Chemosphere xxx (2016) 1–9

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Detection and quantification of acidic drug residues in South African surface water using gas chromatography-mass spectrometry

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HIGHLIGHTS

- Quick easy method for moderately equipped labs in middle income countries.
- Acidic drugs can be detected and quantified in surface and waste water samples.
- Occurrence of acidic drugs in environmental samples in Southern Africa.

ARTICLE INFO

Article history:
Received 13 July 2016
Received in revised form
24 October 2016
Accepted 26 October 2016
Available online xxx

Handling Editor: Klaus Kümmerer

Keywords:
Acidic drugs
Method validation
Derivatization
GC-MS
Surface water
Umgeni river

ABSTRACT

A method was optimized for derivatization, separation, detection and quantification of salicylic acid, acetylsalicylic acid, nalidixic acid, ibuprofen, phenacetin, naproxen, ketoprofen, meclofenamic acid and diclofenac in surface water using gas chromatography-mass spectrometry. For most of the acidic drugs, recovery was in the range 60–110% and the percent standard deviation was below 15% for the entire method, with limits of detection ranging from 0.041 to 1.614 μg L^{-1}. The developed method was applied in the analysis of acidic drugs in Umgeni River system, KwaZulu-Natal South Africa. All of the selected acidic drugs were detected and quantified, their concentration in Umgeni River system ranged from 0.0200 to 68.14 μg L^{-1}.

1. Introduction

In recent years, pharmaceutical compounds have been reported to be present in wastewater effluent, drinking water, rivers and dams in Asia (Saravanan et al., 2014; Shanmugam et al., 2014; Chen et al., 2015; Jindal et al., 2015; Li et al., 2015; Qin et al., 2015), America (Sarmah et al., 2006; Kümmerer, 2009; Caracciolo et al., 2015; Qin et al., 2015), Australia (Sarmah et al., 2006; Watkinson et al., 2009) and Europe (Sarmah et al., 2006; Kümmerer, 2009; Valcárcel et al., 2013; Frederic and Yves, 2014; Net et al., 2015). However, within Africa, there is limited information concerning the occurrence of pharmaceuticals in the environment (Agunbiade and Moodley, 2014; Madikizela et al., 2014; Shanmugam et al., 2014; Matongo et al., 2015). In part, due to the lack of suitable methods that can be used with the limited analytical facilities available.

Pharmaceuticals play an important role in safeguarding people's health (Hotez and Kamath, 2009). However, some, like acidic drugs do not completely degrade in wastewater water treatment plant processes (Lacey et al., 2012), and their presence in the environment can be hazardous towards humans, terrestrial and aquatic organisms, and can disrupt ecosystems (Celiz et al., 2009). For example, Lacey et al. (2012) reported that diclofenac caused vitellogamin in male Japanese medaka fish, and Diniz et al. (2015) reported on the toxicity of pharmaceuticals to zebrafish. Furthermore, some drugs have been found to inhibit seed germination, and crop growth (Caracciolo et al., 2015).

Analytical methods for the quantification and monitoring of

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http://dx.doi.org/10.1016/j.chemosphere.2016.10.105
0045-6535/

pharmaceutical compounds, so as to elucidate their fate and behaviour within the environment are relatively complicated, time consuming and expensive (Iglesias et al., 2012). This is more so in developing countries where state of the art equipment is limited; yet routine analysis of pharmaceuticals at ng L^{-1} levels is of paramount importance (Rozet et al., 2007; Ji et al., 2014; Qiu et al., 2016). Liquid chromatography-mass spectrometry (LC-MS) and gas chromatography (GC-MS) are widely used for environmental analysis of pharmaceuticals (Petrović et al., 2005; Maggioni et al., 2013; Zhao et al., 2014; Cheng et al., 2015). LC-MS is preferred for analysis of polar, non-volatile and acidic analytes, but it can be expensive when used for routine analysis and few organisations can afford such instruments (Kumirska et al., 2015). In contrast, GC-MS is sensitive, selective, cheaper to maintain and is more readily available. Furthermore, GC-MS may be superior to LC-MS for trace analysis of organic compound in matrices of greater complexity, but is limited for non-volatile compounds in aqueous matrices (Hao et al., 2007). Derivatization methods are often used to increase volatility, reduce polarity and enhance detectability of acidic drugs by GC-MS (Lin et al., 2008). The choice of derivatizing reagents is crucial, and more so when developing a routine analytical method (Kumirska et al., 2013).

This work presents optimized methods for the quantification of acidic drugs in water samples using GC-MS. The method developed reduces the retention times 2–3 fold compared to other published methods (Togola and Budzinski, 2007, 2008; Samaras et al., 2010, 2011; Giandomenico et al., 2011; Migowska et al., 2012; Kumirska et al., 2013). In addition, a silylation derivatization was optimized to improve the GC-MS method's application to analysis of acidic drugs in South African waters.

