Development and Application of Analytical Methods for Understanding the Fate and Occurrence of Pharmaceuticals in Freshwater Sediments

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ABSTRACT

There has been a global increase in the use of active pharmaceutical ingredients (APIs) in recent decades due to population growth and population ageing, increasing affluence, changes in disease burdens and the increasing availability of medicines across the world. Numerous studies have been performed into the fate and occurrence of pharmaceuticals in surface waters. However, only a few studies have explored the sediment compartment. Knowledge of the fate of these compounds in sediments is needed in order to determine the risks of pharmaceuticals in the environment. Therefore, this thesis describes the development of new analytical methods and laboratory and field-based studies to understand the fate and distribution of pharmaceuticals in freshwater sediments.

Approaches for prioritising pharmaceuticals in the environment, based on their risk, were initially used to identify candidates for experimental study. Antibiotics, antidepressants and analgesics were identified as the pharmaceutical classes of most concern in surface water, sediment and the terrestrial environment.

New analytical methods were then developed for the extraction and determination of six pharmaceuticals in a range of sediments obtained from the UK and Iraq. Using ultrasonic-assisted extraction (UAE) and high performance liquid chromatography coupled with diode array detector (HPLC–DAD) or liquid chromatography tandem mass spectrometry (LC- MS/MS) for detection. Detection limits ranging from 15 to 58.5 ng g^{-1} and 0.03 to 3.5 ng g^{-1} for water and sediment were achieved, respectively. Best recoveries were obtained for atenolol, amitriptyline, mefenamic acid and diltiazem.

The analytical methods were then utilized to study the sorption, persistence and occurrence of the pharmaceuticals in water-sediment systems. Laboratory-based sorption studies showed that sorption increased in the order: mefenamic acid < cimetidine < atenolol < amitriptyline < diltiazem. Multiple linear regression analysis indicated that the sorption was driven by factors such as the pH-corrected hydrophobicity octanol/water partitioning coefficient (Log Dow) of the study compound and the cation exchange capacity, clay, organic carbon content and exchangeable Ca^{2+} content of the sediment. Dissipation of each pharmaceutical was found to follow first-order exponential decay. Half-lives in the sediments ranged from 9.5 d (atenolol) to 78.8 d (amitriptyline).

Finally, the occurrence and seasonal distribution of pharmaceuticals in water and sediments from River Ouse and River Foss, York, were investigated. All ten pharmaceuticals were detected at concentrations up to 59.7 ng L^{-1} (atenolol) and 18.4 ng g^{-1} (trimethoprim) in water and sediment samples, respectively. Spatial and seasonal distribution profiles revealed different inputs of WWTPs, rivers flow and usage as the main factors responsible for the pharmaceuticals distribution.

Overall, the results show that the fate and occurrence of pharmaceuticals in sediment compartment is driven by chemical use patterns, chemical properties (such as Log Dow) and environmental parameters (such as flow and sediment properties). While this study provides a step forward in understanding some of the key drivers of exposure, further work is needed before we can fully assess the fate and occurrence of pharmaceuticals in sediments at the landscape scale.

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Author Declaration

The work in this thesis was undertaken by me as a PhD student at the Environment department, the University of York. The research was funded by the Ministry of Higher Education and Scientific Research (MOHESR), Iraq. I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this or any other University. All sources are acknowledged as references.

The data reported in Chapter 3 was presented at the SETAC Europe 24th annual meeting, Barcelona, Spain, 2013.

Research from Chapter 5 was presented at the SETAC Europe 25th annual meeting, Nantes, France, 2011.

Chapters 2 to 7 have been written as papers for international peer-reviewed journals. The current publication status of the papers is presented in Table 0.1. All these papers have been reworked so that they can be presented in a consistent style and format in this thesis. For those papers that are published, copyright rests with the publishers.

Table 0.1 Status of the papers presented in this thesis with respect to the publication process

Chapter	Title	Journal	Status and DOI
2	Analysis, Occurrence, Fate and effects of Pharmaceuticals in Freshwater Sediment	-	In preparation
3	Risk-based prioritization of pharmaceuticals in the natural environment in Iraq	Environmental Science and Pollution Research 2016	Published 10.1007/s11356-016-6679-0
4	Determination of pharmaceuticals in freshwater sediments using ultrasonic-assisted extraction with SPE clean-up and HPLC–DAD or LC-ESI-MS/MS detection	Analytical Methods 2017	Published 10.1039/C7AY00650K
5	Impacts of compound properties and sediment characteristics on the sorption behaviour of pharmaceuticals in aquatic systems	Journal of Hazardous Materials 2016	Published 10.1016/j.jhazmat.2016.05.065
6	Effects of sediment properties on the dissipation of pharmaceuticals in freshwater sediments	Environmental Toxicology and Chemistry 2017	Published 10.1002/etc.4015
7	Temporal and Spatial Distribution of Pharmaceuticals in Urban River Environments	Chemosphere	Prepared for submission

Glossary

ACN	Acetonitrile
ADI	Acceptable daily intake
AMR	Antimicrobial resistance
API	Active pharmaceutical ingredient
ASE	Accelerated solvent extraction
BCF	Bioconcentration factor
BMF	Biomagnification factor
BSAF	Biota-sediment accumulation factor
BSTFA	N,O-bis (trimethylsilyl) trifluoroacetamide
CAS	Conventional activated sludge
CEC	Cation exchange capacity
CI	Chemical ionization
DF	Dilution factor
DO	Dissolved oxygen
DOM	Dissolved organic matter
DT50	Times for half of the compound to be dissipate
EC	Emerging contaminant
EI	Electron impact
EMEA	European guideline for environmental risk assessment of human medicines
ESI	Electrospray ionisation
***f*oc**	Organic carbon percentage
FSSPC	Fish steady state plasma concentration
GC/MS	Gas chromatography-mass spectrometry
GC-MS/MS	Gas chromatography tandem mass spectrometry
HLB	Hydrophilic–lipophilic balance
HPLC–DAD	High performance liquid chromatography - Diode array detector
H_TPC	Human therapeutic plasma concentration
IC50	Half maximal inhibitory concentration
IDL	Instrumental detection limit
IQL	Instrumental quantification limit
k	Degradation rate
Kd	Adsorption coefficient
Koc	Organic carbon-based partitioning coefficient
LC- MS	liquid chromatography-mass spectrometry
LC- MS/MS	liquid chromatography tandem mass spectrometry
LC50	Median lethal dose
LC-Ion Trap- MS/MS	Liquid chromatography- ion trap- tandem mass spectrometry
LDTD-APCI-MS/MS	Laser diode thermal desorption (LDTD)/atmospheric pressure chemical ionization tandem mass spectrometry
LOD	Limit of detection
Log Dow	Octanol/water partitioning coefficient corrected to the matrix pH
LogKow	Octanol/water partitioning coefficient
LOQ	Limit of quantification
MAE	Microwave assisted extraction
MAME	Microwave assisted micellar extraction

MAX	Moderate anion exchange
MCX	Medium cation exchange
MDL	Method detection limit
MEC	Measured environmental concentration
MeOH	Methanol
MIC	Minimal inhibitory concentration
MLR	Multiple linear regression
MRM	Multiple reaction monitoring
MSC	Minimal selective concentration
MSPD	Matrix solid-phase dispersion
MSTFA	N-Methyl-N-(trimethylsilyl) trifluoroacetamide
Nd	Non-detected
NOEC	Non-observed effects concentrations
NSAID	Non-steroidal anti-inflammatory drugs
OC	Organic carbon
OM	organic matter
OTC	Over the counter
PAH	Polycyclic aromatic hydrocarbon
PEC	Predicted environmental concentration
PHWE	Pressurized hot water extraction
pKa	Acid dissociation constant
***p*-Kd**	Pseudo-partitioning coefficient
PLE	Pressurized liquid extraction
PNEC	Predicted no-effect concentration
POM	Particulate organic matter
Q–TOF	Quadrupole time-of-flight instrument
RCR	Risk characterization ratios
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals regulatory
REC	Recovery
RSD	Relative standard deviation
S/N	signal-to-noise ratio
SAX	Strong anion exchange
SPE	Solid phase extraction
SRM	Selective reaction monitoring
TGD	Technical Guidance Document
TOF-MS	Time-of-flight mass spectrometry
TPF	triphenylformazane
TTC	2,3,5-Triphenyl-tetrazolium chloride
UAE	Ultrasonic-assisted extraction
UHPLC	Ultra-high-pressure liquid chromatography
UV	Ultra-Violet
WWTP	Waste water treatment plant

Chapter 1

Introduction

1.1 Pharmaceuticals in the Environment

Over the last two decades research into environmental pollutants has moved from investigating the conventional priority pollutants (e.g. polychlorinated biphenyls, and polycyclic aromatic hydrocarbons) towards studies into the fate, effects and risks of the so-called emerging contaminants (Bu et al., 2013). An emerging contaminant (EC) is defined as "a contaminant from a chemical class that so far has not been extensively studied, where the contaminant class may be having an impact on environmental or human health; or where there is a concern that existing environmental assessment paradigms (e.g. exposure models and fate and effects test approaches) are not appropriate for the contaminant class" (Boxall, 2012). ECs include many man-made substances such as pharmaceuticals, detergents, pesticides, personal care products and other chemicals used by society (Jiang et al., 2013; Silva et al., 2011). One EC class of particular interest to researchers and regulators are the pharmaceutical compounds (Beausse, 2004; Krascsenits et al., 2008; Li et al., 2014).

Pharmaceuticals are ubiquitous in the environment since they are extensively used in both human and veterinary medicine (Monteiro and Boxall, 2010). Pharmaceuticals cover a wide spectrum of active ingredients (> 1500 are in use) with designed biological activity and different physicochemical properties (Daughton and Ternes, 1999; Jones et al., 2005). Antibiotics, anti-inflammatories, cardiovascular drugs, hormones, anti-epileptics, lipid regulators and painkillers are among the most highly consumed pharmaceutical classes (Khetan and Collins, 2007; Löffler and Ternes, 2003). Global consumption of pharmaceuticals nowadays is higher than in the past due to the fact that these substances are more readily available and more newly designed pharmaceutical enter the market every year (Depledge, 2011; Kümmerer, 2009a). Across the world, the quantity of pharmaceuticals and personal care products produced every year is believed to be in the region of thousands of tonnes which is similar to the amount of pesticides,

fertilizers and other chemicals used in agriculture (Daughton and Ternes, 1999; Fatta-Kassinos et al., 2011; Monteiro and Boxall, 2010).

Oral administration is the most preferable route of pharmaceutical intake (Jin et al., 2015). After entering the body, pharmaceuticals may be metabolized (e.g. via glucuronidation, demethylation) or remain unchanged before being excreted *via* urine and/or faeces (Dong et al., 2013; Monteiro and Boxall, 2010). The main input route of pharmaceuticals into the environment is from sewage treatment works where parent compounds or their metabolites may discharge to the receiving waterbody through an inadequate (non-specific) treatment process (Daughton and Ternes; 1999, Halling-Sorensen et al., 1998). It is noteworthy to highlight that this route is based on the European and North American systems while in several regions of the world, the connectivity of the population to wastewater treatment technologies is limited (Boxall et al. 2012). Pharmaceuticals may also enter the environment from myriad of routes, such as manufacturing, aquaculture, urban or agricultural runoff as well as releases to soils during biosolid and manure application (Ashton et al., 2004; Boxall et al. 2012). The main sources of pharmaceuticals in the environment in developed countries and the interconnection between different environmental compartments are shown in Figure 1.1.

Some of pharmaceutical active ingredients (APIs) are able to affect ecosystems at concentrations as low as a few nanogrammes per litre (Halling-Sorensen et al., 1998). They have the ability to cause negative effects including impacts on the endocrine system to produce undesired effects such as disruption of homeostasis, short-term and long-term toxicity and antibiotic resistance of microorganisms (Ebele et al., 2017; Fent et al., 2006). For example, a range of chronic and subtle effects, including feminization of male fish and effects on wildlife behaviour, have been observed under laboratory conditions with effects concentrations being similar to those measured in the environment (Bean et al., 2014; Brodin et al., 2014; Kidd et al., 2007).

Over the last few decades there has been considerable activity in the field of environmental risk assessment of chemical products including pharmaceuticals. This has occurred in parallel to the

ubiquitous detection of the APIs and investigations into their hazard and risk in different environmental media (Straub and Hutchinson, 2012). Regulations have been developed regarding the assessment of risks of environmental exposure to APIs (e.g., Center for Drug Evaluation and Research (CDER) 1998 in USA; Committee for Medicinal Products for Veterinary Use (CVMP) 2000, 2004 and Committee for Medicinal Products for Human Use (CHMP) 2006) in Europe). The EMA/CHMP was the first definitive guideline for environmental risk assessment of human medicines that came into effect on 1 December 2006 (EMEA 2006). The guideline describes a tiered procedure, from (Phase I) categorical exclusion or direct referral, to a simple, worst-case exposure estimation of a pharmaceutical active ingredients (APIs) to the (Phase IIA) investigation of fate and effects (i.e. algal growth study, Daphnia reproduction study, and fish early life stage study), up to a refined assessment (Phase IIB) for other environmental compartments (e.g. risks to the terrestrial environment, arising from sludge application to land and if the Koc of the compound is > 10,000) (Boxall, 2012; Straub and Hutchinson, 2012).

In addition to the EMEA 2006 guideline, the European regulation known as the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), was agreed by the EU in 2006 (ECHA, 2006). This requires that any business that manufactures or imports more than 1.0 t of a chemical per year must register it before it be marketed. Although human and veterinary pharmaceuticals are exempted, REACH guidelines are still applicable for intermediate products, manufacturing raw materials and production materials even if they are not contained in the finished pharmaceutical product (Berthod, 2015).

