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ASSESSMENT OF CONVENTIONAL WASTEWATER TREATMENT PLANTS IN REMOVING PHARMACEUTICAL COMPOUNDS: A CASE STUDY OF AL AIN CITY, UAE

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This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Water Resources

Under the Supervision of Professor Munjed Maraqa

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Declaration of Original Work

I, Ahmad Mohammad Hassan AlHalabi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Assessment of Conventional Wastewater Treatment Plants in Removing Pharmaceutical Compounds: A Case Study of Al Ain City, UAE*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Professor Munjed Maraqa in the College of Engineering at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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Abstract

This thesis is concerned with the assessment of conventional wastewater treatment plants (WWTPs) in removing pharmaceutical compounds (PCs). PCs have classes like analgesics, antibiotics, antiseptics, hormones, cosmetic products, personal care products. They are used extensively by humans and they don't have actual guidelines describing their concentration in domestic wastewater discharge. Thus, they may still be present in treated sewage effluent (TSE) or sludge and could consequently pose adverse environmental effect. Limited work has been done to assess the removal of PCs at WWTPs in arid and semi-arid countries including the United Arab Emirates (UAE). As such, this study aimed at investigating the levels of selected PCs in domestic wastewater in Al Ain city before and after treatment. An analytical protocol was developed for identification, quantification, and analysis of 15 PCs using a liquid chromatography tandem mass spectrometry (LC-MS/MS) system. Four batches of used water and sludge samples were collected from different locations at Al Saad WWTP in Al Ain, UAE. Aliquots of each sample were enriched by solid phase extraction (SPE). Results show that phenylephrine, dapsone, noscapine, propyl gallate, genistein, and ketoconazole were present in the raw wastewater at low levels (<0.1 µg/L), while acetaminophen and caffeine were present at high levels (>10 µg/L). The overall removal efficiency of the tested PCs from the water stream in Al Saad WWTP exceeded 99% for cotinine, acetaminophen, caffeine, naproxen, and ibuprofen, but significantly drops (<50%) for phenylephrine, amoxicillin, dapsone, noscapine, spiramycin, noscapine, genistein and ketoconazole. Analysis of the results indicate that, for highly removed PCs, the main mechanism of removal is possibly aerobic biodegradation. However, for tyramine, dapsone, 9-aminoacridine, noscapine, propyl gallate, and ketoconazole sorption onto the mixed liquor suspended solids (MLSS) first occurs in the aeration tank of the activated sludge system followed by removal by anaerobic digestion.

Keywords: Wastewater, pharmaceutical compounds, sludge, Al Saad WWTP, LC-MS/MS, SPE, internal standard, mass balance, Al Ain, UAE.

Title and Abstract (in Arabic)

تقييم محطات معالجة مياة الصرف الصحي التقليدية في إزالة المركبات الصيدلانية: دراسة حالة لمدينة العين، الإمارات العربية المتحدة

الملخص

الهدف من هذه الأطروحة هو تقييم قدرة محطات معالجة مياة الصرف الصحي في إزالة المركبات الصيدلانية. ويوجد العديد من الأنواع من هذه المركبات كالمسكنات، والمضادات الحيوية، والمطهرات، والهرمونات، ومستحضرات التجميل وتلك الخاصة بالعناية الشخصية. ويتم استخدام المركبات الصيدلانية بشكل واسع جدا وبشكل يومي. ولا يوجد حتى الآن حدود لتركيزها في مياة الصرف الصحي المعالجة. وبالتالي قد لا تزال موجودة في مياة الصرف الصحي بعد معالجتها أو في الحمأة الناتجة عن عملية المعالجة مما قد يشكل ضرراًعلى البيئة. ونظرًا لندرة هذا النوع من الأبحاث في البلدان القاحلة وشبه القاحلة، وخاصة في دولة الإمارات العربية المتحدة؛ فقد تم اجراء هذه الدراسة والتي تهدف إلى دراسة مستويات بعض المركبات الصيدلانية في مياه الصرف الصحي في مدينة العين قبل وبعد معالجتها. وقد تم من خلال هذه الدراسة القيام بتطوير بروتوكول تحليلي (analytical protocol) للتعرف على 15 مركب صيدلاني وتحديد تراكيزها باستخدام جهاز مطياف الكتلة المدمج مع سائل فاصل للون عالي الكفائه (LC-MS / MS). وتم جمع أربع دفعات من عينات المياه والحمأة من مواقع مختلفة في محطة الساد لمعالجة المياه العادمة في مدينة العين، الإمارات العربية المتحدة. وتم استخلاص المركبات الصيلانية منها من خلال جهاز استخلاص الطور الصلب (solid phase extraction).

وقد بينت نتائج هذه الدراسة تواجد مركبات فينيليفرين، دابسون، نوسكافين، بروبايل جالات، جينيستين، وكيتوكونازول في مياة الصرف الصحي قبل معالجتها بتراكيز منخفضة (أقل من 0.1 ميكروغرام/ليتر). بينما تواجدت مركبات الأسيتامينوفين، والكافيين بتراكيز عالية تجاوزت 10 ميكروغرام/ليتر . وقد تجاوزت كفاءة الإزالة الكلية للمركبات الصيدلانية من المياه المعالجة في محطة الساد نسبة 99% للمركبات التالية: الأسيتامينوفين، الكافيين، النابروكسين، والإيبوبروفين. لكن إنخفضت الكفاءة بشكل كبير لتصل إلى أقل من 50% لمركبات فينيليفرين، الأموكسيسيلين، الدابسون، النوسكافين، السبيراميسين، جينيستين، والكيتوكونازول. وتشير عملية تحليل النتائج إلى أن آلية الإزالة الكلية للمركبات ذات نسبة الإزالة العالية هي التحلل الحيوي الهوائي (aerobic biodegradation). بينما تمت إزالة التيرامين، الدابسون، 9-أمينو أكريدين،

نوسكافين، بروبايل جالات ، والكيتوكونازول من خلال الامتصاص (sorption) على المواد العالقة في السائل المختلط (mixed liquor suspended solids) ثم عن طريق الهضم اللاهوائي (anaerobic digestion).

مفاهيم البحث الرئيسية: مياة الصرف الصحي، المركبات الصيدلانية، محطة الساد لمعالجة مياة الصرف الصحي، الكروماتوجرفيي السائل، مطياف الكتلة، مدينة العين، الإمارات العربية المتحدة.

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Although this thesis cover shows only my name, many helped directly and indirectly in this outcome. For that I owe them my sincere thanks, especially for those who made this work real and helped in its production.

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Special thanks go to my family especially my parents for their help and support along the way. I tried to make them feel proud of me by finishing my master's degree and thus meeting their expectations. Thanks are also extended to my friends for their support and for the great time that I spent with them.

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Dedication

To my beloved parents and family

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List of Abbreviations

AD	Anaerobic Digestion
AT	Aeration Tank
BOD_5	5-day Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
CS	Coarse Screens
CT	Chlorine Contact Tank
DI W	Deionized Water
EC50	Half Maximal Effective Concentration
ESI	Electrospray Ionization
F	Filter
FE	Final Effluent
FP	Filter Press
FS	Fine Screen
GC	Gas Chromatography
GC-MS/MS	Gas Chromatography Tandem Mass Spectrometry
HPLC	High Performance Liquid Chromatography
IDL	Instrument Limit of Detection
IS	Internal Standard
Ka	Acid Dissociation Constant
Kow	Octanol-Water Partition Coefficient
LC	Liquid Chromatography
LC50	Lethal Concentration
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry

LOD	Limit of Detection
LOQ	Limit of Quantitation
MBR	Membrane Bioreactors
MeCl	Dichloromethane
MLSS	Mixed Liquor Suspended Solids
MRM	Multiple Reaction Monitoring
MS	Mass Spectroscopy
MS/MS	Tandem Mass Spectroscopy
ND	Not Detected
NR	Not Reported
NSAID	Nonsteroidal Anti-Inflammatory Drug
N_{tot}	Total Nitrogen
PCs	Pharmaceutical Compounds
PNEC	Predicted Non-Effect Concentration
PST	Primary Settling Tank
P_{tot}	Total Phosphorus
Q_d	Flow Rate per day
R^2	Coefficient of Determination
RAS	Return Activated Sludge
RE	Removal Efficiency
RQ	Risk Quotient
RW	Raw Wastewater
SBD	Sludge Drying Bed
SGT	Sand & Grease Trap

SMT	Sludge Mixing Tank
SPE	Solid Phase Extraction
SS	Suspended Solids
SST	Secondary Settling Tank
t_R	Retention Time
TSE	Treated Sewage Effluent
UAE	United Arab Emirates
VSS	Volatile Suspended Solid
WWTPs	Wastewater Treatment Plants

Chapter 1: Introduction

1.1 Overview and Statement of the Problem

People usually use pharmaceutical compounds (PCs) as a medicine for their bodies. This leads to higher demand of PCs during the last two decades around the world (Kolpin et al., 2002). PCs have classes like analgesics, antibiotics, antiseptics, hormones, cosmetic products, personal care products (Kolpin et al., 2002; Stackelberg et al., 2004). Many PCs don't have actual guidelines describing their concentration in domestic or industrial wastewater (Löffler et al., 2005). So, during the last decades detection and determination of PCs in wastewater was one of the main concerns especially in Europe, USA and Canada (Anderson et al., 2004; Carrara et al., 2008; Holm et al., 1995). On the other hand, recent work showed that there are some PCs like estrogen that exist at low concentration in water bodies. These compounds come from discharges of wastewater plant effluent and cause problems for aquatic life in water bodies (Jean et al., 2012; Wang et al., 2012).

Since world population is growing exponentially and water resources are limited, people try to recycle, and reuse used water especially municipal wastewater. Usually, treated sewage effluent (TSE) is used in irrigation to reduce potable water consumption (Kolpin et al., 2002). However, effluent water contains some traces of PCs, some of which could have toxicity effect (Mompelat et al., 2009). Some studies indicate the availability of PCs in rivers and streams which will affect human health via a chronic exposure to water or fish from these streams (Lapworth et al., 2012). Other studies indicate the availability of PCs in sludge from wastewater treatment plants (WWTPs) which are used later as soil conditioners (Martín et al., 2012).

Several studies were attempted to identify PCs in wastewater. Heberer et al. (2002) tried to detect ibuprofen and propyphenazone in wastewater form urban areas in Berlin, German, whereas Jean et al. (2012) studied the bioaccumulation of 960 PCs from Civils de Lyon Hospital in France. Furthermore, Reddersen et al. (2002) tried to identify phenazone compound in groundwater in Berlin, Germany.

1.2 Motivation

Limited work has been done to assess the level of PCs in environmental systems in arid and semi-arid countries. In particular, no study has been carried out to assess the type and levels of PCs in domestic wastewater in the UAE. In addition, knowledge of the role of conventional WWTPs in the country in removing PCs from the waste stream is lacking. The research questions that will be addressed in this study are: What are the levels of selected PCs in domestic wastewater in Al Ain City? What is the role of the unit operations employed at the WWTPs in Al Ain in removing these PCs from the water stream? What are the levels of PCs in the generated sludge?

1.3 Objectives of the Study

This research aimed to investigate the presence of selected PCs in domestic wastewater in Al Ain City. The specific objectives of the study were to:

1. Identify and quantify selected PCs in domestic wastewater in Al Ain city, UAE.
2. Assess the role of conventional wastewater treatment methods in the removal of PCs from wastewater.
3. Evaluate the level of selected PCs in sewage sludge produced at wastewater treatment facilities.

1.4 Scope of Work

The selection of the target PCs for this research was done by reviewing the literature to identify the possible PCs that could be present in domestic wastewater. Table 1 shows a short list of PCs that could be present in domestic wastewater.

Table 1: PCs that could be present in domestic wastewater

Aspirin	Naproxen	Ibuprofen	Cotinine	Dapsone
Amoxicillin	Tyramine	phenylephrine	Noscapine	Propyl gallate
L-Ascorbic Acid	Irgasan	ketoconazole	9-aminoacridine	Genisten
Caffeine	Estrone	α-Estradiol	Diethylstilbestrol	Equilin
17α-Ethynyl estradiol	Estriol	Spiramycin		

There are many PCs that could be present in wastewater. However, this study focused only on the analysis of the above mentioned chemicals. Meanwhile, the study was limited to domestic wastewater in Al Ain city. Although it is anticipated that domestic wastewater in the UAE would have similar characteristics in terms of PC levels, such speculation needs to be confirmed by analyzing wastewater samples from different locations in the country.

Another limitation of the study is related to the duration of the sampling protocol which covers the period of September to December, 2018. Within this 4-month duration, any possible seasonal fluctuation in the levels of PCs in the waste stream and in the effectiveness of wastewater treatment plants in removing these compounds would be difficult to assess.

The study is also limited in the sense it assesses the removal of PCs by one treatment plant in the UAE (i.e., Al Saad WWTP). In the UAE, there are many domestic WWTPs that vary in their configuration and design. The major WWTPs in the country employ an activated sludge system with anaerobic digestion of sludge.

However, these plants differ in their reactor design parameters which could influence their removal efficiencies of PCs. Other small plants in the country employ membrane bioreactors (MBR) which could also differ from those with activated sludge system in their removal of PCs

1.5 Approach

In this study, several tasks were undertaken which include review of literature, development of an analytical protocol for the determination of the target PCs, identification and quantification of PCs in domestic wastewater in Al Ain city, assessment of conventional domestic WWTPs in removing PCs from wastewater, investigation of the level of PCs in produced sludge at the treatment plant. The research approach is schematically presented in Figure 1.

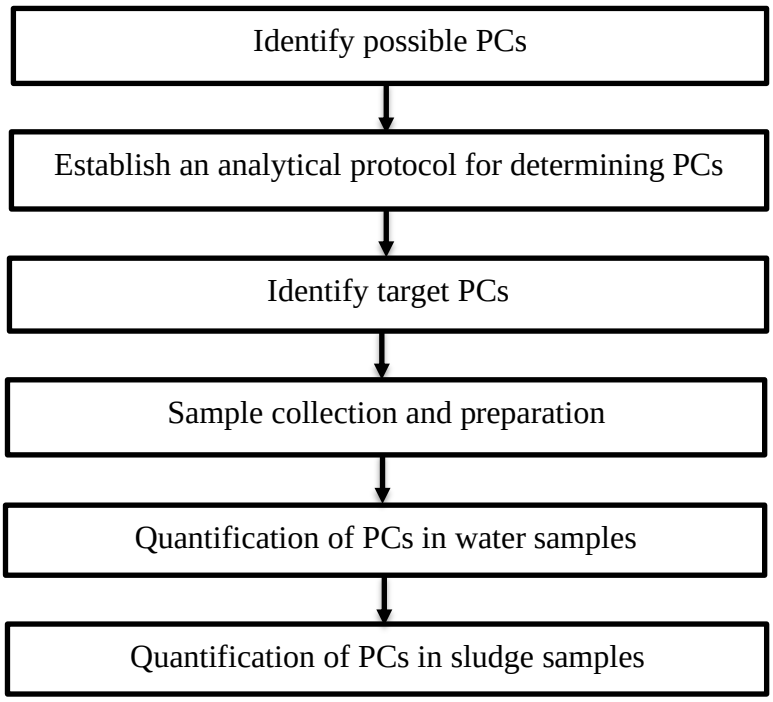


Figure 1: Research approach

1.6 Thesis Structure

This thesis consists of six chapters. Chapter 1 provides a description of the project including project overview and background, statement of the problem, objectives, motivation, scope of work, and approach. Chapter 2 provides an extensive literature review which covers PCs development and types, PCs production worldwide, release of PCs to the environment, effects of PCs on the environment, risk assessment, detection of PCs in wastewater, levels of PCs in wastewater, and the role of conventional WWTPs in removing PCs

Chapter 3 explains in detail the development of an analytical protocol and provides a description of the sampling site (Al Saad WWTP). Chapter 4 presents the results of detection and quantification of the targeted PCs. Chapter 5 presents the levels of PCs in generated domestic wastewater at Al Ain city and their removal through the employed unit operations at Al Saad WWTP. Finally, Chapter 6 provides concluding remarks and gives recommendations for future work.

Chapter 2: Relevant Literature

2.1 Introduction

PCs are compounds that have different classes and treat different types of diseases. PCs could enter to the environment through different sources such as treated sewage effluent (TSE), leakage of sewage networks, septic tanks and landfills. There have been some emphases during the last decade on these compounds as they have been found in different water bodies like rivers, surface water, and groundwater (Fram and Belitz, 2011; Roberts and Thomas, 2006; Yoon et al., 2010). PCs could have an impact on the environment and humans. Therefore, quantification and assessment of the level of PCs in different water bodies and finding ways to remove these compounds become absolute necessity. This chapter provides an extensive literature review regarding the work that has been done related to the analysis and detection of PCs and the effectiveness of WWTPs in removing PCs.

This chapter is organized in six sections. Section 2.2 focuses on the development. It reviews the different types of PCs like antibiotics, antiseptics, analogies, and hormones. Section 2.3 introduces the possible pathways through which PCs could enter the environment. Section 2.4 focuses on the environmental effect of PCs including toxicity and accumulation. Section 2.5 reviews the analytical methods used to detect PCs in wastewater along with their detected levels. Finally, Section 2.6 describes the role of conventional WWTPs in removing PCs.

2.2 PCs Development and Types

In the early days of pharmacology, people used some derivations and treatments from natural plants to create drugs or they found some drugs by serendipity. For example, aspirin was derived from the bark of the willow tree (Mahdi, 2010). In the 1960s, the development of research fields became stronger and made the evolution of medical treatment. As a result, scientists used technology in a drug discovery. They found that drugs can affect the function of cellular receptors, enzymes, and ion channels by activating or inhibiting them which affect diseases status (Takenaka, 2008). To be sure of a drug function, it is tested on rats in a process known as preclinical development. If the drug shows positive results a new step starts which is known as clinical development. This step aims to test the new drug on a sample of people before marketing. In order to develop a new drug, it requires at least 15 years to pass through research, discovery, preclinical and clinical development. As such , a new drug could cost about one billion dollar to develop (Hughes et al., 2011).

Scientists classify drugs and PCs in different classes such as analgesics, antibiotics, antiseptics, hormones, cosmetic products, personal care products (Kolpin et al., 2002; Stackelberg et al., 2004). Table 2 shows the different classes of PCs, list examples, and indicate the general uses of each class.

Analgesics class works as a pain reducer because they depress the central nervous system. Aspirin, ibuprofen, and acetaminophen PCs are considered examples of analgesics because they work in the human body to eliminate sensation (Bell, 2013). They reduce pain, fever, headache, arthritic conditions and inhibit blood clotting (Martin, 2015; Martin and McFerran, 2014; Porta and Last, 2018a).

Antibiotics are another class of PCs which treat and prevent bacterial infection, so they are called antibacterial. Antibiotics decrease the spread of the infection by killing the bacteria or by stopping their growth (Glick, 2016). Dapsone is considered an example antibiotic because it works to treat dermatologic disorders caused by abnormal neutrophil and eosinophil accumulation (Zhu and Stiller, 2001).

Antiseptics contain medications which inhibit or slow the growth of disease-causing microorganisms. They are considered nontoxic to skin and body cells and can be used to antisepsis the skin from bacteria. Hydrogen peroxide and ethanol are two examples of antiseptics which are used to treat minor wounds (Hine and Martin, 2015).

Hormones are chemical compounds that are produced in endocrine glands in the body and transport to specific organs or tissues by blood to regulate their functions. The steroid is the main type of hormones in the body which is responsible for water and salt balance, control inflammation and metabolism, and responsible for the development of sexual characteristics (Porta and Last, 2018b). Diethylstilbestrol, estrogen, and estriol are sexual hormones which are responsible for menstrual cycle and pregnancy in female bodies. In some cases, they can be introduced to female bodies as drugs if it is necessary (Heinonen, 1973).

