
	Influent	Effluent	Influent	Effluent	Raw water	Effluent	(2)	(4)	(5)	(7)
pH	7.29	7.31	7.32	7.24	8.11	7.90	7.71	9.04	9.28	6.57
Conductivity / µS cm^{-1}	418	887	390	494	119	124	286	438	344	331
DO / mg L^{-1}	0.60	0.60	1.98	2.40	5.55	4.40	4.24	8.55	8.21	6.20
Salinity / psu	0.20	0.43	0.19	0.24	0.06	0.06	0.14	0.21	0.16	0.16
TDS / mg L^{-1}	208	444	201	246	59	62	146	219	172	166

DO – dissolved oxygen; TDS – total dissolved solids.

4. Conclusions

In the present study, the selective solid-phase extraction method for naproxen, ibuprofen and diclofenac was applied in the identification and quantification of these acidic drugs from wastewater, DWTP and river water samples. Multi-templates molecularly imprinted polymer was used to enhance the selectivity of the analytical method. The recoveries, detection and quantification limits were determined in order to validate the analytical method. The analytical method was rapid, easy, selective, affordable and sensitive. Target compounds were not detected in DWTP samples, however, all compounds were detected in river water with ibuprofen being the most frequently detected pharmaceutical with maximum concentration of 6.72 μg L^{-1}. Mostly, the concentrations of compounds in wastewater samples were below the limit of quantification except for diclofenac. Poor performance of WWTPs was evident during the analysis of physicochemical properties such as conductivity which demands the urgent need for the upgrade of these facilities. As this study is based on the initial assessment regarding the occurrence of naproxen, ibuprofen and diclofenac in Ladysmith water resources, more analysis needs to be conducted in order to understand the full extent of pollution. Since traces of target compounds were detected, further work in the development of materials such as polymers for the adsorption of such compounds from the environment is required.

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References

1 D.M. Pavlovic, S. Babic, A.J.M. Horvat and M. Kastelan-Macan, Sample preparation in analysis of pharmaceuticals, *TrAC-Trend Anal Chem.*, 2007, **26,** 1062-1075.

2 M.R. Payan, M.A. Bello Lopez, R. Fernandez-Torres, J.L. Perez Bernal and M.C. Mochon, HPLC determination of ibuprofen, diclofenac and salicylic acid using hollow fiber-based liquid phase microextraction (HF-LPME), *Anal Chim Acta.*, 2009, **653,** 184-190.

3 A. Sarafraz-Yazdi, A. Amiri, G. Rounaghi and H. Eshtiagh-Hosseini, Determination of non-steroidal anti-inflammatory drugs in water samples by solid-phase microextraction based sol–gel technique using poly(ethylene glycol) grafted multi-walled carbon nanotubes coated fiber, *Anal Chim Acta.*, 2012, **720,** 134-141.

4 S. Tanwar, M. Di Carro and E. Magi, Innovative sampling and extraction methods for the determination of nonsteroidal anti-inflammatory drugs in water, *J Pharmaceut Biomed.*, 2015 **106,** 100-106.

5 P. Paiga and C. Delerue-Matos, Determination of pharmaceuticals in groundwater collected in five cemeteries' areas (Portugal), *Sci. Total Environ.*, 2016, **569-570,** 16-22.

6 N. Lindqvist, T. Tuhkanen and L. Kronberg, Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters, *Water Res*, 2005, **39,** 2219-2228.

7 J.L. Santos, I. Aparicio, E. Alonso and M. Callejon, Simultaneous determination of pharmaceutically active compounds in wastewater samples by solid phase extraction and high-performance liquid chromatography with diode array and fluorescence detectors, *Anal Chim Acta*, 2005, **550,** 116-122.

8 N. Migowska, M. Caban, P. Stepnowski and J. Kumirska, Simultaneous analysis of non-steroidal anti-inflammatory drugs and estrogenic hormones in water and wastewater samples using gas chromatography–mass spectrometry and gas

chromatography with electron capture detection, *Sci. Total Environ.*, 2012, **441,** 77-88.

9 E. Carmona, V. Andreu and Y. Pico, Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: From waste to drinking water, *Sci. Total Environ.*, 2014, **484,** 53-63.

10 N. Gilart, R.M. Marce, N. Fontanals and F. Borrull, A rapid determination of acidic pharmaceuticals in environmental waters by molecularly imprinted solid-phase extraction coupled to tandem mass spectrometry without chromatography, *Talanta*, 2013, **110,** 196-201.

11 L.M. Madikizela, and L. Chimuka, Determination of ibuprofen, naproxen and diclofenac in aqueous samples using a multi-template molecularly imprinted polymer as selective adsorbent for solid-phase extraction, *J Pharmaceut Biomed.*, 2016, **128,** 210-215.

12 T. Martinez-Sena, S. Armenta, M. de la Guardia and F.A. Esteve-Turrillas, Determination of non-steroidal anti-inflammatory drugs in water and urine using selective molecular imprinted polymer extraction and liquid chromatography, *J Pharmaceut Biomed*, 2016, **131,** 48-53.

13 C. Dai, J. Zhang, Y. Zhang, X. Zhou, Y. Duan and S. Liu, Selective removal of acidic pharmaceuticals from contaminated lake water using multi-templates molecularly imprinted polymer, *Chem Eng J.*, 2012, **211-212,** 302-309.

14 A. Sarafraz-Yazdi and N. Razavi. Application of molecularly-imprinted polymers in solid-phase microextraction techniques, *TrAC-Trend Anal Chem.*, 2015, **73,** 81-90.

15 P. Lacina, L. Mravcova and M. Vavrova, Application of comprehensive two-dimensional gas chromatography with mass spectrometric detection for the analysis of selected drug residues in wastewater and surface water, *J Environ Sci.*, 2013, **25,** 204-212.

16 M. Caban, E. Lis, J. Kumirska and P. Stepnowski, Determination of pharmaceutical residues in drinking water in Poland using a new SPE-GC-

MS(SIM) method based on Speedisk extraction disks and DIMETRIS derivatization, *Sci. Total Environ.,* 2015, **538,** 402-411.

17 L.M. Madikizela, S.F. Muthwa and L. Chimuka, Determination of triclosan and ketoprofen in river water and wastewater by solid phase extraction and high performance liquid chromatography, *S Afr J Chem.,* 2014, **67,** 143-150.

18 L. Patrolecco, N. Ademollo, P. Grenni, A. Tolomei, A.B. Caracciolo and S. Capri, Simultaneous determination of human pharmaceuticals in water samples by solid phase extraction and HPLC with UV-fluorescence detection, *Microchem. J.,* 2013, **107,** 165-171.

19 L.M. Madikizela and L. Chimuka, Synthesis, adsorption and selectivity studies of a polymer imprinted with naproxen, ibuprofen and diclofenac, *J Environ Chem Eng.,* 2016, **4,** 4029-4037.

20 A.E.B. Kermia, D. Fouial-Djebba and M. Trari, Occurrence, fate and removal efficiencies of pharmaceuticals in wastewater treatment plants (WWTPs) discharging in the coastal environment of Algiers, *Comptes Rendus Chimie*, 2016, **19,** 963-970.

21 J. Debska, A. Kot-Wasik and J. Namiesnik, Fate and analysis of pharmaceutical residues in the aquatic environment, *Crit Rev Anal Chem.,* 2004, **34,** 51-67.

22 T.E. Felix-Canedo, J.C. Duran-Alvarez, and B. Jimenez-Cisneros, The occurrence and distribution of a group of organic micropollutants in Mexico City's water sources, *Sci. Total Environ.,* 2013, **454-455,** 109-118.

23 Y. Duan, C. Dai, Y. Zhang and L. Chen, Selective trace enrichment of acidic pharmaceuticals in real water and sediment samples based on solid-phase extraction using multi-templates molecularly imprinted polymers, *Anal Chim Acta,* 2013, **758,** 93-100.

24 F.O. Agunbiade and B. Moodley, Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa, *Environ. Monit. Assess.,* 2014, **186,** 7273-7291.

25 F.O. Agunbiade and B. Moodley, Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi river, KwaZulu-Natal, South Africa, *Environ Toxicol Chem.,* 2016, **35,** 36-46.

26 R. Amdany, L. Chimuka and E. Cukrowska, Determination of naproxen, ibuprofen and triclosan in wastewater using the polar organic chemical integrative sampler (POCIS): A laboratory calibration and field application, *Water SA*, 2014, **40,** 407-414.

27 R. Amdany, A. Moya, L. Chimuka and E. Cukrowska, Optimization of the temperature for the extraction of pharmaceuticals from wastewater by a hollow fiber silicone membrane, *Anal Lett.,* 2015, **48,** 2343-2356.

28 L.M. Madikizela, P.S. Mdluli and L. Chimuka, Experimental and theoretical study of molecular interactions between 2-vinyl pyridine and acidic pharmaceuticals used as multi-template molecules in molecularly imprinted polymer, *React Funct Polym.,* 2016, **103,** 33-43.

29 I. Kaur, S. Rattan, S. Chauhan and N. Gupta, Gamma-radiation-induced grafting of binary mixture of methacrylic acid and 4-vinyl pyridine onto Teflon-FEP film as an effective polar membrane for separation processes, *Nucl Instrum Methods B,* 2010, **268,** 1642-1652.

30 E. Lutge, N. Moodley, A. Tefera, B. Sartorius, T. Hardcastle and D. Clarke, A hospital based surveillance system to assess the burden of trauma in KwaZulu-Natal Province South Africa, *Injury*, 2016, **47,** 135-140.

31 Z. Terzopoulou, M. Papageorgiou, G.Z. Kyzas, D.N. Bikiaris and D.A. Lambropoulou, Preparation of molecularly imprinted solid-phase microextraction fiber for the selective removal and extraction of the antiviral drug abacavir in environmental and biological matrices, *Anal Chim Acta,* 2016, **913,** 63-75.

32 K. Farrington and F. Regan, Investigation of the nature of MIP recognition: The development and characterisation of a MIP for Ibuprofen, *Biosens Bioelectron.,* 2007, **22,** 1138-1146.

33 M.R., Payan, M.A. Bello Lopez, R.F. Torres, M.V. Navarro and M.C. Mochon, Electromembrane extraction (EME) and HPLC determination of non-steroidal anti-inflammatory drugs (NSAIDs) in wastewater samples, *Talanta*, 2011, **85,** 394-399.

34 S. Matongo, G. Birungi, B. Moodley and P. Ndungu, Pharmaceutical residues in water and sediment of Msunduzi River, KwaZulu-Natal, South Africa, *Chemosphere*, 2015, **134,** 133-140.

35 S. Matongo, G. Birungi, B. Moodley and P. Ndungu, Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa, *Environ Sci Pollut Res.*, 2015, **22,** 10298-10308.

36 E. Gracia-Lor, J.V. Sancho, R. Serrano and F. Hernandez, Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia, *Chemosphere*, 2012, **87,** 453-462.

37 P. Paiga, L.H.M.L.M. Santos, S. Ramos, S. Jorge, J.G., Silva and C. Delerue-Matos, Presence of pharmaceuticals in the Lis river (Portugal): Sources, fate and seasonal variation, *Sci. Total Environ.*, 2016, **573,** 164-177.

38 X. Yuan, Z. Qiang, W. Ben, B. Zhu, and J. Liu, Rapid detection of multiple class pharmaceuticals in both municipal wastewater and sludge with ultra high performance liquid chromatography tandem mass spectrometry, *J Environ Sc.*, 2014, **26,** 1949-1959.

39 S. Munyika, V. Kongo, and R. Kimwaga, River health assessment using macroinvertebrates and water quality parameters: A case of the Orange River in Namibia, *Phys Chem Earth*, 2014 **76-78,** 140-148.

40 E.M.M. Wanda, L.C. Gulula, and G. Phiri, Determination of characteristics and drinking water quality index in Mzuzu City, Northern Malawi, *Phys Chem Earth*, 2012, **50-52,** 92-97.

41 E.M.M. Wanda, B.B. Mamba and T.A.M. Msagati, Determination of the water quality index ratings of water in the Mpumalanga and North West provinces, South Africa, *Phys Chem Earth.,* **2016,** 92, 70-78.

42 K. O. K'oreje, L. Vergeynst, D. Ombaka, P. De Wispelaere, M. Okoth, H. Van Langenhove and K. Demeestere, Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya, *Chemosphere*, 2016, **149,** 238-244.

3.3.8 Paper 7

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Chimuka – 20% (supervisor, reviewed the manuscript).

Simultaneous determination of naproxen, ibuprofen and diclofenac in wastewater using solid-phase extraction with high performance liquid chromatography

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ABSTRACT

The occurrence and removal efficiency of naproxen, ibuprofen and diclofenac in two (Kingsburgh and Umbilo) of eThekwini municipality wastewater treatment plants (WWTPs) have been investigated. This study describes a simple method that can be used routinely for the simultaneous determination of such compounds in the influent and effluent of the WWTPs. The method involves the extraction and pre-concentration of target compounds using Oasis MAX solid-phase extraction cartridge (SPE) prior to liquid chromatographic separation. The compounds in wastewater were quantified using photo diode array detection. The method was validated by spiking deionized water with 5 and 50 $\mu g.\ell^{-1}$ of target compounds, in which the recovery range of 76 to 98% was achieved with good precision. The instrument quantification limits obtained were 0.12, 0.42 and 0.39 $\mu g.\ell^{-1}$ for naproxen, ibuprofen and diclofenac, respectively. All the compounds were detected in both influent and effluent in the concentration range of 6.37 to 68.7 $\mu g.\ell^{-1}$ and 0.60 to 4.18 $\mu g.\ell^{-1}$, respectively. Both Kingsburgh and Umbilo WWTPs showed the efficient removal of target compounds in the range of 69 to 97% during the treatment process.

Keywords: solid-phase extraction, wastewater, wastewater treatment plants, ibuprofen, naproxen, diclofenac

Introduction

The presence of pharmaceutical compounds in the environment is a growing concern to the analytical chemists and general public at large. Pharmaceutical compounds such as naproxen, ibuprofen and diclofenac are useful compounds that belong to the group of non-steroidal anti-inflammatory drugs (NSAIDs). These organic compounds are polar and acidic with p*K*a values ranging from 4.2 to 4.9 (Table 1) (Dahane et al., 2013). NSAIDs are widely used by humans for the treatment of rheumatoid arthritis (Mahkam and Poorgholy, 2011). Once consumed, they are subjected to human metabolism, followed by excretion in urine and faeces as metabolites and as unaltered parent compounds which can be subjected to further transformations in wastewater treatment plants (WWTPs) (Parrilla Vazquez et al., 2013).

Overdose or chronic abuse of NSAIDs can lead to toxic side effects (Lagha et al., 2011). Health effects caused by the consumption of acidic pharmaceuticals by animals at low levels is not clear, however, diclofenac has been reported to be the cause of vulture population decline in Asia (Oaks et al., 2004; Taggart et al., 2007). Diclofenac is also known as a compound that affects organ histology and gene expression in fish at a concentration that is as low as 1 $\mu g.\ell^{-1}$ (Cuklev et al., 2012). This information demands the development of a very sensitive analytical methodology for the study of NSAIDs in various sample matrices.

The removal efficiency/rate of naproxen, ibuprofen and diclofenac in wastewater treatment plants (WWTPs) has been reported in many countries. In a municipal sewage treatment system located in South of Sweden, the removal rate of 94 and 99% for naproxen and ibuprofen, respectively, have been reported (Zorita et al., 2009). However, in the same study, diclofenac was not removed during the wastewater treatment process. The removal efficiency for ibuprofen, naproxen and diclofenac in a WWTP located in Germany was

87, 88 and 18%, respectively (Yu et al., 2006). In WWTPs located in Finland, the removal rates were in the ranges of 78 – 100%, 55 – 98% and 9 – 60% for ibuprofen, naproxen and diclofenac, respectively (Lindqvist et al., 2005). All these studies show the incomplete removal of such compounds from WWTPs. As a consequence of this, such compounds have also been detected in river water (Carmona et al., 2014). Carmona et al. (2014) reported the occurrence of ibuprofen, naproxen, diclofenac and other pharmaceutical compounds at ng.ℓ^{-1} levels in tap and mineral water samples collected from Spain. The occurrence of pharmaceutical compounds in WWTP effluents, surface water and drinking water samples in Europe demands a detailed screening of such compounds in a worldwide scale.

Although there is enough evidence on the occurrence of NSAIDs in European water bodies (Yu et al., 2006; Rodil et al., 2012; Togola and Budzinski, 2007), the presence of such compounds in South African environment in not fully known. Most recently, few published reports on the occurrence of NSAIDs in South African WWTPs have emerged (Agunbiade and Moodley, 2014; Amdany et al., 2014; Matongo et al., 2015; Matongo et al., 2015). Therefore, more work is required in order to assess the extent of pollution in South African conditions. This study is based on the determination of selected NSAIDs in Umbilo and Kingsburg WWTPs. To the best of our knowledge, there are currently no available reports on the NSAIDs content in these sites. However, the presence of metals in these sites have been reported (Naidoo et al., 2013).

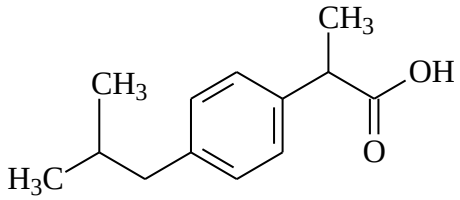
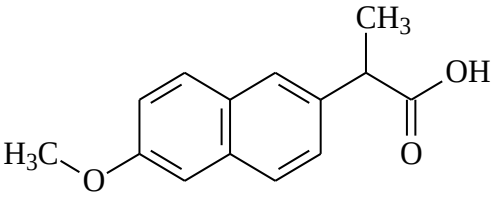
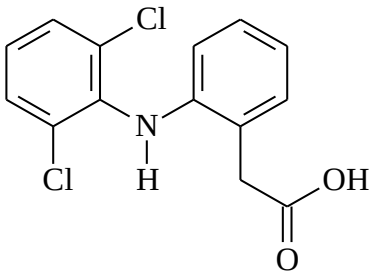
Analytical techniques such as gas and high performance liquid chromatography for trace determination of pharmaceuticals in aqueous samples are well established (Togola and Budzinski, 2007; Rodil et al., 2012). In gas chromatographic analysis, derivatization is employed for the improvement of volatility of target compounds. Togola and Budzinski (2007) derivatized acidic pharmaceuticals with *N*-*M*ethyl-*N*-(trimethylsilyl)trifluoroacetamide prior to gas chromatographic analysis.

Whereas, a simple analysis is usually carried out with a direct injection of samples and compounds into a liquid chromatographic instrument (Rodil et al., 2012). A suitable sample preparation technique is employed prior to the chromatographic separation of target compounds. Solid-phase extraction (SPE) technique using Oasis HLB sorbent is widely used for pre-concentration of NSAIDs and elimination of interfering species (Agunbiade and Moodley, 2014; Amdany et al., 2014; Matongo et al., 2015; Matongo et al., 2015). However, such technique is most suitable when target compounds exhibit both hydrophilic and lipophilic properties (Madikizela et al., 2014). This work focused primarily on the occurrence of hydrophilic compounds in wastewater, hence a solid-phase extraction methodology was developed using Oasis MAX sorbent. Oasis MAX is made of a mixed-mode polymer sorbent with both reversed-phase and anion-exchange functionalities (Lee et al., 2005).

Therefore, this work was carried out in order to achieve three objectives such as; to investigate a suitable extraction technique for the pre-concentration of ibuprofen, naproxen and diclofenac; followed by to study the occurrence of these pharmaceutical compounds in WWTPs located around Durban City, KwaZulu Natal Province in South Africa; and finally to evaluate the removal efficiency of acidic pharmaceuticals from local WWTPs.

Table 1

Physicochemical properties for ibuprofen, naproxen and diclofenac.

Compound	Chemical structure	Water Solubility (mg.ℓ^{-1})	pK_a
Ibuprofen		58	4.9
Naproxen		44	4.2
Diclofenac		10	4.2

Materials and methods

Materials and reagents

Naproxen (98%), ibuprofen (≥98%) and diclofenac sodium salt were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC-grade solvents such as acetonitrile (≥99.9%) and methanol (99.5%) were purchased from Merck (Darmstadt, Germany). Formic acid (approx. 98%) was purchased from Fluka (Steinheim, Germany). Sodium chloride (≥99.5%) and sodium hydroxide

pellets were purchased from Associated Chemical Enterprises (Johannesburg, South Africa).

Oasis MAX 6cc 150 mg solid-phase extraction cartridges were obtained from Waters Corporation (Milford, Massachusetts USA).

Instrumentation and conditions

Chromatographic separation was performed on a high performance liquid chromatography (HPLC) system that consisted of an online mobile phase degasser unit (Model: DGU-20A3), 20 µℓ sample loop, pump (Model: LC-20AB), and photo diode array detector (Model: SPD-M20A), all obtained from Shimadzu Corporation (Kyoto, Japan). The mobile phase used consisted of a mixture of acetonitrile: 0.2% formic acid in water (60:40, v:v) at a flow rate of 0.8 mℓ.min^{-1}. Separation was performed on a Lichrospher C_{18} HPLC column 250 x 4.00 mm x 5 µm obtained from Merck (Darmstadt, Germany). Shimadzu LC solutions software was used for data collection and processing. Photo diode array detection was performed at the wavelengths of 230 nm for naproxen and 200 nm for both ibuprofen and diclofenac.

A Bante900P multi-parameter water quality meter was purchased from Bante instruments (Shanghai, China). Prior to its use, the meter was calibrated using the calibration buffers that were provided by the supplier. Thereafter, the meter was used to monitor the physicochemical properties of the wastewater during the sampling.

For SPE, vacuum manifold purchased from Phenomenex (Carlifonia, USA) was connected to a vacuum pump obtained from Pall Corporation (Fribourg, Switzerland).

Validation of analytical method

A stock solution (100 mg.ℓ^{-1}) containing all target compounds was prepared in acetonitrile, then the solution was diluted in order to prepare working solutions. Each working solution was analyzed using an HPLC system. Limit of detection, limits of quantification and linearity were computed.

Accuracy and precision of the analytical method were determined using deionized water that was spiked with all target compounds at concentration levels of 50 and 5 µg.ℓ^{-1}. The optimized solid phase extraction method was employed for the extraction and pre-concentration of target compounds prior to HPLC quantification.

Sampling

Wastewater samples were collected weekly from the influent and effluent of Umbilo and Kingsburg WWTP using pre-cleaned glass bottles in May 2016. Effect of chlorination on target compounds was investigated by collecting the sample before the disinfection stage. Umbilo and Kingsburg WWTPs are located in the Province of Kwa-Zulu Natal in South Africa. The GPS co-ordinates for the location of Umbilo and Kingsburg WWTPs are S29.84556^0 E30.89103^0 and S30.07445^0 E30.85687^0, respectively. Physicochemical properties of the samples were measured in situ, thereafter, the samples were transported to the laboratory. Samples were filtered through a 0.22 µm nylon syringe filter obtained from Membrane Solutions (Dallas, USA), and stored in the refrigerator (4°C) until analysis.

Solid-phase extraction

Solid-phase extraction method was optimized using a standard solution in order to achieve high extraction efficiency for target compounds. Optimized

parameters were sample pH, sample volume, elution solvent and the effect of salinity.

Pre-optimized conditions were used to treat the collected wastewater samples. The SPE cartridge was conditioned with 5 mℓ of acetonitrile followed by 5 mℓ of acidified deionized water (pH 2.5) both loaded at a flow rate of 1 mℓ.min^{-1}. The acidified (pH 2.5) wastewater sample (100 mℓ) was loaded onto the SPE cartridge at a flow rate of 1 mℓ.min^{-1}. The cartridge was washed with 5 mℓ of methanol:water (10:90%, v:v). Thereafter, the retained compounds were eluted sequentially with 2 mℓ methanol, 2 mℓ mixture of methanol and acetic acid (90:10, v:v) and 2 mℓ of 2 % (v:v) formic acid diluted using a mixture of methanol and acetic acid (40:60, v:v). The volume of the collected extract was reduced to 0.5 mℓ with a gentle stream of nitrogen gas prior to injection into HPLC system.

