PHARMACEUTICALS AND PERSONAL CARE PRODUCTS (PPCPs) AS CONTAMINANTS IN FRESHWATER AQUATIC ENVIRONMENT

By

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MSc, BSc (Hons)

A thesis submitted to the University of Birmingham

for the degree of

DOCTOR OF PHILOSOPHY (Ph.D.)

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August 2019

Acknowledgement

I would like to thank God for giving me the strength that saw me through this program.

Many thanks to my parents; Chief Dr Bestman Paul and Lady Beatrice Anekwe for their endless love, advice, support and sponsorship. Thanks to my siblings most especially Dr Uche Osadolor for her guidance and encouragement. Thanks to Dr Osahon Osadolor for your incentive. This Ph.D. journey could not have come to completion without your prayers and love. I love and appreciate you all.

I am very grateful to my lead supervisor Professor Stuart Harrad for all his support, supervision and invaluable advice throughout this program. Thank you for the exposure and opportunity you gave me to be a part of INTERWASTE PROGRAM during my secondment in Australia. I would never forget the assistance you rendered to me when I had financial difficulty. I honestly appreciate and admire you prof.

My sincere thanks go to Dr Mohamed Abdallah, my co-supervisor. What would I have done without you? Your patience, understanding and encouragement is immeasurable. You have impacted so much knowledge to me and for that I will always be grateful. Indeed, you are a genius.

I would like to thank most especially Dr Daniel Drage, Dr Nuria Ortuno Garcia and Dr Salim Alam for their immense contribution, patience and time spent in answering all my questions. Many thanks to Dr Khanh Hoang Nguyen for your help and support during my method development.

Thanks to all my friends and colleagues in the Public Health Building Affiong Asuquo, Misbah Alghamdi, Chuanzi Gao, Sally Gladwin, Simeon Onoja, Muideen Gbadamosi, Abdalkarim Dawood, Dr William Stubbings, Dr Tuan Vu, Dr Christopher Stark, Nicholas Davidson, Ify Ugbomeh, Alfred Bockarie and Andrew Tongue. This experience would have been way less exhilarating and memorable without you guys.

A very big thank you to professor Jochen Mueller, Dr Jake O'Brien and the amazing team at Queensland Alliance for Environmental Health Sciences at the University of Queensland, Brisbane, Australia. You guys made me feel at home during my secondment. I would also like to say thank you to Dr Temilola Oluseyi and Dr Oluwatoyin Fatunsin for their assistance and friendship during my research visit to University of Lagos, Nigeria. A special thanks go to Emmanuel Ugochukwu Nwoke for some fruitful advice and motivation.

Abstract

The freshwater aquatic environment is under significant pressure via anthropogenic contamination by a variety of emerging contaminants such as pharmaceuticals and personal care products (PPCPs). Knowledge of their presence and potential detrimental effects has increasingly been reported across the globe in the last decade. Wastewater treatment plants have been identified as a major point source of these chemicals to the aquatic environment. Therefore, the main purpose of this thesis is to investigate the fate, occurrence and behaviour of PPCPs in freshwater aquatic environment in developed (United Kingdom and Australia) and developing (Egypt and Nigeria) countries.

Chapter 1 of this thesis contains a detailed introduction/literature review about environmental contamination by PPCPs. This is followed by a description of an analytical method developed for the determination of 30 PPCPs, using ultra high-performance liquid chromatography (UHPLC) coupled to Q-Exactive Orbitrap mass spectrometry in chapter 2. In chapter 3, PPCPs are shown to be ubiquitously detected in Egyptian wastewater and surface water, with higher concentrations found in hospital effluent samples for instance, acetaminophen ranging 980 – 16000 ng/L. In chapter 4, investigation of the occurrence, seasonal variation of and human exposure to PPCPs in surface water, groundwater (boreholes and wells), and drinking water (sachet and bottled) in Lagos State, Nigeria reveals the presence of PPCPs in Lagos State waterways including drinking water in dry (summer) and rainy (winter) season samples. Human exposure to PPCPs via drinking borehole, sachet and bottled water was assessed and results showed 81, 14 and 3 ng/kgBW/day respectively.

In chapter 5, the seasonal and spatial variation of PPCPs was investigated in canal and river water samples from the UK. Results displayed ∑30PPCPs concentration in the canals to be relatively higher in spring than summer season, while in the rivers ∑30PPCPs concentration was higher in summer than spring season. PPCPs concentrations were higher in the rivers due to effluent discharge than the canals. Finally, in chapter 6, per capita release of PPCPs was estimated using effluent samples collected from 66 wastewater treatment plants (WWTPs) on Australian census day. PPCPs consumption was estimated and the result showed average PPCPs consumption to range between 650-4400000 mg/day/1000 people.

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Abbreviations

AGC	Automated gain control
AU	Arbitrary unit
ABS	Australian Bureau of Statistics
ANOVA	Analysis of Variance
BFR	Biofilm reactor
BAS	Batch experiment with activated sludge
BW	Body weight
CA-HA	Carboxyhyratropic acid
CE	Collision energy
CXP	Collision cell exit potential
DES	Diethylstilbesterol
DEET	N, N-diethyltoluamide
DF	Detection frequency
DI	Direct injection
DP	Declustering potential
EIC	Extracted ion chromatograms
EDs	Endocrine disruptors
EP	Entering potential
EU	European Union
ESI	Electrospray ionisation
FWHM	Full Width at Half Maximum
GAC	Granular activated carbon
HCL	Hydrochloric acid
HESI	Heated electrospray ionisation
HDPE	High-density polyethylene

HLB	Hydrophilic-Lipophilic Balance
HPLC-MS/MS	High Performance Liquid Chromatography Tandem Mass Spectrometry
HRMS	High Resolution Mass Spectrometry
HSD	Tukey's honestly significant difference
IDL	Instrument detection limit
IT	Injection time
IS	Internal standard
LOD	Limit of detection
LOQ	Limit of quantification
MCX	Mixed-mode Cation- exchange
MBR	Membrane bioreactor
MeOH	Methanol
MQL	Method quantification limits
Na_2EDTA	Disodium ethylenediaminetetraacetate dehydrate
NF	Nano filtration
NSAIDs	Non-steroidal anti-inflammatory drugs
OH-Ibu	Hydroxyibuprofen
PCBs	Polychlorinated biphenyl
PCPs	Personal Care Products
PEC	Predicted Effect Concentration
PBT	Persistence, bioaccumulation and toxicity
PFCs	Perfluorinated compounds
PBDEs	Polybrominated diphenyl ethers
PPCPs	Pharmaceuticals and Personal Care Products
QC	Quality control
Q1	Precursor ion

Q3	Product ion
R^2	Linear coefficient
RO	Reverse osmosis
RSD	Relative standard deviation
RT	Retention time
RRT	Relative retention time
SEPA	Scottish Environmental Protection Agency
SPE	Solid Phase Extraction
S:N	Signal to Noise
STPs	Sewage treatment plants
TBBPA	Tetrabromobisphenol A
UK	United Kingdom
UPLC	Ultra Performance Liquid Chromatography
USEPA	United States Environmental Protection Agency
WWTPs	Wastewater treatment plants
WFD	Water Framework Directive
WHO	World Health Organization

List of Publications

EBELE, A. J., ABOU-ELWAFA ABDALLAH, M. & HARRAD, S. 2017. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerging Contaminants,* 3**,** 1-16

ABOU-ELWAFA ABDALLAH, M., NGUYEN, K.-H., EBELE, A. J., ATIA, N. N., ALI, H. R. H. & HARRAD, S. 2018. A single run, rapid polarity switching method for determination of 30 pharmaceuticals and personal care products in waste water using Q-Exactive Orbitrap high resolution accurate mass spectrometry. *Journal of Chromatography A*

EBELE, A. J., OLUSEYI, T., DRAGE, D. S., ABOU-ELWAFA ABDALLAH, M. & HARRAD, S. 2020. Occurrence, seasonal variation and human exposure to pharmaceuticals and personal care products in surface water, groundwater and drinking water in Nigeria. *Emerging Contaminants,* 6**,** 124-132

CHAPTER I

Introduction

This chapter contains material taken verbatim from the following peer-reviewed publication: "J. E. Anekwe, M. A.-E. Abdallah, S. Harrad "Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment", *Emerging Contaminants*, 1-16 (2017)."

1.1. Rationale of the Thesis

Freshwater resources on a global level are under increasing pressure in order to satisfy the needs of a growing human population. One way in which this pressure manifests itself is in the potential for contamination of freshwater by "emerging contaminants" such as pharmaceuticals and personal care products (PPCPs). The presence of PPCPs in freshwater has potential significant adverse effects on aquatic organisms as well as humans. Many PPCPs have been demonstrated to persist in the environment even after undergoing water treatment processes, therefore, raising concerns about possible bioaccumulation in aquatic organisms. It is thus crucial that we understand the fate, occurrence and behaviour of some commonly/continuously used PPCPs found in the freshwater aquatic environment.

1.2. PPCPs in freshwater aquatic environment

Pharmaceuticals are defined as prescription, over the counter and veterinary therapeutic drugs used to prevent or treat human and animal diseases, while personal care products (PCPs) are used mainly to improve the quality of daily life (Boxall et al., 2012). Over the past few years, there has been increasing awareness of the presence of PPCPs in various compartments of the aquatic environment (e.g. water, sediments and biota) at concentrations capable of causing detrimental effects to the aquatic organisms. This has become a major concern because PPCPs are extensively and increasingly used in human and veterinary medicine, resulting in their continuous release to the environment (Nikolaou et al., 2007). Priority pollutant lists have been developed both by the European Union (EU) and the United States Environmental Protection Agency (USEPA) identifying

a wide variety of chemicals present in wastewaters and storm water runoff that may pose a threat to receiving water bodies including surface water. An initial list of 33 priority substances was also identified under the EU Water Framework Directive (WFD) 2000/60/EC to be used as a control measure for the next 20 years. In 2007, PPCPs such as diclofenac, iopamidol, musks and carbamazepine were identified as future emerging priority candidates. Ibuprofen, clofibric acid, triclosan, phthalates and bisphenol A are proposed additions to this list (Ellis, 2008). In 2018, the following compounds were included under the watch list; diclofenac, erythromycin, estrone, amoxicillin, venlafaxine etc. (Loos et al., 2018).

Due to their large number and diverse chemical nature, the Environment Agency (EA) of England and Wales proposed a ranking system for PPCPs according to their perceived relative risk, with the aim of identifying substances with great potential to pose a risk to the aquatic environment. This ranking system used a combination of traditional risk assessment procedures, persistence, bioaccumulation and toxicity (PBT) criteria, occurrence data from various countries, availability of suitable analytical methods, and an aim to include compounds representative of different therapeutic classes. Based on this procedure; the top 10 compounds were: Lofepramine, Dextropropoxyphene, Procyclidine, Tramadol, Paracetamol, Clotrimazole, Thioridazine, Mebeverine, Aminophylline, and Tamoxifen (Ashton et al., 2004). Similarly, using the OSPAR selection and prioritisation mechanism for hazardous substances (DYNAMEC), an alternative list of priority substances was identified, including: Lofepramine, Dextropropoxyphene, Procyclidine, Tramadol, Paracetamol, Clotrimazole, Thioridazine, Mebeverine, Aminophylline, Tamoxifen, Fluoxetine, Trimethoprim, Sulfamethoxazole, Fenofibrate1, and Diclofenac (OSPAR Commission, 2002).

Since then, several studies have investigated the concentrations of these priority PPCPs, with others, in the fresh water aquatic environment. This chapter aims to (a) provide an overview of the environmental risk associated with PPCPs; (b) discuss the environmental fate and behaviour of PPCPs in the aquatic system; (c) review the current state-of-knowledge on the levels and trends of PPCPs in various compartments of the fresh water environment; (d) assess the reported methods for analysis of PPCPs in water, fish and sediment; and finally discuss the current research gaps and provide recommendations for future research.

1.3. Environmental risk of PPCPs

The detection of chemicals in any environmental matrix does not necessarily mean that their presence is of concern or may cause harm. However, major concerns arise from the detection of chemicals for which there is evidence that they may adversely affect aquatic life (WHO, 2015).The following sections summarise some of the major concerns about the presence of PPCPs in the freshwater aquatic environment.

1.3.1. Persistence

The physio-chemical properties of many PPCPs, show that many are not easily removed by conventional water treatment processes, as demonstrated by their presence in drinking water (Snyder, 2008). The inability to effect complete removal of PPCPs from waste treatment plant poses a potential risk to aquatic organisms and public health. The overwhelming evidence from monitoring studies is that PPCPs have found their way into the aquatic environment and are ubiquitous (Bu et al., 2013). The high rate and volume of global PPCPs use, coupled with the escalating introduction of new pharmaceuticals to the market is contributing substantially to the environmental existence of these chemicals and their active metabolites in the aquatic environment (Daughton and Ternes, 1999). Moreover, while not all PPCPs are persistent, their continuous use and release to the environment means many are considered "pseudo-persistent". Pseudo-persistent pharmaceuticals may have greater potential for environmental persistence than other organic contaminants like pesticides, because their source continually replenishes even when acted on by environmental processes such as biodegradation, photo degradation and particulate sorption. Hence, pharmaceuticals that may degrade would eventually and effectively behave as persistent compounds because of their constant release into the environment (Houtman et al., 2004). Loffler *et al.* categorised 10 pharmaceuticals and pharmaceutical metabolites into low, moderate and high persistence compounds according to their dissipation time (DT50) in water/sediment samples. Paracetamol, Ibuprofen, 2-hyroxyibuprofen and CBZ-diol were classed as showing low persistence (DT50 = 3.1-7 days), Oxazepam, Iopromide and Ivermectin were deemed moderately persistent (DT50 = 15-54 days) while Clofibric acid, Diazepam, Carbamazepine were rated highly persistent (DT50 = 119-328 days) (Löffler et al., 2005). A more recent study demonstrated the anxiolytic drug (Oxazepam) to persist in freshwater lakes over a long period of time due to past input and growing urban populations (Klaminder et al., 2015).

1.3.2. Bioaccumulation

Although PPCPs are detected in the freshwater environment at relatively low concentrations, many of them and their metabolites are biologically active and can impact non-target aquatic organisms. Several studies have examined the effect of PPCPs on non-target organisms especially fish. The exposure of goldfish (*Carassius auratus*) to waterborne gemfibrozil at an environmentally relevant concentration over 14 days resulted in a plasma bioconcentration factor of 113 (Mimeault et al., 2005). Another study by (Vernouillet et al., 2010), revealed bioaccumulation of the antiepileptic drug carbamazepine (CBZ) by algae - *Pseudokirchneriella subcapitata* and the crustacean - *Thamnocephalus platyurus* with bioaccumulation factors of 2.2 and 12.6 respectively. Furthermore, (Wang and Gardinali, 2013) reported the uptake and depuration of pharmaceuticals in reclaimed water by mosquito fish (*Gambusia holbrooki*). The bioaccumulation factors measured for caffeine, diphenhydramine, diltrazem, carbamazepine and ibuprofen were 2.0, 16, 16, 1.4, and 28 respectively. Oxazepam was detected at high concentrations in Eurasian perch fish with a bioaccumulation factor of 12 (Brodin et al., 2014). Also (Du et al., 2015) revealed the accumulation of fluoxetine in snails with the bioaccumulation factor of 3000. (de Solla et al., 2016) monitored 145 PPCPs in wild and caged mussels from the Grand River, Ontario. Forty-three pharmaceuticals from different classes were detected in mussel tissues, with bioaccumulation factors ranging from 0.66 for metformin to 32 022 for sertraline.

As distinct from pharmaceuticals, PCPs have been detected in algae which comprise the greatest abundance of plant biomass in the aquatic environment. The lipid content of algae provides an entry point for trophic transfer of lipophilic organic contaminants. A study conducted by (Coogan et al., 2007) detected the presence of two widely used antimicrobial agents – triclocarban (TCC), triclosan (TCS) as well as its metabolite methyl-triclosan (M-TCS) in algae samples collected around a wastewater treatment plant (WWTP) in Texas. Concentrations of target PCPs in water samples were low ranging from 50–200 ng/L, while higher levels of 50–400 ng/g fresh weight were detected in algae. The resulting bioaccumulation factors ranged from (700-1500), (900-2100) and (1600-2700) for M-TCS, TCS and TCC respectively.

1.3.3. Toxicity

The major concern about the toxic implications of pharmaceuticals (c.f. polychlorinated biphenyl (PCBs), perfluorinated compounds (PFCs) and polybrominated diphenyl ethers (PBDEs) is that they were designed specifically to maximise their biological activity at low doses and to target certain metabolic, enzymatic, or cell-signalling mechanisms. The evolutionary conservation of these molecular targets in a given species potentially increases the possibility that these pharmaceuticals will be pharmacologically active in non-target organisms. This mode of action (MoA) concept can be applied to all aquatic biota, which are unintentionally exposed to pharmaceuticals in their natural environment, thus raising the risk of ecotoxicological effects (Fabbri and Franzellitti, 2016). The MoA conceptual frame work was tested using the anti-depressant agent Fluoxetine, which targets the serotonin (5-HT) signalling pathway. Because 5-HT is a high-tier physiological controller in aquatic organisms, alterations of the 5-HT pathway by fluoxetine had many adverse outcomes on key physiological functions, including reproduction, metabolism and locomotion in mussels at concentrations approaching or even below environmental levels (Franzellitti et al., 2013, Ford and Fong, 2016).

1.3.4. Endocrine disruption

A major concern raised by the presence of PPCPs in the aquatic environment is their ability to interfere with the endocrine system to produce undesired effects/disruption of homeostasis. The World Health Organization (WHO) defined endocrine disruptors (EDs) as 'exogenous substances or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an organism, its progeny or sub-population'. EDs include a vast group of chemicals from natural (e.g. mycotoxins and phytoestrogens) and synthetic origin (e.g. diethylstilbesterol (DES) and bisphenol A) in varieties of consumer products (e.g. PPCPs, cleaning products, antimicrobials, food preservatives and phthalates) (Wielogórska et al., 2015). Endocrine disrupting pharmaceuticals include sex hormones, glucocorticoids, veterinary growth hormones and few non-steroidal pharmaceutical substances (Figure 1).

Furthermore, toxicity arising from complex mixtures of PPCPs at low concentrations could lead to synergistic interactions. This means that while individual PPCPs may be present at low concentrations that do not elicit significant toxic effects when acting

singly; PPCPs mixtures can still exert considerable ecotoxicity. This was demonstrated by (Cleuvers, 2003), whereby the antiepileptic drug – carbamazepine and the lipid lowering agent clofibric acid (both belonging to different therapeutic classes) exhibited much stronger effects to *Daphnia magna* than single compounds at the same concentration. (Thorpe et al., 2001) also revealed that mixture effect of estradiol (E2) and 4-tert-nonylphenol (NP) can give an additive/synergistic reaction, and consequently induce vitellogenin (Vtg) production in juvenile rainbow trout. A study on the brown trout, a salmonid species native to German rivers, investigated the effect of diclofenac, one of the most prevalent pharmaceuticals in surface water. Results revealed that water-borne diclofenac at levels of 5-50 µg/L affects kidney and gill integrity and selected immune parameters in the fish (Hoeger et al., 2005). A laboratory and field study conducted in France revealed that exposure to 17β-estradiol on a freshwater fish; chub (*Leuciscus cephalus*) resulted in a significant and rapid increase in plasma Vtg in both male and female chub (Flammarion et al., 2000). Mimeault *et al.* also demonstrated that exposure to waterborne gemfibrozil on goldfish (*Carssius auratus*) resulted in reduction on plasma testosterone by over 50% after 14days (Mimeault et al., 2005).

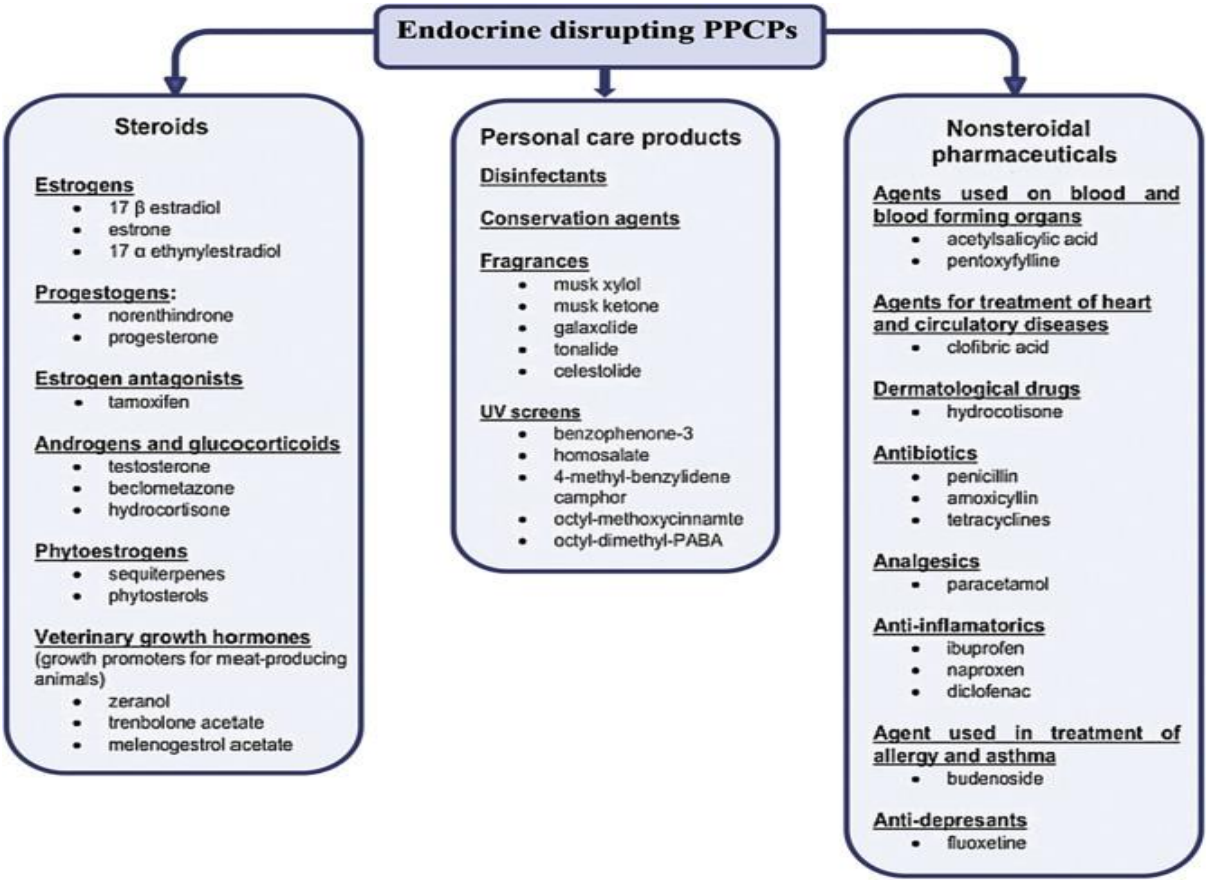


Figure: 1. Summary of endocrine disrupting PPCPs

1.3.5. Antibiotic resistance

Another important concern related to the presence of PPCPs in the environment is the potential creation of antibiotic resistant strains in natural bacterial populations. Extensive use of antibiotics in human medicine and animal husbandry is the major cause for the emergence and spread of antibiotic resistant bacteria, which has become a threat to the effective prevention and treatment of various infectious diseases caused by antibiotic-resistant pathogenic bacteria (WHO, 2015). Six antibiotics (ciprofloxacin, tetracycline, ampicillin, trimethoprim, erythromycin and trimethoprim/sulpha-methoxazole) detected in the effluent of a WWTP in Australia increased the resistance of 2 natural bacterial strains found in the receiving waters (Costanzo et al., 2005). Positive correlations have been found between antibiotic-resistant microorganisms and trace concentrations of aquatic antibiotic contaminants (Novo et al., 2013). Furthermore, the presence of antibiotics could have a detrimental effect on naturally occurring bacteria present in the environment. This was proven by (Davies et al., 2006), who showed that even at sub-inhibitory level concentrations, antibiotics may still exert their biological impact on natural microbial communities by influencing transcription in microbes. Some studies have reported adverse effects on aquatic organisms including: toxicity of ciprofloxacin to green algae (Halling-Sørensen et al., 2000), toxicity of oxolinic acid (a commonly used feed additive in fish farm) to *Daphnia magna*, as well as the toxicity of fluoroquinolone antibiotics (ciprofloxacin, lomefloxacin, ofloxacin, levofloxacin, enrofloxacin and flumequine) on five aquatic organisms, the cynobacterium; *Microcystis aeruginosa*, duckweed; *Lemna minor*, the green alga; *Pseudokirchneriella subcapitata*, the crustacean; *Daphnia magna* and fathead minnow; *Pimephales promelas* (Robinson et al., 2005).

Overall, the toxicity of PPCPs in the aquatic environment extends beyond the acute effects observed when therapeutic levels are reached or exceeded. Recent studies have shown PPCPs toxicity to vary depending on the exposed organism, duration of exposure, contaminant concentration, and developmental window at which exposure occurs. Moreover, the effects of chronic trace-level exposure, especially at certain sensitive stages of development, are more likely to explain observed abnormalities within exposed non-target organisms than acute high dose exposure (Wilkinson et al., 2016). As many pharmaceutical contaminants are introduced into the environment as a result of human

or veterinary use, metabolite concentrations may be more significant than that of parent compounds. For instance, some acetylated metabolites of antibiotics (such as N4-acetylsulfapyridine) were found to be more toxic than the parent compound sulfapyridine in algae (García-Galán et al., 2012). In addition, the presence of active pharmaceutical agents under undesirable conditions in the aquatic environment may alter their toxicological properties. To illustrate, the photo degradation products of naproxen were reported to have more toxic effects than the parent compound on algae, rotifers, and microcrustaceans (Isidori et al., 2005). Acidic pharmaceutical compounds may elicit different toxicological responses at different pH levels in exposed non-target organisms (Fent et al., 2006) and metals shown to accumulate in river biofilms have been shown to increase the toxicity of certain antibiotic contaminants (fluoroquinolones and tetracyclines) in an additive manner (Zhang et al., 2012).

1.4. Environmental fate and behaviour of PPCPs

1.4.1. Sources

Many PPCPs after use find their way into the environment through different routes. The major sources of PPCPs to the environment are via sewage treatment plants (STPs) (Daughton and Ternes, 1999), WWTPs, and landfill leaching. PPCPs are often not completely and consistently removed during conventional wastewater treatment processes, and thus are frequently detectable in reclaimed surface water at concentrations ranging from ng to µg/L (Chen et al., 2013). The contamination of the freshwater environment with pharmaceuticals can occur in various ways (Figure 2) – an important pathway is absorption of PPCPs by the body as a result of medical use, followed by distribution, metabolism and finally excretion; and release into the sewage system or septic tank. After treatment of sewage, the wastewater may be used for irrigation with the bio-solids (treated sludge) potentially applied as fertilizer to agricultural land (Yang and G.S, 2015). Another source of PPCPs to the environment is via the manufacturing processes as the wastewater from the producing facility goes directly into STPs (Fick et al., 2009). After treatment, the sludge is deposited on the soil as fertilizer, with the liquid effluent discharged directly into the freshwater environment. In addition, PPCPs can reach the groundwater through leaching from the soil and this could pose a threat to drinking water. Not only that, pharmaceuticals can also reach freshwater through run-off from land treated with digested sludge for agricultural purposes (Nikolaou et al., 2007).

Veterinary drugs are released into the environment when animal wastes either in solid or liquid states are sprayed on agricultural field as fertilizers. These veterinary drugs together with their metabolites pollute the soil and could enter the food chain. Consequently, agricultural run-off can enter freshwater system and then leach to the groundwater (Farré et al., 2008). Furthermore, Personal care products (PCPs) which are externally applied are mostly discharged through shower waste; bathing, swimming and washing sinks. They can pass through WWTPs, and reach the environment (Peck, 2006).

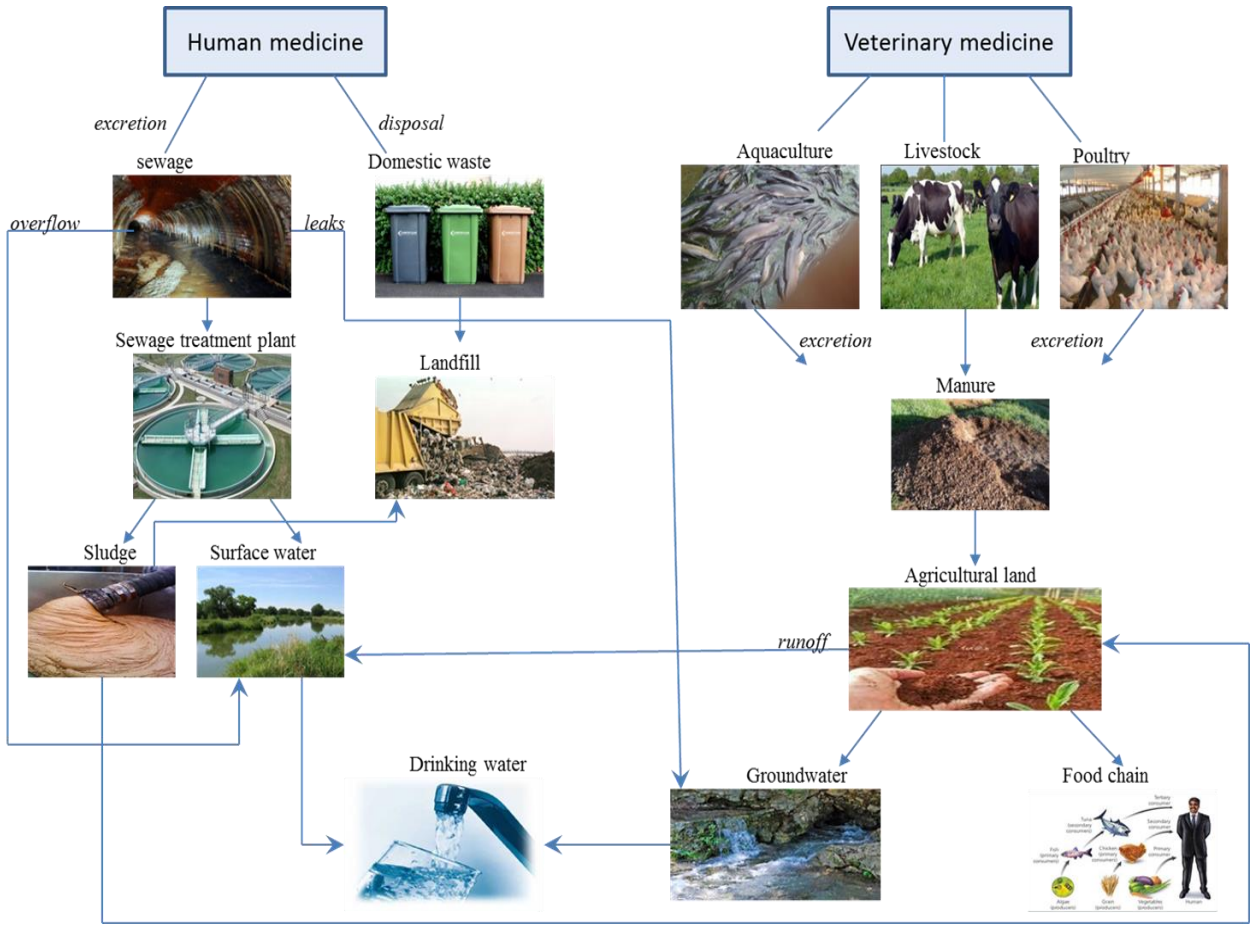


Figure: 2. Illustration of sources of environmental contamination with PPCPs

1.4.2. Transport

Once released into the environment, there is possibility of long-range transport for some PPCPs depending on the physio-chemical properties of the compound and the characteristics of the receiving environment. PPCPs generally have low volatility and are highly polar and hydrophilic in nature, therefore their distribution through the environment will primarily occur through aqueous transport and food chain dispersal (Caliman and Gavrilescu, 2009). Transport of PPCPs between different environmental

media depends on the sorption behaviour of the compound in treatment plants, soil, and the water-sediment system (Boxall, 2004). Several groups of PPCPs can be found in sludge samples of STPs through adsorption. This creates a potential pathway for PPCPs into the environment by direct release or application of sludge to agricultural land as fertilizer (Van Wieren et al., 2012). A study observed that PPCPs were transported into groundwater when biosolids were applied onto agricultural land (Heberer, 2002) as well as fields irrigated with treated wastewater (Pedersen et al., 2005). This resulted in the uptake of PPCPs by, which may constitute a potential pathway of human exposure to PPCPs through dietary intake (Wu et al., 2014, Wu et al., 2015). Runoff from biosolids containing PPCPs either from landfills or applied on agricultural land may be transported into the surrounding surface water or leach into the groundwater (Kleywegt et al., 2007), thereby posing a risk to aquatic life and public health. Sorption in sediment is another mechanism through which PPCPs are transported to the aquatic environment. The sediment acts as a sink and accumulates these environmental contaminants which may be released back to the aquatic environment (Zhao et al., 2013). Several studies have shown some PPCPs (e.g. sulfamethoxazole, carbamazepine, triclosan and ciprofloxacin) to be more persistent in sediment than water (Conkle et al., 2012), (Chenxi et al., 2008). Osenbruck *et al.* identified local river water infiltration, sewer exfiltration, and urban stormwater recharge as the major sources of carbamazepine, galaxolide, and bisphenol A in groundwater underlying the city of Halle (Saale), Germany (Osenbrück et al., 2007).

Nevertheless, the fact that adsorption to sediment or suspended solids may influence concentrations of PPCPs in the receiving water does not necessarily result in a reduction of their bioavailability or toxicity. Several studies have reported the accumulation of PPCPs in different environmental compartments including sediments (Chen and Zhou, 2014, Silva et al., 2011, Azzouz and Ballesteros, 2012). Therefore, there may be possibility of continuous release of these chemical compounds from sediments to the water. This may have adverse effects on benthic organisms that are continuously exposed to these chemicals within the sediments, interstitial water and in overlying water (Gilroy et al., 2012). Tamura *et al.* estimated the combined contribution of triclosan, triclocarban and galaxolide to total river sediment toxicity to be as high as 8.2% using the benthic organism, *Chironomus yoshimatsui* (Tamura et al., 2013). Further understanding of the

toxicological impacts of PPCPs in freshwater sediments appears imperative as sediment acts as a sink for these chemicals.

1.4.3. Environmental degradation and transformation

Biodegradation, photo-degradation and other abiotic transformation processes such as hydrolysis, (Blair et al., 2013) may reduce concentrations of PPCPs in the environment and result in partial loss and mineralization of these compounds (Alexy et al., 2004).

The extent of photo-degradation depends on the intensity of solar irradiation, water depth, organic matter composition, eutrophic conditions, latitude and seasonality. A study conducted by (Chiron et al., 2006), revealed that under artificial estuarine water condition, a photo-degradation product of carbamazepine is acridine. This metabolite has shown to be toxic, mutagenic and carcinogenic. Another study suggested that tetracycline, an antibiotic used widely for animal husbandry, cannot be photodegraded because of its adsorption onto sediment (Tolls, 2001). However, the analgesic diclofenac could be easily and rapidly degraded through direct photolysis with a (pseudo) first-order elimination rate and a short half-life of < 1 h (Buser et al., 1998b). (Robinson et al., 2007) reported 11 – 68 % of propranolol was removed by photo-degradation in US rivers and predicted removal of up to 27 % in the River Aire, UK, during the summer. Similar results were reported for Ibuprofen, Metronidazole, Acetaminophen and several other PPCPs, suggesting photolysis as one of the major degradation pathways of PPCPs in surface waters (Carlson et al., 2015, Boreen et al., 2003).

Biodegradation stems from the reaction with natural microbial flora in the environment. Many PPCPs undergo microbial mediated reactions during WWT processes (Helbling et al., 2010) and in the environment, resulting in the formation of transformation products. Oenesois et al. provided a comprehensive review on biodegradation and removal of PPCPs in treatment systems. They concluded that accurately predicting biodegradability based on a PPCP's intended function may not be possible. Since biodegradation involves enzymatic reactions specific to chemical structures, the biodegradability of PPCPs with different structures grouped in the same therapeutic class is expected to vary, thwarting efforts to observe general trends (Onesios et al., 2009). Microorganisms that utilize PPCP substrates at certain concentration either as a carbon or energy source would be expected to increase in microbial growth and thereby resulting in further degradation of

PPCPs. However, the increase in PPCPs concentrations could inhibit biodegradation, therefore becoming toxic to the natural occurring microorganisms. Despite an initial increasing trend of degradation up to concentrations of 100 µg/L, none of the studied PPCPs including 4-isopropyl-3-methylphenol (biosol), *p*-chloro-*m*-xylenol, gemfibrozil, ketoprofen, and phenytoin achieved their highest degradation at the highest respective concentration of 1000 µg/L, therefore, suggesting enzyme saturation at such high concentrations (Onesios-Barry et al., 2014).

During waste water treatment (WWT), transformation of PPCPs may occur depending on the physiochemical properties of the compound and the conditions of the WWT. During the process, PPCPs may be completely destroyed, or partially transformed to metabolites or in some instances left unchanged (Xia K., 2005). It is important to bear in mind that the breakdown or removal of the parent compounds during WWT does not necessarily mean the removal of toxicity, it is expected that a great number of transformation products with unknown toxicity and persistence may still be present in the final effluent as well as in receiving water bodies (Hughes et al., 2013). Typical examples of the transformation of pharmaceuticals are presented below for the anti-inflammatory/analgesic – ibuprofen, the X-ray contrast media – diatrizole and an antihypertensive drug - valsartan.

(Zwiener et al., 2002) used a biofilm reactor (BFR) and batch experiment with activated sludge (BAS) to study the transformation of ibuprofen. The result revealed hydroxyibuprofen (OH-Ibu) to be the major metabolite of ibuprofen under oxic conditions and carboxyhyratropic acid (CA-HA) under anoxic conditions. Moreover, carboxyibuprofen (CA-Ibu) was identified as a major metabolite under both oxic and anoxic conditions. These transformation products either generated by human metabolism or by microorganisms present in the WWTPs and in the natural environment may increase the probability to finding their presence in the environment (Ferrando-Climent et al., 2012).

In contrast to ibuprofen, diatrizole which is commonly used as X-ray contrast medium, does not metabolize and is excreted unchanged. In WWTPs, it has been shown to be persistent under aerobic conditions. Therefore, diatrizole has been detected at elevated

concentrations in the effluents of WWTPs, surface water, groundwater and even in finished drinking water (Redeker et al., 2014).

(Helbling et al., 2010) reported on the transformation of valsartan - an antihypertensive drug. The transformation products formed followed a sequence of transformation steps in figure 3.

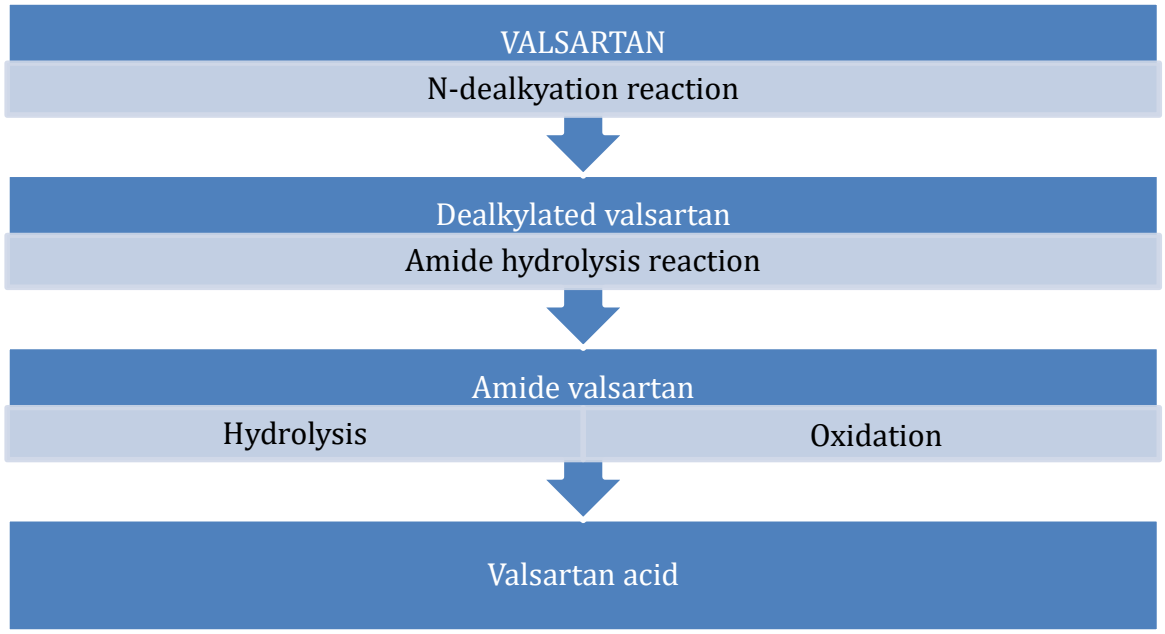


Figure: 3. Degradation pathway of Valsartan

The first reaction was an N-dealkylation reaction, yielding Dealkylated valsartan. This transformation product further transformed to Amino-valsartan by an amide hydrolysis reaction and subsequently another transformation product was formed [2'-(1H-tetrazol-5-yl)biphenyl-4-yl]acetaldehyde (otherwise referred to as valsartan acid), through the hydrolysis and oxidation of Amino-valsartan. These transformation products were suggested to provide the rationale for the environmental persistence of valsartan.

1.5. Levels of PPCPs in the freshwater aquatic environment

The occurrence of pharmaceuticals was first reported in Kansas City, US in 1976 in treated wastewater, where clofibric acid was detected at concentrations ranging from 0.8 – 2 µg/L (Fent et al., 2006). Subsequently, (Richardson and Bowron, 1985) investigated the presence of 25 pharmaceuticals in the river Lee (a source of potable water for North London) with concentrations up to 1 µg/L in 1981. Since then, several studies have detected PPCPs in different environmental compartments across the globe (Hirsch et al.,

1999, Kolpin et al., 2002, Ramirez et al., 2009). Despite the fact that reported concentrations of these PPCPs are low; many of them have the potential to persist in the natural environment for months to years (Boxall et al., 2012). The detection of pharmaceuticals in the environment varies not only between countries but also between different regions of the same country. That is to say, detectable pharmaceuticals in one country or region may not appear in other countries/regions where they are not highly prescribed (Jjemba, 2008). Therefore, levels of different classes of PPCPs reported in the freshwater aquatic environment from each continent will be reviewed separately in the next section.

1.5.1. Measured concentrations in Europe

Wastewater and surface water

In 1981, Richardson and Bowron were the first to detect 25 pharmaceuticals in water samples from the river Lee, UK with concentrations of dextropropoxyphene, erythromycin, sulfamethoxazole, tetracycline and theophylline up to 1 µg/L (Richardson and Bowron, 1985). Subsequently, a study in German municipal STPs and rivers, investigated 32 pharmaceuticals from different classes including antiphlogistics, lipid regulators, psychiatric drugs, antiepileptic drugs, betablockers and β_2-sympathomimetics in discharged effluents, stream and river waters. More than 80% of the selected drugs were detectable in at least one municipal STP effluent with concentrations of carbamazepine up to 6.3 µg/L thus, resulting in the contamination of the receiving waters. The lipid regulator "bezafibrate" showed the highest concentration of 3.5 µg/L in the sampled river waters (Ternes, 1998). Concentrations of ibuprofen detected in influent and effluent samples from various German WWTPs displayed a maximum of 3.5 and 0.3 µg/L respectively (Huppert et al., 1998). Hirsch *et al.* investigated STP effluents and random river water collected in Germany for the presence of antibiotics residues. The results showed frequent detection of erythromycin, roxithromycin and sulfamethoxazole with concentrations up to 6 µg/L (Hirsch et al., 1999). Another German study (Ferrari et al., 2004) reported detection of 6 pharmaceuticals: carbamazepine, clofibric acid, diclofenac, propranolol and sulfamethoxazole at concentrations 6.3, 1.6, 2.1, 0.29 and 2 µg/L in effluent and 1.1, 0.55, 1.2, 0.59, and 0.48 µg/L in surface water, respectively. In addition, carbamazepine, diclofenac, ibuprofen, as well as a variety of antibiotics and lipid regulators were detected

in water samples collected from the River Elbe in 1998 at concentrations ranging between 20 - 140 µg/L (Wiegel et al., 2004). Moreover, a study examined the fate of triclosan and its active transformation product, triclosan-methyl in STPs and surface water (River Ruhr) in Northern Germany. The concentrations of both compounds ranged between <3 and 10 ng/L for triclosan and between 0.3 and 10 ng/L for triclosan-methyl (Bester, 2005).

In the UK, Hilton *et al.* reported the detection of meclofenamic acid, diclofenac, propranolol, erythromycin, trimethoprim and acetyl-sulfamethozole in both downstream discharge of surface water and in effluent samples (Hilton and Thomas, 2003). Ashton *et al.* investigated effluent and surface water samples from Corby, Great Billing, East Hyde, Harpenden and Ryemeads STPs. Ten pharmaceuticals were detected in the STP effluent samples: propranolol (100%, median=76 ng/L), diclofenac (86%, median=424 ng/L), ibuprofen (84%, median=3086 ng/L), meclofenamic acid (81%, median=133 ng/L), dextropropoxyphene (74%, median=195 ng/L), trimethoprim (65%, 70 ng/L), erythromycin (44%, <10 ng/L), acetyl-sulfamethoxazole (33%, median=<50 ng/L), sulfamethoxazole (9%, median=<50 ng/L), tamoxifen (4%, median=<10 ng/L). In the corresponding receiving streams, fewer compounds and lower concentrations were found (Ashton et al., 2004). Another study conducted in the UK by (Thomas and Hilton, 2004) detected clofibric acid, clotrimazole, dextropropoxyphene, diclofenac, ibuprofen, meclofenamic acid, propranolol, tamoxifen and trimethoprim at measurable concentrations in water samples collected from the lower reaches of the rivers Tyne, Tees, Mersey, and Thames as well as Belfast Lough. Clotrimazole appeared to be the most frequently detected in 59 % of all the samples collected at a maximum concentration of 22 ng/L and a mean concentration of 7 ng/L. (Roberts and Thomas, 2006) revealed the result of a survey of wastewater effluent and surface waters of the lower River Tyne, UK. Out of 9 compounds analysed in the raw effluent samples, sulfamethoxazole and acetyl-sulfamethoxazole were detected at concentrations ranging from 11 – 69,570 ng/L. For the surface water samples; clotrimazole, dextropropoxyphene, erythromycin, ibuprofen, propranolol, tamoxifen and trimethoprim were detected at concentrations ranging from 4 – 2370 ng/L.

In the South Wales region of the UK, (Kasprzyk-Hordern et al., 2008d) reported the contamination of the River Taff and the River Ely with PPCPs, illicit drugs and other

endocrine disruptors, which was attributed mainly to the extensive discharge of treated wastewater effluent into the rivers. The investigation suggested that the most frequently detected PPCPs represent the compounds that are highly dispensed in the Welsh community. These include: anti-inflammatories/analgesics (tramadol, codeine, paracetamol, naproxen, ibuprofen and diclofenac), antibacterial drugs (erythromycin, trimethoprim and amoxicillin) and antiepileptic drugs (gabapentin and carbamazepine). Some of these PPCPs (e.g. codeine, erythromycin, valsartan, gabapentin and carbamazepine) were found to be ubiquitous and persistent in the aqueous environment. Illicit drugs were also detected in both rivers at low concentrations. The average daily loads of amphetamine, cocaine and its main metabolite benzoylecgonine were reported at 8, 1 and 39 g/day respectively.

(Zhou et al., 2009), also reported PPCPs such as: propranolol, sulfamethoxazole, carbamazepine, indomethacine and diclofenac were frequently detected in wastewater and river water sampled from three WWTPs in England and the River Ouse. Carbamazepine showed the highest concentrations (up to 2336 ng/L) in WWTP influent samples. Interestingly, (Kugathas et al., 2012) reported as the presence of glucocorticoids (GCs) in the river Thames, in the UK. The total concentrations of 28 GCs ranged between 30 ng/L and 850 ng/L. These concentrations were much higher than those of more extensively studied estrogens especially ethinylestradiol and other steroid hormones. At such concentrations, it is possible to adversely affect aquatic organisms. However, Baker and Kasprzyk-Hordern went further to report occurrence of a comprehensive set of drugs of abuse in river water, untreated and treated wastewater in England, UK. From their study, the top ten pharmaceuticals with the highest median concentration detected from the WWTPs influent and effluent were caffeine (23778.4 ng/L - 1744.2 ng/L), 1,7 dimethylxanthine (20400.4 ng/L – 1219.8 ng/L), nicotine (3919.3 ng/L - 85.7 ng/L), codeine (1255.9 ng/L – 372.2 ng/L), tramadol (1122.6 ng/L – 738.7 ng/L), ephedrine (476.2 ng/L – 35.0 ng/L), nortramadol (397.0 ng/L – 144.8 ng/L), morphine (371.2 ng/L – 59.1 ng/L), dihydrocodeine (226.6 ng/L – 118.2 ng/L), amitriptyline (206.3 ng/L – 66.3 ng/L) respectively. The same high median concentrations were equally seen in these pharmaceutical compounds when river water samples were collected from both upstream and downstream of the WWTPs (Baker and Kasprzyk-Hordern, 2013a).

Furthermore, (Zhou and Broodbank, 2014) reported the occurrence of pharmaceuticals in samples collected from the River Medway, UK in February 2010. Concentrations in water sampled for upstream sewage effluent and effluent discharge sites were: propranolol (8 – 35 ng/L), meso-biliverdin (3 – 11 ng/L), thioridazine (6 – 22 ng/L), carbamazepine (53 - 265 ng/L), tamoxifen (2 – 8ng/L), indomethacine (6 - 28 ng/L) and meclofenamic acid (28 – 176 ng/L). The highest concentration of all the studied compounds was for diclofenac in the effluent discharge site (543 ng/L). Apart from the water samples analysed, high concentrations of pharmaceuticals were also detected in all dry weight samples in the month of June, 2010 with the highest concentration being, diclofenac (58.7 ng/g), carbamazepine (46.5 ng/g), indomethacine (42.6 ng/g), and meclofenamic acid (37.3 ng/g).