Advancement of science led to improved development in the field of medical and treatment technology. Almost all diseases can be stopped by using PCs which increased the demand for these compounds (Kolpin et al., 2002). As a result, PCs improve the standard of living and reduce the death rate.

Table 2: Summary of PCs classes, examples, and their use

PCs Classes	Examples	Usage	Reference
Analgesics / anti-inflammatory	Acetaminophen, ketoprofen, ibuprofen, naproxen	Pain reducer	(Verlicchi et al., 2012)
Antibiotics	Amoxicillin, ciprofloxacin, ofloxacin, spiramycin	Kill bacteria (treat bacterial infections)	
Antifungals	Clotrimazole	Kill fungi (treat and prevent mycosis)	
Antihypertensives	Diltiazem, Enalapril	Lower blood pressure	
Barbiturates	Phenobarbital	Anxiety and insomnia	
β blocker	Acebutolol, Atenolol, betaxolol	Control heart rhythm and treat angina	
Diuretics	Bendroflumethiazide, furosemide	Increase the production of urine	
Lipid Regulators	Bezafibrate, clofibrate, etofibrate	Lower cholesterol	
Psychiatric Drugs	Amitriptyline, carbamazepine, diazepam	Treat mental illness and mental health	
Receptor antagonists	Cimetidine, famotidine, omeprazole	Reduce or block biological response	
Hormones	Estradiol, estriol, estrone	Regulate behavior and physiology	
Beta agonists	Clenbuterol, fenoterol, salbutamol	Breathing disorders	
Antineoplastic	Cyclophosphamide, ifosfamide, tamoxifen	Treat cancer	
Topical products	Crotamiton	Treat skin infections	
Antiseptics	Triclosan	Disinfection	
Antibacterial	Chloramphenicol, clarithromycin, erythromycin	Kill and prevent bacteria from growth	(Oliveira et al., 2015)
Antidiabetics	Glipizide, glyburide, metformin	Lower sugar level in the blood	
Antiepileptics	Carbamazepine, clonazepam, gabapentin	Treat epileptic seizures	
Antihistamines	Chlorpheniramine, loratadine, promethazine	Treat allergic rhinitis	
Antithrombotic	Clopidogrel, warfarin	Reduce blood clots	
Stimulants	Caffeine	Invigorating	(Kosma et al., 2014)

2.3 PCs Production

PCs production increased sharply worldwide during the last decade. The PCs industry and productions are done in different continents at different rates. Some continents such as Europe and Asia spent a lot of money for production, while other continents such as Africa and Oceania didn't spend much. Until 2008, Europe had the highest production rate with US$ 135.1 billion compared to Asia with US$ 119.9 billion. Six years later, Asia production rate exceeded that of Europe to reach US$ 153.9 billion in 2014. Worldwide, the production reached a value of US$ 452.8 billion in 2014. Table 3 summarizes PCs production in different continents between 2006 and 2014 (IFPMA Facts and Figures Report – IFPMA, 2017).

Table 3: PCs industry productions per continents (in billion US$)

Continents	2006	2007	2008	2009	2010	2011	2012	2013	2014
Asia	85.1	94.9	119.9	131.1	148.7	157.2	163.3	148.3	153.9
Europe	104.3	120.9	135.1	130.5	135.1	146.0	134.8	140.9	142.8
North America	95.4	100.4	94.2	110.5	104.9	102.6	105.3	108.3	111.8
Latin America	18.5	20.8	22.7	18.4	20.4	25.2	24.9	21.7	24.6
Africa	3.1	3.4	3.3	4.4	5.0	5.0	5.1	6.2	6.8
Oceania	1.8	2.2	2.1	2.4	3.5	3.2	3.3	3.6	2.7
Worldwide	308.2	342.5	377.3	397.3	417.6	439.2	436.8	428.7	452.8

According to the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) data which were published in 2014, the sales per capita of PCs differ between all countries. Developed countries such as Austria, Switzerland and the USA have higher sales compared to developing countries like India and South Africa. While in the Arabian Gulf region the UAE had the highest sales per capita compared to the KSA, Kuwait, and Oman. Figure 2 shows a comparison of PCs sales between several countries (IFPMA Facts and Figures Report – IFPMA, 2017). A higher number

of PCs sales (per capita) means higher welfare and standard of living and fewer mortality rates especially between children with age less than 10 years.

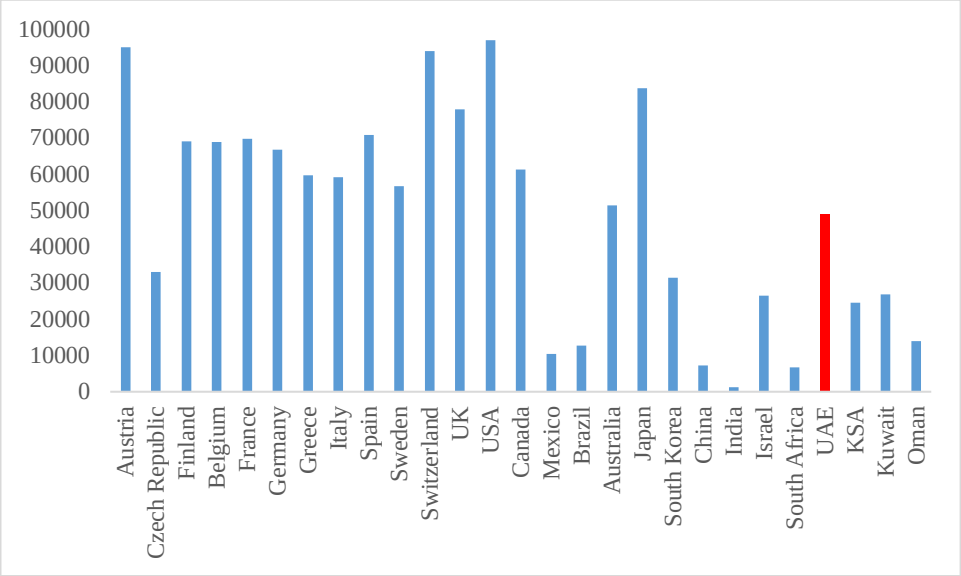


Figure 2: PCs annual per capita sales (in US$) in different countries in 2014 (IFPMA Facts and Figures Report – IFPMA, 2017)

2.4 Release of PCs to the Environment

PCs could enter the environment from different sources. PCs used by humans are released from their bodies in urine and feces to the sewage. Sewage is either discharged through septic tanks or collected in a sewage network and transferred to a WWTP. At a WWTP, sewage is treated to remove certain contaminants but PCs could be released into the environment because of lack of sufficient removal processes at the WWTP (Carrara et al., 2008; Halling et al., 1998). On the other hand, veterinary PCs, which are used to treat animal diseases, increase productivity, and promote growth, are released to the environment through the animal waste (Watanabe et al., 2010). In addition, PCs could be dumped or directly disposed of in a landfill by consumers or producers. Thus, huge quantities of PCs are released to the environment every year. PCs could, thus, exist in surface water and groundwater. The fate of PCs in these

environmental systems will be influenced by possible transfer and transform mechanisms. Ultimately, PCs could become a contaminant of a drinking water source.

Different mechanisms affect the fate of PCs when released into the environment. They could be degraded and converted to carbon dioxide and water. Or PCs could be lipophilic which means like to dissolve in lipids and fats and thus retained in sludge. The third mechanism is when PCs get metabolized to produce more hydrophilic molecules which reach surface and groundwater after sewage treatment. Figure 3 shows the fate and transport of PCs in the environment (Juraj et al., 2017).

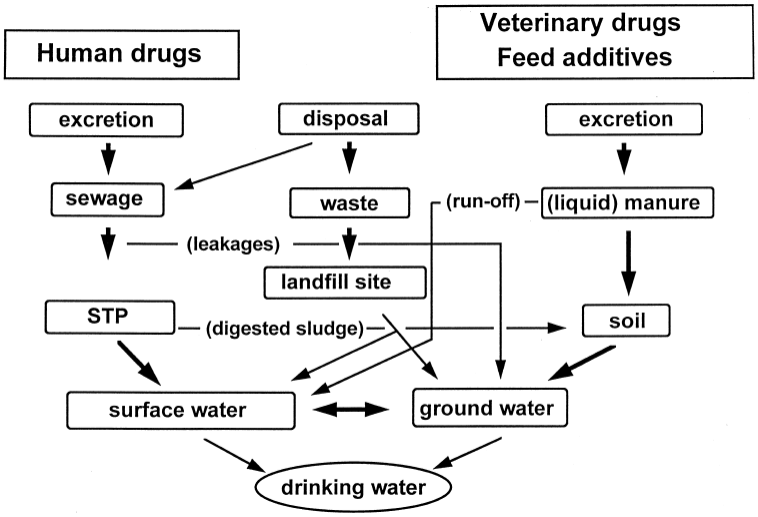


Figure 3: Fate and transport of PCs in the environment (Ternes, 1998)

2.5 Effects of PCs on the Environment

The presence of PCs in the TSE is a challenging issue because they could accumulate in soil, or exist in surface and groundwater, thus posing a threat for indirect potable water reuse. PCs are challenging because of their large number, inability to determine all of them, and the lack of toxicity data and toxicity effect for many of them (Löffler et al., 2005).

Some PCs could have different toxicity effects on flora and fauna in marine and land environments. Lee et al. (1983) showed that PCs dumped in streams could affect some invertebrates' organisms such as *Ampithoe valida.* The authors investigated the *Ampithoe valida* exposure to previous concentration and compared that with a control group. The results showed an increase in the death or reduce fertility for *Ampithoe valida* if chronically exposed to concentration exceeding 1% (v/v). Moreover, Lee et al. (1983) noticed an increase of toxicity effect with the increase of the exposure duration. On the other hand, some PCs such as ethinylestradiol could be present at low concentration in the aquatic environment. Ethinylestradiol causes sexual disturbance for aquatic organisms (Jean et al., 2012), while the presence of estrone and estradiol in the Yellow River in China has been reported to cause fish feminization (Wang et al., 2012).

Aside from the toxicity effect of PCs, these compounds could form by-products after ingestion and excretion. The by-products could have a toxicity effect. However, there is a lack of information of the actual effect of the by-products formed after the release of PCs (Mompelat et al., 2009).

PCs existing in reuse potable water could affect human and plants due to chronic exposure for a long term. They could cause several disorders in respiratory, and reproductive systems for human. Besides, the presence of PCs in the environment could cause breast and testicular cancers, and birth defects. On the other hand, PCs could affect plants by lowering the production rate which leads to a massive death in livestock (Chander et al., 2016).

Limited work has been done to assess the types and levels of PCs in domestic wastewater in arid and semi-arid countries. In these countries, reuse or artificial recharge of TSE is commonly practiced. Since soil and aquifer material in these

countries have low organic matter content, induced PCs due to reuse or artificial recharge of TSE could pose a higher potential to contaminate aquifers.

Some PCs could have the ability to be sorbed onto sewage sludge. Sorption of PCs on sludge depends on the physical and chemical properties of PCs, and sludge characteristics. Martín et al. (2012) investigated the level of PCs in primary, secondary, and digested sludge. Samples collection done from four WWTPs at different locations (north, south, east, west). The authors found that ibuprofen and salicylic acid are present in the different sludge samples at the highest concentration relative to those analyzed as indicated in Table 4. Since sludge could be used as a soil conditioner or fertilizer, its usage in this case could have an ecotoxicological effect on plants. Additionally, PCs could enter the food chain by the uptake of plants.

Table 4: PCs in different sludge samples for four locations (North, South, East, West) (Martín et al., 2012)

PCs	Mean concentration (μg/kg dry matter)											
	Primary sludge				Secondary sludge				Digested sludge			
	N	S	E	W	N	S	E	W	N	S	E	W
Anti-inflammatory drugs												
Diclofenac	< 33.1	< 33.1	< 33.1	< 33.1	< 19.5	< 19.5	< 19.5	< 19.5	< 1.22	< 1.22	< 1.22	< 1.22
Ibuprofen	2988	1425	2728	1683	1889	687	3237	524	1020	1274	1262	1124
Naproxen	40.1	66.6	72.2	23.8	41.4	34.3	32.9	50.4	<7.53	<7.53	<2.38	<7.53
Antibiotics												
Sulfa-methoxazole	<8.87	<8.87	<8.87	<8.87	<10.4	<10.4	<10.4	<10.4	<47.2	<47.2	<47.2	<47.2
Trimethoprim	<53.2	<53.2	<53.2	<53.2	<81.8	<81.8	<81.8	<81.8	<24.6	<24.6	<24.6	<24.6
Antiepileptic drug												
Carba-mazepine	20.3	30.3	<14.8	66.6	259	262	231	460	28.4	18.5	30.8	18.4
β-Blocker												
Propranolol	<1.60	<1.60	<1.60	<1.60	8.58	13.9	32.5	26.6	<1.26	<2.51	<1.26	<1.26
Nervous stimulant												
Caffeine	674	527	401	183	<20.4	<20.4	<20.4	<20.4	<45.4	<45.4	<45.4	116
Estrogens												
17α-Ethynyl estradiol	46.8	28.5	33.0	52.7	105	103	23.8	160	56.8	70.5	19.8	60.2
Estrone	<10.1	<10.1	<10.1	<10.1	<7.68	<7.68	<7.68	<7.68	<4.95	<4.95	<4.95	<4.95
Lipid regulators												
Clofibric acid	<32.9	<32.9	<32.9	<32.9	<36.4	<36.4	<36.4	<36.4	<38.1	<38.1	<38.1	<38.1

N means North, S means South, E means East, and W means West

The intensive use of PCs especially antibiotics as a human therapy could result in the release of these compounds to WWTPs which could lead to the development of antibiotic resistance bacteria. These bacteria could develop during the biological treatment process when they are mixed with antibiotics, leading to less therapeutic potential against pathogens when released into the environment. However, the current knowledge of antibiotic resistance bacteria and their types in the environment are paradoxical and scarce (Rizzo et al., 2013).

The knowledge of the effect of PCs or their derivatives on human and aquatic life is still not clear and further research in this area is needed. Despite the fact that the concentration of released PCs in the environment is very low, the main concern is of the long-term exposure to such levels (Gracia et al., 2012). Table 5 gives a summary for different PCs classes and their ecotoxicological effect on different organisms (Martín et al., 2012).

Table 5: Examples of PCs effect on different organisms (Martín et al., 2012)

PC Class	PC name	Organism effected by PC
Anti-inflammatory	Ibuprofen	*H. attenuata* (invertebrate)
	Ketoprofen	*V. fischeri* (bacteria)
	Naproxen	*H.attenuata* (invertebrate)
Antibiotics	Sulfamethoxazole	*P. subcapitata* (algae)
	Trimethoprim	*D. magna* (invertebrate)
Antiepileptic	Carbamazepine	*D. magna* (invertebrate)
β blocker	Propranolol	*D. subspicatus* (algae)
Stimulants	Caffeine	*Leuciscus Idus* (fish)
Estrogen	Estriol	*S.purpuratus* (invertebrate)
	Estrone	*T. battagliai* (invertebrate)
Lipid Regulators	Clofibric acid	*D. magna* (invertebrate)
	Gemfinrozil	*H.attenuata* (invertebrate)

2.6 Risk Assessment

The impact of PCs on the environment is evaluated by using the risk quotient (RQ) for TSE. RQ is assessed for each PC by dividing its concentration in TSE by the corresponding predicted non-effect concentration (PNEC). Usually, risk assessment uses the worst case of assumption which is the highest concentration that could be available in the environment, while the PNEC is evaluated by using half maximal effective concentration (EC50) or lethal concentration (LC50) for different types of organisms (algae, fish, bacteria and invertebrate). Currently, data related to PNEC for PCs and their effect is limited and this could be considered as a constrain that restricts the ability of full assessment. Table 6 shows the PNEC values for some PCs. Risk analysis generally uses the risk ranking criteria which is applied for RQ; where RQ > 1 indicates high risk, $0.1 \leq RQ \leq 1$ indicates medium risk, and RQ < 0.1 indicates low risk (Ashfaq et al., 2017; Kosma et al., 2014; Oliveira et al., 2015).

Table 6: PNEC values for some PCs

PC	PNEC Value (μg/L)	Species affected	Reference
Ibuprofen	1.65	Fish	(Ashfaq et al., 2017)
	6.6	Green algae	
	0.2	*O. latipes*	
	0.01	*O. latipes*	
Naproxen	2.62	Fish	
	6.6	*C. dubia*	
Amoxicillin	0.0037	Algae	(Verlicchi et al., 2012)
Acetaminophen	1	*Daphnia*	
17α-ethinylestradiol	0.00004	*P. promelas*	(Orias and Perrodin, 2013)
Estriol	0.0075	*O. latipes*	
Estrone	0.00016	*O. latipes*	
Caffeine	0.00005	*X. laevis*	

2.7 Detection of PCs in Wastewater

The determination of PCs in wastewater samples started in 1990. It was very late because it takes time to develop an analytical method for the determination of these compounds in an aqueous matrix such as wastewater. Moreover, many PCs do not have actual guidelines describing their concentration in treated domestic or industrial wastewater (Löffler et al., 2005). However, during the last two decades, more attention was given to the detection and determination of PCs in wastewater especially in Europe, USA and Canada (Anderson et al., 2004; Carrara et al., 2008; Holm et al., 1995).

Generally, the analysis of trace PCs is carried out in a sequence of steps. First, an analytical protocol for the identification and quantification of the target PCs should be developed. Then, extraction of PCs from wastewater samples is done using organic solvents in a process which known as solid-phase extraction (SPE). A new advanced method of extraction known as the direct injection was used recently. This method considered as environment-friendly "green Chemistry" because it decreases the amount of solvent used, costs, and save time compared to other extraction methods (Martínez et al., 2011).

Liquid or gas chromatography (LC or GC) instruments are used for separation, depending on the analyte types. LC or GC is coupled with mass spectrometry (MS) detector for proper identification of the analytes. It should be indicated that SPE, liquid chromatography-tandem mass spectrometry (LC-MS/MS), and gas chromatography-tandem mass spectrometry (GC-MS/MS) are techniques that have been used by others to extract and analyze PCs in wastewater samples (Buser et al., 1998; Busetti et al., 2009).

An extensive monitoring of PCs in wastewater has been performed by many researchers in the last decade. Gracia et al., (2012) studied the presence of PCs in wastewater in Valencia, Spain. The authors collected samples from the influent and effluent of WWTPs in two different seasons. Sample analysis was done by using LC-MS/MS instrument with electrospray ionization (ESI) as a source for ionization. Results showed the availability of 17 PCs such as ibuprofen, naproxen, and acetaminophen. Whereas, Heberer (2002) applied a program to monitor the influent and effluent of WWTPs in Berlin, Germany between 1996 and 2000. Their samples were extracted by SPE then analyzed using GC-MS/MS. Results showed the presence of ibuprofen, caffeine and other PCs at different concentrations in the influent and the effluent.

2.8 Levels of PCs in Wastewater

Levels of PCs have been identified in raw and treated wastewater by different studies in different countries in Europe (Italy, Spain, Greece and Belgium) and US (Bacaloni et al., 2005; Gracia et al., 2012; Kosma et al., 2014; Martín et al., 2012; Oliveira et al., 2015; Van De Steene and Lambert, 2008; Zuccato et al., 2010).

Table 7 lists the concentration of PCs in the influent and effluent of domestic WWTPs along with their range values as reported by others. The table also shows the location for each study, instrument limit of detection (IDL), limit of quantitation (LOQ), and the removal efficiency (RE) for influent and effluent samples. Notice that all PCs were detected by LC-MS/MS instrument. In addition, some values were not detected (ND) in the analyzed wastewater samples and other values were not reported (NR).

