Check for updates

Synthesis and application of a molecularly imprinted polymer in selective solid-phase extraction of efavirenz from water

Sinothando P. Mtolo, Precious N. Mahlambi and Lawrence M. Madikizela

ABSTRACT

Efavirenz is one of the antiretroviral drugs widely used to treat the human immunodeficiency virus. Antiretroviral drugs have been found to be present in surface water and wastewater. Due to complexity of environmental samples, solid-phase extraction (SPE) is used for isolation and pre-concentration of antiretroviral drugs prior to their chromatographic analysis. However, the commercially available SPE sorbents lack selectivity, which tends to prolong the analysis time. Therefore, in this study a molecularly imprinted polymer was synthesized for the specific recognition of efavirenz and then applied as the SPE sorbent for its extraction from wastewater and surface water samples. The imprinted and non-imprinted polymers were synthesized using a bulk polymerization technique where efavirenz was used as the template, 2-vinylpyridine as functional monomer, 1,1′-azobis-(cyclohexanecarbonitrile) as initiator, ethylene glycol dimethacrylate as cross-linker and toluene:acetonitrile (9:1, v/v) as the porogenic solvent mixture. The characterization was performed using Fourier transform infrared spectroscopy, scanning electron microscopy, Brunauer–Emmett–Teller, elemental analysis, and thermogravimetric analysis techniques. Results showed better selectivity of molecularly imprinted polymer to efavirenz than did non-imprinted polymer. The analysis was performed using high performance liquid chromatography equipped with a photo-diode array detector. The analytical method gave a detection limit of 0.41 μg/L and the analyte recovery of 81% in wastewater. The concentrations found in wastewater ranged from 2.79 to 120.7 μg/L, while in surface water they were between 0.975 and 2.88 μg/L. Therefore, the results of this study show a strong need for a detailed screening of efavirenz in major water utilities in the country.

Key words | efavirenz, molecularly imprinted polymer, selectivity, solid-phase extraction

Sinothando P. Mtolo
Precious N. Mahlambi (corresponding author)
Department of Chemistry,
University of KwaZulu-Natal,
Private Bag X01, Scottsville, Pietermaritzburg, 3209,
South Africa
E-mail: *mahlambip@ukzn.ac.za*

Lawrence M. Madikizela
Department of Chemistry,
Durban University of Technology,
P. O. Box 1334, Durban, 4000,
South Africa

INTRODUCTION

Efavirenz is an antiretroviral (ARV) drug, which belongs in the class of non-nucleoside reverse transcriptase inhibitors. ARVs are used for the treatment of human immunodeficiency virus (HIV) worldwide. South Africa has been stated to have the largest antiretroviral therapy in the world with 2.2 million people reported to be receiving HIV treatment in 2012 (World Health Organization 2013). The number of people receiving HIV treatment in South Africa increased by approximately 450,000 in 2013. Therefore, it is anticipated that South Africa utilizes more ARV drugs than any other country in the world (Wood *et al.* 2015). In general, pharmaceuticals are not completely metabolized in the human body and result in excretion in their original or as-metabolized form. The excreted drugs are swept with water into wastewater treatment plants (WWTPs). The majority of WWTPs are not designed for the effective removal of pharmaceuticals; therefore, large quantities of drugs are released into rivers with WWTP effluent. Other sources of pharmaceuticals in the rivers include improper disposal of expired drugs, poor management of hospital waste, waste from pharmaceutical manufacturing facilities, reclaimed water used for artificial groundwater recharge and poor sewerage transport systems (Rimayi *et al.* 2018).

In numerous studies, different classes of pharmaceuticals such as ARVs, antibiotics, non-steroidal anti-inflammatory drugs, among others, have been detected in

doi: 10.2166/wst.2019.054

Downloaded from http://iwaponline.com/wst/article-pdf/79/2/356/616152/wst079020356.pdf
by guest

surface water (Domínguez *et al.* 2011; Agunbiade & Moodley 2014; Colella 2014; Jebiwot 2016; Abafe *et al.* 2018). Among these detected classes of pharmaceuticals, the occurrence of ARVs in water samples has been reported to a lesser extent. Due to the extensive consumption of ARVs in Africa, most studies on their occurrence in the environment have been conducted in African water bodies rather than in other continents (Schoeman *et al.* 2015; Wood *et al.* 2015; K'oreje *et al.* 2016; Schoeman *et al.* 2017; Wooding *et al.* 2017; Abafe *et al.* 2018; Rimayi *et al.* 2018). The impact of ARVs on the ecosystem is currently not well understood; however, it is believed that their prolonged or unintentional consumption that could result from drinking the polluted water may cause resistance towards the drugs (Malawi24 2016). Therefore, it is important to monitor the occurrence of such drugs in the water bodies such as wastewater and river water.

Due to the complexity of the environmental samples and occurrence of pharmaceuticals at trace amounts, solid-phase extraction (SPE) is widely used for their isolation and pre-concentration from water samples (Tamayo *et al.* 2007; Schoeman *et al.* 2015; Zunngu *et al.* 2017; Rimayi *et al.* 2018). The SPE sorbent reported for the extraction of efavirenz from water samples is hydrophilic lipophilic balance (Oasis HLB) (Schoeman *et al.* 2015; Abafe *et al.* 2018). This sorbent is useful for the extraction of multi-analytes; however, it shows poor selectivity when a single compound is targeted. In this study, the application of a molecularly imprinted polymer (MIP) as a selective sorbent in the SPE of efavirenz from water samples has been proposed. MIPs are synthetic polymeric materials that have specific recognition sites which are complementary in shape, size and functional groups to the template molecule, which is usually the target molecule in the analysis (Batlokwa *et al.* 2011). Therefore, the proposal of MIP as a suitable SPE sorbent in the current study was based on its known properties, which include high selectivity, reusability, mechanical strength and resistance against acids, bases and organic solvents (Vasapollo *et al.* 2011). These properties have also been investigated on MIPs synthesized for several pharmaceuticals belonging to the non-steroidal anti-inflammatory group of drugs by our research group (Madikizela *et al.* 2016; Zunngu *et al.* 2017). Therefore, it was in the best interest to synthesize a MIP for efavirenz which has also been recently reported to be present in South African water bodies.

