Verification and Validation of Analytical Methods for Testing the Levels of PPHCPs (Pharmaceutical & Personal Health Care Products) in treated drinking Water and Sewage

Report to the
Water Research Commission

by

Cecilia S. Osunmakinde, Oupa S. Tshabalala, Simiso Dube & Mathew M. Nindi
Chemistry Department, University of South Africa

WRC Report No. 2094/1/13
ISBN 978-1-4312-0441-0

July 2013

Obtainable from:
Water Research Commission
Private Bag X03
GEZINA, 0031

orders@wrc.org.za or download from www.wrc.org.za

DISCLAIMER

This report has been reviewed by the Water Research Commission (WRC) and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the WRC, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

EXECUTIVE SUMMARY

Thousands of metric tons of pharmaceutical and personal care products (PPCPs) are being produced and consumed worldwide per year. This group of emerging contaminants has recently received scrutiny from various communities because their fate and impact on the aquatic environment are not well understood. Since the challenge of water shortages is becoming a reality worldwide, alternatives for protecting the resource such as recycling are being considered. However, such alternatives create concerns relating to the quality of the water especially where the emerging contaminants are not being monitored. There have been reports of the presence of these PPHCPs in water systems although at low concentration levels of microgram to nanogram per litre. It is known though that even at such low concentrations some groups of PPHCPs such as endocrine disrupting compounds may have an adverse effect on aquatic organisms. The developments in analytical methods have enabled scientists to monitor and detect these compounds in water. In this report we (1) provide a literature review of the occurrence, sources, fate of PPHCPs worldwide, (2) discuss the criteria normally used for selecting target analytes, (3) develop a priority list of PPHCPs relevant to the South African context (4) provide analytical methods appropriate for the priority list, and (5) develop, validate and apply analytical methods for determining PPHCPs.

A literature review reveals that the occurrence of pharmaceutical and personal care products in water systems such as surface water, drinking water, and wastewater is on the increase worldwide. Wastewater treatment plants (WWTPs) are viewed as the major routes by which these compounds enter the environment. Other contributors include improper disposal of expired medicines, landfill leachates, residues from manufacturing industry and run off from agricultural effluent. It should be understood that most of the PPHCPS might be found in the environment as metabolites of the parent drugs. Analytical method development should take cognisance of this.

Most countries have their own defined priority target compounds which are selected based on several factors such as the volume of drugs that are used and consumed by the population, toxicity of the analytes and stability of the analytes once discharged into the environment. Stability studies give insight into whether analytes will be easily degraded upon discharge or whether they will be persistent in the environment. Compounds that have longer half-lives and are persistent in the environment normally receive higher priority.

A priority list of target analytes relevant to South Africa was developed based on data collected from the public and private health sectors. Though the list was primarily guided by the prescription volumes of the specific drugs, in some cases stability of the drugs was also considered. The chosen analytes were also those commonly detected in water systems worldwide. The target compounds from a South African perspective fall within six classes, namely hypertension, analgesics, antiretroviral, antibiotics, vitamins and antidiabetic drugs.

Literature review shows that LC-MS, LC-MS/MS and GC-MS are mostly the methods of choice for the determination of PPHCPs in the environment. Usually these methods are used in conjunction with a sample pre-concentration step such as solid phase extraction (SPE) or solid phase microextraction (SPME). Both LC-MS, LC-MS/MS and GC-MS have an advantage of PPHCP sensitivity which allows for the detectability of PPHCPs and their metabolites at low concentrations. GC-MS has a drawback in that most PPHCPs compounds will have to be derivatised prior to analysis which makes the procedure long and tedious. On the other hand very few laboratories are equipped with LC-MS or LC-MS/MS systems due to the cost related to these instruments.

In our experimental work we investigated the applicability of HPLC-Charged Aerosol Detector (CAD) as a cheaper alternative analytical method for the determination of PPHCPs target analytes. This detector was

selected as it is viewed as a 'universal' detector capable of detecting even those compounds that could not be observed on a UV or Fluorescence detector due to lack of chromophores or fluorophores respectively. A separation method was developed for twelve PPHCPs compounds which included antiretroviral drugs (penciclovir, famciclovir and ribavirin), analgesics (paracetamol) and nonsteroidal anti-inflammatory drugs (ketoprofen, diclofenac, fenoprofen, ibuprofen), anticonvulsants (carbamazapine and primidone), antibiotics (sulfamethoxazole) and β blockers (pindolol). All twelve compounds were baseline resolved using a gradient elution mode on a Zorbax Eclipse XDB C18 (4.6 mm x 150 mm x 5 µm). The method showed good linearity (0.9964-0.9994), and good accuracy (84.8 to 129.7%). The limit of detection (LOD) and limit of determination (LOQ) were 0.11-2.04 µg mL^{-1} and 0.36-6.81 µg mL^{-1} respectively. Such high limits might not be ideal for PPHCPs in the environment however it is believed that they may be improved by using sample pre-concentration. Preliminary screening of samples from Daspoort WWTP in Pretoria showed the presence of ribavirin, pindolol, famciclovir, carbamazapine, ketoprofen, fenoprofen and ibuprofen mainly in influent samples. Ribavirin was detected in both influent (19.60 ng/mL) and effluent (0.042 ng/mL) wastewater. Famciclovir, an antiretroviral drug, was also detected in both influent (ca. 19.00 ng/mL) and effluent (0.055 ng/mL) samples.

A second method was developed and validated for the determination of hormones in wastewater. All five hormones and bisphenol A were well resolved on a Zorbax Eclipse XDB C8 column using 70:30 MeOH/water in isocratic mode. Good linearity (r^2 0.9978-0.9991), accuracy (83.9-116%), intra-day (1.69-4.18%) and inter-day precisions of lower than 5% were obtained. As with the method for the PPHCPs, the LODs (0.26-1.4 µg mL^{-1}) and LOQs (0.85-4.5 µg mL^{-1}) were not attractive which means that a good sample preparation method will be required for the method to be more sensitive. Using this method no peaks due to hormones were detected in influent and effluent wastewater. Interestingly the influent had several other peaks that could not be identified. The presence of PPHCPs in influent and effluent wastewater gives an indication of the type of analytes that might enter the environment if not efficiently removed by the WWTP processes. From the data obtained it is apparent that the HPLC-CAD has the ability to detect several analytes without having to derivatise. However further studies are required and an incorporation of a sample preparation method is necessary in order to improve the detection limits to levels that are relevant for environmental analysis. Confirmation of detected analytes using other detectors such as MS is still required.

ACKNOWLEDGEMENTS

The project team wishes to thank the following people for their contributions to the project.

Reference Group	Affiliation
Dr K Murray	Water Research Commission
Prof J Okonkwo	Tshwane University of Technology
Mr C Schoeman	Rand Water
Dr L Chimuka	University of the Witwatersrand
Dr D Odusanya	Department of Water Affairs
Dr EJ Ncube	Rand Water
Dr I Dennis	Independent Consultant
George	Daspoort WWTP in Pretoria

Others	
Peace Nokwethemba Mqadi	Department of Health
Heila Nieuwoudt	Department of Health
Aaron Tshikotshi	UNISA library

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS & ABBREVIATIONS

ARV	Antiretroviral
CAD	Charged aerosol detector
CAD-HPLC	Charged aerosol detector-high pressure liquid chromatography
CAIA	Chemical and Allied Industries
CTX	Co-trimoxazole
DAD	Diode array detector
DLLME	Dispersive liquid-liquid microextraction
EDTA	Ethylenediaminetetraacetic acid
ESI	Electrospray ionisation
GC x GC	2 Dimensional gas chromatography (2 columns)
GS-MS	Gas chromatography-mass spectrometry
HLB	Hydrophilic lipophilic balance
HRT	Hormones replacement therapy
IL-DLLME	Ionic liquid-dispersive liquid-liquid microextraction
LC-MS	Liquid chromatography-mass spectrometry
LC-MS/MS	Liquid chromatography-mass spectrometry/ mass spectrometry
MX	Musk xylene
NSAID	Non-steroid anti-inflammatory drug
PES	Polyether sulfone
POCIS	Polar organic chemical integrative sampler
SMX-TMP	Sulfamethoxazole-trimethoprim
SOP	Standard operating procedure
SPE	Solid phase extraction
WWTP	Wastewater treatment plant

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

Pharmaceutical and personal health care products (PPHCP) are very important for the survival of humans but unfortunately they might have detrimental effects if they find their way into our water systems. PPHCPs include drugs ranging from analgesics, antibiotics, contraceptives, lipid regulators, β blockers, in addition to detergents, perfumes, dental products, etc. There are several review publications on PPHCPs in drinking and treated water which are mentioned in Chapter 2 of this report. Chapter two highlights several methods of identification and quantification of PPHCPs in aquatic environment. Most importantly, common and affordable methods that could be easily utilised within the South African context were identified. Many of the PPHCPs detected in waters around the world are common and also registered in South Africa under the Medicines and Related Substances Act of 1965.

The ever-increasing pharmaceutical industry makes monitoring and detecting of PPHCPs very challenging. There are several hundreds of different forms of drugs that are prescribed every day to human patients alone. In addition to the presence of human drugs in the aquatic environment there are veterinary drugs that add to the complexity. Many countries have therefore created priority lists of PPHCPs that are relevant to their environments. Selecting target compounds to formulate the priority list is a challenge since it requires detailed production and consumption data that can only be obtained from manufacturing and health providers. Unfortunately this kind of data is not always available.

The overall aim of this study was to the verification and validation of analytical methods for testing the levels of PPHCPs (pharmaceutical & personal health care products) in treated drinking water and sewage.

1.2 SPECIFIC PROJECT OBJECTIVES

The following were the objectives of the project:

1. To develop, verify and validate appropriate analytical methods of hormones and related compounds, PPHC and EDC in treated drinking and wastewater.
2. To review and/or develop sampling protocols for treated drinking and wastewater from several WWTPs in and around Gauteng.
3. To develop a cheaper and sensitive method using charged aerosol detector-high pressure liquid chromatography (CAD-HPLC) for hormones, replacement therapy (HRT) related compounds, oral contraceptives (OC), antibiotics, prescription and non-prescription drugs, steroids and PPHCPs
4. To review and/or develop green sample preparation method involving but not limited to hollow fibre supported liquid membrane (HFSLM), dispersive liquid-liquid microextraction (DLLME) and/or ionic liquid-dispersive liquid-liquid microextraction (IL-DLLME) and other suitable methods
5. To compare and validate results from CAD-HPLC against other traditional methods (GC, GC-MS & DAD-HPLC)
6. To identify and quantify the emerging contaminants in the selected water systems of South Africa using the developed sample preparation-CAD-HPLC method
7. Write standard operating procedure (SOPs) for the developed method for all the groups of compounds analyses.

CHAPTER 2: LITERATURE SURVEY

2.1 INTRODUCTION

For decades, heavy metals, pesticides, polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), dioxins and polychlorinated biphenyls (PCBs) have been key compounds of environmental concern and these have established and validated monitoring methods. The past decade has seen a worldwide growth in research interests related to the occurrence, fate and determination of trace levels of pharmaceutical and personal health care products (PPHCPs) in environmental samples such as surface water, drinking water, sediments, sludge, and water treatment influent and effluent (Daughton and Ternes, 1999, Khetan and Collins, 2007). There has been a growing concern regarding the presence and the effects of PPHCPs in the water systems going back to late 1990s. Numerous research publications and reviews in this area in the past decade alone are testimony to growing awareness and interest. This could be attributed to the realisation by the scientific community that there are knowledge gaps concerning the fate, the stability, and impact of PPHCPs and their metabolites to the environment and the methods of analysis. PPHCPs and their metabolites have been recognised as a group of the emerging contaminants in aquatic environment. The emergence of evidence of the endocrine disrupting nature of some of these compounds has significantly contributed to research activity in this area (Solé, 2003, Cleuvers, 2007, Cleuvers, 2006).

Pharmaceutical and personal health care products include over-the-counter and prescription drugs such as antibiotics, analgesics, blood lipid regulators, natural and synthetic hormones, β-blockers, anti-diabetics, antihypertensive and products that are used in everyday life such as surfactants and their degradation products (Yu et al., 2011, Ternes and Siegrist, 2004). The ingredients of soaps and/or detergents, perfumes, skin and hair products and dental care products are part of this very diverse group of compounds. The major concern is the continuous release of these unregulated emerging contaminants into the aquatic ecosystem. PPHCPs have been found in environmental samples in concentrations in the order of ng L^{-1} and µg L^{-1} (Gros et al., 2007, Xagoraraki and Kumar, 2010 & 2010a, López-Roldán et al., 2010).

The fate and presence of PPHCPs in wastewaters has attracted considerable interest worldwide in the scientific community, water stakeholders and the general public. Effluent from wastewater treatment plants (WWTPs) is one of the major routes by which these compounds enter the environment. Other sources include improper disposal of expired medicines, landfill leachates and residue from manufacturing residues. It has been previously reported that WWTPs do not quantitatively remove these compounds since this is outside the scope of their design (Jelic et al., 2009, Ternes, 2001, Zhou, 2009, Camacho-Munoz, 2010, Kasprzyk-Hordern, 2008 & 2008a, Boleda, 2007).

In developed countries such as Germany about 9 000 pharmaceutical preparations and 3 000 different active ingredients are approved for human use. Roughly 31 000 metric tons of pharmaceuticals were prescribed for human use and 800 tons as veterinary drugs. These staggering quantities are just an indication of the amount of PPHCPs that might end up in the water systems. In Africa the quantities of pharmaceuticals are expected to be much lower. However, knowledge of the type and amount of pharmaceutical products used in a region is very important for several reasons. The presence of trace levels of PPHCPs in processed water is dependent on the end use, especially as the demand for water has been increasing steadily worldwide. It is known that for most drugs only a small portion of the active ingredients is excreted unaltered and the rest as metabolites. These metabolites are more polar than their parent pharmaceutical drug and thus they are more soluble in water. This could be problematic if water is targeted for human consumption (USFDA Orange book, Paulekuhn, 2007).

Some of the PPHCPs usually exist as mixtures and have been reported to be ubiquitous in the environment and may cause detrimental effects to aquatic and terrestrial organisms even at trace levels (Camacho-Munoz, 2010). For example, the presence of estrogen and progestogens at concentrations as low as approximately 1 ng L^{-1} have been found to induce endocrine-disrupting effects, such as feminisation and decreased fertility (Solé, 2007, 2007a).

The chemical and physical properties of PPHCPs differ considerably. Most of the PPHCPs and their metabolites are polar while others are neutral and water soluble. Some of these compounds are volatile but several are not. This diversity causes challenges in developing a "universal" analytical method which could be applicable to the determination of most of these compounds. In addition, the nature of the matrix in which these compounds are found makes sample preparation a prerequisite. Therefore, there is a need for multiresidue analytical methods for the detection and quantification of these compounds in the environment. Several methods have been used for the detection and quantification of PPHCPs in water systems (Ternes, 2001, Al-Odaini, 2010), sediments and soils (Yu et al., 2011, Jelic et al., 2009, Chen et al., 2010). Among these analytical methods are hyphenated chromatographic techniques coupled to mass spectrometry. Typical examples are GC-MS (Sebök et al., 2008, Zhao et al., 2009, Soliman et al., 2004, Lopez de Alda, 2008), GC-MS/MS, LC-MS, LC-MS/MS (Lopez de Alda, 2008, Fatta-Kassinos, 2011) techniques that offer high sensitivity and selectivity needed for unequivocal identification. However, the gas chromatographic front end is limited by compound volatility and thermal stability at chosen separation conditions. Another platform that has potential for the analysis of PPHCPs is the GC x GC-MS. This approach could prove to be useful for volatile and semi-volatile PPHCPs (Gomez et al., 2011).

In the past decade several reviews on PPHCPs dealing with exposure, analysis and effects of PPHCPs have been published. Table 1 summarises several reviews on various aspects of PPHCPs including application of LC-MS , analysis of pharmaceutical residues in environmental samples, current state of knowledge and future research of pharmaceutical residue in water and household hazardous waste in municipal landfills to name but a few.

The objective of this chapter is to critically review the fate, occurrence, sources, effects and analysis of PPHCPs in aquatic environment, solid waste and sediments. This would help in identifying major gaps in the current knowledge and future needs of South Africa. While focusing on human pharmaceuticals, veterinary drugs such as anabolics and antibiotics have also been reviewed. The main focus of the project will be to review the presence of PPHCPs in drinking and treated water. The presence of such compounds in sludge and sediments will also have a bearing on the amount found in water. Therefore, the review will also cover such matrices.

2.2 ORIGIN AND OCCURRENCE

PPHCPs as trace environmental pollutants result largely from their worldwide and continual usage by humans and domestic animals through ingestion and excretion as well as the deliberate direct disposal of expired or unwanted drugs (Daughton and Ternes, 1999). There is however, limited knowledge of their potential environmental risks in comparison to other pollutants such as pesticides (Hernandez et al., 2007). Many PPHCPs are extremely bioactive compounds and are unknowingly introduced into the environment as complex mixtures via a number of routes especially sewage effluent (treated and untreated). PPHCPs are discharged continuously into the environment via wastewater treatment plants effluent and are thus present in water bodies such as oceans, groundwater, bank filtrates, and surface waters. In worse scenarios drinking water that has passed through water treatment could still be contaminated with PPHCPs due to the inefficient removal in WWTPs.

The occurrence of PPHCPs and their metabolites or transformed products in the aquatic environment has been investigated in several countries including Austria, Brazil, Canada, Croatia, China, United Kingdom, Germany, Greece, Italy, Spain, Switzerland, Taiwan, The Netherlands, and the USA. PPHCPs are

extensively and increasingly used in human and veterinary medicine and are released continuously into the environment (Daughton and Ternes, 1999). This has resulted in an increasing number of reports on occurrence in environmental samples such as wastewater, seawater, river water, sediments and sludge. Pharmaceuticals were first reported in the 1970s by Tabak. Bunch and Garrison et al. also in 1976 detected the presence of heart drugs, pain killers and birth control medication in wastewater at concentrations of 0.8-2 µg L^{-1}. A survey by the United States Geological Survey that involved more than 50 pharmaceuticals in 139 streams across about 60% of the states was carried out between 1999 and 2000 (Kolpin et al., 2002). Several PPHCPs have been identified in effluent from municipal wastewater treatment plants (Ashton et al., 2004; Roberts and Thomas, 2006). Figure 2.1 shows a typical schematic of the fate and the transport of PPHCPs in the environment (Ternes, 1998). Nineteen PPHCPs were monitored monthly for over a period of a year using twenty-four hour composite sampling in an advanced wastewater reclamation plant (Yang et al., 2011). High monthly concentration averages of 80 000, 80 000 and 1100 ng L^{-1} for caffeine, acetaminophen and ibuprofen respectively were observed in the primary effluent. Table 2.1 shows a typical example of some of the PPHCPs detected in water and wastewater matrices. Several methods of sample preparation were used in the detection of PPHCPs resulting in a wide range of limit of detection (Table.2.1).

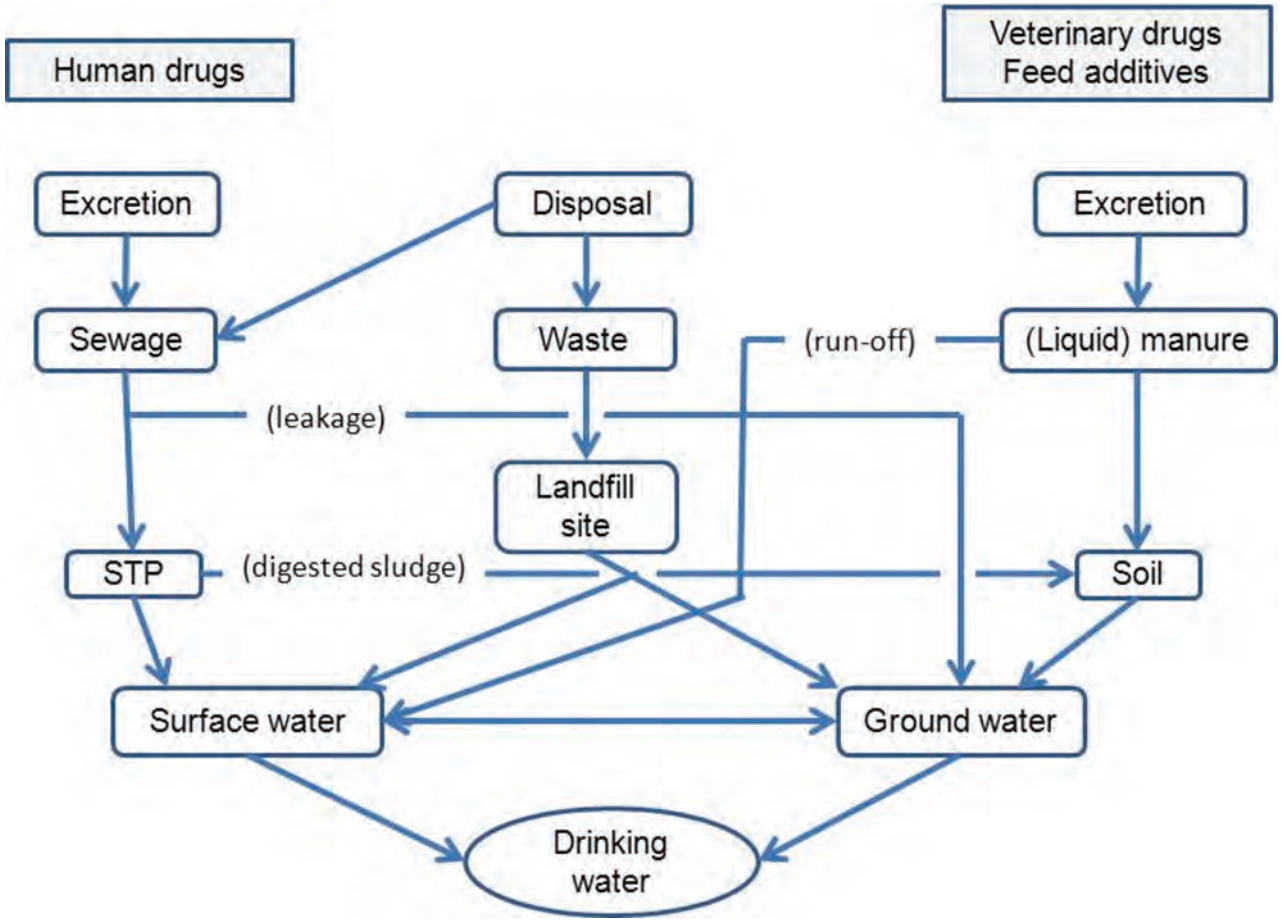


Figure 2-1: Schematic of the wastewater treatment plant (Terns, 1998)

Table 2-1: Examples of the most common pharmaceuticals in water and wastewater matrices

Pharmaceutical Compound/s	Sample Preparation & Detection	Limits of Detection (ng L^{-1})
Ibuprofen, Paracetamol, Phenazone, Carbamazepine, and Nonylphenols	SPME with fibre coating –GC-MS	200-50 000 (Möder, 2000)
Diclofenac, Ibuprofen, Ketoprofen, Indomethane, Naproxen, Fenprofen, Clolibric acid, Bezafibrate, Gemfibrozil, Etofibrate, Fenofibrate, Fenofibric acid, Carbamazepine, Pentoxifylline, Diazepam	RP-C18 SPE with derivatisation with pentaflurobenzyl bromide-GC-MS	3.3-9.6 (Sacher, 2001)
Clolibric acid, Diclofenac, Fenprofen, Flurbiprofen, Gemfibrozil, Ibuprofen, Ketoprofen, Naproxen	RP-C18 SPE with derivatisation with diazomethane for acid compounds-GC-MS	10-4760 (Andreozzi, 2003)
Ibuprofen, Salicylic acid, Gemfibrozil, Naproxen, Ketoprofen, Diclofenac, Indomethacin	Oasis MAX with derivatisation pentafluropropoinic acid anhydride (PFPA & N-methyl-N (tertbutyldimetylsilyl) trifluoroacetamide (MTBSTFA)	10-36 530 (Lee, 2005)
Ibuprofen, Naproxen, Ketoprofen, Diclofenac, Carbamazepine, Clolibric acid	Oasis HLB SPE with derivatisation with tetrabutylammonium salts	1-8 (Lin et al., 2008)
Acetaminophen, Sulfathiazole, Lincomycin, Sulfamethoxazole, Trimethoprin, Sulfamethazine, Chlortetracycline, Oxytetracycline, Enrofloxacin, Ciprofloxacin	SPE-LC-MS/MS	0.0001-0.38 (Koo, 2010)
Atenolol, Sotalol, Metoprolol, Propranolol	Oasis HLB SPE-LC-MS/MS	LOQ (7-83), (Alder, 2010)
Caffeine, Carbamazepine, Sulfamethoxazole, Trimethoprin, Sulfaapyridine, Atenolol, Nadolol, Metoprolol, Acetaminophen, Naproxen, , Naproxen, Gemfibrozil, Citalopram, Desmetyl citapram, N-desmetyl venlafaxine, O-desmetyl venlafaxine, Venlafaxine	POCIS-LC-MS/MS	0.01-33 (Hongxia, 2010)
Caffeine, Carbamazepine, Chlortetracycline, Cimetidine, Ciprofloxacin, Clarithromycin, Clidamycin, Clofibrin acid, Cotinine, Diclofenac, Diltiazem, Gemfibrozil, Salicylic acid, Sulfadimethoxine, Sulfamethazine, Sulfamethizole, Sulfamethoxazole, Sulfathiazole, Sulfisoxazole, Tetracycline	SPE-LC-MS/MS	17-105 (Spongberg, 2008)
Aspirin, Ibuprofen, Naproxen, Paracetamol, Gemfibrozil, Salbutanol, Clenbuterol, Terbutalin, Diclofenac, Diazepam, Caffeine,	Oasis MCX SPE-with MSTFA derivatisation	0.1-2.6 for sample water & 3.2-28.6 for

Pharmaceutical Compound/s	Sample Preparation & Detection	Limits of Detection (ng L^{-1})
Carbamazepine, Amitryptiline, Imipramine, Doxepine, Nordiazepam		wastewater, (Togoa and Budzinski, 2008)
Abacavir, Acyclovir, Lamivudine, Nevirapine, Oseltamivir, Oseltamivir carboxylate, Penciclovir, Ribavirin, Stavudine, Zidovudine	Isolute ENV-LC-MS/MS	5-1800 (Carten et al., 2010)

2.3 COMPOUND CLASSES

2.3.1 Pharmaceuticals

Pharmaceuticals can be divided into antibiotics, hormones, analgesic, anti-inflammatory, endocrine disrupting compounds and chemicals used as disinfectants and for cleaning purposes. Some of these pharmaceuticals are classified as endocrine disruptors because they have the potential to interfere with the normal function of the biological system. Occurrence of hormones in aquatic environments is of major concern since research studies have indicated that this group of compounds can also disrupt the sex in fish and shellfish. The presence of pharmaceutical compounds such as antibiotics in water systems can also be problematic because they can result in resistant bacterial strains. Because we rely heavily on pharmaceutical products it is of paramount importance to understand their occurrence, fate as well as their impact to the environment.

2.3.1.1 β-blockers

β-blockers are β-adrenegenic receptor antagonist drugs belonging to the group of cardiovascular pharmaceuticals and are generally used for treatment of hypertension and cardiac dysfunction. Examples of β-blockers used in some European countries include: salbutamol, atenolol, sotalol, theophylline, propranol, and metoprolol. Two of these drugs, atenolol and metroprolol are reported to account for more than 80% of the total β-blocker consumption in Europe (Alder et al., 2010). In South Africa metoprolol, atenolol, sotalol, metoprolol and propranol are registered for use under the Medicines and Related Substances Act of 1965 (935, September, 2008).

The fate of these drugs depends on structural variations in the substituents of the aromatic ring which determine their pharmacokinetic activity as well as their stability in the environment (Owen et al., 2007). The presence of these drugs in the environment will depend on how they are metabolised in the human body and what forms are excreted. In the case of metoprolol, 30% of the parent drug is excreted from the body and the remaining 70% is metabolised. Atenolol is excreted mainly as the parent compound with approximately equal fractions in faeces and urine (Reeves et al., 1978). Propranol and sotalol are nearly completely absorbed while the rest are extensively metabolised and the metabolites are excreted via urine (Alder et al., 2010). About 10-25% is excreted as propranolol-glucoronide, which is likely to hydrolyse and revert to the parent compound in WWTPs. Once excreted, β-blockers and/or their metabolites are transported through the sewer system to WWTPs where they are partially removed (Cahill et al., 2004). β-blockers such as metoprolol and propranol have been detected in both publicly-owned waste water treatment plants effluent and surface waters. However, the concentrations of these drugs in surface waters were only slightly above the detection limits (Ternes, 1998, Hirsch et al., 1996). Brochodilators such as salbutamol and fenoterol (β_2-symphathomimetics) were also detected in both publicly-owned waste water treatment plants effluent in concentration levels lower than 0.2 µg L^{-1}.

2.3.1.2 Steroids and Hormones

Hormones are an important group of compounds that are essential to both humans and animals. The estrogens are involved in human sexual reproduction and they act as the chemical messengers and are classified as natural and synthetic. Estrogens enter the environment via treated and untreated sewage waste. Their presence in sewage is a result of excretion from females due to natural production and use of contraceptives (synthetic estrogens). Several articles in the literature have focused on steroids and hormones probably due to the fact that they have been implicated as endocrine disrupting agents (Metcalfe et al., 2001; Parrott and Blunt, 2005). With the indiscriminate release of sewage into the environment, estrogens can be found in rivers, lakes, and even in oceans. The presence of estrone (E1), 17-β-estradiol (E2), and 17-α-ethynilestradiol (EE2) in waters is therefore of great concern.

Although 17-α-ethynilestradiol (EE2) (synthetic estrogen) is generally found in low concentrations (< 7 ng L^{-1}) in WWTP effluent there is still a possibility that once combined with 17-β-estradiol (E2) and estrone (E1) it might cause feminisation in male fish (Desbrow et al., 1998). Quintana et al. (Quintana et al., 2004) analysed the concentrations of estrone, 17-β-estradiol (E2) and estriol in influent and effluent of a sewage water plant in Spain and found that estriol was completely removed during the treatment process. However, the removal efficiency of 17-β-estradiol was only 75% whereas the concentration of estrone in influent and effluent was the same indicating that it was not removed at all. The levels of estrone and 17-β-estradiol were 33 ng L^{-1} and 2.9 ng L^{-1} respectively.

Baronti et al. monitored natural and synthetic estrogens in six activated sludge sewage treatment plants (ASSTPs) over two months (Baronti et al., 2000). Their study revealed that the average levels of estrogens entering the plants were 80 ng L^{-1} for estriol, 12 ng L^{-1} for 17-β-estradiol (E2), 3.0 ng L^{-1} for 17-α-ethynilestradiol (EE2) and 52 ng L^{-1} for estrone (E1). ASSTPs were found to remove most of the estrogens efficiently (85-96%) with the exception of estrone (E1) which was only removed by about 61%. They observed that in some cases the levels of estrone were even higher in the effluent than in the influent. In another study in Britain, estrone and 17-β-estradiol were found to be present at concentrations of tens of nanograms per litre while synthetic estrogen 17-α-ethynilestradiol (EE2) was detected at low nanograms per litre levels (Routledge et al., 1998).

