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ESTIMATION OF THE REMOVAL OF CHIRAL PHARMACEUTICAL DRUGS FROM DOMESTIC WASTEWATER USING UPLC-MS/MS

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

Under the Supervision of Professor Mohammed Abdul Rahman Almeetani

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Declaration of Original Work

I, Mohammed Khaled Mohammed Al Tabaji, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Estimation of the Removal of Chiral Pharmaceutical Drugs from Domestic Wastewater Using UPLC-MS/MS*", 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 Mohammed Abdul Rahman Almeetani in the College of Science 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 Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions.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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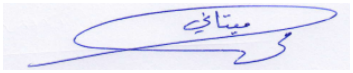
   Signature  Date Nov 30 2020

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Abstract

This thesis is concerned with the estimation of the removal of chiral pharmaceutical compounds (PCs) from domestic wastewater in Al Ain using UPLC-MS/MS. PCs are classified into many classes such as hormones, antibiotics, analgesics, cosmetic products, β blockers, and anti-inflammatory drugs. PCs do not have a guideline to describe their concentrations in treated domestic wastewater. Additionally, there is a very high demand for using PCs around the world, which results in their discharge to wastewater at relatively high masses, which could be harmful to the environment. Furthermore, very limited work has been done to estimate the removal of chiral PCs. Moreover, there is a need to study the removal mechanism of every chiral PC enantiomer alone at wastewater treatment plants (WWTPs). This study is aimed to develop a method for chiral separation and identification of 16 chiral PCs in wastewater; in addition to study their levels and removal selectivity at different locations at Al Saad WWTP in Al-Ain city using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Four batches of wastewater and sludge samples have been grabbed from different locations at Al Saad WWTP, followed by solid-phase extraction (SPE). 15 out of 16 chiral PCs were successfully chirally separated except for tramadol. Calibration curves, instrument limit of detection, instrument limit of quantification, and method limit of detection were successfully identified and prepared. Results show tramadol, atenolol, and o-desmethylfenlafaxine occur at a relatively high concentrations compared to others (>2 µg/L). In general, the removal efficiency exceeded 90% for most of the tested PCs with exception of metoprolol, terbutaline, and fluoxetine (between 30% and 70%), while a low removal was indicated for atenolol (25.9%). Moreover, the treatment process was more selective for the removal of the second enantiomer (E2) of bupivacaine and fluoxetine. In general, results indicate that filtration and disinfection play the main role in the removal of the target PCs. Changes in the PCs behavior at the Return Activated Sludge (RAS), Anaerobic Digester (AD), and Filter Press (FP) units were indicated. Propranolol preferably sorbed to RAS more than the tested PCs, while citalopram and tolperisone preferably sorbed to the AD sludge surface more than other operational units. In addition, E2 of propranolol and mianserin was at the higher

concentration on the sludge than their antipode in the RAS. While RAS was enriched with the first enantiomer of bupivacaine, terbutaline, citalopram, and fluoxetine.

Keywords: Chiral Pharmaceutical Compounds, Enantiomers, Wastewater, Al-Saad WWTP, Sludge, LC-MS/MS, Mass Balance, Internal Standard, Removal Efficiency.

Title and Abstract (in Arabic)

تقدير إزالة العقاقير الصيدلانية اللامتناظرة من مياه الصرف الصحي المحلية باستخدام تقنية كروماتوغرافيا السوائل عالية الكفاءة المرتبط بمطياف الكتل

الملخص

الهدف من هذه الأطروحة هو تقدير إزالة العقاقير الصيدلانية اللامتناظرة (Chiral PCs) من مياه الصرف الصحي باستخدام تقنية كروماتوغرافيا السوائل عالية الكفاءة المرتبط بمطياف الكتل (UPLC-MS/MS). يتم تصنيف العقاقير الصيدلانية إلى العديد من الفئات مثل الهرمونات والمضادات الحيوية والمسكنات ومستحضرات التجميل وحاصرات بيتا والأدوية المضادة للالتهابات. لا يوجد للعقاقير الصيدلانية دليل إرشادي لوصف تراكيزها في مياه الصرف الصحية. بالإضافة إلى ذلك، هناك طلب مرتفع للغاية عليها في جميع أنحاء العالم ، ونتيجة لذلك ، يمكن أن يتم تصريفها في مياه الصرف بكميات كبيرة نسبيًا مما قد يكون ضارًا بالبيئة. علاوة على ذلك، القليل من الدراسات والأبحاث تمت لدراسة و تقييم إزالة العقاقير الصيدلانية اللامتناظرة ودراسة آليات إزالة كل لامتناظر (enantiomer) على حدا في محطات معالجة مياه الصرف الصحي (WWTP). هدفت هذه الدراسة إلى تطوير طريقة للتعرف على 16 مركب من المركبات اللامتناظرة وفصلها. بالإضافة ل دراسة تراكيز هذه المركبات وانتقائية الإزالة في محطة الساد لمعالجة مياه الصرف الصحي في مدينة العين باستخدام تقنية كروماتوغرافيا السوائل عالية الكفاءة المرتبط بمطياف الكتل. تم أخذ أربع دفعات من عينات مياه الصرف الصحي والحمأة من مواقع مختلفة من محطة معالجة الساد، ثم تم استخراج هذه المركبات عن طريق جهاز استخلاص الطور الصلب (solid phase extraction).

15 من أصل 16 من المركبات اللامتناظرة تم فصلها بنجاح باستثناء ترامادول. تم بنجاح تحديد وإعداد منحنيات المعايرة ، وحدود الجهاز للكشف ، وحدود الجهاز للقياس الكمي بالإضافة إلى معرفة حدود طريقة الكشف (method detection limit). أظهرت النتائج أن وجود ترامادول وأتينولول وأو-ديسميثيل فينلافاكسين كان بتركيزات عالية نسبيًا مقارنة بالمركبات الأخرى (< 2 ميكروغرام / لتر). بشكل عام ، كفاءة الإزالة تتجاوز 90٪ لمعظم المركبات اللامتناظرة المختبرة باستثناء ميتوبرولول ، تيربوتالين ، وفلوكستين (بين 30٪ و 70٪). بينما كان هناك إزالة منخفضة للأتينولول (25.9٪). علاوة على ذلك، كانت عملية المعالجة أكثر انتقائية لإزالة اللامتناظر الثاني من بوبيفاكسين وفلوكستين (second enantiomer). بشكل عام ، تشير النتائج إلى أن الترشيح

والتطهير يلعبان الدور الرئيسي في إزالة المركبات اللامتناظرة. تمت ملاحظة تغيير في سلوك المركبات اللامتناظرة في وحدات مياه الحمأة النشطة و مياه الحمأة اللاهوائية و ووحدة ضغط التصفية، البروبرانولول تم امتصاصه على سطح الحمأة في وحدة (RAS) أكثر من الوحدات الأخرى. بينما فضل سيتالوبرام وتولبيريسون أن يتم امتصاصهم على سطح الحمأة في وحدة (AD) أكثر من الوحدات الأخرى. بالإضافة إلى ذلك، كان تركيز (E2) من بروبرانولول وميانسيرين على الحمأة أعلى من تركيز مركباتهم المضادة على سطح الحمأة في وحدة (RAS) . أياً يكن, تم ملاحظة أن سطح الحمأة في وحدة (RAS) كان غنياً ب اللامتناظر الأول ل بوبيفاكسيين وتيربوتالين وسيتالوبرام وفلوكستين.

مفاهيم البحث الرئيسية: المركبات الصيدلانية اللامتناظرة، اللامتناظرات، مياه الصرف الصحي، الساد لمعالجة مياه الصرف الصحي، الحمأة، كروماتوغرافيا السوائل عالية الكفاءة المرتبط بمطياف الكتل ، موازنة الكتلة، المعيار الداخلي، كفاءة الإزالة، العين، الإمارات العربية المتحدة.

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Finally, we are grateful for the Abu Dhabi Sewage Service Company for its support and help in the sample collections.

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
K_{ow}	Octanol-Water Partition Coefficient
LC	Liquid Chromatography
LC_{50}	Lethal Concentration
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry

LOD	Limit of Detection
IQL	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 and 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 Solids
WWTPs	Wastewater Treatment Plants

Chapter 1: Introduction

1.1 Statement of the problem

Chiral PCs are used extensively nowadays to treat many major and minor diseases [1]. In addition, they can be classified as hormones, antibiotics, analgesics, cosmetic products, β blockers and anti-inflammatory drugs [2-5]. Unfortunately, they can reach the groundwater, surface water and rivers in many different ways like treated sewage effluent, septic tanks and landfills [6, 7]. In addition, they don't have a guideline to describe their concentrations in domestic wastewater, so they became an essential problem in the last decades [8]. Moreover, it was reported that chiral PCs, in particular, could behave differently in the environment, as they consist of two enantiomers and every one could be more dangerous and toxic than its antipode [9], [10]. Therefore, separation and quantification of chiral PCs in water is necessary.

As a result of continuously exposing organisms to released PCs in soil and water, many reports have been done to indicate and illustrate the different impacts of different drugs on the environment. These impacts include effects on testicular maturation, impacts on insect behavior, dung decomposition and antibacterial resistance development [11]. Additionally, many drugs could affect aquatic plants such as Ibuprofen, which is considered one of the most common and used drugs. It is reported that ibuprofen plays a role in the inhibition of growth of an aquatic plant called Lemna minor with more than 25%, however, ibuprofen could play an opposite role by stimulating the growth of another aquatic plant called Synechocystis sp. by more than 70% [12].

The enantiomeric composition of some PCs has also been confirmed to be important in the toxicity of different environmental species. S (−)-atenolol and S (+)-

fluoxetine were found to inhibit the growth of a freshwater protozoan called *Tetrahymena thermophila* significantly, more than R(+)-atenolol and R(−)-fluoxetine.

On the other hand, the identification of chiral pharmaceutical drugs is the main concern in wastewater treatment in the last decade, whereas the chiral separation is very difficult and the enantiomers interact differently during the WWTP. Add to that the enantiomers have the same chemical properties, which make them very difficult to be separated using LC and GC methods. However, different studies have been performed to assess the concentrations of chiral PCs in WWTP [9].

1.2 Motivation

Very limited work has been conducted to chirally separate and identify enantiomers in wastewater, especially in arid and semi-arid countries. Specifically, no study has been done before to identify and separate chiral PCs in wastewater in UAE. Moreover, there is a lack of understanding of the mechanism and enantiomers removal selectivity in WWTPs, as well as understanding the enantiomers' behavior in the different stages during the treatment process at the WWTPs.

The research questions that are discussed in this study: what are the chiral PCs concentrations at different stages of treatment at Al Saad WWTP? What are the roles of different units at Al Saad WWTPs in removing the tested PCs? How the chiral PCs enantiomers do behave at every unit?

1.3 Objectives

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

1. To quantitate and estimate 16 chiral pharmaceutical drugs in wastewater using UPLC-MS/MS at different units in Al Saad WWTP.
2. To study the role of different units at Al Saad WWTPs in removing the tested PCs.
3. To study the selectivity and specificity of different operational units in removing enantiomers.

1.4 Scope of work

After reviewing the literature, a group of chiral PCs was selected to be analyzed, identified, and quantified, since they could be enriched and presented in Al Ain domestic wastewater. The Table 1 below shows the selected chiral PCs that were covered in this study.

Table 1: Chiral PCs that will be covered in the study

Bupivacaine	O-Desmethylvenlafaxine	Fluoxetine	Mirtazapine
Amlodipine	Salbutamol	Terbutaline	sotalol
Propranolol	Mianserin	Citalopram	Tramadol
Atenolol	Venlafaxine	Tolperisone	Metoprolol

Many chiral PCs from different types and classes could be available in wastewater. However, only the selected 16 PCs have been studied in this thesis. Moreover, this study is the first of its type, for the analysis of chiral PCs in domestic wastewater in the arid and semi-arid environments such as UAE.

1.5 Approach

Many tasks have been undertaken to complete this study. This includes

literature review, establishing an analytical method for the determination and separation of chiral PCs, sample collection from Al Saad WWTP, extraction and sample preparation, quantifying the selected PCs at different units in the WWTP and studying their removal mechanisms efficiencies, and their removal selectivity. The possible removal mechanisms are sorption, disinfection and transformation reactions

1.6 Thesis structure

Six chapters are included in this thesis. Chapter 1 includes a brief description of the study, including background about the project, statement of the problem, motivation and objectives, approach and scope of work. Chapter 2 includes an extensive relevant literature review that covers PC types and development, PCs production and researches worldwide, how PCs can be released to the environment and their effects, in addition to the recent studies on the detection, identification and stereo selectivity of both chiral and achiral PCs in the environment and wastewater.

Chapter 3 shows the development of the analytical method in detail; moreover, an extensive description of the sampling site is provided. Chapter 4 shows the method parameters optimization, calibration curves, limit of detection and quantification results. Chapter 5 provides an extensive discussion that includes the chiral PCs levels at different units, removal efficiencies, removal mechanisms and unit selectivity toward selected PCs enantiomers. Finally, Chapter 6 includes a brief conclusion and some recommendations and suggestions for future work.

Chapter 2: Relevant Literature

2.1 Introduction

Pharmaceutical drugs are chemical compounds used for the treatment of different types of diseases. They have different classes and types. Unfortunately, they could reach the environment by different routes, like landfills, treated sewage effluent and septic tanks. As a result, they have been found in groundwater, rivers and surface water and became a serious problem to the environment during the last 20 years [6, 7, 13, 14]. Moreover, scientists found that chiral pharmaceutical drugs, in particular, could interact differently in the environment, as they consist of two enantiomers that could react differently with the components of the ecosystems, in addition, it is possible that one of these enantiomers could be more toxic and dangerous than the other one [9, 10, 15]. Therefore, quantification of the level of chiral PCs in groundwater and treated wastewater became a necessity. This chapter contains an extensive review of the work that was done to identify and quantify PCs in a wastewater treatment plant (WWTPs) and its efficiency in removing them.

This chapter is consisting of 8 Sections. Section 2.2 focuses on the types and development of PCs classes such as antiseptics. Antibiotics, and β−blockers. Section 2.3 introduces the research and production steps of PCs classes. Section 2.4 contains the possible pathways through which the PCs could reach the environment. Section 2.5 focuses on how PCs could affect the environment, including accumulation and toxicity. Section 2.6 illustrates the role of the WWTPs in removing PCs. Section 2.7 reviews the stereoselectivity of chiral PCs and how they behave in the environment. Section 2.8 reviews the analytical methods that have been used for chiral separation of

these compounds, and finally, Section 2.9 discusses some research works have been done to detect PCs and their levels in wastewater.

2.2 PCs development and types

The origin of the pharmaceutical drug industry refers to the Middle Ages when the traditional therapies were famous at that time, however, the drug industries, as it is known today, began in the 19th century. For example, aspirin, insulin, and penicillin are considered the most successful drug discoveries at the beginning of the 20th century [16–18]. Later, the drug development industries became stronger when scientists learned more about biological targets, such as receptors, proteins, enzymes, genes, and others [19]. In addition, the process of new drug discovery- starting from the research step to make it commercially available- needs to go through more than one step before it gets approval from health authorities for marketing. It needs to pass through research, discovery, preclinical and clinical development steps. In the preclinical step, the scientists test the drug on animals such as rats, rabbits and/or monkeys; if it gives a positive effect, then they continue the trials of the drug on humans in a step called clinical development. As a result, it needs at least 12 years and cost on average US $2.5 billion before making it commercially available [19–23].

PCs are classified by scientists into different classes such as anti-inflammatory drugs, antihypertensive drugs, cardiac stimulants, blockers, and radiopharmaceuticals [2–5, 24]. Table 2 shows a summary of the PCs classes with some examples for each class, and their medical use.

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

PCs Classes	Usage	Examples	Reference
Receptor antagonists	block biological responses	beta blockers, famotidine, omeprazole	[2, 16, 17, 20, 22, 24, 26]
β blockers	heart rhythm controlling and decreasing blood pressure.	Bisoprolol, Atenolol, alpenolol	
Beta-agonists	Breathing issues	albuterol, fenoterol, salbutamol	
anti-inflammatory drugs	Pain killer	Naproxen, ketoprofen, ibuprofen,	
Lipid Regulating drugs	Cholesterol reducing	Bezalip, Atromid-S	
Antifungal	mycosis preventing	Canesten	
Diuretics (water pills)	Hypertension treatment	Aprinox, furosemide	
Antineoplastic	Cancer treatment	cytophosphane, Ifex, tamoxifen	
Antiseptic drugs	Reduce the possibility of infection	Triclosan	
anti-inflammatory drugs	Pain killer	Naproxen, ketoprofen, ibuprofen,	
Antibiotics	treating bacterial infections	ciprofloxacin, levofloxacin	
Barbiturates	Treating anxiety	Phenobarbital	
Topical products	skin infections treatment	Crotamiton	
Psychiatric Drugs	Treat anxiety and depression	citalopram, carbamazepine, fluoxetine	
Antiepileptics	epileptic seizures Treatment	Tegretol, clonazepam, gabapentin	[32]
Antidiabetics	Lowering glucose level in the blood	Glucotrol, glyburide, pramlintide	
Antithrombotic	blood clots treatment	warfarin	
Antihistamines	Allergies treatment	fexofenadine, loratadine	
Antibacterial drugs	Kill and prevent bacteria from growth	Chloramphenicol, clarithromycin, erythromycin	
Stimulants	Stimulating	Caffeine, methamphetamine	[33]

Non-steroidal anti-inflammatory drugs are one of the most famous classes of PCs. They are used mainly for pain inhabitation. They have multi-side effects such as gastrointestinal bleeding. Ibuprofen, ketoprofen and naproxen are types of drugs under this class [3, 25].

Heart attacks and stroke are two diseases that could cause death. The Heart attack occurs due to the decrease of blood flow to the heart, which could damage the heart muscle. Stroke is a condition that occurs when there is less than enough blood reaching the brain, which causes cell death. To prevent these dangerous diseases, scientists developed antihypertensive drugs. They are among other important PCs classes. Antihypertensive drugs have a very important role in decreasing blood pressure and preventing heart attacks [2, 26]. Diltiazem and nalapril are examples of Antihypertensive drugs [13].

Another interesting class is cardiac stimulant drugs. They are drugs that could stimulants the heart. Either by changing the heart rate (chronotropic) or by changing muscular contraction energy (inotropic) [5]. Dopamine is one of the cardiac stimulants, scientists indicated that dopamine is a stimulant that is responsible for the movement of the body and conveys motivational values. Cocaine which could result in losing communication with reality and methamphetamines that could cause muscle breakdown and brain bleeding when overdosed are other types of cardiac stimulants [5, 27].

Antihypertensive drugs, such as β-blockers, are mainly used to reduce the blood pressure and make the heartbeat slow. They block the effect of a hormone called adrenaline, which causes high blood pressure in the body. Acebutolol, atenolol,

bisoprolol and metoprolol are examples of β blockers [24, 28]. It is worth mentioning that most of this thesis work was done on this group of drugs.

Antibiotics are groups of PCs that are used mainly to stop bacterial infections. Antibiotics eliminate the harm of bacteria by stopping their growth or killing them [29]. Amoxicillin is one of many examples of antibiotics. It has been used to treat different types of bacterial infections such as bronchitis, tonsillitis pneumonia and gonorrhea, or even used to treat ear, nose, and skin infections [30].

A unique class of PCs is radiopharmaceuticals, which are considered the oldest and most common drugs until now. They are used in both the treatment and diagnosis of different diseases. Radiopharmaceuticals contain radioactive isotopes that emit radiation. Calcium-47 and carbon-14 are the most common types of radioactive isotopes used in diagnosis [31, 4].

2.3 PCs research, development and production

Research, development, and production of PCs increased dramatically in the last years. The demand for new treatments and drugs increased worldwide, so people invested a lot of money and time in this kind of research in the last decade. The investment increased by US$ 15 billion between 2005 and 2015 according to Figure 1 [34].

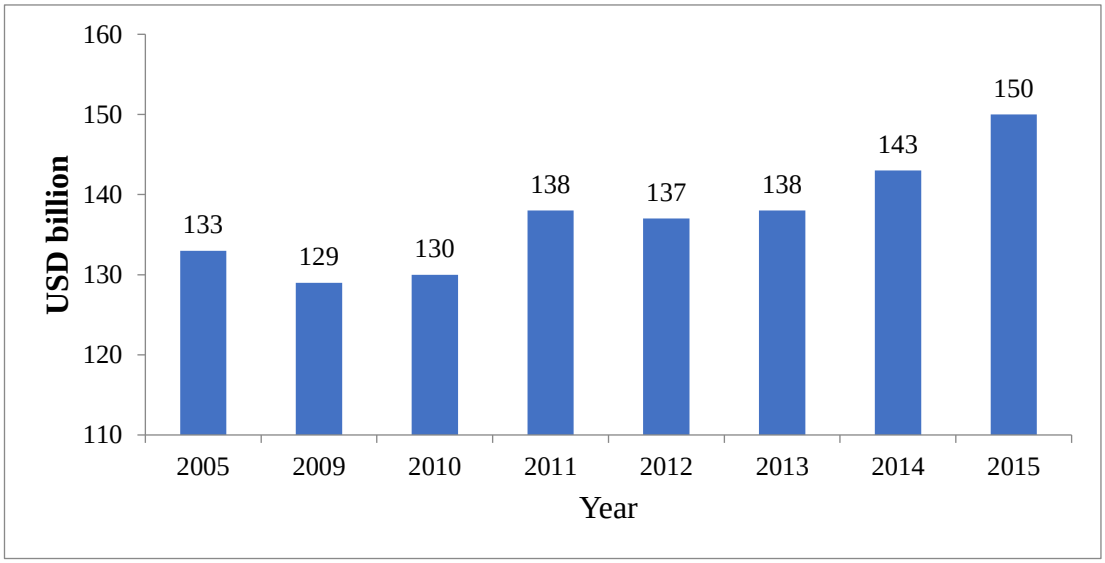


Figure 1: PCs research and development investment between 2005 and 2015 (in billions US$) [35]

The importance of this investment could be noticed when we take a look at the death rate due to HIV between 2005 and 2015, Figure 2 shows that the death rate decreased by more than 1 million during these 10 years [35, 36].

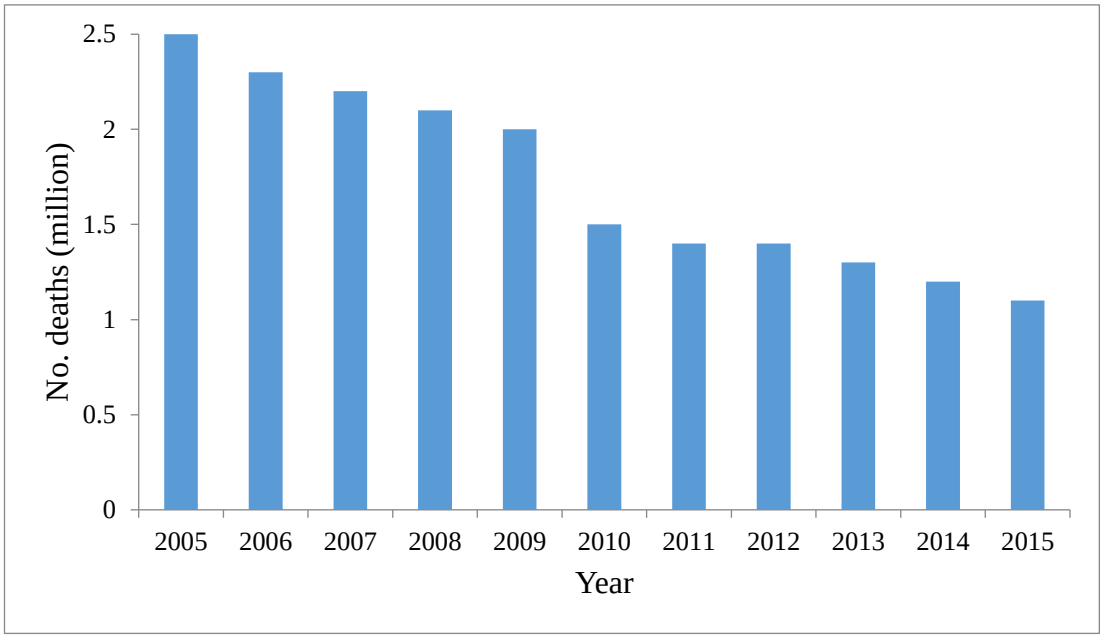


Figure 2: Decline in HIV/AIDS Death Rates [35]

Worldwide, PCs productions differ between continents, according to The International Federation of Pharmaceutical Manufacturers and Associations (IFPMA), Asia and Europe spent the highest amount of money on PCs production compared to other continents. In Table 3, it is noticed that other continents such as Africa, does not exceed US$ 7 billion in PCs production, on the other hand, Asia reached more than US$ 153 billion in 2014. In addition, Europe was the highest continent in PCs production until 2008; then the production of Asia increased sharply from US$ 119.9 billion in 2008 to US$ 131.1 billion in 2009 to become the highest continent in PCs production. Table 3 compares PCs production in different continents between 2006 and 2014 [36].

