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Journal of Environmental Chemistry and Ecotoxicology

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Research Article

**Determination of pharmaceutical compounds in surface and underground water
by solid phase extraction-liquid chromatography** 20

Olatunde James Olaitan, Chimezie Anyakora, Tolulope Bamiro and
Aminat Temitope Tella

Full Length Research Paper

Determination of pharmaceutical compounds in surface and underground water by solid phase extraction-liquid chromatography

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A solid-phase extraction (SPE) followed by high performance liquid chromatography method was used for extraction and analysis of 4 pharmaceutical residues (Diclofenac, Chloroquine, Paracetamol and Ciprofloxacin HCl) in environmental water samples. This method was successfully applied to environmental water samples from Sango-Ota, a high industrial community in Ogun State, Nigeria, for the determination of the targeted pharmaceutical residues. The four calibration curves obtained were all linear with a correlation coefficient ranging from 0.997-0.999. Over-all average concentrations of the targeted pharmaceutical residues were 17.25, 5.01, 2.57 and 0.86 µg/L respectively. These results confirm the presence of such residues in our environment, hence, emphasising the need to bring to the knowledge of people, the danger of pharmaceuticals residues in the environment and the best method for the disposal of unused pharmaceuticals.

Key words: Solid phase extraction, hplc, pharmaceuticals, groundwater, surface water.

INTRODUCTION

Environmental water has been subjected to various pollutants over the years and for much of the last thirty years, research on the effects of chemical pollution of the environment has focused almost exclusively on conventional "priority" pollutants. There exist, however, a group of chemical compounds that have the potential to cause harm but receive relatively little attention as possible environmental pollutants, especially in

developing nations of the world. These are known as Active Pharmaceutical Substances (Oliver et al., 2003; Halling-Sørensen et al., 1997; Najat et al., 2010).

Active pharmaceutical substances are a large class of chemical substances, used by individuals for personal health or health of livestock. They comprise a diverse collection of over three thousand chemical substances which include Prescription Drugs, Over-The-Counter (OTC) drugs, Veterinary Drugs and the excipients which are used in Pharmaceutical formulation and manufacturing

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(EPA, 2010; Daughton and Ternes, 1999). They are disposed or discharged into the environment on a continual basis through a variety of sources. These include: human activities, pharmaceutical industries, hospitals, illicit drugs use, veterinary drug use (especially antibiotics and steroids) and agribusinesses (USEPA, 2002a). Because pharmaceuticals are purposefully designed to have a biological effect at prescribed doses, the potential exists for unexpected impact at low concentrations, e.g. the antineoplastic drugs (USEPA, 2002b; Daughton and Ternes, 1999; Erickson, 2002).

Some pharmaceuticals are known to affect the function of the endocrine system. Adverse reproductive and developmental effects such as decreased fertility, cancers, and other diseases have been reported in humans, wildlife, and laboratory animals (NRC, 1999). Unknown health effects may also occur when individuals are exposed to a mixture of compounds. As many as 38 different organic wastewater compounds have been found in a single water sample (Kolpin et al., 2002). Countries like Malaysia, Canada, Switzerland, England, USA and Italy all have available data of pharmaceutical residues in various water samples (Najat et al., 2010; Ternes et al., 2002; Golet et al., 2002; Buser et al., 1998; Ashton et al., 2004; Kolpin et al., 2002). As at the time of this research, such data is not available in Nigeria.

In Nigeria, analgesics, antibiotics, antimalarials and antihypertensives are among the most consumed classes of compounds. Synthetic steroids are also said to be frequently prescribed but the total amounts annually sold are relatively low (Akande and Ologe 2007; Oshikoya and Ojo 2007; Nwolisa et al., 2006; Odusanya, 2005). Tauxe-Wuersch et al. (2005) also claim that the pharmaceutical compound found in water is a function of how much of it is consumed and discharged in the environment.

This research is aimed at screening the possible presence of pharmaceuticals in water sourced from Sango-Ota, a high pharmaceutical industrial community in Ogun State, Nigeria. The pharmaceuticals of interest in this study included: Paracetamol, Diclofenac, Ciprofloxacin and Chloroquine. They were investigated to detect their possible presence in water samples. These compounds were chosen because of their high usage rate.

