UNIVERSITI PUTRA MALAYSIA

METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN RIVER WATER AND SEWAGE

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By

EUGENIE TAN SIN SING

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

In ever loving memory of my Late Father

To my loving mother, husband and sister

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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December 2012

Chair : Professor Mohamad Pauzi Zakaria, PhD

Faculty : Environmental Studies

Pharmaceuticals and Personal Care Products (PPCPs) are classified as the new emerging class of pollutants by the United States Environmental Protection Agency (U.S. EPA) recently. Its ubiquitous nature coupled with its high persistency in the environment is alarming. Moreover, some PPCPs are endocrine disrupting compounds responsible for feminization of male fishes via production of viltellogenin. Occurrences of antibiotics in the environment induce high bacterial resistance. PPCPs were widely manufactured and administered in Malaysia but little or no quantification was carried out. Lack of data could be attributed to the absence of a recognized, comprehensive and conclusive method for PPCPs analysis. As such, this method aims to provide a robust and sensitive method for identification and quantification of

PPCPs in river water, Sewage Treatment Plant (STPs) influent, intermittent and effluent. This method is specially formulated for simultaneous extraction, detection and quantification of multi-classes PPCPs in a 25 minutes run-time. This is a pioneering method for quantification of acetaminophen, sulfamethoxazole, diclofenac, atenolol, metoprolol, DEET and oxybenzone in Atmospheric Pressure Chemical Ionisation (APCI) mode. Method had been validated for high repeatability and reproducibility; Relative Standard Deviations (RSD) for both was less than 10%. Quantification of PPCPs is often a trace analysis; thus, a good sensitivity is needed. As such, Instrument Quantification Limits (IQLs) for PPCPs were in the range of 0.05-1.0 µg/L; meanwhile, Method Quantification Limits (MQLs) for ultrapure water were within 0.3-15 ng/L. In addition, Solid Phase Extraction (SPE) recoveries were above 75% for most PPCPS demonstrating good accuracy. Lower matrix suppression in APCI mode had enabled quantifying PPCPs in complex matrices producing lower baseline chromatograms and sharper peaks resolutions. Subsequently, the method was applied to investigate environmental occurrences of PPCPs. Twelve out of eighteen PPCPs were detected in river water samples. Five PPCPs were quantified above 1000 ng/L; they were caffeine, estradiol, estriol, estrone and naproxen. On the other hand, three sewage treatment plant (STPs) with different operational mechanisms were sampled. Natural hormones (estradiol, estriol, estrone and progesterone) and personal care products (caffeine, DEET and oxybenzone) constituted majority of influents. Highest detected in influent was caffeine whose mean concentration was 14858.4 ng/L. Thirteen PPCPs were detected in all STP effluents. Highest concentration in effluent was estriol

whose mean concentration was 2160.6 ng/L. In a nutshell, PPCPs were not efficiently removed by Malaysian STPs. Thereafter, Environmental Risk Assessments (ERA) was used to evaluate possible aquatic toxicities in Langat River. High risks of acute toxicities were found for naproxen and sulfamethoxazole. Several PPCPs exhibited high chronic toxicities namely diclofenac, estradiol, ethynylestradiol, estriol, estrone, and sulfamethoxazole. Naproxen exhibited medium risk. Metoprolol and DEET exhibited low chronic risk. Immediate reduction measures were demanded for four steroid hormones; they were estradiol, estriol, estrone and ethynylestradiol.

Keywords: Pharmaceuticals, Personal Care Products (PCPs), water pollution, sewage pollution, High Performance Tandem Mass Spectrometry (HPLC-MS/MS), Atmospheric Pressure Chemical Ionization (APCI), Environmental Risk Assessment (ERA)

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBANGUNAN DAN PENGESAHAN KAEDAH PENENTUAN BAHAN FARMASEUTIKAL DAN PRODUK PENJAGAAN DIRI DALAM AIR SUNGAI DAN SISA KUMBAHAN