Table 3: PCs production per continent (in billion US$) [35, 36]

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

The importance of PCs research and development, in addition to the need of discovering new therapies for dangerous diseases, reflects directly on PCs production worldwide. Figure 3 shows how countries around the world spent more funds between 2006 and 2014 to develop new drugs. They spent more than US$ 452 billion in 2014 [36].

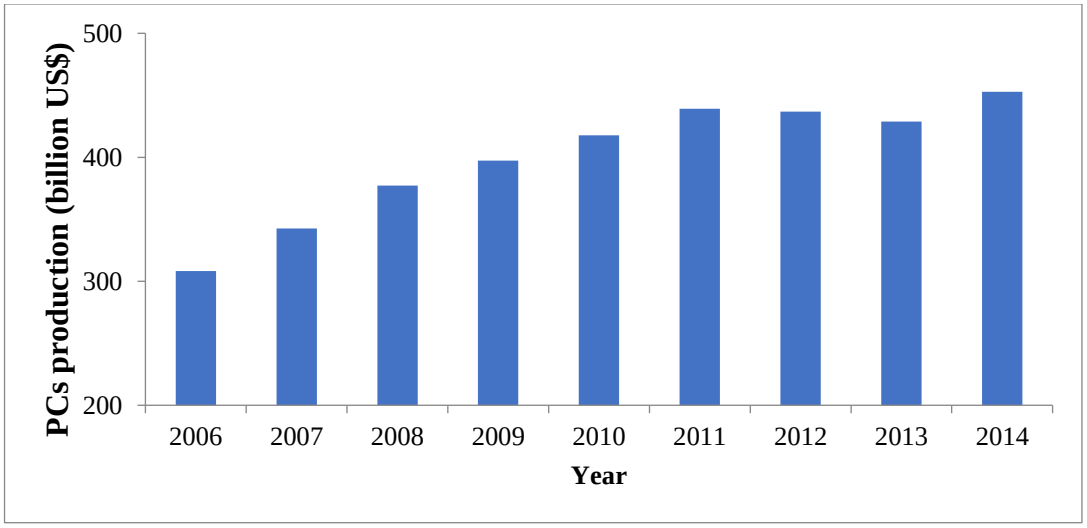


Figure 3: PCs production worldwide between 2006 and 2014 [35, 36]

It is possible to realize that the sales of PCs per capita differ between countries around the world. As a result, it had a direct effect on the mortality rate and standards of living in these countries. Figure 4 illustrates that developed countries such as the

United States, the United Kingdom, and France had higher sales per capita of PCs, which will result in fewer mortality rates and a higher standard of living. On the other hand, many Middle East countries like Oman, Sudan and Jordan have very low sales per capita of PCs compared to developed countries, which is reflected in the higher mortality rates and lower standards of living. The United Arab Emirates had the highest sales of PCs per capita compared to other Middle East countries [36].

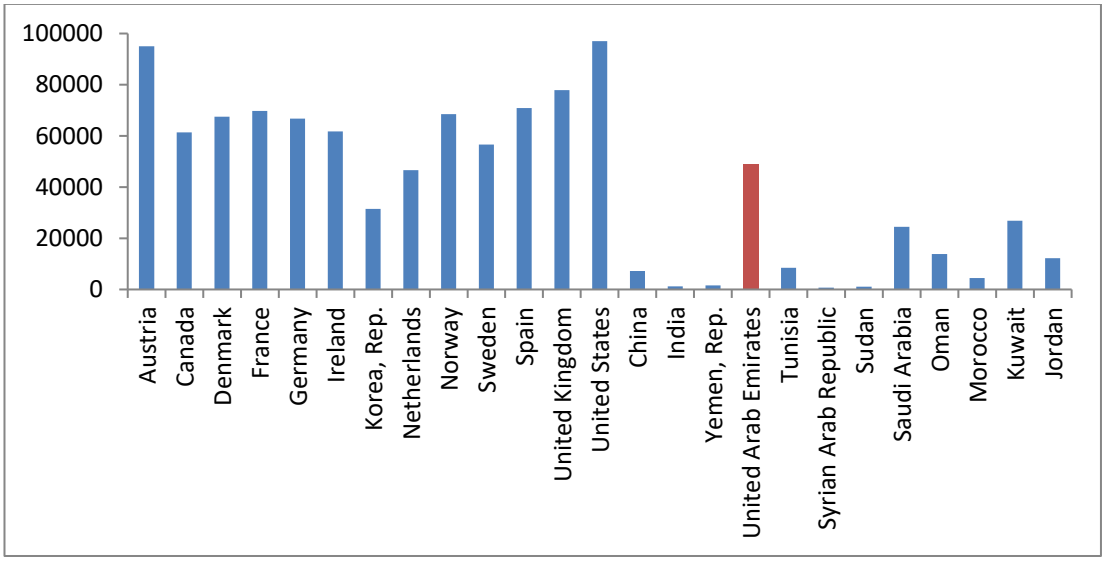


Figure 4: PCs annual per capita sales (in US$) in different countries [36]

2.4 Release of PCs to the environment

Wastewater is considered the main source of pharmaceutical drugs to the environment because they can pass through human bodies to the environment at different concentrations. Usually, these PCs could not be removed completely by WWTP; however, it is possible that some PCs could remain after the treatment process in the treated wastewater [1, 37–39]. Moreover, different types of PCs could be released into the environment by animals. Farmers give such type of drug to the

animals to promote their growth [7, 40]. These PCs will be released to the environment by animals urine and feces, as a result, they may affect the micro-organisms in the soil and water [7, 41–43]. Moreover, the WWTP is not sufficiently effective to remove all PCs residues from wastewater. Thus, a very considerable amount of PCs could reach the environment which makes it a serious problem that needs to be solved. Figure 5 illustrates the different ways of how drug traces could reach the soils and surface waters [44].

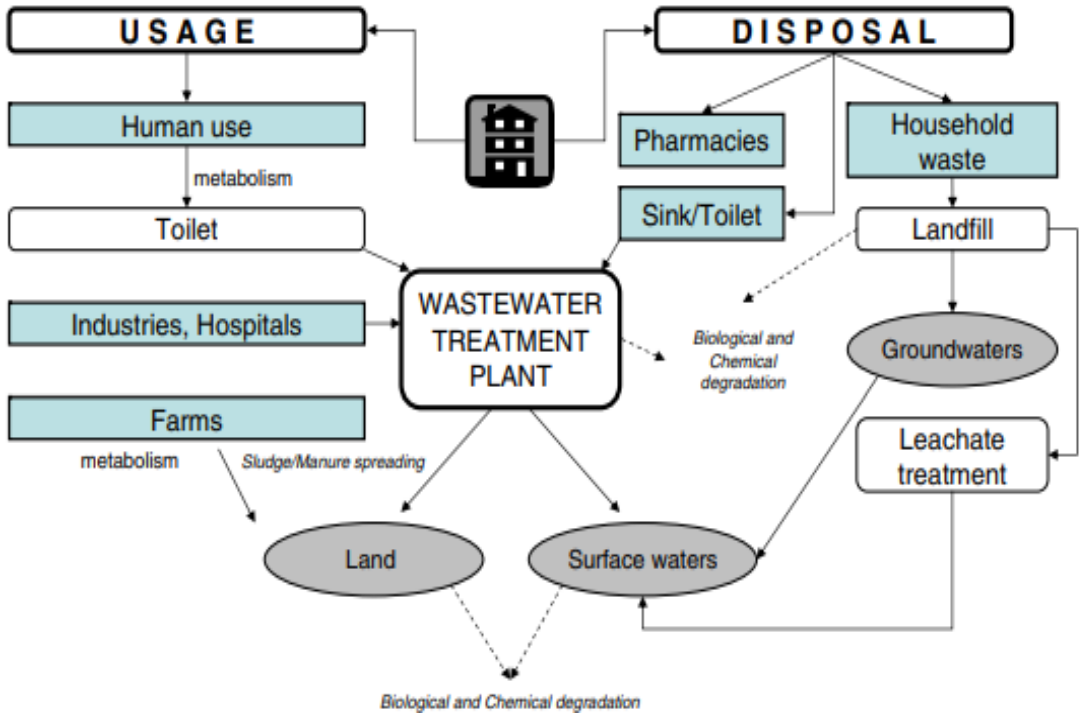


Figure 5: Sources and fate of drugs in the environment [44]

2.5 Effects of PCs on the environment

Nowadays, contaminants that could affect the environment appeared in a very huge amount, ranging from inorganic to organic compounds or nanoparticles. Most of them are released into the environment as a result of human activities. These

contaminants include different classes of PCs that could be very harmful to the environment, thus, it became an important and dangerous problem at the same time that needs a lot of researches and works to be solved [45].

PCs are compounds designed to interact with different enzymes or receptors in human and animal bodies, so they are very active compounds. However, this does not mean that they are not active toward other organisms that live in soils and water; these organisms have receptors that are similar to that in the human body, thus, PCs can interact inside these organisms' bodies similar to that in humans body [11].

As a consequence of the continuous exposure of organisms to the released PCs in soil and water, many reports have indicated and illustrated the diverse impacts of different drugs on the environment. These impacts include effects on testicular maturation, insect behavior, dung decomposition, and antibacterial resistance development [11].

Fenfluramine is a drug under anorexic class, scientists reported that it has a direct effect and an important role in enhancing the release of serotonin hormone in crayfish, which is resulting in larger oocytes. In addition, it accelerates the testicular maturation of crayfish [46]. A parasiticide drug, avermectins, was found to impact both adult and young insects in the environment. For the adult, they found that it decreased impaired mating, disruption of feeding and loss of water balance in the insect body. However, it caused delayed development and physical abnormalities to the younger insects [47].

Additionally, many drugs could affect aquatic plants such as Ibuprofen, which is considered one of the most common and used drugs. They have reported that

ibuprofen played a role in the inhibition of growth of an aquatic plant called Lemna minor with more than 25%, however, ibuprofen could play an opposite role by stimulating the growth of another aquatic plant called Synechocystis sp. by more than 70% [12].

Antibacterials were reported to affect the soil microbes in different ways. For example, veterinary antibacterials may affect sulfate reduction, which is important in the decomposition of dung in the soil [48]. In addition, some PCs when they are released in soils, they could result in antibacterial resistance development. For instance, Sengelov studied the antibacterial resistance in soil, he measured the antibacterial resistance of a drug called tetracycline in soils that containing pig manure slurry. The results indicated that, as the pig manure slurry amount increased in the soil, this will result in increasing the tetracycline antibacterial resistance in that soil [49].

Even though the amount of released PCs in the environment is low, the main concern is the long-term and accumulative exposure to such levels [50]. Apart from the toxicity effect of PCs, these drugs could form by-products after digestion and excretion. These by-products could be more or less toxic than the original compounds. However, the actual effect of by-products needs further research work since there is a lack of information about their effects on the environment. Therefore, the information about the consequences of released PCs or their derivatives on aquatic life and human is still not clear and need more investigation [51]. Table 4 shows some of the PCs and the type of organisms that are affected by these PCs [11].

Table 4: Examples of organisms affected by different PCs

PC Class	PC name	Organism effected by PC
Antibacterial	Tylosin	Soil microbes community
	Erythromycin	cyanobacteria
Synthetic steroid	17a-Ethinylestradiol	Fish
	Methyltestosterone	Snails
Anti-inflammatory	Ibuprofen	cyanobacteria
Stimulants	Caffeine	Fish
Parasiticide	Avermectins	Insects
Lipid Regulators	Clofibric acid	invertebrate
	Gemfinrozil	invertebrate

Chirality plays a very important role in life in general, since enzymes, amino acids, fats, carbohydrates and nucleic acids are chiral compounds. in addition, around 56% of the PCs are chiral and 88% of these drugs are racemic mixture [52]. It will be very interesting to know how chiral pharmaceutical drugs interact with the environment. Are they interacting differently than normal PCs or different enantiomer will behave differently?

Interestingly, experts found that although the enantiomers of chiral drugs have the same structure, molecular weight and shape, most of them exhibit different toxic and biological effects. Many drug enantiomers could be more effective or interact differently than their isomers. For example, the S-enantiomer of the verapamil chiral drug has been used as a calcium channel blocker, however, the R-enantiomer is used in cancer chemotherapy as a multidrug resistance regulator [14].

The enantiomeric composition of some PCs has also been confirmed to be important in the toxicity of different environmental species. For instance, S (−)-atenolol and S (+)-fluoxetine were found to inhibit the growth of a freshwater protozoan called *Tetrahymena thermophila* significantly, more than R(+)-atenolol and R(−)-fluoxetine. At the same time, they found that R(+)-atenolol increases the

mortality rates of algae called *Pseudokirchneriella subcapitata*, significantly more than S(−)-atenolol [53]. Moreover, experts found that propranolol and fluoxetine have enantiomer selective toxicity toward *Pimephales promelas;* they demonstrated that S(−)-propranolol and S(+)-fluoxetine are more toxic than S(+)-propranolol and S(-)-fluoxetine enantiomers [54].

Additionally, many drugs can be very dangerous when they are digested inside the body. Scientists found that drugs such as ibuprofen and fenoprofen can racemize inside the body by converting the inactive stereoisomers to be active enantiomer, resulting in larger dosages of the active isomer than what is expected in the given dose [55, 56]. Moreover, S-(−)-propranolol is found to be more toxic to fathead minnows than its other enantiomer, however, the opposite is true in daphnids drug [54].

2.6 Detection of PCs in wastewater

As was mentioned before, PCs reached the environment as a result of animal and human activities, however, before they reach the water ecosystems, this wastewater is subjected to different biotic and abiotic treatment processes, which are supposed to remove all PCs from wastewater. Furthermore, the problem is that conventional WWTP could be inefficient enough to remove all PCs traces from wastewater. In addition, the removing efficiency depends on the type of treatment process, whereas different types of treatment processes could be used [57].

During the wastewater treatment process, all organic matter including PCs will go through different kinds of abiotic processes, such as sedimentation, adsorption, and photodegradation. Following that, the wastewater containing PCs will go through secondary treatment, where they will be exposed to microbial degradation. Presently,

many development countries use a modern type of wastewater treatment called a tertiary treatment that includes bioremediation using different types of microorganisms such as bacteria and fungi [57]. The results of tertiary wastewater treatment are very promising, thus, treated water after tertiary treatment could be reused for agriculture and drinking.

The presence of chiral PCs in wastewater is an emerging topic that needs to be investigated thoroughly. These Chiral compounds have two enantiomers, when they are exposed to conventional WWTP, they could react and get removed differently than the achiral drugs [58, 59]. In the primary treatment steps, when PCs are exposed to abiotic processes, the handling process is not enantioselective, so both enantiomers of chiral drugs could be removed equally. However, the secondary treatment processes are expected to be enantioselective, and that could result in changing the enantiomeric composition and the two enantiomers will not be at equal quantity in wastewater. Figure 6 shows a typical pathway diagram that depicts the pathway of chiral PCs from different sources to the environment with different treatment processes that may affect the enantiomeric composition of PCs [58, 59].

The composition of chiral drugs enantiomers in a mixture is defined by an enantiomeric fraction (EF). EF could be calculated using the following formula:

$$EF = (S/(S + R))$$

Where [S] is the S-enantiomer and [R] is the R-enantiomer.

In any racemic mixture, the value of EF is 0.5, however, if the EF value was 1 then the S-enantiomer is the only enantiomer available in the mixture, while R-enantiomer will be the only enantiomer available if the EF value was 0.0 [58, 60].

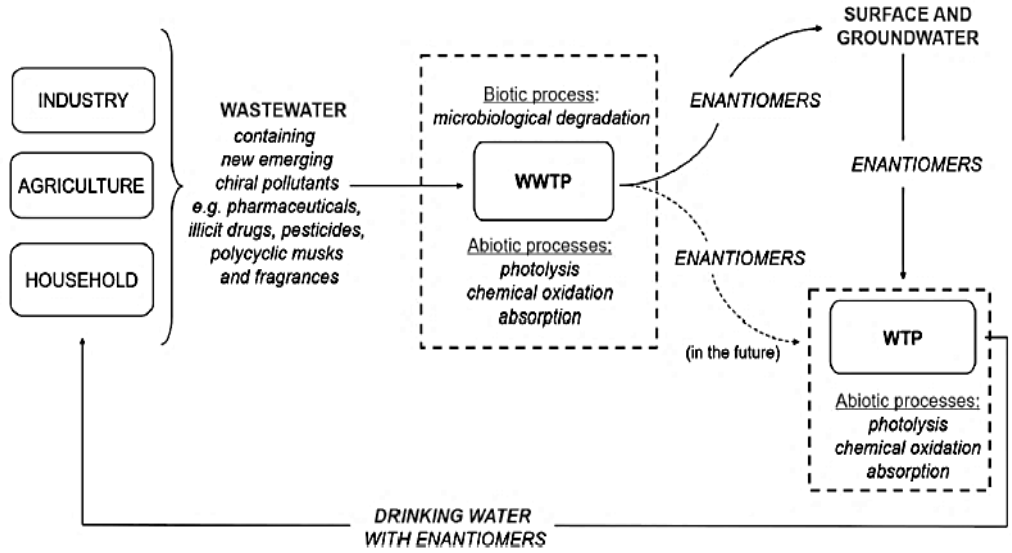


Figure 6: Pathway of chiral PCs from different sources to the environment with different treatment processes that may affect the enantiomeric composition of PCs [9]

2.7 Stereoselectivity of chiral PCs in the environment

Chiral PCs present in the environment could interact and go through different processes. Nevertheless, very few studies have focused on the stereoselectivity of PCs in the environment. Several studies did focus on the enantioselectivity during degradation processes [10, 15]. For example, it was reported that R-ibuprofen is degraded faster in rivers, while S-ibuprofen undergoes biodegradation faster in lake ecosystems [15]. Moreover, it was noticed that an interesting process occurred when such PCs presence in the soil, it called chiral inversion. It is a process in which one of the enantiomers of any chiral compound could be converted to the other enantiomer of that compound. Experts have reported that, a type of bacteria named “Nocardia

coralline" has the capability to produce an enzyme that can invert R-ibuprofen to S-Ibuprofen. Furthermore, it was reported that the chiral inversion of S-naproxen to R-naproxen was observed within activated sludge under laboratory conditions [10].

Interestingly, it was reported that the enantiomeric fractions of many drugs are different in the environment. For instance, many antidepressant drugs like venlafaxine, desmethylcitalopram and fluoxetine were enriched with S- enantiomer in the environment, on the other hand, mirtazapine was found to be enriched with R-enantiomer. In addition, many β-blockers such as metoprolol, atenolol and propranolol were found to be enriched with R-enantiomer. Anti-inflammatory drugs such as Ibuprofen were found to be enriched with S-enantiomer. However, β_2-agonist drugs such as salbutamol were found to be enriched with R- enantiomer [58, 61–63]. Figure 7 illustrates the occurrence of different enantiomers of chiral PCs in surface water.

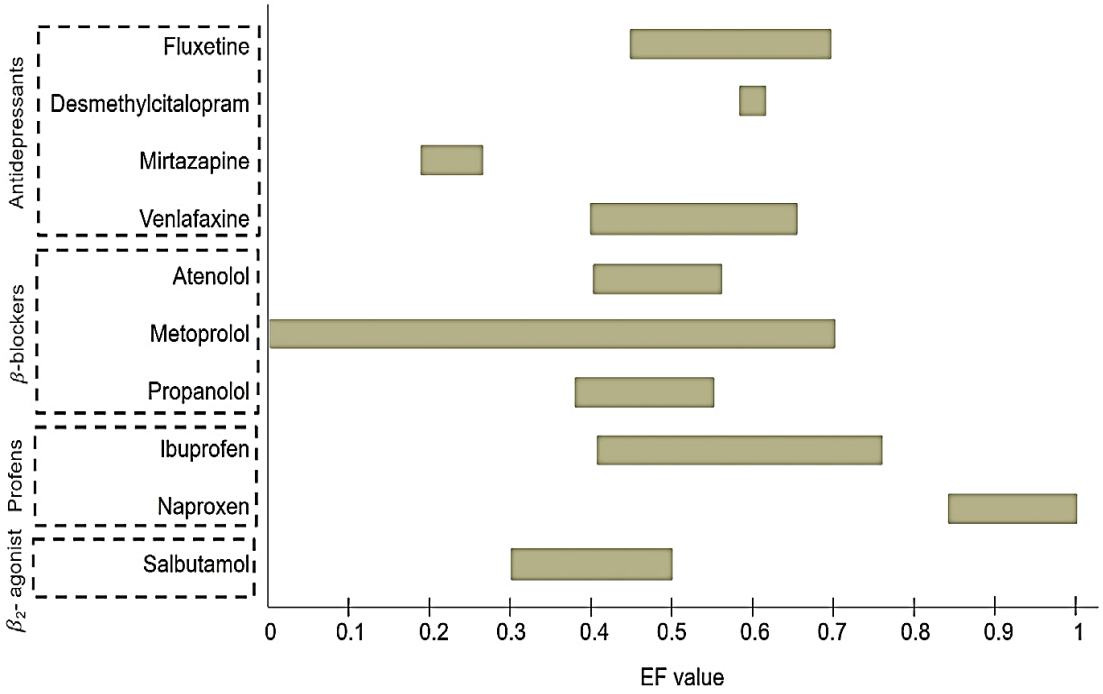


Figure 7: EF values of chiral PCs found in the surface water samples [9]

2.8 Techniques used for the determination of chiral PCs in wastewater

The Identification and separation of chiral PCs enantiomers are a very challenging task, since the two enantiomers have identical properties, for instance, they have the same structure, molecular weight, boiling point, melting point, etc.

Separation of enantiomers have been reported using capillary electrophoresis (CE), gas and liquid chromatography techniques. The analysis could be performed directly on chiral stationary phases that are available in GC and HPLC columns, or using indirect routs either by derivatization with a chiral derivatizing agent or by adding chiral selectors such as cyclodextrins or chiral surfactants to the mobile phase of the HPLC or CE [64–66].

2.8.1 Chiral separation of PCs by liquid chromatography (LC)

Liquid chromatography is the most commonly used technique for the separation of chiral compounds due to the availability of chiral columns. However, there are direct and indirect methods that have been used in LC for enantiomer separation. Indirect methods involve the derivatization process which resulting in forming diastereomers from the enantiomers. Then the two diastereomers can be separated using a normal achiral column. For instance, 2,3,4,6-tetra-O-acetyl-b-glucopyranosyl isothiocyanate has been used as a chiral derivatization reagent for the chiral separation of ammuxetine in dog plasma [67]. In another study, bambuterol enantiomers were separated from human plasma samples using diacetyl-L-tartaric anhydride as a derivatization reagent by LC-MS/MS [64].

Direct methods of chiral separation are preferred over the indirect methods in the environmental analysis since indirect methods are time-consuming and involve many steps for derivative preparation using special reagents and solvents which could be hazardous and toxic chemicals. This can introduce interference and impurities to the sample solution [62, 68].

The most used LC method for chiral separation is the chiral columns (chiral stationary phases). The first chiral stationary phase column has been established in 1981, since then, numerous materials have been used as chiral stationary phases, such as proteins, polysaccharides and macrocyclic antibiotics [69, 70–72]. Recently, the most widely used chiral stationary phases contain Vancomycin and Teicoplanin macrocyclic antibiotics [73, 74].

Chiral mobile phase additives have been widely used to separate enantiomers. Cyclodextrins are considered as very promising chiral mobile phase additives, for instance, hydroxylpropyl-β-cyclodextrin and (2,3,6-tri-O-methyl)-β-cyclodextrin have been used as mobile phase additives for the chiral separation of a group of nonsteroidal anti-inflammatory drugs, including ibuprofen, naproxen, flurbiprofen, ketoprofen, indoprofen, suprofen, carprofen, and cicloprofen [75, 76]. Moreover, Su Zeng and coworkers have used hydroxypropyl-β-cyclodextrin as a chiral mobile phase additive, where they successfully established an HPLC method to separate another eight nonsteroidal anti-inflammatory drugs [76, 77]. Meetani et al. have reported the use of cyclodextrin as an additive for the analysis of Dapsone in treated wastewater. It was possible to detect low concentrations of Dapsone using HPLC fluorescence detection [78].

2.8.2 Chiral separation of PCs using gas chromatography (GC)

Recently, many chiral GC methods have been developed for the separation of volatile and semi-volatile chiral compounds, since they give very high sensitivity, good reproducibility, decent selectivity and rapid analysis [61, 75]. In addition, there is no need to use toxic solvents and hazardous reagents to perform the chiral GC analyses. However, chiral GC is still limited to the analysis of compounds that are volatile and have high thermal stability. Chiral derivatization reagents have been used for nonvolatile compounds to enhance the chiral separation process as well as to improve thermal stability [62, 80].

Chiral separation methods on GC include direct and indirect methods. The direct methods utilize chiral columns. For instance, the presence of Ibuprofen and Naproxen enantiomers in the influent and effluent in wetlands, an activated sludge

wastewater treatment plant, and a sand filter have been evaluated; an astec chiraldex chiral column that was coated with dimethyl-β-cyclodextrin was used [58]. In addition, o-desmethyl naproxen has been determined in urban drain water, river water, sewage effluent and mangrove water. They used a (HYDRODEX beta-6TBDM) chiral column that consist of heptakis-(2,3-di-O-methyl-6-O-t-butyldimethylsilyl)-β-cyclodextrin on the stationary phase [58, 81].

