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RESEARCH ARTICLE

Assessment of nonsteroidal anti-inflammatory drugs by ultrasonic-assisted extraction and GC-MS in Mgeni and Msunduzi river sediments, KwaZulu-Natal, South Africa

Bhekumuzi P. Gumbi[1] · **Brenda Moodley**[1] · **Grace Birungi**[2] · **Patrick G. Ndungu**[3]

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Abstract The occurrence of eight pharmaceuticals was monitored during four seasons (spring, summer, autumn, and winter) along a 250-km stretch of the Msunduzi and Mgeni rivers in KwaZulu-Natal, South Africa. This paper describes an optimized method for the determination of nonsteroidal anti-inflammatory drugs (NSAIDs) in sediments. The method combines ultrasonic, centrifuge, and gas chromatography-mass spectrometry for the detection of these drugs in solid samples. Most of the parameters that affect the extraction step were optimized. Solid samples were placed in a centrifuge tube and extracted with ethyl acetate:acetone (1:1, two cycles), followed by clean-up with Oasis HLB cartridge and derivatization with N, O-bis(trimethylsilyl) trifluoroacetamide (BSTFA). Satisfactory recoveries were obtained ranging from 66 to 130%, depending on the analyte. Precision expressed as RSD (%) ($n = 3$) was less than 20% for all analytes. The LODs and LOQs were in the range of 0.024 to 1.90 ng g^{-1} which allowed to be applied in the analysis solid samples in Msunduzi and Mgeni rivers. In the solid samples analyzed, NSAID concentration ranged from not detected to 221 ng g^{-1}.

Keywords GC-MS · NSAIDs · Ultrasonic-assisted extraction · Emerging pollutants · Biosolids and sediments

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✉ Patrick G. Ndungu
pndungu@uj.ac.za

[1] School of Chemistry and Physics, University of KwaZulu-Natal, Private Bag X 54001, Durban 4000, South Africa

[2] Department of Chemistry, Mbarara University of Science and Technology, Private Bag, 1410 Mbarara, Uganda

[3] Department of Applied Chemistry, University of Johannesburg, Doornfontein, Private Bag 17011, Johannesburg 2028, South Africa

Introduction

The persistence of a drug in sediment or soil mostly depends on its photostability, its binding and sorption capability, degradation rate, and leaching in water (Caracciolo et al. 2015; Gavrilescu et al. 2015; Halling-Sørensen et al. 1998). Strong sorbing pharmaceuticals tend to accumulate in soil or sediment. By contrast, highly mobile pharmaceuticals tend to leach into groundwater and get transported with groundwater (Fairbairn et al. 2015; Jindal et al. 2015; K'Oreje et al. 2016). The environmental persistence of some commonly prescribed drugs is longer than 1 year; for instance, the lifetime of clofibric acid in the environment is 21 years (Buser et al. 1998; Saravanan et al. 2014). But for most drugs, it has environmental fate and risk is poorly understood, because of the lack of analytical standards for their metabolites, the high cost of analysis, and the lack of suitable method for routine monitoring (Petrie et al. 2015). Pharmaceutical drugs have been confirmed to exist in the environment to a greater extent than anticipated (Carmona et al. 2014; Shanmugam et al. 2014). There are over 3000 pharmaceuticals substances accepted for use by humans (Jones et al. 2001). The fate of each substance may be very different, depending on their physicochemical properties and the technology of the wastewater treatment plant (WWTP) receiving sewage (Fairbairn et al. 2015; Qin et al. 2015; Sarmah et al. 2006).

Humans (for treatment and prevention of illness) use pharmacologically active substances regularly. In the animal and fish farming industries, there is a greater dependence on drugs, which are used to prevent diseases and as growth promoters or parasite suppressors (Qin et al. 2015). Most of the drugs available on the market are not for a cure, but are used to limit or control symptoms, with some exceptions including antibiotics and antineoplastic. As a result, the consumption of a number of pharmaceuticals can be continuous and over a long period.

These substances can be excreted as partially metabolized or as active metabolites which eventually enter WWTP (Brewer and Lunte 2015). In addition, unused medicine might be a source of sewage contamination. Kuspis and Krenzelok reported that 35% of people in the USA dump medication down the toilet or sink (Kuspis and Krenzelok 1996). At WWTP, pharmaceuticals are partially removed and escape into the environment (Carmona et al. 2014). The continual input of pharmaceuticals through sewage and partition to sediments or sludge may lead to a long-term exposure of aquatic and terrestrial organisms to harmful substances (Caracciolo et al. 2015; Celiz et al. 2009; Halling-Sørensen et al. 1998).

The acute and chronic toxicities of pharmaceuticals in the environment have been studied by researchers and widely acknowledged by the public (Carmona et al. 2014; Morgan et al. 2011). Because pharmaceuticals are designed to cause biological effects, their occurrence in the environment is no longer only a scientific interest but also a public interest (Sarmah et al. 2006; Trouiller et al. 2002). Currently, a decreasing vulture population in Asia, which is associated with the presence of diclofenac in the environment, has created public awareness about the danger these substances pose in untargeted organisms (Ankley et al. 2007; Swan et al. 2006). Diclofenac is an anti-inflammatory drug that enters vulture ecosystem through the ordinary method of disposing the carcass in Asia. European and Asian lawmakers banned some of the pharmaceuticals drugs because when released into the environments causes ecological disturbance and risks to organisms (Kümmerer 2003; Rico et al. 2012). South Africa has also recorded a significant disappearance of bearded vulture around Maloti-Drakensberg mountain range, which has not been linked to a pharmaceutical drug (Simmons and Jenkins 2007). However, the determination of these compounds in the environment specifically sediments is still scarcely documented in South Africa, due to a lack of suitable analytical methods and government awareness about their occurrence in the environment.