Oleh

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Farmaseutikal dan Produk Penjagaan Diri (FPPD) tergolong sebagai kelas pencemar baru muncul oleh Agensi Alam Sekitar, Amerika Syarikat (A.S. AAS) masa kini. Sifat sentiasa adanya dan pertahanan tingginya dalam persekitaran membimbangkan. Di samping ini, beberapa PPCPs merupakan sebatian pengganggu sistem endokrin yang bertanggungjawab untuk feminisasi ikan-ikan jantan melalui pengeluaran viltellogenin. Kehadiran antibiotik dalam persekitaran juga menimbulkan daya tahan bakteria yang tinggi. FPPD dihasilkan dan digunakan dengan meluas namun pengetahuan serta kesedaran tentang kehadiran FPPD amatlah rendah. Kekurangan data mungkin berpunca daripada ketiadaan satu kaedah yang komprehensif dan

konklusif untuk menganalisis FPPD. Oleh itu, tujuan utama kajian ini ialah untuk menghasilkan satu kaedah sensitif dan terpilih untuk pengenalpastian dan penentuan FPPD dalam air sungai serta air Loji Rawatan Kumbahan (LRK) seperti influen, pertengahan dan efluen. Kaedah ini mempunyai ciri pengekstrakan serentak, pengesanan dan penentuan pelbagai kelas FPPD dalam masa 25 minit. Kaedah in adalah perintis untuk penentuan asetaminofen, sulfamethoxazole, diklofenak, atenolol, metoprolol, DEET dan oxybenzone dalam mod Tekanan Atmosphera Pengionan Kimia (TAPK). Kaedah disahkan mempunyai kebolehulangan dan kebolehasilan semula yang tinggi dengan Sisihan Piawaian Relatif (SPR) yang kurang daripada 10% untuk kedua-duanya. Memandangkan penentuan FPPD merupakan analisis yang khusus, maka kaedah yang mempunyai sensitiviti tinggi diperlukan. Oleh itu, Had Penentuaan Alat (HPA) untuk FPPD ditentukan pada julat 0.05-1.0 µg/L, manakala, Had Penentuaan Kaedah (HPK) untuk air ultra tulen ditentukan pada julat 0.3-15 ng/L. Di samping itu, Ekstrak Fasa Pepejal (EFP) menonjolkan kejituan yang baik dengan nilai pemulihan melebihi 75% untuk kebanyakan FPPD. Penindasan matriks yang lebih rendah dalam mod TAPK meningkatkan kebolehan penentuan FPPD dalam matrik-matrik kompleks; menghasilkan garis tapak kromatogram yang lebih rendah dan kromatogram beresolusi tinggi. Seterusnya, kaedah ini diaplikasi untuk menyiasat kewujudan FPPD dalam sampel air alam sekitar. Dua belas daripada lapan belas FPPD telah dikesan dalam sampel air sungai; dengan lima FPPD berkepekatan melebihi 1000 ng/L iaitu kafein, estradiol, estriol, estron dan naproksen. Tiga loji rawatan kumbahan (LRK) dengan mekanisma- operasi yang berlainan turut disampel. Hormon asli (estradiol, estriol, estron dan

progesteron) serta produk-produk penjagaan peribadi (kafein, DEET and oxybenzone) tergolong sebagai pencemar majoriti dalam influen LRK. Kafein dikesan mempunyai kepekatan tertinggi dalam influen purata 14858.4 ng/L. Tiga belas FPPD telah dikesan dalam semua efluen LRK. Penentuan tertinggi dalam efluen ialah estriol dengan kepekatan puratanya sebanyak 2160.6 ng/L. Ringkasannya, FPPD tidak disingkirkan dengan cekap oleh LRK di Malaysia. Selain itu, Taksiran Risiko Alam Sekitar (TRAS) digunakan untuk menilai kebarangkalian ketoksikan air Sungai Langat. Risiko tinggi ketoksikan mendadak telah ditemui untuk naproksen dan sulfamethoxazole. Beberapa FPPD juga menunjukkan ketoksikan kronik berisiko tinggi iaitu diklofenak, estradiol, ethynylestradiol, estriol, estron, dan sulfamethoxazole; manakala naproksen menunjukkan risiko toksik kronik yang sederhana. Metoprolol dan DEET menunjukkan risiko kronik rendah. Langkah-langkah pengurangan segera diperlukan untuk empat hormon iaitu estradiol, estriol, estrone and ethynylestradiol.