Removal efficiency of target compounds from wastewater treatment plants

The removal efficiency (R) of each compound from both WWTPs was evaluated by employing equation (1):

$$R = \frac{C_{infl} - C_{effl}}{C_{infl}} \times 100 \quad (1)$$

where C_{infl} and C_{effl} are the concentrations obtained for the raw influent and final effluent, respectively (Sari et al., 2014).

Results and discussion

Optimization of extraction conditions

Effect of sample pH

The effect of sample pH on SPE was investigated by loading 10 mℓ of spiked deionized water into the cartridge. The pH of the spiked deionized water sample was varied in the range of 2.5-11. The results obtained (Fig. 1) indicated that the recoveries for all compounds decreased when the pH of water solution was increasing. This was an expected phenomenon for the reversed-phase mode. It is known that when the water sample is acidified to a pH that is less than the p*K*a value (Table 1), the acids are unionized therefore they get adsorbed by the reversed-phase interactions. Therefore in subsequent experiments, the pH in all the sample solutions was adjusted to 2.5.

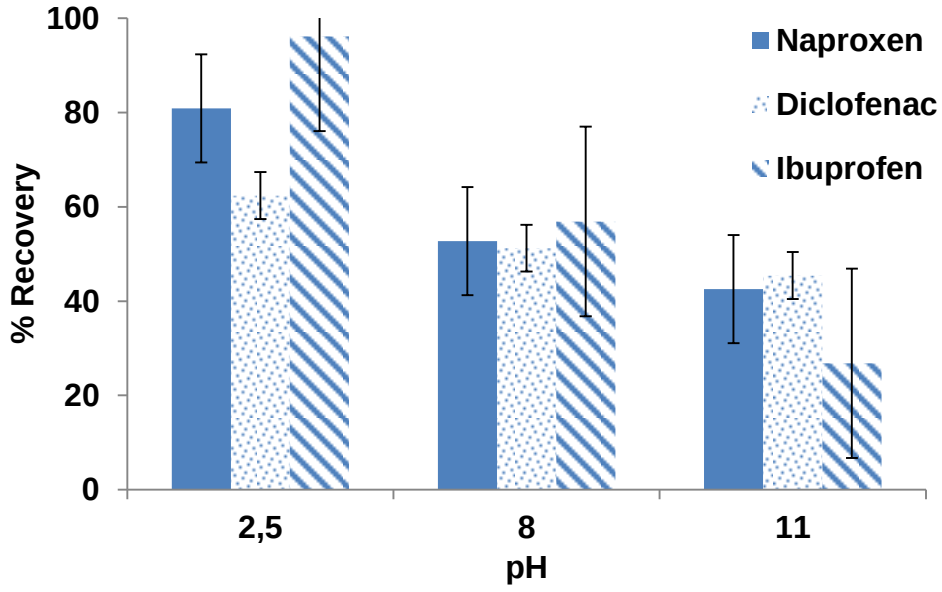


Fig. 1. Effect of sample pH on solid phase extraction

Effect of elution solvent

Different solvent conditions were investigated for the elution of the compounds that were adsorbed on the SPE cartridge. The elution solvents investigated were methanol, acetic acid in methanol (10:90, v:v) and 2% (v:v) formic acid in a mixture of methanol and acetonitrile (40:60, v:v). The results presented in Fig. 2 (a) show clearly that each elution solvent investigated was able to elute a significant amount of a particular compound. The results show that more than 50% of naproxen was eluted with methanol, then the best elution for diclofenac was achieved by using the two solvent mixtures, acetic acid in methanol (10:90, v:v) and 2% (v:v) formic acid in a mixture of methanol and acetonitrile (40:60, v:v), whereas 60% of ibuprofen was eluted with 2% (v:v) formic acid in a mixture of methanol and acetonitrile (40:60, v:v). Therefore a sequential elution was employed, where all the investigated solvents were used in series. It has been documented (Lee et al., 2005) that the elution of compounds retained in Oasis MAX cartridge with methanol only removes the less acidic compounds alongside with other neutral co-extracts which were mainly adsorbed by reversed-phase mechanism. More acidic compounds are usually eluted with 2% formic acid mixture in the unionized form (Lee et al., 2005).

In this study the eluted fractions were further combined and evaporated with a gentle steam of nitrogen to 0.5 mℓ. Results in Fig. 2 (b) show that the recoveries of 74, 85 and 97% were achieved for naproxen, diclofenac and ibuprofen, respectively.

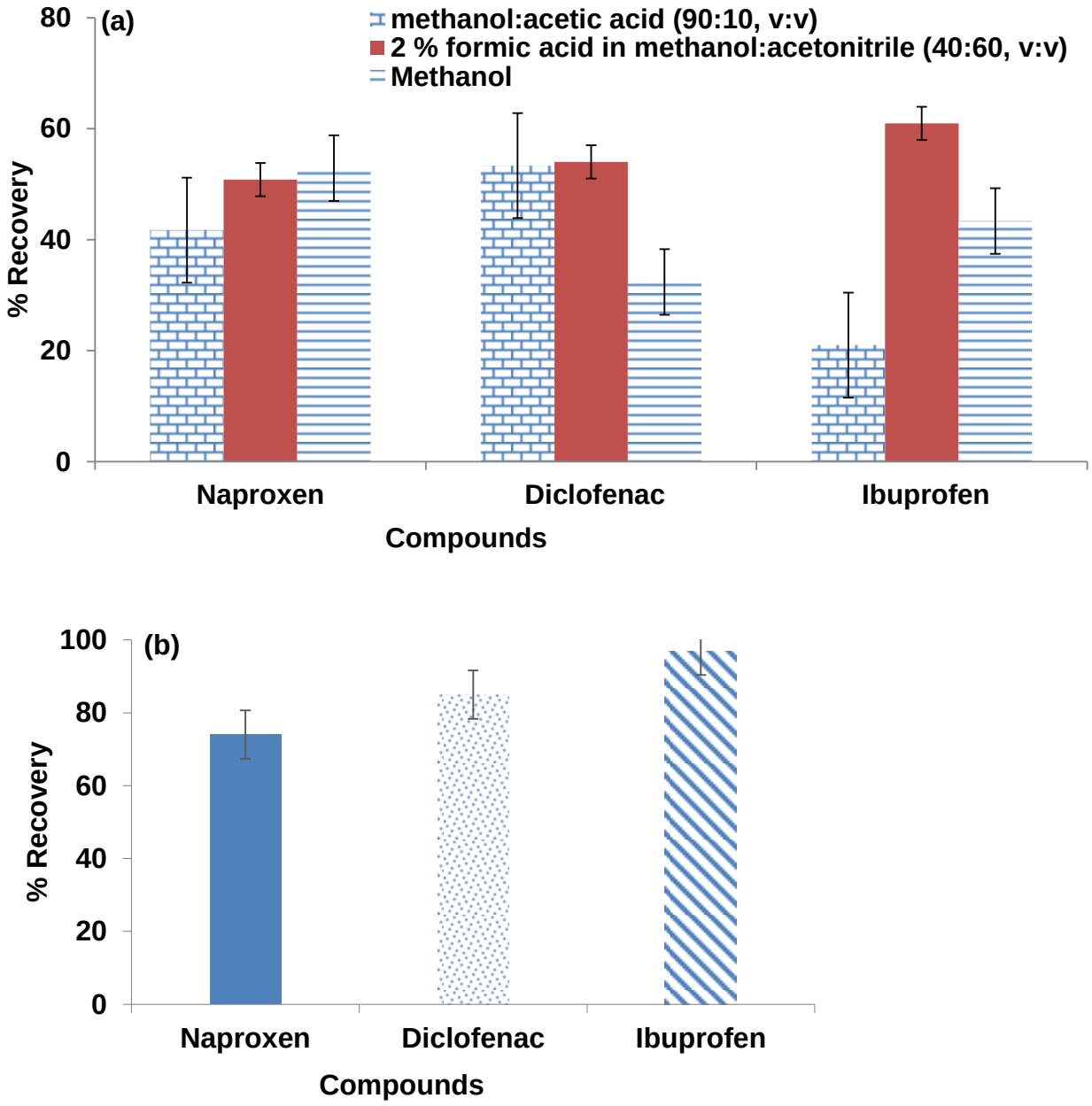


Fig. 2. Effect of elution solvent on solid phase extraction, where (a) shows the % recovery after elution with individual solvents and (b) represent % recovery obtained for the combined eluted fractions.

Effect of salt concentration

The effect of salt content was investigated by spiking a mixture of target compounds in deionized water with different amounts of sodium chloride. The pH of each spiked solution was adjusted to 2.5. Thereafter, 100 mℓ of each solution was loaded into a pre-conditioned SPE cartridge. Therefore, % recovery was determined for each target compound. Addition of salt to the aqueous samples is usually carried out to improve the extraction of several compounds (Sarafraz-Yazdi et al., 2012), however in this study, salt was added in pure compounds to imitate the wastewater contents. Results presented in Fig. 3 showed a decrease in percent recovery as the concentration of sodium chloride increases from 0.1 to 0.4% (m:v). This trend have also been observed elsewhere (Li et al., 2008), where the adsorption amounts of three phthalic acid esters decreased with increasing ionic strength. Sodium chloride dissociates in water, then the competition of sodium ions for adsorption with target compounds takes place that leads to the reduction of adsorption sites.

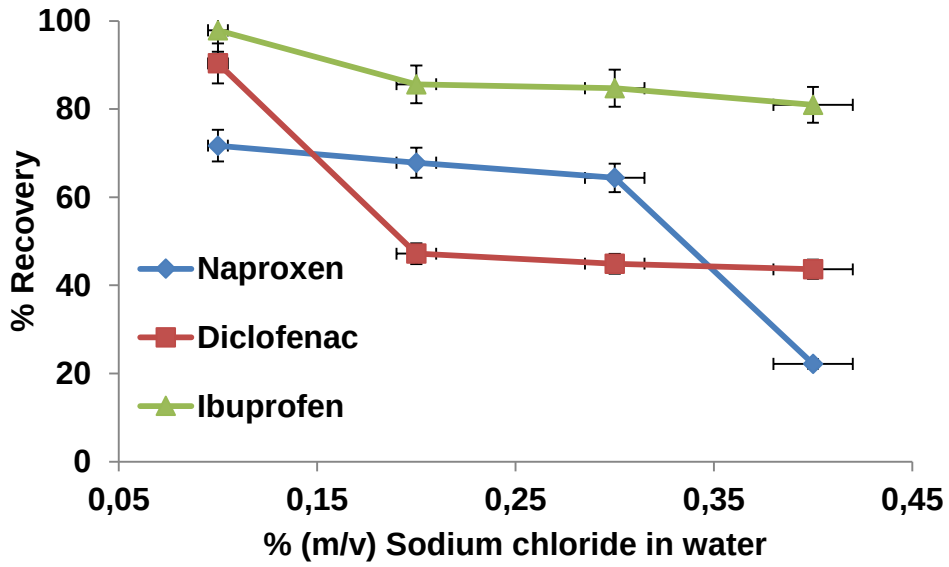


Fig. 3. Effect of salt content on solid phase extraction

Effect of sample volume

Different volumes of deionized water that was spiked with naproxen, ibuprofen and diclofenac at pH 2.5 were loaded into a pre-conditioned SPE cartridge. Percent recovery was determined in each case. Results in Fig. 4 indicated that the percent recoveries increased when the sample volumes was increased. For instance, the recovery for 10 mℓ was low perhaps due to limited sample interaction with sorbent as not enough volume was available to push the whole sample through the cartridge volume. Sample volume was not increased beyond 100 mℓ as this would have resulted in long analysis time. However, in our previous work, it was shown that the percent recoveries tend to decrease when loading high sample volumes into the SPE cartridge (Madikizela et al., 2014). This happens when the capacity of the SPE sorbent have been exceeded, therefore the cartridge becomes overloaded.

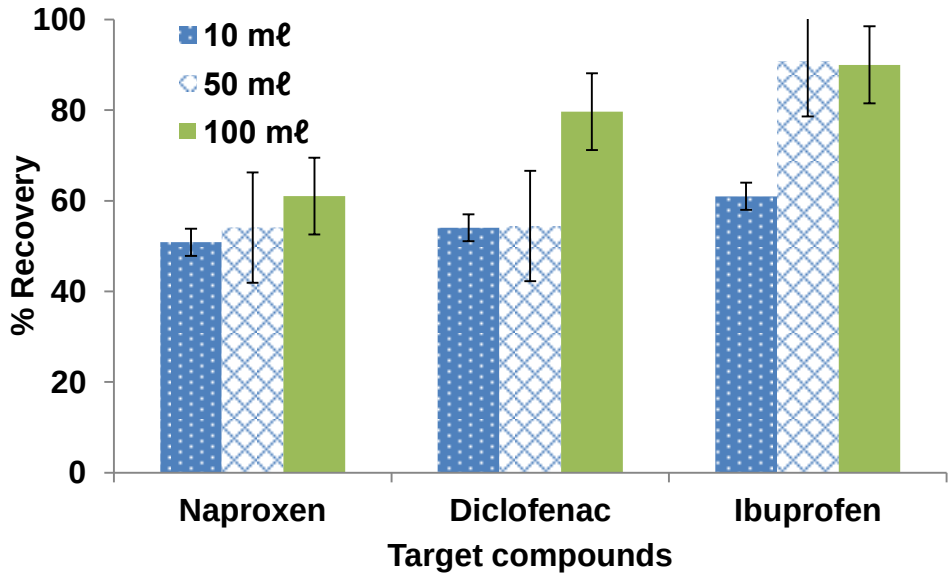


Fig. 4. Effect of sample volume on solid phase extraction

Method validation

The separation of target compounds was achieved on the reverse phase separation column (Fig. 5). The performance of high performance liquid chromatographic method was validated by determining the limit of detection (LOD), limit of quantification (LOQ) and linearity. LOD and LOQ were determined experimentally for each compound where the detectable amounts showed a signal-to-noise ratio of 3 and 10, respectively. The HPLC instrument employed in this study was able to detect up to 0.13, 0.04, 0.12 $\mu g.\ell^{-1}$ for ibuprofen, naproxen and diclofenac, respectively. The LOQ values of 0.42, 0.12 and 0.39 $\mu g.\ell^{-1}$ were obtained for ibuprofen, naproxen and diclofenac, respectively. The LODs obtained (Table 2) were lower than those reported in literature for the analysis of the same compounds using photo diode array detection, which in turn means that the propose method is more sensitive (Payan et al., 2011). Calibration curves were linear for all the compounds with correlation coefficients over 0.99.

Furthermore, the sample preparation method was validated by determining the percent recoveries when the optimized SPE conditions were employed for the deionized water that was spiked with all target compounds at 50 and 5 $\mu g.\ell^{-1}$. Acceptable percent recoveries as indicated in Table 2 were obtained with good precision for all compounds. These results indicate that the sample preparation method proposed in this study was suitable for the extraction and pre-concentration of ibuprofen, naproxen and diclofenac. After successful validation of SPE-HPLC method in deionized water, the same protocol was applied to the analysis of wastewater samples.

Table 2

Limit of detection, limit of quantification, correlation coefficient and percent recovery for ibuprofen, naproxen and diclofenac.

Compound	LOD (µg.ℓ$^{-1}$)	LOQ (µg.ℓ$^{-1}$)	R^2	% Recovery ± %RSD	
				50 µg.ℓ$^{-1}$	5 µg.ℓ$^{-1}$
Ibuprofen	0.13	0.42	0.995	94 ± 1.4	95 ± 0.9
Naproxen	0.04	0.12	0.995	76 ± 2.7	83 ± 11
Diclofenac	0.12	0.39	0.994	81 ± 4.3	98 ± 0.3

Environmental analysis

The optimized SPE conditions were applied for the quantitative analysis of ibuprofen, naproxen and diclofenac in wastewater collected from Kingsburg and Umbilo WWTPs. The treatment process in both plants involves screening for grit removal, settling tanks for sludge production and removal, aeration tanks and chlorination for disinfection. Compounds of interest present in wastewater were identified by using retention time comparison in conjunction with photo diode array detection spectra. The collected data is summarized in Figs. 5-8, and Figs. S1-S3 in support information. Presence of target compounds was observed in each stage of wastewater treatment process (Figs. 6-8). The chromatograms in Fig. 8 clearly show the incomplete removal

of target compounds during the wastewater treatment process. The results (Table 3) further showed that the disinfection with chlorine does not really reduce the amounts of target compounds in wastewater except in the case of diclofenac in Umbilo WWTP, which is in agreement with the results reported elsewhere (Behera et al., 2011). This could be due to the fact that disinfection process is implement for the reduction of pathogenic microorganisms (Behera et al., 2011).

Concentration levels obtained for target compounds in wastewater (Table 3) were comparable to those reported in other studies. For example, mean concentrations for ibuprofen in Goudkoppies and Northern WWTPs influent located in Johannesburg, South Africa, were 39.8 and 111.9 $\mu g.\ell^{-1}$, respectively (Amdany et al., 2014). In another study conducted in North of Spain, ibuprofen and naproxen concentration range in the influent was 2.3 – 42 $\mu g.\ell^{-1}$, whereas a maximum concentration of 5.7 $\mu g.\ell^{-1}$ was reported for the effluent (Fernandez et al., 2014). Few studies that have been conducted in wastewater show the presence of pharmaceuticals in WWTPs located around the city of Durban in South Africa (Agunbiade and Moodley, 2014; Madikizela et al., 2014; Madikizela and Chimuka, 2016; Matongo et al., 2014). In order to fully understand the extent of the problem, it is recommended to conduct the studies on their presence in surface water, aquatic plants and sediments.

The removal efficiency for each compound in WWTPs was determined based on the raw influent and final effluent concentrations. The percentage removal for target compounds in wastewater treatment was in the range of 69 - 97%, which corresponded well with figures reported for a Durban WWTP (Agunbiade and Moodley, 2014). In general, Umbilo and Kingsburgh WWTPs showed a better removal efficiency when compared to some treatment works worldwide. For instance, the removal efficiencies reported for ibuprofen, naproxen and diclofenac in WWTP located in Baltimore, USA, were 87, 88 and 18%, respectively (Yu et al., 2006). Removal rate greater than 90% for naproxen and

ibuprofen has been reported for a WWTP that uses UV for disinfection, however the same plant did not show any removal for diclofenac (Carmona et al., 2014). Poor removal of diclofenac have been reported in a number of WWTPs (Lindqvist et al., 2005; Rosal et al., 2010; Zorita et al., 2009; Samaras et al., 2013). Further work is required in order to investigate the effect of poor removal and to rectify the problem for diclofenac. Although these WWTPs were not specifically designed to remove pharmaceutical compounds, they showed a great potential in this regard.

In general, the performance of the studied WWTPs was evaluated by monitoring pH, conductivity, total dissolved solids, dissolved oxygen, salinity and oxidation reduction potential. The results (Table S1) indicated normal functioning of the WWTPs. This observation was based on the reduction of conductivity, salinity and total dissolved solids from the raw influent to the final effluent. Also the increase in dissolved oxygen from the influent to the effluent was an indication of a working WWTP.

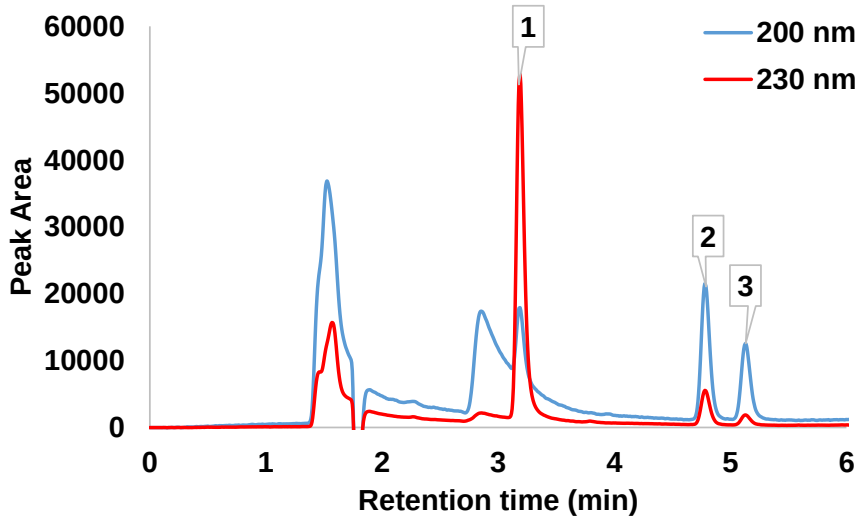


Fig. 5. Chromatograms obtained for the separation of 1000 µg.ℓ^{-1} of ibuprofen, naproxen and diclofenac. Peaks 1, 2 and 3 represent naproxen, diclofenac and ibuprofen.

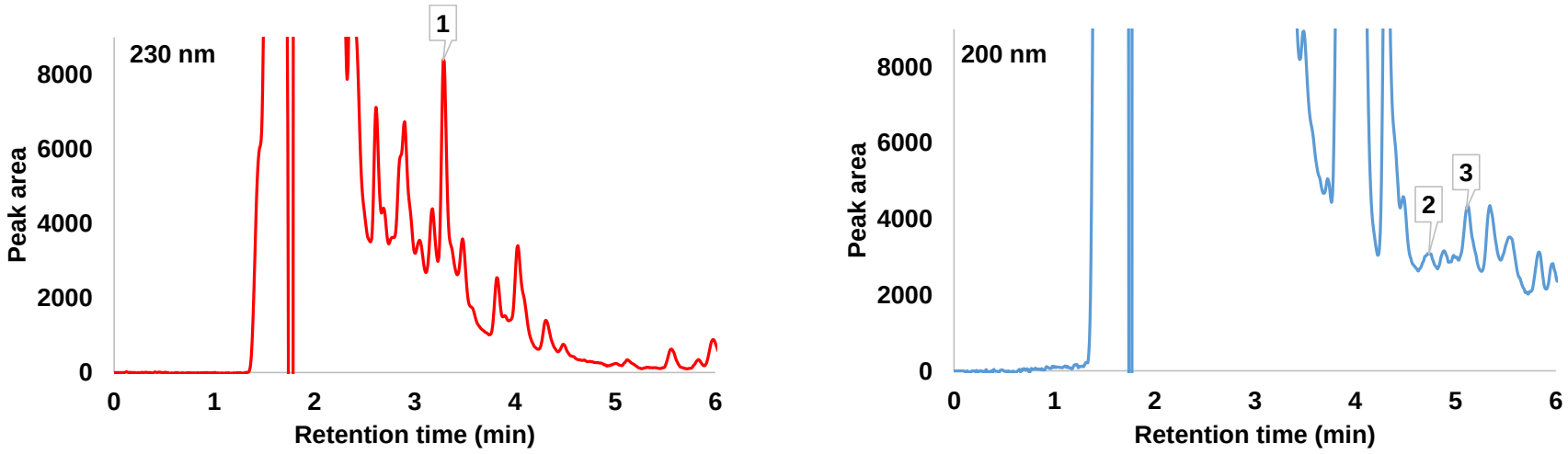


Fig. 6. Chromatograms for raw wastewater influent recorded at 200 and 230 nm, where, Peaks 1, 2 and 3 represent naproxen, diclofenac and ibuprofen.