Environmental samples contain high amounts of interferences as well as low levels of analyte, which require the development of reliable robust analytical methods (Hao et al. 2007). However, most developed methods are not economical for monitoring large and long rivers with several activities, which may constantly introduce contaminants into the river. Sample preparation is a key step in analytical methodologies, and liquid-liquid extraction (LLE) and soxhlet extraction are common techniques still widely used for the extraction of solid environmental samples (Gakuba et al. 2015; Olutona et al. 2016; Vallecillos et al. 2015). Because of the conditions required for the extraction of analytes and cleanup procedures needed in both techniques, most analytes are lost together with solvent waste generated, leading to low recoveries (Xing et al. 2015). Recently, research efforts to simplify solid-liquid extraction techniques have focused on ultra-sonication of solid samples with an appropriate organic solvent (Chen et al. 2015). Ultra-sonication techniques are then normally followed up with cleanup steps with sorbents such as Oasis HLB, C18, and silica gel cartridges (Lacey et al. 2012). Sonication provides efficient contact between the solid and extractant, resulting in the good recovery of most analytes (Chen et al. 2015; Gomez et al. 2007). In the extraction of pharmaceuticals from sediments, the different physicochemical characteristics of the compounds and the matrix may have a significant influence on the optimization of the extraction parameters.

The selection of extraction condition is critical, especially in the development of multi-drugs residues method. Moreover, pharmaceutical compounds present widely differing polarities and pKas. In this paper, a sensitive multi-residue method is proposed for the simultaneous extraction of eight commonly used pharmaceutical drugs from sediments, with many different polarities and pKas. We opted for these drugs because they belong to nonsteroidal anti-inflammatory drug class of compounds frequently detected in many countries.

The specific objectives were the following:

- Optimization of the extraction from sediment using sonication followed by solid phase extraction for the cleanup.
- Validation of the entire analytical method for eight drugs in sediments.
- Application of the method to the occurrence and seasonal variation of these drugs in sediments and biosolids.

Experimental

Chemicals and reagents

All analytical standards were of high purity purchased from Sigma-Aldrich (South Africa). Thirty-seven percent hydrochloric acid (HCl) of analytical grade was bought from Merck (South Africa). Organic solvents, specifically, acetone, acetonitrile, dichloromethane, methanol, and ethyl acetate, were Chromasolv® gradient grade (99.9%) and were purchased from Sigma-Aldrich. Doubly distilled water was obtained using an Aquation Biby A4000D water purification system in our laboratory bought from Biby Sterlin LTD (UK). All carrier gases, including those used for extraction, were of high purity and were bought from Afrox. Derivatization reagent 99% N, O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylsilyl were bought from Sigma-Aldrich. Unless stated, all chemicals and gases were supplied by South African companies.

Apparatus and instruments

All glassware were washed with phosphate-free soap dynachem and soaked in an acid bath for 24 h. After removal from the acid bath, glassware were then rinsed with dichloromethane, acetone, and methanol and baked at 60 °C for 12 h. Small volumes were measured by micropipette plus kit Dragon lab (China) ranging from 0.5 to 1000 μL. All glass fiber Millipore filter papers were bought from Pall Corporation. Mesh used to sieve sediment samples after grinding was bought from KingTest laboratory. Sediment samples were sonicated with a UMC C20 bought from Ultrasonic Manufacturing Company (South Africa), and the centrifuge used was a Hettich® Rotofix 32 A purchased from Labotec (Pty) Ltd. (South Africa). Extraction vacuum manifold and requisite sorbents were used for extraction. The sorbents used were Waters Oasis HLB 6 cc (60 mg) LP bought from Microsep (Pty) Ltd. (South Africa). Gas chromatography-mass spectrometry (GC-MS) used for detection was a Shimadzu QP2010 SE equipped with an auto-injector (AOC-20i) and autosampler (AOC-20s). GC was equipped with a capillary column (intercap SMS/Sil 0.25 mml. D × 30 M df = 0.25 μm), a product of China. Both glassware and instrument were kept at laboratory temperature. Unless stated, all apparatus and instruments were supplied by South African companies.

Preparation of stock solution

Standard stock solutions of the target compounds (1000 μg mL^{-1}) were prepared by dissolving 50 mg of each compound into 50 mL of methanol, and the solutions were stored at 0 °C. A pipette was used to transfer 10 mL of all target analyte stock solutions into 100 mL volumetric and diluted with acetonitrile to the mark. Similar procedure was used for the preparation of multi-drug working stock solution (100 μg mL^{-1}).

Preparation of spiked solid samples

Solid samples (sediments, sand, or biosolids) were dried at room temperature, homogenized with a porcelain mortar and pestle, and then sieved with a mesh (600 to 100 μm). The solid sample (10 g) was weighed accurately into 50-mL screw-top Teflon centrifuge tubes, mixed with 10 mL of acetonitrile/methanol containing an appropriate amount of analyte mixture (100 μg mL^{-1}) to give final concentration in the solid sample at 100-ng-g^{-1} levels, and left for 24 h in order to evaporate the acetonitrile/methanol. The non-spiked samples were also prepared using a similar procedure in order to correct for absolute recoveries.

Optimization of extraction parameters

To optimize the solvent used for extraction, 10 mL of acetonitrile, methanol, ethyl acetate, acetone, water, and their mixtures (1:1 by volume) were added into separate Teflon centrifuge tubes containing 10-g portion of the spiked and non-spiked solid samples, respectively. The tubes were closed and agitated for 5 min using a vortex system. Then the tubes were sonicated for 25 min, and then, the samples were centrifuged for 25 min. The contents of the tubes were decanted into glass vials, and then, the samples were extracted twice with above mentioned solvents. A mixture of acetone:ethyl acetate (1:1) gave higher recoveries and cleaner extracts compared to other solvents. Sonication and centrifugation parameters were optimized by varying time in 5 min intervals from 0 to 30 min. In all cases, extractions were carried out in triplicate.

Cleanup step

Oasis HLB extraction cartridges were used for cleaning up the sediment extracts. In each case, the organic layer was evaporated to less than 0.5 mL under nitrogen stream and diluted to 200 mL with water adjusted to pH 2 using 1 M sulfuric acid. Each cartridge was preconditioned with 5 mL methanol and 5 mL double-distilled water adjusted to pH 2. Then, a diluted sample extract (pH 2) was passed through the cartridge at a flow rate of approximately 5 to 7 mL min^{-1}, using a vacuum manifold system. The cartridges were then allowed to dry under a stream of nitrogen, and then, the analytes were eluted with 8 mL of acetone:ethyl acetate (1:1).

Derivatization procedure

Derivatization was performed using the optimized derivatization procedure described in detail in our previous paper (Gumbi et al. 2017). Briefly, extracts from SPE were evaporated to dryness under a gentle stream of nitrogen. To the dry residues, 50 μL of a mixture of BSTFA +1% TMCS were added. The vials were closed and mixed for 2 min, and then, the derivatization reaction was performed at 70 °C for 30 min. The derivatives were then cooled to room temperature, and diluted to 0.5 mL with acetonitrile and subjected to GC-MS.