Lately, the derivatization of chiral compounds before separation on an achiral GC column has been widely used due to its ability in improving chiral separation as well as enhancing the thermal stability of the targeted compounds [62]. several studies have been conducted using different chiral and achiral derivatizing agents, for instance, a chiral derivatizing agent called (R)-1-phenylethylamine ((R)-1-phenethylamine) and another an achiral derivatizing agent N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) have been used as derivatizing agents for the analysis of ibuprofen, ketoprofen, naproxen, metoprolol and propranolol in surface water, effluent wastewater, and river water [58, 65, 82].

2.8.3 Chiral separation of PCs by capillary electrophoresis (CE)

In CE, Chiral selectors are chiral components that interact with enantiomers to separate them [83, 84]. They are embedded in the buffer solution as a pseudo stationary phase in the electrokinetic chromatography capillary electrophoresis. Electrokinetic chromatography is the chiral selector mode that is used for chiral separation in CE [66, 83, 84].

Recently, liquid chromatography is the most common technique for chiral separation, since 63.2% of the published research papers between 2015-2019 dealing

with chiral separation was conducted using Liquid chromatography,12.7% were conducted using gas chromatography and 16.7% using capillary electrophoresis technique [85]. However, chiral analysis using CE has some advantages such as high resolution and efficiency, low consumption of samples and reagents and short analysis time. In addition, the sensitivity could be highly enhanced using fluorescence or mass spectrometry detectors [85].

Chiral PCs separation using CE has been reported in groundwater, rivers, lakes, and wastewater. For instance, beta-blockers PCs, such as atenolol, pindolol, metoprolol and propranolol, have been determined and chirally separated in surface and groundwater as well as in sewage water using CE with UV detection and methyl-beta-cyclodextrin as a chiral selector [86, 87]. In another report, duloxetine, verapamil, econazole, terbutaline, metoprolol, propranolol and betaxolol drugs have been chirally separated from wastewater samples by CE with UV detector and sulphated-beta-cyclodextrin was used as a chiral selector [88]. Finally, mass spectrometry has been used as a detector for the CE chiral separation of some amphetamines using sulfated-gamma cyclodextrin as a chiral selector [85, 89, 90].

2.9 Levels of PCs in wastewater

The detection and identification of PCs in wastewater began in the 1990s since most of the PCs are newly developed, therefore many of them do not have suitable guidelines that help to describe their levels in treated wastewater [8]. Nevertheless, scientists gave more attention to the identification of PCs in wastewater recently. Different studies were performed to identify and quantify PCs in wastewater. For example, Oleveira and coworkers studied the presence of some PCs and their concentrations in the influent and effluent wastewater in the USA, they found

relatively high concentrations of some drugs like ibuprofen and caffeine in the influent; their levels were in the range of 11.54-33.25 ppm and 73.96-88.33 ppm for Ibuprofen and caffeine, respectively. Though, the removal efficiency was very high, where the effluent concentrations were 0.04 ppm and 0.07 ppm for ibuprofen and caffeine, respectively [32]. It is worth noting that more than one study has confirmed the high levels of both ibuprofen and caffeine relative to the other drugs. They were reported in ranges above 10 ppm and 50 ppm for ibuprofen and caffeine respectively in the influent of different sources of wastewaters [32, 33, 50]. In another interesting study, the authors have investigated the presence of amoxicillin and spiramycin in wastewater using LC-MS/MS. They succeeded to reach to very low limit of quantification that equal to 0.5 ppb [91].

Detection and identification of chiral pharmaceutical drugs in wastewater treatment has gained more interest during the last decade. In addition to the fact that chiral separation of enantiomers is very challenging, the two enantiomers of the same PC could interact differently during the treatment in the WWTP. Many studies have reported the enantiomeric separation using LC and GC methods, very few have been done to study the separation and quantification the levels of these enantiomers in wastewater. Table 5 lists the concentration of chiral PCs in the influent and effluent of domestic WWTPs. Moreover, it shows the instrument limit of detection (IDL) and the limit of quantitation (LOQ). For some, PCs, some concentrations were not detected (ND) in the wastewater samples while for other PCs, the concentrations were not reported (NR).

Table 5: Range of some PCs (μg/L) in the influent and effluent of domestic WWTPs

PCs	Influent	Effluent	LOD	LOQ	Technique	References
Atenolol	971	664	Influent: 0.3-3.7 Effluent: 0.1-0.7	Influent: 1-13 Effluent: 0.2-2.5	LC-MS/MS	[73]
Metoprolol	411	375				
Nadolol	51	20				
Propranolol	10	45				
Salbutamol	20	17				
Amphetamine	24.2-213	ND	Influent: 0.9-3.5 Effluent: 0.9-1.65	Influent: 2.2-11.75 Effluent: 2.4-10.1	LC-MS/MS	[68]
Ephedrine	4-72	2.8-6.4				
Venlafaxine	57-287	80-248				
Bisoprolol	NR	ND	IDL: 163-2868 Method detection limit: 0.65-11.5	Instrument quantification limit: 495-4935 Method quantification limit :1.98-19.7	LC-MS/MS	[60]
Norfluoxetine						
Alprenolol						
Fluoxetine						
Metoprolol						
Salbutamol						
Venlafaxine		40-129				
Ephedrine	NR	NR	Influent:0.4-3.3 Effluent: 0.3-2.5	Influent: 1.3-11.1 Effluent: 1.1-8.4	LC-MS/MS	[92]
Atenolol						
Venlafaxine						
Propranolol and its enantiomers	0.3-0.01	0.16-0.003	LOD: 0.0001-0.001	NR	LC-MS/MS	[93]

Chapter 3: Methodology

3.1 Introduction

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

The 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 that were used in this work. Sections 3.6 to 3.8 give more details about the used LC-MS/MS technique, the procedure used in the analysis, extraction of PCs from wastewater and sludge samples, in addition to 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 review the plant 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, Sections 3.10 and 3.11 show how the removal efficiency and mass balance were calculated for different unit processes.

3.2 Target PCs

In this work, (±)-Cotinine-D_3 has been used as an internal standard (IS) as its structure and molecular weight are close to the studied drug structures, however, 3 hydrogen atoms were replaced by 3 deuterium (D_3) atoms. (±)-Cotinine-D_3 has been chosen as an internal standard because any change that may occur to the PCs in wastewater can be detected since there are no deuterated samples or PCs in wastewater in nature. Table 6 lists the studied PCs and shows their structures and classes, while Table 7 lists the chemical properties of the considered PCs. Note that all targeted PCs are chiral PCs. All of them were bought from Sigma-Aldrich in a standard analytical grade with a purity of (> 99%). The stock solutions have been prepared in methanol and stored in dark at -18°C. The working solutions have been prepared by diluting the stock solution with Methanol solvent.

Table 6: Targeted chiral PCs for LC/MSMS analyses and their structure

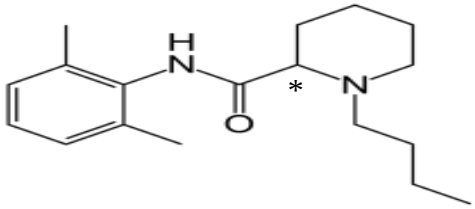
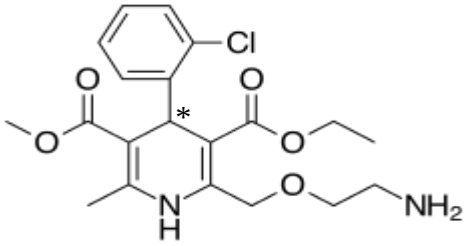
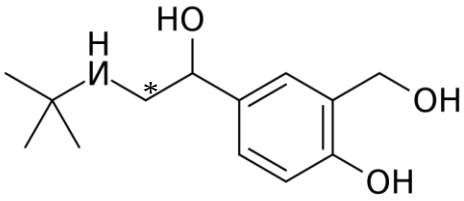
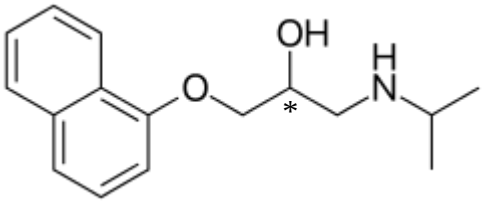
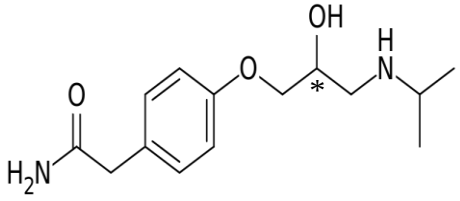
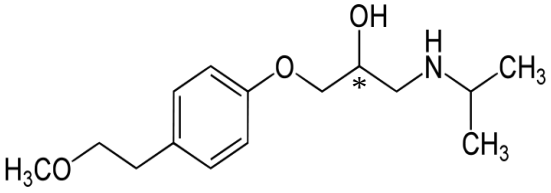
PCs	Class	Chemical structure
Bupivacaine	local anesthetic	
Amlodipine	Calcium channel Blockers	
Salbutamol	β2-adrenergic agonists	
Propranolol	β-blockers	
Atenolol	β-blockers	
Metoprolol	β-blockers	

Table 6: Targeted chiral PCs for LC/MSMS analyses and their structure (continued)

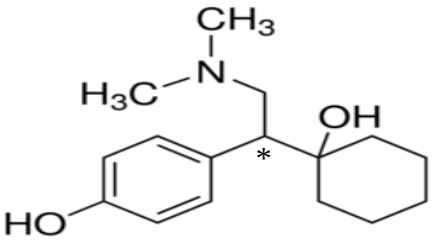
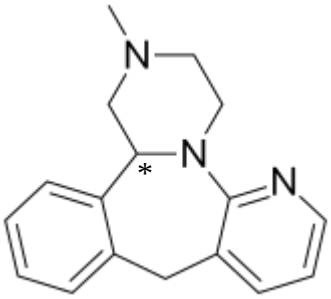
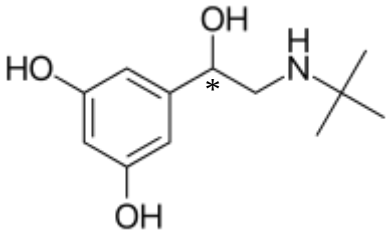
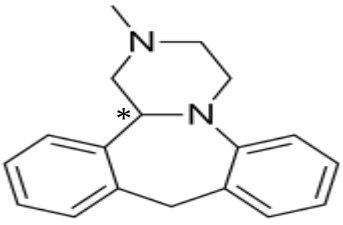
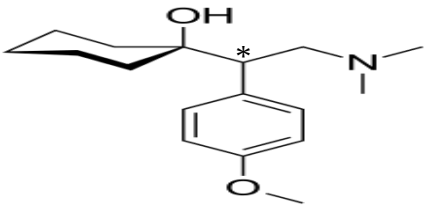
PCs	Class	Chemical structure
O-Desmethylvenlafaxine	Antidepressant	
Mirtazapine	Antidepressant	
Terbutaline	β2- agonists	
Mianserin	Antidepressant	
Venlafaxine	Antidepressant	

Table 6: Targeted chiral PCs for LC/MSMS analyses and their structure (continued)

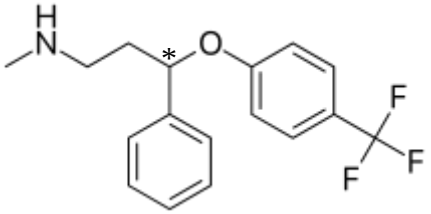
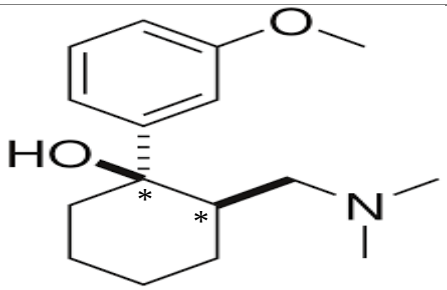
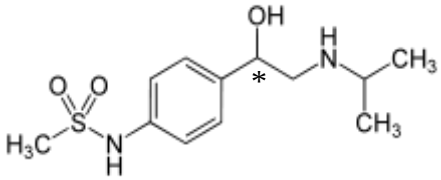
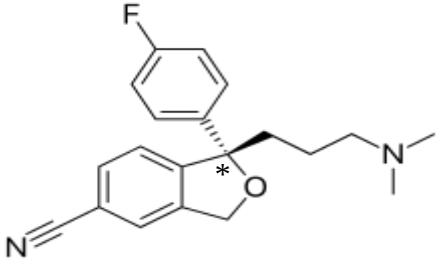
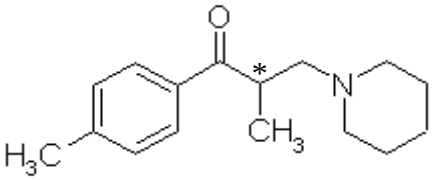
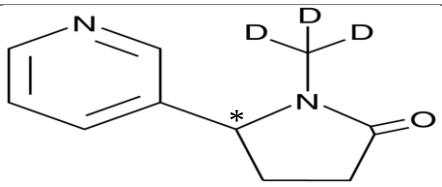
PCs	Class	Chemical structure
Fluoxetine	Antidepressant	
Tramadol	analgesics	
sotalol	β-blockers	
Citalopram	antidepressant	
Tolperisone	Muscle Relaxants	
(±)-Cotinine-D_3	Internal standard	

Table 7: Physicochemical properties of the considered PCs [94], [95]

PCs	Molecular Formula	Molecular Weight (g/mol)	pK_a	Solubility (mg/L)	Log K_{ow}
Bupivacaine	$C_{18}H_{28}N_2O$	288.4	8.1	2400	3.41
Amlodipine	$C_{20}H_{25}ClN_2O_5$	408.9	9.4	0.0074	3.00
Salbutamol	$C_{13}H_{21}NO_3$	239.31	9.2and 10.7	14100	0.64
Propranolol	$C_{16}H_{21}NO_2$	259.34	9.42	61.7	-0.45
Atenolol	$C_{14}H_{22}N_2O_3$	266.34	9.6	13300	0.16
Metoprolol	$C_{15}H_{25}NO_3$	267.36	9.7	1400	1.88
O-Desmethylvenlafaxine	$C_{16}H_{25}NO_2$	263.37	9.45and10.66	3700	2.72
Mirtazapine	$C_{17}H_{19}N_3$	265.3	7.7	1100	3.09
Terbutaline	$C_{12}H_{19}NO_3$	225.2	8.86 and 9.76	5840	-1.8
Mianserin	$C_{18}H_{20}N_2$	264.3	6.92	232	4.24
Venlafaxine	$C_{17}H_{27}NO_2$	277.4	10.09	267	3.20
Fluoxetine	$C_{17}H_{18}F_3NO$	309.3	9.8	1.7	4.65
Tramadol	$C_{16}H_{25}NO_2$	263.3	13.8 and 9.23	750	3.01
sotalol	$C_{12}H_{20}N_2O_3S$	272.3	10.07and 9.43	782	0.24
Citalopram	$C_{20}H_{21}FN_2O$	324.39	9.78	5.88	3.74
Tolperisone	$C_{16}H_{23}NO$	245.36	8.78	176	3.65
(±)-Cotinine-D_3	$C_{10}H_9D_3N_2O$	179.23	4.79	1000000	0.07

[a] K_a is the acid dissociation constant and K_{ow} is the octanol-water partition coefficient.

3.3 Stock solutions preparation

The stock solutions for PCs were prepared as follows: A particular mass of each PC standard in the range of 0.3 – 5 mg was weighed and dissolved. Some of them were dissolved in methanol and the other group was dissolved in dimethylsulfoxide (DMSO) then diluted in methanol to reach a concentration of 200 ppm. The PCs solution vials were moved to an ultrasonic water bath to confirm that they are completely dissolved. The prepared stock solutions were kept in the dark and in the freezer at a temperature lower than -18°C. Fresh working solutions were prepared weekly by dilution of stock solutions to get 100 ppm using methanol solvent.

3.4 Preparation of calibration curve solutions

A particular volume (1 mL) from each working PCs solution (100 ppm) was mixed in one big vial (40 mL). The mixed volumes were then evaporated at room temperature using a vacuum - centrifuge system (CentriVap Concentrator-Labconco). After that, the precipitated PCs powder that remained in the vial was reconstituted in 2 ml methanol to get a final concentration of 50 ppm for each PC. The calibration curve standards were prepared using serial dilutions. The following concentrations were prepared for the calibration curve (50, 30, 25, 20, 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005, and 0.001 ppm).

3.5 Internal standard

(±)-Cotinine-D_3 was used as an internal standard (IS) because it is a deuterated compound, which cannot be found in nature or wastewater. Consequently, (±)-Cotinine-D_3 was used in quantitation during wastewater samples processing steps, which includes sample concentration and cleans up. A stock solution of (±)-Cotinine-

D_3 was prepared by dissolving 5 mg in 5 ml methanol to get a final concentration of 1000 ppm of the compound. It was stored at -18°C. A working solution, 50 ppm, was prepared from the stock in deionized water by proper dilution. A 100 μL of the working solution was spiked into blanks, control, and standard solutions to have 5 ppm of (±)-cotinine-D_3 as a spiked final concentration in each. Likewise, the real samples were spiked with the same volume of the internal standard to have a concentration of 5 ppm in all samples.

3.6 High-performance liquid chromatography technique (HPLC)

HPLC is an efficient, quick, highly accurate, sensitive, and automated technique. It is used to separate and identify different chemical compounds in complex mixtures. The separation of chemical compounds is performed using a solid stationary phase (column) and the liquid mobile phase is used to carry the sample components of different compounds through the column. Usually, the HPLC is attached to a sensitive detector such as triple quadrupole tandem mass spectroscopy using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) interface.

In ESI, the sample is sprayed with nitrogen gas through a capillary tube. Ions will be produced from the sprayed solutions by applying a high voltage on the capillary to create a suspension of the liquid droplets. As the droplet size decreases due to spraying gas and high drying temperature, the electrical charge density on the droplet surface increase. As a result, higher repulsion between charged ions within the droplets is established, then the droplets will explode, which makes the ions to leave the droplets. Figure 8 shows how the ESI process is occurred [96].

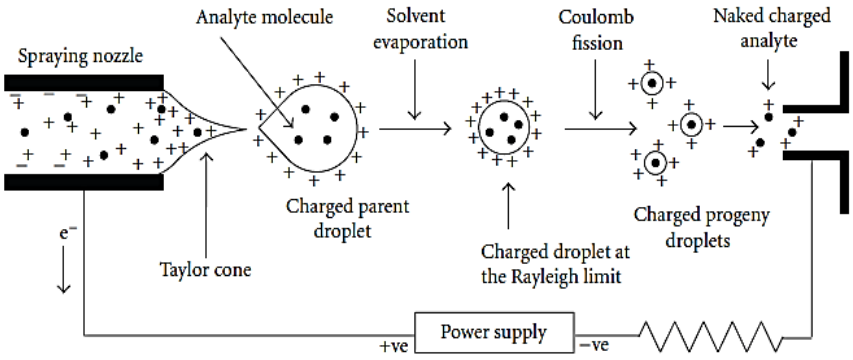


Figure 8: Schematic of the ESI process [96]

Usually, HPLC is attached to different types of detectors, such as UV-VIS spectrophotometer, fluorescence Spectrofluorometer, and mass spectrometer detector. The quadruple tandem mass spectrometry is considered one of the most sensitive and selective detectors attached to the HPLC system. It is used to analyze and identify the masses of a wide range of chemical compounds. Moreover, mass spectrometers can come in different types, such as single quadrupole mass spectrometer, ion-trap mass spectrometer, time of flight mass spectrometer and triple quadruple tandem mass spectrometer (MS/MS) which has been used in this work. Triple quadrupole mass spectrometer (QQQ) utilizes two mass analyzers, the first mass analyzer is a quadrupole one (Q1) which analyzes and filters the precursor ions of the chemical compounds. Then the precursor ion goes through a collision cell which is a quadrupole two (Q2) that will fragment the precursor ion to smaller ions called product ions using an inert collision gas such as Helium, Nitrogen, or Argon, with a controlled high voltage collision energy. Finally, the product ions go through the second mass analyzer, quadrupole three (Q3) in order to analyze and scan the fragment ions. Triple quadrupole mass spectroscopy (MS/MS) is more sensitive and selective than single quadrupole mass spectroscopy (MS). It can reduce the noise and interferences from

the matrix and other chemical compounds by identifying the compound by monitoring its precursor ion and product ions at the same time. Figure 9 shows the Q1, Q2 and Q3 for triple quadrupole mass spectroscopy (MS/MS).

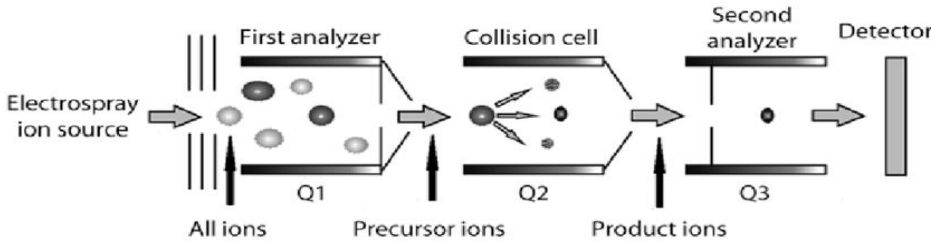


Figure 9: MS/MS with Q1, Q2, and Q3 [97]

3.6.1 LC-MS/MS procedure

The HPLC analyses were done on Nexera-i Liquid Chromatograph (LC-2040C) using Astec CHIROBIOTIC V column (length 250 mm, internal diameter 2.1 mm, and particle diameter 5μm) attached to Astec CHIROBIOTIC V Guard Column (length 20 mm, internal diameter 1 mm, and particle diameter 1 μm). The instrument is coupled to (LCMS-8030) Shimadzu tandem mass spectrometer. Positive ESI mode was used for ionization of the targeted PCs. Table 8 shows a summary of the conditions that have been used for developing the LC-(+)-ESI-MS/MS method, which is used in this work. A flow rate of 0.2 mL/min was used with 55 min of total run time using the instrument that is shown in Figure 10 below. The targeted chiral PCs have been detected by multiple reaction monitoring (MRM) mode using the following steps.

Step 1. Define precursor ion masses in parameter acquisition.

Step 2. Determine the optimum voltage for each fragment.

Step 3. Determine the optimum collision energy and define product ion masses.

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

Table 8: LC-MS/MS method conditions and parameters used for the analysis of PCs

HPLC conditions			
Column	Astec CHIROBIOTIC V column (Supelco) (5µM 25cm X 4.6 mm) attached to Astec CHIROBIOTIC V Guard Column (Supelco) (1µM 2cm X 1 mm)		
Column Temperature	25ºC		
Mobile Phase	4 mM Ammonium acetate dissolved in 100% Methanol, pH 6.8		
Flow rate (isocratic elution)	0.2 mL/min		
Post-Run	3 min		
Total Cycle Time	55 min		
MS Condition			
DL Temperature	250ºC	Heat Block Temperature	400ºC
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		

All selected chiral PCs have precursors and at least 2 product ions. Product ions peaks used for quantifications and qualifications.

Figure 10: LC-MS/MS used to analyze chiral PCs

3.7 Solid phase extraction technique and procedure

Solid-phase extraction (SPE) is a simple technique that was used to prepare samples to be ready for analysis on LC-MS/MS by separating targeted compounds from the sample matrix and concentrating them into smaller volumes. The separation process occurs depending on the compounds' chemical and physical properties [98].

Horizon Technology SPE-DEX® 4790 Automated Extraction system (Figure 11) was used for extracting and concentrating the chiral PCs from the collected wastewater samples. A disk filter (Atlantic® HLB-M SPE, 47 mm) was used to concentrate and collect the PCs from the collected wastewater samples. Initially, the SPE instrument has to be purged before doing any filtration for any sample. Purging steps are shown in Table 9. PCs extraction steps from wastewater samples are shown in Table 10 [99]. Finally, around 40 ml was collected as the final volume of every sample after the SPE process. Afterward, the final volumes were evaporated under a stream of nitrogen until dry. Subsequently, the dried samples were reconstituted by dissolving them in the mobile phase. The samples (1 mL each) have been filtered using Iso-Disc Syringe Filter Unit, PTFE membrane (diameter 25 mm, pore size 0.22 μm), and transferred 1.5 mL vial and placed in the autosampler for LC-MS/MS analysis.

Table 9: The purge method used for purging 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 10: Extraction method used to extract 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	Chloromethane	180	20
Rinse step 3	Chloromethane	60	20
Rinse step 4	Chloromethane	60	60

Figure 11: SPE instrument used for extracting PCs from 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 lowest concentration of analyte that can be identified at a known confidence level. It can be determined by using Equation (1).

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

Where *m* is the slope of the calibration curve, and s_{blank} is the standard deviation of the signal of the blank replicates, usually 20-30 blank replicates are performed under the same instrumental conditions.

The instrument limit of quantification (LOQ), is the minimum concentration at which quantitative measurement can be made. It can be determined by using Equation (2).