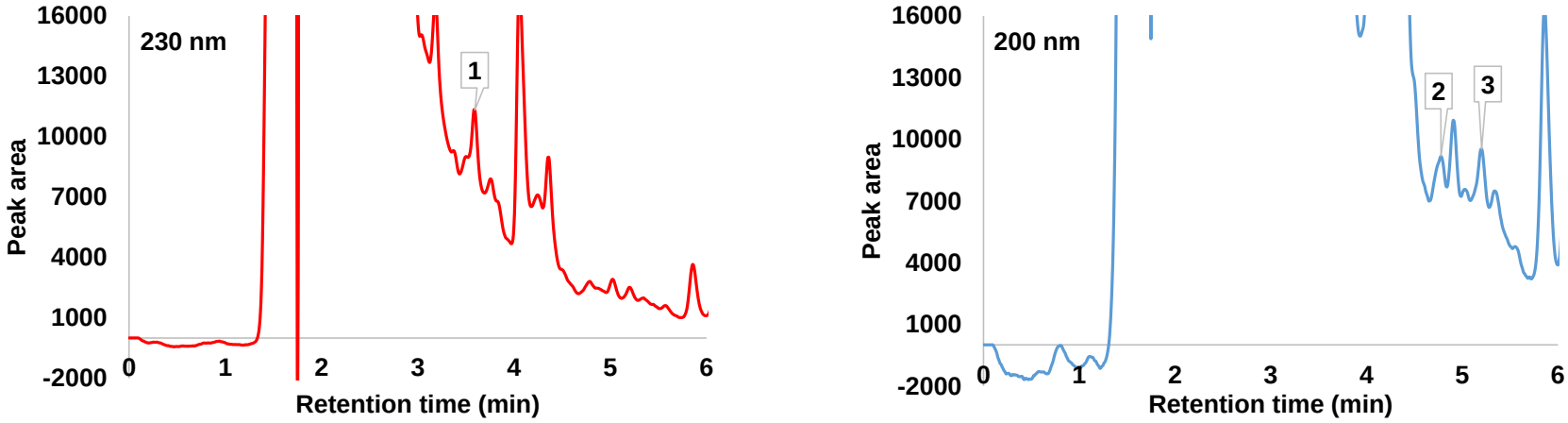


Fig. 7. Chromatograms for wastewater samples collected prior to chlorination. Peaks 1, 2 and 3 represent naproxen, diclofenac and ibuprofen.

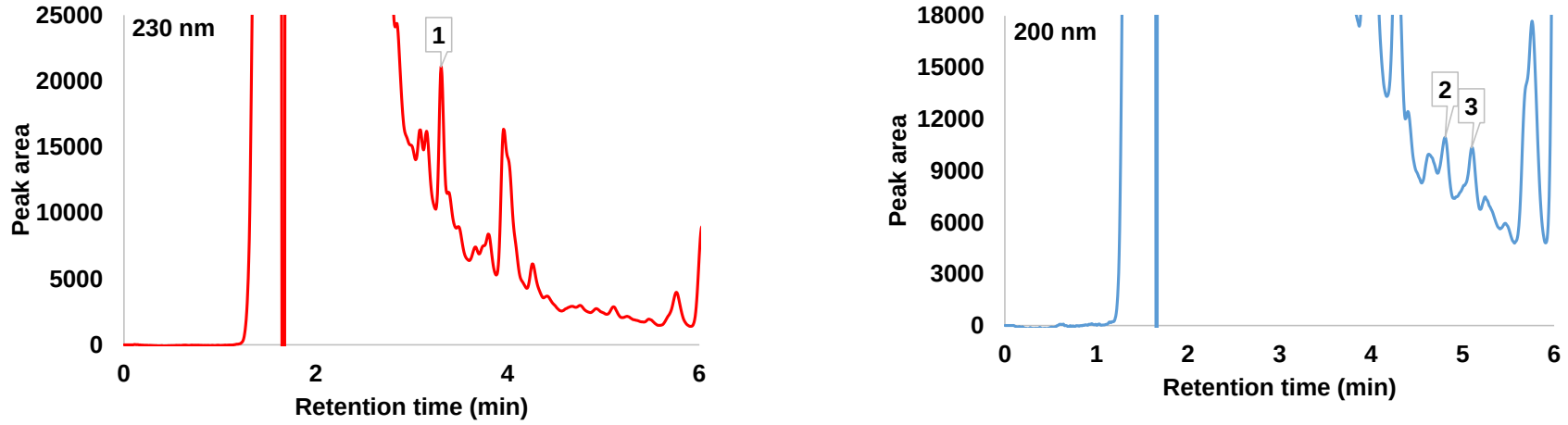


Fig. 8. Chromatograms for wastewater effluent recorded at 200 and 230 nm. Peaks 1, 2 and 3 represent naproxen, diclofenac and ibuprofen.

Table 3

Quantities (µg.ℓ$^{-1}$) of target compounds ± standard deviation (n = 3) detected in WWTPs and removal efficiency (%).

Compound	Kingsburg WWTP				Umbilo WWTP			
	Influent	Mid	Effluent	%R	Influent	Mid	Effluent	%R
Ibuprofen	68.7 ± 6.5	1.90 ± 1.6	2.05 ± 2.9	97	54.5 ± 6.6	5.00 ± 1.3	4.18 ± 1.0	92
Naproxen	20.0 ± 12.9	0.87 ± 0.6	0.60 ± 0.7	97	14.9 ± 11.2	1.30 ± 0.8	1.11 ± 0.7	93
Diclofenac	16.4 ± 6.5	1.67 ± 1.2	1.39 ± 1.1	92	6.37 ± 2.7	5.46 ± 4.9	1.98 ± 0.6	69

Mid: sample collected prior to disinfection, %R: Removal efficiency.

4. Conclusions

The application of advanced high performance liquid chromatography equipped with photo diode array detector to environmental analysis has allowed the determination of ibuprofen, naproxen and diclofenac; and thus permitted more comprehensive assessment of environmental contaminants. Solid-phase extraction method with Oasis MAX offered improved results since the target compounds were extracted and pre-concentrated. Acceptable recoveries that ranged from 76 to 98% were obtained for the target compounds. All compounds were detected in the raw influent and effluent. Both WWTPs were not specifically designed for removal of pharmaceutical compounds, however the percent removal shows that it is possible to remove the drugs to some extent during the treatment process. Therefore, it is recommended to optimize the design of the WWTPs for effective removal of such drugs during wastewater treatment process.

ACKNOWLEDGEMENTS

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REFERENCES

AGUNBIADE FO and MOODLEY B (2014) Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa. *Environ Monit Assess* **186** 7273-7291.

AMDANY R, CHIMUKA L and CUKROWSKA E (2014) Determination of naproxen, ibuprofen, and triclosan in wastewater using the polar

organic chemical integrative sampler (POCIS): A laboratory calibration and field application. *Water SA* **40** 407-414.

BEHERA SK, KIM HW, OH J and PARK H (2011) Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea. *Sci. Total Environ.* **409** 4351-4360.

CARMONA E, ANDREU V and PICO Y (2014) Occurrence of acid pharmaceuticals and personal care products in Turia River Basis: From waste to drinking water. *Sci. Total Environ.* **484** 53-63.

CUKLEV F, FICK J, CVIJOVIC M, KRISTIANSSON E, FORLIN L and JOAKIM LARSSON DG (2012) Does ketoprofen or diclofenac pose the lowest risk to fish? *J. Hazard. Mater.* **229-230** 100-106.

DAHANE S, GIL GARCIA MD, MARTINEZ BUENO MJ, UCLES MORENO A, MARTINEZ GALERA M and DERDOUR A (2013) Determination of drugs in river and wastewaters using solid-phase extraction by packed multi-walled carbon nanotubes and liquid chromatography-quadrupole-linear ion trap-mass spectrometry. *J. Chromatogr. A* **1297** 17-28.

FERNANDEZ M, FERNANDEZ M, LACA A, LACA A and DIAZ M (2014) Seasonal occurrence and removal of pharmaceutical products in municipal wastewaters. *J Environ Chem. Eng.* **2** 495-502.

LAGHA A, ADHOUM N and MONSER L (2011) A molecularly imprinted polymer for the selective solid-phase extraction of ibuprofen from urine samples. *OCBMJ* **4** 7-13.

LEE H, PEART TE and SVOBODA ML (2005) Determination of endocrine-disrupting phenols, acidic pharmaceuticals, and personal-care products in sewage by solid-phase extraction and gas chromatography-mass spectrometry. *J. Chromatogr. A* **1094** 122-129.

LI J, CAI Y, SHI Y, MOU S and JIANG G (2008) Analysis of phthalates via HPLC-UV in environmental water samples after concentration by solid-phase extraction using ionic liquid mixed hemimicelles. *Talanta* **74** 498-504.

LINDQVIST N, TUHKANEN T and KRONBERG L (2005) Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. *Water Res.* **39** 2219-2228.

MADIKIZELA LM and CHIMUKA L (2016) Determination of ibuprofen, naproxen and diclofenac in aqueous samples using a multi-template molecularly imprinted polymer as selective adsorbent for solid-phase extraction. ***J** Pharm Biomed Anal* **128** 210-215.

MADIKIZELA LM, MUTHWA SF and CHIMUKA L (2014) Determination of triclosan and ketoprofen in river water and wastewater by solid-phase extraction and high performance liquid chromatography. *S. Afr. J. Chem* **67** 143-150.

MAHKAM M and POORGHOLY N (2011) Imprinted polymers as drug delivery vehicles for anti-inflammatory drugs. *Nature and Science* **9** 163-168.

MATONGO S, BIRUNGI G, MOODLEY B and NDUNGU P (2015) Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa. *Environ Sci Pollut Res* **22** 10298-10308.

MATONGO S, BIRUNGI G, MOODLEY B and NDUNGU P (2015) Pharmaceutical residues in water and sediment of Msunduzi River, KwaZulu-Natal, South Africa. *Chemosphere* **134** 133-140.

NAIDOO S, VOSLOO D and SCHOEMAN MC (2013) Foraging at wastewater treatment works increases the potential for metal accumulation in an urban adapter, the banana bat (Neoromicia nana). *Afr. Zool.* **48** 39-55.

OAKS JL, GILBERT M, VIRANI MZ, WATSON RT, METEYER CU, RIDEOUT BA, SHIVAPRASAD HL, AHMED S, CHAUDHRY MJI, ARSHAD M, MAHMOOD S, ALI A and KHAN AA (2004) Diclofenac residues as the cause of vulture population decline in Pakistan. *Nature* **427** 630-633.

PARRILLA VAZQUEZ MM, PARRILLA VAZQUEZ P, MARTINEZ GALERA M, GIL GARCIA MD and UCLES A (2013) Ultrasound-assisted ionic liquid dispersive liquid-liquid microextraction coupled with liquid chromatography-quadrupole-linear ion trap-mass spectrometry for simultaneous analysis of pharmaceuticals in wastewaters. *J. Chromatogr. A* **1291** 19-26.

PAYAN MR, LOPEZ MAB, TORRES RF, NAVARRO MV and MOCHON MC (2011) Electromembrane extraction (EME) and HPLC determination of non-steroidal anti-inflammatory drugs (NSAIDs) in wastewater samples. *Talanta* **85** 394-399.

RODIL R, QUINTANA JB, CONCHA-GRANA E, LOPEZ-MAHIA P, MUNIATEGUI-LORENZO S and PRADA-RODRIGUEZ D (2012) Emerging pollutants in sewage, surface and drinking water in Galicia (NW Spain). *Chemosphere* **86** 1040-1049.

ROSAL R, RODRIGUEZ A, PERDIGON-MELON JA, PETRE A, GARCIA-CALVO E, GOMEZ MJ, AGUERA A and FERNANDEZ-ALBA AR (2010) Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. *Water Res.* **44** 578-588.

SAMARAS, VG, STASINAKIS AS, MAMAIS D, THOMAIDIS NS and LEKKAS TD (2013) Fate of selected pharmaceuticals and synthetic endocrine disrupting compounds during wastewater treatment and sludge anaerobic digestion. *J. Hazard. Mater.* **244-245** 259-267.

SARAFRAZ-YAZDI A, AMIRI A, ROUNAGHI G and ESHTIAGH-HOSSEINI H (2012) Determination of non-steroidal anti-inflammatory drugs in

water samples by solid-phase microextraction based sol-gel technique using poly(ethylene glycol) grafted multi-walled carbon nanotubes coated fiber. *Anal. Chim. Acta* **720** 134-141.

SARI S, OZDEMIR G, YANGIN-GOMEC C, ZENGIN GE, TOPUZ E, AYDIN E, PEHLIVANOGLU-MANTAS E and TAS DO (2014) Seasonal variation of diclofenac concentration and its relation with wastewater characteristics at two municipal wastewater treatment plants in Turkey. *J. Hazard. Mater.* **272** 155-164.

TAGGART MA, CUTHBERT R, DAS D, SASHIKUMAR C, PAIN DJ, GREEN RE, FELTRER Y, SHULTZ S, CUNNINGHAM AA and MEHARG AA (2007) Diclofenac disposition in Indian cow and goat with reference to *Gyps* vulture population declines. *Environ. Pollut.* **147** 60-65.

TOGOLA A and BUDZINSKI H (2007) Analytical development for analysis of pharmaceuticals in water samples by SPE and GC-MS. *Anal Bioanal Chem* **388** 627-635.

YU JT, BOUWER EJ and COELHAN M (2006) Occurrence and biodegradability studies of selected pharmaceuticals and personal care products in sewage effluent. *Agric Water Manag.* **86** 72-80.

ZORITA S, MARTENSSON L and MATHIASSON L (2009) Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden. *Sci. Total Environ.* **407** 2760-2770.

3.3.8.1 Paper 7 - Supplementary data

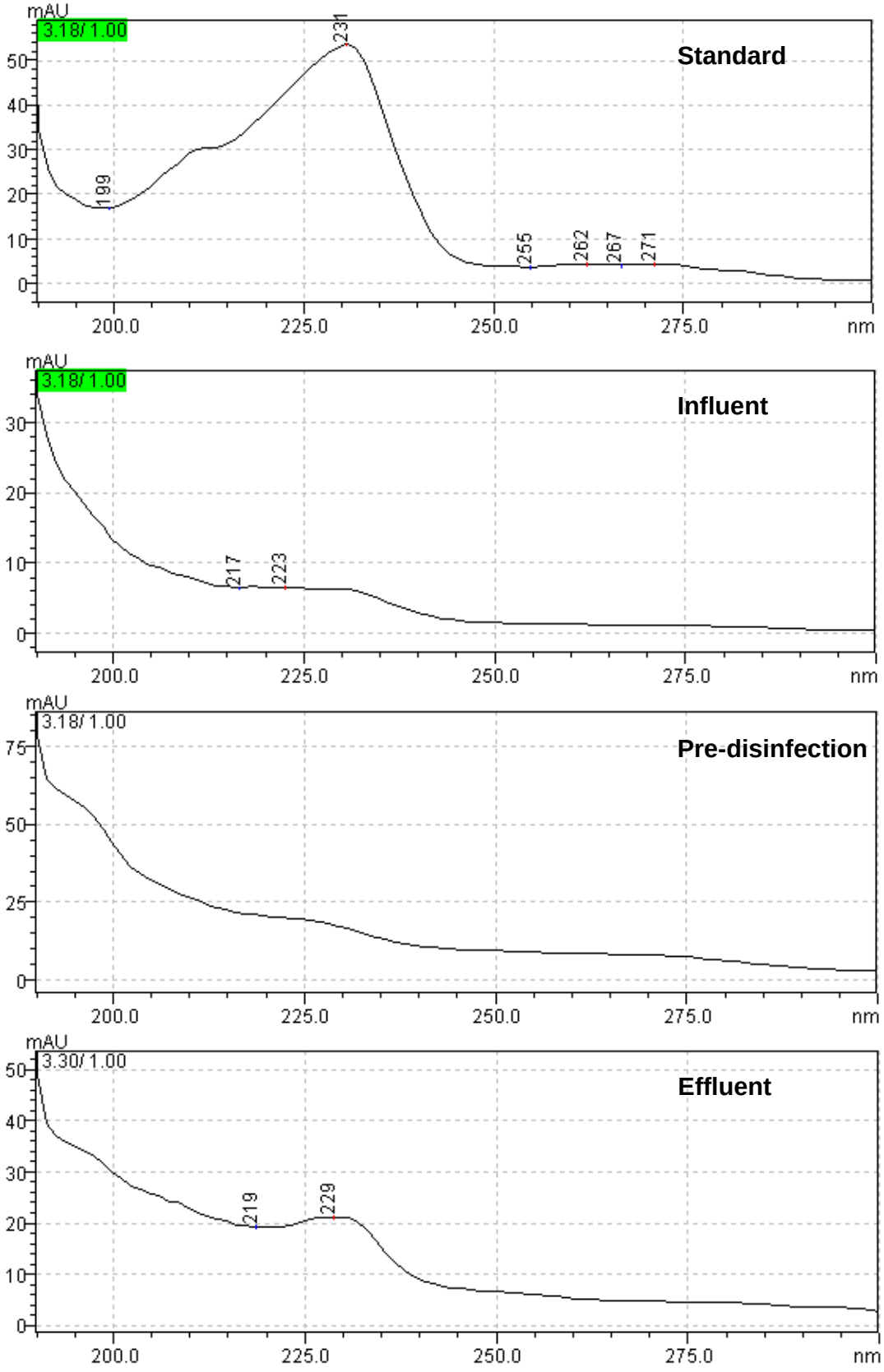


Fig. S1. PDA spectra obtained for naproxen in standard solution and in extracted wastewater samples.

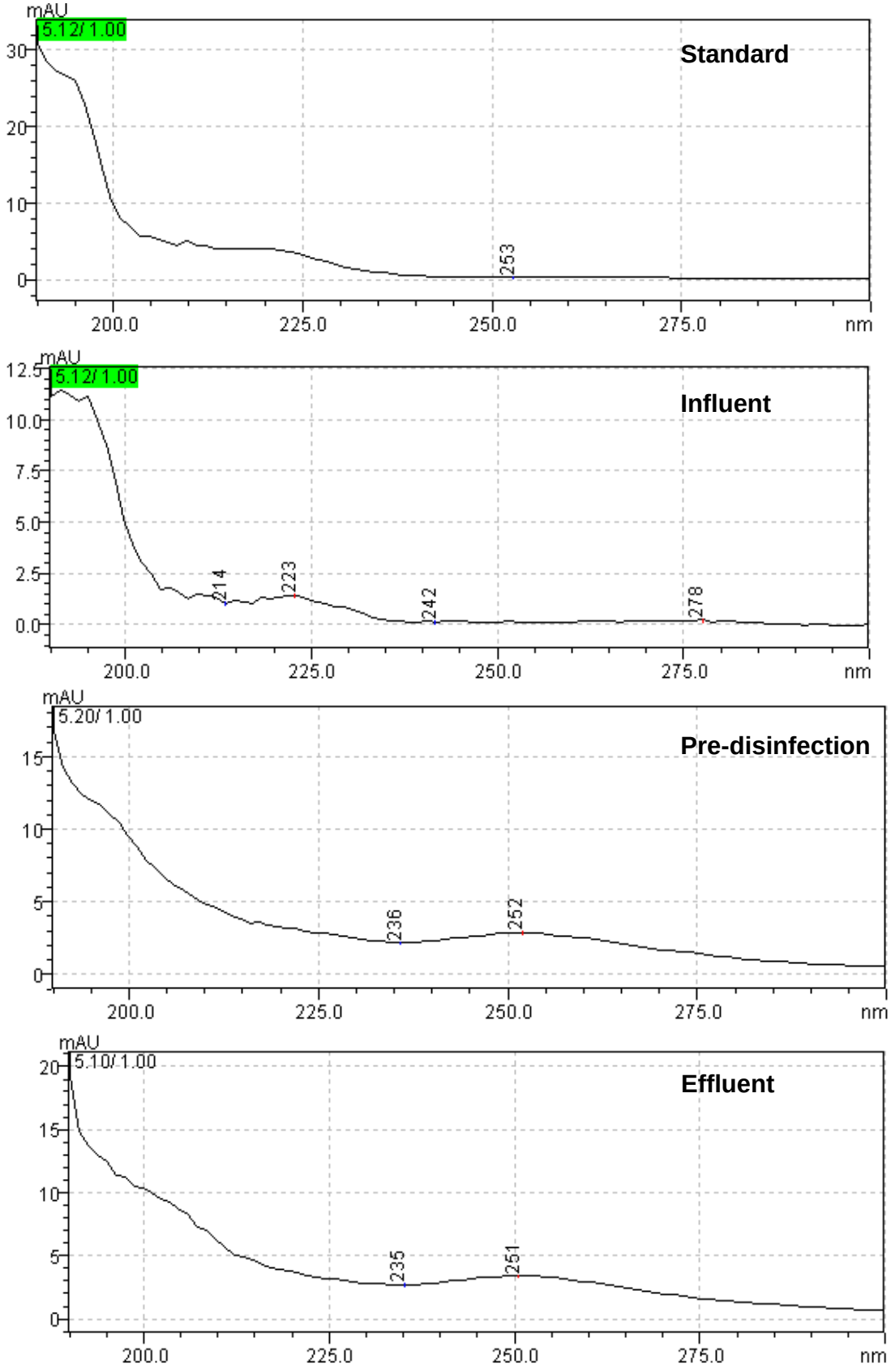


Fig. S2. PDA spectra obtained for ibuprofen in standard solution and in extracted wastewater samples.

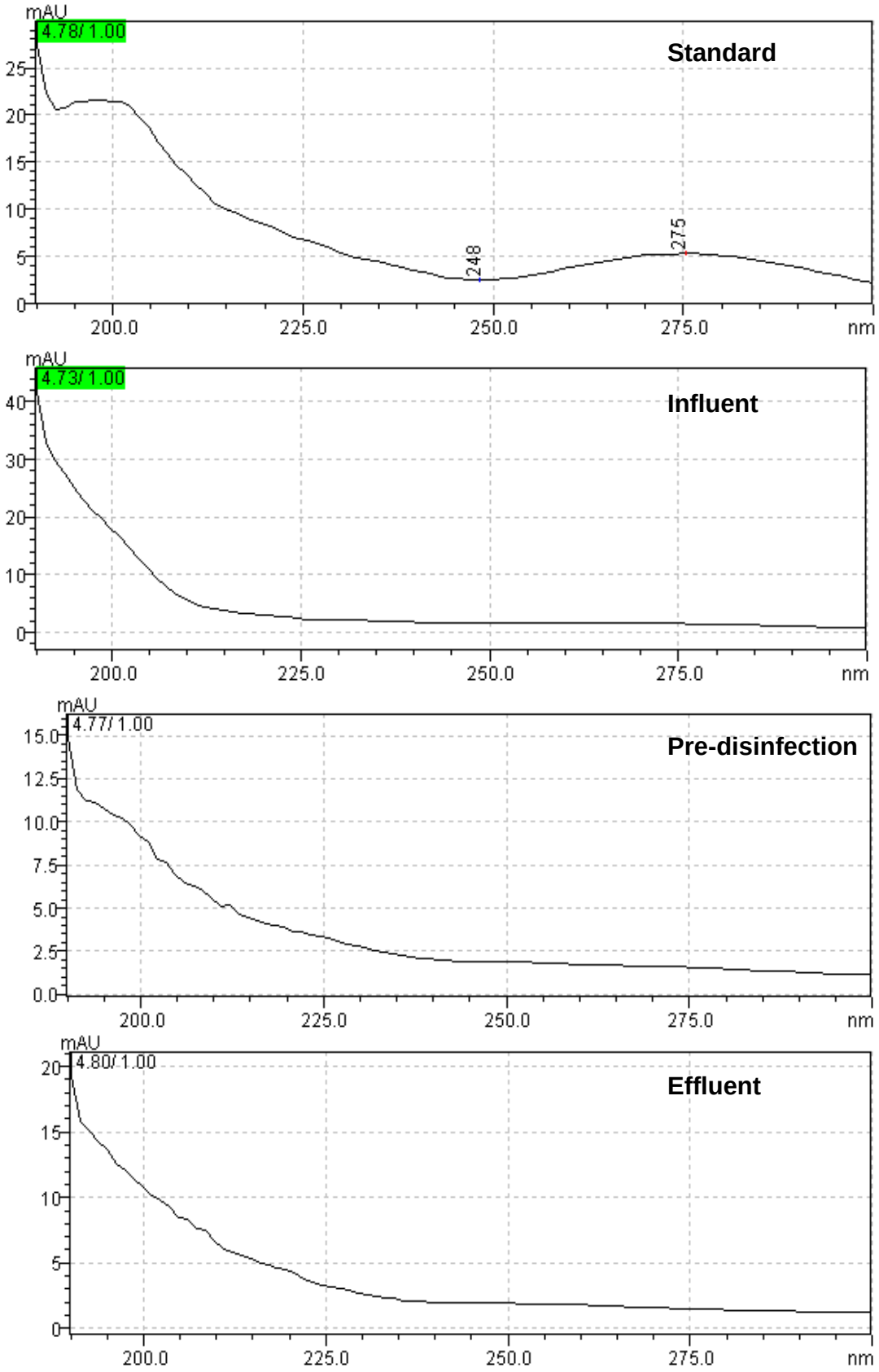


Fig. S3. PDA spectra obtained for diclofenac in standard solution and in extracted wastewater samples.