METHODOLOGY

Chemicals

All chemicals and reagents were of analytical grade and of highest purity possible and were obtained from Fischer Scientific UK. They include Methanol HPLC Grade, Acetonitrile HPLC Grade, Trifluoroacetic acid (TFA) HPLC Grade. Standard Paracetamol (BP), Chloroquine (BP), Diclofenac sodium (BP) and Ciprofloxacin (BP) were supplied by Sigma-Aldrich (Steinheim, Germany). Their chemical structures are shown in Figure 1. Solid Phase Extraction Cartridges that is C18, Si-Cyano, C8-(12 ml, 2 g) were purchased from SiliCycle Inc., Quebec Canada. Table 1 gives the properties of these compounds.

Sample collection

Surface and groundwater samples were collected in triplicate from 4 different locations (an irrigation-canal and 3 wells respectively) in a pharmaceutical industrial area of Sango Ota, Ogun State-Nigeria. The sampling which was carried-out on the 14th of January, 2013 using coherent protocols and procedures designed to obtain a representative water sample using standard depth and width integrating techniques (Shelton, 1994). At each site of collection, composite water sample was collected from approximately 4 to 6 vertical profiles through a stream cross section.

This composite sample was subsequently collected into pre-cleaned amber glass-bottles. These amber glass-bottles were placed in coolers, chilled and maintained at 4°C and then shipped to the laboratory for analysis and were all tested for the presence of the four pharmaceutical compounds. Samples were analyzed within 36 h of collection. To minimize contamination of samples, use of personal care products (that is, insect repellents, colognes and perfumes), caffeinated products, pharmaceutical drugs and tobacco were discouraged during sample collection and processing. This method of sample collection was adapted from Kolpin et al. (2004).

Sample preparation

Within the 36 h of sample collection of the 3 batches, the crude water samples were subjected to a pre-filtration process by passing the sample through a 0.45 µm glass fiber filter. This was necessary to remove the solid particles present in them as these particles can alter the pre-concentration process on SPE cartridges prior to high-performance liquid chromatography (HPLC) analysis. The clean and clear filtrates obtained were respectively collected into clean container and then subjected to solid phase extraction.

Solid-phase extraction

Solid-phase extraction (SPE) procedures were employed to extract the target analytes from the aqueous samples. The extraction which was done in batches was achieved using a vacuum SPE manifold. The manifold accommodated 12 cartridges and extraction was carried out simultaneously. Each of the cartridges were preconditioned with 5 ml of Acetonitrile after which 500 ml of each of the filtrates from the water samples were loaded on the cartridge for the solid phase extraction. Each of the cartridges used has a 12 ml capacity with 2g sorbent. Three different SPE cartridges with diverse 2g sorbents (C18, Si-Cyano, and C8) were combined and the one with the highest recovery was used for each pharmaceutical compound respectively. Next, extracted compounds adsorbed to the sorbents were desorbed with acidified methanol. These compounds whose volume ranged between 109 – 118 µL were slowly reduced to 100 µL under flow of N₂ and then brought to 1 ml by adding 900 µL of aqueous high-performance liquid chromatography mobile phase (Cahill et al., 2004).

Preparation of stock solution of standard

For the HPLC analysis for each of the pharmaceutical compounds, a 200 µg/ml concentration stock solution was prepared using their respective standards to obtain a calibration plot. From the stock solution, 20, 10, 5, 2 and 1 µg/ml concentrations were also made (according to the specification by the International Conference for Harmonization that requires a 5-point calibration curve) using serial dilution.

HPLC analysis

Analyses of the four extracted compounds were quantitatively

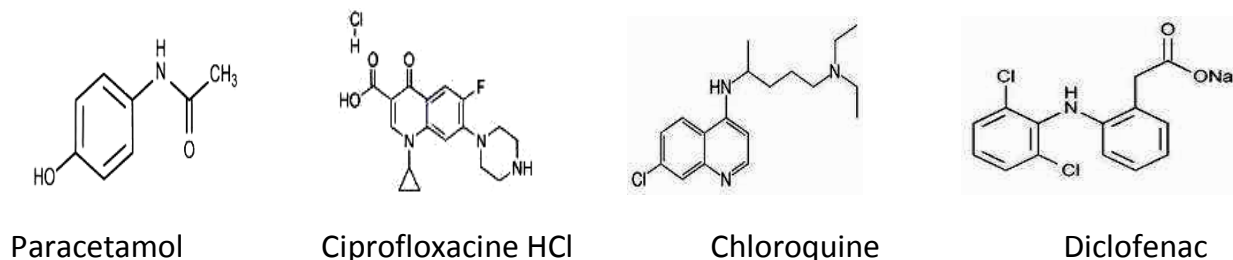


Figure 1. The chemical structures of the studied compounds.