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

Since (±)-Cotinine-D_3 has been used as an internal standard, so it was spiked in the blank samples to be used as a label. Its final concentration was 5 ppm. Consequently, Equations (1) and (2) were modified 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 have been done by analyzing 20 replicates of a blank sample that were spiked with 5 ppm of the IS ((±)-Cotinine-D_3). After that, the blank signal at the same retention time (t_R) of each PC was recorded. Based on the 20 replicates, the standard deviation of each one of the recorded signals was calculated. Then, they have been used with the slope ratio between the IS and every one of the PCs to determine

the IDL and LOQ values. Note that these values have been estimated relative to the internal standard, in addition, the signal used in the calculation was not based on the area but intensity.

3.8.2 Method limit of detection

The method limit of detection (LOD) can be calculated using the IDL value. The method LOD for liquid samples was calculated by dividing the IDL over the sample final volume. On the other hand, for sludge samples, the conversion was computed by dividing the IDL by the extracted dry sludge weight.

3.9 Al Saad WWTP description

3.9.1 Location of Al Saad WWTP

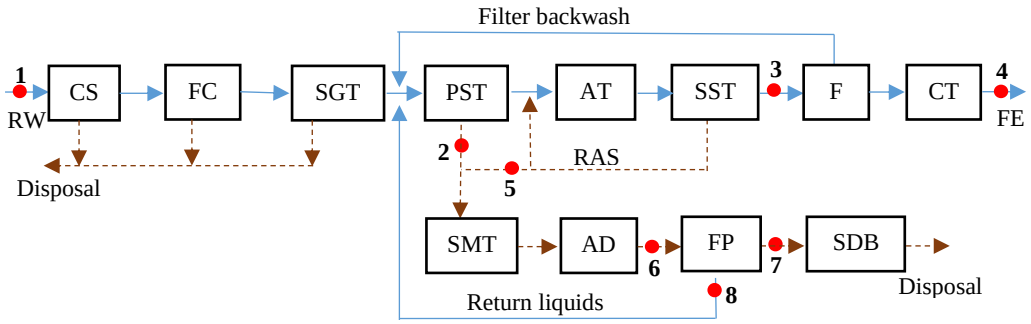
It is a domestic wastewater treatment plant that serves part of Al-Ain city, UAE. It can be found near Al Ain city as shown in Figures 12 and 13. A map view for the location of the plant in Figure 12 while a satellite view for the plant is shown in Figure 13. About 92,000 m^3/d of domestic sewage that the plant receives every day.

Figure 12: Google map view for the location of Al Saad WWTP

Figure 13: Google satellite view for the location of Al Saad WWTP

3.9.2 Al Saad WWTP flow sheet diagram

Figure 14 shows a flow sheet diagram for Al Saad WWTP. The plant contains primary treatment and secondary treatment processes. The primary treatment processes including fine screening, coarse screening, sand and grease trap and finally primary sedimentation. The primary treatment process is used for removing sand, large remains, and a major part of the suspended solids. Then the secondary treatment processes that include aeration and secondary sedimentation take place. The secondary treatment aims to reduce organic matter by employing biological treatment using the activated sludge process. Some of the sludge that is relaxing in the secondary settling tanks will be returned to the aeration tank, however, the rest will be sent for further processing to anaerobic digesters before disposal. Water that comes out from the secondary clarifiers will be filtered before it is purified further 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 14: Basic flow sheet diagram of Al Saad WWTP [100]

3.9.3 Al Saad WWTP design

The design containing different parameters such as flow rate (Q_d), suspended solids (SS), total phosphorus (P_{tot}), total nitrogen (N_{tot}) and biochemical oxygen demand (BOD_5) at different positions at Al Saad WWTP. They are listed below in Table 11. Additionally, the sizes and the number of units are shown in the same table.

3.9.4 Al Saad WWTP historical records

Some of the actual measurements of some parameters for the last 5 years (1st of July 2013 to 31st of March 2018) were obtained from the operator. Parameters including flow rate, recycle flow, volatile suspended solids (VSS), total suspended solids (TSS), total Kjeldahl nitrogen, chemical oxygen demand (COD), alkalinity, nitrate–N, ammonia, and pH. Table 12 presents a summary of these parameter values divided into 3 groups (minimum, average, and maximum).

Table 11: Designed parameters for Al Saad WWTP at different locations

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

Table 12: Summary of 5-year record for some parameters at Al Saad WWTP [100]

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

The samples of sludge and water (1.0 liter each) have been grabbed and collected in glass containers from Al Saad WWTP. They were grabbed from 8 different locations (see Figure 14), they are labeled from (1) to (8) in Figure 14.

Sampling location (1) is the inlet of the plant, it shows the characteristics of the wastewater in Al-Ain. Sampling location (2) was selected to check the adsorption of selected PCs on the sludge in the primary settling tanks, note that there is no biological treatment in this stage. Samples in location (3) were grabbed before the outlet and after the secondary settling tank. Samples in location (4) were collected at the outlet after chlorination, it represents TSE or the effluent which is used for landscaping. Samples in location (5) and (6) were grabbed to analyze the sludge samples before and after the anaerobic digestion (AD) process, respectively. Sampling locations (7) and (8) were intended to analyze the selected PCs in the sludge and water that went out of the filter press unit. A cationic polymer (Corofloc 341, SNF, France) was added to the filter press process unit to make the sludge thick.

Wastewater samples were collected from the selected locations (mentioned above (1-8)) in 4 batches for 2 months. They were collected on the 10th of October, 24th of October, 6th of November, and 25th of November 2018. It is known that composite sampling is better than grab sampling in representing the average of PCs levels per day, however, sample collection was done by grab sampling in this work as a result of the difficulties in collecting samples over 24 hours.

After collection, samples were kept in an icebox and then they were transferred to the lab for analysis. They were preserved in the refrigerator at -17°C, then they have been extracted by SPE. Once extracted, they were frozen at below -25°C then they were analyzed by LC-MS/MS.

3.9.6 Samples preparation

Since some of the wastewater samples contain water, others contain sludge, therefore, the sample preparation and processing have been done in two different ways. For the liquid samples, like influent, the effluent of secondary settling tank (secondary clarifier), liquid filter press samples and final effluent samples, the IS was spiked into these samples as explained in Section 3.5, then they have been extracted and prepared as mentioned in Section 3.7.

However, the sludge samples were filtered and separated from the liquid part of the wastewater samples. This was done for all samples that came from the primary settling tank, returned activated sludge, and effluent of anaerobic digester. The IS has been added to sludge samples before filtration as explained in Section 3.5. The filtration has been done under vacuum using a 9 cm filter paper (Whatman 1 qualitative) and Buchner funnel. After filtration, the extracted water was prepared and

processed as explained in Section 3.7. The remaining solids on the filter papers (filtered sludge) were placed in the oven and heated at 105°C for 4 hours to remove all moisture content from the sludge samples. Then 60 ml of acetone have been added to the sludge samples followed by 2 hours of stirring to perform the manual extraction of PCs, after that the solvent was filtered by using 9 cm filter paper (Whatman 1 qualitative). Then 100 mL of dichloromethane (CH_2Cl_2) has been added to the sample followed by stirring for 2 hours then they have filtered again. The extracted samples in acetone and CH_2Cl_2 were evaporated by nitrogen stream and dissolved again in the mobile phase as it was mentioned in Section 3.7. Finally, after the filter press process, the sludge was prepared manually as explained above for the filtered samples.

3.10 Removal efficiency

In general, PCs removal from wastewater depends on many variables. These variables will not have the same effect on the investigated drugs, for example, drug biodegradability, physiochemical properties like volatilization, adsorption to sludge and water solubility. Many other factors could have a role in removing PCs from wastewater like the treatment unit temperature, the efficiency of removal will be reduced with lower temperature [32, 33].

However, removal efficiency (RE) has been calculated in different units (SST, PST and FE) to understand the removal role of each one. So, RE has been calculated using the following equation (5).

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

3.11 Mass balance

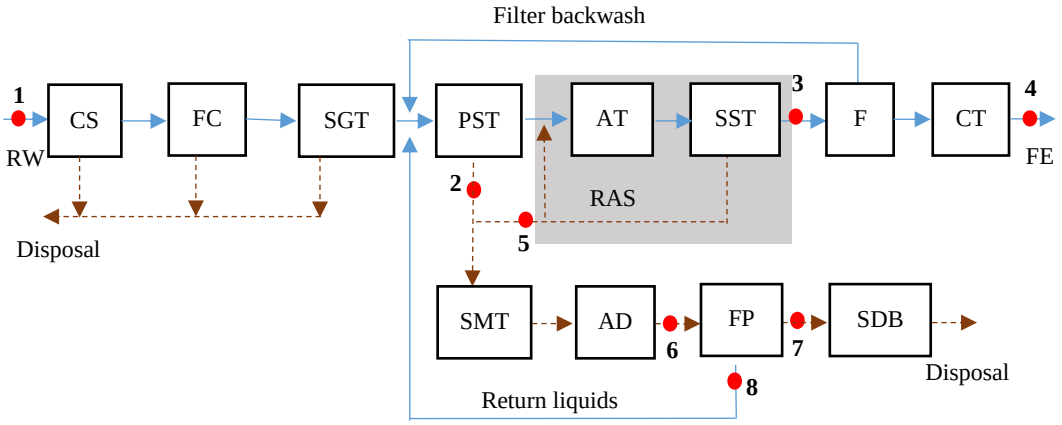
As stated by the conservation of mass law: mass is neither created nor destroyed, so in this part, the mass balance method was applied at different locations of the WWTP to identify the mass loss of PCs. The general mass balance equation is shown below.

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

Therefore, if the rate of mass in is more than the rate of mass out, this indicates a mass loss due to a specific reaction. The reaction (loss of mass) could be a result of biodegradation, adsorption to the sludge or chemical degradation.

3.11.1 Mass balance around the aeration tank part

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 15 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 15: Mass balance for the aeration tank [100]

The removal mechanism has been determined relative to the concentration in the raw wastewater. The removal mechanism after PST was ignored since location (2) has no important role in the treatment process as what was reported in previous work [100] that has been done on the same water samples, where the highest removal efficiency has been found to be equal to 15% or less. However, the removal mechanism after the SST and FE have been determined depending on the PCs concentration in locations (3) and (4) (Figure 15), respectively. See Equation (7) below, which has been used for the calculation to know the mass rate in and out of the RAS system.

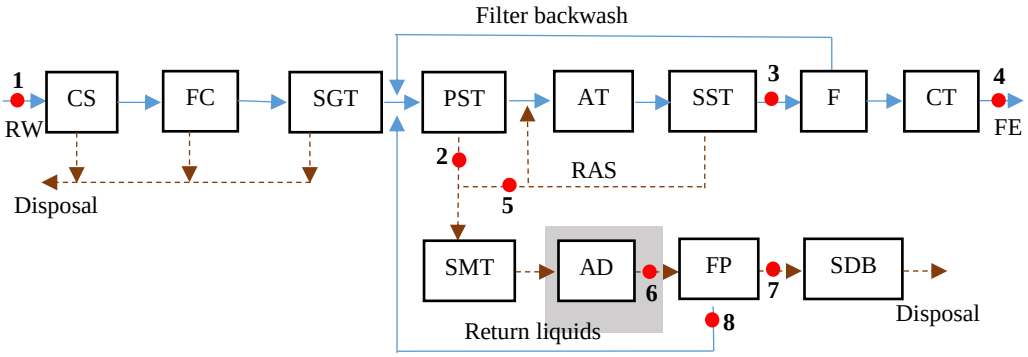
$$(Q_{in} \times C_{in}) + (Q_{FP} \times C_{FP}) = (Q_{out} \times C_{out}) + (SS_{PST} \times S_{PST}) + (Q_{RAS} \times C_{RAS}) + (SS_{RAS} \times S_{RAS}) \quad (7)$$

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 were adsorbed to the sludge or degraded. The shaded unit (grey color) in Figure 16 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 16: Mass balance for the anaerobic digester [100]

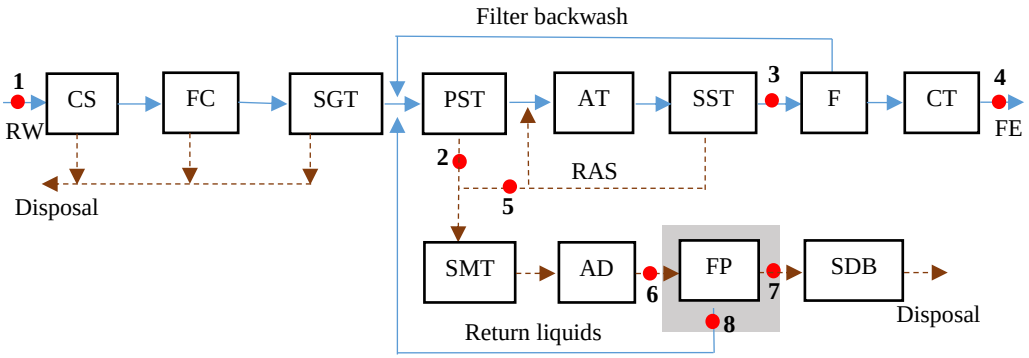
The removal efficiency values were calculated relative to the concentration in RAS in water and sludge at the location (5). The removal efficiency after PST was determined based on mass balance around the PST, while that after the AD was determined based on the concentration of PCs at locations (2) and (6) (Figure 16), 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 the addition of polymer for sludge dewatering. The shaded unit (grey color) in Figure 17 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 17: Mass balance for the filter press [100]

The removal efficiency values were calculated relative to the concentration of AD in water and sludge at location 6. The removal efficiency after the FP unit was determined based on the concentration of PCs in the water at the location **(7)** (Figure 17), 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 Optimization

Optimization of the mass spectrometric detection has been performed for all chiral PCs in the study to identify their precursor ions, product ions, their collision energies, dwell times and their MRM transitions. Table 13 summarizes all mentioned information above for all chiral PCs in this study include bupivacaine, amlodipine, salbutamol, propranolol, atenolol, metoprolol, o-desmethylvenlafaxine, mirtazapine, terbutaline, mianserin, venlafaxine, fluoxetine, tramadol, sotalol, citalopram, tolperisone and (±)-cotinine-D3 which is used as an internal standard.

Table 13: Optimization results and MRM transition for PCs using LC-MS/MS

PCs	Precursor ion (m/z)	Product ion (m/z)	Dwell time (msec)	Collision energy (V)
(±)-Cotinine-D3 (IS)	180.05	80.10	52.0	-25.0
		81.10	52.0	-20.0
		101.00	52.0	-22.0
Bupivacaine	289.25	140.15	100.0	-21.0
		84.15	100.0	-42.0
		97.95	100.0	-37.0
Salbutamol	240.20	148.00	125.0	-19.0
		222.15	125.0	-10.0
		166.10	125.0	-13.0
Terbutaline	226.00	152.05	100.0	-16.0
		107.05	100.0	-30.0
		125.05	100.0	-24.0
(±)-Metoprolol	269.20	116.10	100.0	-19.0
		72.05	100.0	-23.0
Atenolol	268.20	191.05	100.0	-19.0
		74.15	100.0	-24.0
Mianserin	265.10	208.10	100.0	-22.0
		91.00	100.0	-47.0
Mirtazapine	266.15	195.05	100.0	-25.0
		72.15	100.0	-18.0
O-Desmethylvenlafaxine	264.20	246.15	100.0	-12.0
		106.90	100.0	-34.0
Tramadol	264.10	58.05	100.0	-16.0
(±)-Propranolol	261.20	184.05	100.0	-18.0
		116.20	100.0	-18.0
		74.00	100.0	-22.0
Venlafaxine	278.10	58.05	100.0	-24.0
		260.20	100.0	-12.0
		121.10	100.0	-28.0
(±)-Sotalol	273.05	255.10	100.0	-12.0
		133.10	100.0	-27.0
		212.95	100.0	-18.0
Fluoxetine	310.15	44.15	100.0	-13.0
Tolperisone	246.15	98.10	100.0	-20.0
		70.05	100.0	-37.0
		55.05	100.0	-46.0
Amlodipine	409.40	238.00	125.0	-11.0
		294.00	125.0	-11.0
		206.05	125.0	-27.0
citalopram	325.20	109.05	100.0	-28.0
		262.10	100.0	-20.0
		234.00	100.0	-30.0

4.2 Calibration curves

The calibration curves have been prepared and ranged from 30 ppm to 0.001 ppm using 12 concentration levels of prepared standards (30, 25, 20, 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0, 005 and 0.001 ppm). Many selected PCs had shown a very high signal response, where the instrument reached to signal saturation, therefore, in these cases, the calibration curve started from a lower concentration for these PCs. Figure 18 shows the simultaneous LC-MS/MS analysis of a mixture of all chiral PCs standards, 1 ppm each, using MRM mode. Selected ion monitoring was used to show the chromatogram of each chiral PC enantiomers. The retention time (t_R) was ranged from 5.0 minutes for the internal standard "cotinine-d3" to 44.0 min for E2-citalopram. However, many of them eluted at the same t_R but the instrument was able to distinguish between them with the help of the MRM mode. Note that all the selected drugs were chirally separated except one drug called tramadol, so it has been treated as a racemic mixture in the discussion below.

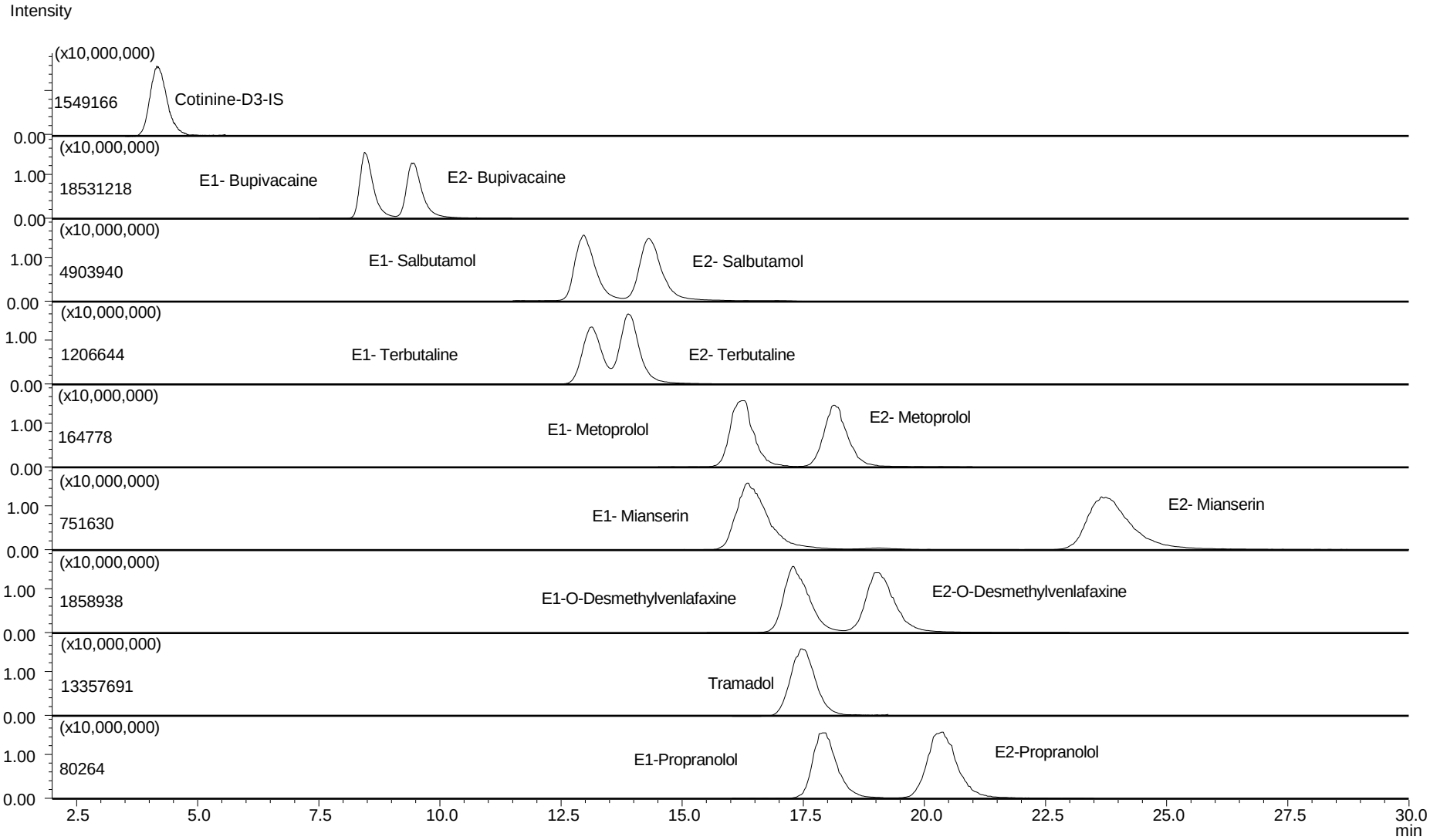


Figure 18: Chiral PCs chromatograms separated by LC-MS/MS using MRM mode

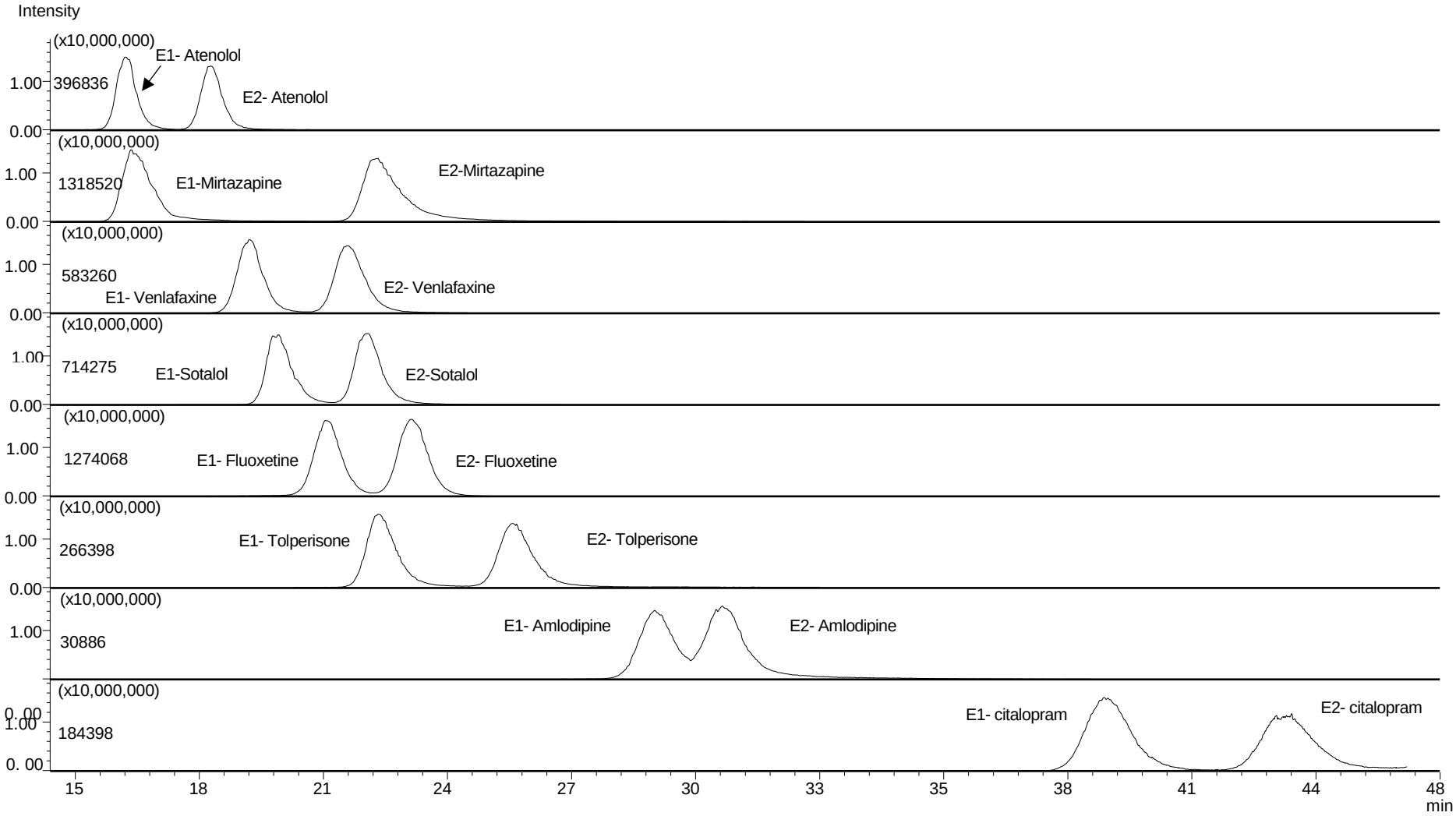
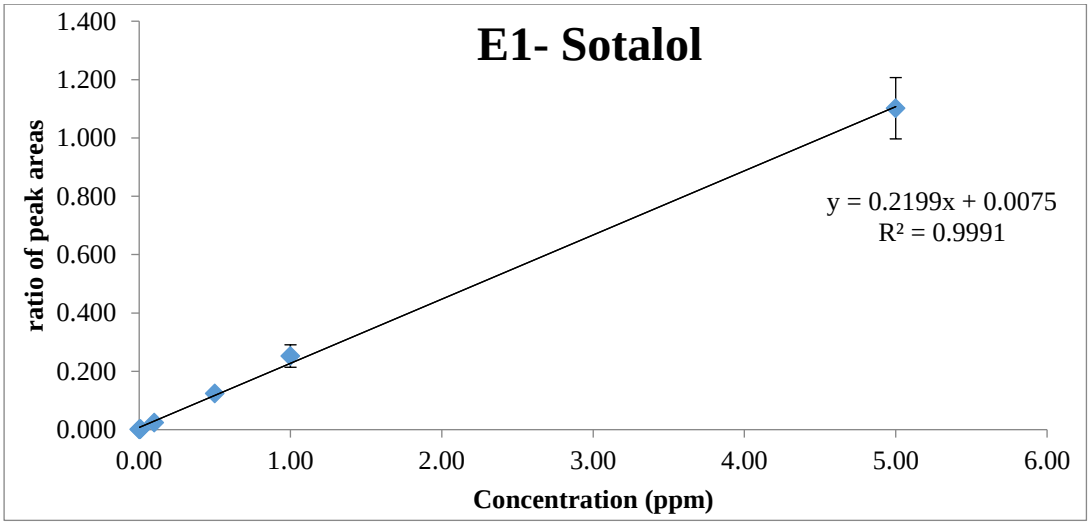


Figure 18: Chiral PCs chromatograms separated by LC-MS/MS using MRM mode (Continued)

Calibration curves for all PCs have been obtained by taking the ratio of the PC chromatographic peak area over the IS chromatographic peak area versus the PC concentration in the x-axis. Figure 19 has shown 2 calibration curves for sotalol drug enantiomers as an example of the prepared calibration curves in methanol. Appendix C shows a complete chromatogram for all PCs together, while appendix A shows the calibration curves for the enantiomers of all 16 PCs.



