Environmentally Persistent Pharmaceutical Pollutants (EPPPs) in Brisbane river sediments and their relationship to urbanisation

Padma Bollapragada

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Science and Engineering Faculty

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Environmentally Persistent Pharmaceutical Pollutants; EPPPs, urbanisation; population growth; ageing population; Brisbane River; sediment pollution; analytical method development.

Abstract

Urbanisation around the world has exerted enormous pressure on water resources and has polluted them with a myriad of pollutants including recently identified Environmentally Persistent Pharmaceutical Pollutants (EPPPs). The EPPPs pollution is widespread in the water column and has even contaminated the sediment bed. The Brisbane River in South East Queensland (SEQ) is no exception to this type of degradation. Being one of the major rivers in SEQ, the Brisbane River is surrounded by urbanisation and is polluted by EPPPs. Additionally, the region is also experiencing population growth and an increase in ageing population which is prone to consume more pharmaceutical drugs. However, there are no past studies of sediment pollution by EPPPs in the Brisbane River, which highlights a knowledge gap to be addressed. The current study was undertaken to address the knowledge gap of EPPPs pollution of the Brisbane River sediments to attain a better understanding of the extent of such pollution. The study was based on the hypothesis that urbanisation and ageing population in SEQ in combination introduce EPPPs into the Brisbane River and accordingly understanding the relationship between urbanisation and occurrences of EPPPs in the river sediments is important to safeguard its environmental values.

The study was initiated with a thorough critical review of research literature that concluded that studies into sediment testing for the presence of EPPPs were very limited compared to surface waters due to the complex nature of the sediment matrix. Furthermore, there is no reported research study into sediment pollution by EPPPs in Australia. This highlighted the need for method development for extraction of EPPPs from sediments and for identification and quantification of EPPPs using the mass spectrometry instrument – Liquid Chromatography-Mass Spectrometer (LCMS/MS). Accordingly, developing methods for extraction and quantification was a primary objective of the research study. As a result, a rapid, reliable Multiple Reaction Monitoring (MRM) method with R^2 value of 0.99 was developed for EPPPs from five therapeutic classes which were selected after critically reviewing the information from Australian Bureau of Statistics (ABS) and Pharmaceutical Benefit Scheme (PBS).

The analysis of target EPPPs confirmed the occurrence of psychoactive drugs in the Brisbane River sediments concluding that the developed methods were reliable. However, it was also concluded that the methods developed required further refinement, in particular, the extraction method, to be able to quantify other target EPPPs.

The discovery of psychoactive drugs in the Brisbane River sediments was a significant finding in this study since these drugs are highly active affecting the central nervous system of living organisms. Studies conducted around the world have concluded that psychoactive drugs do not readily degrade and hence bio-accumulate in aquatic organisms such as fish. This is a serious concern since this creates a potential risk of such drugs entering into the food chain and being consumed by human beings. This study also concludes that wastewater treatment techniques in SEQ are unable to remove EPPPs and therefore are releasing these compounds into the Brisbane River. Data analysis also concluded that consumption of psychoactive drugs by the growing urban population in Queensland is on the rise.

It is hypothesised that wastewater effluents discharged from sewage treatment plants (STPs) situated along the Brisbane River in SEQ to be the primary source for the occurrence of EPPPs. This would mean that recycled water derived from STPs also act as a source of EPPPs. Additionally, the study of sewerage network map of SEQ postulates that leaks from the sewer lines that are located along the length of the Brisbane River could also be acting as a source for EPPPs pollution in the Brisbane River sediments. Therefore, this study has not only addressed an emerging pollution problem but has also contributed new knowledge to the existing knowledge base in relation to EPPPs pollution in the aquatic environment in Australia.

The knowledge derived from this study would be important for future research into EPPPs pollution of the sediment environment. This study also provides baseline information that would contribute to the decision-making process in relation to the use of recycled water for domestic consumption. It can also be concluded from this study that monitoring of EPPPs in Australian aquatic environments is essential in order to implement regulations and guidelines in relation to EPPPs pollution in Australia.

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List of Abbreviations

ASE	Accelerated Solvent Extraction
ABS	Australian Bureau of Statistics
AOP	Activated Oxidation Process
BITRE	Bureau of Infrastructure, transport and Regional Economics
BOM	Bureau of Meteorology
CHO	Chief Health Officer
DE	Diatomeaous Earth
EPPPs	Environmentally Persistent Pharmaceutical Pollutants
ESI	Electrospray Ionisation
LOD	Limit of detection
GCMS/MS	Gas Chromatography Mass Spectrometry
GAC	Granular Activated Carbon
LCMS/MS	Liquid Chromatography Mass Spectrometry
LOQ	Limit of Quantification
MDL	Method detection limit
MeOH	Methanol
MAE	Microwave Assisted Extraction
MRM	Multiple Reaction Monitoring
PBS	Pharmaceutical Benefit Scheme
PIS	Product Ion Scan
QUU	Queensland Urban Utilities
R.C.C	Recovery Corrected Concentration
RSD	Relative standard deviation
SAICM	Strategic Approach to International Chemical Management
STP	Sewage Treatment Plant
SRT	Sludge Retention Time
SPE	Solid Phase Extraction
SEQ	South East Queensland
STD	Standard Deviation

UN	United Nations
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
WWTP	Wastewater Treatment Plant
WHO	World Health Organisation
WWDR	World Water Development Report

Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this institution or any other higher education institution. To the best of my knowledge and belief, the thesis contains no data or material from previously published work except where due reference is made.

QUT Verified Signature

Padma Bollapragada

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Dedication

This dissertation is dedicated to my husband Uday Chandrupatla and our lovely son Arjun Chandrupatla, who have been extremely supportive and understanding, and to my parents, brothers and in-law who encouraged and supported me throughout the research study.

Chapter 1 Introduction

1.1 Background

Research and development in the health sectors have resulted in the manufacture of pharmaceuticals that have eradicated a significant number of diseases around the world. Whilst these pharmaceuticals have been able to improve human health, the occurrence of their residues and transformed products in the environment poses a threat to aquatic flora and fauna (Daughton and Ruhoy, 2008).

The occurrence of pollutants which are termed as environmentally persistent pharmaceutical pollutants (EPPPs) has risen alarmingly, which could be the result of economic developments and associated urbanisation. Today, cities around the world have become the major hubs of economic developments and provide increasing opportunities for wellbeing and growth to billions of people. This has resulted in the exodus of people from rural areas into cities in search of improved livelihoods, enhanced opportunities for employment and education and better access to health care. Such rapidly occurring economic developments have caused greater concentrations of the population giving rise to the conversion of previously agricultural land to urban areas. Urbanisation and economic development advance hand in hand as a country's prosperity improves.

Consequently, land use undergoes changes due to escalating need to provide shelter for the growing influx of population into urban areas. This has resulted in reduced green vegetation due to residential and industrial developments. Activities such as building construction and infrastructure provision are part and parcel of urbanisation that is required to accommodate and satisfy the needs and demands of the burgeoning urban population. These developments and growing urban population have exerted significant stress on natural resources in particular water for domestic and commercial purposes. Water is being extracted in increasing amounts to meet this high and growing demand. Unfortunately, this has threatened the sustainability and quality of this extremely important resource that underpins life on earth.

Australia has been experiencing significant economic and population growth in recent decades. This has resulted in inevitable stress on the surrounding environment (Hatfield-Dodds et al., 2015). It has been estimated that about 90% of Australia's population will be living in urbanised areas by 2020 (WHO, 2015). This highlights the significant stress that Australia's natural resources will be subjected to, particularly the water environment. Being the driest inhabited continent, Australia has experienced significant and long-lasting droughts in its recorded history. Therefore, an ever increasing demand for water for domestic and industrial purposes is likely to exert enormous pressure on the water resources in Australia. Therefore, it is imperative that available water resources are stringently safeguarded.

Water pollution is a serious and major consequence of urbanisation due to the pollutants generated by anthropogenic activities common to urban areas. The developmental activities that are carried out as a consequence of urbanisation result in extensive impervious areas that lead to flushing of pollutants such as heavy metals and hydrocarbons into the receiving water environments (Goonetilleke and Thomas, 2003; Stewart et al., 2014) causing pollution and inhibiting stormwater from percolating back into the ground resulting in groundwater depletion (WWDR, 2015). Another major consequence of urbanisation is the change in lifestyle which demands more resources.

There is a significant change in lifestyle in urban areas compared to rural areas. This lifestyle has been observed to be responsible for the creation of a diversity of organic micro-pollutants commonly referred to as "Emerging contaminants". This a consequence of the affluent urban lifestyle which has resulted in increased consciousness about personal health and grooming which gives rise to an increased use of pharmaceutical drugs like anti-inflammatories, analgesics, psychiatric drugs, lipid regulators, β-blockers and antibiotics (Ellis, 2006). These pharmaceutical compounds are commonly not completely absorbed by the body and part of the compounds are excreted and end up in wastewater effluent (Camacho-Muñoz et al., 2013). Consequently, wastewater effluents act as a source for the occurrence of EPPP compounds in water environments when discharged into receiving waters. Therefore, the increase and multiplication of emerging pollutants such as EPPPs in receiving waters can be attributed to the spread of urbanisation.

Water pollution due to urbanisation continues to remain a serious problem across the globe and the continuing addition of new pollutants such as EPPPs has exacerbated the issue because of the environmental ramifications of these pollutants (Daughton, 2004). In fact, major research projects have been undertaken in Europe and the US in order to understand and expand the fundamental knowledge about EPPPs such as their occurrence, persistence and impacts and to develop better wastewater treatment practices for reducing the loads of such pollutants (Ternes et al., 2004). Similar research projects need to be undertaken in Australia too, to better understand the potential impacts of EPPPs on human and ecosystem health and to develop management strategies for mitigation.

However, currently there is a dearth of information about the potential dangers of EPPPs to the environment which has made these pollutants not being regulated in Australia, which is compounding issue because there is a risk that these compounds being overconsumed and discharged in the environment untreated. Thus, it is very important to understand the behaviour of these pollutants. This chapter discusses the research problem, hypothesis, aims, objectives and scope of the research project undertaken.

1.2 Research problem

Pollution of the Brisbane River has been a problem for many years as a result of rapid urbanisation. Pollutants such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) have been detected in the Brisbane river and its sediments (Kayal and Connell, 1989; Minnery and Barker, 1998; Ogogo, 2013; Shaw and Connell, 1980). Recently a study conducted by Scott et al. (2014) reported the occurrence of EPPPs compounds in the surface waters of the Brisbane River indicating that the Brisbane River is being continuously polluted with numerous pollutants.

The current study was to identify EPPPs in the sediments of the Brisbane River which was hypothesised to be occurring as a result of urbanisation associated with population growth as well as because of the ageing population in Queensland. Urbanisation has played a pivotal role in transforming peoples' lives for good. However, it has left an environmental footprint in the form of pollution caused by

compounds such as pesticides, heavy metals, hydrocarbons and recently recognised 'Emerging contaminants' - EPPPs. These anthropogenic pollutants are being released into the environment via different sources and are occurring throughout the environment in varied concentrations.

The occurrence of EPPPs in the environment, in particular, is a serious problem which needs urgent attention (Murray et al., 2014) because of the following characteristics that these pollutants possess:

i. **Anthropogenic**: because they are formulated organic compounds, they are capable of reacting with the surrounding environment.

ii. **Target specific:** since they are biologically active and designed to target specific tissues, organs, metabolic pathways, eg; cholesterol controlling drugs, painkillers and anti-depressants.

iii. **Low concentrations**: in that they are capable of inducing impacts at very low concentrations, eg. antibiotics

iv. **Accumulate:** as these compounds can bioaccumulate and biomagnify in aquatic organisms (Daughton and Ternes, 1999; Fent et al., 2006; Suárez et al., 2008)

Analysing wastewaters, surface waters and groundwater for EPPPs would provide credible information about the pollution level caused by such pollutants over a particular period of time. Therefore, the analysis of environmental samples is useful in monitoring the pollutants. This is because, when the pollutants are in surface waters, they are likely to dilute and undergo photo-degradation or react with other substances or get flushed away. However, this is less likely in sediments because pollutants are more likely to remain attached to the media.

Sediment analysis would provide wide and comprehensive information about pollution in an aquatic environment. Below are the reasons that explain why sediment analysis is important.

i. Analysing sediments at different depths provide information about the time period of pollution occurring in that area.

ii. Analysing sediments are important as it provides habitat for many aquatic organisms. All the aquatic flora and fauna dwell and breed on the sediments (Choi et al., 2014). Therefore, any contamination in the sediments is likely to have adverse impacts on sediment habitat and eventually affect the flora and fauna in those habitats.

iii. When a polluted sediment bed is disturbed due to natural events like flooding including high flow velocities or anthropogenic activities such as dredging, the pollutants in the sediments can be released back into the environment. Therefore, an understanding of pollutants present in the sediment is important.

iv. EPPP compounds accumulate on sediments and occur in very low concentrations of ppb and ppt (Antonić and Heath, 2007; Camacho-Muñoz et al., 2013; Chen and Zhou, 2014; Silva et al., 2011). Sediments are a sink for such pollutants. Since EPPPs might be potentially harmful (Camacho-Muñoz et al., 2013) even in such low concentrations, analysing sediments for such pollutants becomes important.

As noted above, it is evident that the occurrence of EPPPs in the sediment environment can be significant and poses a potential threat to the health of the aquatic environment. Unfortunately, there has been limited research into EPPPs in Australia and it appears to be limited to surface waters and wastewaters (Birch et al., 2015; Watkinson et al., 2009). The complex nature of the sediments, lengthy sample preparation process and requirement of advanced analytical techniques to quantify the minute concentrations (Kolpin et al., 2002) make analysis of EPPPs in sediments difficult and resource intensive. This could be the reason for a limited number of studies reported in Australia investigating EPPPs. However, in order to understand the full extent of EPPP pollution and its potential impacts to the Australian aquatic environments, it is necessary to investigate EPPPs in sediments.

1.3 Research hypothesis

Australia is one of the most urbanised countries in the world with an appreciable population growth (ABS, 2013). In regards to South East Queensland (SEQ), there

has been rapid urbanisation (BITRE, 2013; QueenslandTreasury, 2016). The region is also experiencing population growth, growth in ageing population and there is an increase in consumption of EPPPs as a result of the increasing ageing population (ABS, 2011, 2015a; Peter Atkins et al., 2015). Additionally, increasing urbanisation has resulted in pollution of the Brisbane River. Along with the above mentioned pollutants, continuous ingestion and excretion of EPPPs by the growing and ageing population in SEQ is likely to be releasing these micropollutants to the Brisbane River via various sources. Accordingly, the research study hypothesised that urbanisation and ageing population in SEQ together introduce EPPPs into the Brisbane River and a robust analytical method capable of quantifying very low concentrations would enable in detecting these micropollutants.

1.4 Aims and objectives

As evident from Sections 1.2 and 1.3, quantifying organic micropollutants such as EPPPs in complex environments such as sediments is a difficult task. Moreover, the lack of research in Australia and unavailability of robust testing methods highlights the need for method development. Therefore, it was important in this study to develop a robust testing method that could extract these micropollutants efficiently from the sediment matrix and quantify in order to achieve the aim of this research study.

Aim: To study the relationship between the occurrence of EPPPs in the Brisbane River sediments and urbanisation using a reliable and robust method for quantification.

Objectives:

The primary objective was to develop a method for extraction and quantification. Development of extraction method for extracting EPPPs from sediment was also equally important for accurate quantification of EPPPs and studying the occurrence and distribution of these emerging pollutants.

1.5 Innovation and contribution to the knowledge

Urbanisation has resulted in a change in lifestyles and has caused the concentration of population in urban areas, thereby exerting stress on natural resources and particularly the water resources. The Brisbane River which is the main waterway running through the major part of the urban area of South East Queensland, is being polluted by numerous kinds of pollutants (eg. trace elements, heavy metals and hydrocarbons) (Kayal and Connell, 1989), the sources of which could be attributed to anthropogenic activities related to urbanisation. Thus, the pollution of the Brisbane River is an ongoing problem.

Recently, the Brisbane River has been investigated and found to be polluted with EPPPs (Scott et al., 2014). The occurrence of these micropollutants could be attributed to urbanisation due to urban lifestyle and ageing population in SEQ consuming EPPPs in significantly high amounts (ABS, 2011, 2014, 2015c), thereby leading to the release of these substances into the River mainly via wastewater. Studies (Antonić and Heath, 2007; Daughton and Ternes, 1999; Ternes et al., 2004) have concluded that current wastewater treatment methods are to be blamed for the occurrence of EPPPs in aquatic environments since these are not capable of removing these substances from wastewaters. EPPP pollution of aquatic environments is concerning because of the ability of these compounds to be active even in minute concentrations (ppb, ppt) and to be able to accumulate and cause detrimental effects on the surrounding aquatic environment (Daughton and Ternes, 1999; Henschel et al., 1997). Therefore, investigating the presence of EPPPs is crucial to understand the extent of pollution caused by these pollutants and the possible environmental impacts these may have on the aquatic ecosystem.

While it is known that the Brisbane River water is polluted with EPPPs, there is no reported study investigating EPPPs in the sediments of the Brisbane River. Investigating EPPPs in the sediments of the Brisbane River is important as this would facilitate in providing a comprehensive overview of the extent of pollution in the waterway, thus, contributing to the enhancement of the knowledge base in relation to EPPP pollution.

Investigation of EPPPs in the sediments of the Brisbane River required the development of test methods for the detection, quantification and extraction which would further contribute to knowledge in method development for EPPPs analysis.

In addition, studying the relationship between urbanisation and EPPPs occurrence in the Brisbane River was also a new approach that would help to understand the entire scenario of EPPP pollution in the Brisbane River.

1.6 Scope

The research study was aimed at investigating the relationship between EPPPs occurrence and urbanisation along the Brisbane River because of two important reasons: first being the potential environmental concerns regarding the occurrence of these emerging organic and biologically active micropollutants in the aquatic environment and second, because of the dearth of information on EPPPs occurrence in Australian aquatic environment. The study reviewed and evaluated research literature and statistical information which led to the conclusion that the growing urban and ageing population due to urbanisation in South East Queensland consumes significant amount of EPPP compounds particularly those belonging to the classes - psychiatric (to treat mental health conditions), β-blockers (to treat high blood pressure), lipid regulators (to control cholesterol levels), anti-inflammatory and analgesics (painkillers), and antibiotics (to treat infections).

Consequently, the study selected target EPPPs that are increasingly consumed in South East Queensland from the respective five therapeutic classes mentioned above and developed test methods for determining these selected EPPP compounds in the sediments. The developed method led to the quantification of the three target EPPP compounds, namely, carbamazepine, diazepam and lorazepam. The developed method can be applied for determining the target EPPPs in the sediment environment.

Urbanisation has a strong influence on the occurrence of EPPPs in the aquatic environment. The knowledge developed on urbanisation and EPPP occurrence has contributed to the knowledge of EPPP pollution in the Brisbane River and provides baseline information for undertaking mitigation measures. Furthermore, the developed knowledge is applicable to other areas of environmental research such as the use of recycled water for domestic consumption.

1.7 Thesis outline

This thesis consists of six chapters. Chapter 1 is the introductory chapter and includes the background and context to the project, and discusses the research project. The chapter also discusses the hypothesis for this research study, its aims and objectives and contribution to knowledge. Chapter 2 presents a critical review of research literature on EPPPs in relation to its occurrence, distribution, sources and impacts on the aquatic ecosystem. Further, the chapter also discusses the relationship between EPPP occurrence and urbanisation associated with population growth and ageing population in South East Queensland.

Chapter 3 discusses the selection of pollutant compounds for this research study, the research design and method development for detection of the selected compounds and the results obtained. The chapter also discusses the statistical methods used to analyse the data. Chapter 4 discusses in detail the study area, selection of sampling areas and sampling.

Chapter 5 summarises the findings of the research study. The chapter has analysed the results obtained and discusses the causes and the likely sources for the occurrence of EPPPs in the Brisbane River.

Chapter 6 summarises the conclusions drawn from Chapter 5 and provides recommendations for future research.

Chapter 2 Environmentally Persistent Pharmaceutical Pollutants (EPPPs) in Urban Water Environment

2.1 Overview

Anthropogenic activities such as industrialisation, urbanisation and along with population growth are part and parcel of the social and economic development of a country. Unfortunately, these developments are not carried out in a sustainable way and therefore result in degradation of resources such as water environments (WWDR, 2015). Water is a precious natural resource and it is essential for life on earth, but anthropogenic activities such as urbanisation, in particular, have resulted in abuse of water resources. Water resources have been exploited to meet the ever increasing demand by the growing population due to urbanisation. Additionally, it is being polluted by harmful and toxic pollutants such as heavy metals, hydrocarbons, nutrients (nitrogen, phosphorus), trace pollutants (organochlorines, pesticides) and EPPPs (WWDR, 2015).

This study was undertaken to test the hypothesis that urbanisation causes EPPP pollution of rivers. Therefore, the focus of the study was on studying the occurrences of EPPPs in the Brisbane river sediments which is exposed to urbanisation. Details about studying the occurrences of EPPPs in the sediments and the selection of the Brisbane River are discussed in Chapter 4. A critical review of research literature was carried out to identify knowledge gaps. The literature review was divided into five sections - (1) EPPPs in the environment (2) Occurrence and distribution of EPPPs around the world; (3) Bioaccumulation and toxicity of EPPPs; (4) Removal of EPPPs (5) Urbanisation and sources of EPPPs(6) Analytical methods for analysis of EPPPs.

2.2 EPPPs in the environment

This section discusses the rising interest in pharmaceuticals as environmental micro-pollutants and the developing research in this area. EPPPs are pharmaceutical drugs that are consumed for the treatment of temporary or chronic health conditions in both, humans and animals (Khetan, 2014). EPPPs constitute of organic chemical compounds, transformed metabolites and preservative chemical compounds from pharmaceutical drugs that are persistent in the aquatic environment and have recently gained considerable attention of the scientific world (Daughton, 2004; Daughton and Ternes, 1999). Jjemba (2006) in his study described EPPPs as natural or manufactured chemicals or materials occurring in the environment that are highly biologically active affecting the biochemical and physiological functions of the body. Accordingly, pharmaceutical substances can be defined as 'Environmentally persistent, biologically active, organic micropollutants derived from anthropogenic sources'.

Study of EPPPs is complex as it spreads across various disciplines and therefore requires expertise from these disciplines – pharmacy, chemistry, environmental engineering, hydrology, toxicology, medicine and social psychology (Daughton, 2009). The occurrence of EPPPs in water affirms the close relationship between human behaviour and the environment (Daughton, 2009). In-depth research into EPPPs commenced in the 1990s and the majority of the research was carried out in the US and Europe (Daughton, 2004, 2009; Daughton and Ternes, 1999). However, recently it has spread to many other countries of the world (Beretta et al., 2014; Ternes et al., 2002; Vazquez-Roig et al., 2010) with studies being conducted into wastewater, soils, sediments, drinking water and marine environments (Arpin-Pont et al., 2014; Pal et al., 2010).

Having being identified and defined as environmental pollutants, pharmaceuticals have been classified under many different terms with one such term classifying these substances are 'Emerging Contaminants (ECs)'. The term 'Emerging contaminants (ECs)' is commonly applied to pharmaceuticals because of their recent emergence as environmental pollutants and due to the dearth of information about their possible adverse environmental impacts (Sauve and Desrosiers, 2014; Silva et al., 2011). ECs is a broad term that encompasses a range of substances including personal care products (PCPs) such as soaps and cosmetics, fragrances, flame retardants, endocrine

disrupting compounds (EDCs) and pharmaceuticals and therefore, does not specifically identify pharmaceuticals. Hence, the use of this term, particularly to this study, was not considered appropriate. In addition, according to Daughton (2004), the word 'emerge' is primarily applied to organic micropollutants that have just been recognised and discovered in the aquatic environment. Most of the target EPPPs that will be investigated in the current study have already been found to have negative impacts on the environment and are therefore of concern. Hence, the use of the term 'ECs' in this study was not considered to be suitable to describe the target EPPPs.

There are many different terms that have been used to describe pharmaceuticals, such as, 'Priority Pollutants', 'persistent bioaccumulative toxics' (PBT), 'persistent organic pollutants'(POPs) and 'Bioaccumulative chemicals of concern' by the EU and US national water pollution control programmes (Ellis, 2006). These terms describe the nature and explain the characteristics of organic pollutants. However, the terms do not specifically relate to pharmaceuticals. For example, the term 'PBT' has been broadened to include pollutants that are persistent, can accumulate in living organisms and are toxic to the environment. Pharmaceutical substances are persistent, but not all of them are bioaccumulative and toxic. In fact, there are limited studies that explain the toxicity or bioaccumulative nature of these compounds which means more research is required to address this knowledge gap. Therefore, this term was not considered relevant to this research study. In their study, Kolpin et al. (2002) classified pharmaceuticals under the term 'Pharmaceutically Active Compounds (PhACs)', whereas Stewart et al. (2014) used 'Organic waste contaminants (OWC)' to describe pharmaceuticals. 'PhACs' highlights pharmaceuticals and encompasses a broad range of pharmaceutical substances such as hormones, whereas 'OWCs' distinguishes pharmaceuticals by pinpointing the source of these compounds, but it is still a broad term and was created according to the focus of the study undertaken.

Again, in other studies, Shareef et al. (2008) assessed pharmaceuticals under the term 'Organic contaminants of Emerging Concern,' whereas Li (2014) termed them as 'Emerging Organic Contaminants,' respectively. Both these terms are quite general that embodies a myriad of pollutants and therefore do not specifically recognise pharmaceuticals. Scott et al. (2014) identified pharmaceuticals as 'Trace Organic

Contaminants' (TrOCs), which is similar to the terms used in previous studies discussed by Shareef et al. (2008) and Stewart et al. (2014). This term again highlights only one characteristic of pharmaceutical micropollutants, which is the organic nature of these compounds. However, the term does not include the persistent nature of these compounds. Therefore, an appropriate term was required to define the target pharmaceutical micropollutants relevant to this study.

Whilst there are many terms used to identify pharmaceuticals in the environment, the term considered by the Strategic Approach to International Chemical Management (SAICM) in 2011 as 'Environmentally Persistent Pharmaceutical Pollutants (EPPP)' (Koekkoek, 2015) fits appropriately to this research study. This term takes into account the two important characteristics associated with pharmaceuticals, namely: (1) it precisely highlights pharmaceuticals as environmental pollutants; and (2) describes the persistence of these compounds in the environment, the most important and concerning characteristic of pharmaceutical pollutants. Accordingly, this term was chosen to describe the target compounds studied in this research study.

EPPP substances are used in the preparation of medicinal drugs. Approximately 3000 different bioactive pharmaceutical substances are present in modern day medicines, such as the lipid regulators, β- blockers, analgesics, psychiatric and antibiotics in the European Union alone (Ellis, 2006; Fent et al., 2006; Nebot et al., 2015; Silva et al., 2011) and the trend to use such substances is escalating globally as a result of increasing concern about personal health and grooming (Ternes et al., 2004). Table 2.1 below presents the different therapeutic classes and the common drugs under each of these classes that are particularly threatening to the environment.

Table 2.1 EPPPs of different therapeutic classes

Therapeutic group of EPPPs	Compounds present in the group
Psychiatric drugs	diazepam, carbamazepine, primidone, lorazepam
Lipid regulators	clofibric acid, bezafibrate, fenofibric acid, etofibrate, gemfibrozil, atorvastatin
β- blockers	atenolol, propranolol, timolol, sotalol, metoprolol
Analgesics and anti-inflammatory	ibuprofen, diclofenac, fenoprofen, acetaminophen, naproxen, acetylsalicylic
Antibiotics	amoxicillin,cefalexin, trimethoprim, erythromycin, lincomycin, chloramphenicol

Source: Adapted from (Ellis, 2006).

The drugs listed in Table 2.1 are among the most widely consumed drugs across the world and have been occurring in wastewaters, surface waters and sediments (Camacho-Muñoz et al., 2013; Shareef et al., 2008; Zhang et al., 2008). Santos et al. (2010) in their review concluded that the occurrence of different types of EPPPs in the environment have negative impacts on non-target organisms such as bacteria, algae, crustaceans and fishes affecting their growth, reproduction and mobility in some cases. Therefore, these drugs have been considered as the pollutants of environmental concern because of their widespread occurrence across the various environmental compartments and their negative impacts on the aquatic ecosystem.

The drugs listed in Table 2.1 are being consumed here in Australia too. National health expenditure on prevailing health conditions – cardiovascular, respiratory, mental, neurological and musculoskeletal has increased over the period from 2004-05 to 2011-12 (Fig 2.1) and the drugs that are consumed to treat these conditions (Table 2.1) are being increasingly prescribed and have been detected in the environment (Birch et al., 2015; Scott et al., 2014). It is understood that the diseases on which the financial resources have been spent are prevalent and increasing in

Queensland as well (CHO Report, 2014). Therefore, Figure 2.1 is also considered to be illustrative of the health condition of Queenslanders and their consumption of EPPP compounds.

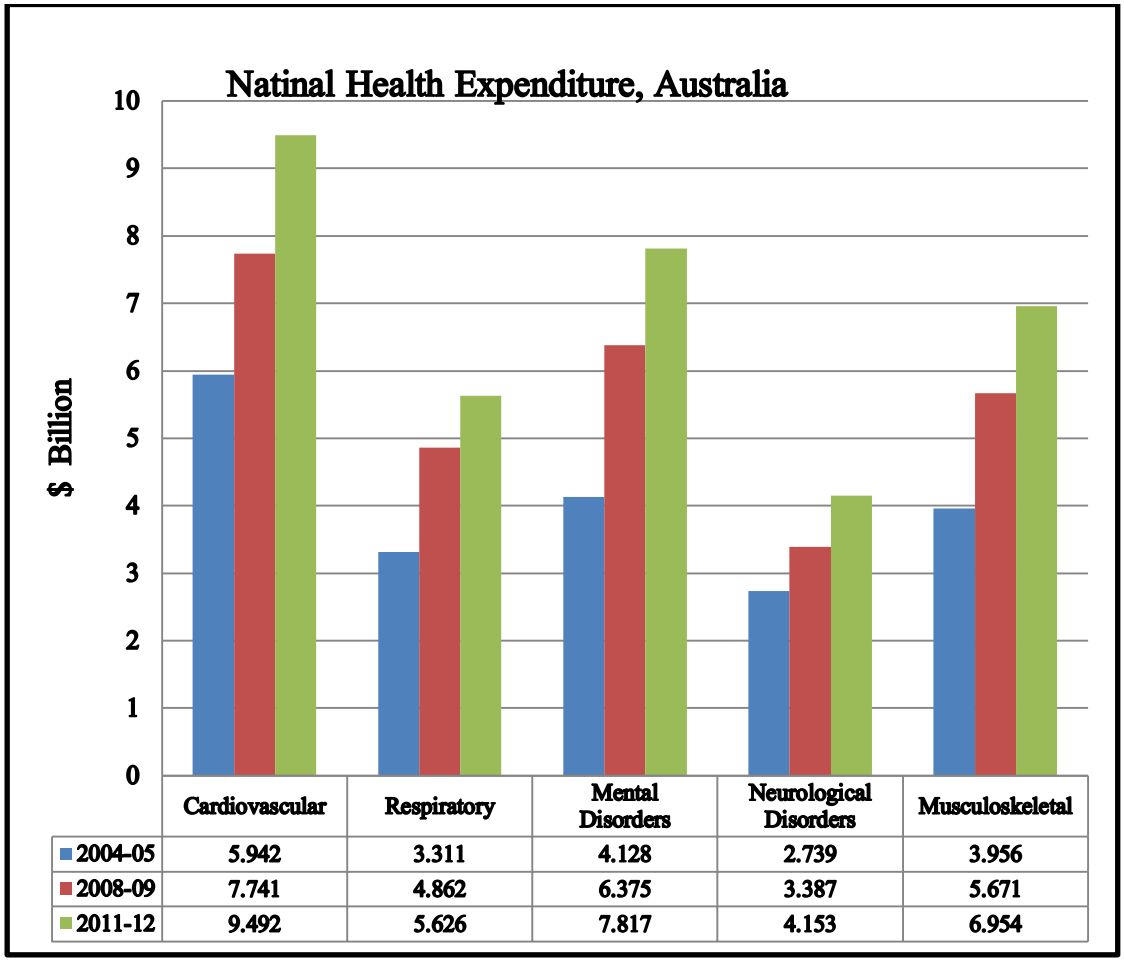


Figure 2.1 Health expenditure on various health conditions during the period 2004-05 to 2011-12.

Source: Adapted from, (CHO Report (2012), 2014))

Figure 2.1 shows a steady increase in the allocated expenditure on the diseases especially cardiovascular, mental and musculoskeletal. This indicates an increase in the consumption of drugs such as β- blockers, psychiatric and anti-inflammatory and analgesics which are sold under a number of different brand names in Australia (Table 2.2). Thus, it can be concluded from Figure 2.1, Table 2.1 and Table 2.2 that the drugs listed are of specific environmental concern and need to be investigated.

Table 2.2 Therapeutic drugs commonly consumed in Australia to treat various health conditions

Therapeutic group	Drugs	Brand names	Health conditions
Psychiatric	Carbamazepine, diazepam, lorazepam	carbamazepine Sandoz, diazepam elixir, ranzepam	Types of seizures, neurological conditions, pain such as trigeminal neuralgia
β-blockers	Propranolol, atenolol	Atenolol Gh, atenolol An, atenolol Rbx, atenolol Sandoz, atenolol aft, Apo-propranolol	high blood pressure and in the prevention of heart attacks
lipid regulator	Atorvastatin	Apo- atorvastatin, Apo-simvastatin, Apo-rousvastatin, Apo-pravastatin	Lowering cholesterol
Anti-inflammatory and analgesics	Ibuprofen, diclofenac	Apo-diclofenac, Diclofenac An, Diclofenac Sandoz, Diclofenac-ga	reduce pain and inflammation
Antibiotics	Amoxicillin, Cephalexin	Amoxicillin –An, Amoxicillin generic health, Amoxicillin Ranbaxy, amoxicillin Sandoz, and Amoxicillin- ga. Apo-cephalexin, Cephalex 250, Cephalex 500, Cephalexin An, Cephalexin Generic health	Treating bacterial infections

Source: Adapted from (Pharmaceutical Benefit Scheme, 2014) .

In addition, health surveys undertaken by the Australian Bureau of Statistics (ABS) have reported that 68% of adults in Australia have been experiencing low

psychological distress and 11.7% population aged 18+ experiencing very high level of psychological distress (ABS, 2015c). This increase in mental health issues suggests that there would be increased consumption of psychiatric drugs to treat these conditions. The survey also reports an increase in musculoskeletal conditions from 11.3% to 18.3% in the past year indicating the rising consumption of anti-inflammatory and analgesics. An increase was also seen in cardiovascular conditions, hypertension and cholesterol, inferring in higher consumption of drugs such as β-blockers and lipid regulators (ABS, 2011-12a, 2015c). Therefore, it can be concluded that mental health, cardiovascular and, musculoskeletal health conditions are increasingly prevalent in the Australian society and that the drugs like β-blockers, lipid regulators, psychiatrics are consumed more frequently and regularly by Australians.

Changing lifestyle associated with urbanisation has been stated as the cause for the increase in the above diseases by the AIHW (2014). According to the AIHW (2014) report, the lifestyle of Australians has become sedentary with very low to no physical activity at all and a significant fraction of the population do not consume a healthy diet, thereby giving rise to chronic and mental health conditions. Only about 43% of Australians undertake moderate physical activity and only 48.5% of Australian adults consume a proper diet. The report (AIHW, 2014) also strengthens the findings of the report by ABS (2015c) that the present population of Australia face emotional, mental and physical stress on an everyday basis, which in turn is directly linked to a range of health conditions. These conditions are considered can be directly extended to the population in South East Queensland.

The notable increase in chronic and mental health illnesses in the past few years (ABS, 2012, 2015c), can be directly attributed as the reason behind the increasing occurrence of EPPP compounds, because the occurrence of any EPPP compound in sediments of aquatic environments is a result of long-term and regular consumption of such drugs (Daughton and Ternes, 1999). It is clear from the above statistics and reported studies (CHO Report, 2014), that mental, cardiovascular and musculoskeletal are the major health conditions that are prevailing in South East Queensland (SEQ) and are likely to be contributing to the pollution by EPPPs of the Brisbane River. Additionally, increase in the prescription of antibiotics such as

amoxicillin (ABS, 2010), would also contribute to the occurrence of such compounds in sediments given that antibiotics are consumed frequently by broader population groups. Therefore, population growth and changing lifestyle associated with urbanisation appears to be the likely cause for the increase in chronic health conditions and furthermore for the increased consumption of EPPPs making these compounds ubiquitous in the environment. Therefore, it can be hypothesised that urbanisation in SEQ is possibly the reason for the occurrence of EPPPs in the Brisbane River sediments.