2.3.1.3 Anti-diabetic drugs

Anti-diabetic drugs have high production volumes throughout the world. Examples of anti-diabetic drugs include glibenclamide, metformin, gilclazide, insulins and analogous metformin hydrochloride. Metformin, glibenclamide and gilclazide have been detected in river waters in Malaysia at concentration levels of 293, 2, and 4 ng L^{-1} respectively. The concentration levels in WWTP effluent were 16 ng L^{-1} for metformin, 5 ng L^{-1} for glibenclamide and 65 ng L^{-1} for gilclazide (Al-Odaini et al., 2010).

2.3.1.4 Antibiotics

Antibiotics include penicillins, tetracyclines, sulfonamides, macrolides, fluoroquinolones and β lactams, and are a very important pharmaceutical group. Antibiotics enter the aquatic environment via human and veterinary therapeutic drugs. The presence and fate of antibiotics in the aquatic environment raises a special concern due to the possible formation of resistant bacterial strains. Sulfamethoxazole for example was detected at concentration levels as low as 2 ng L^{-1} (Daughton and Ternes, 1999, Qui et al., 2008). Hospital effluent has been found to contain 20-80 µg L^{-1} concentrations of β lactams (Van Nuijs et al., 2009). Senta et al., (2008) simultaneously determined sulfonamides, fluoroquinolones, macrolides and trimethoprim in wastewater and river water. The antibiotics were present in low µg L^{-1} concentrations in wastewater and ng L^{-1} concentrations in river water (Senta et al., 2008). Spongberg et al. carried out an investigation of twenty PPHCPs in influent, effluent and biosolids from an urban wastewater treatment plant (Spongberg and

Witter, 2008). Most of the antibiotics investigated in their study, (sulfonamides, fluoroquinolones and tetracycline) were found to be below the limit of quantification for influent, effluent and biosolids. Only sulfamethoxazole and ciprofloxacin were present in concentrations above the limit of quantification. In another study done in German WWTPs effluents and groundwater eighteen antibiotics (macrolides, sulfonamides, penicillins, and tetracyclines) were analysed and sulfonamides and macrolides were detected in µg L^{-1} levels and the rest were not detected (Hirsch et al., 1999). Prescription doses for sulfamethoxazole have been reported in Germany to be 17 003 665 prescriptions. Sulfamethazine could be used as a molecular marker of live-stock contamination (Lin and Yu-Chen, 2009).

2.3.1.5 Analgesics

Analgesic and non-steroidal anti-inflammatory (NSAIDs) drugs are amongst the most prescribed pharmaceuticals worldwide and consequently a frequently-detected group in source waters as well as treated waters. For instance, acetaminophen was highlighted as the most commonly prescribed (578 873 456 doses) drug in Taiwan in 2004 which might explain why it was detected at high concentration levels in surface water (15.7 µg/L), hospital effluent (186.5 µg/L) and pharmaceutical production effluent (417.5 µg/L) (Lin and Yu-Chen, 2009). The other most commonly detected non-steroidal anti-inflammatory drugs (NSAID) in water systems included diclofenac, ibuprofen and naproxen which had doses of 154 486 313, 131 359 432 and 23 822 630 respectively in Taiwan in 2004 (Lin and Yu-Chen 2009). These compounds have also been reported as frequently detected NSAIDs in the USA (Benotti et al., 2009).

2.3.1.6 Antiretroviral drugs

There has been increasing use of antiretroviral (ARV) drugs in recent years and several have been used as part of the standard care of HIV-infected individuals in many countries. Co-trimoxazole (CTX), also known as sulfamethoxazole-trimethoprim (SMX-TMP), is a broad spectrum antimicrobial agent that targets a variety of aerobic Gram-positive and Gram-negative organisms and protozoa. Co-trimoxazole is commonly used in the prevention a number of opportunistic infections and has been shown to significantly reduce mortality among HIV-positive individuals. Antiviral drugs have also attracted interest of the general public due to the pandemic outbreak of the swine influenza virus (Singer et al., 2007). In addition to the treatment of influenza, antivirals are administered against a broad spectrum of viral infections such as HIV, herpes, and hepatitis (De Clercq, 2002; Snoeck and De Clercq, 2002). Similarly to other pharmaceuticals, these compounds are, if not completely metabolised in patients, excreted via faeces or urine. The compounds may enter the environment via wastewater treatment plant discharges. An analytical method was developed for the determination of nine antiviral drugs (acyclovir, abacavir, lamivudine, nevirapine oseltamivir, penciclovir, ribavirin, stavudine, zidovudine) and one active metabolite (oseltamivir carboxylate) in raw and treated wastewater as well as in surface water using electrospray-liquid chromatography-tandem mass spectrometry (ESI-LC-MS/MS) detection (Carsten et al. 2010).

2.3.1.7 Nervous stimulant/illicit drugs

Illicit drugs can include active ingredients from bona fide registered pharmaceuticals having valuable therapeutic uses such as morphine and oxycodone. They can also include active ingredients that are banned from use under various international laws and regulations. Residues of some drugs in the environment have substantial multiple origins making it difficult to monitor levels of illicit use. Morphine residue for example can originate from medical use of morphine itself or from codeine as well as heroine (via O-demethylation) (Huschek and Hansen, 2005).

A small amount of cocaine is excreted in the urine as the parent compound. Most is then excreted in the form of its metabolite, benzoylecgonine (BE). The presence of benzoylecgonine in waste and surface water is therefore an indicator of cocaine usage (Huschek and Hansen, 2005). Examples of illicit drugs most

frequently detected include: cocaine: (benzoylmethecgonine), methamphetamine, heroine, harcotic analgestic: (codine, hydrocodone, morphine, oxycodone, methadone, buprenorphine), benzodiazepines: (alprazolam, clonazepam, diazepam lorazepam) and club drugs: (ketamine, 1-(3-triflurомethylphenyl) piperazine (TFMPP). Cocaine has been detected at concentrations of 25-489 ng L^{-1} and metabolite, benzolyecognine at levels of 22-290 ng L^{-1} (Bones et al., 2007). In the same study morphine, tempazepam and the primary metabolite of methadone were also detected.

2.3.1.8 Antidepressants, anti-anxiety and anti-convulsants

Diazepam and meprobamate are the frequently detected anti-anxiety pharmaceuticals whereas carbamezapime is the most frequently detected anti-convulsant in water systems. Carbamezapime has even been detected in finished treated water in the US (Benotti et al., 2009). Fluotexine is an antidepressant with wide use and has also been frequently detected in water systems.

2.3.1.9 Anti-epileptic

Anti-epileptic drugs are medicines that reduce the frequency of epileptic seizures. Barbiturates were once widely used as sleeping pills and are still used in anaesthesia for surgery. Clonazepam, clorazepate, and diazepine are members of the benzodiazepine group of drugs and are best known for their use as tranquilisers. Phenytoin is used to control epileptic seizures and irregular heartbeats. The most common anti-epileptic drug used today is the carbamazepine. Thirty-three metabolites of carbamazepine have been identified from human and rat urine. Environmental field studies have shown that carbamazepine is one of the most frequently detected pharmaceuticals in sewage treatment plant (WWTP) effluent, in river water and in seawater (Segura et al., 2011) which explains why it has been used to evaluate the efficiency of removal of pharmaceuticals in WWTPs. However, there are no data on the fate of the metabolites of carbamazepine in the environment. Because of the high proportion of carbamazepine metabolites in biological fluids, there is reason to suspect that the metabolites will be present in domestic sewage and in the aquatic environment near WWTP discharges. Carbamazepine has been detected in high concentrations of 2.1 µg L^{-1} in German WWTP effluents and 0.25 µg L^{-1} in river waters (Ternes, 1998). These ubiquitously high concentration levels were attributed to the low removal efficiency (7%) of this drug from the WWTPs. In a separate study carbamazepine and five of its metabolites were detected in WWTP influent and effluent samples (Sebastine and Wakeman, 2003, 66). Only carbamazepine and 10,11-dihydro-10,11-dihydroxycarbamazepine were detected in surface waters with the metabolite existing in all aqueous samples at concentrations three times higher than the parent compound.

2.3.1.10 Anti-depressant

Psychiatric pharmaceuticals, such as anxiolytics, sedatives, hypnotics and antidepressants are among the most prescribed active substances throughout the world. The occurrence of these widely used compounds in the environmental matrices and their high persistence and toxicity to non-target organisms justify the growing concern. A comprehensive and detailed review of psychiatric pharmaceuticals is presented by Calisto et al. (Calisto and Esteves, 2009). From their review it is clear that this group of drugs has been found in a variety of environmental samples such as WWTP influents and effluents, surface waters, drinking water, rivers and stream water, ground water, estuaries and fish muscle. The concentrations reported ranged from low microgram per litre levels (0.00034 µg L^{-1}-5.4 µg L^{-1}) to nanograms per litre levels (0.1-900 ng L-1). Data summarised in the review were obtained for various countries such as Canada, USA, UK, France, Germany, Belgium, Italy and Denmark (Calisto and Esteves, 2009).

Antidepressants are not completely metabolised by the human body and are excreted as the unchanged parent compound or as metabolites or conjugates (e.g. gluconides) in urine or faeces. Benzodiazepines in particular are usually excreted in urine. The process involves extensive metabolisation in the liver to form

pharmacologically inactive glucuronides conjugates. These metabolites are easily decomposed by bacteria and are reconverted to the parent active compound leading to increased quantities in the sewage effluent (Carballa et al., 2008).

2.3.1.11 Antineoplastics

Cytostatic drugs are used in cancer therapy and most of these agents are incompletely metabolised in the body. They can therefore enter hospital wastewater and treatment plants in their active forms via urine and faeces of patients undergoing chemotherapy. Discharges of effluents from hospitals usually reach the municipal sewage system and a small amount via domestic sewages. Hospitals should therefore be considered to be the most important point sources of cytostatic drugs in the aqueous environment. The occurrence of these drugs in hospital effluent can serve as a starting point to monitor their fate in the environment.

Several studies have examined the residues of some cytostatic agents in wastewater treatment plant effluents and surface waters. Oxazaphosphorines, cyclophosphamide and ifosfamide (the most common types) were reported to be present in WWTP effluents at the level of nanograms per litre (Steger-Hartmann et al., 1997). Cyclophosphamide in sewage influent from hospitals reached maximum levels of 143 ng L^{-1} and the levels in the effluent were 17 ng L^{-1}. These concentration levels are several orders of magnitude lower than the concentrations at which acute toxicological effects have been reported for aquatic organisms. However, studies on the chronic effects of these drugs on aquatic organisms are lacking. These compounds have a very potent mechanism of action (i.e. inhibiting the growth and division of cells) and often exhibit carcinogenic, mutagenic characteristics and teratogenic properties, hence their presence in aquatic environment should not be ignored.

2.3.1.12 Blood lipid regulators

Bezafibrate, gemfibrozil, and fenofibric acid, the metabolite of fenofibrate, are persistent pollutants which have also been detected at trace level in sewage effluents and surface water samples. Bezafibrate has been found in influent, effluent and receiving surface waters in samples from most European countries (Miao and Metcalfe, 2003). It has also been quantified in a wastewater irrigation system, soil drainage and spring samples in Mexico (Siemens et al., 2008). Concentration levels of up to 4.6 µg L^{-1} were found in German municipal WWTPs (Ternes, 1998). Gemfibrozil was also found in the WWTP effluents. Polar metabolites such as clofibric acid and fenofibric acid were also detected at concentrations of 1.6 µg L^{-1} and 1.2 µg L^{-1} respectively. However both metabolites were in nanogram concentration levels in river and stream waters (Ternes, 1998). Heberer and Stan reported concentrations of clofibric acid of 4 µg L^{-1} in groundwater and 270 ng L^{-1} in drinking water (Heberer and Stan, 1997). Clofibric acid is most widely used and hence it is usually found at high concentrations most probably because the daily human dosages are generally high.

2.3.2 Personal Care Products (PCPs)

Personal care products are defined as chemicals marketed for direct use by the consumer (excluding over-the-counter medication with documented physiologic effects) and having intended end uses primarily on the human body (products not intended for injection with the exception of food supplements). Most of these chemicals are used as active ingredients or preservatives in cosmetics, toiletries, or fragrances. Although many of these compounds are not necessarily used to treat diseases some may be intended to prevent diseases e.g. sunscreen agents.

2.3.2.1 Retinoids

Retinoids are low molecular weight lipophilic derivatives of Vitamin A and can have profound effects on the development of embryonic systems especially in amphibians in which retinoic acid receptors have been hypothesised to play a role in frog deformities (Daughton, 1999). The studies done in North America described a wide range of deformities which included missing limbs, truncated limbs, extra limbs and skin webbings (Gardiner and Hope, 1999). Different types of retinoic acids and their metabolites were detected in river waters in concentrations ranging from 0.02 to 1.0 ng L^{-1} (Gardiner et al., 2003). Although they are naturally occurring, retinoids have been used for a number of years for a wide array of medical conditions including skin disorders, anti-aging treatments and cancer.

2.3.2.2 Sunscreen agents

Six sunscreen agents (UV filters) have been detected in Germany in Perch with a total concentration as high as 2.0 mg kg^{-1} lipid (Daughton and Ternes, 1999). Methylbenylidene camphor was detected in yet another lake in Germany. These lipophilic sunscreen agents seem to occur widely in fish from small lakes used for recreational swimming. The fact that 2-hydroxy-4-methoxybenzophenone and 2-ethylhexyl-4-methoxycinnamate can be detected in human breast milk shows the potential for dermal absorption and bio-concentration of these compounds in aquatic species.

2.3.2.3 Musk fragrances

Synthetic musk fragrances are very widespread in many homes. They are used as low-cost fragrances in soaps, perfumes, air fresheners, detergents, fabric softeners and other household cleaning products. Many of these products are ultimately disposed into sewers through domestic waste. A large proportion will therefore end up in sewage works and ultimately in rivers, lakes and the seas.

Humans are most at risk from synthetic musk fragrances not only through their exposure from contaminated food species (such as shellfish, fish, etc.), but also through direct absorption via the skin. Synthetic as well as polycyclic musk fragrances have been found in human body fat, with nitro-musks found more in women than men. Some synthetic musks are passed to breast-feeding babies in breast milk. As a result the European Scientific Committee on Cosmetics has concluded that human exposure to musk xylene and musk ketone should be reduced (Daughton and Ternes, 1999).

2.3.2.4 Preservatives

Parabens (alkyl-p-hydroxyybenzoates) are one of the most widely and heavily used antimicrobial preservatives in cosmetics (skin creams, tanning lotions, etc.), toiletries, pharmaceuticals, and even foodstuffs (up to 0.1% w/w). Although the acute toxicity of these compounds is very low, studies have shown that they display weak estrogenic activity. Although the risk from dermal application in humans is unknown, the probable continual introduction of these benzoates into sewage treatment systems and directly to recreational waters from the skin leads to the question of risk to aquatic organism (Daughton and Ternes, 1999).

2.3.2.5 Disinfectants and antiseptics

Triclosan (Irgasan DP 300, a chlorinated diphenyl ether; 2,4,4-trichloro-2-hydroxy-diphenyl ether) is an antiseptic agent that has been widely used for almost 30 years in a vast array of consumer products. Its use as a preservative and disinfectant continues to grow. Triclosan's use in commercial products includes foot wear (in hosiery and insoles of shoes called Odor-Eaters), hospital hand soaps, acne creams, e.g. Clearasil, and rather recently as a slow-release product called microban, which is incorporated into a wide variety of

plastic products such as children's toys. Various disinfectants are used in large amounts in hospitals, domestic households and by livestock breeders. These compounds are often substituted phenolics or compounds such as triclosan. Biphenylol, 4-chlorocresol, chlorophene, bromophene, 4-chloroxylenol and tetrabromo-o-cresol, are some of the active ingredients at percentage volumes of < 1-20% (Daughton and Ternes, 1999).

2.3.2.6 Nutraceuticals and herbal remedies

Nutraceuticals are usually defined as any substance that may be considered food or part of food and provides medical or health benefit, including the prevention and treatment of diseases. Recently, there has been increasing interest in the search for nutritional supplements, codified by the creation of the new term “nutraceuticals” for the subclass of highly bioactive food supplements from natural sources which will be able to provide some additional benefit for human health such as antioxidants, anti-inflammatories and anti-hypertensives. Anti-oxidant compounds are usually employed in the food industry to prevent undesirable changes due to oxidation reactions and also their health benefit. Typical examples of anti-oxidants include butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Nutraceuticals, herbal remedies and spices have been commonly employed as food ingredients to flavour different types of food preparations, since they contain a wide variety of compounds that can also have beneficial health effects and that can have potent physiological effects (Daughton and Ternes, 1999).

2.3.2.7 Insect repellents

N,N-diethyl-m-tolumide (DEET) is the active ingredient of most commercial insect repellents that is most widely used worldwide (Costanzo et al., 2007). DEET has been found to be highly effective against yellow-fever mosquitoes and other species (Qiu and Jun, 1996). DEET works by interfering with the sensory perception of insects to lactic acid on the skin of its hosts, which is the main stimulus used by insects for attraction and location of their hosts.

It has been detected in aquatic water samples from around the world indicating that DEET is both mobile and persistent. The development and use of DEET to combat disease transmission has undoubtedly saved many lives since its introduction as an insect repellent (Khetan 2007; Lopez de Alda, 2003). DEET usually enters aquatic environments via sewage effluent following washing off and absorption and excretion by humans. Studies have demonstrated that a percentage of DEET (< 20%) is absorbed through the skin, largely metabolised and excreted as metabolites (Costanzo et al., 2007). The insect repellent has been detected worldwide in drinking water, streams, open seawater, ground water and treated effluent with DEET concentration ranging from 40-3000 ng L^{-1}. In Australia DEET was detected in 36 sites in coastal waterways at concentration levels of 8 to 1 500 ng L^{-1}.

2.4 BREAKDOWN PRODUCTS AND METABOLITES

The assumption is that most of the active ingredients from the human pharmaceuticals are introduced into the environment as trace levels via the sewage system. Humans dispose of PPHCPs via excretion, mainly urine, faeces and sweat, and also while bathing. The process of excretion usually determines the form in which the active ingredient is excreted. Urine for example, will have portions of PPHCPs that are unmetabolised and conjugates (which might be converted back to the parent) that are susceptible to hydrolysis. However, faeces may contain metabolites excreted via bile in addition to portions of pharmaceuticals that the gut has failed to absorb. The extent of metabolism will also have an effect on what is excreted as unchanged or parent PPHCPs (Buser, 1998; Boreen et al., 2003; Packer et al., 2003;). The majority of pharmaceuticals are conjugated with glucuronic acid or sulfate which leads to a more polar molecule. The polar molecule is easily extracted by the kidney.

Many pharmaceutical compounds undergo different types of breakdown or transformation (Nikolaou, 2007) in the human body and in the environment resulting in the occurrence of a variety of metabolites and breakdown products in the environment (Table 2.2). Most pharmaceuticals are water-soluble, biodegradable and have short lives (Fent, 2006). A significant amount of the parent compound can leave the organism metabolised or unmetabolised via urine or faeces. Many metabolites have been identified (Fent, 2006). However not all pharmaceuticals are transformed in the body so a mixture of pharmaceuticals and their metabolites are released into the environment. Some drugs are either hydrolysed or conjugated; for example ibuprofen and diclofenac are removed by transformation into the carboxyl or hydroxyl derivatives (Nikolaou et al., 2007) in wastewater (Table 2.2). Data on the fate of PPHCPs in the environment is limited, but it is known that most PPHCPs are transported from the terrestrial domain to the aqueous domain.

Table 2-2: Examples of PPHCPs and their metabolitites

Pharmaceutical	Metabolites	References
Ibuprofen	Hydroxy- Ibuprofen, 2- Hydroxy- Ibuprofen, Carboxy- Ibuprofen, Carboxy- Ihydratropic acid	(Daughton and Ternes, 1999; Zwiener et al. 2002; Scheurell et al., 2009)
Naproxen	O-Desmethyl-naproxen	
Acetylsalicylic acid	Salicylic acid, Gentisic acid	
Ketoprofen	3-(Hydroxyl-carboxymethyl)-hydratopic acid 3-(Keto-carboxymethyl)-hydratopic acid	
Carbamazepine	10, 11-Dihydro-10,11-epoxy carbamazepine, 10, 11-Dihydro-10,11-dihydroxy carbamazepine, 2 Hydroxy carbamazepine, 3 Hydroxy carbamazepine, 10 Hydroxy carbamazepine	
Sulfamethoxazole	N_4-Acetysulfamethozazole	
Trimethoprin	α-Hydroxy-trimethoprim Hydroxylated trimethoprim	
Enalapril	Enalaprilat	
Diclofenac	3', 4,5-hydroxyl metabolites,3'-OH-4-methoxy, 4'5-dihydroxy diclofenac	(Gloria Caminal, 2009)
Erythromycin (hydrolysis)	Dehydro-Erythromycin	(Ternes, 1998; Sacher et al., 2001)
Acetyl salicylic acid (ASA) (deacetylation)	Salicylic acid, ortho-hydroxyhippuric acid, Gentistic acid (hydroxylated metabolite)	(Ternes, 1998)

Synthetic steroid hormones, such as 17-ethinylestradiol (EE2), have been reported to be extremely potent, quite persistent in the environment and show estrogenic activity in fish at 1-4 ng L^{-1} or lower (Tan et al., 2008). Natural estrogens such as estrone (E1), 17α or 17β-estradiol (α-EE or β-E2), estriol (E3) and 17 α-ethinylestradiol (EE2) are produced by animals and can also be found in some pharmaceuticals which have been doped (Filby et al 2007; Wang et al 2008). In the aquatic environment E2 is biodegraded to E1, which further degrades to E3. These compounds have been reported to persist in water due to incomplete

removal from WWTPs. The body metabolise estrogens and excrete them in their sulphate and glucuronide conjugates or unchanged (Lopez de Alda, 2001). However, these conjugates are converted back into their free forms during treatment processes and regain their potency (Gentili et al., 2008; Noppe et al., 2007; Petrovic, 2004).

The stability of PPHCPs in the environment will determine the types of analytes that are likely to be detected. Steroid hormones may be removed from the environment by different pathways which include photolytic degradation, sorption and microbial degradation. Photolytic degradation of steroids has been reported by (Liu et al., 2003 and Zou et al., 2003). It is also known that about 80% of 17-β-estradiol undergoes biotransformation to estrone in less than 20 minutes. All these three steroids are thought to undergo photodegradation with a half-life of 2 to 2.3 hours. Such losses of the analytes need to be considered when investigating the occurrence of these emerging contaminants (Yu-Chen et al., 2005). However, some studies have shown that significant concentrations are detectable in water systems despite these compounds being photolabile (Lin et al., 2005).

Within the class of NSAIDs, ketoprofen is extremely photolabile with a photolysis time in natural water systems of a few minutes (Yu-Chen et al., 2005; Lin and Reinhard, 2005). Thus even if this compound was prescribed in high volumes it might not be detectable in the environment due to its unstable nature. Again trace concentration levels that are usually detected for diclofenac in river water samples despite high concentration levels observed in effluents are also related to the fact that this compound is also highly photolabile in the natural environment. Photolability has also been reported for sulfamethoxazole with a half-life ranging between 1 h and 2.4 days (Andreozzi et al., 2003; Lam and Mabury, 2005).

The fate of ibuprofen, naproxen, ketoprofen, diclofenac and bezafibrate) in the influent and effluent from a wastewater treatment plant was observed for winter, spring and summer. The study showed a 3 to 5 fold increase in the concentration of PPHCPs in the influent water during winter (Vieno et al., 2005).

2.5 REMOVAL IN TREATMENT PROCESSES

2.5.1 Wastewater treatment plants

Annually hundreds of tons of approximately 3 000 pharmaceutical active substances are introduced into wastewater treatment plants (WWTPs). These emerging contaminants have been detected in water systems in Brazil, Canada (Lishman 2006), China (Li 2013), Chekolosvakia (Baranowska 2012), France (Vulliet 2008), Germany (Sacher 1998; Ternes 2001), Korea (Behera 2011), Italy (Zuccato 2005), Greece (Koutsouba 2003), Malaysia (Al-Odaini 2010), (Spain (Rodrıguez 2003), U.K. (Rodgers-Gray 2000), Japan (Ying 2002), Taiwan, Switzerland (Buser 1998; Golet 2002), Sweden (Andreozzi 2003), USA (Xu 2008; Kolpin 2002), and Poland (Baranowska 2012). When PPHCPs are consumed, in most cases up to 80% or more of the drug is released through the body without being transformed (Halling-Sorensen 1998). The wastewater treatment plants are unable to completely eliminate some PPHCPs, hormones and their metabolites resulting in the detection of residues in wastewaters, surface waters, sediments and sludge in several countries. PPHCPs and hormones removal at various WWTPs is complex due to many possible mechanisms involved. The removal rate is dependent on the nature of the compounds, season and will also vary from one WWTP to the other. It has been reported that the greatest removal of PPHCPs in conventional WWTPs takes places during secondary treatment such as biological (Richardson 2006). Removal of PPHCPs during primary treatment was reported to be limited (> 20%) for very hydrophobic compounds which exhibited high octane-water partitioning coefficients (log K_{ow} ~ 5.5-6.0). Sorption mechanisms are believed to predominate in the removal of PPHCPs via suspended organic matter which is subsequently extracted by coagulation, flocculation and sedimentation. A review publication by Oulton and co-workers examined the fate of PPHCPs during treatment by WWTPs and their occurrences in the effluent matrices and removal involving various technologies (Oulton et al., 2010). In addition there has been an

increase in the investigations evaluating the fate of PPHCPs at full scale WWTPs and their results have provided an insight to PPHCPs occurrence in wastewater effluent.

Wastewater treatment plants use various methods for the removal of pharmaceuticals. It has been shown that some methods are more efficient and effective than others and their performance depends on the type or group of compounds targeted. For example it has been demonstrated that compounds such as drugs of abuse (cocaine and benzoylecgonine) are poorly removed using conventional methods such dioxychlorination and sand filtration yet these compounds are almost completely removed when using the ozonation process (Berset et al., 2010)). Non-steroidal anti-inflammatory drugs were removed to different extents when treated with a combination of dioxychlorination and sand filtration. Diclofenac was removed with high efficiency of > 99%, naproxen was moderately removed (48%) and ibuprofen was poorly removed at 14%. However ozonation favoured the removal of ibuprofen (>40%) compared to naproxen and diclofenac which were both eliminated by less than 20%. Treatment of waste using dioxychlorination and sand filtration efficiently removes sulfomanides and macrolides (Boleda et al., 2001).

2.5.1.1 Treatment processes

Most WWTPs involve a series of physical, chemical and biological units in their processes and are designated as primary, secondary and tertiary treatment. Primary treatment for example might involve the removal of solids by processes such as coagulation, flocculation and sedimentation. The secondary treatment might involve biological processes, filtration, activated sludge and membrane bioreactors processes. Any treatment beyond the primary and secondary is viewed as tertiary. There are two types of approaches of treatment of wastewater; mainly conventional wastewater treatment process (CWWTP) and advanced wastewater treatment process (AWWTP). The conventional wastewater treatment process include activated sludge (CAS), biological filtration, primary settling, coagulation, and filtration, settling and sand filtration. Advanced wastewater treatment processes include processes such as reverse osmosis, ozonation, UV irradiation, photolysis (UV/hydrogen peroxide), biomembrane, ultrasound and a combination of processes. The physical and chemical properties and diversity of PPHCPs play a role in their removal efficiencies by different treatment processes (Vieno 2007).

Tables 2.3 and 2.4 show data obtained from several countries on the removal efficiencies of conventional wastewater treatment processes and advanced wastewater treatment processes. Activated sludge processes were far more efficient in the removal of PPHCPs than biological processes (Table 2.4). The advanced wastewater treatment processes were generally more efficient (> 97%) than most of the conventional processes.

Table 2-3: Conventional wastewater treatment plant removal efficiencies

Type	Removal range (%)	Source	Country	Reference
Activated sludge	11-99	Raw sewage	Australia	Watkinson (2007)
	7-100	Primary settled sewage	EU, Japan	DWI (2007)
	< 20-80	Primary settled sewage	France	Gabet-Giraud (2010)
	< 40 >80	Primary settled sewage	Europe	Vieno (2007)
	8-98	-	Brazil, EU, Japan	Ziylan (2011)
Biological filtration	6-71	Primary settled sewage	Europe	DWI (2007)
Primary settling	3-45	Not indicated	Brazil, EU, Japan	Ziylan (2011)
Coagulation filtration and settling	5-36	Not indicated		
Sand filtration	0-99	Activated sludge effluent		

Table 2-4: Advanced wastewater treatment plant removal efficiencies

Type	Removal range (%)	Source	Country	Reference
Ozonation	1-99	Activated sludge effluent	Brazil, EU, Japan	Ziylan (2011)
	86-100	Secondary Effluent		Gabet
Ozonation/ultrasound and sonocatalysis	23-45	Not indicated	EU, India, Japan, Turkey, USA	Ziylan (2011)
Ozonation and catalytic ozonation	> 9-100	-	-	
UV irradiation	29	Not indicated	Brazil, EU, Japan	Ziylan (2011)
Photolysis hydrogen peroxide	52-100	Not indicated	EU, India, Japan, Turkey, USA	
Dark & light Fenton	80-100			
UV/TiO_2	> 95			
Biomembrane	23-99	Treated effluent	Brazil, EU, Japan	Ziylan (2011
Reverse osmosis	62-100	Secondary treated effluent	Not indicated	Ziylan (2011
Ultrasound	24-100	Not indicated	EU, India, Japan, Turkey	Ziylan (2011
Grit-biological reactor-clarifier-sand filtration-ozonation-biological activation-carbon-microfiltration-UV-Chlorination	> 90	Wastewater	Australia	Jawad (2007)
Grit-activated sludge/UV-Microfiltration-reverse osmosis	> 90	Wastewater	Australia	Jawad (2007)
Grit-activated sludge-UV-microfiltration-chlorination-reverse osmosis	> 90	Wastewater	Australia	Jawad (2007)

2.5.1.2 Hormones

The removal of hormone 17β-estradiol has been reported to be as low as 47% by biological treatment to as high as 91% (Table 2.5). Ternes reported that the increase in the levels of estrone during the treatment process is due to the oxidation of 17β-estradiol (Ternes 1999). Removal of estrone, 17β estradiol, 17α-ethynyestradiol and 16α-hydroxyestrone from Sussex, U.K. WWTP wastewater by UV photocatalysis ranged from 78-92%; 78-87%, 77-100% and 79-81% respectively (Zhang and Zhou, 2008). Activated sludge treated was reported to efficiently remove EE2 (Baronti 2000). Chinese researchers reported the absence of detectable hormones in the aerated lagoons and effluents and speculated that lagoon treatment may have

been effective in the removal PPHCPs. Previous studies have shown the effectiveness of biodegradation of chemicals under aerobic conditions (Gadd 2010; Yang 2011).

The β form of estradiol is the synthetic hormone used for both human pharmaceutical and animal husbandry. This hormone is not completely removed from WWTPs and persists in the aquatic environment.