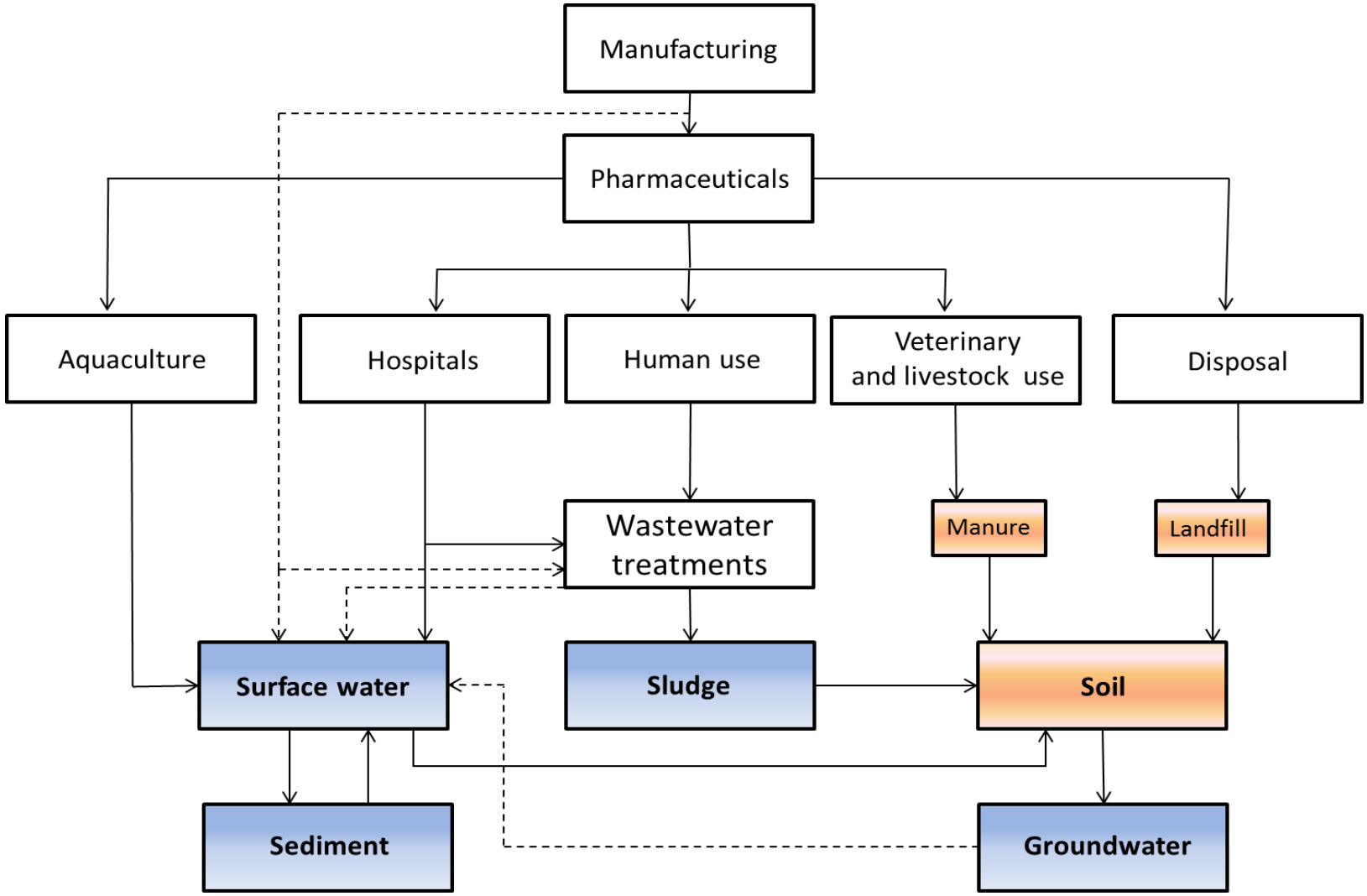


Figure 1.1 The main sources of pharmaceuticals in the environment and the interconnection between different environmental compartment in developed countries

1.2 Aims of the Thesis

The overall aim of the work reported in this PhD thesis was to better understand the factors and processes affecting the fate and behaviour of selected human use pharmaceuticals and their occurrence in the fresh water sediment environment. Sediment can act both as a sink of chemicals through sorption to particles and as a secondary source of contaminants through re-suspension in the aquatic phase. So far, there is only little information about the fate, occurrence and effect of pharmaceuticals in sediments as most of the studies have focused on soil, sludge and water systems. The specific objectives were to:

1. Review the current knowledge regarding the analysis, occurrence, fate and effect of human pharmaceuticals in sediment environment (Chapter 2).
2. Prioritise pharmaceuticals in use based on their potential to enter the aquatic and terrestrial environments and their potential toxic effects on ecosystems, bacterial communities and human health (Chapter 3).
3. Test the most promising and novel analytical methods as identified in 1; and develop and validate a novel analytical method to simultaneously extract and determine pharmaceuticals in sediments with different characteristics (Chapter 4).
4. To characterise physicochemical properties of different sediments, explore the sorption behaviour of pharmaceuticals in sediment-water systems and determine if differences in sorption can be explained by differences in sediment physicochemical properties (Chapter 5).
5. Assess the dissipation and persistence of pharmaceuticals in a wide range of environmental sediment types and determine the influence of sediment properties on the degradation rates of pharmaceuticals (Chapter 6).
6. Establish the seasonal and spatial occurrence and distribution of pharmaceuticals between the water column and sediment phase in a small catchment and evaluate the factors determining the exposure concentrations and distribution in these media (Chapter 7).

1.3 Study Compounds

In this thesis, ten pharmaceuticals from different therapeutic classes including an antidepressant, an antibiotic, anti-histamines, a β-blocker, a calcium channel blocker and non-steroidal anti-inflammatory drugs (NSAID) with different physicochemical properties were used in the experimental investigations. The selection of these pharmaceuticals was done using risk-based prioritisation studies of active pharmaceutical ingredients (APIs) in the UK (Guo et al., 2016) and Iraq (results of Chapter 3). In this thesis, the salt form of amitriptyline hydrochloride, diclofenac sodium, diltiazem hydrochloride and ranitidine hydrochloride have been referred to as amitriptyline, diclofenac, diltiazem and ranitidine to keep the consistency with the published literature. The occurrence of these pharmaceuticals has been investigated in different environmental compartments in different countries in the literature. For instance, the occurrence of the selected compounds was reported in WWTP effluents from South Wales in the UK with maximum concentration up to 9.4 μg L^{-1} for cimetidine followed by atenolol and trimethoprim with maximum concentration of 6.7 and 3.1 μg L^{-1}, respectively (Kasprzyk-Hordern et al., 2009). In the same study, maximum concentrations reported for amitriptyline and diltiazem in WWTPs influent were 5.1 and 3.2 μg L^{-1}, respectively. Recently, cimetidine showed maximum occurrence up to 1.56 μg Kg^{-1} in marine sediments in Korea (Coi et al., 2014). Diclofenac and ibuprofen were reported from WWTP effluents in Portugal at concentrations of 0.67 and 1.37 μg L^{-1} (Pereira et al., 2015). Naproxen has been reported at a maximum concentration occurrence in WWTP effluent of 2.6 μg L^{-1} in Switzerland (Tixier et al., 2003). In soil, trimethoprim was found in concentrations up to 60 μg Kg^{-1} in Malaysia (Ho et al., 2012). Diclofenac and ibuprofen were found at concentrations of 1.16 and 5.03 μg Kg^{-1} in China (Chen et al., 2011). Table 1.1 illustrates the physicochemical properties of the selected pharmaceuticals.

Table 1.1 Physicochemical properties of target pharmaceuticals

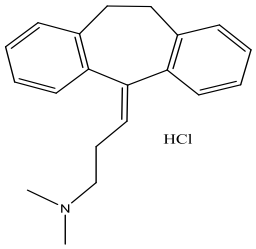
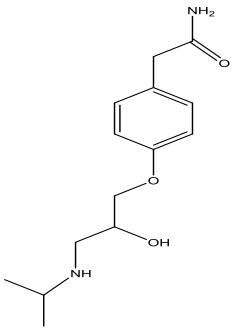
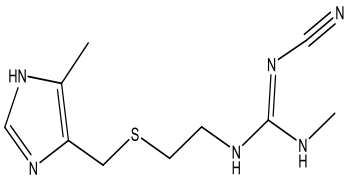
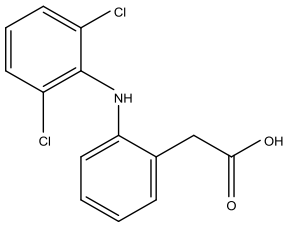
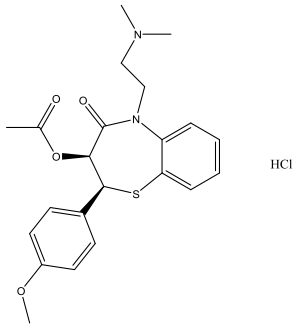
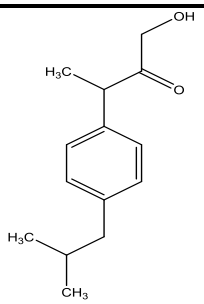
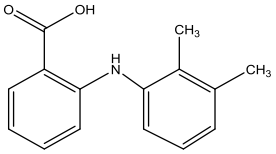
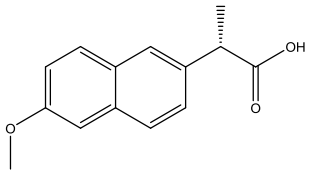
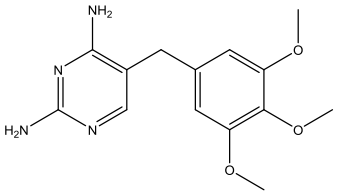
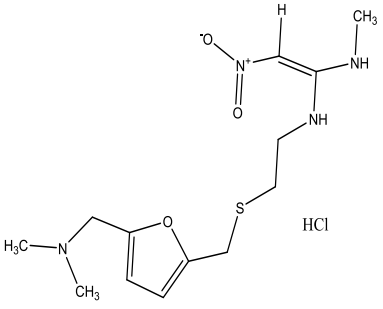
Compound Formula CAS	Therapeutic class	pKa*	Water solubility mg/L*	Log* Kow	Structure
Amitriptyline Hydrochloride $C_{20}H_{24}ClN$ 549-18-8	Anti-depressant	9.4	9.71	4.92	
Atenolol $C_{14}H_{22}N_2O_3$ 29122-68-7	β- Blocker	9.6	1.33E+4	0.16	
Cimetidine $C_{10}H_{16}N_6S$ 51481-61-9	Anti-histamine	6.8**	9380	0.4	
Diclofenac sodium $C_{14}H_{11}C_{l2}NO_2$ 15307-86-5	Non-steroidal anti-inflammatory drug (NSAID)	4.15	2.37	4.51	
Diltiazem Hydrochloride $C_{22}H_{27}ClN_2O_4S$ 33286-22-5	Calcium channel blocker	8.06**	465	2.8	

Table 1.1 (Continued)

Compound Formula CAS	Therapeutic class	pKa*	Water solubility mg/L*	Log Kow*	Structure
Ibuprofen $C_{13}H_{18}O_2$ 15687-27-1	Non-steroidal anti-inflammatory drug (NSAID)	4.91	21	3.97	
Mefenamic acid $C_{15}H_{15}NO_2$ 61-68-7	Non-steroidal anti-inflammatory drug (NSAID)	3.73	20	5.12	
Naproxen $C_{14}H_{14}O_3$ 22204-53-1	Non-steroidal anti-inflammatory drug (NSAID)	4.15	15.9	3.18	
Trimethoprim $C_{14}H_{18}N_4O_3$ 738-70-5	Antibiotic	7.12	400	0.91	
Ranitidine $C_{13}H_{23}N_4O_3SCl$ 66357-35-5	Anti-histamine	8.08	24.7	0.27	

*Drugbank (www.drugbank.ca), **PubChem (www. pubchem.ncbi.nlm.nih.gov)

1.4 Thesis Overview

This thesis is comprised of 8 chapters. A brief description of each chapter is given below:

Chapter 2 synthesises the existing knowledge on the analysis, occurrence, fate and effect of pharmaceuticals in freshwater sediment. This chapter attempts to identify gaps in our current knowledge regarding this environmental compartment and find the trends of occurrence between countries.

Chapter 3 describes the development and implementation of risk-based prioritisation approaches for pharmaceuticals entering the aquatic and terrestrial environments in Iraq. The approach was applied to 99 of the most dispensed pharmaceuticals in the cities of Baghdad, Mosul and Basrah.

Chapter 4 describes the development and validation of analytical methods for the extraction and determination of pharmaceuticals in sediment. Six pharmaceuticals (amitriptyline, atenolol, cimetidine, diltiazem, mefenamic acid and ranitidine) were successfully extracted from sediment matrices using developed ultrasonic-assisted extraction (UAE) followed by clean-up and analytes enrichment step using solid phase extraction (SPE) method. Newly developed analytical methods (HPLC–DAD and LC-MS/MS) were used for the detection and quantification.

Chapter 5 describes laboratory experiments to explore the sorption behaviour of five pharmaceuticals (amitriptyline, atenolol, cimetidine, diltiazem and mefenamic acid) in ten sediments from the UK and Iraq. Statistical analysis was then performed to explore the effects of sediment type on the sorption behaviour. Existing predictive models for ionisable compounds were also evaluated for their suitability for use on pharmaceuticals and improved models developed for estimating sorption of individual pharmaceuticals based on sediment properties.

Chapter 6 describes work to assess the persistence of six pharmaceuticals in sediments under aerobic conditions in sterilised, and non-sterilised treatments for incubation periods of 90 days. Relationships between sediments properties (physicochemical properties and microbial

activities) and sorption coefficients from chapter 5 and degradation behaviour were explored using multiple linear regression (MLR) modelling.

Chapter 7 explores the occurrence of 10 pharmaceuticals (Table 1.1) in water-sediments samples collected from seven sites along rivers around the city of York, UK. The sampling was performed on a seasonal basis from November 2015 to the end of July 2016. Pharmaceuticals in the sediment phase were determined using extraction method presented in chapter 4 and modified analytical method by using highly sensitive LC-ESI-MS/MS method. The results were compared to information on pharmaceutical usage, river flow rates and laboratory-derived sorption data to determine whether these factors explain the monitoring observations.

Chapter 8 highlights and discusses the main findings of the thesis. The broader implications of the reported results and recommendations for future research are also presented.

Chapter 2

Analysis, Occurrence, Fate and effects of Pharmaceuticals in Freshwater Sediment: Literature Review

2.1 Introduction

Pharmaceuticals are a complex class of biologically active compounds used worldwide in human and veterinary medicine which are designed to improve the quality of life *via* prevention and treatment of diseases and for the revitalization or modification of an organs function (Bottoni et al., 2010; Daughton and Ternes, 1999; Monteiro and Boxall, 2010). After entering the body, pharmaceuticals may be metabolized (e.g. via glucuronidation, demethylation) or stay unchanged before being excreted *via* urine and/or faeces (Dong et al., 2013; Monteiro and Boxall, 2010). Following excretion, these compounds typically enter wastewater treatment plants (WWTPs) which represent the main source of pharmaceutical discharge into the aquatic system (Comber et al., 2018; Kümmerer, 2004). Pharmaceuticals and their metabolites may then pass through the WWTP and reach water, underlying sediments and soils (Kümmerer, 2008; Mrozik and Stefanska, 2014). Even though concentrations in the environment are typically very low (i.e. ng L^{-1}) (Buerge et al., 2006; Kümmerer, 2004; Pomati et al., 2008; Zhou and Broodbank, 2014), concerns have been raised over the potential impacts these substances might have on the environment due to the increasing and continuous release and their designed biological activity (Kümmerer, 2008; Valcárcel et al., 2011; Verlicchi et al., 2012). There is, however, still much to be understood about the environmental occurrence, fate and the impact of environmental exposure to pharmaceuticals. While for many compounds, data are available on environmental fate and effects, for a large proportion of pharmaceuticals this is largely unknown (Boxall et al., 2012; Dong et al., 2013; Calisto and Esteves, 2009; Du et al., 2014). For example, a very large body of studies regarding the occurrence, fate and effect antibiotics and hormones in the environment were published in the literature over the last two decades (Daughton, 2016).

Research regarding the occurrence of pharmaceuticals in the environment has increased in last two decades. Improvements in analytical techniques, such as liquid chromatography-tandem mass spectrometry and developments in sample enrichment, have allowed the detection of pharmaceuticals at low concentrations even in highly complex matrices, leading to a rapid increase in the amount of data and knowledge about the levels and toxicity of pharmaceuticals in natural environments (Gómez et al., 2006; Zhou and Broodbank, 2014). A vast volume of literature has reported the occurrence of a wide range of pharmaceutical residues in the environment including analgesics, antibiotics, antiepileptics, antidepressants, anxiolytics, blood-lipid regulators and contraceptives. These compounds have been detected in surface water, ground water and in wastewaters in a number of countries over the last two decades (Al Aukidy et al., 2012; Batt et al., 2008; Focazio et al., 2008; Hernando et al., 2007; Kolpin et al., 2002; Moreno-González et al., 2014; Petrović et al., 2014; Tewari et al., 2013; Weigel et al., 2004). However, despite the importance of sediment as a sink for organic pollutants, limited information is available on the occurrence and behaviour of pharmaceutical in this compartment. An analysis of 1016 original publications and 150 review articles on pharmaceuticals in the environment, found that only 2% of existing environmental studies deal with the occurrence of pharmaceuticals in aquatic suspended solids and bed sediment in comparison to 47% and 40% of studies which have explored occurrence in surface water and wastewater respectively (aus der Beek et al., 2016).