Hence, the aims of this study were to synthesize a MIP that can be used for selective extraction of efavirenz from water samples and to monitor the occurrence of efavirenz in selected WWTPs located in Durban and Msunduzi River in Pietermaritzburg, which are the highly populated regions in the province of KwaZulu-Natal (South Africa). To the best of our knowledge, no work has been conducted on the analysis of efavirenz in the selected sampling sites.

EXPERIMENTAL

Materials

Efavirenz was purchased from J & H Chemical Co. Ltd (Hangzhou Zhejiang, China). 2-vinylpyridine (97%), high performance liquid chromatography (HPLC) grade methanol (99.8%), 1,1′-azobis(cyclohexanecarbonitrile) (98%), ethylene glycol dimethacrylate (98%), toluene (99.7%), fenoprofen (98.00%) and diclofenac (96.5%) 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 (98%) was purchased from Fluka (Steinheim, Germany).

Molecular dynamics simulation for 2-vinylpyridine–efavirenz interactions

Prior to the synthesis of MIP, it was necessary to investigate the functional monomer–template interactions in order to ensure the possibility of entrapping efavirenz using the MIP. In this study, the method described in previous work (Zunngu *et al.* 2017) was adopted and modified where necessary. Molecular dynamics simulations were carried out in canonical ensemble at constant atom number, volume and temperature (NVT). All simulations were executed using the Discover module of Materials Studio (version 7.0). The COMPASS force field was used to calculate the intermolecular interaction between efavirenz and 2-vinylpyridine. 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 100,000 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.

Synthesis of polymers

The procedure for the synthesis of MIP was adopted and modified from the published work (Zunngu *et al.* 2017). This was done by transferring 25 mg of efavirenz (template),

Downloaded from http://iwaponline.com/wst/article-pdf/79/2/356/616152/wst079020356.pdf
by guest

54 μl of 2-vinylpyridine (functional monomer) into a 50 mL round-bottomed flask containing 10 mL porogenic mixture of acetonitrile/toluene (1:9, v/v). The resulting homogeneous solution was stirred at room temperature for 30 min to allow for the monomer–template interactions to occur. This was followed by the addition of 4.77 mL of ethylene glycol dimethacrylate (cross-linking monomer) and 100 mg of 1,1′-azobis-(cyclohexanecarbonitrile) (initiator) into the same reaction vessel. The resulting mixture was de-oxygenated with nitrogen for 10 min, sealed and stirred in an oil bath at 60 °C for 16 h. The temperature was further increased to 80 °C and maintained for 24 h in order to achieve a solid monolith polymer. The resulting polymer was dried at 100 °C, followed by grinding and sieving to achieve solid particles ranging from 25 to 50 μm. The removal of efavirenz from the solid particles was achieved through the application of Soxhlet extraction using a mixture of acetic acid/acetonitrile (1:9, v/v) as the extraction solvent. Soxhlet extraction was repeated until efavirenz could not be detected by HPLC in the washing solutions. Thereafter, the MIP was washed with acetonitrile. The non-imprinted polymer (NIP) was synthesized and treated under similar reaction conditions, using similar reagents and quantities with only efavirenz omitted.

Apparatus

A liquid chromatography (LC) system purchased from Shimadzu (Tokyo, Japan) used for the monitoring of efavirenz in aqueous samples was equipped with a photo-diode array detector (PDA) set at a wavelength of 246 nm. The LC system consisted of an autosampler, a membrane degasser, and a quaternary pump. The chromatographic separation was performed on a Shim-Pack GIST C_{18}-HP (4.6 mm × 150 mm, 3 m) column purchased from Shimadzu (Tokyo, Japan). In each case, a sample volume of 10 μL was injected into the LC system. The mobile phase used consisted of a mixture of acetonitrile and 0.1% formic acid in water at a ratio of 70:30 (v/v) flowing at 0.7 mL/min.

In order to monitor the thermal stability of the MIP and NIP, characterization was done using thermogravimetric analysis (TGA) that was performed using a Mettler Toledo 1 star° TGA system (Columbus, USA). Each polymer was heated at a heating rate of 10 °C/min from 25 to 600 °C under nitrogen atmosphere flowing at 100 mL/min. Further characterization was conducted using Brunauer–Emmett–Teller (BET) analysis on a Flow Prep 060 instrument from Micromeritics (Aachen, Germany) for the measurement of the surface area, total pore volume and average pore diameter of both MIP and NIP. Accordingly, a Fourier transform infrared (FTIR) spectrometer from Perkin Elmer (Llantrisart, UK) equipped with attenuated total reflection was used to study the vibrations of the functional groups present in the synthesized polymers. In addition, the polymer morphology was studied using a scanning electron microscope (SEM), JOEL model 6700F (Tokyo, Japan).

The SPE vacuum manifold was purchased from Sigma Aldrich (Steinheim, Germany). The vacuum pump connected to the SPE vacuum manifold was purchased from Edwards (Munich, Germany). Empty SPE cartridges (3 mL) and frits (10 μm) were purchased from Biotage (Uppsala, Sweden).