Instrument analysis

The samples were analyzed using a GC-MS (QP2010SE Shimadzu) protocol developed from our earlier work in water analysis (Gumbi et al. 2017). Upon the injection of extracts in the GC-MS system, a capillary column separated the analytes. The initial column oven temperature was 70 °C, injection port temperature was kept at 250 °C, and samples were auto-

injected, 2 μL, in splitless mode. The carrier gas was helium at a constant flow rate of 8.0 mL min^{-1} and 61.5 kPa pressure. The oven temperature was initially kept at 70 °C for 1 min, then ramped at 30 °C min^{-1} to 190 °C, held for 1 min, followed by ramping at 15 °C min^{-1} to 230 °C, held for 3 min, and finally ramping at 30 °C min^{-1} to 270 °C which was held for 1 min. The transfer line was set at 200 °C and the ion source at 200 °C. The electron energy for the filament was set at 70 V. The ion trap detector (ITD) setting was as follows: mass range 50–850 *m*/*z* (full scan only) with a start time of 4 min and end time of 14 min. ITD operated in the selected ion monitoring (SIM) mode to enhance detectability of the selected drugs in sediments and biosolids for the quantification of analytes. All target compounds were eluted under 13 min and fragmentations are presented in Table 1.

Results and discussion

GC-MS

The main advantage of GC-MS is its high selectivity and the ability to fragment pollutants in complex matrices. Thus, this makes the mass spectrometry library very useful in the identification of these compounds in the environment. To confirm the presence of the acidic drugs, two parameters are normally employed, the retention and relative abundance of molecular ion peak of the selected drugs shown in Table 1. These parameters were studied in detail and reported elsewhere (Gumbi et al. 2017). With regard to the presence of the metabolites (salicylic and acetylsalicylic acid) and isomers (meclofenamic acid and diclofenac), retention time and fragmentation pattern were used to separate these analytes as shown in Fig. 1, since they all have similar molecular ion peaks.

Optimization of sample extraction

The effects of the extraction solvent were firstly studied using sediments (10 g) spiked with all studied analytes at 100 ng g^{-1} concentration. Ethyl acetate, methanol, acetone, and dichloromethane were selected for this study because these have been used before, for the extraction of pharmaceuticals from solid samples. Extractions were initially performed using 10 mL of extracting solvent and extraction time of 25 min at room temperature. Recoveries below 50% were obtained for all studied analytes. In order to improve the recoveries, sonication and centrifugation time were optimized. Extracting the sample twice with solvents was found to increase the recoveries.

A mixture of solvents gave high recoveries compared to single solvents. The results revealed that the use of a double 25-min cycle of ultrasonic extraction followed by centrifuge with 10 mL (1:1, ethyl acetate:acetone) each time clearly improved the extraction efficiency with recoveries between 66 and 130%, depending on the analyte. In addition, the sample weight could be increased or decreased by 2 g without observing a decrease in the recoveries and improving the sensitivity of the developed procedure in the environmental sample of complexity. Sample cleanup was performed with Oasis HLB as described in our previous paper (Gumbi et al. 2017).

From the obtained results, it was concluded that acidic drugs can be successfully extracted from the studied samples by ultrasonic-centrifugation-assisted extraction with ethyl acetate:acetone, using two cycles of 25 min with 10 mL volume of extraction solvent each time. This extraction method can be used for sediments and biosolids since it exhibited acceptable recoveries as shown in Fig. 2.

Method validation

After optimization, the developed analytical method was evaluated in terms of linearity, precision, accuracy, and detection limits before it was applied in the determination of pharmaceutical drug residues in sediments and biosolids, as per published analytical guidelines (Thompson et al. 2002). The method was validated according to procedures described in

Table 1 Target compounds retention time, molecular fragmentation, and ion monitored

Target compounds	Retention time (minutes)	Main ion fragment (*m*/*z*)	SIM (*m*/*z*)
Salicylic acid	6.30–6.36	73, 155, 193, 200, 267	135, 267
Acetylsalicylic acid	6.40–6.55	65, 73, 120, 195, 210, 268	120, 195
Ibuprofen	6.95–7.15	73, 117, 160, 191, 263, 278	117, 160
Phenacetin	7.6–7.7	53, 109, 137, 179, 209	109, 179
Acetaminophen	7.99–8.10	109, 181, 223	181, 223
Naproxen	10.58–10.65	73, 141, 185, 243, 287, 302	185, 243
Meclofenamic acid	11.90–11.95	73, 152, 208, 223, 298, 313	223, 313
Diclofenac	12.80–12.85	73,151, 214, 242, 277, 367	214, 367

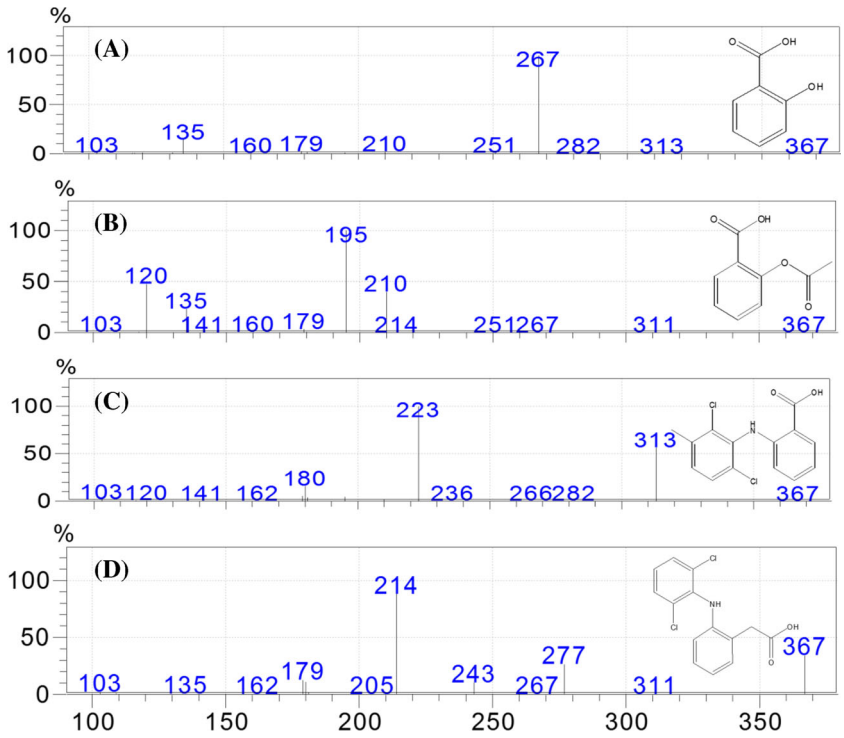


Fig. 1 Mass fragmentation of selected compounds: **a** salicylic acid, **b** acetyl salicylic acid, **c** meclofenamic acid, and **d** diclofenac. These were obtained by injecting extracts from a biosolid solid sample spiked with 300 ng g^{-1} of standard solution

the experimental sections, specifically, the extraction of spiked and non-spiked solid samples.