Table 7: Range of some PCs (μg/L) in the influent and effluent of domestic WWTPs[a]

PCs	Influent	Effluent	IDL	LOQ	RE	Country	Reference
Acetaminophen	1.13-201	ND	NR	0.11-0.09	0.93–1.0	Spain	Gracia et al. (2012)
Ibuprofen	2.28–39.8	ND	NR	0.64-0.25	0.89–0.99		
Naproxen	0.27-3.58	ND-0.72	NR	0.05-0.03	0.8–0.89		
Cotinine	4.28-27.72	0.38-9.53	0.18-0.17	0.21-0.20	0.66–0.91		Martínez et al. (2011)
Phenylephrine	0.89-4.50	0.51-1.99	0.5-0.5	0.5-0.5	0.43–0.56		
Caffeine	18.31-96.15	1.21-53.2	0.2-0.2	0.2-0.2	0.45–0.93		
Amoxicillin	0.018	ND	NR	0.0005-0.002	NR	Italy	Zuccato et al. (2010)
Spiramycin	0.603	0.375	NR	0.0005-0.002	0.38		
Acetaminophen [b]	55.05-91.28	0.03-16.72	NR	NR	0.7–1.0	USA	Oliveira et al. (2015)
Ibuprofen [b]	11.54-33.25	0.07-1.69	NR	NR	0.85–1.0		
Naproxen [b]	6.95-18.39	0.1-1.45	NR	NR	0.79–0.99		
Noscapine [b]	0.01	0.01	NR	NR	0		
Caffeine [b]	73.96-88.33	0.04-11.65	NR	NR	0.86–1.0		
Cotinine [b]	0.82-1.52	0.01-0.14	NR	NR	0.85 – 0.99		
Genistein	0.025-0.053	0.009-0.019	NR	NR	0.64–0.98	Italy	Bacaloni et al. (2005)
Ketoconazole	143	1.12	0.005	0.01	0.99	Belgium	Van De Steene and Lambert (2008)
Ibuprofen	2.8-25.4	0.5-2.6	0.019	0.057	0.82–0.9	Greece	(Osma et al. (2014), (2010)
Caffeine	17.1-113.2	1.9-13.9	0.014	0.043	0.88–0.89		
Naproxen	ND-2.0	ND-0.7	0.043	0.131	0.48–0.62		

[a] ND means not detected and NR means not reported. [b] The range represents average value of four WWTPs.

2.9 Role of Conventional WWTPs in Removing PCs

Domestic WWTPs use physical, chemical, and biological methods to treat the wastewater. WWTPs typically consist of primary treatment processes, followed by secondary treatment and some treatment plants may have units for advanced treatment. A typical flow diagram of a domestic WWTP is shown in Figure 4.

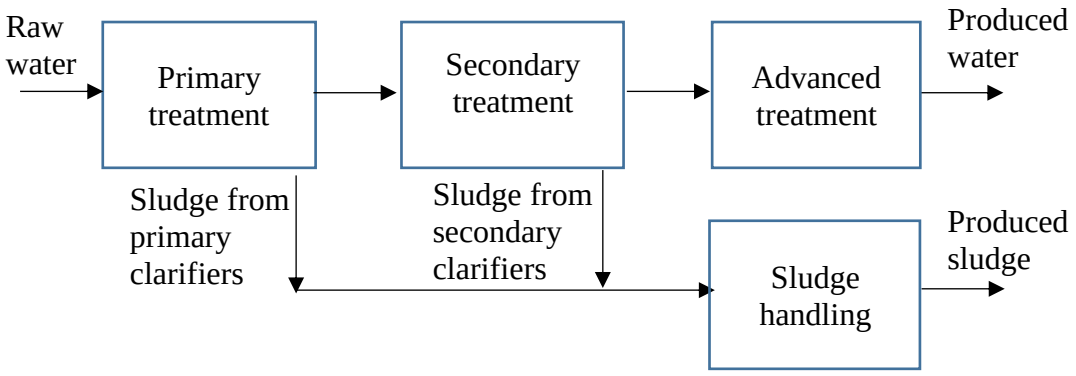


Figure 4: A schematic diagram of a typical domestic WWTP

Primary treatment is used to remove large debris by bar and travelling screen and grit particles by a grit chamber. In most of treatment plants, primary settling is included to remove a portion of the suspended solids (about 60%) and could remove some organic matter (about 30%). After primary treatment, the wastewater is processed by secondary treatment units. Secondary treatment employs biological treatment to reduce organic matter. The commonly used biological system is activated sludge, but other systems such as trickling filters or rotating biological contactors could be employed. The activated sludge system consists of aeration tanks followed by secondary settling tanks (clarifiers). Part of the sludge which settles in the secondary settling tanks is returned to the aeration tanks, while the rest is sent to sludge handling units for further processing before disposal. Advanced treatment could include filtration to reduce suspended solids, activated carbon system for sorption of

contaminants, phosphate removal, or nitrate removal. The final step in the treatment of the water stream is disinfection (usually using chlorine).

Treated wastewater (effluent of a WWTP) is either disposed of or recycled for different usage including landscaping, firefighting, or cooling. Wastewater could also be treated to achieve a drinking water quality (Kolpin et al., 2002).

Generated sludge from primary and secondary treatment is usually treated biologically under either aerobic or anaerobic conditions. The sludge is then dewatered by filter press where cationic polymers are added to enhance the dewatering process. It is then dried by exposure to sunlight in drying bed. Dried sludge is either disposed of in a landfill or utilized as a soil conditioner.

PCs at conventional WWTPs could be subject to transformation and transfer mechanisms that affect their levels in the produced water. They could be sorbed to sludge particles, to the filter media, or in an activated carbon system. PCs could be subject to biological degradation during the secondary treatment and the sludge handling processes. They may also be subject to chemical oxidation when the water is exposed to chlorination, ozonation or ultraviolet radiation (Wang and Wang, 2016).

Chapter 3: Methodology

3.1 Introduction

This chapter is organized into two different parts. The first part describes the development of an analytical protocol which was used to analyze the PCs in wastewater samples, while the second part describes the sampling site (Al Saad WWTP).

Development of an analytical protocol is described in sections 3.2-3.9. Section 3.2 defines the PCs and their classes and lists the physicochemical properties of the studied PCs. Sections 3.3 to 3.5 describe the preparation of stock solutions, preparation of calibration curves, and type of internal standards which were used in this work. Sections 3.6 to 3.8 give more details about the used LC-MS/MS technique, procedure used in the analysis and extraction of PCs from wastewater and sludge samples, and the determination of the limit of detection and limit of quantitation of the analytical method.

The second part (Section 3.9) gives details about the sampling site, Al Saad WWTP. Section 3.9.1 shows the location of the plant, while section 3.9.2 contains a simplified flow sheet diagram for the plant. Sections 3.9.3 and 3.9.4 provide details about the design of each unit process in the plant and and review the palnt historical record for some parameters during the last 5 years. Moreover, sections 3.9.5 and 3.9.6 identify the location of the collected samples and the way they were collected and prepared for analysis. Finally, section 3.10 and 3.11 show how the removal efficiency and mass balance were calculated for different unit processes.

3.2 Target PCs

(±)-Cotinine-D_3 was used as an internal standard (IS) as it is similar in structure to cotinine with the exception that 3 hydrogen atoms have been replaced by 3 deuterium atoms. The selected PCs with their structures and classes are listed in Table 8, while Table 9 lists the physiochemical properties of the target PCs. All PCs were purchased from Sigma-Aldrich in a standard analytical grade purity (> 99%). The stock solutions were prepared in methanol and stored at -18°C. The working solutions were prepared by dilution in deionized water (DI-H_2O) (Milli-Q, Elix Technology Inside).

Table 8: Selected PCs for LC/MSMS analyses and their chemical structure

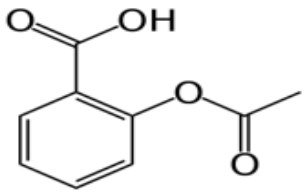
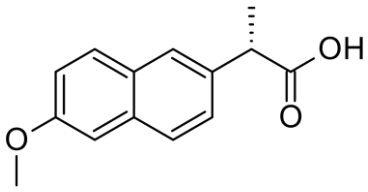
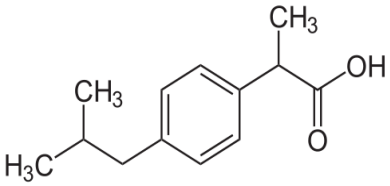
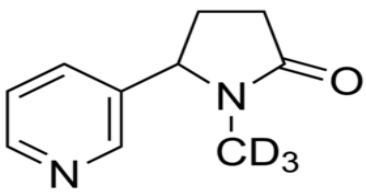
PCs	Class	Chemical structure
Aspirin	Nonsteroidal Anti-Inflammatory Drug (NSAID)	
Naproxen	NSAID	
Ibuprofen	NSAID	
(±)-Cotinine-D3 **IS**	Antidepressant	

Table 8: Selected PCs for LC/MSMS analyses and their chemical structure (continued)

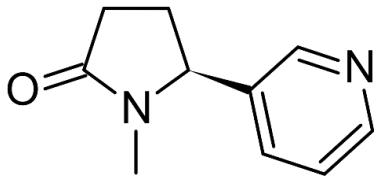
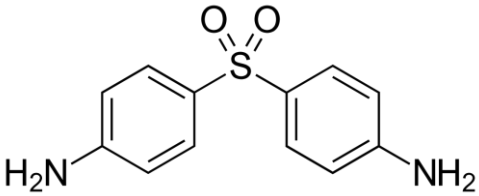
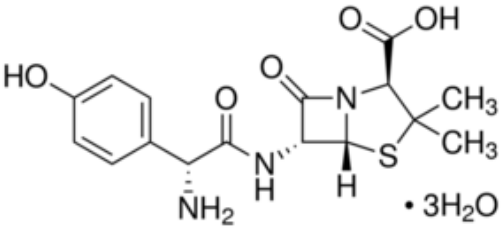
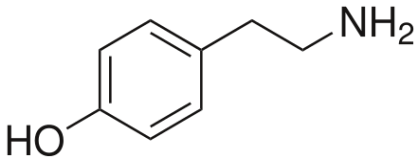
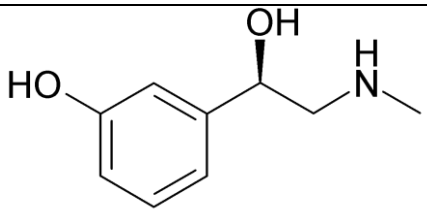
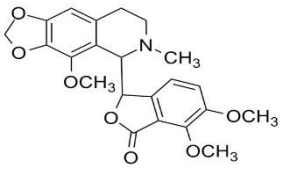
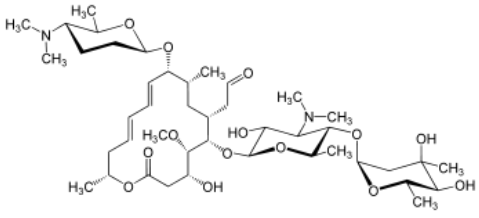
PCs	Class	Chemical structure
Cotinine	Antidepressant	
Dapsone	Antibiotics	
Amoxicillin	Antibiotics	
Tyramine	Antibiotics	
Phenylephrine	Antibiotics	
Noscapine	Antibiotics	
Spiramycin	Antibiotics	

Table 8: Selected PCs for LC/MSMS analyses and their chemical structure (continued)

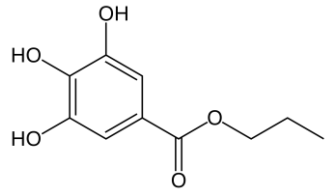
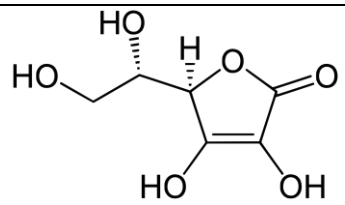
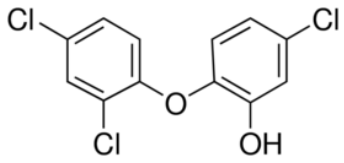
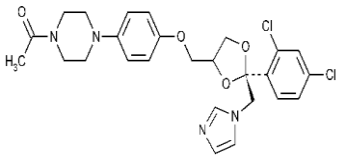
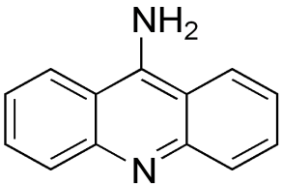
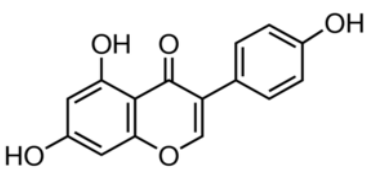
PCs	Class	Chemical structure
Propyl gallate	Antioxidant	
L- Ascorbic acid	Antioxidant	
Irgasan	Antifungal	
Ketoconazole	Antifungal	
9-Aminoacridine	Antiseptic	
Genistein	isoflavones	

Table 8: Selected PCs for LC/MSMS analyses and their chemical structure (continued)

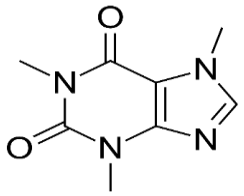
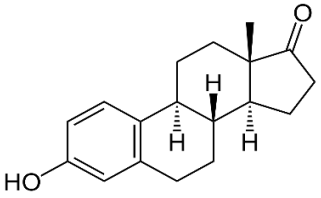
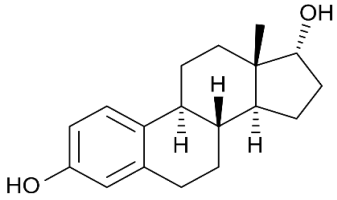
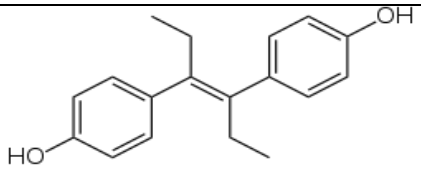
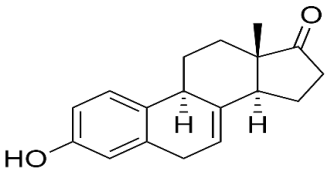
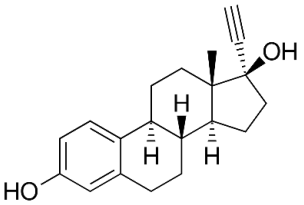
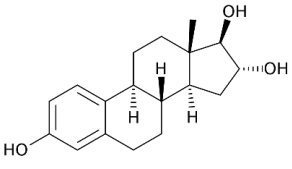
PCs	Class	Chemical structure
Caffeine	Stimulant	
Estrone	Estrogen Hormone	
α-Estradiol	Estrogen Hormone	
Diethylstilbestrol	Estrogen Hormone	
Equilin	Estrogen Hormone	
17α-Ethynylestradiol	Estrogen Hormone	
Estriol	Estrogen Hormone	

Table 9: Physicochemical properties of the target PCs [a] (("Drugs - DrugBank," n.d.; "PubChem," n.d.)

PCs	Molecular Formula	Molecular Weight (g/mol)	Vapor Pressure (mm Hg)	pKa	Solubility (mg/L)	Log Kow
(±)-Cotinine-D3 **IS**	$C_{10}H_9D_3N_2O$	179.23	3.81×10^{-4}	4.79	1000000	0.07
Phenylephrine	$C_9H_{13}NO_2$	167.20	2.2×10^{-15}	8.97	1000000	-0.31
Tyramine	$C_8H_{11}NO$	137.18	3.0×10^{-3}	9.66 & 10.41	10400	-0.72
Cotinine	$C_{10}H_{12}N_2O$	176.21	3.81×10^{-4}	4.79	1000000	0.07
Acetaminophen	$C_8H_9NO_2$	151.16	6.29×10^{-5}	-4.4 & 9.46	14000	0.46
Amoxicillin	$C_{16}H_{25}N_3O_8S$	419.44	4.69×10^{-17}	3.23 & 7.22	3430	0.87
Caffeine	$C_8H_{10}N_4O_2$	194.19	9.0×10^{-7}	- 0.92 & 14	21600	-0.07
Dapsone	$C_{12}H_{12}N_2O_2S$	248.3	2.68×10^{-8}	2.39	380	0.97
9-Aminoacridine	$C_{13}H_{10}N_2$	194.23	NR	9.29	1000	2.74
Spiramycin	$C_{43}H_{74}N_2O_{14}$	843.06	9.9×10^{-31}	7.88, 9.28 & 12.53	196	1.87
Noscapine	$C_{22}H_{23}NO_7$	413.426	NR	6.44 & 14.59	181	2.88
Propyl gallate	$C_{10}H_{12}O_5$	212.201	2.6×10^{-7}	-5.5 & 9	1710	1.8
Genistein	$C_{15}H_{10}O_5$	270.24	5.2×10^{-12}	-5.3 & 6.55	123	2.84
Ketoconazole	$C_{26}H_{28}Cl_2N_4O_4$	531.43	6.41×10^{-14}	3.96, 6.75 & 4.6	0.29	4.35
Naproxen	$C_{14}H_{14}O_3$	230.26	1.89×10^{-6}	-4.8 & 4.19	15.9	3.18
Ibuprofen	$C_{13}H_{18}O_2$	206.28	4.74×10^{-5}	4.9 & 5.3	21	3.97

[a] Ka is the acid dissociation constant and Kow is the octanol-water partition coefficient.

3.3 Stock Solutions Preparation

The preparation of stock solutions for each PC was performed as follows: An exact mass of an individual PC standards in the range of 0.5 – 10 mg was weighted and transferred into a 10.0 mL dark glass vial. The weighted powder of PCs was dissolved in methanol to reach a concentration of 1.0×10^{-3} M. The vials were transferred to an ultra-sonic water bath to assure the complete dissolvation of all solids. The stock solutions were stored in the refrigerator at a temperature below -17°C. A fresh working solution was prepared each month by proper dilution of the stock solutions using ultra-pure water to get 100 ppm.

3.4 Calibration Curves Preparation

An exact volume of 0.50 mL from each stock PCs solution (100 ppm) was measured and mixed together in one 10.0 mL vial. The measured mixed volume was evaporated using the technique of vacuum - centrifuge (CentriVap Concentrator-Labconco) for 1 day. After evaporation, the precipitated PCs solid remaining in the vials were dissolved again by adding 1 mL of mixture of water: methanol: acetonitrile (90:5:5), (v/v/v) to get 50 ppm for each PC. The calibration curve standards (50, 30, 10, 5, 1, 0.5, 0.1, 0.05 ppm) were prepared by serial dilutions using the 50 ppm solution.

3.5 Internal Standard

(±)-Cotinine-D_3 was used as an IS due to its structural similarity with other PCs. Moreover, deuterated compounds can’t be found in wastewater or in nature. Therefore, it can be used for labeling during processing of real wastewater samples. A

stock solution was prepared in dark glass vail by dissolving 5 mg (±)-cotinine-D_3 in 5 ml methanol (1000 ppm) and stored at -17°C. A working solution (50 ppm) was prepared from stock in ultra-pure water using proper dilution. 100 µL working solution (50 ppm) was spiked in each calibration curve standard to have 5 ppm of (±)-cotinine-D_3 as spiked concentration. Additionally, the same volume was spiked in real samples to have the same concentration of 5 ppm in all samples.

3.6 Liquid Chromatography Technique

High-Performance liquid chromatography (HPLC) is a quick, efficient, automated, sensitive, highly accurate and resolving method used to separate and identify certain chemical components in a sample. The separation of chemical compounds on reverse phase stationary phase is based on their polarity by using a liquid mobile phase and a solid stationary phase (Dong, 2013). Usually, the HPLC is combined with single or triple quadruple tandem mass spectroscopy using ESI interface.

In ESI, the sample components are sprayed using nitrogen gas at high speed through a capillary tube. High voltage is applied on the capillary in order to generate ions from solution that are going out in the form of charged droplets of fine spray in a process known as “Taylor cone”. The electric charge density of droplets surface increases as its size decreases. This leads to higher repulsion of the like charges of droplets surface which causes the ions to leave the droplets. The charged droplets evaporate which cause a further decrease in size until they reach a point (Rayleigh limit) to form singly or multiply-charged gaseous ions. The coulomb fission occurs due to smaller surface size of droplets. The produced gaseous ions droplets are smaller

and have a much higher charge to mass ratio. Figure 5 shows a schematic diagram for the ESI process (Banerjee and Mazumdar, 2012).