2. Experimental

2.1. Chemicals and reagents

Aspirin, salicylic acid, nalidixic acid, ketoprofen, Ibuprofen, diclofenac, meclofenac, phenacetin, naproxen, 4,4-Di-*tert*-butylbiphenyl, dichlorodimethylsilane, 99% N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylsilyl chloride (TMSC) were of analytical purity and were purchased from Sigma-Aldrich (South Africa). Cinnamic acid was purchased from BDH chemical Ltd (South Africa). Analytical grade hydrochloric acid (HCl, 37%) was bought from Merck (South Africa). Acetone, acetonitrile, dichloromethane, toluene methanol and ethyl acetate were chromasolv® grade (99.9%) purchased from Sigma-Aldrich (South Africa). Double distilled water was obtained using an Aquation Biby A4000D water purification system (Biby Sterlin LTD (UK)). All carrier gases, including those used for extraction, were of high purity (99.999%) and were purchased from Afrox (Durban, South Africa). All chemicals were used without further purification.

2.2. Apparatus, materials, and instruments

All glassware, including amber bottles used for sampling, were washed with phosphate free soap dynachem (South Africa) and soaked in an acid bath for 24 h. After the acid bath, all glassware's were then rinsed with 5% dichloromethylsilane in toluene and methanol respectively, and then heated at 60 °C for 12 h (except the sampling bottles). Small volumes were measured by micropette plus kit Dragon lab (China) ranging from 0.5 to 1000 μL. All glass fibre Millipore filters were bought from pall corporation (South Africa). Extraction manifold and sorbents used for extraction (oasis HLB 20 cc (1 g) LP, sepak-pak plus CN cartridge and tC18 environmental cartridge sepak-pak) were purchased through Microsep from Waters (United State of America (USA)). GC-MS used was a Shimadzu QP2010 SE equipped with auto injector (AOC-20i) and Auto sampler (AOC-20s) (South Africa, Kyoto Japan, respectively). GC was equipped with a capillary column, Crossbond 5% diphenyl/95% dimethyl polysiloxane (intercap SMS/Sil 0.25 mml. D x 30 M df = 0.25 μm) bought from Restek (USA). Both glassware and instrument were kept at laboratory temperature at 20 °C.

2.3. Preparation of stock solutions

Stock solutions of each compound, internal standard 2-chlorobenzoic acid, surrogate standard 3-phenylprop-2-enoic acid and injector standard 4,4-di-*tert*-butylbiphenyl (1000 μg L^{-1}) were prepared in methanol and stored at 4 °C. Working solution of the standards containing 10 000 μg L^{-1} of each target analyte and IS were also prepared and used in optimizing the derivatization procedure. For the corresponding calibration curves, standard solutions (10 μg L^{-1}–5000 μg L^{-1}) were prepared by diluting a working stock solution that contained all of the target compounds in the appropriate amounts of acetonitrile and stored in the dark at 4 °C. All solutions including samples were evaporated to dryness under a gentle stream of nitrogen, and subjected to derivatization and GC-MS analysis in optimal conditions from 1.5 mL vials.

2.4. Sampling

Sampling was carried out (January 2015; July 2016) along Umgeni River situated in Kwa-Zulu Natal province, South Africa. Umgeni River has a 4418 Km^2 catchment, 257 Km long, contains four large dams, and supports over 4 million people. Fig. 1 presents the locations of various sampling sites.

Samples were collected from Midmar dam (1), Albert Falls (2), Henley dams (3), influent (4) and the effluent (5) of the Darvil wastewater treatment plant (WWTP), Nagle dam (6), Inanda Dam (7, 8), inlet (9) and outlet (10) of the Northern Wastewater Treatment Works, and the joining point between wastewater discharge point and Umgeni estuary (11). Some communities and animals source water directly from both Nagle and Inanda dam (Fig. 1). All composite samples (5 × 500 mL) were collected from an area of 2 m^2, into 2.5 L amber bottles, Environmental parameters (pH, temperature, total dissolve solid, salinity, redox, dissolved oxygen and conductivity) were measured on site and samples were preserved at 4 °C. Samples were transported to the laboratory and stored in a freezer for later analysis.

2.5. Sample preparation

Samples were filtered through 0.45 μm glass fibre (Millipore) filters, and then 1 L of each sample was mixed with a solution of cinnamic acid in acetonitrile, as a surrogate standard (final concentration 100 ng L^{-1}), and then acidified to pH ≤ 2 with HCl. Target analytes were extracted using oasis HLB SPE cartridges. Cartridges were conditioned with 8 mL methanol and then 10 mL of distilled water (pH ≤ 2) at flow rates of 3–6 mL min^{-1}. Then 1 L of water sample was passed through the cartridge at a flow rate of 6–8 mL min^{-1} for approximately 2 h. After, cartridges were left under vacuum for 30 min, and then a gentle stream of nitrogen was passed through for 5 min. Analytes were eluted with 8 mL of acetone/ethyl acetate in ratio of 1:1 and then 1 mL of acetonitrile, at a flow rate of 0.5–1 mL min^{-1}. Sample eluate was then mixed with a 10 μL solution of *o*-chlorobenzoic acid in acetonitrile (10 mg L^{-1}), and then dried under a gentle stream of nitrogen. Samples were derivatized by adding 100 μL of BSTFA and 10 μL TMSC, in a vial sealed with Teflon lined septa and held at 70 °C in a water bath for 30 min. The derivatized sample was partially dried under nitrogen and finally re-dissolved in 1 mL acetonitrile.