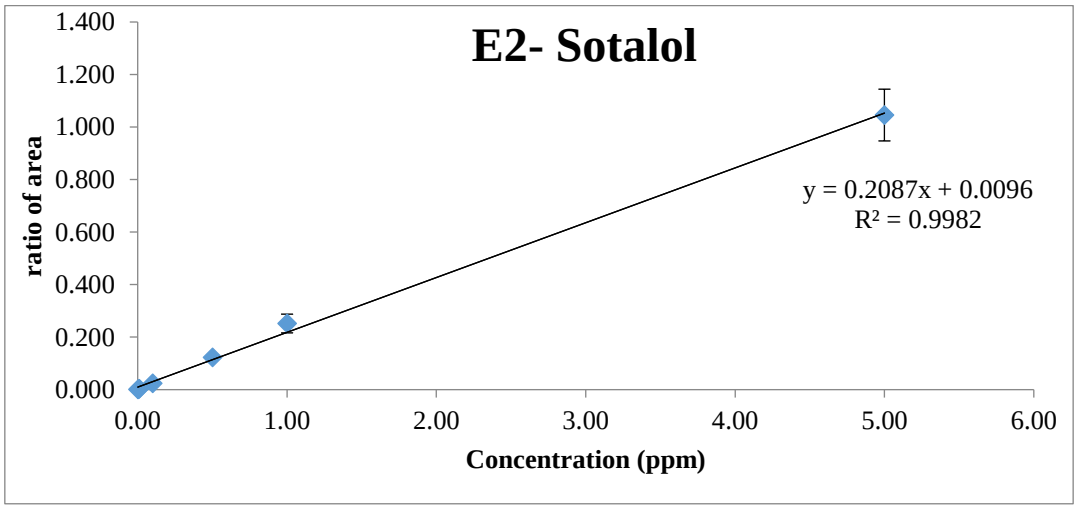


Figure 19: Calibration curve of sotalol enantiomers in methanol as they have been analyzed using the LC-MS/MS

4.3 Instrument detection limit and quantification limit

To determine the instrument detection limit (IDL) and quantitation limit (IQL), experiments have been performed by analyzing 20 replicates of a blank sample that were spiked with 5 ppm of the IS ((±)-Cotinine-D_3) as was explained in Section 3.8.1. The determined values of the IDL and the IQL are listed in Table 14. The IDL values ranged between 0.0003 and 0.0131 ppm, while, the IQL values ranged between 0.0009 and 0.0437 ppm. Moreover, Table 14 gives a summary for PCs retention time, calibration curves equations and coefficient of determination (R^2). The R^2 values are ranged between 0.9932 and 0.9999 for all PCs, which indicates very good linearity for all prepared calibration curves.

Table 14: Calibration curves, retention time, IDL and IQL summary for all PCs

PCs	t_R (min)	Calibration Equation	R^2	IDL (ppm)	IQL (ppm)
E1- Bupivacaine	9.442	y = 0.9835x - 0.0017	0.9996	0.0011	0.0035
E2- Bupivacaine	8.444	y = 1.0302x - 0.0015	0.9999	0.0010	0.0032
E1- Amlodipine	29.340	y = 0.0169x + 0.0018	0.9941	0.0114	0.0378
E2- Amlodipine	30.982	y = 0.0261x - 0.0026	0.9988	0.0057	0.0190
E1- Salbutamol	12.972	y = 0.2382x + 0.035	0.9963	0.0011	0.0037
E2- Salbutamol	14.321	y = 0.2057x + 0.0293	0.9963	0.0007	0.0022
E1-Propranolol	17.902	y = 0.0078x + 0.0005	0.9983	0.0055	0.0185
E2-Propranolol	20.313	y = 0.0105x + 0.0002	0.9993	0.0050	0.0166
E1- Atenolol	16.201	y = 0.0527x + 0.0014	0.9997	0.0012	0.0041
E2- Atenolol	18.152	y = 0.0446x + 0.0026	0.9996	0.0017	0.0058
E1- Metoprolol	16.210	y = 0.0061x + 0.0003	0.9988	0.0084	0.0280
E2- Metoprolol	18.169	y = 0.0046x + 0.0006	0.9932	0.0131	0.0437
E1-O-Desmethylvenlafaxine	17.294	y = 0.1473x + 0.0006	0.9996	0.0016	0.0052
E2-O-Desmethylvenlafaxine	19.030	y = 0.1279x + 0.0059	0.9987	0.0016	0.0052
E1-Mirtazapine	16.365	y = 0.1488x + 0.0112	0.9986	0.0009	0.0030
E2-Mirtazapine	22.426	y = 0.1687x + 0.0088	0.9971	0.0010	0.0033
E1- Terbutaline	13.134	y = 0.2694x + 0.0111	0.999	0.0005	0.0017
E2- Terbutaline	13.903	y = 0.304x + 0.0111	0.9983	0.0008	0.0028
E1- Mianserin	16.362	y = 0.0397x + 0.0034	0.9955	0.0016	0.0054
E2- Mianserin	23.699	y = 0.0533x + 0.0014	0.9995	0.0027	0.0091
E1- Venlafaxine	19.362	y = 0.2733x + 0.0315	0.9965	0.0009	0.0031
E2- Venlafaxine	21.424	y = 0.2757x + 0.0266	0.9972	0.0004	0.0012
E1- Fluoxetine	23.185	y = 0.24x - 0.0268	0.9976	0.0009	0.0029
E2- Fluoxetine	25.069	y = 0.1418x + 0.004	0.9993	0.0008	0.0027
Tramadol	17.457	y = 1.3003x + 0.0041	0.9997	0.0003	0.0009
E1-Sotalol	19.946	y = 0.2199x + 0.0075	0.9991	0.0008	0.0026
E2-Sotalol	22.174	y = 0.2087x + 0.0096	0.9982	0.0013	0.0042

Table 14: Calibration curves, retention time, IDL and IQL summary for all PCs (continued)

PCs	t_R (min)	Calibration Equation	R^2	IDL (ppm)	IQL (ppm)
E1- citalopram	39.160	y = 0.2147x + 0.0155	0.9989	0.0006	0.0019
E2- citalopram	43.536	y = 0.1998x + 0.0172	0.9985	0.0006	0.0021
E1- Tolperisone	22.947	y = 0.2504x + 0.02	0.9984	0.0013	0.0045
E2- Tolperisone	26.012	y = 0.2222x + 0.0184	0.9968	0.0005	0.0016
(±)-cotinine-d3	5.653		IS		

4.4 Method limit of detection

The values of the method detection limit (MDL) for liquid samples were obtained by dividing each IDL value for each drug (see Table 14) over the sample final volume for liquid samples (see Table 15). While for solid samples, the MDL values were obtained by dividing the IDL for every PC over the weight of the extracted sludge sample (see Table 15). An example of the calculated MDL for the solid samples (anaerobic digester) is shown below (Table 17).

The masses of solid samples and volumes of liquid samples for all batches were recorded and listed in Table 15. The sludge samples were heated in the oven at 105°C for 4 hours before recording their masses. However, the liquid samples were filtered before their volumes were recorded.

Table 15: Masses and volumes of collected samples

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

Table 16: MDL values of influent samples for four batches

PCs	LOD (μg/ml) for batch number			
	1	2	3	4
E1- Bupivacaine	0.0011	0.0011	0.0011	0.0011
E2- Bupivacaine	0.0010	0.0010	0.0010	0.0010
E1- Amlodipine	0.0114	0.0114	0.0114	0.0114
E2- Amlodipine	0.0057	0.0057	0.0057	0.0057
E1- Salbutamol	0.0011	0.0011	0.0011	0.0011
E2- Salbutamol	0.0007	0.0007	0.0007	0.0007
E1-Propranolol	0.0055	0.0055	0.0055	0.0055
E2-Propranolol	0.0050	0.0050	0.0050	0.0050
E1- Atenolol	0.0012	0.0012	0.0012	0.0012
E2- Atenolol	0.0017	0.0017	0.0017	0.0017
E1- Metoprolol	0.0084	0.0084	0.0084	0.0084
E2- Metoprolol	0.0131	0.0131	0.0131	0.0131
E1-O-Desmethylvenlafaxine	0.0016	0.0016	0.0016	0.0016
E2-O-Desmethylvenlafaxine	0.0016	0.0016	0.0016	0.0016
E1-Mirtazapine	0.0009	0.0009	0.0009	0.0009
E2-Mirtazapine	0.0010	0.0010	0.0010	0.0010
E1- Terbutaline	0.0005	0.0005	0.0005	0.0005
E2- Terbutaline	0.0008	0.0008	0.0008	0.0008
E1- Mianserin	0.0016	0.0016	0.0016	0.0016
E2- Mianserin	0.0027	0.0027	0.0027	0.0027
E1- Venlafaxine	0.0009	0.0009	0.0009	0.0009
E2- Venlafaxine	0.0004	0.0004	0.0004	0.0004
E1- Fluoxetine	0.0009	0.0009	0.0009	0.0009
E2- Fluoxetine	0.0008	0.0008	0.0008	0.0008
Tramadol	0.0003	0.0003	0.0003	0.0003
E1-Sotalol	0.0008	0.0008	0.0008	0.0008
E2-Sotalol	0.0013	0.0013	0.0013	0.0013
E1- citalopram	0.0006	0.0006	0.0006	0.0006
E2- citalopram	0.0006	0.0006	0.0006	0.0006
E1- Tolperisone	0.0013	0.0013	0.0013	0.0013
E2- Tolperisone	0.0005	0.0005	0.0005	0.0005

Table 17: MDL values of anaerobic digester sludge in AD for four batches

PCs	LOD (μg/g) for batch number			
	1	2	3	4
E1- Bupivacaine	0.0002	0.00003	0.00004	0.00003
E2- Bupivacaine	0.0002	0.00003	0.00004	0.00003
E1- Amlodipine	0.0024	0.00037	0.00043	0.00031
E2- Amlodipine	0.0012	0.00018	0.00022	0.00015
E1- Salbutamol	0.0002	0.00004	0.00004	0.00003
E2- Salbutamol	0.0001	0.00002	0.00003	0.00002
E1-Propranolol	0.0011	0.00018	0.00021	0.00015
E2-Propranolol	0.0010	0.00016	0.00019	0.00013
E1- Atenolol	0.0003	0.00004	0.00005	0.00003
E2- Atenolol	0.0004	0.00006	0.00007	0.00005
E1- Metoprolol	0.0017	0.00027	0.00032	0.00023
E2- Metoprolol	0.0027	0.00042	0.00050	0.00035
E1-O-Desmethylvenlafaxine	0.0003	0.00005	0.00006	0.00004
E2-O-Desmethylvenlafaxine	0.0003	0.00005	0.00006	0.00004
E1-Mirtazapine	0.0002	0.00003	0.00003	0.00002
E2-Mirtazapine	0.0002	0.00003	0.00004	0.00003
E1- Terbutaline	0.0001	0.00002	0.00002	0.00001
E2- Terbutaline	0.0002	0.00003	0.00003	0.00002
E1- Mianserin	0.0003	0.00005	0.00006	0.00004
E2- Mianserin	0.0006	0.00009	0.00010	0.00007
E1- Venlafaxine	0.0002	0.00003	0.00004	0.00002
E2- Venlafaxine	0.0001	0.00001	0.00001	0.00001
E1- Fluoxetine	0.0002	0.00003	0.00003	0.00002
E2- Fluoxetine	0.0002	0.00003	0.00003	0.00002
Tramadol	0.0001	0.00001	0.00001	0.00001
E1-Sotalol	0.0002	0.00003	0.00003	0.00002
E2-Sotalol	0.0003	0.00004	0.00005	0.00003
E1- citalopram	0.0001	0.00002	0.00002	0.00002
E2- citalopram	0.0001	0.00002	0.00002	0.00003
E1- Tolperisone	0.0003	0.00004	0.00005	0.00003
E2- Tolperisone	0.0001	0.00002	0.00002	0.00031

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

5.1 Introduction

This chapter reports the results of chiral PCs levels in Al-Ain domestic wastewater. It discusses the role of different units of operations in the Al-Saad wastewater facility in removing the selected pharmaceuticals under study. It is worth noting that, all the analyzed samples that were taken from the Al-Ain domestic wastewater plant were grab samples and not composite samples. Four grabbed batch samples were taken at different times as mentioned before in Section 3.9.5.

This chapter is organized into four different Sections. Section 5.2 discusses the PCs concentrations in the raw wastewater and compares the occurrence of their enantiomers. Section 5.3 discusses the removal efficiency and mechanism of the PCs in the influent and final effluent. The removal mechanism in the activated sludge, anaerobic digester and filter press unit were presented in Sections 5.4. and 5.5. The enantioselectivity of these three units was discussed in Section 5.6. Finally, the raw data of the calculated concentrations in (μg/L) of all PCs in every unit were listed in appendix B at the end of this thesis.

5.2 Chiral PCs levels in raw wastewater

The average concentrations and standard deviations of all the studied pharmaceuticals in the four batches were calculated and reported in (Appendix B). Some of them were not detected by LC-MS/MS, so their levels were under the method limit of detection, as a result, in this case, the method detection limit of the drug was considered as an actual concentration for that undetectable drug in that

batch in wastewater because these values are needed to do the calculations for mass balance.

Most of the chiral enantiomers showed low standard deviation values. However, o-desmethylvenlafaxine and atenolol enantiomers showed a bit of high standard deviations values that indicate, in general, a bit of high fluctuation in the concentrations of the drug between the four batches. Moreover, all the analyzed samples were garb samples and not composite samples. Thus, these collected data for these samples do not necessarily represent an accurate PCs removal efficiency during WWTP treatment.

As shown in appendix B, the concentrations of the enantiomers in raw wastewater of all selected drugs varied between low level (< 0.1 μg/L) and intermediate level (0.1-3 μg/L), table 18 below shows an example of appendix B results. For instance, mirtazapine, fluoxetine and tolperisone enantiomers were at low levels. However, bupivacaine, amlodipine, salbutamol, propranolol, atenolol, metoprolol, o-desmethylvenlafaxine, venlafaxine, tramadol, and sotalol enantiomers were found at intermediate levels. Interestingly, for some drugs, the levels of the two enantiomers were significantly different. For instance, one of the enantiomers of citalopram, mianserin and terbutaline were at the intermediate level while the other enantiomer of the same drugs was reported at the low level.

Table 18: Average concentrations and standard deviations of Bupivacaine enantiomers at different locations

	E1- Bupivacaine		E2- Bupivacaine	
	Average	Standard deviation	Average	Standard deviation
influent	0.2067	0.33572	0.27795	0.50510
secondary clarifier	0.2126	0.19680	0.03509	0.05427
final effluent	0.0093	0.0165	0.00176	0.00161
Ret.Act.Sludge solid	0.03848	0.0764	0.00059	0.00066
anerobic liquid	0.1307	0.1709	0.15172	0.21186
anerobic solid	0.0122	0.02245	0.00014	0.00015
anerobic liquid final	0.35499	0.59922	0.22444	0.22534
anerobic solid final	0.022728	0.02636	0.00042	0.00067

Figure 20 shows tramadol, atenolol and o-desmethylvelafaxine had high levels that exceed 2 ug/L compared to other compounds. Tramadol has been reported in previous reports to be one of the highest levels in wastewater among other drugs [101], which is consistent with the findings of this study, however, much lower tramadol, atenolol and o-desmethylvelafaxine levels that did not exceed 1.5 ug/L were reported by other studies [73, 76, 92, 101].

Interestingly, in this study, many chiral PCs levels were much higher compared to other reported studies. For instance, citalopram and metoprolol enantiomers levels were twice more than what was reported by others [73, 102]. In addition, venlafaxine, propranolol and salbutamol levels were five times more than what was mentioned by other reports [73, 101, 103]. An exception, however, the same sotalol level was reported by MacLeod et al. (2007) [73]. Meanwhile, mirtazapine and fluoxetine are at very low concentrations which also agreeing with other reports [73, 101, 103].

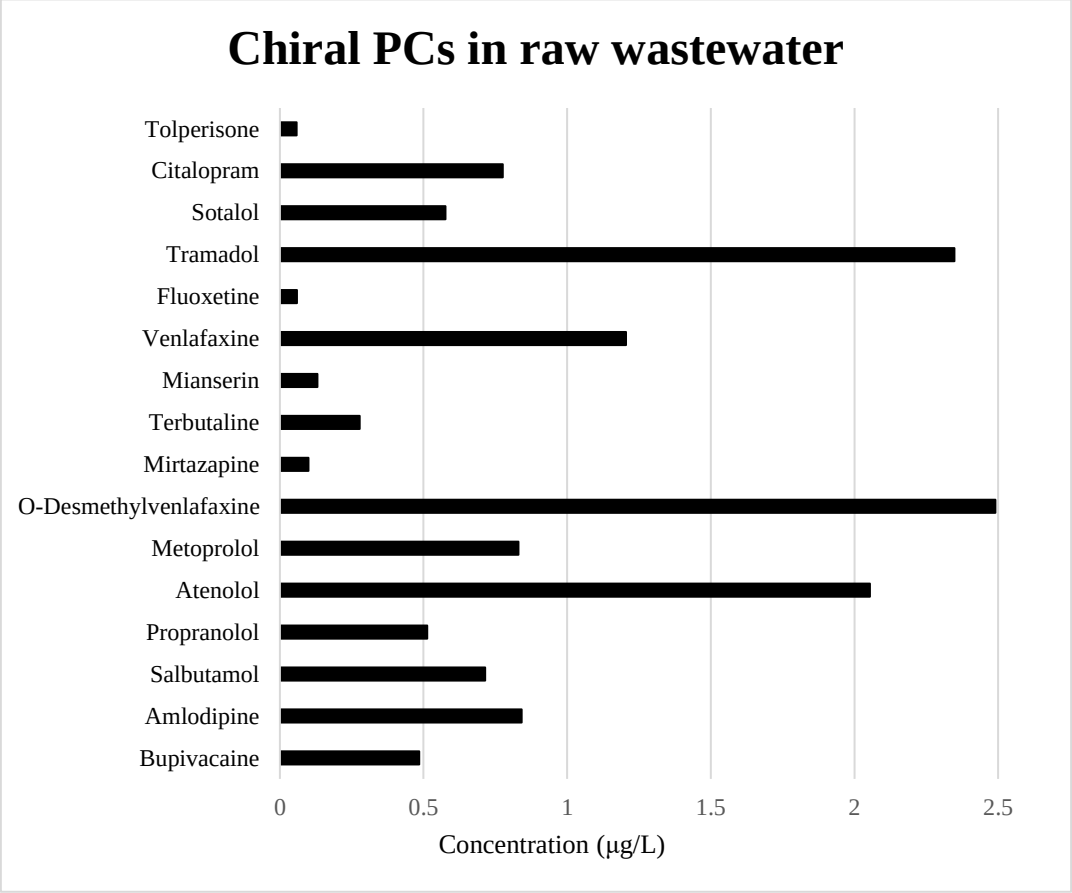


Figure 20: Chiral PCs concentrations in raw wastewater (influent)

The occurrence of chiral drugs enantiomers in wastewater could be as a racemic mixture or as a single enantiomer, depending on many variables, for instance, one of the two enantiomers could be more degradable than the other, or the drug itself could be made and marketed as a single enantiomer and not as a racemic mixture [9, 14].

In this study, most drug enantiomers were found almost at the same level in the raw wastewater. However, citalopram, salbutamol, fluoxetine, mianserin and terbutaline enantiomers were found at different levels as shown below in Figure 21. Interestingly, It was reported that fluoxetine, citalopram and salbutamol enantiomers were at different levels in earlier reports [73, 101, 104]. In addition, all the above five

mentioned drugs are marketed as racemic mixtures [14, 105]; therefore, the differences in enantiomers levels could be a result of selective removal of one of the enantiomers due to either degradation or adsorption more than the other.

Interestingly, atenolol drug was found among the PCs in the raw wastewater as a racemic mixture, Figure 21, meanwhile, it was reported in the literature that the existence of the enantiomers of the atenolol drug is dependent on the WWTP technology. For instance, they found some WWTP to be enriched with S-enantiomer, while some others were enriched with R-enantiomer [92]. Therefore, more studies are needed to understand different removal -either by degradation or adsorption- mechanisms of atenolol enantiomers.