Whilst urbanisation in SEQ is responsible for the occurrence of EPPPs in the aquatic environment, there are other factors contributing to EPPPs occurrence. As stated earlier, changing lifestyle is one of the causes. Together with this, it is understood that the ageing population in Queensland is increasing and is more vulnerable to such chronic health conditions (CHO Report, 2014). The CHO Report (2014) has noted that the number of older population aged above 65 is rising markedly. According to the report by the AIHW (2014), the population of Australia is growing and the growth has been stronger among the older age groups 45-64years and 65+ years than the younger 15-24 years group. The population in the age bracket 65 and above has tripled from 1.1 million to 3.3 million between 1973 and 2013, whereas the growth in population in the age group 85 and above has increased from 73,100 to 439,600. This clearly demonstrates that Australia's population has been ageing over the past half a century. Furthermore, these older Australians are most likely to be affected by chronic diseases such as cardiovascular, elevated blood pressure, musculoskeletal problems and cholesterol issues (AIHW, 2014). It can be concluded that the ageing population of Australia consumes more therapeutic drugs to treat the above said diseases. In the case of SEQ too, the occurrence of EPPPs in the Brisbane River could likely be influenced by both urbanisation and ageing population.

Having discussed the likely reasons for EPPPs occurrence in the Brisbane River, it is also important to understand how these drugs enter the waterways in the first place. According to Daughton (2004), every manufactured drug has the potential to enter the environment from the very first day it is introduced into commerce. Pharmaceutical drugs go through various metabolic reactions after ingestion by humans or animals where they either remain unchanged or are broken down to active

metabolites. Such metabolised drugs are then excreted from the body through urine and faeces into sewage. Some of these drug compounds escape the sewage treatment processes and remain in the effluent and are discharged into the waterways (Daughton and Ternes, 1999; Khetan and Collins, 2007) resulting in the presence of these drugs in the aquatic environment. Prolonged consumption of such drugs causes regular ingestion and excretion from the human body into wastewater effluent, which results in the persistence of EPPPs in the aquatic environment. Therefore, the occurrence of EPPPs in aquatic environments can be attributed to two major reasons: (1) increased consumption of EPPPs; and (2) lack of effective wastewater treatment methods to remove such pollutants.

While increased consumption is the major factor contributing to the occurrence of EPPPs, the chemical and physical properties such as octanol/water partition coefficient (log K_{ow}) and acid dissociation constant (pKa) also play an important role in the persistence of such drugs in the environment. Log K_{ow} can be defined as the ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase of a two phase octanol/water system. Log K_{ow} value of a drug is helpful in correlating its solubility in water, affinity to adsorb to solid matrices and bioaccumulation in aquatic flora and fauna. Therefore, higher the log K_{ow} value, more the adsorption to sediments and the relatively high possibility of bioaccumulation. The pKa value, on the other hand, affects absorption of the drug and determines the acidity of a compound, meaning lower the pKa value, the higher is the absorption and strong acid. This means, the release of EPPPs with higher log K_{ow} value and lower pKa are potentially harmful to the environment since such compounds are most likely to be adsorbed to flora and fauna and environmental matrices.

A clear correlation between the occurrence of EPPPs in sediments and their log K_{ow} could be drawn from the study undertaken by Vazquez-Roig et al. (2010). It was found that the log K_{ow} and pKa values of carbamazepine and lorazepam readily correlated with the occurrence of these compounds in the sediments in the Valencia region in Spain. It is because of such correlation that these values are now used to assess EPPPs in the environment and is required by organisations such as United States Food and Drug Administration (USFDA) in assessing EPPPs (Jjemba, 2006).

While these values are critical in assessment studies and in determining the fate of EPPPs, the direct correlation between the two is not well explained yet and highlights a knowledge gap that needs to be studied.

In order to understand the potential threat posed by EPPPs to the environment, it is necessary to understand how these substances are metabolised. A drug after its absorption in the body is transformed or breaks down into different other intermediate metabolites and this is called 'biotransformation'. Biotransformation of a pharmaceutical compound occurs through two phases: Phase I and Phase II. In Phase, I the transformation occurs through the addition of a functional group such as -OH, -SH,$(>C)_2O$, $-NH_2$, and –COOH, whereas in Phase II conjugations occur to form O-, N- glucuronides, sulphates, acetate esters, carboximides and glutathyionyl products. For example, the clofibric acid in phase II of biotransformation conjugates to form clofibric-O-b-acylglucoronide. Thus, whilst these converted metabolites might not be harmful to the human body, its excretion into the environment might pose risks to the other living organisms because of their biologically active nature (Ionescu and Caira, 2005; Khetan and Collins, 2007).

About 70% of the biotransformed pharmaceutical compounds get excreted through urine and 30% are excreted in the faeces (Khetan and Collins, 2007). For example, 65% of diclofenac is excreted through urine and in the case of ibuprofen, the drug becomes polar on excretion as a result of Phase I and II reactions (Khetan and Collins, 2007). Atenolol and propranolol are excreted in parent form from the human body where atenolol, in particular, can be absorbed fully and pass through the placenta (Heel et al., 1979; Khetan and Collins, 2007; Nałęcz-Jawecki et al., 2008). On the other hand, biotransformation of diazepam results in slow excretion from the body and results in accumulation of the drug in the body (Greenblatt et al., 1983), whereas antibiotics such as amoxicillin and cephalexin can potentially accumulate because of their long half-lives (Khetan and Collins, 2007) suggesting possible bioaccumulation of such compounds in the bodies of the target organisms. Thus, the occurrence of such EPPPs in the Brisbane River sediments is concerning given that these drugs are being consumed widely here in SEQ.

In this discussion urbanisation and ageing population in SEQ have emerged out to be the likely reasons for the increased EPPPs consumption and in turn for their

occurrence in the Brisbane River. It was further understood that the occurrence of EPPP compounds in waterways is resultant of inadequate wastewater treatment methods which leads to the release of such compounds via wastewater effluent. In addition, the critical review of the literature has revealed that physical and chemical properties, increased consumption and metabolic processes all together play a vital role in defining EPPPs as environmental pollutants and their fate in the environment. These findings identify the need to investigate as to how widespread the issue of EPPP pollution is around the world. The next section discusses the occurrence and distribution of EPPPs across different aquatic environments around the world.

2.3 Occurrence and distribution of EPPPs

As pointed out earlier in Section 2.2, EPPPs are being consumed widely around the world. Such continuous and widespread consumption of these formulated products by people has resulted in the common presence of these substances in the environment (Daughton and Ruhoy, 2008; Daughton, 2009). This section discusses the outcomes of the review of literature on the occurrence and distribution of EPPPs. Firstly, derived knowledge on the extent and spread of EPPPs contamination around the world and in various compartments of the aquatic environment, for example, water, sediments, wastewaters and soil. Secondly, to understand the extent of EPPP pollution in the Brisbane River and provide background knowledge for suitable mitigation action to be undertaken.

The interest in research into EPPPs is increasing in the scientific world considering the bioactive nature of these organic pollutants (Daughton and Ternes, 1999). Several recent studies have investigated EPPPs in wastewaters, surface waters such as rivers, lakes, and drinking water (Balmer et al., 2003; Bu et al., 2013; Carmona et al., 2014; Evgenidou et al., 2015) and soil (Andreu Pérez et al., 2011; Jelić et al., 2009), but few studies have been undertaken in sediments (Beretta et al., 2014; Camacho-Muñoz et al., 2013; Silva et al., 2011). This has resulted in the common conclusion that EPPPs are becoming ubiquitous in all compartments of the environment, highlighting the knowledge gap in relation to the study of sediment for EPPPs in Australia. As noted in Section 2.2, the widespread occurrence of such compounds in different compartments of the environment are related to urbanisation and the increased consumption by the population and this finding corresponds to the claims

made by Ternes et al. (2004), Ellis (2006) and Stewart et al. (2014) that the increase in urbanisation and concerns about personal health and grooming has led to increased consumption of EPPPs.

Pollution of aquatic environments by EPPPs is serious given the negative impacts these micropollutants impose on smaller living organisms (Henschel et al., 1997; Santos et al., 2010). Studying the occurrence of EPPPs in various aquatic systems around the world would facilitate in deriving a comprehensive understanding of the extent of EPPP pollution around the world. The discussion below evaluates studies that have been undertaken around the world to investigate EPPPs in water environments.

China is the most populous country in the world and is experiencing tremendous economic growth. However, it is also facing a serious issue of environmental pollution as a result of its large population and growing economic development. Studies conducted in the recent past in China, have confirmed EPPP contamination of the aquatic environments across the eastern regions which are more developed and urbanised (Bu et al., 2013).

For example, studies undertaken by Chen et al. (2012), Dai et al. (2014), Zhu et al. (2013)) have reported frequent detections of trimethoprim, erythromycin A, norfloxacin, ofloxacin, atenolol and diclofenac in the Hangzhou metropolitan and Linan regions of Southeast China along with gemfibrozil, mefenamic acid and trimethoprim in wastewater effluents, hospital effluents and surface waters in Beijing and caffeine (23.8 - 344.7 ng/L) in Qingshan Lake in the east. It was interesting to note that the study areas selected were from urbanised areas. Although Chen et al. (2012) selected areas with varying urbanisation levels, their definition of urbanisation is questionable. Chen et al. (2012) claim that hospital effluents were concentrated sources of EPPPs in the Hangzhou metropolitan and Linan regions of Southeast China, but failed to show any correlation between urbanisation and occurrence of EPPPs.

In another study, Chen and Zhou (2014) reported on the occurrence of antibiotics in sediments in the aquatic environments of Huangpu River, Shanghai, while Chen et al. (2013) demonstrated the occurrence of EPPPs in the sediments of Ou, Min and

Jiulong rivers in Southeast China. It is understood from the above studies from China that EPPPs are common in the various compartments of the aquatic environment including surface waters and sediment beds of the rivers which are important sources of water. A similar scenario is present in Europe and the US where the majority of the research into EPPPs has been carried out.

In the case of European countries, the presence of ~3000 bioactive pharmaceutical compounds in everyday consumed medications explains the ubiquitous presence of EPPPs in different compartments of the aquatic ecosystems. Research into EPPPs in Europe started about two decades ago with the discovery of lipid regulators, analgesics, antibiotics, antiseptics and β- blockers in sewage (Heberer and Stan, 1997). Studies from different parts of Europe (Antonić and Heath, 2007; Castiglioni et al., 2006; Clara et al., 2004; Kosma et al., 2014; Ternes et al., 2002; Zorita et al., 2009) have investigated the occurrence, fate and removal of these compounds in the environment. Many other studies have been reported on method development, analysis and removal from surface waters, sediments, soil, wastewaters and risk assessments of EPPPs in these aquatic environments (Andreu Pérez et al., 2011; Camacho-Muñoz et al., 2013; Carballa et al., 2008; Ginebreda et al., 2010; Moreno-González et al., 2015; Silva et al., 2011; Suárez et al., 2008; Vazquez-Roig et al., 2010) explaining the widespread occurrence of EPPPs. The occurrence of EPPPs from a range of therapeutic classes (anti-inflammatory, psychiatric, lipid regulators, antibiotics, estrogens, nervous stimulants, and β- blockers) were found in surface waters and sediments of Doñana National Park (Camacho-Muñoz et al., 2013) and Ebro River (Silva et al., 2011), demonstrating a strong urban influence.

Research investigations of these pollutants in the US (Kümmerer, 2008) have resulted in many review studies discussing the occurrence, fate, risks and removal of EPPPs (Boxall et al., 2012; Christensen, 1998; Daughton, 2011; Daughton and Ruhoy, 2008; Daughton, 2004, 2009; Daughton and Ternes, 1999). There are studies reporting on the application of the developed methods (Darwano et al., 2014; Ferrer and Furlong, 2002; Schultz and Furlong, 2008; Vanderford et al., 2003) for quantifying EPPPs in the US surface waters, wastewaters, and sediments (Kolpin et al., 2004), which confirms the widespread EPPP pollution in the US.

Apart from Europe and the US, studies have also been reported from other parts of the world e.g. Japan (Azuma et al., 2015) in river water, Taiwan (Jiang et al., 2014) in coastal waters, Brazil (Beretta et al., 2014) in sediments, Hong Kong (Gulkowska et al., 2008) in sewage treatment plants (STPs) and Canada (Khan and Nicell, 2015) in drinking waters. The above studies reveal that EPPPs pollution is widespread in various compartments of the aquatic environments around the world which is a concern. As noted previously, the occurrence of EPPPs in aquatic environments pose negative impacts on the flora and fauna (Henschel et al., 1997; Zhao et al., 2015) and their occurrence in drinking waters is concerning for human health (Carmona et al., 2014; Khan and Nicell, 2015). Therefore, it is important to evaluate studies into fresh and marine waters for a better understanding of the extent of EPPPs pollution across different aquatic environments. EPPPs from STPs often discharged into marine waters via rivers, streams form direct discharge points (Beretta et al., 2014; Gaw et al., 2014), thereby posing potential threats to the marine ecosystem. Marine environments are equally important and need to be studied for EPPPs contamination due to the risk of ingestion of EPPPs through seafood (Gaw et al., 2014).

However, studies investigating EPPPs in the marine environment are limited (Arpin-Pont et al., 2014; Fabbri and Franzellitti, 2015; Gaw et al., 2014) compared to those conducted in other aquatic environments such as freshwater. The possible reason for the limited research into EPPPs in the marine environment is attributed to the low concentrations of EPPPs due to high dilution rate, which makes the analysis a difficult process (Arpin-Pont et al., 2014).

The review undertaken by Bialk-Bielinska et al. (2016) discussed the challenges in developing analytical methods for quantification of EPPPs in marine environments, stating that the analysis of EPPPs in marine sediments and water is a complex process and requires further enhancements. Evaluation of the above review of the literature provided a detailed overview of research investigations carried out in marine environments and confirms the widespread pollution by EPPPs of the marine environment. Research into EPPPs in the marine environment is important particularly in the estuarine areas, coastal and bay areas where most of the world's population resides.

The Brisbane River forms an estuary opening into the South Pacific Ocean and is surrounded by urbanisation and rapidly growing population (BITRE, 2013; Yu et al., 2014). Therefore, it is likely to be polluted by EPPPs. In recent studies undertaken by Borecka et al. (2015), Hedgespeth et al. (2012), Klosterhaus et al. (2013), Spongberg et al. (2011)) and Long et al. (2013), 89 EPPPs were reported highlighting the pollution of the marine environment and the growing interest in this area.

Within Australia, two studies have recently been published that reported on the occurrence of EPPPs in urban influenced estuarine and coastal environments (Birch et al., 2015; French et al., 2015) confirming the strong anthropogenic influences resulting from urbanisation and population growth (Bialk-Bielinska et al., 2016; Gaw et al., 2014). Along with investigative studies into the marine environment which mainly focused on the occurrence and distribution, studies have also confirmed the bioavailability and bioconcentration of EPPPs in marine organisms (Gaw et al., 2014; Gomez et al., 2011; Klosterhaus et al., 2013; McEneff et al., 2014), thereby identifying the bioaccumulative nature of these environmentally concerning compounds. Therefore, it can be concluded from the above analysis of the literature that EPPPs are common in marine aquatic environments and bioaccumulate in aquatic organisms raising environmental concerns. While these findings may not pose an immediate threat to ecosystems and humans, it is likely to cause chronic impacts.

In regards to freshwater environments, studies investigating EPPPs in freshwaters are numerous and this is rightly because rivers provide freshwater for the living beings on earth. Researchers have focused their attention on the occurrence and distribution to understand the fate of these pollutants in freshwater since there are still knowledge gaps about the fate of these pollutants which needs to be addressed. Several studies have been conducted in freshwaters in the past few years studying the occurrence, fate and distribution of EPPPs in drinking waters, lakes, rivers and sediments of the freshwater resources. Pal et al. (2010) in their review have reported 16 pharmaceuticals that are frequently reported in studies carried out during the period 2006-09, thereby concluding the widespread consumption of these drugs across various countries and their presence in the environment. Studies (Proia et al., 2013; Zhao et al., 2015) have reported on the bioaccumulation of analgesics and anti-

inflammatory drugs on autotrophs in Mediterranean rivers and antibiotics in fish tissues, thereby raising environmental concerns and potential toxic impacts on the freshwater ecosystems. The above research literature provides evidence of the widespread occurrence of EPPPs in various compartments of the environment thereby highlighting the need for more research in this area. Research into EPPPs in Australia has been limited. Studies undertaken by Le Corre et al. (2012), Watkinson et al. (2009) have reported on the occurrence of antibiotics in municipal and hospital wastewaters. Recently, studies from Australia (Birch et al., 2015; French et al., 2015; Scott et al., 2014; Watkinson et al., 2009) have reported on the occurrence of EPPPs in Australian estuarine environments and in rivers. EPPPs have also been discovered in marsh frog tadpoles (Melvin et al., 2014), thereby confirming widespread occurrence of EPPPs in Australian surface waters and highlighting bioaccumulation of these pollutants in native organisms.

It can be concluded from the literature review that research into EPPPs in Australia is not as extensive as it is in other parts of the world and it is has just gained momentum in recent years. Another significant finding is that there has been no reported study into the investigation of EPPPs in river sediments which confirms the need to study the presence of EPPPs in the Brisbane River sediments. Such a study, therefore, is critical to obtain a comprehensive knowledge about EPPP pollution in the aquatic environment.

This section has discussed the various regions in the world where EPPPs have been occurring and in the different compartments of the aquatic environment. The most important finding in this section was the lack of research studies into EPPPs in Australia. Whilst there are a few studies reported regarding EPPPs in surface waters, there is no research into EPPPs in the sediment environment in Australia. It is important to investigate the occurrence of EPPPs in sediments in the Brisbane River so as to derive a comprehensive understanding of EPPPs pollution.

2.4 Bioaccumulation and toxicity of EPPPs

As explained earlier, EPPPs, unlike other pollutants, are foreign to the environment. Hence, prolonged exposures to such pollutants are likely to have adverse impacts on the environment. Since EPPPs have been lately discovered and identified as 'emerging' pollutants, there is limited information regarding their potentially harmful effects. The possible reasons for limited research into monitoring and assessment of EPPPs for adverse impacts:

a) Lack of sophisticated and advanced analytical instruments and lack of robust methods for quantification of such pollutants since these occur in very small concentrations (ppb and ppt levels)

b) Lack of substantial evidence of adverse impacts

c) Lack of regulations and legislations relating to the management of such pollutants especially in Australia and

d) The fact that the research is emerging and ongoing with more focus on understanding the fate of EPPPs in various chambers of the environment.

For any given environment, smaller organisms are generally the most vulnerable to the anthropogenic influences. Such organisms have simple cell structures. Nevertheless, they are very important to the ecosystem and for biodiversity. They act as reliable bio- indicators of environmental health for two predominant reasons,

a) These small live forms are most sensitive and susceptible to minute changes in the environment because of their simple cell structures; and

b) The smaller live forms are in abundance in the environment, thereby ensuring easy availability. In addition, frequent sampling with good sample size is possible without threatening the population. For example, microorganisms, aquatic fauna such as fish, biological samples such as cell lines are present abundantly in nature and can be used for assessing environmental health .

Studies conducted around the world have reported on bioaccumulation of certain EPPPs in aquatic organisms (Gaw et al., 2014). Studies undertaken by Gomez et al.

(2011), Klosterhaus et al. (2013), McEneff et al. (2014), Wille et al. (2011) have reported bioaccumulation of pharmaceuticals in marine mussels. Bioaccumulation of EPPPs has also been found in other aquatic organisms such as in fish liver, autotrophs in surface waters (biofilms), wild fish and marsh frog tadpoles and biological tissues (Kwon et al., 2008; Melvin et al., 2014; Proia et al., 2013; Tanoue et al., 2014; Vernouillet et al., 2010; Zhao et al., 2015). Amongst the ecotoxicological studies conducted, carbamazepine appears to be persistent in the environment and highly bioaccumulative in aquatic organisms making it one of the most concerning EPPPs and qualifying as an 'anthropogenic marker'(Clara et al., 2004). Carbamazepine was found to be easily bioaccumulated in algae when exposed to 150mgL^{-1} of concentration (Vernouillet et al., 2010). The bioaccumulation of EPPPs in a range of aquatic organisms demonstrates the persistence of these pollutants in the environment which is primarily due to continuous discharge from wastewater effluents to water environments.

However, apart from the wastewaters, desorption of these pollutants from the sediment environment back into the surface environment also plays an important role in the persistence of these pollutants. The process of desorption of EPPPs from sediments is enhanced by the benthic invertebrates that affect the equilibrium of the desorption process which results in greater bioavailability of these pollutants to the aquatic organisms (Gilroy et al. (2012). A Similar finding was concluded by Goedkoop et al. (2003) in their study where they stated that the burrowing and feeding activity of aquatic organisms affects the sediment environment thereby affecting the sorption/desorption process of EPPPs. Martínez-Hernández et al. (2014) on the other hand concluded that the ionisation of EPPPs affects the sorption/desorption process thereby noting that many factors affect the sorption/desorption processes in the sediments which need to be addressed. The bioavailability and bioaccumulation of EPPPs in the sediment environment further contribute towards the toxicity of the sediment in which they occur (Tamura et al., 2013).

The studies discussed above further confirm the pollution of the marine environment by EPPPs and their accumulation in aquatic life forms. Such studies are important and necessary for regulating the occurrence of EPPPs and would prove beneficial in

providing relevant knowledge for the development of appropriate removal techniques for EPPPs. Additionally, studies on bioaccumulation and bioavailability of EPPPs provide in-depth knowledge about the behaviour of a particular compound which is helpful in assessing the risk and toxicity of the pollutant.

Risk assessment provides vital information about the toxicity of a specific EPPP to the environment, thereby facilitating in implementing appropriate mitigation measures and regulations (Liebig et al., 2014). Values such as EC_{50}- the half maximal effective concentration at which a drug can induce a response after a specified exposure time, RQ – risk quotients and predicted environmental concentration (PEC) are determined to understand the potential risks and harmful effects of an environmental pollutant to its surrounding environment and the living organisms. Henschel et al. (1997) followed the risk assessment procedure according to the EU guidelines (CEC III/ 55004/ 94 draft 4) and calculated the EC_{50} values for paracetamol, clofibric acid and methotrexate towards daphnia, algae and biological samples like fish cell lines and embryos and concluded that paracetamol and clofibric acid are toxic to the environment.

Camacho-Muñoz et al. (2013), Ellis (2006) and Hernando et al. (2006) in their studies assessed the risks of target EPPPs by determining the risk quotients (RQ). The assessed target EPPPs were reported to pose potential risks to the organisms in the aquatic ecosystems where these drugs were detected. Ferrari et al. (2003), on the other hand, determined the environmental risk based on the ratios of predicted environmental concentration (PEC)/predicted no-effect environmental concentration (PNEC), measured environmental concentration (MEC)/PNEC and risk quotients for carbamazepine, clofibric acid and diclofenac. The above risk assessment techniques were applied in assessing risks of pharmaceuticals from domestic wastewater (Backhaus et al., 2014; Kosma et al., 2014), freshwater (Ginebreda et al., 2010; Wu et al., 2014; Zhu et al., 2013) and hospital wastewater (Escher et al., 2011; Hernando et al., 2006). Among the risk assessment studies reviewed, the majority of the research appear to be undertaken in Europe. Unfortunately, unlike in Europe, there are no guidelines in Australia in relation to the occurrence of EPPPs. This could also be another reason for current limited number of studies on EPPPs in Australia.

2.5 Removal of EPPPs

This section discusses the possible alternative wastewater treatment techniques that could be adopted to reduce the load of EPPPs from wastewaters. There is a need to improve the existing wastewater treatment techniques or potentially introduce novel treatment techniques to eliminate EPPPs and prevent their entry into the receiving water environment. Studies (Castiglioni et al., 2006; Lin et al., 2009; Zorita et al., 2009) have compared the concentrations of selected EPPPs from influents, effluents in between the treatment stages to assess the removal rate of EPPPs during the treatment process in wastewater treatment plants (WWTPs). Castiglioni et al. (2006) and Lin et al. (2009) concluded that the treatments were not effective in removing antibiotics and they also found the removal rates for selected EPPPs varied across the different STPs. However, longer retention times during the treatment process were found to be effective in better removal of EPPPs (Lin et al., 2009). Zorita et al. (2009) noted that the tertiary treatment process in sewage treatment plants (STP) in Sweden could eliminate two of the three selected EPPP compounds except for diclofenac. Thus, the current wastewater treatment techniques are not able to fully eliminate all of the EPPPs present in wastewater.

Understanding the inadequacy of the wastewater treatment processes in eliminating EPPPs, research studies have also been undertaken to remove EPPPs from water (Akhtar et al., 2016; Nebout et al., 2016; Omidvar et al., 2015). Akhtar et al. (2016), reviewed the literature relating to different adsorbents that are used to remove EPPPs and the mechanisms of adsorption. The study concluded that while different adsorbents have different removal rates, an effective adsorption of EPPPs actually depends largely on the active functional groups present on these compounds. Nebout et al. (2016), investigated the use of activated carbon, ozonation and the coupling of both methods. They concluded that coupling of ozone/activated carbon resulted in relatively rapid removal of EPPPs. Although the number of EPPPs removed by these techniques was relatively quite low compared to the large numbers of EPPPs present in the environment, studies are ongoing in investigating the efficacy of various techniques.

Research studies have also demonstrated that the sludge retention technique (SRT) which is an important step in wastewater treatment is ineffective for the removal of

pharmaceutical compounds such as carbamazepine and diclofenac, which are highly resistant to degradation (Clara et al., 2004; Zhang et al., 2008). This section discusses different removal techniques such as activated carbon, membrane filtration, sludge treatment, adsorption and oxidation proposed in studies to remove EPPPs (Bu et al., 2013; Li, 2014; Suárez et al., 2008; Westerhoff et al., 2005).

Removal techniques such as granulated activated carbon (GAC), advanced oxidation process (AOP) and activated sludge treatment analysed in studies (Ternes et al., 2004; Ternes et al., 2002; Ternes et al., 2003) were found to be effective in removal of EPPPs. For example, the activated sludge treatment technique has been found to be effective in the removal of diclofenac, 17 α – ethinylestradiol and roxithromycin. The above studies agree with Suárez et al. (2008) about implementing a treatment technique following the conventional wastewater treatment process and go on to propose the above- mentioned removal techniques- (GAC), (AOP) and activated sludge treatment to be effective post-treatment techniques for removal of EPPPs. In the same vein, Tijani et al. (2013) in their review found AOP technique to be more environmentally friendly with no additional chemicals being used and less complex. However, additional research into removal of EPPPs is needed. Ternes et al. (2004) in their study proposed that hospital waste should be treated separately from other wastewaters such as domestic wastewater.

Ozonation treatment proposed by Rosal et al. (2010) was another removal technique stated to be efficient in the removal of compounds such as carbamazepine, diclofenac, atenolol and propranolol indicating another addition to the list of post-treatment measures. While the proposed post- treatment techniques such as ozonation, GAC and AOP have proved effective, the likelihood of implementation of these techniques in the real world is challenging given that these techniques are expensive.

The implementation of post-treatment techniques is difficult considering the cost associated. While this is true, the introduction of better house-keeping practices in hospitals and medical centres, and the controlled administration of prescribed and non-prescribed drugs that are potentially toxic to the environment and generating awareness amongst people about EPPP pollution would help to minimise the risk of EPPP pollution of waterways (Fisher et al., 2013).

On the other hand, it also understood that while some treatment techniques can effectively remove certain harmful pollutants, but cannot guarantee the removal of other potentially environmentally threatening micropollutants into the future. This is because:

a) Research and development are ongoing in the pharmaceutical industry. Therefore, there will be additional new drugs with new discoveries all the time.

b) The removal techniques are a proactive approach to control and potentially stop EPPPs from entering the environment, which is primarily through wastewater effluents. While this is true and can be effectively applied to eliminate such micropollutants, its application is restricted to only wastewaters. Meaning the pharmaceutical compounds that are present in the soil, groundwater and sediments would not be removed. This is a serious concern given the fact that these micropollutants are persistent in the environment and bioaccumulate. For example, the bioaccumulation of antibiotics can result in antibiotic resistant species.

c) Processes such as activated sludge treatment have been successful in eliminating pharmaceutical compounds such as diclofenac. However, application of such sludge to the land, possibly agricultural land, would release these micropollutants back into the environment (Boxall et al., 2012).

d) Processes such as ozonation, activated oxidation process (AOP) causes oxidation of pharmaceutical compounds that can change them into intermediate compounds. These intermediate compounds might not be harmful to human beings, but could be harmful to other living organisms (Suárez et al., 2008).

2.6 Urbanisation and sources of EPPPs

Urbanisation leads to a significant demand for water thereby impacting on the quantity of the resource and affects the quality by causing pollution (Goonetilleke and Thomas, 2003; WWDR, 2015). As discussed in the previous sections,

urbanisation appears to be causing EPPPs pollution in water environments. Thus, it is important to understand how urbanisation causes this pollution and through what sources in the environment. As stated in Sections 2.2 and 2.3, urbanisation around the Brisbane River is increasing markedly and the river has been reported to be polluted by EPPPs (QueenslandTreasury, 2016; Scott et al., 2014). Therefore, it was necessary to investigate the sources of EPPPs in the Brisbane River to understand and study the relationship between the two.

The process of urbanisation results in outcomes such as concentration of population, changing lifestyles and wastewater generation which contribute towards EPPP pollution. Urbanisation causes continuous migration of population into cities mainly for better infrastructure, health, education and employment (Liu et al., 2015). In a recent report by the UN (2014), 54% of the world's population is already residing in urban areas. Such an increase in urban population has given rise to distinct urban lifestyle, which has resulted in increased consciousness about personal health and wellbeing and in the consumption of EPPPs substances (Ellis, 2006; Yuanjia et al., 2007), together with increased generation of wastewater (Sato et al., 2013). Studies (Ellis, 2006; Jiang et al., 2014; Stewart et al., 2014) have reported about the presence of EPPPs in urban-influenced aquatic environments, thereby confirming the role of urbanisation on EPPPs pollution. Therefore, this study which aimed to investigate the relationship between urbanisation and EPPP contamination of the Brisbane River is expected to provide the baseline information that would contribute to the implementation of mitigation measures.

Since, EPPPs present in wastewaters (Heberer, 2002; Zorita et al., 2009) are discharged into the surrounding waterways after conventional treatment (Daughton and Ternes, 1999), it was important to understand the link between water consumption and wastewater generation. Figure 2.2 and 2.3 below illustrate the increase in water consumption and waste generation in Australia.

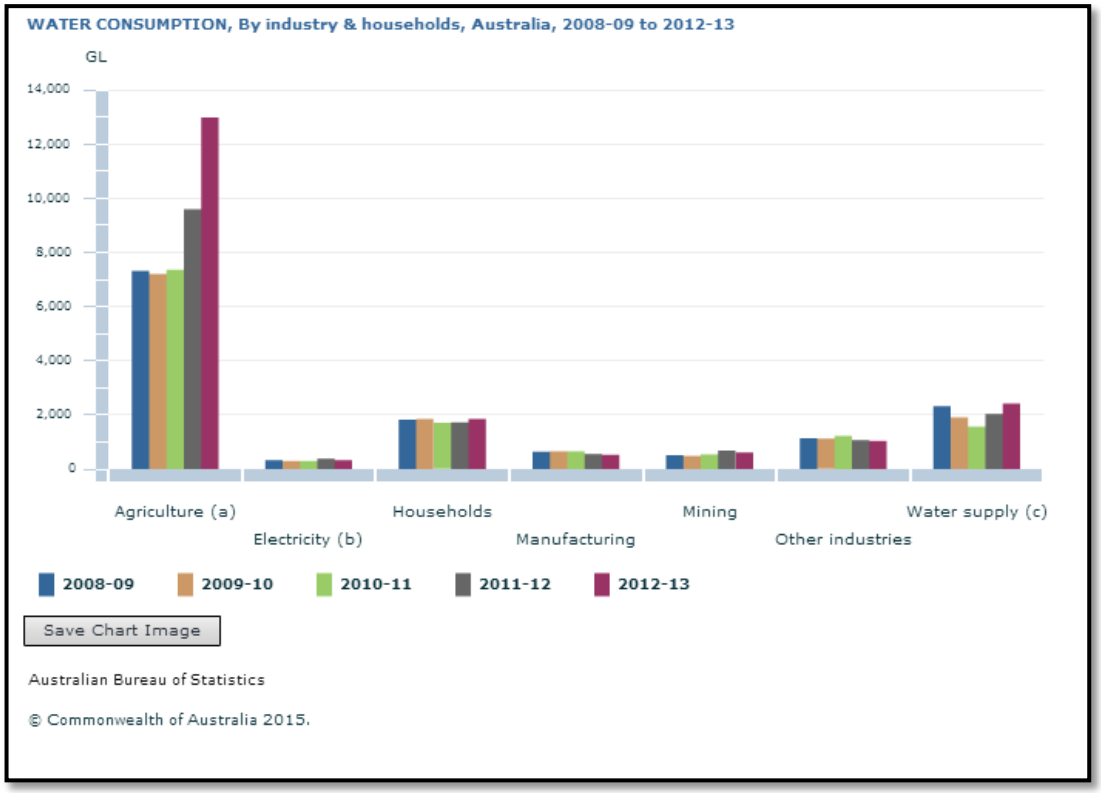


Figure 2.2 water consumption in Australia by industry and households during 2008-09 to 2012-13

Source: (ABS, 2015b)

Water consumption in Australia increased by 23% in 2012-13 compared to the year 2011-12 (Figure 2.2). While the agriculture sector was the largest consumer of water, the water consumption by the household sector in Queensland increased by 6% in past five years from 2008-09 to 2012-13. Queensland was the third among the states in Australia followed by Australian Capital Territory (ACT) and Victoria to show an increase in water consumption, indicating a high demand for water due to population growth (ABS, 2015b).

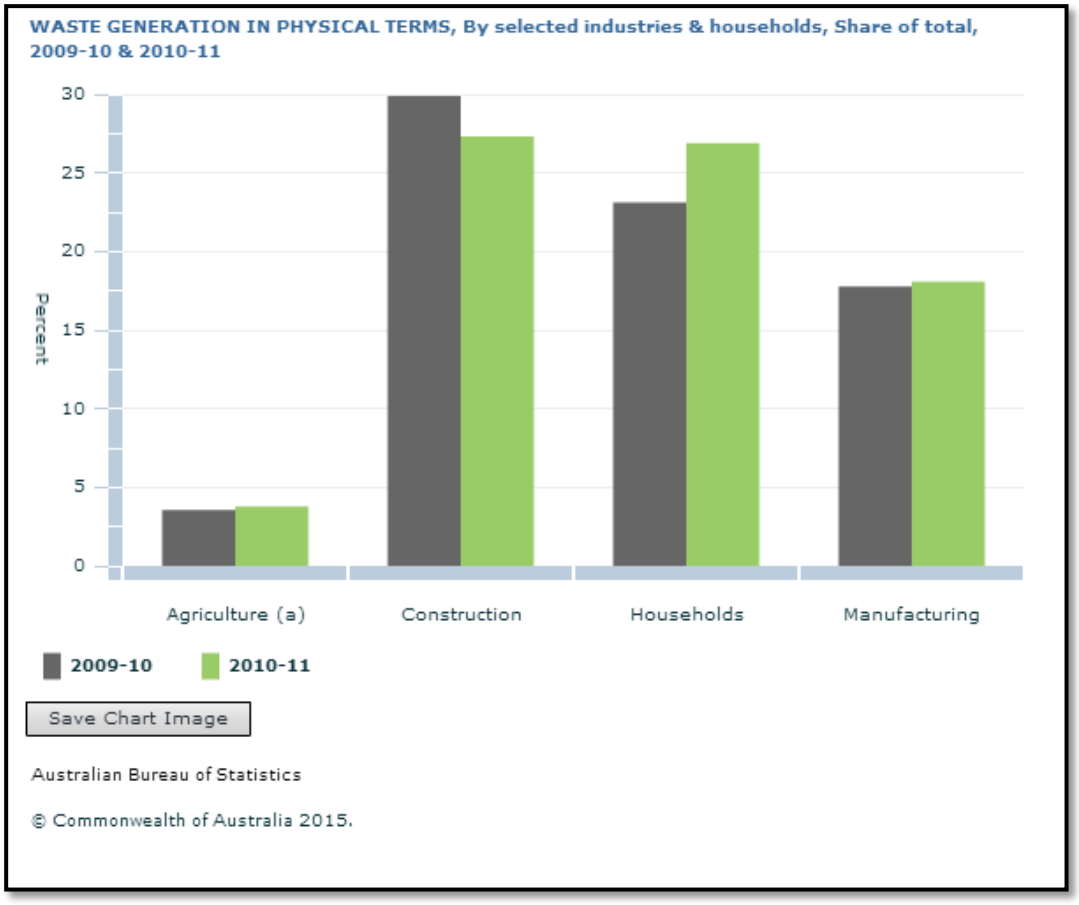


Figure 2.3 waste generation in Australia across major sectors during 2009-10 to 2010-11

Source: (ABS, 2015b)

It can be understood from Figure 2.3 that waste generation has increased in the past few years in all major sectors including households. The increase in household waste generation directly correlates to population growth, which implies an increase in wastewater generation too. Having understood the relationship between urbanisation – population growth – wastewater generation and EPPPs, it was now essential to understand the different sources of EPPPs in the environment. Studies (Fisher et al., 2013; Li, 2014) have separated the sources of EPPPs into two categories: (1) Point sources, and (2) Diffuse sources.