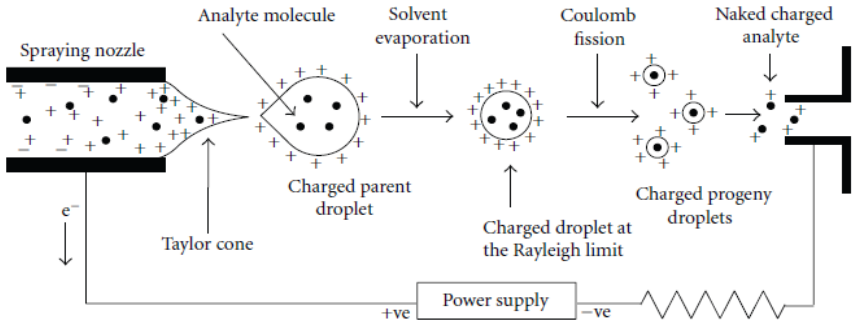


Figure 5: Schematic of the ESI process (Banerjee and Mazumdar, 2012)

The quadruple tandem mass spectroscopy is a mass detector used to identify and analyze the mass of the chemical compounds. It has two types which are single quadruple mass spectroscopy (MS) or triple quadruple tandem mass spectroscopy (MS/MS) which is considered as a combination of two mass analyzers. The first mass analyzer (Q1) works as a mass filter and analyzes the precursor ions. It is followed by a collision cell (Q2) which uses a high energy and an inert gas such as Helium, Argon or Nitrogen to fragment the precursor ions. While the second mass analyzer (Q3) is considered as a mass filter to analyze the product ions. The tandem mass spectrometry (MS/MS) mode has an advantage over the single mass spectrometry (MS) mode. It's more selective and sensitive as well as it reduces the interference and noise from other compounds and the matrix. Figure 6 shows the MS/MS which contains different mass filters.

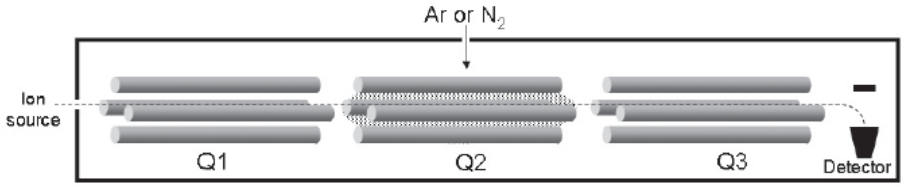


Figure 6: MS/MS which contains different mass filters

3.6.1 LC-MS/MS Procedure

The LC-MS/MS analyses were performed on Nexera-i Liquid Chromatograph (LC-2040C) by using a reversed phase column DISCOVERY HS C18 (Supelco) (length 250 mm, internal diameter 4.6 mm, and particle diameter 5μm). The instrument is coupled to a Shimadzu (LCMS-8030) triple quadruple mass spectrometer. Both positive and negative ESI modes were used for ionization of the PCs (Figure 7). Table 10 gives a summary of the instrumental conditions used for developing an acquisition LC - (+/-) ESI-MS/MS method which is used in this work. An LC flow rate of 0.6 mL/min was used to reduce the elution time of all PCs. The flow was splited and the flow rate becomes 0.05 mL/min for eluate going through the MS detector in order to increase the MS sensitivity. All PCs were detected using a multiple reaction monitoring (MRM) mode following the steps below.

Step 1. Get in acquisition parameters and define precursor ion masses.

Step 2. Determine optimum voltage (maximum response) for each fragment.

Step 3. Define product ion masses and determine the optimum collision energy.

Step 4. Create a batch file for all MRM compounds.

Table 10: LC-MS/MS method parameters and conditions used for the analysis of PCs

Column	DISCOVERY HS C18 (Supelco) (5µM 25cm X 4.6 mm)		
Column Temperature	40 ºC		
Mobile Phase	A: Aqueous solution of 10 mM Ammonium Formate pH 2.5 B: (1:1) ACN: MeOH		
Flow Rate Splitted Flow Rate	0.6 mL/min (0.05 mL/min)		
Gradient elution	Time (min)	% A	% B
	0	99	1
	5	99	1
	30	1	99
	39	1	99
	40	99	1
Post-Run	3 min		
Total Cycle Time	43 min		
MS Condition			
DL Temperature	250 ºC	Heat Block Temperature	400 ºC
Nebulizing Gas Flow	2.5 L/min	Drying Gas Flow	10 L/min
Interface Voltage	0 kV	Detector Voltage	0 kV
IG Vacuum	1.7e-003 Pa	PG Vacuum	1.3e+002 Pa
CID Gas	230 KPa		

Each PC has one precursor ion and at least two product ions. Product ions peaks were used for quantifications and qualifications.

Figure 7:LC-MS/MS used for the analysis of PCs in this study

3.7 Solid Phase Extraction Procedure

SPE is a technique used to prepare samples by separating/concentrating the dissolved or suspended compounds from the liquid mixture. Separation of compounds occurs depending on their chemical and physical properties. SPE is used to purify, concentrate and prepare samples for analysis by isolating compounds from the matrices (Augusto et al., 2013).

Horizon Technology SPE-DEX® 4790 Automated Extraction system (Figure 8) was used for the exaction of PCs from the analyzed samples. It uses a disk filter (Atlantic® HLB-M SPE, 47 mm) to collect and concentrate the PCs from the samples. Initially, the SPE instrument needs to be purged before filtration of any sample. The steps of purging are shown in Table 11. PCs were extracted from wastewater samples as shown in Table 12 (Ferrer and Thurman, n.d.). The volume of the extracted sample was almost equal to 40 mL; It was evaporated under a stream of air until dryness. After that, the dried sample was re-constituted by dissolving it in 0.1 mL of 1:1 mixture of methanol and acetonitrile followed by 0.9 mL of 10 mM ammonium formate buffer. The final ratio was 90:10 (v/v) concentrated samples each of 1 mL. The samples (1 mL each) were filtered by using Iso-Disc Syringe Filter Unit, PTFE membrane (pore size 0.22 μm, diameter 25 mm) then transferred into autosampler vials for LC-MS/MS analysis.

Table 11: The purge method used to clean the SPE instrument

Step	Solvent	Dry Time (sec)
Prewet 1	DI water	15
Prewet 2	Methanol	15
Wash 1	DI water	15
Rinse 1	Methanol	15

Table 12: Extraction method of PCs from the analyzed samples

Step	Solvent	Soak Time (sec)	Dry Time (sec)
Prewet 1	Acetone	30	15
Prewet 2	Acetone	30	15
Prewet 3	DI water	10	2
Prewet 4	DI water	10	2
Process sample			
Air Dry 30 sec			
Rinse step 1	Acetone	180	20
Rinse step 2	MeCl	180	20
Rinse step 3	MeCl	60	20
Rinse step 4	MeCl	60	60

Figure 8: SPE instrument used for purifying wastewater samples

3.8 Limit of Detection and Limit of Quantification

3.8.1 Instrument Limit of Detection and Limit of Quantification

The instrument limit of detection (IDL) is the minimum concentration of analyte that can be detected at a known confidence level. It can be obtained by using the Equation (1)

$$IDL = \frac{3s_{blank}}{m} \qquad (1)$$

Where s_{blank} is the standard deviation of the signal of blank replicates, and m is the slope of the calibration curve.

The instrument limit of quantification (LOQ), on the other hand, is the lowest concentration at which quantitative measurements can be made. It can be obtained by using the Equation (2).

$$LOQ = \frac{10 s_{blank}}{m} \qquad (2)$$

Since the (±)-Cotinine-D_3 was used as IS, so it is spiked in the blank samples to be used as a label with concentration of 5 ppm. This led to some modification in Equations (1) and (2) as shown in Equations (3) and (4)

$$IDL = \frac{3 \times \frac{s_{blank}}{s_{IS}}}{\frac{m_{sample}}{m_{IS}}} \qquad (3)$$

$$LOQ = \frac{10 \times \frac{s_{blank}}{s_{IS}}}{\frac{m_{sample}}{m_{IS}}} \qquad (4)$$

Where s_{blank} is the standard deviation of the signal of blank replicates, $s_{IS}$is the standard deviation of the signal of internal standard replicates, m_{sample} and m_{IS} are the slope ratio of the calibration curve.

The experiments were done by analyzing 20 replicates of a blank sample (spiked with 5 ppm (±)-Cotinine-D_3). Then, the blank signal at the retention time (t_R) of each one of the PCs was recorded. The standard deviation of each one of these signals was calculated based on the 20 replicates. After that, they were used with the slope ratio between the IS and of each of the PCs to find the values of IDL and LOQ. Note that these limits were estimated relative to the IS and the signal used for calculation is based on the intensity not the area.

3.8.2 Method Limit of Detection

The method LOD can be determined using the IDL value. For liquid samples, the conversion is done by dividing the IDL over the final volume of the sample. On the other hand, the method LOD of sludge samples was determined by dividing the IDL over the weight of extracted dry sludge.

3.9 Description of Al Saad WWTP

3.9.1 Al Saad WWTP Location

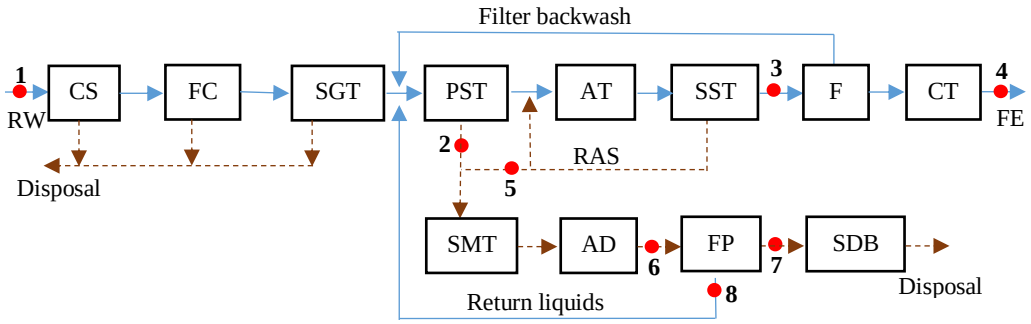
Al Saad WWTP is a domestic wastewater treatment plant that serves part of Al-Ain and it is located near to Al Ain city as shown in Figures 9 and 10. Figure 9 shows a map view for the location of the plant while Figure 10 shows a satellite view for the plant. The plant receives about 92,000 m^3/d of domestic sewage.

Figure 9: Map view of the location of Al Saad WWTP near Al Ain City

Figure 10: Satellite view of the location of Al Saad WWTP

3.9.2 Flow Sheet Diagram of Al Saad WWTP

A simplified flow sheet diagram for Al Saad WWTP is shown in Figure 11. The plant consists of primary treatment processes such as coarse screening, fine screening, sand & grease trap, and primary sedimentation. The primary treatment is used to remove large debris, sand, and a major portion of the suspended solids. Then it is followed by secondary treatment processes which include aeration and secondary sedimentation. The secondary treatment employs biological treatment to reduce organic matter by an activated sludge process. Some parts of the sludge which settles in the secondary settling tanks is returned to the aeration tank, while the rest is sent to anaerobic digesters for further processing before disposal. Water from the secondary clarifiers receives filtration before it is disinfected by chlorine.



RW: Raw wastewater, CS: Coarse screens, FC: Fine screens, SGT: Sand and grease trap, PST: Primary settling tank, AT: Aeration tank, SST: Secondary settling tank, F: Filter, CT: Chlorine contact tank, FE: Final effluent, SMT: Sludge mixing tank, AD: Anaerobic digestion, FP: Filter press, SDB: Sludge drying bed, RAS: Return activated sludge

Figure 11: Simplified flow sheet diagram of Al Saad WWTP

3.9.3 Al Saad WWTP Design

The design parameters including flow rate (Q_d) and associated loading in terms of biochemical oxygen demand (BOD_5), suspended solids (SS), total nitrogen (N_{tot}) and total phosphorus (P_{tot}) at different locations at Al Saad WWTP. These values are listed in Table 13. Moreover, the table shows the number of units and their sizes.

3.9.4 Historical Records

The actual measurement for some parameters including flow rate, recycle flow, wastage flow-SAS, total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), total Kjeldahl nitrogen, nitrate–N, ammonia, alkalinity, and pH of raw wastewater were obtained from the plant operators for the past 5 years (from 1-Jul-2013 to 31-Mar-2018). Table 14 contains a summary of these values (minimum, average, and maximum) during the above-mentioned period. Note that some parameters such as phosphate fraction, inorganic suspended solids, BOD, anions and cations were not measured at the site for the raw wastewater.

Table 13: Designed parameters for Al Saad WWTP at different locations

Location	Q_d (m^3/d)	BOD_5 (kg/d)	SS (kg/d)	N_{tot} (kg/d)	P_{tot} (kg/d)	Units and size
RW	92,000	33,856	40,204	4,830	966	
CS	92,000	33,856	40,204	4,830	966	1+1 Units 40mm bar spacing
FS	92,000	33,856	40,204	4,830	966	2+1 Units 6mm bar spacing
SGT	92,000	33,856	40,204	4,830	966	2 Units V= 2×285 m^3
PST	98,394	26,571	21,350	4,947	960	2 Units V= 2×2,540 m^3
SST	95,619	956	1,316	1,111	675	2 Units V= 4×5,800 m^3
F	91,876	395	459	995	623	5+1 Units Q= 6×1,354 m^3/h
CT	91,876	395	459	995	623	2 Units V= 2×850 m^3
FE	91,876	395	459	995	623	
RAS	2,778	4,335	20,298	1,361	285	
SMT	832	12,684	39,862	1,742	385	2 Units V= 2×430 m^3
AD	832	12,684	27,710	1,738	385	2 Units V= 2×9200 m^3
FP	123	11,620	27,001	1,240	292	2+1 Units Q= 3 × 27 m^3/h
SBD	31.8	11,620	27,001	1,240	292	A= 25,000 m^2

Table 14: Summary of 5-year record for some parameters at Al Saad WWTP

Parameter	Minimum	Average	Maximum
Flow (m^3/d)	67,035	79,988	170,186
Recycle flow (m^3/d)	65,132	77,743	116,138
Wastage flow-SAS (m^3/d)	643	2,374	4,006
TSS (mg/L)	32	196	910
VSS (mg/L)	22	127	550
COD (mg/L)	30	375	1,073
Total Kjeldahl nitrogen (mg/L)	21	34	77
Nitrate–N (mg/L)	0	1	3
Ammonia (mg/L)	1	24	34
Total phosphorus (mg/L)	2	4	35
Alkalinity (mmol/L)	139	224	494
pH	7	7	8

3.9.5 Sample Collection

Grabbed samples of water and sludge (1.0 liter each) were collected in labeled containers from Al Saad WWTP. Samples from eight different locations were collected from the plant as shown in Figure 11 (labeled on the figure from 1 to 8). Location 1 represents the inlet of the plant before treatment. This location represents the characteristics of generated wastewater in Al Ain. Sampling location 2 (before biological treatment) was selected to check adsorption of PCs on the settled sludge in the primary settling tanks. Sampling location 3 is located after the secondary settling tank. Sampling location 4 is located at the outlet of the plant after chlorination, which represents TSE which is usually used for landscaping. Sampling location 5 and 6 were intended to collect and analyze sludge samples before and after anaerobic digestion. Samples were collected from locations 7 and 8 to assess the level of PCs in the water and sludge that leaves the filter press unit. The filter press process requires the addition of a cationic polymer (Corofloc 341, SNF, France) to the sludge to make it thick.

The samples were collected from each location in four batches on different dates. The samples were collected on 10 October, 24 October, 6 November, and 25 November 2018. Although composite sampling will be better than grab sampling in presenting the average of PCs per day, samples collection in this work followed grab sampling due to difficulties in collecting different samples at different locations over a 24-hr period. After collection, samples were preserved in an ice box and transferred to the laboratory for analysis. Samples were placed in the refrigerator at a temperature below -17°C until being extracted by SPE. Once extracted, samples were frozen at a temperature below -25°C till analysis by LC-MS/MS.

3.9.6 Samples Preparation

Since some wastewater samples contain sludge and others do not, so the sample processing was done in two different ways. The first way was by processing the liquid samples which did not contain sludge such as the influent, effluent of secondary settling tank, filtered water of effluent anaerobic digester (filter press) and final effluent samples. IS was spiked into these samples as explained in section 3.5, then they were extracted and prepared as explained in section 3.7.

On the other hand, the sludge samples such as those from the primary settling tank, returned activated sludge, and effluent of anaerobic digester were filtered to separate sludge from the water. IS was added to these samples before filtration as explained in section 3.5. The filtration was done under vacuum by using Buchner funnel and 9 cm filter paper (Whatman 1 qualitative). Then the extracted filtered solution (water) was prepared as explained in section 3.9. The filtered sludge (left on the filter paper) was heated in the oven for 4 hours at 105 °C to remove the moisture content from sludge samples. Then manual extraction was done by adding 60 mL of acetone followed by mixing for 2 hours. Then the solvent was filtered through 9 cm filter paper (Whatman 1 qualitative). After that 100 mL of dichloromethane (MeCl) was added to the sample followed by mixing for 2 hours and filtration. The extracted samples (in acetone and MeCl) were evaporated by an air stream and re-dissolved in the mobile phase as explained in section 3.7. The sludge after the filter press machine was processed manually as explained for the filtered samples above.

3.10 Removal Efficiency

The removal of PCs from wastewater depends on different variables such as their biodegradability and physiochemical properties like water solubility, volatilization, and adsorption to sludge. Other factors could affect the removal such as the temperature of the unit of treatment (lower temperature reduce the efficiency of removal). Other parameters include pH which affects the kinetic of PCs compounds, redox reactions, the hydraulic retention time and the sludge retention time (Kosma et al., 2014; Oliveira et al., 2015).

The removal efficiency (RE) was calculated across different units (PST, SST, and FE) to understand the role of removal of each one. The RE was calculated by using Equation 5.

$$Removal\ Efficiency = \frac{(Cin - Cout)}{Cin} \times 100\% \qquad (5)$$

3.11 Mass Balance

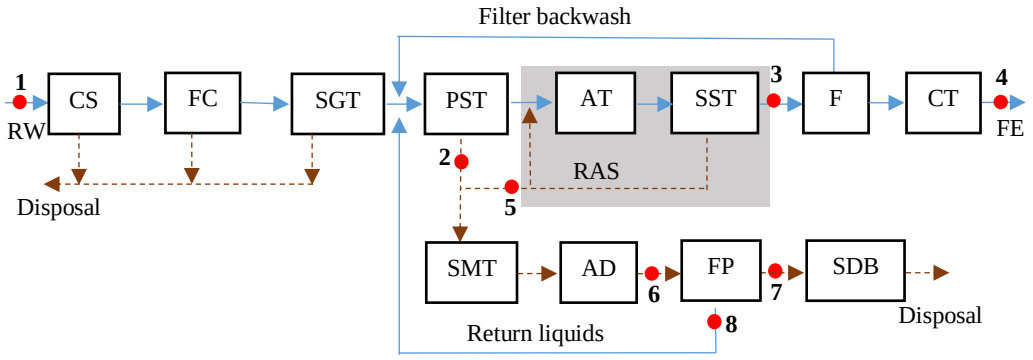
In this part of the study, the mass balance approach was applied for the PCs at different locations within the WWTP. According to the conservation of mass law: mass is neither created nor destroyed. The general mass balance equation (Equation (6)) is shown below.

$$\text{Rate of mass in} = \text{Rate of mass out} + \text{Rate of mass reacted} \qquad (6)$$

So, if the rate of mass out is less than the rate of mass in, it indicates mass loss due to reaction. Loss of mass (reaction) could be due to biodegradation, chemical degradation, or adsorption to the sludge.

3.11.1 Mass Balance Around the Aeration Tank

Mass balance was applied around the aeration tank to separate the role of biodegradation and sorption of PCs on the sludge. The shaded units (grey color) in Figure 12 showed the system of study.