Sediment is often the ultimate reservoir for persistent chemicals which are released from industrial, hospital and domestic effluents or from agriculture and veterinary medicine as diffuse discharges and can therefore be seen as a "secondary source" of contaminants in the environment (Boxall and Maltby, 1995; Gaw et al., 2014; Schnell et al., 2013). Furthermore, sediment contamination is known to play an important role in the longer range transport of contaminants in aquatic environments (Kümmerer, 2008; Löffler and Ternes, 2003; Schnell et al., 2013).

Many anthropogenic chemicals and waste materials including pharmaceuticals eventually accumulate in sediments due to their affinity to solids, metabolic stability and their resistance to

biodegradation in these compartments (Calisto and Esteves, 2009). There are many factors affecting the final concentration of pharmaceuticals in sediments and the impact of sediment-associated pharmaceuticals on aquatic organisms e.g. sorption potential to sediment, biodegradability (aerobic or anaerobic), residence time of pharmaceuticals in sediment and potential for desorption back into the water column (Brodin et al., 2014; Lahti and Oikari, 2011; Silva et al., 2011). Concentrations of accumulated pollutants in sediment may be several times higher than in the water-column; and the partitioning of a compound between overlying water and sediment depends on the physicochemical parameters of both the compound and the sediment (e.g., hydrophobicity, composition of the aqueous phase, salinity, affinity to sediment organic carbon, total organic carbon content, particle size and cation exchange capacity of sediment) (Niedbala et al., 2013; Di toro et al., 1991; Varga et al., 2010; Williams et al., 2009).

Since the sediment compartment has not received as much attention as the water compartment, the volume of data available is lower than for water. Nevertheless in the last decade, a number of studied have been performed regarding the occurrence and fate of pharmaceuticals in the sediment compartment so it is timely to review this existing knowledge. This Chapter therefore explores the most promising analytical methods for quantifications of pharmaceuticals and some of their metabolites in sediment and extraction and clean-up methods that have previously been used. Furthermore, the Chapter synthesises existing knowledge on the occurrence, fate and ecotoxicological effects of pharmaceuticals in sediment and identifies gaps in existing knowledge and future priorities for research.

2.2 Environmental Analysis of Pharmaceuticals in Sediment

2.2.1 Analytical Methods

There is a need for sensitive, reliable and comprehensive analytical methods to identify and quantify pharmaceuticals and their metabolites in the environment to understand the occurrence and fate of these compounds (Huang et al., 2010). For a long time, the approaches used in

routine analysis of traces of pesticides were directly applied to residues of pharmaceuticals (Buchberger, 2007). Significant progress in environmental analytical chemistry in recent years has allowed researchers to detect trace levels of pharmaceuticals from different therapeutic classes in various environmental matrices (Fatta-Kassinos et al., 2011). Despite this improvement, there is still a need for the development of reliable analytical methods for pharmaceutical determination in solid matrices, such as soil and sediment (Kim and Carlson, 2005). Detections as low as a few ng per gram in solid matrices have been recently reported (Jones-Lepp and Stevens, 2007; Wilga et al., 2008). One of the most powerful analytical techniques applied to determine pollutants in environmental matrices is chromatography. Its power comes from the capacity to determine quantitatively many compounds present in a mixture by a single analytical procedure and its low limit of detection (LOD) and low limit of quantification (LOQ). LOD and LOQ are usually defined as the lowest analyte concentrations producing a detectable peak with a signal-to-noise (S/N) ratio of 3 and 10, respectively (Prathap et al., 2013; Radović et al., 2015). In the particular case of determining pharmaceuticals in sediments, the most frequently used techniques to date include gas chromatography-mass spectrometry (GC-MS) or GC tandem mass spectrometry (GC-MS/MS) and liquid chromatography (LC) as (HP)LC–MS or (HP)LC–MS/MS.

GC-MS or GC-MS/MS are reliable techniques offering low LODs and can be used to separate and determine chemical residues in environmental samples at low concentrations. The major advantage of GC-MS is the fact that the usual ionization modes like electron impact (EI) or chemical ionization (CI) are generally less affected by the matrix of the sample and the availability of electron-impact spectral libraries increases the confidence of identification. The disadvantage of the technique is that it can be time-consuming as a derivatisation step is often needed prior to analysis of polar compounds (e.g. pharmaceuticals) with low volatility or low thermal stability. Without this derivatisation step, which is for example conducted by adding reagents like MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide), the majority of pharmaceuticals are not directly compatible with GC (Buchberger, 2007). Incomplete derivatisation in complex samples can also affect the quality of quantification (Brooks et al.,

2012). Furthermore, some of the GC methods reported in the literature are restricted to specific matrices or the methods are not fully described (Floriani et al., 2014; Jones-Lepp and Stevens, 2007) which limits their wider use. In sediment, the GC-MS technique has been used to detect pharmaceuticals and their metabolites. Azzouz and Ballesteros, (2012) developed a method using GC-MS for the determination of residues of 18 pharmaceuticals including analgesics, antibacterial, anti-epileptics, β-blockers, lipid regulators and non-steroidal anti-inflammatories, one personal care product and three hormones in sediments. Even though the developed method includes a derivatisation step, using N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS), the method featured low limits of detection (0.8–5.1 ng kg^{-1}). More recently, Regueiro et al., (2013) applied GC-MS for the quantification of chemicals such as polycyclic aromatic hydrocarbons (PAHs), non-steroidal anti-inflammatory drugs (NSAIDs), polycyclic musk fragrances and pesticides in wetland sediments.

Liquid chromatography (LC), is a powerful and versatile separation technique that has been used to determine pharmaceuticals in sediment, combined with different detectors e.g. Ultra-Violet (UV) detector (Cueva-Mestanza et al., 2008), fluorescence (Fl) detector (Prat et al., 2006), MS detector (López de Alda et al., 2002), or even an MS–MS detector (Klosterhaus et al., 2013). In contrast to GC, the LC technique does not require a derivatisation step. It has been shown to have a better performance in terms of specificity, sensitivity, speed, and robustness and separation capacity from different matrices. HPLC coupled with UV or MS/MS are the most frequently employed methods and are ideally suited for polar compounds. The UV detector which is widely described and probably represents the most common method of detection, when just a few analytes of a certain class are to be analysed, has a lower sensitivity and selectivity compared to the MS detector (Buchberger, 2007; Tadeo et al., 2012).

The MS detector is frequently used, as a powerful and sensitive detection and identification technique, and is one of the most widespread analytical methods for multiresidues analysis in sediment samples (Sosa-Ferrera et al., 2012). Before the introduction of LC-MS, detecting pharmaceutical residues in environmental samples was difficult (Wilga et al., 2008). Although LC-MS offers good sensitivity, in complex samples such as sediment and soil, suffering from

interferences, it can be insufficient in explicitly confirming analytes for the final identification (Pérez and Barceló, 2007). Matrix components have significant effects on the chromatographic response of pharmaceutical analytes with signal suppression or enhancement occurring, predominantly when electrospray ionization (ESI) is used as the ionisation source (Tadeo et al., 2012). The main cause is the presence of undesired components that co-elute in the chromatographic separation and compete for access to the surface of droplets and subsequent "ion evaporation" or change the eluent properties, such as surface tension, viscosity and volatility. Therefore, to obtain accurate results, these matrix effect must be eliminated or compensated for (Hird et al., 2014; Pico et al., 2004). The use of LC-MS/MS overcomes this problem and results in a much higher degree of certainty in the identification of unknowns (Sosa-Ferrera et al., 2012). The use of isotopically labelled analytes as internal standards or the use of standard addition methods can also be used to overcome possible matrix effects (Buchberger, 2007).

Most of the methods found in the literature for pharmaceutical in sediment employed MS/MS as a detector. Löffler and Ternes, (2003) developed one of the early LC-MS/MS analysis methods for the determination of wide range of pharmaceuticals in freshwater sediment and applied this in an investigation of pharmaceutical degradation in a sediment-water system. LC-MS, especially time-of-flight mass spectrometry (TOF-MS), was also used to analyse highly polar compounds, containing ionisable functional groups or high molecular weight environmental contaminants and has been used for the unequivocal confirmation of contaminants in water and sediments by accurate mass measurement of protonated molecules (Ferrer and Thurman, 2003; Minten et al., 2011). The time of flight (TOF) analyser measures the time it takes for the ionised compounds to travel a fixed distance. It has the ability of resolving interferences away from signals of interest with high resolving power. Moreover, the use of a hybrid quadrupole time-of-flight instrument (Q–TOF) allows the most certain confirmation. This confidence is based on the combination of retention time, mass of the quasi molecular ion selected by the quadrupole mass filter, and the complete collision induced mass spectrum obtained by the TOF analyser. The sensitivity of TOF and Q–TOF instruments in relation to triple quadrupole analysers is

however one order of magnitude lower. A comparison of estrogens detection in real river sediment samples showed MS/MS to be much more selective and sensitive (about 13 times) than TOF-MS (Labadie and Hill, 2007). Terzic and Ahel, (2011) investigated the potential of hybrid quadrupole time-of-flight mass spectrometry (Q-TOFMS) coupled to ultra-high-pressure liquid chromatography (UHPLC) for the identification of pharmaceuticals including chlorthalidone, warfarin, terbinafine, torsemide, zolpidem and macrolide antibiotics into sediments affected by discharges from the pharmaceutical industry into sediments. The particular advantage of the applied technique is its capability to detect less known pharmaceutical intermediates and/or transformation products, which have not been previously reported in freshwater sediments. The proposed approach proved to be a useful tool for the initial assessment of contaminated hotspots by providing a basis for the selection of the most critical contaminants to be monitored using target analysis.

Table 2.1 summarises the analytical techniques and approaches that have been used to determine residues of pharmaceuticals in sediments. In order to analyse pharmaceutical residues from complex matrices, preliminary extraction and clean-up procedures need to be employed. A summary of such procedures used, in combination with pharmaceutical detection methods, is given in the following paragraphs.

Table 2.1 Analytical methods used to quantify pharmaceutical concentrations in sediments

Number of pharmaceuticals	Method	Mobile phase	Column	Detection (ng g^{-1})	Purpose of study	Reference
18 pharmaceuticals	GC-MS		DB-5 fused silica capillary column (30m × 0.25mm, 0.25 μm film thickness)	LOD 0.8-5.1	Analytical method and screening	(Azzouz and Ballesteros, 2012)
74 pharmaceuticals and care products	LC-ESI-MS/MS	A 0.1% formic acid and 0.1% ammonium formate in water, B 1:1 acetonitrile: methanol	Atlantis HILIC (100.0 × 2.1, 3.0 μm) Waters Xterra C18, (100.0 × 2.1mm, 3.5 μm)	LOQ 0.1-2600.0	Screening in environmental samples	(Klosterhaus et al., 2013)
12 pharmaceuticals	GS/MS		DB-5 fused silica capillary column (30m × 0.25mm , 0.25 μm film thickness)		Analytical method and qualitative screening	(Regueiro et al., 2013)
4 acidic pharmaceuticals	GC-MS/MS		BPX5 forte capillary: (30 m × 0.25 mm; 0.25 μm)	LOQ 2.0-6.0	Pharmaceuticals screening	(Varga et al., 2010)
9 pharmaceuticals	UHPLC/QToF-MS	A Water: acetonitrile (95:5) B Acetonitrile: water (95:5)	HSS T3 column (100.0 × 2.1mm, 1.8 μm)		Screening of pharmaceutical transformation products	(Li et al., 2014)
7 pharmaceuticals	HPLC-MS/MS	Methanol: water with 0.1% acetic acid	Agilent Eclipse XDB C18 reversed phase column (150.0 × 2.1mm, 5 μm)	LOD 5.0-10.0	Screening and multi-phase distribution	(Duan et al., 2013)

Table 2.1 (Continued)

Number of pharmaceuticals	Method	Mobile phase	Column	Detection (ng g^{-1})	Purpose of study	Reference
10 antibiotics	HPLC–ESI-MS-MS	5.0 mmol L^{-1} ammonium acetate aqueous solution with 0.2% (v/v) formic acid oxalic acid	Agilent Zorbax XRD-C18 column (50.0 × 2.1 mm, 1.8 μm)	LOQ 0.07– 0.22	Screening pharmaceutical in sediment	(Xue et al., 2013)
9 pharmaceuticals	UHPLC-QTOF-MS	A 0.1% formic acid in water B 0.1% formic acid in acetonitrile	BEH C18 (50.0 × 2.1 mm) filled with a 1.7 μm		Analytical method for non-targeted compounds	(Terzic and Ahel, 2011)
3 antibiotics	HPLC–MS	Gradient of A Oxalic acid (10 mM): Methanol (80:20) B acetonitrile: Methanol (80:20)	Spherisorb S3 ODS1(150 × 2.1 mm, 1.8 μm)	LOD 25.0	Degradation of pharmaceutical in sediment	(Delépée et al., 2000)
12 pharmaceuticals	LC-ESI-MS/MS	A 0.1% formic acid in water, B 0.1% formic acid in acetonitrile	Synergy Fusion C18 embedded column (150.0 × 2.0 mm, 4 μm)	LOD 0.125-500	Analytical method	(Mutavdžić Pavlovic et al., 2012)
20 antibiotics	UHPLC–MS/MS	A 0.1% formic acid B acetonitrile containing 0.1% formic acid	HSS T3 column (100.0 × 2.1 mm, 1.8 mm)	LOD 0.01- 0.56	Screening and behaviour of pharmaceutical in sediment	(Chen and Zhou, 2014)
22 antibiotics	HPLC-ESI MS/MS	Methanol–acetonitrile (1:1)	XTerra MS C18 (100.0 × 3.0 mm, 2.0 μm)	LOD 0.02-0.5	Screening of antibiotics	(Li et al., 2012)
7 antibiotics	HPLC-UV	A = 0.05 M phosphoric acid (pH 3.5) : acetonitrile (90:10), B = 0.05 M phosphoric acid (pH 3.5) : acetonitrile (50:50).	Hypersil ODS C18 (100 × 4.6 mm, μ5.0 mm)		Stability in sediment	(Samuelsen et al., 1994)

Table 2.1 (Continued)