Linearity

To determine the linearity of the method, a minimum of six different concentrations were analyzed in triplicates over a wide range of concentration from 1 to 500 ng g^{-1}. The linearity of the calibration curves was estimated using a linear mode, least-square regression in the concentration range studied. For all calibration curves, correction coefficient was above 0.990, and the results are presented in Table 2.

Recovery/accuracy and precision

Recoveries in the different matrix were evaluated by extracting fortified samples of sand, sediment, and biosolid with standards solution (5, 50, and 250 ng g^{-1}) containing all target analytes in triplicate under optimum conditions. These fortified samples were allowed to stand for 4 h to allow solvent evaporation under nitrogen; then, they were analyzed following the GC-MS method described in "Instrument analysis" section. Non-spiked blank samples previously analyzed were subtracted. Recoveries for sand ranged between 92 and 105%, between 66 and 120% for sediments, and between 98 and 130% for biosolids (Table 3). The range of the recoveries achieved is similar to that obtained by other authors in sand, sediments, and biosolids (Chen et al. 2015; Kumirska et al. 2015). The precision of the method expressed by relative standard deviation (RSD) of mean recovery values, when triplicate spiked sand, sediment and biosolid samples were analyzed (within and between days) ranged from 1 to 20% for all matrices (Table 3). The RSD for most targeted drugs were lower than 15% as Table 3 shows, which confirms the good repeatability (Thompson et al. 2002).

Limits of detection and quantification

The limits of detection (LOD) were calculated as three times the signal of the background noise obtained in the lowest spiked sample (1 ng g^{-1}) at the retention times of the corresponding analytes, and the limit of quantification (LOQ) were determined considering a value of ten times the background noise (Thompson et al. 2002). These results are summarized in Table 3, LOQs lower than 0.10 ng g^{-1} were obtained, and therefore, the developed method shows very good sensitivity and selectivity for the determination of target drugs in studied matrices. Hence, the method was suitable for the environmental application.

Application of the developed method in Msunduzi and Mgeni rivers

The developed method was used to assess the occurrence and concentrations of pharmaceutical drugs in various

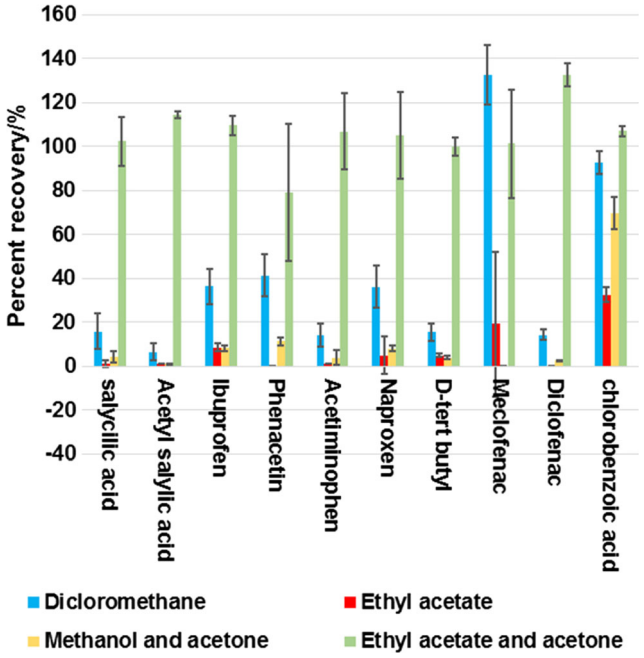


Fig. 2 Selection of extraction solvent for recovery of the indicated analytes (n = 3) using dichloromethane, ethyl acetate, methanol:acetone, and ethyl acetate:acetone. Experimental conditions: sample mass was 10 g, solvent volume was 10 mL (two cycles), analyte concentrations were 100 ng g^{-1}, ultrasonic treatment time was 25 min, centrifuge time used was 25 min, and sample clean-up used an Oasis HLB (60-mg cartridge) conditioned at pH 2

environmental solid samples collected over four seasons along the economically important rivers in KwaZulu-Natal, South Africa. Sampling points are indicated in Fig. 3.

The Mgeni and Msunduzi rivers have five dams and these dams supply over five million people within the Durban and Pietermaritzburg cities. Activities found along these rivers may introduce pharmaceutical drugs; examples of such activities can include hospital effluents, discharges from waste water treatment plants, informal settlements, farming, or animal husbandry. Two WWTPs were also included in this study to determine the occurrence of pharmaceutical in biosolids. These South African WWTPs use conventional processes such as biological and mechanical means to treat wastewater from municipal sewage pipes. The identification of the target compounds in the environmental samples was based on optimized parameters and is presented in Tables 1 and 2, and the quantitative analysis was carried in SIM mode for high sensitivity.

Msunduzi River

Occurrence

All target pharmaceuticals were detected in both sediments and biosolids in the Msunduzi River. In the spring and summer, all drugs were detected in the stream passing by the Edendale Hospital as shown in Table 4. But no drugs were detected in the autumn and winter; this was attributed to the fact that during rainy seasons, hospital drains can overflow and contaminated runoff water might enter close by streams and the contaminant may likely partition to sediments (Jones et al. 2006). Moreover, the concentration of contaminants in sediments depends on the concentration of the contaminants in water (Jones et al. 2001, 2006). Another site where pharmaceuticals were detected frequently was Msunduzi town; this site lies near an informal town in the rural area between Durban and Pietermaritzburg cities with very little proper sanitation. Henley Dam showed the least frequent detection of target analytes; this site is before the Pietermaritzburg city, and thus, this may suggest that residents of Pietermaritzburg Metro are the major contributor of this pharmaceutical in Msunduzi River.