Table S1.

Physicochemical properties measured in wastewater treatment plants. Standard deviations (n = 3) are given in ± values.

Sample	pH	Conductivity ($\mu s.cm^{-1}$)	DO ($mg.\ell^{-1}$)	Salinity (psu)	TDS ($mg.\ell^{-1}$)	ORP (RmV)
Kingsburgh WWTP influent	7.10 ± 0.45	965 ± 176	0.44 ± 0.35	0.47 ± 0.08	477 ± 82	-29.7 ± 8.8
Kingsburgh WWTP prior to disinfection	7.28 ± 0.30	599 ± 27	3.40 ± 0.42	0.29 ± 0.01	300 ± 14	-28.7 ± 3.3
Kingsburgh WWTP effluent	7.39 ± 0.25	589 ± 18	5.12 ± 0.69	0.29 ± 0.02	299 ± 10	-29.9 ± 2.1
Umbilo WWTP influent	7.42 ± 0.26	862 ± 50	0.45 ± 0.22	0.42 ± 0.03	421 ± 34	-30.2 ± 6.4
Umbilo WWTP prior to disinfection	7.22 ± 0.43	780 ± 78	3.34 ± 0.57	0.39 ± 0.05	400 ±47	-24.9 ± 4.7
Umbilo WWTP effluent	7.13 ± 0.41	785 ± 75	5.42 ± 0.85	0.38 ± 0.03	395 ± 37	-25.1 ± 7.3

DO – dissolved oxygen; TDS – total dissolved solids; ORP – oxidation reduction potential.

3.3.9 Paper 8

Madikizela L.M., Muthwa S.F. and Chimuka, L. (2014) Determination of triclosan and ketoprofen in river water and wastewater by solid phase extraction and high performance liquid chromatography. *South African Journal of Chemistry 67, 143-150.* Impact Factor 0.629

Madikizela – 50% (conceptualized the research, planned the research, wrote the manuscript);

Muthwa – 40% (conducted the experiments);

Chimuka – 10% (supervisor, reviewed the manuscript).

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<http://journals.sabinet.co.za/sajchem/>.

Determination of Triclosan and Ketoprofen in River Water and Wastewater by Solid Phase Extraction and High Performance Liquid Chromatography

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ABSTRACT

This paper describes a simple, sensitive and rapid method for the determination of triclosan and ketoprofen in wastewater influent, effluent and river water. The method involves solid phase extraction (SPE) of target compounds using Oasis HLB sorbent. Several extraction parameters such as sample pH, sample volume, SPE cartridge and SPE elution solvent were optimized. The pH of the collected samples was adjusted to 5.5, and then 100 mL of the sample was loaded into an Oasis HLB cartridge. Methanol was used to elute the retained compounds. The eluted compounds were analyzed using reversed-phase high performance liquid chromatography with photo diode array detection (HPLC-PDA). The method was validated by spiking ultra-pure water and wastewater with different concentrations of both compounds ranging from 5 µg L^{-1} to 1000 µg L^{-1}. Recoveries were in the range of 73 % to 104 %, and % RSD ranged from 8 % to 15 %. The method gave good detection limits of 0.01 and 0.08 µg L^{-1} for triclosan and ketoprofen, respectively. Traces of both compounds were detected in all wastewater (influent and effluent) samples at a range of 1.2 to 9.0 µg L^{-1} and in some river water samples.

KEYWORDS

Solid phase extraction, high performance liquid chromatography, wastewater treatment plants, triclosan, ketoprofen.

1. Introduction

The development of suitable quantitative methods for the assessment of environmental pollutants is ongoing. Increasing focus is directed to the analysis of water pollutants. Water pollutants such as triclosan (5-chloro-2-(2,4-dichlorophenoxy)-phenol) and ketoprofen (2,3-benzoyl phenyl-propionic acid) (structures shown in Fig. 1) are among the environmental pollutants that are often detected in water.[1–4] Triclosan is a lipophilic compound that is used as an antibacterial agent in a number of household products such as toothpaste, liquid soap, sponge, plastic cutting boards, etc.[5] Ketoprofen is one of the most widely used acidic pharmaceutical compounds that belongs to the class of non-steroidal anti-inflammatory drugs. It is used as an analgesic in humans and animals.[6]

Both triclosan and ketoprofen have been detected in river water,[7] wastewater influent[7–9] and effluent.[7–9] Triclosan was even detected in human milk.[5] Triclosan inhibits the enoyl-acyl carrier protein reductase enzyme that is responsible for bacterial lipid biosynthesis. Triclosan is known to be toxic to aquatic organisms such as fish, *Daphnia magna* and algae at µg L^{-1} levels.[5] The toxicity of triclosan to humans is low, but it is known as a precursor for the formation of toxic dioxins. Various chlorinated dioxins are formed due to the exposure of triclosan to sunlight and ultra-violet-visible light.[10]

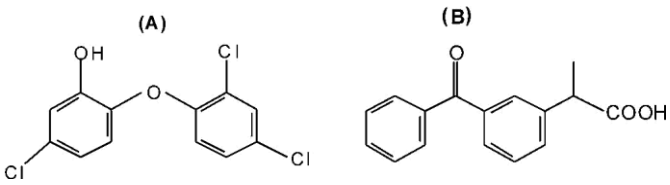


Figure 1 Structure of triclosan (**A**) and ketoprofen (**B**).

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There are no reports found on the toxicity of ketoprofen to aquatic organisms. Presence of these compounds in the aquatic environment can be due to direct disposal to aquatic systems, water run-off from landfill sites and incomplete removal during wastewater treatment.

Chromatographic methods of analysis have been developed for the determination of these compounds in the aquatic systems. Both gas and high performance liquid chromatographic procedures have been reported in the literature.[5–11] When using gas chromatography (GC) for the analysis, derivatization is required to convert the compounds into more volatile forms. Derivatization can lead to the formation of unwanted products and can be time-consuming. High performance liquid chromatography (HPLC) is therefore a preferred technique for non-volatile compounds.

Sample preparation is one of the most important steps when addressing the issue of organic pollutants in the environment. Therefore considerable amount of time has been spent by several researchers in finding a suitable sample preparation technique for the extraction of organics from water samples. Different studies on the analysis of triclosan and ketoprofen[7–9] using solid phase extraction have been reported in foreign countries. How-ever no published reports have been found for the simultaneous determination of these two well-known pollutants using SPE-HPLC-PDA in South African wastewater systems. The aim of this work was to determine a suitable analysis method that is simple, sensitive, accurate, rapid and affordable for the simulta-neous quantification of both lipophilic (triclosan) and hydrophilic (ketoprofen) compounds in water. The proposed method involves

sample pre-treatment using a solid phase extraction technique and quantitation by HPLC with photo diode array detection. The proposed method was applied for the trace determination of both triclosan and ketoprofen in river water and water from a wastewater treatment plant (WWTP).

2. Experimental

2.1. Chemicals, Reagents and Equipment

Ketoprofen (98 %), Triclosan (97 %), HPLC-grade methanol (≥99.9 %) and HPLC-grade ethyl acetate (≥99.7 %) were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC-grade acetonitrile (≥99.9 %) and glacial acetic acid (100 %) were purchased from Merck (Darmstadt, Germany). Sodium hydroxide (≥98 %) and formic acid (approx. 98 %) were purchased from Fluka (Steinheim, Germany). Hydrochloric acid (37 %) was purchased from Merck (Johannesburg, South Africa). Sodium chloride (≥99.5 %) was purchased from Associated Chemical Enterprises (Johannesburg, South Africa). Hexane (60–66 %) was purchased from Honeywell (Muskegon, United States of America). The mobile phase used consisted of a mixture of acetonitrile: 0.2 % formic acid (60:40, v:v) at a flow rate of 1 mL min^{-1}.

A mixture of 100 mg L^{-1} of ketoprofen and triclosan was prepared in acetonitrile. Working standards were prepared from this solution.

Chromatographic separation using a linear solvent system was performed on a high performance liquid chromatography (HPLC) system that consisted of a Waters 600E pump (Waters, Milford, USA), and a photo diode array detector (Shimadzu, Kyoto, Japan). Samples and standards were injected using a Rheodyne 7010 injector equipped with a 20 µL sample loop (California, USA). A Gemini C_{18} HPLC column 150 ×4.60 mm × 5 µm was obtained from Phenomenex (California, USA). Shimadzu LC Solutions software was used for recording of chromatograms. Ketoprofen and triclosan were measured at 255 nm and 200 nm, respectively.

A pH meter (model 691) was obtained from Metrohm (Herizau, Switzerland) and was used for pH reading of all the samples.

The following solid-phase extraction cartridges were investigated; Oasis HLB 6cc 200 mg, Oasis MAX 6cc 150 mg, both obtained from Microsep (Johannesburg, South Africa), and Isolute 500 mg C_{18} was obtained from Separations (Johannes-burg, South Africa).

2.2. Sampling Sites and Sample Collection

Pre-cleaned brown glass bottles were used to collect influent and effluent water samples from a wastewater treatment plant (WWTP) located along the Mbokodweni River south of Durban, South Africa. The grab samples were kept in a refrigerator at 4 °C until SPE-HPLC-PDA analysis. Three sampling points were identified in the Mbokodweni River and grab samples were collected on the upper surface of the river (Fig. 2). Sampling point A was located at approximately 1 km downstream from WWTP, and this point is about 1 km away from the ocean. Sampling points B and C are about 1 km and 3 km, respectively upstream of the WWTP. Monthly collection of samples was done over a three-month period from August to October 2013.

For sample preparation, samples were filtered through 0.45 µm filter paper to remove the suspended solids, and thereafter the pH was adjusted.

2.3. The Optimization of Sample pH, Sorbent Type, Elution Solvent, Sample Volume and Ionic Strength

The optimization of sample pH, sorbent type, elution solvent, ionic strength of the sample and sample volume were carried out on spiked ultra-pure water (1000 µg L^{-1} of each compound) by varying one parameter while keeping others constant. Each optimization procedure was repeated three times (n = 3).

Sample pH was optimized by varying the pH of ultra-pure water spiked with 1000 µg $L^{-1}$of each compound. The pH was adjusted to 2, 4, or 5.5 with HCl (1 mol L^{-1}) and NaOH (0.6 mol L^{-1}). Target compounds were extracted using Oasis HLB

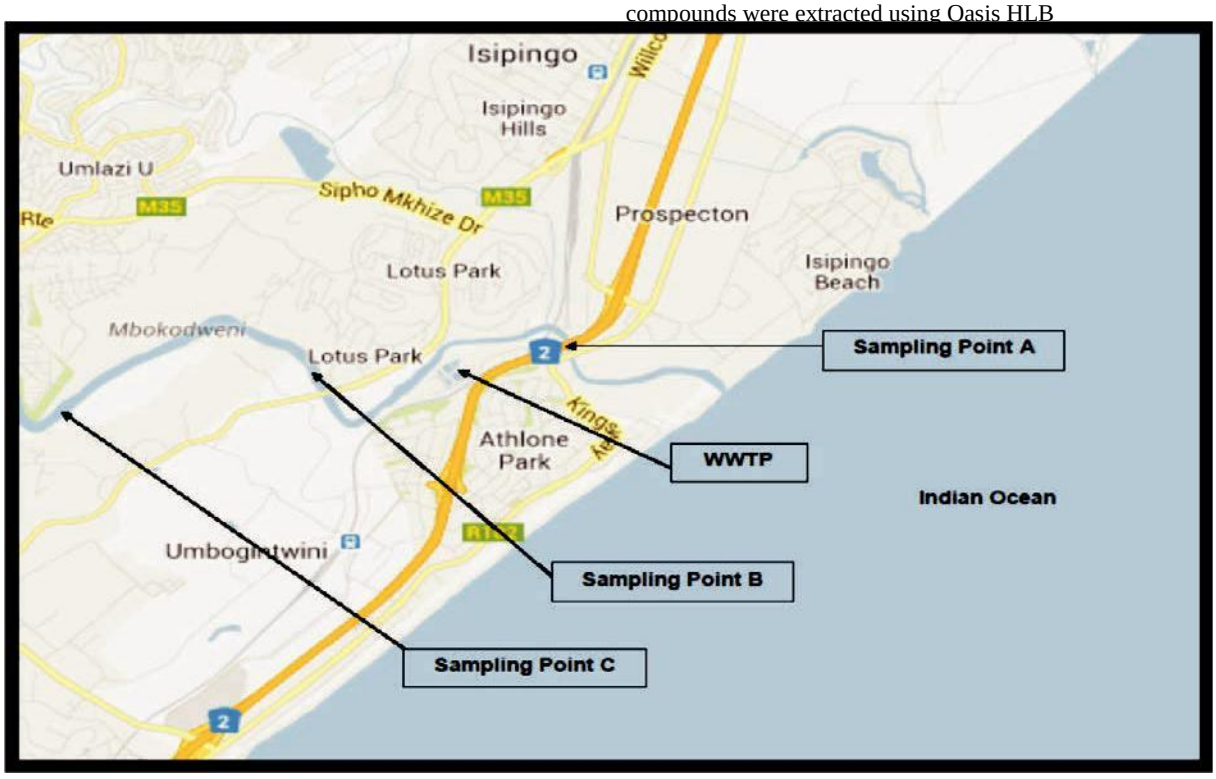


Figure 2 A map showing the sampling sites and areas surrounding the Mbokodweni river.

prior to HPLC-PDA analysis. The following parameters were kept constant while varying the pH of the spiked sample, each cartridge was conditioned with 5 mL methanol and 5 mL of ultra-pure water at a flow rate of 1 mL min^{-1}. A 100 mL volume of the sample was loaded onto the cartridge at a flow rate of 5 mL min^{-1} and sent to waste. The cartridge was vacuum-dried for 30 min and the adsorbed compounds were eluted with 10 ml of methanol at a flow rate of 1 mL min^{-1}.

Flow rates usually vary from 1 to 10 mL min^{-1}, and they are controlled by the use of a vacuum pump.[2,3,4,7] When flow rates are too high, the retention of target compounds might be compromised. For the sample loading step, the flow rate was increased slightly to avoid long sample preparation time.

In order to vary the type of sorbent, three different SPE cartridges were investigated; Oasis HLB (200 mg), Oasis MAX (150 mg) and Isolute C_{18} (500 mg).

Three solvent combinations were tested in order to get the best elution solvent for the Oasis HLB cartridge. These were
(1) methanol, (2) methanol and acetic acid, 9:1 (v/v), and (3) 0.2 % formic acid and acetonitrile, 4:6 (v/v). The other experimental parameters were kept constant while varying the elution solvent.

The effect of sample volume was investigated by passing different volumes of ultra-pure water (pH 5.5) spiked with 1000 µg L^{-1} of each compound through the Oasis HLB SPE cartridge. The volumes of spiked ultra-pure water were in the range of 10 to 300 mL. The compounds retained by SPE cartridge were eluted with methanol. The other experimental parameters were kept constant while varying the sample volume.

The effect of ionic strength of the sample was studied by spiking ultra-pure water with different concentrations of sodium chloride in the range of 0.5–6.0 % (w/v).

2.4. Quality Assurance

Stock solution (100 mg L^{-1}) was diluted with acetonitrile to the desired concentrations until the limit of detection was reached. Four point calibration curves were constructed for both compounds and used for method validation. Recovery, accuracy and precision of the analytical method were determined by spiking ultra-pure water at concentration levels ranging from 5 to 1000 µg L^{-1} and effluent with a concentration of 50 µg L^{-1}. Target compounds were extracted from each spiked sample using the optimized SPE conditions. The analysis was done in triplicate. The extracted compounds were analyzed using HPLC-PDA.

3. Results and Discussion

3.1. Optimization Results

3.1.1. *Sample pH*

The best recoveries for these two compounds were obtained at pH 5.5 (Fig. 3). As a result of these experiments, the optimum pH for selected compounds was chosen as pH 5.5. Low pH is required for the analysis of acidic pharmaceuticals to prevent the dissociation of acidic compounds. However, the pH must not be too low because if the samples are too acidic wastewater interfering substances can also be extracted.[8] Patrolecco *et al.*[7] obtained similar recoveries when extracting ketoprofen and related compounds at pH 3.6 (acidic). Santos *et al.*[8] obtained a mean recovery of 80 % for ketoprofen at neutral pH. In this study this recovery was improved when lowering the pH.

3.1.2. *Sorbent Type*

Three SPE packing materials were investigated in this work. Oasis HLB and Isolute C_{18} cartridges yielded acceptable recoveries (Fig. 4). Oasis HLB exhibits both hydrophilic and lipophilic retention characteristics; therefore it is capable of extracting both polar and non polar compounds.[12] C_{18} cartridges are capable of extracting a wide range of organic compounds from aqueous solutions. Both these cartridges gave acceptable recoveries but extra care needs to be taken when using C_{18} cartridges to ensure that the sorbent stays wet before loading the sample and this is not the case when using Oasis HLB. Therefore Oasis HLB was found to be the best sorbent for this work. Azzouz *et al.*[13] obtained acceptable sorption results when comparing Oasis HLB and C_{18} sorbents. When using Oasis MAX, good recoveries were obtained for triclosan but ketoprofen could not be retained. It is not clear why this is the case; further research needs to be conducted on this.

3.1.3. *Elution Solvent*

Different solvent combinations were tested for the elution of analytes from the SPE cartridge. Due to the polarity of the target compounds, only polar solvents were investigated.

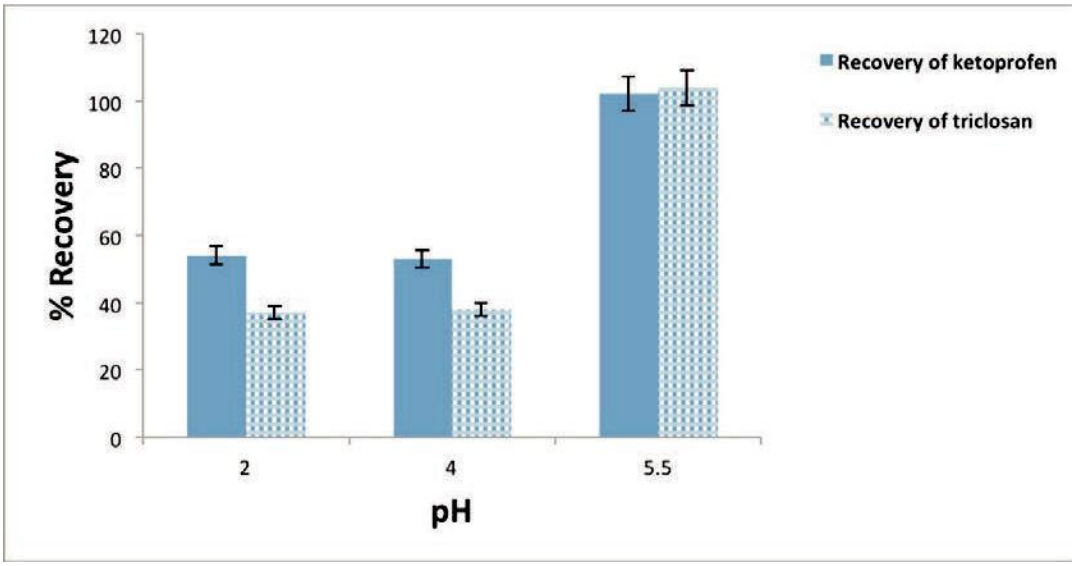


Figure 3 Effect of pH on the recovery of triclosan and ketoprofen. Ultra-pure water was spiked with the target compounds, adjusted to the indicated pHs and 100 mL passed through Oasis HLB. $n = 3$.

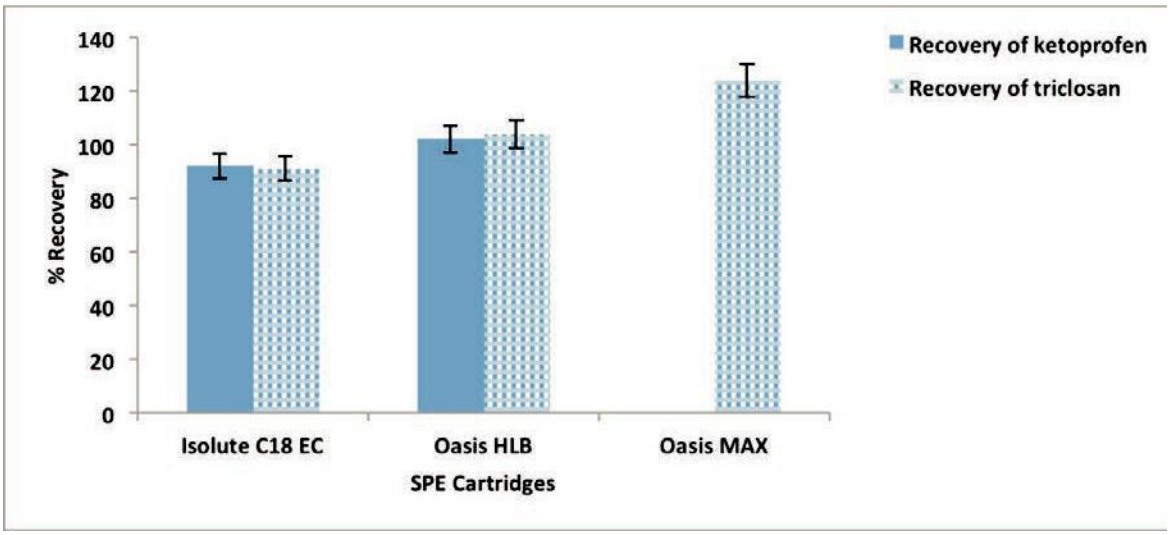


Figure 4 Recovery of triclosan and ketoprofen from different SPE cartridges. Ultra-pure water was spiked with the target compounds, adjusted to pH 5.5 and 100 mL passed through the cartridge. $n = 3$.

Methanol (MeOH) gave the best extraction recoveries compared to other solvents tested. This is in agreement with the results reported in literature.[14,19] Boleda *et al*.[14] obtained recoveries of 86 % and 79 % for triclosan and ketoprofen, respectively, when surface water was spiked with pharmaceutical compounds followed by Oasis HLB extraction and methanol elution of target compounds. Recoveries obtained by Ying *et al*.[19] for effluent samples spiked with 50 ng L^{-1} of each compound were more than 70 % when the target compounds were eluted with methanol from Oasis HLB cartridges. Figure 5 shows the recoveries obtained when using different solvents for SPE elution. To the best of our knowledge, a mixture of formic acid and acetonitrile has not been used as elution solvent for compounds retained on Oasis HLB. It was considered and tried in this study since it is used as a mobile phase, but recoveries were poor. Hexane (non-polar solvent) was tested by Santos *et al*.,[8] and it was discovered that it has a potential of removing lipophilic components although it can remove the hydrophobic interferences as well.

3.1.4. *Effect of Sample Volume*

It was noted that the recoveries of both compounds were affected by the volume of the sample loaded into the SPE cartridge (Fig. 6). This trend was also observed in other study.[15] This is due to capacity of the sorbent being exceeded and has to do with breakthrough volume of the sorbent. A sample volume of 100 mL gave the best recoveries and it was selected for this study. Optimization of sample volume is important when performing the solid phase extraction as higher volumes tend to overload the SPE cartridge and target compounds end up competing for the adsorbing material with matrix interferences. Reduction of ketoprofen recovery to 72.8 % when sample volume was increased to 1000 mL using a molecularly imprinted polymer as SPE sorbent was reported elsewhere[16]. High sample volume can also lead to the saturation of the SPE sorbent and results in poor recoveries.

3.1.5. *Effect of Ionic Strength*

Effect of ionic strength was investigated by spiking ultra-pure water with different concentrations of sodium chloride in the range of 0.5–6.0 % (w/v). It was noted that the presence of sodium chloride affected the retention of target compounds in HLB sorbent (Fig. 7). The recovery of compounds decreased as the amount of sodium chloride increased in the water solution.