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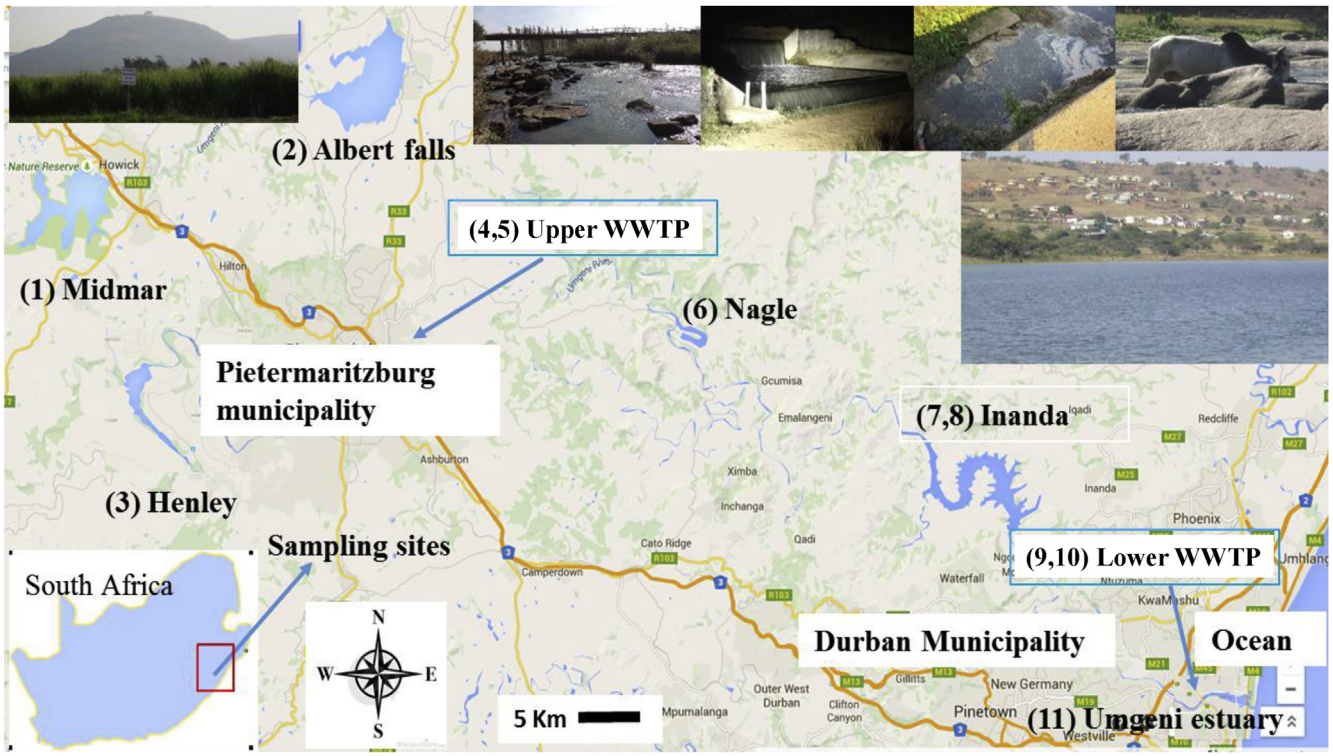

Fig. 1. Location of the sampling sites along the Umgeni river system in Kwazulu-Natal, South Africa.

2.6. GC-MS analysis

Samples were analysed using a GC-MS (QP2010SE Shimadzu) system and separation was performed on a capillary column. The initial column oven temperature was 70 °C, injection port temperature was kept at 250 °C and 2 μL samples were auto-injected 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 kept at 70 °C for 1 min, then ramped at 30 °C min^{-1}–190 °C (held for 1 min), followed by 15 °C min^{-1}–230 °C (held for 3 min) and finally 30 °C min^{-1}–270 °C which was held for 1 min. The transfer line was set at 200 °C and the ion source at 200 °C. Electron energy for the filament was set at 70 V. The ion trap detector (ITD) setting were as follows: mass range 50–850 *m*/*z* (full scan only) with start time of 4 min and end time of 14 min. For quantification of analytes ITD was operated in selected ion monitoring (SIM) mode to enhance detectability of selected drugs in water. Retention times, main fragment ions that were detected, and the two *m*/*z* peaks (one for quantification, and the other for qualitative information) chosen for selected ion monitoring of the analytes are presented in the supplementary material (Table S1).

An independent injector standard 4,4′-Di-tertbutylbiphenyl of 100 μg L^{-1} was prepared and auto-injected into GC-MS 5 times to evaluate stability of the entire instrument. Peak area obtained from each of the 5 injections was constant with a relative standard deviation of less than 2%.

2.7. Validation protocol

To determine LOD, LOQ, inter-day and intra-day precision of the entire method, six independent solutions were prepared in triplicate by spiking river water (Midmar dam, 1 L), final concentration of the solutions ranged from 0.05 to 5 μg L^{-1}. These solutions were analysed with the developed method; extracted, derivatized and detected by GC-MS in SIM mode. Blanks sample were also analysed (non-spiked Midmar sample) and subtracted from spiked sample to determined absolute recoveries. The LOD and LOQ were estimated using Eqs. (1) and (2) respectively (Thompson et al., 2002), (Gustavo González and Ángeles Herrador, 2007):

$$LOD = \frac{3\,s}{b} \quad (1)$$

$$LOQ = \frac{10s}{b} \quad (2)$$

$$Recoveries = \frac{spiked\ peak\ area - blank\ peak\ area}{standard\ peak\ area} \quad (3)$$

Where's' is the standard deviation of ten independent blank samples, and '*b*' is the slope of the calibrations curve. The intra-day and inter-day precision were evaluated at three concentration levels (low: 0.5 μg L^{-1}, medium: 1 μg L^{-1} and high: 5 μg L^{-1}) in triplicate.