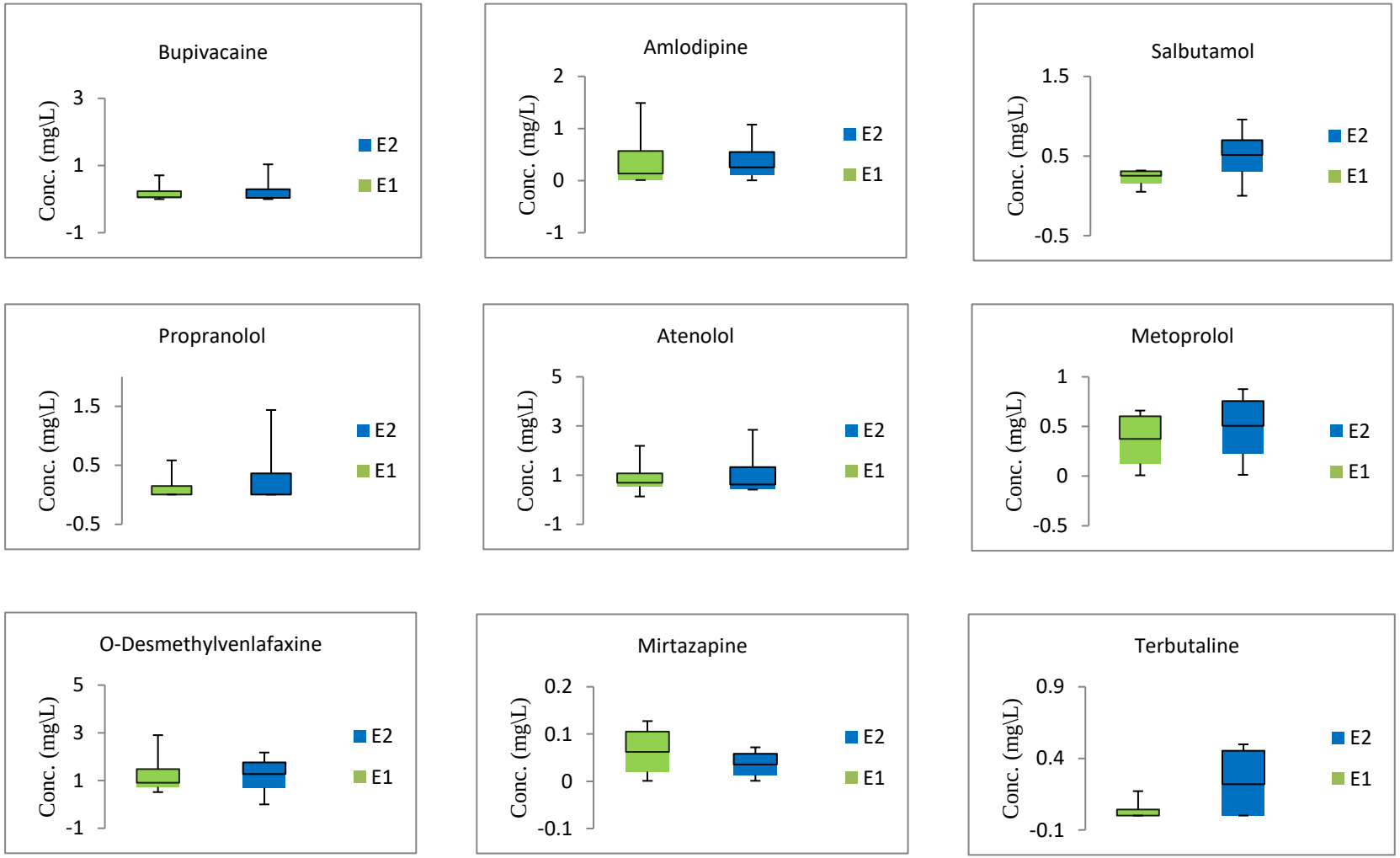


Figure 21: Comparison between the levels of the two enantiomers for each chiral drug found in raw wastewater. E1 represents the first eluted enantiomer, E2 represents the second eluted enantiomer

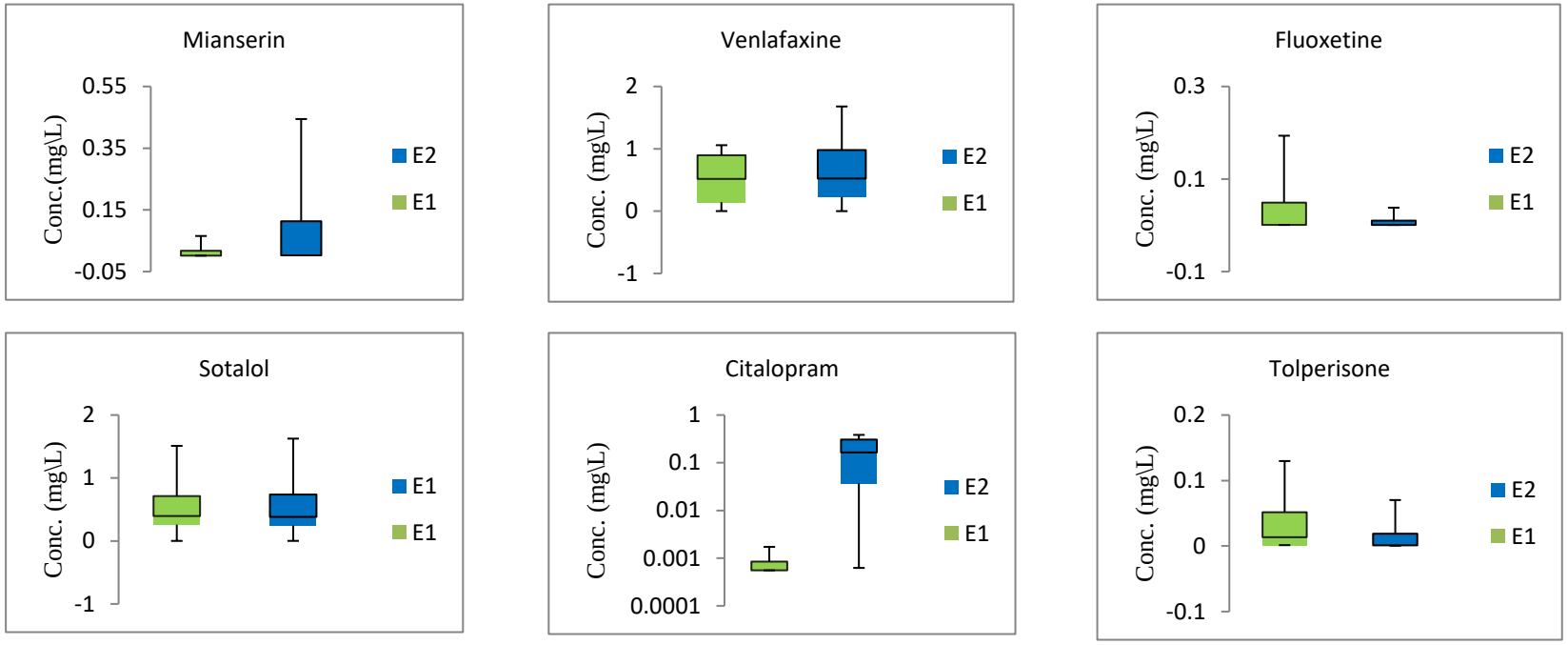


Figure 21: Comparison between the levels of the two enantiomers for each chiral drug found in raw wastewater. E1 represents the first eluted enantiomer, E2 represents the second eluted enantiomer (Continued)

5.3 Removal of PCs between influent and final effluent

The removal of PCs in Al-Saad WWTP can happen due to aerobic and anaerobic processes or/and chlorination process as it was discussed before in Section 3.9. The removal efficiency (RE) values of PCs were calculated relative to the average concentration in the influent as it was discussed earlier in Section 3.10.

For the overall removal, Table 19 shows that there is a high removal for a big group of the studied PCs including bupivacaine, amlodipine, salbutamol, propranolol, mirtazapine, desmethylvenlafaxine mianserin, venlafaxine, tramadol, sotalol, citalopram, and tolperisone (< 90%). However, a small group showed a moderate removal level including metoprolol, terbutaline, and fluoxetine (between 30% and 70%). While a low removal was indicated for atenolol drug (25.9%).

Compared to literature, the RE of highly removed PCs in this study is much higher than what is reported in the literature [73, 101, 102, 106]. In addition, it was reported that metoprolol and fluoxetine were poorly removed, with RE values of < 25% and < 22% , respectively [73, 102]. The RE values for metoprolol and fluoxetine reported in this study were much higher than what was reported in the literature. However, atenolol RE is much lower than what is reported by others [1, 2, 5]. Interestingly, this is the first study that reports the levels of bupivacaine, amlodipine, and tolperisone in the influent and effluent of the wastewater treatment plant, however, they were highly removed and their levels were below the detection limit of the method.

Table 19: Removal efficiency (RE) between influent and final effluent

PCs	Influent (ug/L)	Effluent (μg/L)	RE %
Bupivacaine	0.485	0.011	97.7
Amlodipine	0.841	< 0.017	98.0
Salbutamol	0.715	< 0.002	99.8
Propranolol	0.513	< 0.011	98.0
Atenolol	2.054	1.522	25.9
Metoprolol	0.830	0.530	36.2
O-Desmethylvenlafaxine	2.491	< 0.003	99.9
Mirtazapine	0.099	< 0.002	98.1
Terbutaline	0.278	0.095	65.7
Mianserin	0.131	< 0.004	96.7
Venlafaxine	1.205	< 0.001	99.9
Fluoxetine	0.059	0.031	47.5
tramadol	2.348	< 0.075	96.8
Sotalol	0.576	< 0.002	99.6
Citalopram	0.776	< 0.001	99.8
Tolperisone	0.058	< 0.002	96.9

The sorption coefficient was calculated at location 3 to designate the role of the aeration tank and secondary settling tank in removing PCs, by dividing the average concentrations of the four batches of every PC that was sorbed to the solid material over the average concentration for each left in water. Therefore, the lower the sorption coefficient value, the lower amount of compound sorbed to sludge.

Table 20 shows the sorption coefficient at location 3 of bupivacaine, amlodipine, salbutamol, atenolol, metoprolol, desmethylvenlafaxine, mirtazapine, mianserin, venlafaxine, tramadol, and sotalol that is showing low values, which indicated that these PCs have relatively high concentrations left in water and they were not adsorbed to the sludge. In addition, terbutaline, citalopram, and tolperisone had a relatively low sorption coefficient with a relatively low amount adsorbed to the sludge.

However, the main portions of propranolol and fluoxetine are being adsorbed to sludge.

Generally, the amount of PCs in the wastewater is not being adsorbed to the sludge, instead, they are presented in the liquid phase. On the other hand, high removal RE values was reached at location 4, which indicates that filtration and disinfection play the main role in the PCs removal.

Table 20: Sorption coefficient of tested PCs at location 3

PCs	Solid (ug/L)	Water (µg/L)	Sorption coefficient
Bupivacaine	0.0391	0.2951	0.1324
Amlodipine	0.0045	0.0177	0.2541
Salbutamol	0.0005	0.4736	0.0010
Propranolol	0.1739	0.0875	1.9867
Atenolol	0.0008	1.5325	0.0005
Metoprolol	0.0057	1.0944	0.0052
O-Desmethylvenlafaxine	0.0008	4.6820	0.0002
Mirtazapine	0.0016	0.1487	0.0109
Terbutaline	0.1090	0.3304	0.3297
Mianserin	0.0011	0.0384	0.0298
Venlafaxine	0.0616	3.0583	0.0201
Fluoxetine	0.1082	0.0315	3.4378
Tramadol	0.1146	2.2292	0.0514
Sotalol	0.0005	1.1070	0.0005
Citalopram	0.1451	0.3674	0.3949
Tolperisone	0.0679	0.1911	0.3551

The wastewater treatment process could play an important role in affecting and changing the enantiomers' level of any chiral PC [9, 73, 103]. In this study, the effect of the treatment process on the enantiomers levels was investigated. Figure 22 shows a comparison between the levels of the enantiomers that were available in the influent and the final effluent. Only five PCs that were available in the final effluent samples. The treatment process was more selective for the second enantiomer of bupivacaine

and fluoxetine. Assuming that the second eluted enantiomer of fluoxetine was the R-enantiomer, then the mentioned results agree with the reported literature [104].

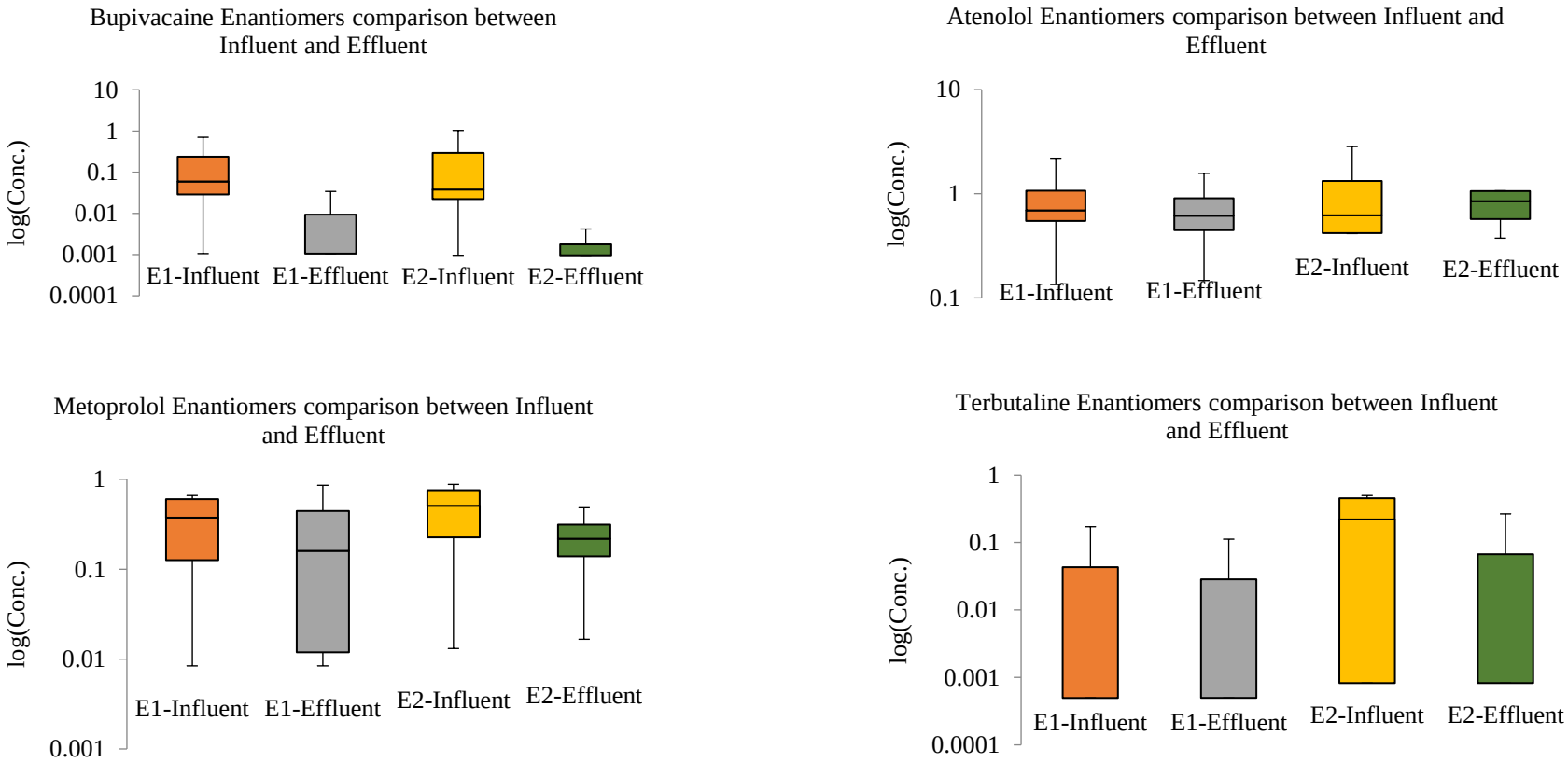


Figure 22: PCs enantiomers levels comparison between influent and effluent

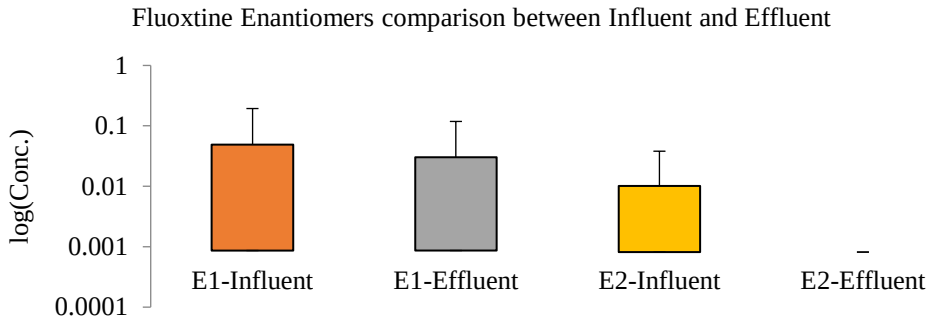


Figure 22: PCs enantiomers levels comparison between influent and effluent (continued)

5.4 Removal PCs mechanisms at the activated sludge system

The decreasing trend for the PCs levels in the activated sludge system could be attributed to degradation or adsorption to the mixed liquor suspended solids (MLSS). However, it is possible that some PCs levels will remain unchanged and they leave the activated sludge system intact. Mass balance was applied for the activated sludge unit to compare and study the mass rate of PCs that goes in and leaves this unit per day. Table 21 shows the comparison between what is entering the unit and what is going out of that unit. Amlodipine had the lowest mass that leaves the SST daily, while sotalol had the highest compared to the others. Moreover, the relative average mass per day in solid for all PCs is between 0.001 and 0.402 mg, however, it ranged between 0.001 and 6.199 mg in water.

The high mass in the effluent of the SST unit for sotalol might be due to the hydraulic retention time effect of the system (4 hrs). On one hand, most tested PCs such as bupivacaine, salbutamol, o-desmethylvenlafaxine and sotalol had low relative average mass adsorbed to sludge (≤0.1), while they were available at relatively high masses in the secondary settling tank effluent. However, they had high removal

efficiency values (>90%), which indicate that they were mainly removed by filtration and disinfection. On the other hand, amlodipine was available at relatively low mass in both secondary settling tank influent and the sludge, and this could be a result of the transformation reactions (i.e. biodegradation, volatilization and photodegradation reactions).

Additionally, propranolol, terbutaline, fluoxetine and tolperisone had a relatively high mass adsorbed to sludge (>0.1), which indicates that they accumulate on the MLSS. However, the RE values of propranolol and tolperisone were more than 90%; Therefore, filtration, disinfection, and adsorption played an important role in removing both of them. While this was not the case for terbutaline and fluoxetine because of their low removal efficiency values.

It was mentioned that propranolol and fluoxetine could get adsorbed to sludge in the activated sludge system [1, 2]. However, atenolol, metoprolol, fluoxetine, and terbutaline were not effectively removed from the wastewater samples.

Table 21: Average daily mass of tested PCs exit the activated sludge system (mg/day)

PCs	Effluent of SST	Mass in water	Mass in solids
Bupivacaine	0.463	0.021	0.021
Amlodipine	0.020	0.001	0.001
Salbutamol	0.690	0.020	0.000
Propranolol	0.530	0.007	0.101
Atenolol	0.707	0.023	0.000
Metoprolol	1.709	0.040	0.001
O-Desmethylvenlafaxine	1.659	0.057	0.000
Mirtazapine	0.767	0.046	0.005
Terbutaline	1.027	0.022	0.276
Mianserin	1.799	0.031	0.003
Venlafaxine	1.799	0.080	0.010
Fluoxetine	0.049	0.011	0.245
tramadol	1.422	0.030	0.011
Sotalol	205.167	6.199	0.024
Citalopram	0.342	0.028	0.056
Tolperisone	2.818	0.133	0.402

Generally, the RAS system could be selective and effective on one of the enantiomers of any chiral drug in a different way than the other due to many reasons such as the microorganisms, sludge characteristics and some chemical aspects like pH and temperature, which can be selective and effective on one of the enantiomers more than the other [1, 12]. For instance, Figure 23 and Table 22 show that the removal process at the RAS system was more selective and efficient in removing the second enantiomer of bupivacaine, terbutaline, propranolol, and mianserin. In addition, E2 of the four PCs were at a relatively low mass adsorbed to sludge and a relatively low mass level at the SST effluent, which can be explained as a result of removal by the transformation reactions. However, it was more efficient in removing the first enantiomer of fluoxetine, tolperisone, and citalopram, while it was not that selective on venlafaxine. Enantiomer 1 (E1) of tolperisone and citalopram were found at a relatively low mass adsorbed to sludge and a relatively low mass level at the SST effluent, so they seem to be removed by the transformation reactions. However,

sorption plays a role in removing E1-fluoxetine because it was found at a relatively high mass adsorbed to sludge and a relatively low mass level at the SST effluent. In addition, E1 of bupivacaine, terbutaline, propranolol, and fluoxetine was found to be more likely sorbed to the sludge. If E1 was the R-enantiomer of fluoxetine then the findings agree with the earlier reports [101]. However, E2 of citalopram and tolperisone were found at higher relative mass on the solid. It was reported that R-citalopram is more likely to be adsorbed to the sludge more than its antipode, which will match this study if E2 was R-citalopram [101].

Lastly, the RAS system was not the highest efficient location in removing PCs; however, it was playing an important role in removing one of the enantiomers more than the other.

Table 22: Average daily mass of some tested PCs enantiomers exit the activated sludge system (mg/day)

PCs	Effluent of SST	Mass in water	Mass in solids
E1-Bupivacaine	0.815	0.037	0.041
E2-Bupivacaine	0.112	0.004	0.000
E1-propranolol	0.840	0.013	0.163
E2-propranolol	0.219	0.002	0.038
E1-Terbutaline	1.421	0.001	0.552
E2-Terbutaline	0.632	0.042	0.000
E1-Mianserin	3.574	0.061	0.005
E2-Mianserin	0.024	0.001	0.001
E1-Venlafaxine	1.899	0.102	0.002
E2-Venlafaxine	1.700	0.057	0.019
E1- Fluoxetine	0.018	0.019	0.485
E2- Fluoxetine	0.080	0.003	0.005
E1-Sotalol	0.828	0.025	0.000
E2-Sotalol	409.505	12.373	0.047
E1- Citalopram	0.015	0.002	0.029
E2- Citalopram	0.668	0.054	0.083
E1- Tolperisone	0.428	0.041	0.006
E2- Tolperisone	5.208	0.225	0.797

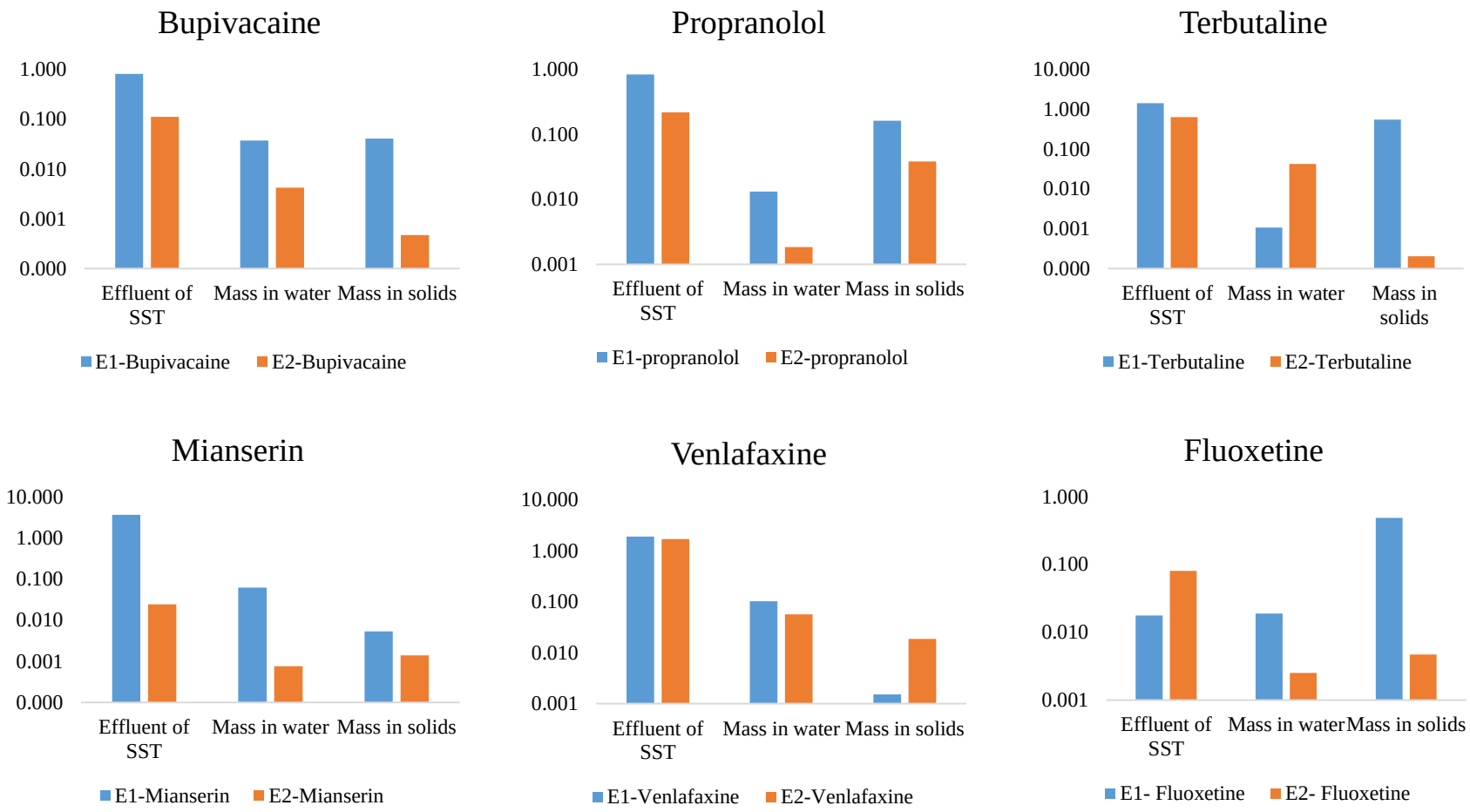


Figure 23: Relative average daily mass of PCs enantiomers leaving the activated sludge system, note that y-axis represents log (concentration in (mg/day))

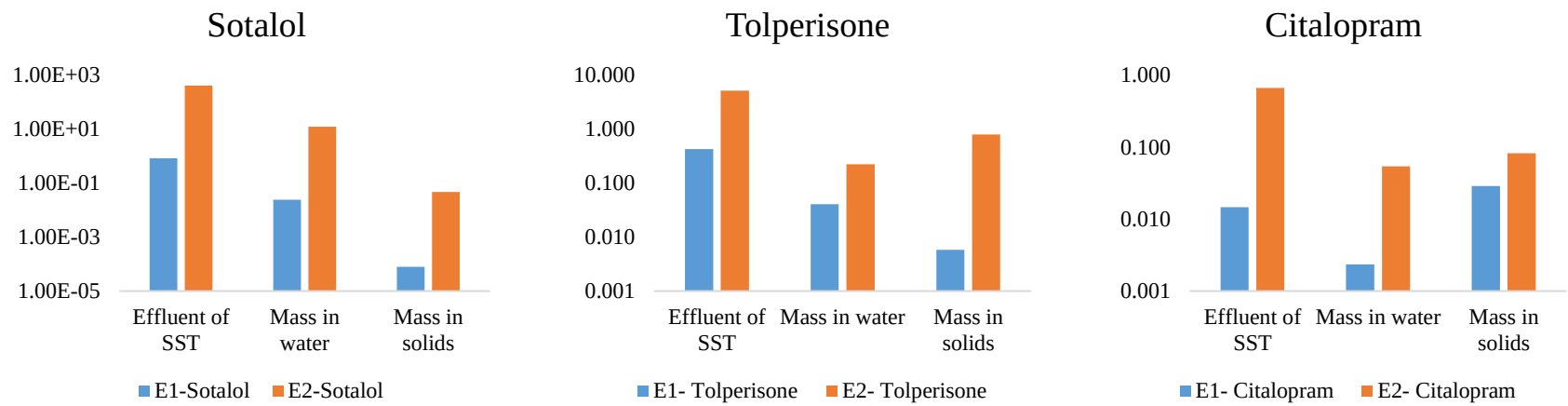


Figure 23: Relative average daily mass of PCs enantiomers leaving the activated sludge system, note that y-axis represents log (concentration in (mg/day)) (continued)

5.5 PCs removal at the AD and FP

Mass balance has been applied for the anaerobic digester (AD) (Figure 16, Section 3.11.2) and the filter press (FP) (Figure 17, Section 3.11.3) units to compare and study the mass rate of chiral PCs that go throw and exit these units. In addition, the cationic organic polymer "Corofloc 341, SNF, France" in the FP unit that aims to remove water out (dewatering). Figure 24 shows the mass rate of tested PCs that goes through the AD unit, exit the AD unit which at the same time inter the FP unit, and exit the FP unit. Some PCs (e.g. propranolol, metoprolol, o-desmethylvelafaxine, venlafaxine, tramadol, sotalol, and citalopram) were effectively removed in the AD system. However, PCs such as amlodipine, mianserin, fluoxetine, and tolperisone were found at a higher mass rate in the effluent of AD more than what is enter the AD unit. This could be due to that the collected samples were grabbed, and not composite samples and they were taken at the same time, so the system retention time effect was not taking into consideration (22 days).