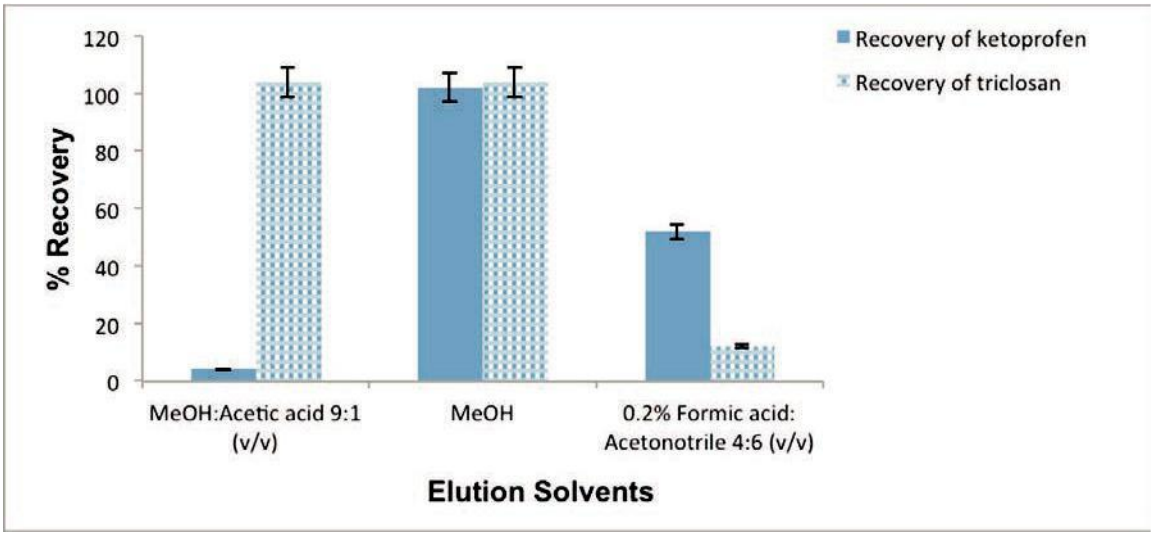


Figure 5 Effect of elution solvent on the recovery of triclosan and ketoprofen. Ultra-pure water was spiked with the target compounds, adjusted to pH 5.5 and 100 mL passed through Oasis HLB. $n = 3$.

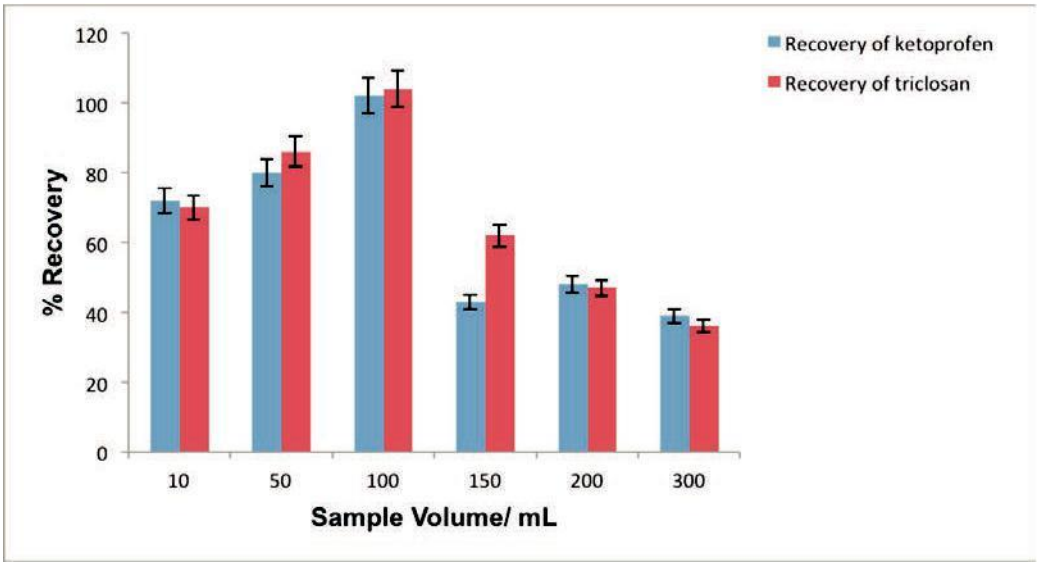


Figure 6 % Recoveries obtained when loading different volumes of spiked ultrapure water onto Oasis HLB cartridge. $n = 3$.

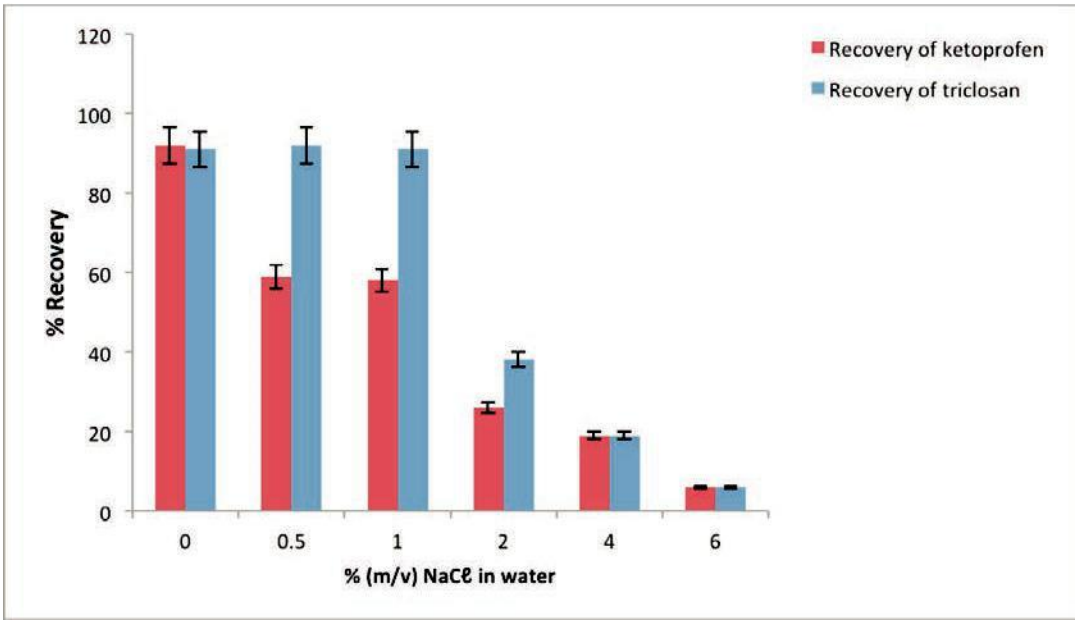


Figure 7 Effect of ionic strength on SPE extraction recoveries.

According to Azzouz *et al.*,[13] the ionic strength of water samples had no effect on adsorption of target compounds onto Oasis HLB on the signals up to 2 mol L^{-1}. Effect of ionic strength was also investigated in another study[17] for the adsorption of pharmaceuticals onto porous silica in the range of 0 to 50 mmol L^{-1} (0 to 0.29 % (w/v)). No significant effect was observed in this range for ketoprofen however triclosan was not included in the study. This was confirmed in the current investigation, and the results were even better when HLB sorbent was used instead of porous silica. From our results, the developed method cannot be applied in the analysis of the same compounds in sea water, because of high levels of salt content in sea water.

3.2. Quality Assurance

To determine the accuracy of the proposed method, validation was carried out by spiking wastewater effluent and ultra-pure water with different concentrations of each compound ranging from 5 µg L^{-1} to 1000 µg L^{-1}. Percentage recoveries obtained after SPE and HPLC-PDA determination are given in Table 1. Calibration curves were found to be linear ($R^2 > 0.99$) for both com-

Table 1 LODs, LOQs, accuracy (% recovery) and repeatability (% RSD, shown as ± values) in ultrapure water spiked at concentration levels ranging from 5 to 1000 µg L^{-1} ($n = 3$) and effluent spiked at 50 µg L^{-1} using optimized SPE conditions.

Compound	LOD/µg L^{-1}	LOQ/µg L^{-1}	% Recovery for ultra-pure water				Effluent
			1000 µg L^{-1}	100 µg L^{-1}	50 µg L^{-1}	5 µg L^{-1}	50 µg L^{-1}
Ketoprofen	0.08	0.26	102 ± 9	83 ± 10	84± 8	85 ± 11	96 ± 14
Triclosan	0.01	0.34	104 ± 8	80 ± 15	73± 10	78 ± 9	75 ± 10

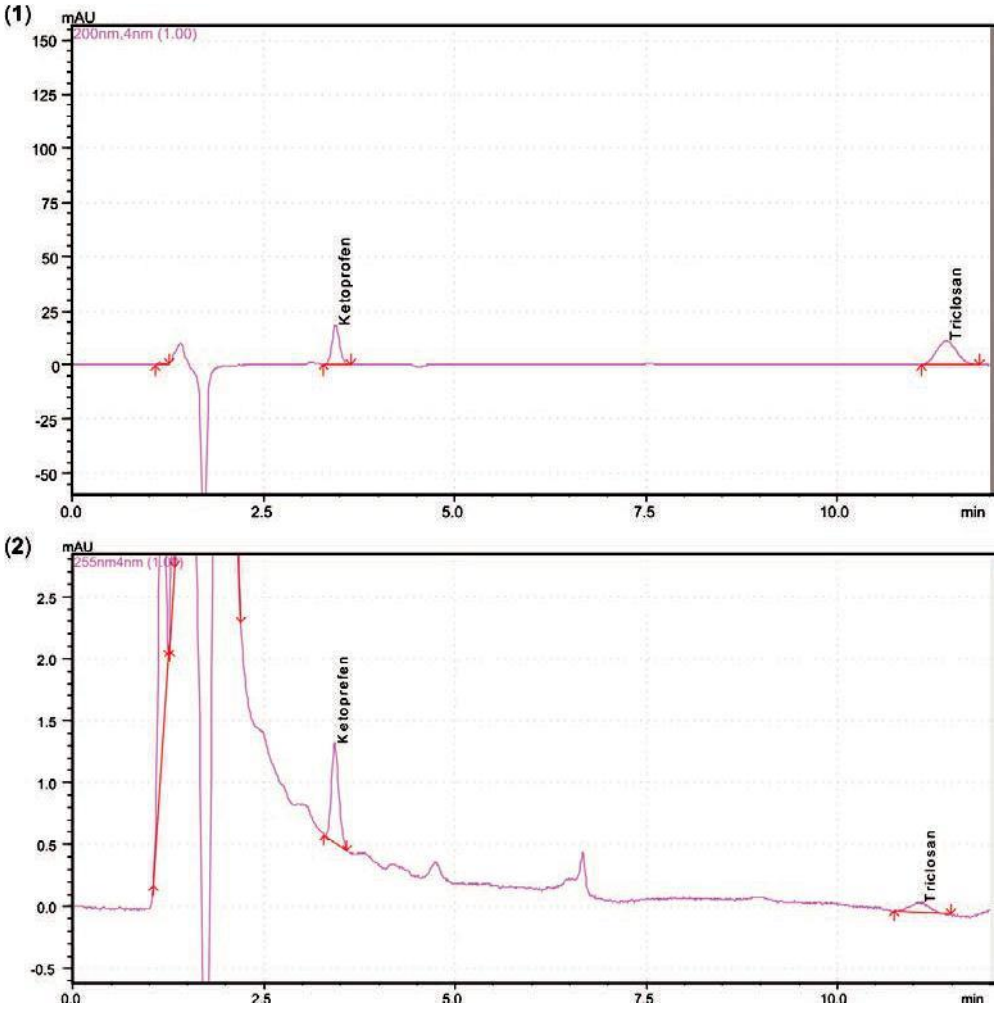


Figure 8 (1) Chromatogram for 1000 µg L^{-1} mixture of both compounds, and **(2)** chromatogram of the effluent sample after SPE.

pounds over a wide concentration range. All methods were conducted in triplicate with % RSD reported as ± values in Table 1. Limit of detection (LOD) and limit of quantification (LOQ) were calculated by a signal to noise ratio of 3 and 10, respectively. From the data obtained (data in Table 1), it can be seen that the proposed method can be applied for the environmental monitoring of these two compounds at sub-µg L^{-1} levels.

3.3. Application of the Method to Environmental Samples

The described method was applied for the qualitative and quantitative determination of triclosan and ketoprofen in wastewater influent, effluent and river water. Identification of compounds present in real samples was based on retention times, and compounds were also confirmed using PDA spectra. Figure 8 shows the chromatograms for the mixture of two compounds and effluent sample and Fig. 9 shows the PDA spectra of both compounds. Both compounds have been detected in all the influent and effluent samples. The WWTP receives approximately 23 000 m^3 d^{-1} of water from two industrial areas (Prospecton and South Gate), and semi-urban areas (Amanzimtoti, Athlone Park, Ispingo, Kwa Makhuta and Folweni). It is well known that WWTPs around the world are not capable of entirely removing the pharmaceutical compounds during the wastewater treatment process.[16,18–,20] The WWTP studied here is not an exception. It has been reported that there is a seasonal variation for the levels of pharmaceuticals in wastewater, where higher concentrations are expected in winter than in summer.[21,22] The explanation for this is that the human consumption of pharmaceuticals is higher during the winter season and compounds degrade faster during summer when temperatures are high.[21,22]

The compounds have been detected in some river samples (Table 2). Mbokodweni River is a long river that cuts in-between Umlazi Township and Kwa Makhuta. One side of the river is

Table 2 Concentrations (µg L^{-1}) of target compounds (n = 3) in river water and wastewater treatment plant.

Sample	Month	Triclosan		Ketoprofen
Influent	August	2.1	± 0.45	1.7± 0.7
	September	9.0	± 2.0	6.4± 2.2
	October	8.5	± 3.0	4.8± 0.90
Effluent	August	1.3	± 0.50	1.2± 0.35
	September	6.4	± 1.0	3.2± 0.80
	October	4.1	± 8.0	4.3± 1.00
MR – A	August	0.9	± 0.22	<LOQ
	September	0.4	± 0.10	1.1± 0.30
	October	<LOQ		2.0± 1.50
MR – B	August	n.d.		n.d.
	September	n.d.		n.d.
	October	n.d.		n.d.
MR – C	August	n.d.		n.d.
	September	n.d.		n.d.
	October	n.d.		n.d.

MR – A: Mbokodweni river-sampling point A; MR – B: Mbokodweni river-sampling point B; MR – C: Mbokodweni river- sampling point C; LOQ result means that the concentration was >LOD but below LOQ; nd means the com-pound was not detected. Sampling points are those explained in section 2.2.

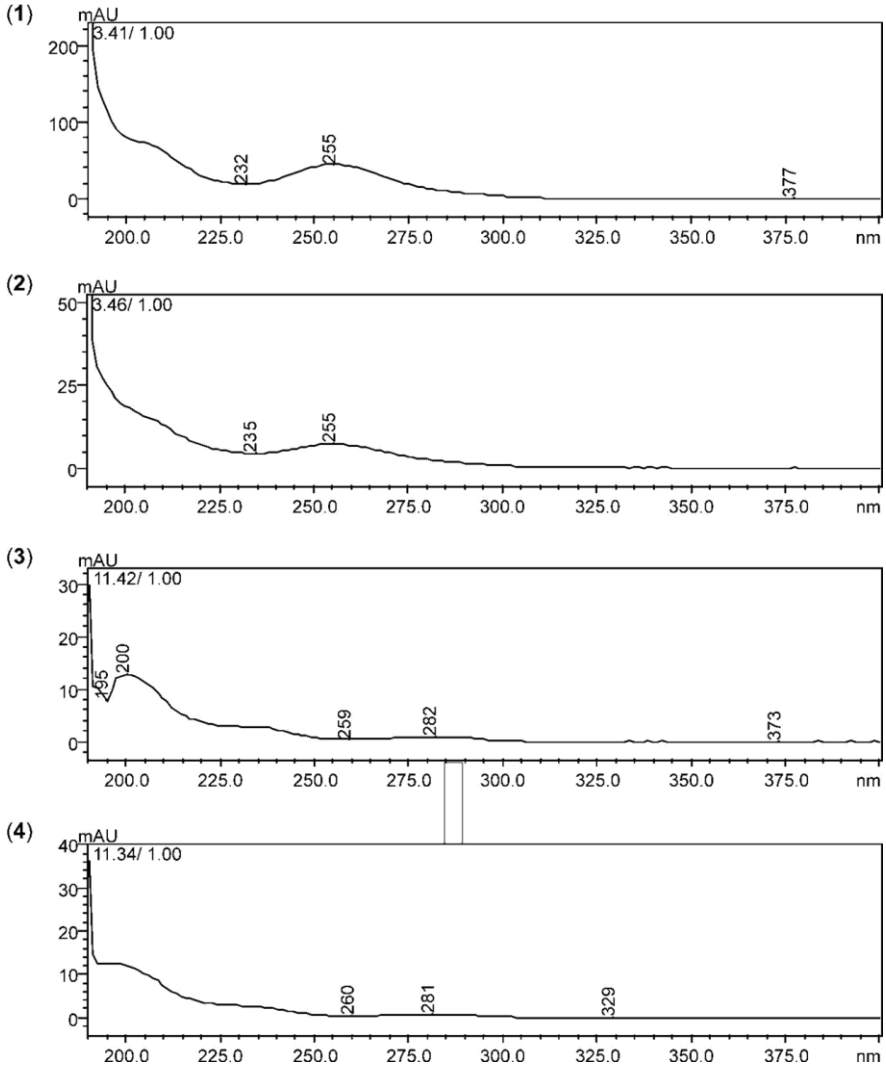


Figure 9 (1) PDA spectrum for ketoprofen standard, **(2)** PDA spectrum for ketoprofen in the effluent sample, **(3)** PDA spectrum for triclosan standard and **(4)** PDA spectrum for triclosan in the effluent sample.

occupied by scattered rural houses. The results obtained for river samples show that as the river flows towards the sea the level of pollutants increases. The samples from upstream of the river were cleaner than the samples downstream, and the target compounds were not detected in upstream samples.

The levels of the compounds obtained in wastewater samples are high compared to what is reported in literature by researchers of other countries (Table 3), therefore something should be done to reduce the levels of these pollutants in South Africa.

4. Conclusions

A simple, sensitive, accurate, rapid and affordable method was developed for the quantitative determination of triclosan and ketoprofen in the aquatic environment. The developed method involves the simultaneous extraction of both compounds using Oasis HLB sorbent. The extracted compounds were pre-concentrated prior to HPLC determination. The method is not suitable for samples that contain high salt content. The described method was applied for the qualitative and quantitative deter-

Table 3 Maximum concentration levels of triclosan and ketoprofen reported in foreign countries.

Compound	Influent/μg L^{-1}	Effluent/μg L^{-1}	Country	Reference
Triclosan	0.8	0.25	United States	23
	0.8	0.15	Korea	24
	0.057	Not reported	Spain	25
	2.42	0.51	Spain	26
	Not reported	0.324	Canada	27
	2.11	0.24	Greece	28
Ketoprofen	1.2	0.28	United States	23
	0.29	0.037	Korea	24
	0.80	0.54	Spain	26
	Not reported	0.210	Canada	27
	1.28	0.12	Greece	28
	0.37	Not reported	Tokyo	29

mination of both compounds in wastewater and river water. High levels of these compounds were detected in wastewater, whereas small residues were occasionally found in river water. The effect of these compounds on aquatic animals of the polluted river is not known. Further research needs therefore to be conducted on health effects of these compounds on aquatic organisms in the Mbokodweni river.

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References

1 S. Bayen, H. Zhang, M.M. Desai, S.K. Ooi and B.C. Kelly, *Environ. Pollut.*, 2013, **182**, 1–8.
2 T.E. Felix-Canedo, J.C. Duran-Alvarez and B. Jimenez-Cisneros, *Sci. Total Environ.*, 2013, **454–455**, 109–118.
3 N.H. Tran, J. Hu and S.L. Ong, *Talanta*, 2013, **113**, 82–92.
4 J. Zhao, G. Ying, L. Wang, J. Yang, X. Yang, L. Yang and X. Li, *Sci. Total Environ.*, 2009, **407**, 962–974.
5 M. Adolfsson-Erici, M. Pettersson, J. Parkkonen and J. Sturve, *Chemosphere*, 2002, **46**, 1485–1489.
6 E. Sagrista, E. Larsson, M. Ezoddin, M. Hildago, V. Salvado and J.A. Jonsson, *J. Chromatogr. A*, 2010, **1217**, 6153–6158.
7 L. Patrolecco, N. Ademollo, P. Grenni, A. Tolomei, A.B. Caracciolo and S. Capri, *Microchem. J*, 2013, **107**, 165–171.
8 J.L. Santos, I. Aparicio, E. Alonso and M. Callejon, *Anal. Chim. Acta*, 2005, **550**, 116–122.
9 Y. Yu and L. Wu, *J. Chromatogr. A*, 2011, **1218**, 2483–2489.
10 J.Y. Shen, M.S. Chang, S. Yang and G.J. Wu, *J. Liq. Chromatogr. Relat. Technol.*, 2012, **35**, 2280–2293.
11 C. Grove, W. Liebenberg, J.L. Du Preeze, W. Yang and M.M. De Villiers, *J. Cosmet. Sci.*, 2003, **54**, 537–550.
12 L.M. Madikizela, *Optimisation of HPLC-based methods for the separation and detection of herbicide glyphosate and its major metabolite in water*, M.Tech. thesis, Durban University of Technology, Durban, South Africa, 2010.
25 A. Azzouz, B. Souhail and E. Ballesteros, *J. Chromatogr. A*, 2010, **1217**, 2956–2963.
26 R. Boleda, T. Galceran and F. Ventura, *J. Chromatogr. A*, 2013, **1286**, 146–158.
27 J. Patsias and E. Papadopoulou-Mourkidou, *J. Chromatogr. A*, 2000, **904**, 171–188.
28 Y. Duan, C. Dai, Y. Zhang and L. Chen, *Anal. Chim. Acta*, 2013, **758**, 93–100.
29 T.X. Bui and H. Choi, *Chemosphere*, 2010, **80**, 681–686.
30 N. Lindqvist, T. Tuhkanen and L. Kronberg, *Water Res.*, 2005, **39**, 2219–2228.
31 M. Farre, I. Ferrer, A. Ginebreda, M. Figueras, L. Olivella, L. Tirapu, M. Vilanova and D. Barcelo, *J. Chromatogr. A*, 2001, **938**, 187–197.
32 G. Ying, R.S. Kookana and D.W. Kolpin, *J. Environ. Monit.*, 2009, **11**, 1498–1505.
33 Y. Yu, L. Wu and A.C. Chang, *Sci. Total Environ.*, 2013, **442**, 310–316.
34 A. Azzouz and E. Ballesteros, *Chemosphere*, 2013, **93**, 2046–2054.
35 J.T. Yu, E.J. Bouwer and M. Coelhan, *Agric. Water Manage.*, 2006, **86**, 72–80.
36 S.K. Behera, H.W. Kim, J. Oh and H. Park, *Sci. Total Environ.*, 2011, **409**, 4351–4360.
37 R. Rodil, J.B. Quintana, E. Concha-Grana, P. Lopez-Mahia, S. Muniategui-Lorenzo and D. Prada-Rodriguez, *Chemosphere*, 2012, **86**, 1040–1049.
38 R. Rosal, A. Rodriguez, J.A. Perdigon-Melon, A. Petre, E. Garcia-Calvo, M.J. Gomez, A. Aguera and A.R. Fernandez-Alba, *Water Res.*, 2010, **44**, 578–588.
39 L. Lishman, S.A. Smyth, K. Sarafin, S. Kleywegt, J. Toito, T. Peart, B. Lee, M. Servos, M. Beland and P. Seto, *Sci. Total Environ.*, 2006, **367**, 544–558.
40 V.G. Samaras, A.S. Stasinakis, D. Mamais, N.S. Thomaidis and T.D. Lekkas, *J. Hazard. Mater.*, 2013, **244-245**, 259-267.
41 N. Nakada, T. Tanishima, H. Shinohara, K. Kiri and H. Takada, *Water Res.*, 2006, **40**, 3297–3303.