3. Results and discussion

3.1. Optimization of derivatization of target compounds

The derivatization protocol was optimized, since it is a crucial step in GC-MS analysis of acidic analytes (Helenkar et al., 2010). A solution of 100 μg L^{-1} containing all target analytes including internal and surrogate standard was prepared and used to optimize the temperature, time, solvent, GC-MS conditions, and concentration of BSTFA. Only one parameter was investigated at a time, while the others were kept constant. Peak areas of the analytes were plotted against parameter, the optimum condition was taken at a point where the peak area was no longer showing significant

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changes.

An independent injector standard 4,4′-Di-tertbutylbiphenyl of 10 μg L^{-1} was prepared and auto-injected into GC-MS 5 times to evaluate stability of the entire instrument. Peak area obtained from each of the 5 injections was constant with a relative standard deviation of less than 2%.

3.1.1. Effect of solvents

Methanol is usually used as a solvent, because most acidic compounds dissolve completely in protic solvents (Shareef et al., 2006). However, upon derivatization in methanol, a labile hydrogen atom on methanol was found to compete with an active hydrogen of the analytes for silylation, which resulted in the incomplete formation of silyl derivatives, and this observation is similar to previous reports (Shareef et al., 2006; Verenitch et al., 2006; Zhou et al., 2007). In some cases methanol formed esters with the analytes, which resulted in additional peaks (impurities) and affected the integration of analytes peaks. Hence methanol was removed completely using nitrogen. Analyte residues were reconstituted into BSTFA + 10% TMSC, because many organic substances are readily soluble in this mixture (Basaglia and Pietrogrande, 2012; Kumirska et al., 2013). After silylation, acetonitrile was then used as a diluent.

3.1.2. Temperature

Heating the solution after addition of silylation reagents is necessary for complete derivatization of analytes (Yu et al., 2007; Schummer et al., 2009; Basaglia and Pietrogrande, 2012). The temperature was varied from 30 °C to 100 °C (10 °C interval) to find optimum conditions. The results obtained (Fig. 2A) showed that there was no significant difference in derivative formation from 70 °C to 100 °C.

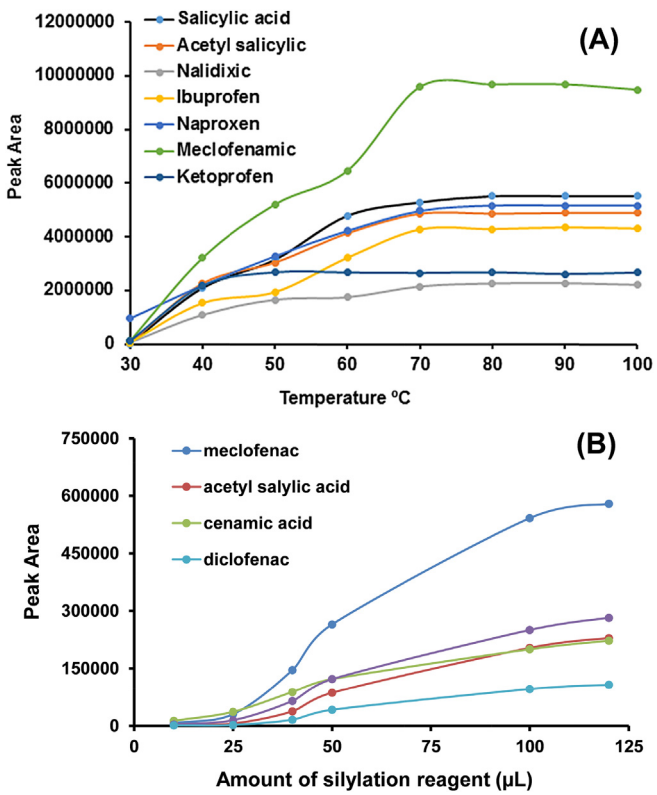

Fig. 2. (A) Temperature optimization for the derivatization of the various drugs using a concentration of 10 mg L^{-1}. (B) Peak area response of the respective derivatized compound, after using varying amounts of the silylation reagent.

3.1.3. Time

The reaction time is an equally important parameter (Yu et al., 2007; Basaglia and Pietrogrande, 2012), and was varied from 10 to 50 min (10 min interval). There was no change observed between 10 and 50 min, but 30 min was chosen for good repeatability.

3.1.4. Optimization of BSTFA concentration

The amount of the derivatizing reagent is important in quantification analysis, and should be in excess (Basaglia and Pietrogrande, 2012). Special attention is needed to prevent silylation reagents from becoming impurities in a chromatogram, which may be mistaken as analyte peaks (Yu et al., 2007; Zhou et al., 2007). The amount needed for derivatization was optimized as shown Fig. 2(B), and there was no significant difference in the silyl derivative formation between 100 μL and 120 μL for all analytes. Thus, 100 μL of BSTFA + 10% TMSC was then used as an optimum amount. For all standards and samples, a gentle stream of nitrogen was used to remove excess silylation reagent followed by dilution with acetonitrile to 1 mL prior to GC-MS analysis.