Quantification

High concentrations of the targeted pharmaceuticals were observed in the biosolid samples at the Pietermaritzburg (Darvill) WWTP, and several drugs were quantified at this site, as well as at the Baynes Spruit site. Acetylsalicylic was found to range from not detected to 221 ng g^{-1} in biosolid, while in sediment, it was found between not

Table 2 Molecular ion peaks of the compounds derivatized with BSTFA, their properties, and linearity

Analytes	MM (g mol^{-1})	Derivatives	pKa	R^2	Linearity (ng g^{-1})
Salicylic acid (SA)	138	282	2.97	0.9972	0.1–500
Acetylsalicylic acid (ASA)	180	252	3.5	0.9956	1–500
Ibuprofen (IB)	206	278	4.91	0.9934	0.1–500
Phenacetin (PN)	179	251	2.2	0.9923	5–500
Acetaminophen (AN)	151	223	9.9	0.9952	0.1–250
Naproxen (NP)	230	302	4.22	0.9901	0.1–500
Meclofenamic acid (MA)	296	368	3.8	0.994	10–250
Diclofenac (DC)	296	368	4.15	0.9957	1–300

Table 3 Results for recoveries, limits of detection and quantification, and precision of spiked different matrices

Analyte	Recoveries			LOD (ng g^{-1})			LOQ (ng g^{-1})			Precision (%)
	Sand	Sediment	Biosolid	Sand	Sediment	Biosolid	Sand	Sediment	Biosolid	All matrices
Salicylic acid (SA)	101	100	105	0.055	0.044	0.170	0.183	0.145	0.565	2–13
Acetylsalicylic acid (ASA)	97.0	91.0	102	0.078	0.020	0.090	0.260	0.065	0.030	1–10
Ibuprofen (IB)	102	92.0	102	0.133	0.048	0.024	0.443	0.161	0.079	1–7
Phenacetin (PN)	75.0	120	98.0	0.069	0.077	0.176	0.231	0.258	0.585	1–15
Acetaminophen (AN)	105	92.0	106	0.087	0.017	0.479	0.291	0.058	1.595	5–20
Naproxen (NP)	93.0	66.0	112	0.312	0.084	0.031	1.039	0.280	0.104	7–20
Meclofenamic acid (MA)	93.0	85.0	121	0.482	0.114	0.137	1.606	0.380	0.456	7–15
Diclofenac (DC)	92.0	103	98.0	0.592	0.092	0.546	1.973	0.305	1.820	3–9

detected to 3.42 ng g^{-1}, and these results are presented in Table 4. The partitioning of pharmaceuticals is influenced by humic acid, and pharmaceuticals are likely to sorb to sludge because of its high organic matter content (Jones et al. 2006). The highest concentrations were found in the winter and most target pharmaceuticals were quantified in the summer season.

Mgeni River

Occurrence

Targeted pharmaceuticals were not frequently detected in Midmar Dam, except acetaminophen which was detected in the summer, autumn, and winter as presented in Table 5.

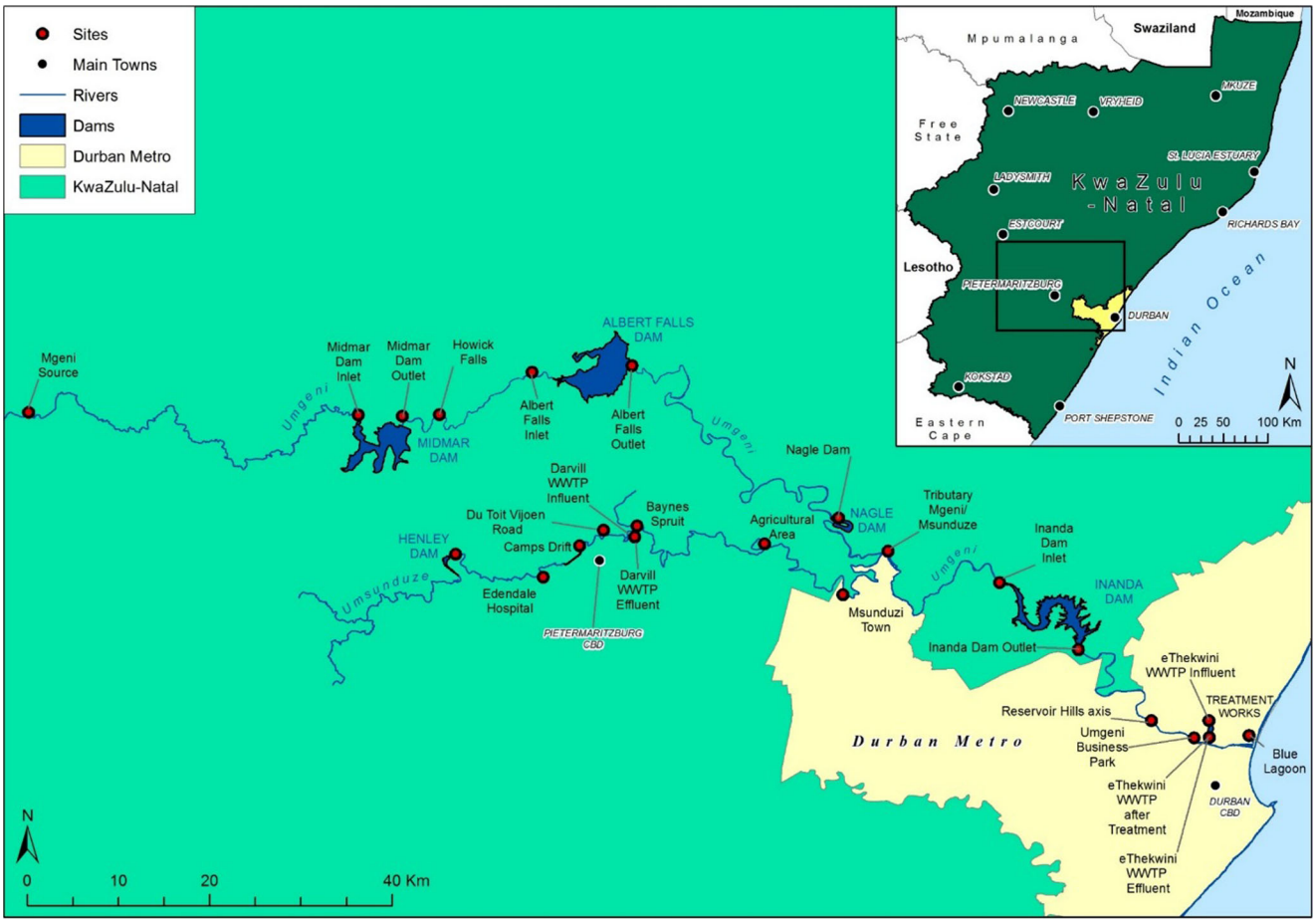


Fig. 3 The selected sampling sites along the Mgeni and Msunduzi rivers (in KwaZulu-Natal province, South Africa) that were used in the current study