Wastewaters originating from hospitals and municipal treatment plants are the main source for EPPPs pollution (Castiglioni et al., 2006; Wu et al., 2015). These sources generate and release large volumes of wastewaters (Li, 2014) which are likely to be discharging high amounts of EPPPs into the aquatic environment. Wastewater

effluents released from municipal wastewater plants contain excreted EPPPs substances (Antonić and Heath, 2007; Camacho-Muñoz et al., 2013; Daughton and Ternes, 1999; Fisher et al., 2013; Jelić et al., 2009; Kolpin et al., 2002; Li, 2014), and when such effluents are released into surrounding water bodies, they release these micropollutants into that environment.

Wastewaters are sent for treatment to the WWTPs before being discharged into waterways. However, the majority of the EPPPs escape this treatment process as current treatment processes are ineffective in eliminating these substances from wastewaters (Daughton and Ternes, 1999; Rosal et al., 2010). Thus, wastewater effluents generated from WWTPs act as point sources of EPPPs pollution (Boyd et al., 2003; Chen et al., 2012; Daughton and Ternes, 1999; Fisher et al., 2013; Li, 2014; Pal et al., 2010; Richardson and Ternes, 2014).

Hospital wastewaters are another important point source. These are more concentrated sources of EPPPs to the environment. Past research studies have determined the load of EPPPs from hospital wastewaters and their potential risks (Escher et al., 2011; Le Corre et al., 2012; Ort et al., 2010), thereby confirming hospital effluents as important point sources of environmentally concerning EPPPs.

Diffuse sources, on the other hand, constitute improper or accidental disposal of pharmaceuticals, agricultural runoff, sewer leaks, leachates from domestic waste landfills and septic tanks, veterinary drugs and their residues from aquaculture and animal carcases (Boxall et al., 2012; Fisher et al., 2013; Kümmerer, 2008). Sewer leaks, leachates from domestic waste landfills and septic tanks could also be releasing EPPPs into the groundwater and to soil (Li, 2014; Ternes et al., 2004), thereby polluting these environments. These diffuse sources contribute fewer amounts of EPPPs, but in concentrated forms. Sewer leaks, leachates from domestic waste landfills and septic tanks could possibly be considered as potential diffuse sources in developed countries such as Australia. Septic tanks and sewerage leakages were stated to be the potential sources for EPPPs occurrence in Ebro River basin in Spain and Sydney estuary in Australia (Birch et al., 2015; Silva et al., 2011), thereby confirming that EPPPs pollution of water environments occurs via diffuse sources. The evaluation of above research literature shows that the spread of urban areas is conducive to more EPPPs use and thus, contributing increasing amounts of EPPPs to

aquatic environments via point and diffuse sources. It can also be concluded that inadequate wastewater treatment processes are also responsible for EPPPs presence in the surrounding aquatic environments.

In regards to Australia, increasing and ageing population and their inclination towards the use of EPPPs suggest that the aquatic systems in Australia are likely to be polluted by these micropollutants. Such contamination is likely to continue to pollute the water bodies. Considering this situation and the fact that EPPPs have been reported in the Brisbane River (Scott et al., 2014; Watkinson et al., 2009) recently, it would be interesting to know whether they occur in the sediments as well.

2.7 Analytical methods for analysis of EPPPs

Interest in research about the fate, occurrence, behaviour and environmental impacts of EPPPs in aqueous environments has gained considerable attention in past 10-15 years (Daughton and Ternes, 1999). Mass spectrometry along with advancements in sample enrichment techniques and coupled with liquid and gas chromatography has enabled the identification and detection of these pollutants in trace amounts (Daughton, 2001; Daughton, 2004; Hernández et al., 2014). As a result, many methods have been developed over the years for determining EPPPs present in various environmental matrices (Borecka et al., 2015; Boyd et al., 2003; Jelić et al., 2009; Löffler and Ternes, 2003; Snow et al., 2013).

Although many methodologies have been developed and applied for the analysis of EPPPs, there are still many challenges to be addressed (Bialk-Bielinska et al., 2016) in order to fully understand the behaviour and fate of these substances in the environment. Limited studies into the analysis of EPPPs present in sediments (Bialk-Bielinska et al., 2016) explain that methods need to be improved and developed because of the complex nature of the matrix. This, in turn, has resulted in the determination of a limited number of pharmaceutical compounds present in the sediment environment. Therefore, method development for the determination of EPPPs in sediments is discussed in this section.

The analysis of EPPPs involves three important stages. These include sample preparation, extraction and clean up and finally, analysis using GCMS/MS or

LCMS/MS. Figure 2.4 below describes the analysis process for EPPPs. After extraction and clean-up, the samples can be analysed using either of the analytical instruments – GCMS/MS or LCMS/MS. However, in some cases of GCMS analysis, target analytes are required to be transformed into similar compound meaning derivatised in order to be quantified.

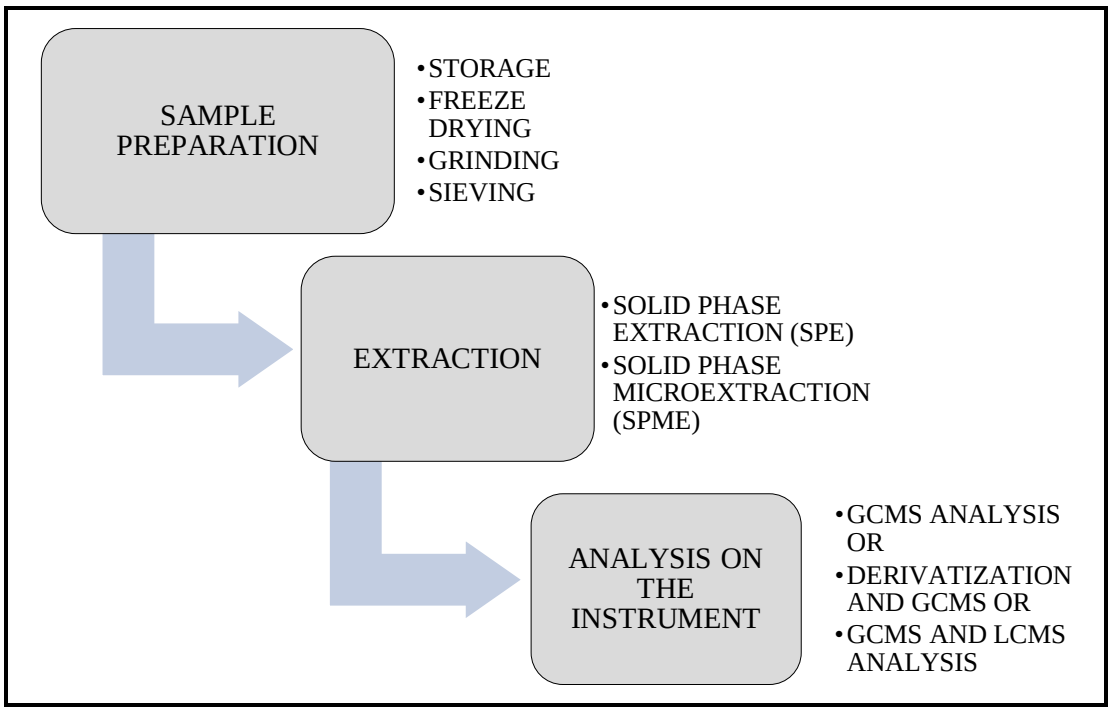


Figure 2.4 Diagrammatic representation of EPPPs analysis.

Source: Adapted from Pietrogrande and Basaglia (2007)

2.7.1 Sample preparation

As described in Figure 2.4 above, sample preparation is the first step in the analysis of EPPPs. It is the most elaborate stage in the analysis of EPPPs and it is the most tedious and time -consuming step. Sample preparation involves sample storage, freeze drying, grinding and sieving. Since these micropollutants are in ppb, ppt concentrations, any sample loss during sample preparation can result in large errors and significantly affects the analytical results (Babic and Pavlovic, 2013). Sample preparation for water samples does not involve the grinding and sieving steps and therefore, the analysis is faster and less complex than that for sediments.

Environmental samples used in the analysis of micropollutants must be stored at sub -zero temperatures such as -20^0C at which the reaction rate is negligible (Darwano et al., 2014). Freeze drying of environmental samples such as sediments is necessary where all the moisture is eliminated under high pressure. This process which is also known as lyophilisation is critical and safe as it facilitates good contact between the target compounds and the matrix (Babic and Pavlovic, 2013). Certain organic micropollutants degrade at high temperature. Therefore, lyophilising sediment matrix is best practice than oven drying (Runnqvist et al., 2010) since thermally sensitive compounds are protected from degrading thereby avoiding sample loss. Grinding and sieving follow the freeze drying step where the sediment matrix is ground into smaller particle sizes allowing more surface area for the compounds to adsorb to the particles (Babic and Pavlovic, 2013). Therefore, the sample preparation stage in the EPPPs analysis is crucial as this step facilitates in retention of the target compounds and proper adsorption to the sediment matrix. Studies conducted into sediments (Bu et al., 2014; Stewart et al., 2014; Vazquez-Roig et al., 2010) have followed the sample preparation steps by storing sediment samples at -20^0C, freeze drying and sieving before extraction. It is concluded that careful sample preparation ensures in minimum sample loss, thereby generating accurate results.

2.7.2 Extraction

Extraction is the next step illustrated in Figure 2.4, which is again a key step because only an efficient extraction will recover most of the EPPP compounds from environmental matrices. This, in turn, facilitate in obtaining accurate results. There

are different extractions methods such as ultrasonication extraction method, soxhlet, and microwave assisted extraction (MAE) and accelerated solvent extraction (ASE). This section has evaluated and compared different extraction methods which enabled the selection of the most appropriate extraction technique for EPPPs analysis in the current study.

Many studies have applied ultrasonication method for extracting EPPPs from different environmental matrices (Beretta et al., 2014; Camacho-Muñoz et al., 2013; Chen and Zhou, 2014; Chen et al., 2015; Langdon et al., 2011; Löffler and Ternes, 2003) with recoveries ranging from 42% -352%. Ultrasonication appears to be a quick and reliable technique for EPPPs extraction. It was recommended in method 1694 developed by the USEPA (2007) for EPPPs analysis. Despite being a standard method, it was concluded that the USEPA method is time -consuming with high possibility of sample loss. Therefore, its application was not considered to be feasible.

A number of research studies, on the other hand have developed what is referred to as quick, easy, cheap, effective, rugged and safe (QuEChERs) methods where EPPPs are extracted with simple vortexing yielding recoveries from 40-90%. Therefore, it is concluded from the above studies that ultrasonication and vortexing are easy extraction methods which yield good recoveries. However, it is to be noted that the application of these techniques is likely to induce more human error and sample loss since it involves a lot of manual handling.

Another extraction method, ASE, which is approved by EPA (Mitra, 2003) for extraction has lately gained popularity as it is quick with minimum chances of error. The method was also found to be efficient, resulting in increased recoveries of EPPPs compared to ultrasonic, soxhlet, MAE extraction methods (Antonić and Heath, 2007). However, according to Antonić and Heath (2007), MAE consumed less solvent and time compared to ASE stating how other factors affect the efficiency of an extraction technique. MAE was chosen for EPPPs analysis in Krka river sediments in Slovenia over ASE. However, it is to be noted that the efficiency of any extraction method depends on other important factors such as the selection of the appropriate organic solvent, temperature, and pressure. For example, Vazquez-Roig et al. (2010) analysed EPPPs using ASE method and used water as an extracting

solvent which is environmentally safe and obtained satisfactory recoveries of 34-105% for the target compounds.

Several studies have reported to have employed ASE for extracting EPPPs from various environmental samples with satisfactory recoveries ranging from 43-116% (A.kinney et al., 2006; Bu et al., 2014; Ferrer and Furlong, 2002; Krogh et al., 2008; Silva et al., 2011; Stewart et al., 2014). Runnqvist et al. (2010) and Vazquez-Roig et al. (2010) state that ASE in tandem with SPE clean-up yields high recoveries and is a preferred method of extraction for sediment samples because it facilitates in proper desorption of EPPP substances from the complex matrix when accompanied by appropriate organic solvents (Bialk-Bielinska et al., 2016). Thus, it was concluded that ASE is an effective extraction method.

As mentioned earlier, ASE method employs organic solvents or water as extracting solvents. Organic solvents such as methanol, acetone and acetonitrile can be used for extractions of polar compounds such as EPPPs (Jelić et al., 2009). Darwano et al. (2014) used a combination of two organic solvents- methanol and acetone (3:1 v/v) for extracting EPPPs from sediment samples using ultrasonication method of extraction and reported recoveries ranging from 40-102% suggesting that combination of organic solvents could also be used in the extraction of EPPPs. The selection of solvent to be used in extraction depends on the polarity of the compound and the instrument on which these compounds are going to be analysed. Besides solvents used in extraction, another factor that affects the efficiency of the process is temperature.

Temperature is a critical factor in extraction processes particularly in the case of the ASE method. High temperature during extraction facilitates good solubility of the analytes in the solvent and aids in complete extraction of the analytes. Runnqvist et al. (2010), stated that a temperature range of 50^0 C-100^0C aids in efficient extraction of analytes thereby demonstrating that increasing temperature improves recoveries of the analytes. However, the stability of the compounds at increased temperatures needs to be considered prior to extraction in order to avoid degradation of the compounds.

High pressure is another parameter which is essential for smooth flowing of the solvent through the cell and proper distribution of the solvent throughout the matrix sample. The pressure is generally kept high to aid in the proper solvent distribution and flow through the system. However, Runnqvist et al. (2010) cites in their review that increase in pressure from 870-2175psi has a negligible effect on the extraction process, but increasing time and static cycle ensures efficient extraction of analytes as it enables the breakdown of the analyte-matrix complex due to increased solvent - analyte contact.

2.7.3 Clean up

The use of SPE method has increased in the last few years mainly because of the increasing need for reducing the use of organic solvents in laboratories due to environmental concerns (Hennion, 1999). Studies around the world have employed SPE method for EPPPs analysis (Boyd et al., 2003; Jiang et al., 2014; Pietrogrande and Basaglia, 2007; Scott et al., 2014; Vazquez-Roig et al., 2010; Zhao et al., 2015). Therefore, SPE was the method chosen for the clean-up of extracts for EPPPs analysis. However, method development is still necessary given the fact that research into EPPPs is still very new, there is a dearth of literature and standardised methods and guidelines for an efficient extraction and clean-up (Hennion, 1999). While SPE is the preferable method of clean-up, the use of cartridges in SPE clean - up plays a vital role in method development for EPPPs analysis. A review by Primel et al. (2012) states that the use of cartridges in SPE clean-up significantly affects the recovery. C18 is the common cartridge used for clean-up of EPPP extracts, but the use of Oasis HLB cartridge by Waters in combination with C18 has been found to result in increased recoveries of EPPP compounds (Bialk-Bielinska et al., 2016; Primel et al., 2012). It was evident from the review of research literature that ASE in tandem with SPE is most efficient and reliable sample preparation technique and use of appropriate cartridges for SPE clean-up is critical for high recoveries.

2.7.4 Analytical instruments

There has been a dearth of literature reporting a standardised analytical method that could be applied to a wide range of EPPP compounds. In addition, the analytical methods developed thus far are sensitive to certain groups of EPPPs only (Comerton

et al., 2009). Therefore, it is required to develop a sensitive analytical method that could be applied across a wide range of compounds which also means it could save a lot of time in terms of analysis. Application of such analytical methods on the high end, sophisticated instruments such as LCMS/MS and GCMS/MS results in sensitive and selective quantification of EPPPs.

Advanced and sophisticated analytical instruments have now facilitated in easy detection and quantification of organic micropollutants such as pharmaceuticals. As stated in Section 2.7, liquid chromatography and gas chromatography in tandem with mass spectrometry has been the preferred choice for the analysis of pharmaceutical compounds. Gas chromatography was initially the first choice for organic micropollutants such as EPPPs. However, the use of gas chromatography gradually became restricted since it could only analyse non-polar and volatile compounds (Ternes et al., 2004). Analysis of polar EPPPs on gas chromatograph is possible but requires derivatisation of EPPP compounds into a volatile form which lengthens the analytical process. This was when the use of liquid chromatography gained momentum as a result of its capability to analyse a varied range of polar EPPP compounds at the same time (Comerton et al., 2009; Primel et al., 2012).

Lately there have been studies that employed LCMS/MS method for the analysis of pharmaceuticals and other organic micropollutants in environmental samples indicating increasing preference and reliability of such instruments (Azuma et al., 2015; Primel et al., 2012; Schultz and Furlong, 2008) because LCMS/MS facilitates in selective and accurate detection the target compounds. Quantification of the target compounds on LCMS/MS is possible by employing the technique of multiple reactions monitoring (MRM) where in the target analytes are smashed into numerous fragments and two transitions are selected unique to the target compound. The two transitions are used as quantitative and qualitative transitions to confirm accurate detection of the compound (Comerton et al., 2009; Hernández et al., 2014; Pietrogrande and Basaglia, 2007).

The accurate selection of target compounds is possible when LCMS/MS is combined with the (Electrospray Ionisation) ESI mode / atmospheric pressure chemical ionisation (APCI) mode. However, the ESI suits better because of its ability to analyse a broad range of compounds – low and high polar and non-volatile organic

compounds (Pietrogrande and Basaglia, 2007). While ESI remains the choice of mode for EPPPs analysis, it is susceptible to matrix effects originating from complex environmental samples such as sediments and soil. The matrix effect, therefore, may result in suppression of signals thus, affecting the sensitivity of the method (Jahnke et al., 2004; Pietrogrande and Basaglia, 2007).

Analytical technique of Multiple Reaction Monitoring (MRM) in positive and negative Electrospray Ionisation (ESI) mode ensures accurate and sensitive determination and quantitation of the target compounds. Developing such MRM technique is again a time -consuming process, but ensures accurate quantification of target analytes. Past research studies (Chen and Zhou, 2014; Chen et al., 2013; Vazquez-Roig et al., 2010) have applied the Multiple Reaction Monitoring (MRM) techniques for EPPPs analysis and claim that the technique is sensitive, accurate and reliable. The MRM technique is explained in detail Section 3.3.2. Accordingly, the analysis of EPPP compounds involves the use of multiple techniques such as ASE, SPE and MRM in tandem with analytical instruments such as GCMS/MS and LCMS/MS.

2.8 Conclusions

It is understood from Sections 2.1 and 2.2 that the aquatic ecosystems around the world are contaminated with various kinds of pollutants. Now there is an addition of a new set of pollutants that include pharmaceuticals. The critical review of research literature led to the adaptation of the term 'Environmentally Persistent Pharmaceutical Pollutants' (EPPPs) proposed by SAICM that was the most relevant to this study. Discussions in Section 2.3 led to the conclusion that EPPPs are ubiquitous in all the compartments of the aquatic environment around the world, including fresh and marine waters. A direct linkage can be noted between urbanisation, population growth, waste generation and increased consumption of pharmaceutical compounds (refer Section 2.6) in Australia and Queensland which was a key finding of this study. This finding provides a strong base for conducting the EPPPs analysis in Brisbane River sediments.

EPPPs are bioaccumulative posing potential threats to aquatic organisms (refer Section 2.4) which implied that the removal of EPPPs from the environment is

important. It can be concluded from Section 2.5 that while the introduction of novel and post treatment techniques such as ozonation, GAC and AOP significantly remove a number of such micropollutants, they are inefficient in removing all the pharmaceuticals present in the environment. Section 2.7 evaluated the various analytical methods used in the EPPPs analysis, which led to the conclusion that investigation of EPPPs occurrence in sediments required developing a method for detection and quantification. Accordingly, it was concluded that the application of ASE-SPE methods and the use of LCMS/MS-ESI results in the sensitive and accurate quantification of EPPP compounds. Method development was therefore, the next and crucial part of this study and it is discussed in Chapter 3.

Chapter 3 Research Design and Methods

3.1 Overview

It was concluded in Chapter 2 that urbanisation, population growth and inefficient wastewater treatment methods are the main causes for the ubiquitous occurrence of EPPPs across different compartments of the aquatic environment. The critical review of research literature in Section 2.2 and 2.3 also concluded that EPPPs analysis in sediment samples is complex requiring sensitive analytical methods. High cost and lack of sophisticated instrumentation are likely to have attracted fewer researchers to study EPPPs and this seems quite possible in the case of Australia in particular and also Queensland.

Evidence from the literature suggests that urbanisation in South East Queensland due to population growth and that population due to ageing is susceptible to cardiovascular, musculoskeletal and mental health issues (ABS, 2015a; BITRE, 2013). It is worth noticing that the population in Queensland is also ageing markedly (CHO Report, 2014) and health expenditure on medicines has increased (ABS, 2011, 2014) thereby implying increased pharmaceutical consumption which has resulted in the occurrence of EPPPs in the Brisbane River (Scott et al., 2014). Literature review in section 2.3 highlighted the lack of study into EPPPs in the aquatic environments of Australia particularly sediments and therefore this study was undertaken to investigate the occurrences of EPPPs in the Brisbane River sediments. The results of the investigation into the occurrence of EPPPs in Brisbane River sediments are discussed in Chapter 4.

3.2 Research design

A robust research design for this study was critical in achieving the aims and objectives. Accordingly, the research design focused on the following six areas:

- Critical review of research literature
- Selection of pharmaceutical compounds for analysis
- Study area and sites for collecting samples for EPPPs analysis

- Sampling, storage and preparation of samples for EPPPs analysis
- Method development and test methods for quantification of EPPPs analysis
- Data Analysis

3.2.1 Critical review of research literature

The critical review of research literature is crucial for a research study in identifying the knowledge gaps and for understanding the research problem and thereby in developing the research methodology. The literature review primarily evaluated research literature in the following key areas;

- EPPPs in the environment
- Their occurrence and distribution around the world
- Risks and removal techniques of EPPPs
- Sources of EPPPs and its link to urbanisation
- Analytical and laboratory test methods for quantification of EPPPs

The critical analysis of research literature enabled in developing an understanding of research problems associated with EPPPs pollution of aquatic environments and facilitated in identifying the knowledge gaps in relation to the occurrence of EPPPs in the Brisbane River sediments. The literature review was beneficial in understanding the linkage between urbanisation around Brisbane, population growth, ageing population and pharmaceutical consumption leading to EPPPs pollution in Brisbane River which further helped in the process of selection of target compounds, study area, sampling sites and test methods for analysis of EPPPs.

3.2.2 Selection of compounds for investigation

A set of target compounds was required for analysis in this research study to investigate the occurrence of EPPPs in the Brisbane River sediments. The selection of target analytes was based on the aim of this study. A critical review of research literature and evaluation of the statistical data from Australian Bureau of Statistics (ABS) and Pharmaceutical Benefit Scheme (PBS), revealed the health status of Queenslanders (Section 2.2). The reports state that Australians aged 65 years and above are more susceptible to chronic health conditions such as cardiovascular

conditions, musculoskeletal conditions and a broader age group of (18-65) is prone to mental health issues. This finding led to the conclusion that drugs used to treat such health conditions are the most likely to be consumed in Australia and thereby in Queensland. In addition, information about expenditure on medicines, the most widely prescribed medicines, urbanisation and population proved essential in substantiating the conclusion and facilitated in establishing a relationship between urbanisation and EPPPs occurrence in the aquatic environment. The information and knowledge obtained, proved decisive in selecting the appropriate EPPP compounds for this study.

3.2.3 Study area and sites for collecting samples for EPPP analysis

A stretch of Brisbane River was selected as the study area and the selection of sampling sites along the selected stretch was based on careful observation of satellite imagery of Brisbane City. Considering the aim of this study and based on the review of the research literature in Section 2.3 and 2.5, this study required careful selection of a study area that exhibits urbanisation and population growth. Inferences drawn from careful observations of satellite images of Brisbane City and the Brisbane River facilitated in the selection of the appropriate sampling sites for collection of representative sediment samples for EPPPs analysis in the Brisbane River. The selection of study area and sampling sites are further discussed in detail in Chapter 4.

3.2.4 Sampling, storage and preparation of samples for analysis of EPPPs

Research literature was critically reviewed for suitable sampling technique to collect representative samples of sediments from the Brisbane River. A literature review revealed that sample storage and sample preparation are the preliminary stages in EPPPs analysis. Storing samples at low temperatures was understood to be critical. The techniques of freeze drying, grinding and sieving in sample preparation explained the significance in the analysis of micropollutants such as EPPPs. This information led to the selection of appropriate measures for storing sediment samples and undertaking sample preparation for analysis of selected target EPPP compounds (Runnqvist et al., 2010). The sample storage and sample preparation of EPPP compounds are further discussed in detail in Section 3.3.3.

3.2.5 Method development and test methods for EPPPs analysis

Analysis of EPPPs in sediment samples was necessary to study the occurrence of EPPPs in the Brisbane River (refer Section 2.2). Further, this analysis was important for studying the relationship between urbanisation and EPPPs occurrence in the Brisbane River. The investigation of EPPPs, however, required the development of an appropriate extraction method and method for detection and quantification for the analytical instrument – LCMS/MS. Critical analysis of research literature (Section 2.6) resulted in the selection and development of MRM method for detection and quantification on Shimadzu LCMS/MS instrument and test methods such as Accelerated Solid Extraction (ASE) and Solid Phase Extraction (SPE) for extraction and cleaning up of sediment extracts for EPPP analysis (Runnqvist et al., 2010). The selected MRM method and test methods are discussed in detail in Section 3.3.2 and 3.3.3, respectively.

3.2.6 Data analysis

The raw MRM data obtained after running the sediment samples on mass spectrometer were processed and analysed for selective identification and quantification of the target compounds using the Skyline software. The Skyline software is used in building MRM methods and analysing resulting mass spectrometer data. Univariate data analysis such as mean, median, minimum, maximum and standard deviation were chosen for analysing the results to obtain information about the occurrence and spread of EPPPs in the Brisbane River.

3.3 Research methods

3.3.1 Selection of EPPP compounds

Selection of target compounds for analysis was crucial to study the occurrence of EPPPs compounds in the Brisbane River sediments and further understand the relationship with urbanisation. This section discusses how the target compounds were selected for this study.

In-depth analyses of research literature led to the conclusion that heart-related conditions, cholesterol, mental health conditions and hypertension are prevalent in Australia (ABS, 2015c) and the drugs used to treat these conditions predominantly belong to therapeutic classes of lipid regulators, psychiatric and β- blockers,

respectively (ABS, 2010, 2011, 2014). According to the statistical data from (ABS (2010), 2011), 2014)) and (CHO Report (2012), 2014)), there has been rise in prescription of the drug atorvastatin (lipid regulator), amoxicillin (antibiotic), cephalexin (antibiotic), propranolol (β- blockers) and ibuprofen (analgesic).

As far as Queensland is concerned, there is no direct information available about medicine consumption. However, it can be implied from the health reports (CHO Report, 2012, 2014), that there has been an increase in consumption of pharmaceuticals given the fact that most of the population is ageing and suffering from cardiovascular, musculoskeletal and mental health conditions. Cardiovascular, respiratory followed by neurological disorders continue to remain the main cause for the rise in health expenditure. In fact the rate of stroke and coronary diseases is 8-9% higher in Queensland compared to Australia (CHO Report, 2014). This information led to selection of pharmaceutical drugs from the therapeutic classes of lipid regulators and β- Blockers.

The risk assessment of EPPP compounds undertaken in studies (Camacho-Muñoz et al., 2013; Henschel et al., 1997) led to the selection of propranolol and clofibric acid. In addition, increased prescription of drugs such as atorvastatin, atenolol, amoxicillin and cephalexin in past years from 2010-2014 (ABS, 2010, 2014) led to the selection these drugs for analysis in this study. Table 3.1 below shows the most widely prescribed drugs in Australia.

Table 3.1 Most commonly used and prescribed pharmaceutical drugs in Australia

Pharmaceutical class	**Name of the drug**	**Reference**
Lipid regulator	Atorvastatin	(ABS, 2011, 2014)
β- Blocker	Atenolol	(ABS, 2010)
Antibiotic	Amoxicillin, Cephalexin	(ABS, 2011, 2014)

It is obvious from the above Table 3.1 that the drugs, namely, atorvastatin, atenolol, amoxicillin and cephalexin are mostly commonly prescribed and therefore widely consumed by the growing population. This suggests that the likelihood of these drugs being released by wastewater effluents into the neighbouring aquatic environments is high. Therefore, these compounds were selected for this study. The following Table 3.2 below displays the therapeutic drugs selected for this study.

Table 3.2 Selected target pharmaceuticals for analysis in this study

Pharmaceutical group	Compounds in the group	Therapeutic use
β – Blockers	Atenolol, Propranolol	High blood pressure, cardiovascular diseases.
Lipid regulators	Atorvastatin, Clofibric acid	High cholesterol.
Antibiotics	Amoxicillin, Cephalexin	Bacterial infections.
Anti-inflammatory and analgesics	Ibuprofen, Diclofenac	Musculoskeletal problems such as back pain.
Psychiatric Drugs	Carbamazepine, Diazepam, Lorazepam	Depression, anxiety, seizures.

Sources: Adapted from (ABS (2010), 2011), 2014), 2015c); Pharmaceutical Benefit Scheme (2015))

A total of eleven target compounds were selected from five different therapeutic classes. The selection of analgesics and anti-inflammatory drugs such as diclofenac and ibuprofen was based on the fact that these drugs are non-prescription drugs and are easily available over the counter in pharmacies and consumed by a wider group of population (Khan and Ongerth, 2004). Diclofenac and ibuprofen are generally consumed to treat the musculoskeletal problem which explains its widespread consumption since it is also available over the counter.

The selection of psychiatric drugs: carbamazepine, lorazepam and diazepam were primarily based on the conclusions drawn from the literature review. Unlike other drugs, there is no current data available about the consumption of psychiatric drugs in Australia or Queensland. However, based on the statistical data from ABS and a limited number of other studies (Hollingworth and Eadie, 2010; Khan and Ongerth, 2004; Roberts et al., 2015; Scott et al., 2014), it was clear that psychiatric drugs are present in the aquatic environments in Australia. Therefore, it was decided to include psychiatric drugs also in this study. Diazepam (psychiatric therapeutic drug) is prescribed to treat severe muscle spasms (Pharmaceutical Benefit Scheme, 2015), which increases its chances of consumption and thereby its occurrence in the Australian aquatic environments.

While the literature review and ABS statistical data were the primary sources in deciding the target compounds, another source; the Pharmaceutical Benefit Scheme (PBS) also proved effective in substantiating the selection of compounds for this study. PBS is a scheme managed by the Australian Government with the objective to provide reliable and affordable access to necessary medicines to Australians (Pharmaceutical Benefit Scheme, 2015). The drugs under this scheme are subsidised for easy affordability. Therefore, the availability of above selected pharmaceutical drugs from the therapeutic classes of lipid regulators, psychiatric, antibiotics and β – Blockers (refer Table 3.2) under the Pharmaceutical Benefit Scheme was considered to be another important factor for the possible occurrence of the target pharmaceutical compounds in the Brisbane river sediments.

The selected target compounds required a selective and sensitive method for identification and quantification. Therefore, the next section discusses the method development and test methods employed in the analysis of the selected target compounds.

3.3.2 Method development for detection and quantification – Multiple Reaction Monitoring (MRM)

The aim of the study was entirely dependent on the method development for EPPPs analysis and therefore formed an important part of this research study. The method development involved developing MRM technique for the detection and quantification of EPPPs that can be applied on a triple quadrupole (QqQ) mass

spectrometers (Kasprzyk-Hordern et al., 2007). An MRM method was developed for the selected compounds on the Shimadzu LCMS-MS 8050 (Figure 3.1) under Electron Ion Spray (ESI) mode for EPPPs analysis.

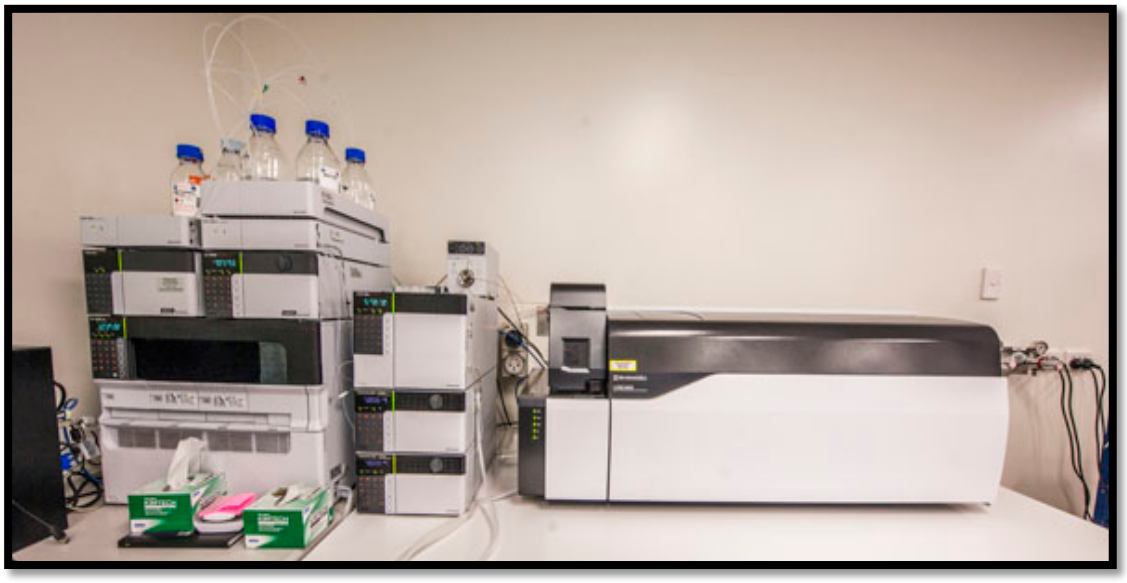


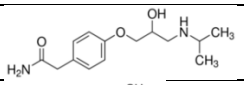
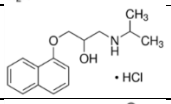
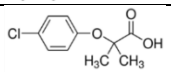
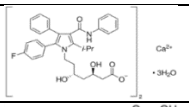
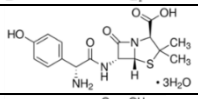
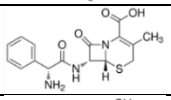
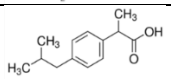
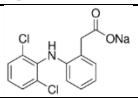
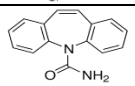
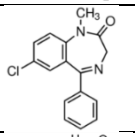
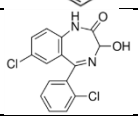
Figure 3.1Shimadzu LCMS/MS 8050 used for EPPPs analysis in this study

The prominent characteristic of MRM is that it allows analysis of multiple precursor ions and all at the same time (Hernández et al., 2014). For this study, a 10 minute quick quantitative and qualitative method using MRM technique was developed on the Shimadzu LCMS-MS 8050, which was robust, sensitive and with less dwell time, making the process of analysis faster. The accuracy of the MRM technique, however, depends on a detailed product ion scan. Performing Product Ion Scan (PIS) during MRM method development ensures sensitive and accurate selection of target analytes (Schultz and Furlong, 2008).

Therefore, the first step in the process of developing the MRM method was performing PIS of each target compound. This required obtaining the molecular formulae of the selected compounds (Table 3.3) and determining their molecular weights since it is a prerequisite in a Product Ion Scan (PIS). The molecular weight is the mass of the compound. During a PIS, each compound is smashed into multiple small fragments, also called transitions. These transitions are monitored and two transitions with strong signal strength are selected. One of the selected transition is used for identification and quantification of the compound hence, called quantitative

transition. The second of the two selected transitions is used for confirming the identified compound that is for qualitative purpose, hence, called qualitative transition. A quantitative and qualitative transition of a compound is unique and therefore, are a part of quality control and assurance procedure.