4. Analytical method development

To obtain maximum detection sensitivity and specificity, base peak chromatogram and distinct peaks of silylated acidic drugs were chosen and used in SIM mode except TMSC $[73]^+$ peak. The $[M+H]^+$ ion was detected for the silylated acetylsalicylic acid, cennamic acid, nalidixic acid, ibuprofen, phenacetin, naproxen, meclofenamic, and diclofenac, but not for ketoprofen. The ion at *m/z* 263 of silylated ibuprofen represented loss of the methyl fragment ion (Fig. 3A). For silylated ketoprofen (Fig. 3B) the base ion was at *m/z* 282, presumably the loss of methyl and the ring opening, silyl derivatives are very strong and likely to be the last to fragment. Also silylated diclofenac (Fig. 3C) has a similar ring opening that results in the *m/z* 308 ion, before TMSC $[73]^+$ fragment. This is a good indication that the silylated acidic compounds are able to pass through the chromatographic column without degradation. The retention times of each of the compounds were also determined. The *m/z* peaks used for quantification are presented in Table S1.

The parameters used in the identification of silylated acidic drugs are provided in the supplementary section (Table S1). Retention times and selected ion were used to develop the method. As predicted, molecular ion $[M]^+$ peak was equal to the theoretical molar mass of the silylated compounds.

Figure S1 displays the chromatogram of the multi-drug standard solution. All silylated acidic drugs were present and their retention times did not change, indicating that there were no interferences among selected acidic dugs. Separation of symmetric peaks was achieved in less than 13 min, and this is shorter than the 25–50 min time frames that were reported previously (Togola and Budzinski, 2008; Migowska et al., 2012; Kumirska et al., 2013). This can be attributed to the different temperature program applied in this developed method.

5. Method validation

The developed method was validated with reference to internationally accepted guidelines for single laboratory validation of method of analysis (Thompson et al., 2002; Trullols Soler, 2006; Peters et al., 2007; Rozet et al., 2007). Linearity, limit of detection (LOD), limit of quantification (LOQ), intra- and inter-day precision, and recoveries were established. All parameters were calculated using the peak area ratio of the target analyte to the internal standard.

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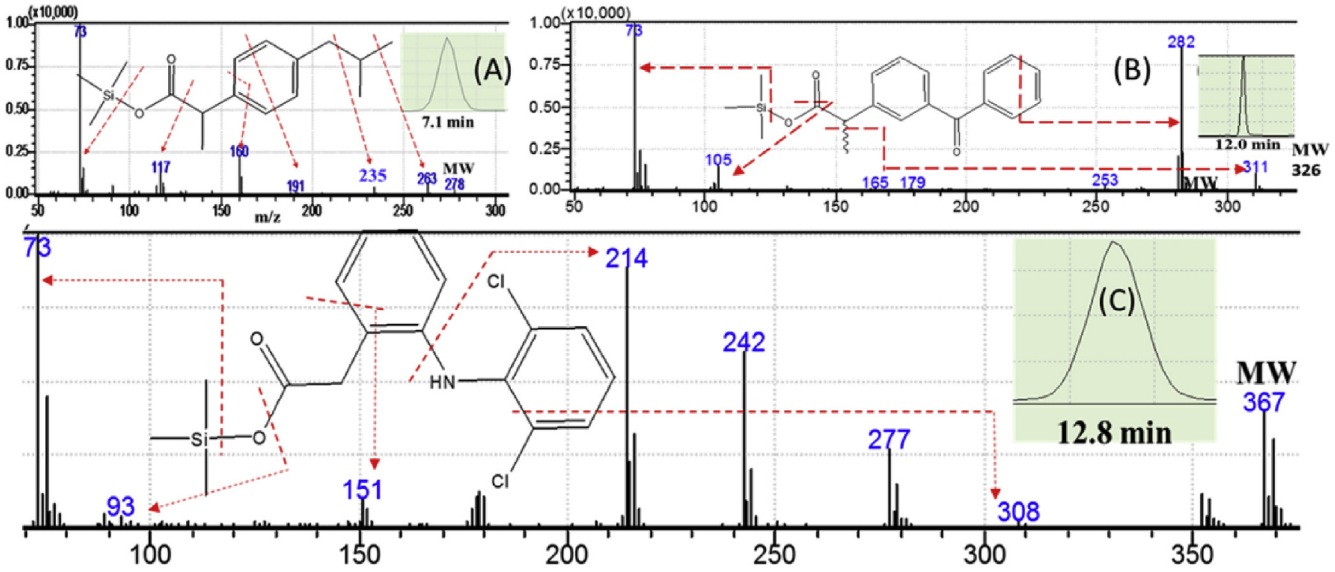

Fig. 3. The profiles of EI mass spectra and tentative fragmentation of the silylated derivatives of (A) Ibuprofen, (B) Ketoprofen and (C) Diclofenac.

5.1. Validation of linearity

To determine the linearity of the method, maximum of 12 different concentrations were analysed in triplicates over a concentration range of 1–8000 μg L^{-1}. Linearity was established within 5–5000 μg L^{-1} for all silylated derivatives, all calibration curves with a minimum of six points and correlation coefficients (R^2) were higher than 0.99 for all analytes (Table S2).

5.2. Limits of detection of the instrument

Lower and upper detection limit of the instrument was determined by taking low and upper concentration standards with percent standard deviation less than 20% (Supplementary Material Table S2). Upper detection limit of the instrument for all target analytes were the same as highest point on the calibration curves.