Buser *et al.* identified clofibric acid concentrations found in various Swiss lakes (the Zurichsee, the Sempachersee and the Greifensee) were in the range of 1 – 9 ng/L (Buser et al., 1998a). Buser *et al.* also studied the occurrence and fate of diclofenac in Swiss lakes and rivers. The concentrations in the lakes ranged from <1 - 12 ng/L, while the highest concentration (11 - 310 ng/L) were observed in the river Aabach, one of the major inflows of Lake Greifensee (Buser et al., 1998b). In 1999, the same research group investigated the presence and behaviour of the chiral pharmaceutical drug, ibuprofen, in surface and wastewater samples. The surface water samples were collected from lakes and rivers in Switzerland and from the North Sea while the wastewater samples were collected from the Swiss WWTPs of Gossau, Pfaffikon and Uster. The concentration of ibuprofen in the influents of the WWTPs was up to 3000 ng/L while in the river and lakes, ibuprofen was detected at concentrations up to 8 ng/L (Buser et al., 1999).

Carbamazepine, atenolol, metoprolol, sulfametoxazole, gemfibrozil and propranolol were detected and demonstrated a high degree of persistence in the Hoje River in Sweden, at concentrations ranging from 160 – 1180 ng/L (Bendz et al., 2005). In a wide survey of more than 100 individual water samples from over 100 European rivers from 27 European Countries, Loos *et al.* identified the most frequently detected and at highest concentration levels, persistent pharmaceuticals as benzotriazole, caffeine and carbamazepine (Loos et al., 2009). A study in Catalonia, Spain also determined the presence of 11 PPCPs in both surface water (Ebro and Llobregat River) and wastewaters with benzophenone-3 having the highest concentration (7 ng/L) (Pedrouzo et al., 2009).

Another study reported the occurrence and distribution of multi-class pharmaceuticals, their active metabolites and transformation products in the Ebro River basin in Spain. Out of the 77 target analytes, the compounds found to be ubiquitous were carbamazepine, clarithromycin, sulfadiazine, propranolol, tamoxifen and salicylic acid. The highest concentration of 1667 ng/L was detected for carbamazepine metabolite (10,11 epoxi-carbamazepine) in a small tributary, Zadorra river (López-Serna et al., 2012).

Calamari *et al.* reported the detection of therapeutic agents such as atenolol, lincomycin, erythromycin, clarithromycin, bezafibrate and furosemide in the River Po and Lambro, Northern Italy at concentration ranging from 0.1 – 250 ng/L (Calamari et al., 2003). A study in Portugal revealed the presence of pharmaceutical active compounds (mostly nonsteroidal anti-inflammatory drugs) ranging from 0.050 – 100 µg/L in the influent and up to 50 µg/L in the effluent of 5 WWTPs. Musks were also detected at concentrations of 11.5 µg/L, 0.9 µg/L and 22.6 µg/L in the influent, effluent and sludge respectively (Salgado et al., 2010). Furthermore, (Meierjohann et al., 2016) monitored seasonal variation of 15 pharmaceuticals during four seasons (February, May, July and November 2010) along a wastewater polluted watercourse, River Rakkolanjoki and Lake Haapajarvi in Eastern Finland. The concentrations ranged from 0-556 ng/L. Out of the 15 studied compounds; carbamazepine had the highest concentrations and was not eliminated during any of the seasons.

In Sediment and sewage sludge

Several studies have shown that pharmaceuticals consumed in large quantities have been detected in the aqueous environment particularly in sediment. Loffler and Ternes reported the detection of acidic pharmaceuticals and their metabolites (clofibric acid, diclofenac, fenoprofen, gemfibrozil, ibuprofen, 2-hydroxy-ibuprofen, indomethacin, ketoprofen, and naproxen), antibiotics (clarithromycin, erythromycin, roxithromycin, sulfadiazine, sulfamethazine, sulfamethoxazole and trimethoprim) and parasiticide ivermectin in sediment from the Wickerbach creek, close to Frankfurt, Germany (Löffler and Ternes, 2003). Martin *et al.* investigated pharmaceuticals in sewage sludge, compost as well as sediment samples collected from the surface water of Guadiamar River in Seville, Southern Spain. The pharmaceuticals detected in the sediment were naproxen, salicylic acid, propranolol, caffeine and 17α-ethinylestradiol at concentrations of 11.2, 9.49, 3.37, 7.21, and 48.1 µg/kg respectively (Martín et al., 2010). A more recent study

examined sediment samples collected along four representative Iberian River basins; Llobregat, Ebro, Jucar and Guadalquivir, Spain. The most widely spread and highly concentrated pharmaceuticals were hydrochlorothiazide (3 ng/g), gemfibrozil (6 ng/g), tetracyclines (6 ng/g), codeine (12 ng/g) azithromycin (24 ng/g) as well as ibuprofen (13 ng/g) (Osorio et al., 2016). Varga *et al.* investigated selected acidic pharmaceuticals: ibuprofen, naproxen, ketoprofen and diclofenac in the Danube river water and sediment in Budapest, Hungary. In the river water, ketoprofen was always below the LOQ, while ibuprofen, naproxen and diclofenac were quantified in the range of 8–50, 2–30, 7–90 ng/L. In sediments, only naproxen and diclofenac were found in the range of 2–20 and 5–38 ng/g, respectively. (Varga et al., 2010). A Scottish study reported concentrations of human pharmaceuticals, illicit drugs and bactericides in sediments and sludge samples provided by the Scottish Environmental Protection Agency (SEPA) and Scottish Water in Scotland. None of the illicit drugs and metabolites were detected in any of the samples but triclosan (up to 5940 ng/g) and triclocarban (up to 2829 ng/g) were present at the highest concentrations (Langford et al., 2011). The study concluded that the drug content of sediment depended on the drug concentration in the aqueous phase and the total organic carbon content of the sediment.

In Biota

Globally, studies have shown that exposure to WWTP effluents containing PPCPs has been associated with a range of deleterious effects on the reproduction in aquatic organisms. (Gibson et al., 2005) revealed in their study bioaccumulation of mixture of estrogenic contaminants in fish tissues, thereby resulting in the induction of vitellogenin (Vtg) and possibly contributing to feminization of wild fish residing in the UK rivers. Pojana *et al.* examined natural and synthetic EDCs in water, sediment and biota of a coastal lagoon in Venice. The result of their study showed that most of the selected compounds were found in water and sediment at concentration ranging from 2.8-211 ng/L and 3.1-289 µg/kg dry weight respectively. The compounds detected in the biota Mediterranean mussel (*Mytilis galloprovincialis*) were 17α-ethinylestradiol and nonylphenol at concentration range 7.2-240 ng/g in dry weight (Pojana et al., 2007). In another study by (Fick et al., 2010), rainbow trout exposed to pharmaceutical sewage effluents showed that levonorgestrel was accumulated in fish blood at concentrations of 8.5 - 12 ng/ml.

Following the analysis of selected PPCPs in fish tissues using pressurized liquid extraction combined with silica gel clean-up, Subedi *et al.* measured galaxolide and tonalide in tilapia and bream fish samples collected from Rhine River, Bimmen at concentrations of 81 and 5.5 ng/g wet weight respectively (Subedi et al., 2011). Alvarez-Munoz investigated the presence of pharmaceuticals in oyster, clam and mussel samples collected from the Ebro delta, Spain. The result revealed that the most ubiquitous compounds detected were the psychiatric drug venlafaxine and the antibiotic azithromycin, with the highest concentrations found in mussel (2.7 ng/g) and oyster (3.0 ng/g) (Alvarez-Muñoz et al., 2015). Another Spanish study examined residual pharmaceuticals in agricultural farm and fish hatchery animals such as pig, veal, lamb and chicken muscle, liver and kidney as well as salmon, sea bass and sole flesh purchased at a local supermarket. The result of the study revealed the most frequently detected analytes were hormones estrone and 17β-estradiol and the antibacterial florfenicol and pyrimethamine (Azzouz et al., 2011).

1.5.2. Measured concentrations in South and North America:

Wastewater and surface water

The occurrence of pharmaceuticals was first reported in Kansas City, US in 1976 in treated wastewater, where clofibric acid was detected at concentrations ranging from 0.8 – 2 µg/L (Fent et al., 2006). In South America, Stumpf *et al.* detected polar drugs residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. In the surface water, clofibric acid, diclofenac and naproxen were frequently detected at low concentrations (0.01 -0.06 µg/L) in the major river used for drinking water production (Stumpf et al., 1999).

(Huang and Sedlak, 2001) reported estrogenic hormone concentrations in four municipal WWTPs effluent in California, USA, surface water from a wetland receiving effluent from only WWTP 4, Colorado River and Sacramento River Delta. The wastewater effluents median concentrations were 1.9 ng/L and - 0.6 ng/L for 17β-estradiol (E2) and 17α-ethinylestradiol (EE2), respectively. The median concentrations for the surface waters were 0.08 ng/L and <0.05 ng/L for E2 and EE2.

(Kolpin et al., 2002) detected various pharmaceuticals in samples from a network of 139 streams susceptible to contamination (i.e. downstream of urban areas and livestock production) across 30 states during 1999 and 2000. The most frequently detected

compounds were coprostanol (faecal steroid), cholesterol (plant and animal steroid), N,N-diethyltoluamide (insect repellent), caffeine (stimulant), triclosan (antimicrobial disinfectant), tri(2-chloroethyl) phosphate (fire retardant), and 4-nonylphenol (non-ionic detergent metabolite). Measured concentrations for this study were generally low and rarely exceeded drinking water guidelines, drinking water health advisories, or aquatic life criteria. Boyd *et al.* investigated the presence of PPCPs in surface water and treated waters of Louisiana, USA and Ontario, Canada. The result of the study revealed that naproxen was detected in Mississippi River and Lake Pontchartrain in Louisiana surface waters at concentrations ranging from 22 – 107 ng/L and in Louisiana STP effluent at concentrations range of 81 – 106 ng/L, while the drinking water treatment plants in Louisiana and Ontario contain naproxen at concentrations ranging from 63-65 ng/L (Boyd et al., 2003). Another study in Montana, USA, reported detection of sulfamethoxazole, atrazine, carbamazepine, dilantin and diclofenac with maximum concentrations of 490 ng/L, 130 ng/L, 420 ng/L, 22 ng/L and 46 ng/L respectively in ground water (Miller and Meek, 2006). Batt *et al.* reported presence of some antibiotics in receiving stream impacted by wastewater discharge in East Aurora and Holland, New York. Ciprofloxacin, sulfamethoxazole and clindamycin (0.043 – 0.076 µg/L) were detected 100m from the discharge point (Batt et al., 2006).

A study in New Orleans, Louisiana, USA, quantified polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and PPCPs in the Mississippi River. The results of the study revealed contamination by PPCPs including: clofibric acid (3-27 ng/L), ibuprofen (<1-34 ng/L), acetaminophen (25-65 ng/L), caffeine (<1-38 ng/L), naproxen (<1-135 ng/L), triclosan (9-26 ng/L), bisphenol A (<1-147 ng/L), carbamazepine (43-114 ng/L), estrone (<1-5 ng/L) and 17β-estradiol (<!-5 ng/L) at the following concentrations (Zhang et al., 2007). A study of a major receiving river, Choptank, Maryland, USA revealed the presence of various antibiotics and hormones at different concentrations in a major agricultural watershed. The most frequently detected antibiotic in the river were sulfamethoxazole and sulfadimethoxine at concentration ranging from 0.005 - 0.007 µg/L (Arikan et al., 2008).

Wu *et al.* reported the occurrence of selected pharmaceuticals in an agricultural landscape, western Lake Erie basin in northern Ohio. The results showed that the most frequently detected compounds were caffeine, carbamazepine, ibuprofen and

paraxanthine with maximum concentrations of 4.2, 1.2, 2.8 and 1.8 µg/L (Wu et al., 2009). Another study examined the distribution and temporal trends of 19 PPCPs including 11 hormones in two WWTPs from Charleston, SC, USA over a period of one year. Acetaminophen, caffeine and ibuprofen showed the highest concentrations in both WWTPs samples, followed by triclosan and triclocarban. In Charleston Harbour surface water, caffeine, cotinine and acetaminophen were frequently detected at 98.6%, 33.3%, and 22.2% respectively (Hedgespeth et al., 2012). (Blair et al., 2013) also reported detection of 32 pharmaceuticals in Lake Michigan and 30 were detected in the sediment. Among the frequently detected were metformin, caffeine, sulfamethoxazole and triclosan.

A study in Cape Cod, Massachusetts, USA, reported pharmaceuticals including sulfamethoxazole, the anticonvulsant – phenytoin, carbamazepine at maximum concentrations of 113 ng/L, 66 ng/L and 72 ng/L, respectively in wells (Schaider et al., 2014). Fairbairn *et al.,* also reported the presence of other emerging contaminants such as atrazine, acetaminophen, caffeine, DEET, trimethoprim, carbamazepine, sulfamethoxazole, erythromycin, cotinine and others in the Zumbro River watershed, Minnesota, USA at concentrations over a period of one year (Fairbairn et al., 2016).

A Canadian study by Metcalfe *et al.* reported the occurrence of neutral and acidic drugs in the effluents of Canadian STPs. The study showed that the lipid regulators such as bezafibrate and gemfibrate were detected in some of the samples of the influent and effluent as well as carbamazepine at concentrations as high as 2.3 µg/L (Metcalfe et al., 2003a). To determine the distribution of acidic and neutral drugs in surface waters near STPs in the lower Great Lake, Metcalfe together with his colleagues examined surface water collected from Lake Ontario and Lake Erie and STPs effluents. The result shows detection of all the acidic drug analytes except ketoprofen in the effluents. However, ibuprofen and gemfibrozil were detected in STP effluents at concentrations that exceeded 1 µg/L (Metcalfe et al., 2003b). Verenitch *et al.* also reported the presence of acidic drugs and caffeine in municipal wastewaters and receiving water on the West coast of Vancouver Island, British Columbia, Canada. Ibuprofen, naproxen as well as salicylic acid were detected in the samples of STP wastewater and also in surface water samples collected near the STP outfall (Verenitch et al., 2006). Hua *et al.* reported on seasonal variations in concentrations of pharmaceuticals and s-triazine herbicides in wastewater effluent and surface water sampled from upper Detroit River, Canada. The study revealed

that 15 pharmaceuticals including carbamazepine, cotinine, caffeine, trimethoprim, and fluoxetine were detected in the effluent samples of the WWTP at concentrations ranging from 1.7 – 1244 ng/L (Hua et al., 2006).

Gibson *et al.* investigated reuse of wastewater for irrigation from the Tula Valley in Mexico. The result of the study revealed that the wastewater used for irrigation contains some pharmaceuticals and potential endocrine disruptors. Concentrations of analytes applied during irrigation of fields were: Ibuprofen (742-1406 ng/L), naproxen (7267-13589 ng/L), and diclofenac (2052-4824 ng/L), while other pharmaceuticals such as gemfibrozil, clofibric acid and ketoprofen were below LOD (Gibson et al., 2010).

Moreover, (Ferreira, 2014a) revealed the presence of ibuprofen in both influent and effluent from WWTP Penha and Ilha do Governador, Rio de Janeiro, Brazil. Ibuprofen was detected in all samples analysed, confirming the low removal efficiency of conventional treatment plants. Another study by same author (Ferreira, 2014b) investigated psychiatric pharmaceuticals in Guandu River, Rio de Janeiro, Southern Brazil. The study revealed the presence of benzodiazepines such as bromazepam, clonazepam and diazepam in all samples of surface water at concentrations of 42 ng/L, 198 ng/L, and 335 ng/L respectively. In a comparative study conducted by the Minnesota Pollution Control Agency: DEET, cotinine, lopamidol, Bisphenol A, metformin and the steroidal hormone androstenedione were frequently detected at maximum concentrations of 103, 42, 510, 36, 18 and 5 ng/L in the lake sampled. In the surface water sample, sixteen chemicals including some antidepressants, antibiotics and antihypertensive pharmaceuticals were detected in the downstream of the WWTPs while six out of 56 PPCPs such as BPA, carbadox, fluoxetine, sulfamethazine, virginiamycin, methylprednisolone, moxifloxacin and triclosan were detected frequently in the upstream (Ferrey, 2015). Nonetheless, in the Caribbean, (Edwards et al., 2015) investigated the concentration of caffeine in surface water and wastewater in Barbados. The result of the study showed that caffeine was detected in all surface water and concentrations ranged from 0.1-6.9 µg/L.

In sediment

The occurrences of PPCPs in freshwater sediment have been documented by several authors. Sediment samples collected from rivers (Mississippi, Sauk, South Fork of the Crow and Grindstone), creeks (Center, Okabena) and lakes (Pepin, Superior, Shagawa) in Minnesota, US, revealed a high level of triclocarban in freshwater sediments at

concentration up to 822 ng/g (Venkatesan et al., 2012). A study conducted by Yang *et al.* investigated pharmaceuticals and organochlorine pesticides in sediments of the Alafia River in Florida, USA. The most frequently detected compounds in sediments were carbamazepine, acetaminophen, diphenhydramine, trimethoprim, caffeine, nicotine, lidocaine and ephedrine at concentrations ranging from 0 – 32.9 ng/g (Yang et al., 2015). A method was applied to surface water, sediment and mussel samples collected from San Francisco Bay, California, USA, an urban estuary that receives direct discharge from 40 municipal and industrial wastewater outfalls. The compounds detected were triclocarban in the sediment, valsartan in the surface water and DEET in mussel at concentrations 33 ng/g, 92 ng/L and 14ng/g respectively (Klosterhaus et al., 2013).

In biota

Furthermore, as reported by Ramirez *et al.* using high performance liquid chromatography-tandem mass spectrometry analysis revealed the presence of norfluoxetine, sertraline, diphenhydramine, diltiazem and carbamazepine in fillet of fish collected from Gila River, New Mexico, USA. In addition, fluoxetine and gemfibrozil were also confirmed in the liver tissue. Sertraline was detected at concentrations as high as 19 and 545 ng/g in fillet and liver tissue, respectively (Ramirez et al., 2009). The same author and his colleagues also reported the concentrations of diphenhydramine, diltiazem, carbamazepine and norfluoxetine detected in muscle tissues from fish collected in Pecan Creek, Denton County Texas, USA. The concentrations ranged from 0.66 - 1.32, 0.11 - 0.27, 0.83 – 1.44, 3.49 – 5.14 ng/g respectively (Ramirez et al., 2007). Mottaleb *et al.* used two screening methods GC-SIM-MS and GC-MS/MS to determine 10 extensively used PCPs and 2 alkylphenol surfactants in fish fillets collected from a regional effluent-dominated stream in Texas, USA. Among the PCPs benzophenone, galaxolide, tonalide and triclosan were detected in all environmental samples at concentrations ranging from 37 – 90, 234 – 970, 26 – 97 and 17 – 31 ng/g respectively (Mottaleb et al., 2009).

Moreover, Foltz *et al.* used GC-MS to detect and quantify selected nitromusks, antimicrobial agent and antihistamine in edible frozen fresh and salt water fish fillets from grocery stores located at Maryvile, Missouri, USA. The compounds consistently detected were galaxolide, tonalide, triclosan and diphenhydramine at concentrations ranging from 0.163 – 0.892, 0.068 – 0.904, 0.189 – 1.182 and 0.942 – 7.472 ng/g

respectively. Musk ketone was not detected in any of the fishes studied (Foltz et al., 2014). A report by Brooks *et al.* revealed pharmaceuticals accumulation in fish of effluent-dominated stream in North Texas, USA. The result of the study showed that selective serotonin reuptake inhibitors (SSRI) fluoxetine and sertraline their metabolites norfluoxetine and desmethylsertraline were detected at level greater than 0.1 ng/g in all tissues examined from fish (Brooks et al., 2005).

Another national pilot study initiated in the US, assessed the accumulation of PPCPs in fish sampled from five effluent-dominated rivers that receive direct discharge from wastewater treatment from Illinois, Texas, Florida, Arizona and Pennsylvania USA. The result of the study revealed presence of galaxolide and tonalide PCPs in fish fillets at every effluent-dominated site with maximum concentrations ranging from 300 - 2100 ng/L and 21 – 290 ng/L respectively. The pharmaceuticals detected both in the liver and fillets include: diphenhydramine, norfluoxetine, sertraline, diltiazem, carbamazepine, fluoxetine, gemfibrozil. With sertraline displaying the maximum concentration in both fillet and liver tissue, up to 19 and 545 ng/L respectively (Ramirez et al., 2009). Schultz *et al.* investigated the occurrence and fate of antidepressant pharmaceuticals in surface water, sediment and selective uptake in native white sucker (*Catostomus commersoni*) samples collected from Boulder Creek, (Colorado) and Fourmile Creek (Iowa). The result of the sediments samples showed detection of all antidepressant fluoxetine, norfluoxetine (degradate), sertraline, norsertraline (degradate), paroxetine, citalopram, venlafaxine, and bupropion except for fluvoxamine and duloxetine from Boulder Creek (Schultz et al., 2010). Another study investigated the uptake of human pharmaceuticals in bull sharks (*Carcharhinus leucas*) inhibiting a wastewater impacted river, Caloosahatchee River. The compounds detected in the plasma of Caloosahatchee River sharks were 17α-ethyinestradiol, citalopram, fluoxetine, fluvoxamine, paroxetine, sertraline and venlafaxine at concentrations ranging from 0.10-6.25 (Gelsleichter and Szabo, 2013).

1.5.3. Measured concentrations in Asia:

Wastewater and surface water

Yamagishi *et al.,* detected synthetic musk xylene and ketone in 100% and 80% respectively of 74 samples from Tama River and Tokyo Bay in Japan (Yamagishi et al., 1983). However, distributions of 12 antibiotics were investigated in the water from the

Mekong Delta, Vietnam and the result were compared with those in the Tamagawa River, Japan. Few antibiotics such as sulfamethoxazole, sulfamethazine, trimethoprim and erythromycin-H_2O were detected in Vietnam at concentrations ranging of 7 - 360 ng/L. While in the Japanese urban river more antibiotics including sulfamethoxazole, sulfapyridine, trimethoprim, erythromycin-H_2O, azithromycin, clarithromycin, and roxithromycin were detected at concentrations ranging from 4 - 448 ng/L (Managaki et al., 2007).

A study (Larsson et al., 2007) investigated the effluent from a WWTP serving drug manufacturers also in Patancheru, near Hyderabad, India. The result of the study revealed extremely high concentration levels of pharmaceuticals, with concentration of ciprofloxacin up to 31000 µg/L. Moreover, (Fick et al., 2009) showed in their study a severe case of contamination of surface, ground and drinking water with pharmaceuticals in Patancheru industrial area in India. Compounds detected include: 1.2 mg/L of cetirizine (an antihistamine) and 6.5 mg/L of ciprofloxacin (an antibiotics) and mg/L of several additional pharmaceuticals.

A study by Choi *et al.* examined the concentrations of several pharmaceutical residues in surface water and STPs of Han River, Korea. The concentrations of the target compounds such as cimetidine, caffeine, acetaminophen, sulfamethoxazole detected in the surface water were 281, 268.7, 34.8 and 26.9 ng/L respectively (Choi et al., 2008). Another study (Kim et al., 2009), detected the following pharmaceuticals and their concentrations (ibuprofen not detected (nd) to 414 ng/L, carbamazepine nd–595 ng/L, atenolol nd-690 ng/L, clarithromycin nd-443 ng/L, mefenamic acid nd–326 ng/L, erythromycin nd–137 ng/L, propranolol nd–40.1 ng/L, indomethacin nd–33.5 ng/L, fluconazole nd-111 ng/L, levofloxacin nd-87.4 ng/L, ifenprodil nd-35.4 ng/L, disopyramide (nd) and triclosan (nd)) in surface water from the Mankyung River, South Korea. Furthermore, same author Kim et al. documented the frequent detection of many pharmaceuticals, hormones, antibiotics as well as flame retardants in three major rivers, the Han River, the Nakdong River and the Youngsan River in South Korea (Kim et al., 2007). Another study by Sim *et al.* investigated the occurrence and distribution of pharmaceuticals in WWTPs located near major river basins of Korea. The result of the study showed that non-steroidal anti-inflammatory drugs, caffeine and carbamazepine were dominant in the influents and the distribution of pharmaceuticals varies with sampling sites and periods (Sim et al., 2011).

Lin *et al.* examined and quantified some pharmaceutical residues such as clofibric acid, ibuprofen, carbamazepine, naproxen, ketoprofen and diclofenac in tap water, groundwater, WWTPs and river water from Fu-Hsing River in China. The result showed that none of the targeted compounds were seen in the tap water and groundwater but in the river water, 30 ng/L concentration of naproxen was seen. For the WWTP effluent concentrations of ibuprofen, carbamazepine and naproxen detected were 30 ng/L, 420 ng/L, and 170 ng/L respectively (Lin et al., 2005). Nonetheless, Xu *et al.* examined selected antibiotics in the Victoria Harbour and the Pearl River, South China and the result were below the limit of quantification (LOD) in the marine water but all of the target compounds except from amoxicillin were detected in the Pearl River at concentrations ranging from 11 – 67 ng/L and 66 – 460 ng/L respectively (Xu et al., 2007).

Also another study conducted in the urban riverine water of the Pearl River Delta at Guangzhou, South China, revealed the presence of estrogenic hormone estrone with maximum concentration of 65 ng/L and acid pharmaceuticals such as salicylic acid, clofibric acid and ibuprofen were detected in most of the water samples with maximum concentrations of 2098, 248, and 1417 ng/L respectively (Peng et al., 2008). Following the uptake of PPCPs by plants, a study in Tianjin, China, most of the target antibiotics including sulfamethoxazole, sulfadoxine, sulfachloropyridazine, choramphenicol, tetracycline, lincomycin, chlortetracycline, ofloxacin, pefloxacin were detected in vegetables at the range of 0.1 – 532 µg/kg (Hu et al., 2010). Luo *et al.* reported the occurrence and transport of tetracycline, sulfaonamide, quinolone and macrolide antibiotics in the Haihe River Basin, China (Luo et al., 2011). The sources of 12 antibiotics studied were likely originated from veterinary application in swine farm and fishponds at concentrations ranging from 0.12-47 µg/L. Following the investigation level of six estrogens including diethylstilbestrol (DES), E1, E2, estriol (E3), EE2 and β-estradiol 17-valerate (EV) in both water and sediment sampled from three rivers in Tianjin area in China. The result showed that concentrations detected of all the six estrogens ranged from 0.98 - 51.6 ng/g dry weight in sediment and varied for each river (Lei et al., 2009). A more recent study in Taihu Lake, China, detected eight pharmaceutically active compounds such as roxithromycin, erythromycin, ibuprofen, diclofenac, propranolol, carbamazepine, E2 and EE2 in surface water and sediment samples with maximum

concentrations in the range of 8.74 - 118 ng/L and 0.78 - 42.5 ng/L dry weight respectively (Xie et al., 2015). Ma *et al.* also examined some pharmaceutically active drugs in Dongting Lake, China. The most frequently detected compound was caffeine followed by diclofenac, DEET, meclofenamic acid, fluoxetine, ibuprofen and carbamazepine with mean concentrations from 2.0 – 80.8 ng/L (Ma et al., 2016).

(Fang et al., 2012) also reported 4 pharmaceutical residues in wastewater STP in Northern Taiwan and in seawater around the effluent discharge area. The pharmaceutical concentrations measured from the influent were clofibric acid (104 - 109 ng/L), diclofenac (152 - 185 ng/L), ibuprofen (724 - 2200 ng/L), ketoprofen (128 - 184 ng/L). For effluent, the concentration for the 4 pharmaceuticals ranges from 95-102 ng/L, 100 - 131 ng/L, 552 - 1600 ng/L, and 68 - 128 ng/L respectively.

In sediment

Lei *et al.* studied level of six estrogens including diethylstilbestrol (DES), estrone (E1), b-estradiol (E2), estriol (E3), 17a-ethynylestradiol (EE2) and b-estradiol 17-valerate (EV) in surface water and sediment sampled from three rivers in Tianjin area, northern China. The concentrations of all six estrogens ranged from 0.98 – 51.6 ng/g in sediment and varied for each river (Lei et al., 2009). Yang *et al.* detected four classes of antibiotics such as sulphonamides, macrolides, fluoroquinolones and tetracyclines in sediment of Pearl River in China. Ofloxacin was found to be the highest concentration of 1560 µg/kg in sediment (Yang et al., 2010). In another study conducted by Liu and his colleagues high concentration of chloramphenicol and oxytetracycline were observed in the sediment of the Nanming River, Guiyang city, China during summer (Liu et al., 2009).

However, Ramaswany *et al.* studied antiepileptic, antimicrobial and preservative compounds in surface water and sediment from the Kaveri, Vallar and Tamiraparani River in the Pichavaram mangrove in India. The maximum concentration reported for the antimicrobial triclosan in sediment in the three rivers Kaveri, Tamiraparani and Vellar were 85.3, 46.87 and 32.1 ng/g respectively (Ramaswamy et al., 2011). A study by Zhou *et al.* reported the occurrence of 4 classes of commonly used antibiotics including sulphonamides, fluoroquinolones, tetracycline and macrolides in the sediments of the Yellow River, Hai River and Liao River in Northern China. Higher concentrations were detected for most antibiotics in the sediment of the Hai River than in sediment of the other rivers. The most frequently detected were norfloxacin, ofloxacin, ciprofloxacin and

oxytetracyclin in the three river with concentrations up to 5770, 1290, 653 and 652 ng/g respectively (Zhou et al., 2011). Another study examined the occurrence of antibiotics in water, sediments, aquatic plants and animals from Baiyangdian Lake in North China. The laboratory analysis revealed that sulphonamides were the dominant antibiotics in the lake while quinolones were prominent in sediments at concentration ranged 0.86 – 1563 ng/L and 65.5 – 1166 µg/kg respectively (Li et al., 2012).

In biota

The catastrophic decline in the vulture population in Pakistan was attributed to vultures consuming diclofenac-treated livestock. The detected diclofenac residue concentrations of 0.051 – 0.643 µg/g was found in the kidneys of 25 out of 25 vultures that died of renal failure (Oaks et al., 2004). Li *et al.* also detected 13 antibiotics in most of the hydrophyte sample (aquatic plants) such as Salvinia natans (Sal), Hydrocharis dubia (Hyd) and Ceratophyllum demersum (Cer) and four crustacean species including crab (*Eriocheir sinensis*), river snail (*Viva parus*), shrimps (*Macrobrachium nipponense*) and lobster (*Palinuridae*), 7 fish species topmouth gudgeon (*Pseudorasbora parva*), loach (*Misgurnus anguillicaudatus*), yellow catfish (*Pelteobagrus fluvidraco*) to mention but few from Baiyangdian Lake. The concentrations of antibiotics in Sal, Cer and Hyd were 1769 µg/kg, 253 µg/kg and 129 µg/kg respectively (Li et al., 2012). Other studies have also reported concentrations of ciprofloxacin in the aquatic plant (*Echinodorus amazonicus*) as high as 795.43 µg/kg (Zhang et al., 2009, Chen et al., 2007).

Liu et al also reported steroid estrogens with concentrations up to 11.3 ng/g dry weight in wild fish species such as crucian carp, carp and silvery minnow from Dianchi Lake in Southern China. Liver had the highest estrogen accumulation, followed by gill and muscle (Liu et al., 2011). Following the study of fluoroquinolones in marine aquaculture environment of the Pearl River Delta, South China, He *et al.* reported the accumulation of fluoroquinolones in *Siganus fuscescens* from Hailing island, *Sparus microcephalus* from Dapengao and *Lutianus argentimaculatus* from Hailing island at concentrations 254.58, 133.15, 5.18 ng/g wet weight respectively with concentrations higher in liver tissues than those in muscle tissues. The level of norfloxacin was higher than ciprofloxacin and enrofloxacin in both muscle tissues (He et al., 2012).

1.5.4. Measured concentrations in Australia:

Wastewater and surface water

There has been published data demonstrating the presence of numerous pharmaceutical compounds in effluents, river systems, marine sediments and sewage sludge in Australia as well as New Zealand. During a national survey of trace organic contaminants in Australian river, the most commonly detected contaminants among others were pharmaceuticals such as salicylic acid, paracetamol, carbamazepine and caffeine at different concentrations 1530 ng/L, 7150 ng/L, 682 ng/L, 3770 ng/L respectively (Scott et al., 2014). A study by (Watkinson et al., 2009) reported the occurrence of antibiotics in three hospital effluents, five WWTPs, six rivers and a drinking water catchment within watersheds of South-East Queensland, Australia. The result of the study found hospital effluent ranging from 0.01 - 14.5 µg/L, WWTP (influent and effluent) up to 64 µg/L and 3.4 µg/L respectively, river samples were up to 2 µg/L and not detected in drinking water.

Apart from the pharmaceuticals, (Ying and Kookana, 2007) detected an antimicrobial triclosan in wastewaters and biosolids from Australia WWTPs. The concentrations of triclosan ranged from 23 ng/L – 434 ng/L, 0.09 mg/kg – 16.79 mg/kg, 75 ng/L for the WWTPs effluents, the biosolids and the surface water respectively. Costanzo *et al.* reported detection of six antibiotics concentrations entering local waterways of South East Queensland Australian. Among the antibiotics investigated, cephalexin had the highest concentration of 2000 ng/L being the second most prescribed antibiotic in Australia (Costanzo et al., 2005).

In sediment

Few studies have been conducted in Australia in terms of monitoring pharmaceuticals in sediments. Sediment sorption is one of the mechanisms by which it is thought that pharmaceuticals may persist in the freshwater aquatic environment. However, Williams and Kookana (2010) investigated isotopic exchangeability to measure the available fraction of carbamazepine in river sediment collected from Mackreath Creek, Scott Creek Conservation Park, South Australia. The study demonstrated isotopic exchangeability as a relatively quick and simple alternative to batch desorption techniques for the assessment of the available fraction of the carbamazepine in sediments following their release into aquatic ecosystems (Williams and Kookana, 2010). A study by Stewart et al. reported the detection of various emerging contaminants including pharmaceuticals in

the estuarine receiving environment around Auckland, New Zealand. 21 out of 46 pharmaceuticals were quantified in one or more estuarine sediments with concentrations ranged from 0.2 – 7.7 ng/g. The highest concentrations were detected for acetaminophen (7.7 ng/g) and naproxen (5.5 ng/g) (Stewart et al., 2014).

1.5.5. Measured concentrations in Africa:

Wastewater and surface water

Currently, very little is known about the occurrence, fate and behaviour of PPCPs in the African freshwater aquatic environment. In most developing countries in Africa where the system of waste disposal is basically through landfill, it is essential that the occurrence of PPCPs be documented since some of these PPCPs are not easily degradable either through biodegradation or photodegradation. Apart from not being easily degradable, reports have shown that they can contaminate groundwater and through runoff may find their way into the aquatic environment, thereby putting the general public and aquatic environment at risk.

A class of pharmaceuticals known as the antimalarial such as artemether and lumefantrine drugs that is widely used in Africa for the treatment of malaria parasite, as well as amoxicillin at concentration ranging from 2.69 - 31.71 µg/L was detected in Tanzania (Miraji et al., 2016). A study in South Africa reported the presence of pharmaceuticals such as erythromycin, chloramphenicol, nalidixic acid, tetracycline, sulfamethoxazole, acetaminophen, atenolol, diclofenac, ibuprofen, caffeine and some others in the Umgeni River used for water supply in KwaZulu-Natal, South Africa. The report revealed that most of the studied compounds were detected in the wastewater at high concentrations while the surface water had the highest concentrations of some of the studied compounds (Agunbiade and Moodley, 2014).

Other authors (Matongo et al., 2015), reported residues of pharmaceuticals in wastewater and Msunduzi River, KwaZulu-Natal, South Africa. From their report, the antipyretic ibuprofen had the highest concentrations of 117 µg/L and 84.60 µg/L in wastewater and surface water respectively. The concentration of antibiotics in surface water were generally lower <10 µg/L but up to 34.50 µg/L in wastewater sampled.

In addition, (K'Oreje et al., 2012) detected 10 pharmaceutically active ingredients (PAIs) in Nairobi River. Out of the pharmaceutical classes detected the analgesic, anti-

inflammatory and antiepileptic were most concentrated PIAs at the range of about 30-35 µg/L, the antibiotics/antimalarial were up to 25-30 µg/L, while the antiretroviral were up to 10-15 µg/L. Another study reported the occurrence of 3 antibiotics such as sulfamethoxazole, trimethoprim, and ciprofloxacin as well as 3 antiretroviral; lamivudine, neirapine and zidovudine in the Nairobi River Basin, Kenya. The maximum (median) concentrations in the river water for both classes of pharmaceuticals were 13800 (1800), 2650 (327), 509 (129), 5430 (1000), 4860 (769), and 7680 (660) respectively (Ngumba et al., 2016).

Pharmaceutical were detected in surface and groundwater collected from an irrigation canal and wells in a pharmaceutical industrial area of Sango Ota, Ogun State, Nigeria. The average concentrations of the targeted pharmaceuticals such as diclofenac, chloroquine, paracetamol and ciprofloxacin HCl were 17.25 µg/L, 5.01 µg/L, 2.57 µg/L and 0.86 µg/L respectively (Olaitan et al., 2014).

To increase the limited information on the occurrence of pharmaceutical compounds in Nigerian environment a monitoring campaign study was conducted. Pharmaceutical residues in wastewater impacted surface waters and sewage sludge from Lagos, Nigeria were quantified using LC-MS/MS. For the surface water, detected concentrations ranging from limits of detection up to 8.84 µg/L for ibuprofen, and sewage sludge were detected with diclofenac concentration up to 1100 µg/kg dry weight (Olarinmoye et al., 2016).

In sediment

Agunbiade and Moodley investigated the occurrence and distribution of acid pharmaceuticals aspirin, diclofenac, ketoprofen and ibuprofen in wastewater, surface water and sediment samples collected from Msunduzi River, KwaZulu-Natal South Africa. The highest concentration in the sediment was observed for aspirin at the range of 212 - 427 ng/g. The concentrations of diclofenac, ketoprofen and ibuprofen detected in sediment were at the range of 57.2 - 309 ng/g, 6.68 – 57.4 ng/g and 4.78 – 11.2 ng/g respectively (Agunbiade and Moodley, 2016). Matongo *et al.* also detected ibuprofen in river sediment of Msunduzi River, KwaZulu-Natal, South Africa at concentrations as high as 659 ng/g (Matongo et al., 2015).

1.6. Human exposure to PPCPs

There are a substantial number of observations of PPCPs as environmental contaminants in surface water, groundwater as well as wastewater. There are two major routes through which human exposure to pharmaceuticals can occur and have totally different consequences and challenges with regards to their control and reduction. The first one is unintended, unexpected exposure through the consumption of drinking water and food containing pharmaceuticals residues which gain access to the environment as a result of their intended use. This implies a potential for unintended human exposure to PPCPs via drinking water supplies (Zuccato et al., 2000) or dietary intake such as fresh produce (Paltiel et al., 2016) irrigated with reclaimed or treated wastewater. Theoretically this route can lead to chronic, unintended exposure to low concentrations of complex mixtures of pharmaceuticals, as illustrated in Figure 2 above. The second route consists of unintended and purposeful ingestion of leftover drug waste which eventually result in acute, high level exposures, and is mostly responsible for significant human morbidity and mortality (Daughton, 2008). The adverse effects of environmental exposure of PPCPs on the general human population are largely unknown; however, significant evidence such as behavioural change (Kubec et al., 2019) and reproductive risks (Mart R Gross, 2005) have been demonstrated in aquatic organisms. Brodin *et al*. reported various effects of pharmaceuticals concentrations on aquatic species (Brodin et al., 2014). Although, depending on the type of WWTP, water treatment reduces the concentrations of these compounds to a reasonable extent, it is important to understand some regions especially developing countries have very few or no access to WWTPs. Therefore, it is paramount to monitor occurrences and understand the long-term accumulation of these chemical compounds as a result of drinking water containing traces of these PPCPs in human population as well as ecological implications for the aquatic organisms.

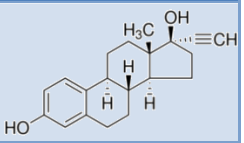
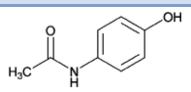
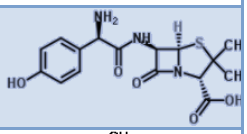
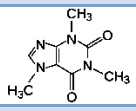
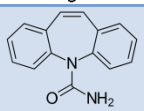
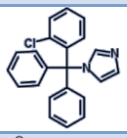
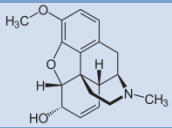
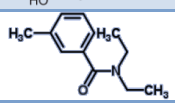
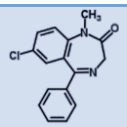
In view of conclusive evidence of PPCPs in various aquatic matrices, a list (Table.1) of most commonly and frequently detected PPCPs of different therapeutic groups, describing their physio-chemical properties were drawn for the purpose of this project. For a molecule to be considered a drug, its solubility and permeability is of importance during drug discovery and development settings. To ascertain that the list of compounds selected for this project are orally available drug, criteria such as the “Lipinski’s Rule of

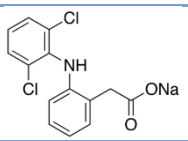
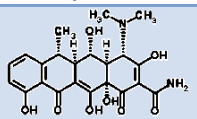
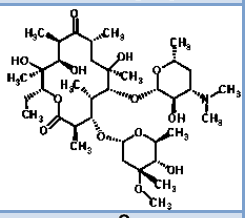
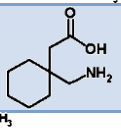
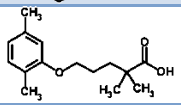
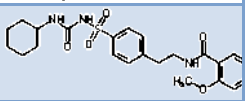
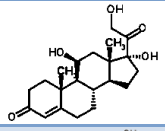
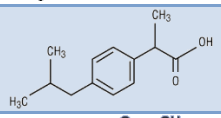
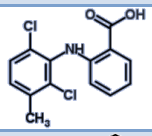
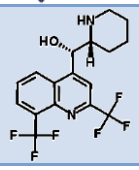
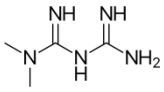
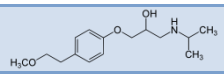
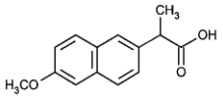
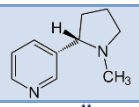
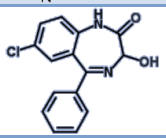
5" was considered. Whereby, poor absorption or permeation are more likely to occur when orally available drugs have;

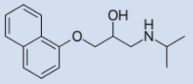
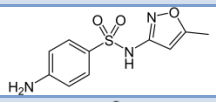
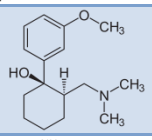
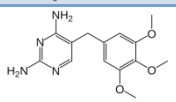
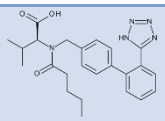
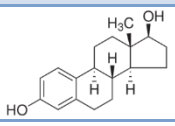
1. Molecular weight over 500 Dalton (Da)
2. Hydrogen bond donors are more than 5
3. Hydrogen bond acceptors are more than 10
4. Log Kow of over 5

Exceptions to the Rule of 5 are compounds that are substrates for biological transporters (Lipinski et al., 2001). Based on these rules, our target PPCPs in Table 1 met the requirements that makes them orally bioavailable except for erythromycin (molecular weight of 734.47 Da), which shows varying oral bioavailability (18-45%).

Table 1: Physio-chemical properties of the studied PPCPs

Compound	Pharmacological activity	Chemical Formula	CAS	Log Kow	pKa	Water Solubility (mg/L)	Chemical structure
17α-Ethynylestradiol	Steroid	$C_{20}H_{24}O_2$	57-63-6	4.12	10.24	116.4	
Acetaminophen	Analgesics	$C_8H_9NO_2$	103-90-2	0.27	9.86	3.04E+04	
Amoxicillin	Antibiotics	$C_{16}H_{19}N_3O_5S$	26787-78-0	0.87	2.40	1.00E+06	
Caffeine	Stimulant	$C_8H_{10}N_4O_2$	58-08-2	0.16	0.52	2.63E+03	
Carbamazepine	Anticonvulsants	$C_{15}H_{12}N_2O$	298-46-4	2.25	13.94	17.7	
Clotrimazole	Antifungal	$C_{22}H_{17}ClN_2$	23593-75-1	6.26	4.70	0.029	
Codeine	Opioid analgesic	$C_{18}H_{21}NO_3$	76-57-3	1.28	13.40	1.22E+04	
DEET	Insect repellent	$C_{12}H_{17}NO$	134-62-3	2.26	<2	173.5	
Diazepam	Sedative, hypnotic	$C_{16}H_{13}ClN_2O$	439-14-5	2.70	3.3	58.8	

Diclofenac sodium	NSAID	$C_{14}H_{10}Cl_2NNaO_2$	15307-79-6	4.02	4.18	4.5	
Doxycycline	Antibiotics	$C_{22}H_{24}N_2O_8$	564-25-0	1.36	3.40	312.9	
Erythromycin	Antibiotics	$C_{37}H_{67}NO_{13}$	114-07-8	3.06	8.8	2.01 E+03	
Gabapentin	Anticonvulsants	$C_9H_{17}NO_2$	60142-96-3	-1.37	3.68	2.11E+05	
Gemfibrozil	Anti-hyperlipidaemic	$C_{15}H_{22}O_3$	25812-30-0	4.77	4.75	8.42	
Glyburide	Antidiabetic	$C_{23}H_{28}ClN_3O_5S$	10238-21-8	4.79	4.32	0.06	
Hydrocortisone	Steroid	$C_{21}H_{30}O_5$	50-23-7	1.62	12.61	219.6	
Ibuprofen	NSAID	$C_{13}H_{18}O_2$	15687-27-1	3.79	4.41	41.1	
Meclofenamic Acid	NSAID	$C_{14}H_{11}Cl_2NO_2$	6385-02-0	6.02	4.21	0.09	
Mefloquine	Antimalarial agent	$C_{17}H_{16}F_6N_2O$	51773-92-3	3.85	8.6	248.89	
Metformin	Antidiabetic	$C_4H_{11}N_5$	1115-70-4	-2.64	12.27	1.00E+06	
Metoprolol	Beta-blocker	$C_{15}H_{25}NO_3$	56392-17-7	1.69	13.89	4.77E+03	
Naproxen	NSAID	$C_{14}H_{14}O_3$	22204-53-1	3.10	4.84	144.9	
Nicotine	Stimulant	$C_{10}H_{14}N_2$	54-11-5	1.00	8.00	1.00E+06	
Oxazepam	Sedative, hypnotic	$C_{15}H_{11}ClN_2O_2$	6801-81-6	3.34	1.7	20.7	

Propranolol	Beta-blocker	$C_{16}H_{21}NO_2$	318-98-9	2.60	13.84	228.0	
Sulfamethoxazole	Anti-bacterial	$C_{10}H_{11}N_3O_3S$	723-46-6	0.48	5.81	3.94E+03	
Tramadol	Opioid analgesic	$C_{16}H_{25}NO_2$	36282-47-0	3.01	14.47	1.15E+03	
Trimethoprim	Anti-bacterial	$C_{14}H_{18}N_4O_3$	738-70-5	0.73	7.04	2.33E+03	
Valsartan	Anti-hypertensive	$C_{24}H_{29}N_5O_3$	137862-53-4	3.65	3.56	1.4	
β-estradiol	Steroid	$C_{18}H_{24}O_2$	50-28-2	3.94	10.27	82.0	

A compound's octanol/water partition coefficient (Kow) which is expressed as Log Kow is the equilibrium ratio of the compound's concentrations in a mixture of two solvents, octanol and water. Both solvents are immiscible and therefore form two phases (Isac-García et al., 2016). Log Kow is an important characteristic of any chemical because of its ability to determine a fate of chemical both inside a living organism and the environment (Hermens et al., 2013). Log Kow values typically range between -3 (very hydrophilic) and +10 (extremely hydrophobic) (Cumming and Rücker, 2017). The Log Kow values of the selected PPCPs in the present study indicate the tendency for most of our target PPCPs to mainly remain in the water phase (highly hydrophilic; ranging from 0.16-3.94) instead of accumulation in sediment, sewage sludge or in aquatic organisms. In addition, a few are moderately (e.g. diclofenac sodium, 17α ethyneylestradiol, gemfibrozil and glyburide) to highly hydrophobic (e.g. clotrimazole and meclofenamic acid) (4.02-6.26), and hence, display a tendency to sorb to sediment or accumulate in aquatic organisms. The log K_{OW} values of our target PPCPs also dictate their binding behaviour to the reversed phase stationary phase used for their chromatographic separation prior to MS detection. The more polar compounds with low log K_{OW} values would have less interaction with the stationary phase and elute with shorter retention times. The reverse is true for less polar PPCPs (higher log K_{OW}), which will have longer retention times.

The influence of the acid-base dissociation constant, pKa on the pharmaceutical properties of drug and other chemical has long been established within the

pharmaceutical and chemical industry. **pKa** is the negative log of the acid dissociation constant (Ka). A lower **pKa** value indicates a stronger acid, which indicates the acid more fully dissociates in water (Kerns and Di, 2004). As the majority of drugs are weak acids and/or bases, knowledge of the pKa helps in understanding the ionic form a molecule will take across a range of pH values. For acidic compounds, the lower the pKa value (molecule predominantly ionized) the stronger is the acid, while the higher the pKa value (molecule predominantly un-ionized) the weaker the acid. The reverse is the case for basic compound, the lower the pKa (molecule predominantly unionized) is a weak base and the higher the pKa (molecule predominantly ionized) a strong base. The pKa of a drug has a great influence on lipophilicity, solubility, protein binding, and permeability which in turn directly affects pharmacokinetic characteristics such as absorption, distribution, metabolism and excretion (Manallack, 2007).

1.6.1. Basic principle of drug metabolism

For any orally administered drug to enter the bloodstream for distribution in the body, it must first be absorbed through the cell lining of the intestinal walls. Therefore, the drug passes through the hydrophobic lipid bilayer of the cell membrane and then through the hydrophilic aqueous cytosol before reaching the bloodstream. Because of the necessity to travel through both hydrophobic and hydrophilic environments, the compound's lipophilicity becomes an important physicochemical property that affects absorption, distribution, metabolism and excretion(Harris and Logan, 2014) as mentioned earlier.