Number of pharmaceuticals	Method	Mobile phase	Column	Detection (ng g^{-1})	Purpose of study	Reference
1 antibacterial	LC-MS/MS	A acetonitrile and B 1 mM ammonium acetate :ACN (90:10)	Gemini-NX C18 column (150.0 × 4.6 mm , 5 µm)	LOD 0.4	Analytical method and screening	(Wagil et al., 2014)
4 antibiotics	LC–ESI-MS/MS	A 0.1% formic acid in water B acetonitrile with 0.1% formic acid	Zorbax Bonus-RP column (150.0. × 2.1 mm, 5.0µm)	LOQ 3.1	Seasonal variation and partitioning	(Cheng et al., 2014a)
1 antibiotic	HPLC-UV	Acetonitrile: sodium phosphate (100 mM) (25:75)	Hypersil ODS C18 (100.0 × 4.6 mm, 5.0µm)	LOD 200.0	Degradation and uptake	(Capone et al., 1996)
5 hormones	HPLC-IT-MS/MS	0.15% ammonium hydroxide in water: acetonitrile	Zorbax Extend C18 (100.0 ×2.1 mm, 3.5µm)	LOD 0.14–0.98	Analytical method	(Matějíček et al., 2007)
6 hormones	GC-MS/MS (EI)		XTI-5 (30 m × 0.25 mm,0.25 mm)	LOD 0.2–4	Analytical method and screening	(Ternes et al., 2002)
3 hormones	LC-DAD-MS	Acetonitrile: water (30:70)	LiChrospher100-RP18 (250.0 × 4.0 mm, 5.0µm)	LOD 0.04–1.0	Analytical method and screening	(López de Alda et al., 2002)
2 antibiotics	HPLC-MS/MS	0.1% formic acid: acetonitrile (90:10)	Luna C8 (50.0 x 2.0 mm, 3 µm)	LOD 0.012-0.061	Screening in sediment and fish	(Lalumera et al., 2004)
20 pharmaceutical	LC–ESI-MS/MS	NI A formic acid 0.1% in methanol B formic acid 0.1% in water, PI acetonitrile/methanol (60:40)	NISunfire C18 (4.6 ×150mm, 3.5 µm) PI Luna C18 (2.0 ×150mm, 3.0 µm)	LOD 0.1-6.8	Analytical method and screening	(Vazquez-Roig et al., 2010)

Table 2.1 (Continued)

Number of pharmaceuticals	Method	Mobile phase	Column	Detection (ng g^{-1})	Purpose of study	Reference
5 pharmaceuticals	LC-ESI-MS/MS UPLC-QTOF/MS	5mM acetic acid and (B) Methanol with 5mM acetic acid A 95% 10mM acetic acid and 5% acetonitrile, B 5% 10mM acetic acid and 95% acetonitrile	Fortis C8 (2.1×100mm, 3.0μm) HSS T3 (2.1×100mm, 1.8μm)	LOQ 0.4–8	Analytical methods	(Minten et al., 2011)
1 pharmaceutical	LC-TOF-MS LC-Ion Trap- MS/MS	A 0.1% formic acid, B acetonitrile with 0.1% formic acid A acetonitrile, B 10mM ammonium formate	MetaChem MetaSil AQ C18, (2.0 × 150 mm, 5μm) Phenom- enex RP18, Torrance, CA (250 × 3.0, 5μm)	LOD 5.0	Analytical method and screening	(Ferrer et al., 2004)
9 pharmaceuticals	LC-MS/MS	A water + 5mMammonium acetate, B methanol + 5 mM ammonium acetate	Thermo Aquasil , (150 ×4.6, 3.0 μm)	LOD 0.1	Screening pharmaceuticals	(Beretta et al., 2014a)
28 pharmaceuticals	LC-ESI-MS/MS	NI (1) A 5 mM formate ammonium in D.I. water, B Methanol PI (2) A 0.1 % formic acid in D.I. water, B methanol PI (3) A 0.1 % formic acid in D.I. water, B 0.1 % formic acid in methanol	Gemini C18 column (2.0 ×100 , 3.0 μm)	LOD 1.0	Screening and distribution	(Yang et al., 2014)

Table 2.1 (Continued)

Number of pharmaceuticals	Method	Mobile phase	Column	Detection (ng g^{-1})	Purpose of study	Reference
7 pharmaceuticals and hormones	LC-ESI-MS/MS	A 0.1 mM ammonium acetate in water, B 0.1 % formic acid in water, C methanol	Kinetex XDB C18 (50.0 × 2.1mm, 1.7 µm)	LOQ 0.5-20.0	Analytical method	(Berlioz-Barbier et al., 2014)
15 pharmaceuticals	LC-ESI-MS/MS	A Water+0.1% formic Acid 10mM ammonium hydroxide B Acetonitrile+0.1% formic acid Acetonitrile C Methanol + 0.1 % formic acid Methanol	Waters BEH-C18 (50 mm, 1.7 µm)	MDL 0.08-0.3	Screening pharmaceuticals	(Cantwell et al., 2017)
10 pharmaceuticals and hormones	LDTD-APCI-MS/MS Laser diode thermal desorption (LDTD)/atmospheric pressure chemical ionization tandem mass spectrometry			MDL 0.7-9.4	Analytical method and screening	(Darwano et al., 2014)
65 pharmaceuticals	LC- ESI-(QqLIT) MS/MS	NI acetonitrile: water PI methanol: 10 mM ammonium formate/formic acid	HSS T3 column (100× 2.1mm, 1.8 µm)	LOD 0.05-25.3	Screening pharmaceuticals	(Moreno-González et al., 2015)
8 pharmaceuticals	LC-UV	Methanol:water (pH 3.0 with acetic acid) mixture	Waters Nova-Pack C18 (150 × 3.9mm, 4 µm)	LOD 6.0-114.0	Analytical method	(Cueva-Mestanza et al., 2008)
45 pharmaceuticals	UPLC-MS/MS	Methanol: 10 mM formic acid/ammonium formate (pH3.2)	C18 Intensity Solo UPLC (100× 2.1mm, 2.0 µm)	LOD <0.01-0.83	Screening pharmaceuticals	(Biel-Maeso et al., 2017)

2.2.2 Extraction and Clean-up

The complexity of an environmental matrix can deeply affect the analysis of a pharmaceutical. Up to 90% of the analysis time can be spent on sample preparation and thus, great effort goes into the development of reliable sample preparation procedures which are as simple as possible in terms of operation and which minimise the number of steps in the process (Zuloaga et al., 2012). One important step in environmental analysis is the choice of suitable extraction and clean-up methods which remove sorbed pharmaceuticals while ensuring high recovery and yield percentages (Aga, 2008). Methods of sediment sample preparation have developed significantly for both occurrence and fate studies of pharmaceuticals in the environment. According to the nature of sediments (having a negatively charged surface) and pharmaceuticals (having polar and ionisable functional groups) and the type of interaction between them, the method and solvents used for extraction need to be adapted (Minten et al., 2011). However, standardised pharmaceutical extraction methods do not exist. Generally, the number of published extraction methods used for trace-analysis of pharmaceuticals in solid matrices is lower than that available for aquatic samples (Varga et al., 2010). This may be due to the fact that sediment methods are time and labour consuming and/ or the importance of water phase as the main driver in environmental risk assessment procedures (Díaz-Cruz et al., 2003; EC, 2003).

In this section, we review the most popular extraction methods and solvents used for extraction of pharmaceuticals from sediment. The most common methods used to extract pharmaceuticals from sediments are solid-liquid extractions (e.g. Gomes et al., 2004; Kim and Carlson, 2007), ultra-sonication (e.g. Blair et al., 2013; Xu et al., 2008; Zhou et al., 2011), pressurized liquid extraction (PLE) (e.g. Li et al., 2012; Vazquez-Roig et al., 2010), which is also known as accelerated solvent extraction (ASE) (Langford et al., 2011) and microwave assisted extraction (MAE) (e.g. (Maskaoui and Zhou, 2010; Matějíček et al., 2007). Antonić and Heath, (2007) compared these methods to each other and recommended MAE as the best method to analyse non-steroidal anti-inflammatory drugs (NSAIDs) in sediment. This was confirmed when MAE was shown to provide higher recoveries than the other extraction methodologies when different pharmaceuticals were extracted from sediment (Liu et al., 2004; Vazquez-Roig et al., 2010).

However, special care is needed when using MAE in order to minimise the temperature and irradiation time which could degrade the analytes. Overall, several major drawbacks are connected to these methods ranging from the large amount of solvent consumption to the complexity and the high cost of the instruments used (Blackwell et al., 2004).

Most methods used in the extraction of solid samples (e.g. sediments) are not selective and therefore part of the environmental matrix may co-extract and interfere with the analysis (Zuloaga et al., 2012). Consequently, the extraction is typically followed by a clean-up step (also known as purification) to remove matrix components (Aga, 2008). The clean-up of sediment samples extracts has been carried out by using different techniques including solid phase extraction (SPE), liquid- liquid extraction (LLE) and gel permeation chromatography (GPC) (Díaz-Cruz et al., 2003). Because of the simplicity of use and lower consumption of solvents, solid phase extraction (SPE) technique is widely used as a purification and pre-concentration procedure (Cueva-Mestanza et al., 2008). SPE generally retains chemicals onto the stationary phase (e.g. C18-sorbent) based on their polarity and allows the subsequent extraction from the stationary phase with a solvent (Kim and Carlson, 2005). Overall, this method is considered as the key for clean-up of extracts for analysis of pharmaceuticals in solid samples (Wilga et al., 2008). SPE cartridges such as hydrophilic–lipophilic balance (HLB), moderate anion exchange cartridge (MAX) and strong anion exchange (SAX) have been frequently used in the clean-up of extracts from sediment samples. The most used stationary phase in SPE is the HLB cartridge. This cartridge is convenient for samples of a wide range of pH values and different properties (Kim and Carlson, 2005). On the other hand, the highest recovery and the cleanest extract can be obtained using the medium cation exchange (MCX) cartridge. This cartridge however needs acidic pH to promote the loading stability of the analyte during extraction (Vazquez-Roig et al., 2013). Furthermore, tandem-SPE methods using SAX or MAX hyphenated to HLB cartridge have been used in the clean-up of extracts of sediment samples. The first cartridge is used to reduce matrix effect of complex co-extracted components and the latter is used to retain the analyte (Vazquez-Roig et al., 2010; Zhou et al., 2011). Table

2.2 lists some of the methods that have been previously used for extraction and clean-up of sediments samples used for sample preparation prior pharmaceutical analysis.

Table 2.2 Extraction and clean-up methods reported in correlation to analysis of pharmaceutical residues in natural and artificial sediments

Number of compounds	Extraction method	Extraction solvent	Recovery %	Clean-up	References
104 pharmaceuticals and personal care products	Solid -liquid extraction	An aqueous phosphate buffer (pH 2.0) or solution of NH_4OH then ACN	20.9-50.9	SPE (Oasis HLB)	(Klosterhaus et al., 2013)
43 pharmaceuticals	PLE	Methanol: water mixture (1:2)	-	SPE (Oasis HLB)	(Silva et al., 2011)
8 Pharmaceuticals	UAE	Acetone: acetic acid (20:1)	61.8-91.2	SPE (Oasis HLB)	(Agunbiade and Moodley, 2015)
35 Pharmaceuticals	ASE	Methanol or methanol: formic acid (100: 0.1)	66.0-131.0	Centrifugation	(Langford et al., 2011)
20 Antibiotics	UAE	Sodium phosphate dodecahydrate, sodium citrate and EDTA in 20 mL of Milli-Q water	44.0- 141.0	SPE (HLB)	(Chen and Zhou, 2014)
14 Pharmaceuticals	PLE	Methanol: water (1:2)	30.7-220	SPE (HLB)	(Moreno-González et al., 2015)
17 Pharmaceuticals	PLE	Na_2–EDTA washed sea sand with water and mixtures of Methanol:water and acetonitrile–water	64.0-110.0	SPE (SAX and HLB)	(Vazquez-Roig et al., 2010)
32 pharmaceuticals	PLE	Methanol with aqueous ammonia solution (0.1 mol L^{-1})	66.0-114.0	SPE (tandem MAX-HLB)	Pérez-Carrera et al.,) (2010
8 Pharmaceuticals	Microwave assisted micellar extraction (MAME)	Methanol	6.0-114.0	SPE (HLB)	Cueva-Mestanza et al.,) (2008

Table 2.2 (Continued)

Compound	Extraction method	Extraction solvent	Recovery %	Clean-up	References
10 pharmaceuticals and metabolites	UAE	1- acetone: acetic acid 20:1, 3x ethyl acetate 2- Methanol: ethyl acetate (50:50), 3x ethyl acetate 3- 2x methanol, 2x acetone	40.0-115.0	SPE (MCX)	(Loffler et al., 2005)
10 antibiotics	UAE	Methanol, Na_2EDTA solution (1mmol L^{-1}) and citrate buffer (0.1mol L^{-1})	58.9-73.6	SPE (SAX) and HLB	(Xue et al., 2013)
30 antibiotics	ASE	50 mMH_2PO_4 (pH= 6): methanol (50:50)	106.1-40.0	Online SPE (HLB)	(Gibs et al., 2013)
3 pharmaceuticals and hormones	PLE	Methanol	70.0-116.0	Centrifugation	(Gilroy et al., 2012)
22 pharmaceuticals and personal care products	MAE	Methanol: water (3:2)	91.0-101.0	SPE (HLB)	(Azzouz and Ballesteros, 2012)
6 hormones	UAE	Acetone: methanol (1:1)	>66	Silica gel and neutral alumina column	(Lei et al., 2009)
2 pharmaceutical and antibacterial	Solid -liquid extraction	Acetone	100.4-102.0	Silica gel	(Ramaswamy et al., 2011)
9 pharmaceuticals	UAE	Methanol	50.8-98.2	SPE (HLB)	(Zhou and Broodbank, 2014)
17 antibiotics	USE	Citric buffer (pH 3) and acetonitrile (50:50)	75.0-198.0	SPE (SAX and HLB)	(Zhou et al., 2011)

Table 2.2 (Continued)

Compound	Extraction method	Extraction solvent	Recovery %	Clean-up	References
22 antibiotics	PLE	Methanol	63.4-132.3	SPE (HLB)	(Li et al., 2012)
5 pharmaceuticals	UAE	Methanol, acetone	74.5±9.3	SPE (ENVI-18)	(Duan et al., 2013)
12 pharmaceuticals	MSPD (matrix solid-phase dispersion)	Acetonitrile :5% oxalic acid (6:4)	37.0-137.1		(Mutavdžić Pavlovic et al., 2012)
6 antibiotics	UAE	0.1% formic acid: methanol	42.8-104.4	SPE (HLB)	(Huang et al., 2010)
1 antimicrobial	Solid -liquid extraction	Water: acetonitrile, HCl	95.2–113.0	SPE (Strata XC)	(Wagil et al., 2014)
4 pharmaceuticals	MAE	Water	95.0–103.0	dispersive matrix extraction (DME) for clean-up and SPE (HLB) for enrichment	(Varga et al., 2010)
7 hormones	UAE	Ethyl acetate and acetone: methanol (1:1)	2.0-122.0	SPE (Florisil)	(Arditsoglou and Voutsa, 2008)
4 pharmaceuticals	MAE	Water	96.0–103.0	SPE (HLB)	(Dobor et al., 2012)
5 pharmaceuticals	LLE and UAE	Acetone: McIlvaine buffer	60.0–75.0	SPE (HLB)	(Minten et al., 2011)
10 pharmaceuticals and hormones	UAE	Methanol: acetone (3:1)	41.0-109.0	SPE (STRATA C18)	(Darwano et al., 2014)

Table 2.2 (Continued)

Compound	Extraction method	Extraction solvent	Recovery %	Clean-up	References
45 pharmaceuticals	pressurized hot water extraction (PHWE)	Milli-Q water	50-140	SPE (Oasis HLB)	Biel-Maeso et al.,) (2017
6 Pharmaceuticals and hormones	Salting-out liquid–liquid extraction	Acetonitrile	37.0-85.0	Dispersive SPE using PSA/GCB (primary and secondary amine exchange/ graphitized carbon black)	Berlioz-Barbier et al.,) (2014
5 antibiotics	Vortex, agitation, UAE, MAE	Methanol Methanol–acetone Methanol–formic acid	8.0-66.0	SPE (HLB)	(Carvalho et al., 2013)
15 pharmaceuticals	Liquid-solid extraction	Acetonitrile modified by acetic acid	94.0-127.0		(Cantwell et al., 2017)

2.3 Occurrence of Pharmaceuticals in Sediments

In recent years, the interest in studying the occurrence and the environmental impact of human pharmaceuticals has increased in parallel with the development of analytical procedures (Monteiro and Boxall, 2010; Xue et al., 2013). In sediments, the occurrence of pharmaceuticals is essential to understand their environmental fate and risk (Brooks et al., 2009). The levels of pharmaceuticals in sediment have been investigated in different parts of the world. There are 23 countries worldwide in which at least 1 pharmaceutical has been reported in sediment. The countries most studied are China, USA and Spain (Figure 2.1).