Table 1. Properties of the pharmaceuticals.

Parameter	Paracetamol	Ciprofloxacin hcl	Chloroquine	Diclofenac
IUPAC	N-(4-hydroxyphenyl)-ethanamide	1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid	(RS)-N'-(7-chloroquinolin-4-yl)-N,N-diethylpentane-1,4-diamine	2-(2-(2,6-dichlorophenyl-amino)phenyl)acetic acid
Formula	C ₈ H ₉ NO ₂	C ₁₇ H ₁₈ FN ₃ O ₃	C ₁₈ H ₂₆ ClN ₃	C ₁₄ H ₁₁ Cl ₂ NO ₂
Molar Mass	151.163 g/mol	331.346 g/mol	319.872 g/mol	296.148 g/mol
Class of drug	Analgesic	Antibiotic	Antimalarial	Analgesic
Metabolism	Liver	Hepatic	Liver	Hepatic
Half life	1 – 4 h	4 h	1-2 months	1.2-2 h
H ₂ O Solubility	In water 12.74 mg/ml (20°C)	1.35e+00 g/L	In water 50 mg/ml (20°C)	2.37 mg/L (25°C)
pKa	9.46	8.65	8.5	4.15

Drugbank.com; Granberg and Rasmuson (1999); Daneshfar and Vafafard (2009).

Table 2. Chromatographic conditions of the pharmaceuticals.

Conditions	Paracetamol	Diclofenac	Chloroquine	Ciprofloxacin HCL
Stationary phase	YMC C18 (100 x 4.6 mm, 5 μ)	YMC C18 (100 x 4.6 mm, 5 μ)	YMC C18 (100 x 4.6 mm, 5 μ)	YMC C18 (100 x 4.6 mm, 5 μ)
Mobile phase	0.1% TFA:ACN (90:10)	0.1% TFA:ACN (30:70)	0.1% TFA:ACN (40:60)	0.1% TFA:MeOH (80:20)
Flow rate	1.5 ml/min	2.0 ml/min	2:0 ml/min	1.5 ml/min
UV detector wavelength	248 nm	278 nm	330 nm	278 nm
Injection volume	10 μl	20 μl	20 μl	10 μl
Run time	2 min	2 min	2 min	3 min

carried out using an Agilent 1100 LC System with UV detector. The analytes were separated with their respective chromatographic conditions stated in Table 2.

RESULT AND DISCUSSION

Calibration curves were obtained using standard concentrations of the standards for the four pharmaceutical compounds. The four calibration curves were all linear with a correlation coefficient ranging from 0.997-0.999. All water samples analyzed largely contains

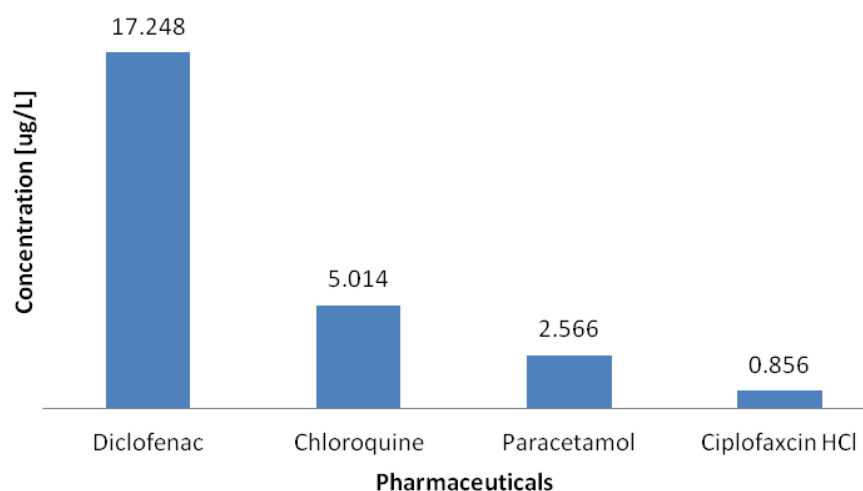
the pharmaceuticals in varying concentrations. Tables 3 to 5 give the summary of the results obtained in this study. The over-all average concentrations of the pharmaceuticals are shown in Figure 2.