Table 2-5: Removal efficiencies of hormones

Hormones	Removal range %	Source	References
Estrone (E1) 17β-Estradiol (E2) Estriol (E3) 17α-ethinyestradiol (17α-EE2) Diethystilbestrol (DES) Ethinyestradiol	86-91	wastewater	Baronti (2000)
E1, E2, E3, EE2. 2OH-E1, 16OH-E1, EQ, E3-3G, 2E-3G, E1-3G, E3-16G, E2-17G, E3-3S, E2-3S, E1-3S	66-100	wastewater	Gentili (2002)
E1, E2, E3, E3-3S, E2-3S, E1-3S, E3-3G, E2-3G, E1-3G, E3-16G, E2-17G	61-97 64-100 84-100 100	wastewater	D'Ascenzo (2003)
E1, E2, 17A-EE2, DES	80-110	wastewater	Benijts (2004)
E1, E2, E3, EE2	80-100	wastewater	Langana (2004)
E1, E2, E3, EE2	52-91	surface water	Beck (2005)

2.5.1.3 Non-hormones

Studies have shown that the removal of analgesic and anti-inflammatory in the WWTP is often incomplete with efficiencies ranging between 30-100% (Table 2.6). One of the most reported pharmaceuticals is ibuprofen with removal from conventional WWTPs of about 70%. Other pharmaceuticals most susceptible to removal via conventional treatment (i.e. solids removal & activated sludge) include paracetamol and aspirin Comparison of the removal efficiencies of pharmaceuticals using different secondary treatment technologies is well treated in a review by Oulton and co-workers (Oulton et al., 2010). Removal of antibiotics by tertiary and AWWTP ranged from < 20 to >95% (Le-Minh, 2010). A study involving 5 WWTPs in one of the largest cities of Korea showed recovery range of 81-100% for acetaminophen, diclofenac, ibuprofen, ketoprofen, naproxen; 23% for carbamazepine and range of 23-65 for metoprolol, antenolol (Behera 2011). Data on the removal rates of atenolol in WWTPs is scarce and inconsistent ranging from 0-79% (Paxeus 2004; Lee 2007).

Table 2-6: Removal efficiencies of non-hormonal PPHCPs

PPHCP	Removal range (%)	Source	Country	Reference
Trimethoprim,		Raw SW		Lindberg (2005)
Norfloxacin, Ofloxacin, Ciprofloxacin,	86-87	Raw SW	Sweden	
Sulfamethoxazole,	42	Raw SW		
Doxycycline		Raw SW		
Sulfamethoxazole,	60	Raw SW	Spain	Carballa (2004)
Antibiotics		effluent	Canada	Miao (2004)
Carbamazepine, Clofibric Acid, Diclofenac Sodium, Naproxen, Primidone, Gemfibrozil, Salicylic Acid, Acetaminophen, Phenytoin	> 90	Raw WW	Australia	Al-Rifai (2007)
Ketoprofen	~ 80			
Sulfadiazine, Sulfamethoxazole, Norfloxacin, Ofloxacin, Ciprofloxacin, Tetracycline, Enalapril, Salbutamol, Famotidine, Ranitidine, Cimetidine, Glibenclamide, Nadolol, Atenolol, Bezafibrate, Gemfibrozil, Atorvastatin, Propyphenazone, Ketoprofen, Naproxen, Ibuprofen, Diclofenac, Acetaminophen, Salicyclic Acid, Furosemide	20-100	wastewater	Spain	Gros (2010)
Analgesics & anti-inflammatories				
Diclofenac	30-100	wastewater	Spain	Gros (2010)
Acetaminophen	96-100	wastewater		
Ibprofen	65-100	wastewater		
Naproxen	60-100	wastewater		
β-Blockers				
Metoprolol	89	wastewater		
Propranolol	93	wastewater		
Atenolol	100	wastewater		
Psychiatric drugs				
Carbamazepine	100	wastewater		
Antibiotics				
Sulfamethoxazole	100	wastewater		

Where Raw SW = Raw sewage water
Raw WW = Raw wastewater

2.5.2 Drinking water treatment plants

Pharmaceutical compounds have been detected in effluents from WWTPs. Some of these plants discharge directly into water bodies (for example rivers, lakes and reservoirs) which might serve as drinking water sources. Drinking water could also be contaminated from leaching pharmaceuticals from either leaking sewages or damage pipes. Most drinking water treatment plants are designed to remove pharmaceuticals efficiently from drinking water sources. These pharmaceuticals exist in trace levels, i.e. ng L^{-1} and have diverse physical and chemical properties such as molecular weight, relative hydrophobicity, aromatic carbon content and functional group composition. The treatment plant must therefore have capacity to remove trace levels of diverse PPHCPs. Drinking water treatment plants can use the following processes;

- Coagulation
- Lime softening
- Powder-activated carbon
- Biofiltration
- Chlorination
- Ozonation
- Membrane treatment

Conversional treatment plants (coagulation, sedimentation and filtration) have been reported to remove less than 25% of most PPHCPs. Efficient removal of PPHCPs is achieved with advanced treatment plants (ozonation, granular activated carbon adsorption, UV irradiation, microfiltration and ultrafiltration). Unfortunately, advanced treatment plants such as ozonation processes are very expensive and most emerging economies would probably not be able to afford these processes at the present time. Microfiltration and ultrafiltration membrane remove PPHCPs from water by sieving, charge, repulsion and adsorption mechanisms.

Exclusion and adsorption mechanism were used to efficiently remove natural hormones by nanofiltration (NF) and reverse osmosis (RO) membranes. Efficient removal of PPHCPs was achieved with a combination of NF and UF membranes. Removal of PPHPCs by UP membranes was predominately by hydrophobic adsorption mechanism whereas in NF membrane it was by hydrophobic adsorption and size exclusion (Yoon, 2006). Cellulose acetate and its modification were successfully used to efficiently remove three pharmaceuticals (carbamazepine, ibuprofen and sulfamethazine) from drinking water (Rana, 2011). Table 2.7 summarises examples of PPHPC removal from drinking water.

Table 2-7: Removal of PPHCPs from Drinking Water

Type	Compounds	Removal range (%)	Country	Reference
		Conventional		
Ferric sulphate	Diclofenac, Ibuprofen, Bezafibrate, carmamazepine, Sulfamethoxazole	36-77%	Finland	Vieno (2006)
Aluminium sulphate & Ferric chloride coagulates	62 PPHCPS	< 25%		Westerhoff (2005)
Coagulates		< 15%		
Filtration (sedimentation)	6 compounds - Phenazone - Propiphenazone - Dimethylamino-phenazone - AMDOPH - AMPH - DMOAS	25-95%	Berlin, Germany	Reddersen et al., (2002)
Coagulates	4 β-Blockers 1 Antiepileptic 1 Lipid regulator 4 Anti-inflammatories 3 Fluoroquinolones	< 15%	Republic of Korea	Kim et al., (2007)
Ferric sulphate	Diclofenac, Ibuprofen, Bezafibrate, carmamazepine, Sulfamethoxazole	36-77%		Vieno (2006)
		Advanced		
NF	Gemfibrozil, Triclosan, Estradiol, Ibuprofen, Progesterone, Oxybenzone, Ethynylestradiol, Testosterone, Estrone, Erythromycin-H2O, Diazepam, Androstenedione, Atrazine, Dilantin, Carbamazepine, Estraol, DEET, TCEP, Trimethoprim, Silfamethozazole, Diclofenac, Meprobamate, Acetaminophen, Pentoxifylline, Caffeine, Iopromide	10-70%	USA	Yeamin et al., (2007)
NF	Naproxen,	< 10%	USA	
NF	Natural hormones,			Nghiem (2006)
UF	Progesterone, Oxybenzone, Estrone, Trimethoprim,	< 30%	USA	

2.6 EFFECTS ON THE ENVIRONMENT

The impact of these organic micropollutants in the environment, their biological activities, degradation and risks to the non-target organisms are the main concerns. The risks are lower for humans because drinking water sources usually receive further treatment which removes some of the pollutants before reaching consumers. Aquatic organisms are usually the most affected in water systems close to sources of PPHCP. Antibiotics are of concern as they could induce bacterial resistance even at low concentrations levels via continuous exposures. Antibiotics are used in animal husbandry for prevention, treatment and growth promotion. Their overuse has resulted in the genetic selection of more harmful bacteria. The bacterial resistance has been observed in wastewater effluent from pharmaceutical industrial plants and hospitals (Petrovic, 2005, Erickson, 2002).

Steroidal chemicals such as sex steroids have the capability of disrupting or modulating endocrine systems. In aquatic systems one can get feminisation of male fish and alteration of the behaviours of either sex at part-per-trillion concentrations. A multitude of other aquatic effects are possible because hormone systems are central to development, functioning and reproduction of most organisms. Although other drugs (e.g. psychoactive agents and street drugs) have potentially significant effects on aquatic systems, aquatic toxicological evidence is lacking.

2.7 ANALYTICAL METHODS

2.7.1 Sample Preparation

Analytical chemists have a mandate to develop methods that are fast, sensitive, selective, reproducible and which have low detection limits. The analysis of PPHCPs is a challenge because the analytes are usually found in complex matrices and at very low concentrations. Therefore sample pre-treatment and/or pre-concentration is a prerequisite to any quantification method. The approach is usually to isolate the analyte of interest from the matrix through various extraction techniques. Because PPHCPs, their metabolites and degradation products exist in diverse forms there is no single extraction method than can be applied across the board.

Ideally sample preparation methods must be robust, simple, cost-effective, reproducible and most importantly they should not generate large volumes of toxic substances that might again impact negatively on the environment. Various greener extraction methods are emerging, an indication that analytical chemists are now sensitive to issues of preserving the environment. In this section, analytical methods that have been used for isolation, clean-up and/or pre-concentration are reviewed.

2.7.1.1 Solid Phase Extraction (SPE)

The dominant approach for extracting PPHCPs from environmental samples is solid phase extraction (SPE). Solid phase extraction offers the advantages of cleaner extracts, higher recoveries, automation and use of smaller volumes of organic solvents compared to liquid-liquid extraction. Reasonably hydrophobic PPHCPs can be pre-concentrated with SPE using reversed phase sorbents. Hormones are usually extracted by octadecyl (C18) bonded silica though use of polymeric adsorbents and graphitised carbon black have also been reported (Ternes, 2009, Gomez, 2011).

Unfortunately the majority of PPHCPs that enter the aquatic environment are highly polar and challenging to extract using the traditional reversed phase sorbents. Poor recoveries observed when polar analytes are extracted using reversed phase SPE have led to the development of other types of sorbents. Thus polar pharmaceuticals have been extracted by functionalised ionic SPE sorbets such as Oasis hydrophilic-lipophilic balance (HLB) (Petrovic, 2005) and mixed cation exchange (MCX). Antibiotics (Al-Odaini, 2010,

Blackwell, 2004), β-blockers (Yu, 2011), anti-epileptic (Chen, 2010, Chen et al., 2010) have been extracted with Oasis HLB/MCX known for its versatility and efficiency for the extraction of a wide range of polarities and pH values of the analytes. Where HLB Oasis SPE fails, MCX SPE (Paulekuhn, 2007) is used, which combines both ion-exchange and reverse phase retention mode. Several workers have reported the use of Waters (HLB) for the clean-up of non-steroidal anti-inflammatory drugs (Soliman et al., 2004, Quintana, 2004), anti-depressants, psychiatric, antineoplastic and antiepileptic (Zhao et al., 2009, Khan and Ongerth, 2004), hormones and β-blockers. Six natural and synthetic estrogens (diethylstilbestrol, estrone, 17-β-estradiol, mestranol, 17-α-ethinylestradiol and estriol) were concentrated using Oasis HLB cartridges and quantification limits between 1-3 ng L^{-1} in sewage water were reported(Quintana, 2004). Senta and co-workers compared the performance of three different SPE sorbents (Envi C18, Strata X and Oasis HLB) in extracting antibiotics (sulfonamides, fluoroquinolones, macrolides, trimethoprim) from wastewater and river water (Senta et al., 2008). In their work they observed that Envi C18 gave low recoveries for most of the antibiotics whereas the results for Oasis HLB and Strata X were comparable. The antibiotics were detected at very low concentration levels of ug L^{-1}.

2.7.1.2 Other sample preparation techniques

Solid phase microextraction (SPME) has also been used significantly in the extraction of PPHCPs from water samples (Huschek and Hansen, 2005). Its advantage over conventional SPE is that it requires less sample volume, it is completely solvent free, allows for even higher enrichment factors and is easily automated. Non-steroid anti-inflammatory drugs have been extracted from water samples using SPME with on-fiber derivatisation. With this approach quantification limits from 12 to 40 ng L^{-1} were achieved (Rodriguez et al., 2004). Liquid phase microextraction (LPME) (Zuccato and Castiglioni, 2005), microwave assisted solvent extraction (MASE) (Qin et al., 2005), pressurised liquid extraction (PLE) (Diaz-Cruz et al., 2006), Soxhlet, ultrasonication, fluid management system (FMS) and supercritical fluid extraction (SFE) have also been used. Morales and co-workers used MASE to extract personal care products from sludge (Quintana, 2004). Pocurul and co-workers used PLE to extract PPHCPs from sewage sludge (Senta, 2008) and another group also extracted antibiotics from sludge (Van Nuijs et al., 2009).

2.7.2 Separation Techniques

Pharmaceuticals and personal care products, their metabolites and degradation products differ considerably in their chemical and physical properties. Such structural diversity of target analytes presents an enormous challenge when attempting to develop a “universal” analytical method for determining PPHCPs. Multi-residue analytical methods which are desirable, would allow for the simultaneous determination of a wider range of pharmaceuticals or personal care products in a “one pot” environment. A desirable method would simultaneously separate all the polar, non-polar and neutral compounds in the so-called “one pot”. Due to the complexity, diversity and wide range of analytes a one pot approach is unrealistic at the moment. The need for sensitive, selective and accurate analytical measurement of these compounds makes mass spectrometry the detection technique of choice.

Several methods for the detection and quantification of PPHCPs and their metabolites in water systems, sediments and soils have been reported. The nature of the analytes and the complexity of environmental sample matrices necessitate the need for highly selective separation techniques coupled to sensitive detectors. Mass spectrometry is the detection technique that fulfils the requirement of high sensitivity and selectivity which is critical for the analysis of trace levels of PPHCPs and their metabolites. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are selective separation techniques for such analytes. Unfortunately only a small percentage of pharmaceutical and personal care products are sufficiently volatile for GC applications without prior derivatisation. Thus the majority of these compounds are polar and non-volatile therefore limiting the use of gas chromatography as the front end technique. LC-MS has become the acceptable analytical technique for a wide spectrum of PPHCPs.

Developments in LC-MS instrumentation such as the use of atmospheric pressure chemical ionisation especially electrospray soft ionisation source and/or interface is the main reason for the maturity of this analytical approach.

2.7.2.1 Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography without doubt is the most useful technique for the determination of volatile and semi-volatile compounds. In addition to the high resolution achieved by the separation technique and availability of a library makes it indispensable. GC has been previously used as a separation method for some polar PPHCPS prior to mass spectrometry detection after derivatisation (Rice and Mitra, 2007) in waste, surface and drinking waters. The use of GC-MS, although attractive due to the presence of a searchable library, is limited by the nature of the majority of the PPHCPs. There is a significant amount of research which has demonstrated the application of gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS) (Mol et al., 2000, Xu et al., 2008, Sang et al., 2011, Lee and Peart, 2005) and gas chromatography-mass spectrometry-mass spectrometry (GC-MS-MS) for the determination of PPHCPs. The incorporation of the derivatisation step makes sample preparation laborious, time consuming and prone to errors. Moreover the derivatisation step might influence accuracy since some analytes might be lost in the process or the reaction might be incomplete. As much as the GC-MS with derivatisation might appear as a daunting or least attractive approach, it has less serious matrix effects due to the electron impact (EI) or chemical ionisation (CI) modes used in MS hyphenated to GC. This allows for lower detection limits to be achieved than on LC-MS using electron spray ionisation (Buchberger, 2011, Pedrouzo et al., 2009). Xu and co-workers used GC-MS via N-methyl-N-(tert-butyldimethylsilyl trifluoroaceamide (MTBSTFA) derivatisation for the detection of non-steroidal anti-inflammatory drugs (Xu et al., 2008, Huschek and Hansen, 2005). Hormones in surface waste and drinking water systems have also been determined using GC-MS (Xu et al., 2008). Reactivity of different silylation reagents with different hydroxyl groups of estrogen compounds have been investigated using GC-MS and GC-MS/MS (Quintana et al., 2004). Recently, the gas chromatography-gas chromatography-mass spectrometry (GC x GC-MS) platform for the analysis of PPHCPs in waste and river waters has been reported (Chen et al., 2010). This platform has demonstrated potential for volatile and semi-volatile PPHCPs (Chen et al., 2010).

2.7.2.2 High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS)

High performance liquid chromatography (HPLC) is without doubt an indispensable separation technique that separates polar and non-polar PPHCPs. In addition HPLC is able to separate volatile and non-volatile PPHCPs. The development of new stationary phases has enabled HPLC to separate a variety of compounds with resolutions comparable to those on GC. It is therefore the most commonly-used separation instrument in most laboratories. HPLC has a consortium of detectors that include, ultraviolet and visible (UV-VIS), diode array (DAD), refractive index (RI), fluorescence (FL), chemilumiscence (CL), electrochemical (EC), nuclear magnetic resonance (NMR), and mass spectrometry (MS). However, mass spectrometry still remains the most appropriate detector for PPHCPs due to its sensitivity, specificity and speed. Recently the development of sub-particle and core shell stationary phases has taken HPLC to a higher level with respect to speed and resolution in the separation of complex analytes. Several research groups have applied HPLC for the separation of PPHCPs in aquatic and sludge samples (Paulekuhn and Dressman, 2007, Zwiener and Frimmel, 2004). The use of Hydrophilic Interaction Liquid Chromatography (HILIC) stationary phase has become very common for PPHCPs. The HILIC column has been used for the separation of anti-diabetic drugs and for estrogen metabolites (Nguyen and Schug, 2008).

2.7.2.3 Capillary Electrophoresis

Very limited work has been reported on the separation of PPHCPs using capillary electrophoresis (CE). The poor sensitivity associated with CE is probably the main reason why there are few applications for

pharmaceuticals and personal care products. Most of these compounds are acidic or basic which make them candidates for CE separation. However the drawback is their very low concentrations in water, requiring detection limits that CE cannot offer. Coupling CE with mass spectrometry has alleviated this limitation to a certain extent, though pre-concentration steps are still required for reasonably low detection limits to be achieved. There are a handful of articles in the literature which demonstrate the application of CE for the determination of PPHCPs in environmental samples. Microemulsion electrokinetic capillary chromatography (MEEKC) has been applied for the determination of NSAIDs in water using a UV diode array detector at 214 nm (Macià A, 2006). Stacking with reversed migrating pseudostationary phase enhanced sensitivity and detection limits in the order of 5-15 µg L^{-1} were achieved. Incorporating a SPE pre-concentration step resulted in even lower limits of detection in 100-230 ng L^{-1} range (Macià A, 2006). Ahrerr and co-workers developed a CE-MS method for the determination of drug residues (NSAIDs, antibiotics, analgesics, anti-epileptics) in water and achieved detection limits of between 4.8 and 19 ng L^{-1} (Ahrer et al., 2001). However, detection limits were only achieved after extensive sample clean-up and pre-concentration using LLE and SPE procedures.

2.7.2.4 Thin Layer Chromatography

A very simple and cost effective thin layer chromatographic method has been reported for the simultaneous determination of veterinary drugs in production wastewater (Babić et al., 200). SPE-HPTLC-Videodensitometry method was developed and validated for the determination of enrofloxacin, oxytetracycline, trimethoprim, sulfamethazine, sulfadiazine, sulfaguanidine and penicillin G/procaine. Acceptable detection limits ranging from 1-200 µg L^{-1} were achieved. Despite the fact that these values are rather high the method was suitable for the particular application considering that the analytes were present in significant amounts in the production wastewater. The authors recommended this method for routine analysis of highly loaded production wastewater as it is inexpensive and provides high sample throughput required when larger sample numbers are to be analysed.

2.7.3 Detection

Several methods have been used for the detection of PPHCPs in water systems, sludge and sediments. Most of the detectors listed for HPLC application can be used for PPHCPs. Generally most of these detectors used for HPLC are less sensitive and/or selective with the exception of mass spectrometry. The development of atmospheric pressure ionisation (API), especially electrospray ionisation in combination with single ion monitoring (SIM) or multiple reaction monitoring (MRM) techniques, has made mass spectrometry the ultimate detector. However, there are limitations of using LC-MS that included sensitivity, matrix including signal suppression especially for aquatic environmental samples. The use of liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS) has minimised some of the limitations and therefore has become the preferred approach. LC-MS-MS configuration is preferred due to its inherent superior selectivity, higher signal-to-noise ratios, and high speed acquisition when in MRM mode. A triple quadrupole configuration (QqQ) is a preferred instrument for quantification of analytes at ng L^{-1} concentration levels. Recently, time of flight (ToF) analysers have evolved such that this analyser can also be used for trace level qualitative and quantitative analysis especially when incorporated in a hybrid configuration such as quadrupole-ToF (Q-ToF) or ion trap-ToF (IT-ToF) mass analysers.

Liquid chromatography-mass spectrometry has been used to determine PPHCPs in fish. The compounds analysed included β-blockers, anti-depressant, antimicrobial, fragrance at ng L^{-1} concentration levels (Julic et al., 2009, Zhou et al., 2009, Ramirez et al., 2009). Ultra-high liquid chromatography incorporation to MS-MS was to determine PPHCPs in surface and wastewater. Monitoring of hormones, natural and synthetic homes from WWTP water was carried on an LC-MS-MS instrument (Baronti et al., 2000).

2.8 CONCLUSIONS

Even if the occurrence, effect and fate of pharmaceuticals and personal care products have been put in the perspective of scientific interest, still little is known about the actual risk to humans, other animals and the environment. Significant gaps still exist in the understanding of the interaction between residues, metabolites and resistance of these compounds, however the consequences of increasing resistance and the diminishing impact of therapeutic drugs reach far beyond tradition and geographic origins and are therefore a global concern.

Several methods of sampling, sample preparation and detections have been used in the determination and quantification of PPHCPs in a variety of matrices worldwide. The detections limits obtained were dependent of the sophistication and the cost of detection method used. A number of countries including China, Mexico and Spain have used Oasis HLB SPE as clean-up and HPLC-DAD/FLD for the determination of PPHCPs. The detection limits of the HPLC-DAD/FLD based methods were in the parts per million (ppm) range. It is therefore possible to use HLB SPE/HPLC-DAD/FLD as an affordable method for simultaneously detection of PPHCPs in the ppm range. There is a need to incorporate per-concentration techniques to enable the use of affordable HPLC-DAD/FLD techniques.

Gas chromatography-mass spectrometry was used by several countries in detecting and quantification of the PPHCPs in water and other samples. However, the GC-MS method requires an additional step of derivatisation to be incorporated. GC-MS has an advantage in that it pushes detection limits to parts per billion (ppb) levels for the PPHCPs and allows for positive identification as it has a library incorporated. Unfortunately, this approach again is also limited not only to the volatility of PPHCPs but also suitability of the volatile derivative product of derivatisation. The most commonly-used method in many countries is LC-MS/MS. This method not only can be used to determine the PPHCPs in their aquatic environment, but also offers the same or better detection limits as the GC-MS. The use of such tools as multi-reaction monitoring (MRM), have extended the detection limit levels but have also added the selectivity of the PPHCPs. However, unlike the GC-MS access to the library is not possible for the LC-MS/MS.

2.9 REFERENCES

Alder AC, Schaffner C, Majewsky M, Klasmeier, and Fenner K (2010). Fate of beta-blocker human pharmaceuticals in surface water: Comparison of measured and simulated concentrations in the Glatt Valley Watershed, Switzerland. Water Research (44) 936-948.

Ahrer W., Scherwenk E. and Buchberger W. 2001). Determination of drug residues in water by the combination of liquid chromatography or capillary electrophoresis with electrospray mass spectrometry, J Chromatogr. A, 910, 69-78.

Al-Odaini NA, Zakaria MP, Yaziz MI and Surif S (2010) Multi-residue analytical method for human pharmaceuticals and synthetic hormones in river water and sewage effluents by solid-phase extraction and liquid chromatography-tandem mass spectrometry. Journal of Chromatography A (1217), 6791-6806.

Babić S., Mutavdžić D., Ašperger D., Horvat A.J.M. and Kaštelan-Macan M (2007). Determination of veterinary pharmaceuticals in production wastewater by HPTLC-videodensitometry. Chromatographia, 65, 105-110

Babic S, Asperger D, Matavdzic (2006). Solid phase extraction and HPLC determination of Veterinary pharmaceuticals in wastewater. Talanta 70, 732-738

Baronti C., Curini R., D'Ascenzo G., di Corcia A., Gentili A and Samperi R (2000). Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. Environmental Science and Technology, 32(24), 5059-5066.

Blackwell PA, Lutzhoft HHH and Ma HP (2004). Fast and robust simultaneous determination of three veterinary antibiotics in groundwater and surface water using tandem solid phase extraction with high performance liquid chromatography-UV detection. Journal of Chromatography A (1045), 111-117.

Boleda MR., Galceran M and Ventura F (2007). Trace determination of cannabinoids and opiates in wastewater and surface water by ultra performance liquid chromatography tandem mass spectrometry. J. Chromatography A, 1175, 38-48.

Boleda M. R., Galceran M.T, Ventura. F (2011) Behaviour of pharmaceuticals and drugs of abuse in a drinking water treatment plant (DWTP) using combined conventional and ultrafiltration and reverse osmosis (UF/RO)treatment. Environmental Pollution 159 1584-1591

Bones J., Thomas K.V., Paull B (2007) Using environmental analytical data to estimate levels of community consumption of illicit drugs of abused pharmaceuticals. Journal of Environmental Monitoring (9) 701-707

Bound J P, Voulvoulis (2005). Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom. Environmental Health Perspective (113) 1705-1711.

Buchberger WW (2011). Current approaches to trace analysis of pharmaceuticals and personal care products in the environment. J Chromatography A., 1218, 603-618.

Cahill JD, Furlong ET, Burkhardt MR, Kolpin D and Anderson (2004). Determination of pharmaceutical compounds in surface- and ground-water samples by solid phase extraction and high performance liquid chromatography-electrospray ionization mass spectrometry. L G, J Chromatography, 1041, 171-180.

Calamari D, Zuccato E, Bagnati R, Fanelli R (2003). Strategic Survey of Therapeutic Drugs in the Rivers Po and Lambro in Northern Italy. Environmental Science and Technology (37) 1241-1248

Calisto V., Esteves V.I (2009) Psychiatric pharmaceuticals in the environment. Chemosphere (77) 1257-1274

Camacho-Munoz D, Martin J, Santos JL, Aparicio I and Alonso E (2010). Occurrence, temporal evolution and risk assessment of pharmaceutically active compounds in Donana Parl (Spain). Journal of Hazardous Materials, 183, 602-608.

Camacho-Munoz D, Santos J. L, Aparicio I, Alonso E (2010) Presence of pharmaceutically active compounds in Donana Park (Spain) Journal of Hazardous Materials 177, 1159-1162

Carballa M., Omil F, Lems J M (2008), Comparison of predicted and measured concentrations of selected pharmaceuticals, fragrances and hormones in Spanish sewage, Chemosphere 72, 1118-1123

Chang HS, Choo KH, Lee B and Choi SJ (2009). The methods of identification, analysis, and removal of endocrine disrupting compounds (ECD) in water. J. Hazardous Materials, 172, (2009), 1-12

Chen F, Ying G G, Yang J F, Zhao J L, Wang L (2010) Rapid resolution liquid chromatography-tandem mass spectrometry method for the determination of endocrine disrupting chemicals (EDCs), pharmaceuticals and personal care products (PPCPs) in wastewater irrigated soils. Journal of Environmental Science and Health Part B (45) 682-693

Chen F, Ying G G, Yang J F, Zhao J L, Wang L (2010). Rapid resolution high chromatography tandem mass spectrometry method for the determination of endocrine disrupting chemicals (ECD), pharmaceutical and personal care products (PPCPs) in waste water irrigated soils. J Environmental Science and Health Part B, 45, 682-693.

Chen ZC, Dillion P and Naidu R (2002). Determination of caffeine as a tracer of sewage effluent in natural water by on-line solid phase extraction and liquid chromatography with diode array detection. Water Research, 36, (2002), 4830-4838

Costanzo S.D., Watkinson A.J., Murby E.J., Kolpin D.W, Sandstrom M.W., (2007) Is there a risk associated with the insect repellant DEET (*N,N*-diethyl-m-toluamide) commonly found in aquatic environments? Science Total Environ (384) 214-220

Daughton C. G., Ternes T (1999) Pharmaceuticals and personal care products in the environment: Agents of Subtle change? Environmental Health Perspective 107, 907-938

Desbrow C, Routledge EJ., Brighty GC, Sumpter JP and Waldock M (1998). Identification of estrogenic chemicals in STW effluent 1: chemical fractionation and in vivo biological screening. Environmental Science Technology 32 (11), 1549-1558.

Diaz-Cruz S, Lopez de Alda MJ and Barcelo D (2006). Determination of antimicrobials in sludge from infiltration basins of two artificial recharge plants by pressurized liquid extraction-liquid chromatography-mass spectrometry. J. Chromatography 1130, 72-82.

Fatta-Kassinos D, Meric S, Nikolaou A (2011). Pharmaceutical residues in environmental waters and wastewater: current state of knowledge. J. Analytical and Bioanalytical Chemistry 399, 251-275

Fent K, Weston AA and Caminada D (2006). Cytotoxicity of pharmaceuticals found in aquatic systems: Comparison of PLHC-1 and RTG-2 fish cell lines. Aquatic Toxicology (76) 114-123

Fent K, Weston AA and Caminada D (2006). Ecotoxicology of human pharmaceuticals. Aquatic Toxicology (79) 122-159

Gardiner DM and Hope DM (1999). Environmentally induced limb malformation in mink frogs (Rana) Septentrionalis). Journal of Environment Zoology, 284 (2), 207-216.

Gardiner D, Ndayibagira A, Grun F and Blumberg B (2003). Deformed frogs and environmental retinoids. Pure and Applied Chemistry, 75 (11-12), 2263-2273

Gardinali PR and Zhao X (2002). Trace determination of caffeine in surface water samples by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS). Environmental International, 28, (2002), 521-528.

Gomez JM, Herrea S, Sole D, Garcia-Calvo E and Fernandez-Alba AR 2011. Automatic searching and evaluation of priority and emerging contaminants in wastewater and river water by stir bar sorptive extraction followed by comprehensive two dimentional gas chromatography time of flight mass spectrometry (ToF-MS). Analytical Chemistry, 83, (2011), 2638-2647.

Gros M, Petrovic M and Barcelo D (2007). Wastewater treatment plants as pathways for aquatic contamination by pharmaceuticals in Ebro river basin (northern Spain). Environmental Toxicology and Chemistry, 26 1553-1562

Heberer T (2002) Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent data. Toxicology Letters (131) 5-17

Heberer T and Stan HJ (1997). Determination of clofibric acid and N-(phenylsulfonyl)-sarcosine in sewage, river and drinking water. International Journal Environmental Analytical Chemistry 67, 113-124.