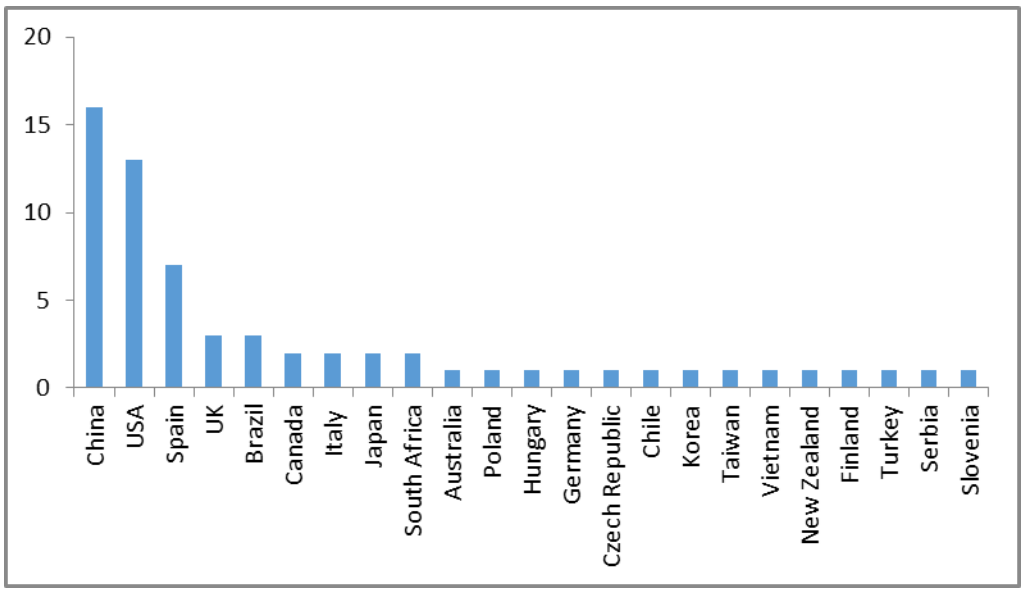


Figure 2.1 Distribution of publications on occurrence of pharmaceuticals in sediments by country of sampling

Sixty-eight studies reporting concentrations of pharmaceuticals in sediments have been published in the last two decades. This number is much lower than the number of studies reporting the occurrence of pharmaceuticals in the water compartment. In most cases, the detected concentrations were in the low ng g^{-1} range. Antibiotics and hormones were the most frequently measured compounds being detected in 53.5% and 35.1% of the studies respectively. This may be due to large amounts of usage as, for example, the annual estimated usage of raw antibiotic materials in China is about 180,000 tonnes (for health, livestock and agricultural use) (Ma et al., 2016; Zheng et al., 2012). Generally, antibiotics are not readily biodegradable and

partially eliminated during wastewater treatment while hormones have been shown to degrade relatively rapidly under aerobic conditions in marine sediment and seawater but are likely to show much longer persistence under anoxic conditions (Luo et al., 2014; Verlicchi et al., 2012; Ying and Kookana, 2003). Hormones typically have a high affinity to adsorb to sediment with the distribution coefficients (Kd) being positively related to organic carbon (OC) and the particle properties (Fei et al., 2017).

Although the detection of antibiotics in sediment has been reported in different parts of the world, the number of publications from China exceeds the number done elsewhere. The antibiotic ingredients most commonly detected in sediments belong to the fluoroquinolone, sulfonamide, macrolide and tetracycline classes (Table 2.3). Oxytetracycline, sulfamethoxazole, tetracycline and erythromycin were the most frequently detected compounds. The highest concentrations recorded were for the sulfonamides (sulfadiazine) with concentrations up to 12300 ng g^{-1} in the sediment of an agricultural watershed of the Dagu River in China (Hu et al., 2012); followed by tetracyclines (oxytetracycline) (Yong-shan, 2011) with reported concentration of 9287.5 ng g^{-1} in the sediments of the main stream around an outfall of a pig farm. A number of studies have determined estrogenic compounds and steroidal estrogens in sediment in the UK (Labadie et al., 2007), the Czech Republic (Matějíček et al., 2007) and Australia (Braga et al., 2005). The earliest study into the occurrence of natural and synthetic hormones in environmental sediment was done in Germany by Ternes et al., (2002). The highest reported concentration was for the synthetic hormone 17 α-ethinylestradiol with concentration of 129.8 ng g^{-1} in marine sediments from Brazil (Froehner et al., 2012, 2011).

In Europe, 18 studies reported the occurrence of pharmaceuticals in sediments. These studies made up to 32% of the total reviewed literatures here and the majority of them were performed in Spain. Silva et al., (2011) reported the occurrence of 43 pharmaceuticals in surface water, suspended solids and sediments in the Ebro River in Spain. The studied pharmaceuticals belonged to different therapeutic groups and included analgesics and anti-inflammatory drugs, antiepileptic drug, psychiatric drugs, anti-ulcer agents, antibiotics, lipid regulators and cholesterol lowering statin drugs, ß- blockers, diuretics and anti-histamines. Amongst the

studied compounds, 30 pharmaceuticals were reported to be detectable in sediment. The highest concentration was reported for acetaminophen with concentrations up to 222.0 ng g^{-1}. In Hungary, Varga et al., (2010) investigated the occurrence of some acidic pharmaceuticals including ibuprofen, naproxen, ketoprofen, and diclofenac and only naproxen and diclofenac were detected in sediment with maximum concentrations of 20 and 38.0 ng g^{-1}, being found respectively. Table 2.3 provides an overview of the concentrations of pharmaceuticals reported around the world. It should be noted that compounds reported as not detected in the viewed publications are not listed in the table, the only exception being when a range of concentrations was reported with non-detected (Nd) being the lower boundary.

Generally, the number of pharmaceuticals reported in sediments from different regions is variable and reflects many factors e.g. the number of pharmaceuticals determined by the analytical methods. For example, some analytical methods have been developed to study the occurrence of only a limited number of pharmaceuticals in sediment while other methods have been developed for multiresidues analysis (e.g. Pérez-Carrera et al., 2010; Silva et al., 2011). Variations in measured concentrations of pharmaceuticals in the environment are likely driven by different variables such as proximity to WWTPs, the density and proximity of agricultural feed operations, discharges of pharmaceuticals manufacturing sites and the hydrology of the study system (Kim and Carlson, 2005; Larsson, 2014).

Table 2.3 Reported data on the occurrence of pharmaceuticals (ng g^{-1}) from sediment samples in different countries

Compound	Concentration ng g^{-1}	Country	Reference
17α-Estradiol	Nd-1.35	Czech Republic, Japan	1, 2
17α-ethinylestradiol	Nd-129.5	Spain, China, UK, Germany, Australia, Canada, Italy , Chile, USA, New Zealand, Brazil	3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
17β-Estradiol	Nd-39.77	Czech Republic, Spain, Canada, Japan , Germany, Australia, UK, USA, China, Chile, New Zealand, Brazil	1, 3, 8, 2, 6, 7, 5, 16, 17, 18, 9, 11, 12, 19,14, 15
Acetaminophen	Nd -222.0	Spain, USA, South Africa, Taiwan, Korea	20, 21, 22, 23, 24, 25, 26, 27
Acetylspiramycin	16-7653	China	28
Amphetamine	0.76-3.21	USA	29
Amlodipine	<MQL	Spain	30
Ampicillin	50.8-369.0	South Africa	31
Amitriptyline	0.45	USA	29
Aspirin	212.0-426.0	South Africa	31
Atenolol	Nd-13.8	Spain, Brazil, USA	20, 32, 33, 68, 19
Atorvastatin	<LOQ- 2.99	Spain	20
Azithromycin	Nd-44.0	USA, China	22, 34, 35, 29
Bezafibrate	Nd -80.3	Spain, South Africa	20, 31
Caffeine	Nd-131.19	Brazil, South Africa, Canada, USA, Taiwan, Spain	32, 23, 36, 16, 24, 26, 33, 9, 68, 19

Table 2.3 (Continued)

Compound	Concentration ng g^{-1}	Country	Reference
Carbamazepine	Nd-46.5	Spain, UK, Brazil, Finland, South Africa, Canada, USA, Serbia	3, 20, 21, 37, 32, 38, 23, 36, 9, 39, 27, 40, 68, 19
Chloramphenicol	Nd-5.35	Spain, China	20, 41, 3, 42, 69
Chlortetracycline	Nd- 1823.6	China, USA	41, 43, 44, 45, 46, 47
Cimetidine	Nd-19.2	Spain, Korea	20, 48, 25
Ciprofloxacin	Nd-1290	China, South Africa, Taiwan , Turkey	28, 49, 50, 31, 24, 45, 51
Citalopram	0.36-14.95	USA	52
Clarithromycin	Nd-130	USA, Spain	22, 20, 35, 53
Clofibric acid	Nd -35.62	Spain, China	20, 21, 54, 9
Clotrimazol	<MDL -22.0	China	55
Codeine	<MDL-4	Spain, USA, Korea	21, 22, 25
Cotinine	<MDL -39.0	USA, Korea	22, 25
Desloratadine	<LOQ	Spain	30
Diazepam	<MDL-48.0	Spain, Brazil , USA, Serbia	21, 32, 39, 40
Diclofenac	Nd-309.0	UK, China, Spain, Hungary , Brazil Finland , South Africa, Canada , Slovenia	37, 54, 3, 20, 56, 32, 38, 31, 36, 9, 57, 27, 19
Diethylstilbestrol	Nd-4.51	China	4
Digoxigenin	<MDL-9.2	USA	22

Table 2.3 (Continued)

Compound	Concentration ng g^{-1}	Country	Reference
Diltiazem	<MDL -5.2	USA, Korea	22, 25
Diphenhydramine	<MDL-160.0	USA, Korea	22, 58, 25, 29
Doxycycline	Nd-1149.5	China, USA	42, 43, 44,45, 46, 47,
Doxycycline hyclate	Nd-21.3	China	41
Econazole nitrate	1-4.0	China	55
Enrofloxacin	Nd-7708	China, USA, Turkey	59, 28, 22, 43, 41, 60, 45, 51
Erythromycin	Nd-385.0	China, USA, Spain, South Africa , Korea, Serbia	59, 22, 41, 60, 49, 35, 20, 43, 50, 23, 25, 45, 47, 40
Estriol	Nd-10.8	China, Spain	4, 61, 9
Estrone	Nd-49.27	Japan, Czech Republic, China , Germany, Australia, UK, Spain , USA, Chile, New Zealand, Brazil	2, 1, 4, 6, 7, 5, 61, 16, 17, 63, 18, 9, 11, 12, 19, 14, 15
Estrone-3-sulfate	Nd-0.41	Czech Republic, Japan	1, 2
Ethynyl estradiol	Nd-86.0	Spain, Canada	61, 36
Famotidine	N d -3.94	Spain	20
Fenofibrate	Nd-10.59	Spain	20, 27
Fenoprofen	Nd-26.0	Spain	27
Fleroxacin	Nd-6.96	China	59
Florfenicol	Nd-1.3	China	41, 42
Flumequine	Nd-578.8	USA, Italy	22, 62

Table 2.3 (Continued)

Compound	Concentration ng g^{-1}	Country	Reference
Flunixin	0.1-0.19	Spain	3
Fluoxetine	<MDL-20.0	USA, Spain	22, 30, 52
Furosemide	Nd -10.9	Spain, USA	20, 53
Galaxolide	2.39-52.5	Brazil	32
Gemfibrozil	Nd -5.2	Spain	20, 9, 19
Gentamicin	Nd- 11.230	China	28
Glibenclamide	Nd -1.76	Spain	20
Hydrochlorothiazide	0.46-27.6	Spain	20
Ibrupoven	Nd-8.8	USA	22
Ibuprofen	Nd-140.0	Spain, Brazil , Finland, South Africa, Slovenia, USA	3, 20, 32, 38, 31, 23, 9, 57, 29, 27, 19
Indomethacin	4.0542.6	UK, Spain	37, 19
Josamycin	Nd-1.27	Spain	20
Ketoconazole	2-5	China	55
Ketoprofen	Nd-320.0	Spain, China, Finland, South Africa, Slovenia	3, 20, 54, 38, 31, 9, 57
Lincomycin	Nd-6.0	USA, Taiwan	22, 24
Lomefloxacin	Nd-298.0	China	59, 45

Table 2.3 (Continued)

Compound	Concentration ng g^{-1}	Country	Reference
Lorazepam	Nd -3.03	Spain	20
Mebeverine	<MQL	UK	37
Meclofenamic acid	37.3	UK	37
Mefenamic acid	Nd -23.0	Spain, USA	20, 53, 27, 19
Mestranol	<LOQ	Germany	6
Metformin	3.8-140	USA	22
Methacycline	3.41	China	43
Metoprolol	Nd-44.0	Spain, USA	20, 53, 68
Metronidazol	Nd -61.93	Spain, South Africa	20, 23, 27
Mevastatin	Nd -99.4	Spain	20
Miconazole	1.5-8.4	USA	22, 29
Minocycline	Nd-5622	China	28
Naproxen	Nd-77.0	Spain, Hungary, Finland , Slovenia	3, 56, 20, 38, 64, 57, 27
Niflumic acid	Nd-0.36	Spain	3
Norethindrone	45.0	Canada	36
Norfloxacin	Nd-5770.0	China, USA, Taiwan, Vietnam	59, 60, 50, 22, 24, 45, 65

Table 2.3 (Continued)

Compound	Concentration ng g^{-1}	Country	Reference
Ofloxacin	Nd-1560.0	China, USA, Spain	59, 28, 22, 43, 41, 60, 49, 50, 21, 45
Omeprazole	4.7-24.3	Spain	48
Oxacillin	<MDL-9.1	USA	22
Oxolinic acid	1.81-426.31	Vietnam	65
Oxytetracycline	Nd-9287.5	China, Italy , USA, Turkey	28, 43, 41, 44, 49, 66, 62, 45, 46, 47, 51, 29
Phenazone	Nd -0.36	Spain	20
Propranolol	3.37-28.5	Brazil , Spain, USA	33, 64, 68
Pyrimethamine	0.017-0.055	Spain	3
Ranitidine	Nd -16.2	Spain, Korea, USA	20, 25, 68
Rifampicin	nd-12370	China	28
Roxithromycin	Nd-1011.0	China, USA, Spain	28, 59, 22, 41, 60, 49, 35, 20, 50, 45, 47
Sarafloxacin	Nd-35.9	USA, China	22, 49
Sertraline	0.27-17.71	USA	52
Sotalol	Nd -1.25	Spain	20
Sulfacetamide	Nd-1.39	China	35, 42
Sulfachloropyridazine	Nd-6310	China, Poland, Spain	28, 49, 67, 42, 19
Sulfadiazine	Nd-12300.0	China, Spain	59, 28, 44, 49, 20, 50, 43, 45, 42

Table 2.3 (Continued)