In the 1st batch as shown in Table 3, the pharmaceutical with the highest individual concentration was Diclofenac with a concentration of 57.16 μg/L (Sample A) and the least was Paracetamol with a concentration of 0.52 μg/L (Sample C). There was no trace of Paracetamol in samples A, B and C. Chloroquine was also not found in sample A. Diclofenac was not found in sample C and D. Also there was no trace OF

Table 3. 1ST batch of water collected.

Pharmaceuticals	Concentration in water samples ($\mu\text{g/L}$)			
	Sample A	Sample B	Sample C	Sample D
Paracetamol	Not detected	Not detected	Not detected	0.69
Chloroquine	Not detected	10.87	2.05	2.12
Diclofenac	57.16	7.47	Not detected	Not detected
Ciprofloxacin HCL	Not detected	1.31	0.52	Not detected

Sample A: Surface water from an irrigation canal, **Sample B:** Underground water from the 1st well, **Sample C:** Underground water from the 2nd well, **Sample D:** Underground water from the 3rd well.

**Figure 2.** Over-all average concentration of each pharmaceutical in the samples**Table 4.** 2ND batch of water collected.

Pharmaceuticals	Concentration in water samples ($\mu\text{g/L}$)			
	Sample A	Sample B	Sample C	Sample D
Paracetamol	1.52	1.00	4.00	0.54
Chloroquine	0.11	8.24	5.60	2.86
Diclofenac	25.20	7.47	11.24	0.154
Ciprofloxacin HCL	0.22	1.44	1.24	0.402

Sample A: Surface water from an irrigation canal, **Sample B:** Underground water from the 1st well, **Sample C:** Underground water from the 2nd well, **Sample D:** Underground water from the 3rd well.

Ciprofloxacin HCl in samples A and D. The average concentrations of Paracetamol, Chloroquine, Diclofenac and Ciprofloxacin HCl in this 1st batch were 0.692, 5.014, 32.316 and 0.916 $\mu\text{g/L}$ respectively.

A similar trend was seen in the 2nd batch of the samples as shown in Table 4, where Diclofenac had the highest average value of 11.01 $\mu\text{g/L}$. The average values of other pharmaceuticals were 4.238, 1.766 and 0.824 $\mu\text{g/L}$ for Chloroquine, Paracetamol and Ciprofloxacin HCl respectively. But the pharmaceutical with the individual

highest and lowest concentration was Diclofenac (25.2 $\mu\text{g/L}$) and Chloroquine (0.112 $\mu\text{g/L}$) as they were both found in sample A.

In the 3rd batch of samples collected, Diclofenac was again found to be the highest in concentration. It had the highest average concentration of 8.424 $\mu\text{g/L}$ (Table 5). The average concentrations of other pharmaceuticals were 5.24, 4.302 and 0.83 $\mu\text{g/L}$ for Paracetamol, Chloroquine and Ciprofloxacin respectively. Diclofenac in sample A had the highest concentration of 20.1 $\mu\text{g/L}$

Table 5. 3RD batch of water collected.

Pharmaceuticals	Concentration in water samples ($\mu\text{g/L}$)			
	Sample A	Sample B	Sample C	Sample D
Paracetamol	1.10	0.102	1.34	18.42
Chloroquine	Not detected	5.08	4.13	8.08
Diclofenac	20.10	13.48	Not detected	0.118
Ciprofloxacin HCL	0.122	1.62	1.58	Not detected

Sample A: Surface water from an irrigation canal, **Sample B:** Underground water from the 1st well, **Sample C:** Underground water from the 2nd well, **Sample D:** Underground water from the 3rd well.

while Paracetamol (Sample B) recorded the lowest concentration of 0.102 $\mu\text{g/L}$. There was no trace of chloroquine in sample A, Diclofenac in sample C and Ciprofloxacin HCl in sample D.

An over-all average concentration of each of the pharmaceutical compounds tested in all three batches collected showed that Diclofenac had the highest concentration of 17.248 $\mu\text{g/L}$. The concentrations of other pharmaceuticals were 5.014 $\mu\text{g/L}$ (Chloroquine), 2.566 $\mu\text{g/L}$ (Paracetamol), 0.856 $\mu\text{g/L}$ (Ciprofloxacin HCl). This is further depicted in Figure 2. There are no available data of environmental pharmaceutical compounds in Nigeria that the result obtained can be related to, as at when this research was conducted. But in a similar previous research by Kolpin et al. (2002), most concentrations recorded exceeded 1 $\mu\text{g/ml}$. However, recent research has documented effects on freshwater algae at concentrations similar to that found in this study (Wilson et al., 2003). Even environmental concentrations of pharmaceutical compounds that are used for medicinal purposes could potentially have injurious effects on non-target organisms (Seiler, 2002).

