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Target and Suspect Screening of Pharmaceuticals and their Transformation Products in the Klip River, South Africa, using Ultra-High–Performance Liquid Chromatography–Mass Spectrometry

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Abstract: In spite of recent reports about the presence of pharmaceuticals in African water bodies, their prevalence has still not been sufficiently quantified. The few available studies have mostly focused on a limited number of pharmaceuticals. In the present study, a suspect screening of 92 compounds (mainly pharmaceuticals and their transformation products) along the Klip River, South Africa was conducted, followed by target monitoring of 21 of the detected pharmaceuticals. The experimental approach was based on solid-phase extraction followed by analysis with ultra-high–performance liquid chromatography–quadrupole time-of-flight–mass spectrometry (UHPLC–QTOF–MS). The results revealed 47 pharmaceuticals, 31 of which were detected for the first time in South African waters. Seven detected pharmaceuticals (propyphenazole, sulfamerazine, levamisole, tryptophan, dibucaine, albuterol, and fenpropimorph) are not approved medications in South Africa. Six pharmaceutical metabolites were detected for the first time in South Africa. Pharmaceuticals with the highest concentrations in river water were flumequine (0.257 μg L^{-1}), oxolinic acid (0.355 μg L^{-1}), and acetaminophen (0.432 μg L^{-1}). Oxolinic acid presented the highest hazard quotient, 48.6, indicating a risk of toxicity to aquatic organisms. Hazard quotients for other pharmaceuticals were below 1, except that of flumequine, which reached 1.285. These results suggest a need for further research into the fate of pharmaceuticals in surface waters, and a quantification of the risks associated with the identified drugs because they are likely to accumulate in the tissues of fish/aquatic organisms, thus affecting humans. *Environ Toxicol Chem* 2022;41:437-447. © 2021 SETAC

Keywords: Suspect screening; Target screening; Pharmaceutical metabolites; Klip River; Water pollution

INTRODUCTION

In recent years, there has been an increase in the number of studies reporting the occurrence of pharmaceuticals in surface water bodies in Africa (Madikizela et al., 2017, 2020; Ngqwala & Muchesa, 2020). The release of pharmaceuticals into the environment is mostly linked to wastewater treatment plant (WWTP) effluents and direct disposal of unused medications (Mlunguza et al., 2019). It has been reported worldwide that WWTP processes do not eliminate pharmaceutical residues and thus quite a large number of pharmaceuticals have been detected in WWTP effluents (Liu & Wong, 2013; Tran et al., 2018). The human population may then be exposed to pharmaceuticals through drinking water from contaminated sources (Furlong et al., 2017). From an African perspective, many communities including those in urban areas do not have access to proper sanitation facilities, leading to direct release of excreta and sewerage into surface water bodies (Madikizela et al., 2017). As a result, much higher concentrations of pharmaceuticals in the environment have been reported for Africa compared with Europe (Archer et al., 2017; Faleye et al., 2018; Fekadu et al., 2019). In addition, various other

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point sources of pharmaceuticals common in developing countries such as dumpsites, seepages from septic tanks, landfill leachates, and WWTP sludge used for fertilizer have been reported in the literature (Madikizela et al., 2017; Patel et al., 2019; Philip et al., 2018).

Monitoring and environmental risk assessment studies are fairly new in Africa, and most studies have targeted a small number of pharmaceuticals (Madikizela et al., 2020). Thus the creation of databases of priority lists and compounds of potential concern has been limited. The recording of priority lists and compounds of potential concern in the European Union, Canada, and the United States, for example, has been possible largely due to screening and compilation of exhaustive lists of pharmaceuticals (Furlong et al., 2017; Lee & Choi, 2019). The frameworks vary by region or country based on the specific data obtained. Such databases have also enabled the development of regulations governing the discharge of effluents from the pharmaceutical industry, WWTPs, domestic solid waste, and disposal of unused or expired medications (Patryn et al., 2011). Furthermore, approval of pharmaceuticals that can be legally prescribed to humans is guided by clearly defined directives that demand strict approval protocols. It is clear from the foregoing that limited screening and databases can not only have far-reaching implications for both the environmental and human health; they constitute a basic requirement for the development of regulations.

However, in developing countries, the capacity for continuous monitoring studies as a basis for pharmaceutical regulation frameworks is limited. Furthermore, there is noncompliance with policies governing sewage disposal, and the ability of WWTPs to remove pharmaceuticals from sewage in Africa is often poor. In the few cases in which such policies exist, there is no evidence of measures to ensure compliance. This implies that the potential human health and aquatic risks of pharmaceuticals in Africa is largely unknown. Urban dwellings in Africa are characterized by informal settlements with limited access to water and sanitation services, which creates a serious health and environmental concern.

Current priority lists and regulations are based on what has been detected and/or prescribed in developed countries. The production and the consumption pattern of drugs are not necessarily the same across the world, and hence the detection levels and profiles of drug residues in water bodies are different. For example, more than 70% of people living with HIV/AIDS reside in Africa (Ncube et al., 2018), which would make antiretroviral drugs more of an environmental risk problem in Africa than elsewhere. Furthermore, recent reviews of studies on pharmaceuticals that have been detected in surface water sources in Africa (Faleye et al., 2018; Gwenzi & Chaukura, 2018; Madikizela et al., 2017, 2020) have observed that the numbers and classes of pharmaceuticals targeted in each study were limited. Two previous studies done separately on two South African river water systems have included pharmaceuticals in a list that also included personal care products, illicit drugs, and pesticides (Mhuka et al., 2020; Rimayi et al., 2019). The study by Mhuka and colleagues along the Apies River in Pretoria, South Africa reported 24 compounds that were detected for the first time in South Africa, of which 10 were pharmaceuticals (Mhuka et al., 2020).