Kata kunci: Farmaseutikal, Produk Penjagaan Diri (PPD), pencemaran air, pencemaran kumbahan, Kromatografi Cair Prestasi Tinggi- Spekrometri Jisim Bersama (KCPT-SJ/SJ), Tekanan Atmosphera Pengionan Kimia (TAPK), Taksiran Risiko Alam Sekitar (TRAS)

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I certify that a Thesis Examination Committee has met on 14th December 2012 to conduct the final examination of Eugenie Tan Sin Sing on her thesis entitled "Method Development and Validation for Determination of Pharmaceuticals and Personal Care Products in River Water and Sewage" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosphy.

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DECLARATION

I declare that this thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and/or concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

EUGENIE TAN SIN SING

Date: 14 December 2012

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µg/L	Microgram per Litre
AC	Activated Carbon
ACN	Acetonitrile
ACR	Acute-to-chronic ratio
AL	Aerated Lagoon
AOP	Advanced Oxidation Processes
APCI	Atmospheric Pressure Chemical Ionisation
APPI	Atmospheric Pressure Ionisation
arb	Arbitrary
AS	Activated Sludge
CAFO	Concentrated Animal Feeding Operations
CCL	Contaminant Candidate List
CID	Collision Induced Dissociation
CITAC	Cooperation and International Traceability in Analytical Chemistry
CNS	Central Nervous System
DCM	Dichloromethane
DDD	Defined Daily Dose
DEET	*N, N-diethyl-m-toluamide*
DMDCS	Dichlorodimethylsilane
DWTP	Drinking Water Treatment Plan
EC_{50}	Half-maximal effective concentration
EDC	Endocrine Disrupting Compounds
ELISA	Enzyme Linked Immunosorbent Assay

EMEA	European Medicines Agency
ERA	Environmental Risk Assessment
ESI	Electrospray Mode
EURACHEM	Analytical Chemistry in Europe
GAC	Granular Activated Carbon
GC-MS	Gas Chromatography Mass Spectrometry
GC-MS/MS	Gas Chromatography- Tandem Mass Spectrometry
GF	Glass Fibre Filter
HACA	Hierarchical Agglomerative Cluster Analysis
HLB	Hydrophilic-Lipophilic Sorbent
HPL	High Pollution Location
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonisation
IDEA	Intermittent Decanting Extended Aeration
IDL	Instrument Detection Limit
IQL	Instrument Quantification Limit
IS	Surrogate/ Internal Standard
IUPAC	International Union for Pure and Applied Chemistry
IWK	Indah Water Konsortium
K_{ow}	Octanol-water Partition Coefficient
LC_{50}	Half-maximal lethal concentration
LC-MS	Liquid Chromatography Mass Spectrometry
LC-MS/MS	Liquid Chromatography – Tandem Mass Spectrometry
LLE	Liquid-liquid extraction
LOD	Limit of Detections

LOEC	Lowest-observed effects concentration
LOQ	Limit of Quantification
LPL	Low Pollution Location
m/z	Mass to charge ratio
Max	Maximum
MCX	Mixed-cation exchange
MDL	Method Detection Limit
ME	Matrix Effects
MEC	Measured Environmental Concentration
mg/L	Milligram per Litre
Min	Minimum
min	Minute
MPL	Moderate Pollution Location
MQL	Method Quantification Limit
MRM	Multiple Reactions Monitoring
MRSA	Methicillin Resistant *Staphylococus aureus*
MTBE	Methyl-tert-butyl-ether
ng/L	Nanogram per Litre
nm	Nanometer
NOEC	No-observed effects concentration
NOM	Natural Organic Matter
OECD	Organisation for Economic Co-operation and Development
PAC	Powder Activated Carbon
PCA	Principal Component Analysis
PCP	Personal Care Products