5.3. Optimization and validation of the solid phase extraction conditions

The solid phase extraction (SPE) step was optimized by studying pH and sorbents independently. In each study 1 L double-distilled water was spiked to provide a final concentration of 1 μg L^{-1}. Each study was done in triplicate, thus, enabling an estimation of repeatability.

5.3.1. Effect of pH

Before extraction, water samples were adjusted to three different pH values 2, 7 and pH 9 with HCl (4 M) or NaOH (2 M). Results obtained showed that pH 2 favoured the extraction of acidic drugs and these results are presented in Fig. 4. The optimum pH of 2, is in agreement with previous work in the literature (Agunbiade and Moodley, 2014; Matongo et al., 2015). Ethyl acetate has been reported to be suitable for extraction of pharmaceuticals where silylation is employed. However, this step was optimized by comparing methanol, acetone/ethyl acetate (1:1) and gradient elution (Acetone 4 mL, ethyl acetate 4 mL and methanol 1 mL) as the solvent systems. A mixture of acetone/ethyl acetate (1:1) was found to have higher recoveries than the other solvents, and thus the results using an optimum pH of 2, and elution with acetone/ethyl acetate are presented in Fig. 4.

5.3.2. Type of cartridge and matrix

Three different types of cartridges; specifically, cyno, environmental C_{18} and oasis HLB, were compared using optimum pH and elution solvents. The results presented in Fig. 4 show the extraction recoveries for the three different cartridges under optimum conditions. Recoveries ranged from 0 to 140% (for cyno) and 0–160% (for environmental C_{18}). Also, chromatograms of blank solutions from the cyno and environmental C_{18} cartridges had some peaks which directly interfered with integration of target analytes peaks. Hence, recoveries of these cartridges were not acceptable according to acceptable guidelines (Thompson et al., 2002). Furthermore, salicylic, nalidixic and naproxen were not recovered by the cyno cartridge. The cyno cartridge is recommended by the manufacturer to be suitable for extraction of pharmaceutical drugs in blood samples. Since it is cheaper than the HLB oasis cartridge, it was tested as an alternative cartridge for environmental samples. However, the optimization procedures used in this study were unable to adapt the cartridge for environmental work. Environmental C_{18} cartridges did show high recoveries for ibuprofen and ketoprofen, and maybe due to interferences. Oasis HLB recoveries ranged from 60 to 120% for all target acidic drugs, and there were no extraneous peaks observed. Therefore it was selected for the extraction of acidic drugs, at pH 2 with flow rate between 4 and 10 mL min^{-1} and eluted with 9 mL (1:1, v/v) mixture of acetone/ethyl acetate. Matrix effect was determined by spiking deionised water, a river water sample (final concentration 1 μg L^{-1}) and a wastewater sample (final concentration 50 μg L^{-1}) followed by extraction with optimum conditions. The obtained results were not significantly different in terms of recoveries and percent standard deviations.

5.4. LOD, LOQ, inter- and intra-day studies

The LOD and LOQ of the target analytes presented in Table 1 were similar to those reported in the literature for GC-MS and LC-MS techniques (Lin et al., 2005; Hao et al., 2007; Helenkar et al., 2010; Lolic et al., 2015) and slightly better when compared to published HPLC methods (Weigel et al., 2004; Hao et al., 2007; Agunbiade and Moodley, 2014; Madikizela et al., 2014; K'Oreje et al., 2016). For all target analytes, precision results had a percent standard deviation lower than 15%, as seen in Table 1.

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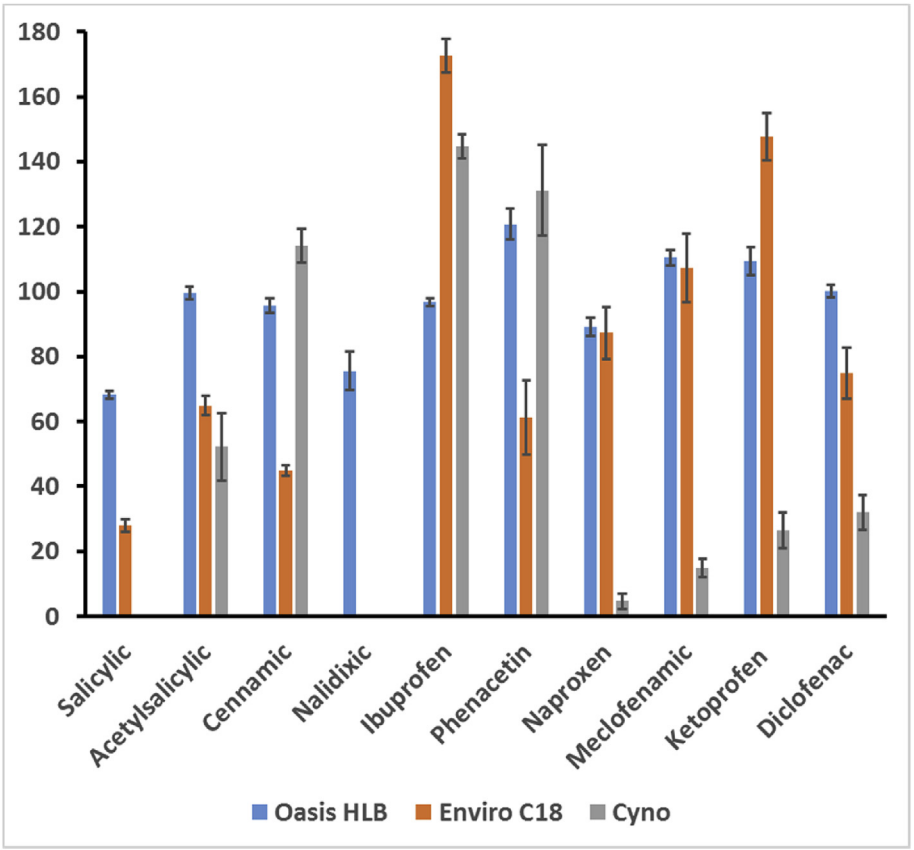