Sampling

Samples were collected from two highly populated regions (Durban and Pietermaritzburg) of the KwaZulu-Natal Province in South Africa using dark brown bottles. Four WWTPs located around the city of Durban were sampled together with surface water along Msunduzi River in Pietermaritzburg. The exact locations of the WWTPs and sampling points along Msunduzi River based on global positioning system (GPS) co-ordinates are given in Tables S1 and S2 (available with the online version of this paper). Samples were collected in the months of May and October, representing the cold and hot seasons of South African weather, respectively. During each sampling campaign, the physico-chemical properties of water samples (Tables S3–S5, available online) were measured in each sampling site using a Bante900P multi-parameter water quality meter purchased from Bante Instruments (Shanghai, China). All samples were preserved by storing in cold conditions during their transportation into the laboratory. In the laboratory, the samples were stored at 4 °C until further treatment and analysis.

Packing of SPE cartridges and application

Each empty SPE cartridge was packed with 50 mg of the MIP. Two frits were used to safeguard against sorbent losses. In this case, one frit was placed at the bottom of the empty SPE cartridge prior to packing the cartridge with the sorbent. A second frit was used above the sorbent in the cartridge in order to keep the sorbent bed fixed.

Each packed cartridge was conditioned with 2 mL of organic solvent containing a mixture of acetonitrile and methanol at a ratio of 1:1 (v/v). Thereafter, equilibration of the sorbent was conducted using 2 mL of deionized

Downloaded from http://iwaponline.com/wst/article-pdf/79/2/356/616152/wst079020356.pdf
by guest

water. Each water sample (50 mL at pH 2.5) after filtration on 0.45 μm filter paper was percolated on the conditioned cartridge. After sample loading, the cartridge was washed with 2 mL of 10% (v/v) methanol in water followed by drying under vacuum for 5 minutes. Finally, the adsorbed analyte was eluted using 2 mL of acetonitrile and injected into the HPLC system for analysis. In all cases, the samples and solutions were loaded at a flow rate 1 mL/min.

Method validation

The validation of the proposed analytical method was based on linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). Five standard solutions of efavirenz in the concentration range of 0.2–1.0 mg/L were prepared in acetonitrile by diluting the stock solution of 100 mg/L. These standard solutions were analysed with the LC-PDA system and used for the construction of the calibration curve. The accuracy and precision of the analytical method were validated using a water sample spiked with 10 μg/L of efavirenz and subjected to the SPE prior to LC-PDA separation and quantification. The sensitivity of the analytical method was measured based on LOD and LOQ that were computed from the signal to noise ratios of 3 and 10, respectively.

RESULTS AND DISCUSSION

Synthesis of molecularly imprinted polymer and computational investigation

In this study, a MIP was synthesized using a bulk polymerization technique that is known for its ability to convert all the reagents into a solid polymer (Madikizela *et al.* 2018). This technique is relatively greener when compared to precipitation and suspension polymerization methods which require excess amount of porogenic solvent and result in generation of liquid waste (Madikizela *et al.* 2018). MIPs mostly interact with analytes and adsorb them from different matrices using the functionalities of their monomers. Therefore, the choice of the suitable functional monomer is always essential for the synthesis of MIPs as it is responsible for the extraction of the analyte from the sample matrix. In this study, 2-vinylpyridine, which has been a functional monomer of choice in MIPs for acidic pharmaceuticals (Madikizela *et al.* 2016), was computationally investigated for its ability to interact with efavirenz. Computational investigation was conducted in the gas phase assuming no solvent was interfering with the interactions between efavirenz and 2-vinylpyridine. The results indicated the existence of the hydrogen bonding between the hydrogen atom of the amine group from efavirenz and the nitrogen atom from 2-vinylpyridine (Figure 1). In Figure 1, the dotted line indicates the hydrogen bonding with a bond distance of 1.857 Å. The binding energy of the complex that results from the interaction of efavirenz and 2-vinylpyridine was calculated using Equation (1).

$$\Delta E = E_{(complex)} - E_{(template)} - E_{(2\text{-vinylpyridine})} \quad (1)$$

A strong complex between efavirenz and 2-vinylpyridine resulted in the binding energy of −18 kcal/mol. The complex formed in this work indicates that the synthesized MIP was expected to bind strongly with efavirenz. The results of the present study indicate that the complex between efavirenz and 2-vinylpyridine was stronger than the complexes reported by Madikizela *et al.* (2016), which were based on interactions of acidic pharmaceuticals, namely naproxen, diclofenac, and ibuprofen, with 2-vinylpyridine.

Characterization of both molecularly and non-imprinted polymers

FTIR spectroscopy was used to study the functional groups present in the synthesized polymers (Figure 2). Spectral similarity of the MIP to the NIP was observed due to identical synthetic conditions. Characteristic bands for MIP were observed at the wavenumbers of 1,150, 1,730, and 2,942 cm^{-1}, which corresponded to the presence of C-O, C=O and N-H bonds, respectively. There was a slight shift

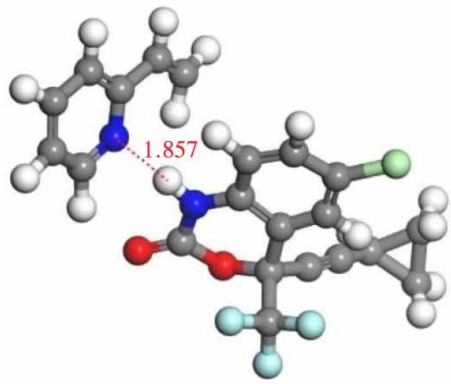

Figure 1 | Computational representation of the binding of efavirenz to 2-vinylpyridine where carbon, hydrogen, oxygen, nitrogen, fluorine, and chlorine are represented by grey, white, red, blue, light blue, and green round images, respectively. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2019.054.