PEC	Predicted Environmental Concentration
pH	Minus the decimal logarithm of the hydrogen ion activity
pK_a	Acid Dissociation Constant
PNEC	Predicted No Effects Concentration
PPCP	Pharmaceuticals and Personal Care Products
QC	Quality Control
QSAR	Quantitative Structure Activity Relationship
R^2	Coefficient of determination
RF	Response Factors
RQ	Risk Quotient
RR	Relative Response
RSD	Relative Standard Deviation
RW	River water sampling location
S/N	Signal to noise ratio
SBR	Sequential Batch Reactor
SD	Standard Deviation
SPE	Solid Phase Extraction
SRM	Selected Reaction Monitoring
STP	Sewage Treatment Plant
TSQ	Thermo Scientific Quantum
U.S. EPA	United States Environmental Protection Agency
VF	Varimax Factor
VTG	Vitellogenin
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Pharmaceuticals are referred as medicine, medication or medicament, can be loosely defined as any chemical substance intended for medical diagnosis, cure, treatment, or prevention of disease. Personal-care products (PCPs) are synthetic organic chemicals derived from commercial products such as toiletries (soaps, lotions, toothpaste, etc.) cosmetics, household goods and many more. Together they constitute the chemical class termed as Pharmaceutical and Personal Care Products (PPCPs).

PPCPs is the emerging class of pollutants in the past decade due to its ubiquitous nature, toxicity, and persistency in the environment. The term “emerging pollutants” describe (1) entrance or generation of pollutants into the environment in appreciable amount, (2) having significant degree of persistency and (3) exhibiting detrimental effects on organisms (Khetan & Collins, 2007).

A national reconnaissance was created when the occurrence of PPCPs was investigated in 139 streams across 30 states in the United States by Kolpin *et al.,* (2002). PPCPs were detected in 80% of the streams sampled. Most frequently detected PPCPs include caffeine and DEET.

Scientific interests on PPCPs have escalated in the past decade; with significant increase of studies conducted yearly. These studies are focused on distribution, degradation and ecotoxicities to aquatic organisms as well as their end-point in potable drinking water. Fewer studies on soils, sludge and sediments were reported (Caliman & Gavrilescu, 2009).

PPCPs are constantly discharged into the environment in large quantities due to their manufacture, use and disposal yet extremely little are known about them. Pharmaceuticals ingested are not completely metabolized; thus significant amount of them are excreted via urine and feces. Subsequently they constitute in wastewater. Unused or expired medicines are discarded as household waste or flushed down the sewer. Thus, landfills experience abundance of PPCPs. Surface run-off from landfills flow into the nearby streams. Meanwhile, infiltration of landfill leachate poses a threat to groundwater contamination. Other sources of PPCPs include concentrated animal feeding operations (CAFO), discharge from recreational activities such as washing, swimming and bathing activities as well as aquaculture. Some PPCPs have short half life but their continual discharge into the environment have lead to widespread of PPCPs developing “pseudo-persistence” (Daughton Christian, 2001b)

Pharmaceuticals are designed for therapeutic purposes such as capable of eliciting biological responses in intended recipients particularly human and livestock. On the other hand, personal care products (PCP) are manufactured solely for human use. Occurrence of PPCPs in terrestrial and

aquatic environment have exposed PPCPs to non-target organisms. Halling Sorensen *et al.,* (1998) reviewed different toxicities across various tropic levels such as microorganisms, phytoplanktons and crustaceans/copopods. Ecotoxicities of PPCPs reported include (1) developments of antibacterial resistance in microorganisms against *Staphylococus aureus*, (2) growth inhibitions and retardations in phytoplanktons exposed to antibacterials as well as (3) smaller adults, reduced egg productions and abnormal growths in copopods. The said antibacterial resistance developed was attributed by three factors; (1) mutation in genes, (2) transfer of resistant genes and (3) increase of selective pressure. As such, traditional or commonly applied treatment regimes are compromised further complicating treatment for infections.

Several PPCPs were classified as potential endocrine disrupting compounds (EDCs). They are estradiol, estrone, ethynylestradiol, progesterone, oxybenzone, acetaminophen, naproxen and diclofenac. Although EDCs do not pose specific acute or chronic toxicities, they indirectly interfere with endocrine system resulting in enhancements or suppressions of hormones. EDCs are capable of causing (1) sexual underdevelopments, (2) infertilities, (3) altering or reducing sexual behaviours, (4) attention deficiencies or hyperactivity, (5) altering thyroid or adrenal cortical function, (6) increased incidences of certain cancers and (7) birth defects (Caliman & Gavrilescu, 2009).