Compound	Concentration ng g^{-1}	Country	Reference
Sulfadimethoxine	Nd-0.2	China, Poland	59, 67
Sulfadimidine	Nd-0.7	China	35
Sulfamerazine	Nd-3.67	China	59, 41, 42
Sulfamethoxazole	Nd-1700	China, UK, South Africa , Canada , Poland , Taiwan, Spain , Vietnam	59, 41, 49, 37, 35, 23, 36, 67, 24, 45, 9, 65
Sulfameter	Nd-56.65	China	42
Sulfamethazine	Nd-248.0	China, Spain, Poland, USA	59, 41, 60, 20, 50, 67, 45, 47, 42, 19
Sulfamonomethoxine	Nd-7.0	China	59, 43, 42
Sulfapyridine	Nd-6.6	China	59, 41, 50
Sulfaquinoxaline	0.08-0.9	China	41
Sulfathiazole	Nd-5.94	China , Poland	59, 67, 42
Sulfisoxazole	Nd-1.71	China, Poland	41, 59, 6
Sulfisoxazole	Nd-0.71	China	59
Tamoxifen	<LOQ-7.0	UK, USA	37, 53
Triamterene	0.25-0.82	USA	29
Tetracycline	Nd- 1794.2	China, USA, Turkey	60, 44, 49, 50, 43, 41, 68, 45, 46, 47, 51, 42

Table 2.3 (Continued)

Compound	Concentration ng g^{-1}	Country	Reference
Tonalide	2.81-27.9	Brazil	32
Thiamphenicol	Nd-1.3	China	41
Triclocarban	Nd-510	USA	22
Trimethoprim	Nd-734.61	Spain, China, Poland, Korea, Vietnam	30, 49, 20, 67, 25, 45, 9, 65, 19
Tylosin	Nd-20	USA	22
Tylosin A	Nd -71.1	Spain	20
Verapamil	Nd-9.4	USA	29, 68
β-estradiol	0.71–9.70	China	4
β-estradiol17-valerate	Nd-9.45	China	4

1- Matějíček et al., (2007) 2- Isobe et al., (2006) 3- Azzouz and Ballesteros, (2012) 4- Lei et al., (2009) 5- Liu et al., (2004) 6- Ternes et al., (2002) 7- Braga et al., (2005) 8- Robinson et al., (2009) 9- Martín et al., (2010) 10- Pojana et al., (2007) 11- Bertin et al., (2011) 12- Wang et al., (2012) 13- Stewart et al., (2014) 14, 15- Froehner et al.,(2012, 2011) 16- Kolpin et al., (2013) 17- Gong et al., (2011) 18- Labadie et al., (2007) 19- (Biel-Maeso et al., 2017). 20- Silva et al., (2011) 21- Vazquez-Roig et al., (2010) 22- Blair et al., (2013) 23- Matongo et al., (2015) 24- Yang et al., (2014) 25- Coi et al., (2014) 26- Fairbairn et al., (2015) 27- Pintado-Herrera et al., (2013) 28- Hu et al., (2012) 29- Edward R Long et al., (2013) 30- Moreno-González et al., (2015) 31- Agunbiade and Moodley, (2015) 32- Beretta et al., (2014) 33- de Sousa et al., (2015) 34- Gibs et al., (2013) 35-Xue et al., (2013) 36- Darwano et al., (2014) 37- Zhou and Broodbank, (2014) 38- Lindholm-Lehto et al., (2015) 39- Maruya et al., (2012) 40- Radović et al., (2015) 41- Chen and Zhou, (2014) 42- Na et al., (2013) 43- Zhou et al., (2012) 44- Yong-shan, (2011) 45- Zhou et al., (2011) 46- Pei et al., (2006) 47- Kim and Carlson, (2007) 48- Pérez-Carrera et al., (2010) 49- Luo et al., (2011) 50-Yang et al., (2010) 51-Okay et al., (2012) 52-Schultz et al., (2010) 53- Lara-Martín et al., (2014) 54-Duan et al., (2013) 55- Huang et al., (2010) 56-Varga et al., (2010) 57-Antonić and Heath, (2007) 58-Ferrer et al., (2004) 59-Li et al., (2012) 60- Liang et al., (2013) 61-López de Alda et al., (2002) 62-Lalumera et al., (2004) 63-Mibu et al., (2004) 64-Martin et al., (2010) 65-Le and Munekage, (2004) 66-Li et al.,(2010) 67- Siedlewicz et al., (2016) 68- Cantwell et al., (2017).

2.4 Fate of Pharmaceuticals in Sediment

Once pharmaceuticals are introduced into surface water, they may undergo biodegradation, hydrolysis or photodegradation, as well as partition to natural solid matter such as suspended solids and bed sediments and be taken up by organisms (Boxall, 2012; Liang et al., 2013). In general, the major processes that govern the fate of pharmaceuticals in the sediment environment are sorption and degradation (Yamamoto et al., 2009). The next sections provide an overview of the fate processes for pharmaceuticals in sediment.

2.4.1 Sorption

Sorption is the process through which chemicals become associated with solid phases and/or move inside the sorbent particle (Berg et al., 2001; Gilroy et al., 2012). The sorption of pharmaceuticals in sediment provides useful insight into processes that control the partitioning, mobility and bioavailability in the environment (Lei et al., 2009; Thiele-Bruhn, 2003). To date these processes have been less studied for pharmaceuticals in sediment than other solid matrices such as soil so our understanding of the factors and processes affecting sorption of pharmaceuticals in sediments is limited (Löffler et al., 2005; Yamamoto et al., 2009). Unlike neutral organic compounds, where differences in partitioning typically occurs through van der Waals interactions with sorbent organic carbon and is correlated to the hydrophobicity of the compound, the sorption of pharmaceuticals, which are typically ionisable compounds, to environmental solids is thought to be through a combination of interactions e.g. hydrogen bonds, electrostatic interactions, ionic exchange and hydrophobic interactions (Brooks et al., 2009; Stein et al., 2008; Williams et al., 2009).

The extent to which pharmaceuticals bind to sediment particles is widely described by a water-solid distribution coefficient Kd, which is defined as the ratio of the concentration of a chemical remained in the aqueous phase to the amount sorbed by solid phase at equilibrium and is calculated using Equation 2.1 (Wegst-Uhrich et al., 2014) :

$$\mathbf{Kd = Qe_{sediment}/Ce_{solution}} \qquad \mathbf{(2.1)}$$

Where: Qe (mg Kg^{-1}) and Ce (mg L^{-1}) are the concentrations in solid and water phase, respectively.

A high Kd would suggest strong affinity for sediment whilst a lower Kd would infer that these pharmaceuticals are more mobile and could be released back into the aqueous phase (Krascsenits et al., 2008). Sorption behaviour of pharmaceuticals varies significantly for the same compound on different solid phase types (Tolls, 2001). The physicochemical properties of the pharmaceuticals such as water solubility and hydrophobicity and the sediments properties including organic matter content, clay content and ion exchange capacity influence the sorption behaviour of pharmaceuticals (Carballa et al., 2008; Díaz-Cruz et al., 2003; Drillia et al., 2005). Properties of the surrounding environmental system, such as pH, ionic strength, temperature, organic matter (OM) including particulate organic matter (POM) and dissolved organic matter (DOM) and presence of complexing metal oxides (e.g. Ca^{2+}, Mg^{2+}, Al^{3+} or Fe^{3+}) are also important (Boxall and Ericson, 2012; Lapworth et al., 2012; Pal et al., 2010; Spark and Swift, 2002). Moreover, long contact times could lead to increased sorption over extended periods (Xu et al. 2008; Vieno et al. 2005; Gomez et al. 2006). It is also noteworthy that in most sorption studies Kds were obtained from the difference between initial and equilibrium solution concentrations and this can often lead to an overestimation of sorption if loss from solution is due to processes other than sorption, such as degradation and/or volatilization (Sarmah et al., 2006).

A literature search revealed a wide range of adsorption coefficient (Kd) values for pharmaceuticals in sediments which ranged between 0.2-12465 L Kg^{-1} (Table 2.4). Large variations in Kd can be observed for single compounds. For example, Ramil et al., (2010) found atenolol to have Kd values ranging from 1.13 to 3.1 L Kg^{-1}. While the value reported by Martínez-Hernández et al., (2014) was 2 to 6 times higher. This was explained due to the difference in cation exchange capacity (CEC) between the investigated sediments. CEC is considered as the predominant factor controlling the sorption of atenolol. In another study, Kd values calculated for some antibiotics in sediments showed high variability ranging from 18 to 1818 L kg^{-1} for chloramphenicol and from 2 to 536 L kg^{-1} for oxytetracycline. Such variability

likely results from differences in sediment particle size and composition and the degree of water-sediment interaction (Chen and Zhou, 2014). Moreover, partitioning of organic contaminants has been demonstrated to depend on the water to solid-ratio and the organic matter content of the solid due to the intrinsic hydrophobic nature (Chen and Zhou, 2014).

An organic carbon-based partitioning coefficient (Koc), obtained from normalizing Kd to organic carbon content (Equation 2.2) is used as an alternative to describe the affinity and tendency of a given substance (particularly neutral hydrophobic organic chemicals) to adsorb to and accumulate in sediment (Caliman and Gavrilescu, 2009). For pharmaceuticals, the use of Koc is acceptable when non-specific lipophilic interactions have occurred while the use of this parameter is probably inappropriate when partitioning occurs with special parts of organic matter (OM) or with minerals (Carballa et al., 2008; Liebig et al., 2005).

$$\mathbf{Koc = Kd \times 100 / } f_{oc} \qquad \mathbf{(2.2)}$$

Where $f$oc is the organic fraction percentage in sediment.

Yamamoto et al. (2009) reported wide ranges of Koc values for ibuprofen in sediments ranging from 1.8-120.2 L Kg^{-1} and for acetaminophen between 169.8-12882.5 L Kg^{-1}. The variability in Koc values showed that another factor (e.g. CEC) might drive the partitioning across selected sediments. The findings suggest that variation in sorption cannot be only explained by interaction with organic content but that additional mechanisms may also be in operation such as partitioning of pharmaceuticals residues to DOM in pore water which reduces hydrophobic interactions with the POM (Aga, 2008; Boxall and Ericson, 2012; Lees et al., 2016).

For neutral organic molecules, the octanol–water partition coefficients (Kow), is commonly used to estimate the distribution of a chemical between water and an organic phase (Lahti, 2012). Kow is a useful indicator of the partitioning behaviour of chemicals (Caliman and Gavrilescu, 2009). The majority of pharmaceuticals are polar and hydrophilic, exhibiting low Kow suggesting that they have low binding to the organic carbon in sediment compared to other

organic compounds (e.g. PAHs, pesticides). However, experimentally-derived sorption coefficients (Kd) of pharmaceuticals are typically greater than those predicted from the log Kow (Scheytt et al., 2005). This mismatch is due to the fact that the sorption of most pharmaceuticals does not only result from hydrophobic interactions between solid organics and the pharmaceutical (Boxall and Ericson, 2012; Chiouet al., 1983; Scheytt et al., 2005; Yamamoto et al., 2005). In addition, functional groups within the structure of pharmaceuticals can provoke significant deviations between measured and predicted partitioning coefficients, based on Kow, as they only bind to a specific part of the organic matter or other parts of the solid phase (Stein et al. 2008). Therefore, when trying to model sorption of pharmaceuticals, there is a need for considering the properties of the functional groups of the molecules at the pH of the system of interest, as suggested by several researchers (Drillia et al., 2005; Goss and Schwarzenbach, 2001; Yamamoto et al., 2005).

By introducing at least one of the partitioning coefficients above, the distribution of pharmaceuticals into sediments has been studied for a number of pharmaceuticals in recent years. For example, the sorption of different pharmaceuticals onto natural organic matter and the inorganic surfaces of natural sandy loam sediment were quantified separately by Martínez-Hernández et al., (2014). The findings showed that the partitioning was based on the pharmaceuticals charge, degree of ionisation, octanol–water partitioning coefficient (Kow) and the sediment organic carbon fraction ($f$oc). The sorption of cationic species onto the sediment was higher than that of anionic species while the sorption of neutral species was negligible since they are uncharged at natural pH and most of the adsorption was suggested to occur on charged surfaces of the study sediments (80% of pharmaceuticals are ionisable at environmental pH (Lees et al., 2016)). Moreover, Pal et al., (2010) and Zhou and Broodbank, (2014) suggested that compounds with higher molecular weight have the affinity to adsorb to sediment. On the other hand, pharmaceutical properties and functional group were also found to affect sorption behaviour; for example, carboxylic pharmaceuticals with low pKa showed low sorption tendency to sediment (Fent et al. 2006; Williams et al. 2009; Scheytt et al. 2005).

2.4.2 Persistence and Degradation

Alongside sorption, persistence is an important process determining the fate of pharmaceuticals in the environment. Persistence is often expressed as a residence time or half-life (DT50), which refers to the time for a substance to be degraded by 50% (Kah et al., 2007). Persistence in sediment differs amongst the pharmaceuticals. Some are known to be readily biodegradable through use of the ready biodegradation test - the first step or tier in biodegradation screening. This test utilises estringent (low biomass) test conditions where positive test results (pass) of readily biodegradability' indicate the chemical will undergo rapid and complete mineralisation. The Ready Biodegradation test is available in several standard options that accommodate DOC, dissolved oxygen (DO), CO_2 evolution and O_2 uptake as endpoints and for chemicals within a 10-day window of the 28-day test (OECD 301), while others appear to be more persistent (Conkle et al., 2012). Wide ranges in DT50 values for single pharmaceuticals in sediment have been reported in laboratory studies and this variability may due to differences in experimental protocol and the adopted laboratory conditions (Sarmah et al., 2006). Due to the complex nature of sediment it is believed that the depletion of pharmaceutical concentration is not only related to degradation but rather to the formation of non-extractable residues (Boxall and Ericson, 2012; Brooks et al., 2009; Höltge and Kreuzig, 2007). It is also important that we begin to understand the factors affecting persistence of commonly occurring pharmaceuticals in sediments. Environmental and physical-chemical properties such as hydrophobicity and degree of dissociation (Beausse, 2004), environmental conditions as temperature, pH, salinity and composition of the sediment and abundance of bacteria are all thought to be factors controlling persistence of pharmaceuticals (Bakal and Stoskopf, 2001; Boxall and Ericson, 2012). The persistence of pharmaceuticals in the environment (regardless of their entry mode) is determined by the susceptibility of the compound to biodegrade, photolyse, hydrolyse, adsorption affinity and the how strongly bound they are (Boxall and Ericson, 2012; Calisto and Esteves, 2009; Yamamoto et al., 2009). In sediment, photochemical degradation is not likely to take place due to a lack of light beneath the water column (Kümmerer, 2008). Therefore, biodegradation in addition to sorption is expected to be the main proposed elimination pathways of

pharmaceuticals and hormones in sediment (Gröning et al., 2007; Kunkel and Radke, 2008; Ying and Kookana, 2003).