The present study focused on screening 92 pharmaceutical residues and some important transformation products in an urban river impacted by industrial and domestic activities. The Klip River was chosen as a representative urban river; it passes through the major township of Soweto in Johannesburg, South Africa (Murray et al., 2013). The township has various informal dwellings and receives effluents from three WWTPs and leachates from a municipal dumpsite (Freeman et al., 1997). Water from the river is at times used by local communities for irrigation of vegetables and crops while at the same time fishing activities along the river contribute to the availability of food for some residents. Our study therefore seeks to contribute to identification of pharmaceuticals that can be prioritized from a South African perspective. Our primary aim was to do an initial screening of different classes of pharmaceuticals for the purpose of contributing to the identification and prioritizing of pollutants relevant to South Africa. The list of screened pharmaceuticals covers those detected previously in South Africa and Africa as a whole, those detected in other developing countries, and those detected and listed as priority pharmaceuticals in developed nations.

MATERIALS AND METHODS

Chemicals and preparation of solutions

Pharmaceutical standards of high-purity chemicals (more than 95%) and formic acid (98% or greater) were purchased from Sigma-Aldrich. The high-performance liquid chromatography (HPLC)-grade solvents acetonitrile (99.9% or greater) and methanol (99.5% or greater) were both obtained from Merck Chemicals (Johannesburg, South Africa). Deionized water used in solid-phase extraction (SPE) and dilution of standard solutions was purified from a Milli-Q-RO4 system from Millipore (Bedford, MA, USA). A stock solution containing a mixture of 21 pharmaceuticals was prepared by dissolving each pharmaceutical in 10 ml of acetonitrile, which resulted in a final concentration of 100 mg L^{-1} for each compound. A stock solution was preserved by storing it at less than 4 °C in a refrigerator when not in use. The stock solution was used within a month. Individual solutions of each pharmaceutical were also prepared and stored in the same manner. The calibration standards were prepared by dissolving the stock solution in water and were analyzed within 1 day.

Study site

The presence of various classes of pharmaceuticals in the Klip River, which traverses Soweto Township and also receives effluents from three WWTPs, was examined. The Klip River catchment lies to the west of Johannesburg, with most tributaries converging in Soweto Township. The river flows through Soweto Township in a southerly direction, and then eastward toward the three WWTPs before turning south again toward a major South African river, the Vaal River. Running for approximately 1120 km (Dikio et al., 2010), it supplies water to the

country's major economic, agricultural, industrial, and mining hubs, and to a population of approximately 12 million people (Groffen et al., 2018). It is also the largest tributary of the Orange River in South Africa.

Water samples were collected at points where the Klip River passes through Orlando East and Orlando West within 100 m of the Orlando stadium in Soweto (Site 1). Site 2 was at a road across a wetland in Soweto. Soon after leaving Soweto, the Klip River is joined by small streams originating from three WWTPs, Olifantsvlei, Goedkoppies, and Bushkoppies. At each point where the river meets each stream, there are large artificial wetlands designed to remove pollutants. Sampling points (Site 1 - 6) are shown in Figure 1. Importantly, samples were collected at the exit of selected wetlands (Sites 3 and 5). Site 3 was located at the Golden Highway crossing point after receiving effluent from Olifantsvlei WWTP, and Site 4 at the N1 road crossing just before the river meets a stream with effluents from Goedkoppies and Bushkoppies WWTPs. Samples were also collected just after the wetland where the river meets with combined effluents from two WWTPs (Site 5) and further down the river, toward the Vaal River (Site 6).

Sampling and sample preparation

Grab sampling was used for sample collection. To circumvent the disadvantage associated with grab sampling, 100-ml samples were collected at 30-min intervals over a 4-h period between 10 a.m. and 2 p.m. South African time. A previous study has observed an increase of pharmaceutical concentrations from midday toward the afternoon in a WWTP located in Johannesburg (Amdany et al., 2015). In addition, the sampling period of 10 a.m. to 2 p.m. was selected in consideration of the many informal dwellings with no modern ablution facilities, which is likely to result in direct contamination of river water. Furthermore,