Table 4 Detection and quantification of targeted pharmaceutical in Msunduzi River

Season	Analytes	Pharmaceutical concentration along Msunduzi River (ng g^{-1})							
		Henley Dam	Hospital (stream)	Baynes Spruit	Du toit	Camp Drift	Darvill WWTP biosolid	Agriculture (rural area)	Msunduzi town
Spring	SA	ND	3.43 ± 0.28	BQL	ND	ND	6.89 ± 0.71	ND	ND
	ASA	ND	94.5	163 ± 10.0	ND	BQL	124 ± 11.1	BQL	8.00 ± 0.15
	IB	ND	BQL	BQL	ND	ND	1.96 ± 0.50	ND	BQL
	PN	BQL	BQL	ND	ND	ND	0.120 ± 0.030	BQL	BQL
	AN	BQL	BQL	ND	ND	10.12	6.90 ± 0.58	BQL	BQL
	NP	ND	BQL	BQL	ND	ND	2.52 ± 0.350	ND	BQL
	MA	ND	BQL	BQL	ND	ND	ND	ND	BQL
	DC	ND	BQL	BQL	ND	ND	9.53 ± 0.83	ND	BQL
Summer	SA	ND	1.01 ± 0.01	0.240 ± 0.110	ND	0.447 ± 020	48.7 ± 5.01	ND	ND
	ASA	ND	0.322 ± 0.050	BQL	BQL	0.308 ± 0.031	33.2 ± 2.1	BQL	ND
	IB	ND	1.32 ± 0.15	0.505 ± 0.122	0.302 ± 0.012	0.737 ± 0.082	27.20 ± 3.02	0.182 ± 0.231	ND
	PN	ND	0.651 ± 0.12	0.323 ± 0.105	0.302 ± 0.010	0.582 ± 0.003	35.0 ± 5.03	0.321 ± 0.051	0.289 ± 0.010
	AN	0.264 ± 0.005	0.671 ± 0.010	0.334 ± 0.010	0.318 ± 0.010	0.702 ± 0.010	2.15 ± 0.092	0.272 ± 0.010	0.350 ± 0.010
	NP	BQL	BQL	BQL	BQL	BQL	12.9 ± 0.520	BQL	BQL
	MA	BQL	2.83 ± 0.10	1.78 ± 0.01	BQL	1.05 ± 0.01	46.2 ± 6.03	BQL	BQL
	DC	BQL	8.10 ± 0.99	3.77 ± 0.089	BQL	2.17 ± 0.034	181 ± 10	BQL	BQL
Autumn	SA	ND	ND	BQL	ND	BQL	2.84 ± 0.35	ND	BQL
	ASA	ND	ND	BQL	BQL	ND	ND	ND	ND
	IB	ND	ND	BQL	ND	BQL	16.6 ± 0.7	ND	BQL
	PN	ND	ND	ND	ND	BQL	ND	ND	BQL
	AN	BQL	BQL	2.60 ± 0.52	BQL	BQL	3.67 ± 0.19	ND	1.70 ± 0.10
	NP	ND	ND	ND	ND	ND	ND	ND	ND
	MA	ND	ND	ND	ND	ND	ND	ND	ND
	DC	ND	ND	BQL	ND	ND	ND	ND	ND
Winter	SA	ND	ND	ND	ND	ND	ND	ND	BQL
	ASA	ND	ND	ND	ND	11.5 ± 0.28	221 ± 23	ND	ND
	IB	ND	ND	6.87 ± 0.19	ND	BQL	3.08 ± 0.21	BQL	BQL
	PN	ND	ND	ND	ND	BQL	ND	BQL	BQL
	AN	ND	ND	0.303 ± 0.016	0.260 ± 0.060	3.24 ± 0.29	BQL	1.29 ± 0.029	BQL
	NP	ND	ND	BQL	BQL	ND	ND	BQL	ND
	MA	ND	ND	BQL	BQL	ND	54.86 ± 4.15	BQL	ND
	DC	ND	ND	ND	ND	ND	ND	ND	ND

ND not detected, *BQL* detected but below quantification limit

Table 5 Detection and quantification of targeted pharmaceutical in Mgeni River, seasonal concentration variation in Mgeni River ng g^{-1}