As shown in Table 2.4, only a handful publications have explored the degradation of pharmaceuticals in sediments. On the other hand, many studies have explored the degradation of pharmaceuticals in wastewater (e.g. Joss et al., 2006; Quintana et al., 2005), sludge (e.g. Carballa et al., 2007; Li and Zhang, 2010; Radjenović et al., 2009) and soils (e.g. Li et al., 2013; Monteiro and Boxall, 2009; Xu et al., 2011, 2009; Yu et al., 2013). Persistence of pharmaceuticals in sediment systems can range from days to years. For example, within a chemical class, Jurgens et al., (2002) reported that 17β-estradiol (E2) degraded in 0.11 and 0.66 d when incubated under aerobic and anaerobic conditions in bed sediment, respectively, by the transforming to estrone (E1), while 17α-ethinylestradiol is very persistence with a DT50 of 81.0 d (Ying and Kookana, 2003). The longest half-life estimated (346.57 day) was reported for sulfamethoxazole in sterilised sediment (Xu et al., 2011). In wetland sediments, Conkle et al., (2012) reported the half-lives of ibuprofen and gemfibrozil to be <20.0 d under aerobic conditions, while carbamazepine showed half-lives between 165.0-264.0 d. These half-lives increased under anaerobic conditions by factors of 1.5-2.5 for carbamazepine and of 11–34 for ibuprofen and gemfibrozil. Table 2.4 illustrates the distribution coefficients (Kd) and half-lives (DT50) under aerobic or anaerobic conditions for a range of pharmaceuticals in different sediments.

Table 2.4 Available literature values for partitioning coefficients and DT50 for pharmaceuticals in sediment

Compound	Matrix	DT_{50} (day)	Conditions	Kd (L/kg)	References
17α-Ethinylestradiol	Marine sediment	20	Aerobic	-	(Ying and Kookana, 2003)
	Aquifer sediment	81	Aerobic	-	(Ying et al., 2003)
17β-estradiol	River sediment	0.11	Aerobic	-	(Jurgens et al., 2002)
		0.66	Anaerobic	-	
	Marine sediment	4.4	Aerobic	-	(Ying and Kookana, 2003)
	Aquifer sediment	2-70	Aerobic		(Ying et al., 2003)
Acebutolol	River sediment	2.4	Aerobic	1900	(Lin et al., 2010)
Acetaminophen	River sediment	-		2.6-10	(Yamamoto et al., 2009)
	Sandy loam sediment	-		0.5	(Martínez-Hernández et al., 2014)
		-			
	Reservoir sediment	-		1.0-54	(Williams et al., 2009)
	Stream sediment	-		316.3	(Fairbairn et al., 2015)
	River sediment			5	(Lin et al., 2010)
Atenolol	Streams and rivers sediment	2.3-3	Aerobic	1.13-3.1	(Ramil et al., 2010)
		-		7.93	(Martínez-Hernández et al.,2014)
	Sandy loam sediment	-		>1.54	
	Aquifer sediment	-		0.8-3.48	(Burke et al., 2013)
	River sediment	-		1.3-8.1	(Schaffer et al., 2012a)
	River sediment				(Yamamoto et al., 2009)
Azithromycin	River sediment	23.1	Aerobic	-	(Ericson, 2007)
Bezafibrate	River sediment	2.5-3.4	Aerobic	-	(Kunkel and Radke, 2008)
Bisoprolol	Streams and rivers sediment	3.9-8.4	Aerobic	2.0-6.5	(Ramil et al., 2010)
Caffeine	Sandy loam sediment	-		17.86	(Martínez-Hernández et al., 2014)
	Stream sediment	-		20	(Fairbairn et al., 2015)
	River sediment	1.5	Aerobic	250	(Lin et al., 2010)
Carbamazepine	Well sediment	-		0.21-5.32	(Scheytt et al., 2005)
	River sediment	-		1.7-12.3	(Stein et al., 2008)
	Sandy loam sediment	-		0.4	(Martínez-Hernández et al., 2014)
	River sediment	328	Anaerobic	1.3	(Loffler et al., 2005)
	Wetland sediment	165-264	Aerobic	2.93-15.11	(Conkle et al., 2012)
	River sediment	-		0.085-1.8	(Yamamoto et al., 2009)
	River sediment	-		26.68-	(Krascsenits et al., 2008)
	Reservoir sediment	-		54.92	(Williams et al., 2009)
	Stream sediment	-		0.1-20	(Fairbairn et al., 2015)
				0.01	
Celiprolol	Streams and rivers sediment	23.9-67	Aerobic	2.11-7.4	(Ramil et al., 2010)
Chlorpheniramine	Reservoir sediment	-		11-370	(Williams et al., 2009)

Table 2.4 (Continued)

Compound	Matrix	DT_{50} (day)	Conditions	Kd (L/kg)	References
Ciprofloxacin	Wetland sediment	23.24	Aerobic	-	(Thuy and Loan, 2014)
Clofibric acid	River sediments	26	Anaerobic	0.3	(Loffler et al., 2005)
Chloramphenicol	River sediment	-		18-1818	(Chen and Zhou, 2014)
Codeine	River sediment	-		2.1-14.1	(Stein et al., 2008)
Diazepam	River sediment	-		1.9-24.8	(Stein et al., 2008)
	Aquifer sediment	-		0.25	(Burke et al., 2013)
		192	Anaerobic	3	(Loffler et al., 2005)
Diclofenac	Well sediment	-		0.55-4.66	(Scheytt et al., 2005)
	Wetland sediment	3.2-8.5	Aerobic	-	(Kunkel and Radke, 2008)
	River sediments	-		0.2–1.4	(Dobor et al., 2012)
	Reservoir sediment	-		1-18	(Williams et al., 2009)
	River sediment	-		3.66-4.73	(Krascsenits et al., 2008)
Dihydrocodeine	River sediment	-		1.4-6.5	(Stein et al., 2008)
Estriol	Sediment	-		479	(López de Alda et al., 2002)
Estrone	River sediment	0.42-14.3	Aerobic	-	(Jurgens et al., 2002)
Exemestane	River sediment	15.1	Aerobic	-	(Ericson, 2007)
Florfenicol	Marine sediment	1.7	Aerobic	-	(Hektoen et al., 1995)
		7.3	Anaerobic		
Flumequine	Marine sediment	60	Aerobic	-	(Hektoen et al., 1995)
		>300	Anaerobic		
Fluoxetine	River sediment	-		180-4300	(Yamamoto et al., 2009)
	Wetland sediment	Nd-5.6	Aerobic	-	(Kunkel et al., 2008)
	Wetland sediment	15-22	Aerobic	0.26-20.11	(Conkle et al., 2012)
	River sediment	-		9.9-12.57	(Krascsenits et al., 2008)
Griseofulvin	Wetland sediment	31-39	Aerobic	-	(Thuy and Loan, 2014)
Ibuprofen	Well sediment	-		0.18-1.69	(Scheytt et al., 2005)
	River sediment	1.2-2.5	Aerobic	-	(Kunkel et al., 2008)
	Wetland sediment	7.0-19	Aerobic		(Conkle et al., 2012)
	River sediment	-		0.08-2.62	(Yamamoto et al., 2009)
	River sediments	-		0.093-0.91	(Dobor et al., 2012)
	Reservoir sediment	-		0.1–0.4	(Williams et al., 2009)
	River sediment	-		0.1-11	(Krascsenits et al., 2008)
				4.86-5.16	
Ifenprodil	River sediment	-		31-1400	(Yamamoto et al., 2009)
Imipramine	Reservoir sediment	-		44.0-7333	(Williams et al., 2009)
Indomethacin	River sediment	-		0.12-6.8	(Yamamoto et al., 2009)
ketoprofen	River sediments	-		0.2–1.2	(Dobor et al., 2012)
	Reservoir sediment			0.1-10	(Williams et al., 2009)

Table 2.4 (Continued)

Compound	Matrix	DT_{50} (day)	Conditions	Kd (L/kg)	References
Mefenamic acid	River sediment	-		5.6-20	(Yamamoto et al., 2009)
Metronidazole	Forest stream sediment	3.0-104		-	(Ingerslev et al., 2001)
Metoprolol	Streams and rivers sediment	4.1-8.7	Aerobic	1.75-7.3	(Ramil et al., 2010)
		-		>2.47	(Burke et al., 2013)
	Aquifer sediment	-		1.94-2.51	(Schaffer et al., 2012a)
	River sediment				
Morphine	River sediment	-		3.1-21.5	(Stein et al., 2008)
N- acetylsulfa methoxazole	River sediment	-		0.013-0.7	(Stein et al., 2008)
Nadolol	Streams and rivers sediment	3.1-3.7	Aerobic	1.54-6.7	(Ramil et al., 2010)
Naproxen	River sediment	5.6-6.9	Aerobic	-	(Kunkel et al., 2008)
	River sediments	-		0.2–0.7	(Dobor et al., 2012)
	Sandy loam sediment	-		1.86	(Martínez-Hernández et al., 2014)
	Reservoir sediment	-		0.2-17.0	(Williams et al., 2009)
Norfloxacin	Lake sediment	-		4493-47093	(Cheng et al., 2014)
	River sediment			66.6-288.0	(Liang et al., 2013)
Norethindrone	Sediment	-		128.0	(López de Alda et al., 2002)
Ofloxacin	Lake sediment	-		5925.0-12465.0	(Cheng et al., 2014a)
Olaquindox	Forest stream sediment	4.0-21.5	Aerobic	-	(Ingerslev et al., 2001)
Oxazepam	River sediment	-		2.0-23.5	(Stein et al., 2008)
	Aquifer sediment			0.19	(Burke et al., 2013)
		156.0		2.2	(Loffler et al., 2005)
Oxolinic acid	Fish farm sediment	151	Aerobic	-	(Hektoen et al., 1995)
		300	Anaerobic		
Oxytetracycline	Lake sediment	-		277.0- 1800	(Cheng et al., 2014)
	Marine fish farm sediment	13.0-16.0	Aerobic		(Coyne et al., 1994)
	River sediment	-		2.0-356.0	(Chen and Zhou, 2014)
	Forest stream sediment	42.0–45.9	Aerobic	-	(Ingerslev et al., 2001)
Pindolol	Streams and rivers sediment	0.12-0.5	Aerobic	0.51	(Ramil et al., 2010)
Progesterone	Marine sediment	-		204.0	(López de Alda et al., 2002)
Promethazine	Reservoir sediment	-		206 -1.575	(Williams et al., 2009)
Propranolol	Streams and rivers sediment	0.4-1.8	Aerobic	4.55-12.0	(Ramil et al., 2010)
		-		>2.47	(Burke et al., 2013)
	Aquifer sediment	-		0.6-129.0	(Williams et al., 2009)
	River sediment	2.2	Aerobic	270	(Lin et al., 2010)
	River sediment	-		2.2-160	(Yamamoto et al., 2009)
	River sediment				

Table 2.4 (Continued)

Compound	Matrix	DT_{50} (day)	Conditions	Kd (L/kg)	References
Rifampicin	Wetland sediment	25	Aerobic	-	(Thuy and Loan, 2014)
Sarafloxacin	Marine sediment	151 >300	Aerobic Anaerobic	-	(Hektoen et al., 1995)
Sotalol	Streams and rivers sediment Aquifer sediment	7.6-8.2 -	Aerobic	1.41-3.9 >0.43	(Ramil et al., 2010) (Burke et al., 2013)
Sulfadiazine	Marine sediment	50 100 -	Aerobic Anaerobic	- 1.67-10.37	(Hektoen et al., 1995) (Zhong et al., 2013)
Sulfamethoxazole	River sediment River sediment Sandy loam sediment Reservoir sediment River sediment River sediment River sediment River sediment -	- - - 10.66 346.57 - -	Non sterile-aerobic Sterile-aerobic	7.66-83.3 0.2-0.9 4.25 0.05-15.0 1.25-3.73 8.5-273.0 0.1-24	(Zhong et al., 2013) (Stein et al., 2008) (Martínez-Hernández et al., 2014) (Williams et al., 2009) (Xu et al., 2011) (Real et al., 2012) (Radke et al., 2009) (Chen and Zhou, 2014)
Sulfamethoxine	River sediment	-		1.58-7.52	(Zhong et al., 2013)
Sulfamethazine	River sediment	-		0.011-0.071	(Zhong et al., 2013)
Temazepam	River sediment	-		5.6	(Stein et al., 2008)
Tetracycline	Lake sediment	-		768-1227	(Cheng et al., 2014)
Tramadol	River sediment	-		2.4-7.7	(Stein et al., 2008)
Trimethoprim	Marine sediment	75 100	Aerobic Anaerobic	-	(Hektoen et al., 1995)
Tylosin	Sediment	15.5-95.0	Aerobic		(Ingerslev et al., 2001)
Varenicline	River sediment	24.8	Aerobic	-	(Ericson, 2007)
Verapamil	Reservoir sediment	-		1.341-5.876	(Williams et al., 2009)

2.5 Uptake into Sediment-dwelling organisms

Recently, there has been increasing interest in understanding the environmental levels of pharmaceuticals in aquatic and terrestrial systems and their uptake and potential effects in aquatic and terrestrial organisms (Karlsson et al., 2015). To date, most of the studies have focused on determining and understanding the uptake and depuration of pharmaceuticals into plants (e.g. Boxall et al., 2006), earthworms (e.g. Carter et al., 2014) and aquatic organisms *via* the water column (e.g. Meredith-Williams et al., 2012). Only limited work has been done to understand the uptake of pharmaceuticals from sediment. The occurrence of pharmaceuticals in aquatic sediment raises concerns over the potential of these compounds to be taken up by sediment-dwelling organisms. This section discusses the available information on the uptake of pharmaceuticals in sediment dwelling organisms and the factors and processes that influence uptake and accumulation.

Most of our understanding regarding exposure routes for sediment-dwelling organisms is based on studies that have investigated the behaviour of neutral organics compounds *via* pore water where the octanol/water partition coefficient (Kow) is the main uptake indicator (Karlsson et al., 2015). However, for the uptake of pharmaceuticals, Kow may not be a good descriptor since most of these compounds are ionisable (Meredith-Williams et al., 2012). Furthermore, the variability of pH, which affects the uptake by influencing the degree of dissociation, leads to high variability in uptake of pharmaceuticals in the environment (Kim et al., 2014; Nakamura et al., 2008). Recently, Karlsson et al., (2017) demonstrated the importance of exposure medium pH for predicting the uptake and depuration of ionisable pharmaceuticals into sediment-dwelling organisms. Moreover, it was found that a relationship between physicochemical properties and biological diversity and ecology (i.e. life cycle, size and habitat) can be established to understand the uptake from sediment (Meredith-Williams et al., 2012). However, it is difficult to develop a clear relationship between pharmaceutical properties and their uptake as some of pharmaceuticals are taken up variably between organisms (according to physiology of organisms) and across different environments. This is perhaps not surprising as data for other environmental processes (e.g. sorption) show that not only hydrophobic interaction is

responsible factor but also related to a range of factors including CEC, clay content and complexation (Boxall and Ericson, 2012). On the other hand, food availability and feeding behaviour have been shown to enhance the uptake into sediment-dwelling organisms when the availability and quality of food are high and sediment ingestion is a feeding route (Granberg and Forbes, 2006; Kaag et al., 1997).