Table 3.3 Molecular formula and structures of selected target compounds

Therapeutic group	PPCP compound	Formula	Structure
β- Blockers	Atenolol	$C_{14}H_{22}N_20_3$	
	Propranolol	$C_{16}H_{21}NO_2.ClH$	
Lipid Regulators	Clofibric acid	$C_{10}H_{11}ClO_3$	
	Atorvastatin	$C_{33}H_{34}FN_2O_5.0.0.5Ca.1.5H_20$	
Antibiotics	Amoxicillin	$C_{16}H_{19}N_3O_5S.3H_20$	
	Cephalexin	$C_{16}H_{17}N_3O_4S$	
Analgesics and Anti-inflammatory	Ibuprofen	$C_{13}H_{18}O_2$	
	Diclofenac	$C_{14}H_{10}ClNNaO_2$	
Psychiatric	Carbamazepine	$C_{15}H_{12}N_20$	
	Diazepam	$C_{16}H_{13}ClN_2O$	
	Lorazepam	$C_{15}H_{12}Cl_2N_2O_2$	

(Source: Adapted from http://www.sigmaaldrich.com/catalog/product/fluka/34228?lang=en&region=AU)

However, prior to PIS, 10µl injections of the mix stock of higher concentration (10000ppb) were injected using ESI mode. In the ESI source, the compounds were scanned through Q1 quadrupole for most intense signals in both modes – positive and negative. Compounds, carbamazepine, lorazepam, diazepam, amoxicillin, cephalexin, atorvastatin, propranolol and atenolol gave intense signals in positive mode whereas ibuprofen, diclofenac and clofibric acid gave intense signals in

negative mode. Table 3.4 shows the modes in which the target compounds have been analysed. The classifications of target compounds in the positive and negative mode were key steps in the PIS scan.

After optimising the ESI as a source, the next step in the process of PIS was injecting a mixed stock of target compounds through the mass spectrometer for monitoring the different fragments or transitions of each compound. 1µl injections of mix stock (conc 10,000 ppb) of compounds prepared in 5% Acetonitrile –Methanol in 10mM Ammonium acetate solution were sprayed under high voltage in the ESI mode through to the three quadrupoles where the molecules were scanned in Scanning quadrupole (Q1) and then smashed into Collision cell quadrupole (Q2).

The preparation of solution - 5% Acetonitrile –Methanol in 10mM Ammonium acetate was prepared by mixing the mobile phases A (acetonitrile – methanol 60/40v/v) and B (10mM ammonium acetate) used on the LCMS/MS because these mobile phases facilitated in selective quantification of target compounds in the standards by enhancing the signals. Additionally, the methanol concentration was reduced to <5% (Jelić et al., 2009) to achieve better signals. Later this solution was also used to resuspend the SPE cleaned sediment extracts for analysis using the LCMS/MS.

The PIS process ended with scanning in quadrupole (Q3) where all the transitions of the target compounds were monitored. The transitions were monitored at different collision energies. The collision energies were optimised for intense transition signals. Then the optimised collision energies for each compound was recorded and used in the MRM method. Table 3.4 displays the optimised collision energies for each target compound. Amongst all the transitions, two transitions of higher mass and unique to each target compound (refer Table 3.4) were selected and monitored in the MRM method for accurate and sensitive detection of target compounds.

Table 3.4 Qualitative and Quantitative transitions of the target compounds at optimum collision energies on the Shimadzu LCMS/MS

Compound name	Quantitative transition	Collision energy (eV)	Retention time	Qualitative transition	Collision energy (eV)	ESI mode
Amoxicillin	398.25>349.15	-22	1.602	398.25>160.0	-22	Positive
Cephalexin	348.00>158.00	-12	1.635	348.00>160.00	-18	Positive
Atenolol	267.25>190.10	-19	1.569	267.25>225.2	-16	Positive
Atenolol-d7	274.25>190.10	-19	1.566	274.25>225.2	-17	Positive
Clofibric acid	213.20>127.10	16	2.02	213.20>85.00	10	Negative
Propranolol	260.25>74.00	-22	2.156	260.25>155.0	-22	Positive
Carbamazepine	237.20>194.20	-19	2.46	237.20>192.1	-23	Positive
Diclofenac	294.20>249.80	12	2.548	294.20>214.2	19	Negative
Lorazepam	321.00>303.00	-15	2.614	321.00>275.0	-15	Positive
Atorvastatin	559.15>440.15	-23	2.82	559.15>466.1	-17	Positive
Ibuprofen	205.30>161.20	10	2.927			Negative
Ibuprofen d3	208.30>164.20	10	2.928			Negative
Diazepam	285.00>154.00	-26	3.34	285.00>193.00	-32	Positive

The sign ">" in Table 3.4 is used to point out the quantitative and qualitative transition selected for that particular compound. For example, in the case of diazepam, the mass of diazepam is 285.00. During the MRM process, diazepam was smashed into fragments also called 'transitions'. Further, the obtained transitions were monitored and two transitions of 154.00 and 193.00 that gave strong signal strength were selected. These transitions were used for quantification and qualitative purpose. Therefore, in Table 3.4 the quantitative transition of diazepam is represented as 285.00>154.00 and qualitative transition for diazepam is represented as 285.00>193.

The two transitions act as quantitative and qualitative transitions. The detection of both transitions confirms the detection of the compound. Thus, a sensitive, selective MRM technique was developed for the selected compounds. Once the MRM method was developed, calibration standards of the target compounds were prepared to obtain a calibration curve. The purpose behind a obtaining a calibration curve for

each compound was to check the reliability and sensitivity of the MRM method and to establish a range within which the target compounds will be quantified. The developed MRM method was successful in the quantification of psychiatric compounds. However, in order to derive a comprehensive knowledge of EPPPs pollution in the Brisbane River, further refining and development of the MRM method was imperative.

The MRM technique followed PIS. In the MRM method, two transitions unique to each target compound were selected. Then, the selected transitions were monitored for detection and quantification of target compounds during the sediment sample analysis. Figure 3.2 below explains the process of product ion scan (PIS) and multiple residues monitoring (MRM). Figure 3.2 (A) describes the PIS process where the multiple transitions of a compound are monitored following ionisation in the ESI source, scan in the Q1 and smashing in Q2. Two transitions of higher masses compared to other transitions and unique to the target compound were selected from the PIS scan. Following the PIS, only the two selected transitions for each target compound were then monitored for detection and quantification in MRM as described in Figure 3.2 (B).

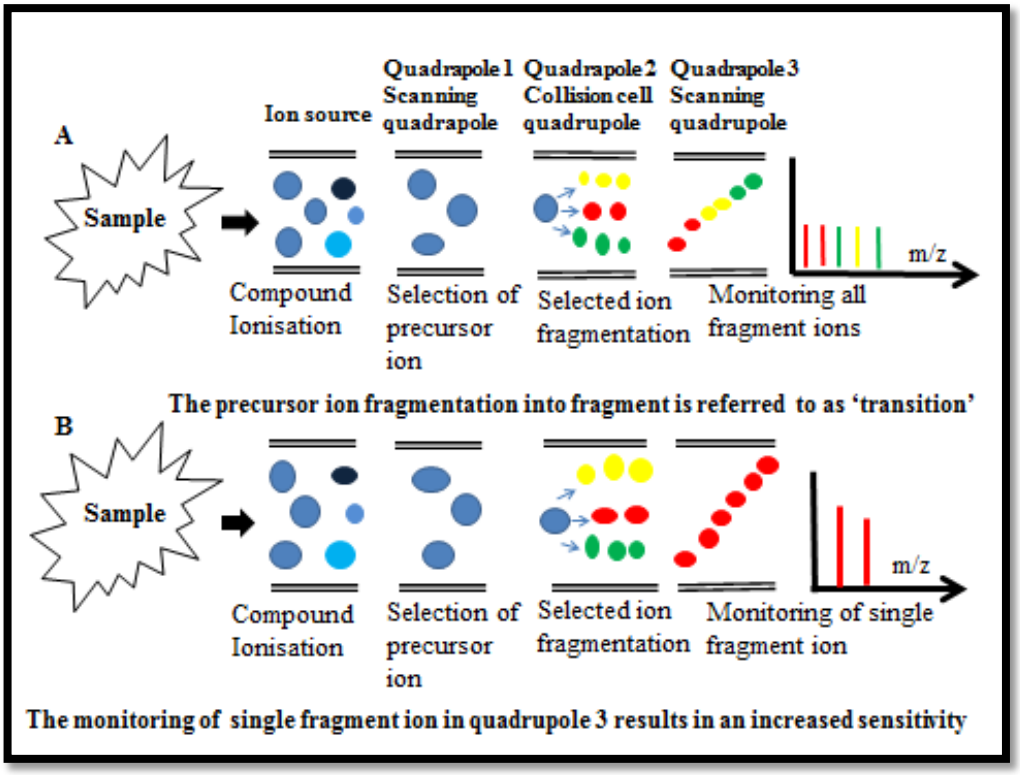


Figure 3.2 (A) PIS technique (B) MRM technique (Adapted from source: http://science.sciencemag.org/content/sci/312/5771/212/F1.large.jpg)

Considering the limited research in Australia on EPPPs, a broad calibration range was selected for this study similar to the study by Vazquez-Roig et al. (2010) of 0.1 ppb – 300ppb. The calibration curves developed had R^2 values ranging from 0.4 to 1. Table 3.5 shows the calibration equations for all the target compounds.

Table 3.5 Linear equations and R2 values of the target compounds

Compound	Equation	R^2 value
Amoxicillin	y = 33.933x + 175.07	0.9966
Cephalexin	y = 11.619x + 2672.9	0.4135
Propranolol	y = 730.06x + 679.68	0.9986
Carbamazepine	y = 6218.2x + 4261.6	0.9997
Lorazepam	y = 9986.6x - 48774	0.998
Atorvastatin	y = 13985x - 7394	1
Diazepam	y = 22513x + 57471	0.9985
Atenolol	y = 812.06x + 5193.3	0.9999
Atenolol D7	y = 3093.6x + 3636.2	0.9998
Clofibric acid	y = 125.19x + 481.35	0.9993
Diclofenac	y = 122.56x + 1092.8	0.995
Ibuprofen	N/A	N/A
Ibuprofen D3	y = 225.53x - 122.12	0.9993

x - concentration and y - peak area

After developing the MRM method, the next step in method development was to develop an extraction method for extracting EPPPs from the sediment samples. An efficient and working extraction method should be able to extract most of the target analytes adsorbed to the sediment particles (Babic and Pavlovic, 2013). Such an extraction method could be employed in the analysis of the samples. The extraction method development is discussed in the Section 3.3.3 in detail.

3.3.3 Test methods

This section explains the test methods for sample preparation, extraction and clean up in the analysis of EPPPs from the Brisbane River sediments.

- Sample storage and preparation
- Accelerated Solvent Extraction and
- Solid Phase Extraction (Clean-up method)

3.3.3.1 Sample storage and preparation

The sediment samples collected from the Brisbane River were stored in eskys containing ice during transportation to the laboratory. Then the samples were stored at -20^{0}C in the laboratory until analysis. The stored sediment samples were then subjected to freeze drying for at least 2-4 days depending on the moisture content in the sample. The sediment samples were transferred into wide mouth glass containers of the freeze dryer Christ Alpha 1- 4 by John Morris Scientific (Figure 3.3) and then fitted with suction caps. The freeze drying was carried out for 2 days initially and then continued for 2 more days to remove any traces of moisture at -50^{0}C.

Following freeze drying, the next step in sample preparation was grinding the frozen sediment samples and then sieving. Since the collected sediment samples contained dried foliage and lumps of clay and gravel, they were sieved. A sieve of size 250µm was used to obtain fine particles of sediment samples. The sieved samples were stored back at -20^{0}C until extraction.

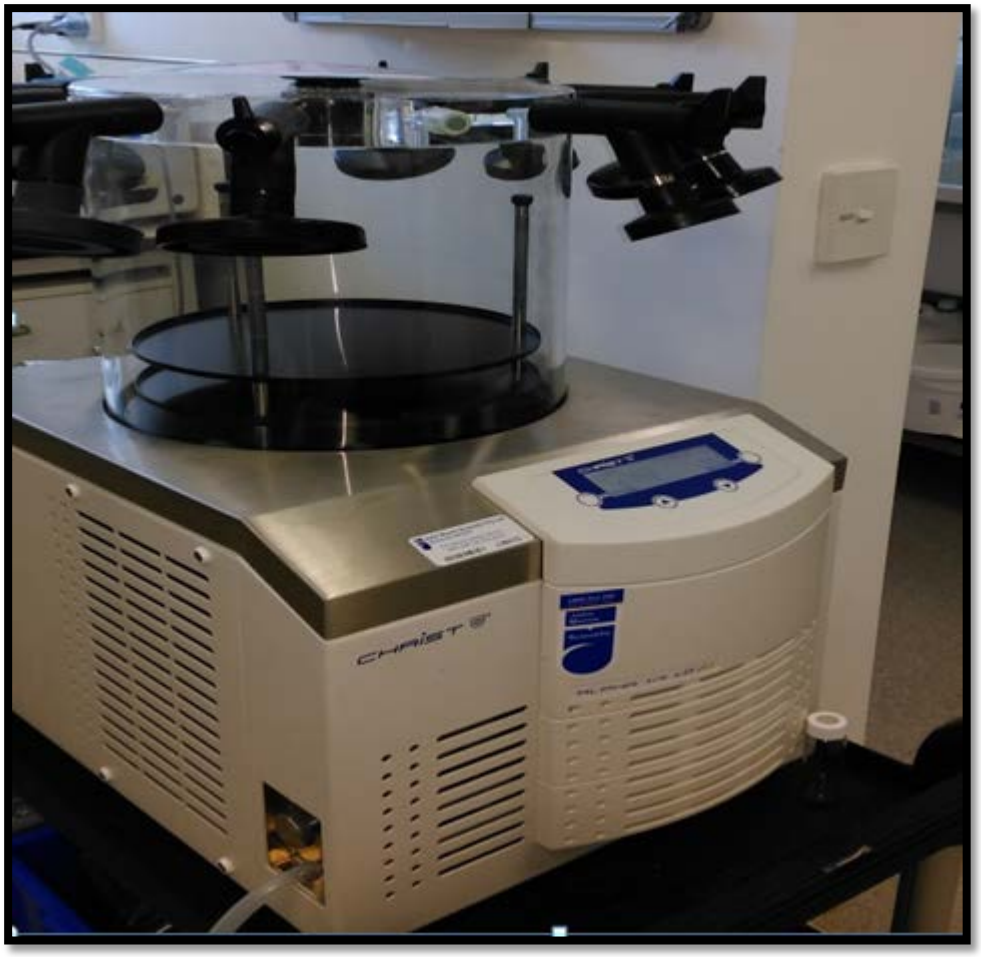


Figure 3.3Christ 1-4 Freeze dryer

3.3.3.2 Accelerated solvent extraction

As stated in Section in 3.3.3, the analysis of EPPPs in this study also involved developing an extraction method. Extraction is an additional, but critical step in the analysis of sediments for micropollutants such as EPPPs where the desired compounds are desorbed from the matrices with the help of solvent under increased temperature and pressure. In this study, the extraction of EPPPs from sediments was carried out using Dionex ASE 350 (Figure 3.4).

Figure 3.4 Dionex ASE 350 instrument used in extraction of target compounds in this study

Although the instrument is capable of extracting different pollutants at high pressure and temperature, it was necessary to determine the appropriate temperature that could aid in the optimum recovery of all selected EPPP compounds. Therefore, an extraction method suitable for selected target compounds was required for this study. To begin with the extraction method development, extraction methods developed by Jelić et al. (2009) and Vazquez-Roig et al. (2010)) were attempted. Section 3.3.3.4

discusses the attempted extraction methods to obtain optimum recoveries for the target compounds in this study.

3.3.3.3 Solid phase extraction (clean-up method)

In a SPE technique, the extracts obtained from the extraction process are subjected to clean up, to remove any unwanted co-extracts from the sample using different types of cartridges as cited in the review by Babic and Pavlovic (2013). A SPE was performed after the ASE extraction, using Phenomenex strata SAX and Oasis HLB cartridges.

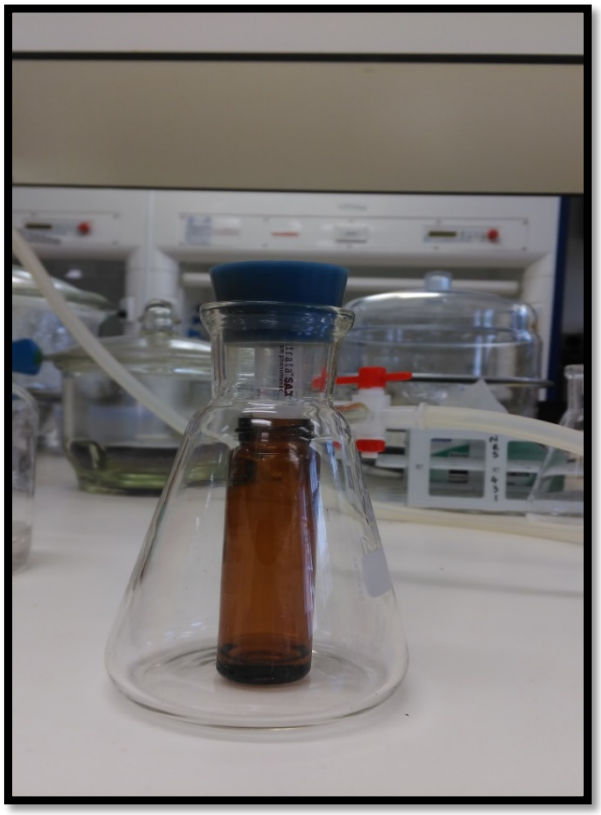


Figure 3.5 SPE clean -up experiment set-up.

Figure 3.5 above illustrates the experimental set up for SPE clean-up. The EPPP extracts obtained after extractions (approximately 100 mL) were first subjected to vacuum concentration where the volume of the extract was reduced by evaporating the organic solvent to approximately 10mL. Then the reduced extracts were cleaned using a SAX cartridge followed by HLB cartridge. The clean-up was performed by applying vacuum which facilitated in slow filtration of the extracts. Slow filtration (dropwise filtration) was maintained by adjusting the maximum contact between

analytes in the extract and cartridge which allow trapping of unwanted co-extracts and release only EPPPs analytes.

The next step in the analysis involved concentrating the SPE cleaned extracts under a gentle stream of nitrogen and filtering it through PTFE filters (0.20µm). Then the filtered extracts were resuspended in the solution (5% acetonitrile - methanol in 10mM ammonium acetate) in which calibration standards were prepared.

3.3.3.4 Attempted extraction experiments for pharmaceuticals

This section discusses the different extraction methods that were attempted. The preparations for the extraction experiments were done by studying and reviewing research literature.

The procedure involved a mixed standard of known concentration (100ppb) of target compounds that was used to spike acid cleaned sand. Acid washed sand was used as a representative of the sediment sample. A 5gm of the representative sand sample was weighed into a glass beaker and then spiked with 1mL of the mixed standard (conc - 100ppb). The sample size of 5gms was chosen against 3gms as in the study by Vazquez-Roig et al. (2010) and after studying the USEPA method for easy calculations and assuming that a bigger sample size would allow extraction of compounds in minute concentrations.

Following the spike, 5gms of diatomeaous earth (DE) was weighed and added to the above mixture. The purpose of adding the DE was to allow the even distribution of the sand particles throughout the cell. Such an even distribution of the sand particles facilitates sufficient contact between each sand particle and extracting solvent, thereby allowing efficient extraction of the compounds.

A 33 mL stainless steel (ss) cell was used for extraction. The cell was initially washed with detergent and cleaned with acetone to remove any residual organics and dried at approximately 110^0C for one hour to be used for transferring the above mixture. The mixture of spiked sand and DE was then transferred into the 33mL ss cell fitted with glass fibre filters at the bottom. The lid of the cell was closed tightly and labelled accordingly. The cell was then placed in the instrument along with a collection bottle (250mL) placed corresponding to the cell number.

Calibration standards and quality control (QC) samples were run with every batch of the extracted samples from each extraction method attempt as part of quality control. The batch of extraction method attempt samples on the mass spectrometer was set with at least 3 runs of QC samples followed by calibration standard run and then sample run. A QC sample was randomly placed in between the samples to check the accuracy and performance of the mass spectrometer. Each extraction method was run in triplicate to check the consistency of the extraction method. The extraction method did not involve the use of internal standards.

Extraction method 1

The first attempt at extraction was according to the extraction method applied in the study conducted by Vazquez-Roig et al. (2010).

The extraction method was then loaded on the ASE instrument with required settings given in Table 3.6. An SPE clean-up using the SAX and HLB cartridges was carried out for all the extracts. The clean-up samples were then filtered using a 0.2µm filter to eliminate any suspended particles, if any. The recoveries of the extraction method obtained are given in Table 3.6 below.

The extraction method gave low recoveries <50% for all the target compounds. The recoveries for cephalexin (41.7%) and diclofenac (31.7%) was comparatively higher than for the rest of the target compounds while still <50%.

Whilst the extraction method was followed exactly as in the study by Vazquez-Roig et al. (2010), the analysis of the compounds on the instrument varied with respect to the selection of the mobile phase on the LCMS-MS and the instrument itself. For the sake of simplicity and quick analysis, only one mobile phase of Acetonitrile – Methanol (60/40 v/v) and 10mM Ammonium acetate was used in both positive and negative modes. However, the choice of using only mobile phase may have possibly resulted in poor signals for the compounds and in turn showed low recoveries. Secondly, the matrix effect could also be a likely cause for the suppression of signals of the target compounds.

The choice of the extraction solvent could also be a reason as water has a neutral pH. Since the target compounds have different polarities, the neutral pH of water might

possibly have resulted in low recoveries. The lack of internal standards for correcting the loss was also be considered to be a likely cause for low recoveries in this attempt.

Table 3.6 Recoveries obtained from the Extraction Attempt 1

Extraction method 1 : Solvent - Water; Temperature - 90^0C; Heat - 5min; Static - 7 min; Cycles - 3								
Volume flush - 100%; Purge - 60s.								
SPE Clean up : SAX and HLB cartridge								
Name	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background	Concentration	Recovery
Amoxicilin	398.25	349.1495	QUAN	1.32	17	0	4.84	4.84
Cephalexin	347.9995	157.9995	QUAN	1.34	233	0	41.77	41.77
Propranolol	260.2495	73.99945	QUAN	1.78	32	87	0.73	0.73
Carbamazepine	237.1995	194.1995	QUAN	1.97	1363	6748	0.54	0.54
Lorazepam	320.9995	302.9995	QUAN	1.96	3194	3761	1.91	1.91
Atorvastatin	559.1495	440.1495	QUAN	2.14	18	0	-0.32	-0.32
Diazepam	284.9995	153.9995	QUAN	2.31	31	0	-2.18	-2.18
Atenolol	267.2495	190.0995	QUAN	1.33	8374	520	16.67	16.67
Clofibric acid	213.1995	127.0995	QUAN	1.52	90	40	-0.11	-0.11
Diclofenac	294.1995	249.7995	QUAN	1.89	4444	3187	31.78	31.78
Ibuprofen	205.2995	161.1995	QUAN	1.86	647	1060	5.50	5.50

Extraction method 2

The second attempt at extraction and SPE clean-up was according to the study by Jelić et al. (2009). The SPE clean-up of the extracts obtained from this extraction was performed using only the HLB cartridge. The cleaned extracts were filtered through 0.22µm (PTFE) filters. Table 3.7 below shows the parameters for this extraction and the recoveries obtained.

The recoveries obtained for this extraction attempt were low. The possible reasons for the low recoveries could be attributed to the same reasons as stated in extraction attempt 1. Though the pH was not changed in this extraction, the pH was slightly acidic due to the addition of methanol to the water and its influence on the recoveries. The extraction method resulted in recoveries which were < 70%. Hence, this extraction method was rejected as it failed to recover all the target compounds.

Table 3.7 Recoveries obtained from Extraction attempt 2

Extraction method 2 : Solvent - MeOH:Water (1/2= v/v) ; Temperature - 100^0C ; Heat - 5min;									
Static - 7min; Cycles - 3:volume flush - 100%: Purge - 60s									
SPE Clean up - HLB cartridge									
Name	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background	Peak Rank	concentration	Recovery
Amoxicilin	398.25	349.149451	QUAN	1.44	54	0	2	3.39	3.39
Cephalexin	347.9995	157.999451	QUAN	1.36	348	0	1	18.53	18.53
Propranolol	260.2495	73.999451	QUAN	1.94	11	27	2	-38.27	-38.27
Carbamazepine	237.1995	194.199451	QUAN	1.89	25636	1237	1	8.71	8.71
Lorazepam	320.9995	302.999451	QUAN	1.99	17811	9449	1	4.60	4.60
Atorvastatin	559.1495	440.149451	QUAN	1.99	16737	96	1	3.37	3.37
Diazepam	284.9995	153.999451	QUAN	2.29	28992	374	1	3.43	3.43
Atenolol	267.2495	190.099451	QUAN	1.38	13156	572	1	9.18	9.18
Clofibric acid	213.1995	127.099451	QUAN	1.49	4161	261	1	16.65	16.65
Diclofenac	294.1995	249.799451	QUAN	1.98	497	553	1	5.09	5.09
Ibuprofen	205.2995	161.199451	QUAN	2.07	893	1975	1	9.59	9.59

Extraction method 3

In this extraction attempt, the temperature of the extraction process was reduced to 90^0C with the ratio of methanol MeOH: Water as (1:1). The remaining parameters were the same as in extraction attempt 2. The method was modified by changing the temperature to 90^0C. The solvent ratio of MeOH: Water was also changed to 1:1 with the objective that the equal volumes of the solvents would facilitate an efficient extraction. After SPE clean-up using HLB, the sample was filtered through a 0.2µm filter.

This extraction method (Table 3.8) also failed to yield good recoveries for the target compounds except for clofibric acid (62.3%). It was concluded that the combination of MeOH: Water in the ratio 1:1 at 90^0C probably does not facilitate extraction of the target compounds. Due to low recovery, the investigation of different extraction methods was continued.

Table 3.8 Recoveries obtained from Extraction Attempt 3

Extraction method 3: Solvent - MeOH:Water(1:1); Temperature - 90^0C ; Heat - 5min;								
Static - 7min; Cycles - 3; Volume Flush - 100%; Purge - 60s								
SPE Clean-up - HLB cartridge								
Name	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background	Concentration	Recovery
Amoxicilin	398.25	349.1495	QUAN	1.29	254	1	24.25	24.25
Cephalexin	347.9995	157.9995	QUAN	1.51	76	0	5.87	5.87
Propranolol	260.2495	73.99945	QUAN	1.8	332	0	1.64	1.64
Carbamazepine	237.1995	194.1995	QUAN	1.94	171	1042	0.15	0.15
Lorazepam	320.9995	302.9995	QUAN	1.96	7721	4790	5.49	5.49
Atorvastatin	559.1495	440.1495	QUAN	2.21	14	0	-0.32	-0.32
Diazepam	284.9995	153.9995	QUAN	2.33	203	0	-2.16	-2.16
Atenolol	267.2495	190.0995	QUAN	1.33	7732	530	15.22	15.22
Clofibric acid	213.1995	127.0995	QUAN	1.49	13604	562	62.32	62.32
Diclofenac	294.1995	249.7995	QUAN	1.89	1746	1484	2.42	2.42
Ibuprofen	205.2995	161.1995	QUAN	1.91	255	580	-10.28	-10.28

Extraction method 4

The fourth extraction attempt consisted of a combination of organic solvents of methanol and acetonitrile in the ratio 60/40 v/v. The MeOH-ACN combination is the mobile phase used to analyse the standards on the LCMS-MS. MeOH-ACN ratio 60/40 v/v was used as mobile phase in the negative mode in the analysis of clofibric acid, diclofenac and ibuprofen (Vazquez-Roig et al., 2010). The parameters for extraction in the ASE were the same as in the extraction attempt 3. Table 3.9 shows the parameters chosen for the extraction and the recoveries obtained for the compounds. The SPE clean-up procedure also remained unchanged using SAX and HLB cartridges and later filtered through a 0.2µm filter.

It is evident from Table 3.9 that the choice of mobile phase as an extracting solvent yields good recoveries for most of the target compounds. While the recoveries were still low (<70%), it indicated that the use of acetonitrile facilitates extraction of the target compounds. Since this solution was able to detect and give good signals for the target compounds, it was chosen as an extracting solvent so as to see whether it aids in extracting the target compounds.

Table 3.9 Recoveries obtained from Extraction attempt 4

Extraction method 4 : Solvent - MeOH:ACN (60/40 v/v); Temperature - 90^0C ; Heat - 5min;								
Static - 7min; Cycles - 3; Volume Flush - 100%; Purge - 60s								
SPE Clean-up - SAX and HLB cartridge								
Name	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background	Concentration	Recovery
Amoxicillin	398.25	349.149451	QUAN	1.33	253	0	24.17	24.17
Cephalexin	347.999451	157.999451	QUAN	1.52	111	0	13.87	13.87
Propranolol	260.249451	73.999451	QUAN	1.91	18494	340	56.91	56.91
Carbamazepine	237.199451	194.199451	QUAN	1.9	136555	2684	44.98	44.98
Lorazepam	320.999451	302.999451	QUAN	2	65679	17552	51.31	51.31
Atorvastatin	559.149451	440.149451	QUAN	2.03	91921	1048	17.50	17.50
Diazepam	284.999451	153.999451	QUAN	2.33	447197	5475	46.60	46.60
Atenolol	267.249451	190.099451	QUAN	1.33	13730	632	28.74	28.74
Clofibric acid	213.199451	127.099451	QUAN	1.49	7827	197	35.63	35.63
Diclofenac	294.199451	249.799451	QUAN	1.79	3332	1074	19.68	19.68
Ibuprofen	205.299451	161.199451	QUAN	1.9	1746	1794	49.74	49.74

Extraction method 5

The extraction attempt 5 was an in-cell extraction using methanol and acetonitrile (60/40 v/v) using the same set of parameters as in extraction attempt 4. This extraction method was quick since the clean-up was in- cell using Florosil. The recoveries obtained from this extraction also yielded good recoveries for a few of the compounds ranging from 10% - 96%. However, the recoveries obtained from this extraction were found to be inconsistent. Table 3.10 shows the recoveries for the target compounds.

Table 3.10 Recoveries obtained from Extraction Attempt 5

Extraction method 5 : Solvent - MeOH:ACN (60/40= v/v) ; Temperature - 90^0C ; Heat - 5min;								
Static - 7min; Cycles - 3; Volume Flush - 100%; Purge - 60s								
SPE Clean up - Florosil								
Name	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background	Concentration	Recovery
Amoxicillin	398.25	349.149451	QUAN	1.22	57	0	8.12	8.12
Cephalexin	347.999451	157.999451	QUAN	1.33	54	0	0.84	0.84
Propranolol	260.249451	73.999451	QUAN	1.91	20276	162	62.33	62.33
Carbamazepine	237.199451	194.199451	QUAN	1.9	172773	3064	56.89	56.89
Lorazepam	320.999451	302.999451	QUAN	2.01	56543	25689	44.09	44.09
Atorvastatin	559.149451	440.149451	QUAN	2.04	57371	560	10.80	10.80
Diazepam	284.999451	153.999451	QUAN	2.33	659588	7799	69.77	69.77
Atenolol	267.249451	190.099451	QUAN	1.33	14712	755	30.96	30.96
Clofibric acid	213.199451	127.099451	QUAN	1.5	18714	732	85.92	85.92
Diclofenac	294.199451	249.799451	QUAN	1.88	4178	2398	28.88	28.88
Ibuprofen	205.299451	161.199451	QUAN	1.9	2911	2357	96.63	96.63

Extraction method 6

The sixth extraction attempt used only acetonitrile as the extracting solvent with the rest of the parameters set exactly as in extraction attempt 4. The extract obtained was cleaned up using SPE technique through SAX and HLB cartridges. The sample was filtered through a 0.2um filter and analysed and checked for recoveries. The selection of acetonitrile as a solvent of extraction resulted in improved and higher recoveries (above 70%) for 3 of the 11compounds analysed.

Table 3.11 shows that the extraction method yielded acceptable recoveries of >70% for psychiatric drugs such as the Carbamazepine, Lorazepam and Diazepam. It also worked well for atenolol (19%) and propranolol (43%). Therefore, the above extraction method was chosen in the analysis of EPPPs.

Table 3.11 Recoveries obtained from Extraction attempt 6

Extraction method 5 : Solvent - Acetonitrile ; Temperature - 90^0C ; Heat - 5min;								
Static - 7min; Cycles - 3; Volume Flush - 100%; Purge - 60s								
SPE Clean-up - SAX and HLB cartridge								
Name	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background	Concentration	Recovery
Amoxicillin	398.25	349.1495	QUAN	#N/A	#N/A	#N/A	#N/A	#N/A
Cephalexin	347.9995	157.9995	QUAN	1.36	4765	0	-43.3051	-43.30508
Propranolol	260.2495	73.99945	QUAN	1.83	42828	1208	43.29975	43.299755
Carbamazepine	237.1995	194.1995	QUAN	1.91	741499	8292	70.17141	70.17141
Lorazepam	320.9995	302.9995	QUAN	2.02	634406	23513	66.11259	66.112593
Atorvastatin	559.1495	440.1495	QUAN	2.1	411	251	-0.36844	-0.368442
Diazepam	284.9995	153.9995	QUAN	2.34	2156011	21218	72.05639	72.05639
Atenolol	267.2495	190.0995	QUAN	1.33	24333	718	19.07783	19.077827
Clofibric acid	213.1995	127.0995	QUAN	#N/A	#N/A	#N/A	#N/A	#N/A
Diclofenac	294.1995	249.7995	QUAN	#N/A	#N/A	#N/A	#N/A	#N/A
Ibuprofen	205.2995	161.1995	QUAN	2.16	227133	267875	#N/A	#N/A

It was understood from the above extraction attempts that the use of acetonitrile significantly improved the recoveries of the target compounds (Table 3.9 and Table 3.10). However, the use of only acetonitrile as an extracting solvent yielded better recoveries of above 70% for psychiatric group of compounds (Table 3.11). Whilst the recoveries improved with the introduction of acetonitrile, the method still had two major limitations in its application.

The extraction method resulted in recoveries for psychiatric compounds. Therefore, although the method was selected for the analysis of EPPPs, it required major refinement and improvement so as to obtain better recoveries for all the target compounds. The second limitation of applying this method is the use of acetonitrile for extraction because acetonitrile is highly toxic to humans and the environment. Therefore, utmost care is required to be taken while handling the solvent and in its disposal.

It is to be noted that although this extraction method resulted in extraction of only psychiatric compounds, the recoveries for these compounds were high and stayed consistent when the extraction method was repeated three times. On the other hand, the previous extraction methods although extracted more target compounds, were resulting in recoveries that were inconsistent. This was one of the major reasons for the selection of extraction attempt 6 for analysing EPPPs. The extraction solvents, temperature and clean-up procedures were the only factors that were modified and changed in order to derive better recoveries of the target compounds. The rest of the factors such as pressure, pH, static cycles, volume flush and purge remained the same. The pH did change slightly with the change in the extraction solvent. However, it was not changed manually.

3.4 Pharmaceutical analysis

The current study was able to detect and quantify three of the crucial target EPPPs – carbamazepine, lorazepam and diazepam by applying the developed methods. This section, discusses the occurrence and distribution of the target compounds in the Brisbane River sediments. Table 3.12 below shows the method detection limit (MDL), limit of detection (LOD) and limit of quantification (LOQ) of the quantified analytes and relative standard deviation (RSD). The lower MDLs of the target compounds suggest that the method is reliable for the analysis of the compounds quantified below.

Table 3.12 MDL, LOD, LOQ values of target compounds

Target compound	**MDL ngg^{-1}**	**LOD ngg^{-1}**	**LOQ ngg^{-1}**	**RSD %**
Carbamazepine	0.22	0.67	2.23	3.03 – 40.4
Diazepam	0.26	0.78	2.59	0.2-113
Lorazepam	0.04	0.12	0.39	3.3-33

The MDL values in Table 3.12 indicated that the developed method was sensitive in detecting the target compounds. Compared to the MDL values reported by Jelić et al. (2009) in their study for lorazepam (3.20 ngg^{-1}), diazepam (0.8 ngg^{-1}) and carbamazepine (0.03 ngg^{-1}), the MDL value for lorazepam (0.04 ngg^{-1}) in the current study was higher whereas that for carbamazepine (0.22 ngg^{-1}) and diazepam were lower. On the other hand, the MDL value for diazepam was found to be higher in comparison to the MDL value reported by Vazquez-Roig et al. (2010) in their study as 0.8ngg^{-1}. The MDL value of carbamazepine in the current study was found to be lower by 0.02 ngg^{-1} than that reported in the study (Vazquez-Roig et al., 2010) as 0.2 ngg^{-1}. MDL for lorazepam was significantly higher than that reported by Jelić et al. (2009), confirming that the method developed is more sensitive in detecting lorazepam. It was therefore concluded that the method developed was sensitive and selective and was applicable for the analysis of the above detected target compounds.

In addition to MDL values, the RSD which explains the variation in the data for the above three compounds were also low, which means that the results obtained are reliable. The RSD values of < 20% demonstrate the reproducibility and reliability of the developed method. However, the RSD values were higher (>20%) for a few of the sites, the concentrations of which need to be considered cautiously. The developed method, therefore, was applied to 39 sediment samples. The sediment samples were analysed under total gradient flow of 4000mL/min and with oven temperature at 60^0C using Phenomenex kinetex 100A 100 x 2.1 µm column. The concentrations of carbamazepine, diazepam, and lorazepam in the sediment samples are listed below in Table 3.13.