Drug metabolism is mainly the chemical alteration of a drug in the body. The liver plays an important role in drug metabolism in the body as well as other organs such as the kidney, skin and gastrointestinal tract (Ilowite and Laxer, 2011). During metabolism, drugs are usually converted to more polar metabolites to facilitate their excretion and this process is catalysed by enzymic reactions. The reactions of drug metabolism occur in two phases. Typically, enzymes known as cytochrome P450 (CY450s) found in virtually all forms of life modulates the bioavailability of the drug molecule (Celiz et al., 2009). Phase 1 reactions of drug metabolism involve oxidation, reduction, or hydrolysis of the parent compound, thereby resulting in its conversion to a more functional polar molecule that either facilitate excretion or further metabolism. While the phase 2 reaction involves conjugation by linking the drug or its metabolites to another molecule with high energy cofactors such as glucuronidation, acylation, sulfate or glicine (Lu and Xue, 2019). Drug

metabolism could result in either development of less toxic metabolites or formation of more toxic compounds (Shehata, 2010). The metabolites formed during these processes are generally more hydrophilic which are eventually excreted either as metabolites (less toxic/toxic) and/or unchanged parent compounds through urine, faeces, sweat, sliver, tears, and breast milk (Kapusta, 2007), travel through the sewer networks and end up in WWTPs.

1.7. Wastewater based epidemiology

Wastewater based epidemiology (WBE) is an emerging tool for assessing chemicals such as pharmaceuticals used by large populations of people. Assessing human exposure to environmental contaminants can be very costly and time-consuming. Therefore WBE provides a potential alternative to conventional socio-epidemiological methods such as population surveys, crime statistic, medical record and surveillance (Castiglioni et al., 2013a). Wastewater analysis has been proven to be a promising monitoring technique to estimate different therapeutic drug consumption including illicit drugs use at community level (Lai et al., 2011). This technique is based on the principle that some proportions of the active parent compounds and/or their metabolites of any substance in the human body are excreted through urine and/or faeces into the urban sewer networks (Castiglioni et al., 2013a) and as a result end up in wastewater. Wastewater networks are municipal; therefore, the wastewater on its own contains different chemical residues representing whole communities. Wastewater analysis is representative of whole communities and can serve as a non-invasive approach to monitoring exposure and consumption of chemicals. This approach has been applied in a number of researches conducted in Australia coupled with Bayesian inference which allows any kind of information relating to population size, to estimate the population contributing to the wastewater using samples collected on census day (O'Brien et al., 2014, Lai et al., 2016). The major advantages of using data generated from wastewater analysis is that it provides a near-real-time estimate of drug use as well as the ability to measure average drug use in both small and large population in a given wastewater catchment. Apart from the aforementioned, it also offers flexibility to address emerging problems and identify previously unknown drug threats and consumption patterns (Commission, 1 March 2017).

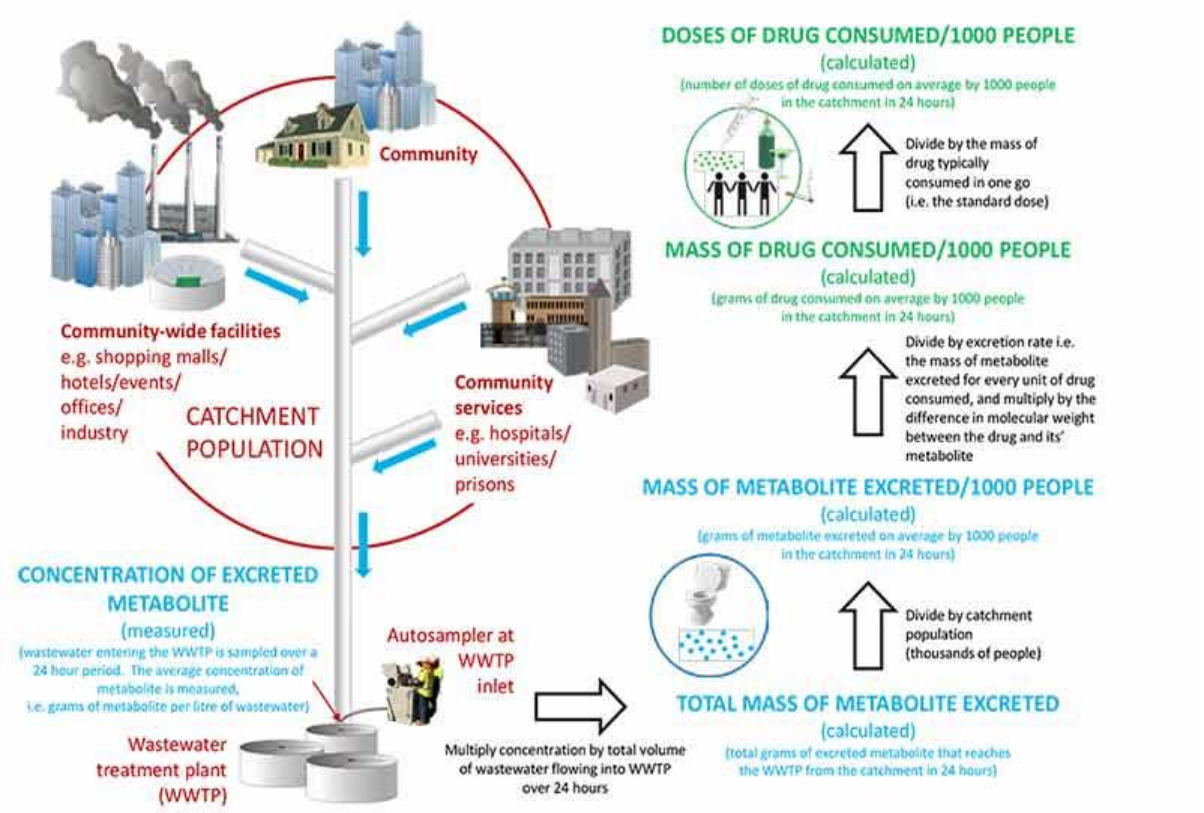


Figure 4: Schematic of the population catchment area and methodology employed to convert measure concentration of substances in wastewater to mass loads or doses consumed per day normalised population(Commission, 2017).

The idea of using this approach to determine collective drug usage at community level as well as to provide exposure data for aquatic biotic was suggested by (Daughton, 2001). This approach was first implemented in several Italian cities (Zuccato et al., 2005, Calamari et al., 2003), in Brussels, Belgium (van Nuijs et al., 2011). In the United Kingdom (UK), the same approach has been applied by many scientists (Baker and Kasprzyk-Hordern, 2013b, Kasprzyk-Hordern et al., 2008d, Archer et al., 2017b, Petrie et al., 2017).

1.7.1. Back-calculating chemical exposure/consumption from wastewater analysis

To back-calculate chemical exposure/consumption, several parameters are taken into account such as: (1) the concentrations of the target PPCPs (ng/L), (2) the flow rate of the WWTPs (Mega litre per day) in order to transform the concentrations into daily mass load and (3) the number of the population contributing to a given wastewater catchment.

Therefore, to calculate the chemical consumed on a per capita basis; measured concentrations of target compounds (C_i) in this case PPCPs in wastewater samples which are representative of whole days, multiplied by the daily flow rate (F), divided by the normalised population (P) number within a catchment (1000 people) and corrected for excretion factor (E), multiplied by molecular weight (Mw) of compound.

$$\textit{Daily chemical consumption}\ (\text{mg/day/1000 people}) = \frac{\boldsymbol{C_i \times F}}{\mathbf{P \times E}} \times \boldsymbol{Mw\ PPCP}$$

1.7.2. Uncertainties associated with wastewater-based epidemiology

Data generated using WBE involves many stages and each stage comes with its own uncertainty which often affects the overall quality of the final data. These uncertainties are related to the sampling (Ort et al., 2010), stability of the compounds, the analytical uncertainty driven by environmental and analytical chemists (van Nuijs et al., 2014). High uncertainty may also be related to the fluctuation of flow rate into WWTPs over 24 hours due to rainfall. Actual number of the population contributing to WWTPs may change due to commuting of people, events, tourism, as well as migration of labour forces in a certain catchment. Another sources of uncertainty may relate to mass of excreted metabolite/mass of drug consumed when back-calculating consumption data (Castiglioni et al., 2013b, Daughton, 2012).

The mass of excreted metabolites and parent drug consumed can be subject to huge uncertainty; because conjugated drug metabolites have the potential to undergo deconjugation during WWT processes and transform back to the original form of the parent drug consumed. Therefore, resulting in increased concentrations of the parent pharmaceuticals over time. A possible transformation of N4-acetylsulfamethoxazole back to its parent compound sulfamethoxazole leading to high variability of sulfamethoxazole elimination was reported (Göbel et al., 2007). The same observation was reported by Topp et. al., explain the increase in ibuprofen and acetaminophen in runoff of PPCPs following application of biosolids to an agricultural field (Topp et al., 2008).

1.8. Research Aims and Objectives

The selection of 30 PPCPs studied as representatives of different therapeutic groups listed in Table 1 were based on the priority pollutant lists developed by the EU under the Water Framework Directives (WFD), as well as the USEPA. Other selection criteria were based on frequent environmental occurrence, persistence and reported toxicity to aquatic organisms.

The selected PPCPs were investigated for their occurrence, fate and behaviour in freshwater samples from four different geographical locations. These includes two developed countries (Australia and United Kingdom) and two developing countries (Egypt and Nigeria). The rationale for selecting these countries was to understand the PPCPs concentrations/profiles in environmental waters in both developed and developing countries; as this may vary significantly depending on geographical location. For instance, the need for antimalaria drugs in countries (e.g. Nigeria) susceptible to malaria may not necessarily be needed in countries (e.g. Egypt) with no occurrence of malaria.

The number and type of samples collected in each of the studied countries (Egypt, Nigeria, UK and Australia) varied according to accessible sample locations, duration of sampling campaign and the objectives of each study in the specified country (i.e. seasonal variability in Nigeria vs. investigating the presence of PPCPs in wastewater for the first time in Egypt).

To achieve this aim, we develop an analytical method based on Q-Exactive Orbitrap high resolution accurate mass spectrometry that allows determination of a wide range of PPCPs in wastewater and surface water samples in a single chromatographic run. Using this method, we:

1. Determine concentrations of 30 pharmaceuticals and personal care products in wastewater and surface water from Egypt.
2. Investigate the occurrence, seasonal variation and human exposure to pharmaceuticals and personal care products in surface water, groundwater and drinking water in Nigeria.

3. Study the seasonal and spatial variation in concentrations of pharmaceuticals and personal care products in water from UK Rivers and canals.
4. Apply a WBE approach to estimate the population contributing to wastewater using samples collected on census day in Australia.

CHAPTER II

Analytical Methods

This chapter contains material taken verbatim from the following analytical paper publication: "ABOU-ELWAFA ABDALLAH, M., NGUYEN, K.-H., EBELE, A. J., ATIA, N. N., ALI, H. R. H. & HARRAD, S. 2018. "A single run, rapid polarity switching method for determination of 30 pharmaceuticals and personal care products in waste water using Q-Exactive Orbitrap high resolution accurate mass spectrometry." Journal of Chromatography A"

2.0. Introduction

Different analytical methods have been applied for the detection of PPCPs in environmental matrices by various scientists. The two most reported have been the use gas chromatography (GC) and liquid chromatography (LC) for detection of PPCPs in the environment. These two techniques are usually coupled to mass spectrometry (MS) methods which may use single-quadrupole MS, magnetic sector tandem MS/MS, triple quadrupole (QqQ)MS/MS, ion-trap MS (IT-MS), time-of-flight MS (TOP-MS), or hybrid quadrupole-TOF-MS (qTOF-MS) in the analysis of PPCPs (Hao et al., 2007).

Prior to analysis in either GC-MS, GC-MS/MS or LC-MS, LC-MS/MS environmental samples are prepared using extraction techniques such as liquid-liquid extraction (LLE) and solid phase extraction (SPE). The use of SPE has replaced traditional LLE as it allows the extraction of samples and clean-up at the same time. To extract target PPCPs, SPE cartridges packed by various sorbents such as ion-exchange phase, C-18, non-polar phase, and polymeric phase have been used by various authors (Stolker et al., 2004, Rodriguez-Mozaz et al., 2004, Batt and Aga, 2005). The use of Waters Oasis HLB (Hydrophilic-Lipophilic Balanced) and Oasis MCX (Mixed-Cation Exchange) has also been reported for preconcentration of both polar and non-polar compounds. Oasis MCX was employed in this thesis for multi-residue analysis of selected PPCPs. Section 2.4 provides further details on the SPE procedure used.

Most PPCPs are polar, non-volatile and thermally labile compounds with exception of some neutral drugs and fragrance ingredients (musks), therefore, making the use of GC separation unsuitable for analysis. The use of LC-MS analysis has a greater advantage

because of challenges associated with derivatization of hydroxly- and carboxyl- groups prior to GC-MS analysis of PPCPs. Derivatization of sample or analyte prior to GC-MS analysis is typically performed to alter the analyte properties for better separation as well as enhancing the method sensitivity (Moldoveanu and David, 2018). Derivatization involves the use of reagents for silylation, alkylation, or acylation reactions. These reagents react with active hydrogens for silylation reaction, active hydrogens on amine and acidic hydroxyl groups for the alkylation reaction, and with polar functional groups for the acylation reaction (Kyle, 2017). Some of the noted disadvantages of derivatization are formation of multiple derivatives, incomplete reaction, complexity of the method which could lead to low recovery analyte, time consuming as well as poor chromatographic reproducibility (Choi and Dong, 2005).

2.1. Overview

The analytical method for the determination of PPCPs in this thesis comprises the following steps: sampling, and analytical procedures i.e. filtration, extraction/clean-up and instrumental analysis. Samples of freshwater including surface water, groundwater, drinking water and wastewater were collected across the globe and analysed for identification and quantification of PPCPs. Details of sampling strategy and sample collection methods are given in later chapters. In this chapter, the analytical procedures as well as the materials used are described in detail. Method validation, quality assurance and quality control measures employed in the studies are also presented.

2.2. Materials

The analytical solvents used in the studies reported in this thesis were purchased from Fisher Scientific™ (Loughborough, UK) and were of UPLC grade. Individual standards of 30 PPCPs, in addition to isotope-labelled Caffeine-D9, Codeine-D3, Carbamazapine-D10, Estone-D4 and 4-Chlorophenol-2,3,5,6-D4 used as internal (surrogate) standards were purchased from Sigma-Aldrich™ (Irvine, UK) at the highest possible purity (>99 %). ^{13}C-tetrabromobisphenol A (^{13}C-TBBPA) and tris (2-chloroethyl) phosphate-D12 (TCEP-D12) used as recovery (syringe standards) were obtained from Wellington Laboratories (Guelph, ON, Canada). All standard stock solutions were prepared and further diluted in methanol. Oasis MCX and Oasis HLB cartridges (6 cm_3, 150 mg sorbent per cartridge) were obtained from Waters™ (Hertfordshire, UK). Ammonium formate (NH_4COOH), ammonium hydroxide (NH_4OH, 30 %), ammonium fluoride (NH_4F) and formic acid

(HCOOH) were obtained from Sigma-Aldrich™ (Gillingham, UK). Milli-Q water was used for cleaning and sample preparation purposes.

2.3. Glassware Preparation

To reduce any form of contamination of samples and standards, all glassware used during the experimental work were cleaned by washing with CleanPro™ washing up liquid (UK) and subsequently, rinsed thoroughly with Milli-Q water and dried up in an electric oven at 120°C. Prior to use, all glassware were rinsed at least three times with methanol (MeOH) to remove any organic residues, and then allowed to air dry in a fume cupboard.

2.4. Sample preparation and extraction

Individual and mixture stock solutions (0.5 g/L) of the targeted PPCPs (Table 1) were prepared in methanol and stored in dark amber vials at −20 °C. Working solutions were prepared fresh daily by diluting the stock solutions to the required final concentration and were stored at 4 °C for a maximum of 24 h. The isotope labelled internal standards were prepared and mixed separately at 1 ng/µL in methanol and kept in dark amber vials at −20 °C.

Extraction of environmental water samples was carried out by solid phase extraction (SPE) using Oasis MCX 6 ml cartridges and Waters™ 20-port controlled pressure vacuum manifold equipped with 50 Hz vacuum pump (Waters, Hertfordshire, UK). The SPE cartridges were pre-conditioned with 3 mL of methanol and equilibrated with 3 mL of Milli-Q water. 250 mL of the water sample were spiked with 100 ng of isotopically-labelled internal standards mixture and treated with 500 mg Na_2EDTA to release the free form of target analytes (e.g. Doxycycline) from potential complexes with Ca^{+2} and Mg^{+2} in environmental waters (Kasprzyk-Hordern et al., 2007). The samples were loaded onto the pre-conditioned cartridges at a flow rate of 5 mL/min. The cartridges were washed with 3 mL of 0.5 % HCOOH in Milli-Q water (3 mL/min). After drying, PPCPs were eluted with 5 mL of methanol following by 5 mL of 5 % NH_4OH in methanol. The combined eluate was dried under a gentle stream of nitrogen using a TurboVap II® evaporator (Biotage™, Sweden) and reconstituted in 100 µL of methanol containing 25 pg/µL of ^{13}C-TBBPA and TCEP-D12 used as recovery (syringe) standards for QA/QC purposes.

2.5. Instrumental Analysis

Extracted samples were analysed on a UPLC - Q Exactive Orbitrap™ - HRMS system (Thermo Fisher Scientific™, Bremen, Germany) composed of a Dionex Ultimate 3000

liquid chromatograph equipped with an HPG-3400RS dual pump, a TCC-3000 column oven and a WPS-3000 auto sampler. The UPLC is coupled to a Q-Exactive Plus Orbitrap mass spectrometer equipped with a heated electrospray ionisation (HESI) ion source.

The Q-Exactive Orbitrap mass spectrometer (Figure 5) was used because of its high and ultra-high mass resolution offered by the Orbitrap mass analyser. Q-Exactive Orbitrap MS uses accurate mass for identification of compounds at high scanning speed and rapid polarity switching of the heated electrospray ionisation source. The Q-Exactive provides unique low mass error (<1 ppm), as well as highly stable accurate mass calibration over a broad mass range. Orbitrap mass analyser has the ability to resolve analytes of interest from interferences by high resolution, accurate mass discrimination between ions of interest and interfering ions in the very low and low mass-to-charge (m/z) ranging from m/z 50-300 and m/z 300-1000, respectively. Another added advantage is its capability of accommodating a larger number of analytes in a single run as well as post acquisition screening of non-targeted analytes in a full scan mode (Bromirski, 2018).

Figure 5: Thermo Fisher Scientific Q-Exactive Orbitrap Mass Spectrometer (UoB workstation)

Chromatographic separation was achieved on an Accucore RP-MS column (100 x 2.1 mm, 2.6 µm) with 2 mM NH_4COOH/2 mM NH_4F in water (mobile phase A) and 0.5 % formic acid in methanol (mobile phase B). A gradient method at 400 µL/min flow rate was applied as follows: start at 2 % B, stay for 1 min; increase to 98 % B over 11 min, held for 1 min; then decrease to 2 % B over 0.1 min; maintained constant for a total run time of 16 min. Injection volume was 5 µL. The Orbitrap™ parameters were set as follows: alternate switching (-)/(+) ESI, sheath gas flow rate 50 AU (arbitrary unit), auxiliary gas flow rate 15 AU, spray voltage ± 4.5 kV, capillary temperature 275 ^oC, probe heater temperature 300 ^oC. The optimal MS parameters were: S-lens RF-level 50, resolution 17,500 FWHM (Full Width at Half Maximum) and scan 57 range 125 to 750 m/z. In each scan, the automated gain control (AGC) target in the C-trap was set at 1 x 10^6 ions and the maximum injection time (IT) was 50 ms.

For quantification purposes, isotope-labelled internal standards were used for proper method calibration. Isotope-labelled internal standards have the same physico-chemical properties of the native chemical, except for the difference in mass caused by the heavier isotope (e.g. deuterium, ^2H). Therefore, the isotope-labelled internal (surrogate) standards exhibit similar behaviour to the analytes during all stages of analysis. This allows for successful compensation of losses during the extraction and sample preparation step and any matrix effects in the MS ionisation source (Bodnar-Broniarczyk et al., 2019, Kasprzyk-Hordern et al., 2008c). While the isotope-labelled internal standard usually co-elutes with the unlabelled analyte/target compound because of the similarity of their chemical and physical properties (Figure 6), they can be easily separated based on the mass difference caused by the heavier isotope atoms (e.g. deuterium, ^2H) (Wang et al., 2007).

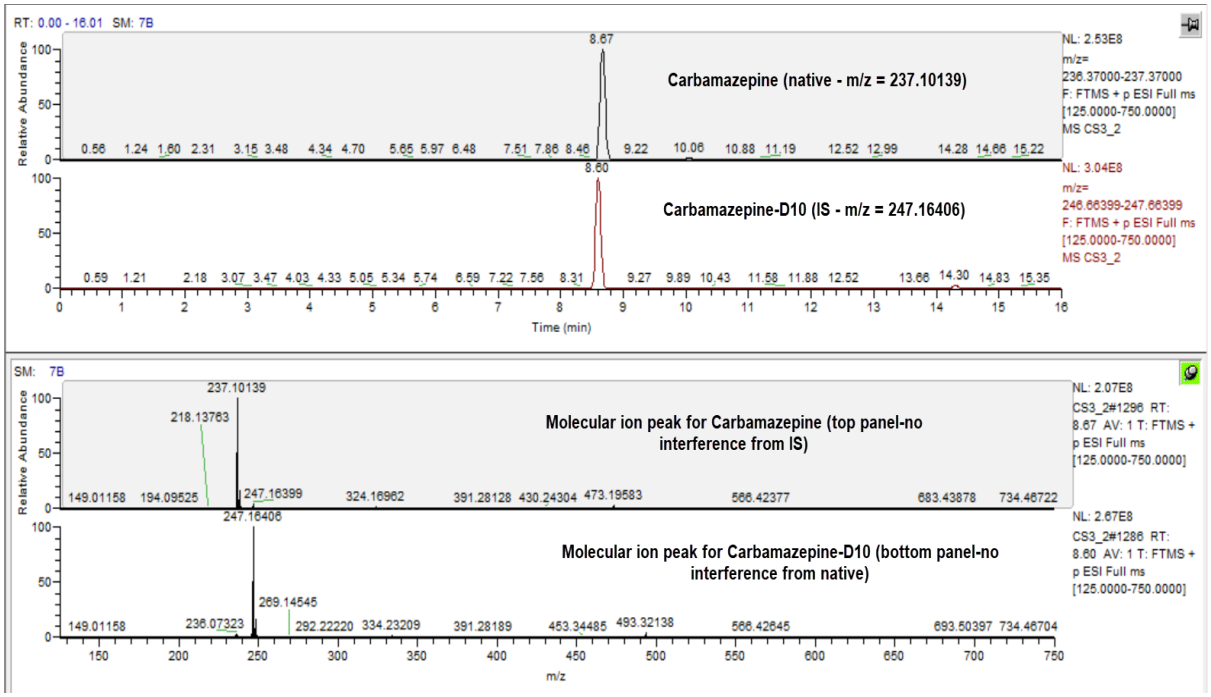


Figure 6: Representative example showing the chromatographic co-elution of Carbamazepine and its isotope labelled internal standard and their complete separation based on their accurate mass difference.

The use of Isotope labelled internal standard for each compound to compensate for variations in instrument response, ion-enhancement/suppression during analysis that behave exactly like the target compound during extraction and instrument analysis (isotope dilution method) would be ideal. However, this was not feasible in this project due to: (1) high prices for 30 compounds and some methods up to >100 chemicals, (2) unavailability of isotope-labelled standards for some target analytes, and (3) further complexity of the produced chromatograms/mass spectra and subsequent data analysis (García-Galán et al., 2016, Wu et al., 2013).

2.6. Method Validation parameters

Method linearity is the ability to obtain test results which are directly proportional to the concentration of analyte in the sample. Hence, this method was used to test the linear relationship between analyte signals and the concentrations in the calibration samples. In this case, a straight line was used to describe the relationship between LC-MS signal and analyte concentration (Seyed and Sussan, 2018). The method's linearity was investigated via triplicate injections of 6 points calibration standards for each of the studied analytes over a concentration range of 1–1,000 ng/mL, using a fixed

concentration of 100 ng/mL of the isotope labelled IS. Linearity was evaluated through the linearity coefficients (R^2) of the obtained calibration curves.

Other method validation parameters were calculated using Milli-Q water spiked with the target PPCPs at 3 concentration levels (10, 250 and 750 ng/mL).

Accuracy was estimated as the percentage recovery of target analytes and evaluated through the percent deviation from the known spiked concentration level.

Precision was calculated as relative standard deviation (RSD %) for inter- and intra-day multiple injections. Nine injections covering the 3 concentration levels (3 injections each) were used for assessment of precision. Further validation of method precision was performed via triplicate analysis of 3 different samples (spiked tap water, surface water from the River Nile and effluent sample A).

Limit of detection (LOD) and **limit of quantification** (LOQ) were estimated using the signal to noise (S:N) approach. Instrumental detection limit (IDL) was calculated as the lowest concentration that gives a S:N ratio of 3:1, while instrumental quantification limit (IQL) was calculated as the lowest concentration that gives a S:N ratio of 10:1. Method quantification limits (MQL) were determined by repeated injection of tap water samples spiked at low concentrations of target compounds. The concentration that produces a S:N ratio of 10:1 (+2 standard deviation of 5 replicate injections) was estimated as the MQL.

2.7. Quality assurance/quality control (QA/QC) Criteria

None of the target compounds were detected in method blanks (one blank for every 5 samples; each blank is composed of 250 mL Milli-Q water treated like a sample). Therefore, no blank correction of the results was required.

QC acceptance criteria for method accuracy and precision evaluation were adapted from US EPA method 1694 for PPCPs analysis in water by HPLC/MS/MS, whereby the RSD must be smaller than 30% and recovery must be within 55-120% (USEPA, 2007). High percent recoveries (>70 %) of all five internal standards were obtained indicating good overall performance of the method.

A calibration standard containing all the target compounds (500 ng/mL) and IS (100 ng/mL) was injected before and after each sample batch. For a given peak to be identified as a target analyte in a sample; the relative retention time (RRT) of the peak in the sample must be within ± 0.1 min of the average value determined for the same analyte in the 2 calibration standards ran before and after that sample batch.

2.8. Extraction method Optimisation

Several authors have documented the use of SPE as the method of choice for PPCPs in wastewater samples using various sorbent beds (Renita et al., 2017). In this present study, two of the most widely reported sorbent beds for extraction of various PPCPs were tested, namely: Oasis MCX and Oasis HLB. A paired t-test for comparison of means revealed no significant differences between the recoveries of target analytes in water samples spiked with 500 ng/L of all target PPCPs, n=3) from both solid phases (Figure 7). However, it was generally observed that a higher chromatographic baseline and more spectral interference occurred in real effluent samples extracted with HLB cartridges compared to MCX (Figure 8). This is in good agreement with the results reported by Petrie et al. (Petrie et al., 2016) and this can be attributed to the non-selective nature of the hydrophilic-lipophilic balance of reversed-phase HLB sorbent bed, which can cause significant matrix-related interferences when using ESI mode (Petrie et al., 2013). Therefore, Oasis MCX was applied for the extraction of all the samples in this project because it was built upon HLB copolymer with mixed mode cation-exchange and reversed phase interaction.

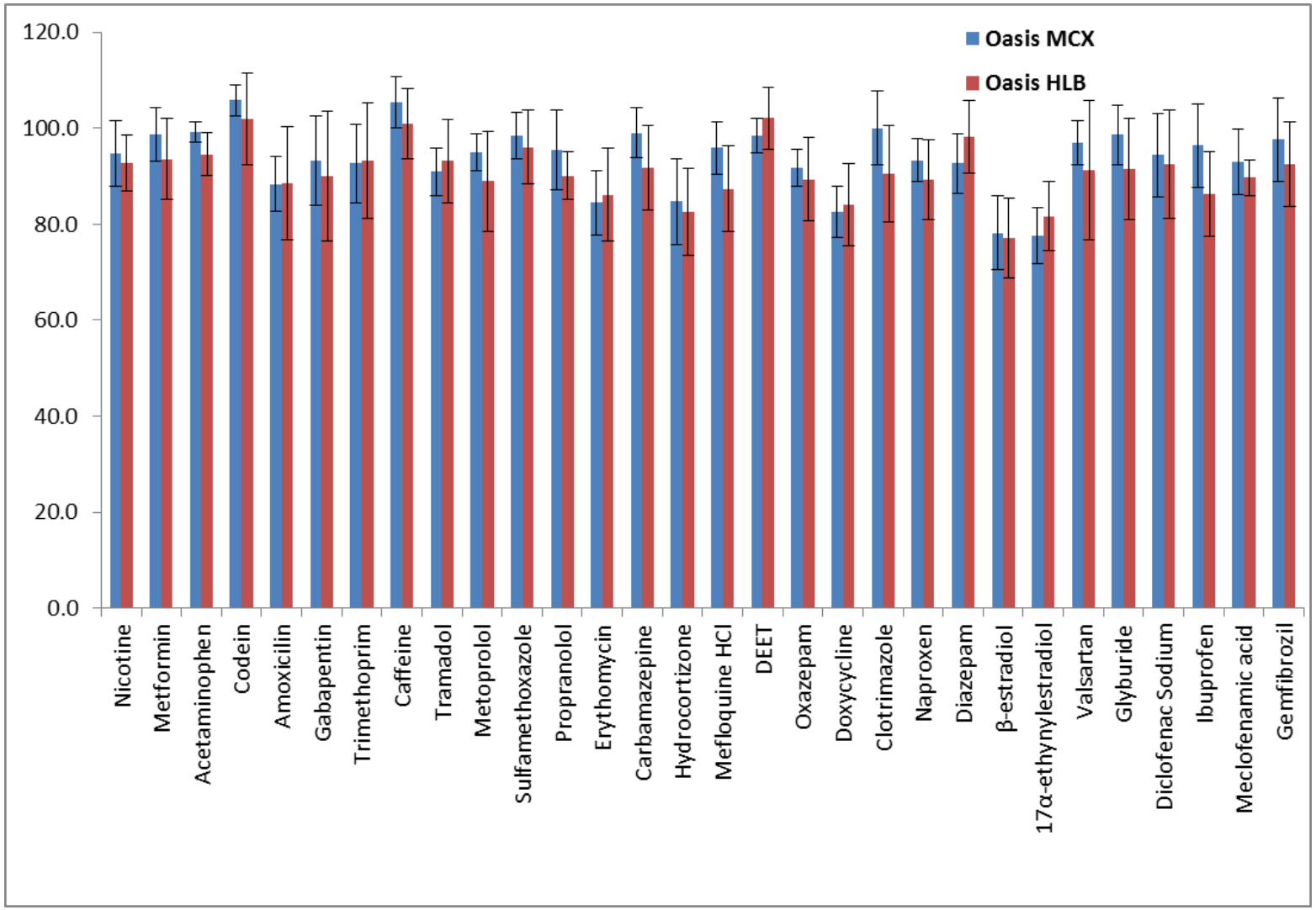


Figure 7: Average percent recoveries (n=3) of target PPCPs following SPE of spiked tap water (500 ng/L) samples using Oasis HLB and Oasis MCX cartridges. Error bars represent 1 standard deviation.

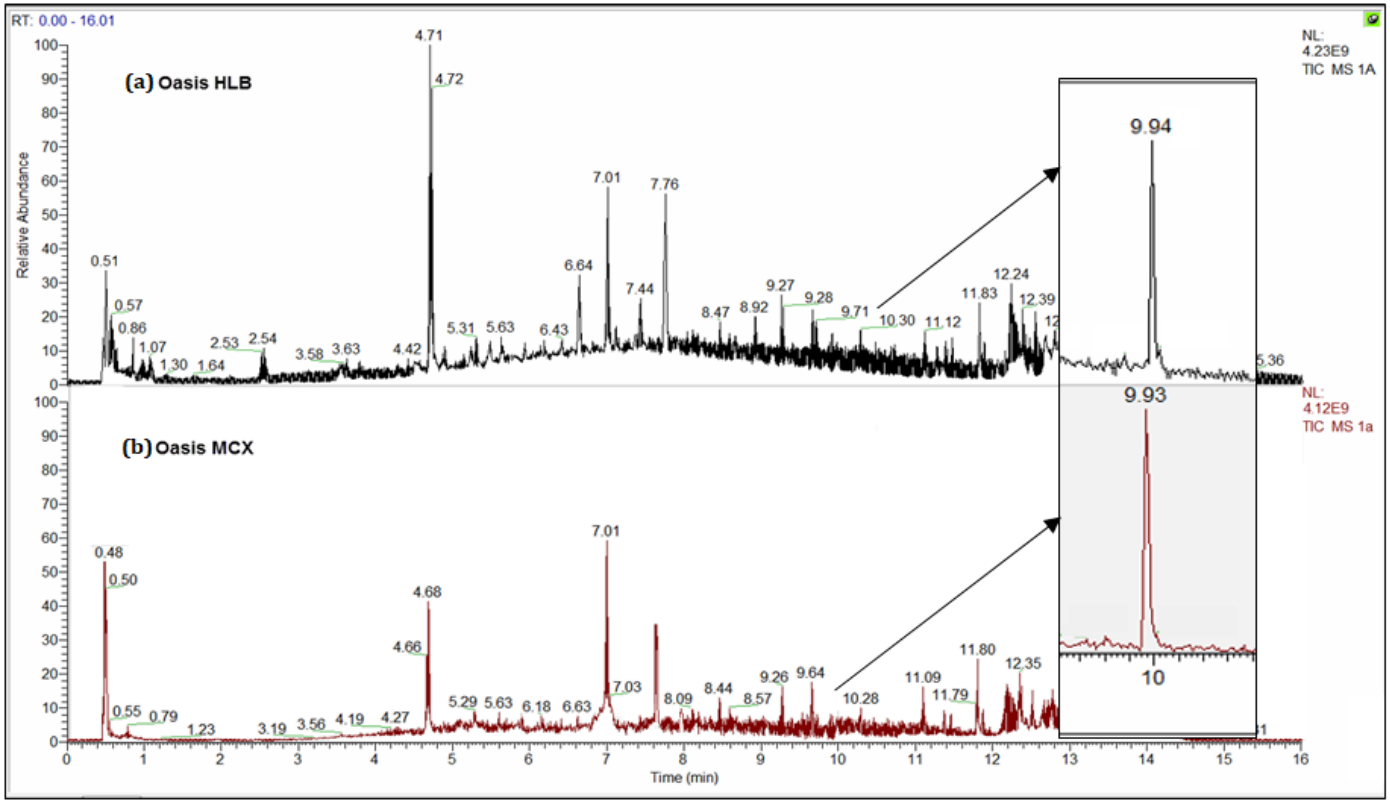


Figure 8: Total ion chromatogram of effluent sample 1A following SPE on (a) OASIS HLB crtridge (higher baseline) and (b) Oasis MCX (lower baseline). Inset shows the extracted ion chromatogram for Diazepam at m/z = 285.07928 (representative example) with a higher baseline in the Oasis HLB sample.

Oasis HLB is made specifically from a ratio of two monomers; hydrophilic N-vinylpyrrolidone and lipophilic divinylbenzene which provides superior reversed-phase capacity with a neutral polar "hook" for enhanced retention of polar analytes. However, Oasis MCX provides dual modes of retention; cation exchange and reversed phase on a single, clean, stable, high-surface-area, organic co-polymer that is stable from pH 0-14 (Waters Corporation, 2010).

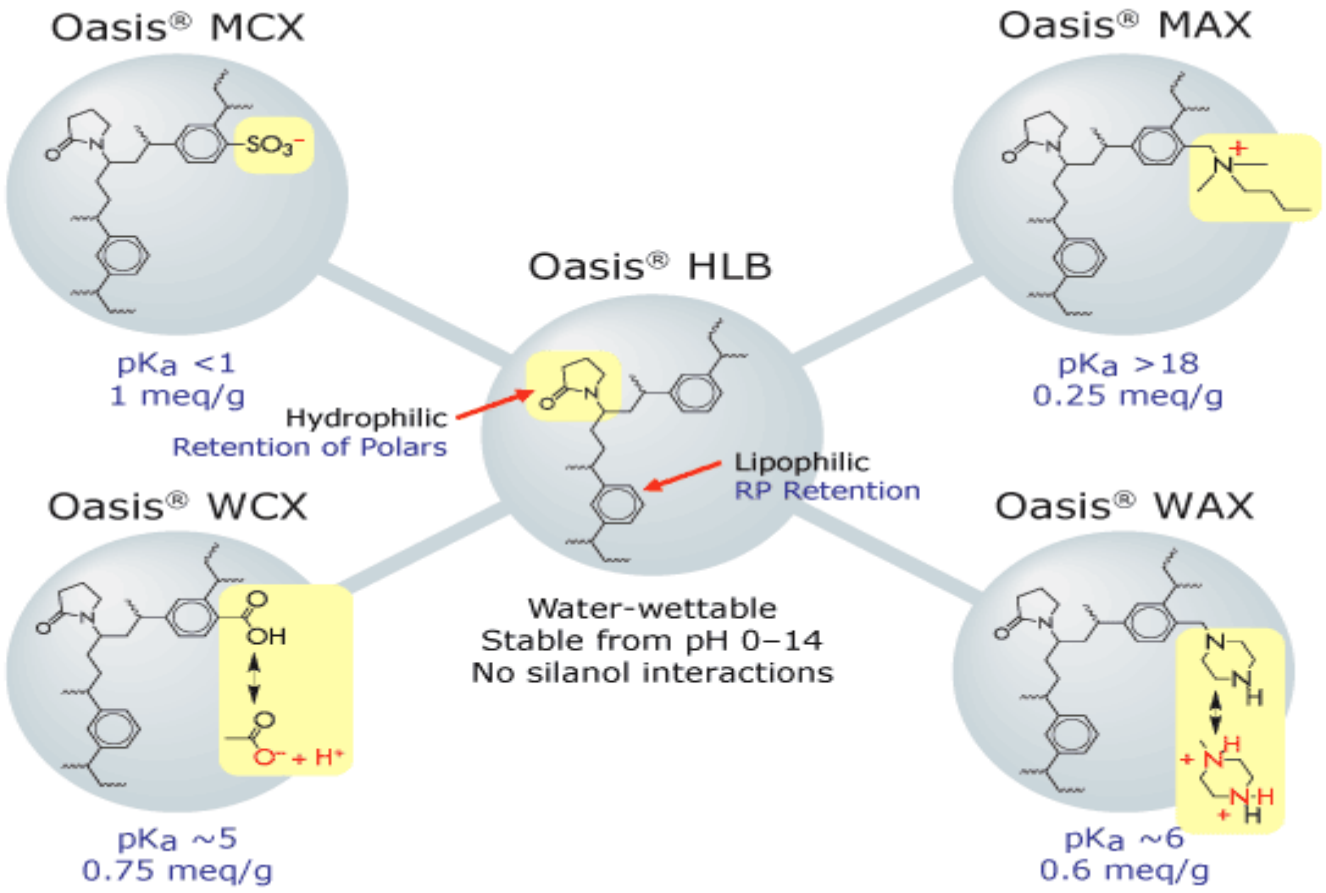


Figure 9: Chemical structures of different members of the Oasis "family" of SPE products

Table 2: Recovery of target PPCPs from spiked tap water samples (n=3) (500 ng/L of all target compounds).

	Exp.1	Exp.2	Exp.3	Average		SD	Recovery %
Metformin	487	498	437	474.0	±	32.5	94.8
Nicotine	514	462	506	493.8	±	27.6	98.8
Acetaminophen	484	497	505	495.5	±	10.3	99.1
Codeine	548	516	523	529.0	±	16.9	105.8
Amoxicillin	469	437	419	441.8	±	25.2	88.4
Gabapentin	460	426	513	466.3	±	43.8	93.3
Trimethoprim	447	436	507	463.1	±	38.1	92.6
Caffeine	531	553	496	526.8	±	28.5	105.4
Tramadol	480	437	447	454.5	±	22.6	90.9
Metoprolol	457	494	473	475.1	±	18.5	95.0
Sulfamethoxazole	498	512	466	491.8	±	23.7	98.4
Propranolol	444	520	468	477.2	±	39.3	95.4
Erythromycin	454	400	412	422.2	±	28.2	84.4
Carbamazepine	515	466	505	495.3	±	25.7	99.1
Hydrocortisone	408	395	467	423.5	±	38.2	84.7
Mefloquine	450	500	488	479.5	±	26.1	95.9
DEET	485	480	513	492.6	±	17.6	98.5
Oxazepam	471	467	438	458.7	±	18.1	91.7
Doxycycline	394	437	408	413.0	±	22.2	82.6
Clotrimazole	529	456	515	500.3	±	38.6	100.1
Naproxen	445	468	487	466.8	±	21.1	93.4
Diazepam	497	449	444	463.4	±	29.0	92.7
β-estradiol	379	425	368	390.5	±	30.0	78.1
17α-ethynylestradiol	370	414	381	388.4	±	22.7	77.7
Valsartan	489	505	460	484.7	±	22.6	96.9
Glyburide	489	465	526	493.2	±	30.3	98.6
Diclofenac sodium	439	518	458	471.8	±	41.2	94.4
Ibuprofen	474	527	445	482.0	±	41.7	96.4
Meclofenamic acid	454	501	440	464.9	±	31.9	93.0
Gemfibrozil	523	441	501	488.3	±	42.4	97.7

2.9. Results and Discussion

2.9.1. Chromatographic separation and mass spectrometry

Although baseline chromatographic separation of all analytes was not targeted due to subsequent MS analysis (Figure 9), the method was optimised towards achieving a better peak shape and higher intensity in subsequent ESI ionisation. Therefore, a simple mobile phase gradient based on H_2O and methanol was chosen due to the observed overall reduction of ESI signal intensity when using acetonitrile compared to methanol. This may be attributed to the reduced charge status of ionised species in the electrospray droplets by the neutral vapour of acetonitrile in the atmospheric region of the source (Hopper et al., 2012).

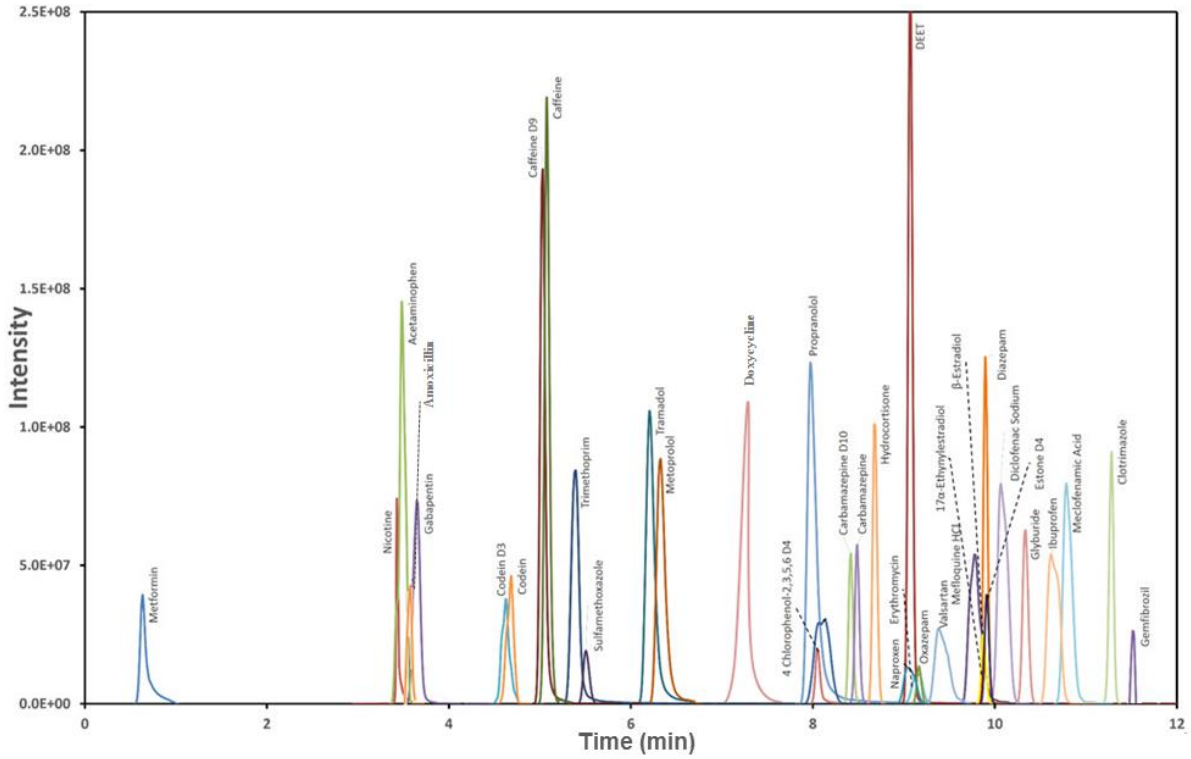


Figure 10: Reconstructed UPLC-Q-Exactive™ Orbitrap/MS chromatogram of target PPCPs and IS (500 ng/mL in methanol).

Acetic acid buffer had a substantial effect on enhancing the peak shape and signal intensity of basic analytes (Figure 11) via promoting their protonation in ESI positive mode (Petrovic et al., 2005). Moreover, the use of NH_4F as a mobile phase additive resulted in significant enhancement of signal intensity for the steroid hormones 17α-ethinylestradiol and β-estradiol by 360 % and 480 %, respectively. Petrie et al. reported

more than 400 % increase in the signal intensity for the steroid hormones E1 and E2 upon using NH_4F as a mobile phase additive for LC-ESI(-ve)-MS/MS analysis (Petrie et al., 2016).

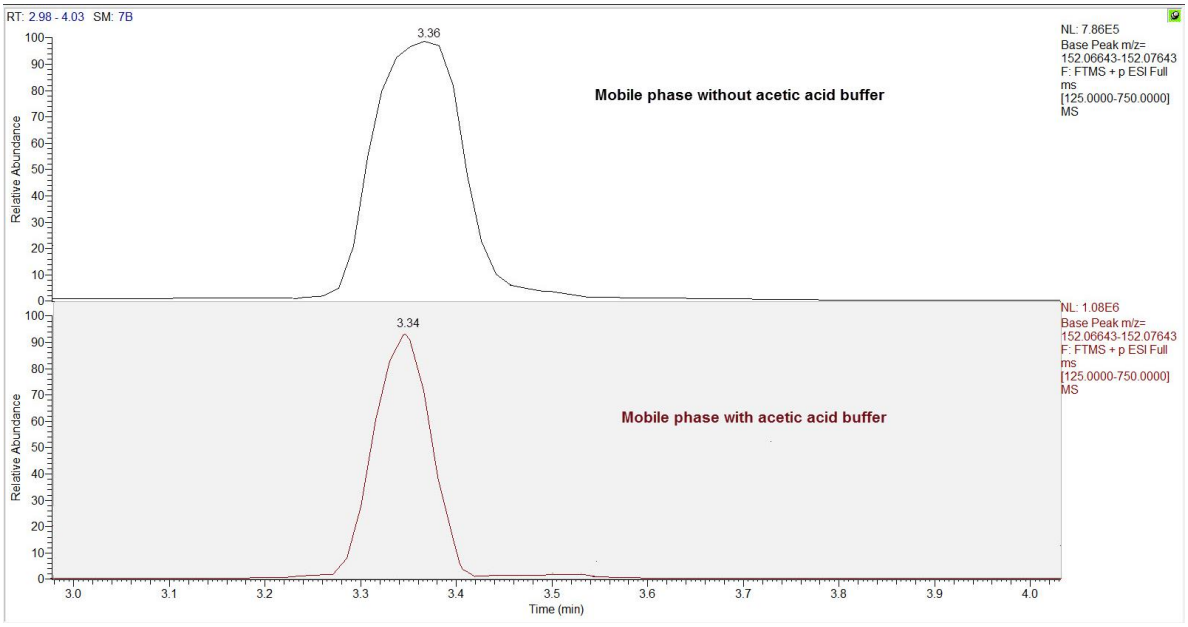


Figure 11: Extracted ion chromatograms (EICs) showing the effect of acetic acid buffer on the peak width of Acetaminophen as representative example of basic analytes.

Similarly, Carmona et al. reported NH_4F to improve the peak shape and signal intensity compared to ammonium formate for LC-ESI(-ve)-MS/MS analysis of various PPCPs including indomethacin, ibuprofen, diclofenac and gemfibrozil (Carmona et al., 2014). This may be explained by the strong electronegativity of the $[F]^-$ anion, which enhances deprotonation of the acidic analytes in ESI negative mode (Carmona et al., 2014). In the current study, NH_4F was used mainly due to significant enhancement of the signals for steroid hormones, while its influence on other acidic analytes (e.g. ibuprofen, diclofenac and gemfibrozil) was less evident (Figure SI-4) in the appendix II.

Several mass spectrometric parameters were optimised to maximise method sensitivity and achieve the highest signal/noise (S/N) ratio for the studied compounds. Individual standards of target PPCPs were directly infused into the Orbitrap mass spectrometer using a syringe pump at 20 µL/min in order to identify the most abundant ions and their respective ionization modes for each PPCPs and ISs. Most of the compounds studied

ionised abundantly in either positive or negative pseudo molecular ion: $[M+H]^+$ or $[M-H]^-$.

Out of the 29 PPCPs studied, it was observed that 17 of the compounds ionised in positive mode and 5 of them ionised in negative mode. Interestingly, 7 of the PPCPs were found to ionise well in both positive and negative mode and these include: naproxen, sulfamethoxazole, oxazepam, valsartan, meclofenamic acid, diclofenac sodium and glyburide (Table 3).