Season	Analyte	Sampling sites												
		Midmar inlet	Midmar outlet	Howick Falls	Albert Falls inlet	Albert Falls outlet	Tributary Mgeni/ Msundui	Nagle Dam	Inanda Dam inlet	Inanda Dam outlet	Reservoir hills	Business park	Durban WWTP biosolid	Umgeni estuary
Spring	SA	ND	ND	ND	BQL	ND	2.57	1.01	BQL	BQL	1.36 ± 0.31	ND	BQL	BQL
	ASA	ND	ND	53.7 ± 8.88	ND	ND	191 ± 27	31.7 ± 4.1	200 ± 35	ND	178 ± 14	ND	40.9 ± 3.8	92.7 ± 10.0
	IB	ND	ND	ND	ND	ND	BQL	BQL	ND	BQL	BQL	ND	BQL	2.29 ± 0.597
	PN	ND	ND	ND	BQL	ND	BQL	BQL	ND	BQL	BQL	ND	BQL	ND
	AN	ND	ND	ND	BQL	0.804 ± 0.102	BQL	BQL	17.3 ± 3.41	ND	0.333 ± 0.024	ND	0.392 ± 0.045	ND
	NP	ND	ND	ND	ND	ND	BQL	BQL	ND	BQL	ND	ND	BQL	ND
	MA	ND	ND	ND	ND	ND	BQL	BQL	ND	BQL	BQL	ND	BQL	ND
	DC	ND	ND	ND	ND	ND	BQL	BQL	ND	BQL	BQL	ND	BQL	ND
Summer	SA	ND	ND	ND	0.263 ± 0.010	ND	ND	ND	ND	ND	ND	BQL	55.3 ± 10.8	ND
	ASA	ND	ND	ND	0.184 ± 0.010	ND	0.181 ± 0.012	2.10 ± 0.33	0.373 ± 0.003	BQL	ND	ND	24.4 ± 1.0	ND
	IB	0.239 ± 0.02-0	0.237 ± 0.01-0	0.476 ± 0.08-0	0.3253 ± 0.025	ND	ND	BQL	0.353 ± 0.052	ND	ND	0.318 ± 0.00-4	20.2 ± 0.1	0.100 ± 0.020
	PN	BQL	BQL	BQL	BQL	BQL	BQL	ND	BQL	ND	ND	0.318 ± 0.00-7	40.8103 ± 8.03	0.31 ± 0.0
	AN	0.200 ± 0.01-0	0.27 ± 0.04-0	0.266 ± 0.05	0.476 ± 0.008	BQL	0.342 ± 0.070	ND	0.221 ± 0.010	0.360 ± 0.010	0.376 ± 0.010	0.365 ± 0.01-0	6.00 ± 0.14	0.272 ± 0.070
	NP	ND	ND	ND	ND	ND	ND	ND	15.1 ± 1.0	1.40 ± 0.02	ND	ND	ND	ND
	MA	ND	ND	ND	ND	ND	ND	4.01 ± 0.30	0.983 ± 0.120	ND	ND	ND	46.7 ± 1.0	ND
	DC	ND	ND	ND	BQL	ND	ND	0.912 ± 0.05	3.75 ± 0.01	BQL	BQL	1.07 ± 0.15	206 ± 15	BQL
Autumn	SA	1.92 ± 0.05	BQL	BQL	BQL	BQL	3.99 ± 0.12	BQL	BQL	ND	ND	BQL	ND	BQL
	ASA	ND	ND	ND	ND	ND	ND	BQL	BQL	93.3 ± 5.0	ND	ND	ND	ND
	IB	BQL	BQL	ND	BQL	BQL	2.25 ± 1.17	BQL	BQL	2.52 ± 0.32	ND	BQL	ND	ND
	PN	ND	BQL	ND	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	ND	BQL
	AN	0.130 ± 0.07-0	BQL	ND	0.238 ± 0.060	0.517 ± 0.130	4.60 ± 1.20	BQL	BQL	BQL	BQL	6.18 ± 1.56	ND	1.03 ± 0.15
	NP	BQL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 5 (continued)

Season	Analyte	Sampling sites												
		Midmar inlet	Midmar outlet	Howick Falls	Albert Falls inlet	Albert Falls outlet	Tributary Mgeni/ Msundui	Nagle Dam	Inanda Dam inlet	Inanda Dam outlet	Reservoir hills	Business park	Durban WWTP biosolid	Umgeni estuary
	MA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	DC	ND	ND	ND	ND	ND	ND	ND	BQL	ND	ND	ND	ND	ND
Winter	SA	ND	ND	ND	BQL	BQL	ND	1.15 ± 0.17	ND	ND	ND	ND	1.81 ± 0.29	43.2 ± 5.0
	ASA	ND	ND	ND	BQL	BQL	64.8 ± 14.4	ND	32.9 ± 0.94	97.09 ± 11.5	129 ± 4.81	ND	ND	BQL
	IB	ND	ND	ND	BQL	BQL	0.160 ± 0.010	ND	0.228 ± 0.033	0.471 ± 0.100	0.300 ± 0.072	ND	ND	13.4 ± 0.4
	PN	ND	ND	ND	BQL	BQL	ND	ND	ND	ND	ND	ND	ND	BQL
	AN	8.30 ± 0.40	ND	ND	BQL	BQL	BQL	ND	BQL	9.12 ± 1.13	1.25 ± 0.139	ND	0.670 ± 0.014	BQL
	NP	ND	ND	ND	BQL	BQL	ND	ND	BQL	ND	ND	ND	ND	4.31 ± 0.40
	MA	ND	ND	ND	BQL	BQL	BQL	ND	BQL	BQL	ND	ND	ND	5.40 ± 0.98
	DC	ND	ND	ND	BQL	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 6 Comparison of the results obtained on the Msunduzi and Mgeni rivers with selected examples from the literature

Analytes	Spring (ng g^{-1})	Summer (ng g^{-1})	Autumn (ng g^{-1})	Winter (ng g^{-1})	References
Salicylic acid	ND	-	ND	-	Moreno-González et al. 2015
	BQL–6.89	BQL–55.3	BQL–3.99	BQL–43.2	Proposed method
Acetyl acetylsalicylic	-	-	212–427	-	Agunbiade and Moodley 2016
	BQL–200	BQL–33.2	BQL–93.3	BQL–221	Proposed method
Ibuprofen	ND	-	-	-	Antonic and Heath 2007
	-	-	4.76–9.56	-	Agunbiade and Moodley 2016
	BQL–2.29	ND–20.1	BQL–16.6	BQL–13.4	Proposed method
Phenacetin	-	-	-	-	No study
	BQL–0.109	BQL–40.8	BQL–ND	BQL–ND	Proposed method
Acetaminophen	ND–222	-	-	-	Paiga et al. 2016
	BQL–10.13	BQL–6.00	BQL–6.18	BQL–9.13	Proposed method
Naproxen	ND–60	-	-	-	Antonic and Heath 2007
	ND–LOQ	-	-	-	Paiga et al. 2016
	ND–12.0	4.00–20.0	4.0–10.0	9.00–20.0	Varga et al. 2010
	BQL–2.52	BQL–15.1	BQL–ND	BQL–4.31	Proposed method
Meclofenamic acid	8.00–13.0	35.0–45.0	-	18.0–28.0	Zhou and Broodbank 2014
	BQL–ND	BQL–47.0	BQL–ND	BQL–5.40	Proposed method
Diclofenac	10.0–15.0	55–65	-	15.0–25.0	Zhou and Broodbank 2014
	ND	-	-	-	Antonic and Heath 2007
	BQL–2.65	-	-	-	Paiga et al. 2016
	5.00–12.0	14.0–24.0	ND–22.0	10.0–38.0	Varga et al. 2010
	BQL–9.53	BQL–206	BQL–222	BQL–ND	Agunbiade and Moodley 2016
			BQL–ND		Proposed method