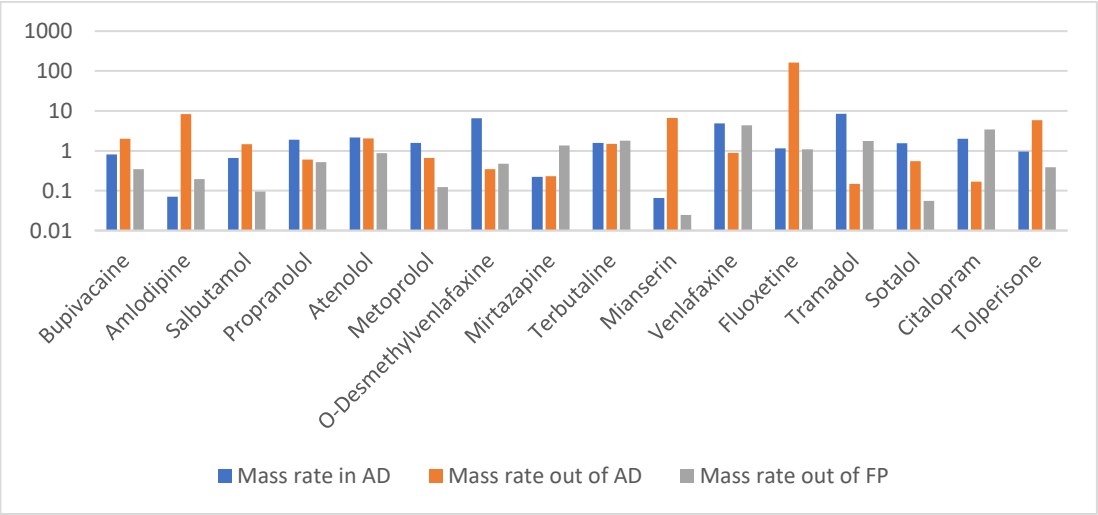


Figure 24: Mass Rate of tested PCs (mg/day) in the AD and the FP unit, note that the y-axis represents log (concentration in (mg/day))

In addition, the rate of mass out of the AD unit (FP influent) was much higher than the rate of mass that leaves the FP for some of the PCs like bupivacaine, amlodipine, salbutamol, mianserin, fluoxetine, and tolperisone. However, what causes this decreasing trend is not clear, it could be due to that organic chemicals like to make strong interactions with the organic polymer therefore it will leave the water, or the polymer is accumulating the solid particles.

Moreover, some PCs were available at higher mass rates in the effluent of the FP unit more than what is there in the influent, such as mirtazapine, tramadol, citalopram and venlafaxine. Since there is an organic polymer in the water, it seems that it makes the water more hydrophobic, as a result, it was suggested hydrophobic PCs like to get desorbed out of the sludge and leaves with water, which will result in higher concentrations of these PCs.

5.6 Enantioselectivity of RAS, AD and FP

RAS system was more selective and efficient in removing one of the enantiomers of many drugs more than the others as it was depicted in Section 5.4. The removing process could be attempted either by transformation reactions or sorption process to the solid particles depending on many variables related to the location environment or some chemical and physical aspects like pH, temperature or chemical nature and structure of the drugs.

However, the sorption coefficient was calculated for the PCs in RAS, AD and FP units, as was mentioned before in Section 5.3. For the case of the RAS unit, Table 23 shows some of the PCs that were not affected and their behavior was constant, they

were not getting sorbed to solids during the three stages, such as the two enantiomers of metoprolol, salbutamol and venlafaxine.

Interestingly, a huge change in propranolol enantiomers' behavior was designated, where they were at a relatively high concentration on the sludge at the RAS units and then they got desorbed or degraded at the other units. Moreover, tolperisone and citalopram favored being sorbed more in the AD unit while fluoxetine was highly sorbed at both AD and FP units

Table 23: Sorption coefficient of return activated sludge, anaerobic digester and filter press units

PCs	RAS	AD	FP
E1-Bupivacaine	0.150	0.094	0.034
E2-Bupivacaine	0.015	0.001	0.001
E1-Amlodipine	0.254	0.010	0.011
E2-Amlodipine	0.254	0.002	0.076
E1-Salbutamol	0.002	0.000	0.000
E2-Salbutamol	0.001	0.000	0.000
E1-Propranolol	1.699	0.027	0.056
E2-Propranolol	2.836	0.020	0.091
E1-Atenolol	0.000	0.175	0.126
E2-Atenolol	0.001	0.112	0.061
E1-Metoprolol	0.004	0.002	0.001
E2-Metoprolol	0.006	0.003	0.001
E1-O-Desmethylvenlafaxine	0.000	0.149	0.062
E2-O-Desmethylvenlafaxine	0.000	0.117	0.032
E1-Mirtazapine	0.003	0.542	0.862
E2-Mirtazapine	0.024	0.120	0.103
E1-Terbutaline	70.404	2.326	0.009
E2-Terbutaline	0.001	0.009	0.012
E1-Mianserin	0.012	0.001	0.076
E2-Mianserin	0.254	0.003	0.001
E1-Venlafaxine	0.002	0.001	0.003
E2-Venlafaxine	0.045	0.008	0.006
E1- Fluoxetine	3.526	39.204	1.151
E2- Fluoxetine	0.254	33.167	5.678
E1-Sotalol	0.000	0.000	0.000
E2-Sotalol	0.001	0.000	0.000
E1- Citalopram	1.684	6.377	0.182
E2- Citalopram	0.209	19.856	0.077
E1- Tolperisone	0.020	6.124	0.636
E2- Tolperisone	0.485	8.714	0.331

Some PCs enantiomers preferred to be selectively sorbed more than their antipodes to the sludge, regardless of that they have the same structure, molecular weight, boiling point etc. For instance, E2 of propranolol and mianserin was at a higher concentration on the sludge than their antipode in the RAS unit. While RAS solid was enriched with the first enantiomer of bupivacaine, terbutaline, citalopram and fluoxetine. In addition, the first enantiomer of mirtazapine, terbutaline, and fluoxetine preferred to be sorbed more in the AD unit compared to their enantiomers while the opposite happened with citalopram and tolperisone.

In general, the FP unit had the lowest sorption coefficient for all PCs except for mirtazapine. This is could be explained by the hydrophobic–hydrophobic interaction between the mirtazapine and the organic polymer so it will leave the aqueous medium and move to the organic medium available in the polymer. Another explanation for PCs' behavior of low sorption could be that the polymer is accumulating the solid particles, so it will be very difficult for PCs to leave the sludge and move to water.

Chapter 6: Conclusion and Recommendations

6.1 Conclusion

Pharmaceuticals' presence in wastewater is increasing at a constant base due to the increase in the human population and consequent increase in the consumption of medicinal drugs. Therefore, relatively high concentrations of these PCs are released to the environment, and they are presented in treated wastewater soil and groundwater. Therefore, there is a need to develop new and advanced techniques to measure released PCs levels and assess their risk on the environment.

In this study, 16 chiral PCs were investigated, among which 15 were chirally separated successfully. They were detected in wastewater and filtered sludge samples using UPLC-ESI-MS/MS. (±)-Cotinine-D_3 was used as an internal standard in the calibration curve preparation. The instrument detection limit and quantification limit were successfully determined in addition to the method detection limit. The samples were grabbed from Al Saad WWTP from different locations. Solid-phase extraction was applied to extract and concentrate the PCs in the grabbed samples. The mass balance calculations were performed to understand the removal mechanism in different locations.

Results showed that the occurrence of tramadol, atenolol, and o-desmethylfenlafaxine was at a relatively high concentration compared to others. In addition, generally, the concentrations of most of the tested PCs were at much higher concentrations compared to others. In addition, citalopram, salbutamol, fluoxetine, mianserin, and terbutaline enantiomers were found at different levels in raw wastewater.

In general, the amount of selected PCs that were detected in the wastewater are not getting sorbed to the sludge, relatively they are presented in the liquid phase. On the other hand, high removal RE values were reached at the final effluent, which indicates that filtration and disinfection processes play the main role in these PCs removal. In addition, the effect of the treatment process on the enantiomers levels was studied, the treatment process was more selective for the removal of the second enantiomer of bupivacaine and fluoxetine. Finally, atenolol, metoprolol, fluoxetine, and terbutaline were not effectively removed from the wastewater.

The removal process at the RAS unit was more selective and efficient in removing the second enantiomer of bupivacaine, terbutaline, propranolol, and mianserin. However, it was more efficient in removing the first enantiomer of fluoxetine, tolperisone and citalopram, while it was not selective for the case of venlafaxine drug.

A group of investigated PCs including propranolol, metoprolol, o-desmethylvelafaxine, venlafaxine, tramadol, sotalol, and citalopram were effectively removed in the AD system. In addition, bupivacaine, amlodipine, salbutamol, mianserin, fluoxetine and tolperisone were at much lower levels after adding the cationic organic polymer. However, what causes this decline is not clear. It could be due to those organic chemicals like to make a strong bond with the polymer since it is an organic polymer so it will leave the water, or it could be as a result that the polymer is accumulating the solid particles.

Finally, changes in the PCs behavior at the RAS, AD and FP units were indicated; for instance, propranolol was preferred to be sorbed on RAS sludge more than the other PCs, while citalopram and tolperisone preferred adsorption on the AD

sludge surface more than other units. In addition, E2 of propranolol and mianserin was at a higher concentration on the sludge than their antipode in the RAS unit. While RAS solid was enriched with the first enantiomer of bupivacaine, terbutaline, citalopram, and fluoxetine. In general, the FP unit had the lowest sorption coefficient for all PCs except for mirtazapine.

6.2 Recommendations

A lot of chiral PCs need to be investigated and quantified in wastewater besides what has been investigated in this study, since many of them have not been investigated, identified and quantified, especially in UAE wastewater. In addition, there is a lot of WWTPs facilities in the UAE, so, a comparison between their removal efficiency and mechanisms could be carried out, taking into consideration the retention time inside every unit.

More studies could be performed to understand the selectivity and the behavior of chiral PCs enantiomers inside every unit of the WWTP. Moreover, the risk assessment of that result of such selectivity should be considered in the evaluation of the possible risks to the environment.

Additionally, the possibility of the formation of intermediate products from the parent PCs needs to be studied. Since they could be more harmful to the environment. Moreover, more studies are needed to be conducted in a possible way to degrade these PCs and identify/test the corresponding transformation products that resulted in the WWTPs.

A study could be performed to better understand the role of filter press unit and the addition of organic polymer to the water in removing PCs. the study could

investigate the reason behind the increase of some PCs concentrations in the PF effluent.

The sample preparation step consumed a lot of solvents and time, especially in the extraction step. In addition, many difficulties have been faced to separate the sludge of the water. So, it was a time and effort consuming and a very costly process. However, nowadays, new highly sensitive LC-MS/MS instruments are available. These instruments can use a very small amount of aqueous samples and auto-extract the analyte directly from the collected sample. As a result, it is reducing the time and effort as well as reducing the cost and solvents waste.

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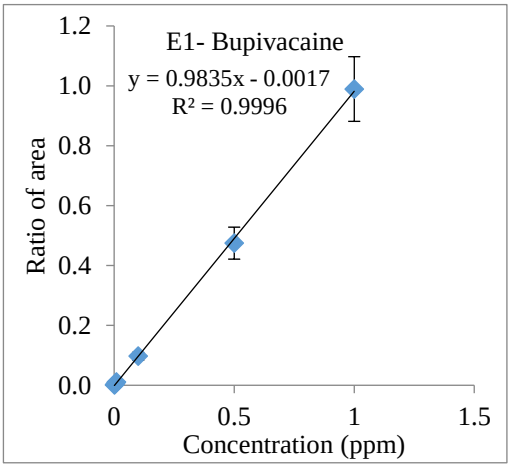
Appendices

Appendix A: Calibration curves for PCs as analyzed by LC-MS/MS



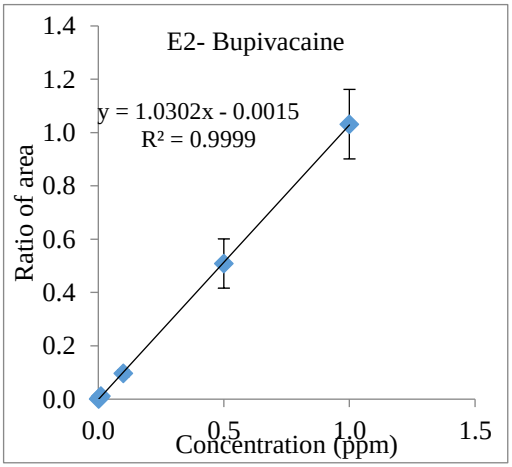
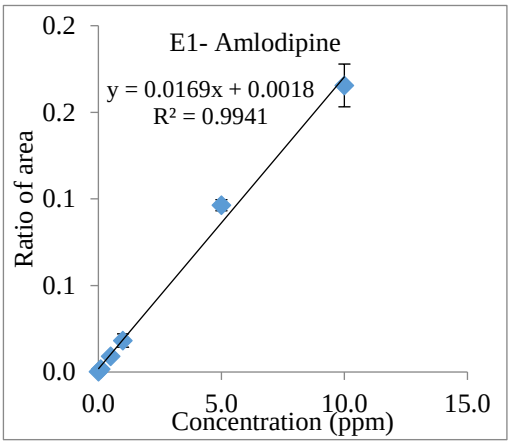


Figure A1: Bupivacaine enantiomers calibration curves



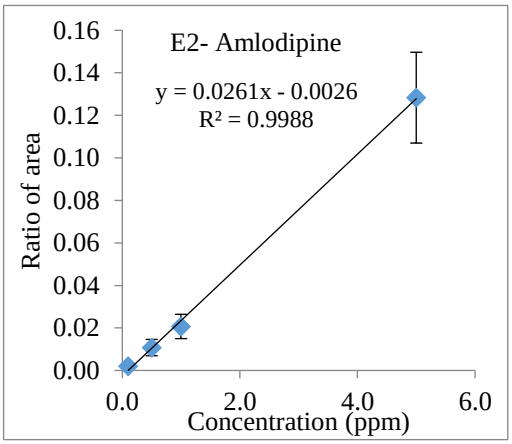


Figure A2: Amlodipine enantiomers calibration curves

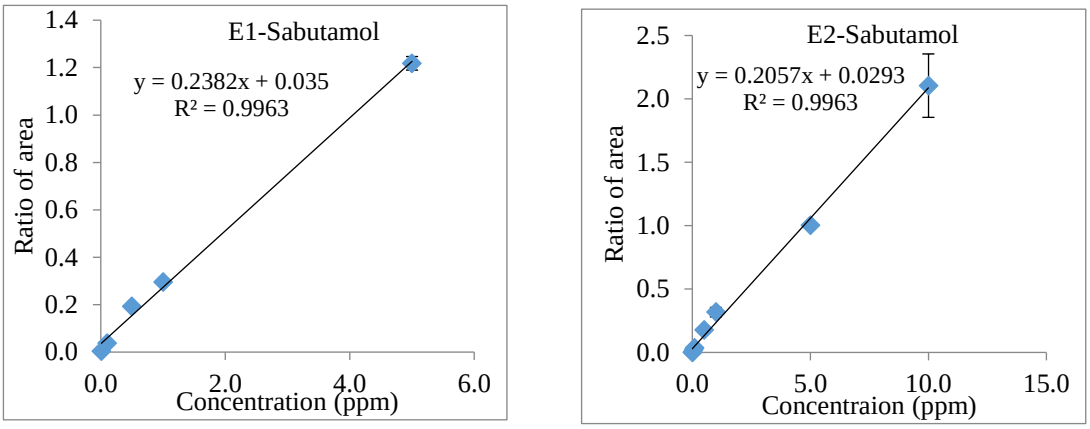


Figure A3: Salbutamol enantiomers calibration curves

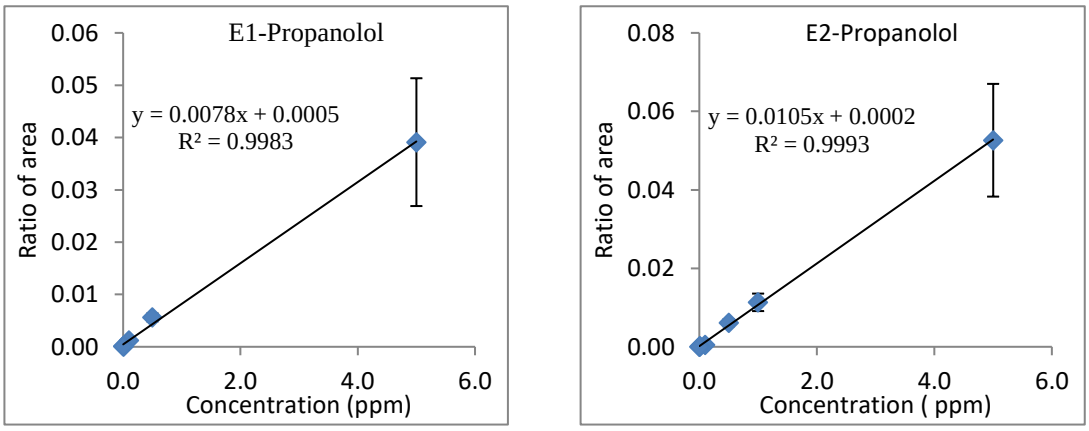


Figure A4: Propranolol enantiomers calibration curves

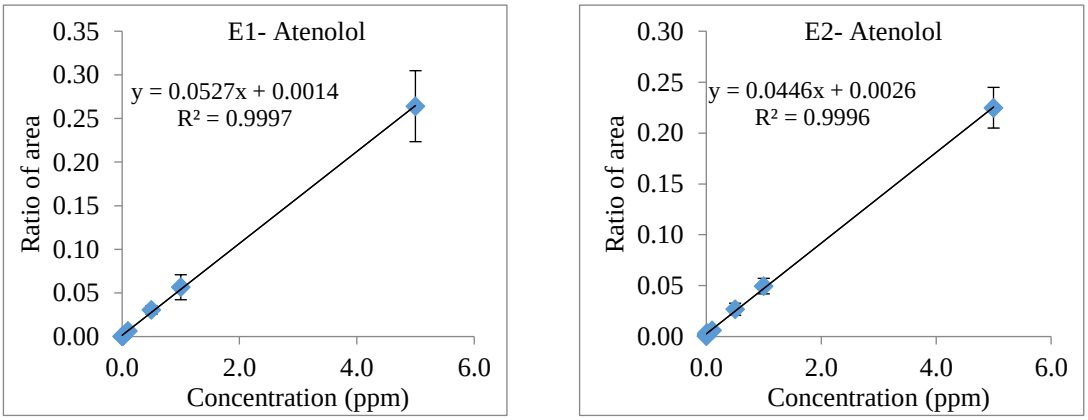


Figure A5: Atenolol enantiomers calibration curves

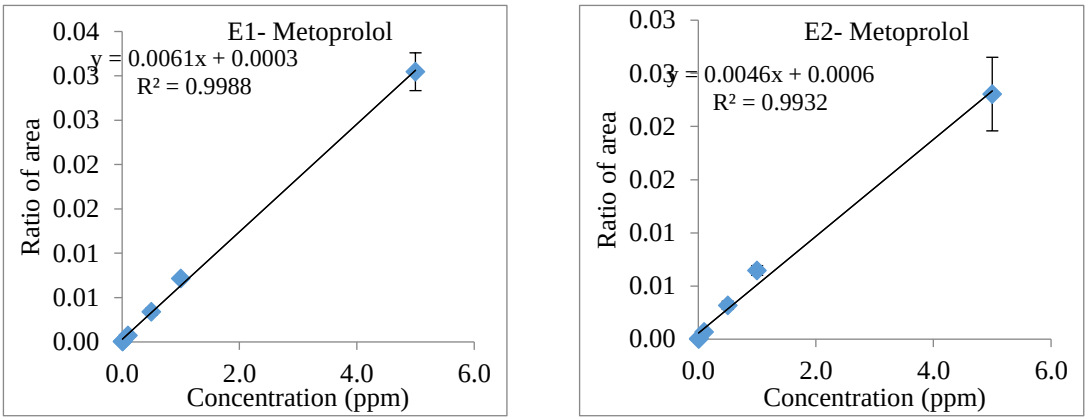


Figure A6: Metoprolol enantiomers calibration curves

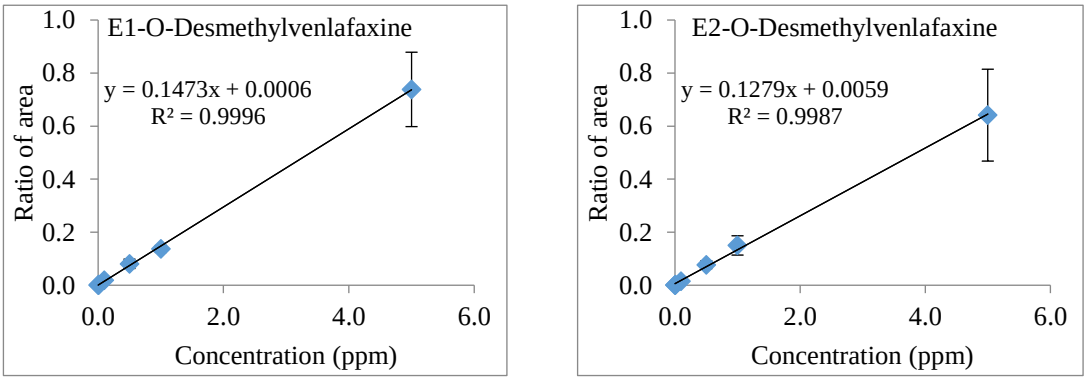


Figure A7: O-Desmethylvenlafaxine enantiomers calibration curves

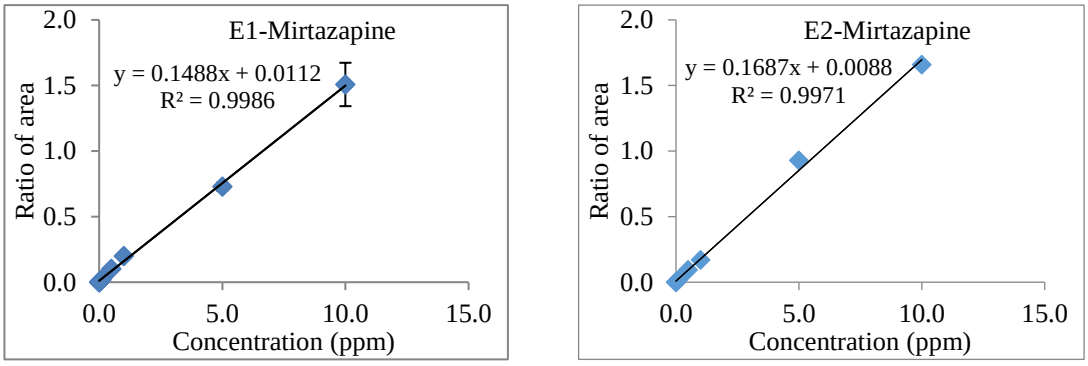
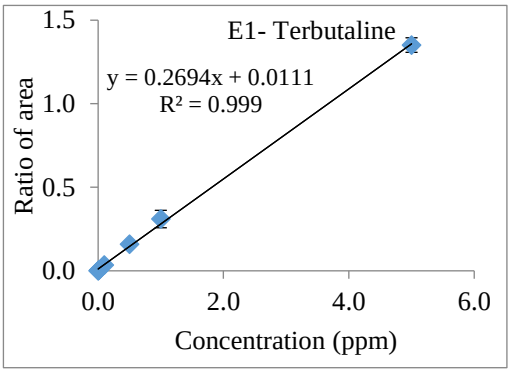