Table 3: Identification of PPCPs by LC-Orbitrap HRMS

Name	Therapeutic group	Chemical formula	Ionisation	Mass (Da)	t_R (min)	Internal standard
Metformin	Anti-diabetic	$C_4H_{11}N_5$	+ve	130.10884	0.64	Codeine-D3 (t_R= 4.63 min)
Nicotine	Stimulant	$C_{10}H_{14}N_2$	+ve	163.12318	3.43	Codeine-D3
Acetaminophen	Analgesic	$C_8H_9NO_2$	+ve	152.07143	3.46	Codeine-D3
Amoxicillin	Antibiotic	$C_{16}H_{19}N_3O_5S$	+ve	366.09687	3.53	Codeine-D3
Gabapentin	Anti-convulsant	$C_9H_{17}NO_2$	+ve	172.13417	3.65	Codeine-D3
Codeine	Narcotic analgesic	$C_{18}H_{21}NO_3$	+ve	300.16089	4.69	Codeine-D3
Caffeine	Stimulant	$C_8H_{10}N_4O_2$	+ve	195.08862	5.17	Caffeine-D9 (t_R= 5.13 min)
Trimethoprim	Anti-bacterial	$C_{14}H_{18}N_4O_3$	+ve	291.14540	5.40	Codeine-D3
Sulfamethoxazole	Anti-bacterial	$C_{10}H_{11}N_3O_3S$	+ve	254.05949	5.50	Caffeine-D9
Tramadol	Narcotic analgesic	$C_{16}H_{25}NO_2$	+ve	264.19584	6.20	Codeine-D3
Metoprolol	Beta-blocker	$C_{15}H_{25}NO_3$	+ve	268.19076	6.33	Codeine-D3
Doxycycline	Antibiotic	$C_{22}H_{24}N_2O_8$	+ve	445.14963	7.47	Codeine-D3
Propranolol	Beta-blocker	$C_{16}H_{21}NO_2$	+ve	260.16433	7.97	Codeine-D3
Carbamazepine	Anti-convulsant	$C_{15}H_{12}N_2O$	+ve	237.10333	8.49	Carbamazepine-D10 (t_R= 8.49 min)
Hydrocortisone	Steroid	$C_{21}H_{30}O_5$	+ve	363.21686	8.67	Carbamazepine-D10
Naproxen	NSAID	$C_{14}H_{14}O_3$	-ve	229.08824	9.05	4 Chlorophenol-D4 (t_R= 8.05 min)
DEET	insect repellent	$C_{12}H_{17}NO$	+ve	192.13931	9.07	Carbamazepine-D10
Erythromycin	Antibiotic	$C_{37}H_{67}NO_{13}$	+ve	734.47192	9.14	Carbamazepine-D10
Oxazepam	Sedative, hypnotic	$C_{15}H_{11}ClN_2O_2$	+ve	287.05860	9.17	Carbamazepine-D10
Valsartan	Anti-hypertensive	$C_{24}H_{29}N_5O_3$	-ve	434.22117	9.56	4 Chlorophenol-D4
Mefloquine	Anti-malarial	$C_{17}H_{16}F_6N_2O$	+ve	379.12231	9.78	Carbamazepine-D10
17α-ethynylestradiol	Steroid	$C_{20}H_{24}O_2$	-ve	295.17047	9.87	Estone-D4 (t_R= 9.91 min)
β-estradiol	Steroid	$C_{18}H_{24}O_2$	-ve	271.16998	9.88	Estone-D4
Diazepam	Sedative, hypnotic	$C_{16}H_{13}ClN_2O$	+ve	285.07928	9.89	Carbamazepine-D10
Diclofenac Na	NSAID	$C_{14}H_{10}Cl_2NNaO_2$	-ve	294.01031	10.06	4 Chlorophenol-D4
Glyburide	Anti-diabetic	$C_{23}H_{28}ClN_3O_5S$	-ve	492.13818	10.34	4 Chlorophenol-D4
Ibuprofen	NSAID	$C_{13}H_{18}O_2$	-ve	205.12297	10.61	4 Chlorophenol-D4

Meclofenamic acid	NSAID	$C_{14}H_{11}Cl_2NO_2$	-ve	294.01031	10.78	4 Chlorophenol-D4
Clotrimazole	Anti-fungal	$C_{22}H_{17}ClN_2$	+ve	345.11676	11.28	Carbamazepine D10
Gemfibrozil	Anti-hyperlipidaemic	$C_{15}H_{22}O_3$	-ve	249.15001	11.54	4 Chlorophenol-D4

For quantification purpose of compounds that ionizes in both positive and negative mode, the mode that produces higher intensity ions was selected. The compounds targeted eluted within the retention time range (0.64 – 11 mins) showing their broad polarity range. A 16 mins total runtime was applied to each sample in order to allow a reasonable time for column equilibration as well as avoid potential ion suppression due to co-elution of analytes.

Several mass spectrometric parameters were optimised to maximise method sensitivity and achieve the highest signal/noise (S/N) ratio for the studied compounds. While the Q-Exactive Orbitrap™ enables very high mass resolution (up to 280,000 FWHM), the scan (dwell) time increases with increasing mass resolution. Long dwell time per scan cycle results in broad chromatographic peaks due to few data points acquired per each peak as it elutes from the column. This ultimately leads to reduced overall sensitivity and lower quantitative reproducibility of the analytical method. Therefore, a minimum of 8-10 data points across an LC peak is required to define its shape and enable reproducible quantitation based on area under the peak, while an optimum of 15-20 points are required to expose subtle peak-shape features (Niessen, 1998). Another unique feature of the Orbitrap MS is the automatic gain control (AGC), which defines the maximum number of ions (from 2×10^4 - 4×10^6) to be injected into the mass analyser within a specified injection time (IT). To optimise for these multiple parameters, we adopted a systemic approach for each target analyte by studying the concomitant impact of mass resolution (up to 280,000 FWHM) and AGC (up to 4×10^6) on the peak area of the studied compound (Figure 9) with defined IT of 50 milliseconds and a minimum of 15 data points per peak. Despite few non-significant variations for a few compounds, results revealed the optimum MS parameters for the overall method as: resolution = 35000 FWHM, AGC target = 1×10^6 ions and IT = 50 ms.

Method selectivity and minimisation of potential interferences from co-extracted molecules in real samples were achieved via monitoring the molecular ion peak for each of the target compounds using its specified accurate mass (Table 3) with the following filters applied: maximum mass tolerance of 5 ppm, retention time window of 20 seconds

and relative retention time (to the designated labelled IS) window of 5 seconds. The extracted ion chromatograms (EIC) according to these filters showed well-defined correctly identified and appropriately integrated peaks in real water samples.

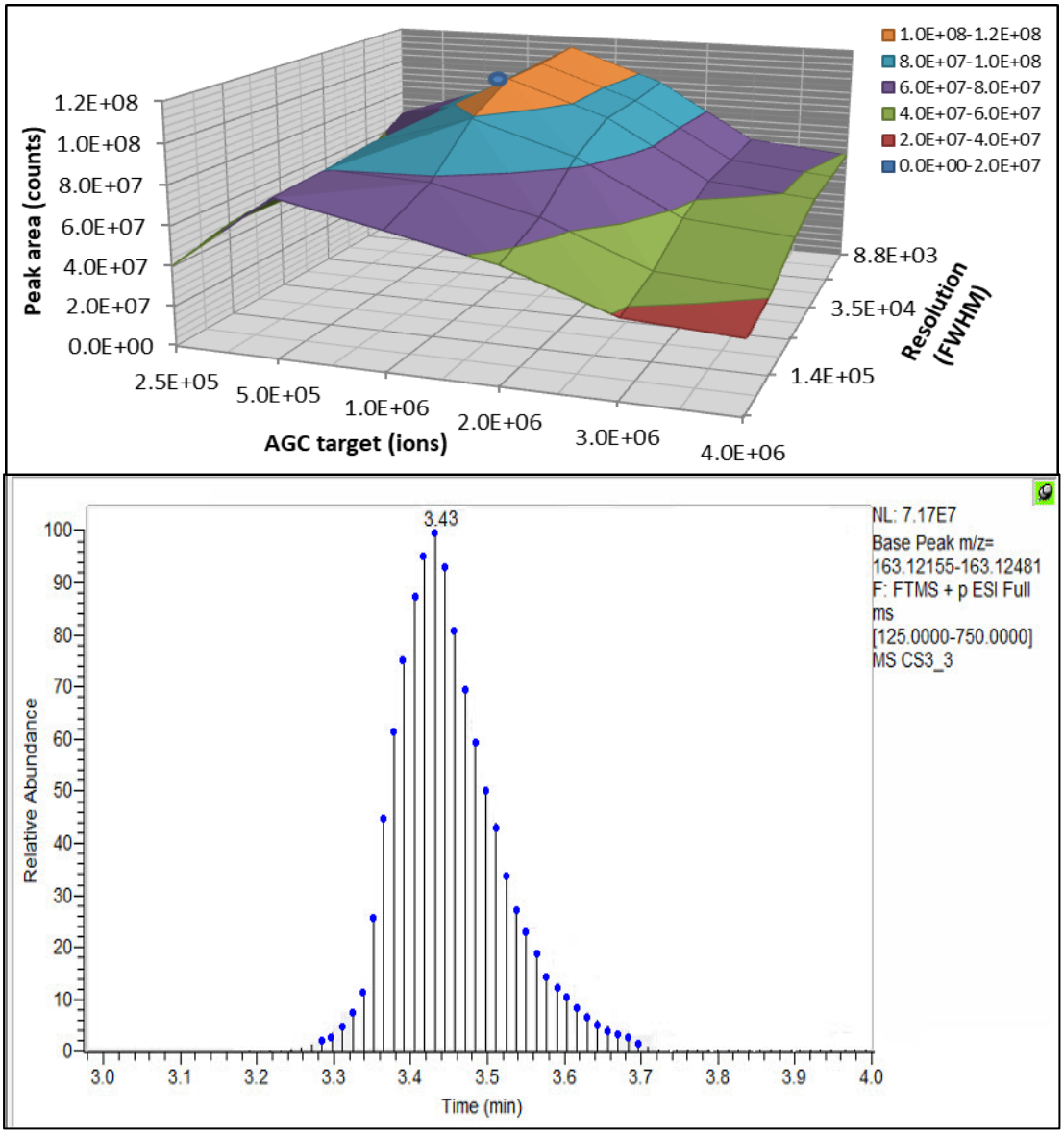


Figure 12: Representative example showing the impact of mass resolution (FWHM), Automatic gain control target (ions) on the peak area of Nicotine (750 ng/mL) and the number of data points per selected peak.

2.9.2. Validation of the Analytical Method

2.9.2.1. Method Linearity

The calibration plots displayed good linearity of the method over a wide concentration range of all target compounds. This was achieved by plotting the concentration of each target PPCP against the peak area ratio of each compound as well as its corresponding internal standard. The linearity coefficient (R^2) exceeded 0.99 for all target PPCPs with exception of 6 analytes where it ranged from 0.95 – 0.98 (Table 4). Figure 13 and 14 shows the linearity of nicotine and acetaminophen.

Table 4: Linear coefficient (R^2) and calibration equation for PPCPs analysis by UPLC-HRMS in this study

Name	R^2 - Linear	Slope	Equation – Linear
Nicotine	0.999	2.83E-03	Y = 0.0145297 + 0.00283335*X
Metformin	0.9972	1.96E-03	Y = 0.0108054 + 0.00195786*X
Acetaminophen	0.9938	4.05E-04	Y = 0.0166712 + 0.000405021*X
Gabapentin	0.9951	2.17E-03	Y = 0.00985349 + 0.00217336*X
Codeine	0.9984	2.05E-03	Y = 0.0343788 + 0.00204988*X
Caffeine	0.9951	1.89E-03	Y = 0.0385984 + 0.00189125*X
Trimethoprim	0.9975	2.68E-03	Y = 0.0494513 + 0.00268347*X
Sulfamethoxazole	0.9957	2.64E-03	Y = 0.0825096 + 0.00264225*X
Tramadol	0.9958	2.26E-02	Y = 0.336885 + 0.0226217*X
Metoprolol	0.9992	2.01E-02	Y = 0.182446 + 0.0201201*X
Propranolol	0.9957	2.85E-02	Y = 0.552799 + 0.0284973*X
Clofibric acid	0.9979	9.30E-03	Y = -0.0595296 + 0.00929926*X
Carbamazepine	0.9749	1.41E-03	Y = 0.156625 + 0.00140982*X
Hydrocortisone	0.9856	2.37E-04	Y = 0.014941 + 0.000236982*X
Naproxen	0.9629	9.16E-05	Y = 0.00795388 + 9.16462e-005*X
DEET	0.9524	5.59E-03	Y = 0.810959 + 0.0055861*X
Erythromycin	0.992	9.17E-04	Y = 0.00903905 + 0.000916565*X
Oxazepam	0.9923	4.46E-04	Y = 0.0157737 + 0.000445531*X
Valsartan	0.9951	2.90E-04	Y = 0.000961776 + 0.000289576*X
Mefloquine Hydrochloride	0.9937	1.63E-04	Y = -0.00259393 + 0.000163415*X
17a-ethynylestradiol	0.9952	9.16E-04	Y = 0.0290476 + 0.000915875*X
β-estradiol	0.9951	1.11E-03	Y = 0.0483223 + 0.00110658*X
Diazepam	0.9739	3.35E-03	Y = 0.190931 + 0.00334533*X
Diclofenac Sodium	0.9944	1.73E-04	Y = 0.00124698 + 0.000172746*X
Glyburide	0.9951	2.09E-04	Y = 0.00162146 + 0.000208631*X
Ibuprofen	0.9949	3.98E-04	Y = -0.00142091 + 0.000397643*X
Meclofenamic acid	0.9994	1.35E-04	Y = -0.00172738 + 0.000134729*X

Clotrimazole	0.9619	1.78E-04	Y = 0.0105712 + 0.000178402*X
Gemfibrozil	0.9906	2.91E-05	Y = -0.000708347 + 2.90714e-005*X

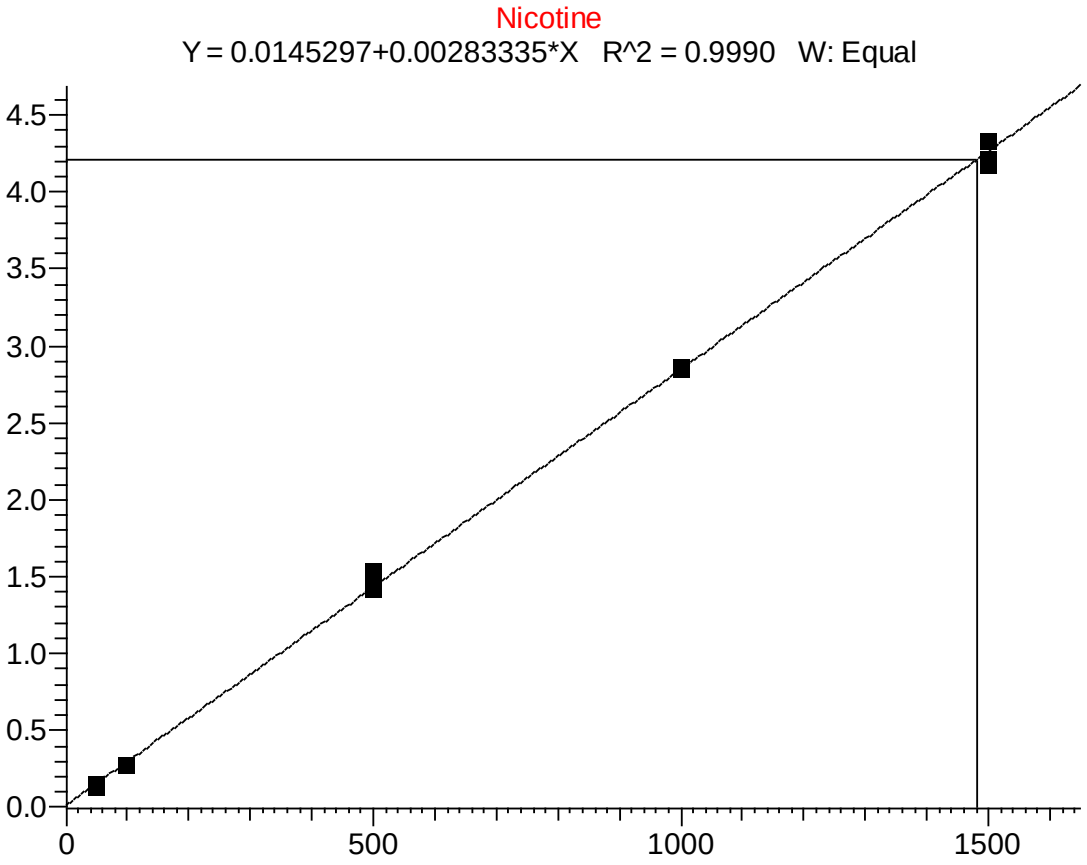


Figure 13: Schematic diagram showing linearity of Nicotine

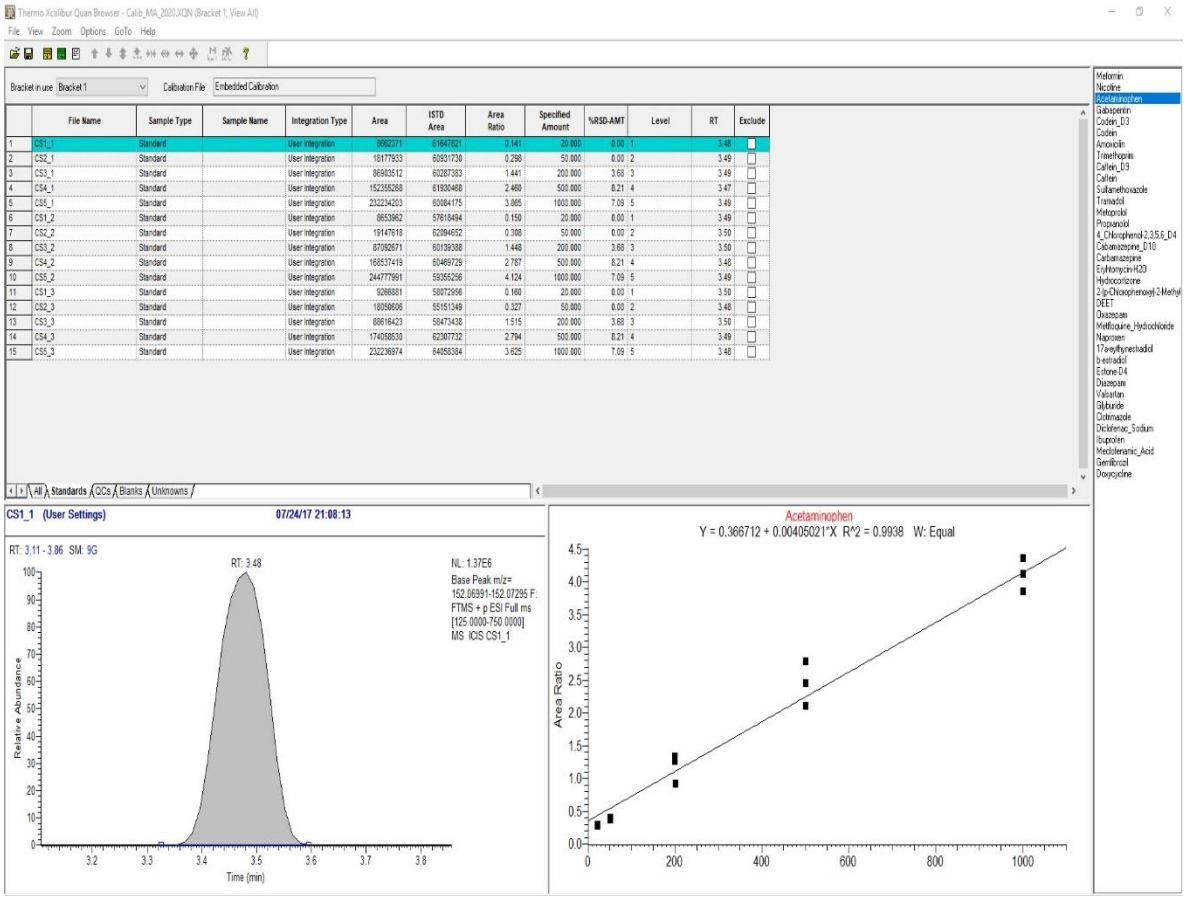


Figure 14: Quan bowser showing 5 point calibration for Acetaminophen

2.9.2.2. Method Accuracy and Precision

The accuracy of the method was estimated by the percentage recovery of target analytes and evaluated through the percentage deviation from spiked Milli-Q water samples ranged between 76.2 - 103.4 % at 3 concentration levels (Table 5). There was no significant difference observed between the recoveries of each analyte at the different concentration level, with the RSD all below 10 %, indicating good accuracy of the method.

Table 5: Method accuracy expressed as % recovery (±SD; n=3) from spiked Milli-Q water samples at 3 concentration levels.

	10 ng/mL	250 ng/mL	750 ng/mL	Mass accuracy (ppm)
Metformin	91.5 ± 6.6	90.8 ± 8.0	94.2 ± 2.9	1.1
Nicotine	91.2 ± 7.2	93.1 ± 8.6	95.3 ± 2.7	1.2
Acetaminophen	93.4 ± 7.8	98.1 ± 2.8	97.6 ± 2.8	1.6
Amoxicillin	88.1 ± 3.1	88.2 ± 3.3	89.5 ± 2.8	2.4
Gabapentin	90.7 ± 7.6	88.1 ± 3.7	92.6 ± 6.0	0.9
Codeine	92.7 ± 3.2	92.2 ± 3.8	91.2 ± 2.5	1.3

Caffeine	103.4 ± 5.9	101.1 ± 4.6	99.7 ± 4.2	0.9
Trimethoprim	95.6 ± 6.6	96.8 ± 2.6	96.4 ± 3.1	1.7
Sulfamethoxazole	93.2 ± 3.1	92.3 ± 3.1	93.0 ± 2.8	1.6
Tramadol	89.6 ± 5.8	93.8 ± 2.5	92.2 ± 3.5	2.0
Metoprolol	92.0 ± 2.9	93.6 ± 3.6	93.6 ± 3.3	1.2
Propranolol	95.2 ± 9.4	93.7 ± 5.6	97.8 ± 2.6	0.7
Doxycycline	86.3 ± 4.2	85.1 ± 4.0	85.7 ± 4.1	1.1
Carbamazepine	87.7 ± 2.8	88.0 ± 3.5	88.7 ± 3.3	0.8
Hydrocortisone	82.4 ± 5.1	84.0 ± 5.8	84.2 ± 3.7	1.5
Naproxen	89.0 ± 5.4	90.2 ± 4.2	91.4 ± 4.6	0.8
DEET	87.1 ± 8.1	95.8 ± 3.0	99.3 ± 2.1	1.5
Erythromycin	81.9 ± 4.2	85.6 ± 3.8	83.1 ± 3.2	2.7
Oxazepam	92.6 ± 6.8	96.5 ± 4.2	94.9 ± 4.8	2.1
Valsartan	86.6 ± 9.3	92.8 ± 8.0	98.2 ± 4.5	1.5
Mefloquine	85.7 ± 4.3	87.2 ± 5.1	87.2 ± 4.2	0.9
17α-ethynylestradiol	79.0 ± 6.2	78.4 ± 5.0	79.2 ± 3.9	1.7
β-estradiol	77.8 ± 5.1	76.6 ± 4.9	76.2 ± 5.7	1.4
Diazepam	93.4 ± 9.7	93.7 ± 7.1	97.5 ± 3.5	1.4
Diclofenac Sodium	89.9 ± 3.4	88.2 ± 4.3	89.9 ± 3.9	2.2
Glyburide	86.5 ± 4.8	87.9 ± 3.9	90.5 ± 4.7	1.5
Ibuprofen	91.7 ± 2.9	90.4 ± 3.5	90.7 ± 3.5	2.1
Meclofenamic acid	86.7 ± 5.7	85.7 ± 4.6	85.9 ± 3.9	2.3
Clotrimazole	102.4 ± 4.9	100.7 ± 3.5	102.4 ± 3.5	1.6
Gemfibrozil	89.0 ± 8.0	92.1 ± 7.6	95.8 ± 4.2	1.2

The precision of the methods was evaluated for repeatability (intra-day precision) and reproducibility (inter-day precision) at 3 concentration levels and the result (Table 6) showed a relative standard deviation value <15 % for all studied analytes.

Table 6: Method precision expressed as relative standard deviation (RSD %) of triplicate analysis of spiked Milli-Q water samples at 3 concentration levels

	Intra-day precision (Milli-Q water)			**Inter-day precision (Milli-Q water)**		
	10 ng/ml	**250 ng/ml**	**750 ng/ml**	**10 ng/ml**	**250 ng/ml**	**750 ng/ml**
Metformin	3.2	9.1	1.8	7.9	9.2	2.8
Nicotine	2.3	6.8	3.2	7.2	8.8	3.1
Acetaminophen	3.1	3.1	3.8	8.4	2.8	2.9
Amoxicillin	1.6	3.7	1.1	3.5	3.8	3.1
Gabapentin	8.8	6.0	8.4	8.4	4.2	6.5
Codeine	2.8	1.7	3.6	3.4	4.1	2.7
Caffeine	2.2	4.8	5.1	5.7	4.6	4.2
Trimethoprim	2.3	4.1	4.3	6.9	2.7	3.2

Sulfamethoxazole	1.4	1.5	4.2	3.3	3.3	3.1
Tramadol	2.2	2.8	5.4	6.5	2.7	3.8
Metoprolol	2.1	0.4	3.5	3.1	3.8	3.6
Propranolol	13.5	5.6	1.2	9.8	5.9	2.6
Doxycycline	4.6	4.1	5.2	4.8	4.7	4.8
Carbamazepine	2.2	5.6	3.9	3.1	4.0	3.8
Hydrocortisone	6.1	4.8	4.4	6.2	6.8	4.3
Naproxen	2.8	3.3	4.0	6.1	4.6	5.0
DEET	7.4	3.5	2.5	9.3	3.1	2.2
Erythromycin	5.4	1.8	2.4	5.1	4.4	3.9
Oxazepam	11.0	2.3	5.5	7.3	4.4	5.0
Valsartan	12.9	10.5	4.0	10.8	8.7	4.6
Mefloquine	5.2	8.0	5.8	5.0	5.9	4.8
17α-ethynylestradiol	8.2	2.9	4.8	7.9	6.4	4.9
β-estradiol	7.9	2.0	4.0	6.5	6.4	7.4
Diazepam	3.7	6.7	3.6	10.4	7.6	3.6
Diclofenac sodium	2.4	6.5	5.9	3.8	4.9	4.3
Glyburide	2.6	3.4	6.6	5.5	4.4	5.2
Ibuprofen	2.6	3.8	4.0	3.2	3.9	3.9
Meclofenamic acid	1.3	6.1	4.3	6.5	5.3	4.6
Clotrimazole	6.8	3.3	1.1	4.8	3.5	3.4
Gemfibrozil	11.0	4.6	6.6	8.9	8.3	4.3

2.9.2.3. Evaluation of matrix effects

This is the effect of co-elution of chemical components that of not of interest on the ionization of the target compound in the ESI source, therefore resulting in either signal suppression/enhancement or overlap of chromatographic peaks(Ohoro et al., 2019). This can negatively affect the reproducibility and accuracy of the development of the analytical method (King et al., 2000, Dams et al., 2003). This is why isotopically labelled internal standards were used to compensate for any matrix effects. It's also worth mentioning that the high analytical capabilities of the Q-Exactive orbitrap allows for the use of accurate mass with minimal mass error (< 5ppm) across the whole mass range, which greatly minimises matrix effects on the baseline of the chromatogram. Matrix suppression of the ESI signal for target analytes in real samples was evaluated using the matrix-matched calibration method described by Kasprzyk-Hordern et al. (2008). In summary, effluent sample (1A) and surface water sample (2G) were spiked with 500-1500 ng/L of each

target PPCP then the signal suppression for each target analyte was calculated in comparison to Milli-Q according to the equation:

$$Signal\ suppression\ (\%) = \left(1 - \frac{I_s - I_0}{I_{MQ}}\right) x\ 100$$

Where, IS is the PPCP peak area in the spiked effluent or surface water sample; I_0 is the PPCP peak area in the unspiked effluent or surface water sample; and I_{MQ} is the PPCP peak area in Milli-Q water spiked at the same concentration level as the effluent or surface water samples.

Table 7: Signal suppression (%) of target PPCPs in effluent and surface water.

Name	Signal Suppression (%)		Name	Signal Suppression (%)	
	Effluent	Surface water		Effluent	Surface water
Metformin	49	44	**Naproxen**	16	13
Nicotine	7	5	**DEET**	38	27
Acetaminophen	32	26	**Erythromycin**	36	32
Amoxicillin	43	41	**Oxazepam**	28	21
Gabapentin	24	22	**Valsartan**	26	25
Codeine	22	15	**Mefloquine Hydrochloride**	23	17
Caffeine	26	21	**17α-ethynylestradiol**	14	12
Trimethoprim	31	24	**β-estradiol**	17	11
Sulfamethoxazole	33	23	**Diazepam**	30	23
Tramadol	41	28	**Diclofenac Sodium**	37	24
Metoprolol	35	22	**Glyburide**	22	19
Propranolol	24	21	**Ibuprofen**	22	17
Doxycycline	29	26	**Meclofenamic acid**	29	19
Carbamazepine	26	29	**Clotrimazole**	31	30
Hydrocortisone	12	10	**Gemfibrozil**	22	18

The method precision was further evaluated on aquatic matrices including spiked tap water (at 500 ng/L), river water and effluent sample collected from a waste water treatment plant. The results (Table 8) revealed that effluent sample had the highest RSD, followed by the river water and the lowest was spiked tap water for the PPCPs detected.

Table 8: Precision for complex matrices expressed as RSD % for triplicate analysis of target PPCPs

Compounds	Spiked tap water	Surface water	Effluent sample
Nicotine	6.8	10.9	9.3
Metformin	5.6	6.9	11.6
Acetaminophen	2.1	5.9	5.1
Amoxicillin	3.8	7.4	12.2
Gabapentin	9.5	9.9	<MQL
Codeine	6	9.7	<MQL
Caffeine	8.2	8.3	5.4
Trimethoprim	1.5	7.6	11.2
Sulfamethoxazole	4.9	3.3	13.4
Tramadol	4.2	9.9	10.8
Metoprolol	4.8	<MQL	<MQL
Propranolol	8	11.9	15.1
Doxycycline	4	<MQL	<MQL
Carbamazepine	5.2	13.8	16.3
Hydrocortisone	6.2	7.5	12.6
Naproxen	5.4	<MQL	<MQL
DEET	3.6	<MQL	<MQL
Erythromycin	5.1	<MQL	8.5
Oxazepam	3.4	<MQL	<MQL
Valsartan	4.5	8.2	10.3
Mefloquine hydrochloride	6	12.1	<MQL
17α-ethynylestradiol	6.3	<MQL	<MQL
β-estradiol	4.9	<MQL	<MQL
Diazepam	6.3	<MQL	<MQL
Diclofenac Sodium	4.7	6.9	8.9
Glyburide	6.2	3.6	3.5
Ibuprofen	8.7	<MQL	11.3
Meclofenamic acid	8.8	6.8	6.3
Clotrimazole	6.9	8.9	13.5
Gemfibrozil	8.7	9.6	<MQL

This is not surprising as effluent samples contain a more complex cocktail of chemicals as well as more organic matter than river water. The result demonstrated similar precision values were obtained in comparison to the spiked Milli-Q water analysis.

Therefore, this method can be applied to other complex aquatic matrices without sacrificing precision and accuracy.

2.9.2.4. Method detection and quantification limits

The sensitivity of the method was illustrated by the limits of detection and quantification (Table 8). The IDLs that gave a S:N ratio of 3:1 were generated for the PPCPs studied and range from 0.02 to 1.21 ng/mL, while IQL values representing S:N ratios of 10:1 range from 0.07 – 4.05 ng/mL. Two possible explanations for the variation between the two detection limits were: 1. the polarity mode and/or variable ionization efficiency for different compounds; and 2. matrix effects or co-elution of analytes at a particular retention time. These two conditions could suppress the signal and therefore affect the instrument sensitivity. The method quantification limits generated here are in line with those for a previously reported UPLC-MS/MS method for the analysis of PPCPs in environmental water (Primel et al., 2012). Our data were obtained by analysis of spiked tap water containing target PPCPs at concentrations ranging from 2.4 - 83.8 ng/L (Table 9).

Table 9: Summary of IDLs, IQLs and MQLs for the developed PPCP analysis

Compounds	IDL (ng/mL)	IQL (ng/mL)	MQL (ng/L)
Nicotine	**0.50**	**1.67**	**13.30**
Metformin	0.10	0.33	9.50
Acetaminophen	0.10	0.33	2.80
Amoxicillin	1.10	3.67	22.40
Gabapentin	0.28	0.93	5.20
Codeine	0.23	0.77	5.00
Caffeine	0.80	2.80	7.20
Trimethoprim	0.04	0.12	2.40
Sulfamethoxazole	0.06	0.20	3.40
Tramadol	0.17	0.56	4.60
Metoprolol	0.02	0.07	2.70
Propranolol	0.04	0.14	4.70
Doxycycline	0.24	0.79	22.90
Carbamazepine	0.02	0.07	2.50
Hydrocortisone	0.34	1.13	37.80
Naproxen	0.09	0.30	4.70
DEET	0.11	0.37	5.70
Erythromycin	0.25	0.84	22.00
Oxazepam	0.15	0.49	6.30
Valsartan	0.32	1.05	8.60
Mefloquine	0.30	0.99	24.70
17α-ethynylestradiol	1.21	4.05	83.80

β-estradiol	1.16	3.87	81.00
Diazepam	0.13	0.43	4.70
Diclofenac sodium	0.15	0.50	9.80
Glyburide	0.30	0.99	12.90
Ibuprofen	0.12	0.41	8.90
Meclofenamic acid	0.17	0.57	10.30
Clotrimazole	0.36	1.19	16.30
Gemfibrozil	0.31	1.05	14.50

2.9.3. Wastewater-based epidemiology method

2.9.3.1. Analytical standards

A native standard stock solution mixture containing only high purity standards of PPCPs (Acesulfame, Atenolol, Caffeine, Carbamazepine, Citalopram, Codeine, Cotinine, N,N-Diethyl-*meta*-toluamide (DEET), Desmethyl citalopram, Gabapentin, Hydrochlorothiazide, Hydroxycotinine, Ibuprofen, Iopromide, Naproxen, Nicotine, Paracetamol, Paraxanthine, Salicylic acid, Tramadol, Triclosan, Venlafaxine, Verapamil) was prepared at concentration of 1000 nanogram per millilitre (ng/mL). This mixture was further diluted for calibration standards of different concentrations with Milli-Q water (Millipore, 0.22 µm filtered, 18.2 mΩ cm^{-1}). Labelled analytical standards were purchased from different suppliers and prepared at a concentration of 1000 ng/mL. Analytical grade hydrochloric acid 32% was purchased from Univar (Ingleburn, Australia). Liquid chromatography grade methanol was purchased from Merck (Darmstadt, Germany). Liquid chromatography grade acetic and formic acid were purchased from Sigma Aldrich (Castle Hill, Australia).

2.9.3.2. Chemical analysis

Analysis of wastewater containing many PPCPs is usually prepared for instrumental analysis by solid phase extraction and subsequent concentration of the eluate. For the purpose of WBE, we focussed on PPCPs that could be measured directly in filtered wastewater without the need for extraction and concentration. PPCPs were determined in waste water by HPLC-MS/MS using an AB/Sciex API6500^{+} Qtrap mass spectrometer (AB/Sciex, Concord, Ontario, Canada) equipped with an electrospray (TurboV) interface coupled to a Shimadzu Nexera HPLC system (Shimadzu Corp., Kyoto, Japan). Effluent wastewater samples were filtered (0.45 µm, 47 mm nylon filter membranes (Phenomenex, Lane Cove, Australia)). Labelled analogues of some analytes (Table 10)

were added to a 1 mL sample of filtered, acidified wastewater and 5 µL injected into the column.

Table 10: List of labelled internal standards

Labelled analogues	QI	Q3	RT	DP	EP	CE	CXP
Acesulfame D4	166	86	0.8	-25	-10	-20	-9
Ibuprofen D3	208.1	164	6.36	-30	-10	-10	-15
Hydrochlorothiazide 13CD2	298.9	269.9	2.54	-115	-10	-27	-21
Caffeine 13C3	198.3	140.1	4.65	60	10	24	17
Codeine d3	303.3	152	4.17	96	10	89	15
D10 Carbamazepine	247.2	204.1	5.9	65	10	28	18
Paracetamol-D4	156.1	114.1	1.46	80	10	22	13
DEET-D7	199.2	126.1	6.03	60	10	24	21
Venlafaxine D6	284.2	64	5.4	40	10	48	9
Gabapentin D10	182.1	164	3.4	45	10	19	14
Cotinine-D3	180.1	80	3.47	100	10	34	10

Q1: precursor ion, Q3: product ion, RT: retention time, ID: analyte, LOD/LOQ: limit of detection/limit of quantification, DP: declustering potential, EP: entrance potential, CE: collision energy, CXP: collision cell exit potential.

The instrument was optimized for each analyte of interest in manual tune mode. The instrument voltages affecting ion path was adjusted to result in maximum signal intensity. Optimization of the DP, EF, CE and CXP involves gradually changing the voltage range while monitoring the signal intensity of the compound.

The declustering potential (DP) maximizes analytes' entry into the MS optics without causing analyte fragmentation. This potential ranges from 0-300 V in positive mode and -300-0 V for negative mode.

The entrance potential (EP) guides and focuses the ion through the high pressure Q0 region. This parameter has a minor effect on compound optimization and typically set at 10 V for positive ions or -10 V for negative ions. It could also be set at default values without impact on the analyte detection limits.

The collision energy (CE) controls the potential difference between the first and second quadrupole Q0 and Q2. Basically, it is the amount of energy that the precursor ions receive as they accelerate into the collision cell, where they collide with gas molecules and fragment. This ranges from -300-300 V.

The exit cell potential (CXP) is the potential difference between the focusing lenses and filter positioned at Q2. It is only applicable in MS/MS-type scans, where ions are transmitted into Q3 (SCIEX, 2015).

Separation was achieved using a 2.6 micron, 50 mm x 2.0 mm Phenomenex Kinetic Biphenyl column (Phenomenex, Torrance, CA) run at 45 ^{o}C, and a flow rate of 0.3 mL min^{-1} with a linear gradient starting at 5% B ramped to 100% B in 5.2 minutes, held at 100% for 4.3 minutes followed by equilibration at 5% B for 4.5 minutes. (A = 1% methanol in HPLC grade water, B = 95% methanol, both containing 0.1% acetic acid). A 50 mm x 4.6 mm, 5 micron EVO C18 column (Phenomenex, Torrance, CA) was inserted between the pumps and autosampler to trap mobile phase contaminants. The mass spectrometer was operated in the positive/negative ion switching, scheduled multiple reaction-monitoring mode using nitrogen as the collision gas. Mass spectrometer parameters were set as Table 11.

Table 11: Target analyte optimization parameters

Target analytes	Q1	Q3	ESI	DP	EP	CE	CXP	RT
Acesulfame	162	82	-	-25	-10	-38	-8	0.85
Caffeine	195.1	138.1	+	71	10	26	16	4.66
Carbamazepine	237.2	194	+	86	10	29	14	5.9
Citalopram	325.3	109	+	70	10	36	14	5.87
Codeine	300.2	215.1	+	60	10	35	19	4.19
Cotinine	177.1	80	+	90	10	33	12	3.5
DEET	192.1	119	+	86	10	24	14	6.1
Desmethyl citalopram	311.3	109	+	60	10	33	10	5.88
Gabapentin	172.1	154	+	45	10	19	14	3.5
Hydrochlorothiazide	296	269	-	-90	-10	-26	-20	2.58
Hydroxycotinine	193.1	134.1	+	50	10	30	15	1.4

Ibuprofen	205.1	161	-	-32	-10	-9.5	-15	6.37
Iopromide	792	573.1	+	80	10	33	34	0
Naproxen	231.2	185.1	+	64	10	21	15	6.2
Nicotine	163.1	132	+	65	10	22	20	1.35
Paracetamol	152.1	110	+	56	10	22	14	1.5
Paraxanthine	181	124	+	40	10	27	14	3.92
Salicylic acid	137	93	-	-25	-10	-22	-8	4.1
Tramadol	264.2	58	+	40	10	42	8	5.01
Triclosan	287	35	-	-30	-10	-33	-15	6.76
Venlafaxine	278.2	58	+	40	10	48	9	5.42
Verapamil	455.2	183	+	110	10	35	19	6.23

Positive samples were confirmed by retention time and by comparing transition intensity ratios between the sample and an appropriate concentration standard from the same run. Sample were reported as positive if the two transitions were present, retention time was within 0.15 minutes of the standard and the relative intensity of the confirmation transition was within 20% of the expected value. The value reported was that for the quantitation transition. Data acquisition and quantification was performed using the Sciex software package Analyst Software 1.6 and MultiQuant 3.0 respectively.

2.9.3.3. Limit of detection and limit of quantification

Limits of detection (LOD) and limits of quantification (LOQ) were calculated based on replicate injections of 0.2ug/L spikes in MilliQ, saline and bore water (8 injections of each) and reported in Table 12. Calibration was linear from 0. 1 to 40 µg/L depending on sensitivity of compounds analysed.

Table 12: Limit of detection and quantification (µg/L)

PPCP	LOD	LOQ
Paraxanthine	0.02	0.05
Caffeine	0.02	0.05
Carbamazepine	0.02	0.05
Citalopram	0.04	0.1
Codeine	0.04	0.1

DEET	0.02	0.05
Desmethyl Citalopram	0.04	0.1
Gabapentin	0.02	0.05
Iopromide	0.04	0.1
Naproxen +ve	0.04	0.1
Paracetamol	0.02	0.05
Tramadol	0.04	0.1
Venlafaxine	0.04	0.1
Salicylic acid	0.04	0.1
Acesulfame	0.02	0.05
Ibuprofen	0.2	0.5
Verapamil	0.04	0.1
Nicotine	0.06	0.2
Cotinine	0.04	0.1
Hydroxycotinine	0.04	0.1
Triclosan	0.04	0.1
Hydrochlorothiazide	0.04	0.1

2.9.3.4. Quality assurance and quality control

To ensure accurate quantification of target PPCP, rigorous quality assurance and quality control was implemented. All standards and samples were treated equally, analysed in duplicate and the relative difference of the replicate samples were less than 15%. For the laboratory blank, three Milli-Q water samples were analysed in the same manner as the effluent samples. A set of calibration standards was run at the beginning and end of the sequence with additional calibration standards after every tenth sample. The influence of matrix effects was minimised by using deuterated internal standards.

2.9.3.5. The Australian Bureau of Statistics (ABS) Population Count

A population census in Australia takes place every five years. Sampling for this study was carried out either on or around the 2016 ABS census day. The WWTPs studied provided a geographical map with boundaries of the catchment they serve. With the help of the ABS, the population connected to each WWTP was determined by intersecting georeferenced census data available at the administrative level "Statistical Area 1" with the catchment maps. The data returned by the ABS was for both *Enumerated Population* (count based on where people spent most of their time on census day; hence *de facto*

population) and *Usual Population* (count based on residential addresses; hence *de jure* population). *De facto* population provided by the ABS was used for the purpose of this study.

2.9.4. Back calculations

2.9.4.1. Daily mass load

For back calculations, knowledge of several parameters is taken in to account such as; (1) the concentrations of the target PPCPs (ng/L), (2) the daily flow rate of the WWTPs (Mega litre/day) in order to transform the concentrations into daily mass load. Back calculation steps have been described and applied widely in literatures (Zuccato et al., 2005, Zuccato et al., 2008, Castiglioni et al., 2013a) and this was achieved by multiplying the measured concentrations of PPCPs by the daily flow rate of the WWTPs provided by the WWTPs operator.

Daily mass load (mg/day) = concentration x WWTPs flow rate

2.9.4.2. Relationships between PPCPs mass loads with population

To determine the relationship between the chemical mass loads with the *de facto* population two criteria were selected to identify these chemicals. Criteria 1: in all of the samples collected, the chemical must be measurable by means of direct injection on the LC-MS/MS. Criteria 2: there must be a correlation between the chemical mass load and the population size. On those grounds, the calculated mass loads for the chemicals measured in the samples were then plotted against the population provided by the Australian Bureau of Statistics (ABS).

CHAPTER III

Application of developed method to Egyptian surface water and wastewater

3.1. Synopsis

The developed analytical methodology in chapter 2 of this thesis was successfully applied for the determination of 30 PPCPs (Table 3) in the Egyptian freshwater aquatic environment for the first time. The aim of this work was to investigate the occurrence and concentrations of PPCPs in river water and effluent samples of waste water treatment plants (WWTPs) collected from the Egyptian city of Assiut. The presence of PPCPs in environmental waters is unintentional and this has been attributed to inefficient removal of these chemical compounds during conventional waste water treatment processes (Abou-Elwafa Abdallah et al., 2018). Therefore, we hypothesize that concentrations of PPCPs in WWTP effluent samples will be higher than those in the Nile river water samples. Since this study is the first of its kind in Egypt, PPCPs concentrations in the studied water samples will be compared to reported PPCPs levels from other African cities. This will facilitate understanding the significance of PPCPs as a class of emerging contaminants in the Egyptian environment within the context of other developing countries in Africa.

3.2. Sampling

The sampling campaign was conducted by researchers at the faculty of pharmacy, Assiut University, Egypt between 15th October to 15th November 2017. Effluent samples of 1 L each were collected from 5 waste water treatment plants (WWTPs) in Assiut governorate, Egypt. These include 3 major WWTPs in Assiut city (Al Helaly, Nazalat-Abdellah and El Walidiyaah), the water treatment plant of Sodfa town, in addition to the water treatment plant of Assiut University hospitals. Assiut governorate is one of Egypt's ancient governorates, belonging to the Middle Upper Egypt region. It lies between two mountains with a very hot and dry climate. Assiut has a population of over 4 million people with majority of the population (3.1 million people) living in the rural areas with a further 1.1 million people in the urban areas. Surface water samples were also collected from the River Nile and El-Ebrahmiya canal in Assiut city. These were grab samples collected upstream of the WWTP discharge point in clean glass bottles and immediately

transferred to the lab in ice boxes. The samples were stored at 4 °C until extraction within 48 hrs of collection following the SPE procedure mentioned in section 2.4. The SPE cartridges were couriered in an iced-pack to University of Birmingham, UK for further extraction and instrumental analysis. On arrival, cartridges were placed in a freezer at –20 ˚C until further processing within 24 hours of receipt. Each cartridge was eluted with 5 mL MeOH followed by 5 mL of 5% ammonium hydroxide in methanol (NH_4OH). The eluate was evaporated to dryness under a gentle stream of nitrogen and reconstituted in 200 µL of 8:2 water/methanol solution before instrumental analysis on UPLC - Q Exactive Orbitrap MS.

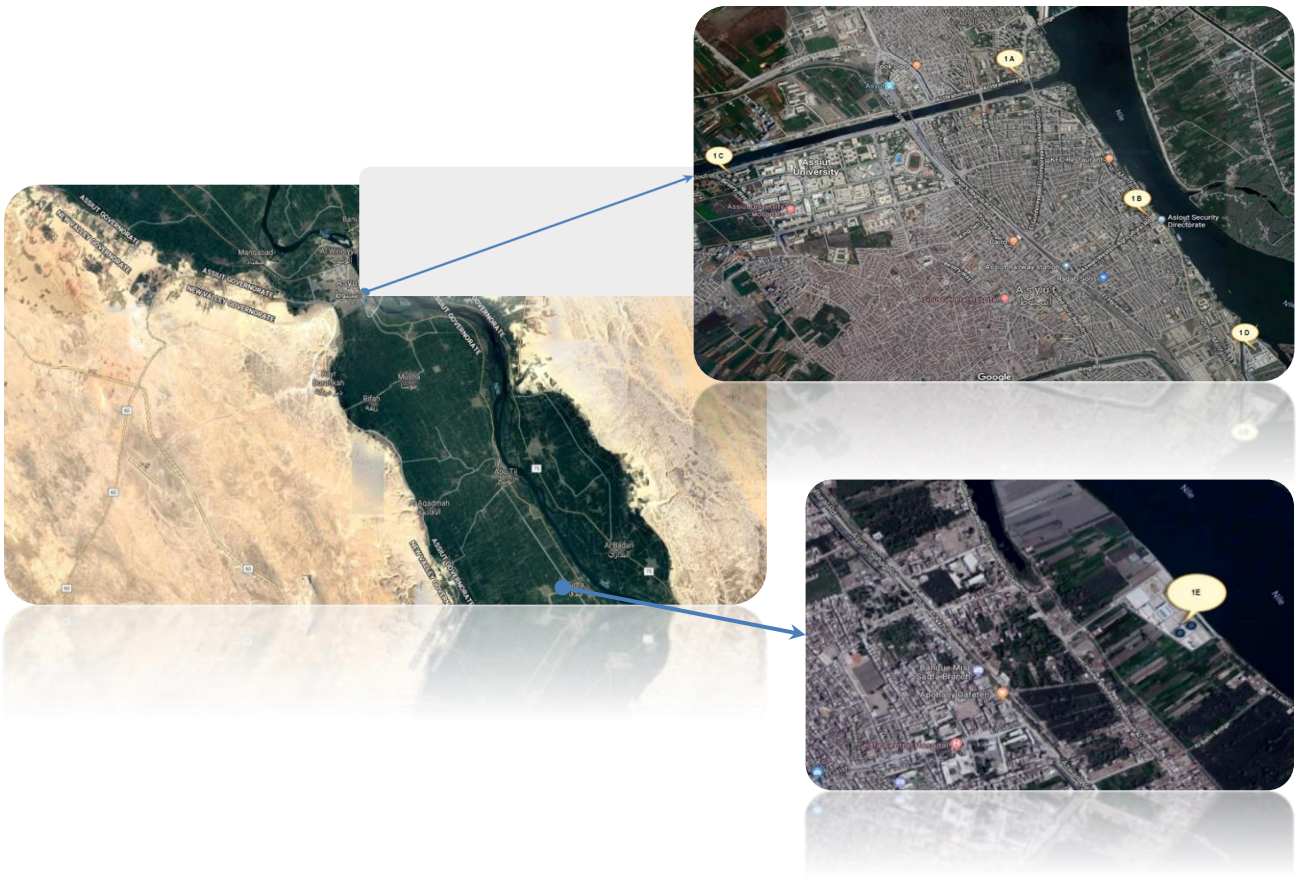


Figure 15: Google map of Assiut Governorate, Egypt showing the locations of the WWTPs sampled in the current study.

3.3. Results and discussions

All target PPCPs were successfully detected and quantified in the analysed samples, with exception of the anti-malarial drug mefloquine hydrochloride and the insect repellent DEET. Mefloquine hydrochloride and DEET concentrations were less than half MDL. Concentrations of PPCPs in effluent samples exceeded those in surface water samples (Table 12A and 12B). This finding is similar to previous studies (Ebele et al., 2017, Kasprzyk-Hordern et al., 2008c, Ternes, 1998). Agunbiade and Moodley reported that concentrations of antipyretics in Kwazulu-Natal, South Africa surface water samples were much lower than in effluent wastewater. They found concentrations of ibuprofen to range between 1060 and 1380 ng/L in effluent samples while concentrations in the surface water ranged from 445 to 689 ng/L (Agunbiade and Moodley, 2016). A similar finding was observed in a German study, where the maximum concentration found in effluent (6300 ng/L) was much higher than that of river water (1100 ng/L) (Ternes, 2001).