As such, PPCPs need to be evaluated in details. PPCPs are complex mixtures of varying chemical and physical properties. The process of extraction and quantification for a group of PPCPs are often extensive in technicality, labour and cost. Several analytical approaches had been developed for different combinations of PPCPs including use of immunoassay (Estévez *et al.,* 2005), gas chromatography- mass spectrometry (GC-MS) (Farré *et al.,* 2007), gas chromatography-tandem mass spectrometry (GC-MS/MS) (Hibberd *et al.,* 2009), liquid chromatography-mass spectrometry (LC-MS) (Barel-Cohen *et al.,* 2006) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Lagana *et al.,* 2000).

All these have lead to development of a new discipline termed Environmental Pharmacology which deals with presence of pharmaceuticals in the environment (Rahman & Khan, 2006). Environmental Pharmacology and ecopharmacology are often used interchangeably. Ecopharmacology had been used to describe monitoring and detection of PPCPs environmental risk assessment and many more. It is then said, the term ecopharmacology is too broad and largely undefined. Pharmacoenvironmentology is proposed to further define ecopharmacology as it seeks to deal with environmental impact of drugs given to humans and animals at therapeutic doses (Rahman *et al.,* 2007). Recently, another jargon PharmEcovigilance was introduced by (Daughton & Ruhoy, 2011). PharmEcovigilance covers issues such as origins of problem, exposure hazards, direct disposal, environmental exposure, source distribution, ecological effects, human exposure, pollution

control, and many more. As to date, literature reviews lack specific definitions and defined scopes of various jargons. Therefore this research adopts the broader perspectives of environmental pharmacology as it studys methodology for extraction and detection, environmental risk assessment as well as conventional and advance treatment mechanisms for PPCPs.

1.2 Problem Statement

The awakening for studies on PPCPs contamination in lakes, rivers, streams and groundwater began in the late 1990s. The number of studies escalated in recent years to include behaviour, fate, removal mechanisms and ecotoxicities. Despite significant number of studies, there were no uniform lists of PPCPs to be studied. Many of the analyzed PPCPs were not detectable in the environment. Global researches are constantly altering their selection of PPCPs with the aim to develop a conclusive method. To further complicate matters, different countries have different patterns of medicinal consumptions as well as range of personal care products. One apparent example is the continual use of DEET (insecticide) in our country; however, it is only use during summer in temperate countries. Hence, list of PPCPs is individualistic. Extraction and detection methods are constantly improving to provide lower detection limits as PPCPs are often found in the environment at nanogram and microgram ranges. Matrix effect is another constant struggle as environmental samples like sewage, sludge and soils are complex. PPCPs were reported to disrupt endocrine functions, inhibit growth and develop antibacterial resistance in non-target organisms (Khetan & Collins, 2007). Human population are not sparred as concentration of PPCPs

were also detected in drinking water, thus posing significant risk such as antibiotic resistance and possible synergistic effects of mixtures (Al-Odaini, 2010). Owing to alarming effects of PPCPs on aquatic biota and human health, the occurrence of PPCPs in Malaysian waters needs to be documented. Hence this calls for development of simultaneous extraction and detection of PPCPs for Malaysia and any country whose consumption or pattern of use are similar. Subsequently, the said developed method can be applied to investigate the presence of PPCPs in environmental waters. Therefore, this study aims to address the following research questions:

- What are levels of PPCPs in Malaysian environmental waters?
- How can these pollutants be measured?
- Are the current wastewater treatment mechanisms adequate for removing PPCPs?
- Are environmental levels of PPCPs eliciting any harm?

1.3 Significance of Study

Protecting the integrity of water resources is the epitome of importance owing to the scarcity of sources for potable drinking water. Therefore , development of method for detecting and monitoring water resources is apply; as it could notify and alert for possible ecotoxicities. Detections create awareness for detailed investigations on performance evaluation of current removal mechanisms, possible acute or chronic toxicities as well as development for improved removals of PPCPs. Nevertheless, developed method must be selective, sensitive, precise and accurate for quantifying PPCPs in dirty and complex matrices. Labour, time and cost effective measures are also

incorporated to encourage routine environmental monitoring of emerging pollutants.