Hirsch R, Ternes TA, Harberer K. Mehlich A, Ballwanz F and Kratz KL (1996). Determination of antibiotics in different water compactment via liquid chromatography electrospray mass spectrometry. J Chromatography 815, 213-223.

Hirsch R, Ternes TA., Harberer K and Kratz KL (1999). Occurrence of antibiotics in the aquatic environment, Science of the Total Environment, 225, 109-119-118.

Huschek G and Hansen PD (2006). Ecotoxicological classification of the Berlin river system using bioassay in respect to the European Framework Directive. Environmental Monitoring and Assessment, 121, (2005), 15-31.

Jelic A, Petrovic M and Barcelo D (2009). Multi-residue method for trace level determination of pharmaceutical in soiled samples using pressurized liquid extraction followed by liquid chromatography-Quadrupole linear ion trap mass spectrometry. Talanta, 80, 363-371.

Jones OAH, Voulvoulis N and Lester JN (2004). Potential ecological and human Health Risk Associated with the presence of Pharmaceutically active compounds in the Aquatic environment. Critical Reviews in Toxicology 34(4), 335-350.

Kasprzyk-Hordern B, Disdale RM and Guwy A (2008). Occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South wales, UK. Water Research, 42, 3498-3518.

Kasprzyk-Hordern B, Disdale RM and Guwy AJ (2008). The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Research, 43 (2008) 363-380

Kemper N (2008) Veterinary antibiotics in the aquatic and terrestrial environment. Ecological Indicators (8) 1-13

Ketan SK and Collins TJ (2007). Human pharmaceutical in the aquatic environment: A challenge to green chemistry. Chemical Review, 107, 2319-2364.

Khan SJ and Ongerth SE (2004). Modelling of pharmaceutical residue in Australian sewage by quantities of use and fugacity calculations. Chemosphere 54, 355-367.

Kümmerer, K (2001). Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. Chemosphere 45, 957-969.

Lee HB, Peart TE and Svoboda ML (2005). Determination of endocrine-disrupting phenols, acidic pharmaceuticals, and personal care products in sewage by solid phase extraction and gas chromatography-mass spectrometry. J. Chromatography A, 1094,122-129.

Lopez de Alda MJ, Diaz-Cruz S, Petrovic M and Barcelo D (2003). Liquid chromatography-(tandem) mass spectrometry of selected emerging pollutants (steroid sex hormones, drugs and alkylphenolic surfactants) in the aquatic environment. J. Chromatography 1000, (2003), 503-526.

López-Roldán R, de Alda ML, Gros M, Petrovic M, Martín-Alonso J and Barcelo D (2010). Advanced monitoring of pharmaceutical and estrogens in Llobregat River basin (Spain) by liquid chromatography-triple Quadrupole tandem mass spectrometry in combination with ultra performance liquid chromatography Time of Flight mass spectrometry. J, Chemosphere, 80, 1337-1344.

Macià A, Borrull F., Calull M and Aguilar C (2006). Analysis on nonsteroidal anti-inflammatory drugs in water samples using microemulsion electrokinetic capillary chromatography under pH-suppressed electroosmotic flow with an on-column preconcentration technique. Chromatographia, 63, 149-154.

Medicines and Related Substances Act, 1965 (Act 101 of 1965): 935, September 5, 2008

Miao XS and Metcalfe CD (2003). Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography-electrospray tandem mass spectrometry. Analytical Chemistry, 75 (15), 3731-3738.

Mol HGJ, Sunarto S and Steijger OM (2000).Determination of endocrine disruptors in water after derivatization with N-methyl-N-(tert.-butyldimethyltrifluoroacetamide) using gas chromatography-mass spectrometry detection. Chromatography 879, 97-112.

Morales S, Canosa P, Rodriguez I and Cela R (2005). Microwave assisted extraction followed by gas chromatography with tandem mass spectrometry for the determination of triclosan and two related chlorophenol in sludge and sediments. J Chromatography A, 1082, 68-81.

Musson SE and Townsend TG (2009). Pharmaceutical compounds content of municipal solid waste. J. Hazardous Materials, 162, 730-735.

Nguyen HP and Schug KA (2008). The advantages of ESI-MS in conjunction with HILIC mode separations: Fundamentals and applications. J Separation Science 31, 1465-1480.

Oulton RL, Kohn T and Cwiertny DM (2010). Pharmaceutical and personal care products in effluent matrices: A survey of transformation and removal during wastewater treatment and implications for wastewater management. J Environmental Monitoring, 12, 1956-1978.

Owen SF, Giltrow E, Hugget DB, Hutchinson TH, Saye J, Winter MJ and Sumpter JP (2007). Comparative physiology pharmaceuticology and toxicology of beta blockers mammals versus fish. Aquatic Toxicology 82, 145-162.

Paulekuhn GS, Dressman JB and Saal C (2007). Trends in active pharmaceutical ingredient salt selection based on analysis of the orange book Database. J. Medical Chemistry, 50, 6665-6672.

Pedrouzo M, Borrull F, Marce RM, Pocurull E (2009). Ultra-high-performance liquid chromatography-tandem mass spectrometry for determining the presence of eleven personal care products in surface and wastewaters. J Chromatography A., 1216, 6994-7000.

Petrovic M, Dolores Hermando M, Diaz-Cruz M S, Barcelo D, (2005) Liquid chromatography-tandem mass spectrometry for the analysis of pharmaceutical residues in environmental samples: a review. J. Chromatography (1067) 1-14

Qin F, Zhao YY, Sawyer MB and Li XF (2008). Hydrophilic interaction liquid chromatography-tendem mass spectrometry determination of estrogen conjuagtes in human urine. Analytical Chemistry 80, 3404-3411

Quintana J.B., Carpinteiro J., Rodriguez I., Lorenzo R.A., Carro AM., Cela R. (2004). Determination of natural and synthetic estrogens in water by gas chromatography with mass spectrometry detection. J. Chromatogr. A, 1024 (2004), 177-185

Qiu H and Jun HW (1996). Solid-phase extraction and liquid chromatographic quantitation of insect repellent N,N-diethyl-m-toluamide in plasma. J Pharmaceutical and Biomedical Analysis, 15, 241-250.

Ramirez AJ, Brain RA, Usenko S, Mottaleb MA, Hurtado PP, Dobbins LL, Brooks BW, Chambliss, (2009), J Chromatography A, 1216, 815-823.

Reeves P R, McAinsh J, Mcintosh D A, Winrow M J, Xenobiotica, 8, (1978), 313-320

Rice S. L., Mitra S, Analytical Chimica Acta, 589, (2007), 125-132

Richardson B J, Lam P K S, Martin M (2005) Emerging chemicals of concern: Pharmaceuticals and personal care products (PPCPs) in Asia, with particular reference to Southern China. Marine Pollution Bulletin 50, 913-920

Rosal R, Rodea-Palomares I, Bolts K, Fernandez-Pinas F, Leganes F, Gonzalo S, Petre A, Environmental Science Pollution research, 17, (2010), 135-144

Routledge E.J., Sheanan D., Desbrow C., Brighty G.C., Waldock M., Sumpter J.P., Environ. Sci. Technol. 32 (11), (1998), 1559-1565

Rodriguez I., Carpinteiro J., Quintana, J.B., Carro A.M., Lorenzo R.A., Cela R., J. Chromatogr. A, 1024 (2004), 1-8

Sang Y, Xiong G, Maser E, Chemico-Biological Interactions, 191, (2011), 89-94

Santos J. L, Aparicio I, Alonso E (2009) Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain). Journal of Hazardous Materials (164) 1509-1516

Sebök À, Vasanits-Zsigrai A, Palkó Gy, Záray Gy, MolnárPerl I (2008) Identification and quantification of ibuprofen, naproxen, ketoprofen and diclofenac present in waste-waters, as their trimethylsilyl derivatives, by gas chromatography mass spectrometry. Talanta (76) 642-650

Sebastine I M, Wakeman R J, Process Safety and Environmental Protection, 81, (2003), 229-235

Segura P A, MacLeod S L, Lemoine P, Sauve S, Gagnon C, Chemosphere (2011) *in Press*

Senta I., Terzic S., Ahel Marijan, Chromatograpia, 68, (2008) 747-758

Slack R. J, Gronow J. R, Voulvoulis (2005) Household hazardous waste in municipal landfills: contaminants in leachate. Science of the Total Environment (337) 119-137

Solé M, Raldua D, Piferrer F, Baeceló D, Porte C (2003) Ferminization of wild carp, Cyprinus carpio, in a polluted environment: plasma steroid hormones, gonadal morphology and xenobiotic metabolized system. Comparative Biochemistry and Physiology Part C (136) 145-156

Solé M, Raldua D, Baeceló D, Porte C, Ecotoxicology and Environmental Safety, 56, (2003), 373-380

Soliman M A, Pedersen J A, Suffet I H (2004) Rapid gas chromatography-mass spectrometry screening methods for human pharmaceuticals, hormones, antioxidants and plasticizers in water. Journal of Chromatography A (1029) 223-237

Spongberg, A.L., Witter, J.D., Science of the Total Environment, 397, (2008), 148-157.

Segura P A, MacLeod S L, Lemoine P, Sauve S, Gagnon C, Chemosphere (2011) *in Press*

Siemens J., Huschek, G., Siebe C., Kaupenjohann M., Water Research, 42(8-9) (2008), 2124-2134

Steger-Hartmann T., Kümmerer K., Hartmann A., Ecotoxicol Environ Safety, 36, (1997), 174-179.

Ternes T.A, Siegrist H, Environmental Science and Technology, 38, (2004), 392A-399A

Ternes T, Trends in Analytical Chemistry, 20, (2001), 419-434

Ternes T.A., Water Res., 32(11), (1998), 3245-3260

U.S. Food and Drug Administration Orange Book, http://www.fda.gov/cder/ob/.

Vanderford B J, Pearson R A, Rexing D J, Snyder S A, Analytical Chemistry, 75, (2003), 6265-6274

Van Nuijs A L N, Pecceu B, Theunis L (2009) Cocaine and metabolites in waste and surface water across Belgium. Environmental Pollution (157) 123-129

Wang C, Shi H, Adams C D, Gamagedara S, Stayton I, Timmons T, Ma Y, Waters Research, 45, (2011), 1818-1828

Weigel S, Kuhlmann J, Hühnerfuss H (2002) Drugs and personal care products as ubiquitous pollutants: occurrence and distribution of clofibric acid, caffeine and DEET in the North Sea. Science of the Total Environment (2002) 131-141

Wu J, Qian X, Yang Z, Zhang L, J Chromatography A, 1217, (2010), 1471-1475

Xagoraraki I, Kumar A, Science of the Total Environment, 408 (2010), 5972-5989

Xagoraraki I, Kumar A, Regulatory Toxicology, 57 (2010), 146-156

Xu J, Wu L, Chen W, Cheng A.C, J Chromatography A, 1202, (2008), 189-195

Yu Y, Huang Q, Cui J, Zhang K, Analytical Biochemistry Chemistry 399 (2011) 891-902

Zhao J. L, Ying G G, Wang Li, Yang J. F (2009) Determination of phenolic endocrine disrupting chemicals and acidic pharmaceuticals in surface water of the Pearl Rivers in South China by gas chromatography-negative chemical ionization-mass spectrometry. Science of the Total Environment (407), 962-974.

Zhou J L, Zhang Z. L., Banks E, Gover, D, Jiang J. Q, J. Hazardous Materials, 166 (2009) 655-661

Zwiener C, Frimmel F H (2004) LC-MS analysis in the aquatic environment and in water treatment – a critical review. J. Analytical and Bioanalytical Chemistry (378) 862-874

Zwiener C, Frimmel F H (2004) LC-MS analysis in the aquatic environment and in water treatment – a critical review. J. Analytical and Bioanalytical Chemistry (378) 851-861

Zuccato E, Castiglioni S, J. Hazardous Materials, 122, (2005), 205-209

Zuccato E, Castiglion S, Fanelli R, Reitano G, Bgnati R, Chiabrando C, Pomati F, Rossetti C, Calamari D (2006) Pharmaceuticals in the environment in Italy: Causes and occurrence. Environmental Science and Pollution Research (13) 15-21

CHAPTER 3: SOUTH AFRICAN MOST-PRESCRIBED DRUGS

3.1 INTRODUCTION

There are four possible routes of discharge of PPHCPs into the environment;

1. Municipal wastewater treatment plants
2. Hospital dumping of unused pharmaceuticals
3. Manufacturers production waste
4. Manure containing veterinary pharmaceuticals

However, data are not available on the concentrations of PPHCPs discharged in these ways. Therefore, in this study we examined prescription volumes in the private and public health sectors in South Africa.

3.2 PRESCRIPTION VOLUMES

Table 3.1 and 3.2 show the top 50 most-prescribed drugs and their classes in the private and public sectors. The data show an interesting pattern with respect to classes of drugs prescribed. There is a distinct difference between the private and the public health systems. Although both health systems heavily prescribe analgesics, in the private health system a combination of the analgesic and non-steroid anti-inflammatory drugs (NSAIDs) are the most prescribed group of drugs followed by antihistamine, decongestants and asthmatic group of drugs respectively. Table 3.2 shows an interesting public health sector prescription pattern which consists of the hypertensives being the most prescribed class, followed closely by analgesic drugs. ARV and antibiotic classes of drugs are the next most prescribed in the third and fourth place respectively. The high levels of hypertensive, ARV and antibiotics drug groups prescribed in the public health system should be noted. The prescription pattern could be an indicator of the environmental occurrence of these compounds.

Table 3-1: Top 50 most prescribed drugs in the public health sector

	Product	Drug	Class	Prescriptions
1	RIDAQ	Hydrochlorothiazide	Hypertension	12 119 557
2	AUSTELL-PARACETAMO	Paracetamol 500 mg	Analgesic	10 712 781
3	PHARMAPRESS	Enalapril maleate & Hydrochlorothiazide	Hypertension	9 751 575
4	VITAMIN B CO (UNB)	Vitamins	Vitamins	7 335 796
5	CO-TRIMOXAZOLE (UN)	Co-trimoxazole	ARV	6 555.480
6	PACIMOL	Paracetamol 500 mg	Analgesic	6 424 452
7	METHYL SALICYL (UN)	Methyl salicylate	NSAID	5 759 858
8	METFORMIN	Metformin Hydrochloride	Diabetic	5 543 892
9	AMOXYCILLIN	Amoxycillin	Antibiotic	5320.452
10	PARACETAMOL (UNB)	Paracetamol 500 mg	Analgesic	5 243709
11	AUSTELL-AMLODIPINE	Amlodipine as besylate, mesylate	Hypertension	5 141 771
12	ADALAT	Nifedipine	Hypertension	4 281 022
13	ADCO-ATENOLOL	Atenolol	β-blocker	3 979 057
14	PREXUM	Perindopril	Hypertension	3 706 965
15	MULTIVITAMIN (UNB)	Vitamins	Vitamins	3 345 026
16	WATER FOR INJ (FK2)	Water	water	3 345 026
17	TRIPHASIL	Levonorgestrel & Ethinyloestradiol	Contraceptive	3 162729
18	VIREAD	Tenofovir disoproxil fumarate	Hepatitis B virus	3 025 638
19	NUR-ISTERATE	Norethisterone enantate	Contraceptive	2 939 375
20	FOLIC ACID (A&D)	Vitamins	Vitamins	2 476 826
21	RANAMP	Ampicillin	Antibiotics	2 380 188
22	PAINBLOK	Paracetamol 500 mg	Analgesic	2 375 900
23	MEDROXYPROGEST-FRE	Medroxyprogesterone Free	Hypertension	2 372 561
24	GULF PARACETAMOL	Paracetamol 500 mg	Analgesic	2 369 300
25	ASPEN LAMIVUDINE	Lamivudine	Antiviral	2 232 857
26	SIMVOTIN	Simvastatin	Cholesterol	2 203 242
27	POVIDONE IODIN (UN)	Povidone-iodine	Surgical infesion	2 144 193
28	SONKE-LAMIVUDINE	Sonke-Lamivudine	ARV	2 004 389
29	NORDETTE 28	Levonorgestrel	Contraceptive	1 959 696
30	PANADO CO	Paracetamol 500 mg, codeine	Analgesic	1 959 471
31	COCILLANA COMP	Cocillana Compound Syrup	Cough syrup	1 880 751
32	ALLERGEX	Chlorphenoxamine hydrochloride	Anti-Allergic	1 861 470
33	ASTHAVENT	Salbutamol Sulphate	Asthma	1 857 698
34	MIST TUSSI INFANS	Fams mist tussi infans	Cough syrup	1 847 307
35	SABAX SODIUM CHLOR	sodium chloride for IV	IV	1 802 455
36	CEFTRIAXONE	Ceftriaxone	Antibiotics	1 766 351
37	ADCO-EFAVIRENZ	Adco Efavirenz	ARV	1 706 909
38	PREVENAR	Pneumococcal 7-valent conjugate	Vaccine	1 700 790
39	AUGMENTIN	Amoxycillin (50 mg)	Antibiotic	1 687 550
40	ASPEN EFAVIRENZ	ASPEN EFAVIRENZ (600 MG)	ARV	1 645 745
41	MYLOCORT	Hydrocortisone acetate	Anti-itch	1645.348
42	ASPEN STAVUDINE	Aspen Stavudine	ARV	1 636 949
43	AUSTELL-FUROSEMIDE	Austell-Furosemide	Anti-Microbials	1 634 441
44	CHLORAMPHENICO	Chloramphenicol	Anti-bacteria	1 626 134
45	ZENTEL	Albendazole	Anthelmintic	1 613 990
46	LIQUID PARAFFI (UN)	LIQUID PARAFFI (UN)	Laxative	1 561 855
47	GLYGARD	Gliclazide (80 mg)	Diabetic	1 499 644
48	SONKE-STAVUDINE	SONKE-STAVUDINE	ARV	1 496 839
49	HUMULIN 30/70	regular insulin and 70 units of IIHB	Diabetics	1 452 452
50	OVRAL 28	Norgestrel and Ethinyl Estradiol	Contraceptive	1 350 853

Table 3-2: Top 50 most prescribed drugs in the private health sector.

	Product	Drug	Class	Prescriptions
1	ADCO-DOL	Paracetamol, 450 mg; Codeine, Caffeine	Analgesic	6 670 821
2	ALCOPHYLLEX	Diphenhydramine, theophyline & etofyline	Expectorate	6 257 534
3	ALLERGEX	Chlorpheniramine maleate	Antihistamines	6 014 431
4	DPH	Diphenhydramine	Antihistamines	3 944 118
5	PANAMOR	Diclofenac sodium (25-100 mg)	Anti-Inflammator	3 724 619
6	PANADO	Paracetamol 500 mg, codeine (8 mg)	Analgesic	2 880 919
7	BRONCLEER WITH COD	Diphenhydramine 125 mg, Codeine, 10 mg;	expectorants	2 859 364
8	CORENZA C	Aspirin, Chlorprophenpyridamine;	Cold and Flu	2 334 721
9	SINUCON	Phenylpropanolamine	Antihistamines	2 158 663
10	SINUEND	Chlorpheniramine and phenyltoloxamin	Antihistamines	2 098 621
11	ASTHAVENT	Salbutamol 100 µg	Asthmatic	2 068 270
12	MAYOGEL	Aluminium oxide, Magnesium oxide	Antacids	1 825 719
13	GEN-PAYNE	Ibuprofen, paracetamol and codeine	Analgesic	1 697 405
14	THEOPHEN COMPOUND	Theophylline, β-Hydroxyethyl Theophylline,	Expectorants	1 634 112
15	MYBULEN	Ibuprofen, paracetamol; and codeine	Analgesic	1 625 431
16	BENYLIN FOUR FLU	Paracetamol, diphenhydramine HCl	Analgesic	1 620 013
17	PERSIVATE	Etamethasone (5 mg)	Corticosteroid	1 570 532
18	STILPANE	Paracetamol, Codeine, Caffeine	Analgesic	1 515 511
19	ADCO-SIMVASTATIN	Simvastatin, Butylhydroxyanisol	Anti-cholesterol	1 513 178
20	NAPAMOL	Paracetamol, 500 mg	Analgesic	1 451 078
21	ACC 200	N-acetylcysteine (200 mg)	Mucolytic agaent	1 448 168
22	FLUTEX	Paracetamol, Caffeine, SCP	Colds & influenza	1 409 604
23	DISPRIN	Acetylsalicylic acid (Aspirin)	NSAIDs	1 408 014
24	MYPRODOL	Ibuprofen; codeine and paracetamol	Analgesic	1 392 375
25	PURBAC	Trimethoprim, Sulphamethoxazole	Antibiotics	1 376 298
26	ADCO-LINCTOPENT	Bromhexine HCl, orciprenaline	Expectorant	1 360 582
27	VENTEZE	Salbutamol	Asthmatic	1 337 780
28	BACTROBAN	Mupirocin	Antibiotics	1 303 609
29	GLUCOPHAGE	Metformin Hydrochloride (500 mg)	Diabetic	1 297 591
30	MYLAN DICLOFENAC	Diclofenac sodium	NSAIDs	1 293 478
31	GAVISCON	Alginic acid and bicarbonate	Anti-acid	1 227 850
32	SINUTAB	Paracetamol & pseudoephedrine HCl	AND	1 227 631
33	CENTRUM	Multivitamin supplement	Vitamins	1 197 453
34	ILIADIN	Oxymetazoline HCl & Benzalkonium chloride	Decongestants	1 173 866
35	LENAZINE	Codeine, Ephedrine HCl, Promethazine HCl	Expectorants	1 165 048
36	BPYN	Paracetamol, Codeine, Caffeine,	Analgesic	1 163 355
37	ANDOLEX-C	Benzydamine HCl, Chlorhexidine	NSAIDs	1 144 247
38	CATAFLAM	Diclofenac KCaPO3	NSAIDs	1 138 718
39	ADCO-SINAL CO	Paracetamol, Phenylpropanolamine, Codeine	NSAIDs	1 126 793
40	HYOSPASMOL	Hyoscine Butylbromide	antispasmodics	1 115 273
41	FLUSIN	Paracetamol, Pseudoephedrine	AAA	1 113 084
42	AUGMENTIN GSK	Amoxicillin	Antibiotic	1 105 783
43	VERMOX	Mebendazole	Anthelmintics	1 090 839
44	CRESTOR	Rosuvastatin calcium	Anticholesterol	1 076 578
45	SYNDOL	Paracetamol, Codeine, Caffeine	Antihistamine	1 050 326
46	STREPSILS	Amylmetacresol	Antiseptics	1 034 799
47	CHLORAMEX	Chloramphenicol (10 mg)	Antibiotics	1 029 357
48	ASPAVOR	Atorvastatin	Anticholesterol	1 021 177
49	YASMIN	Drospirenone and ethinyl estradiol	Contraceptive	1 002 940
50	NORFLEX CO	Orphenadrine, Aspirin, and Caffeine	Arthritis	978 804

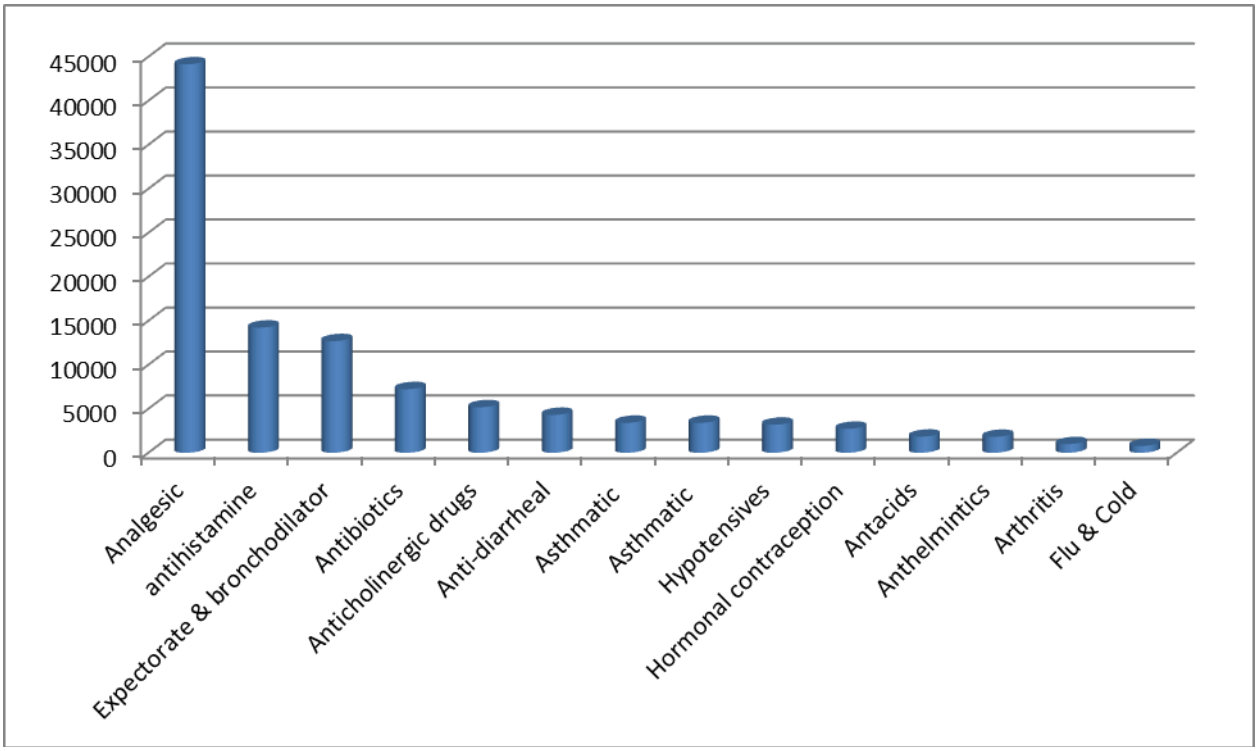


Figure 3-1: Most prescribed classes of drugs in the private health sector

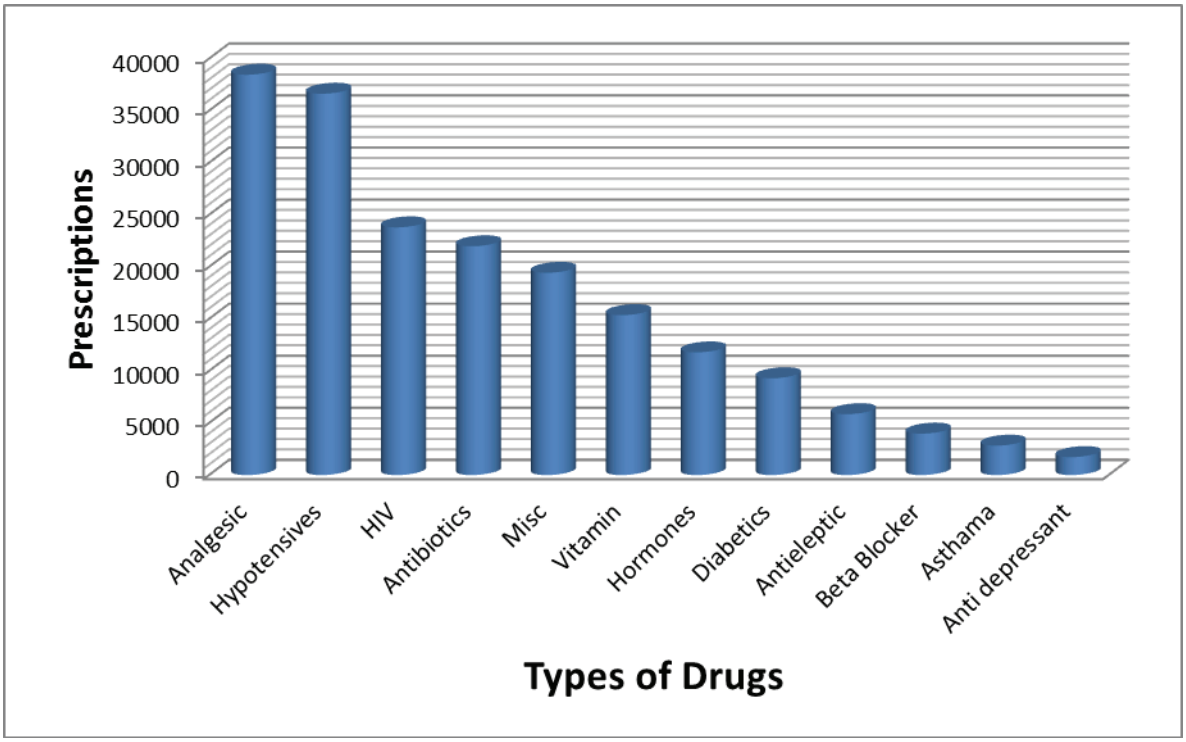


Figure 3-2: Most prescribed classes of drugs in the public health sector

The overall picture indicates the possibility of finding large quantities of analgesic and anti-inflammatory drugs observed in both health systems in our water systems. Amongst the analgesics, paracetamol features more frequently and is usually combined with codeine and caffeine.

In the absence of in situ measurements, the data on prescription volumes can only provide one perspective on what might be expected in the water systems. A closer look at the data reveals an even more complex pattern. Table 3.3 shows the top 20 most prescribed products by both health sectors. These included the following; paracetamol, hydrochlorothiazide in combination with enalapril maleate, vitamins, trimethoprim and sulfamethoxole, methyl salicylate, metformin hydrochloric, amoxicillin, diphehydrazine, chloropheniramine and diclofenac sodium. The most prescribed individual drug is paracetamol followed by hydrochlorothiazide. As noted in Chapter 2 of this report, the presence or absence of an individual drug will depend on a number of parameters which include prescribed volumes, stability of the drug in the environment, metabolic pathways and its removal efficiency in treatment plants.

Table 3-3: 20 most prescribed drugs in both private and public sectors

	Product	Drug	Class	Prescriptions
1	Ridaq	Hydrochlorothiazide	Hypertension	12119557
2	Austell Paracemol	Paracetamol 500 mg	Analgesic	10712781
3	Pharmapress	Enalapril maleate & Hydrochlorothiazide	Hypertension	9751575
4	Vitamin B complex	Vitamins	Vitamins	7335796
5	Co-trimoxazole	Trimethoprim and sulfamethoxazole	ARV	6555480
6	Pacimol	Paracetamol 500 mg	Analgesic	6424452
7	Methyl Salicyl	Methyl salicylate	NSAIDs	5759858
8	Metformin	Metformin Hydrochloride	Antidiabetic	5543892
9	Amoxycillin	Amoxycillin	Antibiotics	5320452
10	Paracetamol	Paracetamol 500 mg	Analgesic	5243709
11	Adco-Dol	Paracetamol, 450 mg; Codeine, Caffeine	Analgesic	6670821
12	Alcophyllex	diphenhydramine, theophyline & etofyline	Cough mixture	6257534
13	Allergex	Chlorpheniramine maleate	Antihistaminic	6014431
14	DPH	Diphenhydramine	Antihistaminic	3944118
15	Panamor	Diclofenac sodium	Analgesic	3724619
16	Panado	Paracetamol 500 mg, codeine (8 mg	Analgesic	2880919
17	Broncleer with Cold	Diphenhydramine 125 mg, Codeine	Cough syrup	2859364
18	Corenza C	Aspirin, Chlorprophenpyridamine	Colds & flu	2334721
19	Sinucon	Phenylpropanolamine	Antiitchiness	2157663
20	Sinuend	Chlorpheniramine and phenyltoloxamin	Antihistamines	2086681

3.3 SELECTION OF ANALYTICAL METHODS

Knowledge of the PPHCP distribution is important as this would inform us of the expected quantities within municipality wastewater treatment plants. In addition the pattern observed in **Figure 3.3** has a bearing on the analytical techniques needed for detection and quantification. The analytical methods needed must be capable of detecting trace levels and diverse PPHCPs in treated and drinking water. The analgesic and anti-inflammatory drugs for example vary from acidic, neutral to basic and would require separation on a stationary phase that can accommodate such diversity. The challenges for the analysis of PPHCPs is that many of the compounds are either very polar or non-volatile therefore limiting their direct determination by gas chromatography. The pharmaceutical personal care products have in the past been determined by several analytical techniques that incorporated a separation method that included, gas chromatography with FID, gas chromatography-mass spectrometry (GC-MS), gas chromatography-mass spectrometry (GC-MS),

gas chromatography-mass spectrometry/mass spectrometry (GC-MS/MS) (Mottaleb et al., 2009) GCxGC-MS (Gómez et al., 2011), Liquid chromatography-mass spectrometry (LC-MS) (Li et and Tsai, 2009), Liquid chromatography-mass spectrometry/ mass spectrometry (LC-MS/MS) (Ramirez et al., 2007) with electrospray ionisation.