Out of the 30 PPCPs studied here, acetaminophen, tramadol, ibuprofen, metformin, glyburide, trimethoprim, valsartan, hydrocortisone, caffeine and nicotine were detected in all samples, indicating their high consumption and prescription frequencies in the studied area. The PPCPs present at the highest levels were acetaminophen (140-16000 ng/L), ibuprofen (30-6700 ng/L), metformin (20-5600 ng/L), glyburide (250-4200 ng/L), diclofenac sodium (40-3600 ng/L), and trimethoprim (270-2700 ng/L). These concentration ranges are similar to those reported in other African countries such as South Africa (Archer et al., 2017a, Madikizela et al., 2017) and Nigeria (Olarinmoye et al., 2016). In comparison with the present study, Madikizela et al. reported concentrations for naproxen, ibuprofen and diclofenac in influent, effluent and river water samples collected from Kwa-Zulu Natal in South Africa. The highest concentrations found were Ibuprofen in effluent (12000-67900 ng/L) and the Mbokodweni River (not detected-11400 ng/L)(Madikizela et al., 2017). In Kenya, K'Oreje et al. reported the highest concentration of acetaminophen (5500 ng/L) in the Nzoia River basin (K'Oreje et al., 2018), while the highest concentration in surface water in the present study was 954 ng/L. Our result shows significant difference between the concentrations in effluent and surface water for the following compounds; nicotine (p value=0.005), tramadol (p value

=0.05), hydrocortisone (p value =0.04) and valsartan (p value =0.02) with higher concentrations in the effluent samples.

However, it is well-documented that PPCPs concentrations and profiles in environmental waters may vary significantly between different geographical regions (Ebele et al., 2017). Therefore, the absence of anti-malaria drugs in the samples studied may be attributed to the very low occurrence or incidence of malaria in Egypt compared to other African countries (WHO, 2005).

Table 13A: Concentrations (ng/L) of PPCPs in Effluent samples

	Effluent sample concentrations (ng/L)						
	1A	1B	1C	1D	1E	Median	Average
Metformin	220	590	5600	1100	170	590	1500
Nicotine	370	740	570	840	420	570	590
Acetaminophen	1500	980	16000	3000	1600	1600	4600
Amoxicillin	<11	130	2000	<11	30	130	720
Gabapentin	<3	40	280	<3	<3	160	160
Codeine	60	<3	470	30	<3	60	170
Caffeine	80	1700	860	120	70	120	570
Trimethoprim	1100	270	2700	460	650	650	1000
Sulfamethoxazole	<2	<2	20	<2	<2	20	20
Tramadol	350	500	1100	190	280	350	480
Metoprolol	30	220	1100	67	57	67	300
Propranolol	8	20	190	60	<2	40	70
Doxycycline	<12	<12	30	<12	<12	30	30
Carbamazepine	60	150	340	<1	<1	150	180
Hydrocortisone	40	80	130	80	50	80	80
DEET	<3	<3	<3	<3	<3	<3	<3
Mefloquine	<12	<12	<12	<12	<12	<12	<12
Naproxen	<2	30	90	<2	10	<2	40
Erythromycin	50	10	280	110	10	50	90
Oxazepam	<3	<3	39	<3	10	<3	30
Valsartan	110	260	590	320	290	110	310
β-estradiol	<41	<41	170	<41	<41	<41	170
17α-ethynylestradol	<42	<42	220	<42	100	<42	160
Diazepam	<2	20	60	<2	<2	<2	40
Diclofenac sodium	270	80	3600	170	200	270	860
Glyburide	2100	800	4200	550	1400	2100	1800

Ibuprofen	1500	1700	6700	810	1100	1500	2400
Meclofenamic acid	20	<5	50	<5	<5	20	40
Clotrimazole	30	<8	230	<8	40	30	100
Gemfibrozil	<7	<7	110	40	<7	<7	80

Table 13A shows that the highest concentrations of PPCPs in effluent samples were found in location 1C compared to other locations (1A, 1B, 1D and 1E). This sample from location 1C was effluent from a hospital WWTP and therefore, had the highest concentrations for most of the target PPCPs. This is not surprising, as one would expect high usage of pharmaceuticals in a hospital along with other pharmaceuticals such as caffeine and nicotine that are indicative of daily lifestyle. The sum measured concentrations of the most abundant pharmaceuticals were acetaminophen (23000 ng/L), trimethoprim (5200 ng/L), and ibuprofen (12000 ng/L) in effluent samples in the present study are relatively lower than reported total measured concentrations in Nairobi and Kasumu city, Kenya (K'Oreje et al., 2016). The concentration of acetaminophen of 16000 ng/L found in our hospital effluent sample is comparable to that found in hospital effluents samples in Almeria, southeast of Spain for which the average concentration was 16020 ng/L (K'Oreje et al., 2016). However, the concentrations of pharmaceuticals (carbamazepine, diclofenac, erythromycin and trimethoprim and β-estradiol) found in our hospital effluent sample exceeded the average concentrations in Spanish hospital effluent samples (Gómez et al., 2006). Moreover, the concentrations of acetaminophen and ibuprofen in our Egyptian hospital effluent sample exceeded those reported in Ngaka Modiri Molema District, in South Africa, where concentrations of acetaminophen (6100 and 1240 ng/L) and ibuprofen (5250 and 500 ng/L) were detected in 2 WWTPs effluent samples from a hospital and a clinic (Kanama et al., 2018).

Table 13B: Concentrations of PPCPs in surface water samples

Surface water samples concentrations (ng/L)							
	2F	2G	2H	2I	2J	Median	Average
Metformin	30	60	20	20	40	30	34
Nicotine	120	90	270	380	100	120	190
Acetaminophen	950	140	210	390	780	390	490
Amoxicillin	<11	20	<11	<11	30	25	25
Gabapentin	<3	8	<3	10	<3	9	9
Codeine	<3	20	10	20	20	20	18
Caffeine	10	40	20	7	50	20	25
Trimethoprim	230	120	210	220	180	210	190
Sulfamethoxazole	<2	<2	<2	<2	<2	<2	<2
Tramadol	40	90	60	30	60	60	56
Metoprolol	20	8	5	9	10	9	10
Propranolol	<2	6	<2	7	<2	7	7
Doxycycline	<12	<12	<12	<12	<12	<12	<12
Carbamazepine	<1	6	<1	<8	<1	6	6
Hydrocortisone	40	40	60	40	40	40	40
DEET	<3	<3	<3	<3	<3	<3	<3
Mefloquine	<12	<12	<12	<12	<12	<12	<12
Naproxen	<2	6	<2	<2	8	7	7
Erythromycin	<11	<11	<11	30	60	45	45
Oxazepam	<3	<3	<3	<3	<3	<3	<3
Valsartan	60	60	100	60	40	60	64
β-estradiol	<41	<41	<41	<41	<41	<41	<41
17α-ethynylestradol	<42	<42	<42	<42	<42	<42	<42
Diazepam	<2	<2	<2	9	<2	9	9
Diclofenac sodium	40	<2	70	40	<2	40	50
Glyburide	330	630	390	370	250	370	390
Ibuprofen	50	30	90	60	30	50	52
Meclofenamic acid	<5	10	<5	<5	<5	10	10
Clotrimazole	<8	20	<8	20	<8	20	20
Gemfibrozil	<7	20	<7	20	20	20	20

Furthermore, PPCPs were ubiquitous in the surface water samples with highest average concentration at 490 ng/L acetaminophen; followed by glyburide (390 ng/L), trimethoprim (190 ng/L) and nicotine (190 ng/L). Two antibiotics (sulfamethoxazole and doxycycline), the hormones (β-estradiol and 17α-ethynylestradiol), DEET,

mefloquine HCl and oxazepam were less than MDL in the surface water while the rest of the studied compounds were measured at concentrations < 100 ng/L. In comparison with other studies in Africa, a similar average concentration was found for nicotine (154 ng/L) in upstream of a WWTP in Gauteng, South Africa (Archer et al., 2017a), while much higher concentrations were reported for acetaminophen (1740 ng/L), diclofenac (3404 ng/L), ibuprofen (591 ng/L), naproxen (224 ng/L), erythromycin (<10 ng/L), trimethoprim (383 ng/L), carbamazepine (380-1650 ng/L) and caffeine (812 ng/L) in African surface water (Agunbiade and Moodley, 2014, Agunbiade and Moodley, 2016, Matongo et al., 2015).

In addition to the above findings on concentrations of PPCPs in Egyptian wastewater and surface water, it was observed that using the rapid, high resolution full scan MS analysis provided an added advantage, as it permitted post-acquisition independent data analysis. This allows screening for non-target compounds. This is beneficial for monitoring of PPCPs in environmental matrices due to the diverse nature of this group of contaminants. Post-acquisition screening of our effluent and surface water samples revealed the potential presence of bisphenol A. Bisphenol A is a common environmental contaminant that was not targeted in our method. To confirm the identity of bisphenol A we thus compared the accurate mass, retention time and molecular ion cluster of the peak suspected to be bisphenol A with those of an authentic standard. Further confirmation of compound identity was achieved via sample fortification with an authentic bisphenol A standard, which resulted in increased peak area for the designated chromatographic peak Figure 16. Investigation of our method blanks showed the presence of bisphenol A in 3 out of 8 blanks at peak areas < 5% of those detected in real water samples. In conclusion, although accurate quantification of bisphenol A in our samples was not possible, these observations demonstrates the possibility for combined target/non-target approaches to PPCP analysis using the high resolution, full scan mode of the Orbitrap.

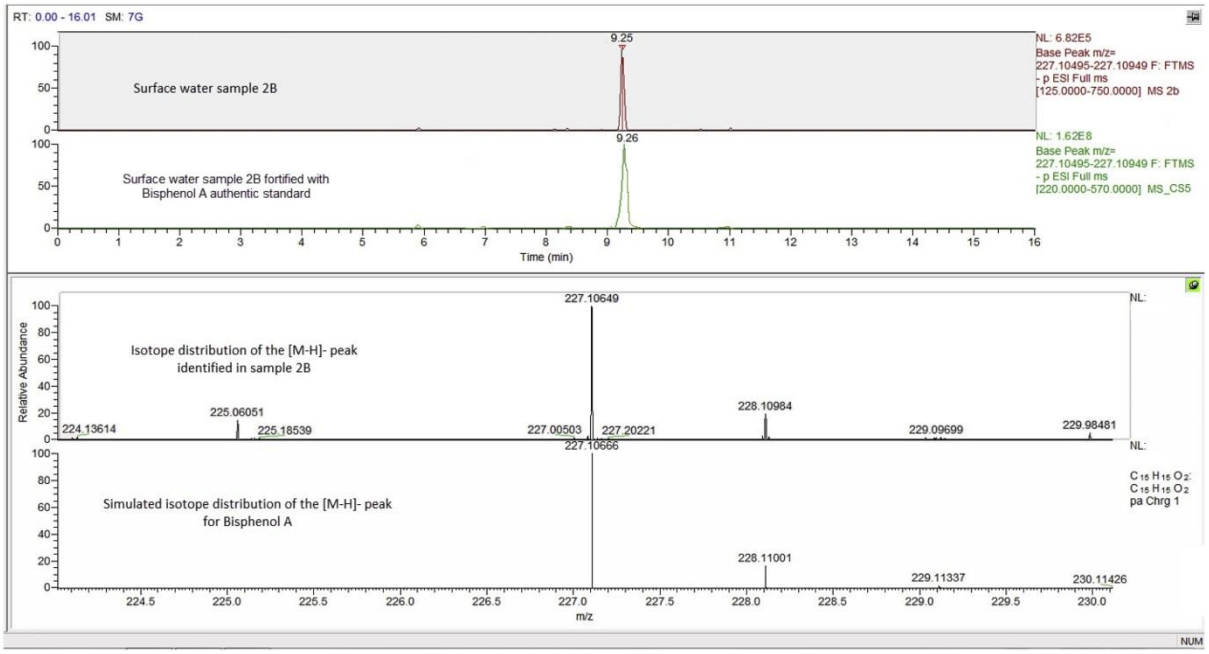


Figure 16: Post-acquisition identification of non-targeted Bisphenol A in the studied water samples through its accurate mass, isotope cluster and confirmation by fortification with Bisphenol A standard.

In summary, the results of this study support those of previous investigations that demonstrate the modest efficiency of conventional wastewater treatment plant protocols in removing PPCPs.

CHAPTER IV

Occurrence, seasonal variation and human exposure to pharmaceuticals and personal care products in surface water, groundwater and drinking water in Nigeria

This chapter contains material taken verbatim from the following paper publication: "EBELE, A. J., OLUSEYI, T., DRAGE, D. S., ABOU-ELWAFA ABDALLAH, M. & HARRAD, S. "Occurrence, seasonal variation and human exposure to pharmaceuticals and personal care products in surface water, groundwater and drinking water in Nigeria". Emerging Contaminants, 6**,** 124-132 (2020)

4.0. Synopsis

In developed countries such as the United Kingdom, United States of America, Australia and Japan, several studies have been conducted to better understand the occurrence, behaviour and fate of PPCPs in the environment. However, very few studies have addressed this area of research in developing countries in Africa and South America (Ebele et al., 2017, Archer et al., 2017a). The insufficient data on the occurrence and behaviour of PPCPs in Nigeria, with a population of nearly 200 million people, compounded with an incomplete sewage treatment system (probably direct discharge into waterways) represent a cause for concern and a significant research gap. Most urban communities in Nigeria, with the exception of the capital Abuja and limited areas in Lagos, have no sewage system. Consequently, the sewage and sullage either lie stagnant or are disposed through the storm water drainage system (Henley, 2000). This is supported by the scarce data available on PPCPs in the Nigerian environment, where high concentrations of 1–20 µg/L of paracetamol, chloroquine, diclofenac and ciprofloxacin were detected in four surface- and groundwater samples collected from an industrial area of Sango Ota, Ogun State, Nigeria (Olaitan et al., 2014).

Dilution and degradation factors were suggested as natural mechanisms capable of reducing PPCPs concentrations in Nigerian surface water in the absence of wastewater treatment processes (Inam et al., 2015). Several studies have demonstrated that environmental conditions (weather/season), source characteristics, as well as water flow rates are significant factors that control the detection frequency and concentrations of PPCPs in natural waters

(Daneshvar et al., 2010, Meierjohann et al., 2016). Nigeria has only two major seasons (dry and rainy season). The dry season, known locally as the "Harmattan" is accompanied by predominant influences of dust-laden air mass from the Sahara Desert. It usually starts from late October and lasts to early March with peak dry conditions between early December and late February. The rainy season starts in April and lasts until early October with peak wet conditions in June. This season is influenced by wind from the South Atlantic Ocean mostly known as the South West wind. At the peak of the rainy season, the weather in Lagos is wet about half the time (Olawale, 2017). Despite these stark seasonal differences in precipitation, there exists no information on the seasonal influence on the concentrations of PPCPs in Nigerian freshwater (surface and ground water). Moreover, due to the incomplete sewage treatment and water disposal systems, many Nigerian households rely upon boreholes as their main source of non-potable water and 97% of households use bottle or sachet water as their main drinking water source (Capstick et al., 2017). The use of sachet water, which is packaged in individual units of 500 mL high density polyethylene sachets, is common in Nigeria with an estimated daily consumption of up to 60 million units (Dada, 2009). The major of sachet water is from treated or untreated borehole system, only few brands are from spring water. It is a fast-growing source of drinking water for some African nations especially in the Western part, for instance Nigeria and Ghana (Stoler et al., 2012, Dzodzomenyo et al., 2018). Sachet water - popularly known in Nigeria as "Pure water"- is regulated by some government agencies such as the Standard Organisation of Nigeria (SON), the National Agency for Food and Drugs Administration and Control (NAFDAC) and other regulatory agencies in Nigeria with expectation to meet the Nigeria Industrial Standard (NIS)(Augustine et al., 2019). Most sachet water commercially sold is branded with NAFDAC registration number, to show that they at least meet the World Health Organisation Standard; free from physical, chemical and microbial substances that could pose as threat to human. However, due to the lucrative nature of sachet water production, many people have ventured into the business without following due diligence. The Director-General of NAFDAC expressed great disappointment because of the unhygienic conditions some of the sachet water producers operated in, some of them had not registered with the agency, while some had labelled fake NAFDAC registration number on the sachet water (Victor, 2016).

Figure 17: An illustration of Sachet water sold in Nigeria

Other forms of packaged water that are available in Nigeria include bottle-packaged water (patronised by citizens in the higher income brackets) and 'ice water' which is mainly consumed by lower income individuals. The 'ice water' type is usually prepared by withdrawing water from nearby piped or vended water sources, filtered over a piece of cloth and thereafter put into transparent low-density polyethylene (LDPE) plastic mini-bags. Therefore, 'ice water' has been almost completely phased out for hygienic reasons and due to the advent of the better packaged and purified Sachet water (Omole et al., 2015). Therefore, only bottled and sachet water were investigated in the current study. The average cost difference between sachet-packaged ($0.03) and bottled ($0.3) water is 1000%. Thus, sachet water is perceived as clean and affordable to a wide spectrum of middle and low-income earners in Nigeria (Omoniyi and Abu, 2012). It's also worth mentioning that a small fraction of low-income Nigerian households may use water from local boreholes for drinking, despite the public health advice against it (Capstick et al., 2017).

Against this backdrop, the main objectives are (1) to investigate the occurrence and concentrations of 30 used PPCPs in Lagos State waterways, groundwater and drinking water.

(2) evaluate seasonal variability in concentrations of target PPCPs in the studied water samples during the Nigerian dry and wet seasons; and (3) use the measured concentrations in drinking water to estimate unintended human exposure to the studied PPCPs via drinking water. The driven hypothesis to this chapter is that, there will be reduction in measured PPCPs concentrations during rainy season compared to dry season due to extensive rainfall/degradation (photodegradation); and also, possible exposure on drinking traces of PPCPs contaminants in sachet water.

4.1. Sampling locations and preparation

Water samples (one litre each) were collected at different locations comprising 17 surface water (rivers, canals, and lagoons), 12 groundwater (4 wells and 8 boreholes) and 8 drinking water samples (4 brands of sachet water and 4 brands of bottled water) within the mega city of Lagos, Nigeria. One sample was collected from each location during the dry season (December 2017- February 2018) and the rainy season (June - July 2018), except for the bottled water samples (n=4), which were only collected during the rainy season sampling campaign.

The sampling locations represent some of the major waterways that travel across the city and flow down into the Atlantic Ocean. These sampling locations are in close proximity to both residential areas, as well as industrial and commercial activities (Figure 18). Collected samples were stored in ice-packed containers and transported back to the chemistry department laboratory at the University of Lagos, where they were stored at -18^{o}C until extraction within a maximum of 48 hours from collection.

One challenging key factor was observed; whereby the SPE cartridges were getting blocked during the extraction procedure. The blockages were mainly due to high turbidity found in the surface water and the groundwater sampled. To resolve this, sample filtration was done to all the samples collected before spiking with isotopically labelled internal standard mix. The blockage issue was not fully resolved; we ended up using 2-3 cartridges per 500 mL of sample. 500 mL of the sachet and bottled water sample each passed through the cartridges without any blockage, therefore, there was no need for filtration of the drinking water samples. Sample extraction was conducted according to a recently reported method (Abou-Elwafa Abdallah et al., 2018). Briefly, 500 mL of each sample were spiked with 50 ng of isotope-labelled internal standards mixture (Caffeine-D9, Codeine-D3, Carbamazapine-D10

and 4-Chlorophenol-2,3,5,6-D4) then passed onto Oasis MCX cartridges that were pre-conditioned with 2 mL of MeOH and equilibrated with 2 mL of deionized water. Each of the cartridges were carefully labelled and wrapped in aluminium foil before been couriered to University of Birmingham, UK for further extraction and instrumental analysis. On arrival to University of Birmingham, cartridges were placed in a freezer at –20 ˚C until further processing within 24 hours of receipt. Each cartridge was eluted with 5 mL MeOH followed by 5 mL of 5% ammonium hydroxide in methanol (NH_4OH). The eluate was evaporated to dryness under a gentle stream of nitrogen and reconstituted in 200 µL of 8:2 water/methanol solution containing 25 pg/µL of ^{13}C-TBBPA and TCEP-D12 used as recovery (syringe) standards for QA/QC purposes. The same process was applied to the cartridges containing one sample, each of the cartridges were eluted, dried in one text tube and eventually reconstituted. Extracted samples were analysed on UPLC- coupled to a Q-Exactive Plus Orbitrap mass spectrometer.

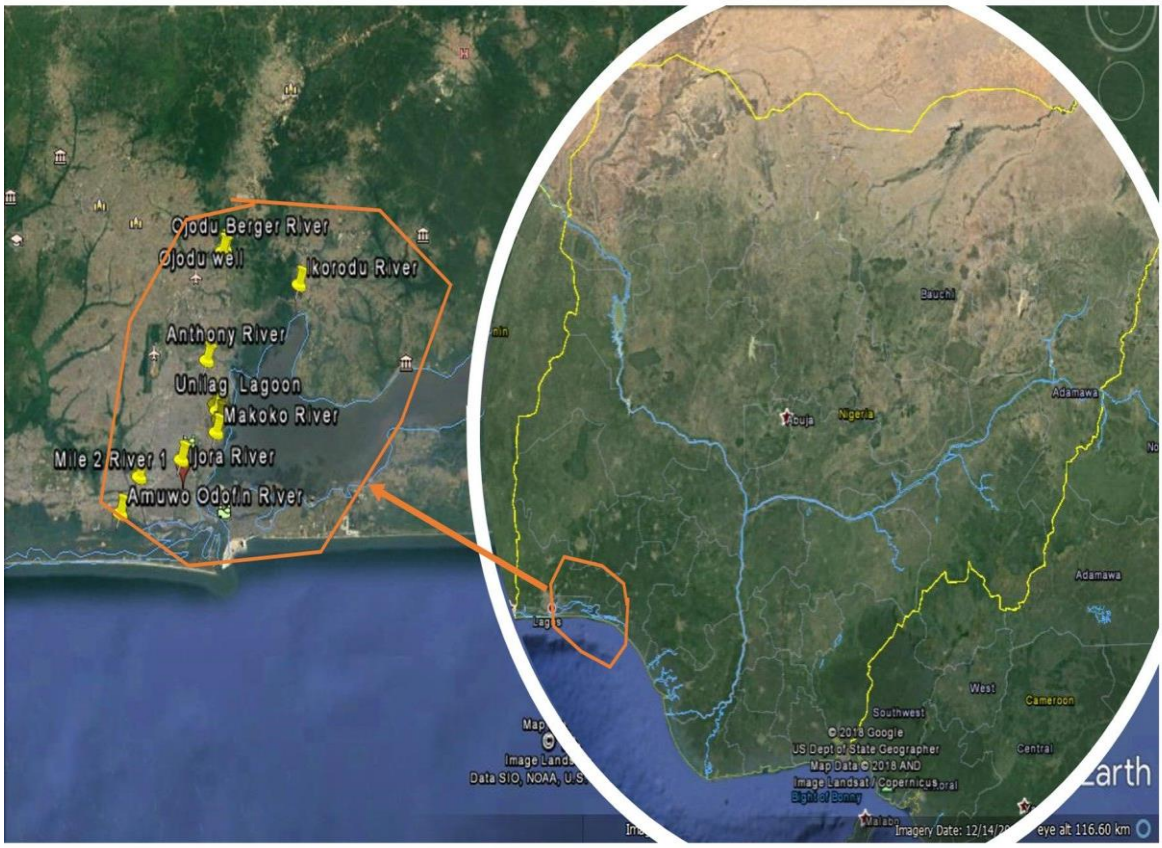


Figure 18: Map depicting sampling locations in Lagos State Southwest of Nigeria

4.2. Statistical analysis

Statistical analysis was conducted using IBM SPSS statistics 22.0 software package for Windows 10. Initially, sample distribution was investigated using the Kolmogorov-Smirnov test combined with visual inspection of the Q-Q plot for data distribution. Results revealed the generated datasets to be normally distributed. Therefore, parametric tests were further applied for comparison of sample means (student t-test to compare between two datasets) and Analysis of Variance (ANOVA). ANOVA in combination with Tukey's honestly significant difference (HSD) post-hoc test was applied for pair-wise comparison among 3-8 datasets to assess statistically significant differences. For statistical analysis purposes, concentrations below the method detection limit (MDL; Appendix II Table SI-1) were assigned a value of 0.5 MDL, except in cases of a detection frequency below 50% where a value of MDL multiplied by the detection frequency was assigned to minimise statistical bias (e.g. 0.35 MDL for compound-X with a detection frequency of 35%). P values < 0.05 were considered significant

4.3. Results and discussion

Out of the 30 target PPCPs studied, 27 were detected in at least one sample of surface water, 24 in groundwater and 14 in sachet drinking water. The antibiotic Doxycycline was not detected in any sample. Data for the hormones β-estradiol and 17α-ethynylestradiol were erratic because the instrument was unable to identify the internal standard (Estone-D4) for better quantification. The loss of Estone-D4 in virtually all the samples is suspected to be during the extraction process, therefore resulting to the systematic errors of determination of their concentration measurements.

4.3.1. PPCPs profiles in Lagos surface water, groundwater and sachet drinking water

Most of the chemical compounds of different therapeutic groups studied were detected in both dry and rainy season samples, with higher detection frequencies in rainy season samples. The average concentrations for $\sum_{30}$PPCPs analysed during the dry season were approximately 28000 ng/L, 2100 ng/L and 380 ng/L, while the rainy season average concentrations were 10000, 2900, 710 ng/L, for surface water, groundwater and sachet drinking water samples respectively (Table 14). Statistical analysis revealed no significant

differences ($P > 0.05$) in $\sum_{30}$PPCPs concentrations among the three types of water samples studied.

Table 14: Statistical summary of concentrations of $\sum_{30}$PPCPs (ng/L) detected in Nigerian surface, ground and sachet water samples collected during the dry and rainy seasons.

		Dry season	Rainy season
Surface water (n=17 from each season)	Min	500	2500
	Median	5000	6700
	Max	280000	30000
	Average	28000	10000
	StDev	70000	8000
Ground water (n=12 from each season)	Min	130	180
	Median	1000	1200
	Max	6700	9100
	Average	2100	2900
	StDev	2200	3200
Sachet drinking water (n=4 from each season)	Min	150	530
	Median	400	640
	Max	530	1000
	Average	380	710
	StDev	170	220

The two most frequently detected compounds in the current study were amoxicillin and acetaminophen. Amoxicillin was detected in all surface water samples at an average concentration 13000 ng/L (range: 90-270000). The detection of amoxicillin, a widely-used antibiotic, was not surprising as it is regularly dispensed in Nigeria as an over the counter drug, which does not require a prescription (Eshiet et al., 2015, Kamaldeen et al., 2013). Nevertheless, this is of concern because previous studies have shown continuous input of antibiotics such as amoxicillin, into the environment may lead to significant long term, irreversible impacts such as the development of antibiotic-resistant microbial strains (Sanderson et al., 2004).

Acetaminophen was detected in 90% of the samples at average concentrations of 510 ng/L (range: 3-12000), 25 ng/L (range: 3-190) and 2 ng/L (range: <1-10) in surface, groundwater and sachet water respectively. This high detection frequency is in line with previous studies from both African and non-African countries, and is consistent with the common over-the-counter use of this analgesic to relieve fever, aches and pain. Acetaminophen was reported as the third most consumed drug (4.3 tonnes) in Kenya and was detected at relatively high concentrations up to 16000 ng/L in the Nairobi River basin (K'Oreje et al., 2012). It was also detected in water from the Umgeni River, South Africa at concentrations ranging from 5800 - 58700 ng/L (Agunbiade and Moodley, 2014). In the river Leine, Germany, the average acetaminophen concentration was 1992 ng/L (Nödler et al., 2010), while in the river Taff, UK; acetaminophen was present at indicative concentrations of 1013 – 1388 ng/L (Kasprzyk-Hordern et al., 2007).

DEET was also detected in 90% of the samples studied at average concentrations 190 ng/L (Range: 5-1400), 18 ng/L (range: <3-60) and 6 ng/L (range: <3-10) in surface, ground and sachet water, respectively. The use of DEET as an insect repellent is common in Nigeria, as one of the recommended protective measures against malaria (WHO, 2017a). The number of publications reporting the presence of DEET in surface water from tropical and sub-tropical countries has increased over the last decade. The concentration of DEET detected in surface water in the current study is similar to that reported in Australian urban waterways where concentrations ranging from 8-1500 ng/L were reported (Costanzo et al., 2007, Brausch and Rand, 2011, Aronson et al., 2012) but were lower than the exceptionally high DEET concentrations reported in surface waters from Jakarta, Indonesia (30 - 24000 ng/L) (Dsikowitzky et al., 2014).

Table 15: Concentrations (ng/L) and detection frequency (DF) of PPCPs in surface water, groundwater and sachet water in Lagos State, Nigeria.

	Surface water			Groundwater			Sachet water		
Compounds	**Range**	**Median**	**DF (%)**	**Range**	**Median**	**DF (%)**	**Range**	**Median**	**DF (%)**
Metformin	<1-1800	4	41	<1-350	<1	8	<1	<1	0
Acetaminophen	1-12000	24	100	<1-190	7	71	<1-10	1	25
Gabapentin	<1-67	9	59	<1-40	1	21	<1	<1	0
Nicotine	<7-9300	910	85	<7-3500	12	50	<7-140	10	50

Codeine	<2-1800	420	85	<2-2400	8	50	<2-310	50	50
Sulfamethoxazole	<1-3200	6	59	<1-60	<1	25	<1-7	<1	25
Caffeine	<4-1100	120	74	<4-170	<4	33	<4-50	8	63
Trimethoprim	2-390	30	79	<1-20	<1	33	<1	<1	0
Amoxicillin	90-270000	1600	100	40-6500	240	100	90-500	360	100
Tramadol	<2-850	56	79	<2-880	14	71	<2-6	<2	25
Metoprolol	<1-170	8	50	<1-50	<1	21	<1-4	<1	13
Propranolol	<1-10	<1	12	<1	<1	0	<1	<1	0
Carbamazepine	<1-340	9	53	<1-50	<1	29	<1	<1	0
Hydrocortisone	<3-470	<3	9	<3	<3	0	<3	<3	0
Erythromycin-H2O	<1-280	<1	3	<1	<1	0	<1	<1	0
DEET	5-1400	82	91	<4-60	11	54	<4-10	6	50
Oxazepam	<2-1200	<2	38	<2-30	<2	13	<2-7	<2	13
Mefloquine HCl	<1-60	<1	12	<1-60	<1	4	<1	<1	0
Naproxen	<3-2100	19	76	<3-20	<3	38	<3-20	<3	13
Valsartan	<1-3300	27	74	<1-80	<1	13	<1	<1	0
Diazepam	<1-40	<1	18	<1-30	<1	8	<1	<1	0
Glyburide	<3-330	18	53	<3-40	<3	33	<3-30	<3	25
Diclofenac	<1-200	12	56	<1-40	<1	13	<1	<1	0
Ibuprofen	<4-2700	300	85	<4-2300	32	63	<4-50	12	50
Clotrimazole	<1-620	<1	26	<1-190	<1	4	<1	<1	0
Meclofenamic acid	<1-2000	76	71	<1-40	<1	13	<1	<1	0
Gemfibrozil	<4-550	158	79	<4-730	90	83	<4-30	<4	25

Nicotine, codeine, caffeine, valsartan, tramadol, ibuprofen, naproxen, gemfibrozil and meclofenamic acid were detected in more than 70% of the surface water samples. Codeine was detected at median concentrations of 420, 8, 50 ng/L in surface water, groundwater and sachet water respectively. Similar median concentrations of codeine (9–320 ng/L) were reported in the River Ely, UK (Kasprzyk-Hordern et al., 2008d) and the river Llobregat, Spain (30 - 39.5 ng/L) (Boleda et al., 2009). Archer *et al.* reported average concentration of 129 ng/L of codeine in the Gauteng River in South Africa (Archer et al., 2017a). Given misuse of codeine by Nigerian youth for recreational purposes has been previously highlighted (Kazeem and

Chutel, 2018); its detection in sachet drinking water, albeit at low concentrations, raises concern due to the addictive nature of this chemical.

Average concentrations of caffeine in surface water, groundwater and sachet water were 120 ng/L (<4-1100), 4 ng/L (<4-170) and 8 ng/L (<4-50) respectively. Higher concentrations were reported for caffeine in South African surface water (average = 2078 ng/L (Archer et al., 2017a) and ranged from 1170–60530 ng/L (Agunbiade and Moodley, 2014), while concentrations of caffeine in three urban rivers in the Yangpu District of Shanghai, China, ranged from 66–8571 ng/L (Zhou et al., 2016). Another study in California reported a maximum concentration of 290 ng/L caffeine in groundwater (Fram and Belitz, 2011).

Another stimulant, nicotine, was detected in more than 50% of the studied samples at average concentrations of 2000 ng/L (<7-9300), 290 ng/L (<7-3500), and 30 ng/L (<7-140) in surface, ground, and sachet water, respectively. A much lower average concentration of 190 ng/L was reported in Egyptian surface water samples (Abou-Elwafa Abdallah et al., 2018). Archer *et al.* reported an average concentration at 246 ng/L of Nicotine in water samples collected downstream of a WWTP in Gauteng Province of South Africa, while the upstream samples contained an average nicotine concentration of 154 ng/L (Archer et al., 2017a).

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently detected pharmaceuticals in the freshwater aquatic environment due to their high consumption rate (Ebele et al., 2017). Four NSAIDs were investigated in the current study, namely Naproxen (DF = 76 %), Ibuprofen (DF = 83 %), Diclofenac (DF = 56 %) and Meclofenamic acid (DF = 71 %) (Table 15). The concentrations of Naproxen in our surface water samples is comparable to those detected in Poland (12-76 ng/L) (Kasprzyk-Hordern et al., 2008a) and Sweden (13-87 ng/L) (Kosjek et al., 2005). Madikizela reported average concentrations (ng/L) ± standard deviations for naproxen, ibuprofen and diclofenac in South African surface water of 2770 ± 1.66, 6720 ± 1.23 and 2580 ± 1.38, respectively (Madikizela et al., 2017). Olarinmoye et al. reported a broad range of concentrations for Naproxen (<20-1030 ng/L), Ibuprofen (<20-8840 ng/L) and Diclofenac (<20-270 ng/L) in surface water samples collected from Lagos, Nigeria in 2015 (Olarinmoye et al., 2016). These are generally in line with the concentrations reported in the current study (Table 15). Meclofenamic acid was detected at concentration of 2380 ng/L in South African surface water (Gumbi et al., 2017), which is relatively higher than the average concentration of 230 ng/L (range; <1- 2000 ng/L) in the present study.

4.3.2. Seasonal variation

Nigeria is located in the tropical zone, with variable rainy and dry seasons. Based on this seasonal variability, we hypothesise that the concentrations of PPCPs in surface and ground water will be lower in the rainy season than the dry season due to a dilution effect. To test this hypothesis, concentrations of target PPCPs in samples collected from the same locations in dry and rainy seasons were statistically compared (Table 16).

Results revealed seasonal variation in some PPCPs concentrations between the dry and rainy season samples. Concentrations of Glyburide (an antidiabetic) in groundwater were significantly higher during the dry season (p = 0.03). Similarly, Caffeine was detected at higher concentrations (p = 0.02) in surface water samples collected in the dry season (average = 340 ng/L) than rainy season (average = 150 ng/L). Likewise, the non-steroidal anti-inflammatory drugs, Naproxen and Diclofenac, displayed significantly higher concentrations in groundwater and surface water, respectively during the dry season, which is in agreement with our initial hypothesis of dilution by the extensive rainfall during the wet season in Nigeria.

In contrast, the average concentration of nicotine in surface water was significantly higher (p= 0.002) in the wet season (3300 ng/L) than in the dry season (650 ng/L). This was in contrast with the expected reduction in PPCPs concentration due to dilution during the rainy season. Further inspection of potential sources revealed that nicotine is predominantly found in tobacco and cocoa as well as in lower quantities in plants such as tomatoes, potatoes and capsicum. The high concentrations of nicotine observed in this case could thus be due to the presence of tobacco cultivation areas around the sampling locations(Ibrahim et al., 2017). The heavy flooding during the rainy season could wash out nicotine from tobacco plants; therefore, contributing to the high concentrations observed in the rainy season samples.

Similarly, the average concentration of codeine was also significantly higher ($p<0.05$) in the wet season (900 ng/L) than the dry season (100 ng/L) in surface water samples. This is difficult to explain but may be attributed to the increased use of cold and flu medication during the wet season (Foley et al., 2018). Most of the cold and flu medicines dispensed in Nigeria contain codeine combination products (such as codeine combined with aspirin) or Codeine linctus (up to 2mg/mL) and are sold over-the-counter without a medical prescription (Akande-Sholabi et al., 2019).

Table 16: Concentrations (ng/L) of target PPCPs in water samples collected during the dry (D) and wet (W) seasons in Lagos, Nigeria

Compound		Surface water				Groundwater				Sachet water			
		Range		Median	Average	Range		Median	Average	Range		Median	Average
Metformin	D	<1-	26	3	4	<1-	3	<1	3	<1		<1	<1
	W	<1-	1800	37	260	<1-	350	<1	52	<1		<1	<1
Acetaminophen	D	3-	270	18	61	<1-	190	8	31	<1-	3	<1	3
	W	7-	12000	55	950	<1-	95	4	19	<1-	11	2	4
Gabapentin	D	<1-	49	11	14	<1-	10	<1	3	<1-		<1	<1
	W	<1-	67	2	14	<1-	41	2	10	<1		<1	<1
Nicotine	D	<7-	5000	67	650	<7-	73	9	14	<7-	10	9	9
	W	110-	9300	2100	3300	9-	3500	66	560	<7-	140	21	51
Codeine	D	<2-	450	18	99	<2-	5	3	3	<2-	3	3	3
	W	68-	1800	770	900	13-	2400	87	490	<2-	310	130	160
Sulfamethoxazole	D	<1-	3200	8	330	<1-	64	<1	10	<1-	7	<1	3
	W	<1-	130	6	30	<1-	16	<1	3	<1-	5	<1	3
Caffeine	D	<4-	1100	280	340	<4-	160	<4	35	<4-	50	9	18

	W	<4-	480	51	150	<4-	170	<4	22	<4-	48	6	16
Trimethoprim	D	<1-	140	53	49	<1-	21	<1	4	<1		<1	<1
	W	<1-	390	19	62	<1-	10	<1	3	<1		<1	<1
Amoxicillin	D	87-	270000	460	24000	44-	6500	140	1300	86-	440	340	300
	W	230-	7600	1800	2700	58-	6500	320	1300	330-	500	410	410
Tramadol	D	<2-	850	56	240	<2-	880	44	150	<2-	6	<2	4
	W	<2-	480	30	140	<2-	320	10	56	<2-	5	<2	3
Metoprolol	D	<1-	1	<1	1	<1-	8	<1	2	<1-	1	<1	1
	W	<1-	170	70	73	<1-	54	<1	11	<1-	4	<1	2
Propranolol	D	<1-	9	<1	2	<1		<1	<1	<1		<1	<1
	W	<1-	1	<1	1	<1		<1	<1	<1		<1	<1
Carbamazepine	D	<1-	340	36	71	<1-	46	<1	8	<1		<1	<1
	W	<1-	20	1	7	<1-	50	<1	6	<1		<1	<1
Hydrocortisone	D	<3-	470	<3	34	<3		<3	<3	<3		<3	<3
	W	<3-	70	<3	7	<3		<3	<3	<3		<3	<3
	D	<1-	280	<1	17	<1		<1	<1	<1		<1	<1

Erythromycin-H2O	W	<1-	1	<1	<1	<1		<1	<1	<1		<1	<1
DEET	D	<4-	1400	87	240	<4-	63	7	19	<4-	13	6	7
	W	<4-	640	67	150	<4-	60	13	18	<4-	10	6	6
Oxazepam	D	<2-	1200	19	130	<2-	27	<2	5	<2-	7	<2	3
	W	<2-	12	<2	2	<2-	11	<2	<2	<2-	3	<2	<2
Mefloquine HCl	D	<1-	31	<1	7	<1-	2	<1	2	<1		<1	<1
	W	<1-	58	<1	5	<1-	56	<1	7	<1		<1	<1
Naproxen	D	<3-	2100	8	140	<3-	9	<3	4	<3-	3	<3	3
	W	<3-	130	22	33	<3-	17	<3	7	<3-	17	<3	6
Valsartan	D	<1-	3300	43	320	<1-	84	<1	12	<1		<1	<1
	W	<1-	110	17	28	<1-	10	<1	5	<1		<1	<1
Diazepam	D	<0.3-	42	<1	6	<1-	26	<1	3	<1		<1	<1
	W	<0.3-	1	<1	<1	<1-	5	<1	<1	<1		<1	<1
Glyburide	D	<3-	330	33	57	<3-	39	10	17	<3-	30	<3	12
	W	<3-	240	6	28	<3-	14	<3	7	<3-	34	<3	13

Diclofenac Na	D	<1-	1900	130	390	<1-	42	<1	9	<1		<1	<1
	W	<1-	34	<1	9	<1-	4	<1	4	<1		<1	<1
Ibuprofen	D	<4-	1700	290	490	<4-	2300	48	260	<4-	34	13	17
	W	<4-	2700	360	910	<4-	1100	19	130	<4-	50	22	25
Clotrimazole	D	<1-	620	<1	63	<1-	3	<1	1	<1		<1	<1
	W	<1-	120	<1	15	<1-	190	<1	18	<1		<1	<1
Meclofenamic acid	D	<1-	2000	140	400	<1-	43	<1	10	<1		<1	<1
	W	<1-	170	21	49	<1-	5	<1	<1	<1		<1	<1
Gemfibrozil	D	<4-	440	180	150	<4-	730	110	180	<4-	31	<4	16
	W	<4-	550	84	160	<4-	480	79	110	<4-	32	<4	16

***D Dry season**
***W Wet season**

No statistically significant seasonal variability was observed in the concentrations of other target PPCPs in the studied surface and ground water samples. This may be attributed to several factors including the small sample size and the low detection frequency for some of the target PPCPs. However, it is evident that factors other than dilution by rain water contribute to the concentrations of some PPCPs in the Nigerian aquatic environment (e.g. agricultural sources for Nicotine). To our knowledge, this is the first study to investigate seasonal variation of PPCPs in the Nigerian freshwater environment, while only limited information is available on the factors affecting seasonal variation of PPCPs in surface and ground water worldwide. A recent study investigated the seasonal variation of 11 PPCPs in the Huangpu River, Shanghai, China. The results indicated that most target PPCPs exhibited higher frequencies of detection and concentrations during the dry season than those during the wet season, which was mainly attributed to the influence of rainfall (Mei et al., 2018). Similarly, a study of 5 PPCPs in 6 Indian lakes, reported higher concentrations of Ciprofloxacin and Caffeine in summer (April to June) and winter seasons (October to February) compared to the rainy season (July to September) (Archana et al., 2017). While these two studies focused mainly on investigating the influence of meteorological conditions (i.e. rain and temperature), another study from California attributed some of the observed PPCPs seasonal variation in surface water to increased use of insect-repellents and sunscreens in the summer. Other factors affecting PPCPs temporal variability in California included rainfall and the flow rates in both the Colorado River and the San Joaquin Delta (Loraine and Pettigrove, 2006). Therefore, further investigation of both environmental and anthropogenic factors influencing seasonal variability of different PPCPs in different geographical locations is recommended.

4.3.3. Human exposure to PPCPs

There has been a considerable interest regarding inadvertent human exposure to PPCPs as an emerging class of environmental contaminants. Such exposure can occur via drinking water with detectable concentrations of various PPCPs. It is worth mentioning that wastewater treatment processes are not sufficient to completely remove PPCPs even in developed countries like the United Kingdom, Canada, Australia and USA, which utilise advanced water treatment methods such as ozone or granular activated carbon (GAC) (Ebele et al., 2017). In developing countries like Nigeria, the supply of water by the state water board is not always reliable; therefore, households or the local communities will rely on borehole water that in

most cases does not undergo any treatment. Using our concentrations of target PPCPs in borehole water, as well as in bottled and sachet drinking water, equation (1) was applied to estimate inadvertent adult exposure to $\sum_{30}$PPCPs s via drinking water in Nigeria.

$$\textit{Daily exposure} = \frac{\sum_{30}\textit{PPCPs concentration x estimated daily water intake (2L)}}{\textit{Average body weight of adult (80kg)}}$$

It is often presumed by the public that commercially-vended water (bottled or sachet) is highly purified and free from environmental pollutants. However, several target PPCPs were detected in bottled and sachet water samples bought from local shops in Lagos, Nigeria (Table 17).

Table 17: Statistical summary of $\sum_{30}$PPCPs (ng/L) in Bottled water, Sachet water and Borehole water and the resulting estimates of inadvertent Nigerian adult exposure to ΣPPCPs via drinking water.

Parameter	**$\sum_{30}$PPCPs concentrations (ng/L)**		
	Bottled water (n=4*)	Sachet water (n=8**)	Borehole water (n=16$^{\$}$)
Average	110	560	3200
Median	99	550	2500
Min	61	170	160
Max	180	1000	9200
	Estimated adult exposure (ng $\sum_{30}$PPCPs Kg BW/ day)		
Average	3	14	81
Median	2	14	61
Min	2	4	4
Max	5	26	230

* collected during the rainy season.
** 4 samples collected in each of the rainy and the dry seasons.
$^{\$}$ 8 samples collected in each of the rainy and the dry seasons.

A study in Shanghai also detected an antibiotic (Florfenicol) used for veterinary purpose in bottled water purchased from supermarkets at concentrations of 0.6 ng/L, 0.76 ng/L and 1 ng/L in the same brand of bottled water (Wang et al., 2016).

The average concentrations of $\sum_{30}$PPCPs in the current study were 3200, 560, 110 ng/L resulting in average estimated Nigerian adult exposures of 81, 14 and 3 ng $\sum_{30}$PPCPs/kg BW/day via consumption of borehole, sachet water and bottled water, respectively (Table 17).

The concentrations of PPCPs identified in drinking-water in the current study are typically orders of magnitude less than the lowest therapeutic doses as shown in table 18. While this may indicate low risk from exposure to single compounds, there are no toxicity endpoints or tolerable daily intakes of regulatory standing for inadvertent exposure to mixtures of PPCPs via drinking water or other exposure pathways (WHO, 2017b). This raises concern because while the estimated exposure may be lower than the reported toxic dose for a single pharmaceutical compound, concurrent exposure to such a complex cocktail of pharmaceutically active ingredients may result in unexpected toxic effects resulting from potential drug-drug interactions. More toxicological research into the potential adverse effects of such inevitable exposure to PPCPs is urgently required for accurate risk assessment of this emerging class of environmental contaminants.

Table 18: Therapeutic dose, Toxic dose, Conc. Ranges in Groundwater and Sachet water

Compounds	Daily therapeutic dose (mg)	Toxic dose (mg/kg)	Conc. Range Groundwater (ng/L)	Conc. Range Sachet water (ng/L)
Metformin	500-2000**	1000***	<1-350	<1
Nicotine	4-64	50***	<7-3500	<7-140
Acetaminophen	1000-4000**	1944***	<1-190	<1-10
Codeine	120-240	427***	<2-400	<2-310
Amoxicillin	750-1500**	>15000***	40-6500	90-500
Gabapentin	900-3600**	>8000***	<1-40	<1
Trimethoprim	100-600**	>5300***	<1-20	<1
Caffeine	200-400**	192***	<4-170	<4-50

Tramadol	50-100**	350***	<2-880	<2-6
Metoprolol	200-400**	5500***	<1-50	<1-4
Sulfamethoxazole	400-800	6200***	<1-60	<1-7
Propranolol	160-320	466***	<1	<1
Erythromycin	250-1000	4600***	<1	<1
Carbamazepine	800-1600	3750***	<1-50	<1
Hydrocortisone	20-240	5000***	<3	<3
Mefloquine	100	880***	<1-60	<1
Oxazepam	15-25**	>8000***	<2-30	<2-7
Doxycycline	100-200**	1700*	nd	nd
Clotrimazole	500**	708***	<1-190	<1
Naproxen	500-1000**	248***	<3-20	<3-20
Diazepam	5-10**	249***	<1-30	<1
Valsartan	80-320**	>2000***	<1-80	<1
Glyburide	1.25-2.5**	>3200***	<3-40	<3-30
Diclofenac sodium	50-150**	53***	<1-40	<1
Ibuprofen	200-600**	636***	<4-2300	<4-50
Meclofenamic acid	50-100**	225***	<1-40	<1
Gemfibrozil	900-1200**	1414***	<4-730	<4-30

*Pfizer Ltd
**NHS
***Cayman chemicals
nd non detects

4.4. Study limitations

It is important to recognise the limitations associated with the limited sampling resources for the PPCPs seasonal variation study. One sample was collected from each location during the sampling campaign period comprising one month in each season. Single sampling events that represent an entire seasonal behaviour might have some bias in the results. This may be attributed to daily- and/or sub-daily variation in chemical concentrations in contaminated waterways, especially during the rainy season. Therefore, the current study findings provide useful “snapshot” information on seasonal variability, rather than a continuous monitoring

programme. Nevertheless, our results provide first evidence on seasonal variation of PPCPs concentrations in Nigerian surface, ground and drinking water. This may be a necessary initial step to establish long monitoring programmes with multiple sampling events over several years to further investigate such seasonal variability in different countries.

CHAPTER V

Seasonal and spatial variation of pharmaceuticals and personal care products in UK Rivers and Canals

5.1. Synopsis

This study investigates the occurrence, concentrations and seasonal–spatial distribution characteristics of 30 pharmaceuticals and personal care products (PPCPs) in 2 canals and 2 rivers in the West Midlands, UK. WWTPs discharge contribute immensely to high concentration level in environmental water. Therefore, we hypothesized that PPCPs concentrations will be much higher in the Rivers than the Canals. This is mainly due to discharge of secondary effluents into rivers and other environmental conditions (seasons)/sources such as runoffs from agricultural lands into the nearby rivers. The objectives of this study are (1) to investigate the occurrence and concentrations of 30 commonly used PPCPs. (2) evaluate seasonal variability in concentrations and spatial distribution of studied PPCPs.

5.2. Sampling

Grab samples were collected each month from 2 rivers (the River Severn and the River Tame) and 2 canals (the Worcester and Birmingham canal and Coventry canal) in the UK. Samples were collected between March and August 2018 (24 samples in total). Figure 19 below shows the sampling locations within the West Midlands, UK.