RW: Raw wastewater, CS: Coarse screens, FC: Fine screens, SGT: Sand and grease trap, PST: Primary settling tank, AT: Aeration tank, SST: Secondary settling tank, F: Filter, CT: Chlorine contact tank, FE: Final effluent, SMT: Sludge mixing tank, AD: Anaerobic digestion, FP: Filter press, SDB: Sludge drying bed, RAS: Return activated sludge

Figure 12: Mass balance for the aeration tank

The removal efficiency values were calculated relative to the concentration in the raw wastewater. The removal efficiency after PST was determined based on a mass balance around the PST, while that after the SST and for the FE were determined based on the concentration of PCs at locations 3 and 4 (Figure 11), respectively. Equation 7 was used for calculation as shown below.

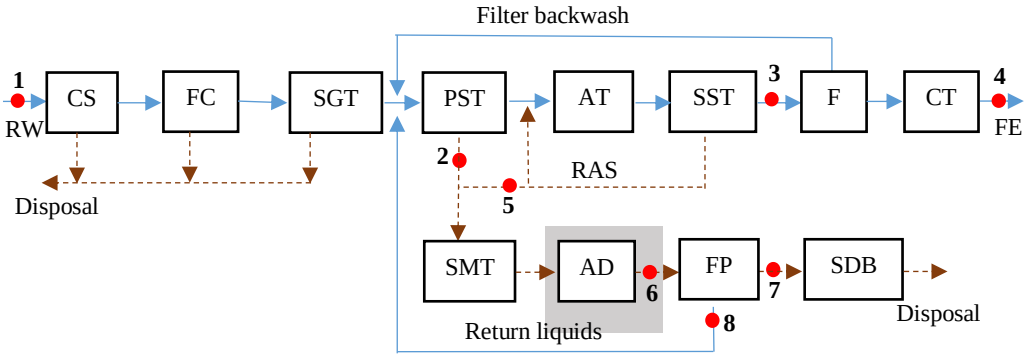
$$(Q_{in} \times C_{in}) + (Q_{FP} \times C_{FP}) = (Q_{out} \times C_{out}) + (SS_{PST} \times S_{PST}) + (Q_{RAS} \times C_{RAS}) + (SS_{RAS} \times S_{RAS}) \quad (7)$$

Where Q_{in} is the flow rate of raw wastewater (m^3/d), C_{in} is the concentration of PCs in the raw wastewater (µg/L), Q_{FP} is the flow rate of filter press (m^3/d), C_{FP} is the concentration of PCS in the effluent of the filter press water (µg/L), Q_{out} is the final

effluent flow rate (m^3/d), C_{out} is the concentration of PCs in the final effluent (µg/L), SS_{PST} is the rate of suspended solids in the PST (kg/d), S_{PST} is the concentration of PCs adsorbed to PST solids (µg/kg), Q_{RAS} is the flow rate of RAS (m^3/d), C_{RAS} is the concentration of PCs in RAS water (µg/L), SS_{RAS} is the rate of suspended solids in RAS water (kg/d), and the S_{RAS} is the concentration of PCs adsorbed to RAS solids (µg/kg).

3.11.2 Mass Balance for the Anaerobic Digester

The mass balance approach was applied around the anaerobic digester to check if some traces of PCs adsorbed to the sludge or degraded. The shaded unit (grey color) in Figure 13 showed the system of study.



RW: Raw wastewater, CS: Coarse screens, FC: Fine screens, SGT: Sand and grease trap, PST: Primary settling tank, AT: Aeration tank, SST: Secondary settling tank, F: Filter, CT: Chlorine contact tank, FE: Final effluent, SMT: Sludge mixing tank, AD: Anaerobic digestion, FP: Filter press, SDB: Sludge drying bed, RAS: Return activated sludge

Figure 13: Mass balance for the anaerobic digester

The removal efficiency values were calculated relative to the concentration in RAS in water and sludge at location 5. The removal efficiency after PST was determined based on mass balance around the PST, while that after the AD was determined based on the concentration of PCs at locations 2 and 6 (Figure 12),

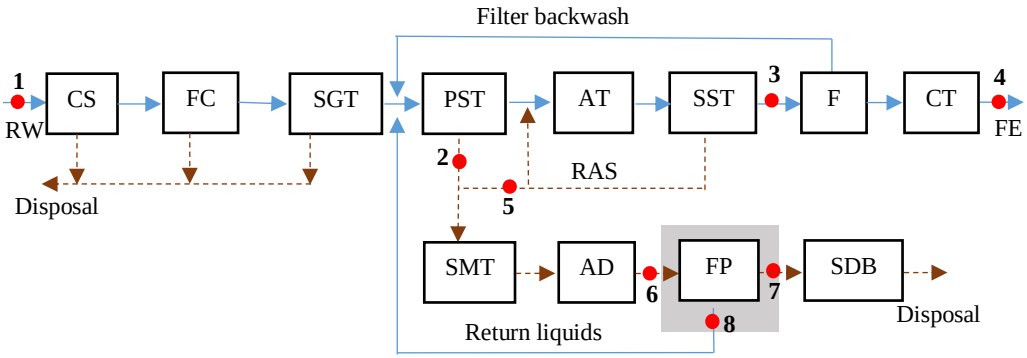
respectively. The mass balance equation for the anaerobic digester is given by Equation 8:

$$(Q_{RAS} \times C_{RAS}) + (SS_{RAS} \times S_{RAS}) + (SS_{PST} \times S_{PST}) = (Q_{AD} \times C_{AD}) + (SS_{AD} \times S_{AD}) \quad (8)$$

Where Q_{RAS} is the influent flow rate of RAS (m^3/d), C_{RAS} is the concentration of PCs in RAS water (μg/L), SS_{RAS} is the suspended solids in RAS water (kg/d), S_{RAS} is the concentration of PCs adsorbed to RAS solids (g/d), SS_{PST} is the suspended solids in the PST (kg/d), S_{PST} is the concentration of PCs adsorbed to PST solids (μg/kg), Q_{AD} is the flow rate of AD (m^3/d), C_{AD} is the concentration of PCs in AD water (μg/L), SS_{AD} is the suspended solids in AD water (kg/d), and the S_{AD} is the concentration of PCs adsorbed to AD solids (μg/kg).

3.11.3 Mass Balance for the Filter Press

Mass balance was also applied around the filter press unit to check the effect of addition of polymer for sludge dewatering. The shaded unit (grey color) in Figure 14 showed the system of study.



RW: Raw wastewater, CS: Coarse screens, FC: Fine screens, SGT: Sand and grease trap, PST: Primary settling tank, AT: Aeration tank, SST: Secondary settling tank, F: Filter, CT: Chlorine contact tank, FE: Final effluent, SMT: Sludge mixing tank, AD: Anaerobic digestion, FP: Filter press, SDB: Sludge drying bed, RAS: Return activated sludge

Figure 14: Mass balance for the filter press

The removal efficiency values were calculated relative to the concentration in AD in water and sludge at location 6. The removal efficiency after the FP unit was determined based on the concentration of PCs in water at location 7 (Figure 12), whereas the effect of the return liquids (point 8) was ignored due to its small flow rate. Equation 9 is used for the calculation as shown below.

$$(Q_{AD} \times C_{AD}) + (SS_{AD} \times S_{AD}) = (Q_{FP} \times C_{FP}) + (SS_{FP} \times S_{FP}) \quad (9)$$

Where Q_{AD} is the flow rate of AD (m^3/d), C_{AD} is the concentration of PCs in AD water (µg/L), SS_{AD} is the suspended solids in AD water (kg/d), S_{AD} is the concentration of PCs adsorbed to AD solids (µg/kg), Q_{FP} is the flow rate of AD (m^3/d), C_{FP} is the concentration of PCs in the FP water (µg/L), SS_{FP} is the suspended solids in FP water (kg/d), and the S_{FP} is the concentration of PCs adsorbed to FP solids (µg/kg).

Chapter 4: Development of an Analytical Protocol

4.1 Introduction

This chapter presents the results of the developed analytical protocol for the determination of PCs in wastewater. Section 4.2 describes the optimization process of PCs by ESI and their results of PCs, while section 4.3 shows the results of calibration for PCs. Sections 4.4 and 4.5 show the results of the IDL and LOQ of LC-MS/MS instrument and the method LOD.

4.2 Optimization

Each PC has one precursor ion and at least two product ions. Product ions peaks were used for quantifications and qualifications. Table 15 lists the precursor ion, product ions, dwell time, and collision energy for two detected PCs. The table also provides a summary for MRM transitions. Note that, out of the 23 originally selected PCs (Table 8) only 15 PCs were optimized by the ESI and could be detected in this study. Detected PCs by the developed analytical protocol include phenylephrine, cotinine, tyramine, amoxicillin, acetaminophen, caffeine, 9-aminoacridine, dapsone, spiramicin, noscapine, propyl gallate, genistein, ketoconazole, naproxen, and ibuprofen.

Table 15: Qualification and quantification MRM transition for PCs in wastewater using LC-MS/MS

PCs	Precursor ion (m/z)	Product ion (m/z)	Dwell time (msec)	Collision energy (V)
Phenylephrine	167.90	150.10	8.0	-13.0
		91.10	8.0	-22.0
		42.05	8.0	-28.0
Tyramine	137.90	121.10	8.0	-13.0
		77.00	8.0	-29.0
		51.05	8.0	-45.0
Cotinine	176.85	80.05	8.0	-23.0
		98.05	8.0	-22.0
		53.05	8.0	-45.0
(±)-Cotinine-D3 IS	180.05	80.1	8.0	-25.0
		81.1	8.0	-20.0
		101	8.0	-22.0
Acetaminophen	151.9	110.1	15	-17.0
		65.1	15	-31.0
		93.1	15	-23.0
Amoxicillin	365.95	349.15	40	-10.0
		114.05	40	-20.0
		208	40	-14.0
Caffeine	194.9	138.05	12	-19.0
		42.1	12	-35.0
		110	12	-23.0
Dapsone	248.9	156	12	-17.0
		92.1	12	-17.0
		108.05	12	-28.0
9-Amino acridine	194.95	93.1	12	-38.0
		77.05	12	-40.0
		50.95	12	-55.0
Spiramycin	843.7	174.05	8	-38.0
		101	8	-47.0
		142.15	8	-39.0
Noscapine	413.95	220.1	7	-24.0
		205.05	7	-48.0
		353.1	7	-26.0
Propyl gallate	210.7	124.15	7	23.0
		125.2	7	21.0
		78	7	38.0
Genistein	270.85	91.15	7	-39.0
		152.95	7	-28.0
		64.95	7	-52.0
Ketoconazole	532.95	82.05	9	-46.0
		491	9	-32.0
		246	9	-36.0
Naproxen	229.15	169.25	15	29.0
		170.25	15	15.0
		185.2	15	6.0
Ibuprofen	205.2	161.3	42	8.0
		140.9	42	13.0

4.3 Calibration Curves

The calibration range was 50 – 0.05 ppm with eight levels (50, 30, 10, 5, 1, 0.5, 0.1, and 0.05 ppm). Instrument responses at these concentrations were used to plot the calibration curve for each PCs. Figure 15 shows the chromatogram for all PCs standards at 30 ppm measured by LC-MS/MS through the MRM mode. As shown, the retention time (t_R) for these PCs ranges between 12.787 to 32.041 minutes. Some PCs could have the same t_R such as (±) cotinine D3 (IS), phenylephrine, tyramine, and cotinine, but the LC-MS/MS instrument can recognize each PC by using the MRM mode. The t_R values for all PCs are listed in Table 16.

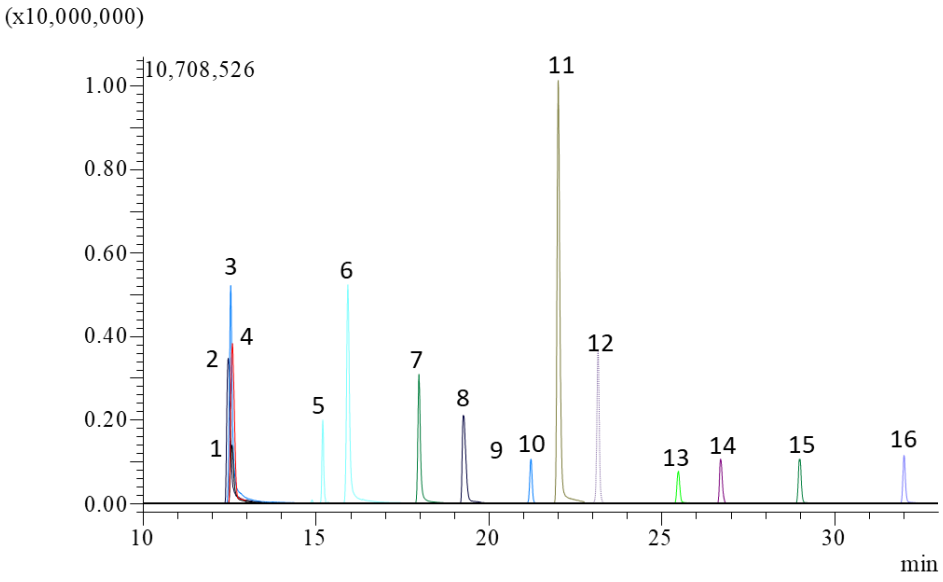


Figure 15: Spectrum for detected PCs. 1 (±) cotinine D3 (IS), 2 phenylephrine, 3 cotinine, 4 tyramine, 5 amoxicillin, 6 acetaminophen, 7 caffeine, 8 9-aminoacridine, 9 dapsone, 10 spiramicin, 11 noscapine, 12 propyl gallate, 13 genistein, 14 ketoconazole, 15 naproxen, 16 ibuprofen

Generated calibration curve for each PC has been done by calculating the ratio of chromatographic peak area between the PC and the IS versus the concentration of the PC. Figure 16 shows an example of a calibration curve for phenylephrine in a

mixture of water: methanol: acetonitrile (90:5:5), (v/v/v) solvent. Appendix A illustrates the calibration curves of the 15 PCs in the same solvent.

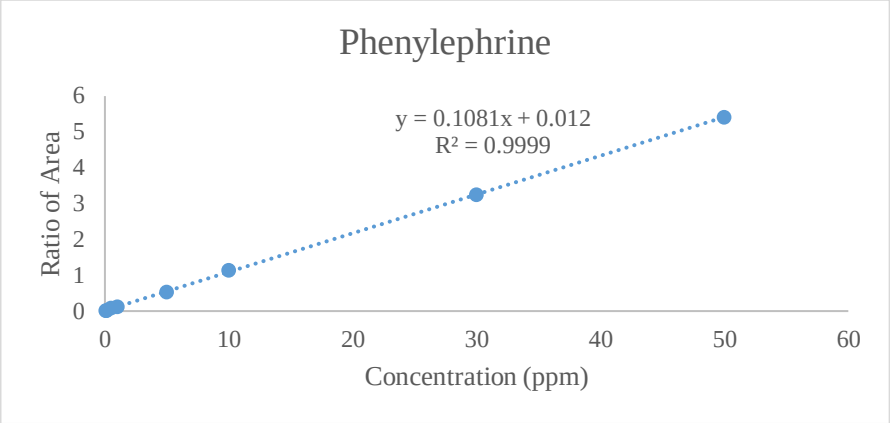


Figure 16: Calibration curve of phenylephrine in a mixture of water: methanol: acetonitrile (90:5:5), (v/v/v) solvent as analyzed by the LC-MS/MS instrument

4.4 Instrument Limit of Detection and Limit of Quantification

The IDL is calculated after analyzing 20 replicates of blank samples spiked with 5 ppm (±)-Cotinine-D_3 as explained in section 3.8.1. Values of the IDL and LOQ are listed in Table 16. As shown in Table 16, the IDLs values for all PCs range between 0.0004 and 0.5215 ppm, while the LOQ values range between 0.0015 and 1.7384 ppm.

Table 16 gives a summary for all PCs calibration curves, coefficient of determination (R^2), and t_R values. As shown in Table 16, the R^2 values range between 0.9831 and 1.0 for all PCs. This range indicates excellent linearity for the tested concentrations. Moreover, the t_R values vary between PCs.

Table 16: Calibration curves, retention time, IDL and LOQ summary for all PCs

PCs	t_R (min)	Calibration Equation	R^2	IDL (ppm)	LOQ (ppm)	Method LOD (µg/L) Liquid	Method LOD (µg/g) Sludge
Phenylephrine	12.803	y = 0.1081x + 0.012	0.9999	0.0121	0.0403	0.0120-0.0302	0.0002-0.0037
Tyramine	13.032	y = 0.1032x - 0.0436	0.9989	0.0093	0.0312	0.0093-0.0234	0.0001-0.0029
Cotinine	12.824	y = 0.101x + 0.0109	1	0.0114	0.0382	0.0114-0.0286	0.0002-0.0035
(±) cotinine D3	12.787	IS					
Acetaminophen	15.912	y = 0.1077x - 0.0555	0.9987	0.0056	0.0189	0.0056-0.0142	0.0001-0.0017
Amoxicillin	15.22	y = 0.0001x - 4E-05	0.999	0.5215	1.7384	0.5215-1.3038	0.0106-0.1618
Caffeine	17.977	y = 0.0577x - 0.028	0.9991	0.1728	0.5762	0.1728-0.4321	0.0035-0.0536
Dapsone	19.776	y = 0.0104x - 0.0037	0.9995	0.0398	0.1329	0.0398-0.0997	0.0008-0.0123
9-Amino acridine	19.198	y = 0.0525x + 0.0502	0.9958	0.1804	0.6016	0.1804-0.4512	0.0036-0.0559
Spiramycin	21.248	y = 0.0037x - 0.0066	0.9831	0.0158	0.0526	0.0158-0.0395	0.0003-0.0049
Noscapine	21.946	y = 0.1928x - 0.0741	0.9994	0.0056	0.0189	0.0056-0.0142	0.0001-0.0017
Propyl gallate	23.067	y = 0.0789x - 0.0454	0.998	0.0014	0.0047	0.0014-0.0035	2.89E-05-0.0004
Genistein	25.42	y = 0.0166x + 0.0001	0.9976	0.0564	0.1882	0.0564-0.1412	0.0011-0.0175
Ketoconazole	26.534	y = 0.0211x - 0.0198	0.9965	0.0071	0.0239	0.0071-0.0179	0.0001-0.0022
Naproxen	28.892	y = 0.0018x - 4E-05	0.9972	0.0004	0.0014	0.0004-0.0010	8.94E-06-0.0001
Ibuprofen	32.041	y = 0.001x - 0.0002	0.9971	0.0016	0.0054	0.0016-0.0041	3.36E05-0.0005

4.5 Method Limit of Detection

Table 17 shows the volumes and masses of liquid and solid samples for all collected batches of samples. The volume of liquid samples were recorded after filtration. On the other hand, the sludge mass was recorded after heating in the oven for 4 hours at 105 °C as explained in section 3.9.6.

Table 17: Volumes and masses of collected samples

Type of sample	Sample Name	Batch number			
		1	2	3	4
Liquid samples volume (mL)	RW	1000	1000	1000	1000
	SST	1000	1000	1000	1000
	FE	1000	1000	1000	1000
	RAS	960	965	980	940
	AD	400	840	845	860
	FP	1000	1000	1000	1000
Sludge samples mass (g)	PST	49.011	43.878	48.636	47.569
	RAS	3.799	4.379	3.930	3.223
	AD	4.831	30.975	26.131	36.994
	FP	9.843	18.683	10.094	12.704

The method LOD for all PCs can be obtained using the instrument IDL value for each PC (Table 16). For liquid samples, the method LOD was obtained by dividing the instrument IDL over the final volume of the sample (Table 17). Table 18 shows an example of calculation of LOD for liquid samples (influent). On the other hand, the method LOD for all PCs for sludge samples was determined by dividing the instrument IDL for each PC (Table 16) over the weight of the extracted dry sludge (Table 17). Table 19 shows an example of calculation of the method LOD for sludge samples (PST). Note that, the method LOD values for all PCs in liquid and sludge samples were calculated in the same manner as described above. The range of the method LOD for all the liquid and solid samples is shown in Table 16.