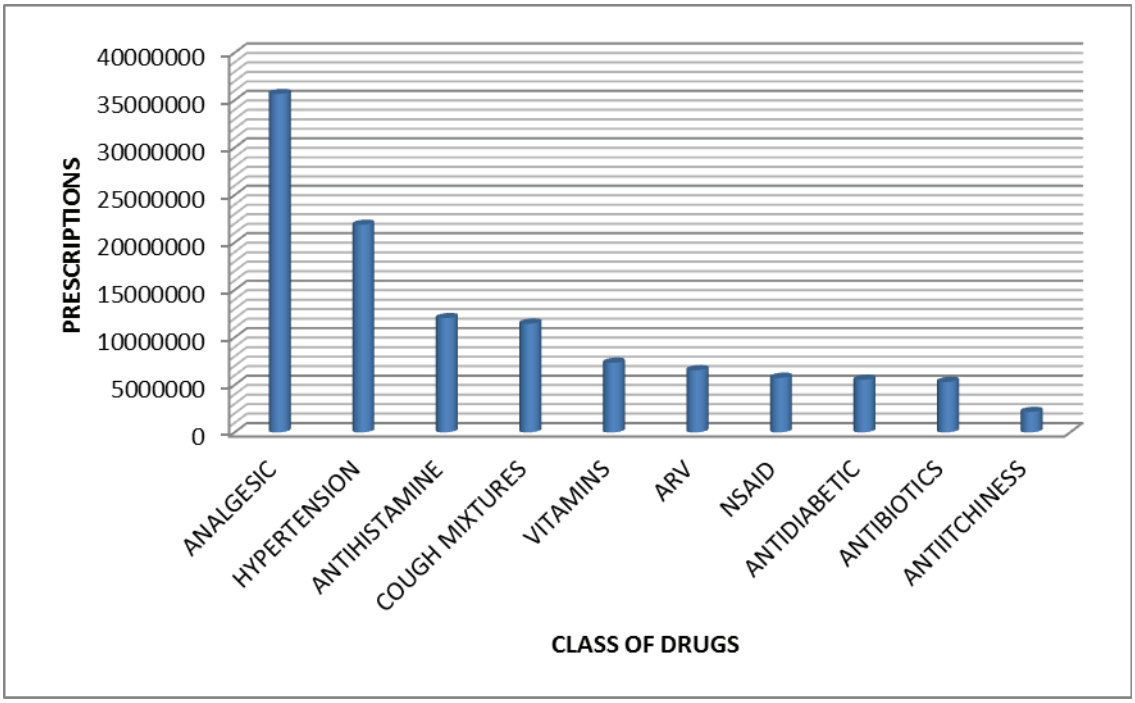


Figure 3-3: Distribution of the top 20 prescribed classes of drugs in South Africa

The liquid chromatography-linear ion quadrupole trap mass spectrometry/mass spectrometry (LC-QLIT-MS2) has been used to quantify several classes of PPHCPs that included analgesic/inflammatory, lipids regulators, psychiatric, antibiotics such as macrolides and sulfonamides in WWTP water. The LC-MS methods are direct and preferred to the GC-MS methods which are indirect and hence require derivatisation step for the analysis of most PPHCPs.

3.4 CONCLUSIONS

The list of compounds of interest in the South African context has been drawn primarily based on information of prescription volumes though we appreciate that other parameters could influence the presence of PPHCPs in the water systems. The most prescribed compound that is likely to be found in the South African environment fall within six classes which are hypertension, analgesics, antiretroviral, antibiotics, vitamins and antidiabetic drugs. These consisted of the following drugs; hydrochlorothiazide, paracetamol, enalapril maleate & hydrochlorothiazide, methyl salicylate, metformin hydrochloride, amoxycillin, paracetamol in combination with codeine and caffeine, diphenhydramine in combination with theophyline and etofyline, chlorpheniramine maleate, diphenhydramine, diclofenac sodium, aspirin in combination with chlorprophenpyridamine, phenylpropanolamine and chlorpheniramine in combination with phenyltoloxamin. Large prescription volumes of these classes of drugs might imply their occurrence in water systems. Further experimental work which will take into consideration parameters such as stability, removal of PPHCPs in wastewater treatment plant, should provide a clearer picture and thus refine the list of selected compounds.

CHAPTER 4: ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

4.1 INTRODUCTION

Analytical methods needed to analyse and quantify each of the six classes (hypertension, analgesic, ARV, anti-diabetic, antibiotics, vitamins) are presented in this chapter. Wherever possible, the USA Environmental Protection Agency Method 1694 will be used as is or modified (EPA Method 1694, 2007). The selected methods should include:

- Sampling and sample preparation
- Extraction and/or preconcentration
- Separation and detection

In Chapter 3 the list of interest for South African PPHCPs was drawn based on the prescription data provided from the public and private health systems. The most prescribed compounds included six classes which are hypertension, analgesics, antiretroviral, antibiotics, vitamins and antidiabetic drugs. In our preliminary experimental work we selected a few PPHCPs which were representative of the most prescribed groups; Anti-convulsants (carbamazapine and primidone), antiretroviral drugs (penciclovir, famciclovir and ribavirin), β blockers (pindolol), antibiotics (sulfamethoxazole), analgesics and non-steroidal anti-inflammatory (paracetamol, ketoprofen, diclofenac, fenoprofen, ibuprofen).

4.2 SAMPLING AND SAMPLE PREPARATION

Sampling and sample preparation will be common to the six classes of drugs. The following are two approaches to sampling;

1. **Grab sampling** is the most common and cheapest approach. It involves drawing water from a selected point and depth. The water is collected in 500 mL or 1 L silanised amber bottles and stored in an ice cooler box for transportation to the laboratory. Sodium azide (10 mL of 2 M) is added to each sample to prevent any bacterial growth as well as sample degradation. The sample is stored at 4ºC until filtration and extraction. Once in the laboratory, water is filtered through a 0.45 µm and 0.22 µm cellulose acetate membrane. For antibiotics the sample is further adjusted to pH 3 or less and 1.00 mg/L of EDTA is added to reduce complexation of antibiotics with Ca^{2+} or Mg^{2+}. The limitation of this approach is that several samples have to be collected from a given location over the entire period of sampling in order to have an overall view. This is not only time consuming, but also expensive.

2. **Passive sampling** involves the measurement of the concentration of the analyte of interest as a weighted average based only on the sampling and/or extraction time (Macleod SL. 2007; Wong, Charles S. 2009) In addition passive samplers are suitable for long-term collection of the analyte. Waters Oasis HLB sorbent (also known as sequestering medium) is best for the classes of pharmaceuticals of interest in this work. Methanol is used to clean aluminium foil to be used as working surface and polyether sulfone (PES) membrane. A sequestering medium (Oasis LHB) 0.200 g per sampler is weighed accurately to ± 0.001 g on a weighing paper. The medium is secured between the two halves of the sampler and placed into a protective cage for sampling.

Wastewater treatment plants samples, collected using either grab or passive sampling methods usually require further clean up and/or pre-concentration. The solid phase extraction (SPE) step is carried out prior to the chromatographic and detection steps. The Water Oasis® HLB SPE cartridges exhibit both hydrophilic

and lipophilic retention and hence are commonly utilised for pharmaceuticals prior to gas and liquid chromatography. The HLB SPE is used where sampling was via Grab approach. In our present work HLB-Disk was used in combination with with POCIS (see Appendix C).

4.3 ANALYTICAL METHODS AND DETECTION

There are two approaches to the analysis and quantification of twenty PPHCPs compounds selected for South Africa. The first approach is based on gas chromatography-mass spectrometry (GC-MS) available in most South African laboratories. This method will involve derivatisation since most compounds on the priority list are non-volatile. The second approach is based on liquid chromatography with diode array detection (DAD), fluorescence, charged aerosol detection (CAD) and mass spectrometry. LC-MS and LC-MS/MS methods are direct and are capable of detecting all PPHCPs. Unfortunately LC-MS instrumentation is expensive and not affordable by many of the laboritories. The use of HPLC with other non-mass spectrometry detection, although cheap, suffers limited application due to the fact that some of the PPHCPs are non-UV/Vis absorbing and non-fluorescent and hence not detectable. In this work we intend to explore HPLC with charged aerosol detector as an alternative and cheaper approach for the detection of PPHCPs. Charge aerosol detector is perceived as a universal detector with a potential of detecting their compounds of interest in environmental samples.

4.4 HPLC-CAD

Preliminary studies on the capabilities of charged aerosol detectors for the compounds of interest are presented in this section. Out of the six classes of drugs that we had selected only analgesics, hypertension, antibiotics and antiretroviral drugs have been considered in this current report. We have also included the analysis of steroid hormones and anticonvulsants even though they were not necessarily part of the most prescribed drugs.

The principle of charged aerosol detector is based on charging of aerosol particles by corona discharge and subsequent measurement of the charged particles using an electrometer, similar to an atmospheric chemical ionisation mass spectrometry (APCI/MS). The principle and operation of CAD has been reported previously (Dixon and Peterson 2002). The response for CAD is based on electrical aerosol detection technology, which has been demonstrated to be sensitive even for very small particles (<100 nm) (Dixon and Peterson, 2002). Higher sensitivity for various ranges of particle sizes also provides better reproducibility and linearity over wide ranges. CAD also has the advantage of lower operational cost, as it does not require optical components for detection as in the case of evaporative light scattering detector (ELSD).

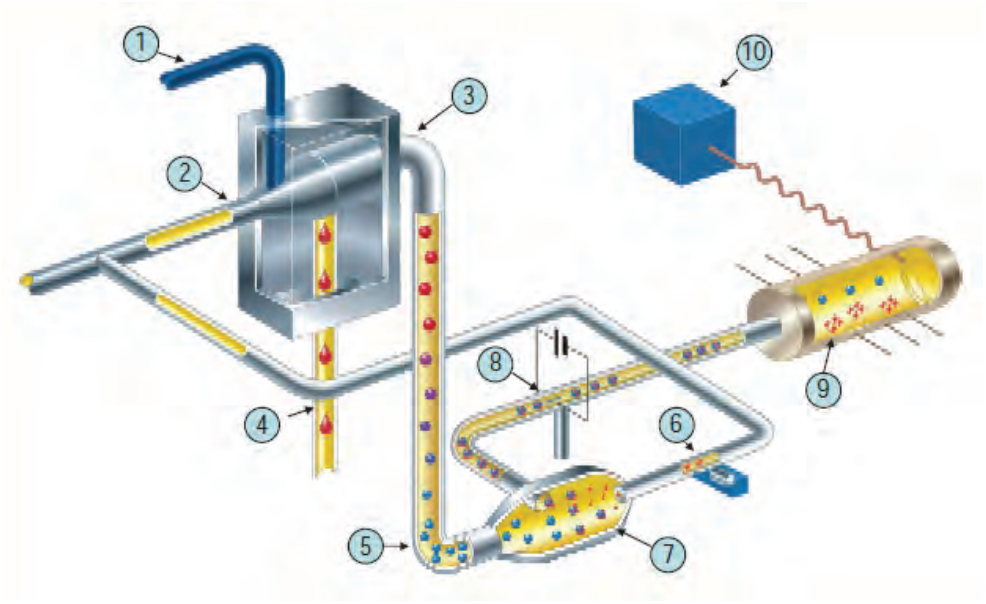


Figure 4-1: Schematic of a charged aerosol detector

The CAD system operates as follows: (1) Liquid eluent enters from HPLC system, (2) Pneumatic nebulisation occurs, (3) Small particle droplets enter drying tube, (4) Large droplets exit to drain, (5) Dried particles enter mixing chamber, (6) Gas stream passes over Corona needle, (7) Charged gas collides with particles and transfer charge, (8) High mobility species are removed, (9) Remaining charged particles measured, and (10) Signal transferred to chromatographic software.

4.5 EXPERIMENTAL CONDITIONS

4.5.1 Procedures for non-hormones

4.5.1.1 HPLC-CAD procedures

An HPLC-CAD method was developed for the simultaneous separation and determination of different classes of drugs which included analgesics, antibiotics, anticonvulsant, hypertension and antiretrovirals. The analgesic group consisted of nonsteroidal anti-inflammatory drugs (fenoprofen, ibuprofen, diclofenac, and ketoprofen) and paracetamol. Sulfamethoxazole was the only antibiotic included in the mixture. ARVs such as penciclovir, famciclor and ribavirin were also included in the mixture. Pindolol (hypertension β-blocker) and two anticonvulsants (primidone and carbamazepine) were also part of the study. All analytes were prepared by dissolving appropriate amounts in a 50:50 mixture of methanol and water to give a desirable concentration range 0.1 to 75 µg mL^{-1} for calibration standards. The mixture of drugs was then separated on a Zorbax Eclipse XDB C18 (4.6 mm x 150 mm x 5 µm) using acetonitrile (A) and 0.01% acetic acid (B) mobile phase in a gradient elution mode. Conditions for the step gradient elution mode and CAD are summarised below and in Table 4.1.

The Corona_Ultra Detector parameters were set as follows;

Nitrogen gas	=	35 psi
Filter	=	None
Current range	=	500 pA
Nebuliser heater	=	25^{o}C
HPLC Parameters		

Solvent phase A	=	Acetonitrile
Solvent phase B	=	0.1% Acetic Acid
HPLC Column	=	Zorbax C18 (5 µm x 4.6 mm x 150 mm)
Flow rate	=	1.0 mL/min
Run time	=	30 min
Injection volume	=	10 µL
Column temperature	=	Ambient

Table 4-1: Gradient program conditions used for the separation of pharmaceuticals

Time (min)	% A	% B
0.00	5	95
2.50	5	95
5.00	20	80
7.50	40	60
10.00	50	50
15.00	60	40
20.00	70	30
25.00	70	30
26.00	5	95
30.00	5	95

4.5.1.2 Procedure for SPE extraction

A wastewater sample was initially filtered through a Whatmat No.1 filter membrane paper to get rid of any particulate matter. It was then filtered through 0.45 µm and 0.22 µm cellulose acetate membrane to obtain cleaner filtrates. The filtrate was then passed through a Water Oasis® HLB SPE cartridge for clean-up and pre-concentration. The procedure for SPE is outlined in the following steps:

1. The SPE catridge was conditioned with 6 mL of methanol followed by 6 mL of water.
2. 90 mL of water sample were loaded at a low flow rate .
3. The cartidge was then washed with 8 mL of 5% methanol, 2 mL of 2% acetic acid, 8 mL of 5% methanol, 2 mL of 2% ammonium hydroxide and finally 8 mL 65% methanol.
4. The cartridge was dried under nitrogen flow for 30 mins.
5. Analytes were then eluted from the catridge using 8 mL of 65% methanol
6. The extracted sample was dried with gentle stream of nitrogen and reconstituted to 3 mL with methanol

4.5.2 Procedures for hormones

4.5.2.1 HPLC-CAD procedure

A mixture consisting of five steroidal hormones estradiol, β estradiol, 17 α estradiol, testosterone and progesterone was prepared in a 50:50 solution of methanol and water at a concentration of 100 ppm. Bisphenol A was also added to this mixture. The analytes were separated using an isocratic mode on a Zorbax Eclipse XDB C8 column and detected on a charged aerosol detector. Details of the separation and detector conditions are listed in Table 4.2 below.

Table 4-2: Conditions for separation and detection of steroid hormones on HPLC-CAD

	Corona_Ultra Detector	
	Gas pressure	35 psi
	Filter	Noon
	Range	100 pA
	Nebuliser	ON
HPLC	Mobile phase	Methanol/water (70/30, v/v)
	Column	Zorbax Eclipse XDB C8 (4.6 mm x 150 mm x 5 µm)
	Flow rate	1.2 mL/min

4.5.2.2 Pre-concentration of hormones on SPE

A Water Oasis® HLB SPE cartridge (1 cc, 30 mg sorbent cartridge) was used to clean and pre-concentrate the hormones from the wastewater sample that had been filtered previously as described in section 4.5.1.2. The SPE procedure is described in the following steps:

1. The cartridge was conditioned with 6 mL of H_2O and MeOH
2. 90 mL of the sample was loaded onto the cartridge at a low flow rate
3. The loaded cartridge was washed with 8 mL 5% MeOH, 2 mL 2% acetic acid, 8 mL 5% MeOH, 2 mL 2% ammonium hydroxide and 8 mL 65% MeOH
4. The cartridge was dried under vacuum for 30 min
5. Elution was done using with 10 mL of MeOH
6. The eluted sample was dried under N_2 and reconstituted with 0.5 mL (ACN/H_2O)

4.5.3 Sampling procedures for wastewater

Wastewater was sampled from Daspoort WWTP which is located on southern banks of Apies River, on the north-west edge of Pretoria central business district. There were two sampling influent points, the old (#1) and newer (#2) works. The two influent samples were collected after undergoing mechanical and grit removal. The third sample was collected from the effluent just before discharging into the river. Two sampling approaches were used in this work, the grab and passive sampling. Figure 4.2 shows some of the sampling points.

Figure 4-2: Daspoort sampling points; A & B show influent sampling point #2 and C is effluent sampling point

4.5.3.1 Grab sampling

Wastewater samples were drawn from three selected points (2 influents and 1 effluent). The water was collected in 2.5 L silanised amber bottles and stored in an ice cooler box for transportation to the laboratory. Sodium azide (10 mL of 2 M) was added to each sample to prevent any bacterial growth as well as sample degradation. Once in the laboratory, samples were filtered through No.1 Whatman filter first to remove suspended solids and then 0.45 µm and 0.22 µm cellulose acetate membrane. For antibiotics the wastewater sample was further adjusted to pH 3 or less and 1.00 mg L^{-1} of EDTA was added to reduce complexation of antibiotics with Ca^{2+} or Mg^{2+}.

4.5.3.2 Passive sampling

Figure 4.3 shows units that were used for sampling wastewater in this work. Each of the samplimg units contained three samplers. One sampler contained Waters Oasis HLB sorbent (also known as sequestering medium) and the other two contained HLB SPE disks. A sequestering medium (Oasis HLB) (0.200 g per sampler) was weighed accurately to ± 0.001 g on a weighing paper. The medium was secured between the two halves of polyether sulfone (PES) membrane of the sampler and placed into a protective cage for sampling. This formed the bases of Polar Organic Chemical Integrative Sampler (POCIS) used for PPHCPs.

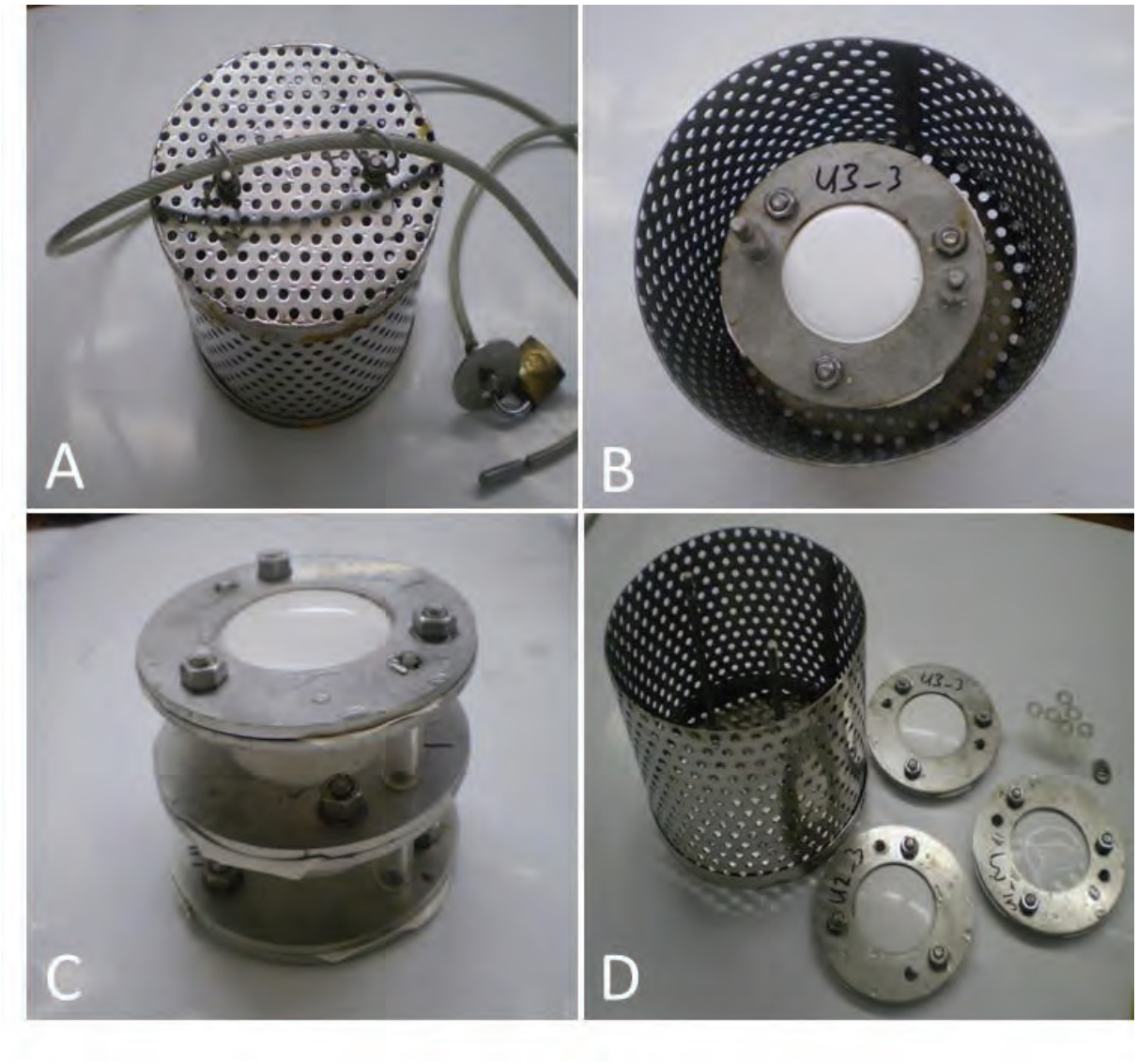


Figure 4-3: POCIS sampler used in this work. A = cage used to protect the samplers; B = samplers inside the cage; C= 3 samplers and D = cage and 3 samplers

4.6 DETERMINATION OF PPHCPS IN WATER USING HPLC-CAD

4.6.1 Validation of HPLC-CAD method for determination of PPHCPs in aquatic systems

An HPLC-CAD method was developed and validated for the determination of twelve selected PPHCPs in aquatic systems. The selected compounds are representative of the top 20 most prescribed PPHCPs identified in South Africa. The groups of drugs included in this study were analgesics, non-steroidal anti-inflammatory drugs, ARVs, β-blockers and antibiotics. Figure 4.4 shows a typical chromatogram of a mixture of twelve PPHCPs compound separated on a Zorbax Eclipse XDB C18 column using a gradient elution programme summarised in Table 4.3. All compounds were successfully detected using the charged aerosol detector. CAD is known to be very sensitive to changes in the mobile phase composition which could be problematic if gradient elution is to be employed. The results however demonstrate that it is possible to use gradient elution mode without severe baseline drift. Validation parameters which were considered in this study were linearity range, limit of detection (LOD), limit of quantification (LOQ), and accuracy.

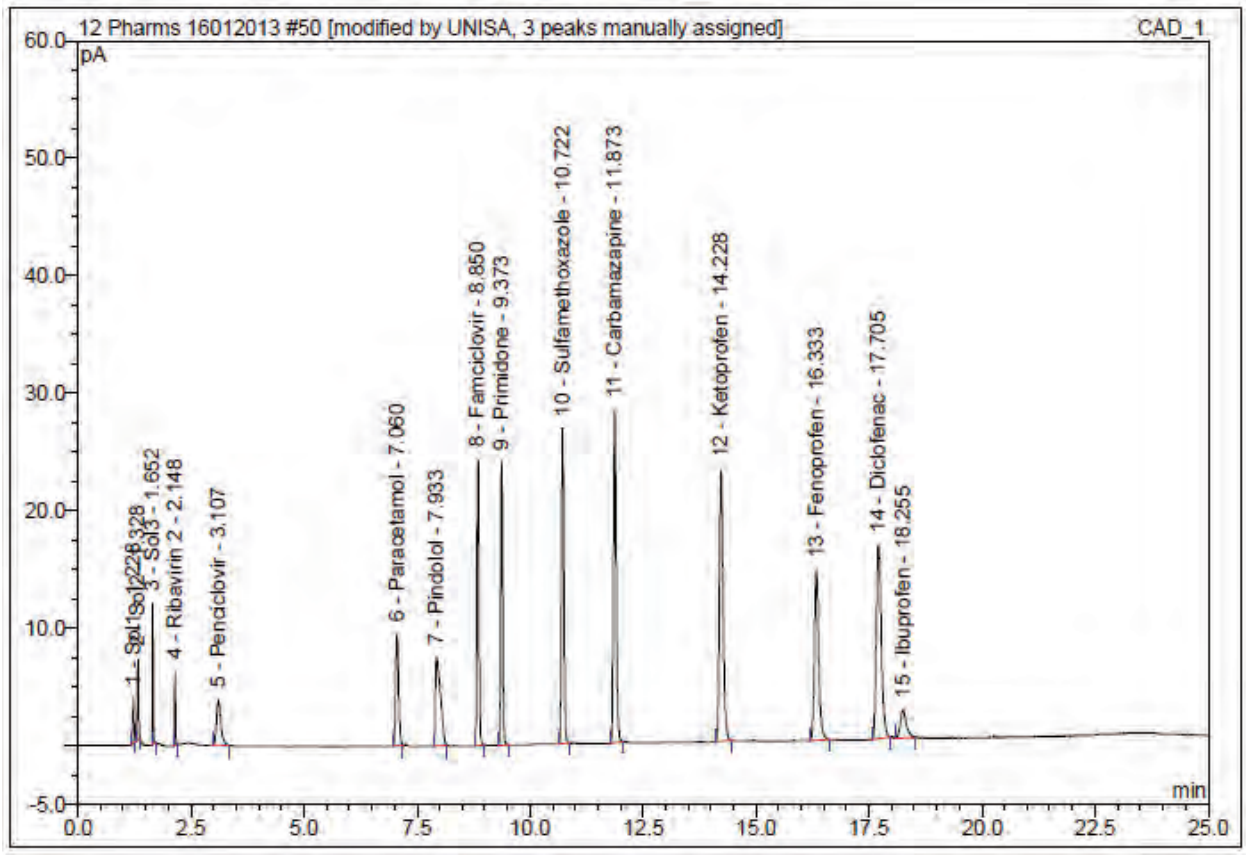


Figure 4-4: Chromatogram of 12 pharmaceutical compounds on a Zorbax C18 column. The compounds of interest are (4) Ribavirin, (5) Penciclovir, (6) Paracetamol, (7) Pindolol, (8) Famciclovir, (9) Primidone, (10) Sulfamethoxazole, (11) Carbamazepine, (12) Ketoprofen, (13) Fenoprofen, (14) Diclofenac, and (15) Ibuprofen. Peaks 1 to 3 are due to the solvent.

4.6.1.1 Linearity range

Calibration curves were constructed using calibration standards at ten concentration levels to cover a range of 0.1 to 75 ug mL^{-1}. Each concentration level was prepared in six replicates and each of the replicates was run three times. Table 4.3 shows the linearity range, regression equation and correlation of determination (r^2) for each analyte. Seven of the analysed drugs had a linearity range of 0.1-20 µg mL^{-1} with good linearity as indicated by the correlation of determination of 0.9964 to 0.9994. Ribavirin, famciclovir and pindolol had a linearity range of 0.1-10 µg mL^{-1}, 0.1-15 µg mL^{-1} and 0.1-40 µg mL^{-1} respectively. Ibuprofen gave a narrow linear range of 0.1-1.0 µg mL^{-1}. Generally all compounds gave acceptable linearity of 0.9958 and above. For a wider concentration range of 0.1 to 75 µg mL^{-1} it was noted that all analytes exhibited polynomial calibration curves which is expected of a mass detector such as the charged aerosol detector where the relationship of the signal and amount of the analyte is nonlinear (Vehovec and Obreza, 2010). Details of both linear and polynomial calibration curves may be found in Appendix A and a comparison of r^2 values is presented in Table 4.3.