High-density polyethylene (HDPE) plastic bottles were first rinsed 3 times with water sample from each sampling site, which was discarded. Afterwards, 1 litre of water was collected and the caps were replaced on the sample bottles. Samples were then transported back to the University of Birmingham in ice-boxes and stored in a cold, dark room with temperature set at 3-5 ˚C. Samples were extracted within 3 days of collection, applying the protocols described in Section 2.4.

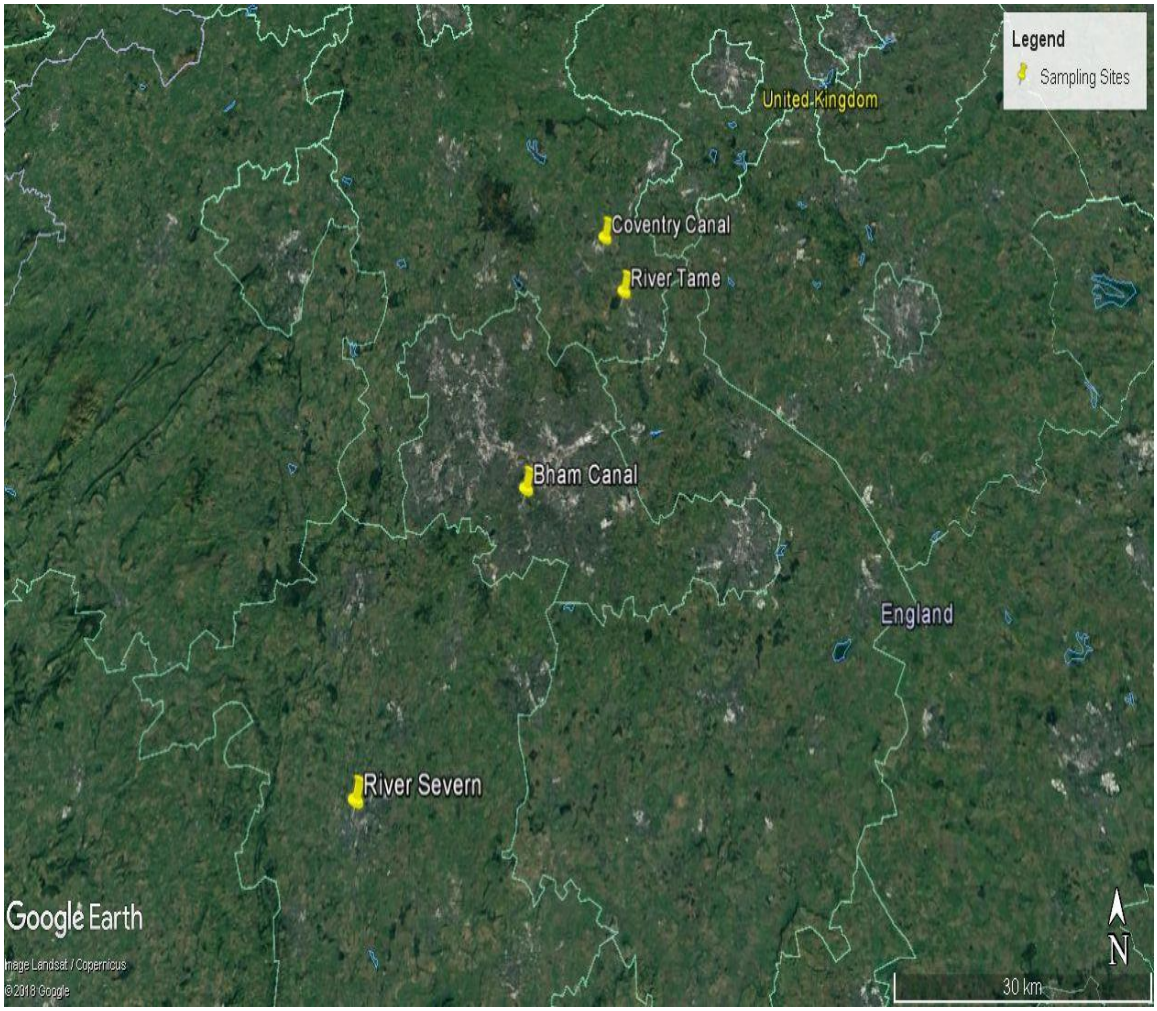


Figure 19: Map of sampling locations in the West Midlands, UK.

5.2.1. Statistical analysis

IBM SPSS version 22.0 software package for windows 10 was used for the statistical analysis. Parametric tests were applied for comparison of sample means (Student t-test to compare between two datasets for spring and summer results). ANOVA in combination with Tukey's HSD post-hoc test was applied for pair-wise comparison among multiple datasets to test for statistically significant differences. For statistical purposes, concentrations below the MDL were assigned a value of half the MDL. P values <0.05 were considered significant.

5.3. Results and discussions

The occurrence of 30 PPCPs was investigated in canals and rivers based on their concentrations and seasonal-spatial distribution characteristics. 30 of the compounds studied were detected in measurable concentrations, with few below detection limits. β-

estradiol, acetaminophen, doxycycline, clotrimazole, valsartan and mefloquine hydrochloride were not detected in any of the studied samples in both seasons. The absence of the antimalarial mefloquine hydrochloride is expected due to little or no occurrence of malaria in this part of the globe. (Ebele et al., 2017, Abou-Elwafa Abdallah et al., 2018). The most abundantly detected PPCPs were metformin, amoxicillin and nicotine at concentrations exceeding 1000 ng/L. Caffeine and oxazepam were below the limit of detection in the two canals and River Severn; however, in the River Tame caffeine and oxazepam were detected. An average concentration 6 ng/L was found for diazepam at the Coventry canal in summer sample, the rest of the sites were below detection limit. PPCPs average concentrations in spring and summer seasons are presented in table 19.

Table 19: Average concentrations (ng/L) in UK surface water during spring and summer season

	Average concentrations (ng/L) in UK Surface water							
Target PPCPs	**Birmingham canal**		**Coventry canal**		**River Severn**		**River Tame**	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
Metformin	5200	1900	770	1100	1400	4500	8000	14600
Gabapentin	230	5	120	3	210	3	440	3
Codeine	450	670	670	430	783	400	390	260
Nicotine	1800	1900	1100	960	153	2400	760	900
Trimethoprim	3	6	3	6	35	49	110	210
Caffeine	4	4	4	4	4	4	640	1300
Sulfamethoxazole	4	2	12	2	14	2	14	2
Amoxicillin	1300	2600	1900	1600	3700	1500	2000	1600
Tramadol	5	2	14	19	39	92	370	620
Metoprolol	2	1	1	1	1	1	8	24
Doxycycline	11	11	11	11	11	11	11	11
Propranolol	2	2	2	2	2	4	22	38
Carbamazepine	16	1	23	15	47	120	240	390
Erythromycine-H2O	11	11	11	11	29	40	100	150

Hydrocortisone	19	31	19	19	34	19	21	19
DEET	23	28	13	70	23	74	48	74
Oxazepam	3	3	3	3	3	3	5	11
Clotrimazole	8	8	8	8	8	8	8	8
Mefloquine HCl	12	12	12	12	12	12	12	12
Naproxen	8	95	8	2	21	60	33	97
Diazepam	2	2	2	6	2	2	2	2
Valsartan	4	4	4	4	4	4	9	4
17-α-ethinylestradiol	47	42	42	42	130	110	42	42
Glyburide	16	20	16	17	18	34	24	28
Diclofenac sodium	5	5	5	5	5	9	49	48
Ibuprofen	4	4	4	4	65	450	400	11
Meclofenamic acid	5	5	5	5	10	26	56	75
Gemfibrozil	7	24	41	16	140	17	7	7

5.3.1. The canals PPCPs profile

Birmingham canal (Bham canal)

The canal which runs next to the University of Birmingham train station is known as the Worcester and Birmingham Canal, hence will be referred to as Bham canal. Bham canal runs from the centre of Birmingham through the country side to the heart of the cathedral City of Worcester. This canal is 29.5 miles long with 58 locks, intersecting with 2 other canals, meeting Stratford-upon-Avon at Kings Norton Junction and meeting the Droitwich canal at Hanbury Junction (CanalandRiverTrust). The canal serves as a major cruising route for boating and a walking and cycling path for people. Our result shows that out of the 30 PPCPs studied, 16 were measurable and quantifiable either in spring and/or summer samples. The $\sum_{30}$PPCPs concentrations measured in Bham Canal in spring and summer were 27000 and 22000 ng/L respectively (table 21). PPCPs were ubiquitously detected in Bham canal in both seasons spanning a broad concentration range. Metformin (300-12000 ng/L), codeine (260-1000 ng/L), nicotine (240-4400 ng/L), amoxicillin

(900-4200 ng/L), naproxen (2-140 ng/L), DEET (5-64 ng/L), glyburide (7-43 ng/L) and trimethoprim (2-11 ng/L) were detected in 100% of the samples. On the other hand, Caffeine, propranolol, oxazepam, clotrimazole, diazepam, valsartan diclofenac and meclofenamic acid were below the limit of detection. The results from this site showed no significant difference between the concentrations detected in the spring and summer samples. Table 20A provides a summary of individual PPCPs concentrations in both seasons in Bham canal.

Table 20A Summary of concentrations (ng/L) of target PPCPs in Bham canal

Bham Canal	Metformin	Gabapentin	Codeine	Nicotine	Trimethoprim	Caffeine	Sulfamethoxazole	Amoxicillin	Tramadol	Metoprolol	Doxycycline	Propranolol	Carbamazepine	Erythromycine-H2O
Spring	12000	310	480	3900	2	4	6	1500	10	1	11	2	44	11
	1100	370	610	540	4	4	3	1500	3	2	11	2	1	11
	2800	3	260	830	2	4	2	900	3	1	11	2	1	11
Summer	300	11	1000	240	11	4	2	4200	3	1	11	2	1	11
	760	3	560	890	3	4	2	2200	2	1	11	2	1	11
	4700	3	410	4400	3	4	2	1400	2	1	11	2	1	11
	Hydrocortisone	**DEET**	**Oxazepam**	**Clotrimazole**	**Mefloquine HCl**	**Naproxen**	**Diazepam**	**Valsartan**	**17a-eythynestradiol**	**Glyburide**	**Diclofenac sodium**	**Ibuprofen**	**Meclofenamic acid**	**Gemfibrozil**
Spring	19	27	3	8	12	2	2	4	42	15	5	4	5	7
	19	11	3	8	12	19	2	4	42	21	5	4	5	7
	19	31	3	8	12	2	2	4	58	12	5	4	5	7
Summer	57	5	3	8	12	139	2	4	42	9	5	4	5	7
	19	14	3	8	12	49	2	4	42	7	5	4	5	7
	19	64	3	8	12	98	2	4	42	43	5	4	5	59

Coventry canal

The second canal studied is the Coventry canal which runs from Coventry and ends 38 miles north at Fradley Junction, north of Lichfield with 13 locks, intersecting with the Trent and Mersey canal. Samples from this site were collected around the village of Hopwas along Lichfield road in Tamworth (CanalandRiverTrust). This canal serves as home for species of birds, boating activities and small local businesses such as pubs and restaurants along the canal. A total of 14 compounds were detected in either spring and/or summer samples. The $\sum_{30}$PPCPs concentrations measured in spring and summer samples were 14000 and 13000 ng/L respectively. Amoxicillin (740-2700 ng/L), nicotine (200-2300 ng/L), metformin (110-1900 ng/L), codeine (220-750 ng/L), tramadol (6-25 ng/L) were detected in all of the samples in both seasons (Table 20B). The concentrations detected for trimethoprim and DEET were significantly higher ($P = 0.02$ and 0.04, respectively) in the summer compared to the spring, indicating higher usage of these compounds during summer season. This is not surprising for DEET as consumers' use of DEET-containing insect repellents increases during this period. Knepper reported similar findings of higher concentrations of DEET in the summer and decreasing concentrations during spring and fall in the river Nadda, Nied, Germany (Knepper, 2004). Table 21 provides a statistical summary of $\sum_{30}$PPCPs concentrations measured in both canals investigated. This shows a much higher detection of these chemicals in the spring than the summer season however, no significant difference was found between $\sum_{30}$PPCPs level in both seasons and canals.

Table 20B Summary of concentrations (ng/L) of target PPCPs in Coventry canal

Coventry Canal	Metformin	Gabapentin	Codeine	Nicotine	Trimethoprim	Caffeine	Sulfamethoxazole	Amoxicillin	Tramadol	Metoprolol	Doxycycline	Propranolol	Carbamazepine	Erythromycine-H2O
Spring	970	3	670	2300	1	4	7	2100	24	1	11	2	29	11
	110	350	750	860	2	4	27	2000	6	1	11	2	17	11
	1300	3	580	200	6	4	2	1500	11	1	11	2	22	11
Summer	1200	3	220	980	5	4	2	740	19	1	11	2	27	11
	170	3	330	710	4	4	2	1400	14	1	11	2	18	11
	1900	3	740	1200	8	4	2	2700	25	1	11	2	1	11
	Hydrocortisone	DEET	Oxazepam	Clotrimazole	Mefloquine HCl	Naproxen	Diazepam	Valsartan	17a-eythynestradiol	Glyburide	Diclofenac sodium	Ibuprofen	Meclofenamic acid	Gemfibrozil
Spring	19	27	3	8	12	2	2	4	42	25	5	4	5	100
	19	8	3	8	12	20	2	4	42	17	5	4	5	7
	19	3	3	8	12	2	2	4	42	6	5	4	5	10
Summer	19	90	3	8	12	2	2	4	42	24	5	4	5	15
	19	41	3	8	12	2	5	4	42	10	5	4	5	12
	19	79	3	8	12	2	10	4	42	19	5	4	5	22

5.3.2. PPCPs profile in UK rivers

Unlike the canals, the PPCPs concentrations detected in the two rivers under investigation are relatively higher in concentrations during summer than spring.

The River Severn

The River Severn is the longest river in Britain. It runs for 220 miles from the Welsh mountains through the Shropshire and Worcestershire countryside. The length of the river is about 42.6 miles with 6 locks. The river is home to various aquatic species including fishes and duck. Rowing, cruising and fishing are some of the activities offered by the river (CanalandRiverTrust). Our results revealed that River Severn had a total of 23 PPCPs detected in either spring and/or summer samples, with the most abundant being: metformin (500-5600 ng/L), codeine (290-1700 ng/L), amoxicillin (1000-8500 ng/L), ibuprofen (4-1200 ng/L), trimethoprim (19-55 ng/L), tramadol (22-110 ng/L), carbamazepine (13-130 ng/L), DEET (3-94 ng/L), glyburide (14-59 ng/L) and meclofenamic acid (8-37 ng/L)(table 20C). The ΣPPCPs concentrations in spring and summer samples were 20000 and 30000 ng/L (Table 22). There was no significant difference between the seasons for majority of the compounds. However, nicotine was found to be significantly higher in summer than spring ($p < 0.05$).

High concentrations (1900, 3400, and 2000 ng/L) of nicotine were detected in 100% of the summer samples. Nicotine average concentration was ~15 times higher in the summer (2400 ng/L) than the spring (150 ng/L) samples. This could be partly due to relatively lower water volume (less rainfall observed during the summer 2018, which was mostly influenced by heat wave), therefore making the dilution and flow processes slower compared to the spring. The average rainfall volume for Worcester during the spring and summer months were 78 mm and 31 mm, respectively. These data were provided by the National Meteorological Library and Archive – Met Office, UK (NationalMeteorologicalLibraryandArchive).

There was no significant difference found for the antibiotic amoxicillin in the spring and summer samples and both seasons had concentrations exceeding 1000 ng/L. The average concentrations of amoxicillin in spring and summer samples were 3700 and 1500 ng/L respectively. This is not surprising as amoxicillin is a commonly used antibiotic for treatment of wide variety of bacterial infections in humans as well as animals. Amoxicillin has a low metabolism rate in humans and 80-90% of it is excreted unchanged, therefore

making it one of the most frequently detected PPCPs in the environment (Hirsch et al., 1999, Pérez-Parada et al., 2011).

The antidiabetic drug metformin was also detected at high average concentrations in spring and summer 1400 and 4500 ng/L respectively. Not only is it used for diabetic treatment, it is also used in treatment of other medical conditions such as: treatment of cancer (Ben Sahra et al., 2010), as well as treatment for polycystic ovarian syndrome (Tang et al., 2012). This could also influence the concentrations detected in this case. The high concentrations measured in this study are also most likely to be due to effluent discharge in the river/high consumption combined with the fact that metformin is not metabolised in the liver and is excreted unchanged in urine (Gong et al., 2012). Scheurer *et al.* found a similar average concentration of metformin (3000 ng/L) in the river Schwarzbach, Germany. Such relatively high concentrations were attributed to the impact of wastewater effluent from a WWTP at the mouth of the river, together with the relatively dry weather conditions (summer) during sampling (Scheurer et al., 2012).

Table 20C Summary of concentrations (ng/L) of target PPCPs in River Severn

River Severn	Metformin	Gabapentin	Codeine	Nicotine	Trimethoprim	Caffeine	Sulfamethoxazole	Amoxicillin	Tramadol	Metoprolol	Doxycycline	Propranolol	Carbamazepine	Erythromycine-H2O
Spring	500	3	1700	450	53	4	36	8500	22	1	11	2	13	39
	620	630	380	7	19	4	4	1000	23	1	11	2	37	11
	3000	3	290	7	33	4	2	1600	70	1	11	2	92	37
Summer	5600	3	330	1900	50	4	2	1100	64	1	11	2	130	46
	4300	3	370	3400	43	4	2	1300	110	1	11	3	130	19
	3700	3	510	2000	55	4	2	2000	99	2	11	7	100	55
	Hydrocortisone	**DEET**	**Oxazepam**	**Clotrimazole**	**Mefloquine HCl**	**Naproxen**	**Diazepam**	**Valsartan**	**17a-eythynestradiol**	**Glyburide**	**Diclofenac sodium**	**Ibuprofen**	**Meclofenamic acid**	**Gemfibrozil**
Spring	65	3	3	8	12	2	2	4	90	18	5	4	10	400
	19	43	3	8	12	39	2	5	42	17	5	150	8	7
	19	23	3	8	12	22	2	4	240	21	5	46	13	9
Summer	19	94	3	8	12	47	2	4	42	30	5	1200	12	38
	19	68	3	8	12	31	2	4	230	14	16	110	37	7
	19	60	3	8	12	100	2	4	42	59	7	12	28	7

The River Tame

The River Tame is the main river of the West Midlands conurbation and is one of the main tributaries of the River Trent. The River Tame is about 59 miles long, flowing from its source near Oldbury down to where it joins the River Trent at Alrewas. The river receives three major sewage works outflows – Coleshill, Tamworth and Minworth sewage treatment works, which is the largest in Europe. 23 out of 30 compounds were detected in the River Tame. The highest concentrations detected were; metformin ranging from (4100-20300 ng/L), followed by amoxicillin (950-3600 ng/L), nicotine (<7-2700 ng/L), gabapentin (<3-1300 ng/L), caffeine (490-1500 ng/L), ibuprofen (<4-1100 ng/L), tramadol (200-700 ng/L), codeine (75-570 ng/L) carbamazepine (110-480 ng/L), trimethoprim (36-220 ng/L), erythromycin-H_2O (24-210 ng/L), meclofenamic acid (23-120 ng/L) and naproxen (<2-110 ng/L) (Table 20D).

The ∑PPCPs concentrations of the target compounds in spring and summer samples were 41000 and 61000 ng/L respectively (table 22). Statistical analysis showed that average concentrations of caffeine were significantly higher ($p = 0.03$) in summer 1300 ng/L (range: 910-1500 ng/L) than spring 640 ng/L (490-810 ng/L). These concentrations are not surprising and suggest strong anthropogenic influence. Caffeine has a widespread usage in food, beverages, as well as pharmaceuticals. The detection of caffeine in environmental water reflects its consumption in a community, which can be used to track the lifestyle within that community. Other studies have identified caffeine as a good population marker, which was significantly correlated to the population number served by a certain WWTP (O'Brien et al., 2014, Moldovan et al., 2008).

Naproxen concentrations were significantly higher ($p < 0.05$) in summer (average = 97 ng/L, range = 86-110 ng/L) than spring samples (average = 33 ng/L, range = <2-77 ng/L) in the river Tame. Despite high input or consumption of this pharmaceutical used for different clinical conditions such as musculoskeletal disorders and short and long term pain (McGettigan and Henry, 2013), lower naproxen concentrations during spring may be due to dilution effects. Also, direct photo transformation and biodegradation processes might contribute to attenuation in concentrations (Tixier et al., 2003, Luo et al., 2014) as well as performance of the treatment plants discharging into the rivers (Munro et al., 2019).

Table 20D Summary of concentrations (ng/L) of target PPCPs in River Tame

River Tame	Metformin	Gabapentin	Codeine	Nicotine	Trimethoprim	Caffeine	Sulfamethoxazole	Amoxicillin	Tramadol	Metoprolol	Doxycycline	Propranolol	Carbamazepine	Erythromycine-H2O
Spring	11000	3	570	1500	110	810	40	3600	200	1	11	14	110	97
	4100	1300	310	830	36	620	2	950	350	1	11	12	220	24
	9400	3	290	7	180	490	2	1500	560	21	11	42	400	190
Summer	16000	3	75	7	200	1500	2	1300	590	21	11	42	330	130
	7700	3	370	7	190	1500	2	1600	700	29	11	31	480	100
	20000	3	340	2700	220	910	2	1700	560	22	11	40	360	210
	Hydrocortisone	**DEET**	**Oxazepam**	**Clotrimazole**	**Mefloquine HCl**	**Naproxen**	**Diazepam**	**Valsartan**	**17a-eythynestradiol**	**Glyburide**	**Diclofenac sodium**	**Ibuprofen**	**Meclofenamic acid**	**Gemfibrozil**
Spring	25	60	3	8	12	2	2	18	42	27	33	120	76	7
	19	35	3	8	12	21	2	4	42	7	61	1100	23	7
	19	51	9	8	12	77	2	4	42	37	52	4	68	7
Summer	19	100	11	8	12	86	2	4	42	20	38	26	55	7
	19	31	13	8	12	96	2	4	42	18	48	4	53	7
	19	87	10	9	12	110	2	4	42	47	56	4	120	7

Ibuprofen is one of the most widely used analgesics. In the River Tame, ibuprofen was measured at average concentrations of 400 (range <4-1100 ng/L) and 10 ng/L (range <4-26 ng/L) in spring and summer respectively. The concentrations detected for ibuprofen in this present study is not surprising, because of the effluent discharge in the river. In this case ibuprofen was detected more in spring, which could be due to higher consumption to prevent or relieve colds that likely occur more frequently in spring. Previous studies have reported similar/higher concentrations, detection frequencies of PPCPs, as well as seasonal variations in concentrations (Focazio et al., 2008, Bound and Voulvoulis, 2006, Hughes et al., 2013, Ngubane et al., 2019, Letsinger et al., 2019).

In comparison with the present study, Letsinger et al. investigated the spatial and temporal occurrence of five pharmaceuticals including; ibuprofen, paracetamol, diclofenac, trimethoprim and citalopram in UK estuaries. Their result revealed consistent detection of ibuprofen (666 ng/L) and paracetamol (89 ng/L) at average concentrations that could adversely affect aquatic organisms (Letsinger et al., 2019). This was based on the acute and chronic ecotoxicity data in environmental risk assessment of pharmaceuticals proposed by European Medicines Agency. If the predicted effect concentration (PEC) in surface water is below 10 ng/L, then the pharmaceutical is unlikely to represent a risk for the environment (Vestel et al., 2016). Based on this, further investigations and PPCPs monitoring is required especially in the rivers studied as most of our target PPCPs were above the stated guideline of PEC in surface water.

Table 21: Statistical summary of concentrations of $\sum_{30}$PPCPs (ng/L) detected in the Bham and Coventry canals

	Seasons	Conc.(ng/L)	Σ30PPCPs	Average	Min	Median	Max
Bham Canal	Spring	18000	27000	1600	2	44	12000
		4200		1100	2	20	1500
		4900		540	2	58	2800
Bham Canal	Summer	6000	22000	540	3	57	4200
		4500		560	3	300	2200
		11000		1300	3	98	4700

Coventry Canal	Spring	6200	14000	620	7	67	2300
		4200		347	2	23	2000
		3600		450	6	110	1500
Coventry Canal	Summer	3300	13000	330	5	59	1200
		2700		240	4	18	1400
		6800		680	8	52	2700

Table 22: Statistical summary of $\sum_{30}$PPCPs (ng/L) detected in Rivers in this study

Sites	Seasons	Conc. (ng/L)	ΣPPCPs	Average	Min	Median	Max
River Severn	Spring	11900	20000	790	3	53	8500
		3000		210	4	38	1000
		5500		370	5	37	3000
	Summer	11000	30000	710	3	64	5600
		10000		600	1	68	4300
		8700		550	2	60	3700
River Tame	Spring	17800	41000	990	14	100	11000
		10000		590	7	220	4100
		13400		840	9	130	9400
	Summer	20400	61000	1200	11	86	16000
		13000		810	13	99	7700
		27700		1500	10	1600	20000

5.3.3. Spatial variations of PPCPs in UK surface water

PPCPs were detected in most of the sites studied. Relatively higher concentrations were found in the River Tame for metformin, trimethoprim, tramadol, metoprolol, propranolol, carbamazepine, erythromycin, oxazepam, diclofenac, and meclofenamic acid compared to other sites. This higher contamination seen in the River Tame could be influenced by environmental conditions as well as proximity of the site sampled to an input source (i.e. effluent discharge from a WWTP) (Letsinger et al., 2019). Our samples from the River Tame were collected 1.2 miles downstream of major sewage treatment plant in Lichfield road, Tamworth (Severn Trent Water, 2019) serving 50000 - 200000 equivalent population. The River Tame receives secondary treated sewage effluent which explains the detected concentrations of these pharmaceuticals in the river. Table 20 and 21 above shows that the River Tame had highest $\sum_{30}$PPCPs than the other three sites investigated. As pointed out earlier in section 1.1.1 of this thesis, many PPCPs are not efficiently removed by conventional wastewater treatment processes; therefore, supporting our finding. However, studies have shown higher removal efficiencies using tertiary treatment that utilises membrane bioreactor (MBR) treatment combined with reverse osmosis (RO) or nano filtration (NF) membrane treatment (Wang et al., 2018, Zhao et al., 2014, Kumar et al., 2019).

Naproxen concentrations were significantly ($p = 0.01$) higher in the River Tame than the Coventry canal. This could be attributed to sewage effluent discharge to the river. Other studies have identified WWTPs as a major source of pharmaceuticals contamination in the freshwater or marine aquatic environment (Daughton and Kummerer, 2004, Kolpin et al., 2002, Ebele et al., 2017). Another non-steroidal anti-inflammatory drug, Diclofenac was significantly higher in concentration in the River Tame compared to other sites where it was found below the detection limit. Letsinger *et al.* reported the absence of diclofenac and citalopram in UK estuaries (Letsinger et al., 2019). The present study found average concentrations of diclofenac in the River Tame, 49 ng/L and 48 ng/L in spring and summer respectively. This is likely to be due to the WWPT nearby and also, its detection mainly in the River Tame could be due to regional prescriptions in the area.

17-α-ethinylestradiol was frequently detected in the River Severn. It is a synthetic hormone with many uses including; as an oral contraceptive, hormone replacement therapy, veterinary medicine for growth enhancement and athletic performance

enhancement by a large population. Its source into the environment is likely to be primarily the result of human/animal excretion and inappropriate disposal of pharmaceuticals into sewage (Swart and Pool, 2007). Agricultural runoff was highlighted by another author as major source of synthetic hormone in the environment (Viglino et al., 2008). 17 α-ethynylestradiol has been identified as one of the major potent estrogenic pollutants in effluent discharge from WWTPs and has the potential to cause detrimental effect on aquatic organism (Miyagawa et al., 2016, Aris et al., 2014). The average concentrations found in the River Severn in spring and summer samples were 130 ng/L and 110 ng/L respectively. The samples from the River Severn were collected upstream of a water treatment plant that serves a population of approximately 200000 people. In close proximity to the river sampling location is an animal farm which could contribute to the concentrations measured. The hormone was below detection limit in the River Tame and Coventry canal, while in Bham canal a single detection of 58 ng/L was reported in a spring sample.

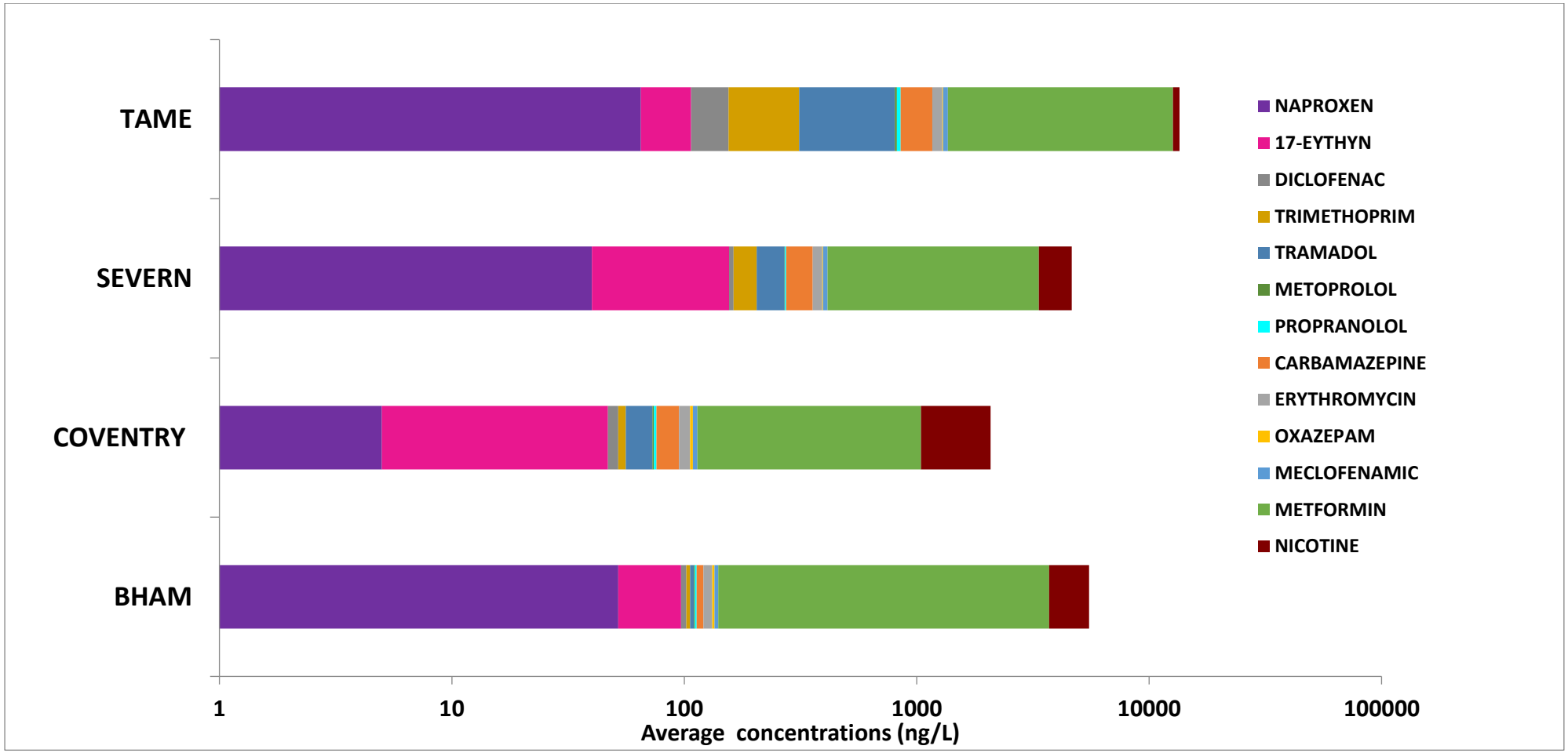
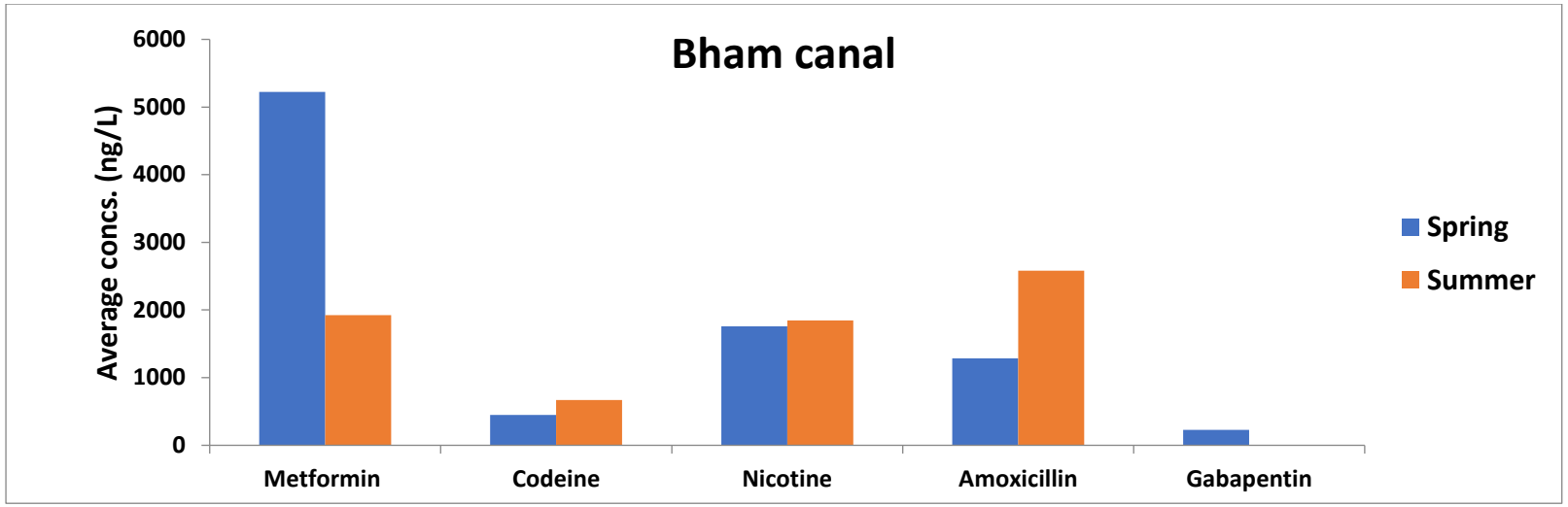


Figure 20: PPCPs Spatial variation across the studied locations.



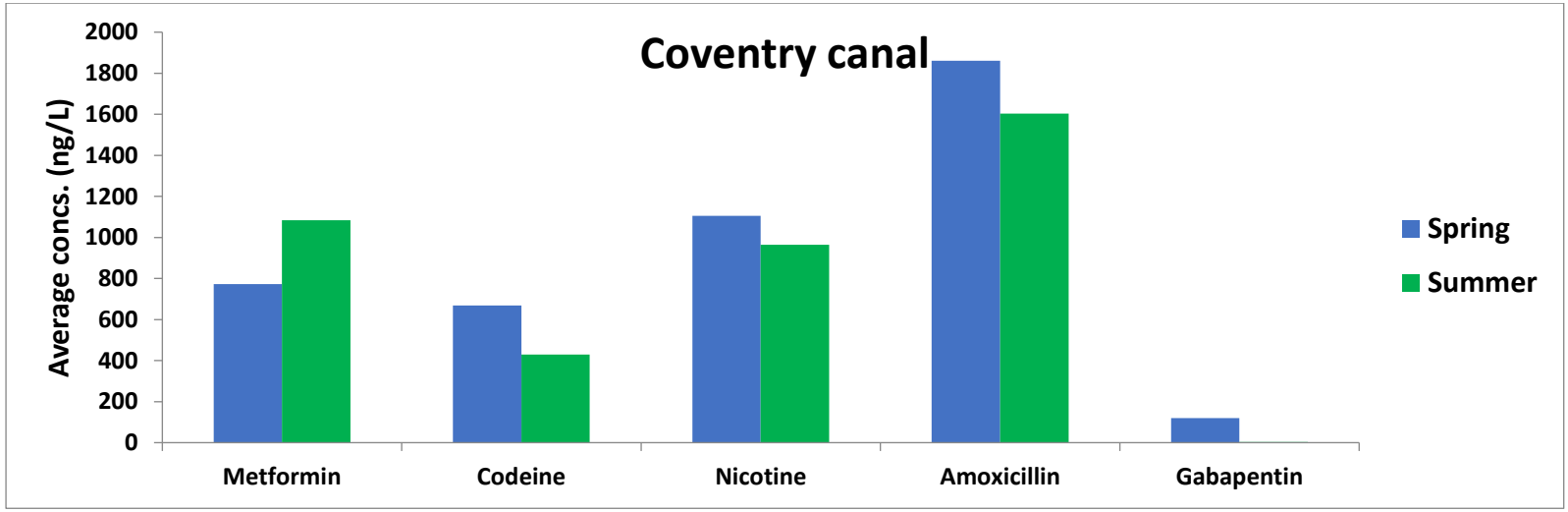
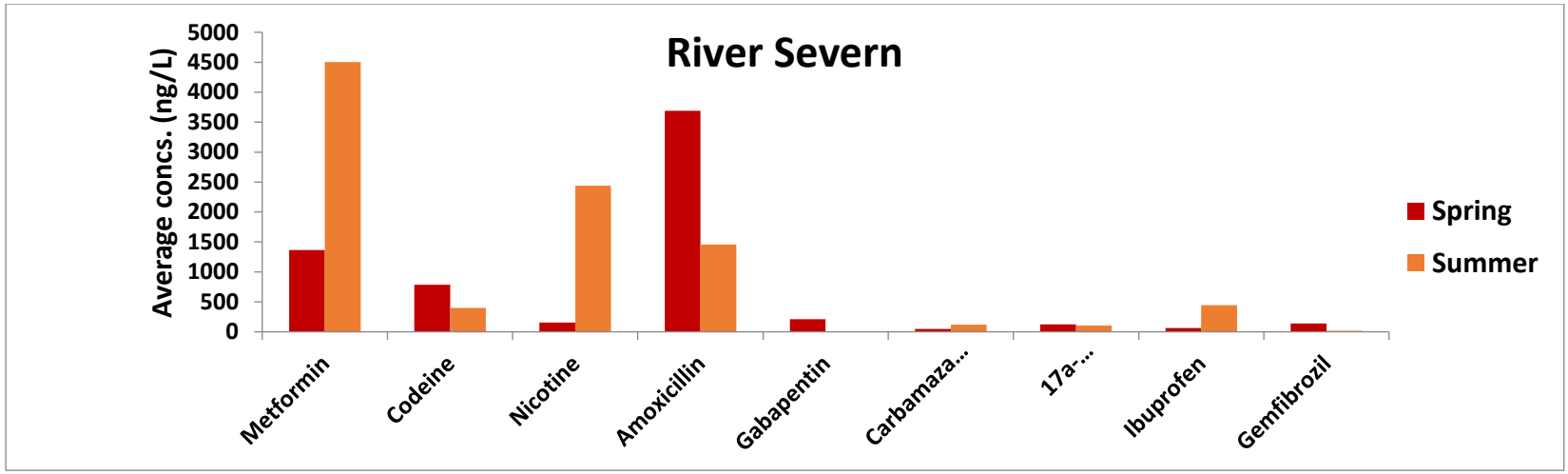


Figure 21A: Seasonal variation of PPCPs in canals



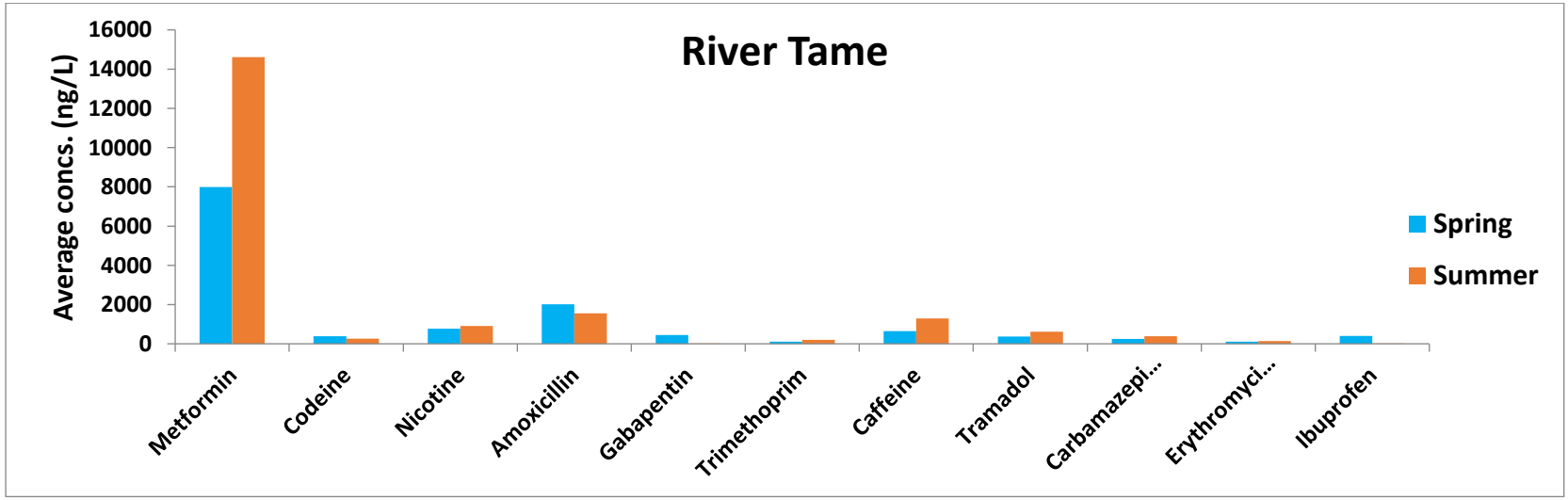


Figure 21B: Seasonal variation of PPCPs in the Rivers

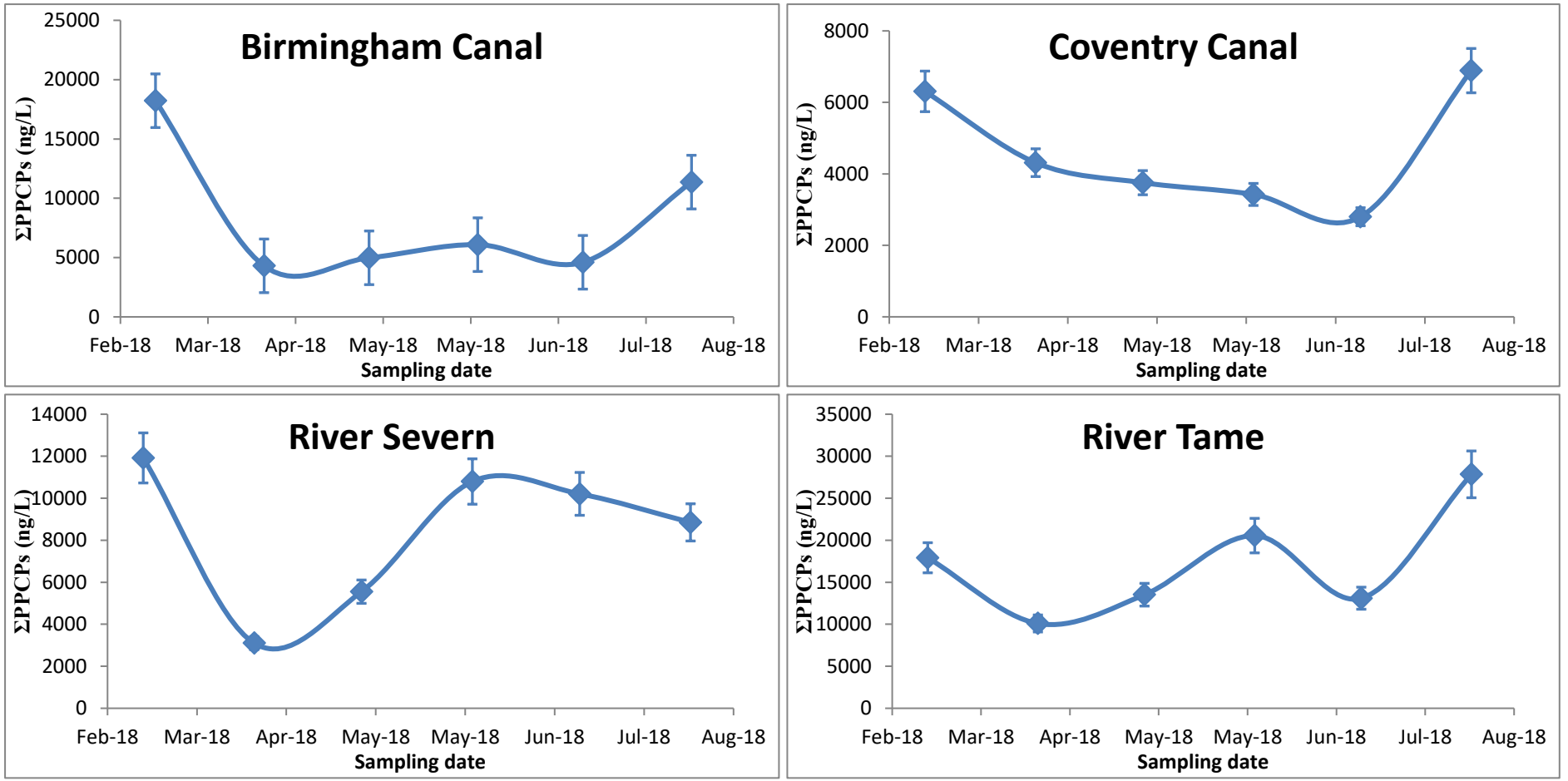


Figure 22: Seasonal variation of $\sum_{30}$PPCPs measured in the studied rivers and canals

The present study investigated seasonal and spatial variation of most freqently detected PPCPs in aquatic environment. Most of our target PPCPs were quantified in samples collected in spring and/or summer at four sampling locations (2 canals and 2 rivers). The $\sum_{30}$PPCPs concentrations in the canals were comparatively higher in spring than summer season, whereas in the rivers, concentrations were higher in the summer season.

PPCPs were ubiquitously detected in the canals and rivers with relatively higher detection in the River Tame as a result of secondary effluent discharge in the river. Further, environmental risk assessment of PPCPs is needed in the River Tame compartments such as the sediment and aquatic organisms (fish). This is important as sediment could act as a sink for these chemicals (Schultz et al., 2010, Devarajan et al., 2015) and subsequently releases to the river water. The presence of these biologically active pharmaceuticals in the river water could lead to the uptake of these chemicals by aquatic biota as mentioned in section 1.3.2.

Finally, advancement in WWTPs processes and technology is required for effective removal of pharmaceutical residues.

CHAPTER VI

Wastewater-based epidemiology for assessing Australian population consumption of PPCPs on census day

6.1. Synopsis

A major uncertainty when estimating per capita consumption of PPCPs by wastewater analysis (which in most cases referred to as wastewater-base epidemiology) relates to size/variability of the De facto population in the catchment area of interest. This is important to verify whether variations observed in PPCPs mass load are due to changes in usage habits such as purity of product, increased/decreased consumption, effect of drug policies or as a result of variations in population size. This study aims to utilise wastewater-based epidemiology data obtained from wastewater sampling campaign on census day to estimate Australian population usage of certain PPCPs. The target PPCPs in this study were based on the most frequently detected compounds based on the previous study of influent samples reported by a researcher in University of Queensland, Australia. Effluents samples collected at a unique opportunity, that is, on census day in Australia were used to estimate the number of people contributing to a given wastewater sample (per capita release of PPCPs to WWTPs). Mass loads of PPCPs of different therapeutic groups were quantified in the effluents of 66 WWTPs serving populations ranging from approximately 400 – 1600000. We investigated the relationship/correlation between the chemical mass load for individual PPCPs and the population contributing to a given WWTP. We hypothesized that concentrations of PPCPs detected in the effluent samples will correlate with the population. A "snapshot" information on the mass of drug excreted per 1000 people was estimated. Individual PPCPs consumption was also estimated with human excretion factor accounted.

6.2. Wastewater sampling

The sampling campaign was conducted by researchers at the University of Queensland, Australia between the 4th and 15th of August 2016. 24 hours composite samples were collected from the effluent outlets of 66 WWTPs across Australia. The samples were collected at this time to coincide with Australian census day. These WWTPs cover urban, semi-urban as well as rural catchments with populations ranging from 400 - 1600000 (estimation provided by STP workers). All samples were preserved immediately after

sampling by acidifying to pH 2 with 2 M hydrochloric acid and then transported back to the laboratory on ice. Prior to analysis, the samples were stored at -20 ˚C in freezers. Figure 23 shows a map of Australia and the number of WWTPs sampled in each state. Details of the WWTPs were kept confidential such as name of the sites and the type of treatment. Each site was allocated a unique code (001-100) and day sampled (Day 1-7).