Downloaded from http://iwaponline.com/wst/article-pdf/79/2/356/616152/wst079020356.pdf
by guest

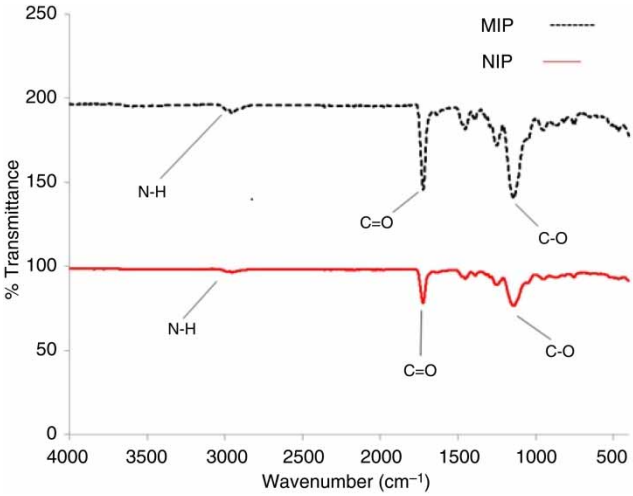

Figure 2 | FTIR spectra for the MIP and NIP.

observed in the wavenumbers of the same peaks in the NIP due to minor structural variations that could have occurred during the template removal from MIP. Therefore, the peaks corresponding to the presence of C-O, C=O and N-H in NIP were observed at 1,160, 1,734 and 2,968 cm^{-1}, respectively. Furthermore, the peaks observed in the MIP were more intense than the corresponding peaks in NIP, which could be due to the overlapping of bonds of the MIP with the template as reported in literature (Chrzanowska *et al.* 2015; Wang & Cao 2015; Madikizela *et al.* 2016).

Thermal stability studies of MIP and NIP were conducted using TGA. The two polymers (MIP and NIP) had a weight loss of about 4% around 35 °C (Figure 3) which was assumed to be due to evaporation of the acetonitrile used as the washing solvent, which was trapped within the pores of the polymers. It was further observed that the polymers had similar thermal stability patterns due to similar reagents and conditions used during their synthesis. The backbone of both MIP and NIP collapsed at 250 °C with

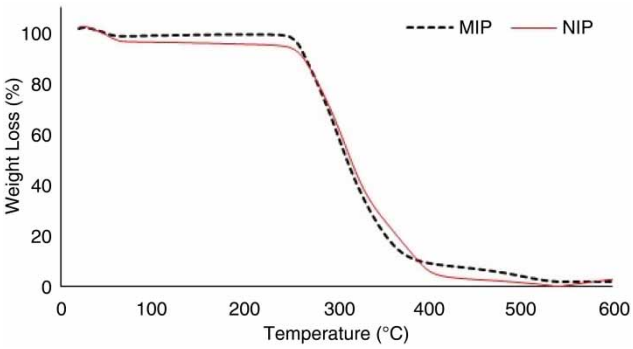

Figure 3 | TGA curves for MIP and NIP.

significant weight loss of approximately 90%. The backbones of polymers reported in literature that were synthesized under the same conditions using bulk polymerization technique collapsed at comparable temperatures of 290 °C (Zunngu *et al.* 2017) and 294 °C (Wang & Cao 2015).

The porous features of the polymers were studied using BET. The results revealed that the MIP had a higher surface area compared to the NIP (Table 1). This is in agreement with the results presented for MIP synthesized for selective extraction of acidic pharmaceuticals from water bodies and its corresponding NIP (Madikizela *et al.* 2016). This usually results in MIP having greater adsorption efficiency or binding capacity than the NIP. The average pore diameters obtained for both polymers fall within the range of 2–50 nm, which means that the structure of both polymers is mesoporous (Farrington & Regan 2007; Chrzanowska *et al.* 2015; Madikizela *et al.* 2016). The pore volume and diameter were similar for both polymers.

The morphology of the polymers was studied using a SEM. The SEM images showed that the MIP surface is more rough than the NIP (Figure 4), which could imply that MIP has more cavities (binding sites) than the NIP.

The elemental composition of the polymers was evaluated and the results showed that both polymers had similar percentage composition of carbon, hydrogen, nitrogen and oxygen (Table 2). This could be due to similar synthetic conditions that involved the same reagents and their quantities with only efavirenz omitted in the synthesis of NIP. Hence, similar composition of these elements could also be an indication that there was a complete removal of the template from the MIP during washing.

Solid-phase extraction studies

The synthesized MIP was applied as sorbent in the SPE of efavirenz from water. NIP was excluded in these experiments, as previous work showed that it yields poor adsorption results when compared to MIP (Madikizela *et al.* 2016, Zunngu *et al.* 2017). These results are usually evidenced by poor recoveries, low adsorption capacity or poor selectivity for NIP, even though the synthetic reagents and conditions are similar to

Table 1 | BET results for MIP and NIP

Polymer	Surface area (m^2/g)	Total pore volume (cm^3/g)	Average pore diameter (nm)
MIP	420	0.86	8.36
NIP	409	0.87	8.87

Downloaded from http://iwaponline.com/wst/article-pdf/79/2/356/616152/wst079020356.pdf
by guest