Fig. 4. Extraction recovery percentages obtained with different SPE cartridges using pH 2 and eluted with 9 mL of acetone/ethyl acetate (1:1). River water samples were spiked and the final concentration of the solutions were 1 μg L^{-1}.

Table 1
LOD, LOQ, intra-day precision and inter-day for the entire method.

Acidic analytes	LOD μg L^{-1}	LOQ μg L^{-1}	Intra-day precision %RSD			Inter-day precision %RSD		
			Low	Med	High	Low	med	High
Salicylic acid	0.041	0.135	1.16	3.63	3.25	5.52	11.98	0.06
Acetylsalicylic acid	0.285	0.950	0.08	4.02	2.95	1.11	8.26	0.50
Cennamic acid	0.117	0.390	0.12	3.15	2.07	14.74	9.80	2.42
Nalidixic acid	0.186	0.620	2.19	11.58	20.00	11.23	4.56	1.71
Ibuprofen	0.143	0.477	1.04	3.52	1.35	8.20	10.59	0.97
Phenacetin	0.345	1.151	20.00	20.00	9.75	7.10	2.86	6.10
Naproxen	0.075	0.248	8.66	1.88	4.42	8.21	14.17	4.04
Meclofenamic	0.082	0.272	3.79	1.03	3.25	6.39	8.40	1.76
Ketoprofen	0.130	0.400	6.27	2.80	6.64	6.47	14.47	8.49
Diclofenac	0.484	1.614	6.68	3.63	4.39	6.13	11.73	2.88

Low (0.5 μg L^{-1}), med (1 μg L^{-1}) and high (5 μg L^{-1}).

The results presented in Table 1 showed that the developed method is suitable for the analysis of environmental samples. Furthermore, Table 2 show the environmental concentration levels of pharmaceutical in African countries, these concentration levels reported by other authors are within the detection range of our developed method. South Africa has high concentration of pharmaceuticals compared to other African countries. This may be attributed to the use of conventional wastewater treatment plants in South African municipalities, where treatment partially removes pharmaceutical compounds and this may lead to environmental exposure. Zambia had the least concentration of pharmaceutical when compared to Kenya and Nigeria. This trend is attributed to the number of people connected to municipality sewage pipes, and the ability to recycle and treat the sewage (infrastructure).

6. Analysis of samples from the Umgeni river system.

All selected acidic drugs were detected in the Umgeni River system (Table 3). Meclofenamic was not detected in summer season, perhaps due to its almost complete degradation prior to excretion (Metcalfe et al., 2003); but in winter it was detected in four sites. In South Africa people may buy more medication in winter, and they may dump them to their sinks, eventually reaching the environment through wastewater treatment plants. None of the selected acidic drugs were quantified at Midmar dam in summer season, perhaps due to its location surrounded by rural/farms and far from possible sources of contamination (sewage discharge). But three drugs were quantified in winter, this was attributed to drought currently going on in South Africa that has led to reduced

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Table 2
Environmental concentration levels of pharmaceutical in the African waters.

Pharmaceutical	Extraction/matrix	Country	Instruments	Environmental concentration μg L^{-1}	Reference
Acidic and antibiotic	SPE/water	Kenya	HPLC – MS	ND – 30.0	(K'Oreje et al., 2012)
Acidic and antibiotic	SPE/water	South Africa	HPLC – MS/MS	ND - 117	(Matongo et al., 2015)
Acidic and antibiotic	SPE/water	South Africa	HPLC – DAD	ND – 61.0	(Agunbiade and Moodley, 2014, 2016)
Acidic and antibiotic	SPE/water	Nigerian	HPLC – MS/MS	ND – 8.84	(Olarinmoye et al., 2016)
Acidic/personal care products	SPE/water	South Africa	HPLC – PDA	ND - 221	(Madikizela et al., 2014; Madikizela and Chimuka, 2016)
Stimulants/personal care products	Liquid – liquid/water	Zambia	GC – MS	ND – 1	(Sorensen et al., 2015)
Acidic drugs	SPE/water	South Africa	Derivatization – GC – MS	ND – 68.3	Current study

Table 3
Application of the developed method to real water samples collected in Umgeni system, values are in μg L^{-1}.