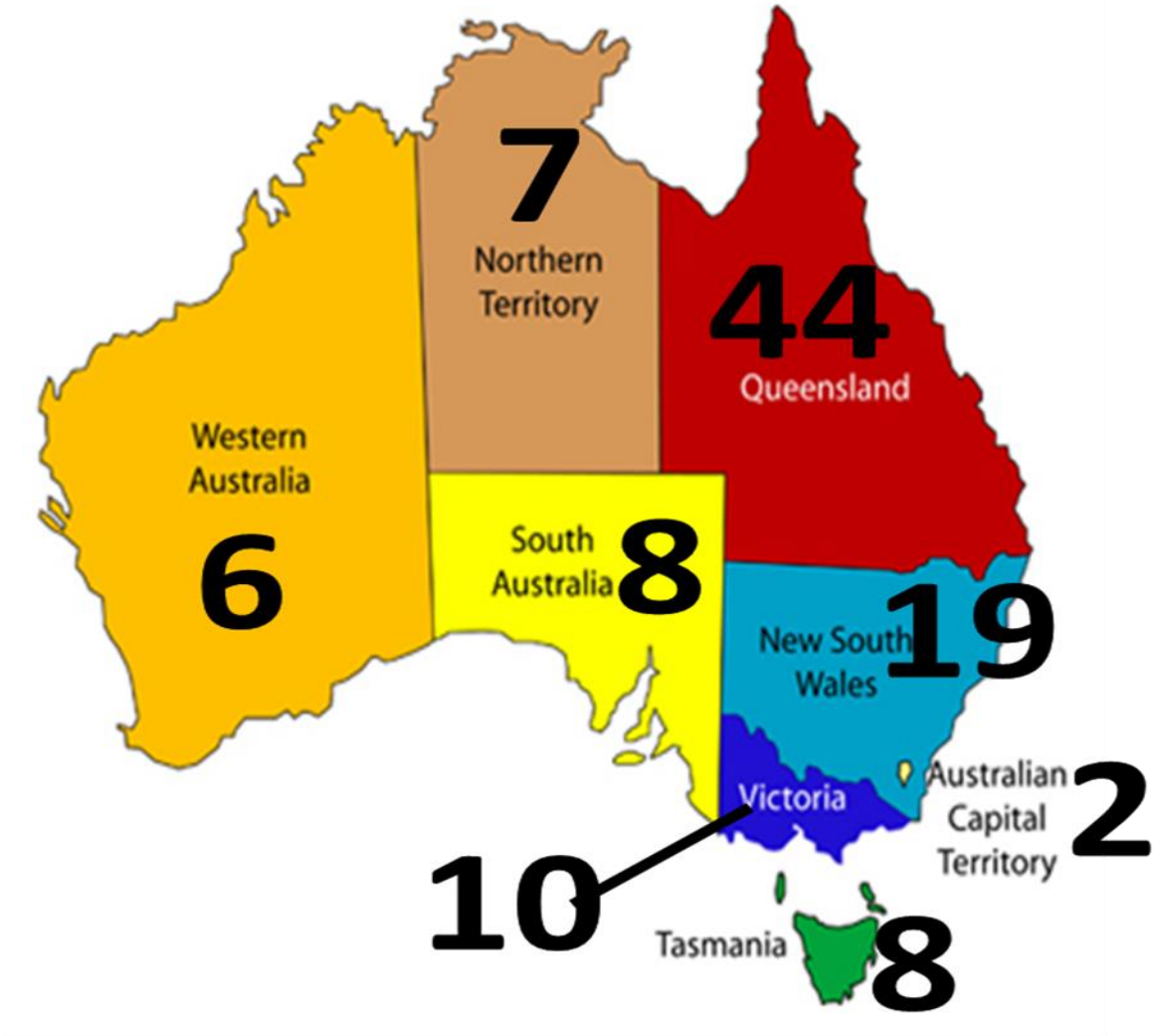


Figure 23: Map of Australia showing number of WWTPs sampled in each state

6.2.1. Sample preparation

Samples were thawed, filtered and aliquoted into 1mL vials for direct injection on to HPLC-MS/MS using an ABSciex API6500+ Qtrap for the determination of the 23 PPCPs listed in section 2.9.3.1 of this thesis. Each of the 1 mL vials were spiked with 5 µL of deuterated labelled analogues mix (Table 10) prior to injection of 5 µL into the LC column.

50 mL aliquots of unfiltered effluent wastewater samples were also spiked with the same concentration of labelled analogues mix prior to clean-up via a previously developed SPE procedure (Abou-Elwafa Abdallah et al., 2018) to enrich the concentrations of target compounds; followed by instrumental analysis as described in section 2.9.3.2 of this thesis.

6.3. Results and discussion

6.3.1. Measured PPCPs concentrations in effluent samples (Direct Injection Method)

Twenty-two out of the 23 PPCPs targeted were measured and quantified using LC-MS/MS Sciex QTrap 6500+. Gabapentin, venlafaxine, and tramadol were detected in 80% of the wastewater effluent samples at average concentrations of 4000, 1200 and 3400 ng/L respectively. Carbamazepine and desmethylcitalopram were detected in more than 70% of the samples at average concentrations of 720 and 300 ng/L respectively. Salicylic acid, acesulfame, hydrochlorothiazide, iopromide and citalopram were detected in 60% of the samples. In 30% – 40% of the samples, caffeine, ibuprofen, paraxanthine, naproxen and DEET were detected. Others such as codeine, paracetamol, nicotine, cotinine, hydroxycotinine, verapamil and triclosan were found in 9% - 28% of samples (Table 23). Atenolol - a beta blocker medication - was not detected in any of the effluent samples.

Table 23: Effluent average concentrations (ng/L) and detection frequencies (DF - %)

Compounds	Average Concentrations	DF (%)
Paraxanthine	5600	42
Caffeine	7500	41
Carbamazepine	720	72
Citalopram	290	59
Codeine	390	29
DEET	1200	39
Desmethylcitalopram	300	71
Gabapentin	4000	80
Iopromide	9600	61
Naproxen	790	38
Paracetamol	8900	18
Tramadol	3400	80
Venlafaxine	1200	80
Salicylic acid	6100	62
Acesulfame	4500	65
Ibuprofen	2800	42
Triclosan	49	9
Hydrochlorothiazide	700	68
Nicotine	220	14
Cotinine	200	18
Hydroxycotinine	300	18
Verapamil	45	12

Average concentrations were calculated based on all samples (including those where the PPCP is present below the LOQ - such samples were replaced with an assumed concentration of LOQ/2)

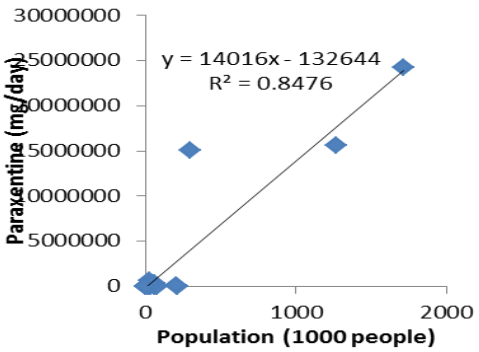
6.3.2. Relationship between chemical mass load and population

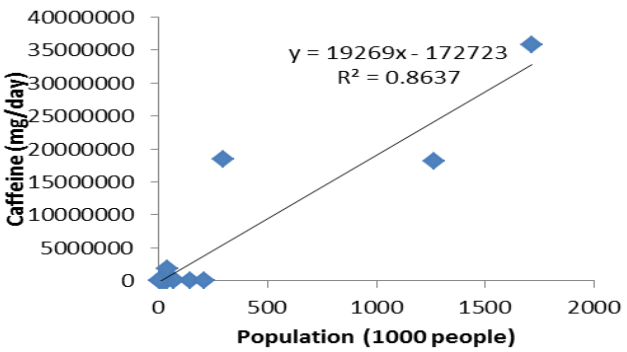
Concentrations were converted to daily mass load by multiplying the measured concentrations by daily flow rate in each of the 66 WWTPs.

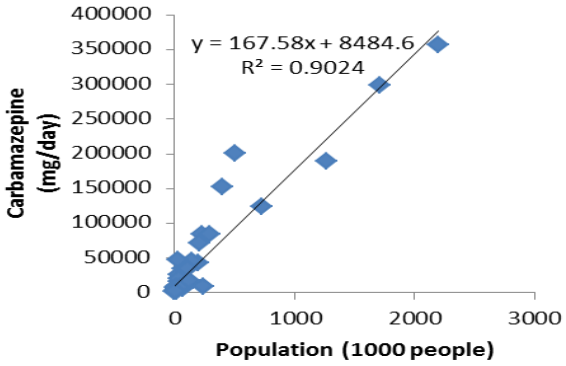
Equation: Daily mass load (mg/day) = concentration (ng/L) x WWTPs flow rate (ML/day)

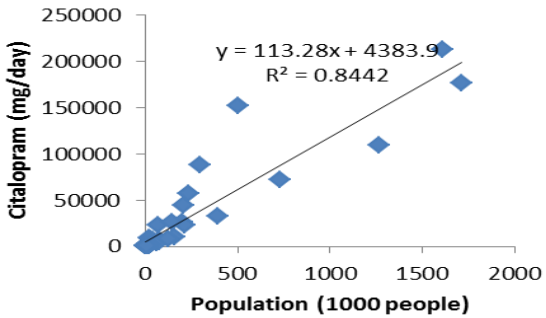
**ML=megaliters = 1 Million litres.*

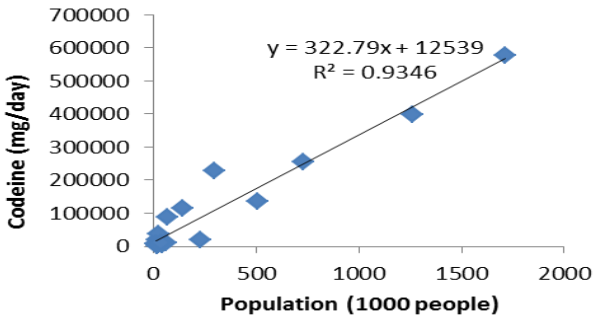
The data were plotted against the equivalent population served by the WWTPs to check for any correlation. All of the selected chemicals showed strong correlation between population and daily mass load with the exception of these compounds, iopromide, salicylic acid and acesulfame (figure 24). The compounds that showed strong correlation could be used as chemical markers for population size and usage pattern of the respective drug (O'Brien et al., 2014). A study in Valencia identified caffeine and nicotine as biomarker for population assessment (Rico et al., 2017). Good correlation was found between caffeine, its metabolite, nicotine and its metabolite mass load and population size in wastewater analysis in 13 WWTPs in Italy (Senta et al., 2015).

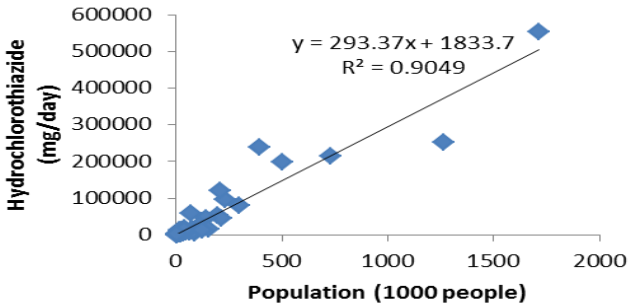

y = 14016x - 132644
R² = 0.8476
Paraxentine (mg/day)
Population (1000 people)

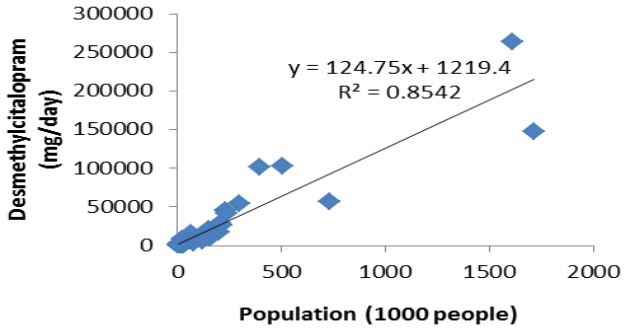

y = 19269x - 172723
R² = 0.8637
Caffeine (mg/day)
Population (1000 people)

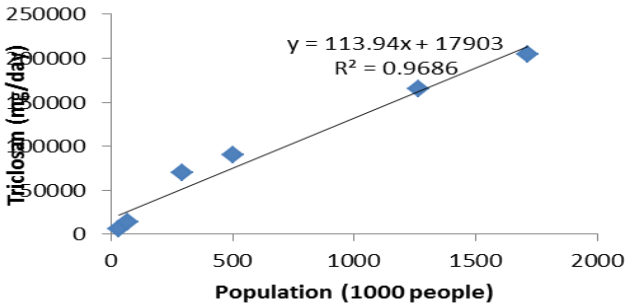

y = 167.58x + 8484.6
R² = 0.9024
Carbamazepine (mg/day)
Population (1000 people)

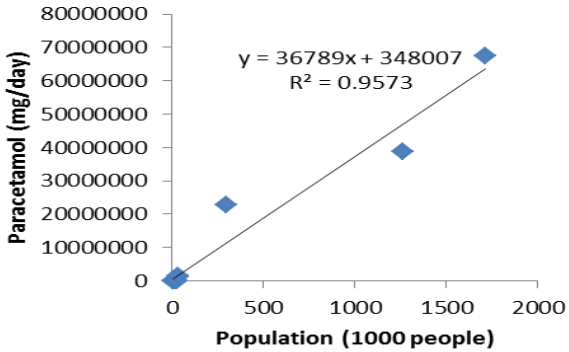

y = 113.28x + 4383.9
R² = 0.8442
Citalopram (mg/day)
Population (1000 people)

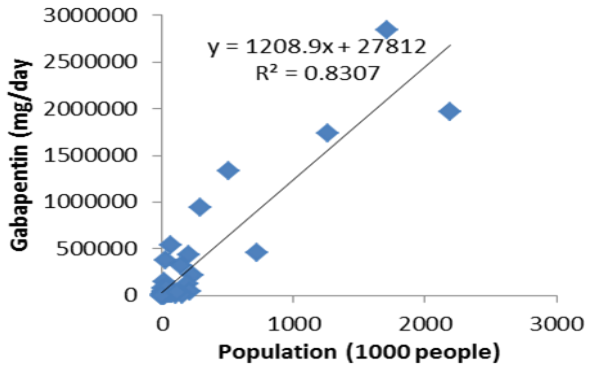

y = 322.79x + 12539
R² = 0.9346
Codeine (mg/day)
Population (1000 people)

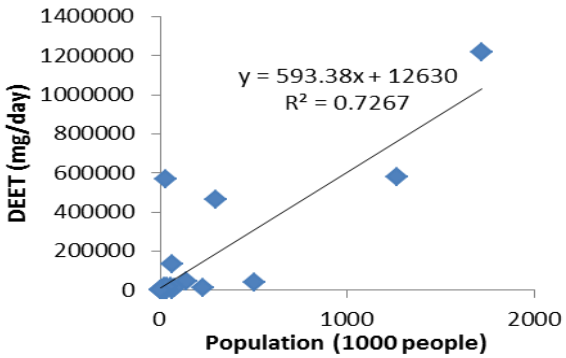

y = 293.37x + 1833.7
R² = 0.9049
Hydrochlorothiazide (mg/day)
Population (1000 people)

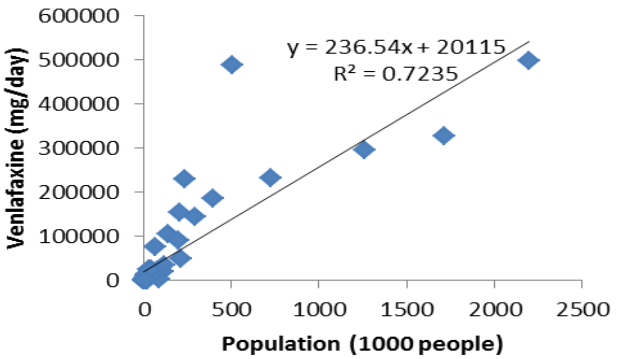

y = 124.75x + 1219.4
R² = 0.8542
Desmethylcitalopram (mg/day)
Population (1000 people)

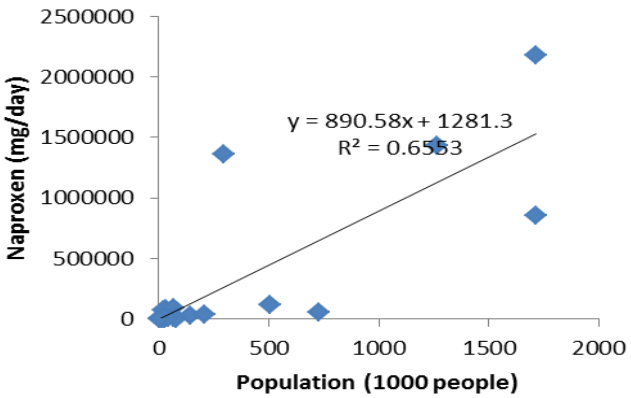

y = 113.94x + 17903
R² = 0.9686
Triclosan (mg/day)
Population (1000 people)

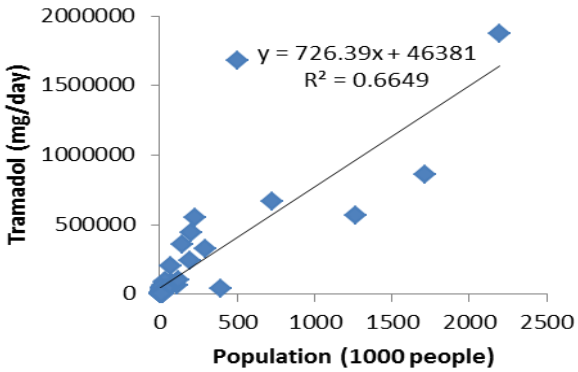

y = 36789x + 348007
R² = 0.9573
Paracetamol (mg/day)
Population (1000 people)

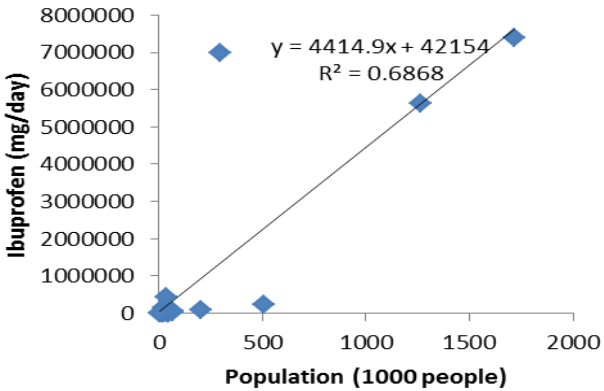

Gabapentin (mg/day
y = 1208.9x + 27812
R² = 0.8307
Population (1000 people)

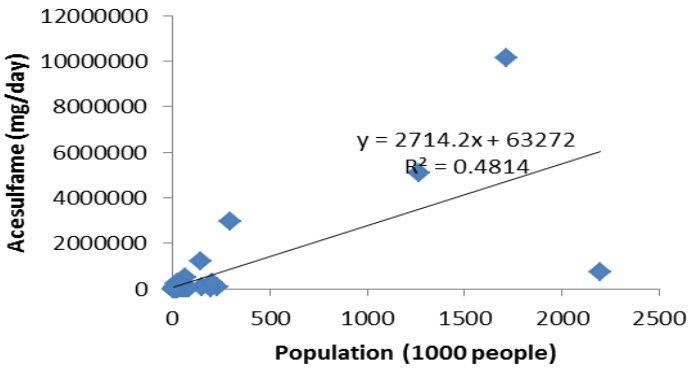

DEET (mg/day)
y = 593.38x + 12630
R² = 0.7267
Population (1000 people)

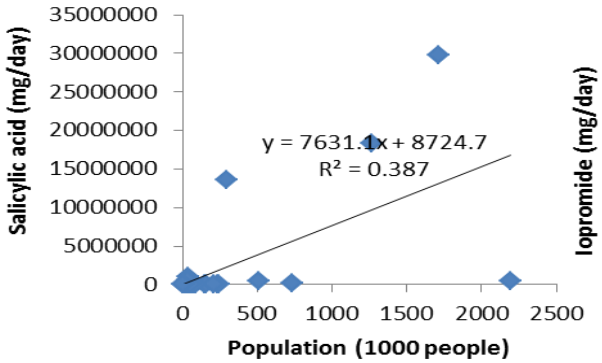

Venlafaxine (mg/day)
y = 236.54x + 20115
R² = 0.7235
Population (1000 people)

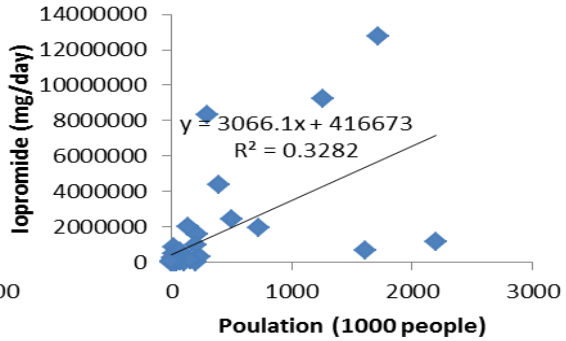

Naproxen (mg/day)
y = 890.58x + 1281.3
R² = 0.6553
Population (1000 people)


Tramadol (mg/day)
y = 726.39x + 46381
R² = 0.6649
Population (1000 people)


Ibuprofen (mg/day)
y = 4414.9x + 42154
R² = 0.6868
Population (1000 people)


Acesulfame (mg/day)
y = 2714.2x + 63272
R² = 0.4814
Population (1000 people)


Salicylic acid (mg/day)
y = 7631.1x + 8724.7
R² = 0.387
Population (1000 people)


Iopromide (mg/day)
y = 3066.1x + 416673
R² = 0.3282
Poulation (1000 people)

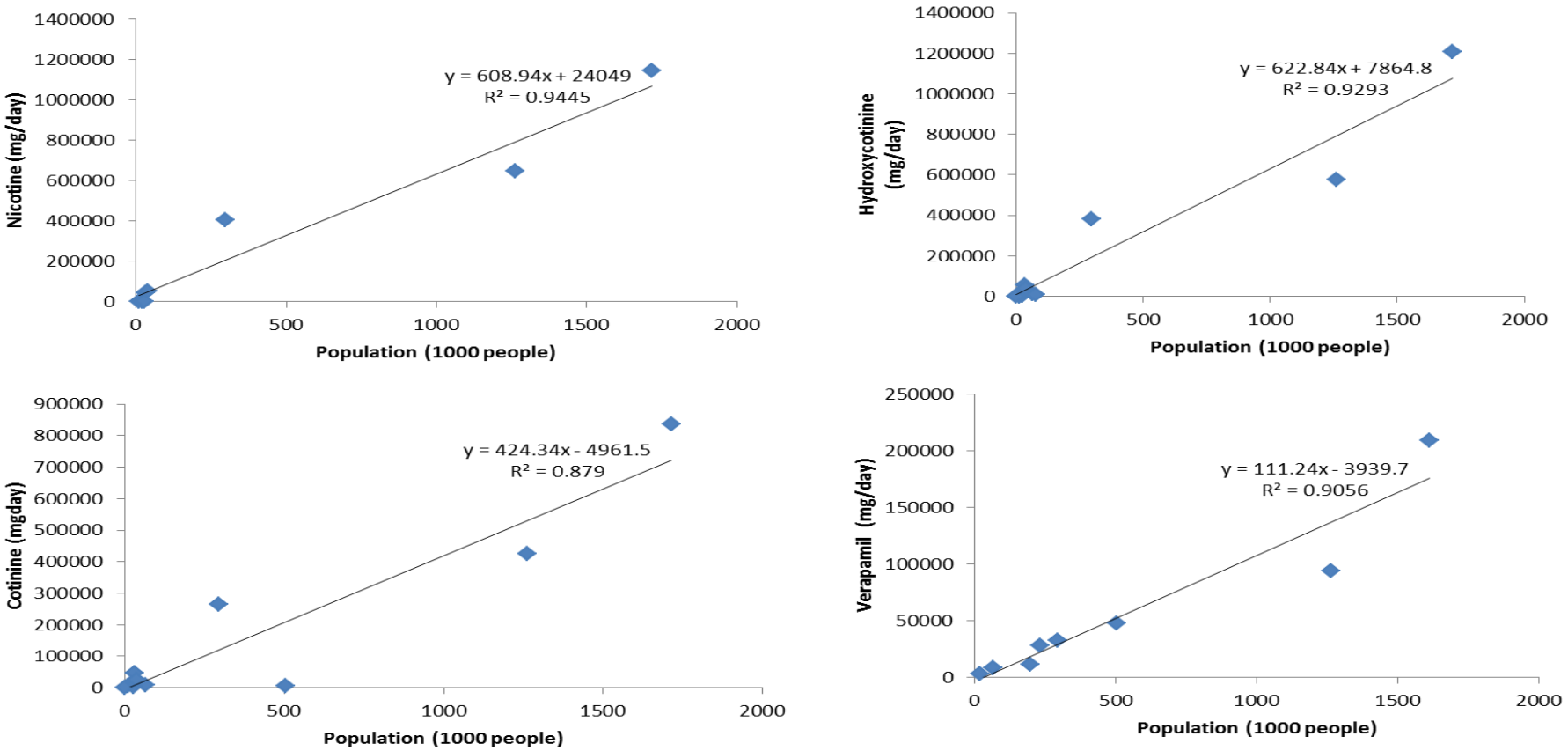


Figure 24: Population versus daily mass load of selected chemicals

The X-ray contrast media iopromide displayed the weakest correlation (R^2 = 0.32) with population. The correlation between the mass load of iopromide and population was weak compared to other chemicals investigated as a result of its variable spatiotemporal usage pattern. O'Brien et al. found a similarly weak correlation for iopromide (R^2 = 0.38) in influent samples collected on a previous census period in Australia (O'Brien et al., 2014). Iopromide is excreted from the human body without undergoing any form of metabolism (Bourin et al., 1997). It has also shown to be resistant to microbial degradation during the activated sludge process in WWTPs and consequently, released into the aquatic environment unchanged (Pérez et al., 2009); which could be an explanation for the high concentrations measured in the present study.

The following compounds displayed the strongest positive correlations at (R^2=0.9); codeine, paracetamol, triclosan, hydrochlorothiazide, carbamazepine, hydroxycotinine, nicotine and verapamil. Therefore, these compounds could be used as wastewater indicator compounds to predict population size and usage pattern of a particular chemical (Kankaanpää et al., 2016, Gunnar and Kankaanpää, 2019). A study in Sydney used WBE to estimate population usage of illicit drugs. The result of the study shows methamphetamine to be the most used illicit drug in Sydney wastewater (Bannwarth et al., 2019). In-sewer degradation of PPCPs also revealed salicylic acid and furosemide to be degradable; therefore, should not be used as best practice for chemical markers (O'Brien et al., 2017). In our study, salicylic acid also displayed a weak correlation (R^2=0.39).

6.3.3. Mass of drug excreted per 1000 people

The mass of pharmaceuticals excreted per 1000 people was estimated by dividing the daily mass load of chemical by the actual population and multiplying by 1000. Figure 25 A and 25B shows the average mass of drug excreted per 1000 people across Australia and expressed as mg/day/1000 people.

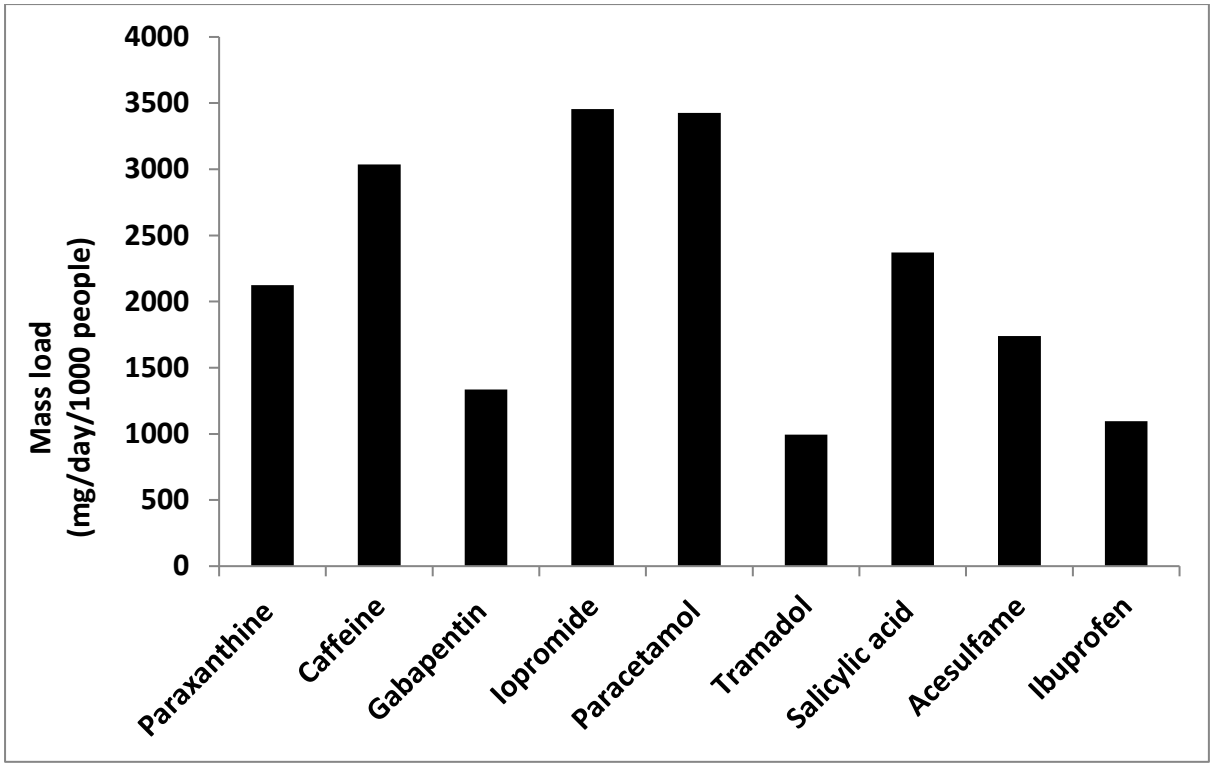


Figure 25A: Mass of drugs excreted per 1000 people across Australia

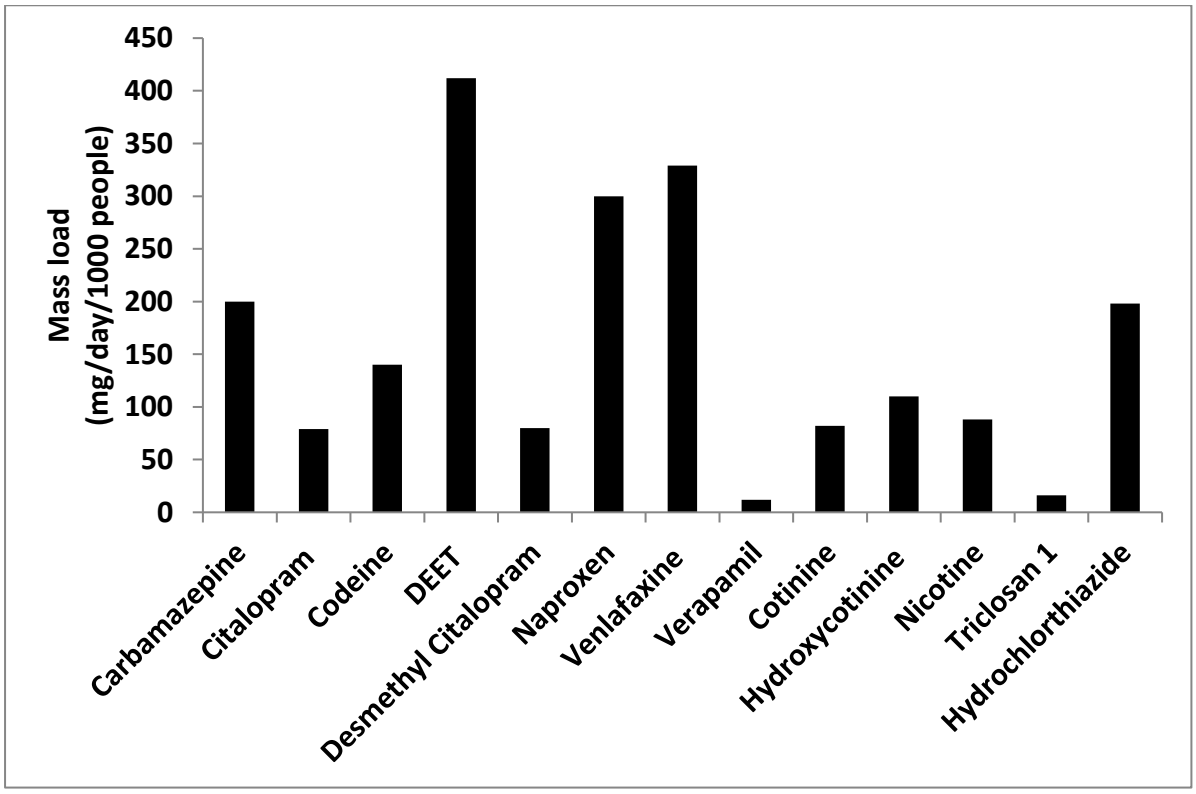


Figure 25B: Mass of drugs excreted per 1000 people across Australia

6.3.4. Estimation of PPCPs daily consumption in Australia

We estimated the consumption of selected PPCPs by dividing the population normalised mass load (Concentration x Flow) by the percentage of drug excreted after consumption (excretion factor), multiplied by the molecular weight of the parent compound or metabolite.

Equation: Daily chemical consumption (mg/day/1000 people) = $\frac{C_i \times F}{P \times E}$ **x Mw PPCP**

Where Ci is the PPCP or metabolite concentration in waste water; F is flow rate of waste water from the WWTP; P is the population served by the WWTP divided by 1000; E is the excretion factor of the PPCP; and Mw is the molecular weight of pharmaceutical or its metabolite.

Estimation of PPCPs consumption indicates the most commonly used substances which may be easily measured in wastewater and could be associated with health status, lifestyle and behaviour of the population investigated (Nakada et al., 2017, Thai et al., 2018, Gao et al., 2016, O'Brien et al., 2014, Daughton, 2018).

6.3.4.1. Consumption of PPCPs across Australia

PPCPs consumption was estimated in effluent wastewater across Australian states. Table 24 below displays a statistical overview of 14 of our PPCPs studied with human excretion factors accounted for. The anticonvulsant gabapentin and iopromide were found to be the most highly consumed pharmaceuticals. This may be due to high concentration dosage administered during the course of therapeutic treatment. For instance, iopromide is used in a range of doses such as 150 mg/mL, 240 mg/mL, 300 mg/mL and 370 mg/mL (Drugs.com) depending on the radiographic imaging required.

Most of the population in the 66 WWTPs catchments studied had high population normalised consumption with the exception of 13 sites for gabapentin and 26 sites for iopromide, where they were not detected. Consumption of gabapentin ranged from 8400 to 1900000 mg/day/1000 people in site 033 and site 019, while iopromide ranged from 83000 to 32000000 mg/day/1000 people in site 011 and site 004. The high output of gabapentin and iopromide could be due to 80% and 97% excretion of unchanged parent compounds respectively (Vollmer et al., 1986, Law et al., 2013).

Caffeine and acesulfame which are common additives found in a wide range of soft drinks and snacks could reflect the expenditure on snack food and soft drinks in the population as lifestyle markers. Consumption of acesulfame was relatively much higher, ranging from 13000 to 1700000 mg/day/1000people than caffeine ranged from approximately 68 to 290000 mg/day/1000 people across the Australia (Table 24). The high output seen for acesulfame is because this chemical is not readily metabolised and is excreted essentially unchanged (excretion rate 99%) after its consumption (Gao et al., 2016). One of the properties of acesulfame is its stability when heated; this explains why it is found in many baked goods and other products (West, 2017). A typical example of acesulfame containing product is diet coke, a 330 mL can of which contains 45 mg acesulfame. In addition, acesulfame is relatively persistent in wastewater and surface water, therefore its concentration in these media increases with population (Buerge et al., 2009).

Table 24: Statistical summary of daily population normalised consumption estimates for PPCPs (mg/day/1000 people) calculated for 66 Australian WWTPs

Compound	Minimum	Median	Maximum	Average	StDev
Caffeine	68	4900	290000	35000	72000
Carbamazepine	63	470	4000	650	610
Citalopram	900	4300	17000	5300	3700
Codeine	12000	46000	200000	66000	54000
Gabapentin	8400	120000	1900000	230000	330000
Paracetamol	170	4600	230000	57000	77000
Tramadol	4700	71000	260000	95000	68000
Venlafaxine	320	4800	16000	5700	3600
Acesulfame	13000	82000	1700000	430000	530000
Salicylic acid	290	1200	190000	16000	38000
Naproxen	610	7200	110000	18000	27000
Iopromide	83000	2200000	32000000	4400000	6400000
Ibuprofen	1900	15000	440000	48000	93000
Hydrochlorothiazide	6700	53000	200000	62000	43000

Population normalised consumption was estimated for 3 non-steroidal anti-inflammatory drugs, ibuprofen, naproxen and salicylic acid. Out of the 66 WWTPs ibuprofen was detected in 27 sites, naproxen in 25 sites and salicylic acid in 41 sites. These drugs metabolise rapidly after ingestion with only 9%, 10% and 3% excreted as parent drugs respectively (Mills et al., 1973, Vree et al., 1993, Von Lehmann et al., 1973). The average estimated daily population normalised consumption across Australia ranged from (1900-440000 mg/day/1000 people) for ibuprofen, (610-110000 mg/day/1000 people) naproxen, and (290-190000 mg/day/1000 people) salicylic acid. The population at site 008 had the highest estimated consumption of the non-steroidal anti-inflammatory drugs ibuprofen (440000 mg/day/1000people), naproxen (110000 mg/day/1000people) and salicylic acid (190000 mg/day/1000people). The high consumption of non-steroidal anti-inflammatory drugs could be attributed to hospital waste within the catchment population. Another possible explanation could also be the type of wastewater treatment processes used, which may not efficiently remove these compounds.

Codeine and tramadol (both opioid analgesics) were also investigated for their population normalised consumption. At the 66 WWTP sites, codeine and tramadol were detected in a total of 19 sites and 53 sites respectively with estimated consumption ranging from 12000-200000 mg/day/1000 people (codeine) and 4700-260000 mg/day/1000 people (tramadol). Interestingly, a smaller population in site 019 had a relatively higher consumption of codeine compared to a much larger population in site 002. The same pattern is seen for tramadol; and the high concentration has been linked to hospital effluent contribution to WWTPs (Munro et al., 2019). Although it was detected in most of the sites, those with smaller population appear to have higher consumption rates of tramadol. Another possible explanation of the high consumption rate seen in small population catchments may be due to the type of WWTPs in use in these catchments that may be less efficient at removing these chemicals, as well as hospital waste inputs.

Carbamazepine which is relatively persistent in wastewater and environmental water was found in 48 sites at an average concentration 720 ng/L across all the sites. The consumption of this pharmaceutical appears moderate across most of the catchment population but still showed a much higher consumption at site 053 with a population of 27158. The population normalised consumption for carbamazepine ranged from 63-

4000 mg/day/1000 people. Table 25 provides detailed PPCPs consumption in Australia with human excretion factors accounted (mg/day/1000people)

Table 25: PPCPs consumption estimated for 66 individual WWTPs in Australia with human excretion factors accounted for (mg/day/1000people)

WWTPs	Population (1000people)	Caffeine	Carbamazepine	Citalopram	Codeine	Gabapentin	Paracetamol	Tramadol	Venlafaxine	Acesulfame	Salicylic acid	Naproxen	Iopromide	Ibuprofen	Hydrochlorothiazide
001_day 6	1611.22			5200									330000		
002_day 7	231.64		850	9500	12000	130000		180000	14000	43000	630				87000
003_day 6	1715.78	98000	410	4000	46000	230000	120000	38000	2700	950000	72000	29000	5700000	80000	69000
004_day 4	20.57	42000	710	17000	130000	970000	630	230000	9600	1700000	15000	85000	32000000	140000	130000
005_day 7	504.53		940	12000	37000	370000		260000	13000		4100	5300	3700000	8900	84000
006_day 6	1263.53	67000	350	3400	43000	190000	94000	35000	3300	640000	60000	26000	5600000	82000	43000
007_day 4	728.76		400	3900	48000	87000		70000	4400		940	1700	2000000		63000
008_day 6	295.55	290000	670	12000	110000	440000	230000	84000	6800	1600000	190000	110000	22000000	440000	57000
009_day 6	395.46		910	3200				7600	6600				8500000		130000
010_day 6	32.76	120000	450	5100	67000	340000	99000	52000	4600	1400000	130000	59000	660000	250000	36000
011_day7	198.24		500	5200		83000		92000	6400	27000			83000		56000
012_day5	238.44		70								620		930000		
013_day 4	212.31			4300		28000			3200				5600000		44000
014_day 6	3.67			2000		15000									
016_day 4	40.77	1700	1400		13000	300000		160000	5100	440000	1600		3300000	5900	
017_day 5	13.11		850	4300		160000		130000	9300		600				73000
018_day 4	29.49	7400				85000	5600	51000		710000	6400		4700000	30000	
019_day 3	27.06	9500	820	12000	200000	1900000		250000	13000	1600000	6700	19000	14000000	20000	110000
020_day 6	19.39	250				14000	170	18000	1800	19000	660			2600	26000
021_day 6	66.98	7800	1200	14000	180000	1100000		230000	16000	1200000	6000	31000	5800000	19000	190000
022_day 4	13.04		510	4500		56000		100000	5800				130000		93000
023_day 6	1.3		230	2400		19000		33000	1500						25000
024_day 6	43.92		770	6200		230000		110000	8100	46000		4500	1200000		55000
025_day 6	142.78	430	740	7200	110000	300000		190000	10000	1400000	660	4800	11000000		69000
026_day 6	4.73			4300		62000		60000	3900	16000	1900				
028_day 7	61.96		560	2500		43000		67000	4400						22000

*The blank cells are non-detects.

*The values were approximated to two significant figures.

WWTPs	Population (1000people)	Caffeine	Carbamazepine	Citalopram	Codeine	Gabapentin	Paracetamol	Tramadol	Venlafaxine	Acesulfame	Salicylic acid	Naproxen	Iopromide	Ibuprofen	Hydrochlorothiazide
029_day 6	61.09									56000	650		2800000		
030_day 5	5.14												1200000		
031_day 6	3.17		1700	3300		19000		120000	3000						37000
032_day 5	65.32	13000	200		23000	130000		56000	2800	82000	1100	2400	2600000	12000	24000
033_day 7	109.79		710			8400		45000	2700				120000		53000
034_day 6	18.81	150		1800		66000		22000	2600	79000	1400			2800	
035_day 5	151.84									58000	530				20000
036_day 3	3.24	1500	460	2500		570000	250	130000	5700	67000	1000	7200		5200	56000
037_day 7	205.52	2200	810	8300		290000		170000	10000	190000	1500	3900	3700000	7700	130000
038_day 4	10.88	22000			78000	450000	3600	110000	6200	1300000	23000	21000		68000	
039_day 4	17.77	880	230			120000		48000	5000	520000	2200	2500		2200	
040_day 6	22.05		360	4600		160000		120000	5500	26000	930				200000
041_day 4	13.03	2100	1000	8000	74000	830000	730	250000	13000	600000	2200	9700	14000000	10000	200000
042_day 6	77.6		510	3400		94000		66000	4000	45000	410	3100	6000000		43000
043_day 6	21.46	6800	200	2900	15000	99000		61000	5200	24000	1000	12000		32000	27000
044_day 7	13.86		190	2100		16000		38000	3200	32000	390			1900	15000
045_day 6	29.41	68	450	4400		160000		71000	4800	42000			150000		40000
046_day 6	32.19		450	3000		110000		79000	5100	58000			590000		46000
047_day 3	43.6														
048_day 4	16.08	4600	470		33000	350000		98000	4000	580000	2900	8500		22000	
049_day 7	84.94		210						700	13000	450		1300000		15000
051_day 6	5.06		130			160000		4700		80000			200000		
052_day 6	20.11		160						320		310		150000		
053_day 6	27.16	4900	4000		16000	150000	2200	110000	6200	740000	7800	1800	4100000	18000	59000
054_day 7	79.62		370	2600		37000		75000	4000	14000		940	450000		28000
055_day 6	0.48														

*The blank cells are non-detects.

*The values were approximated to two significant figures.

WWTPs	Population (1000people)	Caffeine	Carbamazepine	Citalopram	Codeine	Gabapentin	Paracetamol	Tramadol	Venlafaxine	Acesulfame	Salicylic acid	Naproxen	Iopromide	Ibuprofen	Hydrochlorothiazide
056_day 4	38.53		1100			97000		180000	9300				220000		95000
057_day 6	11.71	1400	1100			110000		98000	8700	350000			670000	5100	74000
058_day 3	22.43	6200				110000		16000		230000	17000	7200		39000	
059_day 4	155.6			2600		12000							600000		
062_day 6	15.84		1200	6700		160000		210000	10000	94000	1200				120000
063_day 4	25.95	220	340	1900		67000		62000	3700	36000				2700	49000
064_day 6	47.59		360			64000		47000	3100	30000	1200		130000	3800	30000
066_day 3	37.66	220000			39000	300000	130000	82000	8100	1000000	74000	5400	2300000	20000	64000
067_day 6	2196.38		380			120000		65000	3200	56000	970		410000		
073_day 6	121.08		330	2600		40000		65000	3900						23000
075_day 1	4.16		63	900				24000	1500		290	610			6700
087_day 5	3.53	490	390					22000	1500	270000	660		7300000		41000
098_day 4	9.47							33000	2000						
100_day 7	1.35					26000								8500	

*The blank cells are non-detects.

*The values were approximated to two significant figures.

6.3.4.2. Uncertainties

The result of wastewater-based epidemiology demonstrates the feasibility of using wastewater analysis to estimate daily PPCPs consumption in the national population. However, these data may be associated with a number of uncertainties as mentioned in chapter 1.7.2. These uncertainties could affect the quality of the data presented here. One of the main factors that are anticipated to contribute to the uncertainty in this case could be the quality of the population data. Uncertainty associated with population may arise due to the two population definitions per catchment. These are: the actual number of people that reside in the catchment (known as the *de jure* population) and the people present in the catchment during the census period which includes people working around there, holidaying there, as well as exclusion of the traditional residents not present during the period (*de facto* population). The present study utilised the *de facto* population. Due to dynamic nature of populations, the data generated during census could easily be outdated. Apart from the dynamic changing nature of the population, contributions from industrial/institutional wastewater such as discharge of pharmaceutical-containing wastewater either from manufacturing companies or hospitals could affect the quality of the population estimates in any given catchment. Aside from the aforementioned, uncertainty in the population could also arise from the misuse and overuse of certain PPCPs, particularly drugs of abuse (e.g. Codeine and Tramadol). Limited data are available about the excretion factors of these chemicals and this may affect the accuracy of the study when estimating consumption of PPCPs.

6.4. Measured PPCPs concentrations in effluent samples (SPE method)

Effluent samples from 68 WWTPs were analysed for 17 PPCPs using SPE-enriched extracts. Most of the compounds were detected above the limits of quantification in the 68 effluent samples. Detected in 100% of the effluent samples were nicotine, its metabolite cotinine and DEET at average concentrations (and ranges) of 120000 (3000-1900000), 12000(1200-150000) and 2000(30-30000) ng/L respectively. In more than 90% of the samples were caffeine at average concentration, ranged 33000(30-1500000 ng/L), followed by; iopromide 89000(50-580000 ng/L), gabapentin 13000(30-62000 ng/L), desmethyl citalopram 6000(50-16000 ng/L), citalopram 9000(50-34000 ng/L), and paraxanthine 6000(30-50000 ng/L). Pharmaceuticals found in 80% of the effluent samples were tramadol 34000(50-130000 ng/L), paracetamol 11000(30-230000 ng/L), carbamazepine 3000(30-14000 ng/L), venlafaxine 3000(50-11000 ng/L).

Hydroxycotinine was detected in 72% of the samples at average concentration 8000(50-80000 ng/L), while naproxen and verapamil were in 65% of the samples at 3000(50-26000 ng/L) and 1000(50-8000 ng/L). However, it is worth mentioning, that the measured concentrations for 14 compounds (with the exception of codeine, naproxen and cotinine) in some sites were semi quantitative or erratic, which apparently could underestimate the measured concentrations reported in table 26.

Table 26: Statistical summary of solid phase extracted effluent samples (ng/L)

Target compounds	Average	Median	Min	Max	Stdev	DF (%)
Paraxanthine	6000	720	30	50000	12	90
Caffeine	33000	620	30	1500000	120	99
Carbamazepine	3000	3000	30	14000	3	87
Citalopram	9000	8000	50	34000	7	90
Codeine	3000	500	50	16000	4	68
DEET	2000	700	30	30000	4	100
Desmethyl Citalopram	6000	5000	50	16000	4	96
Gabapentin	13000	10000	30	62000	12	97
Iopromide	89000	15000	50	580000	140	97
Naproxen +ve	3000	1000	50	26000	5	65
Paracetamol	11000	200	30	230000	38	84
Tramadol	34000	34000	50	130000	27	88
Venlafaxine	3000	3000	50	11000	2	87
Verapamil	1000	700	50	8000	2	65
Cotinine	12000	4000	1000	150000	24	100
Hydroxycotinine	8000	2000	50	80000	16	72
Nicotine	120000	19000	3000	1900000	330	100

The highest concentrations of nicotine (1900000 ng/L) and caffeine (1500000 ng/L) were measured in site 066, with a population of 37656 people while site 055 with a population estimate of 476 people had concentrations for both compounds 10000 ng/L and 130 ng/L respectively. The frequent detection of caffeine has been identified and quantified in wastewater, surface water, groundwater by researchers, therefore, making caffeine a suitable anthropogenic marker for wastewater contamination of surface water (Buerge et al., 2003). The concentration of caffeine in the present study is much higher compared to the reported concentrations in municipal WWTPs in Zurichsee Switzerland

(range 30-9500 ng/L), Boston USA (60-80 ng/L), Sweden (2000 ng/L) (Buerge et al., 2003, Siegener and Chen, 2002, Glassmeyer et al., 2005). The high concentration seen in this case strongly suggests that the predominant sources are associated with human domestic emissions.

The average concentration of nicotine found in the 68 effluent samples was 120000 ng/L (range 3000 - 1900000 ng/L) in the present study. In comparison with other studies, Huerta-Fontela *et al.* reported concentrations of nicotine and caffeine in effluent samples in North-Eastern Spain that ranged between 800 - 31900 ng/L and 30 - 43500 ng/L respectively (Huerta-Fontela et al., 2008). In Northern Nevada, United States, caffeine concentrations in sewage effluent samples were reported as 60 and 80 ng/L (Seiler et al., 1999). A study in Beijing, China, reported the concentration of caffeine in secondary effluent to range between 2.2 and 320 ng/L in effluent samples (Sui et al., 2010). The caffeine concentration in the present study is much higher than other studies, therefore indicating that the Australian population consume more caffeinated drinks (coffee mostly) than other countries. For instance, the Chinese population consume drinks such as green tea and black tea which are lower in caffeine than coffee (Gao et al., 2016). A 1995 nutritional survey estimated high caffeine use in Australia, with adult caffeine use ranging from 190-410 mg/person/day (Chen et al., 2002). This is somewhat higher than estimations of caffeine usage in this study (0.068 – 290 mg/person/day, Table 25). This is likely to be due to the temporal difference between the 1995 and this study as well as different methodological approaches. A more recent survey reveals the average consumption of coffee which is a major source of caffeine in Australia to be around 1.92 kg/person in 2017 (Statista Research Department, 2019). Based on the caffeine content of 8390 mg/kg in coffee beans (Payne, 2019), this is the equivalent of a caffeine intake of 44.1 mg/person/day, which is similar to the average of 35 mg/person/day estimated in the present study (table 24).