In a risk assessment test conducted by Tauxe-Wuersch et al. (2005) it is assumed that diclofenac or its degradation products are less or as toxic as the parent substance itself. This assumption was proved to be wrong by Schmitt-Jansen et al. (2006), who found a 5-6 times increase in toxicity of a degradation product compared to diclofenac itself. Fish exposed to diclofenac displayed significantly reduced haematocrit levels after 7 and 14 days of exposure. After 21 days, trout were examined for histopathological alterations, whereby diclofenac exposure resulted in increased monocyte infiltration in the liver, telangiectasis in gills, and the occurrence of interstitial hyaline droplets, interstitial proteinaceous fluid and mild tubular necrosis in trunk kidney. Also, continuous presence of Paracetamol in water can contribute to the damage of the liver of aquatic organism.

The potential presence of Ciprofloxacin and other antibiotics for instance in drinking water sources is of major concern due to the health effects of chronic low-level exposure over a lifetime if they persist in consumers' drinking water. This drug causes unpleasant

odors and skin disorders, and may cause microbial resistance among pathogenic organisms or the death of microorganisms which are effective in wastewater treatment (Budyanto et al., 2008).

The resistant bacteria may also cause diseases that cannot be treated by conventional antibiotics (Andersons, 2003). For these reasons, antibiotic contamination of drinking water needs to be eliminated or minimised. The resistance of *Plasmodium falciparum* parasite to Chloroquine, the cheapest and most widely available antimalaria medicine could also be attributed to its continual presence in drinkable water as Chloroquine has already lost its effectiveness in most parts of Africa.

In addition, study has shown that selected chemical combinations can exhibit additive or synergistic toxic effects (Thorpe et al., 2001) even when individual chemicals are present at concentrations below their NOEC (no-observed-effects concentration) values (Silva et al., 2002) and for compounds with different modes of action (Porter et al., 1999).

The results from this research provide evidence that supports the case that pharmaceutical compounds do indeed exist in the environment. But what is not explicitly known, however, is if these chemicals and their metabolites can elicit cumulative physiologic effects, at low concentrations at which they are observed, on human and other organisms living in Sango community, even as previous researches have confirmed that Pharmaceuticals in water leads to aqua-toxicity which has been related to quite a whole lots of health challenges which include drug resistance, allergic effects, disruption of the endocrine system, mutagenic and carcinogenic conditions (Jorgensen and Halling-Sorensen, 2000; Sorsa et al., 1985; Ehlhardt et al., 1988; Giuliani et al., 1996; Sheryl, 2004).

While a substantial pharmacological and clinical testing was obtained for pharmaceutical substances, information on the ecotoxicity of these biologically active substances is generally limited. Acute toxicity values were in the mg/l range for most of the pharmaceuticals detected in the environment (Halling-Sorensen et al., 1998; Webb, 2001). Reports however, has it that concentration levels in surface water for example are at least three orders of magnitude below the mg/L levels which cause acute

toxicity. Even at that, it is believed that a cumulative effect of these substances could have long-term effect. For example, the development of antibiotics resistance is in recent times favoured by pollution or concentrations of antibiotics in waters or sediments which had led to the increased design and use of antibiotics during the last five decades (Kümmerer and Henninger, 2003; Leff et al., 1993; Attrassi et al., 1993; Jorgensen and Halling-Sorensen, 2000).

Nevertheless, according to Kümmerer (2004), there is insufficient information available to reach a final conclusion on the impact of antibiotics on bacterial populations in the environment.

Conclusion

The findings from this research further supports observations and conclusions of a number of previous studies outside Nigerian borders that suggests pharmaceutical compounds are present in ground and surface water (Godfrey et al., 2007; Godfrey and Woessner, 2004; Szabo et al., 2004; Verstraeten et al., 2002). So far there is very limited data available in Nigeria on the level of pharmaceutical residues in ground and surface water, and this poses adverse threat on the environment as so many are ignorant of their presence in the water system. In an attempt to preserve the ecosystem in this part of the world, there is a need to enforce modern treatment plants for pharmaceutical waste water before discharge. It also calls for a need to enlighten people on how best to dispose unused pharmaceuticals in our homes, healthcare centers and other related places.


Conflict of Interests

The authors have not declared any conflict of interests.

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