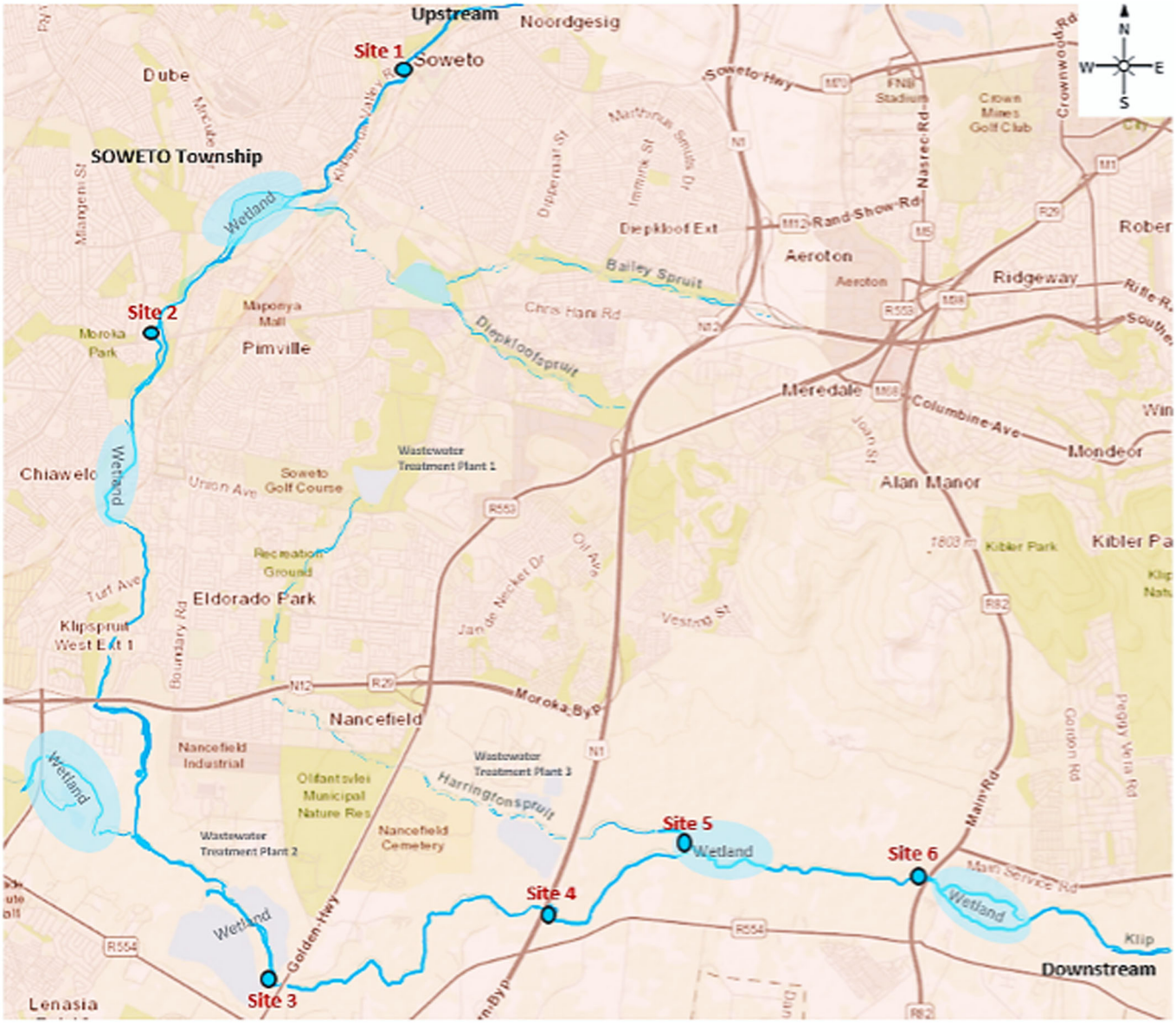

FIGURE 1: Sampling sites along the Klip River in Soweto Township, South Africa.

fishing activities mostly occur at the same time. The samples were immediately filtered through 0.45-μm hydrophilic polypropylene membrane filters obtained from Pall into a 2-L brown bottle. Subsequent samples were added to the same bottle.

Extraction and preconcentration of pollutants

All the collected samples were subjected to filtration through the 0.45-μm membrane filters prior to extraction of pollutants and their preconcentration using SPE. The SPE was performed on the automated Dionex™ Auto Trace™ 280 SPE instrument from Thermo Fisher Scientific using preconditioned 200-mg Oasis HLB cartridges (6 cc) supplied by Waters. Conditioning of the SPE cartridges was performed with 5 ml of methanol, followed by equilibration with 5 ml of ultra-high-purity water, both flowing at 1 ml min^{-1}. Thereafter, 500 ml of water samples was loaded at 15 ml min^{-1}. This was followed by rinsing the SPE cartridges with 2 ml of ultra-high-purity water at 5 ml min^{-1}. Cartridges were dried with a stream of nitrogen gas for 10 min. Retained compounds were eluted with two portions of 5 ml of methanol at a flow rate of 1 ml min^{-1}. These two portions were combined and evaporated to near dryness under a gentle stream of nitrogen gas. This was followed by reconstituting the dried extracted samples in 1 ml of 0.1% formic acid in methanol. At this stage, the sample was ready for analysis using ultra-high–performance liquid chromatography–quadrupole time-of-flight–mass spectrometry (UHPLC–QTOF–MS).