ND not detected, - not considered in study, *BQL* detected but below quantification limit

Midmar Dam is located in a mountainous area away from most sources of contamination near the source of Mgeni River. Most target analytes were detected after the joining of Msunduzi River to Mgeni River, which might suggest that Msunduzi River is also a source of contamination into the Mgeni River. Also, it can be noted that Mgeni River passes through various remote and rural locations, which are sparsely populated, and thus, the possibility of contamination is minimal. However, when the Mgeni River approaches the Durban city, the detection of pharmaceuticals along the various sampling sites was observed. Most target pharmaceuticals were detected in biosolids at Durban WWTPs and Mgeni River estuary. Six drugs were detected in the estuary; this area is used for recreational activities (fishing and boat sports) and vulnerable to contamination through dumping during major events. In addition, Mgeni estuary is located after Durban WWTPs and pharmaceuticals are likely settle to sediment, simply due to the tides experienced at the estuary.

Quantification

Most target pharmaceuticals in Mgeni River were quantified in the summer season; results were presented in Table 5. The site showed high concentration was WWTPs with diclofenac ranging from not detected to 209 ng g^{-1}. Acetylsalicylic acid was quantified in most site in the winter, but its highest concentration was found in tributary point at 178 ng g^{-1} in the spring season and further confirm the contribution of Msunduzi River toward the concentrations of pharmaceutical in Mgeni River.

In general, the winter showed high-level concentration pharmaceutical and this was attributed to the scenario that in the winter the source of water for this river is likely to be recycled water from WWTPs with high concentrations of pharmaceuticals entering river system. Naproxen, meclofenamic acid, and diclofenac were not quantified in the spring and autumn in Mgeni River.

Comparison of the results between rivers and the literature

Considering the number of pharmaceuticals detected per season, most drugs were detected in the summer followed by the winter, then the spring, and finally, the autumn which had the least occurrence of pharmaceuticals in the environment in both rivers as shown in Tables 4 and 5. Salicylic acid and

acetaminophen were detected in all seasons, and acetaminophen was frequently detected in the autumn. More drugs were detected in Msunduzi than in the Mgeni River with exception of Inanda Dam and Mgeni estuary sampling sites.

The concentration of these drugs was found to be higher in biosolids than in sediments. For example, the highest concentration of diclofenac in biosolid was 209 ng g^{-1} whereas in sediment, it was 8.1 ng g^{-1} at the sampling site closest to the hospital. Generally, Msunduzi River exhibited high concentration of the selected pharmaceutical drugs than Mgeni River. Msunduzi River flows through Pietermaritzburg city and informal settlements, while the Mgeni River, along the various inland sampling points, flows through small towns and sparsely populated areas. Toward the ocean, the Mgeni River enters the Durban metropolitan area, and the level of pharmaceutical drugs started to increase and more were detected. Salicylic acid, naproxen, and meclofenamic acid were not frequently detected within the Mgeni River sediments except in the spring, and the salicylic acid concentrations ranged from not detected to 50 ng g^{-1}. Salicylic acid is a metabolite of acetylsalicylic acid and is prone to hydrolysis. Meclofenamic acid is known to be completely degraded by our bodies (Samaras et al. 2010). The high concentration of pharmaceuticals detected in Msunduzi River can be attributed to the scenario that pharmaceutical enters Msunduzi River through main pathways, such as human disposal or direct from human excretion because of proximity to these activities.

However, old and malfunctioning sanitation systems have been reported to cause outbreaks all over the world (Blom 2015). Sanitation is generally inadequate in rural areas and more so in developing countries. The current study has shown that poor sanitation in rural areas, malfunctioning or improper hospital drainage systems, and the conventional treatment methods used by South African WWTPs are contributing to the occurrence of pharmaceuticals in the Mgeni and Msunduzi rivers. WWTPs, in South Africa, mainly focus on stabilizing biological active substances and to some extent heavy metals, while chemical compounds such as emerging contaminants are mainly ignored. New technologies or methods are needed to manage the treatment of waste water in South Africa. The occurrence of pharmaceuticals in the site close to a hospital highlights the need for physical barriers, such as water seals for drainage, which can prevent storm water from entering any nearby temporary or permanent streams during the rainy season. Proper fecal management, the installation of suitable sanitation facilities in rural areas, and informal settlements might also help to prevent the pollution of these rivers at these various points.

The concentration of these drugs in the matrix studied is similar to other studies done elsewhere using different analytical methods (Carmona et al. 2014) and also in comparison with Table 6. Table 6 shows that most studies focus on one or two seasons specifically the winter and summer. There is little information available on the four seasonal variation of the year for better comparison of our proposed method. However, Varga et al. 2010 did on four seasons and their results showed similar patterns with our current work that occurrence of pharmaceutical is frequently in the winter and summer with respect to diclofenac and naproxen drugs studied. Moreover, our proposed method showed better recovery of these drugs in the environment. In the winter, they found high concentrations of these drugs compared to the summer. The spring was the season of choice for most researchers reviewed as depicted in Table 6. The developed method was able to detect targeted drugs in different matrices in the environment and WWTP biosolids.

Conclusion

In this study, we developed an analytical method for the detection and quantification of eight pharmaceutical drugs in sediment and biosolid matrices, based on derivatized GC-MS method. The use of ultrasonic treatment and centrifugation for extraction followed by derivatization provides low LOQs, and therefore, this developed method is useful for the determination of acidic drugs in sediments and biosolids at trace levels. The validation results showed that the method can be used to study pharmaceuticals in environmental solid samples. This method was successfully applied for the determination of eight pharmaceutical drugs in environmental samples collected along Mgeni and Msunduzi rivers, over a year, located in the province of KwaZulu-Natal, South Africa. These pharmaceuticals displayed high levels in biosolids compared to sediments, and Msunduzi River had high concentrations of targeted drugs compared to Mgeni River. Acetylsalicylic and ibuprofen were frequently detected in the various sampling sites selected, while diclofenac was found to be predominantly at WWTP (209 ng g^{-1}) in the summer.

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