Sampling sites	Acid	Acetylsalicylic acid	Nalidixic acid	Ibuprofen	Phenacetin	Naproxen	meclofenamic	Ketoprofen	Diclofenac
Summer season									
Midmar dam	D	nd	nd	nd	nd	nd	nd	nd	nd
Albert falls dam	D	nd	nd	D	nd	nd	nd	nd	nd
Henley dam	nd	nd	nd	nd	D	nd	nd	nd	nd
PMB STP inlet	nd	nd	nd	3.000 ± 0.065	1.95 ± 0.005	D	nd	D	nd
PMB STP outlet	nd	nd	nd	D	D	nd	nd	nd	nd
Nagle dam	nd	nd	nd	D	nd	nd	nd	nd	nd
Inanda dam inlet	D	1.13 ± 0.0658	2.53 ± 0.430	D	2.34 ± 0.204	D	nd	0.620 ± 0.0903	D
Inanda dam outlet	nd	nd	nd	nd	nd	nd	nd	nd	nd
Durban STP inlet	D	D	nd	D	nd	nd	nd	D	nd
Durban STP outlet	D	nd	nd	D	nd	nd	nd	nd	nd
Umgeni estuary	nd	nd	nd	D	8.14 ± 0.474	nd	nd	nd	nd
Winter season									
Midmar dam	nd	D	nd	D		D	0.849 ± 0.091	0.443 ± 0.0001	nd
Albert falls	D	D	nd	D	D	D	nd	nd	nd
Henley dam	nd	D	nd	2.13 ± 0.002	nd	nd	2.38 ± 0.579	9.22 ± 1.81	–
PMB STP inlet	0.823 ± 0.125	D	nd	7.38 ± 0.46	nd	nd	nd	D	D
PMB STP outlet	nd	D	nd	D	nd	nd	nd	nd	nd
Nagle dam	nd	D	nd	0.868 ± 0.064	nd	nd	1.62 ± 0.119	0.443 ± 0.00014	1.01 ± 0.366
Inanda dam inlet	nd	nd	nd	D	68.3 ± 7.00	nd	nd	D	nd
Inanda dam outlet	nd	D	nd	0.524 ± 0.047	nd	nd	nd	D	nd
Durban STP inlet	nd	D	nd	17.6 ± 0.850	D	59.3 ± 2.38	D	D	10.2 ± 0.250
Durban STP outlet	6.60 ± 0.001	nd	nd	D	D	nd	nd	D	nd
Umgeni estuary	nd	nd	nd	2.57 ± 0.410	nd	nd	nd	nd	nd

D – detected but below quantification limit, - below detection limit.
nd - not detected.

dam capacity, hence the observed concentration of pharmaceuticals. Ibuprofen was frequently detected at all sites but was only quantified at Pietermaritzburg (PMB) STP inlet in summer, where it was found at a concentration of 3 μg L^{-1}. In summer, Durban STP inlet also showed a high concentration of Ibuprofen. Phenacetin concentration increased as river flows toward the ocean, i.e. 1.95 μg L^{-1}, 2.35 μg L^{-1}, and 8.14 μg L^{-1} at the PMB STP inlet, Inanda dam and the Umgeni estuary respectively in summer.

The Inanda dam inlet was found to be more polluted compared to the other sites, because of high concentration of phenacetin. It has been shown in other studies that Inanda dam is vulnerable to pollutants other than pharmaceuticals (Papu-Zamxaka et al., 2010). These results maybe of serious concern because the bulk of drinking water consumed by Durban municipality comes from this dam. Most selected drugs were detected in winter as compared to summer. This trend is similar to what has been observed by other researchers in African waters, in Table 2. Furthermore, the environmental concentration levels found in this work, are within the range what has been found elsewhere in African continent as shown in Table 2.

The presence of salicylic acid in the Durban STP outlet sample can be related to presence of the drug acetylsalicylic acid. Approximately, 10% of a low dose of acetylsalicylic (aspirin) is released as salicylic acid in the urine (Huerta et al., 2015). Also, acetylsalicylic acid under STP conditions degrades to salicylic acid and can result in the observed increase at the inlet. Generally the present results are also comparable to world-wide studies (Helenkar et al., 2010; Carmona et al., 2014; Lee et al., 2014; Kumirska et al., 2015). The results showed that the sensitivity of the developed analytical method is sufficient to detect most of the compounds studied and able to quantify them in the environmental samples seasonally.

7. Conclusions

The developed method was able to detect target analytes at the μg L^{-1} level, and all selected acidic drugs were detected in surface water and wastewater samples. Sample preparation and derivatization was fast, simple and analyses of derivatives was achieved in less than 13 min. As demonstrated in method validation for linearity, LOD, LOQ, precision and confirmation of sorbent, the developed method was sensitive and repeatable over established calibration ranges. Winter season had high concentration of pharmaceuticals compared to summer season. The detection of the selected acidic drugs in Inanda dam needs further study, and the findings add to the growing data on pharmaceuticals in the environment on the African continent.

Acknowledgements

Funding for this work was provided by the Water Research Commission of South Africa. BP Gumbi acknowledges the NRF

Please cite this article in press as: Gumbi, B.P., et al., Detection and quantification of acidic drug residues in South African surface water using gas chromatography-mass spectrometry, Chemosphere (2016), http://dx.doi.org/10.1016/j.chemosphere.2016.10.105

South Africa for awarding a bursary.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.10.105.

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Please cite this article in press as: Gumbi, B.P., et al., Detection and quantification of acidic drug residues in South African surface water using gas chromatography-mass spectrometry, Chemosphere (2016), http://dx.doi.org/10.1016/j.chemosphere.2016.10.105