Table 18: Method LOD values for four batches of influent samples

PCs	LOD (μg/L) for batch number			
	1	2	3	4
Phenylephrine	0.012107	0.012107	0.012107	0.012107
Tyramine	0.009377	0.009377	0.009377	0.009377
Cotinine	0.011472	0.011472	0.011472	0.011472
Acetaminophen	0.005681	0.005681	0.005681	0.005681
Amoxicillin	0.521541	0.521541	0.521541	0.521541
Caffeine	0.172863	0.172863	0.172863	0.172863
Dapsone	0.039882	0.039882	0.039882	0.039882
9-Aminoacridine	0.18048	0.18048	0.18048	0.18048
Spiramycin	0.015803	0.015803	0.015803	0.015803
Noscapine	0.005691	0.005691	0.005691	0.005691
Propyl gallate	0.001421	0.001421	0.001421	0.001421
Genistein	0.056485	0.056485	0.056485	0.056485
Ketoconazole	0.007189	0.007189	0.007189	0.007189
Naproxen	0.000438	0.000438	0.000438	0.000438
Ibuprofen	0.001647	0.001647	0.001647	0.001647

Table 19: Method LOD values for four batches of settled sludge in PST

PCs	LOD (μg/g) for batch number			
	1	2	3	4
Phenylephrine	0.000247	0.000276	0.000249	0.000255
Tyramine	0.000191	0.000214	0.000193	0.000197
Cotinine	0.000234	0.000261	0.000236	0.000241
Acetaminophen	0.000116	0.000129	0.000117	0.000119
Amoxicillin	0.010641	0.011886	0.010723	0.010964
Caffeine	0.003527	0.00394	0.003554	0.003634
Dapsone	0.000814	0.000909	0.00082	0.000838
9-Aminoacridine	0.003682	0.004113	0.003711	0.003794
Spiramycin	0.000322	0.00036	0.000325	0.000332
Noscapine	0.000116	0.00013	0.000117	0.00012
Propyl gallate	2.9E-05	3.24E-05	2.92E-05	2.99E-05
Genistein	0.001152	0.001287	0.001161	0.001187
Ketoconazole	0.000147	0.000164	0.000148	0.000151
Naproxen	8.94E-06	9.99E-06	9.01E-06	9.22E-06
Ibuprofen	3.36E-05	3.75E-05	3.39E-05	3.46E-05

Chapter 5: PCs in Domestic Wastewater and their Removal at Al Saad WWTP

5.1 Introduction

This chapter discusses the results of PCs in Al Ain domestic wastewater and the role of different unit operations at Al Saad WWTP in removing these compounds from the waste stream. The chapter is organized in four sections. Section 5.2 lists the levels of PCs in the raw wastewater. Section 5.3 compares the RE of PCs for different units at Al Saad WWTP. The mechanisms of removal for PCs in the activated sludge system, AD system, and FP unit where presented and discussed in sections 5.4 and 5.5. Appendix B lists the raw data for all PCs at different units as determined by the employed analytical protocol.

5.2 PCs in Raw Wastewater

The concentration, average, and standard deviation of PCs in raw wastewater are listed in Table B1 (Appendix B) for four collected batches on different days. As shown, some PCs were not detected by the LC-MS/MS instrument, so their concentration were lower than the method LOD in one or more batches. For instance, phenylephrine had a lower concentration than its method LOD in batch 1 and 4. However, the average concentrations and standard deviations were calculated by setting non-detects at the corresponding method LOD. Some PCs such as acetaminophen and caffeine showed a high standard deviation value. This indicates a generally high fluctuation in the concentration among the different batches. However, there is no trend of increase or decrease in the level of PCs during the sampling period.

Based on the results presented in Table C1, the concentration of PCs varies between low (<0.1 µg/L), intermediate (i.e., 0.1-10 µg/L) and high (>10 µg/L). Specifically, phenylephrine, dapsone, noscapine, propyl gallate, genistein, and ketoconazole are present in the raw wastewater at low levels. On the other side, acetaminophen, and caffeine are present at high levels, while tyramine, cotinine, amoxicillin, 9-amioacridine, spiramycin, naproxen, and ibuprofen, exist at intermediate levels.

A comparison had been done between the average value of PCs found in this study and those reported in other studies (Table 7, section 2.8). In general, the concentration of PCs in this study is consistent with the findings of others. For example, acetaminophen had high levels which exceed 10 µg/L in some reported studies (Gracia et al., 2012; Oliveira et al., 2015). Furthermore, caffeine had a high level and generally exceeds 20 µg/L as reported by Kosma et al. (2010), Martínez et al.(2011), and; Oliveira et al. (2015). An exception, however, is a higher phenylephrine level reported by Martínez et al. (2011), a higher naproxen level reported by Oliveira et al. (2015), and a much higher ketoconazole level reported by Van De Steene and Lambert (2008). Meanwhile, ibuprofen, in our case, is at the lower end of the values reported by others (Gracia et al., 2012; Kosma et al., 2010; Oliveira et al., 2015).

5.3 Removal Efficiency of PCs

The RE values of PCs were calculated at different locations in Al Saad WWTP. Specifically, the RE values of each PC were calculated for PST, SST, and FE. Values of RE were calculated relative to the concentration in RW. The RE values for the PST were calculated based on applying mass balance around the PST (section 3.11). On the

other hand, RE values were calculated for SST and FE based on the concentration of PCs at locations 3 and 4 (Figure 11 – section 3.9.2), respectively.

As shown in Table 20, the PST had the lowest RE compared to SST and FE which indicates that it does not play a significant role in removing PCs from the waste stream. In other words, the mass of PCs in the raw wastewater are not adsorbed to the suspended solids, rather they are available in the liquid phase. Tyramine and caffeine both showed an exception with slightly average RE of 6.9% and 10.5%, respectively. Our results are in agreement with those of others (Gracia et al., 2012; Kosma et al., 2010; Oliveira et al., 2015) and showed that primary treatment plays a poor role in removing PCs.

Table 20: RE of PCs after PST, SST, and FE

PCs	RE-PST (%)			RE-SST (%)			RE-FE (%)		
	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg
Phenylephrine	0.1	16.4	4.3	0.0	88.8	33.0	0.0	88.8	33.0
Tyramine	0.0	19.8	6.9	0.0	99.8	71.4	0.0	99.9	70.1
Cotinine	0.7	1.6	1.1	98.3	99.6	99.3	98.2	99.8	99.3
Acetaminophen	0.0	0.2	0.1	99.7	100	99.9	100	100	100
Amoxicillin	0.4	0.5	0.4	0.0	17.1	4.3	0.0	17.1	4.3
Caffeine	4.5	14.4	10.5	99.6	99.8	99.8	99.6	100	99.8
Dapsone	0.2	10.2	4.5	0.0	58.4	29.2	0.0	58.4	24.6
9-Aminoacridine	0.4	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Spiramycin	0.0	0.4	0.3	0.0	98.9	24.7	0.0	98.9	24.7
Noscapine	0.5	2.0	1.3	0.0	83.4	41.7	0.0	83.4	44.2
Propyl gallate	0.5	1.4	0.8	17.3	98.5	71.3	98.2	98.5	98.4
Genistein	0.1	0.5	0.4	2.8	71.7	37.3	2.8	71.7	37.3
Ketoconazole	0.0	0.5	0.2	0.0	93.4	46.2	0.0	93.4	46.2
Naproxen	0.0	0.0	0.0	47.4	100	83.4	99.7	100	99.9
Ibuprofen	0.2	6.8	2.1	98.4	100	99.5	98.4	100	99.5

As shown in Table 20, the RE values determined at location 3 and location 4 are almost the same for most of the PCs. The similarity of RE at both locations indicates that the main process responsible for the removal of PCs is the activated

sludge system and that filtration and disinfection generally do not contribute to the removal of PCs. Propyl gallate and naproxen both showed an exception with an increase in the RE value for propyl gallate from 71.3% to 98.4% and for naproxen from 83.4% to 99.9%. Boyd et al. (2005) indicated that naproxen could be removed by chlorination.

In conclusion, the activated sludge system is the main unit operation that affects the removal of the tested PCs. However, there are variations in the removal of PCs in the activated sludge system among the different tested compounds. A very high percent removal (>99%) values were found for cotinine, acetaminophen, caffeine, and ibuprofen, while for phenylephrine, amoxicillin, dapsone, noscapine, spiramycin, genistein, and ketoconazole the percent removal values dropped significantly (<50%). For the other tested PCs (i.e., tyramine, propyl gallate, and naproxen) the removal efficiency by the activated sludge system ranges between 70 and 85%.

For the overall removal of the tested PCs at Al Saad WWTP, cotinine, acetaminophen, caffeine, naproxen, and ibuprofen showed a very high removal (>99%), and tyramine and propyl gallate showed a high removal (70-99%). However, the removal of phenylephrine, noscapine, genistein, and ketoconazole was moderate (30-70%), while that for amoxicillin, dapsone, 9-aminoacridine, and spiramycin it was low (<30%).

In general, our removal efficiency values are comparable to those reported by others (Figure 17). However, the values for highly removed compounds fall in the upper range of those reported in the literature (Gracia et al., 2012; Kosma et al., 2010; Martínez et al., 2011; Oliveira et al., 2015; Van De Steene and Lambert, 2008). On the other hand, the RE values for moderately removed PCs in this study are slightly lower

than those reported by others (Bacaloni et al., 2005; Martínez et al., 2011; Zuccato et al., 2010).

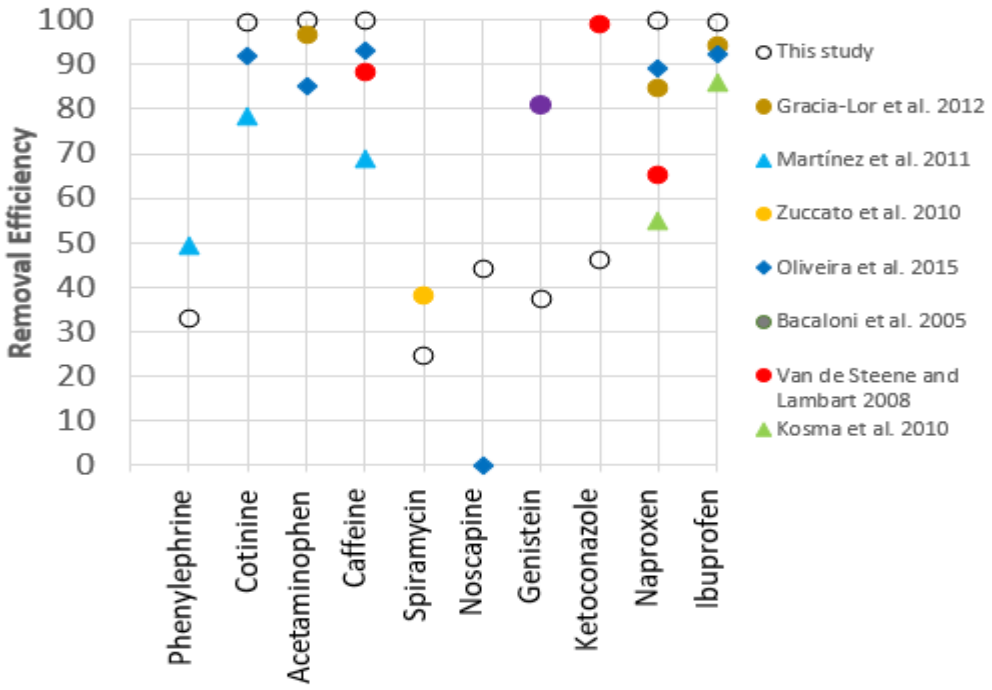


Figure 17: Comparison of PCs removal with the literature data

5.4 Removal Mechanisms of PCs in the Activated Sludge System

PCs in the aeration tank of the activated sludge system could be sorbed to the mixed liquor suspended solids (MLSS), they could be degraded, or they could remain unaltered and leave the system in the clarified water after the SST. Table 21 shows the relation between the input and output of relative average masses of PCs per day that leave the activated sludge system. As shown in Table 21, 9-aminoacridine had the highest relative mass per day that leaves the SST while acetaminophen had the lowest compared to the other PCs. In addition, the relative average mass per day in water for all tested PCs ranges between 0.001 and 0.769, while that for the solids exiting the system ranges between 0.001 and 1726. The higher mass in the solids for some PCs

such as tyramine and dapsone could be due to the effect of the hydraulic retention time of the system. It is clear from results in Table 21 that the highly removed PCs (>99%) such as cotinine, acetaminophen, caffeine, naproxen, and ibuprofen had the lowest relative average mass in sludge (≤0.1) compared to the other tested PCs. The lowest relative mass in sludge for these compounds could be due to the transformation reactions (i.e., volatilization, photodegradation, and biodegradation). Biodegradation is considered as the main transformation reaction that alter PCs in the activated sludge system (Verlicchi et al., 2012). Apparently, the operational conditions of the activated sludge system at Al Saad WWTP with a hydraulic retention time of 4 hrs, sludge retention time of 5.2 d, and the nitrification process play an important role in the cometabolism of cotinine, acetaminophen, caffeine, and ibuprofen.

Table 21: Relative average daily mass of PCs leaving the activated sludge system (n=4)

PCs	Effluent of SST	Mass in water	Mass in solids
Phenylephrine	0.699	0.022	0.042
Tyramine	0.303	0.012	1726
Cotinine	0.007	<0.001	0.095
Acetaminophen	<0.001	<0.001	0.043
Amoxicillin	0.960	0.030	0.056
Caffeine	0.003	<0.001	0.103
Dapsone	0.796	0.035	7.3
9-Aminoacridine	1.003	0.036	15.7
Spiramycin	0.754	0.769	0.046
Noscapine	0.685	0.024	18.4
Propyl gallate	0.281	0.021	4.3
Genistein	0.850	0.022	0.774
Ketoconazole	0.540	0.023	27.7
Naproxen	0.001	0.004	<0.001
Ibuprofen	0.005	0.001	<0.001

As also shown in Table 21, some PCs (i.e., tyramine, dapsone, 9-aminoacridine, noscapine, propyl gallate, and ketoconazole) had a relative mass in the exiting sludge of more than 0.1. The relative average daily mass range in the sludge

for these PCs range between 4.3 for propyl gallate and 1726 for tyramine. Such high values indicate that these compounds accumulate on the MLSS. Among the tested PCs, tyramine had the highest adsorption ability to the solids. Finally, the remaining PCs (i.e., phenylephrine, amoxicillin, spiramycin, and genistein) were not effectively removed from the waste stream.

5.5 Removal of PCs in the AD and FP Units

Mass balance was applied for the AD (Figure 13 – section 3.11.2) and the FP (Figure 14 – section 3.11.3) units to study and compare the rate of mass of PCs that enter and leave these units. As indicated before, a cationic polymer (Corofloc 341, SNF, France) is added in the FP unit for dewatering.

Figure 18 compares the rate of mass of PCs that enters the AD unit, leaves the AD unit (enters the FP unit), and the one that leaves the FP unit. As shown, PCs such as tyramine, dapsone, 9-aminoacridine, noscapine, propyl gallate, and ketoconazole were significantly removed (92.3-99.9%) in the AD system. In addition, caffeine and acetaminophen are removed by more than 90% in the AD system. The effluent rate of mass is much smaller than the influent rate of mass for all PCs in the AD unit. An exception is for phenylephrine where a higher rate of mass exists the unit as compared to the one that enters. This could be to attributed to the adopted sampling protocol of grab samples at a given time, which does not consider the effect of the system detention time (22 d).

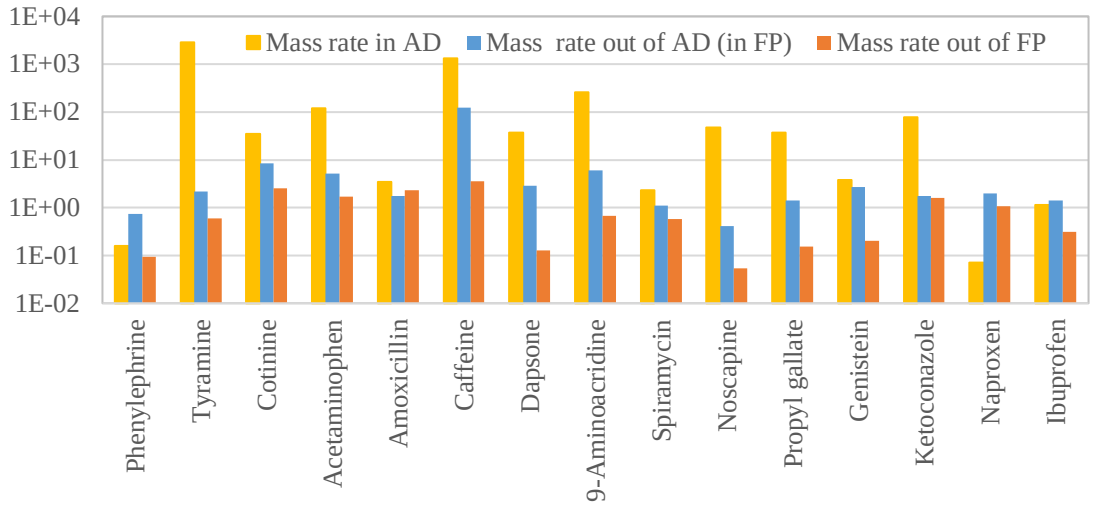


Figure 18: Rate of mass of PCs (g/d) as affected by the AD and the FP unit

Meanwhile, the effluent rate of mass is much smaller than the influent rate of mass for all PCs in the FP unit. While the reason behind this reduction is not clear, it could be due to agglomeration of the solid particles by the polymer or possibly the formation of strong bonds between the polymer and the PCs, making it difficult for the PCs to be released during solid-phase extraction.

Chapter 6: Conclusion and Recommendations

6.1 Conclusion

The development of new and advance techniques for extraction and measurements of PCs increased widely during the last decades. This allowed measurement of these compounds at the micro and nano levels, which enabled researchers to carry on assessment of the risk they could pose when released into the environment.

In this work, a method was developed to detect 15 PCs out of 23 in wastewater and sludge samples using LC-MS/MS instrument equipped with a triple quadrupole mass spectrometer detector. Calibration curves were prepared for each PC with (±)-Cotinine-D_3 used as an internal standard. The IDL and LOQ were determined and were used to determine the method LOD. Wastewater and sludge samples were collected form Al Saad WWTP at different locations to measure the levels of PCs. The extraction and preparation of all samples were conducted by using solid phase extraction with acetone and dichloromethane being used as organic solvents. Mass balance was applied at different locations within the WWTP to understand the mechanisms of removal of PCs.

Results showed that acetaminophen and caffeine exist at levels that exceeds 10 µg/L in the raw wastewater. However, tyramine, cotinine, amoxicillin, 9-amioacridine, spiramycin, naproxen, and ibuprofen, exist at intermediate levels (i.e., 0.1-10 µg/L). Whereas, the level of phenylephrine, dapsone, noscapine, propyl gallate, genistein, and ketoconazole was low (<0.1 µg/L).

The removal of PCs in the WWTP is mainly carried out in the biological reactors (i.e., the aeration tank and the anaerobic digester). The role of other treatment processes such as primary settling, filtration and chlorination was found to be either negligible or not significant.

Cotinine, acetaminophen, caffeine, and ibuprofen are highly removed during treatment due to possibly aerobic degradation in the activated sludge system. On the other hand, the removal of tyramine, dapsone, noscapine, and ketoconazole is moderate and the mechanisms of removal could be attributed to accumulation of these compounds on the MLSS in the activated sludge system followed by degradation in the anaerobic digesters.