Chapter 4 - CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

In this chapter, major conclusions are highlighted that were drawn from the presented publications and manuscripts. These conclusions are accompanied by the suggestions and recommendations for future research.

A multi-templates MIP was synthesized for the selective extraction of naproxen, ibuprofen and diclofenac from water samples. The synthesized MIP was able to adsorb naproxen, ibuprofen and diclofenac selectively in both organic and aqueous solvents. For the selective adsorption in organic phase, both high polar and less polar solvents were investigated in the presence gemfibrozil which was used as a competitor. The selective adsorption in aqueous phase was investigated in the presence of gemfibrozil and fenoprofen as competitors. The adsorption in water occurred better in acidic pH conditions. This was a result of strong hydrogen bonding interactions between target compounds and MIP that occurred at low pH when naproxen, ibuprofen and diclofenac were protonated.

Molecularly imprinted polymer was able to adsorb naproxen, ibuprofen and diclofenac from the contaminated wastewater and river water. Unlike the commercial available sorbents, MIP can be recycled by washing with appropriate organic solvents and re-used. This minimises the production of solid waste and cost of the MIP sorbent. However, the disadvantage of re-using MIPs in this analysis is the production of liquid waste that contains the organic solvents used in sorbent regeneration and target compounds.

The application of MIP as a selective sorbent in the solid-phase extraction led to the development of highly sensitive, cheap and rapid method for the quantification of naproxen, ibuprofen and diclofenac in water bodies. This method was highly accurate and precise. The method was used to quantitatively determine the concentrations of naproxen, ibuprofen and diclofenac in samples collected from WWTPs located in eThekwini Municipality, as well as Mbokodweni River. In comparison with a commercial available sorbent (Oasis MAX) for SPE, MIP was more selective. The molecularly imprinted solid phase extraction method was further applied in an initial assessment of naproxen, ibuprofen and diclofenac in water samples collected from Ladysmith WWTPs, river and drinking water treatment facility.

Regarding the environmental assessment, the concentrations of naproxen, ibuprofen and diclofenac detected in samples collected near the city of Durban in eThekwini Municipality were higher than the levels observed in Ladysmith

samples. The contamination of rivers with pharmaceuticals was evident in all studied sites.

Therefore, future studies should involve the assessment regarding the contamination of aquatic species such as fish. Such studies should concentrate on quantitative and qualitative determination of pharmaceuticals in aquatic animals. Also focus should be directed to the analysis of aquatic plants and sediments. Development of polymers or materials for remediation process needs to be investigated. Also, the use of mass spectrometry detection system to verify the results obtained using photo diode array detector is recommended in future studies.

REFERENCES

Agunbiade, F.O., Moodley, B. (2014), Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa, *Environmental Monitoring and Assessment*, 186, pp. 7273-7291.

Agunbiade, F.O., Moodley, B. (2016), Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi River, KwaZulu-Natal, South Africa, *Environmental Toxicology and Chemistry*, 35, pp. 36-46.

Al-Hadithi, N**.,** Saad, B., Grote, M. (2011), A solid bar microextraction method for the liquid chromatographic determination of trace diclofenac, ibuprofen and carbamazepine in river water, *Microchimica Acta*, 172, pp. 31-37.

Amdany, R. (2013), Passive samplers: development and application in monitoring organic micropollutants in South African water bodies and wastewater, PhD thesis, University of the Witwatersrand, South Africa.

Amdany, R., Cukrowska, E., Chimuka, L. (2014), Determination of naproxen, ibuprofen and triclosan in wastewater using the polar organic chemical integrative sampler (POCIS): A laboratory calibration and field application, *Water South Africa*, 40, pp. 407-414.

Amdany, R., Moya, A., Cukrowska, E., Chimuka, L. (2015), Optimization of the temperature for the extraction of pharmaceuticals from wastewater by a hollow fiber silicone membrane, *Analytical Letters*, 48, pp. 2343-2356.

Andrade-Eiroa, A., Canle, M., Leroy-Cancellieri, V., Cerda, V. (2016), Solid-phase extraction of organic compounds: A critical review (Part I), *TrAC Trends in Analytical Chemistry*, 80, pp. 641-654.

Antuna-Jimenez, D., Carmen Blanco-Lopez, M., Miranda-Ordieres, A.J., Jesus Lobo-Castanon, M. (2014), Artificial enzyme with magnetic properties and peroxidase activity on indoleamine metabolite tumor marker, *Polymer*, 55, pp. 1113-1119.

Araujo, L., Wild, J., Villa, N., Camargo, N., Cubillan, D., Prieto, A. (2008), Determination of anti-inflammatory drugs in water samples, by in situ derivatization, solid phase microextraction and gas chromatography–mass spectrometry, *Talanta*, 75, pp. 111-115.

Arditsoglou, A., Voutsa, D. (2008), Passive sampling of selected endocrine disrupting compounds using polar organic chemical integrative samplers, *Environmental Pollution*, 156, pp. 316-324.

Arsenault, J.C. (2012), Beginner's guide to SPE solid-phase extraction, Waters Corporation, pp. 191-199.

Azzouz, A., Souhail, B., Ballesteros, E. (2010), Continuous solid-phase extraction and gas chromatography–mass spectrometry determination of pharmaceuticals and hormones in water samples, *Journal of Chromatography A*, 1217, pp. 2956-2963.

Bialk-Bielinska, A., Kumirska, J., Borecka, M., Caban, M., Paszkiewicz, M., Pazdro, K., Stepnowski, P. (2016), Selected analytical challenges in the determination of pharmaceuticals in drinking/marine waters and soil/sediment samples, *Journal of Pharmaceutical and Biomedical Analysis*, 121, pp. 271-296.

Bagheri, H., Piri-Moghadam, H., Naderi, M. (2012), Towards greater mechanical, thermal and chemical stability in solid-phase microextraction, *Trends in Analytical Chemistry*, 34, pp. 126-139.

Batlokwa, B.S., Mokgadi, J., Nyokong, T., Torto, N. (2011), Optimal template removal from molecularly imprinted polymers by pressurized hot water extraction, *Chromatographia*, 73, pp. 589-593.

Behera, S.K., Kim, H.W., Oh, J., Park, H. (2011), Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea, *Science of The Total Environment*, 409, pp. 4351-4360.

Bones, J., Thomas, K., Nesterenko, P.N., Paull, B. (2006), On-line preconcentration of pharmaceutical residues from large volume water samples

using short reversed-phase monolithic cartridges coupled to LC-UV-ESI-MS, *Talanta*, 70, pp. 1117-1128.

Caban, M., Lis, E., Kumirska, J., Stepnowski, P. (2015), Determination of pharmaceutical residues in drinking water in Poland using a new SPE-GC-MS(SIM) method based on Speedisk extraction disks and DIMETRIS derivatization, *Science of the Total Environment*, 538, pp. 402-411.

Camino-Sanchez, F.J., Rodriguez-Gomez, R., Zafra-Gomez, A., Santos-Fandila, A., Vilchez, J.L. (2014), Stir bar sorptive extraction: Recent applications, limitations and future trends, *Talanta*, 130, pp. 388-399.

Caro, E., Marce, R.M., Borrull, F., Cormack, P.A.G., Sherrington, D.C. (2006), Application of molecularly imprinted polymers to solid-phase extraction of compounds from environmental and biological samples, *Trends in Analytical Chemistry*, 25, pp. 143-154.

Caro, E., Marce, R.M., Borrull, F., Cormack, P.A.G., Sherrington, D.C., Borrull, F. (2004), A new molecularly imprinted polymer for the selective extraction of naproxen from urine samples by solid-phase extraction, *Journal of Chromatography B*, 813, pp. 137-143.

Cormack, P.A.G., Elorza, A.Z. (2004), Molecularly imprinted polymers: synthesis and characterisation, *Journal of Chromatography B*, 804, 173-182.

Cuklev, F., Fick, J., Cvijovic, M., Kristiansson, E., Forlin, L., Joakim Larsson, D.G. (2012), Does ketoprofen or diclofenac pose the lowest risk to fish?, *Journal of Hazardous Materials*, 229-230, pp. 100-106.

Dahane, S., Gil Garcia, M.D., Martinez Bueno, M.J., Ucles Moreno, A., Martinez Galera, M., Derdour, A. (2013), Determination of drugs in river and wastewaters using solid-phase extraction by packed multi-walled carbon nanotubes and liquid chromatography–quadrupole-linear ion trap-mass spectrometry, *Journal of Chromatography A*, 1297, pp. 17-28.

Dai, C., Zhang, J., Zhang, Y., Zhou, X., Duan, Y., Liu, S. (2012), Selective removal of acidic pharmaceuticals from contaminated lake water using multi-templates molecularly imprinted polymer, *Chemical Engineering Journal*, 211-212, pp. 302-309.

Duan, Y., Dai, C., Zhang, Y., Ling-Chen. (2013), Selective trace enrichment of acidic pharmaceuticals in real water and sediment samples based on solid-phase extraction using multi-templates molecularly imprinted polymers, *Analytica Chimica Acta*, 758, pp. 93-100.

Farrington, K., Regan, F. (2007), Investigation of the nature of MIP recognition: The development and characterisation of a MIP for ibuprofen, *Biosensors and Bioelectronics*, 22, pp. 1138-1146.

Felix-Canedo, T.E., Duran-Alvarez, J.C., Jimenez-Cisneros, B. (2013), The occurrence and distribution of a group of organic micropollutants in Mexico City's water sources, *Science of The Total Environment*, 454-455, pp. 109-118.

Gilart, N., Cormack, P.A.G., Marce, R.M., Fontanals, N., Borrull, F. (2014), Selective determination of pharmaceuticals and illicit drugs in wastewaters using a novel strong cation-exchange solid-phase extraction combined with liquid chromatography–tandem mass spectrometry, *Journal of Chromatography A*, 1325, pp. 137-146.

Gilart, N., Marce, R.M., Fontanals, N., Borrull, F. (2013), A rapid determination of acidic pharmaceuticals in environmental waters by molecularly imprinted solid-phase extraction coupled to tandem mass spectrometry without chromatography, *Talanta*, 110, pp. 196-201.

Gracia-Lor, E., Martinez, M., Sancho, J.V., Penuela, G., Hernandez, F. (2012), Multi-class determination of personal care products and pharmaceuticals in environmental and wastewater samples by ultra-high performance liquid-chromatography-tandem mass spectrometry, *Talanta*, 99, pp. 1011-1023.

Haginaka, J., Sanbe, H., Takehira, H. (1999), Uniform-sized molecularly imprinted polymer for (*S*)-ibuprofen: Retention properties in aqueous mobile phase, *Journal of Chromatography A,* 857, pp. 117-125.

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sanchez, R., Ventura, F., Petrovic, M., Barcelo, D. (2011), Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment, *Water Research,* 45, pp. 1165-1176.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J. (2008), The effect of signal suppression and mobile phase composition on the simultaneous analysis of multiple classes of acidic/neutral pharmaceuticals and personal care products in surface water by solid-phase extraction and ultra performance liquid chromatography–negative electrospray tandem mass spectrometry, *Talanta,* 74, pp. 1299-1312.

Kermia, A.E.B., Fouial-Djebbar, D., Trari, M. (2016), Occurrence, fate and removal efficiencies of pharmaceuticals in wastewater treatment plants (WWTPs) discharging in the coastal environment of Algiers, *Comptes Rendus Chimie*, 19, pp. 963-970.

Koutsouba, V., Heberer, T., Fuhrmann, B., Schmidt-Baumler, K., Tsipi, D., Hiskia, A. (2003), Determination of polar pharmaceuticals in sewage water of Greece by gas chromatography-mass spectrometry, *Chemosphere,* 51, pp.69-75.

Kostopoulou, M., Nikolaou, A., (2008), Analytical problems and the need for sample preparation in the determination of pharmaceuticals and their metabolites in aqueous environmental matrices, *Trends in Analytical Chemistry*, 27, pp. 1023-1035.

K'oreje, K.O., Demeestere, K., De Wispelaere, P., Vergeynst, L., Dewulf, J., Van Langenhove, H., (2012), From multi-residue screening to target analysis of pharmaceuticals in water: Development of a new approach based on magnetic sector mass spectrometry and application in the Nairobi River basin, Kenya, *Science of the Total Environment*, 437, pp. 153-164.

K'oreje, K.O., Vergeynst, L., Ombaka, D., De Wispelaere, P., Okoth, M., Van Langenhove, H., Demeestere, K. (2016), Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya, *Chemosphere*, 149, pp. 238-244.

Kummerer, K. (2009), The presence of pharmaceuticals in the environment due to human use – present knowledge and future challenges, *Journal of Environmental Management*, 90, 2354-2366.

Larsson, N., Petersson, E., Rylander, M., Jonsson, J.A. (2009), Continuous flow hollow fiber liquid-phase microextraction and monitoring of NSAID pharmaceuticals in a sewage treatment plant effluent, *Analytical Methods*, 1, pp. 59-67.

Laven, M., Alsberg, T., Yu, Y., Adolfsson-Erici, M., Sun, H. (2009), Serial mixed-mode cation- and anion-exchange solid-phase extraction for separation of basic, neutral and acidic pharmaceuticals in wastewater and analysis by high-performance liquid chromatography–quadrupole time-of-flight mass spectrometry, *Journal of Chromatography A*, 1216, pp. 49-62.

Le, M.D., Duong, H.A., Nguyen, M.H., Saiz, J., Pham, H.V., Mai, T.D. (2016), Screening determination of pharmaceutical pollutants in different water matrices using dual-channel capillary electrophoresis coupled with contactless conductivity detection, *Talanta*, 160, pp. 512-520.

Li, Z., Qin, C., Li, D., Hou, Y., Li, S., Sun, J. (2014), Molecularly imprinted polymer for specific extraction of hypericin from *Hypericum perforatum* L. herbal extract, *Journal of Pharmaceutical and Biomedical Analysis*, 98, pp. 210-220.

Lindqvist, N., Tuhkanen, T., Kronberg, L. (2005), Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters, *Water Research*, 39, pp. 2219-2228.

Mahkam, M., Poorgholy, N. (2011), Imprinted polymers as drug delivery vehicles for anti-inflammatory drugs, *Nature and Science*, 9, pp. 163-168.

Mahugo-Santana, C., Sosa-Ferrera, Z., Torres-Padron, M.E., Santana-Rodriguez, J.J. (2011), Application of new approaches to liquid-phase microextraction for the determination of emerging pollutants, *Trends in Analytical Chemistry*, 30, pp. 731-748.

Manrique-Moreno, M., Garidel, P., Suwalsky, M., Howe, J., Brandenburg, K. (2009), The membrane-activity of ibuprofen, diclofenac, and naproxen: A physico-chemical study with lecithin phospholipids, *Biochimica et Biophysica Acta (BBA) – Biomembranes*, 1788, pp. 1296-1303.

Manrique-Moreno, M., Heinbockel, L., Suwalsky, M., Garidel, P., Brandenburg, K. (2016), Biophysical study of the non-steroidal anti-inflammatory drugs (NSAID) ibuprofen, naproxen and diclofenac with phosphatidylserine bilayer membranes, *Biochimica et Biophysica Acta (BBA) – Biomembranes*, 1858, pp. 2123-2131.

Martin, J., Camacho-Munoz, D., Santos, J.L., Aparicio, I., Alonso, E. Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal, *Journal of Hazardous Materials*, 239-240, pp. 40-47.

Martinez-Sena, T., Armenta, S., de la Guardia, M., Esteve-Turrillas, F.A. (2016), Determination of non-steroidal anti-inflammatory drugs in water and urine using selective molecular imprinted polymer extraction and liquid chromatography, *Journal of Pharmaceutical and Biomedical Analysis*, 131, pp. 48-53.

Matongo, S., Birungi, G., Moodley, B., Ndungu, P. (2015[a]), Pharmaceutical residues in water and sediment of Msunduzi River, KwaZulu-Natal, South Africa, *Chemosphere*, 134, pp. 133-140.

Matongo, S., Birungi, G., Moodley, B., Ndungu, P. (2015[b]), Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa, *Environmental Science and Pollution Research*, 22, 10298-10308.

Migowska, N., Caban, M., Stepnowski, P., Kumirska, J. (2012), Simultaneous analysis of non-steroidal anti-inflammatory drugs and estrogenic hormones in water and wastewater samples using gas chromatography–mass spectrometry and gas

chromatography with electron capture detection, *Science of the Total Environment*, 441, pp. 77-88.

Mijangos, I., Navarro-Villoslada, F., Guerreiro, A., Piletska, E., Chianella, I., Karim, K., Turner, A., Piletsky, S. (2006), Influence of initiator and different polymerisation conditions on performance of molecularly imprinted polymers, *Biosensors and Bioelectronics*, 22, pp. 381-387.

Moraes de Oliveira, A.R., Cesarino, E.J., Bonato, P.S. (2005), Solid-phase microextraction and chiral HPLC analysis of ibuprofen in urine, *Journal of Chromatography B*, 818, pp. 285-291.

Nawala, J., Czuprynski, K., Popiel, S., Dziedzic, D., Beldowski, J. (2016), Development of the HS-SPME-GC-MS/MS method for analysis of chemical warfare agent and their degradation products in environmental samples, *Analytica Chimica Acta*, 933, pp. 103-116.

Oaks, J.L., Gilbert, M., Virani, M.Z., Watson, R.T., Meteyer, C.U., Rideout, B.A., Shivaprasad, H.L., Ahmed, S., Chaudhry, M.J.I., Arshad, M., Mahmood, S., Ali, A., Khan, A.A. (2004), Diclofenac residues as the cause of vulture population decline in Pakistan, *Nature*, 427, pp. 630-633.

Olarinmoye, O., Bakare, A., Ugwumba, O., Hein, A. (2016), Quantification of pharmaceutical residues in wastewater impacted surface waters and sewage sludge from Lagos, Nigeria, *Journal of Environmental Chemistry and Ecotoxicology*, 8, pp. 14-24.

Ollers, S., Singer, H.P., Fassler, P., Muller, S.R. (2001), Simultaneous quantification of neutral and acidic pharmaceuticals and pesticides at the low-ng/l level in surface and waste water, *Journal of Chromatography A*, 911, pp. 225-234.

Paiga, P., Santos, L.H.M.L.M., Ramos, S., Jorge, S., Gabriel Silva, J., Delerue-Matos, C. (2016), Presence of pharmaceuticals in the Lis river (Portugal): Sources, fate and seasonal variation, *Science of The Total Environment*, 573, pp. 164-177.

Parrilla Vazquez, M.M., Parrilla Vazquez, P., Martinez Galera, M., Gil Garcia, M.D., Ucles, A. (2013), Ultrasound-assisted ionic liquid dispersive liquid-liquid microextraction coupled with liquid chromatography-quadrupole-linear ion trap-mass spectrometry for simultaneous analysis of pharmaceuticals in wastewaters, *Journal of chromatography A*, 1291, pp. 19-26.

Pawliszyn, J. (2000), Theory of solid-phase microextraction, *Journal of Chromatographic Science*, 38, pp. 270-278.

Payan, M.R., Bello Lopez, M.A., Fernandez Torres, R., Bernal, J.L.P., Callejon Mochon, M. (2009), HPLC determination of ibuprofen, diclofenac and salicyclic acid using hollow fiber-based liquid phase microextraction (HF-LPME), *Analytica Chimica Acta*, 653, pp. 184-190.

Payan, M.R., Bello Lopez, M.A., Fernandez Torres, R., Villar Navarro, M., Callejon Mochon, M. (2011), Electromembrane extraction (EME) and HPLC determination of non-steroidal anti-inflammatory drugs (NSAIDs) in wastewater samples, *Talanta*, 85, pp. 394-399.

Payan, M.R., Bello Lopez, M.A., Fernandez Torres, R., Callejon Mochon, M., Gomez Ariza, J.L. (2010), Application of hollow fiber-based liquid-phase microextraction (HF-LPME) for the determination of acidic pharmaceuticals in wastewaters, *Talanta*, 82, pp. 854-858.

Petrie, B., Youdan, J., Barden, R., Kasprzyk-Hordern, B. (2016), Multi-residue analysis of 90 emerging contaminants in liquid and solid environmental matrices by ultra-high-performance liquid chromatography tandem mass spectrometry, *Journal of Chromatography A*, 1431, pp. 64-78.

Pichon, V., Chapuis-Hugon, F. (2008), Role of molecularly imprinted polymers for selective determination of environmental pollutants- A review, *Analytica Chimica Acta*, 622, pp. 48-61.

Piri-Moghadam, H., Ahmadi, F., Pawliszyn, J. (2016) A critical review of solid phase microextraction for analysis of water samples, *TrAC Trends in Analytical Chemistry*, 85, pp. 133-143.

Quero-Pastor, M.J., Garrido-Perez, M.C., Acevedo, A., Quiroga, J.M. (2014), Ozonation of ibuprofen: A degradation and toxicity study, *Science of The Total Environment*, 466-467, pp. 957-964.

Quintana, J.B., Rodil, R., Muniategui-Lorenzo, S., Lopez-Mahia, P., Prada-Rodriguez, D. (2007), Multiresidue analysis of acidic and polar organic contaminants in water samples by stir-bar sorptive extraction–liquid desorption–gas chromatography–mass spectrometry, *Journal of Chromatography A*, 1174, pp. 27-39.

Quintana, J.B., Rodil, R., Reemtsma, T. (2004), Suitability of hollow fibre liquid-phase microextraction for the determination of acidic pharmaceuticals in wastewater by liquid chromatography–electrospray tandem mass spectrometry without matrix effects, *Journal of Chromatography A*, 1061, pp. 19-26.

Ramelow, U.S., Pingili, S. (2010), Synthesis of ethylene glycol dimethacrylate-methyl methacrylate copolymers, determination of their reactivity ratios, and a study of dopant and temperature effects on their conductivities, *Polymers*, 2, pp. 265-285.

Rodriguez, I., Carpinteiro, J., Quintana, J.B., Carro, A.M., Lorenzo, R.A., Cela, R. (2004), Solid-phase microextraction with on-fiber derivatization for the analysis of anti-inflammatory drugs in water samples, *Journal of Chromatography A*, 1024, pp. 1-8.

Rostamizadeh, K., Vahedpour, M., Bozorgi, S. (2012), Synthesis, characterization and evaluation of computationally designed nanoparticles of molecular imprinted polymers as drug delivery systems, *International Journal of Pharmaceuticals*, 424, pp. 67-75.

Saad, D. (2013), Development and application of functionalized polymeric materials for heavy metal ions recovery from industrial and mining wastewaters, PhD thesis, University of the Witwatersrand, South Africa.

Santos, J.L., Aparicio, I., Alonso, E., Callejon, M. (2005), Simultaneous determination of pharmaceutically active compounds in wastewater samples by

solid phase extraction and high-performance liquid chromatography with diode array and fluorescence detectors, *Analytica Chimica Acta*, 550, pp. 116-122.

Sarafraz-Yazdi, A., Amiri, A., Rounaghi, G., Eshtiagh-Hosseini, H. (2012), Determination of non-steroidal anti-inflammatory drugs in water samples by solid-phase microextraction based sol–gel technique using poly(ethylene glycol) grafted multi-walled carbon nanotubes coated fiber, *Analytica Chimica Acta*, 720, pp. 134-141.

Sharifi, V., Abbasi, A., Nosrati, A. (2016), Application of hollow fiber liquid phase microextraction and dispersive liquid–liquid microextraction techniques in analytical toxicology, *Journal of Food and Drug Analysis*, 24, pp. 264-276.

Silva, A.R.M., Portugal, F.C.M., Nogueira, J.M.F. (2008), Advances in stir bar sorptive extraction for the determination of acidic pharmaceuticals in environmental water matrices: Comparison between polyurethane and polydimethylsiloxane polymeric phases, *Journal of Chromatography* A, 1209, pp. 10-16.

Stancova, V., Zikova, A., Svobodova, Z., Kloas, W. (2015), Effects of the non-steroidal anti-inflammatory drug(NSAID) naproxen on gene expression of antioxidant enzymes in zebrafish (*Danio rerio*), *Environmental Toxicology and Pharmacology*, 40, pp. 343-348.