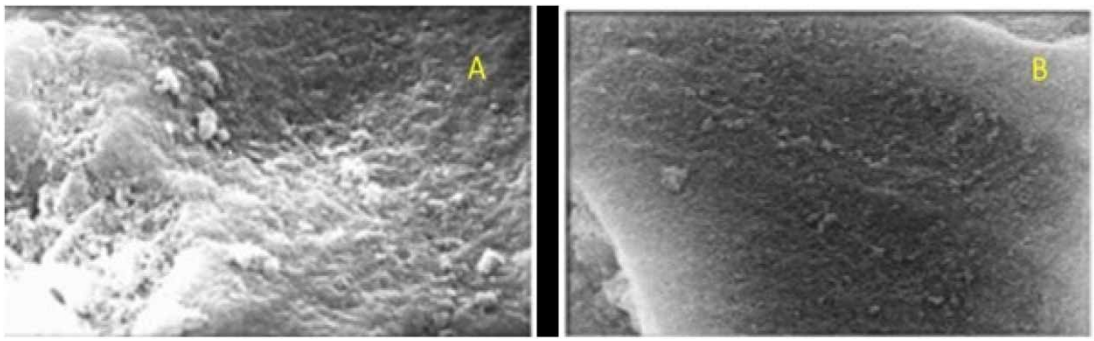

Figure 4 | SEM images for MIP (A) and NIP (B).

Table 2 | The elemental composition of MIP and NIP

	Elemental composition (%)	
Element	**MIP**	**NIP**
Nitrogen	0.6	0.9
Carbon	59.8	59.0
Hydrogen	7.1	7.0
Oxygen	29.1	29.4

those used for MIP with the exception of template exclusion. Prior to application in real samples (wastewater and surface water samples), the SPE method was optimized for maximum extraction of efavirenz from water.

Optimization of solid-phase extraction

In this study, the optimized conditions were only the conditioning solvent and sample volume. For the conditioning step, the solvents that were investigated were acetonitrile, methanol and a mixture of acetonitrile:methanol at a ratio of 1:1 (v/v). The results obtained (Figure 5) showed that acceptable recoveries were achieved, regardless of the applied conditioning solvent; however, they were higher for the mixture of solvents. The maximum recovery of 120% was achieved when the mixture of acetonitrile and methanol (A + M) was used as the conditioning solvent. This is an indication that the mixture of the two solvents was more effective in activating the functional groups of the sorbent and thus increased its surface area, which could have resulted in strong interactions between efavirenz and the sorbent.

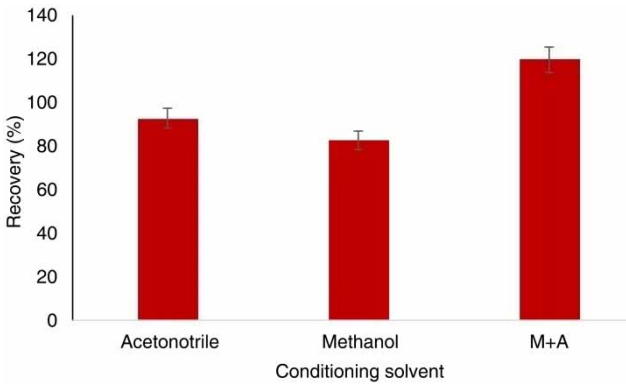

Figure 5 | The effect of the conditioning solvent on the recoveries.

The effect of sample volume was investigated as large sample volumes lead to high pre-concentration factors which subsequently lead to better sensitivity of the analytical method. However, at the same time it should be noted that large sample volumes prolong the analysis time. In this study, the sample volumes investigated were 25, 50 and 100 mL. The results showed that the analyte recovery increased from the sample volume of 25 mL to 50 mL and then slightly decreased at 100 mL (Figure 6). The lower recoveries achieved when 25 mL sample volume was percolated through the sorbents could be due to the limited amount of efavirenz available for interactions with the sorbents. The highest recoveries were achieved when the sample volume was 50 mL; therefore, this was regarded as optimum and all the subsequent experiments were conducted using this volume. The slight decrease of recoveries when the volume of the sample was increased to 100 mL could be due to reaching the breakthrough volume, which resulted in poor adsorption of efavirenz. This could also imply that the surface of the MIP was saturated during the loading of a sample exceeding 50 mL; therefore, there were no binding sites available for further adsorption of efavirenz from water. There is also a possibility that the adsorbed efavirenz started to be eluted by the excess volume of water sample loaded into the SPE cartridge. This trend was also observed in literature (Zunngu *et al.* 2017), where a sample volume of 50 mL gave the highest recovery for MIP and corresponding NIP, which resulted in its selection as the optimum volume.

Downloaded from http://iwaponline.com/wst/article-pdf/79/2/356/616152/wst079020356.pdf
by guest

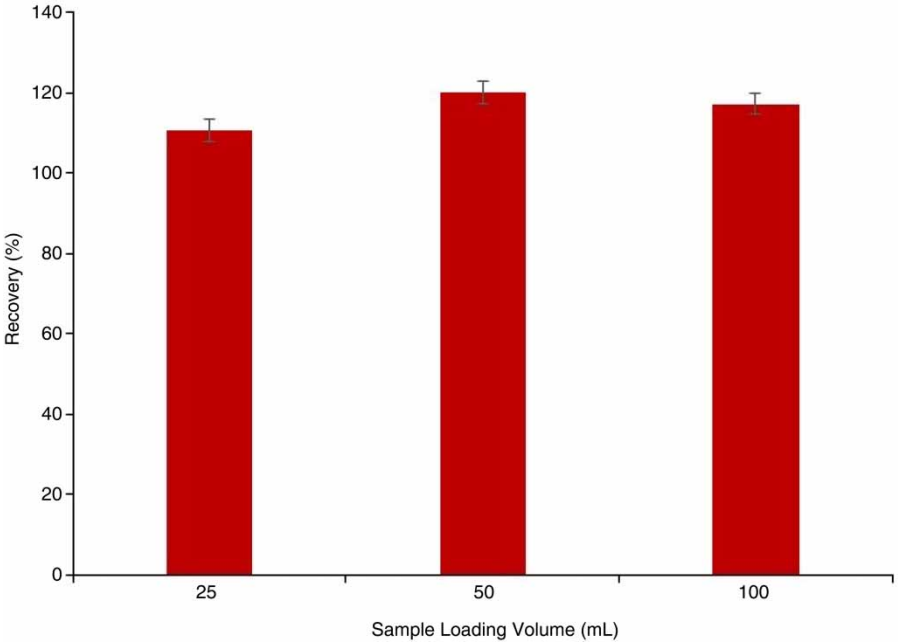

Figure 6 | The effect of the sample loading volume on the recoveries.