Table 3.13 Concentrations of target analytes in per gram of sediment sample.

Results of samples in ng/g of sediment			
Sampling batch/sites	Carbamazepine	Diazepam	Lorazepam
B1-1	1.99	<MDL	0.22
B1-2	1.26	<MDL	<MDL
B1-3	1.01	<MDL	0.31
B1-4	4.56	<MDL	0.13
B1-5	2.43	<MDL	0.10
B1-6	1.07	<MDL	0.17
B1-7	1.93	<MDL	0.30
B1-8	0.96	<MDL	0.11
B1-9	<MDL	<MDL	0.10
B1-10	0.56	<MDL	<MDL
B1-11	<MDL	<MDL	0.17
B1-12	0.96	<MDL	0.15
B1-13	<MDL	<MDL	0.23
B2-1	1.07	<MDL	0.50
B2-2	6.39	<MDL	0.10
B2-3	3.54	<MDL	0.21
B2-4	2.52	<MDL	0.12
B2-5	0.98	<MDL	0.26
B2-6	2.73	<MDL	0.08
B2-7	0.53	<MDL	0.21
B2-8	0.25	<MDL	0.32
B2-9	1.01	<MDL	0.74
B2-10	0.96	<MDL	0.12
B2-11	0.84	<MDL	0.18
B2-12	1.04	<MDL	1.78
B2-13	<MDL	<MDL	0.07
B3-1	0.84	<MDL	0.31
B3-2	4.90	<MDL	0.25
B3-3	2.09	<MDL	0.24
B3-4	1.99	<MDL	0.14
B3-5	1.21	<MDL	0.22
B3-6	0.83	<MDL	0.30
B3-7	0.58	<MDL	0.39
B3-8	0.45	<MDL	0.16
B3-9	0.43	<MDL	0.31
B3-10	0.59	<MDL	0.25
B3-11	1.20	<MDL	0.27
B3-12	1.77	<MDL	0.98
B3-13	<MDL	<MDL	0.21

3.5 Data analysis

3.5.1 Skyline software for raw data analysis

Skyline is mostly employed in the field of biology, particularly in peptide MRM data analysis. The functionality of Skyline is its ability to pick the peaks of multiple target compounds and optimise the collision energy, simultaneously. The raw MRM data obtained after running the calibration standards, extracts of attempted extractions and the sediment samples were imported into Skyline software. Such raw MRM data imported from the LCMS-MS into skyline was then analysed for optimising the collision energies for each transition of the target compounds.

Figure 3.6 and 3.7 below demonstrate the collision energy optimisation and MRM data analysis in skyline software.

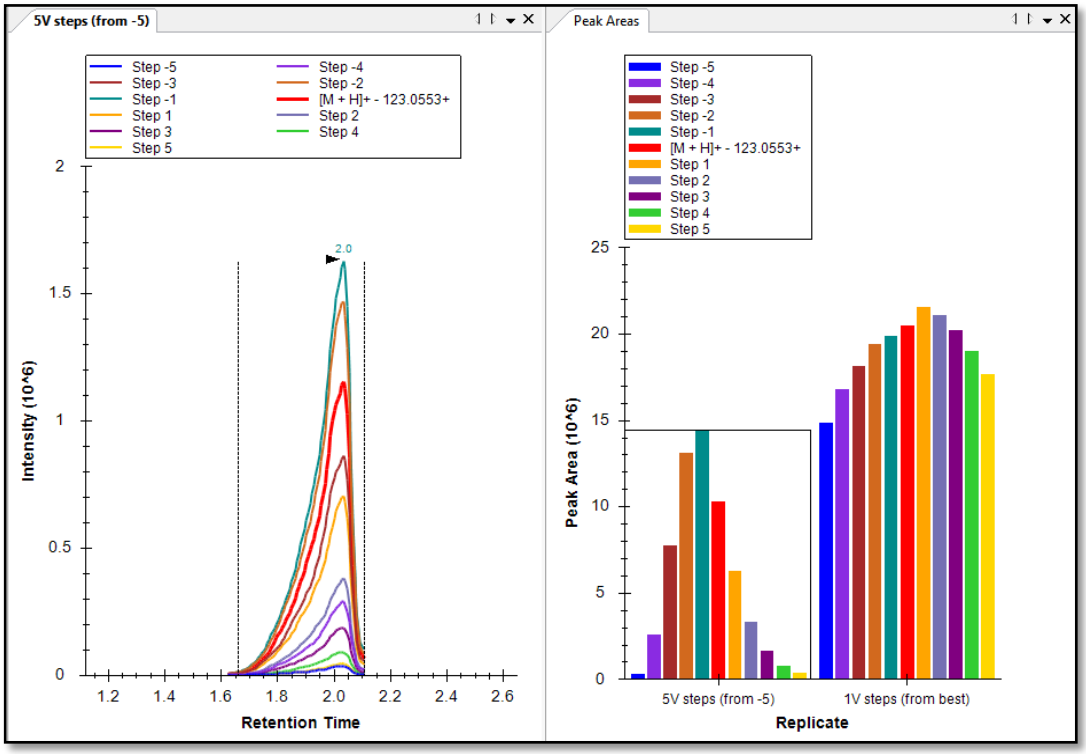


Figure 3.6 Collision energy optimisation in skyline

When the raw MRM data from the LCMS/MS is imported into Skyline, it optimises the collision energies at which each transition of the target compounds gives a better signal in order to obtain a clear chromatogram.

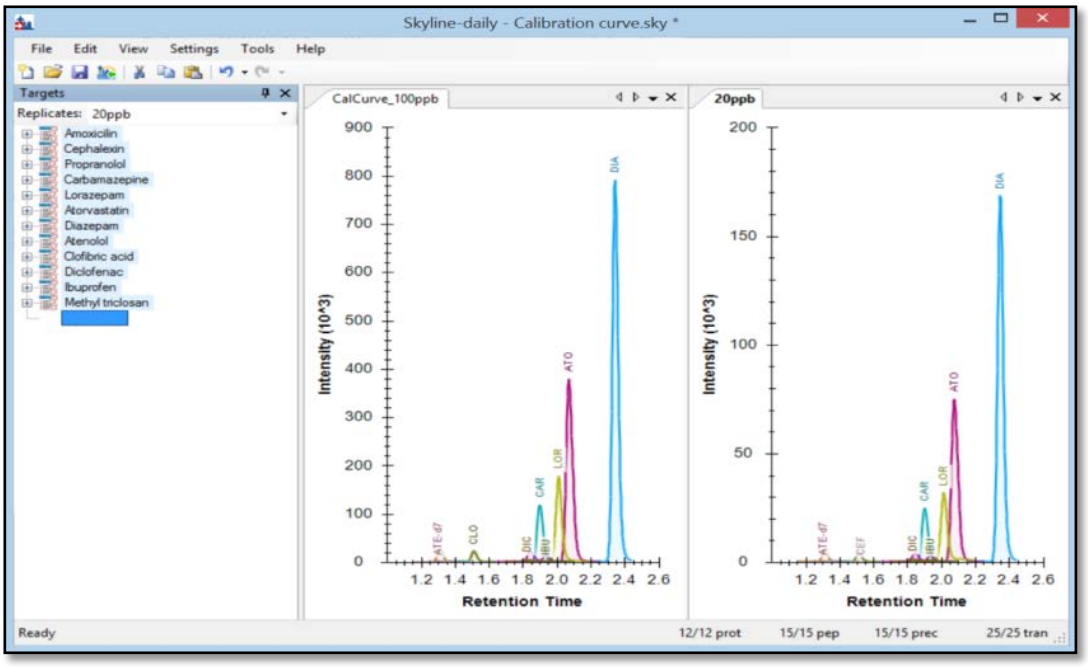


Figure 3.7 MRM data in skyline

The qualitative and quantitative transitions obtained for each target compound were checked at different collision energies simultaneously by Skyline (Figure 3.6). The process ended up in the selection of collision energies at which both the transitions gave intense signals thus, resulting in selective and sensitive quantification of target compounds (Figure 3.7).

In the case of calibration standards, the MRM data after collision energy optimisation in skyline was exported into Microsoft excel to obtain values. These values were then used to develop calibration curves for each target compound (Refer Table 3.5). The raw MRM data derived from the sediment sample analysis using the LCMS/MS was imported into Skyline. This raw MRM data of the sediment samples were compared with the MRM data of calibration standards in Skyline.

The MRM data of each target compound was compared with its respective MRM data of the calibration standard. For example, the MRM data of carbamazepine derived after sediment sample testing was compared with the MRM data of its calibration standard for retention times. The standard sample of carbamazepine elutes between retention times 1.88-1.9. If the MRM data from the tested sediment sample shows a peak between retention times 1.88-1.9, it confirms the presence of carbamazepine compound in the sample.

The identification of each target compound was confirmed in a similar way by comparing the MRM data with their respective MRMs of standards. The MRM data of the confirmed target compounds were then exported into Microsoft excel to obtain values. These values were further used in the developed calibration equations (Refer Table 3.5) to calculate the concentrations in the sediment samples. The concentrations were expressed in ngg^{-1}.

3.5.2 Univariate analysis of the results

The results obtained from the analysis of sediment samples were analysed using univariate analysis such as median, minimum (min) and maximum (max), standard deviation (STD). The largest and smallest values in the data were explained by minimum and maximum values.

3.6 Summary

This chapter has reviewed the two key aspects of this study. These are the selection of the compounds and the method development. The chapter started with discussing the design of the research project which included field study and laboratory work in Section 3.2. Selection of target EPPPs compounds which were one of the key aspects in this chapter was discussed in detail in Section 3.3.1. Section 3.3.2 further discussed the second important aspect, method development. The Chapter has explained the necessity for the development of a method for target EPPPs analysis in this study. The development of the MRM method applied in the quantification of target EPPPs was explained in detail. The selected test methods namely, sample preparation, ASE, SPE, were explained in detail in Sections 3.3.3.

The analysis of the data obtained from testing the sediment samples was discussed in Section 3.4. The section explained the use of skyline software for raw data analysis which was obtained from testing the sediment samples. The section further discussed the analysis of results using Univariate methods.

Chapter 4 Study Area and Sample Collection

4.1 Overview

Brisbane is Australia's third largest city after Sydney and Melbourne. It is also among the fastest growing cities in Australia (BITRE, 2013; Stimson and Taylor, 1999). With a subtropical climate, good infrastructure, business and career opportunities, Brisbane is rapidly undergoing urbanisation. According to the CHO Report (2014), the population of Queensland has grown rapidly in the past years. Figure 4.1 below shows the population trends in Queensland by age group from 1971. While the population of Queensland appears to be increasing, it is interesting to note that the ageing population has also increased noticeably and the trend continues to be increasing.

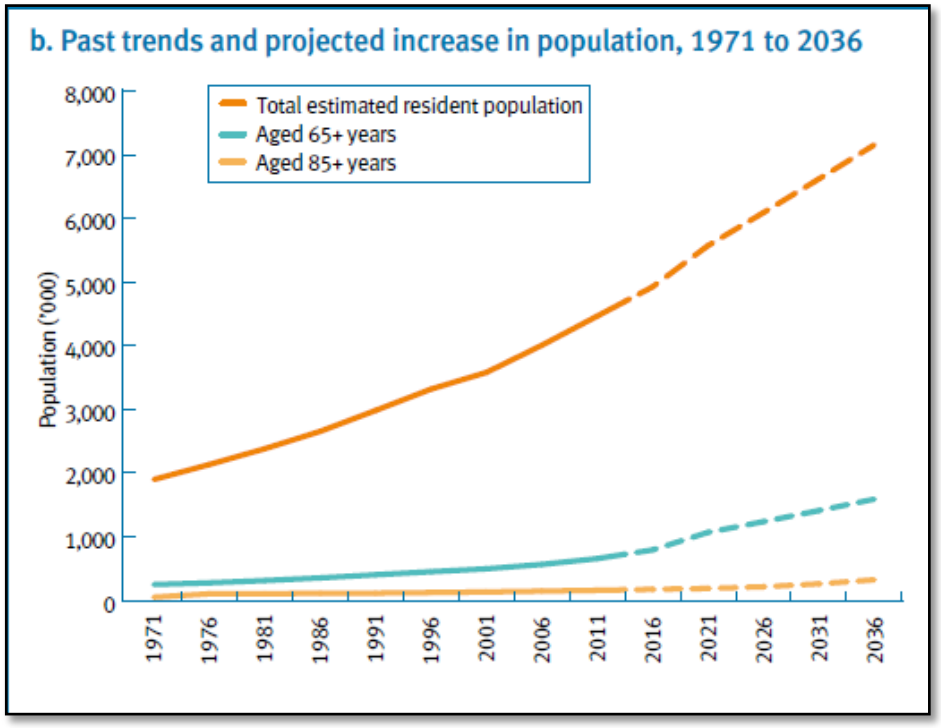


Figure 4.1 Population trends in Queensland - the past and projected increase from 1971-2036 (Source: CHO Report (2014)

The population of Queensland has grown in the past five years from 4.4 million in 2011 to 4.6 in 2013 (CHO Report, 2012, 2014). Between June 2012 and 2013, Queensland's population increased by 2% making it the third fastest growing city in

Australia (CHO Report, 2014). Consequently, the dwelling trends have also increased to accommodate the burgeoning population. According to the Queensland Treasury (2016) report, dwelling units approval rose by 1.1% in December 2015. Figure 4.2 below shows the increasing trend in dwelling approvals in past five years from 2010 – 2015. In fact, the dwelling approval trends rose only in Victoria (1.6%) and Queensland (1.1%) in the last year, whereas it fell in other states (ABS, 2016), thereby highlighting significant urbanisation in Queensland.

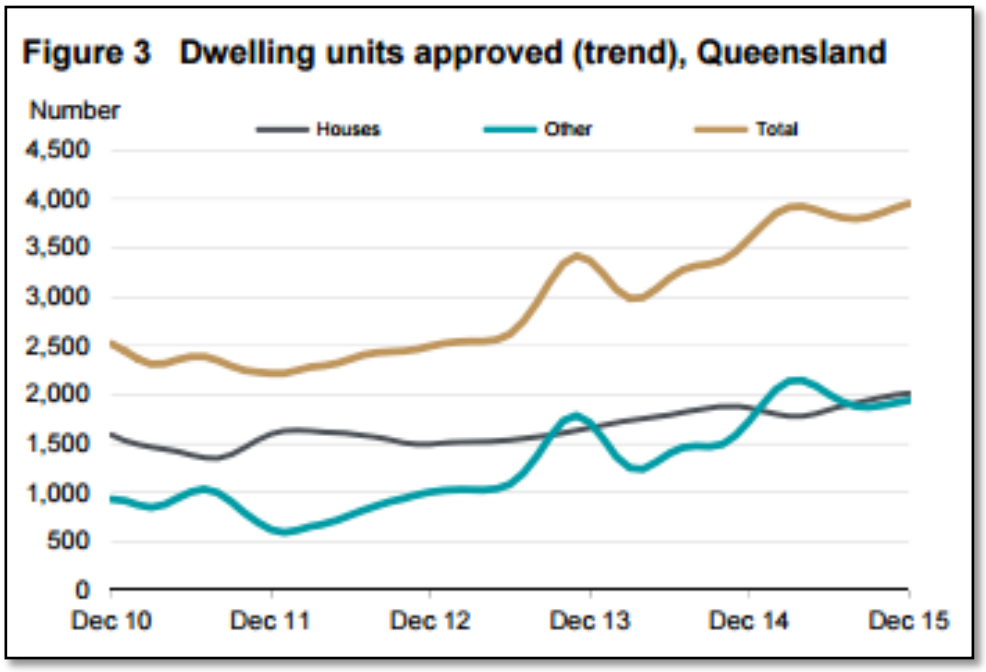


Figure 4.2 Dwelling units approval trend in Queensland (Decemner 2010- 2015) (Source: Queensland Treasury (2016).

In addition, the review of research literature undertaken in Chapter 2 also concluded that population growth associated with urbanisation in Queensland and ageing population is responsible for the increased consumption of pharmaceutical drugs. Brisbane River is the major river flowing right through the urbanised areas of Brisbane (Figure 4.4). Therefore, it is likely that the Brisbane River and its sediments would be polluted with EPPPs. This chapter, therefore, is focused on the selection of study area and sample collection in the Brisbane River.

4.2 Selection of study area

The Brisbane River is the major river in SEQ and used for transport and recreational activities. It flows through the Brisbane City and finally meets the Moreton Bay. Hence, significant urban and residential complexes and businesses can be seen along both the banks of the river. Such an exposure to urbanisation and population growth is likely to have impacted the aquatic environment of the Brisbane River and the sediment bed. Figure 4.4 below shows the existing urban areas in Brisbane. According to the estimates by BITRE (2013), about 88% of population growth and 89% of dwelling approvals occurred within the existing urban footprint boundary, thereby highlighting the significant urbanisation along the selected study area of the Brisbane River.

Population growth associated with urbanisation in SEQ has led to an increase in population density in the existing urban area during 2001-2011. Figure 4.3 below shows the change in population density in SEQ from 2001-2011. An increase in population and density has occurred in the urbanised area along the Brisbane River, thereby resulting in the increase in the urban area.

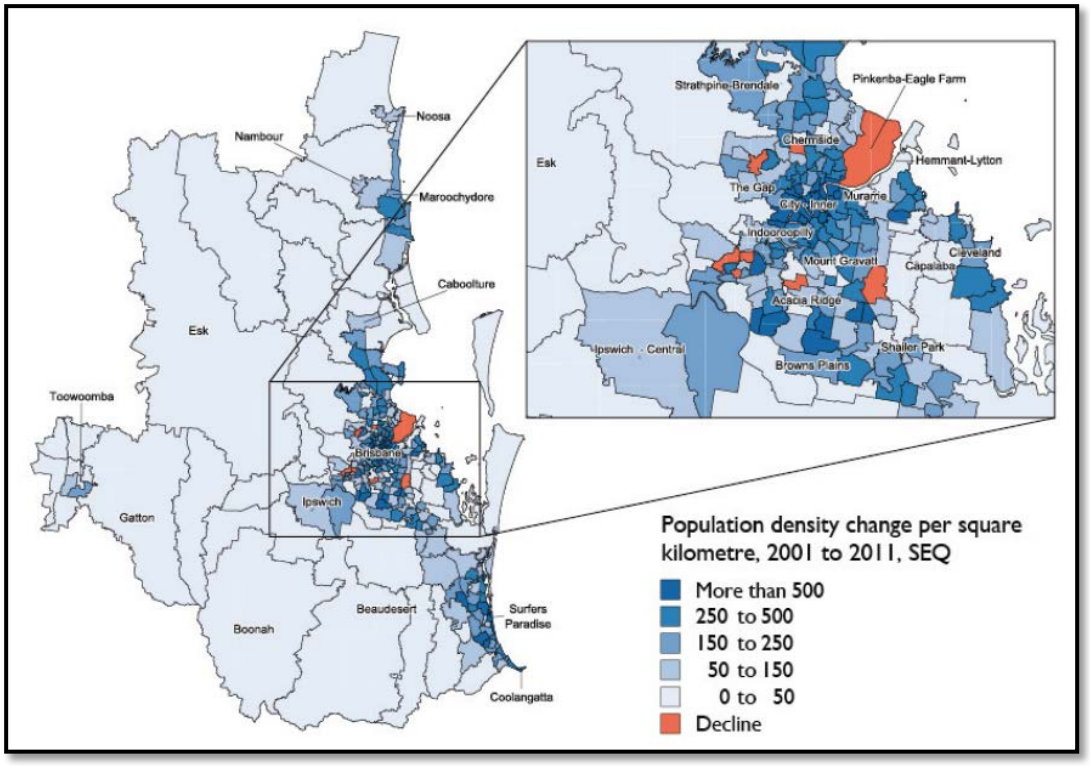


Figure 4.3 Change in population density in SEQ during the period 2001-2011 (Source: BITRE (2013).

A stretch of Brisbane River that is subjected to urbanisation and population growth (Figure 4.4) was thus selected for sediment sampling in order to achieve the aim and objectives of this study.

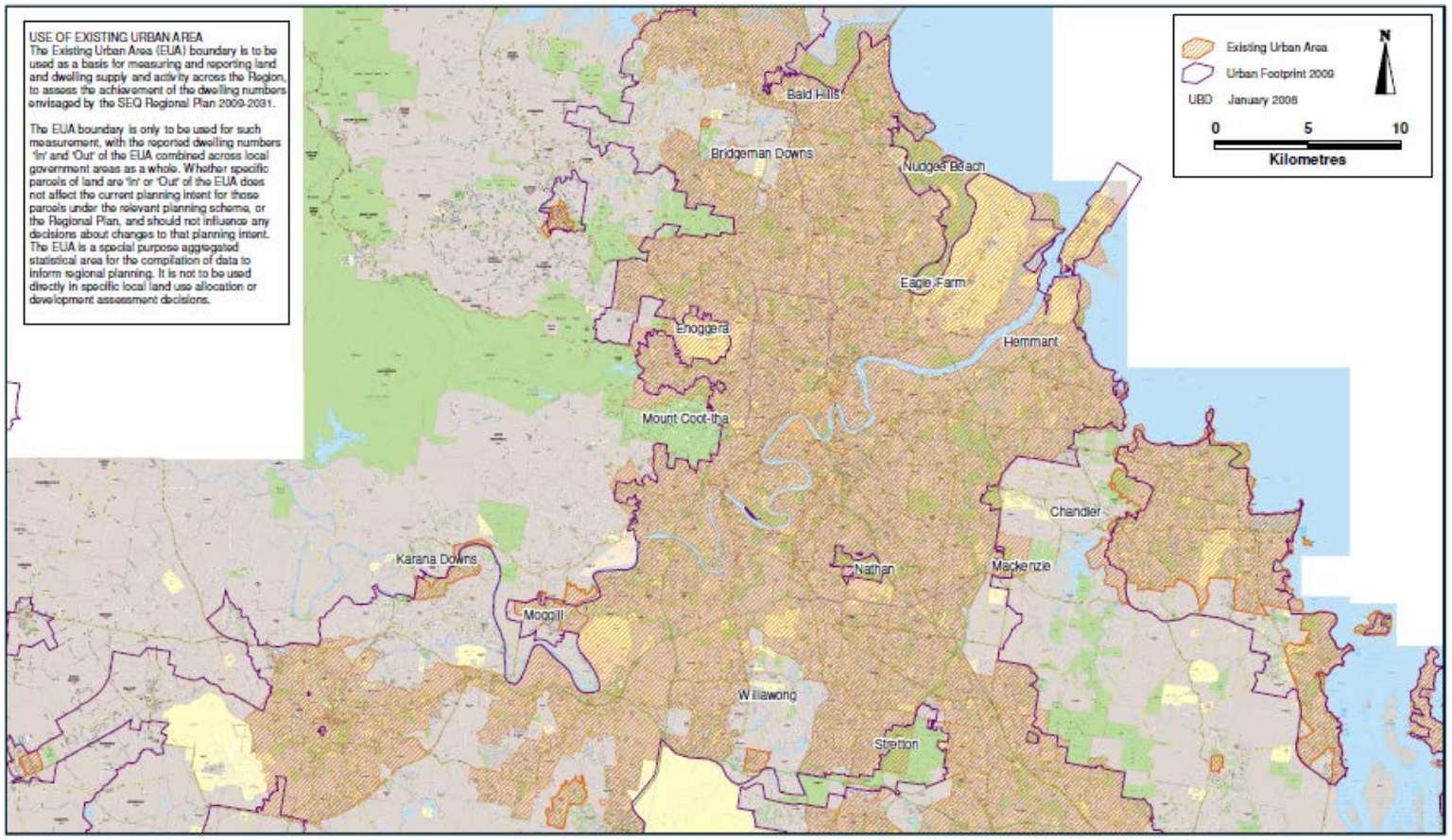


Figure 4.4 Existing urban areas along the Brisbane River

(Source: http://www.qgso.qld.gov.au/about-statistics/existing-urban-area/index.php)

It can be concluded from the above Figure 4.4, that urbanisation is relatively less upstream towards Karana Downs and increases significantly downstream of the river, thereby implying greater population density downstream than upstream of the Brisbane River. Therefore, such an extensive urbanisation along the Brisbane River is likely to cause EPPPs pollution in the river as noted by Ellis (2006) and Stewart et al. (2014). Investigating the sediments for the presence of EPPPs was crucial in order to derive a holistic view of EPPPs pollution in the Brisbane River. Hence, after the critical review of the literature and assessment of Figure 4.3, a length of the Brisbane River stretching from upstream near Karana Downs to downstream to the mouth of the river was selected. The selected stretch was 56km in length.

After finalising the study area, sampling sites were selected. In total 13 sites were selected and numbered from upstream to downstream as shown in Figure 4.5.

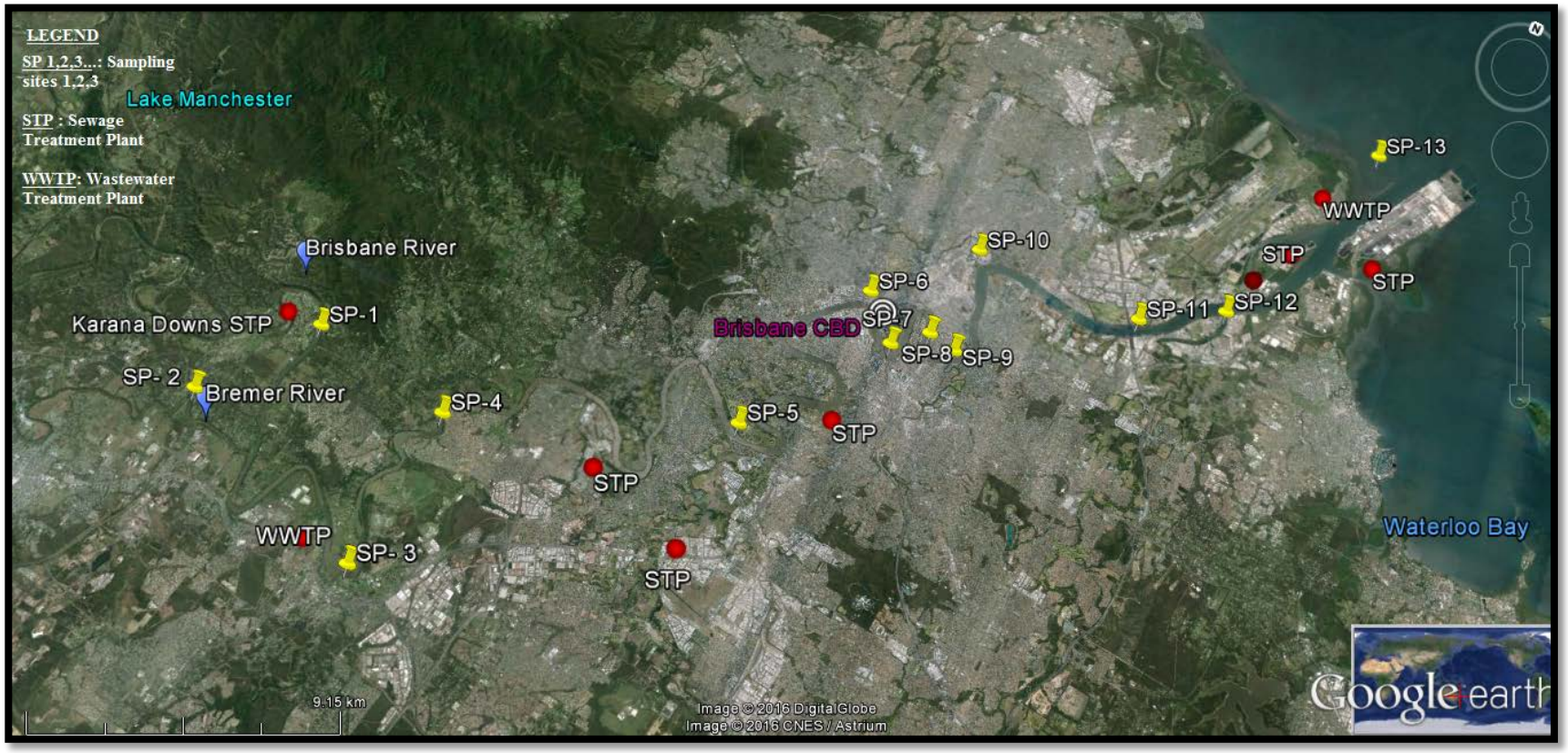


Figure 4.5: Digital map of Brisbane city (Source: Google earth).

4.3 Selection of sampling sites

The sampling sites for this study were selected to obtain representative sediment samples of the entire study area. The sampling sites were chosen such that they showed varying degree of urbanisation, less upstream and increasing downstream. This was to understand the possible influence that urbanisation has on the occurrence of EPPPs. The coordinates of the sites were obtained for easy location of the sites for sampling sediments. The list of the sampling sites is given below in Table 4.1.

Table 4.1 Sampling sites along the Brisbane River

Name	Code	Latitude	Longitude	Land use characteristics
Karana Downs	SP1	27°32'10.13"S	152°50'53.15"E	Suburban area
Confluence of Bremer and Brisbane River	SP2	27°34'36.33"S	152°51'4.46"E	Suburban area
Confluence of Woogaroo Creek and Brisbane River	SP3	27°36'20.05"S	152°54'10.65"E	Urban area
Confluence of Pullen Creek and Brisbane River	SP4	27°33'12.89"S	152°54'6.45"E	Urban area
Confluence of Oxley Creek and River	SP5	27°31'36.03"S	152°59'38.65"E	Urban/ industrial area
West end	SP6	27°28'7.31"S	153° 1'0.60"E	Urban/ commercial area
QUT GP	SP7	27°28'48.95"S	153° 1'54.24"E	Urban/ commercial area
Story Bridge	SP8	27°28'36.60"S	153° 2'41.17"E	Urban/ commercial area
Confluence of Norman Creek and Brisbane River	SP9	27°28'45.69"S	153° 2'58.27"E	Urban/ commercial area
Confluence of Breakfast creek and Brisbane River	SP 10	27°28'21.16"S	153° 3'12.68"E	Commercial area
Near the Gateway Motorway Bridge	SP11	27°26'13.07"S	153° 6'56.40"E	Commercial area
Confluence of Bulimba creek and Brisbane River	SP12	27°26'26.49"S	153° 7'27.70"E	Commercial/ industrial area
Mouth of Brisbane River	SP 13	27°21'42.13"S	153° 9'21.08"E	Commercial/ industrial area

Sites SP1 to SP5 are extended throughout the upstream of the study area until the Brisbane CBD area, whereas sites SP6 to SP13 are located downstream of the river from the CBD area to its estuary. Sites SP6, SP7, SP8, SP9, and SP10 are located in Brisbane CBD area whereas, sites SP11, SP12and SP13 are around the Brisbane Port area. Sites SP3, SP4, SP5, SP9, SP10 and SP12 were selected near the confluence of creeks (Table 4.1) and the Brisbane River to identify any possible sources for the EPPPs loads.

4.4 Sampling

In any environmental study, sampling is an important stage. Collection of samples such as sediments is difficult since it is the deepest section of the water column (Cowgill, 1994). A sediment bed acts as a sink for the pollutants discharged to the water environment (Antonić and Heath, 2007), and it also provides habitat for the lowest aquatic flora and fauna (Choi et al., 2014). Therefore, the occurrence of pollutants such as EPPPs in the sediment environment is concerning as these micropollutants are likely to interfere with the lifecycles of the organisms dwelling in such environments causing negative impacts (Choi et al., 2014; Santos et al., 2010).

Studying sediments of the Brisbane River was therefore particularly crucial after a recent study (Scott et al., 2014) which showed the occurrence of EPPPs in the surface waters of Brisbane River. In addition, the literature review in Chapter 2 highlighted the knowledge gap that there has been no study into sediments of the Brisbane River for EPPPs. Thus, understanding the potential threats of EPPPs to the environment and the fact that Brisbane River is polluted by EPPPs, this study was therefore focused on investigating the occurrence of EPPPs in the sediments of the Brisbane River. The aim was to obtain a holistic picture of EPPPs pollution in the urbanisation influenced stretch of the Brisbane River system. Sampling of sediments was carried in three episodes; in July 2014, September 2014 and in December 2014. The sampling events spread across dry and wet seasons in order to study any possible seasonal effects. Sediments from the uppermost layer are the most exposed to pollution, thereby acting as a sink for pollutants. Thus, the collection of sediment samples from this top layer is suitable for environmental studies (Cowgill, 1994), such as this study. Collection of sediment samples is possible using appropriate sampling techniques and sampler.

The collection of the sediment samples was carried out by applying a grab sampling technique as used in the studies undertaken by (Chen and Zhou (2014); Vazquez-Roig et al. (2010)). This is a preferred technique for investigative analysis in environmental studies and in particular for sampling surface sediments (Cowgill, 1994). Accordingly, the selected sampling sites were accessed using a boat and the sediments were collected using a Van Veen Grab Sampler (Figure 4.6). A Van Veen

Grab sampler is stainless steel equipment designed to collect sediment samples from the depths of the waterbodies. The equipment is heavy for the easy descent into the sediment bed. The grab sampler has two jaws that are locked while being dropped into the water. As the grab sampler hits the sediment bed, the jaws open allowing the sediment to be collected inside the sampler. As the sampler is raised, the jaws close preventing the collected sediment from escaping (Figure 4.7). The jaws are opened again for transferring the collected sediment sample into the container (Figure 4.8).

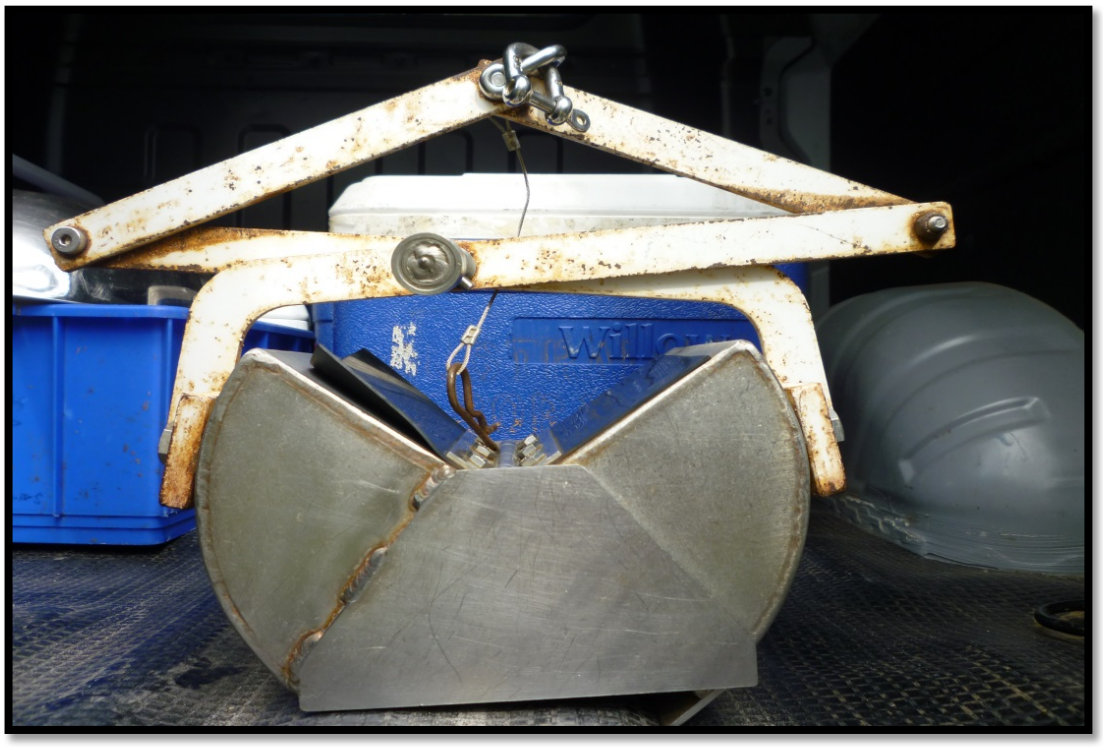


Figure 4.6 The stainless steel Van Veen Grab sampler used in sediment sampling

Figure 4.7 Sampling of sediment using the Van Veen Grab sampler

Figure 4.8 Opened jaws of the Van Veen Grab Sampler

The collected sediment samples were transferred into a clean stainless steel container and then using a clean stainless steel spoon, the samples were transferred into 250mL wide mouth clean glass jars (Figure 4.9). The glass jars were labelled appropriately including the sampling location, date and time of sampling. Gloves were worn and changed after each sediment sampling to avoid cross contamination.

Figure 4.9 Transferring the collected sediment sample into acetone washed wide mouth glass containers

The glass jars were immediately freeze stored in ice in eskys (Figure 4.10) and during entire transportation to the laboratory where they were stored at -20^{o}C in a freezer until further analysis.

Figure 4.10 Storage of the sediment sample bottles during the sampling event.