The addition of a cationic polymer for sludge dewatering at Al Saad WWTP appears to have a positive effect on reducing the level of PCs in the sludge. Although the reason behind this reduction is not clear, it could be due to agglomeration of the solid particles by the polymer or possibly the formation of strong bonds between the polymer and the PCs, making it difficult for these compounds to be released during solid-phase extraction.

6.2 Recommendations

The number of PCs that could be present in domestic wastewater are much more than the ones investigated in this study. This study provides a preliminary assessment of the levels of some of the PCs in domestic wastewater produced in the UAE, however, additional studies are needed to tackle the issue in a more comprehensive manner that allows inclusion of other PCs and consideration of possible temporal and spatial variability in wastewater characteristics.

Another point of research that could be carried out is to compare the removal of PCs among different WWTPs in the country. Plants that employ activated sludge systems could be assessed for the removal of PCs with consideration of the effect of hydraulic residence time, sludge age, type of activated sludge system, etc. Also, comparison could also be made between the performance of WWTPs with activated sludge systems and those that employ membrane bioreactors.

Another point of research that is linked to the above suggested point is to carry on risk assessment to evaluate the compound(s) that could pose adverse impact on the environment.

As concluded in this study, a major mechanism that affect the fate of PCs during wastewater treatment is biodegradation. It is not clear if the degraded PCs undergo complete degradation or form intermediate products. Thus, it is recommended to monitor the formed by products as in some cases the products could be more harmful than the parent compounds.

A major part of treated wastewater in the UAE is utilized for landscaping. Despite that no study has been done to assess soil and groundwater contamination in the country by PCs present in the used treated effluent. A study of this nature should be initiated and will be important to protect valuable water resources from contamination.

One of the observations made in this study is related to a drop in the PCs level following sludge dewatering. It is speculated that the reduction in the level of PCs in the sludge after dewatering could be due to agglomeration of the solid particles by the

polymer or the formation of strong bonds between the polymer and the PCs. Such speculations need to be experimentally confirmed.

The analytical technique developed in this study is time consuming and costly as it requires the use of a large quantity of solvents for extraction. New manufactured LC-MS/MS instruments with high sensitivity are available nowadays. These instruments should be used in future studies to reduce the effort and cost of analysis as well as to reduce the amount of generated solvent waste. Another advantage of such instruments is that they require a small aqueous sample (less than 10 mL) as compared to the less sensitive ones that rely on SPE for aliquot concentration. Nonetheless, SPE would still be needed to for the analysis of sludge samples.

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Appendix A: Calibration Curves for PCs as Analyzed by LC-MS/MS