Table 4-3: Coefficient of determination, LOD and LOQ values for 12 PPHCPs

Compounds	Linearity range (µg/mL)	Regression equation	Correlation of determination (R^2)	Standard error on estimation (Se)	SD of slope (Ss)	SD of intercept (Si)	LOD (µg/mL)	LOQ (µg/mL)
Ribavirin	0.1-10	0.0026x+0.0014	0.9958	0.00081	0.00010	0.00048	0.9518	3.1727
Penciclovir	0.1-20	0.0068x-7e-05	0.9990	0.00182	0.00010	0.00010	0.7996	2.6652
Paracetamol	0.1-20	0.0112x-0.0001	0.9994	0.00230	0.00012	0.00124	0.6161	2.0536
Pindolol	0.1-40	0.0191x-0.0079	0.9978	0.01299	0.00034	0.00617	2.0424	6.8082
Famciclovir	0.1-15	0.0233x+0.0043	0.9964	0.00950	0.00070	0.00532	1.2178	4.0593
Primidone	0.1-20	0.0226x+0.0077	0.9966	0.01130	0.00059	0.00607	1.5007	5.0022
Sulfamethoxazole	0.1-20	0.0313x+0.0067	0.9972	0.01420	0.00074	0.00763	1.3622	4.5408
Carbamazapine	0.1-20	0.0025x+0.0016	0.9986	0.01400	0.00073	0.00755	1.1597	3.8656
Ketoprofen	0.1-20	0.0397x+0.0006	0.9983	0.01420	0.00074	0.00763	1.0723	3.5743
Fenoprofen	0.1-20	0.0249x+0.0107	0.9966	0.01260	0.00066	0.00678	1.5187	5.0622
Diclofenac	0.1-20	0.0321x+0.0058	0.9986	0.01040	0.00054	0.00561	0.9739	3.2464
Ibuprofen	0.1-1	0.0028x+0.001	0.9991	0.00118	0.00184	0.00119	0.1083	0.3609

Table 4-4: Comparison of Linear and Polynomial Equation

Compounds	Conc. range (µg/mL)	Linear Equation	R^2	Polynomial Equation Conc. range (0.1 to 75µg/mL)	R^2
Ribavirin	0.1- 10	y = 0.0026x + 0.0014	0.9958	$y = 2411x^2 + 283.39x + 0.1494$	0.997
Penciclovir	0.1-20	y = 0.0068x - 7E-05	0.9990	$y = 165.8x^2 + 118.41x + 0.562$	0.998
Paracetamol	0.1-20	y = 0.0112x - 0.0001	0.9994	$y = 67.942x^2 + 74.555x + 0.3986$	0.998
Pindolol	0.1-40	y = 0.0191x - 0.0079	0.9978	$y = 60.651x^2 + 29.359x + 0.9995$	0.998
Famciclovir	0.1-15	y = 0.0233x + 0.0043	0.9964	$y = 30.293x^2 + 29.273x + 0.537$	0.998
Primidone	0.1-20	y = 0.0226x + 0.0077	0.9966	$y = 31.418x^2 + 28.893x + 0.4913$	0.998
Sulfamethoxazole	0.1-20	y = 0.0313x + 0.0067	0.9972	$y = 15.866x^2 + 21.542x + 0.5229$	0.998
Carbamazapine	0.1-20	y = 0.0025x + 0.0016	0.9986	$y = 9.3674x^2 + 20.413x + 0.3466$	0.999
Ketoprofen	0.1-20	y = 0.0397x + 0.0006	0.9983	$y = 6.9428x^2 + 19.067x + 0.6165$	0.997
Fenoprofen	0.1-20	y = 0.0249x + 0.0107	0.9966	$y = 10.286x^2 + 35.776x - 0.1039$	0.997
Diclofenac	0.1-1	y = 0.0321x + 0.0058	0.9986	$y = 8.5625x^2 + 23.599x + 0.554$	0.998
Ibuprofen		y = 0.0028x + 0.001	0.9991	$y = 650.72x^2 - 13.878x + 0.7445$	0.995

4.6.1.2 Limit of Detection (LOD) and Limit of Quantification (LOQ) of PPHCPs

Limits of detection (LOD) and quantification (LOQ) were estimated using regression statistical analysis according to ISO11843 method where both parameters are defined as follows:

$$LOD = \frac{3s_{y/x}}{b} \text{ and } LOQ = \frac{10s_{y/x}}{b}$$

Equation 1

where; $s_{y/x}$ is the standard error of regression and b is the slope of the curve. The limit of detection ranged from 0.11-2.04 µg mL^{-1} while the LOQ was between 0.36-6.81 µg mL^{-1}. For this method the LODs and LOQ were not as low as desirable for environmental samples. However, it is expected that incorporating a sample clean-up and pre-concentration step should lower the detection limits.

4.6.1.3 Accuracy

Accuracy was determined by calculating recoveries after spiking analytes into ultra-high purity water at three concentration levels (20, 40 and 60 µg mL^{-1}). Each concentration level was prepared in six replicates. Average recovery values are summarised in Table 4.6. Most PPHCPs in this study had good recoveries at the three concentrations which ranged from 84.8 to 129.7%. Ibuprofen however gave very low recoveries of 29.8 and 17.3% at 40 and 60 µg mL^{-1} respectively. Pindolol on the other hand gave exceptionally high recoveries of 134.7 and 162.1% at these concentrations.

The relative standard deviation of less than 15% for most analytes indicates good precision for the developed method. Exceptionally high RSD values of 22 .8 and 16.1% were observed for penciclovir and sulfamethoxazole respectively.

4.6.2 Application to real wastewater samples

The developed and validated method was then applied for the determination of PPHCPs in a real wastewater sample. The wastewater sample had been preconcentrated using SPE following the procedure described in section 4.5.1.2. As can be observed in Figure 4.5 a chromatogram of unspiked wastewater sample exhibited several peaks most of which could not be identified. Only six peaks with retention times corresponding to ribavirin, pindolol, famciclovir, carbamazapine, ketoprofen, fenoprofen and ibuprofen were identified. Ribavirin, an antiretroviral drug was detected in both influent (19.60 ng mL^{-1}) and effluent (0.042 ng mL^{-1}) wastewater. Famciclovir, also an antiretroviral drug, was also detected in both influent (ca. 19.00 ng mL^{-1}) and effluent (0.055 ng mL^{-1}) samples (Table 4.6). It should be noted that since CAD responds to a number of analytes we can only speculate that the analytes identified based on retention times matched analytes under investigation. A positive identification can only be confirmed using LC-MS and/or LC-MS/MS.

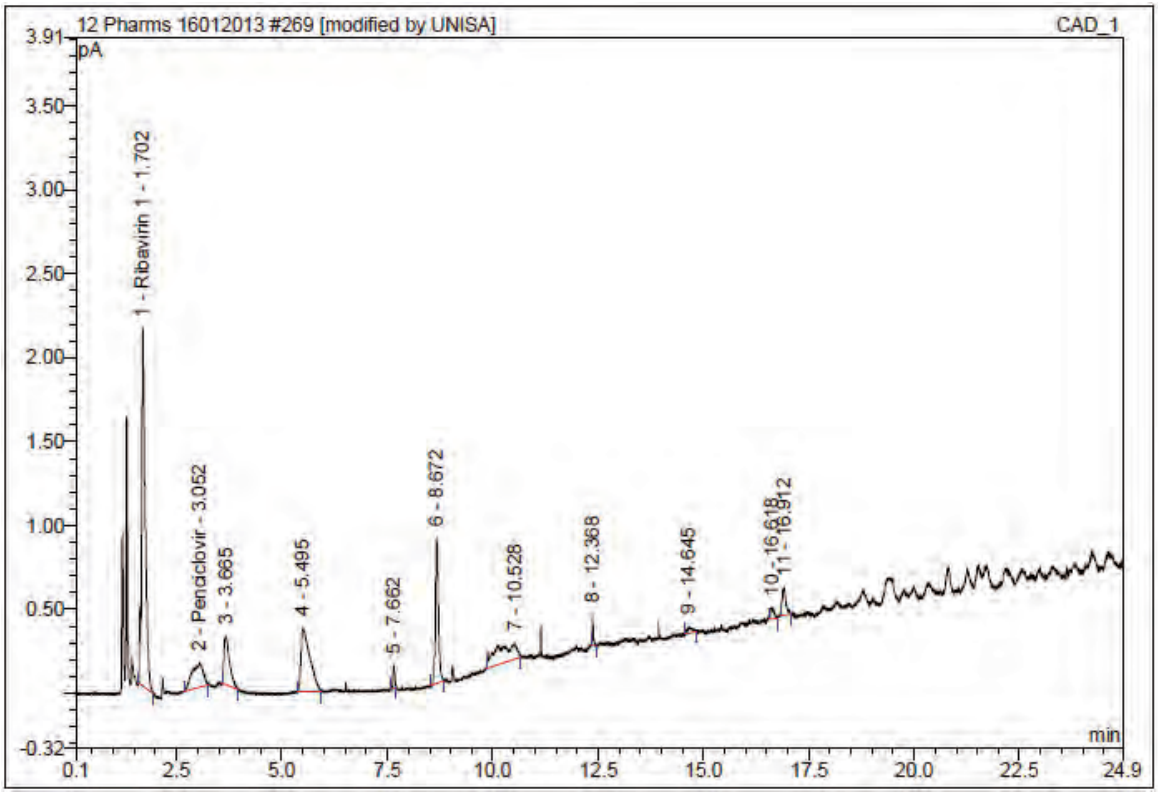


Figure 4-5: Chromatogram of of Influent wastewater by HPLC-CAD using conditions above

Table 4-5: Recoveries from spiked samples (n = 6)

Analyte	Concentration (ug/ml)	Experimental (ug/ml)	% Recovery	SD	% RSD
Ribavirin	20	22.93	114.6	3.114	13.6
	40	40.68	101.7	0.008	9.3
	60	67.39	112.3	0.005	4.3
Penciclovir	20	21.30	106.5	4.860	22.8
	40	39.23	98.1	0.033	11.9
	60	64.27	107.1	0.016	4.6
Paracetamol	20	20.66	103.3	1.605	7.8
	40	37.22	93.1	0.04	9.5
	60	59.31	98.8	0.034	6.4
Pindolol	20	19.48	97.4	9.820	-
	40	53.86	134.7	0.116	14.5
	60	97.28	162.1	0.067	6.4
Famciclovir	20	21.21	106.1	2.244	10.6
	40	46.63	116.6	0.086	10.3
	60	77.80	129.7	0.053	4.4
Primidone	20	20.45	102.3	1.785	8.7
	40	43.42	108.5	0.074	8.9
	60	69.53	115.9	0.051	4.7
Sulfamethoxazole	20	11.04	55.2	1.773	16.1
	40	44.76	111.9	0.099	8.7
	60	72.42	120.7	0.073	4.7
Carbamazapine	20	10.03	50.2	1.424	14.2
	40	43.12	107.8	0.128	9.5
	60	69.85	116.4	0.094	5.1
Ketoprofen	20	19.93	99.7	1.077	5.4
	40	40.52	101.3	0.161	10.6
	60	59.52	99.2	0.004	0.2
Fenoprofen	20	19.01	95.1	0.246	1.3
	40	33.93	84.8	0.087	9.1
	60	51.18	85.3	0.084	7.7
Diclofenac	20	19.58	97.9	0.781	4.0
	40	39.72	99.3	0.127	9.8
	60	64.20	107	0.113	6.7
Ibuprofen	20	19.18	95.9	2.611	13.6
	40	11.92	29.8	0.023	8.3
	60	10.36	17.3	0.011	7.9

Table 4-6: Detection of PPHCPs in influent wastewater by HPLC-CAD

Analyte	Influent # 1 (ng/mL)	Influent # 2 (ng/mL)	Efluent # 3 (ng/mL)
Ribavirin	ND	19.59	0.042
Penciclovir	ND	ND	ND
Paracetamol	ND	ND	ND
Pindolol	0.043	34.26	0.028
Famciclovir	0.071	19.00	0.056
Primidone	ND	ND	ND
Sulfamethoxazole	ND	ND	ND
Carbamazapine	0.081	12.39	ND
Ketoprofen	0.190	22.16	0.14
Fenoprofen	ND	ND	ND
Diclofenac	ND	ND	ND
Ibuprofen	0.181	22.8	ND

ND = Not detected

4.6.3 Validation for hormone methods

Figure 4.6 shows a typical separation chromatogram of a mixture of five steroidal hormones which included estradiol, β estradiol,17 α estradiol, testestorone and progesterone plus bisphenol A was separated on a Zorbax Eclipse XDB C8 column and detected on a charged aerosol detector. Details of the separation and detector conditions are listed in Table 4.4 above. These results show that CAD is capable of detecting steroid hormones and could thus be employed for the determination of this group of compounds in environmental samples.

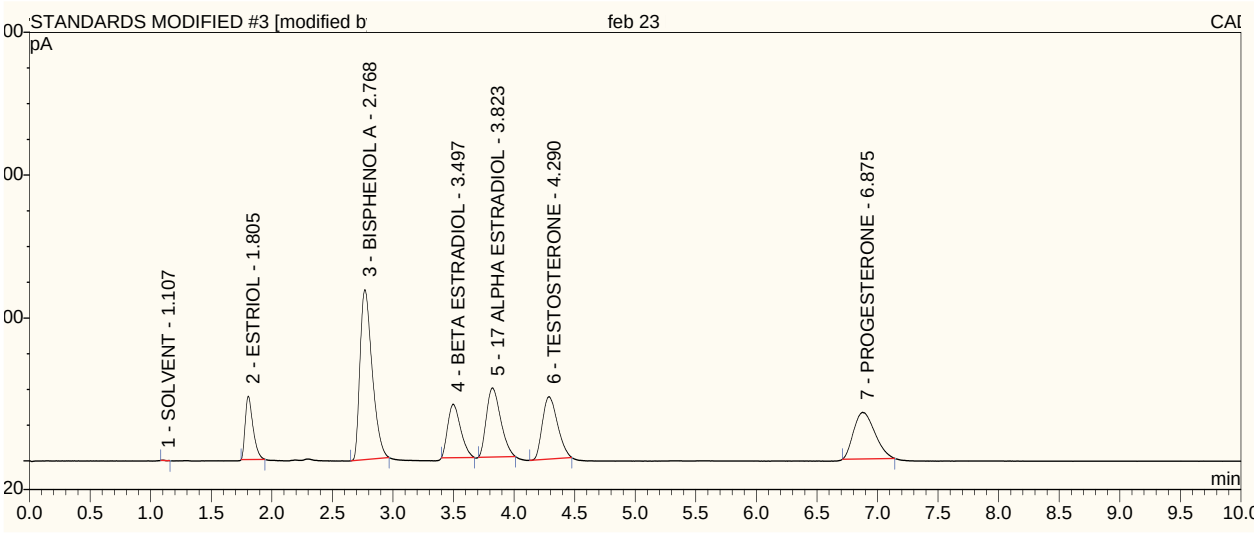


Figure 4-6: Chromatogram of five steroid hormones separated on Zorbax Eclipse XDB C8 using conditions given in section 4.5.2.1

4.6.3.1 Linearity range

The calibration curves were constructed with eight concentrations to cover 0.3 to 100 µg mL^{-1} and each level had six replicates which were run three times. Table 4.8 shows good linearity for all analytes in the range of 0.3 to 50 µg mL^{-1} as indicated by the coefficient of determination which was between 0.9978 and 0.9991. The relative standard deviation (1.1 to 1.7%) also indicates good precision for the developed method. Polynomial calibrations were observed for wider concentration range of 0.3 to 100 µg mL^{-1} for reasons already discussed in section 4.6.1.1. Linear and polynomial calibration curves are attached as Appendix B.

4.6.3.2 Limit of detection and limit of quantification of steroid hormones

Limit of detection and limit of determination values were calculated as given in Equation 1 using data from six replicates. LOD values ranged from 0.26 to 1.4 µg mL^{-1} and LOQs were between 0.85 and 4.5 µg mL^{-1} as shown in Table 4.8. Obviously the sensitivity of the method in the absence of a pre-concentration step is not attractive for environmental samples where the analytes are usually reported to be detected in ng L^{-1} levels. However we believe that the incorporation of an appropriate pre-concentration procedure should improve the sensitivity of this method.

4.6.3.3 Accuracy

The method was evaluated for accuracy by spiking 5, 10 and 25 µg mL^{-1} standard solutions into MilliQ, tap and wastewater and determining the percentage recovery as an average of six replicates. Table 4.9 shows data from two concentration levels (5 and 10 µg mL^{-1}) in ultra-high purity (UHP) water, drinking and wastewater. The recoveries from UHP water ranged from 81.4-101%; 85.5-101% and 95.1-106% for 5 µg mL^{-1}, 10 µg mL^{-1} and 25 µg mL^{-1} respectively. In drinking water the recoveries ranged from 83.9-104%; 95.2-116% and 101-110% for 5, 10 and 25 µg mL^{-1} respectively. The recoveries of 5 compounds at 5 µg mL^{-1} from the three difference water systems was acceptable at %RSD of less than 15% with the exception of progesterone which was found to be greater than 22%. At higher concentration levels (10 and 25 µg mL^{-1} the recoveries were at acceptable levels, i.e. ≤ 15% (Appendix C).

4.6.3.4 Precision

Intra- and inter-day precision were evaluated over a period of five days by using a 25 µgmL^{-1} standard prepared in five replicates. For the intra-day precision the replicate standards were injected ten times and the average peak areas and relative standard deviations were calculated. Table 4.10 shows good intra-day precision as illustrated by the %RSD for the five hormones ranging from 1.69 to4.18%. Inter-day precision results also indicated good method precision with all data falling within acceptable %RSD of 5% or less.

Table 4-7: Coefficient of determination, LOD and LOQ values for hormones

Compounds	Linearity range (µg/mL) n = 6	Regression equation	Correlation of determination (R^2) n = 6	Standard error on estimation (Se) n = 6	SD of slope (Ss) n = 6	SD of intercept (Si) n = 6	LOD (µg/mL) n = 6	LOQ (µg/mL) n = 6
Estriol	0.3-50	y = 0.0207x + 0.0223	0.9991	0.011	0.00025	0.0050	0.8485	2.8284
Bisphenol A	0.3-50	y = 0.0226x + 0.0206	0.9986	0.015	0.00031	0.0063	0.8485	2.8284
β Estradiol	0.3-50	y = 0.0241x + 0.002	0.9986	0.017	0.00037	0.0075	0.5109	1.7032
α Estradiol	0.3-50	y = 0.0197x + 0.022	0.9978	0.017	0.00038	0.0076	0.2541	0.8468
Testosterone	0.3-50	y = 0.0209x – 0.0055	0.9994	0.010	0.0022	0.0044	1.3626	4.521
Progesterone	0.3-50	y = 0.016x + 0.0012	0.9979	0.014	0.00030	0.0061	0.9630	4.2101

Table 4-8: Accuracy of HPLC-CAD method as determined by recovery studies from UHP water, drinking water and wastewater

Compounds	UHP water (5 µg/mL) Mean Recovery % n = 5	Tap water (5µg/mL) Mean Recovery % n = 5	Wastewater (5 µg/mL) Mean Recovery % n = 5	% RSD n = 5	UHP water (10 µg/mL) Mean Recovery % n = 5	Tap water (10 µg/mL) Mean Recovery %, n = 5	Wastewater (10 µg/mL) Mean Recovery %, n = 5	% RSD n = 5
Estriol	81.4	83.9	88.7	4.1	100	116	98.2	5.1
Bisphenol A	95.2	99.0	101.4	4.2	101.2	112	94.1	8.3
β Estradiol	96.4	100.2	99.9	9.3	108	107.2	95.2	7.1
α Estradiol	96.8	99.1	99.7	5.5	85.5	98	92.4	7.1
Testosterone	85.0	88.2	90.1	5.7	98.5	95.2	98.4	6.1
Progesterone	101.0	104.1	110.1	22.4	98.5	101	110.2	12.1

4.6.4 Application of HPLC-CAD for determination of hormones in real wastewater samples

Only a qualitative study of hormones in wastewater was carried out. Both influent and effluent wastewater samples that had been pre-concentrated using OASIS HLB SPE cartridges were analysed using the developed and validated HPLC-CAD method. No peaks were detected in the effluent sample. However, the

influent sample yielded several peaks that we could not identify (data not included). Further work is necessary to confirm the presence of the hormones in wastewater using HPLC-CAD.

4.7 CONCLUSIONS

Two HPLC-CAD methods were successfully developed and validated for the determination of PPHCPs and hormones in water. Preliminary screening of samples from Daspoort WWTW in Pretoria showed the presence of ribavirin, pindolol, famciclovir, carbamazapine, ketoprofen, fenoprofen and ibuprofen mainly in influent samples. Ribavirin was detected in both influent (19.60 ng mL^{-1}) and effluent (0.042 ng mL^{-1}) wastewater. Famciclovir, an antiretroviral drug, was also detected in both influent (ca. 19.00 ng/mL) and effluent (0.055 ng mL^{-1}) samples. HPLC-CAD method of hormones requires further work.

LC-MS and LC-MS/MS methods are attractive because of their ability to do multi-residue analysis. However they are expensive and are limited only to a few laboratories in the country. GC-MS methods have also been used widely for PPHCPs that fall within the SA most precribed list. However they are time consuming due to the inherent derivatisation step. HPLC-CAD methods that we have developed for steroid hormones and 12 pharmaceuticals (hypertension, ARVs, antibiotics, analgesics and anticonvulsants) show potential to be used in monitoring of the target compounds. Although the detection limits are presently reported in mg L^{-1} levels, incorpoaration of sample preparation and/or preconcentration is expected to bring the concentration levels to µg L^{-1}. This part of the work is still on going.

CHAPTER 5: CONCLUSIONS & RECOMMENDATIONS

5.1 CONCLUSIONS

- A list of most prescribed drugs relevant to South Africa was developed based on data from the public and private health sector. Though the list was drawn primarily from the prescription volumes of the specific drugs.

- The list of most prescribed drugs falls within six classes which are hypertension, analgesics, antiretroviral, antibiotics, vitamins and antidiabetic drugs. They include the following; hydrochlorothiazide, paracetamol, enalapril maleate & hydrochlorothiazide, methyl salicylate, metformin hydrochloride, amoxycillin, paracetamol in combination with codeine and caffeine, diphenhydramine in combination with theophyline and etofyline, chlorpheniramine maleate, diphenhydramine, diclofenac sodium, aspirin in combination with chlorprophenpyridamine, phenylpropanolamine and chlorpheniramine in combination with phenyltoloxamin.

- Two methods were developed and validated for the determination of pharmaceuticals and hormones.

- Sampling of Pretoria tap drinking water and wastewater from Daspoort Wastewater Treatment Plant was carried out and the developed and validated. HPLC-CAD methods were used.

- Preliminary screening of samples from Daspoort WWTP in Pretoria showed the presence of ribavirin, pindolol, famciclovir, carbamazapine, ketoprofen, fenoprofen and ibuprofen mainly in influent samples. Ribavirin, an antiretroviral drug was detected in both influent (19.60 ng/mL) and effluent (0.042 ng/mL) wastewater. Famciclovir, also an antiretroviral drug, was also detected in both influent (ca. 19.00 ng/mL) and effluent (0.055 ng/mL) samples.

- The developed HPLC-CAD method for hormones will need to be further investigated when used together with an optimised sample preparation method in order to reduce the detection limits to levels that are relevant for wastewater analysis. Not suitable for drinking water.

- Overall the HPLC-Charged Aerosol Detector (CAD) demonstrated the potential as a cheaper alternative analytical method for the determination of PPHCPs in wastewater.

5.2 RECOMMENDATIONS

- The preliminary detection of some pharmaceutical in one of South Africa's wastewater warrants a need to expand the project to include a systematic screening of WWTPs for the top 20 PPHCPs.

- Having demonstrated the presence of a range of PPHCPs in South African wastewaters it is recommended that there be further research to fully understand their possible impacts

- This study did not cover all the sampling and sample-preparation approaches due to limited time and resources. There is a need to expand the sample preparation and pre-concentration to bring the analytes within the detection level of the HPLC-CAD.

- A national survey of WWTPs in wastewaters should be considered using HPLC-CAD, LC-MS/MS and/or GC-MS.

- There is a need to strengthen the capacity of WWTPs laboratories. This would allow the laboratories to be used in the proposed national survey.

REFERENCES

Al-Odaini NA, Zakaria MP, Yaziz MI, Surif S (2010). Multi-residue analytical method for human pharmaceuticals and synthetic hormones in river water and sewage effluents by solid-phase extraction and liquid chromatography-tandem mass spectrometry. Journal of Chromatography A, 6791-6806.

Alder AC, Schaffner C, Majewsky M, Klasmeier, Fenner K (2010). Fate of beta-blocker human pharmaceuticals in surface water: Comparison of measured and simulated concentrations in the Glatt Valley Watershed, Switzerland. Water Research (44) 936-948.

Andreozzi R, Raffaele M and Nicklas P. (2003). Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. Chemosphere, 50(10), 1319-1330.

Ashoton D, Hilton M, Thomas KV (2004). Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. Science of the Total Environment, 333:167-184.

Beausse J (2004). Selected drugs in solid matrix: a review of environmental determination, occurrences and properties of principles substances. Trac-Trends in Analytical Chemistry, 23,753-761.

Behera SK, Kim HW, Oh JE, Park HS (2011). Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea. Science of the Total Environment, 409, 4351-4360.

Boreen A, Arnold W, and McNeill K (2003). Photodegradation of pharmaceuticals in the aquatic environment: A review RID A-9834-2009. Aquatic Sciences, 65(4), 320-341.

Benotti MJ, Trenholm, RA, Vanderford BJ, Holady JC Stanford BD and Synder SA (2009). Pharmaceuticals and Endocrine Disrupting Compounds in US Drinking Water Environmental Science & Technology, 43:,597-603.

Baronti C, Curini R, D'Ascenzo G, Di Corcia A, Gentili A, Samperi (2000). Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. Environmental Science & Technology, 34, 5059-5066.

Beck IC, Bruhn R, Gandrass, Ruck W (2005). Liquid chromatography-tandem mass spectrometry analysis of estrogenic compounds in coastal surface water of the Baltic Sea. J. Chromatography A 1090, 98-106.

Benijts T, Lambert W, De Leenheer A (2004). Analysis of multiple endocrine disruptors in environmental waters via wide-spectrum solid-phase extraction and dual-polarity ionization LC-ion trap-MS/MS. Analytical Chemistry 76, 704-711.

Buchberger WW (2007). Novel analytical procedures for screening of drug residues in water, waste water, sediment and sludge, Analytica Chimica Acta, 593, 129-139.

Buser, H, Poiger, T., & Muller, M. (1998). Occurrence and fate of the pharmaceutical drug diclofenac in surface waters: Rapid photodegradation in a lake. Environmental Science & Technology, 32(22), 3449-3456.

Carballa M, Omil F, Lema JM, Llompart M, Garcia-Jares C, Rodriguez I, Gomez M, Ternes T (2004). Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. Water Research, 38, 2918-2926.

Christensen FM (1998). Pharmaceutical in the environment – a human risk? Regulatory Toxicology and Pharmacology, 28, 212-221.

Crane M, Watts C and Boucard T (2006). Chronic aquatic environmental risks from exposure to human pharmaceuticals Science of the Total Environment, 367:23-41.

Daughton C and Ternes T (1999). Pharmaceutical and personal care products in the environments: Agents of subtle change. Environmental Health Perspectives, 107, 907-938.

Daughton CG (2003). Environmental Stewardship of Pharmaceuticals: The Green Pharmacy; In: Proceedings of the 3rd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, National Ground Water Association, 19-21 March, Minneapolis, Minnesota, USA

D'Ascenzo G, Di Corcia A, Gentili A, Mancini R, Mastropasqua R, Nazzari M, Samperi R (2003). Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. Science of Total Environment, 302, 199-209.

Dixon RW and Peterson DS (2002). Development and testing of a detection method for liquid chromatography based on aerosol charging. Analytical Chemistry, 74:2930-2937.

Erickson BE (2002). Analyzing the ignored environmental. Environmental Science & Technology, 36: 140A-145A.

Fent K, Weston AA and Caminada D (2006). Ecotoxicology of human pharmaceuticals. Aquatic Toxicology, 76, 122-159.

Foodstuffs, Cosmetics and Disinfectants Act, 1972, (Act 54 of 1972).

Fertilizers, Farm Feeds, Agriculture Remedies and Stock Remedies Act, 1947 (Act 36 of 1947).

Filby AL. Neuparth T, Thorpe KL, Owen R, Galloway TS Tyler CR (2007). Health impacts of estrogens in the environment, considering complex mixture effects. Environmental Health Perspectives. 115, 12, 1704-1710.

Gadd JB, Northcott GI, Tremblay LA (2010). Passive secondary biological treatment systems reduce estrogens in dairy shed effluent. Environmental Science & Technology, 44, 7601-7606.

Garrison AW, Pope JD, Allen FR (1976). GC-MS analysis of organic compounds in domestic wastewaters. In Keith, CH (Ed.), Identification and Analysis of Organic Pollutants in Water. Ann Arbor Science Publishers, Ann Arbor, pp 517-556 Chapter 30.

Gentili A, Perret D, Marchese S, Perret D, Mastropasqua R, Curini R, Di Corcia A, (2002). Analysis of free estrogens and their conjugates in sewage and river waters by solid-phase extraction then liquid chromatography-electrospray-tandem mass spectrometry. Chromatographia, 56, 25-32.

Gentili A, Marchese S, Perret D (2008). MS techniques for analyzing phenols, their metabolites and transformation products of environmental interest. Trends in Analytical Chemistry 27, 888-902.

Golet EM, Xifra I, Siegrist H, Alder AC, Giger W (2003). Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. Environmental Science & Technology, 37, 3243-3249.

Golet EM, Alder AC, Giger W (2002). Environmental exposure and risk assessment of fluoroquinolone antibacterial agents in wastewater and river water of the Glatt Valley Watershed, Switzerland. Environmental Science & Technology, 36 (17), 3645-3651.

Gómez MJ, Herrera S, Solé D, García-Calvo E and Fernández-Alba AR (2011), Automatic searching and evaluation of Priority and emerging contaminants in wastewater and river water by stir bar sorption extraction followed by comprehensive two-dimensional gas chromatography-time of flight mass spectrometry. Analytical Chemistry, 83, 2638-2647.

Gros M, Petrović M, Ginebreda A, Baeceló (2010). Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environment International, 36, 15-26.

Halling-Sorensen B, Nielsen SN, Lanzky, Ingerslev F, Holten Lützhoft HC, Jorgensen SE (1998). Occurrence, Fate and Effects of Pharmaceutical Substances in the Environment – A Review. *Chemosphere,* 36(2), 357-393.

Janex-Habibi ML, Huyard A, Esperanza M, Bruchet A (2009). Reduction of endocrine disruptor emission in the environment: the benefit of wastewater treatment. Water Research, 43(6), 1565-1576.

Al-Rifai JH, Gabelish CL, Schäfer AI, Occurence of pharmaceutically active and non-steroidal estrogenic compounds in three different wastewater recycling schemes in Australia. Chemosphere, 69, 803-815.

Kasprzyk-Hordern B, Dinsdale RM and Guwy AJ, (2008). The effect of signal suppression and mobile composition on the simultaneous analysis of multiple classes of acidic/neutral pharmaceutical and personal care products in surface water by solid-phase extraction and ultra performance liquid chromatography-negative electrostray tendem mass spectrometry. Talanta, 74, 1299-1312.

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. Environmental Science & Technology, 36 (6), 1202-1211

Koo SH, Jo CH, Shin SK, Myung SW (2010). Simultaneous Determination and Occurrences of Pharmaceuticals by Solid-Phase Extraction and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) in Environmental Aqueous Samples, Bull Korean Chemical Society, 31, 1192-1198.

Koutsouba V, Heberer Th Fuhrmann B, Schmidt-Baumler K, Tsipi D, Hiskia A (2003). Determination of polar pharmaceuticals in sewage water of Greece by gas chromatography – mass spectrometry. Chemosphere 51, 69-75.

Lagana A, Bacaloni A, De Leva I, Faberi A, Marino A (2004). Analytical methodologies for determining the occurrence of endocrine disrupting chemicals in sewage treatment plants and natural waters high-performance liquid chromatography for determination of estrogens in environment. Analytica Acta, 501, 79-88.

Lam M. and Mabury S. (2005). Photodegradation of the pharmaceuticals atorvastatin, carbamazepine, levofloxacin, and sulfamethoxazole in natural waters. Aquatic Sciences, 67(2), 177-188.

Lee HB, Peart TE, Svoboda ML (2005). Determination of endocrine-disrupting phenols, acidic pharmaceuticals, and personal-care products in sewage by solid-phase extraction and gas chromatography – mass spectrometry, Journal of Chromatography A, 1094, 122-129.

Lee, Hing-Biu, Sarafin K, Peart, TE (2007). Determination of beta-blockers and beta(2)-agonists in sewage by solid-phase extraction and liquid chromatography-tandem mass spectrometry. J. Chromatography A 1148, 158-167.