4.5 Summary

This Chapter discussed the importance of selecting Brisbane River as the study area which was an essential requirement in order to achieve the aim of this study. The study area in the Brisbane River stretched approximately to a distance of 56 km where significant urbanisation was evident. In addition, the selected stretch also included the confluence of Bremer River from southwest and creeks that originate from the urban areas. The sampling sites selected were located along the selected stretch of the river which facilitated in obtaining representative sediment samples.

Analysis of sediment samples was intended to provide an in -depth understanding of the occurrence and distribution of the target EPPPs in the Brisbane River sediments. The sediment samples from the selected sampling sites were collected using a grab sampling technique which was found to be the most appropriate technique for this type of investigative study. The application of grab sampling technique using Van Veen Grab sampler resulted in the collection of representative sediment samples for

this study. The collected sediment samples were then analysed for target EPPPs and the results obtained have been discussed in the following Chapter 5.

Chapter 5 Results and Discussions

5.1 Overview

EPPPs are the 'emerging contaminants' of concern. These contaminants have been receiving considerable attention from the scientific community lately (Beretta et al., 2014; Chen and Zhou, 2014; Choi et al., 2014; Kolpin et al., 2004; Scott et al., 2014; Vazquez-Roig et al., 2010). Although these micropollutants have been occurring in very low concentrations (ppb, ppt) in different environmental compartments, they are potentially harmful and toxic to the environment (Daughton and Ternes, 1999; Hernando et al., 2006). EPPPs substances can bioaccumulate in aquatic flora and fauna (Henschel et al., 1997; Vernouillet et al., 2010), thereby posing a potential risk to the entire aquatic ecosystem.

In Australia, research into EPPPs has been limited to surface waters. In fact, there is only one reported study by Scott et al. (2014) which demonstrates EPPPs occurrence in the surface waters of the Brisbane River. However, in order to understand the EPPPs pollution in the entirety of the Brisbane River system and to assess its potential risks to its aquatic environment, it is critical to study their occurrence in sediments. Hence, this study aimed at detection and quantification of target EPPPs in the Brisbane river sediments. This chapter discusses the analysis of the target EPPPs in the Brisbane River sediments and the results obtained.

5.2 Pharmaceutical analysis

As pointed out previously, there has been no investigation of EPPPs in the Brisbane River sediments, probably because sediments are complex matrices (Babic and Pavlovic, 2013), which makes an analysis of EPPPs difficult. Therefore, in order to investigate the occurrence of EPPPs in the Brisbane River sediments, this research required method development for quantification of the target EPPPs. Thus, an extraction and MRM method was developed with R^2 values of 0.99 which mean the method was reliable. The method was applied to 39 sediment samples which resulted

in quantification of psychiatric compounds of carbamazepine, diazepam and lorazepam.

The raw data obtained after testing the sediments was analysed using the skyline software. The identification of the target compounds were confirmed by comparing the retention times, quantitative and qualitative peaks of the target compounds with their respective calibration standards. The confirmed target analytes were then quantified using the calibration equations. The relative standard deviation (RSD) of the concentrations of the detected target analytes ranged from <20% for most of the samples as reported in other studies (Chen et al., 2013; Moreno-González et al., 2015; Vazquez-Roig et al., 2010), thereby demonstrating the precision of the instrument, repeatability and reliability of the extraction method for the detected target EPPPs. However, the RSD values for a few samples, particularly diazepam were high, with up to 113% indicating possible matrix effect. This means that the concentration results for these samples are required to be considered cautiously. Higher RSD values were reported in a study conducted by Nebot et al. (2015) wherein the RSD values ranged from 0-464% for the target analytes.

Statistical analysis of the results of the detected target EPPPs was performed using univariate analysis which resulted in minimum, maximum and mean concentrations of the target analytes. Table 5.1 below shows the minimum, maximum concentrations of the detected target compounds.

Table 5.1 Minimum, maximum and mean concentrations in ngg^{-1}of detected target EPPPs in the Brisbane River sediments

Compound	Minimum	Maximum	Mean
Carbamazepine	<MDL	6.39	1.07
Diazepam	<MDL	<MDL	<MDL
Lorazepam	<MDL	1.78	0.22

The values given in Table 5.1 were compared with the values reported in other studies conducted around the world in order to understand the extent and seriousness of EPPPs pollution in the Brisbane River sediments. Table 5.2 below shows the concentrations of detected target compounds reported by studies conducted in

different environmental compartments around the world. The occurrence and distribution of target compounds namely; carbamazepine, diazepam and lorazepam in various environmental matrices demonstrates that these EPPPs are ubiquitous in the environment.

Table 5.2 Occurrence and distribution of target compounds in various environmental compartments around the world

Author	EPPPs	Concentrations	Media	Region/country
(Jelić et al., 2009)	Carbamazepine,	10.1- 12.7ngg^{-1}	WWTP Sludge	Tudela, Arazuri -Spain
	Diazepam	3.2 – 8.5 ngg^{-1}		
(Azuma et al., 2015)	Carbamazepine	22 ngl^{-1}	Stream(surface water)	Yodo River basin, Kansai-Japan
		25 ngl^{-1}	Tributary (surface water)	
		96 ngl^{-1}	STP effluent	
		2 ngl^{-1}	STP effluent (after ozonation)	
(Beretta et al., 2014)	Carbamazepine,	0.41 ngg^{-1}	Sediments	Todo os Santos Bay and North coast Salvador, Bahia,Brazil
	Diazepam	0.39 ngg^{-1}		
(Birch et al., 2015)	Carbamazepine	< LOD – 2.7 ngl^{-1}	Sydney estuary	Sydney,Australia
(Darwano et al., 2014)	Carbamazepine	3 ngg^{-1}	River	Montreal, Canada
		<MDL	Sediment	
		70 ngg^{-1}	WWTP influent	
		147 ngg^{-1}	WWTP effluent	
(Du et al., 2014)	Carbamazepine	140-160 ngl^{-1}	WWTP influent	Waco, Texas, USA
(Kasprzyk-Hordern et al., 2007)	Carbamazepine	<MDL – 9 ngl^{-1}	River Taff	Wales,UK and Poland
		311 – 794 ngl^{-1}	River Warta	
(Ferguson et al., 2013)	Carbamazepine	0.5 -10 ngl^{-1}	Lake Michigan	Michigan city, USA
(French et al., 2015)	Carbamazepine	0.1- 0.5 ngl^{-1}	Wastewater and surface water	Darwin, Australia
(Roberts et al., 2015)	Carbamazepine	57 g/day	STP Effluent load	Canberra, Australia
(Jiang et al., 2014)	Carbamazepine	Not detected – 3.83 ngl^{-1}	seawater	Southwestern Taiwan
(Klosterhaus et al., 2013)	Carbamazepine,	5.2- 44.2 ngl^{-1}	Surface Water	San Francisco, USA
		Not detected ngg^{-1}	Sediments	
		1.3- 5.3 ngg^{-1}	Mussels	
	Diazepam	<reporting limit – 0.5	Surface Water	
		< reporting limit	Sediments	
		< reporting limit	Mussels	
(Kolpin et al., 2004)	Carbamazepine	0.002-0.263	Surface Water	Iowa, USA

Table 5.2 Occurrence and distribution of target compounds in various environmental compartments around the world (continued from previous page)

Author	EPPPs	Concentrations	Media	Region/country
(Kosma et al., 2014)	Carbamazepine	< detection limit – 54 ngl^{-1}	WWTP influents	Greece
(Vazquez-Roig et al., 2010)	Carbamazepine	1.43-5.77 ngg^{-1}	Soil	Valencia, Spain
		1.43 – 6.85 ngg^{-1}	Sediment	
	Diazepam	4.65 ngg^{-1}	Soil	
		2.50 – 3.72 ngg^{-1}	Sediment	
(Wu et al., 2015)	Carbamazepine	45 ngl^{-1}	WWTP Influent	Huangpu River, China
		35 ngl^{-1}	WWTP Effluent	
		25 ngl^{-1}	River water	
		<LOQ	Drinking water	
	Diazepam	9.5 ngl^{-1}	WWTP Influent	
		9.7 ngl^{-1}	WWTP Effluent	
		24.3 ngl^{-1}	River water	
		1.9 ngl^{-1}	Drinking water	
	Lorazepam	35.8 ngl^{-1}	WWTP Influent	
		<LOQ	WWTP Effluent	
		4.0 ngl^{-1}	River water	
		Not detected	Drinking water	

The mean concentration of carbamazepine (Table 5.1) in the current study was higher than the mean concentrations reported by Beretta et al. (2014) in sediments and by Moreno-González et al. (2015) in surface waters. On the other hand, the mean concentrations for lorazepam was again found to be higher when compared with the mean concentration in surface waters reported by Moreno-González et al. (2015), whereas the mean concentration of diazepam was found to be below MDL compared to 0.39ngg^{-1} reported in the study by Beretta et al. (2014). The increased mean concentrations of carbamazepine and considerably lower values for lorazepam and diazepam suggest possible increased consumption of carbamazepine here in Queensland. Additionally, the higher mean concentrations of carbamazepine also demonstrate resistance of the drug to wastewater treatment, thereby remaining persistent in the environment as stated by Ternes et al. (2004) and Clara et al. (2004).

The obtained concentrations were then displayed on the selected study area of the Brisbane River (Figure 5.1) in order to study the occurrence and distribution of the target EPPPs along with the degree of urbanisation. The target compounds were distributed along the entire length of the study area surrounded by urbanisation from

upstream to downstream indicating urban influence on the occurrence of the compounds.

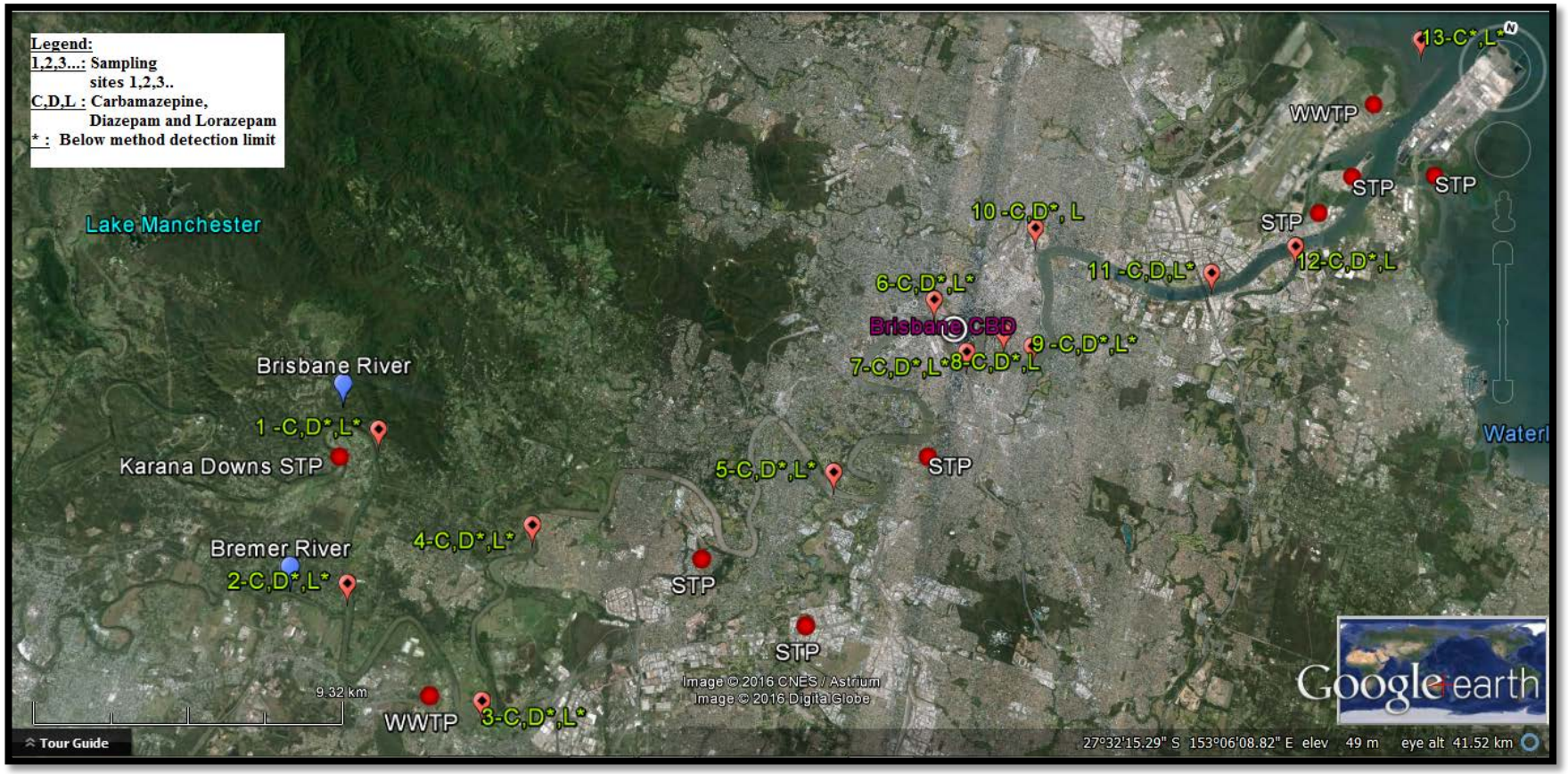


Figure 5.1 Occurrence and distribution of target analytes in the selected stretch of the Brisbane River sediments.

Source: Google earth

The detection of the psychiatric compounds suggested EPPPs occurrence in the Brisbane river sediments and this addresses the knowledge gap about their presence in the river. Moreover, it can be concluded from the literature review (chapter 2) and Section 3.3.1 that occurrence of psychiatric compounds in the Brisbane river sediments raises concerns about potential toxicity to the aquatic flora and fauna in the River.

5.3 Discussion

The objective of this research was to detect and quantify the target EPPPs in the Brisbane River sediments to achieve the aim about its relationship to urbanisation. As stated in Figure 4.3, the population density increased significantly in inner city and middle suburbs (east and west) which are located around the sampling sites in the study area of the current research. Figures 5.2 and 5.3 below show the Brisbane sectors and regions and the population density changes in these areas from 2001-2011. The changes indicate rapid urbanisation and population growth along the Brisbane River.

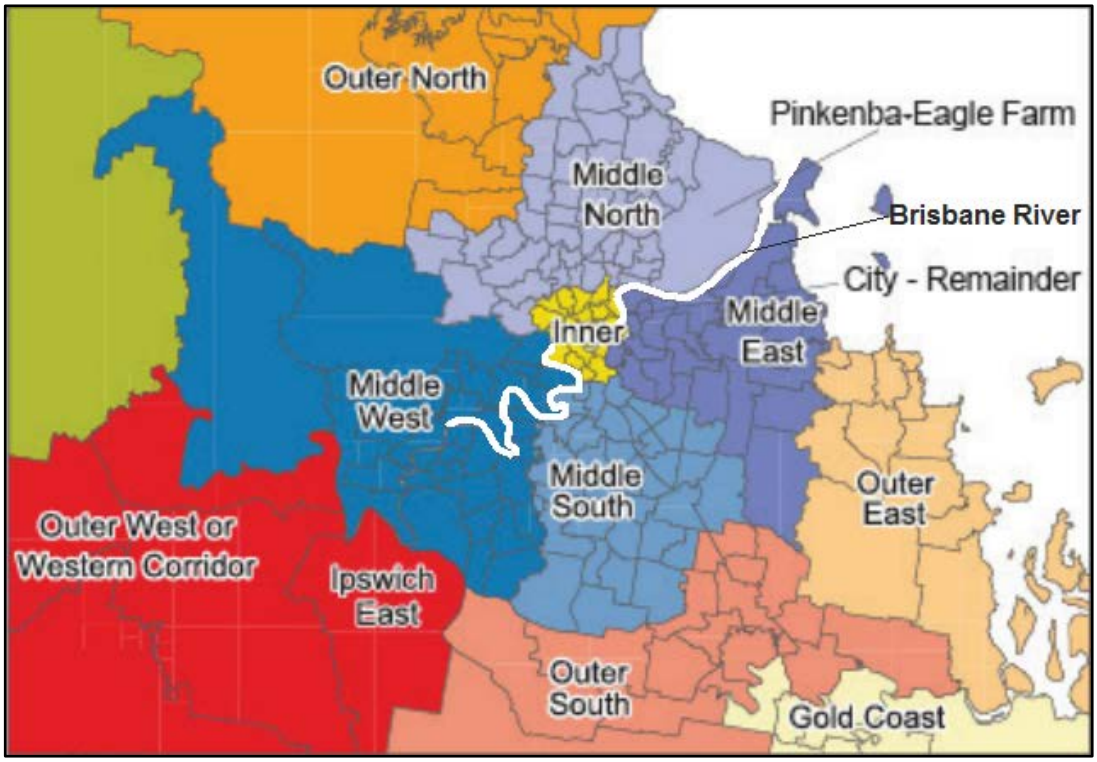


Figure 5.2 Regions, sectors, suburbs and statistical areas in South East Queensland (Source: Adapted (BITRE, 2013).

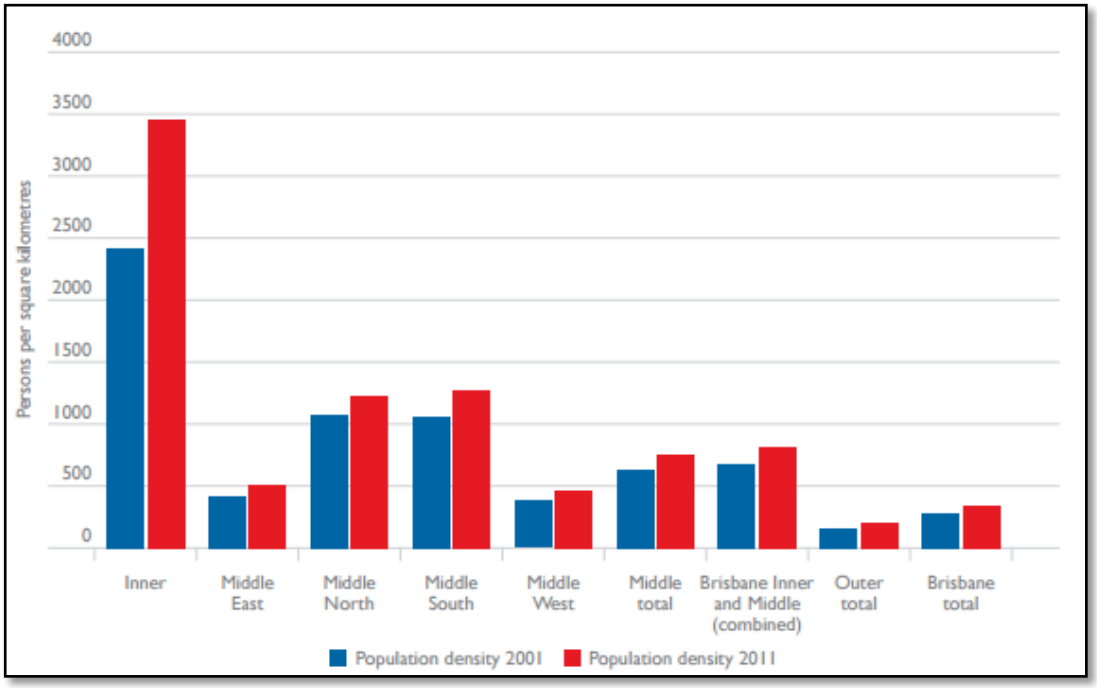


Figure 5.3 Population density in Brisbane sectors from 2001-2011 Source: (BITRE, 2013)

The study area of the Brisbane River falls right in this urbanised areas. Therefore, the occurrence of target EPPPs in the sites exposed to such increased population density shows a direct correlation between urbanisation and EPPPs occurrence thus, achieving the aim of the current research.

While urbanisation plays a crucial role in EPPPs occurrence, the release of these pollutants is not direct. In fact, there are many factors and sources that release these micropollutants and affect its occurrence and distribution. Therefore, the discussion of the results is divided into two sections according to two major hypotheses.

Literature review confirmed that weather events such as rainfall and tides impact the occurrence of EPPPs in aquatic environments. Since EPPPs occur in low concentrations of ppb and ppt, any change in the environment is likely to impact its fate in the environment. Thus, the first section discusses the possible impacts of weather events on the occurrence and distribution and the second part discusses the possible impacts of sewerage system on the occurrence and distribution of the target EPPPs. Studying the sewerage system of Brisbane was appropriate since wastewaters in the sewerage system are a point source for excreted unmetabolized EPPPs into the aquatic environment (Fent et al., 2006; Kosma et al., 2014).

5.3.1 Impacts of weather events on distribution of detected target EPPPs in the Brisbane River sediments

The target EPPP compound carbamazepine was detected in all the sites along the Brisbane River (Figure 5.1). The concentrations of carbamazepine in the current study was found to be in line with the concentrations stated by Stewart et al. (2014) in their study (<2 ngg^{-1}) on the sediments of urban environments in New Zealand thereby suggesting an urban influence on the occurrence of such EPPPs here in Brisbane River sediments. In regards to Australia, there is no data about recent consumption rate of carbamazepine, but according to the statistical information from ABS (2015c), information from CHO Report (2012; 2014)), and from above Figure 5.1, it can be concluded that carbamazepine is a widely prescribed and consumed drug to treat mental health problems in Australia. Such widespread consumption by the population associated with urbanisation is likely to be the reason behind its

occurrence in the Brisbane River sediments. Furthermore, it also demonstrates that urbanisation acts as a catalyst in the EPPPs pollution.

In regards to diazepam, the concentrations were quite low (<MDL) compared to the concentrations reported by Vazquez-Roig et al. (2010) in sediments in Spain, whereas it was not detected in sediment samples analysed by Silva et al. (2011) and Moreno-González et al. (2015). The low concentrations could mean that diazepam is comparatively less consumed and therefore gets diluted leaving very limited opportunity for accumulation in sediments. However, there is no information about diazepam consumption in Australia to support this hypothesis. Moreover, diazepam is photodegradable (Calisto et al., 2011; Straub, 2008). Therefore, although it might be present in the surface waters of the Brisbane River, it is highly likely that diazepam might have been degraded in surface waters under solar radiation leading to its dilution and therefore limited availability for accumulation in sediment. Hence, this could be the possible reason for <MDL concentrations in the Brisbane River sediments.

Similar reasoning could be true for the low concentrations of lorazepam in the Brisbane River sediments given it belongs to the same class as diazepam (benzodiazepines) and it is photodegradable (Calisto et al., 2011). Lorazepam concentrations were found to be higher as compared to the results of the studies by Jelić et al. (2009), Moreno-González et al. (2015) and Silva et al. (2011), where lorazepam was not even detected in the sediments. It can be therefore concluded that diazepam and lorazepam are significantly consumed in Australia compared to other countries, but possibly less compared to carbamazepine. Additionally, their susceptibility to photodegradation might also have impacted on its occurrence in the Brisbane River sediments. While these drugs are susceptible to photodegradation, the efficiency of the process is dependent on seasons and other constituents in the water (Fent et al., 2006) and therefore, more information is required to support this hypothesis.

The occurrence of psychiatric drugs in the Brisbane River sediments also reflects on the health of the Queenslanders and the impact of urbanisation. As pointed out in the literature review, psychiatric compounds are a serious threat to the environment

(Fent et al., 2006; Straub, 2008), particularly with carbamazepine being so ubiquitous since it is bioaccumulative and lethal to smaller living organisms in aquatic environments (Clara et al., 2004; Vernouillet et al., 2010). Having concluded the fact that the Brisbane River is polluted by EPPPs, it was now important to understand the variation in the occurrence of these compounds in the sediments.

It was interesting to note that out of the three sampling episodes conducted in the current study, the concentration of carbamazepine decreased from upstream to downstream suggesting dilution of the target EPPPs. The concentrations also decreased with every sampling episode suggesting a possible effect of weather conditions on the occurrence of these pollutants. Sampling of the sediments was carried out in July 2014, September 2014 and December 2014. Each sampling event fell into different seasons of winter, spring and summer, respectively. Figure 5.4 illustrates the influence of rainfall events in different seasons on the distribution of the detected target EPPPs along the Brisbane River. The Y axis (Figure 5.4) represents the concentrations of carbamazepine, diazepam and lorazepam. Figures 5.5, 5.6 and 5.7 show the average rainfalls in Queensland in the respective months of July, September and December 2014.

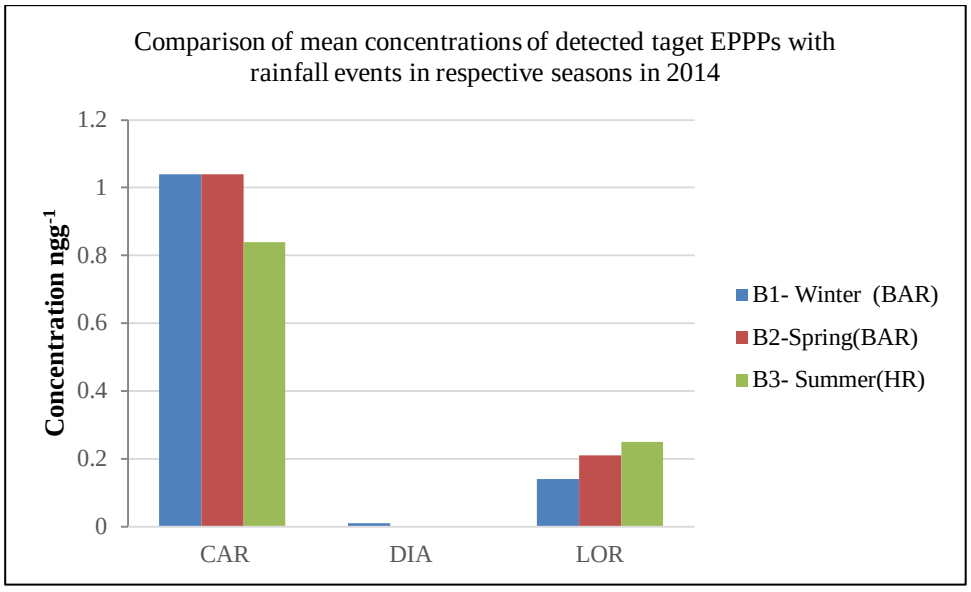


Figure 5.4 Comparison of mean concentrations of detected target EPPPs with rainfall events in respective seasons

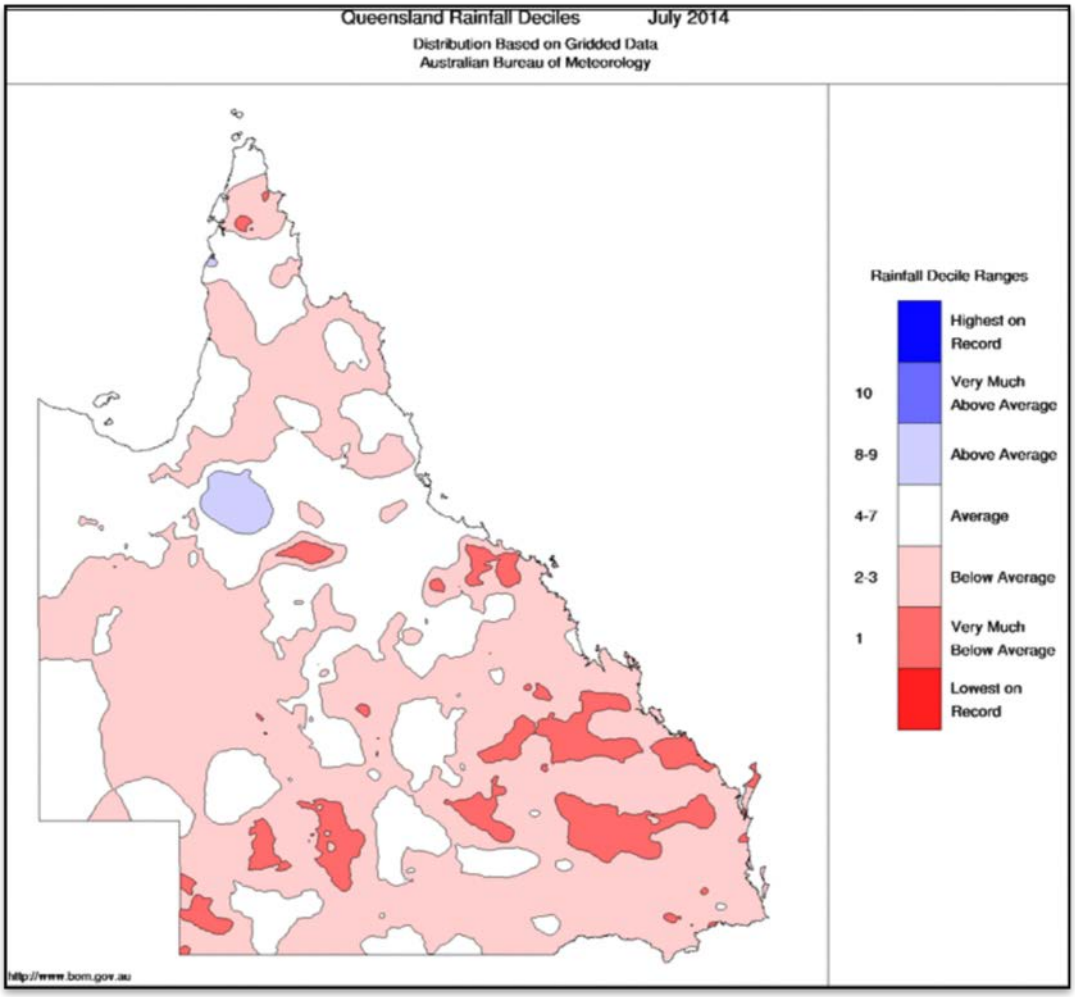


Figure 5.5 Rainfall in South East Queensland July 2014

Source: http://www.bom.gov.au/web03/ncc/www/awap/rainfall/decile/month/colour/history/qd/2014070120140731.gif

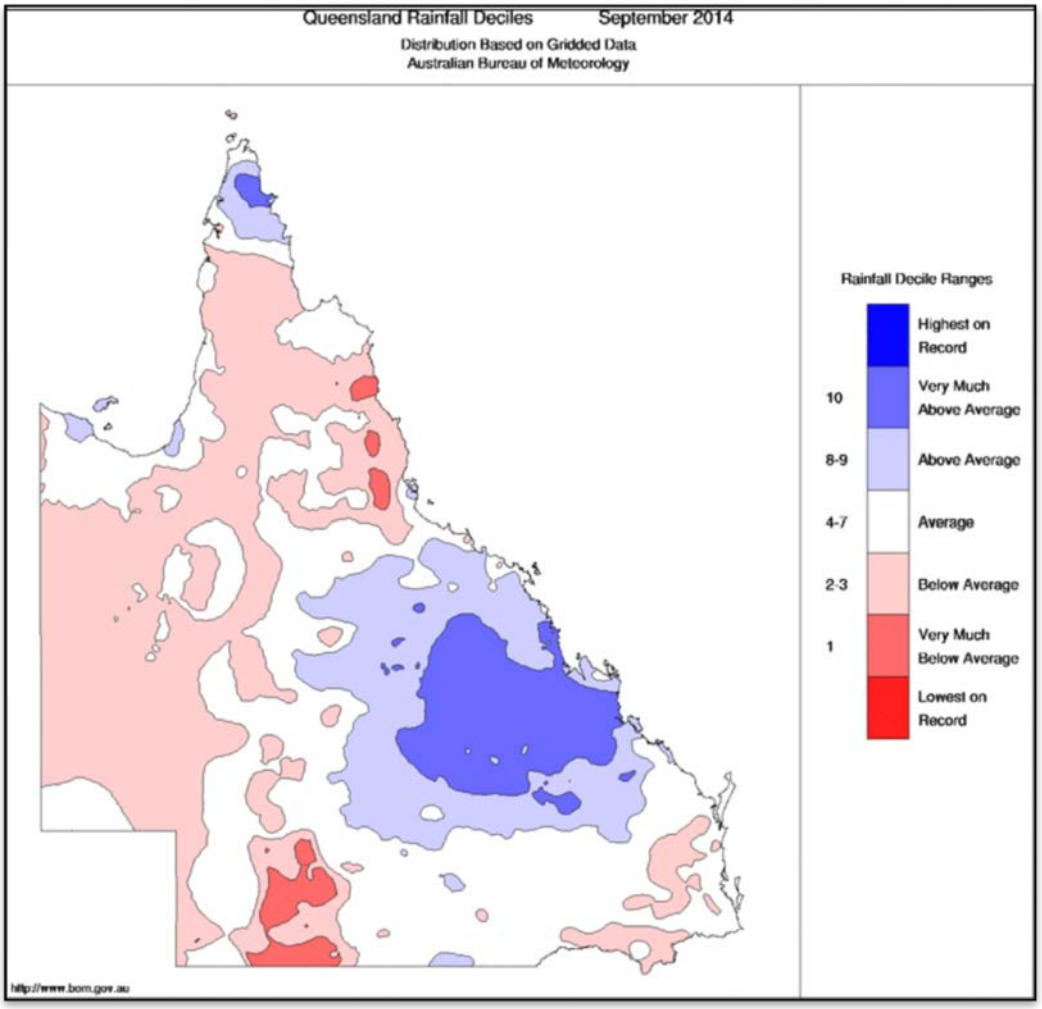


Figure 5.6 Rainfall in Queensland in September 2014

Source: http://www.bom.gov.au/web03/ncc/www/awap/rainfall/decile/month/colour/history/qd/2014090120140930.gif

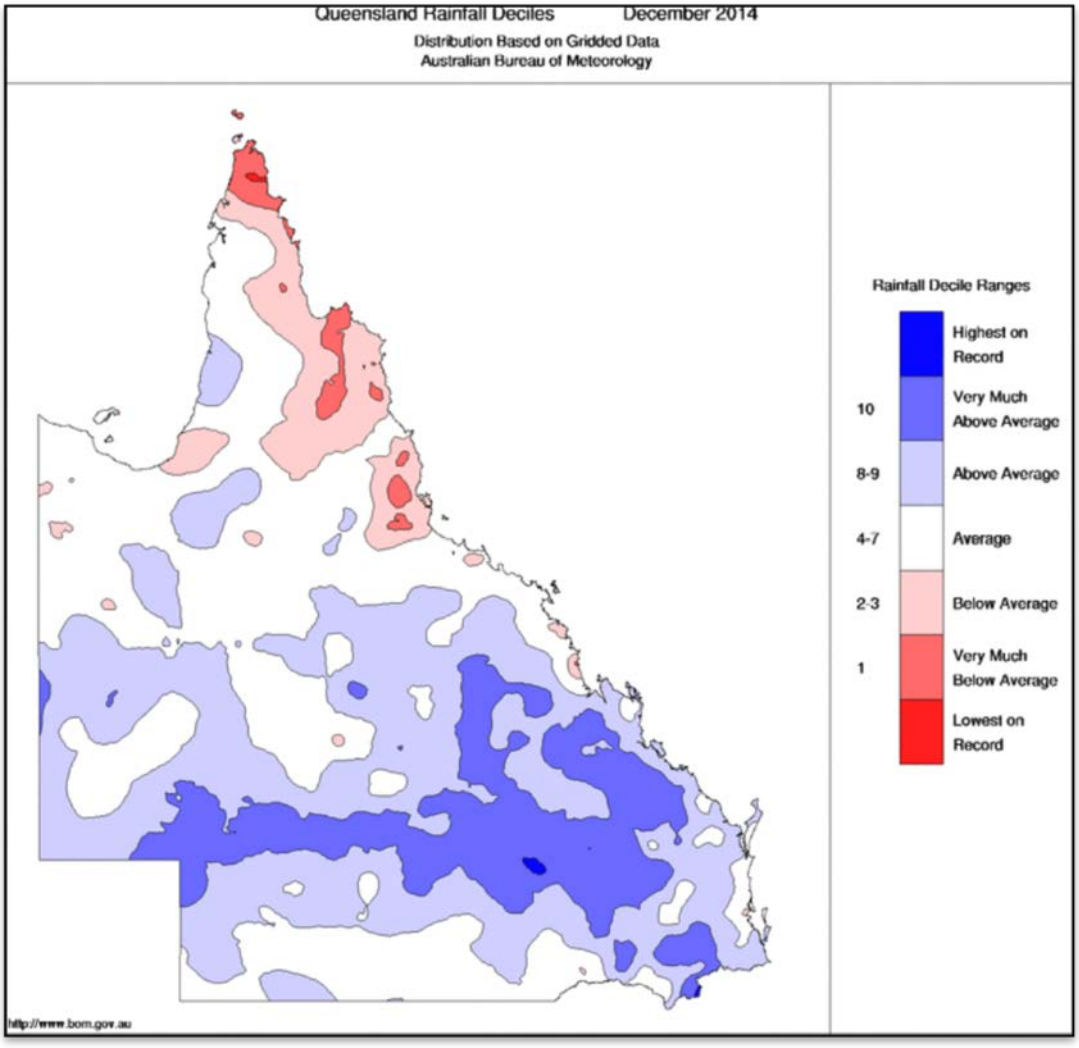


Figure 5.7 Rainfalls in Queensland in December 2014

Source: http://www.bom.gov.au/web03/ncc/www/awap/rainfall/decile/month/colour/history/qd/2014120120141231.gif

It can be understood from Figures 5.5 and 5.6 that rainfall in Queensland was much below average suggesting less dilution which compliments with the higher concentrations of the target EPPPs, particularly carbamazepine in those months compared to third sampling in December 2014. Figure 5.7, on the other hand, shows significant rainfall in December compared to July and September which might have played a role in the dilution of the target EPPPs in the Brisbane River resulting in relatively lower concentrations.