LC–MS

Chromatographic separation of treated samples, identification, and quantitation of pharmaceuticals and transformation products present in water samples from the Klip River were performed using a Dionex Ultimate 3000 UHPLC system from Thermo Scientific. The chromatographic instrument was attached to a QTOF–MS detector supplied by Bruker Daltonics. The mass spectrometric detection was operated in positive electrospray ionization mode. In each case, 5 μl of each sample extract or standard solution was injected into the chromatographic system using a built-in autosampler, followed by separation of compounds on a Luna® Omega C_{18} column (50 mm × 4.6 mm × 3 μm) from Phenomenex. The mobile phase used was a mixture of 0.1% (v/v) formic acid in water (solvent A) and 0.1% (v/v) formic acid in acetonitrile (solvent B) operated under the gradient mode. A gradient elution program was initiated by using 5% solvent B in the first 0.5 min, which was ramped to 90% in the next 27 min and re-equilibrated to initial conditions after each analysis. An initial screening approach involved searching the chromatograms for 92 pharmaceuticals including their transformation products based on their chemical formula, exact masses, and fragmentation patterns. The screened pharmaceuticals were selected based on frequency of occurrence in environmental waters around the world. A list of the suspected pharmaceuticals and the transformation products is given in the Supporting Information, Table S1. The suspect compounds included pharmaceuticals of different therapeutic classes, including those not approved for usage, sale, or consumption as South African medications.

Peak detection and compound identification

Data processing was performed using MZmine 2.53, an open source software in which the MS data were converted to mzXML files (Pluskal et al., 2010). Optimal MZmine parameters for detection of peaks were set according to a previously reported method based on centroid algorithms in a three-step procedure involving mass detection, a chromatograph builder, and peak deconvolution (Ncube et al., 2021). The isolated *m/z* value for each peak was then converted to its neutral value and subjected to identification and confirmation using three online database resources (METLIN, KEGG, and Mass Bank). Only the neutral *m/z* values relating to the suspect list of 92 pharmaceuticals were loaded to the three search engines. Optimal candidates were screened based on specific tolerance levels (Ncube et al., 2021) and isotopic similarities of the detected and predicted patterns. Finally, the RANdom SAmple Consensus (RANSAC) algorithm was used to match identified compounds across all sampling sites.

Validation of analytical method for target screening of pharmaceuticals in river water

The performance of the analytical method we used for the quantitation of selected pharmaceuticals was validated in terms of linearity, accuracy, precision, sensitivity, and matrix effects. For linearity, a chromatographic analysis of a mixture of pharmaceuticals at different concentrations ranging from 100 to 1000 μg L^{-1} was performed. Calibration curves were constructed for each pharmaceutical by plotting a concentration of each compound against its corresponding peak area from the UHPLC–QTOF–MS analysis. Other parameters were validated using river water samples spiked with a mixture of 21 pharmaceuticals at three spiking concentrations (1, 10, and 100 μg L^{-1}) for each compound. Pharmaceuticals were extracted from the spiked water samples using SPE and analyzed with UHPLC-QTOF-MS. Both extraction and analysis were done in triplicate. Recovery of each analyte was computed and used as a measure of accuracy of the analytical method. Precision was determined by calculating the relative standard deviation (RSD) using the ratio of standard deviation to the mean of three replicates. Sensitivity of the analytical method was determined based on the limit of detection (LOD) and limit of quantification (LOQ) values, which were calculated as the concentration of each analyte where its chromatographic signal-to-noise ratios were found to be 3 and 10, respectively. Matrix effects were determined from Equation (1) using slopes of matrix-matched calibration (S_{matrix}) and the solvent standard calibration ($S_{solvent}$; Yao et al., 2021).

$$\text{Matrix effects (\%)} = (S_{matrix} - S_{solvent})/S_{solvent} \times 100 \quad (1)$$

Risk assessment

Risk assessment was conducted for the pharmaceuticals that were detected and quantified in the Klip River. This was

performed by computing hazard quotients (HQs), which were calculated using Equation (2) based on the maximum environmental concentration (MEC) in $\mu g\,L^{-1}$ of each detected pharmaceutical and the predicted no-effect concentration (PNEC) values ($\mu g\,L^{-1}$) reported for aquatic organisms (Lin et al., 2008; Mutiyar & Mittal, 2014; Rivera-Jaimes et al., 2018; Rodriguez-mozaz et al., 2020; Zounkova et al., 2011).

$$HQ = MEC / PNEC \quad (2)$$

In Equation (2), HQ is the hazard quotient, MEC is the maximum environmental concentration ($\mu g\,L^{-1}$), and PNEC is the predicted no-effect concentration ($\mu g\,L^{-1}$).

RESULTS AND DISCUSSION

Initial screening of pharmaceuticals and their transformation products in the Klip River

Out of the 92 compounds in the suspect list (Supporting Information, Table S1), 47 compounds were detected from at least one sampling site of the Klip River (Supporting Information, Table S2). The number of identified pharmaceuticals in our study was greater than the 33 pharmaceuticals identified from a suspect list of 2200 contaminants in the Mediterranean River basin (Ccanccapa-cartagena et al., 2019). In the present study, the detected compounds included 40 pharmaceuticals of different therapeutic classes, as well as seven transformation products. Two of the detected transformation products are the metabolites of carbamazepine, an antiepileptic drug that has been found in numerous South African surface waters (Khulu et al., 2022; Mhuka et al., 2020; Ojemaye & Petrik, 2021). Most of the detected compounds from the suspect list (34 of them) were found at sampling Site 1. The number of pollutants in the Klip River decreased downstream, with the last sampling site (Site 6) having just 10 of the screened pollutants. This means the wetlands along the river played a crucial role in reducing pollution in the river.