Le-Minh N, Khan SJ, Drewes JE, Stuetz RM (2010). Fate of antibiotics during municipal water recycling treatment processes. Water Research 44, 4295-4323.

Li AYu-C and Tsai Yu-T, (2009). Occurrence of pharmaceutical in Taiwan's surface waters: Impact of waste streams from hospitals and pharmaceutical production facilities, Science of Total Environment 407, 3793-3802

Li X, Zheng W, Kelly WR (2013). Occurrence and removal of pharmaceutical and hormones contaminants in rural wastewater laggons. Science of Total Environment, 445, 22-28.

Lin WC, Chen HC, Ding WH (2005). Determination of pharmaceutical residues in waters by solid-phase extraction and large-volume on-line derivatization with gas chromatography – mass spectrometry, Journal of Chromatography A, 1065, 279-285

Lindberg RH, Wennberg P, Johansson MI, Tysklind M, Andersson, BAV (2005). Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. Environmental Science & Technology, 39, 3421-3429.

Lishman L, Smyth SA, (Sarafin, K; Kleywegt, S, Toito, J, Peart, T, Lee, B, (Servos, M, Beland, M, Seto, P (2006). Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada. Science of the Total Environment, 367, 544-558.

Marco-Urrea E, Perez-Trujilo M, Vicent T and Caminal G (2008). Ability of white-rot fungi to remove selected pharmaceutical and identification of degradation products of ibuprofen by trametes versicolor. Chemosphere 74, 765-772.

Medicine and Related Substances Act, 1965 (Act 101 of 1965).

Miao XS, Bishay F, Chen M, Metcalfe CD (2004). Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada. Environmental Science & Technology, 38, 3533-3541.

Moeder M, Schrader S, Winkler M, Popp P (2000). Solid-phase microextraction-gas chromatography-mass spectrometry of biologically active substances in water samples, Journal of Chromatography A, 873, 95-106.

Mottaleb MA, Usenko S, O'Donnell JG, Ramirez AJ, Brooks BW and Chambliss CK, (2009). Gas chromatography-mass spectrometry screening methods for selected UV-filters, synthetic musks, alkylphenols, an antimicrobial agents and an insect repellent in fish, J Chromatogrraphy A, 1216, 815-823.

Monthly Index of Medical Specialities, Volume 42 (1), March 2002.

Monthly Index of Medical Specialities, Volume 50 (3), March 2010.

Nikolaou A, Meric S and Fatta D (2007). Occurrence patterns of pharmaceutical in water and waste water. Analytical and Bioanalytical Chemistry 387, 1225-1234. Journal of Chromatography A, 109, 122-129.

Noppe H, Verslycke T, De Wulf E, Verheyden K, Monteyne E, Van Caeter P, Peter J, Janssen C, De Brabander H (2007). Occurrence of estrogens in the Scheldt estuary: A 2-year survey. Ecotoxicology Environmental Safety 66, 1-8.

Packer J., Werner J., Latch, D McNeill, K and Arnold, W. (2003). Photochemical fate of pharmaceuticals in the environment: Naproxen, diclofenac, clofibric acid, and ibuprofen RID A-9834-2009. Aquatic Sciences, 65(4), 342-351.

Paxeus N (2004). Removal of selected non-steroidal anti-inflammatory drugs (NSAIDs), gemfibrozil, carbamazepine, beta-blockers, trimethoprim and triclosan in conventional wastewater treatment plants in five EU countries and their discharge to the aquatic environment. Water Science and Technology 50, 253-260.

Peng, X, Yu YJ, Tang CM,(Tan, JH,; Huang QH Wang ZD (2008). Occurrence of steroid estrogens, endocrine-disrupting phenols, and acid pharmaceutical residues in urban riverine water of the Pearl River Delta, South China, Science of The Total Environment, 397, 158-166 .

Petrovic M, Hernando MD, Diaz-Cruz MS, Barcelo D. Liquid chromatography-tandem mass spectrometry for the analysis of pharmaceutical residue in environmental samples: a review. J. Chromatography A 1067:1-14.

Ramirez AJ, Mottaleb MA, Brooks BW, Usenko S, Chambliss CK, (2007). Analysis of pharmaceutical in fish using liquid chromatography-tandem mass spectrometry. Analytical Chemistry 79:3155-3163.

Roberts PH and Thomas KV (2006). The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Chemosphere 49, 539-153.

Rodgers-Gray TP, Jobling S, Morris S, Kelly C, Kirby S, Janbakhsh A, Harris JE, Waldock JP, Sumpter JP, Tyler CR (2002). Long term temporal effects on fish. Environmental Science & Technology, 34, 1521-1528.

Rodr´ıguez I, Quintana JB, Carpinteiro J, Carro AM, Lorenzo RA, Cela R, (2003). Determination of acidic drugs in sewage water by gas chromatography-mass spectrometry as *tert*.-butyldimethylsilyl derivatives. Journal of Chromatography A, 985,265-274.

Rodriquez-Mozaz S, de Alda, MJL); Barcelo, D (2004). Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction-liquid chromatography-mass spectrometry. J. of Chromatography A 1045 (1-2), 85-92.

Sacher F, Lang F, Brauch H and Blankenhorn I (2001). Pharmaceutical in groundwaters-analytical methods of monitoring program in Baden-Wurttenberg, Germany. J. chromatography A 938, 199-201.

Scheurell M, Franke S, Shah RM, Huehnerfuss H (2009) Occurrence of diclofenac and its metabolites in surface water and effluent samples from Karachi, Pakistan, Chemosphere 77, 870-876.

Singer AC, Nunn MA, Gould EA and Johnson AC (2007). Potential risks associated with the proposed widespread use of Tamiflu. Environmental Health Perspectives.115 (1), 102-106.

Snyder SA, Trenholm RA, Pleus RC, Bruce GM, Snyder EM and Hemming JCD (2008). Toxicological Relevance of EDCs and Pharmaceuticals in Drinking Water, Awwa Research Foundation and WaterReuse Foundation: Denver, CO, p443.

Spongberg AL and Witter JD (2008). Pharmaceutical compounds in wastewater process stream in Northwest Ohio. Science of the Total Environment 397, 148-157.

Tabak HH and Bunch RL (1970). Steroid hormines as water pollutants. I Metabolism of natural and synthetic ovulation-inhibing hormones by microorganism of activated sludge and primary settled sewage. Development in Industrial Microbiology, 11, 367-376.

Tan B, Hawker D, Muller J, Tremblay, Chapman H (2008). Stir bar sorptive extraction and trace analysis of selected endocrine disruptors in water, biosolids and sludge samples by thermal desorption with gas chromatography-mass spectrometry. Water Research 42, 404-412.

Ternes TA (1998). Water Research 32, 3245-3260.

Ternes TA, Kreckel P and Mueller J (1999). Behaviour and occurrence of estrogens in municipal sewage treatment plants – II. Aerobic batch experiments with activated sludge. Science of the Total Environment. 228, 91-99.

Ternes TA, Hirsch R, Mueller, J, Haberer (2001). Methods for the determination of neutral drugs as well as beta-blockers and beta(2)-sympathomimetics in aqueous matrices using GC/MS and LC/MS/MS. Fresenius Journal of Analytical Chemistry, 362 (3), 329-340.

Togola A and Budzinski (2008). Multi-residue analysis of pharmaceutical compounds in aqueous samples, Journal of Chromatography A, 1177, 150-158.

Vehovec T and Obreza A (2010). Review on operating and application of the charged aerosol detector. Journal of Chromatography A, 1217, 1549-1556.

Vieno, N, Tuhkanen, T, and Kronberg, L. (2005). Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. Environmental Science & Technology, 39(21), 8220-8226.

Vieno N, Tuhkanen T, Kronberg L, (2007). Elimination of pharmaceuticals in sewage treatment plants in Finland. Water Research, 41, 1001-1012.

Vulliet E, Wiest L, Baudot R, Grenier-Loustalot MF (2008). Multi-residue analysis of steroids at sub-ng/L levels in surface and ground-waters using liquid chromatography coupled to tandem mass spectrometry. Journal of Chromatography A, 1210, 84-91.

Wang S, Huang W, Fang G, He J, Zhang Y 2008. On-line coupling of solid-phase extraction to high-performance liquid chromatography for determination of estrogens in environment. Analytica Chimica Acta, 606, 194-201.

Watkinson AJ, Murby EJ, Costanzo SD (2007). Removal of antibiotics in conventional and advanced wastewater treatment: implication for environmental discharge and wastewater recycling. Water research, 41 (18) 4164-4176.

WHO (2011). Pharmaceuticals in Drinking Water. World Health Organisation.

Xu J, Wu L, Chen W, Chang AC (2008), Simultaneous determination of pharmaceuticals, endocrine disrupting compounds and hormone in soils by gas chromatography-mass spectrometry. Journal of Chromatography A, 1202, 189-195

Yang X, Flowers RC, Weinberg HS and Singer PC, (2011). Occurrence and removal of pharmaceutical and personal care products (PPCPs) in an advanced wastewater reclamation plant. Water Research 4545, 5218-5228.

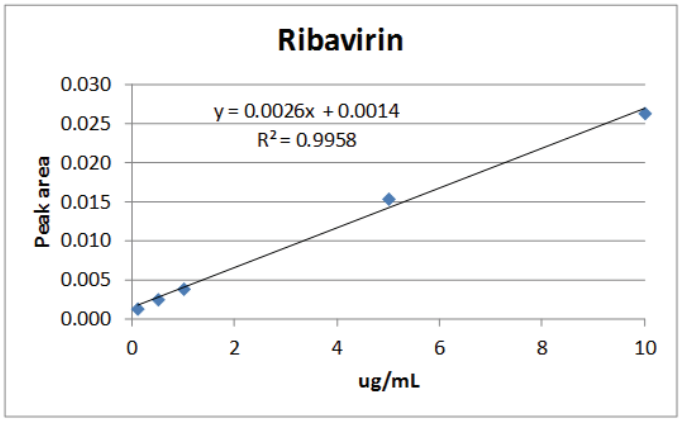
Yang YY, Pereyra LP, Young RB, Reardon KF, Borch T (2011). Testosterone-mineralizing culture enriched from swine manure: characterization of degradation pathways and microbial community composition. Environmental Science & Technology, 45, 6879-6879.

Zhang Y and Zhou JL (2008). Occurrence and removal of endocrine disrupting chemicals in wastewater. Chemosphere 73, 848-853.

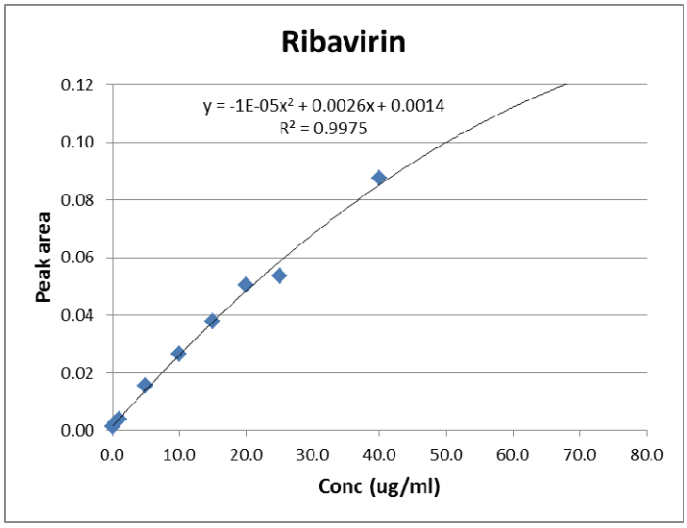
Ying GG, Kookana RS, Ru YJ (2002). Occurrence and fate of hormones steroids in the environment. Environmental International 28, 545-551.

Zuccato E, Castiglioni S, Fanelli 5 (2005). Identification of the pharmaceuticals for human use contaminating the Italian aquatic environment. Journal of Hazardous Materials, 122 (3), 205-209.

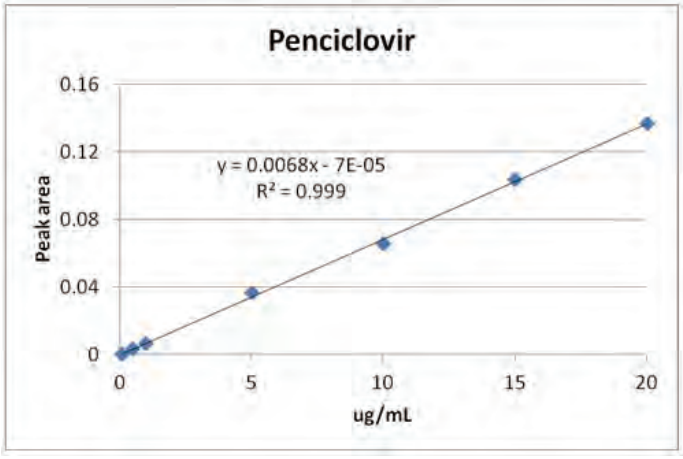
APPENDIX A: LINEAR AND POLYNOMIAL CALIBRATION CURVES



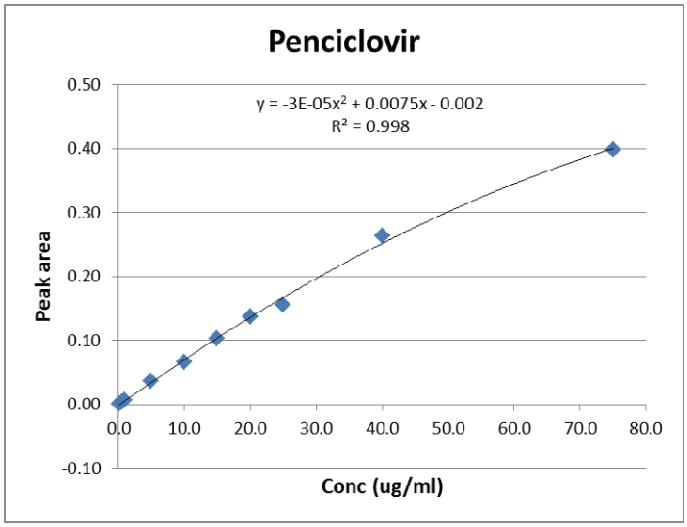
A1: Linear range of ribavirin, 0.1-10 ug/mL



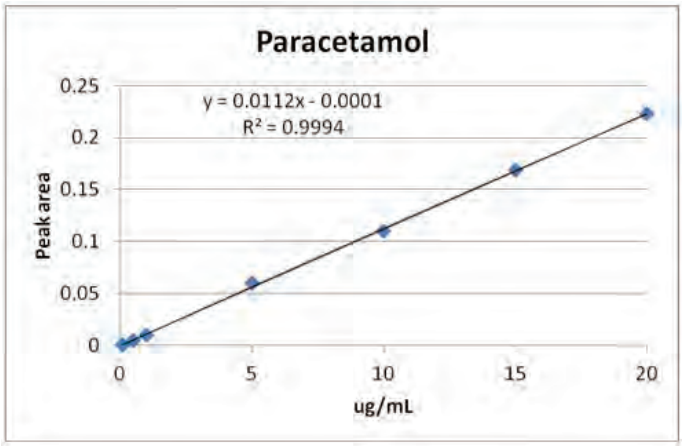
A2: Polynomial range from 0.1 to 75 ug/mL of Ribavirin



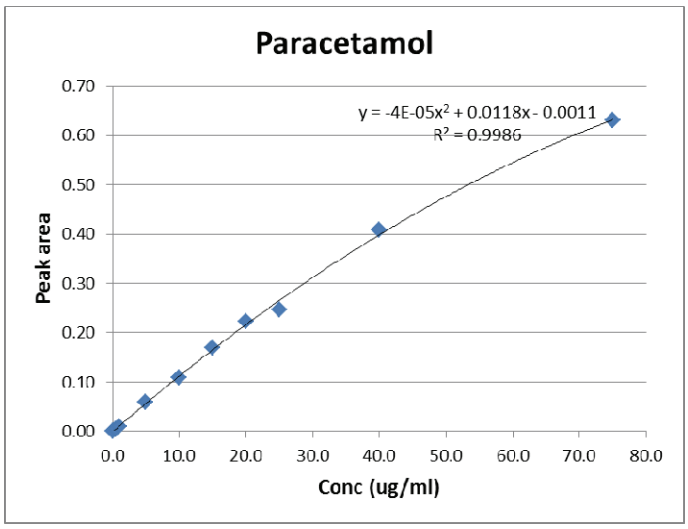
A3 Linear range of Penciclovir, 0.1-20 ug/



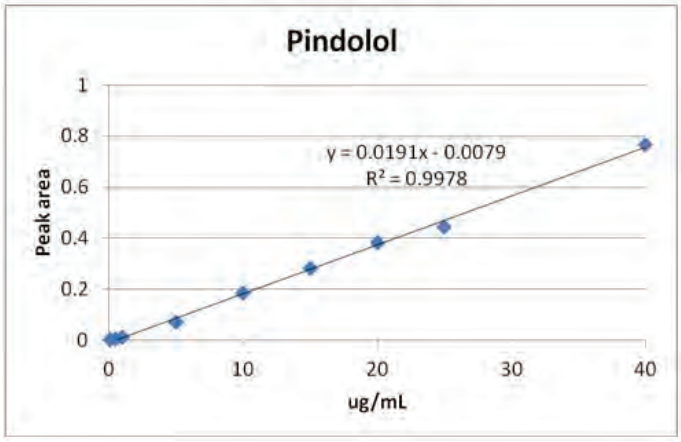
A4: Polynomial range from 0.1 to 75 ug/mL of Penciclovir



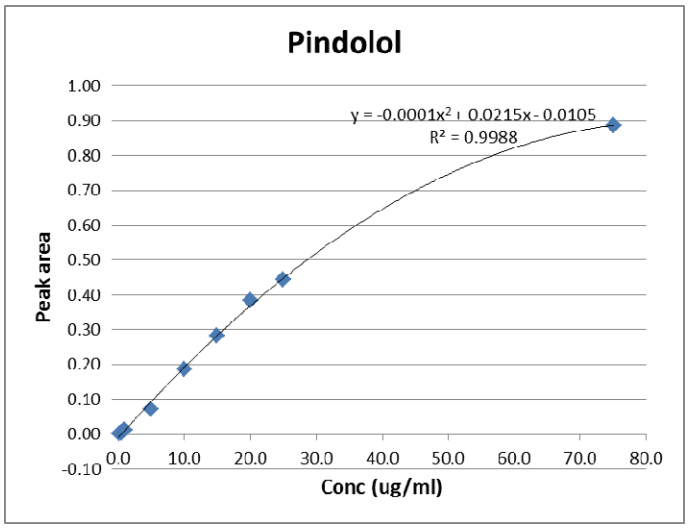
A5 Linear range of paracetamol, 0.1-20 ug/mL



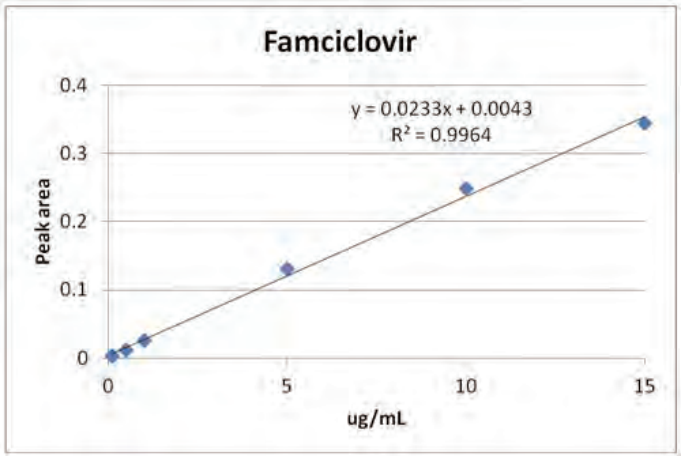
A6: Polynomial range from 0.1 to 75 ug/mL of Paracetamol



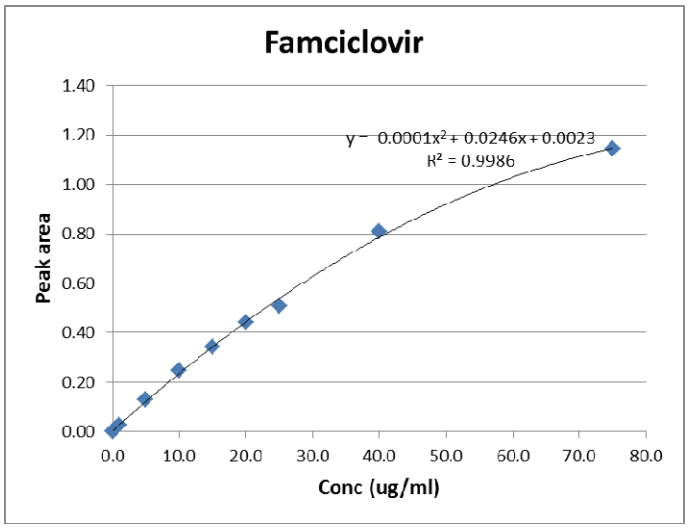
A7 Linear range of pindolol, 0.1-40 ug/Ml



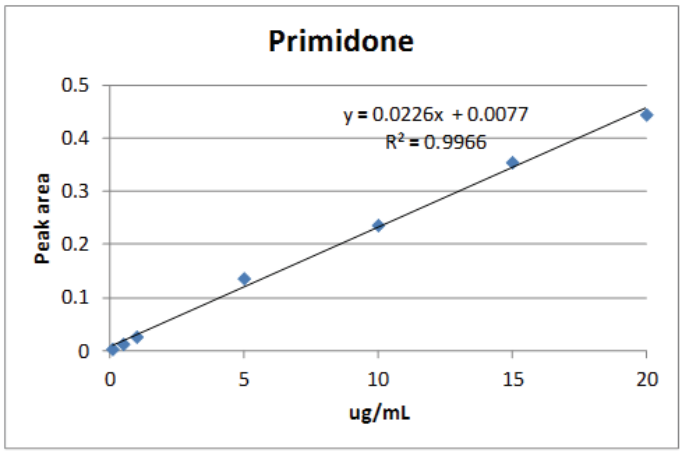
A8: Polynomial range from 0.1 to 75 ug/mL of Pindolol



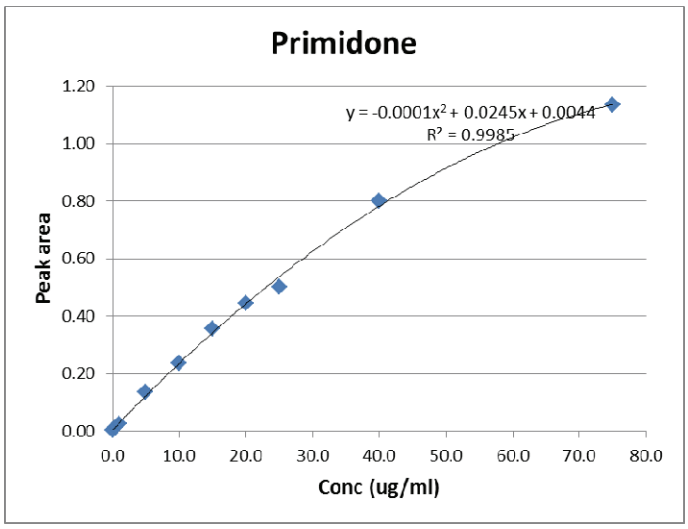
A9 Linear range of famciclovir, 0.1-15 ug/Ml



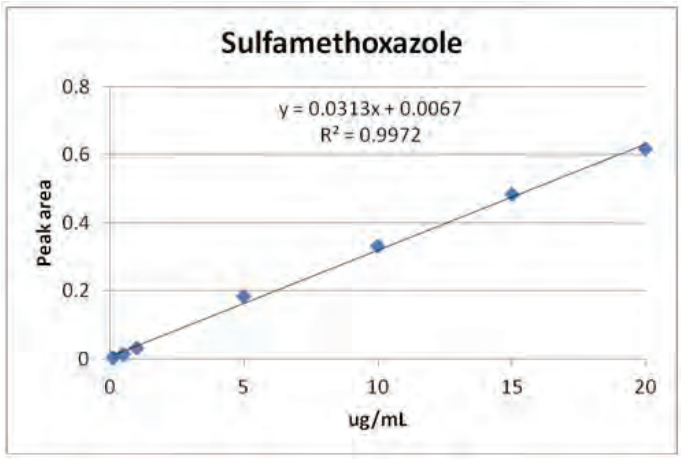
B10: Polynomial range from 0.1 to 75 ug/mL of Famciclovir



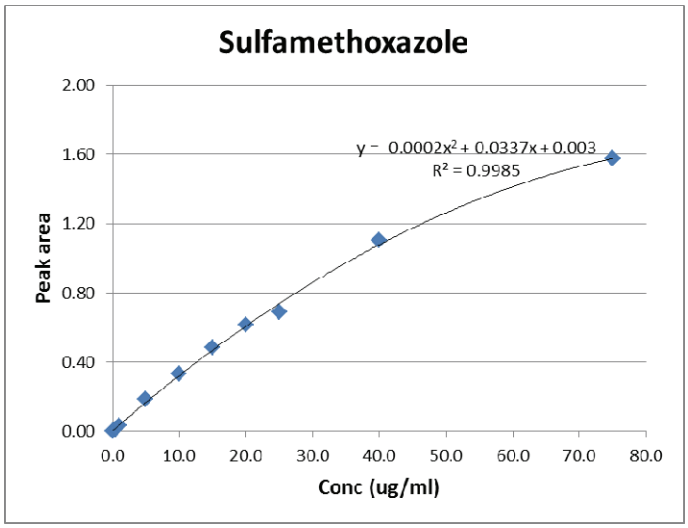
A11 Linear range of primidone, 0.1-20 ug/mL



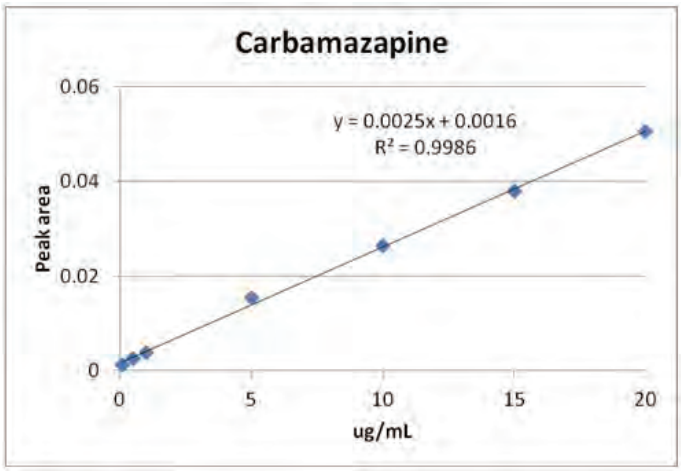
B12: Polynomial range from 0.1 to 75 ug/mL of Primidone



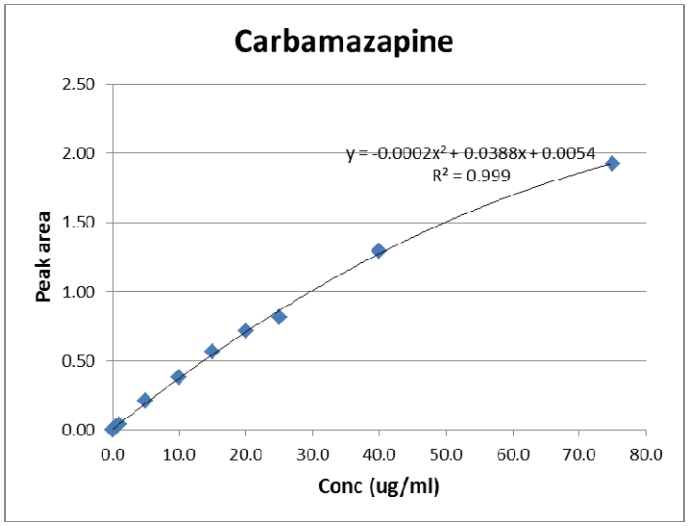
A7: Linear range of sulfamethoxazole, 0.1-20 ug/mL



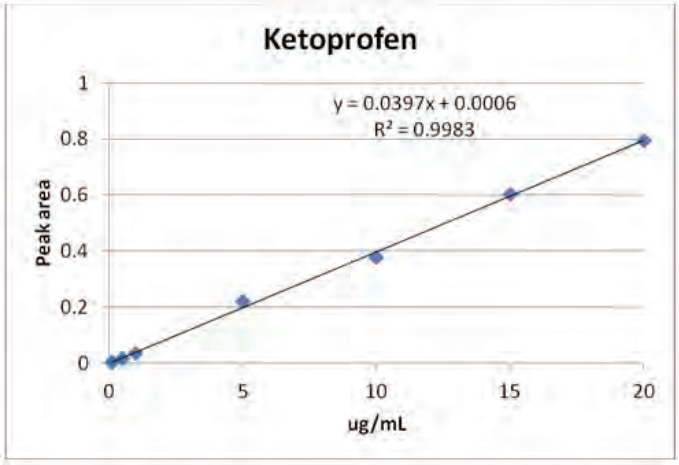
A14: Polynomial range from 0.1 to 75 ug/mL of Sulfamethoxazole



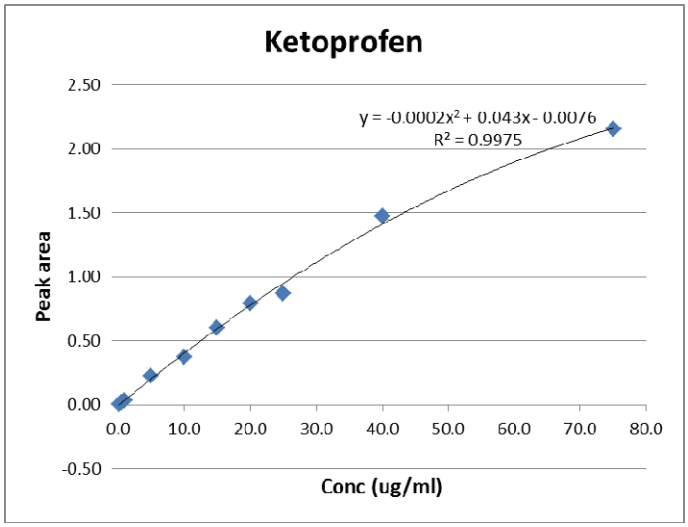
A15: Linear range of carbamazapine, 0.1-20 ug/mL



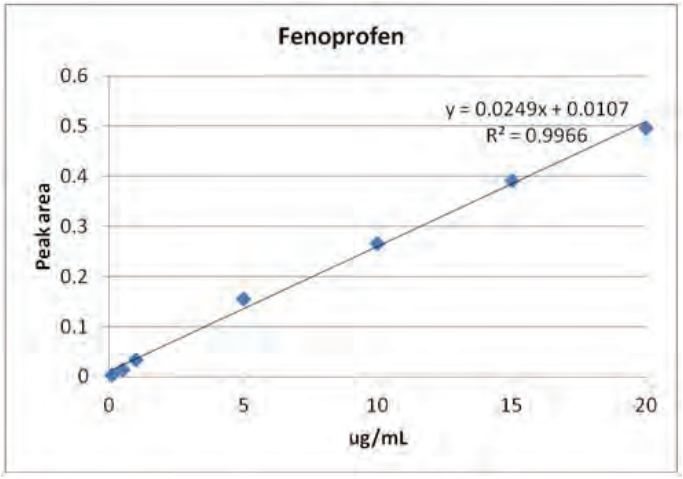
A16: Polynomial range from 0.1 to 75 ug/mL of Carbamazapine