Stulten, D., Zuhlke, S., Lamshoft, M., Spiteller, M. (2008), Occurrence of diclofenac and selected metabolites in sewage effluents, *Science of The Total Environment*, 405, pp. 310-316.

Suchara, E.A., Budziak, D., Martendal, E., Costa, L.L.F., Carasek, E. (2008), A combination of statistical and analytical evaluation methods as a new optimization strategy for the quantification of pharmaceutical residues in sewage effluent, *Analytica Chimica Acta*, 613, pp. 169-176.

Sun, Y., Chen, Z., Wu, G., Wu, Q., Zhang, F., Niu, Z., Hu, H. (2016), Characteristics of water quality of municipal wastewater treatment plants in China:

implications for resources utilization and management, *Journal of Cleaner Production*, 131, pp. 1-9.

Sun, Z., Schussler, W., Sengl, M., Niessner, R., Knopp, D. (2008), Selective trace analysis of diclofenac in surface and wastewater samples using solid-phase extraction with a new molecularly imprinted polymer, *Analytica Chimica Acta*, 620, pp. 63-81.

Taggart, M.A., Cuthbert, R., Das, D., Sashikumar, C., Pain, D.J., Green, R.E., Feltrer, Y., Shultz, S., Cunningham, A.A., Meharg, A.A. (2007), Diclofenac disposition in Indian cow and goat with reference to *Gyps* vulture population declines, *Environmental Pollution*, 147, pp. 60-65.

Terzopoulou, Z., Papageorgiou, M., Kyzas, G.Z., Bikiaris, D.N., Lambropoulou, D.A. (2016), Preparation of molecularly imprinted solid-phase microextraction fiber for the selective removal and extraction of the antiviral drug abacavir in environmental and biological matrices, *Analytica Chimica Acta*, 913, pp. 63-75.

USEPA method 1694. (2007), Pharmaceuticals and personal care products in water, soil, sediment and biosolids by HPLC/MS/MS. Available from URL: https://www.epa.gov/sites/production/files/2015 10/documents/method_1694_2007.pdf. Accessed on 05/02/17.

Vera-Candioti, L., Gil Garcia, M.D., Martinez Galera, M., Goicoechea, H.C. (2008), Chemometric assisted solid-phase microextraction for the determination of anti-inflammatory and antiepileptic drugs in river water by liquid chromatography–diode array detection, *Journal of Chromatography A*, 1211, pp. 22-32.

Vergeynst, L., Haeck, A., De Wispelaere, P., Van Langenhove, H., Demeestere, K. (2015), Multi-residue analysis of pharmaceuticals in wastewater by liquid chromatography-magnetic sector mass spectrometry: Method quality assessment and application in a Belgium case study, *Chemosphere*, 119, pp. S2-S8.

World Health Organization, (2011), Pharmaceuticals in drinking water. Available from URL:

http://www.who.int/water_sanitation_health/publications/2011/pharmaceuticals_20110601.pdf. Accessed on 07/02/17.

Yuan, X., Qiang, Z., Ben, W., Zhu, B., Liu, J. (2014), Rapid detection of multiple class pharmaceuticals in both municipal wastewater and sludge with ultra high performance liquid chromatography tandem mass spectrometry, *Journal of Environmental Sciences*, 26, pp. 1949-1959.

Yu, Y., Wu, L. (2011), Comparison of four extraction methods for the analysis of pharmaceuticals in wastewater, *Journal of Chromatography A*, 1218, pp. 2483-2489.

Zhao, J., Ying, G., Wang, L., Yang, J., Yang, X., Yang, L., Li, X. (2009), Determination of phenolic endocrine disrupting chemicals and acidic pharmaceuticals in surface water of the Pearl Rivers in South China by gas chromatography-negative chemical ionization-mass spectrometry, *Science of the Total Environment*, 407, pp. 962-974.

Zhu, W., Xu, L., Zhu, C., Li, B., Xiao, H., Jiang, H., Zhou, X. (2016), Magnetically controlled electrochemical sensing membrane based on multifunctional molecularly imprinted polymers for detection of insulin, *Electrochimica Acta*, 218, pp. 91-100.

Zorita, S., Martensson, L., Mathiasson, L. (2009), Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden, *Science of The Total Environment*, 407, pp. 2760-2770.

APPENDIX

1 Referred Conference Outputs

These are directly related to the research work discussed in the current document. All the work was presented by myself in a form of poster or oral in local and international conferences.

1.1 **L.M. Madikizela*,** S.F. Muthwa and L. Chimuka

Evaluation of triclosan and ketoprofen in river water and wastewater using solid phase extraction and high performance liquid chromatography. ANALITIKA 2014, Parys, 7-11 September 2014. **Poster Presentation**.

1.2 **L.M. Madikizela***, P.S. Mdluli and L. Chimuka

Theoretical and experimental studies of molecularly imprinted polymer for ibuprofen, naproxen and diclofenac. IUPAC 2015 (45th IUPAC World Chemistry Congress), Bexco, Busan, Korea, 9-14 August 2015. **Poster Presentation**.

1.3 **Lawrence Mzukisi Madikizela*,** and Luke Chimuka

Synthesis, characterization and molecular structure of multi-template molecularly imprinted polymer for selected acidic pharmaceuticals. South African Chemical Institute (42nd National Convention), Elangeni hotel, Durban, South Africa, 29 November - 4 December 2015. **Oral Presentation**.

1.4 **Lawrence Mzukisi Madikizela*,** and Luke Chimuka

Synthesis, characterization, adsorption and selectivity studies of a multi-template molecularly imprinted polymer. MIP 2016 (The 9th International Conference on Molecular Imprinting), Elite Hotel Ideon, Lund, Sweden, 26 June - 30 June 2016. **Poster Presentation**.

1.5 **Lawrence Mzukisi Madikizela***, and Luke Chimuka

Molecularly imprinted solid-phase extraction coupled with high performance liquid chromatography for determination of naproxen, ibuprofen and diclofenac in wastewater treatment plants. ChromSAAMS2016, Riverside Sun, Vanderbijlpark, Gauteng, 11–14 September 2016. **Oral Presentation**.

2 Other Conference Outputs

The following work was presented in local conferences by a MSc student, Ms. S.S. Zunngu, under my co-supervision. The MSc project was my original idea. The MSc project involves the synthesis of molecularly imprinted polymer for ketoprofen that was designed using computational tools. The synthesized MIP was characterized and applied as SPE sorbent in the quantitative analysis of ketoprofen in wastewater.

2.1 Silindile Senamile Zunngu*, **Lawrence Mzukisi Madikizela,** Luke Chimuka and Phumlane Selby Mdluli

Computational design and synthesis of molecularly imprinted polymer for Ketoprofen. South African Chemical Institute (42nd National Convention), Elangeni hotel, Durban, South Africa, 29 November - 4 December 2015. **Poster Presentation.**

2.2 Silindile Senamile Zunngu*, **Lawrence Mzukisi Madikizela,** Luke Chimuka and Phumlane Selby Mdluli

Synthesis and application of molecularly imprinted polymer in the solid-phase extraction of ketoprofen from wastewater. ChromSAAMS2016, Riverside Sun, Vanderbijlpark, Gauteng, 11–14 September 2016. **Poster Presentation.**

3 Paper 9

The following paper emanated from MSc project conducted by Ms. S.S. Zunngu was published in Comptes Rendus Chimie Journal. In Press, http://dx.doi.org/10.1016/j.crci.2016.09.006

Silindile Senamile Zunngu*, **Lawrence Mzukisi Madikizela,** Luke Chimuka and Phumlane Selby Mdluli

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Full paper/Mémoire

Synthesis and application of a molecularly imprinted polymer in the solid-phase extraction of ketoprofen from wastewater

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ABSTRACT

Ketoprofen is a nonsteroidal anti-inflammatory drug widely consumed by humans as it possesses analgesic activities. A selective molecularly imprinted polymer (MIP) for ketoprofen was synthesized and applied as a solid-phase extraction sorbent. MIP was synthesized using 2-vinylpyridine, ethylene glycol dimethacrylate, 1,1′-azobis(cyclohexanecarbonitrile), toluene/acetonitrile (9:1, v/v), and ketoprofen as a functional monomer, cross-linker, initiator, porogenic mixture, and template, respectively. The polymerization was performed at 60 °C for 16 h, and thereafter the temperature was increased to 80 °C for 24 h to achieve a solid monolith polymer. Nonimprinted polymer was synthesized in a similar manner with the omission of ketoprofen. Characterization with thermogravimetric analysis and X-ray diffraction showed that the synthesized polymers were thermally stable and amorphous. Solid-phase extraction cartridges packed with MIP were used with high-performance liquid chromatography for quantitative analysis of ketoprofen in wastewater. The analytical method gave detection limits of 0.23, 0.17, and 0.09 μg/L in wastewater influent, effluent, and deionized water, respectively. The recovery for the wastewater influent and effluent spiked with 5 μg/L of ketoprofen was 68%, whereas 114% was obtained for deionized water. The concentrations of ketoprofen in the influent and effluent samples were in the ranges of 22.5–34.0 and 1.14–5.33 μg/L, respectively. Overall, the analytical method for the analysis of ketoprofen in wastewater was rapid, affordable, accurate, precise, sensitive, and selective.

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1. Introduction

Ketoprofen, also known as [(*RS*)-2-(3-benzoylphenyl)-propionic acid], is a commonly used pharmaceutical drug which possesses anti-inflammatory and analgesic activities because of its ability to inhibit cyclooxygenase enzymes that promote inflammation [1]. Ketoprofen is widely used in medical care because it is able to treat inflammatory diseases and musculoskeletal injury [2]. Because of the large quantity of ketoprofen consumed by humans, the compound is widely detected with other nonsteroidal anti-inflammatory drugs (NSAIDs) in wastewater and surface water [3–5]. Once consumed, 80% of ketoprofen is eliminated as unchanged drug and its degradation in wastewater treatment plants (WWTPs) depends on the biological treatment efficiency [6]. It has been previously reported that WWTPs are the primary source of pharmaceuticals in river water [5].

To date, many reports have emerged on the occurrence of NSAIDs such as ibuprofen, naproxen, and diclofenac in South African environment [7–13]. However, there are

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currently few studies that have reported on the presence of ketoprofen in South African aquatic conditions [11–13]. With the view of preserving the precious resource such as water, there is a need to understand the extent of all widely used pharmaceuticals in the environment. South Africa has a large number of WWTPs that are mainly used for domestic wastewater treatment and their potential for removal of pharmaceuticals such as ketoprofen is not known. There is currently a lack of data on the toxicity of ketoprofen in aquatic life. To understand the risk of aquatic life and water consumers from suffering the health effects caused by pollutant levels, it is necessary to fully evaluate the occurrence of ketoprofen in water resources.

To address this problem, the development of highly sensitive and selective methods for trace determination of compounds such as ketoprofen in complex wastewater matrix is required. One of the most suitable methods of ketoprofen analysis is the use of chromatographic methods that are equipped with a very sensitive mass spectrometry detector [14,15]. However, the operation, maintenance and cost, of mass spectrometry detector is expensive. Therefore, some laboratories had opted for the use of a cheap and readily available UV–visible detector. The sensitivity of UV–visible detector is usually improved by the use of solid-phase extraction (SPE) for cleanup and preconcentration of target compounds [16].

In SPE, the most widely used extraction media for ketoprofen are Strata X, C_{18}, and Oasis hydrophilic lipophilic balanced (HLB) sorbents [17,18]. Although the application of these sorbents leads to the improvement of sensitivity, they often lack selectivity and their single use results in massive generation of solid waste. Nowadays, molecularly imprinted polymers (MIPs) are developed for SPE applications because of their properties that include high selectivity, reusability, mechanical strength, and resistance against acids, bases, and organic solvents [19]. The development and application of MIPs for the selective analysis of single NSAID such as ibuprofen and diclofenac is well documented [20,21]. The use of multitemplate MIPs for ketoprofen and several NSAIDs in wastewater analysis has been explored [22]. MIPs synthesized using the multitemplate approach are usually selective toward a group of compounds. However, these may not be useful in the analysis of a single analyte as it is important to obtain cleaner chromatograms which subsequently lead to more accurate measurements. Currently, there is a lack of available information for the synthesis of MIP that can selectively extract ketoprofen from aqueous samples.

Ketoprofen has been identified as one of the pharmaceutical drugs that contaminate Umgeni River which is found in the northern part of Durban city in South Africa [11,13]. With the exception of the work published by Madikizela et al. [12], there are currently no reports on the occurrence of ketoprofen in the southern region of Durban. Apart from these studies [11–13], there is currently no other South African study that has focused on the analysis of ketoprofen in water resources. Therefore, the aims of this study were to evaluate the occurrence and removal efficiency of ketoprofen in WWTPs located in the southern part of Durban city, South Africa. To achieve these aims, an MIP was synthesized, characterized, and applied in selective SPE of ketoprofen from wastewater before high-performance liquid chromatographic quantification.

2. Experimental section

2.1. Materials

Ketoprofen (≥98%), triclosan (≥97%), 2-vinylpyridine (97%), high-performance liquid chromatography (HPLC) grade methanol (≥99.9%), 1,1′-azobis-(cyclohexanecar bonitrile) (98%), ethylene glycol dimethacrylate (98%), and toluene (99.7%) were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC grade acetonitrile (≥99.9%) and glacial acetic acid (100%) were purchased from Merck (Darmstadt, Germany). Formic acid (approximately 98%), fenoprofen (≥97%), and HPLC grade triethylamine (≥99%) were purchased from Fluka (Steinheim, Germany).

2.2. Synthesis of MIP

Published work was adopted with slight modifications for the synthesis of MIP for ketoprofen [23,24]. Synthesis was carried out by mixing 25 mg of ketoprofen with 54 µL of 2-vinylpyridine. The mixture was stirred at room temperature in a 250 mL round-bottomed flask containing 10 mL of a acetonitrile/toluene (1:9, v/v) porogenic mixture for 30 min. Thereafter, the reaction vessel was placed on ice to prevent unwanted polymerization. Ethylene glycol dimethacrylate (4.77 mL) and 100 mg of 1,1′-azobis-(cyclohexanecarbonitrile) were added. The mixture was purged with nitrogen gas for 10 min, sealed, and stirred in an oil bath at 60 °C for 16 h to initiate polymerization. After 16 h, the temperature was increased to 80 °C and maintained for 24 h to achieve a solid monolith polymer. The polymer was dried to constant mass at 80 °C followed by grinding and sieving. Particles ranging from 25 to 90 µm were collected and washed repeatedly with a mixture of acetic acid/acetonitrile (1:9; v/v) until ketoprofen could not be detected in high-performance liquid chromatographic system. Nonimprinted polymer (NIP) was synthesized and treated likewise with the omission of ketoprofen in the reaction mixture.

2.3. Apparatus

HPLC system consisting of an online mobile phase degasser unit (model DGU-20A5), 20 µL sample loop, pump (model LC-20AT), and UV–visible detector (model SPD-20A) obtained from Shimadzu Corporation (Kyoto, Japan) was used. The mobile phase conditions consisted of a mixture of acetonitrile and 0.2 formic acid in water (60:40, v/v) at a flow rate of 1 mL/min. Separation was performed on a Gemini C_{18} HPLC column of 150 × 4.6 mm × 5 µm obtained from Phenominex (California, USA). Shimadzu liquid chromatography (LC) solutions software was used for data collection and processing. Detector wavelength was set at 255 nm.

For characterization, thermal analysis was performed using thermogravimetric analysis/differential scanning

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calorimetry (TGA/DSC) 1 Star^e system obtained from Mettler Toledo (Columbus, USA). Thermograms were recorded using a heating rate of 10 °C/min from 25 to 700 °C, under nitrogen atmosphere at a rate of 100 mL/min.

Diffraction patterns (10–90°) of the synthesized polymers were determined using an X-ray diffraction (XRD) equipped with XRD commander for data collection and Eva software for processing. XRD system was obtained from Bruker AXS (Karlsruhe, Germany). Cu Kα radiation source at a rate of 2°/min was used.

Elemental analysis was performed using Series II carbon, hydrogen, nitrogen, sulfur and oxygen (CHNS/O) analyzer 2400 from Perkin Elmer (Llantrisant, United Kingdom).

For SPE, manifold was purchased from Phenominex (California, USA), and it was connected into vacuum pump that was obtained from Merck Millipore (Massachusetts, USA).

2.4. Sampling

Samples analyzed consisted of raw influent collected after wastewater screening for solid removal and final effluent sampled after disinfection of treated water with chlorine. Wastewater samples were collected once per week in the month of May in 2016 from Amanzimtoti (Global positioning system (GPS), S30.00749° E30.91720°), Kingsburgh (GPS, S30.07445° E30.85687°), and Umbilo (GPS, S29.84556° E30.89103°) WWTPs located around the city of Durban in KwaZulu-Natal Province of South Africa. The samples were transported into the laboratory, where they were filtered through a 0.22 μm syringe filter; thereafter, pH was adjusted to 5. Samples were refrigerated at 4 °C until analysis.

2.5. Preparation and application of SPE

Empty SPE cartridges (1 mL) purchased from Supelco (Bellefonte, USA) were mounted into the manifold and washed with methanol before their use. Thereafter, 14 mg of MIP particles were packed in between two polypropylene frits.

Water samples were percolated through the packed cartridge using the preoptimized SPE conditions. This was achieved by conditioning the cartridge with 1 mL of methanol followed by equilibration with 1 mL of deionized water. Fifty milliliters of wastewater sample (pH 5) was loaded at 1 mL/min. The cartridge was vacuum dried for 10 min, followed by washing with 1 mL of 5% (v/v) triethylamine in water. The retained ketoprofen was eluted with 1 mL of methanol and injected into HPLC.

After each use, the cartridges were regenerated by washing with 3 mL of deionized water and 3 mL of methanol.

2.6. Quality assurance

A stock solution of 100 mg/L ketoprofen was prepared in acetonitrile. The stock solution was further diluted to prepare the working standards. The working standards were analyzed using HPLC. Calibration curve, limit of detection (LOD), and limit of quantification (LOQ) were computed. The accuracy and precision of analytical method were validated using deionized water and wastewater samples that were spiked with ketoprofen at concentration levels ranging from 5 to 1000 μg/L.

3. Results and discussion

3.1. Characterization of polymers

Thermogravimetric analysis was performed for the washed MIP and NIP (Fig. 1). At 40 °C, the washed polymers had a mass loss of approximately 4%. This was probably because of the adsorbed methanol used in the template removal step [25]. Further thermal decomposition of polymers was observed at 290 °C which was marked as the temperature where the polymer backbone collapses. In a separate study, the polymer backbone collapsed at 250 °C for an MIP that was synthesized for 1,3-diisopropylurea [25]. It was further observed that at 425 °C, there was 100% thermal decomposition for the NIP, whereas the corresponding mass loss was 90% for the MIP. The difference might have been caused by structural variations that could have happened during template removal process.

Differential scanning calorimetry thermograms (Fig. 2) of the MIP and NIP were similar with endothermic peak at 355 °C which is associated with the temperature where there is complete thermal decomposition of the polymers. Similarities in the thermograms could be because of similar structural arrangements of the MIP and NIP. Structural similarities were further confirmed with X-ray diffractograms (Fig. S1). The lack of peaks in diffractograms is an indication of amorphous polymers.

The synthesized polymers were analyzed in terms of their carbon, nitrogen, and hydrogen contents using an elemental analyzer. As indicated in Table 1, the carbon and hydrogen contents were identical because of similar conditions in the synthesis. Also, this is an indication of a successful template removal as the presence of ketoprofen in the MIP could result in higher carbon and hydrogen contents. The sources of nitrogen in the polymers are the initiator and functional monomer used in synthesis. The differences in the nitrogen contents of polymers can be explained by the possible disruptions in the chemical

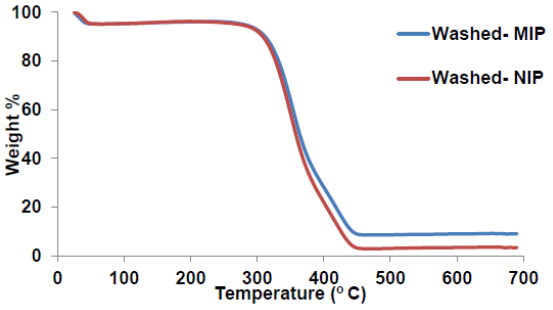


Fig. 1. Thermogravimetric analysis of the synthesized polymers.

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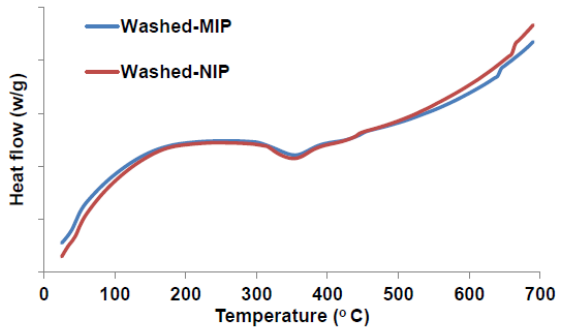


Fig. 2. DSC curves for the polymers.

Table 1
Measured percentage by mass of carbon, hydrogen, and nitrogen of the polymers using CHN analyzer.

Polymer	Carbon (%)	Hydrogen (%)	Nitrogen (%)
Washed MIP	58.28	7.39	0.33
Washed NIP	59.68	7.33	1.0

structure of the MIP during excess washing while removing ketoprofen. This was evident in the chromatograms obtained during template removal where there were unknown peaks recorded for the MIP solutions. Oxygen is the only other element (not quantified) known to be present in both polymers. The sources of oxygen in polymers are cross-linker and template used in polymerization.

3.2. SPE studies

3.2.1. Optimization

Optimization was carried out by using deionized water spiked with 1 mg/L of ketoprofen as the sample. SPE parameters investigated include sample pH, sorbent mass, sample volume, and washing solvents. Because the target compound is polar, methanol was used for its elution from the SPE cartridge. During optimization, only one parameter was changed at a time whereas keeping other parameters constant. Each experiment was conducted in triplicate, where average results are discussed.

For the adsorption of acidic pharmaceuticals, it has been reported that the extraction is based on hydrogen binding of the target compound and functional monomer (2-vinylpyridine) [26]. In this context, pH of the water solutions was adjusted to promote the monomer–template interactions. pH was investigated in the range of 3–7, where the highest recovery was obtained at pH 5 (Fig. 3). Therefore, pH 5 was selected as optimum and used in all subsequent experiments. At selected pH, the recovery for ketoprofen using NIP was 69.5% which could be because of the adsorption based on nonspecific interactions, whereas for the corresponding MIP, 88% was obtained because of molecular recognition. pH values greater than 7 were not investigated because of the deprotonation of ketoprofen which could lead to unavailability of adsorption sites. This behavior is somewhat similar to the behavior of other acidic pharmaceuticals on the MIP [27].

The quantity of the polymer for the optimum extraction of ketoprofen was investigated. It was discovered that low mass of the polymers resulted in poor recoveries which could be a result of maximum occupation of the binding sites. However, the accepted recovery was achieved by using 14 mg of the polymer (Fig. 4). It should be noted that this work was investigated using 1 mg/L of ketoprofen which is much higher than the concentrations expected in wastewater samples. Therefore, the amounts of polymers beyond 14 mg could not be investigated.

High sample volumes are often required in environmental analysis as they tend to lead to high preconcentration factors which in turn produce better sensitivity. The results in Fig. 5 show that the recovery increased from the sample volume of 10–20 mL for both polymers because of limited analyte interaction with the polymer as not enough volume was available to percolate the whole sample through the sorbent. Accepted recoveries (>70%) were achieved in the sample volumes of 20 and 50 mL for the MIP. However, 50 mL was selected as optimum as it leads to higher preconcentration factor. Recoveries decreased beyond the sample volume of 50 mL for the MIP which could be because of the capacity of the polymers being exceeded. Because of limited binding sites in the NIP, maximum recovery was obtained when the sample volume was 20 mL.