Flumequine was the only antibiotic present at all sampling sites. In South Africa, this pharmaceutical was previously detected in the Apies River (Mhuka et al., 2020). The other most common compounds appearing at five of the six sampling sites were oxcarbazepine, propyphenazone, ketorolac, clonidine, and atenolol (Supporting Information, Table S2). Of interest among these frequently detected pharmaceuticals was the presence of ketorolac, oxcarbazepine, propyphenazone, and clonidine because this is the first report of their occurrence in South African water bodies. In a different qualitative monitoring study conducted in South Africa, atenolol was detected at all study sites (Rimayi et al., 2019), and in 88% of the monitored samples in the Umgeni River water system of South Africa (Agunbiade & Moodley, 2014). This suggests that it is widespread in South African surface waters.

Compounds detected for the first time in the South African aquatic environment

Main pharmaceutical drugs. The Supporting Information, Table S3 provides a list of pharmaceuticals and their metabolites that were identified for the first time in the South African environment. Thirty-one pharmaceuticals belonging to different therapeutic classes were found in the Klip River. In addition, a word search for each drug conducted in recent review articles published by South African–based authors (Gani et al., 2020; Madikizela et al., 2017, 2020; Ngqwala & Muchesa, 2020) and articles focusing on multiresidue screening of pharmaceuticals in South African waters (Mhuka et al., 2020; Rimayi et al., 2019) suggested that the present study is the first to report the occurrence of these pharmaceuticals in South African water bodies. Previously, Mhuka et al. (2020) reported 10 other pharmaceuticals (lidocaine, procaine, salbutamol, terbutaline, metoprolol, pindolol, bufexamac, indomethacin, phenacetin, and ifosfamide) along the Apies River for the first time in South Africa.

Interestingly, we observed that some of the detected pharmaceuticals are not registered for use as medications in South Africa based on the information extracted on March 26, 2021 from the South African Health Products Regulatory Authority (2021) website. These drugs may have entered the country illegally or come into South Africa with travelers from other countries. Study Site 1 is close to a soccer stadium, and within 1 km from Vilakazi Street, a major tourist attraction area. The main international airport is also located approximately 46 km from the first sampling site. These two factors may explain the occurrence of pharmaceuticals in the Klip River not registered for use as medications in South Africa. Mhuka et al. (2020) have also reported a banned analgesic, phenacetin, along the Apies River. Phenacetin was also detected in the present study. Importantly, these results, which stress a need for stringent regulations, suggest that unregistered pharmaceuticals are in circulation in South Africa. There is a need for more environmental monitoring studies focusing on analysis of pharmaceutical compounds including those not registered in the country.

Metabolites and transformation products. As noted previously, monitoring of pharmaceutical byproducts in the South African environment is limited. In the present study, six pharmaceutical metabolites were detected for the first time in South African surface water (Supporting Information, Table S3). In fact, to the best of our knowledge, ours is the first study to report the occurrence of these metabolites in environmental waters from the African continent. Among the detected metabolites, both demethyl-dextrorphan and dextrorphan originate from an antitussive, dextromethorphan (also detected in the present study), which is not registered by the South African Health Products Regulatory Authority (2021). These two metabolites are formed through the hydrolysis of glucuronide metabolites in wastewater that are excreted via urine (Thurman & Ferrer, 2012). An antiepileptic drug, carbamazepine, has been monitored in South African surface waters, at concentrations reaching $\mu g\text{-}L^{-1}$ levels (Hlengwa & Mahlambi, 2020; Mhuka et al., 2020). Hlengwa and Mahlambi (2020) recently reported a concentration of $3.79\,\mu g\,L^{-1}$, which is the highest reported to date. However, there are no records indicating the monitoring of its metabolites in South African surface waters.

Nor-citalopram, which we detected, is known to escape most WWTP processes. This metabolite has also been reported in treated wastewater from Athens, Greece (Ibáñez et al., 2017).

Although trace amounts of parent compounds have been found in the environment, in a number of cases they have not been detected (Hlengwa & Mahlambi, 2020; Wanda et al., 2017). The results of our study suggest that when the parent drugs are not detected or when their levels are low in the environment, it is quite possible that metabolic reactions and environmental conditions have converted these compounds into metabolites or transformation products (Escher & Fenner, 2011; Hernández et al., 2019). For example, in the present study, carbamazepine was found only in water from study Site 1, whereas its two transformation products, 10-hydroxy-carbamazepine and dihydro-carbamazepine, were detected at study Sites 1–4. Therefore, environmental monitoring of pharmaceuticals should include the corresponding metabolites. This is also important because the concentrations of metabolites can sometimes exceed those of the parent drugs. Jaén-Gil et al. (2019) reported higher concentrations of metoprolol acid in urban wastewaters than those of metoprolol, its parent compound.