Additionally, low rainfalls in SEQ impacted the flows of the major rivers in SEQ which include Bremer and Brisbane Rivers (BOM, 2014). The flow in the rivers

might have resulted in comparatively higher concentrations of target EPPPs in the first two sampling events. Such phenomenon was noted by Silva et al. (2011) where the concentrations of target EPPPs were found be lower downstream due to high flow when compared to upstream with low flow suggesting that concentrations of EPPPs were possibly impacted by flowrate. In regards to the current research, the higher concentrations of the target EPPPs could be attributed to the lower than average flowrate in the Brisbane and Bremer rivers in the year 2013-14 owing to low rainfall (Figure 5.8, 5.9).

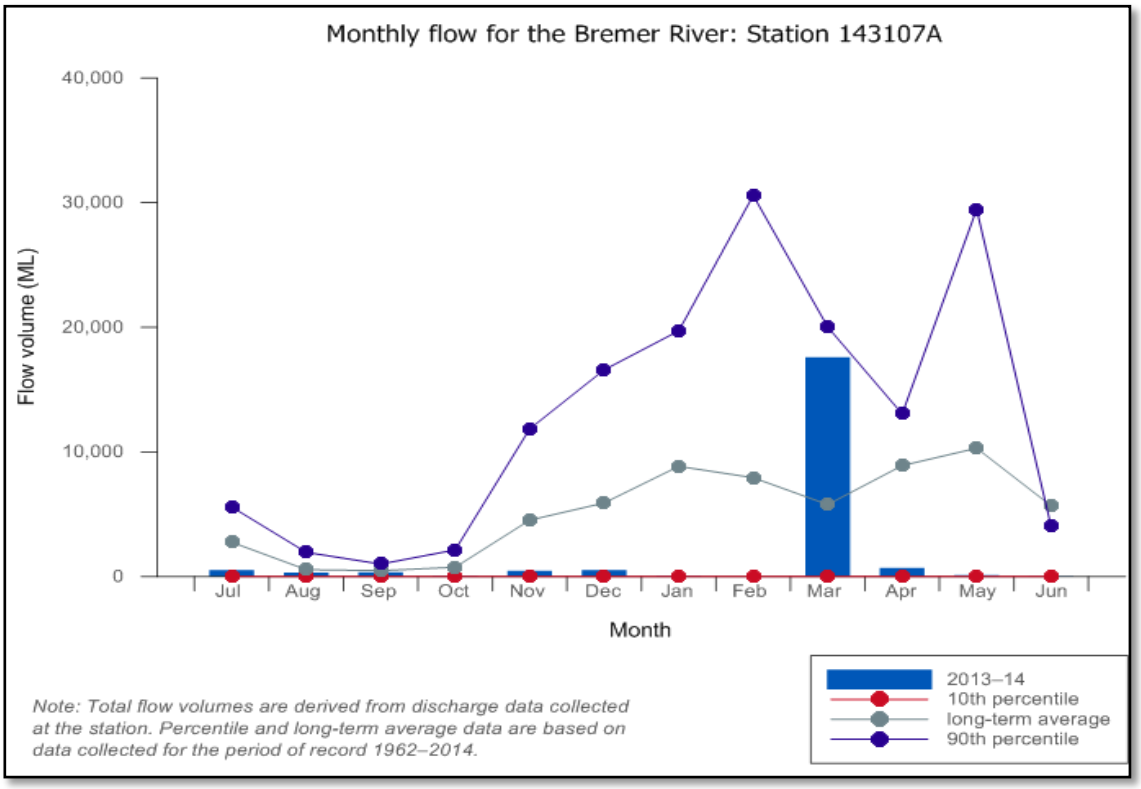


Figure 5.8 Monthly flow along Bremer River during the year 2013-14

Source: http://www.bom.gov.au/water/nwa/2014/seq/contextual/wateroverview.shtml#major_water_initiatives

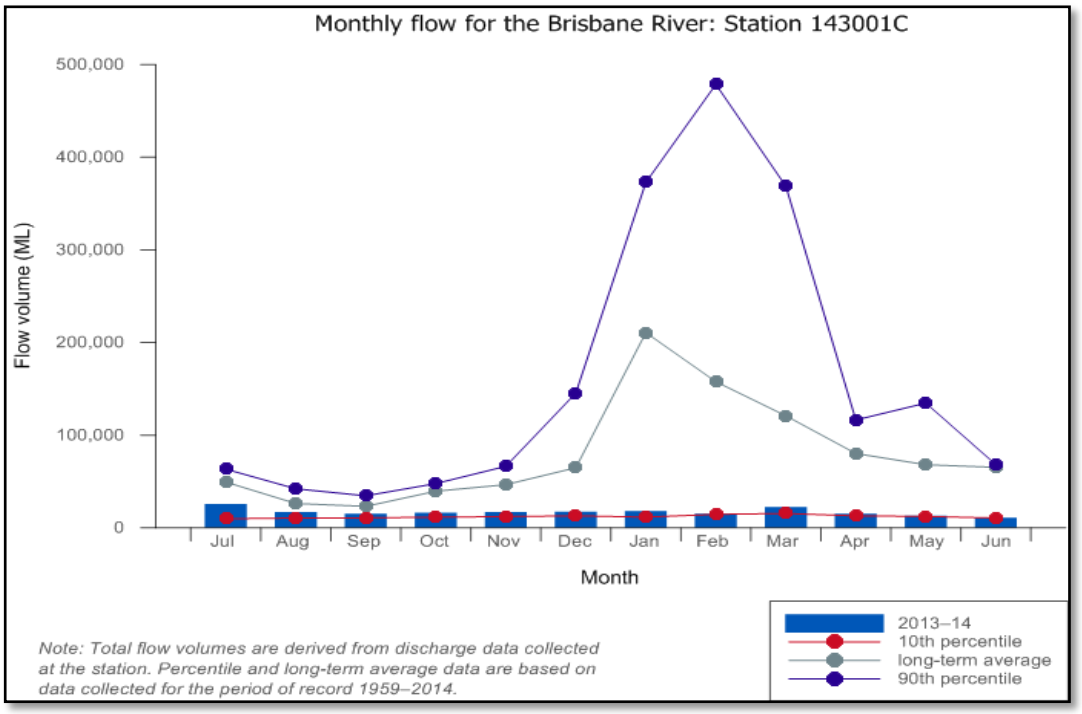


Figure 5.9 Monthly flow along the Brisbane River during the year 2013-14

Source: http://www.bom.gov.au/water/nwa/2014/seq/contextual/wateroverview.shtml#major_water_initiatives

Figure 5.8 and 5.9 show lower than average flow for the Bremer and Brisbane rivers which might have impacted on the occurrence of target EPPPs in the Brisbane River sediments. Thus, it can be stated from the above discussions that record low rainfalls in SEQ associated with lower flowrates in the Bremer and Brisbane Rivers resulted in high concentrations during the initial sampling events upstream of the study area. However, despite the decreasing trend in concentrations, the concentration of carbamazepine at B2- SP2 was significantly high (6.39 ngg^{-1}) compared to the first sampling event, the occurrence of which remains unexplained. The next section discusses the other possible sources such as the sewerage network contributing towards the occurrence of these target EPPPs in the Brisbane River sediments.

5.3.2 The sewerage network of Brisbane and EPPPs occurrence

It is evident from the discussion in Section 5.3.1 that EPPPs are present in the Brisbane River sediments which leads to the conclusion that these pollutants are continuously being released into the river. Therefore, it was important to know what sources contribute to the persistence of these target EPPP pollutants. As pointed out in Section 2.5, wastewater effluents originating from the WWTPs act as point sources of EPPPs pollution in aquatic environments whereas sewer leaks act as diffuse sources. In addition, the evidence produced in Section 2.5 also confirmed that the increasing and ageing population in urbanised Queensland consumes target EPPPs regularly. These facts, therefore, conclude that the target EPPPs are being discharged via wastewater effluents from neighbouring WWTPs and septic tanks into the Brisbane River.

While the above interpretation was logical, it was essential to study the sewerage network which would support this inference. Thus, the Brisbane sewerage network was studied (Figure 5.10), which covers the entire Brisbane region. On careful observation of the network, it could be stated that wastewater effluents could be the possible cause for EPPPs occurrence in the Brisbane River sediments. Besides, the STPs are also built close to the Brisbane River.

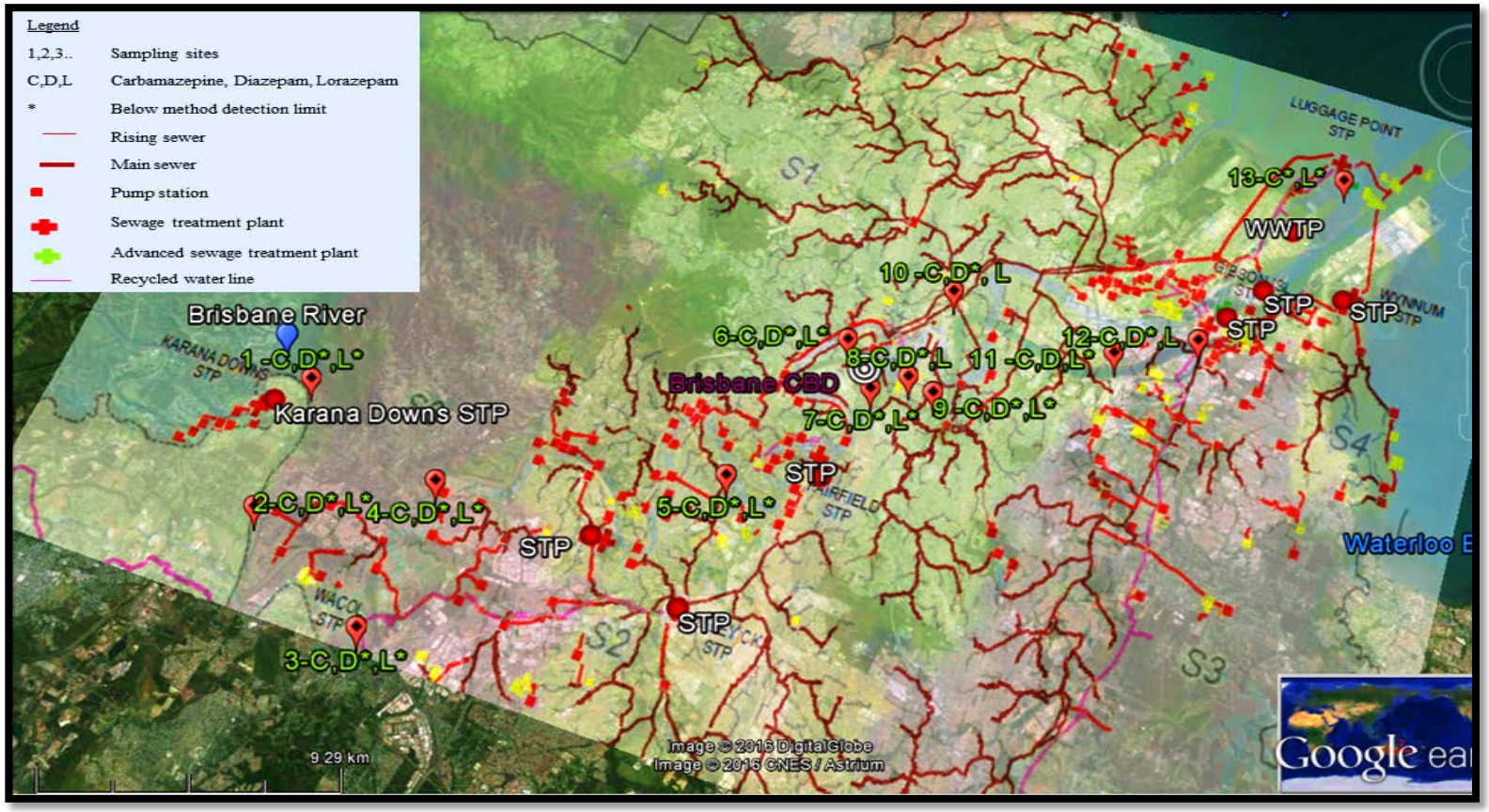


Figure 5.10 The sewerage network of Brisbane along the Brisbane River (Source: (QUU, 2014), Google earth).

The sewerage network of Brisbane shows seven WWTPs and two advanced STPs that are situated along the Brisbane River (QUU, 2014). Studies undertaken by Moreno-González et al. (2015), Silva et al. (2011) and Stewart et al. (2014) report the occurrence of EPPPs in sites in proximity to WWTPs. The Brisbane River is also in proximity of the sewerage network and therefore it can be implied that the sewerage system of Brisbane could likely be releasing EPPP pollutants to the Brisbane River.

While the WWTPs are point sources of EPPPs pollution, the likelihood of these treatment plants releasing these pollutants to the Brisbane River is quite low because there are no discharge points for release into the Brisbane River. Instead, the sewers carry the treated wastewater to the mouth of the river where they get released into the Pacific Ocean (Figure 5.10). However, there could be leaks in the sewers that could possibly be releasing sewage into the Brisbane River and this seems quite possible since EPPPs could be seen present in the sites, particularly, around Brisbane CBD (SP7, SP8, SP9, SP10, and SP11) which has no WWTPs in its proximity.

The target EPPPs were quite spread out in the River and there was variation in the concentrations of these pollutants which was critical to study. The occurrence of the target EPPPs upstream particularly at sites SP1, SP2 and SP3 could be attributed to three possible sources – Karana Downs WWTP, Bremer River, sewer leaks and leaks in the pipelines carrying recycled water. The sewer leaks and the location of WWTP at Karana Downs could possibly be the source for the occurrence of target EPPPs at site SP1.

The Bremer River, which flows upstream from Ipswich and joins Brisbane River at site SP2 could be carrying the wastewaters from the WWTPs upstream of the river and releasing it into the Brisbane River thereby possibly adding to the EPPPs released upstream of the River. In addition, residential properties upstream of the Brisbane and Bremer rivers have septic tanks and these are likely to release EPPPs into the groundwater which leach into the river carrying the pollutants downstream. While this could be the case, the possibility of sewer leaks cannot be denied. The concentration of carbamazepine at SP2 from the second sampling episode was the

maximum at 6.39ngg^{-1}, which could be possibly from a sewer leak. Figure 5.11 below shows the maximum concentration of carbamazepine detected at site SP2.

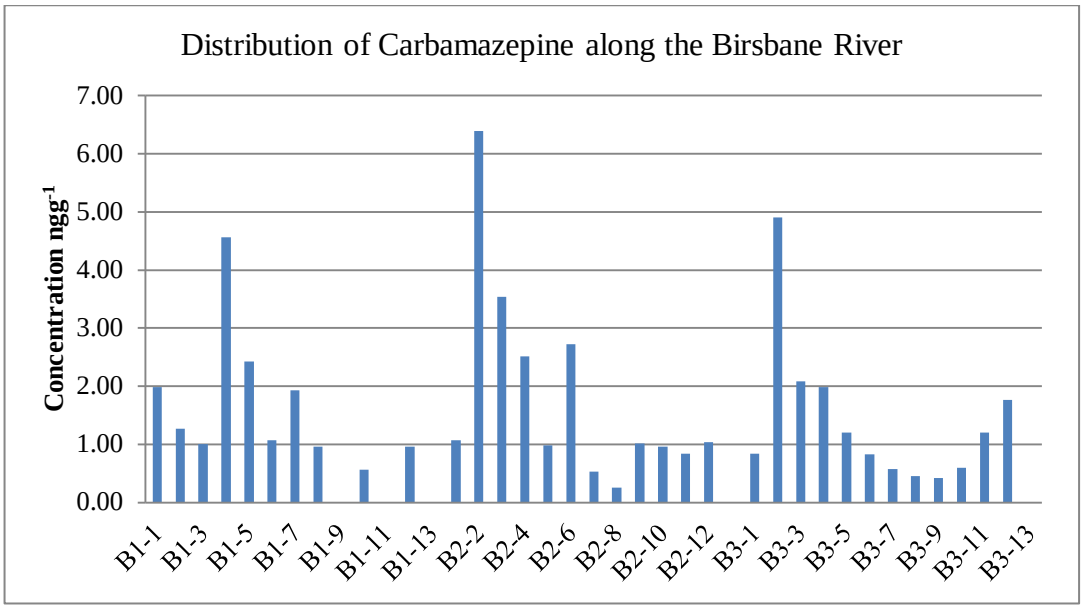


Figure 5.11 Concentrations in ngg^{-1} of carbamazepine in the Brisbane River sediments during the three sampling event. (B1- First sampling batch; B2 – Second Sampling batch; B3- Third Sampling batch)

The concentrations of carbamazepine were also higher than its mean concentration at sites SP4 in first and second sampling, SP3 in second sampling. Site SP4 lies in proximity to sewer lines and a pump station that pumps the collected sewage into the sewers. Possible leaks from the sewers and pump station or accidental outflow due to high load of sewage could be the likely cause for higher than mean concentrations at this site. Site SP3, on the other hand, is downstream of the WWTP. Therefore, any leaks or accidental outflow of effluents from this WWTP might have resulted in higher than mean concentrations of carbamazepine at this site. Additionally, downstream to Karana Downs WWTP, EPPPs occur at sites SP3 and SP4. Adjacent to these sites there are pipelines carrying recycled water (Figure 5.9). Recycled water will also contain EPPPs since the treatment methods are not designed to remove such pollutants (Antonić and Heath, 2007). Therefore, these pipelines could also be leaking out EPPPs.

Carbamazepine was quantified <MDL at sites SP9 (at the confluence of Norman creek and Brisbane River), SP11 (near Gateway Motorway) during the first sampling

event (Figure 5.10), which could possibly be because of dilution of these pollutants at these sites. However, more information is required to support this hypothesis. However, the concentration of carbamazepine remained <MDL at site SP13 in all the sampling episodes suggesting possible dilution since this site lies at the mouth of the river. The mean, minimum and maximum concentrations for diazepam were <MDL at all the sites which is likely to be because of previously discussed reasons of low consumption, dilution along the river and photodegradation.

Although the EPPPs have been detected throughout the length of the River, their concentrations have been found to be decreasing downstream. Downstream of the river, from the CBD to the mouth of the River, there is no WWTP in the proximity of the sites SP7, SP8, SP9, and SP10. This implies that the occurrence of target EPPPs could possibly be because of leaks from the sewers and pump stations. In addition to this, another possible reason for the occurrence of target EPPPs could be dilution of these pollutants along the river from upstream to downstream as stated in section 5.3.1 and in a review by Gaw et al. (2014), where it was noted that dilution of the discharged effluents also affects the occurrence of EPPPs.

Although sewer leaks, which are diffuse sources, the possible continuous infusion of these pollutants through leaks into the river might have led to the persistence of EPPPs in the Brisbane river sediments. In addition, the lower than average flow of the river might have impacted the dilution which led to the detection of these target EPPPs.

In regards to lorazepam, Figure 5.12 below shows the distribution of the pollutant along the Brisbane River. Site SP12 has the highest concentration at 1.78 ngg^{-1}.

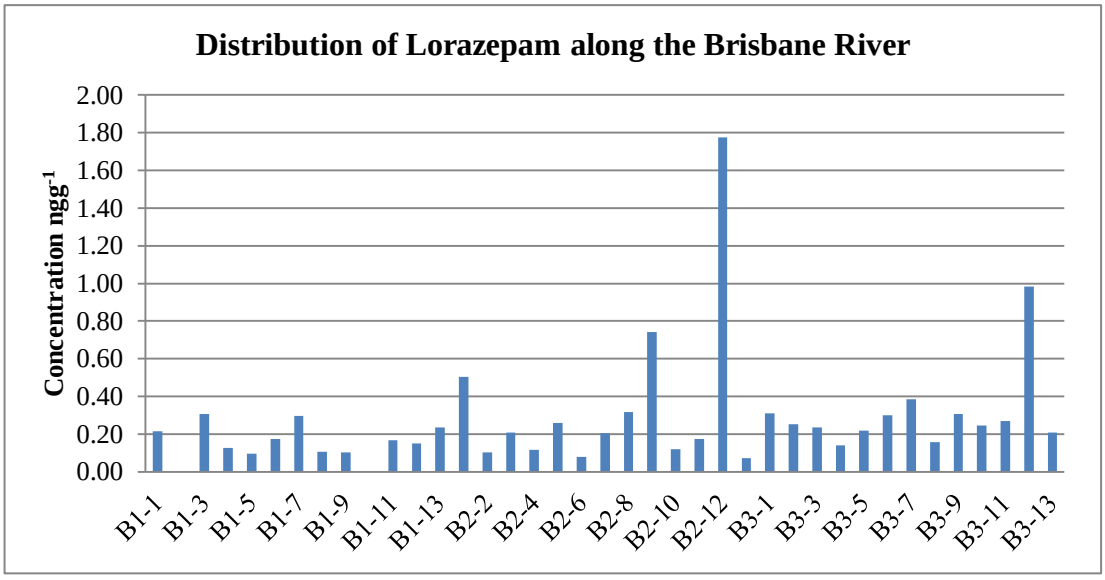


Figure 5.12 Distribution of Lorazepam in ngg^{-1}along the Brisbane River (B1-First Sampling batch; B2-Second Sampling batch; B3- Third Sampling batch)

B2-12 shows the maximum concentration of lorazepam during the second sampling event and B3-12 shows higher than mean concentration in third sampling event. It is to be noted that there are two STPs in proximity to the site SP12. Higher concentrations of EPPPs in the wastewater effluents would result in high concentrations in the waters where it is released (McEneff et al., 2014) and higher outflow of effluents could also be releasing high loads of pollutants (Gaw et al., 2014). Therefore, considering the hypothesis that higher outflow of effluents from these two STPs near site SP12, this could possibly be the reason for increased concentrations of lorazepam. In other words, higher concentrations of lorazepam in the released effluents could also be likely due to higher consumption.

Although these hypotheses may be true, leaks from the sewers originating from these WWTPs could also be considered as a source for the increase in concentrations. Interestingly, site SP12 lies at the confluence of Bulimba creek and Brisbane River where there is a main sewer that carries the sewage to the neighbouring STPs. A possible leak in this main sewer might also have resulted in higher than mean concentrations of lorazepam. The lowest concentration of lorazepam was detected at site SP13, the site after SP12 in the second sampling event suggesting rapid dilution at this site. The dilution at SP13, which is located at the mouth of the river seems to be high due to its proximity to the ocean.

5.4 Summary

This chapter has confirmed the occurrence of the three target EPPPs pollutants in the Brisbane River sediments. It has also established a direct correlation between urbanisation and EPPPs occurrence in the Brisbane River sediments thereby achieving the aim of the current research. The concentrations of the target EPPPs are comparable with the concentrations reported in other studies (Beretta et al., 2014; Vazquez-Roig et al., 2010). The occurrence and distribution of EPPPs in the Brisbane River sediments were discussed considering the hypotheses - sewer leaks, proximity to WWTPs, weather events, flow rate and photodegradation. Sewer leaks and proximity to WWTPs appear to be the major sources for EPPPs occurrence in the Brisbane River which complements with the findings of Birch et al. (2015) in their study in Sydney estuary. Furthermore, this hypothesis was explained in detail with the sewerage network plan of Brisbane (Fig 5.10). While sewer leaks seem to be the likely cause in this particular research study, other factors that contribute to EPPPs pollution – flow of the river, effluent outflow and weather events such as rainfall were also discussed and considered as possible causes for the occurrence of target EPPPs in the Brisbane River sediments.

An interesting inference that was made in this chapter was of recycled water being a possible source of target EPPPs occurrence in the upstream sites of the Brisbane River. This conclusion came after careful observation of the sewerage network (Figure 5.10) and critical review of the literature (Chapter 2), which suggests that recycled water can contain EPPPs. In this particular study, flow volume in the river and rainfall appear to be the second most likely sources of EPPPs occurrence and distribution in the Brisbane River sediments. It can be implied from the discussions that urbanisation is the reason for the occurrence of EPPPs. Increasing urbanisation along the Brisbane River has resulted in increased consumption of EPPPs and continuous ingestion and excretion of such drugs has consequently resulted in their occurrence in the sewage and thereby in the Brisbane River sediments.

Chapter 6 Conclusions and Recommendations

6.1 Conclusions

This research has confirmed EPPPs pollution in the Brisbane River sediments. The study succeeded in establishing a relationship between urbanisation and EPPPs pollution, thereby achieving the aim of this research. The study identified three target EPPPs that were quantified by applying a method that was developed during this study. This research has addressed the knowledge gap about the lack of research on EPPPs in Brisbane River sediments and thus, has provided comprehensive knowledge about EPPPs pollution of Brisbane River by extracting important information about urbanisation, population growth, an ageing population and pharmaceutical consumption. This information was crucial and supported the hypothesis of this study that pharmaceuticals are present in the Brisbane River sediments. The study, therefore, involved selecting target EPPPs for analysis of the sediments, developing a method for extraction, detection and quantification of target pharmaceuticals from the Brisbane River sediments.

Selecting target analytes for analysis in the sediment samples was a primary and crucial requirement of the study, which involved a critical review of the literature, studying of statistical data on pharmaceutical consumption and population. In addition, critical information on drug use and health conditions was obtained from PBS and CHO reports from Queensland Government, respectively. All of this extracted information was vital and proved decisive in the selection of eleven target EPPP compounds from five therapeutic classes. The selected target compounds were then analysed by applying the method developed.

Method development was one of the objectives of this study. The developed method resulted in linear equations for all the target EPPP compounds with R^2 values being 0.99 for most of the compounds. The application of this method led to the detection of three important target EPPPs. The concentrations of the detected target EPPPs have been reported with 95% confidence level. The quantification of the target

EPPPs was carried out by applying Skyline software for analysis of the raw MRM data for the standards and sediment testing. The developed method was quick, selective and sensitive in the quantification of target analytes – carbamazepine, diazepam and lorazepam.

It can be concluded from the findings that carbamazepine is the most commonly occurring psychiatric drug resistant to degradation and removal techniques (Clara et al., 2004; Ternes et al., 2004), while on the other hand, diazepam and lorazepam despite belonging to the same therapeutic class, are susceptible to photo-degradation (Calisto et al., 2011) to some extent. This is possibly why carbamazepine was found to be occurring in all the sites of the Brisbane River compared to the other two compounds. Diazepam and lorazepam were also detected in most of the sites below MDL and in concentrations lower than that of carbamazepine. Proximity to WWTPs and sewer lines and sewer leaks are claimed to be the major sources for the occurrence of these EPPPs pollutants in the Brisbane River sediments.

The analysis undertaken in this study has contributed to the existing knowledge of EPPP pollution here in Australia. The methods used in the current study could be applied elsewhere for the analysis of psychiatric compounds. However, a major limitation of the developed method was its inability to quantify other target analytes which led to the conclusion that the method still needs to be further developed and improved so as to be able to quantify the remaining target analytes of environmental concern. The next section, therefore, discusses the recommendations for further method development.

6.2 Recommendations for further research

In addition to knowledge created in relation to EPPPs pollution scenarios in the Brisbane River sediments, this research study also identified several opportunities for future research. Further research in these areas can potentially contribute to the knowledge base created by the current study.

Further research is necessary in the area of method development. The method requires to be further refined, such that it enables the investigation of the remaining target EPPPs of environmental concern. Therefore, the study needs to be extended

using an improved method that could quantify the remaining target EPPPs and contribute to the existing knowledge of EPPPs pollution. Figure 6.1 displays additional suggestions for further developing the current method that was employed in this study.

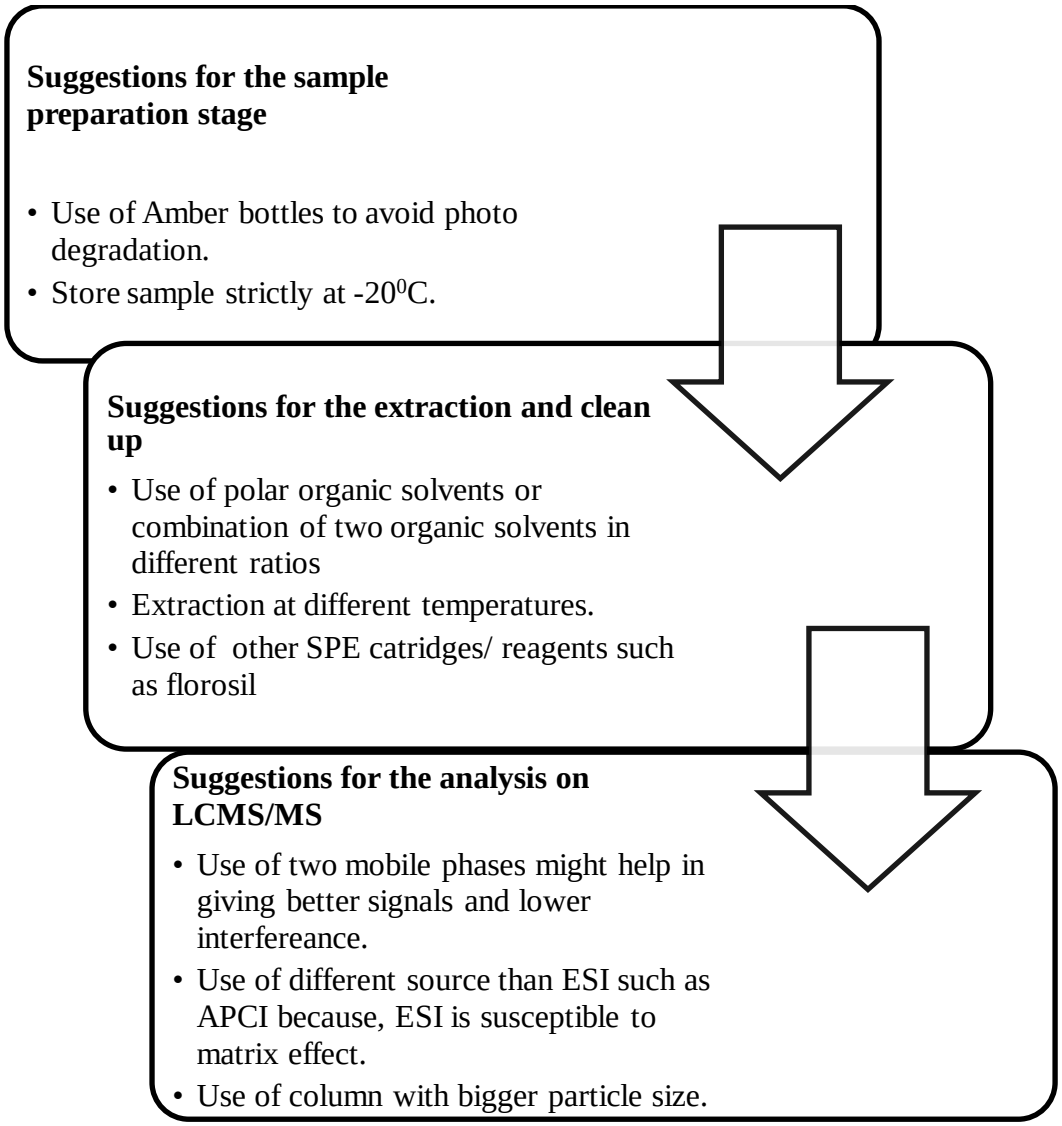


Figure 6.1 Recommendations for further method development at different stages of sediment analysis

It was understood that developing a robust method for EPPPs analysis is quite complex particularly when it is designed to quantify pharmaceuticals across multiple classes. This is because the chemical and physical properties of the pharmaceuticals differ with each group. Figure 6.1 shows some of the suggestions that would help

refine the method. Since, these compounds are highly active organics, preservation of these compounds in amber bottles at -20^0C is recommended because some of the compounds are sensitive to light. Storing the standards in such a way would minimise the degradation and therefore, loss of the target compound which means the concentrations of the standards remain constant. Having the concentrations steady and constant is critical during method development on the LCMS/MS because the instrument (LCMS/MS) should be able to record the right concentration.

The next stage for refinement is the extraction method. There are various factors that affect the extraction of the EPPPs such as temperature, pH and organic solvents. In this study, extraction recovery was largely affected by the choice of the solvents used. Therefore, it is recommended to try different organic solvents and combination of organic solvents as a step towards achieving better recovery of EPPP compounds. While the selection of organic solvents might help improve the recovery, experimenting with other factors such as temperature and pH might also prove critical in deriving better recovery.

The Clean-up stage in the EPPPs analysis is also important. The clean-up process is followed up immediately by the extraction process. Therefore, it is difficult to state whether the extraction or clean-up process requires to be refined. This is because SPE cartridges in the clean-up process allows selective filtration of desired target EPPP compounds from the extracts derived from the extraction method. It is, therefore, recommended to test the extracts after extraction for the concentration of target EPPP compounds in order to check the efficiency of extraction method first and then proceed with clean-up. Performing such test would facilitate in determining which of the processes are working and efficient and further allow direction for refinement.

In regards to the quantification of the EPPPs compounds using the LCMS/MS, it is recommended to use a column of larger particle size. This recommendation needs to be considered seriously, in particular, while working with a complex matrix such as sediment. Use of one column is highly recommended because the change in the column affects the efficiency of the MRM method which is critical particularly when dealing with minute concentrations such as that of EPPPs (ppb and ppt). The

sediment extracts have a large number of impurities as compared to other extract samples. Using a column of larger particle size would allow these impurities to pass through the column and not block it, thereby saving the column.

Although the above recommendations would enable in developing an improved method, it is important to note that the method development, particularly, in the case of pharmaceuticals, is an ongoing process due to the following reasons which need to be accounted for in research studies for method development.

a) Addition of new drugs

Research in finding a cure to diseases and drug designing will produce new and modified drugs. This would either replace the existing ones or add to the already available range of medicines. Therefore, the introduction of any new drugs into the market is ultimately going to be released into the waterways and being introduced into the environment. Analysis of such newly added drugs would require further method development so that the method could be applied across multiple classes of pharmaceuticals.

b) Studies on the impacts of toxicity of drugs on the environment

At present, there are limited toxicological studies about pharmaceuticals in the environment. Part of this limited work could be related to the lack of guidelines and regulations surrounding this issue. Some drugs that are not identified as toxic now, might be toxic at a later date. The quantification of such drugs would be important which then means refining the method again to accommodate such drugs.

Therefore, developing a method that could quantify EPPPs across multiple therapeutic classes would be an important area for further investigation. Such a method would help investigate a broad range of EPPPs in a single analysis and facilitate in further research such as toxicological studies. In addition, such a method would save a lot of time which otherwise is the case in the analysis of such micropollutants.

In regards to data analysis in this study, it is recommended that further and future research would require use of other parameters such as land use characteristics,

rainfall runoff parameters, data regarding the river flow and population density. Such information is necessary for studying the distribution and occurrence in the river adjoining an urban environment.