Figure A8: Mirtazapine enantiomers calibration curves



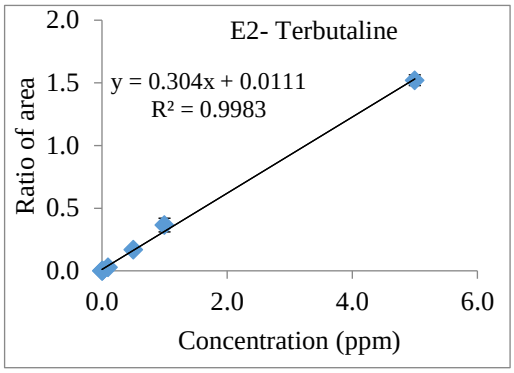
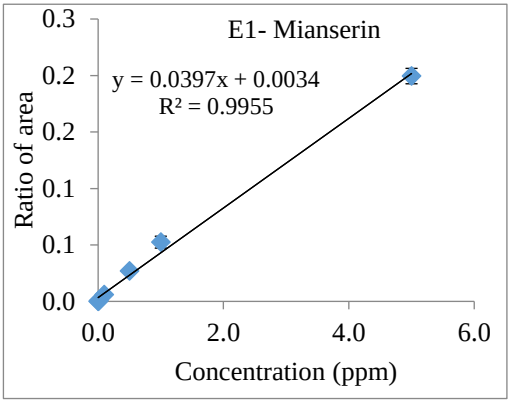


Figure A9: Terbutaline enantiomers calibration curves



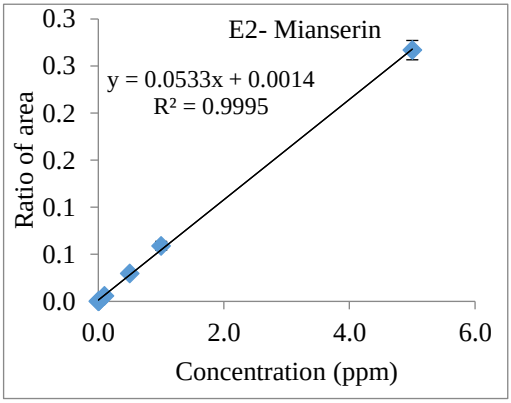
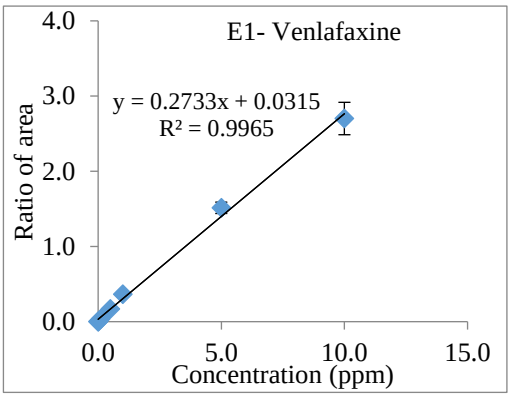


Figure A10: Mianserin enantiomers calibration curves



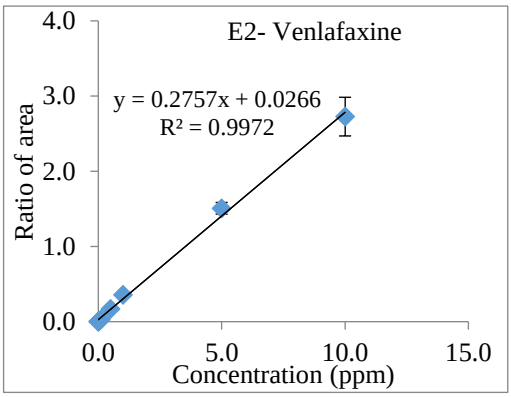


Figure A11: Venlafaxine enantiomers calibration curve

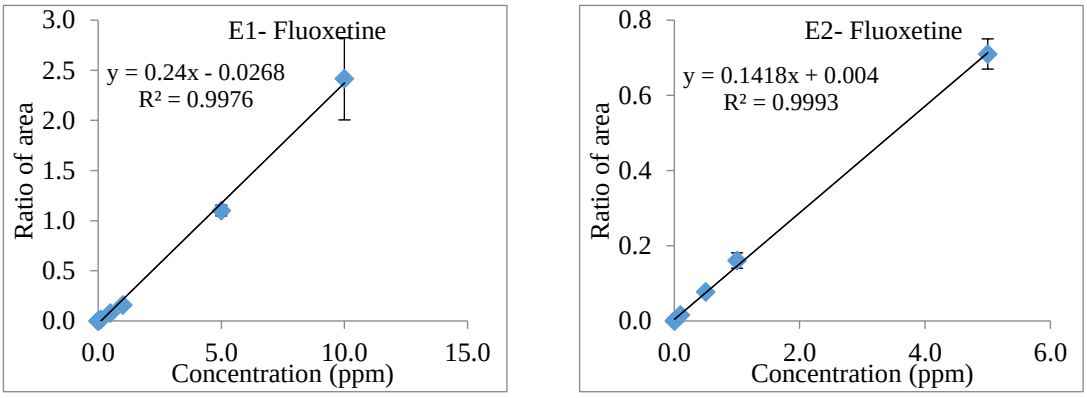


Figure A12: Fluoxetine enantiomers calibration curve

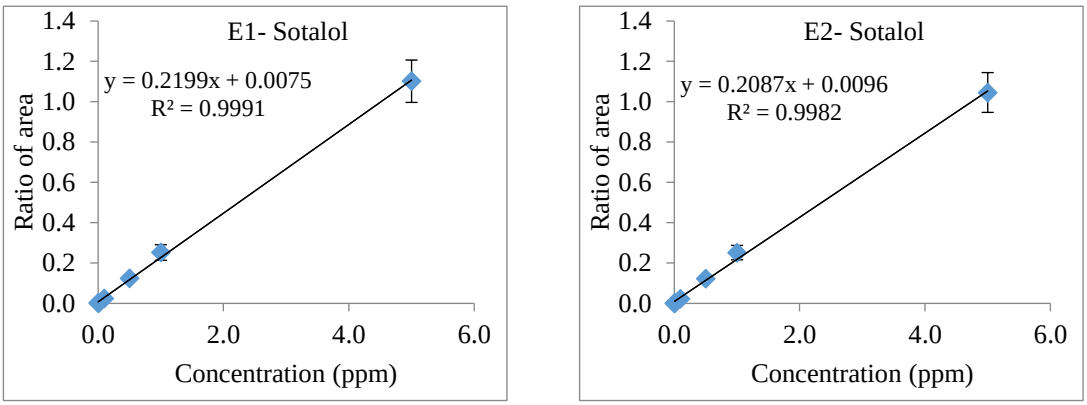


Figure A13: Sotalol enantiomers calibration curves

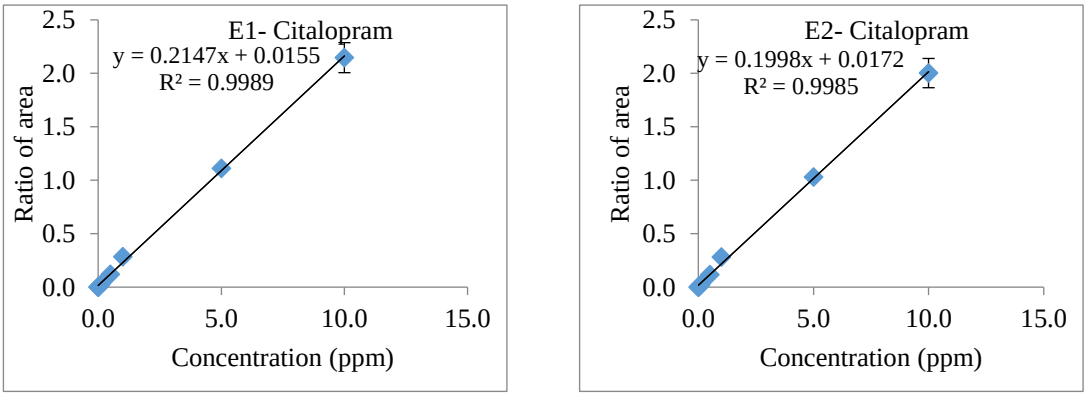


Figure A14: Citalopram enantiomers calibration curves

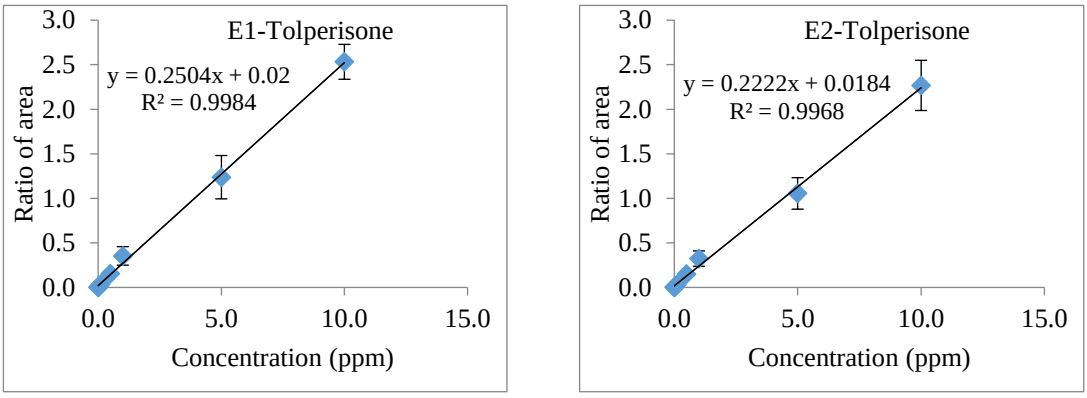


Figure A15: Tolperisone enantiomers Calibration Curves

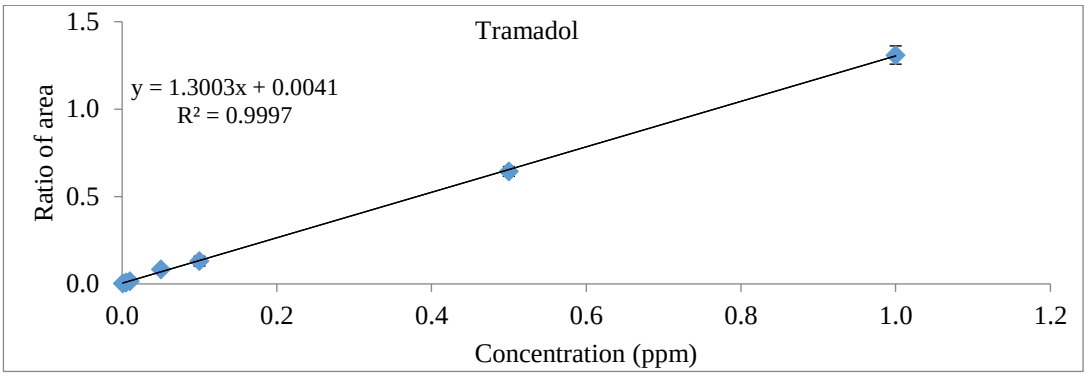


Figure A16: Tramadol Calibration Curve

Appendix B: Concentrations of chiral PCs in Al Saad WWTP at different locations at (mg/L)

	E1- Bupivacaine		E2- Bupivacaine	
	Average	Standard deviation	Average	Standard deviation
influent	0.2067	0.33572	0.27795	0.50510
secondary clarifier	0.2126	0.19680	0.03509	0.05427
final effluent	0.0093	0.0165	0.00176	0.00161
Ret.Act.Sludge solid	0.03848	0.0764	0.00059	0.00066
anerobic liquid	0.1307	0.1709	0.15172	0.21186
anerobic solid	0.0122	0.02245	0.00014	0.00015
anerobic liquid final	0.35499	0.59922	0.22444	0.22534
anerobic solid final	0.022728	0.02636	0.00042	0.00067

* NA= Not available

	E1- Amlodipine		E2- Amlodipine	
	Average	Standard deviation	Average	Standard deviation
influent	0.44356	0.70745	0.39766	0.47616
secondary clarifier	<0.01158	0.00028	<0.00580	0.00014
final effluent	<0.01135	NA	<0.00569	NA
Ret.Act.Sludge solid	<0.00300	0.00039	<0.00150	0.00019
anerobic liquid	0.04142	0.05429	0.25530	0.23792
anerobic solid	0.00040	0.00008	<0.00043	0.00050
anerobic liquid final	0.03638	0.05005	<0.00569	NA
anerobic solid final	0.00069	0.00043	0.01368	0.01655

* NA= Not available

	E1- Salbutamol		E2- Salbutamol	
	Average	Standard deviation	Average	Standard deviation
influent	0.21846	0.12383	0.49632	0.39972
secondary clarifier	0.14275	0.14869	0.34501	0.31803
final effluent	<0.00111	NA	<0.00065	NA
Ret.Act.Sludge solid	<0.00029	0.00004	<0.00017	0.00002
anerobic liquid	0.19934	0.23118	0.49721	0.57484
anerobic solid	<0.00008	0.00010	<0.00005	0.00006
anerobic liquid final	0.54332	0.39152	0.97484	0.99008
anerobic solid final	<0.00009	NA	<0.00005	0.00001

* NA= Not available

	E1- Propranolol		E2- Propranolol	
	Average	Standard deviation	Average	Standard deviation
influent	0.1502	0.2893	0.3631	0.7163
secondary clarifier	0.0959	0.1796	0.0510	0.1051
final effluent	<0.0055	NA	<0.0050	NA
Ret.Act.Sludge solid	0.1110	0.1289	0.0629	0.1231
anerobic liquid	0.3153	0.6115	0.3016	0.5900
anerobic solid	0.0086	0.0095	0.0061	0.0113
anerobic liquid final	0.1550	0.2988	0.0667	0.1235
anerobic solid final	0.0367	0.0726	<0.0004	0.0001

* NA= Not available

	E1- Atenolol		E2- Atenolol	
	Average	Standard deviation	Average	Standard deviation
influent	0.9281	0.8835	1.1255	1.1624
secondary clarifier	0.7766	0.3751	0.7074	0.3943
final effluent	0.7370	0.6003	0.7849	0.3399
Ret.Act.Sludge solid	<0.0003	NA	<0.0005	0.0001
anerobic liquid	0.6276	0.8738	0.6820	0.7697
anerobic solid	0.1098	0.2195	0.0763	0.1525
anerobic liquid final	0.8746	0.6261	1.2433	1.2116
anerobic solid final	0.0210	0.0416	0.0334	0.0665

* NA= Not available

	E1- Metoprolol		E2- Metoprolol	
	Average	Standard deviation	Average	Standard deviation
influent	0.3544	0.3171	0.4757	0.3930
secondary clarifier	0.6012	0.4676	0.6454	0.5390
final effluent	0.2962	0.3988	0.2337	0.1935
Ret.Act.Sludge solid	<0.0022	0.0003	<0.0035	0.0004
anerobic liquid	0.3983	0.7769	0.3322	0.6335
anerobic solid	<0.0006	0.0007	<0.0010	0.0011
anerobic liquid final	0.7934	0.9628	0.8268	0.9426
anerobic solid final	<0.0007	0.0002	<0.0011	0.0003

* NA= Not available

	E1- O-Desmethylvenlafaxine		E2- O-Desmethylvenlafaxine	
	Average	Standard deviation	Average	Standard deviation
influent	1.3096	1.0816	1.1815	0.9380
secondary clarifier	2.2511	0.7419	2.1553	0.7577
final effluent	<0.0016	NA	<0.0016	NA
Ret.Act.Sludge solid	<0.0004	0.0001	<0.0004	0.0001
anerobic liquid	0.5189	0.9202	0.1739	0.1989
anerobic solid	0.0773	0.1249	0.0204	0.0292
anerobic liquid final	1.2560	0.8398	0.6437	0.7469
anerobic solid final	0.0263	0.0523	<0.0001	NA

* NA= Not available

	E1- Mirtazapine		E2- Mirtazapine	
	Average	Standard deviation	Average	Standard deviation
influent	0.0632	0.0593	0.0360	0.0325
secondary clarifier	0.0676	0.0679	0.0438	0.0496
final effluent	<0.0009	NA	<0.0010	NA
Ret.Act.Sludge solid	<0.0002	NA	0.0014	0.0022
anerobic liquid	<0.0014	0.0007	0.0110	0.0196
anerobic solid	0.0008	0.0014	0.0013	0.0025
anerobic liquid final	<0.0009	NA	0.0128	0.0236
anerobic solid final	0.0379	0.0757	0.0628	0.0725

* NA= Not available

	E1- Terbutaline		E2- Terbutaline	
	Average	Standard deviation	Average	Standard deviation
influent	0.0432	0.0853	0.2345	0.2709
secondary clarifier	0.0317	0.0736	0.2389	0.2695
final effluent	0.0284	0.0558	0.0670	0.1323
Ret.Act.Sludge solid	0.1087	0.2172	<0.0002	0
anerobic liquid	<0.0008	0.0004	0.9059	0.9907
anerobic solid	0.0018	0.0028	0.0077	0.0083
anerobic liquid final	0.1948	0.3392	0.6314	0.7279
anerobic solid final	0.0360	0.0543	0.0935	0.0770

* NA= Not available

	E1- Mianserin		E2- Mianserin	
	Average	Standard deviation	Average	Standard deviation
influent	0.0176	0.0319	0.1132	0.2209
secondary clarifier	0.0492	0.0928	<0.0028	0.0001
final effluent	<0.0016	NA	<0.0027	NA
Ret.Act.Sludge solid	<0.0004	0.0001	<0.0007	0.0001
anerobic liquid	0.1853	0.3669	0.0665	0.1203
anerobic solid	<0.0001	0.0001	<0.0002	0.0002
anerobic liquid final	<0.0016	NA	0.3198	0.3934
anerobic solid final	<0.0001	NA	<0.0002	0.0001

* NA= Not available

	E1- Venlafaxine		E2- Venlafaxine	
	Average	Standard deviation	Average	Standard deviation
influent	0.5233	0.5072	0.6813	0.7325
secondary clarifier	1.3162	0.8452	1.2239	1.0195
final effluent	<0.0009	NA	<0.0004	NA
Ret.Act.Sludge solid	0.0036	0.0058	0.0580	0.0884
anerobic liquid	1.7274	1.7648	1.2639	1.3641
anerobic solid	0.0009	0.0015	0.0104	0.0051
anerobic liquid final	0.3116	0.2288	1.8081	0.7706
anerobic solid final	0.1072	0.0971	0.2057	0.1099

* NA= Not available

	E1- Fluoxetine		E2- Fluoxetine	
	Average	Standard deviation	Average	Standard deviation
influent	0.0490	0.0964	0.0101	0.0186
secondary clarifier	0.0157	0.0420	<0.0008	NA
final effluent	0.0302	0.0587	<0.0008	NA
Ret.Act.Sludge solid	0.1080	0.2155	<0.0002	NA
anerobic liquid	<0.0014	0.0007	0.0024	0.0028
anerobic solid	0.0538	0.1024	0.0790	0.1574
anerobic liquid final	0.0468	0.0918	0.0139	0.0262
anerobic solid final	0.0441	0.0530	0.0364	0.0516

* NA= Not available

	E1- Sotalol		E2- Sotalol	
	Average	Standard deviation	Average	Standard deviation
influent	0.5756	0.6515	0.5976	0.7097
secondary clarifier	0.4728	0.3924	0.6395	0.5719
final effluent	<0.0008	NA	<0.0013	NA
Ret.Act.Sludge solid	<0.0002	NA	<0.0003	NA
anerobic liquid	0.2693	0.5368	0.3299	0.6569
anerobic solid	<0.0001	0.0001	<0.0001	0.0001
anerobic liquid final	0.3318	0.3876	0.5334	0.6168
anerobic solid final	<0.0001	NA	<0.0001	NA

* NA= Not available

	E1- Citalopram		E2- Citalopram	
	Average	Standard deviation	Average	Standard deviation
influent	0.0008	0.0006	0.1782	0.1831
secondary clarifier	0.0276	0.0413	0.2202	0.2463
final effluent	<0.0006	NA	<0.0006	NA
Ret.Act.Sludge solid	0.0781	0.1560	0.0669	0.0408
anerobic liquid	<0.0009	0.0005	<0.0010	0.0005
anerobic solid	0.0056	0.0107	0.0197	0.0238
anerobic liquid final	0.0310	0.0609	0.2550	0.2360
anerobic solid final	0.1331	0.1030	0.1172	0.0770

* NA= Not available

	E1- Tolperisone		E2- Tolperisone	
	Average	Standard deviation	Average	Standard deviation
influent	0.0395	0.0612	0.0182	0.0347
secondary clarifier	0.0351	0.0593	0.1172	0.1427
final effluent	<0.0013	NA	<0.0005	NA
Ret.Act.Sludge solid	0.0010	0.0014	0.0668	0.1304
anerobic liquid	0.0105	0.0177	0.0078	0.0145
anerobic solid	0.0640	0.1276	0.0683	0.1366
anerobic liquid final	0.1006	0.1985	0.2067	0.3432
anerobic solid final	<0.0001	NA	0.0273	0.0328

* NA= Not available

	Tramadol	
	Average	Standard deviation
influent	2.3481	1.2303
secondary clarifier	1.5543	0.7969
final effluent	0.0750	0.0628
Ret.Act.Sludge solid	0.1146	0.1333
anerobic liquid	0.3178	0.4502
anerobic solid	0.0135	0.0103
anerobic liquid final	1.5253	0.7800
anerobic solid final	0.0581	0.0393

* NA= Not available

Appendix C: Complete chromatogram for chiral PCs

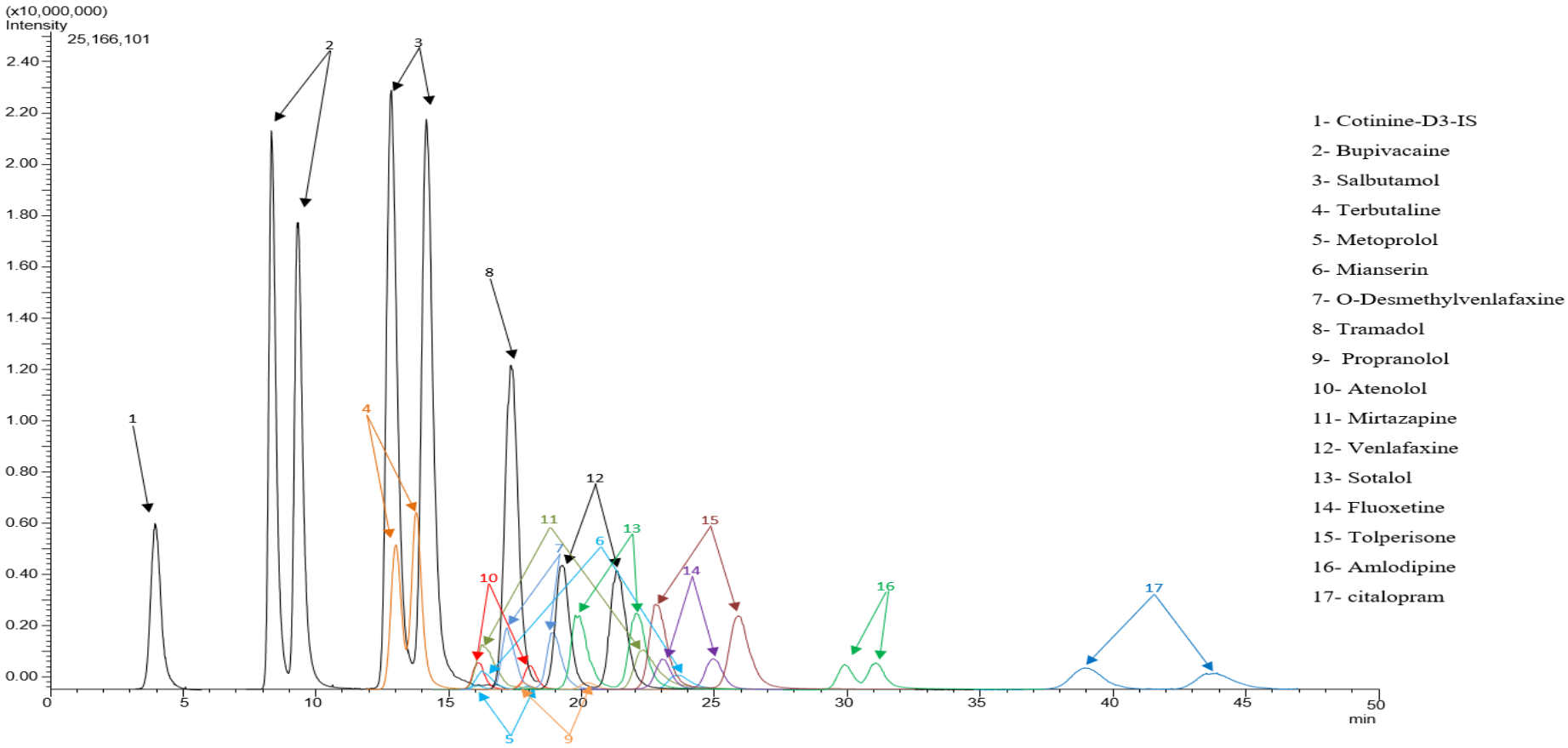


Figure A17: Complete chromatogram for all chiral PCs at 1 ppm concentration