Chapter VII

Summary and Conclusions

The magnitude of interest on issues surrounding the presence and potential adverse effects of pharmaceuticals and personal care products (belonging to a group of emerging contaminants) in the aquatic environment has grown in recent decades. This is evidenced by the increasingly growing number of scientific papers addressing this topic. The use of pharmaceuticals and personal care products is indispensable for human and animal health. Human pharmaceuticals and veterinary drugs are used to prevent or treat diseases, while personal care products are used to maintain and improve the quality of daily life. Their extensive and increasing use has led to their ubiquitous presence in the aquatic environment. This has become a general public health concern due to their environmental persistence, bioaccumulation capacity, established toxicity to aquatic organisms, disruption of the endocrine system and potential development of antibiotic resistant strains as stated in chapter 1.3.

The environmental occurrence of PPCPs is predominantly as result of input of treated and untreated sewage effluent into the aquatic environment. After use, many PPCPs find their way into the freshwater aquatic environment through different routes with the major source being through WWTPs. A proportion of ingested pharmaceuticals and their metabolised products (metabolites) are excreted via urine and faeces into the sewer network. Detailed descriptions on their sources and transport into the environment have been explicitly discussed in chapter 1.4. After treatment processes, generated wastewater is discharged into the nearby surface water. Some of the excreted drugs and their metabolites have been shown to escape degradation during wastewater treatment processes and are hence discharged to receiving surface waters or find their way to groundwater via leaching from landfill sites. Studies have measured relatively high concentrations of these chemicals in various aquatic compartments.

The principal objective of this thesis was to gain understanding of the occurrence and behaviour of PPCPs in different geographical environmental matrices, using state-of-the-art high-resolution mass spectrometry (ultra-performance liquid chromatography coupled to Orbitrap high resolution mass spectrometry). A successful method was

developed for simultaneous determination and quantification of commonly used and frequently detected PPCPs in environmental samples in a single run.

7.1. Key findings

In chapter 2

- Using alternate switching (+)/(-) ESI-UPLC-Q-Exactive high resolution mass spectrometry method, a high analytical throughput was achieved for the determination and quantification of 29 PPCPs in a single run analysis. The method accuracy, precision, repeatability and reproducibility were successfully acquired with SPE recovery ranged between 76.2-103.4%.
- PPCPs were also determined in wastewater using another sophisticated analytical instrument HPLC-MS/MS using an AB/Sciex API6500^{+} Qtrap mass spectrometer, equipped with an electrospray (TurboV) interface coupled to a Shimadzu Nexera HPLC system by means of direct injection of wastewater into the column without the need for sample concentration.

In chapter 3

- The developed PPCPs method was applied to environmental samples collected from Assiut city, Egypt to determine the level of these chemicals in both effluent wastewater and surface water samples. Our result shows multiple detections of PPCPs in Egyptian water samples. In the effluent samples, we found antidiabetics (metformin and glyburide), antibiotic (trimethoprim), analgesics (acetaminophen), opioid analgesic (tramadol), NSAIDs (diclofenac and ibuprofen), anti-hypertensive (valsartan), beta-blocker (metoprolol), stimulants (caffeine and nicotine), and steroid (hydrocortisone) in all samples. Approximately, acetaminophen had the highest concentration (range 980-16000 ng/L), followed by ibuprofen (810-6700 ng/L), metformin (170-5600 ng/L), glyburide (550-4200 ng/L), diclofenac sodium (80-3600 ng/L), and trimethoprim (270-2700 ng/L), amoxicillin (<MDL-2000 ng/L). The high concentrations seen for some of these chemicals especially for acetaminophen, ibuprofen and metformin are

not surprising as the samples were collected from a hospital wastewater treatment plant. These elevated concentrations are indicative of high usage of these pharmaceuticals and may attest also to the ineffectiveness of the WWTPs in removal of PPCPs which was pointed out in chapter one of this thesis.

- In the surface water samples, the average concentrations measured for the following PPCPs were in the order; amoxicillin (730 ng/L), acetaminophen (500 ng/L), glyburide (390 ng/L), trimethoprim (190 ng/L) and nicotine (190 ng/L).
- The findings in chapter 3 of this thesis supports our hypothesis that measured PPCPs concentrations in the effluents will be much higher than the measured concentration in the surface water. This is due to the insufficient removal of PPCPs in WWTPs and thereby plants acting as major point sources to environmental contamination.

In chapter 4

- We measured the concentrations of PPCPs in surface water, groundwater and drinking water (sachet and bottled water) samples collected from Nigeria during the dry and wet seasons. Results revealed the average concentrations for Σ30PPCPs during the dry season were 28000 ng/L, 2100 ng/L and 380 ng/L, while the rainy season average concentrations were 10000ng/L, 2900 ng/L, 710 ng/L, respectively, for surface water, groundwater and sachet water. No significant difference was found in the Σ30PPCPs concentrations between the three types of water samples studied.
- In surface water samples, the highest average concentration was recorded for the antibiotic amoxicillin (13000 ng/L), followed by nicotine (2000 ng/L), ibuprofen (700 ng/L), acetaminophen (510 ng/L), codeine (500 ng/L), caffeine (250 ng/L), meclofenamic acid (230 ng/L), diclofenac sodium (200 ng/L), DEET (190 ng/L), tramadol (190 ng/L), sulfamethoxazole (180 ng/L), valsartan (170 ng/L), gemfibrozil (160 ng/L) and metformin (130 ng/L).

- In groundwater samples the highest average concentrations of PPCPs measured were in the order of: amoxicillin (1300 ng/L), nicotine (290 ng/L), codeine (250 ng/L), ibuprofen (200 ng/L), gemfibrozil (150 ng/L) and tramadol (100 ng/L).
- In sachet water, amoxicillin had the highest average concentration of 360 ng/L, followed by; codeine (84 ng/L), nicotine (30 ng/L) and ibuprofen (20 ng/L).
- In bottled water samples, amoxicillin average concentration was 79 ng/L, glyburide (14 ng/L), acetaminophen (10 ng/L), caffeine (9 ng/L), naproxen (4 ng/L) and sulfamethoxazole (2 ng/L).
- PPCPs concentrations were assessed for variability between the dry and wet season samples. A t-test was applied, revealing;
    - Surface water - Higher concentrations of metformin, nicotine, codeine, and metoprolol were found in the wet season than the dry season with p-values of 0.04, 0.001, 0.0001, 0.001 respectively. However, concentrations of propranolol, carbamazepine, diclofenac and meclofenamic acid were significantly higher in the dry season than the wet season with p-values of 0.05, 0.01, 0.02 and 0.04 respectively.
    - Groundwater – concentrations of naproxen and glyburide were significantly higher in the wet season with p-values 0.05 and 0.03 respectively.
    - Sachet water – codeine concentrations were significantly higher in the wet season than the dry season samples with a p-value of 0.04.
- We estimated daily human exposure to ∑30PPCPs via drinking borehole, sachet and bottled water. The result shows that average daily exposure to Σ30PPCPs concentration to the Lagos State population via drinking borehole, sachet and bottled water are 81, 14, and 3 ng/kg/day respectively.

In chapter 5

- In chapter 5, the seasonal and spatial variation of PPCPs in UK Rivers (River Severn and River Tame) and canals (Bham canal and Coventry canal) was assessed. Samples were collected in spring and summer. The ΣPPCPs concentrations measured in both canals are as follows;

- **Bham canal** – the ΣPPCPs concentrations measured in spring and summer were 27000 ng/L and 2200 ng/L respectively. PPCPs were predominantly found in both seasons in the order of; metformin (300-12000 ng/L), nicotine (240-4400 ng/L), amoxicillin (900-4200 ng/L), codeine (260-1000 ng/L), DEET (5-64 ng/L), glyburide (7-43 ng/L) and trimethoprim (2-11 ng/L). Statistical analysis showed no significant difference between the two seasons for this site.
- **Coventry canal** – the ΣPPCPs concentrations in spring and summer were 14000 ng/L and 13000 ng/L respectively. Amoxicillin (740-2700 ng/L), nicotine (200-2300 ng/L), metformin (110-1900 ng/L), codeine (220-750 ng/L), tramadol (6-25 ng/L) were detected in 100% of the samples in both seasons. Measured concentrations for trimethoprim and DEET were significantly higher in the summer than spring season with p-values of 0.02 and 0.04 respectively.

- In both canals, PPCPs concentrations were relatively higher in spring than summer samples.
- In both rivers, measured PPCPs concentrations were much higher in summer than spring. The ΣPPCPs concentrations measured in both rivers are as follows;
  - **The River Severn** – had ΣPPCPs concentrations of 20000 ng/L and 30000 ng/L in spring and summer respectively. Amoxicillin was the most abundant in the River Severn (range 1000-8500 ng/L), followed by metformin (500-5600 ng/L), nicotine (450-3400 ng/L), codeine (290-1700 ng/L), ibuprofen (12-1200 ng/L), carbamazepine (13-130 ng/L), tramadol (22-110 ng/L), DEET (3-94 ng/L), glyburide (14-59 ng/L) and meclofenamic acid (9-37 ng/L). Nicotine concentrations were significantly higher in summer than spring.
  - **The River Tame** – ΣPPCPs concentrations in spring and summer samples were 41000 and 61000 ng/L respectively. The River Tame had the highest PPCPs concentrations detected for 23 compounds; metformin (4100-20300 ng/L), amoxicillin (950-3600 ng/L), nicotine (<7-2700 ng/L), gabapentin (<3-1300 ng/L), caffeine (490-1500 ng/L), ibuprofen (<4-1100 ng/L), tramadol (200-700 ng/L), codeine (75-570 ng/L) carbamazepine (108-475 ng/L), trimethoprim (36-220 ng/L), erythromycin-H_2O (24-205

ng/L), meclofenamic acid (23-120 ng/L), naproxen (<2-110 ng/L), DEET (31-100 ng/L), diclofenac (33-61 ng/L), glyburide (7-47 ng/L), propranolol (12-42 ng/L), sulfamethoxazole (<2-40 ng/L), metoprolol (<1-29 ng/L), hydrocortisone (<19-25), valsartan (<4-18ng/L), oxazepam (<3-11 ng/L), clotrimazole (<8-9 ng/L). Caffeine and naproxen concentrations were significantly higher in summer than spring with p-value at 0.03 and 0.05 respectively.

- The higher concentrations seen in the two rivers compared to the canals are thought to be a result of wastewater effluent discharge to the receiving water.
- Most of the pharmaceuticals especially metformin, trimethoprim, tramadol, metoprolol, propranolol, carbamazepine, erythromycin, oxazepam, diclofenac, and meclofenamic acid investigated were ubiquitous in the River Tame.
- Two NSAIDs naproxen and diclofenac were relatively higher in the River Tame compared to other sites studied.
- The synthetic hormone 17-α-ethynylestradiol was detected more in the River Severn at average concentrations 125 ng/L and 106 ng/L in spring and summer samples respectively. In comparison, it was not detected in the River Tame and the Coventry canal, and detected in only 1 sample at 58 ng/L in the Birmingham canal.

In chapter 6

- The wastewater epidemiology technique was used to assess per capita release of PPCPs from WWTPs in Australia. We estimated the mass of drug excreted per 1000 people. The relationship between the daily mass load of chemicals and the contributing populations were investigated. The daily consumption of measured PPCPs was estimated.
- The daily mass load of chemicals was generated by multiplying the flow rate of the WWTPs by the concentrations measured for each chemical compound.
- The mass of drug excreted per 1000 people was calculated by dividing the daily mass load of chemical by the actual population and multiplying by 1000 people.
- Average concentrations of PPCPs studied ranged from 45 ng/L–9600 ng/L.

- Daily chemical mass loads were plotted against the population serving each WWTP. The graphs obtained showed strong correlations between the mass load and the population for most chemicals except for iopromide, salicylic acid and acesulfame. Those chemicals displaying strong correlations are suggested to be useful chemical markers for estimation of population size and usage pattern of a given chemical (Thai et al., 2018, Gao et al., 2016, O'Brien et al., 2014).
- Consumption of caffeine and acesulfame was highlighted as a lifestyle marker as they reflect population expenditure on a wide range of snack food and soft drinks.
- Average PPCPs consumption for some of the compounds studied are as follows: Iopromide (4400000 mg/day/1000people), acesulfame (430000 mg/day/1000 people), gabapentin (230000 mg/day/1000 people), tramadol (95000 mg/day/1000 people), codeine (66000 mg/day/1000 people), hydrochlorothiazide (62000 mg/day/1000 people), paracetamol (57000 mg/day/1000 people), ibuprofen (48000 mg/day/1000 people), caffeine (35000 mg/day/1000 people), naproxen (18000 mg/day/1000 people), salicylic acid (16000 mg/day/1000 people), venlafaxine (5700 mg/day/1000 people), citalopram (5300 mg/day/1000 people) and carbamazepine (650 mg/day/1000 people).

Our data collectively confirm the ubiquitous nature of PPCPs in the aquatic environment and that these chemicals must be considered important environmental pollutants. Measured concentrations were found to vary depending on the season and geographical location. Although there is limited legislation regulating the presence of some pharmaceuticals in the aquatic environment, other PPCPs that are continuously and constantly detected should be further assessed, and if deemed necessary, regulated.

7.2. Research gaps and future perspectives

This thesis highlights that the current database on PPCPs in the aquatic environment is still small for a group of emerging contaminants so continuously and extensively used in everyday life. Intrinsically, the PPCPs investigated are biologically active and thus their presence in the aquatic environment could potentially influence aquatic organisms and human health. Yet very little or no data is available about the public and aquatic health implications of their environmental presence.

Several studies have detected PPCPs in various environmental compartments as well as demonstrating their detrimental impacts, though little is currently known about their concentrations in sediments. Sorption has an important role in determining the fate of PPCPs in the freshwater aquatic environment; since sediment acts as a sink for some compounds. Hence further research is needed to understand their fate in freshwater sediments. The effects of many PPCPs and their transformation products are not understood and require further investigation.

Antibiotic resistance has been identified as one of the major human concerns, therefore risk assessments of antibiotics in aquatic ecosystems should incorporate strategic monitoring of amoxicillin as it is continuously detected in most environmental waters even in drinking water.

The inefficiency of WWTPs in removal of PPCPs has been highlighted by many researchers, therefore, there is need to understand the fate and behaviour of PPCPs undergoing treatment before the discharge of effluent in receiving water. This is important as some metabolites can undergo deconjugation during treatment process and be transformed back to its original form as a parent compound. There is also need to use advanced WWTP technologies in developing countries such as Egypt and Nigeria.

Based on a review conducted in this thesis of the global occurrence of PPCPs, it has been observed that not much research has been conducted in places like Australia and Africa. Future studies are needed on both sediments and aquatic biota in order to understand the fate of these contaminants in these regions.

A standardized model is needed in wastewater epidemiology studies to minimise uncertainties when estimating actual populations served by a given WWTP as well as the

uncertainty arising from the actual flow measurements. In estimating PPCPs consumption, there seems to be a gap in knowledge regarding exact values of human excretion factors for most PPCPs.

Although most risk assessments indicate that the very low concentrations of PPCPs found in drinking water are unlikely to pose any appreciable risks to human health, a knowledge gap still exists in this area. These include the risk assessment to human health associated with long term exposure to low concentrations of PPCPs and the possible combined effects of cocktail/mixtures of PPCPs.

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Appendix I

Table SI-4: Method precision expressed as relative standard deviation (RSD) of triplicate analysis of spiked Milli-Q water samples at 3 concentration levels, spiked tap water sample (500 ng/L of all target compounds), effluent sample (1A) and surface water sample (2G).

	Intra-day precision (Milli-Q water)			Inter-day precision (Milli-Q water)			Spiked Tap water	Effluent (1A)	Surface water (2G)
	10 ng/ml	250 ng/ml	750 ng/ml	10 ng/ml	250 ng/ml	750 ng/ml			
Metformin	3.2	9.1	1.8	7.9	9.2	2.8	6.8	9.3	10.9
Nicotine	2.3	6.8	3.2	7.2	8.8	3.1	5.6	11.6	6.9
Acetaminophen	3.1	3.1	3.8	8.4	2.8	2.9	2.1	5.1	5.9
Amoxicillin	1.6	3.7	1.1	3.5	3.8	3.1	3.8	12.2	7.4
Gabapentin	8.8	6.0	8.4	8.4	4.2	6.5	9.5	<MQL	9.9
Codeine	2.8	1.7	3.6	3.4	4.1	2.7	6.0	<MQL	9.7
Caffeine	2.2	4.8	5.1	5.7	4.6	4.2	8.2	5.4	8.3
Trimethoprim	2.3	4.1	4.3	6.9	2.7	3.2	1.5	11.2	7.6
Sulfamethoxazole	1.4	1.5	4.2	3.3	3.3	3.1	4.9	13.4	3.3
Tramadol	2.2	2.8	5.4	6.5	2.7	3.8	4.2	10.8	9.9
Metoprolol	2.1	0.4	3.5	3.1	3.8	3.6	4.8	<MQL	<MQL
Propranolol	13.5	5.6	1.2	9.8	5.9	2.6	8.0	15.1	11.9
Doxycycline	4.6	4.1	5.2	4.8	4.7	4.8	4.0	<MQL	<MQL
Carbamazepine	2.2	5.6	3.9	3.1	4.0	3.8	5.2	16.3	13.8
Hydrocortisone	6.1	4.8	4.4	6.2	6.8	4.3	6.2	12.6	7.5
Naproxen	2.8	3.3	4.0	6.1	4.6	5.0	5.4	<MQL	<MQL
DEET	7.4	3.5	2.5	9.3	3.1	2.2	3.6	<MQL	<MQL
Erythromycin	5.4	1.8	2.4	5.1	4.4	3.9	5.1	8.5	<MQL
Oxazepam	11.0	2.3	5.5	7.3	4.4	5.0	3.4	<MQL	<MQL

Valsartan	12.9	10.5	4.0	10.8	8.7	4.6	4.5	10.3	8.2
Mefloquine	5.2	8.0	5.8	5.0	5.9	4.8	6.0	<MQL	12.1
17α-ethynylestradiol	8.2	2.9	4.8	7.9	6.4	4.9	6.3	<MQL	<MQL
β-estradiol	7.9	2.0	4.0	6.5	6.4	7.4	4.9	<MQL	<MQL
Diazepam	3.7	6.7	3.6	10.4	7.6	3.6	6.3	<MQL	<MQL
Diclofenac sodium	2.4	6.5	5.9	3.8	4.9	4.3	4.7	8.9	6.9
Glyburide	2.6	3.4	6.6	5.5	4.4	5.2	6.2	3.5	3.6
Ibuprofen	2.6	3.8	4.0	3.2	3.9	3.9	8.7	11.3	<MQL
Meclofenamic acid	1.3	6.1	4.3	6.5	5.3	4.6	8.8	6.3	6.8
Clotrimazole	6.8	3.3	1.1	4.8	3.5	3.4	6.9	13.5	8.9
Gemfibrozil	11.0	4.6	6.6	8.9	8.3	4.3	8.7	<MQL	9.6

Table SI-5: Optimised MS/MS transitions for determination of target PPCPs in parallel reaction monitoring (PRM) mode.

Name	Precursor ion (m/z)	Fragment 1 (m/z)	Collision energy (eV)	Fragment 2 (m/z)	IQL (ppb)
Metformin	130.10884	60.06740	50	70.98956	0.26
Nicotine	163.12318	130.04927	40	116.99036	1.55
Acetaminophen	152.07143	110.10027	30	92.87368	0.25
Amoxicillin	366.09687	160.02719	20	207.09893	3.58
Gabapentin	172.13417	154.09628	20	137.03517	0.68
Codeine	300.16089	215.08376	50	152.11047	0.59
Caffeine	195.08862	138.07064	50	110.06993	2.35
Trimethoprim	291.1454	230.19694	30	123.09217	0.12
Sulfamethoxazole	254.05949	156.11271	30	92.18916	0.17
Tramadol	264.19584	120.68591	30	58.02061	0.41
Metoprolol	268.19076	116.08391	40	121.10312	0.07
Propranolol	260.16433	183.08693	40	116.08196	0.1

Doxycycline	445.14963	427.92017	20	409.91526	0.52
Carbamazepine	237.10333	194.08807	40	179.10467	0.11
Hydrocortisone	363.21686	121.18312	30	331.10375	1.15
Naproxen	229.08824	185.01425	20	170.02417	0.33
DEET	192.13931	119.12148	20	91.08922	0.22
Erythromycin	734.47192	158.11032	30	558.18592	0.56
Oxazepam	287.0586	241.09516	40	268.93511	0.25
Valsartan	434.22117	350.08532	30	179.07934	0.85
Mefloquine	379.12231	321.13571	40	271.14162	0.42
17α-ethynylestradiol	295.17047	144.88213	40	158.07256	2.92
β-estradiol	271.16998	144.90211	40	182.93627	2.55
Diazepam	285.07928	193.17213	40	153.90352	0.25
Diclofenac Sodium	294.01031	250.02168	20	235.92611	0.36
Glyburide	492.13818	369.18214	20	169.08516	0.85
Ibuprofen	205.12297	161.47241	20	-	0.35
Meclofenamic acid	294.01031	258.02169	20	-	0.6
Clotrimazole	345.11676	165.01174	30	276.90314	1.4
Gemfibrozil	249.15001	121.01169	20	126.92217	1.12

Evaluation of matrix suppression

Matrix suppression of the ESI signal for target analytes in real samples was evaluated using the matrix-matched calibration method described by Kasprzyk-Hordern et al. (2008)(Kasprzyk-Hordern et al., 2008b). In summary, effluent sample (1A) and surface water sample (2G) were spiked with 500-1500 ng/L of each target PPCP then the signal suppression for each target analyte was calculated in comparison to Milli-Q according to the equation:

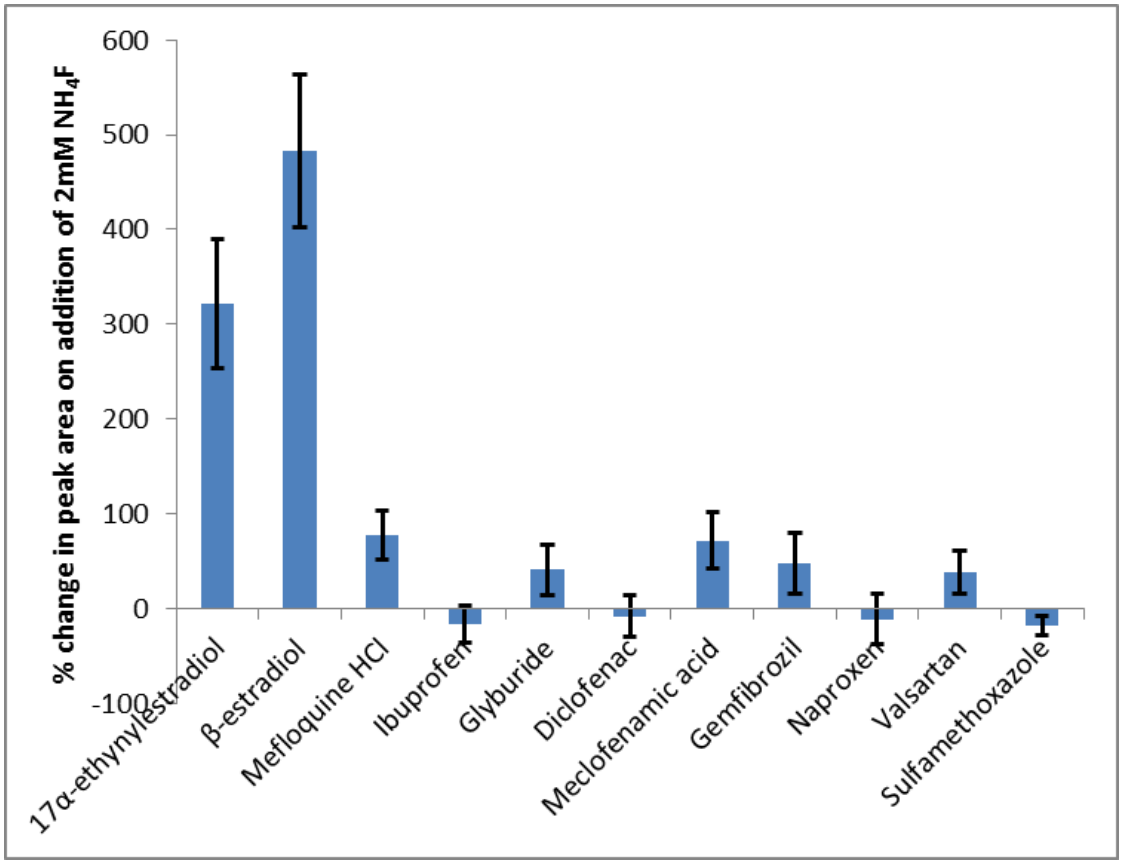
$$Signal\ suppression\ (\%) = \left(1 - \frac{I_s - I_0}{I_{MQ}}\right) x\ 100$$

Where, I_S the PPCP peak area in the spiked effluent or surface water sample; I_0 is the PPCP peak area in the unspiked effluent or surface water sample; and I_{MQ} is the PPCP peak area in Milli-Q water spiked at the same concentration level as the effluent or surface water samples.

Table SI-6: Signal suppression (%) of target PPCPs in effluent and surface water.

Name	Signal Suppression (%)		Name	Signal Suppression (%)	
	Effluent	Surface water		Effluent	Surface water
Metformin	49	44	Naproxen	16	13
Nicotine	7	5	DEET	38	27
Acetaminophen	32	26	Erythromycin	36	32
Amoxicillin	43	41	Oxazepam	28	21
Gabapentin	24	22	Valsartan	26	25
Codeine	22	15	Mefloquine Hydrochloride	23	17
Caffeine	26	21	17α-ethynylestradiol	14	12
Trimethoprim	31	24	β-estradiol	17	11
Sulfamethoxazole	33	23	Diazepam	30	23
Tramadol	41	28	Diclofenac Sodium	37	24
Metoprolol	35	22	Glyburide	22	19
Propranolol	24	21	Ibuprofen	22	17
Doxycycline	29	26	Meclofenamic acid	29	19
Carbamazepine	26	29	Clotrimazole	31	30
Hydrocortisone	12	10	Gemfibrozil	22	18

Figure SI-4: The effect of mobile phase NH_4F (2mM) on the signal response (expressed as % change in peak area) for ESI (-ve) ionised analytes. Error bars represent one standard deviation (n=3).



Appendix II

Table SI-1: Summary of method validation parameters.

Compound	R^{2*}	Accuracy# (% Recovery ±SD)	Precision (RSD %) *				MDL† (ng/L)
			Matrix Spikes	Surface water	Ground water	Sachet water	
Metformin	0.9972	93.2 ± 6.1	6.6	6.8	9.3	10.9	0.5
Nicotine	0.999	92.2 ± 5.8	6.4	5.6	11.6	6.9	7
Acetaminophen	0.9938	96.4 ± 4.5	4.7	2.1	5.1	5.9	1
Amoxicillin	0.9924	88.6 ± 3.1	3.5	3.8	12.2	7.4	18
Gabapentin	0.9951	90.4 ± 5.8	6.4	9.5	<MQL	9.9	1
Codeine	0.9984	92.0 ± 3.1	3.4	6.0	<MQL	9.7	2
Caffeine	0.9951	101.4 ± 4.9	4.8	8.2	5.4	8.3	4
Trimethoprim	0.9975	96.3 ± 4.1	4.3	1.5	11.2	7.6	1
Sulfamethoxazole	0.9957	92.8 ± 3.0	3.2	4.9	13.4	3.3	1
Tramadol	0.9958	91.8 ± 4.0	4.3	4.2	10.8	9.9	2
Metoprolol	0.9992	93.1 ± 3.3	3.5	4.8	<MQL	<MQL	1
Propranolol	0.9957	95.6 ± 5.8	6.5	8.0	15.1	11.9	1
Doxycycline	0.9979	85.7 ± 4.1	4.8	4.0	<MQL	<MQL	20
Carbamazepine	0.9749	88.2 ± 3.2	3.6	5.2	16.3	13.8	1
Hydrocortisone	0.9856	83.5 ± 4.8	5.8	6.2	12.6	7.5	3
Naproxen	0.9629	90.2 ± 4.7	5.2	5.4	<MQL	<MQL	3
DEET	0.9524	94.1 ± 4.4	4.9	3.6	<MQL	<MQL	4
Erythromycin	0.9920	83.5 ± 3.7	4.5	5.1	8.5	<MQL	1
Oxazepam	0.9923	94.7 ± 5.3	5.6	3.4	<MQL	<MQL	2
Valsartan	0.9951	92.5 ± 7.3	9.9	4.5	10.3	8.2	1
Mefloquine	0.9937	86.7 ± 4.5	5.2	6.0	<MQL	12.1	1
Diazepam	0.9739	94.9 ± 6.8	7.2	6.3	<MQL	<MQL	0.3
Diclofenac Sodium	0.9944	89.4 ± 3.9	4.3	4.7	8.9	6.9	1
Glyburide	0.9951	88.3 ± 4.5	5.1	6.2	3.5	3.6	3
Ibuprofen	0.9949	90.9 ± 3.3	3.7	8.7	11.3	<MQL	4
Meclofenamic acid	0.9994	86.1 ± 4.7	5.5	8.8	6.3	6.8	1
Clotrimazole	0.9619	101.8 ± 4.0	3.9	6.9	13.5	8.9	1
Gemfibrozil	0.9906	92.3 ± 6.6	7.2	8.7	<MQL	9.6	4

* Linearity co-efficient over a range of 1-1500 ng/ml.
# Recovery % of triplicate measurements of 5 matrix spikes in Milli-Q water.
$ Relative standard deviation (RSD%) of triplicate measurements in PPCPs matrix spikes in Milli-Q water and 3 real samples.
† MDL Method detection limit

Table SI-2: Summary of concentrations (ng/L) of target PPCPs in surface water samples from Lagos, Nigeria.

Compound ⇨	Metformin		Acetaminophen		Gabapentin		Nicotine		Codeine		Sulfamethoxazole		Caffeine		Trimethoprim		Amoxicillin		Tramadol	
ID ⇩	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet
Lg_SW1	<MDL	51.0	9.9	<MDL	9.9	<MDL	17.2	1290.3	4.7	583.1	435.5	109.6	303.3	56.7	56.3	41.4	243.9	1463.6	769.6	46.5
Lg_SW2	<MDL	<MDL	8.5	22.5	8.5	22.5	<MDL	1474.9	5.7	620.0	379.2	38.1	116.5	<MDL	73.6	13.2	402.9	1039.0	240.8	28.7
Lg_SW3	<MDL	<MDL	49.4	47.8	49.4	47.8	83.9	108.3	17.7	67.9	<MDL	<MDL	280.2	360.4	99.0	98.3	230.8	229.0	492.1	481.8
Lg_SW4	<MDL	12.4	18.7	9.5	18.7	9.5	72.3	2020.6	11.2	504.2	<MDL	5.6	68.7	<MDL	87.5	<MDL	848.8	1088.4	515.2	13.0
Lg_SW5	<MDL	<MDL	<MDL	67.4	<MDL	67.4	<MDL	6904.3	<MDL	769.8	5.3	<MDL	<MDL	<MDL	<MDL	<MDL	110.4	1752.3	16.6	<MDL
Lg_SW6	<MDL	1760.2	15.3	<MDL	15.3	<MDL	2324.9	1328.6	60.6	767.4	291.9	18.3	282.9	360.9	84.2	30.0	272149.8	2211.6	16.8	450.1
Lg_SW7	<MDL	10.0	<MDL	<MDL	<MDL	<MDL	31.4	545.3	<MDL	689.1	5.8	<MDL	24.3	<MDL	<MDL	9.1	87.2	2646.5	46.2	<MDL
Lg_SW8	<MDL	74.7	<MDL	<MDL	<MDL	<MDL	<MDL	4142.8	<MDL	575.0	<MDL	<MDL	36.0	<MDL	7.3	<MDL	104.2	1535.0	3.2	<MDL
Lg_SW9	<MDL	36.9	23.3	<MDL	23.3	<MDL	109.1	756.1	91.3	415.1	<MDL	<MDL	760.3	<MDL	53.4	<MDL	40157.4	1116.2	<MDL	<MDL
Lg_SW10	<MDL	860.7	11.1	<MDL	11.1	<MDL	79.5	4595.8	376.0	1778.0	284.8	38.5	683.8	354.7	37.2	107.8	1693.7	5771.6	<MDL	226.3
Lg_SW11	<MDL	363.7	18.0	<MDL	18.0	<MDL	840.2	9334.9	432.5	1407.3	271.7	26.9	351.9	113.0	16.6	86.7	2626.2	4173.1	<MDL	203.6
Lg_SW12	<MDL	<MDL	23.6	18.2	23.6	18.2	39.4	984.2	28.6	880.2	<MDL	42.5	350.3	482.4	144.0	19.4	460.3	1533.0	852.5	42.5
Lg_SW13	<MDL	10.8	16.2	7.7	16.2	7.7	66.6	2442.9	26.0	1477.2	466.0	<MDL	900.7	50.5	88.3	54.7	3704.4	3222.5	765.9	29.7
Lg_SW14	25.5	654.1	10.3	<MDL	10.3	<MDL	2453.0	4205.6	446.9	1481.7	3180.6	89.2	509.6	346.7	69.2	388.4	78517.7	7624.8	164.1	432.9
Lg_SW15	<MDL	538.8	17.8	35.5	17.8	35.5	4948.6	5563.5	172.6	1325.7	219.4	132.2	1082.8	415.7	2.9	194.3	4922.2	5169.9	73.3	359.0
Lg_SW16	<MDL	12.3	<MDL	<MDL	<MDL	<MDL	<MDL	2081.8	<MDL	777.4	<MDL	3.3	13.3	<MDL	<MDL	<MDL	96.4	1816.1	53.2	10.6
Lg_SW17	<MDL	97.6	<MDL	<MDL	<MDL	<MDL	<MDL	8931.8	<MDL	1152.4	<MDL	<MDL	35.1	<MDL	<MDL	2.4	407.0	3714.9	56.1	9.0

Compound ⇨	Propranolol		Carbamazepine		Hydrocortisone		Erythromycin-H2O		DEET		Oxazepam		Mefloquine HCl		Naproxen		Valsartan		Diazepam	
ID ⇩	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet
Lg_SW1	<MDL	<MDL	8.3	<MDL	<MDL	<MDL	<MDL	<MDL	55.0	119.3	9.6	<MDL	<MDL	<MDL	8.2	20.1	27.9	16.9	<MDL	<MDL
Lg_SW2	<MDL	<MDL	8.7	<MDL	<MDL	<MDL	<MDL	<MDL	47.0	27.8	8.6	<MDL	<MDL	<MDL	9.5	13.9	42.9	8.8	<MDL	<MDL
Lg_SW3	<MDL	<MDL	16.3	20.1	<MDL	<MDL	<MDL	<MDL	118.4	117.8	12.4	12.0	26.0	<MDL	27.7	27.0	79.0	6.8	<MDL	<MDL
Lg_SW4	<MDL	<MDL	43.1	<MDL	<MDL	<MDL	<MDL	<MDL	192.4	67.1	20.0	<MDL	<MDL	<MDL	27.6	18.6	103.2	9.0	<MDL	<MDL
Lg_SW5	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	33.6	<MDL	<MDL	<MDL	<MDL	<MDL	22.4	<MDL	22.5	<MDL	<MDL
Lg_SW6	7.2	<MDL	341.7	12.5	470.9	<MDL	<MDL	<MDL	498.8	50.2	1221.9	<MDL	<MDL	<MDL	<MDL	<MDL	1483.4	<MDL	42.4	<MDL
Lg_SW7	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	74.3	<MDL	7.1	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	128.7	<MDL	<MDL	<MDL	<MDL
Lg_SW8	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	7.8	16.2	<MDL	<MDL	<MDL	<MDL	5.6	32.6	26.5	<MDL	<MDL	<MDL
Lg_SW9	<MDL	<MDL	61.1	<MDL	<MDL	<MDL	<MDL	<MDL	84.8	<MDL	291.2	<MDL	<MDL	<MDL	2115.9	8.9	3327.4	<MDL	<MDL	<MDL
Lg_SW10	5.0	<MDL	95.7	17.8	56.8	69.7	<MDL	<MDL	253.1	444.7	162.8	<MDL	<MDL	<MDL	60.5	54.7	76.6	59.6	9.2	<MDL
Lg_SW11	6.3	<MDL	95.2	16.5	<MDL	<MDL	<MDL	<MDL	413.8	636.4	34.5	<MDL	<MDL	<MDL	<MDL	32.6	27.5	64.6	<MDL	<MDL
Lg_SW12	<MDL	<MDL	51.3	<MDL	<MDL	<MDL	<MDL	<MDL	108.7	78.7	21.2	<MDL	<MDL	<MDL	29.4	11.4	50.3	50.3	7.3	<MDL
Lg_SW13	<MDL	<MDL	35.7	<MDL	<MDL	<MDL	<MDL	<MDL	87.3	65.7	18.9	<MDL	<MDL	<MDL	34.8	53.7	17.8	48.1	10.7	<MDL
Lg_SW14	<MDL	<MDL	260.3	19.2	<MDL	<MDL	274.7	<MDL	784.1	281.9	253.3	<MDL	26.3	<MDL	<MDL	32.4	69.9	29.1	13.0	<MDL
Lg_SW15	8.9	<MDL	184.8	17.1	53.7	<MDL	<MDL	<MDL	1347.9	359.1	86.0	<MDL	30.9	<MDL	19.5	12.0	93.6	24.4	5.4	<MDL
Lg_SW16	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	11.3	23.7	<MDL	<MDL	<MDL	58.3	<MDL	14.7	<MDL	11.3	<MDL	<MDL
Lg_SW17	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	12.3	155.3	<MDL	<MDL	<MDL	<MDL	<MDL	70.0	<MDL	106.3	<MDL	<MDL

Compound ⇨	Glyburide		Diclofenac Sodium		Ibuprofen		Clotrimazole		Meclofenamic acid		Gemfibrozil		Metoprolol	
ID ⇩	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet
Lg_SW1	22.7	<MDL	87.4	<MDL	593.4	1260.8	19.7	<MDL	89.8	40.1	205.1	37.6	<MDL	61.3
Lg_SW2	32.7	<MDL	97.8	<MDL	645.7	221.7	18.3	<MDL	101.3	13.7	223.4	58.5	<MDL	46.2
Lg_SW3	<MDL	<MDL	121.8	33.7	861.7	2351.3	<MDL	<MDL	125.8	122.0	212.8	310.4	<MDL	13.7
Lg_SW4	24.8	<MDL	258.5	<MDL	1682.6	300.4	103.1	<MDL	267.0	11.7	306.1	48.1	57.2	<MDL
Lg_SW5	72.0	<MDL	<MDL	<MDL	61.8	<MDL	<MDL	<MDL	<MDL	<MDL	203.8	42.7	<MDL	53.2
Lg_SW6	<MDL	238.1	1930.4	<MDL	34.5	984.7	215.7	<MDL	2004.1	20.8	10.8	47.8	<MDL	73.3
Lg_SW7	325.7	<MDL	<MDL	<MDL	73.2	<MDL	<MDL	122.2	<MDL	<MDL	174.2	31.1	<MDL	48.2
Lg_SW8	43.1	<MDL	13.6	<MDL	156.4	<MDL	<MDL	<MDL	14.0	<MDL	128.5	84.2	<MDL	127.0
Lg_SW9	29.5	37.1	1891.9	<MDL	295.4	<MDL	617.9	<MDL	1938.5	<MDL	10.8	10.8	<MDL	20.8
Lg_SW10	39.2	<MDL	271.1	15.2	394.3	2275.6	<MDL	<MDL	281.5	165.5	10.8	415.9	<MDL	69.6
Lg_SW11	32.4	<MDL	130.6	11.0	<MDL	2068.5	<MDL	<MDL	136.3	115.9	10.8	216.2	17.2	110.3
Lg_SW12	54.6	<MDL	532.9	14.1	1660.9	1285.5	29.8	<MDL	562.4	74.2	435.4	199.0	<MDL	48.3
Lg_SW13	80.5	<MDL	404.9	<MDL	290.1	355.8	39.5	94.4	420.4	116.2	310.8	298.3	71.8	<MDL
Lg_SW14	13.1	82.9	714.3	11.3	1316.8	1478.4	<MDL	<MDL	729.4	78.1	10.8	162.7	<MDL	86.8
Lg_SW15	<MDL	37.1	144.1	21.4	42.7	2737.0	<MDL	<MDL	150.2	53.3	10.8	152.9	18.6	168.2
Lg_SW16	38.0	<MDL	<MDL	<MDL	72.3	29.1	<MDL	<MDL	<MDL	<MDL	179.1	57.4	<MDL	109.4
Lg_SW17	140.1	<MDL	<MDL	<MDL	134.6	40.7	<MDL	<MDL	<MDL	<MDL	181.9	551.9	<MDL	78.7

Table SI-3: Concentrations (ng/L) of target PPCPs in individual ground water samples from Lagos, Nigeria.

Compound ⇨	Metformin		Acetaminophen		Gabapentin		Nicotine		Codeine		Sulfamethoxazole		Caffeine		Trimethoprim		Amoxicillin		Tramadol	
ID ⇩	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet
Lg_GW1	5.6	348.6	7.4	31.3	<MDL	41.3	19.1	3533.4	26.4	344.1	16.7	<MDL	<MDL	<MDL	<MDL	<MDL	44.0	851.5	235.8	16.3
Lg_GW2	<MDL	<MDL	187.6	<MDL	10.1	<MDL	10.4	89.1	<MDL	188.0	6.2	<MDL	150.7	<MDL	<MDL	<MDL	151.4	717.9	882.6	148.1
Lg_GW3	<MDL	<MDL	10.3	3.4	9.5	12.7	8.2	54.2	<MDL	13.4	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	58.0	57.8	<MDL	<MDL
Lg_GW4	<MDL	<MDL	7.3	6.1	<MDL	<MDL	<MDL	86.0	<MDL	99.7	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	109.2	246.6	31.1	5.4
Lg_GW5	2.6	<MDL	95.0	95.3	<MDL	<MDL	<MDL	9.1	13.6	2021.4	<MDL	<MDL	163.5	166.1	4.4	4.5	5080.1	5199.3	56.8	57.0
Lg_GW6	7.8	250.5	<MDL	62.4	<MDL	<MDL	33.1	2670.0	<MDL	522.0	5.1	<MDL	<MDL	<MDL	<MDL	<MDL	123.8	1458.0	10.1	8.8
Lg_GW7	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	73.4	37.3	<MDL	36.9	15.2	15.6	41.3	39.6	6.0	6.1	367.5	384.0	326.7	323.2
Lg_GW8	<MDL	<MDL	17.0	5.4	<MDL	<MDL	<MDL	47.5	<MDL	20.8	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	69.1	68.7	84.3	85.2
Lg_GW9	<MDL	<MDL	9.2	<MDL	<MDL	<MDL	11.2	105.9	<MDL	70.7	64.3	<MDL	12.1	<MDL	21.0	<MDL	363.2	150.1	154.2	<MDL
Lg_GW10	3.9	<MDL	<MDL	<MDL	<MDL	40.9	<MDL	78.8	<MDL	73.8	<MDL	<MDL	<MDL	<MDL	<MDL	9.8	2854.7	229.2	<MDL	<MDL
Lg_GW11	4.6	<MDL	<MDL	2.9	<MDL	<MDL	<MDL	22.4	43.5	2442.5	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	6487.1	6472.9	<MDL	<MDL
Lg_GW12	<MDL	<MDL	26.9	9.3	<MDL	<MDL	<MDL	17.0	<MDL	16.0	<MDL	<MDL	24.5	22.1	2.5	2.5	62.0	62.2	11.7	12.1

Compound ⇨	Propranolol		Carbamazepine		Hydrocortisone		Erythromycin-H2O		DEET		Oxazepam		Mefloquine HCl		Naproxen		Valsartan		Diazepam	
ID ⇩	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet
Lg_GW1	<MDL	<MDL	4.0	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	14.8	<MDL	<MDL	<MDL	<MDL	<MDL	13.6	<MDL	<MDL	<MDL	<MDL
Lg_GW2	<MDL	<MDL	25.4	2.5	<MDL	<MDL	<MDL	<MDL	63.4	25.6	<MDL	10.9	<MDL	<MDL	4.8	5.7	84.4	<MDL	25.8	4.9
Lg_GW3	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Lg_GW4	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	10.8	22.8	<MDL	<MDL	<MDL	<MDL	<MDL	14.4	<MDL	<MDL	<MDL	<MDL
Lg_GW5	<MDL	<MDL	<MDL	6.5	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	9.1	9.4	<MDL	<MDL	<MDL	<MDL
Lg_GW6	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	11.4	<MDL	<MDL	<MDL	55.6	5.9	16.7	<MDL	10.4	<MDL	<MDL
Lg_GW7	<MDL	<MDL	46.4	53.3	<MDL	<MDL	<MDL	<MDL	14.2	14.0	26.8	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Lg_GW8	<MDL	<MDL	1.0	<MDL	<MDL	<MDL	<MDL	<MDL	59.4	68.7	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL

Lg_GW9	<MDL	<MDL	6.5	<MDL	<MDL	<MDL	<MDL	<MDL	10.9	<MDL	12.1	<MDL	<MDL	<MDL	<MDL	<MDL	19.3	<MDL	<MDL	<MDL
Lg_GW10	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.9	4.9	<MDL	<MDL	<MDL	<MDL
Lg_GW11	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Lg_GW12	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	43.6	42.8	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL

Compound ⇨	**Glyburide**		**Diclofenac Sodium**		**Ibuprofen**		**Clotrimazole**		**Meclofenamic acid**		**Gemfibrozil**		**Metoprolol**	
ID ⇩	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet
Lg_GW1	39.3	<MDL	11.9	<MDL	105.9	<MDL	<MDL	<MDL	12.3	<MDL	35.3	26.9	<MDL	50.5
Lg_GW2	32.3	<MDL	41.5	<MDL	341.7	<MDL	<MDL	<MDL	42.9	<MDL	244.7	89.1	8.0	<MDL
Lg_GW3	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Lg_GW4	25.3	<MDL	<MDL	<MDL	2245.2	1142.4	38.2	<MDL	<MDL	<MDL	729.8	197.4	<MDL	18.4
Lg_GW5	13.8	13.5	<MDL	<MDL	40.9	43.1	<MDL	<MDL	<MDL	<MDL	466.3	483.1	<MDL	<MDL
Lg_GW6	27.1	6.0	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	184.3	124.9	<MDL	53.9
Lg_GW7	<MDL	<MDL	<MDL	<MDL	54.5	53.9	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Lg_GW8	<MDL	<MDL	<MDL	<MDL	186.4	189.2	<MDL	190.5	<MDL	<MDL	67.1	68.5	<MDL	<MDL
Lg_GW9	24.3	13.9	22.3	<MDL	23.8	6.5	<MDL	<MDL	23.0	<MDL	124.4	46.0	<MDL	<MDL
Lg_GW10	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	75.2	25.7	<MDL	5.0
Lg_GW11	<MDL	<MDL	<MDL	<MDL	6.5	21.0	23.1	<MDL	<MDL	<MDL	144.4	155.5	<MDL	<MDL
Lg_GW12	<MDL	<MDL	<MDL	<MDL	78.2	85.9	<MDL	<MDL	<MDL	<MDL	93.3	90.5	<MDL	<MDL

Table SI-4: Concentrations (ng/L) of target PPCPs in individual drinking water samples from Lagos, Nigeria.

Compound ⇨	Metformin		Acetaminophen		Gabapentin		Nicotine		Codeine		Sulfamethoxazole		Caffeine		Trimethoprim		Amoxicillin		Tramadol	
ID ⇩	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet
Lg_SaW1	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	20.4	<MDL	147.8	6.5	6.6	51.3	47.9	<MDL	<MDL	441.3	449.8	<MDL	<MDL
Lg_SaW2	<MDL	<MDL	<MDL	11.0	<MDL	<MDL	<MDL	143.5	<MDL	304.7	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	85.8	495.0	<MDL	<MDL
Lg_SaW3	<MDL	<MDL	3.4	<MDL	<MDL	<MDL	22.2	<MDL	<MDL	112.5	<MDL	<MDL	7.6	<MDL	<MDL	<MDL	350.2	365.2	<MDL	<MDL
Lg_SaW4	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	16.1	92.9	<MDL	<MDL	<MDL	10.2	8.4	<MDL	<MDL	330.8	334.3	<MDL	<MDL
Lg_BoW1	<MDL		3.10		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		65.01		<MDL	
Lg_BoW2	1.34		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		87.03		<MDL	
Lg_BoW3	1.51		<MDL		1.47		<MDL		<MDL		4.96		<MDL		<MDL		39.83		<MDL	
Lg_BoW4	2.90		31.46		<MDL		<MDL		<MDL		<MDL		8.73		<MDL		123.74		<MDL	

Compound ⇨	Propranolol		Carbamazepine		Hydrocortisone		Erythromycin-H2O		DEET		Oxazepam		Mefloquine HCl		Naproxen		Valsartan		Diazepam	
ID ⇩	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet
Lg_SaW1	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	7.3	2.2	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Lg_SaW2	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.6	16.6	<MDL	<MDL	<MDL	<MDL
Lg_SaW3	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	8.7	8.6	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Lg_SaW4	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	10.4	10.1	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Lg_BoW1	<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		6.26		<MDL	
Lg_BoW2	<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		3.41		<MDL		<MDL		<MDL	
Lg_BoW3	<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL	
Lg_BoW4	<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		<MDL		1.1	

Compound ⇨	Glyburide		Diclofenac Sodium		Ibuprofen		Clotrimazole		Meclofenamic acid		Gemfibrozil		Meclofenamic acid	
ID ⇩	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet	dry	wet
Lg_SaW1	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Lg_SaW2	33.7	29.6	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	1.9	3.7
Lg_SaW3	<MDL	<MDL	<MDL	<MDL	34.4	38.2	<MDL	<MDL	<MDL	<MDL	30.6	32.3	<MDL	<MDL
Lg_SaW4	<MDL	<MDL	<MDL	<MDL	20.5	49.6	<MDL	<MDL	<MDL	<MDL	10.8	8.6	<MDL	<MDL
Lg_BoW1	<MDL		<MDL		<MDL		<MDL		<MDL		3.81		<MDL	
Lg_BoW2	21.21		<MDL		6.02		<MDL		<MDL		9.74		<MDL	
Lg_BoW3	12.92		<MDL		<MDL		<MDL		<MDL		5.76		<MDL	
Lg_BoW4	16.71		<MDL		<MDL		1.47		<MDL		8.35		<MDL	

*Lg_SW (Lagos Surface water)

*Lg_GW (Lagos Groundwater)

*Lg_SaW (Lagos Sachet water)

*Lg_BoW (Lagos Bottled water)