Target screening of pharmaceuticals in river water

Method performance for target screening of pharmaceuticals in river water. The validation results for analytical method performance are summarized in Table 1. The linear responses with R^2 exceeding 0.965 (Table 1) were satisfactory for all the analytes except for efavirenz, which had an R^2 value of 0.905. These results agree with those reported in related studies for monitoring pharmaceuticals and personal care products in water (Abdallah et al., 2019; Asghar et al., 2018). Water samples from the Klip River were spiked at 1, 10, and 100 $\mu g\ L^{-1}$ of each pharmaceutical and subjected to SPE prior to chromatographic analysis. As a measure of the accuracy of the analytical method, analyte recoveries were determined for each pharmaceutical by comparing the concentration of each compound obtained after chromatographic analysis of the solid-phase extract (actual concentration) and the spiked concentration (theoretical concentration). The background concentration of each pharmaceutical in the water sample used in the method validation was subtracted from the actual concentration. Acceptable recoveries in the range of 83%–107% were achieved for all the investigated pharmaceuticals. This was an indication that the proposed analytical method for the analysis of targeted pharmaceuticals in river water is highly accurate. Furthermore, the RSD values achieved after triplicate analysis of each spiked water sample were below 20% (except for efavirenz, with an RSD of 22%), which is an indication of acceptable precision. The proposed analytical method was highly sensitive to all the target pharmaceuticals, having attained LOQ values that were below 0.4 $\mu g\ L^{-1}$.

Matrix effects were also evaluated to measure the potential of the environmental matrix to affect the accuracy of the proposed method in the analysis of pharmaceuticals in river water. The presence of nontargeted substances in environmental waters such as natural organic matter, salts, ion-pairing agents, and nontarget contaminants could affect the LC–MS analysis of pharmaceuticals (Rivera-Jaimes et al., 2018). This could be due to matrix components interfering with the electrospray ionization processes, thus leading to signal suppression or signal enhancement and eventually affecting the accuracy as well as the sensitivity of the analytical method (Rivera-Jaimes et al., 2018; Vergeynst & Langenhove, 2014). The results given in Table 1 show the negative values of matrix effects for all analytes. Negative values are indicative of signal suppression, and signal enhancement is represented by positive values (Borecka et al., 2013; Conley et al., 2008). The absence of matrix effects is indicated by a value of 0% (Borecka et al., 2013). In the present study, only five analytes had a signal suppression less than 10% (Table 1). Ibuprofen was the most affected by matrix effects, registering up to 90% signal suppression. Due to the influence of matrix effects, quantitation of all analytes was done using matrix-matched calibration curves.

Occurrence and quantitation of selected pharmaceuticals in the Klip River. Target screening and quantitation of 21 pharmaceuticals in water from sampling sites along the Klip River was also conducted. This was done for those pharmaceuticals for which we had high purity standards available in our laboratories. Based on preliminary research showing their previous detection in the country and their registration status as active ingredients in South African medications, these pharmaceuticals are considered to be most relevant in the South African context. The same 21 pharmaceuticals were also included in the suspect list. As shown by the results presented in Table 2, 9 of the 21 targeted pharmaceuticals were detected in the Klip River. However, levels of three of these pharmaceuticals (carbamazepine, efavirenz, and ketoprofen) were below their quantitation limits of 0.094, 0.050, and 0.018 $\mu g\ L^{-1}$, respectively. The occurrence of these pharmaceuticals in South African surface waters at trace levels has been reported in the literature (Madikizela et al., 2017). Access to carbamazepine and efavirenz is by prescription, which limits their availability, resulting in their low levels in their environment. In a recent study conducted in South African surface waters, carbamazepine concentrations varied from below detection to 3.8 $\mu g\ L^{-1}$ (Hlengwa & Mahlambi, 2020). Similarly, ketoprofen concentrations in South African rivers were found to mostly range from below detection to low $\mu g\ L^{-1}$ levels (Agunbiade & Moodley, 2016; Gumbi et al., 2017; Madikizela et al., 2014). The maximum concentrations recorded for ketoprofen in South African rivers such as the Mbokodweni, the Umgeni, and the Msunduzi Rivers were 2.0 $\mu g\ L^{-1}$ (Madikizela et al., 2014), 0.62 $\mu g\ L^{-1}$ (Gumbi et al., 2017), and 0.44 $\mu g\ L^{-1}$ (Agunbiade & Moodley, 2016), respectively. The highest concentration of the antiretroviral drug, efavirenz, found in the Umgeni River was 0.14 $\mu g\ L^{-1}$ (Rimayi et al., 2018). We suggest that, to improve detection of these compounds in South African surface waters, the use of high sample volumes to achieve high preconcentration factors and better sensitivity of the analytical methods is necessary.

TABLE 1: Performance of the analytical method for targeted pharmaceuticals in the Klip River