Selectivity

The selectivity of the MIP was investigated using deionized water spiked with a mixture of efavirenz, diclofenac and fenoprofen. Both diclofenac and fenoprofen were selected as competitors as they have similar functional groups as efavirenz (Table S6, available with the online version of this paper). Both competitors are pharmaceutical drugs which are commonly detected in the aquatic environment (Fatta *et al.* 2007; Patrolecco *et al.* 2013). The recovery obtained for efavirenz in the presence of both competitors was 97% (Table 3), when MIP was used as SPE sorbent. Higher recovery of MIP towards efavirenz than towards both competitors indicates that MIP was more selective. Even though it can interact with the other compounds in a similar manner as efavirenz, it binds strongly to efavirenz due to molecular recognition.

Method validation

The method was validated based on linearity, sensitivity, precision and accuracy. The computed calibration curve showed good linearity with R^2 value of 0.9986 in the efavirenz concentration range of 0.2–1.0 mg/L. The LOD and LOQ were used as the quantitative measure of the sensitivity. The results obtained are summarized in Table 4. The LOD and LOQ were found to be 0.41 and 1.39 µg/L, respectively. This high sensitivity for the method could be due to the ability of MIP to eliminate most of the matrix effects from the samples. The overall recovery found was 81%, which is an indication of high accuracy. The lower relative standard deviation (RSD) value indicated that the method is precise.

Application of the optimized methods on real water samples

Determination and removal of efavirenz in wastewater

The optimized SPE method was applied for the extraction of efavirenz from wastewater and river water samples. The eluate from the SPE was injected into the LC-PDA system for separation and quantification of extracted compounds.

Table 3 | Recovery percentages from the selectivity studies of MIP

Pharmaceutical	MIP recovery (%)
Efavirenz	97
Diclofenac	88
Fenoprofen	61

Table 4 | The LOD, LOQ and recovery values ($n = 3$) obtained for water sample

Parameter	MIP
LOD (µg/L)	0.41
LOQ (µg/L)	1.39
Recovery (%) ± % RSD	81 ± 0.27

Downloaded from http://iwaponline.com/wst/article-pdf/79/2/356/616152/wst079020356.pdf
by guest

Samples for analysis were collected over two seasons, Spring (hot season) and Autumn (cold season), of the same year. Results obtained for wastewater samples are given in Table 5. Higher concentrations of efavirenz were observed in the hot season rather than the cold season. These results are in disagreement with those reported by Colella (2014), where the author observed similar concentrations of target pharmaceuticals in all the seasons of the year. In another study, the concentrations of detected pharmaceuticals in water were higher during the cold seasons due to the ability of the cold weather to decrease the biodegradation of the target compounds, thus resulting in higher concentrations (Kot-Wasik *et al.* 2016). The variations in concentrations of efavirenz observed in this work over two seasons could be due to different amounts of efavirenz arriving in the WWTPS, which could depend on numerous factors such as the frequency of its use and its resistance to biodegradation as well as the excretion of un-metabolized drug (Nikolaou *et al.* 2007). Furthermore, the discrepancies in the volume of water in each sampling site over two different sampling campaigns could cause significant differences in concentration of drugs present in water. For example, low water volume, especially if there is no rainfall, is expected to result in pre-concentration of water pollutants. In this study, it was observed that water levels in most WWTPs was low during the hot season. Therefore, this could have resulted in pre-concentration of efavirenz as the excretion rate is expected to be similar throughout the entire year. Another possible reason for these seasonal variations could be due to the collection of wastewater samples at different times of the day in both seasons. Also, variations in environmental conditions such as temperature of water could influence the degradation of efavirenz as well as its water solubility, which can subsequently affect its ability get adsorbed on solid particles.

It was also observed that the concentrations of efavirenz found in WWTP influents were higher than those in the effluents. This was possibly due to the retention of efavirenz onto the sludge as a result of its large Log K_{OW}. Therefore, the sludge removal in the early stages of the WWTP process could have resulted in significant amounts of efavirenz removal from water. A similar observation was made for other WWTPs investigated in another study (Abafe *et al.* 2018).

The maximum concentrations found during this study are higher than the concentration of efavirenz previously reported to be present in WWTPs in South Africa. Maximum concentrations of efavirenz previously reported in South African WWTPs are 34 μg/L (Abafe *et al.* 2018), 14 μg/L (Schoeman *et al.* 2017) and 17.4 μg/L (Schoeman *et al.* 2015). In Kenya the maximum concentration found for efavirenz was 1.02 μg/L (K'oreje *et al.* 2016).

The removal efficiency (%) of efavirenz in WWTPs was calculated using Equation (2):

$$\text{Removal efficiency} = \frac{C_{\text{influent}} - C_{\text{effluent}}}{C_{\text{influent}}} \times 100 \quad (2)$$

where C_{influent} and C_{effluent} are the concentration obtained in the raw influent and final effluent, respectively.