This research is expected to provide a robust and reliable method for quantifying multi-classes PPCPs which is of great importance to unveil the status of PPCPs pollution in Malaysian waters. To the author's best knowledge, this study features several pioneering efforts; (1) extraction of levonorgestrel and norethindrone (both synthetic hormones) using solid phase extraction (SPE) Hydrophilic-Lipophilic Sorbent (HLB) and (2) quantifications of seven PPCPs using liquid chromatography tandem mass spectrometry (LC-MS/MS) in atmospheric pressure chemical ionisation (APCI) namely acetaminophen, sulfamethoxazole, diclofenac, atenolol, metoprolol, DEET and oxybenzone.

Given completion of method developments and validations; studies on occurrence of PPCPs in Langat river basin and removals of PPCPs in current sewage treatment plant (STP) are made possible. As to date, there are no regulations for PPCPs in environmental waters. However, the United States Environmental Protection Agency (U.S. EPA) had just listed several PPCPs in their Contaminant Candidate List (CCL) during their recent stakeholder meeting on the 16th of June 2011. These CCLs are unregulated contaminants which may require national water legislations or guidelines drinking in the future. Natural estrogens (estradiol, estriol and estrone) and synthetic estrogens (ethynylestradiol) are listed as CCLs. They can be detected and quantified using the developed method. CCLs are selected for causing

adverse effects to human, having verified occurrences in environmental waters and meaningful opportunity for risk reduction (U.S. EPA, 2011).

Results from this study will provide pioneering baseline information for (1) health and environmental legislators, (2) aquatic toxicologists, (3) sewage treatment plant (STP) and drinking water treatment plant (DWTP) operators as well as (4) commercials products manufacturer (bottled mineral water, bottled drinking water, house water filters and etc.).

1.4 Objectives of the Study

The primary objective of this study is to develop simultaneous extraction and quantification for PPCPs in water matrices using SPE and LC-MS/MS. Performance of the developed method will be validated using river water, STP influent and effluent. Upon successful validation, it will be applied to investigate (1) occurrences of these pollutants in Langat river, (2) wastewater from selected STPs in Langat basin and(3) performance of current STPs in removing PPCPs.

Specific objectives of this study are:

1. To develop a selective, sensitive, precise and accurate method for simultaneous extraction, determination and quantification of PPCPs using SPE and LC-MS/MS in Atmospheric Pressure Chemical Ionization (APCI) mode.
2. To validate the developed method in environmental waters such as river water, STP influent and effluent.

3. To identify and quantify PPCPs in river water and wastewater (influent, intermittent and effluent) from selected STP plants in Langat River Basin.
4. To access aquatic toxicity based on Environmental Risk Assessment (ERA).

1.5 Thesis Synopsis

This thesis focuses on development of LC-MS/MS method for detection and quantification of PPCPs in river water and wastewater for Malaysia. Following sections provide initial previews on each chapter and elaborate their linkages between chapters.

Chapter 2 provides literature reviews which cover definitions of pharmaceuticals and personal care products (PPCPs). Origins and sources of PPCPs as well as their fate in the environment are discussed. In addition, various analytical methodologies are summarized and compared. The occurrence of PPCPs in the environment has lead to ecotoxicities in aquatic and terrestrial non-target organism. Ecotoxicities of various PPCPs are discussed. This chapter also reviews conventional and advanced treatment technologies as well as various pollution control methodologies.

Chapter 3 describes the development and optimization of SPE and LC-MS/MS for simultaneous extraction and quantification. The sample preparation method and optimization of different experimental parameters are discussed in details. This is followed by validation procedures of the

developed method in environmental water samples as discussed in Chapter 4. The method is validated for sensitivity (detection and quantification limits), selectivity, precision (inter-day and intra-day precision) and accuracy in ultrapure water, river water samples, STP influent and effluent.

Chapter 5 discusses the application of develop method to study occurrence of PPCPs in twelve river water sampling locations and three STP plants [Aerated Lagoon (AL), Sequential Batch Reactor (SBR) and Intermittent Decanting Extended Aeration (IDEA)]. Results of analysis are discussed in details. Environmetrics are also applied to further interpret the data in the manner of providing descriptions of sampling locations and source apportionment for PPCPs in Langat river basin.

Chapter 6 summaries the research and discusses overall findings, evaluations and contributions of this study. Further experimental works and future endeavors arising from the outcome of this research are proposed.

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