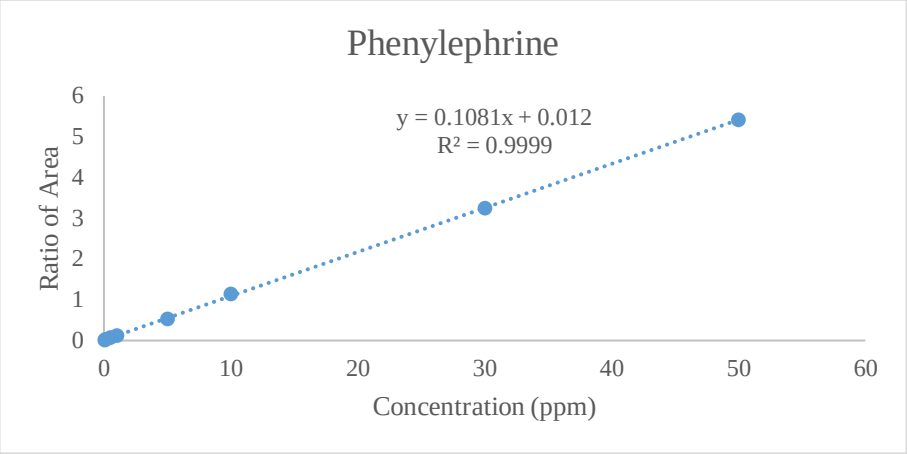

Figure A1: Phenylephrine Calibration Curve

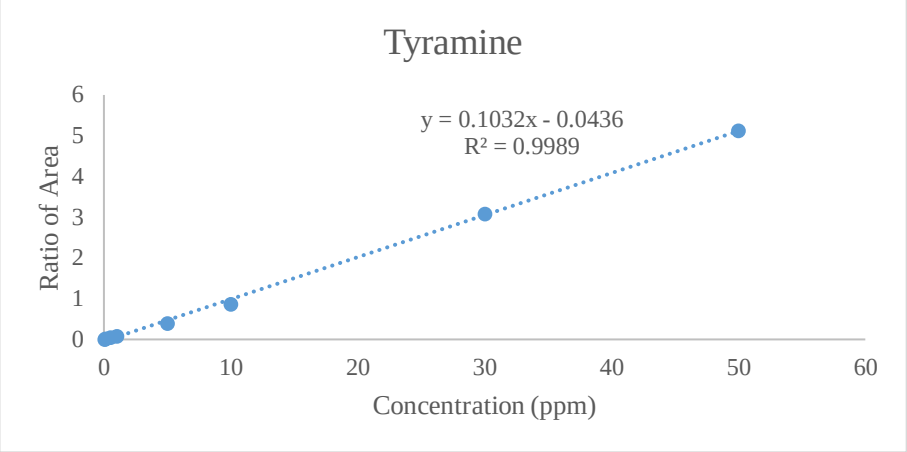

Figure A2: Tyramine Calibration Curve

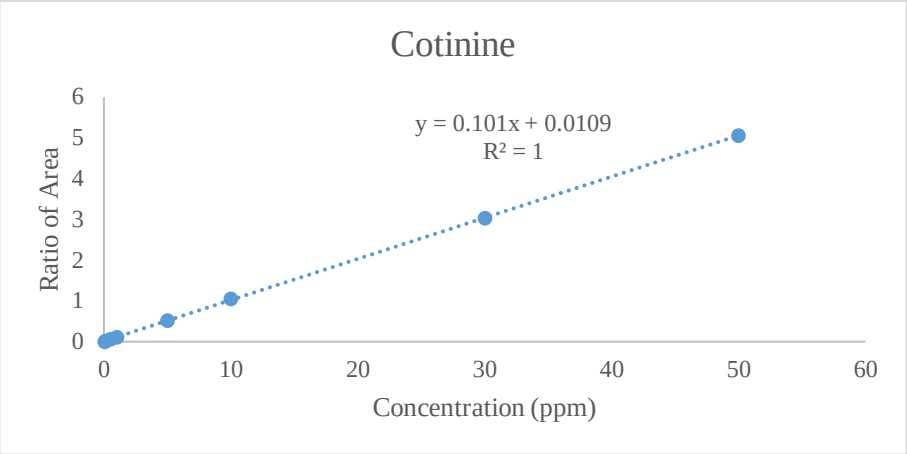

Figure A3: Cotinine Calibration Curve

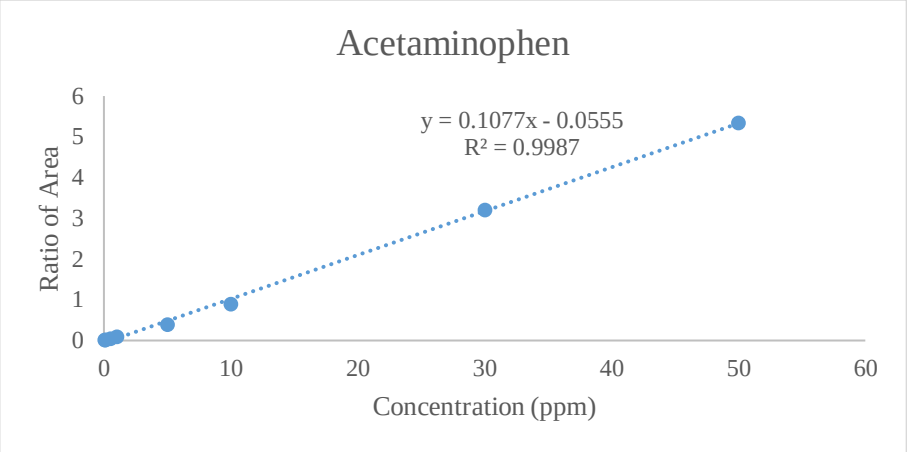

Figure A4: Acetaminophen Calibration Curve

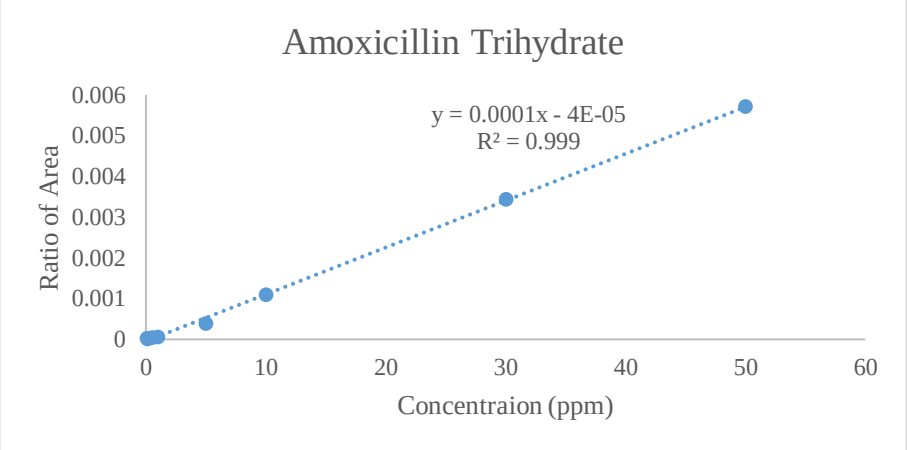

Figure A5: Amoxicillin Trihydrate Calibration Curve

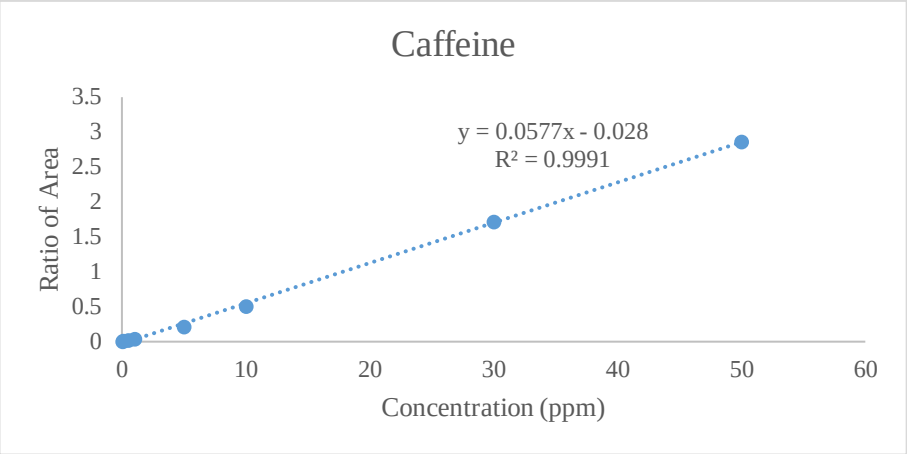

Figure A6: Caffeine Calibration Curve

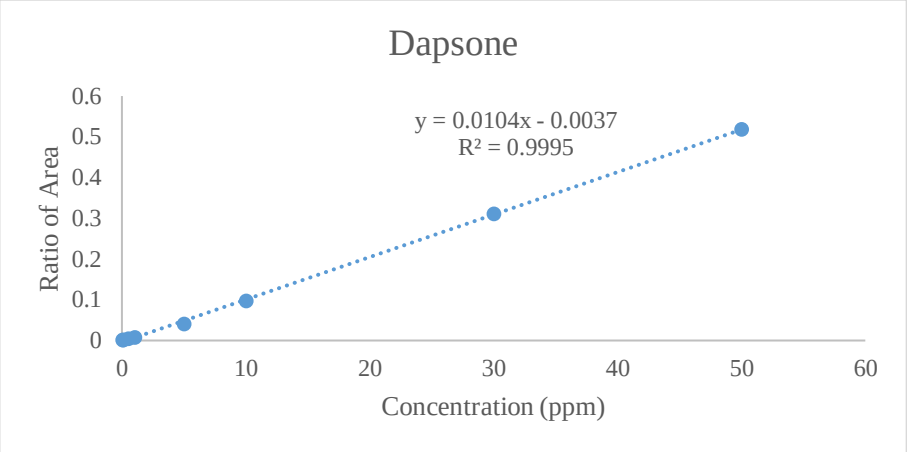

Figure A7: Dapsone Calibration Curve

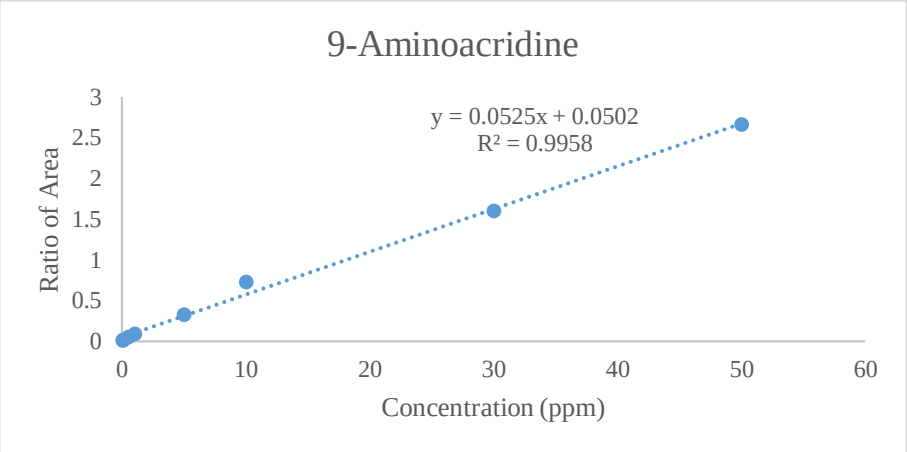

Figure A8: 9-Aminoacridine Calibration Curve

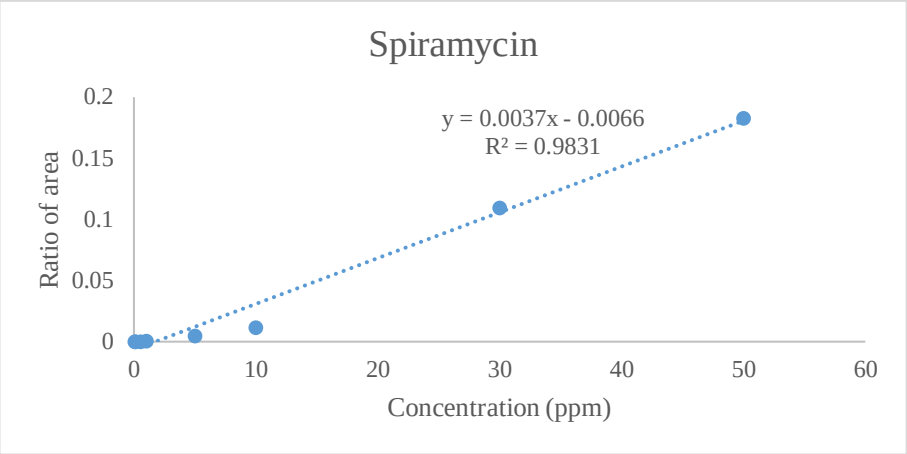

Figure A9: Spiramycin Calibration Curve

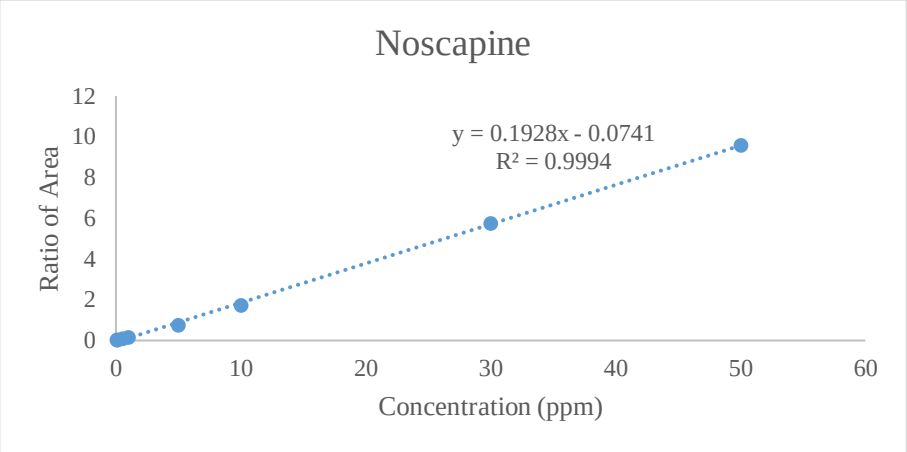

Figure A10: Noscapine Calibration Curve

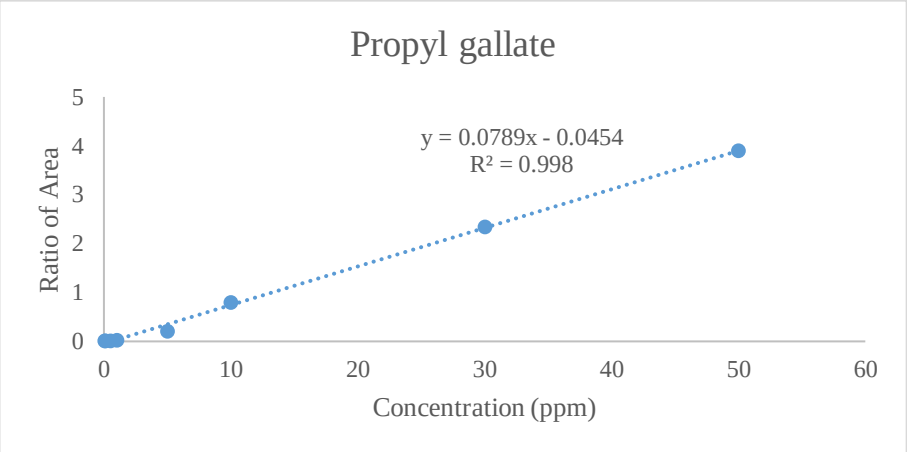

Figure A11: Propyl gallate Calibration Curve

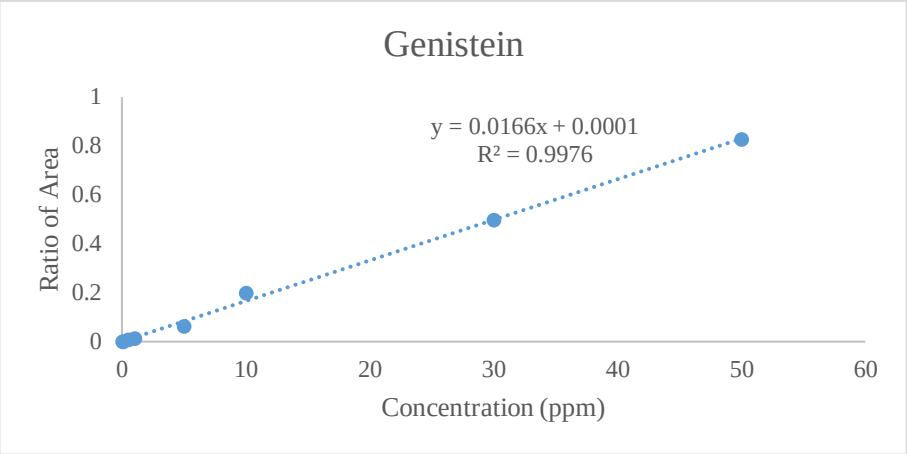

Figure A12: Genistein Calibration Curve

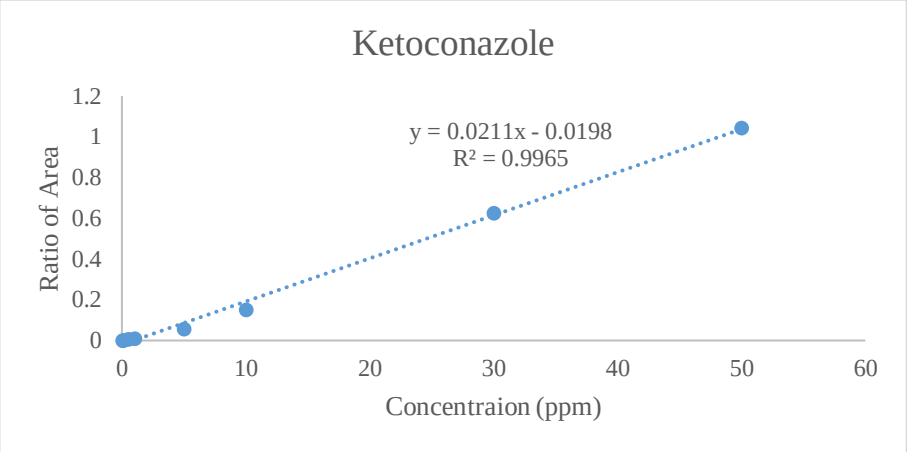

Figure A13: Ketoconazole Calibration Curve

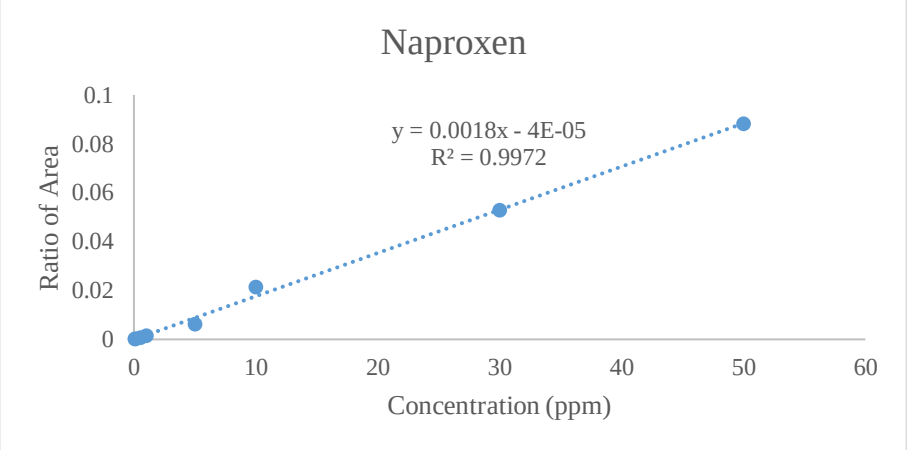

Figure A14: Naproxen Calibration Curve

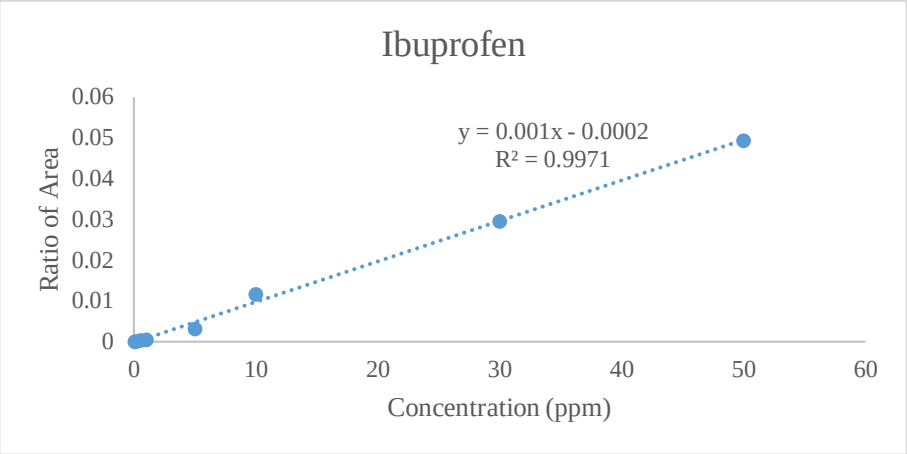

Figure A15: Ibuprofen Calibration Curve

Appendix B: Raw Data for 4 Batches from Al Saad WWTP at Different Locations

Table B1: Concentration (μg/L) of PCs in the raw wastewater (sampling point 1)*

PC	Batch 1	Batch 2	Batch 3	Batch 4	Average	Standard Deviation
Phenylephrine	<0.0121	0.0213	0.1077	<0.0121	0.0383	0.0464
Tyramine	0.1206	0.0807	1.6521	<0.0093	0.4657	0.7922
Cotinine	5.4794	3.2662	4.9475	2.9686	4.1654	1.2354
Acetaminophen	42.3362	29.8037	15.3142	18.7783	26.5581	12.1988
Amoxicillin	<0.5215	0.6298	<0.5215	<0.5215	0.5484	0.05387
Caffeine	113.4778	61.2848	68.0099	47.7143	72.6217	28.5154
Dapsone	0.0497	0.0958	0.0884	0.0398	0.0684	0.0277
9-aminoacridine	<0.1804	<0.1804	<0.1804	<0.1804	<0.1804	NA
Spiramycin	<0.0158	1.4667	<0.0158	<0.0158	0.3785	0.7254
Noscapine	0.0276	0.0341	0.0282	<0.0056	0.0239	0.0125
Propyl gallate	0.0821	0.0969	0.0955	0.078	0.0881	0.0095
Genistein	0.0513	0.0581	0.1995	0.0546	0.0909	0.0724
Ketoconazole	0.1086	0.0828	<0.0071	<0.0071	0.0514	0.0521
Naproxen	0.8635	0.1354	0.4904	0.905	0.5985	0.3606
Ibuprofen	2.0978	0.1025	1.5651	4.1000	1.966	1.653

* NA= Not available

Table B2: Concentration (μg/g) of PCs in the settled sludge of the PST (sampling point 2)*

PC	Batch 1	Batch 2	Batch 3	Batch 4	Average	Standard Deviation
Phenylephrine	<0.0002	0.0160	<0.0002	<0.0002	0.0041	0.0068
Tyramine	0.1093	0.4586	0.0689	<0.0001	0.1592	0.1771
Cotinine	0.1866	0.2382	0.1951	0.1725	0.1981	0.0245
Acetaminophen	0.1258	0.2342	0.1392	0.0314	0.1327	0.0718
Amoxicillin	<0.0106	<0.0118	<0.0107	<0.0109	0.0110	NA
Caffeine	74.9744	27.3635	42.0853	9.7369	38.5401	23.9508
Dapsone	<0.0008	<0.0009	0.0412	0.0132	0.0140	0.0164
9-aminoacridine	<0.0036	<0.0041	<0.0037	<0.0037	0.0038	NA
Spiramycin	<0.0003	<0.0003	<0.0003	<0.3810	0.0955	NA
Noscapine	0.0022	0.0030	0.0012	<0.0001	0.0016	0.0011
Propyl gallate	0.0028	0.0032	0.0021	0.0049	0.0033	0.0010
Genistein	<0.0011	<0.0012	<0.0011	<0.0011	0.0011	NA
Ketoconazole	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	NA
Naproxen	<8.94E-06	<9.99E-06	<9.01 E-06	<9.22E-06	9.29E-06	NA
Ibuprofen	0.1136	0.0318	0.012	0.0575	0.0537	0.0381

* NA= Not available

Table B3: PCs in the effluent of the SST (μg/L) (sampling point 3)*

PC	Batch 1	Batch 2	Batch 3	Batch 4	Average	Standard Deviation
Phenylephrine	<0.0121	<0.0121	<0.0121	<0.0121	0.0121	NA
Tyramine	0.0030	<0.0093	0.0026	<0.0093	0.0061	0.0037
Cotinine	0.0933	<0.0114	0.0252	<0.0114	0.0353	0.0392
Acetaminophen	0.0714	<0.0056	0.0518	<0.0056	0.0336	0.0333
Amoxicillin	<0.5215	<0.5215	<0.5215	<0.5215	0.5215	NA
Caffeine	0.1859	<0.1728	0.1174	<0.1728	0.1622	0.0305
Dapsone	0.0523	<0.0398	0.1228	<0.0398	0.0637	0.0398
9-aminoacridine	<0.1804	<0.1804	<0.1804	<0.1804	0.1804	NA
Spiramycin	<0.01580	<0.0158	<0.01580	<0.01580	0.0158	NA
Noscapine	0.0286	<0.0056	0.0305	<0.0056	0.0176	0.0138
Propyl gallate	0.0850	<0.0014	0.0789	<0.0014	0.0417	0.0465
Genistein	<0.0564	<0.0564	<0.0564	<0.0564	0.0564	NA
Ketoconazole	<0.0071	<0.0071	<0.0071	<0.0071	0.0071	NA
Naproxen	0.1176	<0.0004	0.2579	<0.0004	0.0941	0.1224
Ibuprofen	<0.0016	<0.0016	<0.0016	<0.0016	0.0016	NA

* NA= Not available

Table B4: PCs in the FE (μg/L) (sampling point 4)*

PC	Batch 1	Batch 2	Batch 3	Batch 4	Average	Standard Deviation
Phenylephrine	<0.0121	<0.0121	<0.0121	<0.0121	0.0121	NA
Tyramine	<0.0093	<0.0093	0.0015	<0.0093	0.0074	0.0038
Cotinine	0.0114	0.0587	0.0114	0.01147	0.0232	0.0236
Acetaminophen	<0.0056	0.0117	<0.0056	<0.0056	0.0071	0.0030
Amoxicillin	<0.5215	<0.5215	<0.5215	<0.5215	0.5215	NA
Caffeine	0.3829	0.0269	<0.1728	<0.1728	0.1889	0.1465
Dapsone	<0.0398	<0.0398	0.0708	<0.0398	0.0476	0.0154
9-aminoacridine	<0.1804	<0.1804	<0.1804	<0.1804	0.1804	NA
Spiramycin	<0.0158	<0.0158	<0.0158	<0.0158	0.0158	NA
Noscapine	0.0287	<0.0056	0.0143	<0.0056	0.0136	0.0108
Propyl gallate	0.0879	<0.0014	<0.0014	<0.0014	0.0230	0.0432
Genistein	<0.0564	<0.0564	<0.0564	<0.0564	0.0564	NA
Ketoconazole	<0.0071	<0.0071	<0.0071	<0.0071	0.0071	NA
Naproxen	<0.0004	<0.0004	<0.0004	<0.0004	0.0004	NA
Ibuprofen	<0.0016	<0.0016	<0.0016	<0.0016	0.0016	NA

* NA= Not available

Table B5: PCs in the effluent of RAS water (μg/L) (sampling point 5)*

PC	Batch 1	Batch 2	Batch 3	Batch 4	Average	Standard Deviation
Phenylephrine	<0.012	<0.0125	<0.0123	<0.0128	0.0125	NA
Tyramine	0.0368	<0.0097	0.0965	<0.0099	0.0382	0.0408
Cotinine	0.0572	<0.0118	0.0263	<0.0122	0.0269	0.0213
Acetaminophen	0.1413	<0.0058	0.0660	<0.0060	0.0548	0.0642
Amoxicillin	<0.5432	<0.5404	<0.5321	<0.5548	0.5426	NA
Caffeine	0.2257	<0.1791	0.1151	<0.1838	0.1759	0.0456
Dapsone	0.1256	<0.0413	<0.0406	<0.0424	0.0625	0.0420
9-aminoacridine	0.3013	<0.1870	<0.1841	<0.1920	0.2161	0.0568
Spiramycin	1.5704	<0.0163	<0.0161	<0.0168	0.4049	0.7770
Noscapine	0.0478	<0.0058	<0.0058	<0.0060	0.0164	0.0209
Propyl gallate	0.1560	<0.0014	0.0803	<0.0015	0.0598	0.0741
Genistein	0.0244	<0.0585	<0.0576	<0.0600	0.0501	0.0171
Ketoconazole	0.0904	<0.0074	<0.0073	<0.0076	0.0282	0.0414
Naproxen	0.1171	<0.0004	0.2088	<0.0004	0.0817	0.1010
Ibuprofen	0.1240	<0.0017	0.1442	<0.0017	0.0679	0.0769

* NA= Not available

Table B6: PCs in the effluent of RAS sludge (μg/g) (sampling point 5)*

PC	Batch 1	Batch 2	Batch 3	Batch 4	Average	Standard Deviation
Phenylephrine	<0.0031	<0.0027	<0.0030	<0.0037	0.0031	NA
Tyramine	30.1072	123.7522	131.2717	275.3946	140.1315	101.23
Cotinine	1.1208	4.8751	<0.0029	<0.0035	1.5006	2.3105
Acetaminophen	0.5511	20.7158	<0.0014	1.4365	5.6762	10.043
Amoxicillin	<0.1372	<0.1191	<0.1327	<0.1618	0.1377	NA
Caffeine	14.5764	60.7373	13.4354	17.45314	26.5506	22.8537
Dapsone	1.7880	2.9102	<0.0101	2.4613	1.7924	1.2745
9-aminoacridine	5.3009	31.4520	11.4741	2.9331	12.790	12.9517
Spiramycin	<0.0041	<0.0036	<0.0040	<0.0049	0.0041	NA
Noscapine	0.3235	8.6620	<0.0014	0.3529	2.3349	4.2210
Propyl gallate	1.5912	5.4626	<0.0003	0.1231	1.7943	2.5501
Genistein	0.7091	<0.0016	<0.0143	<0.0022	0.1818	0.3515
Ketoconazole	1.3565	11.2560	<0.0018	2.5342	3.7871	5.0856
Naproxen	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	NA
Ibuprofen	<0.0004	<0.0003	<0.0004	<0.0005	0.0004	NA

* NA= Not available

Table B7: Concentration (μg/L) of PCs in the effluent of the AD water (sampling point 6)*

PC	Batch 1	Batch 2	Batch 3	Batch 4	Average	Standard Deviation
Phenylephrine	1.0266	0.0016	<0.0143	0.7058	0.4371	0.5125
Tyramine	0.4591	0.1063	0.0358	0.01589	0.1543	0.2068
Cotinine	2.7027	4.2396	3.6573	3.2899	3.8422	0.4518
Acetaminophen	2.7027	0.1520	0.1676	0.1112	0.7834	1.2797
Amoxicillin	<1.3038	<0.6208	<0.6172	<0.6064	0.7870	NA
Caffeine	1.7753	0.8032	0.8368	0.4282	0.9609	0.5736
Dapsone	0.9145	0.0746	0.3699	<0.0463	0.3513	0.4029
9-aminoacridine	0.2034	<0.2148	<0.2135	<0.2098	0.2104	0.0051
Spiramycin	5.0532	<0.0188	<0.0187	<0.0183	1.2772	2.5173
Noscapine	0.7098	0.0343	0.0325	<0.0066	0.1958	0.3429
Propyl gallate	1.8160	0.1019	0.0622	<0.0016	0.4954	0.8813
Genistein	<0.1412	0.0951	<0.0668	<0.0656	0.0922	0.0353
Ketoconazole	<0.0179	<0.0085	<0.0085	<0.0083	0.0108	NA
Naproxen	5.6575	3.9299	<0.0005	<0.0005	2.3971	2.8558
Ibuprofen	1.0039	1.0872	4.5779	<0.0019	1.6677	2.0018

* NA= Not available

Table B8: Concentration (μg/g) of PCs in the effluent of the AD sludge (sampling point 6)*

PC	Batch 1	Batch 2	Batch 3	Batch 4	Average	Standard Deviation
Phenylephrine	<0.0025	0.0402	0.0113	0.0005	0.0136	0.0183
Tyramine	<0.0019	0.2605	0.0277	0.0079	0.0745	0.1245
Cotinine	0.3580	0.1571	0.1411	0.0846	0.1852	0.1193
Acetaminophen	0.5075	0.0956	0.0396	0.0087	0.1629	0.2325
Amoxicillin	<0.1079	<0.0168	<0.0199	<0.0140	0.0397	NA
Caffeine	<0.03578	0.3744	0.6413	0.1867	0.3095	0.2609
Dapsone	0.3404	0.0160	0.0093	<0.0010	0.0917	0.1659
9-aminoacridine	0.8056	0.0135	0.0061	<0.0048	0.2075	0.3987
Spiramycin	<0.0032	<0.0005	<0.0006	<0.0004	0.0012	NA
Noscapine	0.0315	0.0021	0.0013	0.0009	0.0089	0.0150
Propyl gallate	0.1321	0.0054	0.0035	3.841E-05	0.0352	0.0646
Genistein	0.3704	0.0061	<0.0021	<0.0015	0.0950	0.1836
Ketoconazole	0.1450	0.0154	0.0845	0.0047	0.0624	0.0654
Naproxen	<9.07E-05	<1.42E-05	<1.68E-05	<1.19E-05	3.34E-05	NA
Ibuprofen	<0.0003	<5.32E-05	<6.30E-05	<4.45E-05	0.0001	NA

* NA= Not available

Table B9: Concentration (µg/g) of PCs in the effluent of the FP sludge (sampling point 7)*

PC	Batch 1	Batch 2	Batch 3	Batch 4	Average	Standard Deviation
Phenylephrine	0.0056	0.0032	<0.0011	<0.0009	0.0027	0.0021
Tyramine	0.0525	0.0138	<0.0009	0.0014	0.0171	0.0243
Cotinine	0.2770	0.0615	<0.0011	0.0083	0.0870	0.1295
Acetaminophen	0.2248	0.0059	0.0023	0.0036	0.0592	0.1104
Amoxicillin	<0.0529	<0.0279	<0.0516	<0.0410	0.0434	NA
Caffeine	0.3307	0.0314	0.0121	0.0512	0.1063	0.1504
Dapsone	0.0057	<0.0021	<0.0039	<0.0031	0.0037	0.0015
9-aminoacridine	0.0393	<0.0096	<0.0178	<0.0142	0.0202	0.0131
Spiramycin	<0.0016	0.0787	<0.0015	<0.0012	0.0207	0.0386
Noscapine	0.0044	0.0014	<0.0005	<0.0004	0.0017	0.0018
Propyl gallate	0.0139	0.0042	<0.0001	<0.0001	0.0046	0.0065
Genistein	<0.0057	0.0023	<0.0055	<0.0044	0.0045	0.0015
Ketoconazole	0.2037	<0.0003	<0.0007	0.0086	0.0533	0.1003
Naproxen	<4.45E-05	0.1166	0.0373	<3.45E-05	0.0385	0.0549
Ibuprofen	<0.0001	0.0323	<0.0001	<0.0001	0.0082	0.0161

* NA= Not available

Table B10: Concentration (µg/L) of PCs in the effluent of the FP water (sampling point 8)*

PC	Batch 1	Batch 2	Batch 3	Batch 4	Average	Standard Deviation
Phenylephrine	<0.0121	0.0746	<0.0121	<0.0121	0.0277	0.0312
Tyramine	<0.0093	0.7113	<0.0093	0.0066	0.1841	0.3514
Cotinine	<0.0114	0.3851	<0.011	0.641	0.262282	0.3078
Acetaminophen	<0.0056	0.2218	0.2324	0.071	0.132746	0.1123
Amoxicillin	<0.5215	4.6783	<0.5215	<0.5215	1.560737	2.0783
Caffeine	<0.1728	2.7988	0.4896	0.133	0.898577	1.2768
Dapsone	<0.0398	<0.039	<0.0398	0.038	0.0394	0.0009
9-aminoacridine	<0.1804	<0.180	<0.1804	<0.1804	0.18048	NA
Spiramycin	<0.0158	<0.0158	<0.0158	<0.015	0.015	NA
Noscapine	<0.0056	0.0271	<0.0056	<0.0056	0.0110	0.0107
Propyl gallate	<0.0014	0.087	<0.001421	0.0780	0.0420	0.0471
Genistein	<0.0564	0.2906	<0.0564	<0.0564	0.1150	0.1171
Ketoconazole	<0.0071	0.8116	<0.0071	<0.0071	0.2083	0.4022
Naproxen	<0.0004	0.0603	<0.0004	0.1190	0.0450	0.0568
Ibuprofen	<0.0016	<0.0016	<0.0016	0.4826	0.1219	0.2405

* NA= Not available