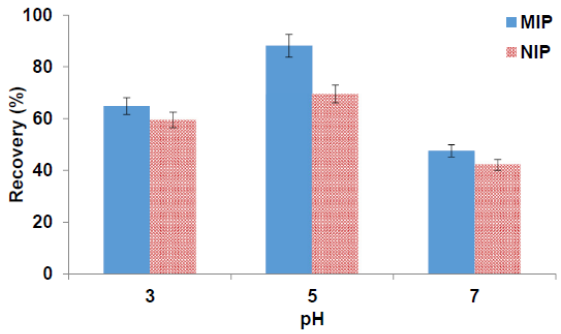


Fig. 3. Effect of pH on the recovery of ketoprofen from the polymers. Extraction conditions were as follows: sample volume, 20 mL; washing solvent, 1 mL of deionized water; and elution solvent, 1 mL methanol.

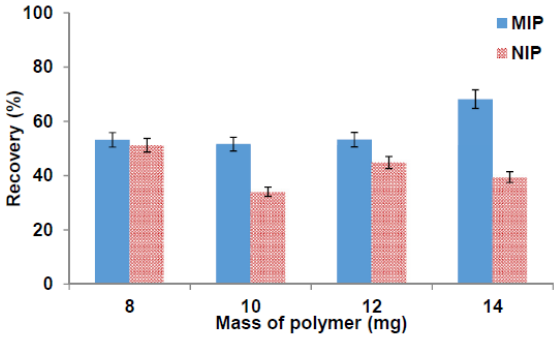


Fig. 4. Effect of polymer amount on the recovery of ketoprofen. Extraction conditions were as follows: sample volume, 50 mL; washing solvent, 1 mL of deionized water; elution solvent, 1 mL methanol; and pH, 5.

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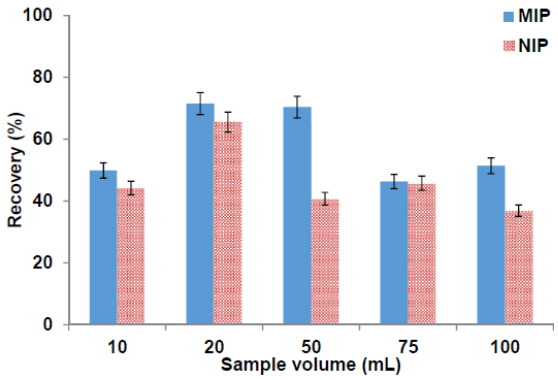
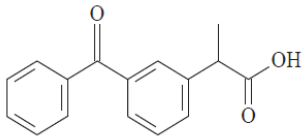
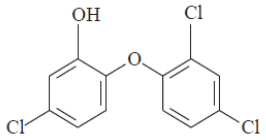
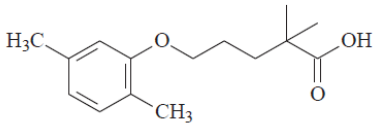
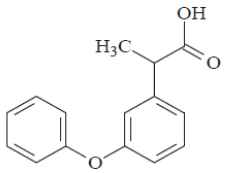


Fig. 5. Effect of sample volume on the recovery of ketoprofen. Extraction conditions were as follows: washing solvent, 1 mL of deionized water; elution solvent, 1 mL methanol; sorbent mass, 14 mg; and pH, 5.

Table 2
Molecular structures of ketoprofen and competitors.

Compound	Molecular structure
Ketoprofen	
Triclosan	
Gemfibrozil	
Fenoprofen	

3.2.2. Selectivity

The selectivity of the polymers was investigated using deionized water that was spiked with ketoprofen, fenoprofen, gemfibrozil, and triclosan. Fenoprofen and gemfibrozil are acidic pharmaceuticals that contain similar functional groups as ketoprofen (Table 2). These two pharmaceuticals are usually present in wastewater solutions that contain ketoprofen [28,29]. Triclosan is an antibacterial compound usually detected in wastewater with ketoprofen [12,30]. These competitors were expected to be adsorbed by the MIP through the formation of hydrogen bonds because of the presence of hydroxyl groups in their molecules.

The results show low recoveries (<20%) for all competitors which may be because of differences in their molecular shapes and sizes compared to ketoprofen. Therefore, the prepared polymers were highly selective toward ketoprofen (Tables S1 and 3). However, high ketoprofen recovery in the NIP could indicate a nonspecific adsorption which could be problematic considering the complex wastewater matrix. Therefore, selective washing was investigated in this case. Ten percent was recovered for triclosan when the MIP cartridge was washed with water, 17% was obtained for the corresponding NIP. Further washing solvents were investigated for selective removal of competitors which could result in cleaner chromatograms when the method is applied in wastewater analysis. Addition of organic solvents in the washing solutions resulted in elution of ketoprofen together with competitors for the NIP cartridges. This is a result of nonspecific binding. Therefore, the cartridges packed with NIP were discarded because of poor selectivity. However, in the case of the MIP, only competitors were removed during washing as a result of molecular imprinting. The introduction of acetonitrile in the washing solvent resulted in the reduction of ketoprofen recovery in the case of the MIP as well.

Table 4
LOD, LOQ, recovery (%), and RSD (%) values (n=3) achieved for the deionized water and wastewater influent and effluent spiked with ketoprofen.

Spiked sample	LOD (μg/L)	LOQ (μg/L)	Recovery (%) ± RSD (%)		
			1000 μg/L	25 μg/L	5 μg/L
Deionized water	0.09	0.30	68 ± 3	80 ± 17	114 ± 16
Influent	0.23	0.78	_	_	68 ± 10
Effluent	0.17	0.55	_	_	68 ± 15

3.3. Validation of the analytical method

The chromatographic analysis of ketoprofen was achieved on a reverse-phase stationary phase (Fig. S2). The performance of the proposed analytical method that uses MIP as a selective sorbent for SPE was validated based on

Table 3
Effect of washing solvent in SPE of ketoprofen using the MIP.

Compound	% Recovery				
	H_2O	MeOH/H_2O (5:95, v/v)	TEA/ACN (1:99, v/v)	TEA/H_2O (1:99, v/v)	TEA/H_2O (5:95, v/v)
Ketoprofen	69.06	67.61	33.39	107.85	103.98
Fenoprofen	5.81	1.98	2.49	1.17	2.68
Gemfibrozil	0.96	1.03	0.59	1.85	0.15
Triclosan	9.93	9.56	2.31	13.76	12.64

ACN, acetonitrile; H_2O, water; MeOH, methanol; TEA, triethylamine.

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Table 5
Removal rates and concentrations (μg/L) ± standard deviations ($n = 3$) of ketoprofen in wastewater samples.

WWTP	Influent	Effluent	%Removal
Amanzimtoti	28.4 ± 1.1	3.50 ± 2.4	88
Kingsburgh	28.2 ± 12	3.40 ± 3.1	88
Umbilo	27.3 ± 0.57	2.90 ± 2.5	90

sensitivity, accuracy, and precision. A linear plot with R^2 greater than 0.99 was achieved for a seven-point calibration curve in the concentration range of 0.001–10 mg/L. On the basis of LOD and LOQ results shown in Table 4, the developed method is highly sensitive. LOQ and LOD are defined as concentrations that gave the signal-to-noise ratio of 10 and 3, respectively. Greater preconcentration factor in the proposed method led to better method sensitivity as compared with the data in the literature [31]. The results in Table 4 show that the analytical method was highly accurate as indicated with recoveries in the range of 68–114%. The relative standard deviation (RSD) given as ± values indicate that the developed method is precise.

3.4. Wastewater analysis

The developed procedure was applied in the SPE of ketoprofen from wastewater before high-performance liquid chromatographic quantification. Ketoprofen was detected in all wastewater samples (Table 5) with concentrations greater than 2 μg/L in the effluent, whereas the levels were much higher in the influent. Chromatograms were cleaner for the effluent when compared with influent samples because of effective wastewater treatment process (Fig. 6). Detected levels of ketoprofen were compared with previous concentrations reported locally and in other countries. Previous work [12] reported the concentration range of 1–6 μg/L in Amanzimtoti WWTP influent, whereas the present study reports the average concentration of 28.4 μg/L. The differences could be because of seasonal variations as the samples were collected from August to October in the previous work [12], whereas the samples of the present study were collected in May. Also, the variations in population dynamics might be another factor to be considered. Overall, the concentrations of ketoprofen

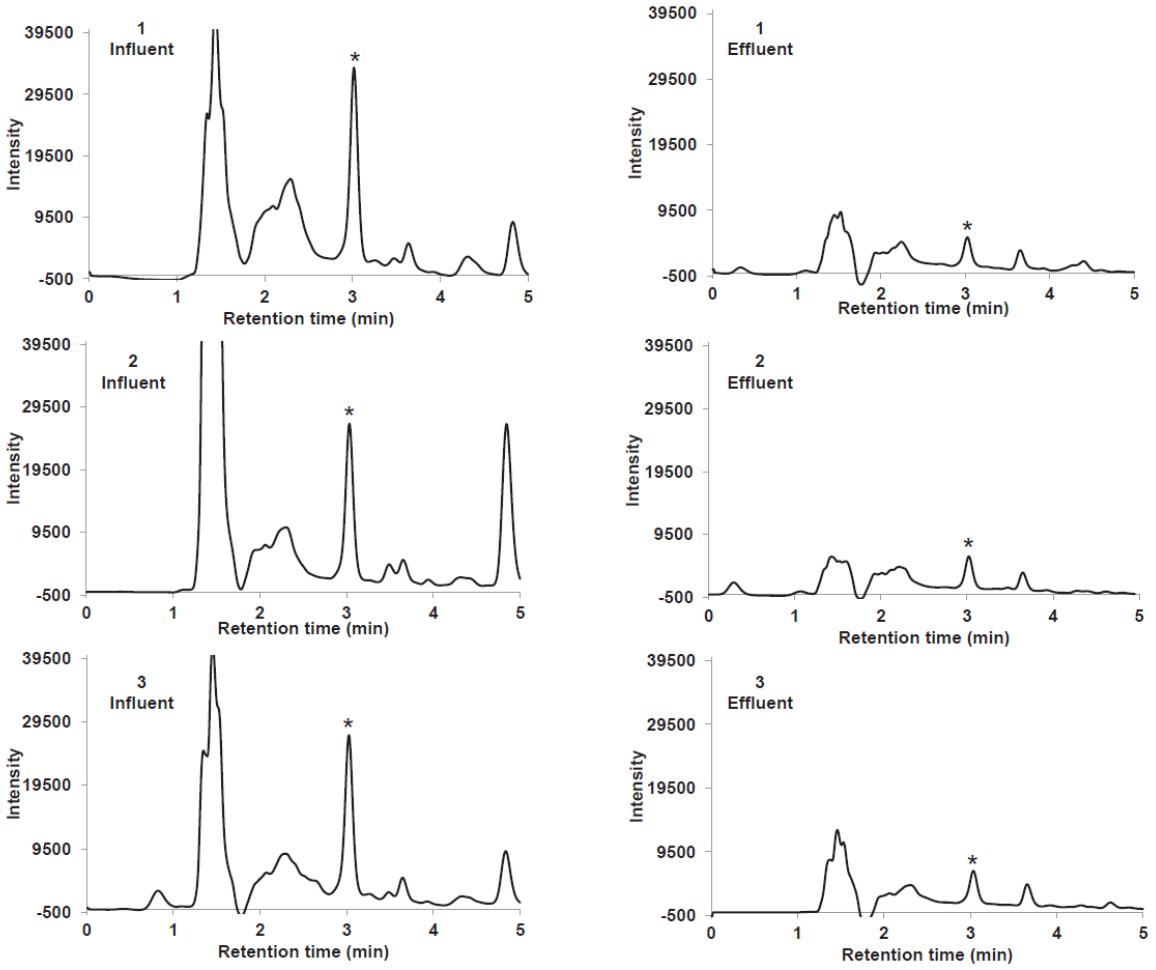


Fig. 6. Typical chromatograms obtained for wastewater influent and effluent collected from Kingsburgh (1), Umbilo (2), and Amanzimtoti (3) WWTPs. *Peak for the target compound (ketoprofen).

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detected in these plants were higher than the levels reported in South Africa and the rest of the world (Table S2) because of differences in WWTP designs and consumption of the drug in foreign countries [32–36]. Most recently, ketoprofen is one of the pharmaceuticals that have been detected in wastewater and surface water from several countries including Algeria and China [6,37,38].

To the best of our knowledge, this is the first detailed study based on the occurrence and removal rate of ketoprofen in Amanzimtoti, Kingsburgh, and Umbilo WWTPs. Removal rates were determined based on the concentrations present in both influent and final effluent. Therefore, the performance of these WWTPs in terms of reducing ketoprofen during treatment process was compared with various plants in the world (Table S3). It was observed that the sampled WWTPs perform in a similar manner and sometimes better when compared with various plants for the reduction of ketoprofen during the wastewater treatment [30,39–41].

4. Conclusions

For the first time, a selective MIP for ketoprofen was synthesized and applied as the SPE sorbent. SPE technique was optimized and used with HPLC for the quantitative determination of ketoprofen in Umbilo, Amanzimtoti, and Kingsburgh WWTPs. For wastewater analysis, ketoprofen was detected in all samples. Concentrations of ketoprofen found in this study were higher when compared with what has been reported for WWTPs located in Europe. The removal rate of ketoprofen during domestic wastewater treatment was in the range of 88–90%. These results call for a detailed screening of ketoprofen in other South African water bodies including river and dam water. The analytical methodology used for wastewater analysis was fast, highly accurate, sensitive, and selective and gave results with good precision.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.crci.2016.09.006.

References

[1] N.H. Hashim, S.J. Khan, J. Chromatogr., A 1218 (2011) 4746–4754.
[2] Y. Zhuang, H. Song, J. Pharm. Biomed. Anal. 44 (2007) 824–828.
[3] Y. Yu, L. Wu, A.C. Chang, Sci. Total Environ. 442 (2013) 310–316.
[4] M. Laven, T. Alsberg, Y. Yu, M. Adolfsson-Erici, H. Sun, J. Chromatogr., A 1216 (2009) 49–62.
[5] A. Togola, H. Budzinski, Anal. Bioanal. Chem. 388 (2007) 627–635.
[6] A.E.B. Kermia, D. Fouial-Djebbar, M. Trari, C. R. Chimie 19 (2016) 963–970.
[7] R. Amdany, L. Chimuka, E. Cukrowska, Water SA 40 (2014) 407–414.
[8] L.M. Madikizela, L. Chimuka, J. Pharm. Biomed. Anal. 128 (2016) 210–215.
[9] S. Matongo, G. Birungi, B. Moodley, P. Ndungu, Environ. Sci. Pollut. Res. 22 (2015) 10298–10308.
[10] S. Matongo, G. Birungi, B. Moodley, P. Ndungu, Chemosphere 134 (2015) 133–140.
[11] F.O. Agunbiade, B. Moodley, Environ. Toxicol. Chem. 35 (2016) 36–46.
[12] L.M. Madikizela, S.F. Muthwa, L. Chimuka, S. Afr. J. Chem. 67 (2014) 143–150.
[13] F.O. Agunbiade, B. Moodley, Environ. Monit. Assess. 186 (2014) 7273–7291.
[14] J. Zhao, G. Ying, L. Wang, J. Yang, X. Yang, L. Yang, X. Li, Sci. Total Environ. 407 (2009) 962–974.
[15] M. Farre, I. Ferrer, A. Ginebreda, M. Figueras, L. Olivella, L. Tirapu, M. Vilanova, D. Barcelo, J. Chromatogr., A 938 (2001) 187–197.
[16] R. Cueva-Mestanza, Z. Sosa-Ferrera, M.E. Torres-Padron, J.J. Santana-Rodriguez, J. Chromatogr., B 863 (2008) 150–157.
[17] L. Patrolecco, N. Ademollo, P. Grenni, A. Tolomei, A.B. Caracciolo, S. Capri, Microchem. J. 107 (2013) 165–171.
[18] J.L. Santos, I. Aparicio, E. Alonso, M. Callejon, Anal. Chim. Acta 550 (2005) 116–122.
[19] B.B. Prasad, G. Rai, Spectrochim. Acta, Part A 105 (2013) 400–411.
[20] K. Farrington, F. Regan, Biosens. Bioelectron. 22 (2007) 1138–1146.
[21] Z. Sun, W. Schussler, M. Sengl, R. Niessner, D. Knopp, Anal. Chim. Acta 620 (2008) 73–81.
[22] Y.P. Duan, C.M. Dai, Y.L. Zhang, L. Chen, Anal. Chim. Acta 758 (2013) 93–100.
[23] V. Pakade, S. Lindahl, L. Chimuka, C. Turner, J. Chromatogr., A 1230 (2012) 15–23.
[24] M.B. Gholivand, N. Karimian, G. Malekzadeh, Talanta 89 (2012) 513–520.
[25] G. Szekely, J. Bandarra, W. Heggie, F.C. Ferreira, B. Sellergren, Sep. Purif. Technol. 86 (2012) 190–198.
[26] L.M. Madikizela, P.S. Mdluli, L. Chimuka, React. Funct. Polym. 103 (2016) 33–43.
[27] V. Manzo, K. Ulisse, I. Rodriguez, E. Pereira, P. Richter, Anal. Chim. Acta 889 (2015) 130–137.
[28] Q. Sun, M. Lv, A. Hu, X. Yang, C. Yu, J. Hazard. Mater. 277 (2014) 69–75.
[29] S.S. Verenitch, C.J. Lowe, A. Mazumder, J. Chromatogr., A 1116 (2006) 193–203.
[30] S.K. Behera, H.W. Kim, J. Oh, H. Park, Sci. Total Environ. 409 (2011) 4351–4360.
[31] L. Ascar, I. Ahumada, A. Lopez, F. Quintanilla, K. Leiva, J. Braz. Chem. Soc. 24 (2013) 1160–1166.
[32] J. Martin, D. Camacho-Munoz, J.L. Santos, I. Aparicio, E. Alonso, J. Hazard. Mater. 239 (2012) 40–47.
[33] R. Rosal, A. Rodriguez, J.A. Perdigon-Melon, A. Petre, E. Garcia-Calvo, M.J. Gomez, A. Aguera, A.R. Fernandez-Alba, Water Res. 44 (2010) 578–588.
[34] Q. Sui, J. Huang, S. Deng, G. Yu, Q. Fan, Water Res. 44 (2010) 417–426.
[35] J.T. Yu, E.J. Bouwer, M. Coelhan, Agric. Water Manage. 86 (2006) 72–80.
[36] E. Larsson, S. al-Hamimi, J.A. Jonsson, Sci. Total Environ. 485–486 (2014) 300–308.
[37] Q. Sun, Y. Li, M. Li, M. Ashfaq, M. Lv, H. Wang, A. Hu, C. Yu, Chemosphere 150 (2016) 596–604.
[38] Q. Sun, M. Li, C. Ma, X. Chen, X. Xie, C. Yu, Environ. Pollut. 208 (2016) 371–381.
[39] P. Lacina, L. Mravcova, M. Vavrova, J. Environ. Sci. 25 (2013) 204–212.
[40] M. Gros, M. Petrovic, A. Ginebreda, D. Barcelo, Environ. Int. 36 (2010) 15–26.
[41] N. Nakada, T. Tanishima, H. Shinohara, K. Kiri, H. Takada, Water Res. 40 (2006) 3297–3303.

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3.1 Paper 9 - Supplementary data

Supporting Information Figures and Tables

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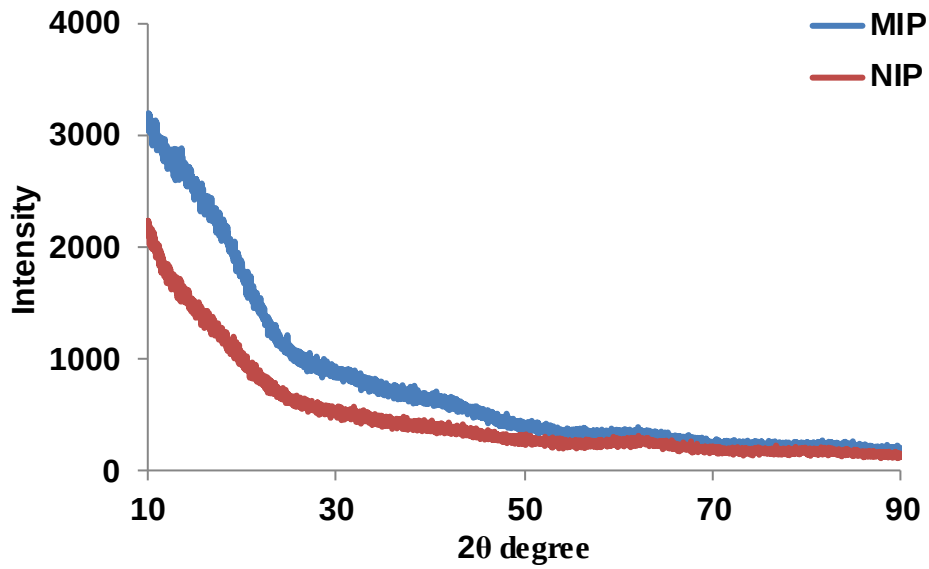


Fig. S1. X-ray diffractograms for washed MIP and NIP

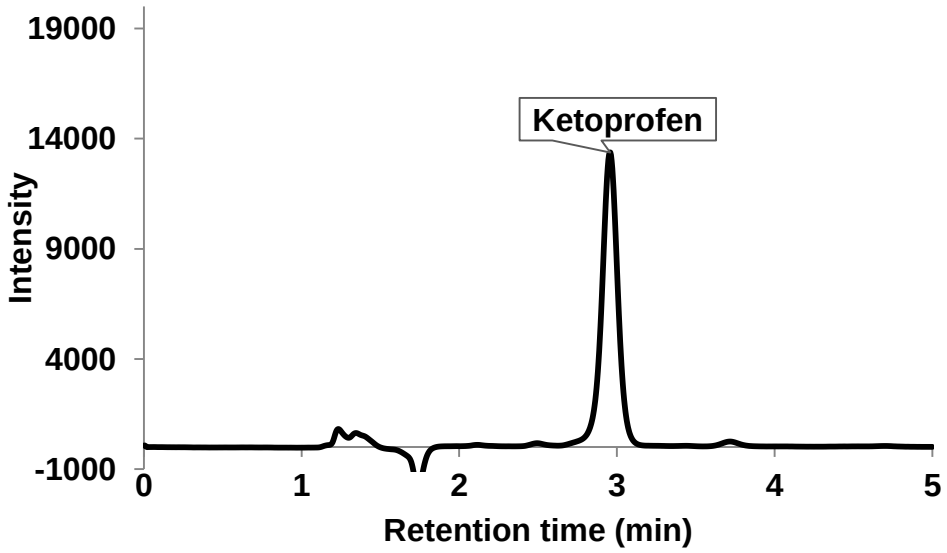


Fig. S2. Chromatographic analysis of 1000 µgL^{-1} ketoprofen standard solution.

Table S1

Effect of washing solvent in solid-phase extraction of ketoprofen using NIP

	% Recovery				
Compound	H_2O	MeOH/H_2O (5:95, v/v)	TEA/ACN (1:99, v/v)	TEA/H_2O (1:99, v/v)	TEA/H_2O (5:95, v/v)
Ketoprofen	87.44	61.63	2.01	50.34	5.61
Fenoprofen	5.43	0.99	2.35	1.34	2.09
Gemfibrozil	0.90	0.90	0.57	1.77	0.08
Triclosan	17.59	10.12	3.31	14.39	12.93

MeOH: methanol; TEA: triethylamine; ACN: acetonitrile; H_2O: water

Table S2

Concentrations (µg/L) of ketoprofen reported in literature

WWTP – City - Country	Influent	Effluent	Reference
Darvill – Pietermaritzburg – South Africa	3.15	0.38	11
Amanzimtoti – Durban – South Africa	8.6	1.55	12
Northern – Durban – South Africa	<10	1.0	13
Baltimore Back River – MD – United States	1.20	0.28	35
Kallby – Lund - Sweden	1.35	0.48	36

Table S3

Literature data on the removal rates of ketoprofen from WWTPs

Country	%Removal	Reference
Korea	94	29
Czech Republic	72	39
Spain	40 - 100	40
Tokyo	45	41