Pharmaceutical	Pharmaceutical class	Molecular formula (*m/z*)	Retention time (min)	Linearity (R^2)	Matrix effects (%)	Recovery (%) ± RSD	LOD (μg L^{-1})	LOQ (μg L^{-1})
Acetaminophen	Analgesic	$C_8H_9NO_2$ (152.073)	0.81	0.992	−8	105 ± 8	0.051	0.171
Tramadol	Analgesic	$C_{16}H_{25}NO_2$ (264.197)	18.87	0.998	−28	107 ± 7	0.010	0.032
Carbamazepine	Antiepileptic	$C_{15}H_{12}N_2O$ (237.102)	21.79	0.999	−9	97 ± 18	0.028	0.094
Gabapentin	Antiepileptic	$C_9H_{17}NO_2$ (172.133)	10.97	0.976	−8	99 ± 14	0.073	0.242
Digoxigen	Cardiac glycoside	$C_{23}H_{34}O_5$ (391.248)	26.46	0.992	−12	86 ± 17	0.021	0.070
Efavirenz	Antiretroviral	$C_{14}H_9ClF_3NO_2$ (316.032)	15.27	0.905	−32	87 ± 22	0.015	0.050
Ibuprofen	NSAID	$C_{13}H_{18}O_2$ (205.123)	17.22	0.967	−90	86 ± 5	0.008	0.025
Ketoprofen	NSAID	$C_{18}H_{14}O_3$ (255.102)	21.75	0.998	−40	95 ± 13	0.005	0.018
Oxolinic acid	Antibiotic	$C_{13}H_{11}NO_5$ (262.071)	26.98	0.982	−36	92 ± 6	0.034	0.112
Sarafloxacin	Antibiotic	$C_{20}H_{17}F_2N_3O_3$ (386.131)	19.94	0.973	−37	90 ± 11	0.049	0.162
Lomefloxacin	Antibiotic	$C_{17}H_{19}F_2N_3O_3$ (352.147)	0.86	0.991	−34	98 ± 12	0.047	0.158
Ciprofloxacin	Antibiotic	$C_{17}H_{18}FN_3O_3$ (332.141)	15.49	0.970	−38	86 ± 11	0.019	0.064
Flumequine	Antibiotic	$C_{14}H_{12}FNO_3$ (262.087)	26.98	0.984	−47	96 ± 9	0.034	0.112
Sulfamethazine	Antibiotic	$C_{12}H_{14}N_4O_2S$ (279.091)	10.93	0.984	−19	97 ± 12	0.078	0.261
Sulfadimethoxine	Antibiotic	$C_{12}H_{14}N_4O_4S$ (311.081)	6.60	0.997	−19	83 ± 8	0.069	0.229
Sulfamerazine	Antibiotic	$C_{11}H_{12}N_4O_2S$ (265.075)	11.50	0.987	−15	91 ± 10	0.106	0.353
Sulfamethizole	Antibiotic	$C_9H_{10}N_4O_2S_2$ (271.133)	0.89	0.984	−4	89 ± 5	0.054	0.180
Sulfamethoxazole	Antibiotic	$C_{10}H_{11}N_3O_3S$ (254.058)	0.90	0.993	−18	94 ± 13	0.050	0.167
Trimethoprim	Antibiotic	$C_{14}H_{18}N_4O_3$ (291.145)	0.82	0.999	−17	94 ± 11	0.025	0.082
Thiabendazole	Antifungal	$C_{10}H_7N_3S$ (202.043)	0.74	0.999	−7	94 ± 7	0.038	0.127
Triclocarban	Antiseptic	$C_{13}H_9Cl_3N_2O$ (314.984)	0.80	0.999	−50	94 ± 8	0.106	0.352

RSD = relative standard deviation; LOD = limit of detection; LOQ = limit of quantitation; NSAID = nonsteroidal anti-inflammatory drug.

TABLE 2: Concentrations of targeted pharmaceuticals found in different study sites along the Klip River

Pharmaceutical	Detected concentration (μg L^{-1} ± RSD)					
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Acetaminophen	0.343 ± 0.207	0.367 ± 0.321	0.432 ± 0.016	Nd	Nd	0.350 ± 0.078
Carbamazepine	<LOQ	Nd	Nd	Nd	Nd	Nd
Efavirenz	<LOQ	Nd	<LOQ	Nd	<LOQ	Nd
Ibuprofen	0.113 ± 0.020	Nd	Nd	Nd	Nd	Nd
Ketoprofen	<LOQ	Nd	<LOQ	Nd	Nd	Nd
Oxolinic acid	0.349 ± 0.09	0.355 ± 0.085	Nd	Nd	Nd	Nd
Lomefloxacin	0.386 ± 0.219	0.365 ± 0.050	Nd	Nd	Nd	Nd
Flumequine	0.229 ± 0.106	0.245 ± 0.045	0.257 ± 0.023	0.231 ± 0.018	0.227 ± 0.072	0.235 ± 0.149
Sulfamerazine	<LOQ	0.400 ± 0.008	Nd	Nd	Nd	Nd

RSD = relative standard deviation; Nd = not detected; <LOQ = the pharmaceutical was detected but concentrations were below the quantitation limit.

Flumequine (an antimicrobial agent) was the most ubiquitous pharmaceutical in the Klip River, followed by acetaminophen (an analgesic) as shown in Table 2. Flumequine was detected at all sampling sites (highest concentration: 0.257 μg L^{-1}). Indeed, flumequine concentrations along the Klip River remained almost constant at 0.227–0.257 μg L^{-1}. These concentrations are higher than those reported in surface waters elsewhere (Kim et al., 2018; Tamtam et al., 2008). This pharmaceutical is mainly administered as an additive to food pellets used in veterinary medicine (Dinh et al., 2017).

Acetaminophen was found at four of the six sites (highest concentration: 0.432 μg L^{-1}). The detected levels are comparable to those reported in surface waters from Egypt, where acetaminophen concentrations ranged from 0.14 to 0.95 μg L^{-1} (Abdallah et al., 2019). In two separate Spanish studies, acetaminophen was among the most frequently detected pharmaceuticals at highest concentrations in surface waters, with concentrations up to 0.20 μg L^{-1} (Fonseca et al., 2020) and 1.9 μg L^{-1} (Gracia-lor et al., 2011).