In all the investigated WWTPs, the Northern WWTP had the highest removal efficiency (92%) of efavirenz (Table 6). This observation could be due to higher biodegradation or adsorption of the compounds on sludge, which resulted in higher removal of efavirenz from water (Martin *et al.* 2012). Elsewhere, it has been reported that the degradation of compounds in WWTPs is dependent on the biological treatment efficiency (Madikizela 2017), which is an indication that pharmaceutical transformation is possible in WWTPs. Also, some of the compounds can resist biodegradation, resulting in lower removal efficiency, and hence can be discharged with wastewater effluent into the rivers. In this case, a mathematical model of water quality rehabilitation where rainwater can be utilized as reported elsewhere (Wu & Chau 2006) can be investigated for the purpose of improving the quality of water.

Table 5 | Concentrations of efavirenz in Durban WWTPs detected using MIP sorbents

	Concentration (μg/L)			
	Hot season		Cold season	
Sample (WWTP)	Influent	Effluent	Influent	Effluent
Umhlathuzana	140.4	86.9	9.63	2.79
Amanzimtoti	96.9	78.8	11.1	8.69
Umbilo	114.4	93.1	37.4	7.19
Northern	89.8	7.46	28.04	12.98

The extraction was based on the MIP as the sorbent.

Table 6 | Removal efficiencies for efavirenz in WWTPs located in Durban

	Detected concentration (μg/L)		
WWTP	Influent	Effluent	% Removal efficiency
Umhlathuzana	140.4	86.9	38
Amanzimtoti	96.9	78.8	19
Umbilo	114.8	93.1	19
Northern	89.8	7.46	92

Downloaded from http://iwaponline.com/wst/article-pdf/79/2/356/616152/wst079020356.pdf
by guest

Determination of efavirenz in surface water

Over the years, there has been a great demand for a detailed evaluation of the quality of river water for the purpose of safeguarding public health and protection of fresh water resources (Wang *et al.* 2014). In this context, the occurrence and quantitation of efavirenz was conducted in Msunduzi River. The highest concentration of efavirenz obtained in Msunduzi River was 2.45 μg/L (Table 7), which was found in the Bishopstowe sampling point. This could be influenced by the nearby Darvill WWTP which could be releasing efavirenz into the river. This is elaborated in literature where WWTPs are identified as the main source of water pollutants (Chau 2005) including pharmaceutical contamination in rivers (Schoeman *et al.* 2017). This maximum concentration detected in Msunduzi River is higher than the levels previously reported in other rivers found in South Africa and Kenya. The maximum concentrations of efavirenz that have been previously reported in South African rivers are 0.138 μg/L (Rimayi *et al.* 2018) and 0.696 μg/L (Wood *et al.* 2017), whereas in a Kenyan river, 0.560 μg/L has been found for the same drug (K'oreje *et al.* 2016). This could be triggered by differences in the number of people that are consuming efavirenz for ARV treatment in various regions.

CONCLUSION

Both MIP and NIP were synthesized successfully via bulk polymerization approach. The characterization results showed that the two polymers (MIP and NIP) are structurally similar with MIP showing surface roughness and higher surface area. The SPE with the MIP sorbent was optimized and applied in the extraction of efavirenz from wastewater influent and effluent as well as surface water. A recovery of 120% in distilled water and 81% in wastewater was obtained. Efavirenz was detected and quantified in all wastewater samples; however, for surface water, in most of the samples the analyte was below the quantification limit. The concentrations found in wastewater (2.79–140.4 μg/L) were higher than the concentrations in the surface water, where a maximum of 2.45 μg/L was observed. Efavirenz in most surface water samples could not be quantified due to its trace amounts in the environment. In this case, a more sensitive analytical instrumentation such as LC equipped with mass spectrometry for detection should be investigated in future for the analysis of efavirenz. The primary focus of the study was on water analysis and efavirenz was detected in some samples; therefore, future work should involve the analysis of solid samples including sewage sludge, aquatic plants and biota. In order to fully understand the extent of water pollution in South Africa caused by efavirenz, its analysis should be conducted throughout the country. The results of this study indicated that the treatment technology used in the investigated WWTPs was not effective for the removal of efavirenz from water. Overall, this study has demonstrated that MIP can be used for selective analysis of ARV drugs in water samples.

Table 7 | The concentrations of efavirenz detected in Msunduzi River, Pietermaritzburg

Sampling site	Concentration (μg/L) MIP
College Road	n.q
Baynespruit	n.q
Bishopstowe	2.45
YMCA	n.q
Barnsley Road	n.q

n.q – detected but below the LOQ.

ACKNOWLEDGEMENTS

The authors acknowledge National Research Foundation (NRF) of South Africa under Thuthuka grant (grant number: 107091) and Competitive Program for Unrated Researchers (grant number: 114415) for financial support given to P. N. Mahlambi and L. M. Madikizela, respectively.

REFERENCES

Abafe, O. A., Späth, J., Fick, J., Jansson, S., Buckley, C., Stark, A., Pietruschka, B. & Martincigh, B. S. 2018 LC-MS/MS determination of antiretroviral drugs in influents and effluents from wastewater treatment plants in KwaZulu-Natal, South Africa. *Chemosphere* **200**, 660–670.

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.

Batlokwa, B. S., Mokgadi, J., Nyokong, T. & Torto, N. 2011 Optimal template removal from molecularly imprinted polymers by pressurized hot water extraction. *Chromatographia* **73**, 589–593.

Chau, K. W. 2005 Characterization of transboundary POP contamination in aquatic ecosystems of Pearl River delta. *Marine Pollution Bulletin* **51**, 960–965.