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Appendix A Raw Data on the Detected Target Compounds

Table A.1 Carbamazepine data from first sampling batch

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Carbamazepine	237.199	194.2	QUAN	1.9	46282	4559	1445.18	STD			
Carbamazepine	237.199	194.2	QUAN	1.9	47650	8233	47701.00	AVG	B1-1	6.99	9.95
Carbamazepine	237.199	194.2	QUAN	1.89	49171	7523	3.03	RSD			
Carbamazepine	237.199	194.2	QUAN	1.9	29499	6334	2038.16	STD			
Carbamazepine	237.199	194.2	QUAN	1.88	32897	8036	31848.00	AVG	B1-2	4.44	6.32
Carbamazepine	237.199	194.2	QUAN	1.9	33148	7477	6.40	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	26836	14118	1636.02	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	24420	12886	26265.00	AVG	B1-3	3.54	5.04
Carbamazepine	237.199	194.2	QUAN	1.89	27539	15268	6.23	RSD			
Carbamazepine	237.199	194.2	QUAN	1.88	98000	7659	5620.35	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	104388	9748	103864.00	AVG	B1-4	16.02	22.82
Carbamazepine	237.199	194.2	QUAN	1.9	109204	9114	5.41	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	53307	13586	3808.20	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	57361	14436	57195.33	AVG	B1-5	8.51	12.13
Carbamazepine	237.199	194.2	QUAN	1.89	60918	20070	6.66	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	25032	6768	2693.87	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	30403	9765	27595.00	AVG	B1-6	3.75	5.35

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Carbamazepine	237.199	194.2	QUAN	1.89	27350	12930	9.76	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	47477	9350	2192.66	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	47962	12218	46461.33	AVG	B1-7	6.79	9.67
Carbamazepine	237.199	194.2	QUAN	1.89	43945	15590	4.72	RSD			
Carbamazepine	237.199	194.2	QUAN	1.88	24273	13224	814.87	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	25237	12451	25134.33	AVG	B1-8	3.36	4.78
Carbamazepine	237.199	194.2	QUAN	1.89	25893	13623	3.24	RSD			
Carbamazepine	237.199	194.2	QUAN	1.9	7294	8516	1311.42	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	8957	7583	8711.00	AVG	B1-9	0.72	1.02
Carbamazepine	237.199	194.2	QUAN	1.89	9882	11058	15.05	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	15033	9455	2666.78	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	15031	13134	16571.67	AVG	B1-10	1.98	2.82
Carbamazepine	237.199	194.2	QUAN	1.89	19651	14743	16.09	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	7388	12176	1200.93	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	9362	12145	8770.00	AVG	B1-11	0.72	1.03

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Carbamazepine	237.199	194.2	QUAN	1.9	9560	11578	13.69	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	27454	21982	2332.17	STD			
Carbamazepine	237.199	194.2	QUAN	1.9	25605	17287	25293.33	AVG	B1-12	3.38	4.82
Carbamazepine	237.199	194.2	QUAN	1.89	22821	13990	9.22	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	6073	8133	1127.47	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	4569	7107	4836.00	AVG	B1-13	0.09	0.13
Carbamazepine	237.199	194.2	QUAN	1.89	3866	12203	23.31	RSD			

Table A.2 Carbamazepine data from second sampling batch

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Carbamazepine	237.199	194.2	QUAN	1.89	27589	4166	1372.47016	STD			
Carbamazepine	237.199	194.2	QUAN	1.88	29070	6954	27662.3333	AVG	B2-1	3.76	5.36
Carbamazepine	237.199	194.2	QUAN	1.89	26328	8561	4.96151261	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	133624	12672	9259.33498	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	145345	13751	143623.333	AVG	B2-2	22.41	31.94
Carbamazepine	237.199	194.2	QUAN	1.89	151901	15317	6.44695731	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	78306	12796	4475.4078	STD			
Carbamazepine	237.199	194.2	QUAN	1.9	86541	12094	81411	AVG	B2-3	12.41	17.68
Carbamazepine	237.199	194.2	QUAN	1.89	79386	14767	5.49730111	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	54770	14989	4064.99606	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	59920	15452	59161	AVG	B2-4	8.83	12.58
Carbamazepine	237.199	194.2	QUAN	1.89	62793	13980	6.87107396	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	25245	21027	1028.113	STD			

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Carbamazepine	237.199	194.2	QUAN	1.89	24813	19352	25609.3333	AVG	B2-5	3.43	4.89
Carbamazepine	237.199	194.2	QUAN	1.89	26770	18001	4.01460273	RSD			
Carbamazepine	237.199	194.2	QUAN	1.88	56903	12196	5957.39291	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	66444	12404	63733.6667	AVG	B2-6	9.56	13.63
Carbamazepine	237.199	194.2	QUAN	1.89	67854	16302	9.34732493	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	12624	13412	2881.72194	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	16534	14647	15801.3333	AVG	B2-7	1.86	2.64
Carbamazepine	237.199	194.2	QUAN	1.89	18246	17526	18.2372074	RSD			
Carbamazepine	237.199	194.2	QUAN	1.88	5193	13440	3943.75473	STD			
Carbamazepine	237.199	194.2	QUAN	1.88	11803	18784	9740.33333	AVG	B2-8	0.88	1.26
Carbamazepine	237.199	194.2	QUAN	1.89	12225	22827	40.4889093	RSD			

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Carbamazepine	237.199	194.2	QUAN	1.88	24767	10393	1371.36769	STD			
Carbamazepine	237.199	194.2	QUAN	1.88	27121	10908	26350.3333	AVG	B2-9	3.55	5.06
Carbamazepine	237.199	194.2	QUAN	1.89	27163	9574	5.20436561	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	25906	12618	1259.77419	STD			
Carbamazepine	237.199	194.2	QUAN	1.88	23763	11183	25217	AVG	B2-10	3.37	4.80
Carbamazepine	237.199	194.2	QUAN	1.89	25982	13510	4.99573378	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	24519	12723	1936.57559	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	22369	13253	22514	AVG	B2-11	2.94	4.18
Carbamazepine	237.199	194.2	QUAN	1.88	20654	16244	8.60165047	RSD			
Carbamazepine	237.199	194.2	QUAN	1.88	26751	19978	1141.189	STD			
Carbamazepine	237.199	194.2	QUAN	1.88	26048	14442	27026.3333	AVG	B2-12	3.66	5.22
Carbamazepine	237.199	194.2	QUAN	1.89	28280	14641	4.22250768	RSD			
Carbamazepine	237.199	194.2	QUAN	1.91	4735	18256	926.680276	STD			
Carbamazepine	237.199	194.2	QUAN	1.87	6118	30671	5782.66667	AVG	B2-13	0.24	0.35
Carbamazepine	237.199	194.2	QUAN	1.9	6495	25523	16.0251373	RSD			

Table A.3 Carbamazepine data from third sampling batch

Compound	Precursor	Product	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Carbamazepine	237.199	194.2	QUAN	1.89	20195	11672	2092.14372	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	24079	8775	22586.3333	AVG	B3-1	2.95	4.20
Carbamazepine	237.199	194.2	QUAN	1.89	23485	13486	9.26287453	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	106834	9569	4878.26735	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	116462	14459	111192.333	AVG	B3-2	17.19	24.50
Carbamazepine	237.199	194.2	QUAN	1.89	110281	11955	4.38723355	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	47010	9331	3873.77882	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	54228	6205	49806.3333	AVG	B3-3	7.32	10.44
Carbamazepine	237.199	194.2	QUAN	1.89	48181	6340	7.7776832	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	40538	5442	6159.84488	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	51115	8716	47650	AVG	B3-4	6.98	9.94
Carbamazepine	237.199	194.2	QUAN	1.89	51297	7648	12.9272715	RSD			
Carbamazepine	237.199	194.2	QUAN	1.88	31294	12364	30606.6667	AVG	B3-5	4.24	6.04
Carbamazepine	237.199	194.2	QUAN	1.89	33244	9982	9.9319829	RSD			

Compound	Precursor	Product	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Carbamazepine	237.199	194.2	QUAN	1.89	23295	12458	2739.12127	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	19215	16318	22310.3333	AVG	B3-6	2.90	4.14
Carbamazepine	237.199	194.2	QUAN	1.89	24421	16634	12.277366	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	12927	10377	3852.27327	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	20625	14412	16867.6667	AVG	B3-7	2.03	2.89
Carbamazepine	237.199	194.2	QUAN	1.89	17051	14573	22.8382108	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	16588	16930	2861.37735	STD			
Carbamazepine	237.199	194.2	QUAN	1.88	10988	11729	14128.3333	AVG	B3-8	1.59	2.26
Carbamazepine	237.199	194.2	QUAN	1.89	14809	21766	20.2527593	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	10473	15840	2769.14644	STD			

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Carbamazepine	237.199	194.2	QUAN	1.89	14377	12378	13559	AVG	B3-9	1.50	2.13
Carbamazepine	237.199	194.2	QUAN	1.89	15827	13194	20.42294	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	15276	10292	1925.34361	STD			
Carbamazepine	237.199	194.2	QUAN	1.88	19126	18147	17222	AVG	B3-10	2.08	2.97
Carbamazepine	237.199	194.2	QUAN	1.89	17264	11853	11.1795587	RSD			
Carbamazepine	237.199	194.2	QUAN	1.88	29205	14784	1245.79867	STD			
Carbamazepine	237.199	194.2	QUAN	1.88	31679	13496	30527.3333	AVG	B3-11	4.22	6.02
Carbamazepine	237.199	194.2	QUAN	1.89	30698	12739	4.08092859	RSD			
Carbamazepine	237.199	194.2	QUAN	1.89	42426	23401	1475.34075	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	44552	21128	42898.3333	AVG	B3-12	6.21	8.85
Carbamazepine	237.199	194.2	QUAN	1.89	41717	21350	3.43915634	RSD			
Carbamazepine	237.199	194.2	QUAN	1.9	4985	12148	1300.22934	STD			
Carbamazepine	237.199	194.2	QUAN	1.89	5717	9371	6071.33333	AVG	B3-13	0.29	0.41
Carbamazepine	237.199	194.2	QUAN	1.9	7512	14936	21.415878	RSD			

Table A.4 Diazepam data from first sampling batch

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Diazepam	284.9995	153.999	QUAN	2.33	1382	187	133.2454	STD			
Diazepam	284.9995	153.999	QUAN	2.33	1163	352	1316.333	AVG	B1 -1	0.06	0.08
Diazepam	284.9995	153.999	QUAN	2.33	1404	298	10.12247	RSD			
Diazepam	284.9995	153.999	QUAN	2.33	861	153	92.42474	STD			
Diazepam	284.9995	153.999	QUAN	2.32	742	107	760.6667	AVG	B1-2	0.03	0.05
Diazepam	284.9995	153.999	QUAN	2.33	679	104	12.15049	RSD			
Diazepam	284.9995	153.999	QUAN	2.34	1898	143	237.7485	STD			
Diazepam	284.9995	153.999	QUAN	2.33	1431	354	1690.333	AVG	B1-3	0.07	0.10
Diazepam	284.9995	153.999	QUAN	2.33	1742	0	14.06518	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	4652	419	368.4725	STD			
Diazepam	284.9995	153.999	QUAN	2.32	4746	431	4488	AVG	B1-4	0.20	0.27
Diazepam	284.9995	153.999	QUAN	2.33	4066	595	8.210172	RSD			
Diazepam	284.9995	153.999	QUAN	2.33	1892	319	120.3786	STD			

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Diazepam	284.9995	153.999	QUAN	2.32	1684	145	1823	AVG	B1-5	0.08	0.11
Diazepam	284.9995	153.999	QUAN	2.32	1893	9	6.603323	RSD			
Diazepam	284.9995	153.999	QUAN	2.18	510	348	536.2558	STD			
Diazepam	284.9995	153.999	QUAN	2.32	1516	121	905.6667	AVG	B1-6	0.04	0.06
Diazepam	284.9995	153.999	QUAN	2.34	691	199	59.21117	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	1728	590	471.1946	STD			
Diazepam	284.9995	153.999	QUAN	2.32	2320	555	1812.333	AVG	B1-7	0.08	0.11
Diazepam	284.9995	153.999	QUAN	2.32	1389	979	25.99933	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	645	107	89.83874	STD			
Diazepam	284.9995	153.999	QUAN	2.33	604	187	574	AVG	B1-8	0.03	0.03
Diazepam	284.9995	153.999	QUAN	2.32	473	193	15.65135	RSD			

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Diazepam	284.9995	153.999	QUAN	2.16	327	160	183.6773	STD			
Diazepam	284.9995	153.999	QUAN	2.49	65	1	270.3333	AVG	B1-9	0.01	0.02
Diazepam	284.9995	153.999	QUAN	2.33	419	52	67.94473	RSD			
Diazepam	284.9995	153.999	QUAN	2.13	253	59	61.40304	STD			
Diazepam	284.9995	153.999	QUAN	2.34	354	176	283.3333	AVG	B1-10	0.01	0.02
Diazepam	284.9995	153.999	QUAN	2.16	243	291	21.67166	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	663	17	260.1621	STD			
Diazepam	284.9995	153.999	QUAN	2.33	502	0	439.6667	AVG	B1-11	0.02	0.03
Diazepam	284.9995	153.999	QUAN	2.13	154	345	59.17258	RSD			
Diazepam	284.9995	153.999	QUAN	2.33	683	86	93.12536	STD			
Diazepam	284.9995	153.999	QUAN	2.32	750	365	666.3333	AVG	B1-12	0.03	0.04
Diazepam	284.9995	153.999	QUAN	2.32	566	216	13.97579	RSD			
Diazepam	284.9995	153.999	QUAN	2.14	125	0	29.1376	STD			
Diazepam	284.9995	153.999	QUAN	2.52	68	0	93	AVG	B1-13	0.00	0.01
Diazepam	284.9995	153.999	QUAN	2.25	86	3	31.33076	RSD			

Table A.5 Diazepam data from second sampling batch

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Diazepam	284.9995	153.999	QUAN	2.12	89	31	541.8213	STD			
Diazepam	284.9995	153.999	QUAN	2.31	1044	56	714.3333	AVG	B2-1	0.03	0.04
Diazepam	284.9995	153.999	QUAN	2.32	1010	81	75.84993	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	4993	118	418.6884	STD			
Diazepam	284.9995	153.999	QUAN	2.32	4323	478	4803	AVG	B2-2	0.21	0.29
Diazepam	284.9995	153.999	QUAN	2.32	5093	298	8.717227	RSD			
Diazepam	284.9995	153.999	QUAN	2.33	5502	65	14.29452	STD			
Diazepam	284.9995	153.999	QUAN	2.33	5474	347	5489.667	AVG	B2-3	0.24	0.33
Diazepam	284.9995	153.999	QUAN	2.32	5493	125	0.26039	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	1647	261	267.9409	STD			
Diazepam	284.9995	153.999	QUAN	2.33	2159	251	1857.333	AVG	B2-4	0.08	0.11
Diazepam	284.9995	153.999	QUAN	2.32	1766	335	14.42611	RSD			
Diazepam	284.9995	153.999	QUAN	2.33	534	35	209.2136	STD			
Diazepam	284.9995	153.999	QUAN	2.25	140	0	377.6667	AVG	B2-5	0.02	0.02

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Diazepam	284.9995	153.999	QUAN	2.33	459	43	55.39637	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	1724	478	429.7895	STD			
Diazepam	284.9995	153.999	QUAN	2.32	1973	219	1611	AVG	B2-6	0.07	0.10
Diazepam	284.9995	153.999	QUAN	2.32	1136	661	26.67843	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	754	50	55.80621	STD			
Diazepam	284.9995	153.999	QUAN	2.13	728	179	709.6667	AVG	B2-7	0.03	0.04
Diazepam	284.9995	153.999	QUAN	2.32	647	127	7.863722	RSD			
Diazepam	284.9995	153.999	QUAN	2.23	126	43	275.2278	STD			
Diazepam	284.9995	153.999	QUAN	2.33	587	140	269.6667	AVG	B2-8	0.01	0.02
Diazepam	284.9995	153.999	QUAN	2.33	96	86	102.0622	RSD			

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Diazepam	284.9995	153.999	QUAN	2.11	103	105	720.4506	STD			
Diazepam	284.9995	153.999	QUAN	2.32	1536	277	863	AVG	B2-9	0.04	0.05
Diazepam	284.9995	153.999	QUAN	2.34	950	336	83.4821	RSD			
Diazepam	284.9995	153.999	QUAN	2.33	1337	414	568.3933	STD			
Diazepam	284.9995	153.999	QUAN	2.14	216	313	831	AVG	B2-10	0.04	0.05
Diazepam	284.9995	153.999	QUAN	2.33	940	269	68.39872	RSD			
Diazepam	284.9995	153.999	QUAN	2.11	584	329	199.8533	STD			
Diazepam	284.9995	153.999	QUAN	2.32	596	211	474.6667	AVG	B2-11	0.02	0.03
Diazepam	284.9995	153.999	QUAN	2.43	244	0	42.10392	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	550	380	34.0196	STD			
Diazepam	284.9995	153.999	QUAN	2.32	482	513	516.6667	AVG	B2-12	0.02	0.03
Diazepam	284.9995	153.999	QUAN	2.35	518	592	6.584439	RSD			
Diazepam	284.9995	153.999	QUAN	2.57	73	1	27.51363	STD			
Diazepam	284.9995	153.999	QUAN	2.15	99	0	100	AVG	B2-13	0.00	0.01
Diazepam	284.9995	153.999	QUAN	2.3	128	20	27.51363	RSD			

Table A.6 Diazepam data from third sampling site

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Diazepam	284.9995	153.999	QUAN	2.31	1168	0	296.1064	STD			
Diazepam	284.9995	153.999	QUAN	2.33	764	137	841	AVG	B3-1	0.04	0.05
Diazepam	284.9995	153.999	QUAN	2.33	591	113	35.20885	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	4821	192	53.10681	STD			
Diazepam	284.9995	153.999	QUAN	2.32	4740	367	4760.667	AVG	B3-2	0.21	0.29
Diazepam	284.9995	153.999	QUAN	2.32	4721	407	1.115533	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	4852	274	339.9799	STD			
Diazepam	284.9995	153.999	QUAN	2.32	4339	706	4724.333	AVG	B3-3	0.21	0.29
Diazepam	284.9995	153.999	QUAN	2.33	4982	83	7.196357	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	1948	152	29.36551	STD			
Diazepam	284.9995	153.999	QUAN	2.32	1890	236	1916.333	AVG	B3-4	0.08	0.12
Diazepam	284.9995	153.999	QUAN	2.32	1911	74	1.53238	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	1095	79	165.004	STD			

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Diazepam	284.9995	153.999	QUAN	2.31	817	148	904.6667	AVG	B3-5	0.04	0.06
Diazepam	284.9995	153.999	QUAN	2.32	802	251	18.23921	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	771	225	283.3284	STD			
Diazepam	284.9995	153.999	QUAN	2.27	416	265	466	AVG	B3-6	0.02	0.03
Diazepam	284.9995	153.999	QUAN	2.11	211	134	60.80009	RSD			
Diazepam	284.9995	153.999	QUAN	2.13	118	148	556.6929	STD			
Diazepam	284.9995	153.999	QUAN	2.35	1007	13	756	AVG	B3-7	0.03	0.05
Diazepam	284.9995	153.999	QUAN	2.32	1143	400	73.63663	RSD			
Diazepam	284.9995	153.999	QUAN	2.35	523	368	257.405	STD			
Diazepam	284.9995	153.999	QUAN	2.21	51	186	227.6667	AVG	B3-8	0.01	0.01
Diazepam	284.9995	153.999	QUAN	2.13	109	91	113.0622	RSD			

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C
Diazepam	284.9995	153.999	QUAN	2.31	521	86	80.01458	STD			
Diazepam	284.9995	153.999	QUAN	2.31	363	167	449.3333	AVG	B3-9	0.02	0.03
Diazepam	284.9995	153.999	QUAN	2.33	464	6	17.8074	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	474	209	214.3113	STD			
Diazepam	284.9995	153.999	QUAN	2.63	70	49	313.3333	AVG	B3 -10	0.01	0.02
Diazepam	284.9995	153.999	QUAN	2.1	396	349	68.39722	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	1311	879	333.7669	STD			
Diazepam	284.9995	153.999	QUAN	2.31	1090	604	1018.667	AVG	B3-11	0.04	0.06
Diazepam	284.9995	153.999	QUAN	2.32	655	812	32.76507	RSD			
Diazepam	284.9995	153.999	QUAN	2.33	1821	305	468.5968	STD			
Diazepam	284.9995	153.999	QUAN	2.33	884	558	1358	AVG	B3-12	0.06	0.08
Diazepam	284.9995	153.999	QUAN	2.33	1369	199	34.50639	RSD			
Diazepam	284.9995	153.999	QUAN	2.32	250	37	89.93887	STD			
Diazepam	284.9995	153.999	QUAN	2.31	107	30	147	AVG	B3-13	0.01	0.01
Diazepam	284.9995	153.999	QUAN	2.24	84	0	61.1829	RSD			

Table A.7 Lorazepam data from first sampling batch

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C	R.C.C.B	F.C.C
Lorazepam	320.9995	302.9995	QUAN	2	16218	15785	3057.136	STD					
Lorazepam	320.9995	302.9995	QUAN	2	18979	22576	19173.33	AVG	B1-1	6.80	10.29	9.21	1.09
Lorazepam	320.9995	302.9995	QUAN	2	22323	23119	15.94473	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	11289	9288	2025.701	STD					
Lorazepam	320.9995	302.9995	QUAN	2	11365	9609	12496.33	AVG	B1-2	6.14	9.28	9.21	0.07
Lorazepam	320.9995	302.9995	QUAN	2	14835	16729	16.21036	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	23048	17090	2083.774	STD					
Lorazepam	320.9995	302.9995	QUAN	2	23612	21837	22138	AVG	B1-3	7.10	10.74	9.21	1.53
Lorazepam	320.9995	302.9995	QUAN	2	19754	18323	9.412659	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	15196	21055	1481.428	STD					
Lorazepam	320.9995	302.9995	QUAN	2	15421	21401	16161.33	AVG	B1-4	6.50	9.84	9.21	0.63
Lorazepam	320.9995	302.9995	QUAN	2.01	17867	21938	9.166499	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	13019	16038	2912.785	STD					

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C	R.C.C.B	F.C.C
Lorazepam	320.9995	302.9995	QUAN	2	14127	22546	15224	AVG	B1-5	6.41	9.69	9.21	0.49
Lorazepam	320.9995	302.9995	QUAN	2	18526	26136	19.13285	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	19468	28361	1705.008	STD					
Lorazepam	320.9995	302.9995	QUAN	2	17772	32589	17766	AVG	B1-6	6.66	10.08	9.21	0.87
Lorazepam	320.9995	302.9995	QUAN	2	16058	34863	9.597028	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	18125	39104	5291.248	STD					
Lorazepam	320.9995	302.9995	QUAN	2	27831	51205	21760.67	AVG	B1-7	7.06	10.68	9.21	1.48
Lorazepam	320.9995	302.9995	QUAN	2	19326	35800	24.31565	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	15549	18285	797.1357	STD					
Lorazepam	320.9995	302.9995	QUAN	2	16243	10266	15481.67	AVG	B1-8	6.43	9.73	9.21	0.53
Lorazepam	320.9995	302.9995	QUAN	2	14653	20701	5.148901	RSD					

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C	R.C.C.B	F.C.C
Lorazepam	320.9995	302.9995	QUAN	2	14686	17167	1523.486	STD					
Lorazepam	320.9995	302.9995	QUAN	2	17192	24458	15438.67	AVG	B1-9	6.43	9.73	9.21	0.52
Lorazepam	320.9995	302.9995	QUAN	2	14438	18386	9.867989	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	10796	26534	1322.267	STD					
Lorazepam	320.9995	302.9995	QUAN	2	12987	27379	12319	AVG	B1-10	6.12	9.25	9.21	0.05
Lorazepam	320.9995	302.9995	QUAN	2	13174	25498	10.73355	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	16760	13548	3384.432	STD					
Lorazepam	320.9995	302.9995	QUAN	2	14631	14353	17550.33	AVG	B1-11	6.64	10.05	9.21	0.84
Lorazepam	320.9995	302.9995	QUAN	2	21260	19592	19.28415	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	16457	27628	2913.667	STD					
Lorazepam	320.9995	302.9995	QUAN	2	14384	20757	16992.67	AVG	B1-12	6.59	9.96	9.21	0.75
Lorazepam	320.9995	302.9995	QUAN	2	20137	29753	17.14662	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	20936	8173	1384.296	STD					
Lorazepam	320.9995	302.9995	QUAN	2	18225	8748	19742.67	AVG	B1-13	6.86	10.38	9.21	1.17
Lorazepam	320.9995	302.9995	QUAN	2	20067	14267	7.011695	RSD					

Table A.8 Lorazepam data from second sampling batch

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C	R.C.C.B	F.C.C
Lorazepam	320.9995	302.9995	QUAN	2	27747	9409	2158.156	STD					
Lorazepam	320.9995	302.9995	QUAN	2	27079	10271	28644	AVG	B2-1	7.75	11.73	9.21	2.52
Lorazepam	320.9995	302.9995	QUAN	2	31106	10389	7.53441	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	10907	20886	4322.393	STD					
Lorazepam	320.9995	302.9995	QUAN	2	15976	23217	15463	AVG	B2-2	6.43	9.73	9.21	0.52
Lorazepam	320.9995	302.9995	QUAN	2	19506	24761	27.95313	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	17799	13265	2328.473	STD					
Lorazepam	320.9995	302.9995	QUAN	2	21620	21902	18941	AVG	B2-3	6.78	10.26	9.21	1.05
Lorazepam	320.9995	302.9995	QUAN	2	17404	12370	12.2933	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	13849	15358	2380.12	STD					
Lorazepam	320.9995	302.9995	QUAN	2	15319	14164	15891	AVG	B2-4	6.48	9.79	9.21	0.59
Lorazepam	320.9995	302.9995	QUAN	2	18505	20553	14.97779	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	20439	15243	2116.876	STD					

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C	R.C.C.B	F.C.C
Lorazepam	320.9995	302.9995	QUAN	2	22823	12954	20621	AVG	B2-5	6.95	10.51	9.21	1.30
Lorazepam	320.9995	302.9995	QUAN	2	18601	12364	10.26563	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	14528	18397	937.2653	STD					
Lorazepam	320.9995	302.9995	QUAN	2	13821	26086	14675.67	AVG	B2-6	6.35	9.61	9.21	0.40
Lorazepam	320.9995	302.9995	QUAN	2	15678	29351	6.386527	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	15706	12962	3195.39	STD					
Lorazepam	320.9995	302.9995	QUAN	2	18685	16166	18827.67	AVG	B2-7	6.77	10.24	9.21	1.03
Lorazepam	320.9995	302.9995	QUAN	2	22092	13288	16.97178	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	21690	19137	757.9743	STD					
Lorazepam	320.9995	302.9995	QUAN	2	23195	13344	22495	AVG	B2-8	7.14	10.79	9.21	1.59
Lorazepam	320.9995	302.9995	QUAN	2	22600	12510	3.369523	RSD					

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C	R.C.C.B	F.C.C
Lorazepam	320.9995	302.9995	QUAN	1.85	44666	31650	12159.09	STD					
Lorazepam	320.9995	302.9995	QUAN	1.85	42375	30330	36531.67	AVG	B2-9	8.54	12.92	9.21	3.71
Lorazepam	320.9995	302.9995	QUAN	2	22554	40766	33.28371	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	18681	29310	3225.031	STD					
Lorazepam	320.9995	302.9995	QUAN	2	12392	28153	15950	AVG	B2-10	6.48	9.80	9.21	0.60
Lorazepam	320.9995	302.9995	QUAN	2	16777	25468	20.21963	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	21159	35493	3033.82	STD					
Lorazepam	320.9995	302.9995	QUAN	2	15236	27631	17817.33	AVG	B2-11	6.67	10.09	9.21	0.88
Lorazepam	320.9995	302.9995	QUAN	2	17057	29663	17.02735	RSD					
Lorazepam	320.9995	302.9995	QUAN	1.85	69279	36633	2932.552	STD					
Lorazepam	320.9995	302.9995	QUAN	1.85	68574	34991	70607.33	AVG	B2-12	11.95	18.08	9.21	8.88
Lorazepam	320.9995	302.9995	QUAN	1.86	73969	41433	4.153324	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	15033	11419	902.6367	STD					
Lorazepam	320.9995	302.9995	QUAN	2	14890	11510	14442	AVG	B2-13	6.33	9.57	9.21	0.37
Lorazepam	320.9995	302.9995	QUAN	2	13403	9782	6.250081	RSD					

Table A.9 Lorazepam data from third sampling batch

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C	R.C.C.B	F.C.C
Lorazepam	320.9995	302.9995	QUAN	2	20674	6836	2525.331	STD					
Lorazepam	320.9995	302.9995	QUAN	2	20890	10513	22238.67	AVG	B3-1	7.11	10.76	9.21	1.55
Lorazepam	320.9995	302.9995	QUAN	2	25152	7104	11.35559	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	18857	16083	3388.38	STD					
Lorazepam	320.9995	302.9995	QUAN	2	24283	26139	20398	AVG					
Lorazepam	320.9995	302.9995	QUAN	2	18054	17732	16.61134	RSD	B3-2	6.93	10.48	9.21	1.27
Lorazepam	320.9995	302.9995	QUAN	2	17067	12347	3996.82	STD					
Lorazepam	320.9995	302.9995	QUAN	2	24373	23479	19783.67	AVG					
Lorazepam	320.9995	302.9995	QUAN	2	17911	18200	20.20262	RSD	B3-3	6.86	10.38	9.21	1.18
Lorazepam	320.9995	302.9995	QUAN	2	17983	16407	1421.107	STD					
Lorazepam	320.9995	302.9995	QUAN	2	15145	10137	16608.67	AVG	B3-4	6.55	9.90	9.21	0.70
Lorazepam	320.9995	302.9995	QUAN	2	16698	10299	8.556421	RSD					
Lorazepam	320.9995	302.9995	QUAN	2	18538	13490	642.5398	STD					

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C	R.C.C.B	F.C.C
Lorazepam	320.9995	302.9995	QUAN	2	19510	11941	19266.67	AVG					
Lorazepam	320.9995	302.9995	QUAN	2	19752	15710	3.334981	RSD	B3-5	6.81	10.31	9.21	1.10
Lorazepam	320.9995	302.9995	QUAN	2	21049	30548	786.6857	STD					
Lorazepam	320.9995	302.9995	QUAN	2	21955	20191	21873.33	AVG					
Lorazepam	320.9995	302.9995	QUAN	2	22616	23166	3.596551	RSD	B3-6	7.07	10.70	9.21	1.49
Lorazepam	320.9995	302.9995	QUAN	2	23581	12034	3043.351	STD					
Lorazepam	320.9995	302.9995	QUAN	2	22494	18570	24766.33	AVG					
Lorazepam	320.9995	302.9995	QUAN	2	28224	20674	12.28826	RSD	B3-7	7.36	11.14	9.21	1.93
Lorazepam	320.9995	302.9995	QUAN	2	14639	12574	2299.846	STD					
Lorazepam	320.9995	302.9995	QUAN	2	18960	17696	17254.67	AVG					
Lorazepam	320.9995	302.9995	QUAN	2	18165	16216	13.32883	RSD	B3-8	6.61	10.00	9.21	0.79

Compound	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background			SP	Concentration ngg^{-5}	R.C.C	R.C.C.B	F.C.C
Lorazepam	320.9995	302.9995	QUAN	2	23371	18868	1378.743	STD					
Lorazepam	320.9995	302.9995	QUAN	2	20676	19689	22192	AVG					
Lorazepam	320.9995	302.9995	QUAN	2	22529	15165	6.212794	RSD	B3-9	7.11	10.75	9.21	1.54
Lorazepam	320.9995	302.9995	QUAN	2	19613	38193	2867.49	STD					
Lorazepam	320.9995	302.9995	QUAN	2	23214	41362	20125	AVG					
Lorazepam	320.9995	302.9995	QUAN	2	17548	23442	14.2484	RSD	B3-10	6.90	10.44	9.21	1.23
Lorazepam	320.9995	302.9995	QUAN	2	21724	53517	1178.199	STD					
Lorazepam	320.9995	302.9995	QUAN	2	21548	41903	20957.67	AVG					
Lorazepam	320.9995	302.9995	QUAN	2	19601	41236	5.621803	RSD	B3-11	6.98	10.56	9.21	1.36
Lorazepam	320.9995	302.9995	QUAN	1.85	43427	35106	1308.984	STD					
Lorazepam	320.9995	302.9995	QUAN	1.85	44136	37843	44509	AVG					
Lorazepam	320.9995	302.9995	QUAN	1.85	45964	36370	2.940942	RSD	B3-12	9.34	14.13	9.21	4.92
Lorazepam	320.9995	302.9995	QUAN	2	19791	11072	992.0546	STD					
Lorazepam	320.9995	302.9995	QUAN	2	17832	10189	18902.33	AVG					
Lorazepam	320.9995	302.9995	QUAN	2	19084	11804	5.248318	RSD	B3-13	6.78	10.25	9.21	1.04

Appendix B Method Detection Limit, Limit of Detection and Quantification of Detected Target Compounds

Table B.1 MDL, LOD, LOQ values of Carbamazepine

CARBAMAZEPINE: MDL,LOD,LOQ									
Name	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background	Peak Rank	Concentration ngg^{-1}	
Carbamazepine	237.1995	194.19945	QUAN	1.91	3383	1534	1	0.07	
Carbamazepine	237.1995	194.19945	QUAN	1.91	2355	1297	1	-0.26	
Carbamazepine	237.1995	194.19945	QUAN	1.89	2447	648	1	-0.23	
Carbamazepine	237.1995	194.19945	QUAN	1.89	2118	1054	1	-0.33	
Carbamazepine	237.1995	194.19945	QUAN	1.89	1359	1060	1	-0.58	
Carbamazepine	237.1995	194.19945	QUAN	1.91	1083	684	1	-0.67	
Carbamazepine	237.1995	194.19945	QUAN	1.76	842	656	1	-0.75	
							STD	0.22	MDL
							STDx3	0.67	LOD
							STDx10	2.23	LOQ

Table B.2 MDL, LOD, LOQ values of Diazepam ngg^{-1}

DIAZEPAM : MDL, LOD, LOQ									
Name	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background	Peak Rank	concentration ngg^{-1}	
Diazepam	284.9995	153.999451	QUAN	2.33	3300	1095	2	0.19	
Diazepam	284.9995	153.999451	QUAN	2.32	3130	872	1	0.16	
Diazepam	284.9995	153.999451	QUAN	2.57	49	223	2	-0.49	
Diazepam	284.9995	153.999451	QUAN	2.33	2899	619	2	0.11	
Diazepam	284.9995	153.999451	QUAN	2.33	1506	374	2	-0.19	
Diazepam	284.9995	153.999451	QUAN	2.32	936	544	2	-0.31	
Diazepam	284.9995	153.999451	QUAN	2.3	1793	721	2	-0.12	
							STD	0.26	MDL
							STDx3	0.78	LOD
							STDx10	2.59	LOQ

Table B.3 MDL, LOD, LOQ values of Lorazepam

LORAZEPAM: MDL,LOD,LOQ									
Name	Precursor Mz	Product Mz	Fragment Ion	Retention Time	Area	Background	Peak Rank	Conc. ngg^{-1}	
Lorazepam	320.999451	302.999451	QUAN	2	10789	5878	2	5.96	
Lorazepam	320.999451	302.999451	QUAN	2	11447	6080	1	6.03	
Lorazepam	320.999451	274.999451	QUAL	2	11602	2353	2	6.05	
Lorazepam	320.999451	274.999451	QUAL	1.99	11525	2799	2	6.04	
Lorazepam	320.999451	302.999451	QUAN	2	10820	10079	2	5.97	
Lorazepam	320.999451	302.999451	QUAN	2	10879	7226	2	5.97	
Lorazepam	320.999451	274.999451	QUAL	2	10735	2812	2	5.96	
							STD	0.04	MDL
							STDx3	0.12	LOD
							STDx10	0.39	LOQ

Table B.4 Results of EPPPs analysis in Brisbane River sediments from first sampling batch

Results for sampling from first batch			
Analytes concentration= ng/g of sediment			
SAMPLING SITES	CARBAMAZEPINE	DIAZEPAM	LORAZEPAM
B1-1	1.99	0.01	0.22
B1-2	1.26	0.01	0.01
B1-5	1.01	0.01	0.31
B1-7	4.56	0.04	0.13
B1-12	2.43	0.02	0.10
B1-13	1.11	0.01	0.15
B1-14	1.07	0.00	0.17
B1-15	1.93	0.00	0.00
B1-16	0.96	0.01	0.11
B1-18	0.56	0.00	0.01
B1-19	0.21	0.00	0.17
B1-20	0.96	0.01	0.15
B1-21	0.20	0.00	0.10
B1-22	0.02	0.03	0.23

Table B.5 Results of EPPPs analysis in the Brisbane River sediments from second sampling batch

Results for sampling from second batch			
Analytes concentration= ng/g of sediment			
SAMPLING SITES	CARBAMAZEPINE	DIAZEPAM	LORAZEPAM
B2-1	1.07	0.01	0.50
B2-2	6.39	0.04	0.00
B2-5	3.54	0.05	0.21
B2-7	2.52	0.02	0.12
B2-12	0.98	0.00	0.26
B2-14	2.73	0.00	0.08
B2-15	0.53	0.01	0.21
B2-16	0.00	0.00	0.32
B2-17	1.01	0.00	0.00
B2-18	0.00	0.00	0.12
B2-19	0.84	0.01	0.18
B2-20	1.04	0.00	1.78
B2-22	0.07	0.00	0.07
B2-8	4.65	0.03	0.27
B2-9	2.76	0.00	0.26

Table B.6 Results of EPPPs analysis in the Brisbane River sediments from third sampling batch

Results for sampling from third batch			
Analytes concentration= ng/g of sediment			
SAMPLING SITES	CARBAMAZEPINE	DIAZEPAM	LORAZEPAM
B3-1	0.84	0.00	-0.36
B3-2	4.90	0.04	0.25
B3-5	2.09	0.04	0.24
B3-7	1.99	0.02	0.14
B3-12	1.21	0.01	0.22
B3-14	0.83	0.00	0.30
B3-15	0.58	0.00	0.39
B3-16	0.45	0.00	0.16
B3-17	0.43	0.00	0.31
B3-18	0.59	0.00	0.25
B3-19	1.20	0.00	0.27
B3-20	1.77	0.00	0.98
B3-22	0.08	0.00	0.21