Other pharmaceuticals detected in the present study with concentrations above the method quantitation limits were ibuprofen, oxolinic acid, lomefloxacin, and sulfamerazine. Ibuprofen at a concentration of 0.113 μg L^{-1} was detected at Site 1, which is at the center of Soweto Township. The detection of ibuprofen in these samples was not unexpected because this nonsteroidal anti-inflammatory drug is easily accessible as an over-the-counter medication. Thus, ibuprofen is one of the most consumed medications in South Africa, with its concentration in surface water known to reach 84.6 μg L^{-1} (Matongo et al., 2015). Concentrations of oxolinic acid, lomefloxacin, and sulfamerazine (all antibiotics) reached 0.355, 0.386, and 0.400 μg L^{-1}, respectively. All of them were detected at Sites 1 and 2, at the center of Soweto Township. In fact, these two sampling sites (Sites 1 and 2) contained more pharmaceutical residues than the other sites. This may suggest high usage by Soweto residents, or improper disposal of pharmaceuticals, resulting in surface water contamination.

Approximately 12 of the targeted pharmaceuticals we investigated were not detected in river water samples. More studies should be conducted to confirm this result for the Klip River because most of these pharmaceuticals have been reported in other similar waters on the African continent (Madikizela et al., 2017, 2020).

Risk assessment

The results of the risk assessment analyses are presented in Table 3. It has been suggested that HQ values in the range of 0.1–1 signal a low or negligible risk, whereas values between 1 and 10 indicate a medium risk, and finally, values exceeding 10 indicate a high ecological risk (Rivera-Jaimes et al., 2018). The results of the present study show negligible risk for acetaminophen, ibuprofen, lomefloxacin, and sulfamerazine, as well as low risk to the selected species due to the presence of these pharmaceuticals in surface water. Nevertheless, their presence in the environment may have considerable consequences. Rivera-Jaimes et al. (2018) showed that the presence of ibuprofen and acetaminophen in surface water posed ecotoxicological risks at HQ values of 111 and 2.19, respectively.

A low-to-medium risk can be expected from flumequine, which was found to have an HQ value of 1.285 at study Site 3. Further investigation into the occurrence of flumequine in South Africa is needed, because previous studies conducted elsewhere in the world have reported mainly low risks, with HQ values below 0.20 (Nieto-Juárez et al., 2021; Zounkova et al., 2011). The concentration of 0.355 μg L^{-1} for oxolinic acid,

TABLE 3: Hazard quotients (HQs) calculated from the detected concentration of various pharmaceuticals in the Klip River

Pharmaceutical	MEC (μg L^{-1})	Study site	PNEC (μg L^{-1})	HQ
Acetaminophen	0.432	3	6.92[a]	0.062
Ibuprofen	0.113	1	0.2[b]	0.565
Oxolinic acid	0.355	2	0.0073[c]	48.6
Lomefloxacin	0.386	1	20[d]	0.019
Flumequine	0.257	3	0.2[e]	1.285
Sulfamerazine	0.400	2	0.68[f]	0.588

Data for PNEC values:
[a]*Streptocephalus proboscideus*.
[b]*Oryzias latipes*.
[c]*Vibrio fisheri*.
[d]*Selenastrum capricornutum* (Mutiyar & Mittal, 2014).
[f]*Lemna minor* (Orias & Perrodin, 2013).
[e]Median effect concentration (EC50; Zounkova et al., 2011).
MEC = maximum environmental concentration; PNEC = predicted no-effect concentration.

which corresponds to an HQ value of 48.6, presents a high ecological risk, particularly in the heart of Soweto Township. This HQ value may also be attributed to the lower PNEC value of 0.0073 μg L^{-1} used for oxolinic acid. A previous study conducted in Europe found a lower HQ, representing a negligible risk (Rodriguez-mozaz et al., 2020). We contend that differences in access to water and sanitation, as well as to pharmaceuticals, influenced the exposure risk in the present study. Overall, however, we surmise that the maximum concentration of oxolinic acid has the potential to result in acute toxicity to the flora and fauna of the Klip River. It is of most concern that the Klip River drains into the Vaal River, which serves a population of approximately 12 million people. Therefore, it is imperative to safeguard the Vaal River against pollution.

CONCLUSIONS

We have reported on the screening of 92 compounds belonging to different therapeutic classes of pharmaceuticals, as well as their transformation products, 47 of which were detected in the Klip River, South Africa. Flumequine, at a concentration as high as 0.257 μg L^{-1}, was the most prominent drug in the Klip River. Other pharmaceuticals frequently detected along the river were oxcarbazepine, propyphenazone, ketorolac, clonidine, and atenolol, and some of those detected in the Klip River are not registered for use as medications in South Africa. The results also suggest a high risk of toxicity to aquatic organisms from oxolinic acid, which had a maximum concentration of 0.355 μg L^{-1} and the highest HQ, of 48.6. Based on these results, we recommend further investigations of Klip River waters and those of other South African rivers to evaluate the fate of pharmaceuticals in the environment. It would be prudent for researchers to include the compounds we have identified in future studies of pharmaceuticals and their transformation products in South African waters. Our study is significant because it provides valuable baseline information for environmental risk assessments, as well as policy development for the regulation of human pharmaceuticals in South Africa. Such screening of a wide range of pharmaceuticals can be particularly useful for countries considering the development of environmental risk assessment systems for human pharmaceuticals, in place of approaches focusing on a few or specific pharmaceuticals.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5265.

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