Chrzanowska, A. M., Poliwoda, A. & Wieczorek, P. P. 2015 Characterization of particle morphology of biochanin A molecularly imprinted polymers and their properties as a

Downloaded from http://iwaponline.com/wst/article-pdf/79/2/356/616152/wst079020356.pdf
by guest

potential sorbent for solid-phase extraction. *Materials Science and Engineering: C* **49**, 793–798.

Colella, K. 2014 *Time Trends of Pharmaceuticals in Wastewater Treatment Plant Effluent with Sources from Pharmaceutical Manufacturing Facilities and Hospitals*. MSc thesis, Stony Brook University.

Domínguez, J. R., González, T., Palo, P. & Cuerda-Correa, E. M. 2011 Removal of common pharmaceuticals present in surface waters by Amberlite XAD-7 acrylic-ester-resin: influence of pH and presence of other drugs. *Desalination* **269**, 231–238.

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**, 1138–1146.

Fatta, D., Achilleos, A., Nikolaou, A. & Meric, S. 2007 Analytical methods for tracing pharmaceutical residues in water and wastewater. *TrAC Trends in Analytical Chemistry* **26**, 515–533.

Jebiwot, K. F. 2016 *Occurrence and Removal of Pharmaceutical Residues in Kenyan Wastewater Treatment Plants*. MSc thesis, University of Gent.

K'oreje, K., 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.

Kot-Wasik, A., Jakimska, A. & Śliwka-Kaszyńska, M. 2016 Occurrence and seasonal variations of 25 pharmaceutical residues in wastewater and drinking water treatment plants. *Environmental Monitoring and Assessment* **188**, 661.

Madikizela, L. M. 2017 *Determination of Selected Acidic Pharmaceutical Compounds in Wastewater Treatment Plants*. PhD thesis, University of Witwatersrand.

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., Tavengwa, N. T., Tutu, H. & Chimuka, L. 2018 Green aspects in molecular imprinting technology: from design to environmental applications. *Trends in Environmental Analytical Chemistry* **17**, 14–22.

Malawi24 2016 https://malawi24.com/2016/07/09/drinking-water-contaminated-arv/

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.

Nikolaou, A., Meric, S. & Fatta, D. 2007 Occurrence patterns of pharmaceuticals in water and wastewater environments. *Analytical and Bioanalytical Chemistry* **387**, 1225–1234.

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

Rimayi, C., Odusanya, D., Weiss, J. M., De Boer, J. & Chimuka, L. 2018 Contaminants of emerging concern in the Hartbeespoort Dam catchment and the uMngeni River estuary 2016 pollution incident, South Africa. *Science of the Total Environment* **627**, 1008–1017.

Schoeman, C., Mashiane, M., Dlamini, M. & Okonkwo, O. 2015 Quantification of selected antiretroviral drugs in a wastewater treatment works in South Africa using GC-TOFMS. *Journal of Chromatography & Separation Techniques* **6**, 1–7.

Schoeman, C., Dlamini, M. & Okonkwo, O. 2017 The impact of a wastewater treatment works in Southern Gauteng, South Africa on efavirenz and nevirapine discharges into the aquatic environment. *Emerging Contaminants* **3**, 95–106.

Tamayo, F., Turiel, E. & Martín-Esteban, A. 2007 Molecularly imprinted polymers for solid-phase extraction and solid-phase microextraction: recent developments and future trends. *Journal of Chromatography A* **1152**, 32–40.

Vasapollo, G., Sole, R. D., Mergola, L., Lazzoi, M. R., Scardino, A., Scorrano, S. & Mele, G. 2011 Molecularly imprinted polymers: present and future prospective. *International Journal of Molecular Sciences* **12**, 5908–5945.

Wang, Z. & Cao, X. 2015 Preparation of core–shell molecular imprinting polymer for lincomycin and its application in chromatographic column. *Process Biochemistry* **50**, 1136–1145.

Wang, W., Xu, D., Chau, K. & Lei, G. 2014 Assessment of river water quality based on theory of variable fuzzy sets and fuzzy binary comparison method. *Water Resource Management* **28**, 4183–4200.

Wood, T. P., Duvenage, C. S. & Rohwer, E. 2015 The occurrence of anti-retroviral compounds used for HIV treatment in South African surface water. *Environmental Pollution* **199**, 235–243.

Wood, T. P., Du Preez, C., Steenkamp, A., Duvenge, C. S. & Rohwer, E. 2017 Database driven screening of South African surface water and the targeted detection of pharmaceuticals using liquid chromatography-high performance resolution mass spectrometry. *Environmental Pollution* **230**, 453–462.

Wooding, M., Rohwer, E. R. & Naudé, Y. 2017 Determination of endocrine disrupting chemicals and antiretroviral compounds in surface water: a disposable sorptive sampler with comprehensive gas chromatography–time-of-flight mass spectrometry and large volume injection with ultra-high performance liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A* **1496**, 122–132.

World Health Organization 2013 *Global Update on HIV Treatment 2013: Results, Impact and Opportunities*, World Health Organization, Geneva, Switzerland.

Wu, C. L. & Chau, K. W. 2006 Mathematical model of water quality rehabilitation with rainwater utilization – a case study at Haigang. *International Journal of Environment and Pollution* **28**, 534–545.

Zunngu, S. S., Madikizela, L. M., Chimuka, L. & Mdluli, P. S. 2017 Synthesis and application of a molecularly imprinted polymer in the solid-phase extraction of ketoprofen from wastewater. *Comptes Rendus Chimie* **20**, 585–591.

First received 15 October 2018; accepted in revised form 26 January 2019. Available online 11 February 2019

Downloaded from http://iwaponline.com/wst/article-pdf/79/2/356/616152/wst079020356.pdf
by guest