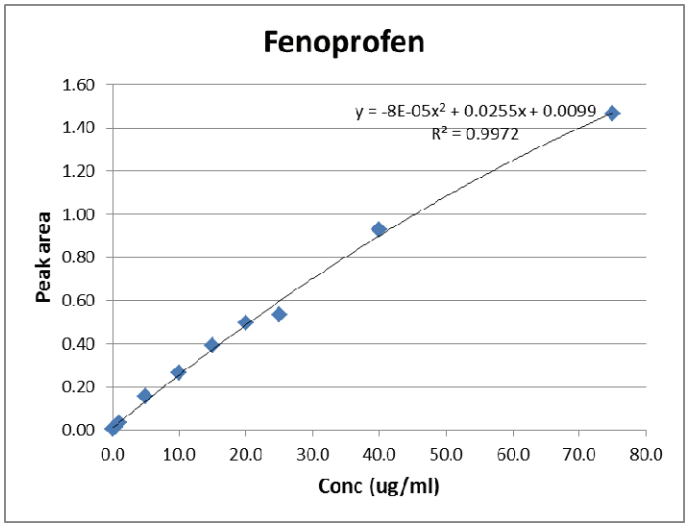
A17: Linear range of ketoprofen, 0.1-20 ug/mL



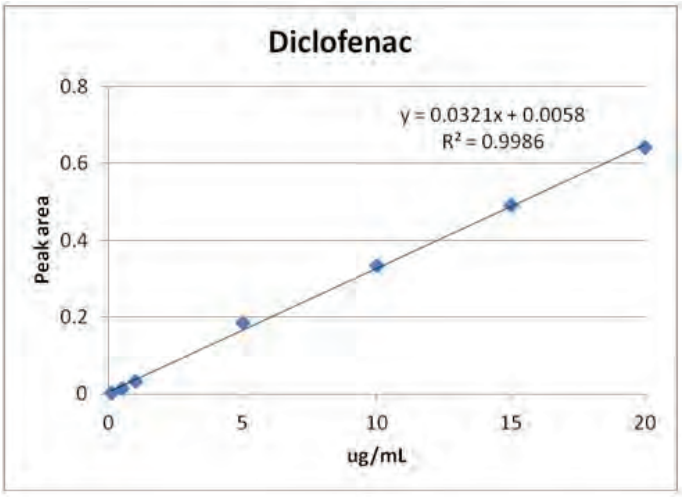
B9: Polynomial range from 0.1 to 75 ug/mL of Ketoprofen



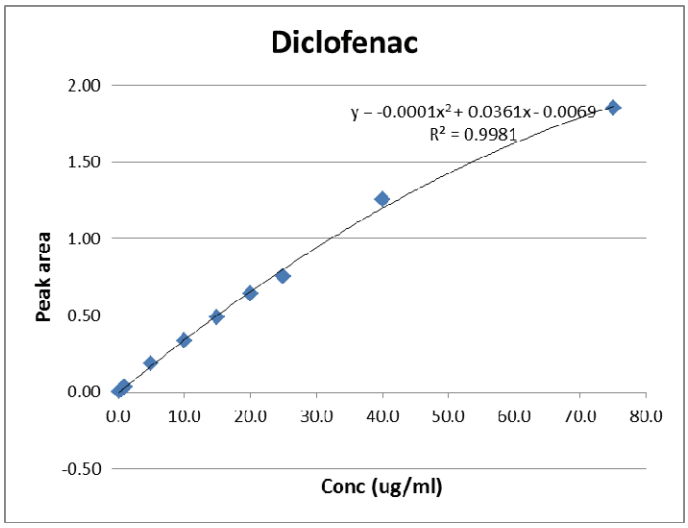
A19: Linear range of fenoprofen, 0.1-20 ug/mL



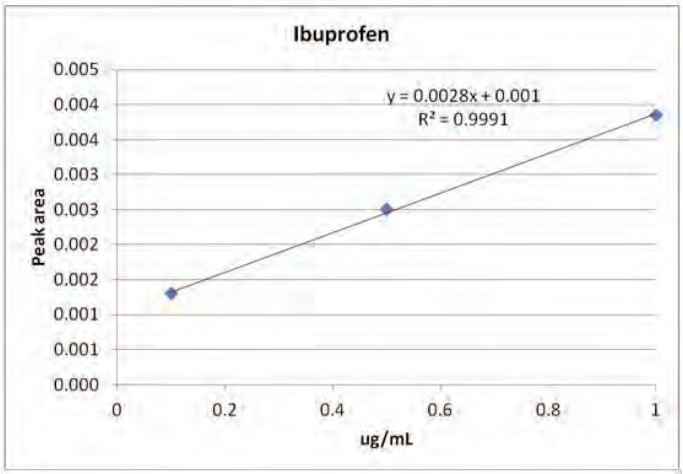
A20: Polynomial range from 0.1 to 75 ug/mL of Fenoprofen



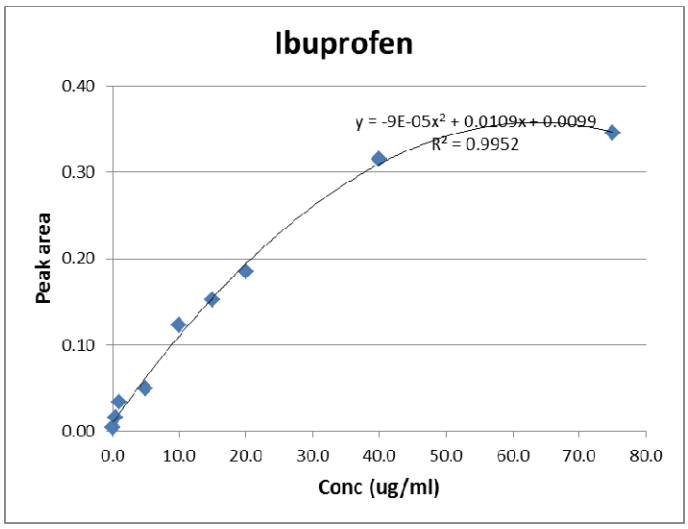
A21: Linear range of diclofenac, 0.1-20 ug/Ml



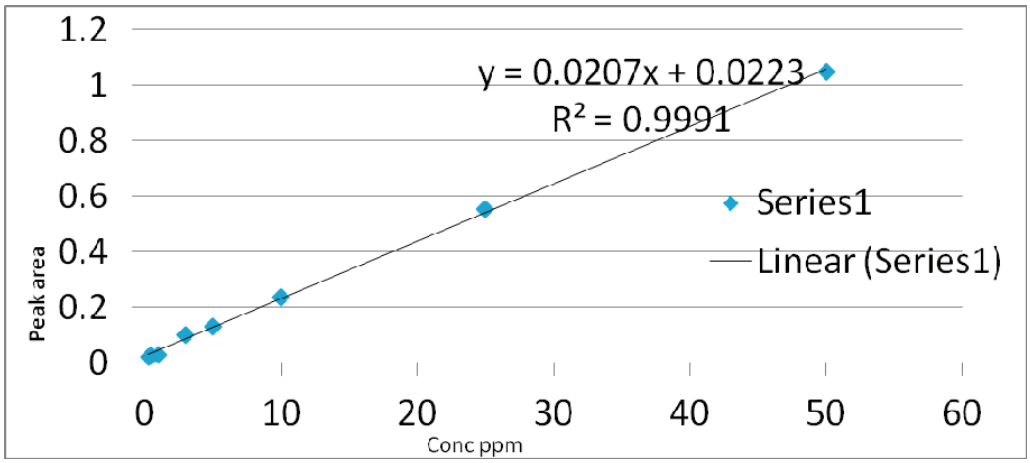
A22: Polynomial range from 0.1 to 75 ug/mL of Diclofenac



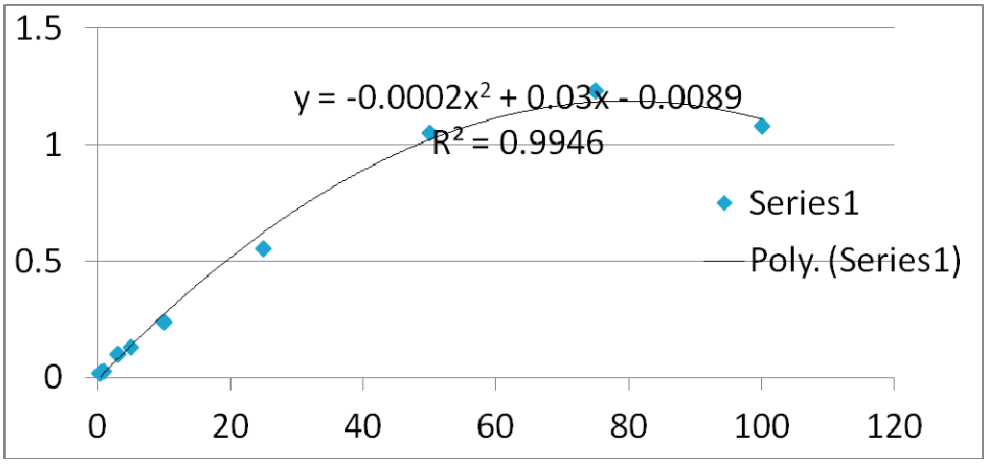
A23: Linear range of ibuprofen, 0.1-1 ug/Ml



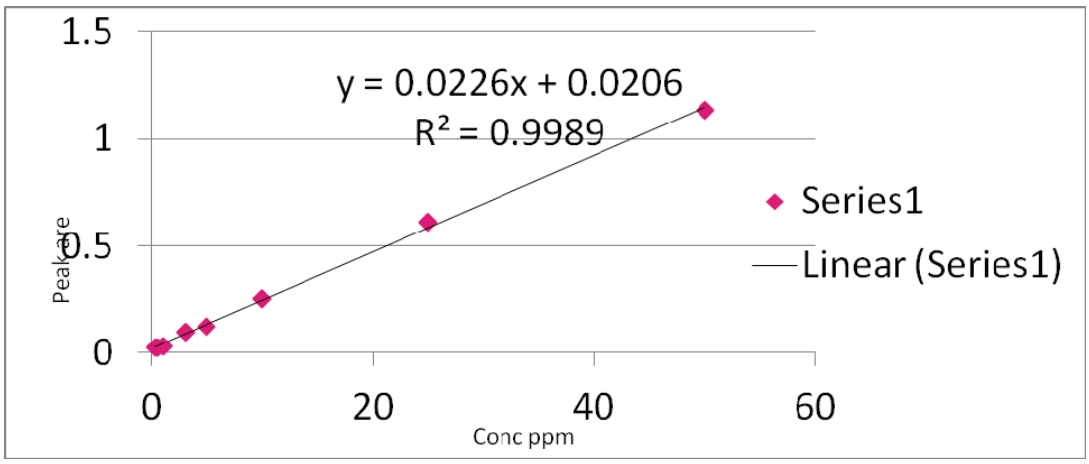
A24: Polynomial range from 0.1 to 75 ug/mL of Diclofenac



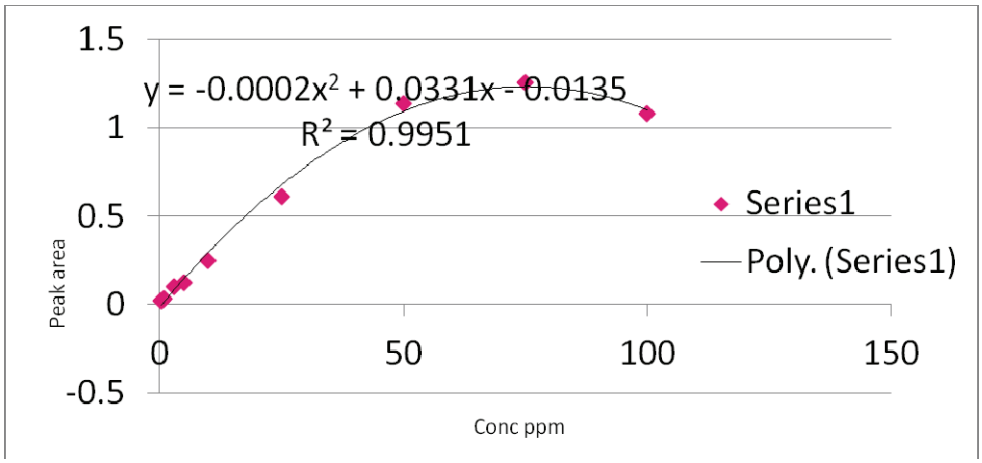
B1: Linear Calibration Curve for Estriol



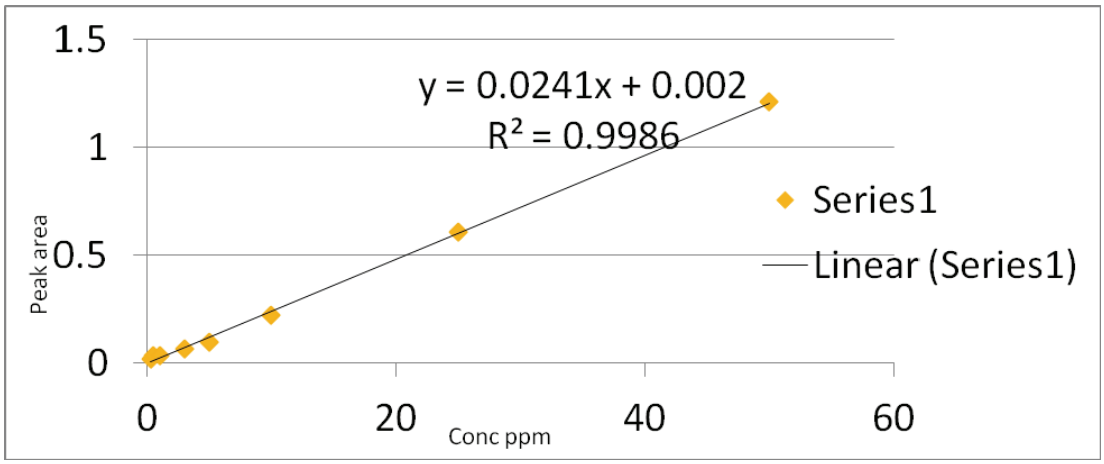
B2: Polynomial Calibration Curve for Estriol



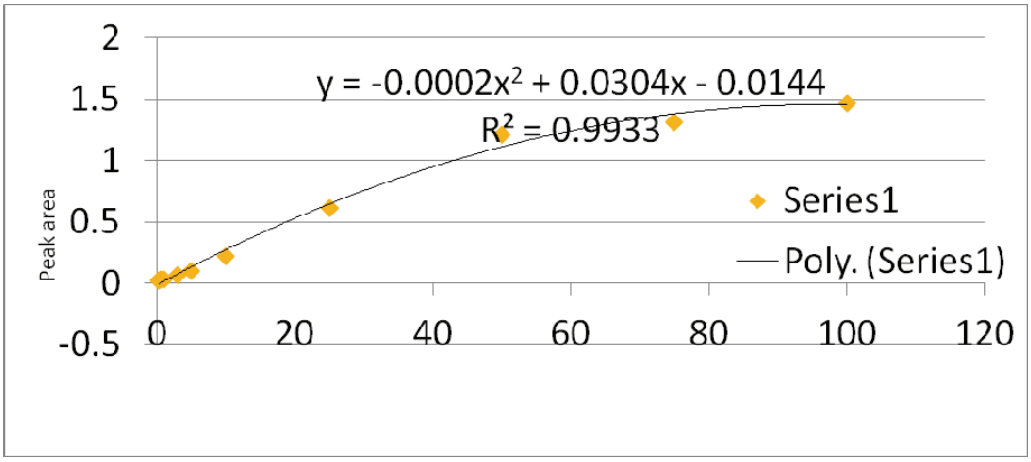
B3: Linear Calibration Curve for Bisphenol A



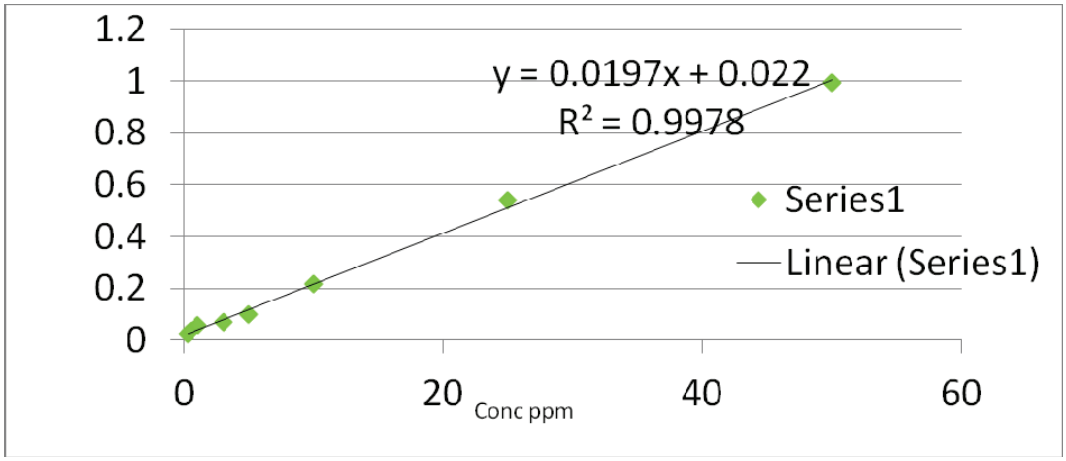
B4: Polynomial Calibration Curve for Bisphenol A



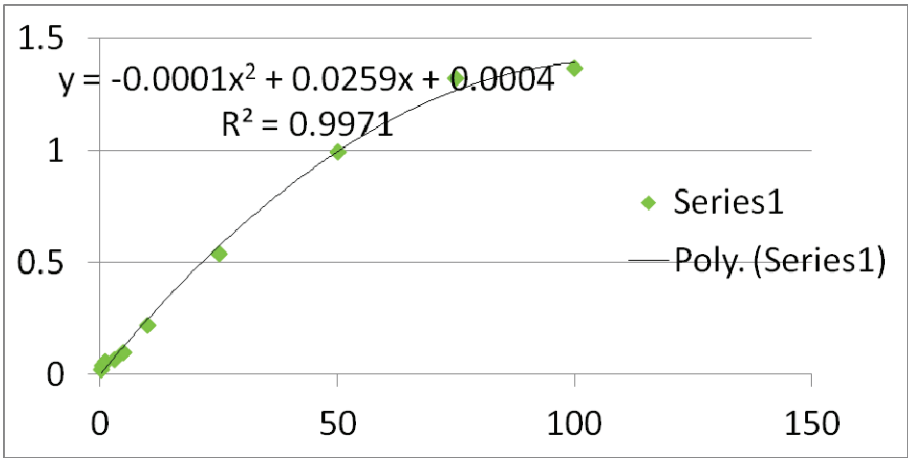
B5: Linear Calibration Curve for B-Estradiol



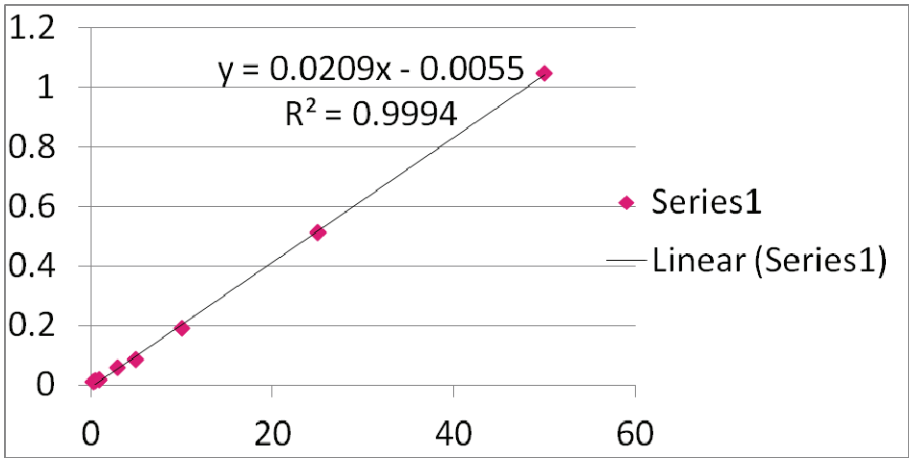
B6: Polynomial Calibration Curve for B-Estradiol



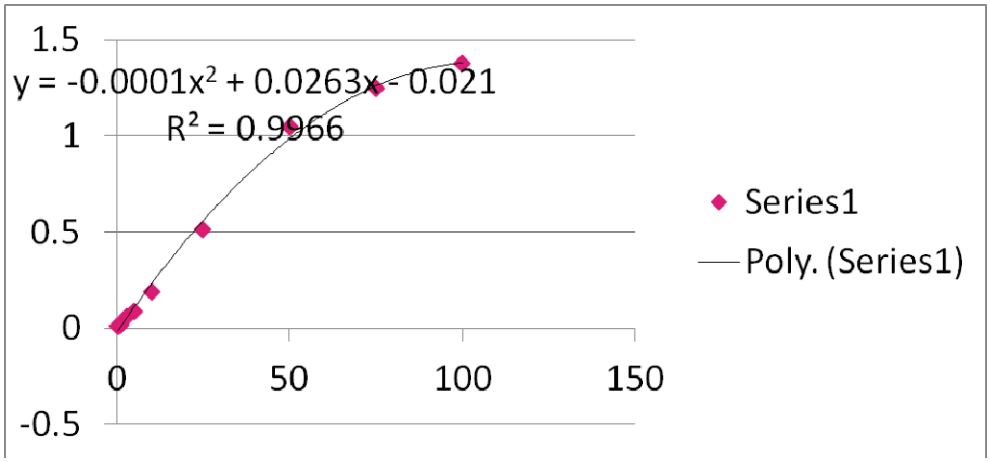
B7: Linear Calibration Curve for A-Estradiol



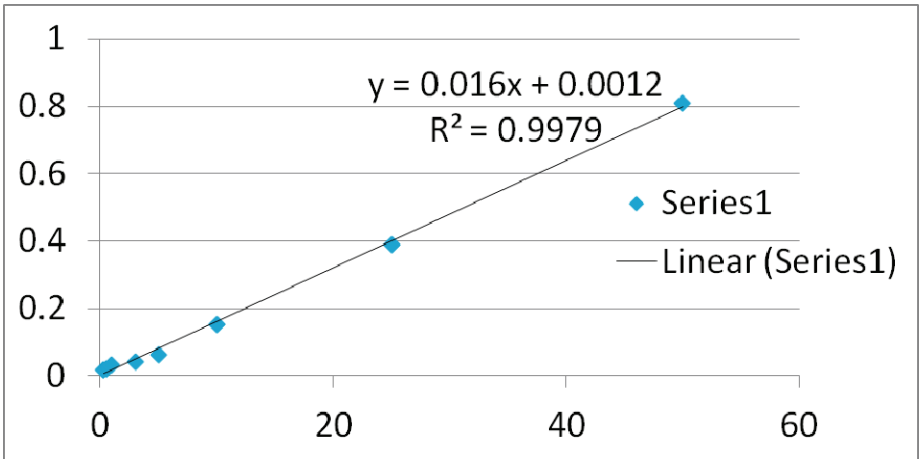
B8: Polynomial Calibration Curve for Estradiol



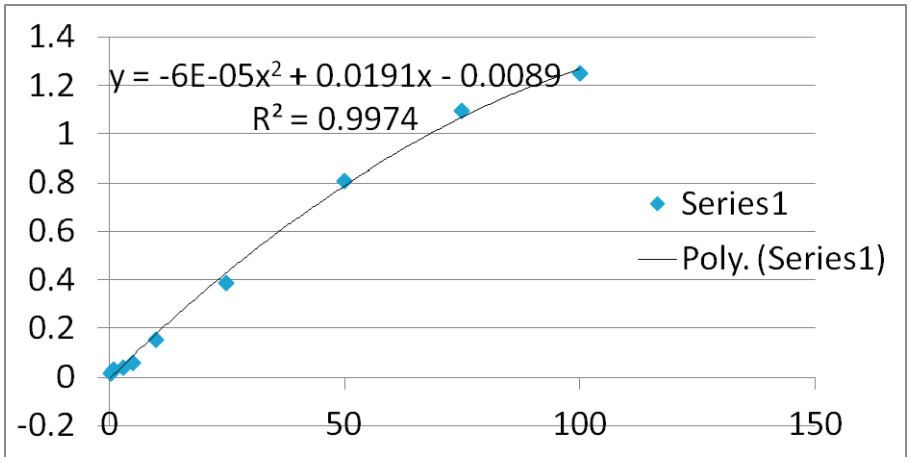
B9: Linear Calibration Curve for A-Testosterone



B10: Polynomial Calibration Curve for Testosterone



B11: Linear Calibration Curve for α-Testosterone



B12: Polynomial Calibration Curve for Testosterone

APPENDIX B: ANALYTICAL PROCEDURES

POCIS

The procedure for extracting the sequestration medium from the Polar Organic Chemical Integrative Sampler (POCIS)

1. Cover work area with solvent rinsed aluminum foil.
2. Fit stopcock to chromatography column and, using glass rod, firmly seat a plug of glass wool (1-2 cm) on top of the stopcock. Place chromatography columns (one per POCIS) in holder, add funnel, and rinse column with methanol. Allow to drain into a beaker and discard waste. Place 125 mL flat bottom flask under column.
3. Using gloves hold the POCIS horizontally to remove the hardware.
4. Hold POCIS over the funnel and separate the washers.
5. Use tweezers to separate the membranes and wash the sequestration medium into the column with the wash bottle of methanol. Rinse the funnel with methanol (the rinse portion is not included in the amount of extraction solvent used).
6. Add another plug of glass wool on top of sample to prevent it from washing up the sides of the column when adding the extraction solvent(s).
7. Extraction of OASIS HLB (pharmaceuticals) is done with 40 mL methanol.
8. Close stopcock and add appropriate solvent. Open stopcock and allow extract to drip at a slow but steady rate into the 125 mL flask.
9. Roto-vap, SpeedVac or evaporate (over UHP nitrogen) the extract to a volume of 1 to 2 mL and filter through glass fiber filter paper using methanol as the transfer solvent.

SPE

Treated wastewater is passed through a solid phase extraction (SPE) for clean-up and pre-concentration. In addition to Water Oasis® HLB SPE cartridges (see section 2.12), Isolute ENV cartridges (6 ml, 500 mg) have also been used (Carsten, 2010). The conditions of use of Isolute ENV SPE are as follows;

1. Conditioning of SPE with 1 x 2 mL n-heptane, 1 x 2 mL acetone, 3 x 2 mL methanol, followed 4 x 2 mL acetone.
2. Apply the water sample at a flow rate of about 5 mL/min.
3. Dry cartridge with nitrogen.
4. Elute analyte with 5 x 2 mL methanol/acetone (1:1 + 0.2% formic acid).

The extracted sample is dried with gentle stream of nitrogen and reconstituted to 1 mL with aqueous buffer (5 mM ammonium formate) at pH 5.6.

SPE DISKS

Two types of Atlantic HLB SPE Disks (Medium and High) were used for sampling and/or preconcentration.

1. A litre of water collected in brown bottle is used for either acid or base fraction.
2. 80 mg of sodium thosulfate is added to each 1 L water fraction
3. The acid fraction is acidified with HCl to pH 2 and
4. The basic fraction is basified with NH4OH to a pH of 10
5. To each fraction, 500 mg Na_4EDTA is added.

6. Shake the mixture and allow to equilibrate for 90 minutes

The extraction of PPHCPs is dependent on their acidity or basicity.

a) Acidic PPHCPs;
   1 The HLB-M/HLB-H Disk are placed on to 47 mm solvent exatrction unit
   2 The disks are wahed and equilibtrated using acid method
   3 Apply 500 ml water sample to the HLB disk at low flow rate to allow for intercation of the analyte with active sites of the disk
   4 Air dry the disk for about 15.00 minutes
   5 Wash with methanol
   6 Eluate with 1:1 Acetone/Methanol

b) Basic PPHCPs
   1. The HLB-M/HLB-H Disk are placed on to 47 mm solvent exatrction unit
   2. The disks are wahed and equilibtrated using acid method
   3. Apply 500 ml water sample to the HLB disk at low flow rate to allow for intercation of the analyte with active sites of the disk
   4. Air dry the disk for about 15.00 minutes
   5. Wash with methanol
   6. Eluate with 2% formic acid

APPENDIX C: ALTERNATIVE PROCEDURES

Analgesics

GC-MS

Paracetamol and ibuprofen are derivatised with bis(trimethylsilyl-trifluoroacetamide (BSTFA) (Heberer et al., 1997). Pentafluorobenzyl bromide can also be used for derivatisation of these two analgesics. The following procedure is followed;

1. 200 µL of 2% solution of pentafluorobenzyl bromide in cyclohexane is used to dissolve solid.
2. 2 µL of triethylamine is then added into the solution.
3. Within 2 hours the solution is dried in an oven at 100 ºC.
4. Products are then analysed by GC-MS.

The following conditions are set for the GC-MS;

1. DB 35 column (30 m x 0.25 mm x 0.25 µm)
2. UHP Helium is used as a carrier gas
3. Temperature program used for separation: 65 ºC held for 2 min, raised at 30 °C/min to 180 ºC, then at 5 ºC/min to 300 ºC and held at 300 ºC for 12 min.
4. The injection and detector temperature are set at 300 ºC and 200 ºC respectively.

LC-MS/MS

Liquid chromatography-mass spectrometry with electrospray ionisation is used. This method is limited to few South African laboratories and will therefore not be considered in this work.

Antiretrovirals (ARVs)

Several methods have been developed for the analysis of ARV in a variety of matrices. These methods can be extended to treated wastewater. Hydrophilic interaction liquid chromatography (HILIC) stationary phase with electrospray LC-MS/MS has been used for ARVs and their metabolites (Carsten, 2010).

The ARV pharmaceuticals are separated using the following conditions;

1. RP column (150 mm x 3 mm x 4 um).
2. Solvent system consists of A; (5 mM ammonium formate) and B; (methanol).
3. Oven temperature of 40 °C and flow rate of 0.40 ml/min.
4. Gradient elution conditions with respect to Mobile Phase A as follows; 0-4 min, 100%; 7 min, 30%; 17 min, 10%; 18 min, 100%.

The detection is by electrospray MS/MS using multi reaction monitoring (MRM).

Hypertension drugs

The GC-MS method given above is also used for the hypertension drugs. Slight modification of separation conditions is implemented if monitoring is focused on one class.

Anti-diabetics

As much as GC-MS is a more robust routine method for certain classes of pharmaceuticals it should not be necessarily be replaced by HPLC in all cases of drugs. Naturally, the most suitable method for the detection and monitoring of antidiabetics is the LC-MS/MS (Kasprzyk-Hordern, 2008; Spongberg and Witter, 2008). A number of HPLC methods with diode array detector (DAD), fluorescence (FLD) and charged aerosol detector will be investigated. The separation column used for antidiabetic drugs is similar the column used for the ARV class of drugs.

Antibiotics and vitamins

Antibiotics and the vitamins are also determined using either GC-MS or HPLC similar to the antidiabetics class of compounds.