Generally, available literature data on the uptake from sediments are limited to a number of pharmaceuticals in limited species (Fent et al., 2006; Vasquez et al., 2014). In a 35 day study, the uptake of radio labelled synthetic steroid C^{14}17α-ethinylestradiol into *L. variegatus* was found to be high, resulting in a lipid normalised biota-sediment accumulation factor (BSAF) of 75. The accumulation of total radioactivity measured in this study was higher than expected from other bioaccumulation studies with oligochaetes exposed to lipophilic compounds with comparable Kow's. For example, the BSAF of 16 polycyclic aromatic hydrocarbons (PAHs; logKow: 3.4–6.4) in *L. variegatus* and found BSAFs between 0.97 and 5.3 and between 1.0 and 8.8 for laboratory-exposed and field-collected animals, respectively (Brunson et al., 1998; Burton and Burton, 2002; Liebig et al., 2005). Later, sediment was shown to play a negligible role in the bioaccumulation of 17 α-ethinylestradiol in two benthic invertebrates (*C. tentans* and *H. azteca*) when exposure was via spiked sediments with BSFA of 0.8 and 0.3, respectively. *C. tentans* showed greater 17 α-ethinylestradiol accumulation than *H. azteca* in water with BSAF values of 215.0 and 142.0, respectively (Dussault et al., 2009a). Recently, Karlsson et al., (2015) studied the 48-h uptake of ^{14}C-labeled ingredients (diclofenac, fluoxetine and a personal care antibacterial, triclosan) from sediment with *L. variegatus*. The study explored the importance of uptake route into organisms and found the uptake of diclofenac (BSAF=0.3) and fluoxetine (BSAF=0.5) were *via* sediment pore water while triclosan (BSAF=9.0) was taken up *via* ingestion. The study suggested that particle ingestion of sediment contaminated with chemicals with log Kow>5 can lead to an enhanced rate of uptake into oligochaete worms. Ingestion, may therefore need to be considered in the future in order to develop approaches to better assess uptake from sediment.

2.6 Ecotoxicity of Pharmaceuticals to Sediment-dwelling organisms

The field of ecotoxicology is rapidly growing as the scientific community starts to be aware of many possible pollutants that have entered the environment, and which may be hazardous to non-target organisms. One is pharmaceuticals which can be expected to have the potential to pose a risk to a wide range of organisms in the natural environment (Franzellitti et al., 2013; Petersen et al., 2014). A diverse range of effects of pharmaceuticals on organisms and changes in physiological functions have been reported in different environmental compartment (Cuklev et al., 2011; Fent et al., 2006; Liu et al., 2014). However, not much is known about the adverse toxicological effects of these substances in sediment (Li and Randak, 2009; Oetken et al., 2005). Sediments act as a sink for pharmaceuticals, and provide a continuous source of these compounds to sediment-dwelling organisms, including invertebrates and also act as a reservoir from which chemicals can be remobilized by resuspension or desorption (Crane et al., 2006; Oetken et al., 2005).

The organisms commonly used in toxicity tests on pharmaceuticals in sediment are *C. riparius, C. dilutes, C. tentans, H. limbata, P. nubifer, H. Azteca* and *L. variegatus* (Brooks et al., 2003b; Dussault et al., 2009a; Gilroy et al., 2012; Oetken et al., 2005). The oligochaete worm, *L. variegatus*, which feeds by ingesting the whole sediment and serves as food for predators in aquatic ecosystems, particularly fish, is a common subject for toxicity tests which are recommended by the American Society for Testing and Materials (ASTM) as a standard organism (Nentwig et al., 2004; Nentwig, 2007). Different regulatory frameworks have recommended toxicity tests for the sediment compartment, including the mortality rate of amphipods exposed to sediment for 10 days, as a useful tool to evaluate marine and estuarine sediment quality in the setting of sediment quality guidelines like CEDEX 1994, European Sediment Network (Sed-Net) 2003 and USEPA 2001 (Maranho et al., 2014; Ramos-Gomez et al., 2011).

In sediment, acute and chronic toxicity tests have been used to investigate the effects of pharmaceuticals contaminated sediment on a variety of benthic dwelling organisms (Table 2.5). Exposure to carbamazepine in the range of 160 to 280 μg kg^{-1} *via* sediment was found to cause

a blocking of pupation and decreased emergence of *C. riparius* (Oetken et al., 2005). Gilroy et al., (2012) studied the toxicity of atorvastatin, carbamazepine, and 17α-ethinylestradiol to benthic invertebrates, *C. dilutus* and *H. azteca*, in spiked sediment and showed that the compounds are unlikely to cause larval mortality the No-Observed-Effect Concentrations (NOEC) ranging from 1.2 to 56.5 mg kg^{-1} (dw). The maximum NOEC's of 35 and 56.5 mg kg^{-1} were observed for carbamazepine in *C. dilutus* and *H. azteca*, respectively. In another study, exposure of *C. tentans* and *H. azteca* to fluoxetine showed a significant reduction in the growth with lowest observed effect concentrations (LOECs) of 1.3 and 5.6 mg kg^{-1} respectively, being obtained (Brooks et al., 2003a, 2003b). It is noteworthy that the few published monitoring studies report measured sediment concentrations of pharmaceuticals that are smaller than the highest concentration investigated in the reviewed studies, for which no toxicity or growth effects is expected to be observed at environmentally relevant concentrations.

A short-term toxicity study based on enzyme and receptor mode of action of organisms to assess possible effects of pharmaceuticals accumulated in sediment has been recently performed (Maranho et al., 2015). Carbamazepine, fluoxetine and propranolol were found to cause mortality to 20% of the amphipod *Ampelisca brevicornis* at concentrations up to 186.52 ng g^{-1}. Moreover, the testing of some biomarkers during long-term exposure to environmentally relevant levels of pharmaceuticals showed caffeine at concentration 1.5 ng g^{-1} and propranolol at concentrations of 50, 5, 0.5, and 0.05 ng g^{-1} to cause oxidative stress to the benthic biota; while DNA strand breaks were observed after exposure to sediment spiked with ibuprofen, fluoxetine, propranolol, caffeine, and 17α-ethinylestradiol at environmental levels of 0.5, 0.05 ng g^{-1} (Maranho et al., 2015).

A10-day exposure of *C. tentans* to fluoxetine in sediment resulted in an LC50 value of 17 mg kg^{-1}. LC50 for *H. Azteca* was >43 mg kg^{-1} in acute toxicity test while survival was not affected by the highest treatment level tested (43 mg kg^{-1}) in long term test (Brooks et al., 2003a). Furthermore, feeding activity and growth of *C. teleta* in fluoxetine spiked sediment was studied over 18-day and no significant effect was observed through the exposure to 0, 0.001, 0.03, 0.3 and 3.3 mg g^{-1}. On the other hand, high level of fluoxetine was found in males with abnormal

genital spines (Méndez et al., 2013a). Investigations into whether exposure of *C. riparius* and *L. variegatus* to carbamazepine and fluoxetine in spiked sediment showed effects which were not revealed by aqueous exposure were performed (Nentwig et al., 2004; Nentwig, 2007).

In sediment, the extensive exposure to antibiotics has been shown to affect bacterial community composition in fish farm systems and even to provoke the formation of resistance in bacteria (Chelossi et al., 2003; Hansen et al., 1993; Madureira et al., 2012, 2011). For example, a culture-based method was used by Akiayma and Savin (2010) to determine antibiotics (ampicillin, tetracycline, trimethoprim, and sulfamethoxazole) resistance in *E. coli* bacteria in sediment and reported resistance promotion of about 15% towards sulfamethoxazole. Furthermore, Kristiansson et al., (2011) investigated exposure of bacterial communities in river sediment to waste water from the production plant of antibiotics in India. Eight fluoroquinolones and forty six sulfonamides and sulphonamide- like compounds were investigated and were shown to promote resistance genes in bacterial communities.

Table 2.5 Available literature data on toxicity effects of pharmaceuticals on organisms in sediment

Compound	Organism	Toxicological endpoint	Concentration	Sediment Physicochemical properties	Reported effect	Reference
14C-17α-ethinylestradiol	Lumbriculus variegatus	NOEC	31.6 μg g–1	TOC= 2.4%	Did not affect the reproduction and growth of the worms	(Liebig et al., 2005)
17 α -ethinylestradiol	V. fischeri	5 min-IC50	57.8 ng g^{-1}	TOC= 1.2%	Bioluminescence inhibition	(Maranho et al., 2015)
		15 min-IC50	52.9 ng g^{-1}			
		30 min-IC50	39.4 ng g^{-1}			
	Ampelisca brevicornis	10d- sub lethal responses	100 and 0.01 ng g^{-1}	TOC= 1.2%	Decrease in etoxification metabolism, glutathione S-transferase (GST).	(Maranho et al., 2015)
			100, 1.0, 0.01 ng g^{-1}		Decrease in glutathione peroxidase (GPX), lipid peroxidation (LPO), and DNA strand breaks	
	Chironomus dilutes	NOEC	7.6 ± 3.96 ng g^{-1}	pH= 8.17, TOC= 1.9%	Unlikely to cause mortality with survival >72.5%	(Gilroy et al., 2012)
	Hyalella azteca	NOEC	1.2 ± 0.21 ng g^{-1}			
Atorvastatin	*Chironomus dilutes*	NOEC	3.7 ng g^{-1}	pH= 8.17, TOC= 1.9%	Unlikely to cause mortality	(Gilroy et al., 2012)
	Hyalella azteca		2.7 ng g^{-1}			

Table 2.5 (Continued)

Compound	Organism	Toxicological endpoint	Concentration	Sediment Physicochemical properties	Reported effect	Reference
Caffeine	V. *fischeri*	5 min-IC50	735.0 ng g^{-1}	TOC= 1.2%	Bioluminescence inhibition	(Maranho et al., 2015)
		15 min-IC50	646.5 ng g^{-1}		Bioluminescence inhibition	
		30 min-IC50	507.6 ng g^{-1}		Bioluminescence inhibition	
	Ampelisca brevicornis	10d- sub lethal responses	0.15 ng g^{-1}	TOC= 1.2%	Decreased ethoxyresorufin O-deethylase (EROD) and caused neurotoxicity to amphipods.	(Maranho et al., 2015)
			0.15-1500.0 ng g^{-1}		Decreased in glutathione peroxidase (GPX), increase DNA strand breaks	
Carbamazepine	*Chironomus dilutes*		56.5 ± 10.21 ng g^{-1}	pH= 8.17, TOC= 1.9%	Unlikely to cause mortality with survival >70.0%	(Gilroy et al., 2012)
	Hyalella azteca		35.0 ± 3.53 ng g^{-1}			
	Chironomus riparius	28d-EC50	160.0-280.0 ng g^{-1}	TOC= 0.85%, 1.36%	A blockade of pupation and emergence in the nonbiting midge	(Oetken et al., 2005)
		28d-LOEC	140.0-234.0 ng g^{-1}			
		28d-NOEC	33.0-140.0 ng g^{-1}			
		28d-EC10	70.0-210.0 ng g^{-1}			
	Chironomus riparius	28d-NOEC	0.625-0.8 μg g^{-1}	TOC= 1%, 1.6%	No evidence for a potential hazard	(Nentwig et al.,2004)
		28d-LOEC	1.25-4.0 μg g^{-1}			
	Lumbriculus variegates	28d-EC50	0.16, 0.8, 4, 20, and 100 μg g^{-1}		Did not show negative effects	

Table 2.5 (Continued)

Compound	Organism	Toxicological endpoint	Concentration	Sediment Physicochemical properties	Reported effect	Reference
	V. fischeri	5 min-IC50	412.8 ng g^{-1}	TOC= 1.2%	Bioluminescence inhibition	(Maranho et al., 2015)
		15 min-IC50	205.0 ng g^{-1}			
		30 min-IC50	95.6 ng g^{-1}			
	Paracentrotus lividus	Spermiotoxicity			Fecundation failure	
	Isochrysis galbana	Embryotoxicity			abnormal larval development	
	Tetraselmis chuii	LC50	21.0 ng g-1		Growth rate inhibition	
		LC50	84.61 ng g-1		Growth rate inhibition	
	Ampelisca brevicornis	10d-Mortality	5.0 ng g^{-1}	TOC= 1.2%	20.69% mortality	(Maranho et al., 2015)
		10d-LC20	186.52 ng g^{-1}		Activated glutathione reductase (GR) and decreased lipid peroxidation (LPO)	
		10d- sub lethal responses	5.0, 0.05 ng g^{-1}			
Diclofenac	*Hyalella azteca*	72-h LC50	467.0 ng g^{-1}	TOC= 10%	Low acute toxicity	(Oviedo-Gomez et al., 2010)
Fluoxetine	*Capitella teleta*	18d-EC50	0, 0.001, 0.03, 0.3 and 3.3 ng g^{-1}	TOC= 7.3%	No effect on egestion rates, body weight and size-specific egestion rates	(Méndez et al., 2013b)

Table 2.5 (Continued)

Compound	Organism	Toxicological endpoint	Concentration	Sediment Physicochemical properties	Reported effect	Reference
	Chironomus tentans	10d- LOEC	1.3 μg g^{-1}	TOC= 2.2%	A significant reduction in the growth	(Brooks et al., 2003a, 2003b)
		10d-LC50	15.2 μg g^{-1}			
	Hyalella Azteca	10d-LC50	> 43 μg g^{-1}			
		10d-LOEC	5.6 μg g^{-1}		A significant reduction in the growth	
	Chironomus riparius	28d-LOEC	1.12 μg g^{-1}	TOC= 1%, 1.6%	Reduction in emergence	(Nentwig, 2007)
	Lumbriculus variegatus	28d-EC50	0.94 and 2.34 μg g^{-1}		Slight increase in reproduction	
	V. fischeri	5 min-IC50	54.4 ng g^{-1}	TOC= 1.2%	Bioluminescence inhibition	(Maranho et al., 2015)
		15 min-IC50	67.0 ng g^{-1}			
		30 min-IC50	36.1 ng g^{-1}			
	Ampelisca brevicornis	10d-Mortality	10.0 ng g^{-1}	TOC= 1.2%	37.93% mortality	(Maranho et al., 2015)
		10d-LC20	62.28 ng g^{-1}		-	
		10d- sub lethal responses	50.0, 5.0, 0.05 ng g^{-1}		Decreased in glutathione peroxidase (GPX), decreased DNA strand breaks and caused neurotoxicity to amphipods	

Table 2.5 (Continued)

Compound	Organism	Toxicological endpoint	Concentration	Sediment Physicochemical properties	Reported effect	Reference
	Chironomus riparius	LOEC	0.17 ng g^{-1}	TOC= 0.16%	Reduced adults emergence	(Sánchez-Argüello et al., 2009)
Ibuprofen	V. fischeri	5 min-IC50	271.1 ng g^{-1}	TOC= 1.2%	Bioluminescence inhibition	(Maranho et al., 2015)
		15 min-IC50	217.8 ng g^{-1}			
		30 min-IC50	100.6 ng g^{-1}			
propranolol	V. fischeri	5 min-IC50	272.8 ng g^{-1}	TOC= 1.2%	Bioluminescence inhibition	(Maranho et al., 2015)
		15 min-IC50	206.1 ng g^{-1}			
		30 min-IC50	163.9 ng g^{-1}			
	Ampelisca brevicornis	10d-LC20	5.0 ng g^{-1}	TOC= 1.2%	Decreased of DNA strand breaks	(Maranho et al., 2015)
		10d- sub lethal responses	50.0, 5.0, 0.05 ng g^{-1}			

TOC= Total organic carbon

2.7 Conclusion

Pharmaceutically active substances are widely used around the world. Antibiotics, analgesics, anti-inflammatory drugs and beta-blockers are the most heavily used pharmaceutical classes. To date, there is a gap in our understanding of the occurrence, fate and effects of pharmaceuticals and their metabolites in sediment. The research in the area of pharmaceuticals occurrence in sediment has lagged behind that of the water phase. Several trends are identifiable from this review that point to the future developments in the study of pharmaceuticals in the sediment. Most of the studies that have reported detectable concentrations of pharmaceuticals in sediment have focused on only a limit number of pharmaceuticals from similar therapeutic class while only a handful of studies reported more data on the occurrence of pharmaceuticals from different therapeutic classes. Over the past ten years, different methods have been proposed for the monitoring of pharmaceutical residues in sediment samples. Many analytical methods suffer limitations due to problems arising from co-extraction and matrix interferences which are sometimes more abundant than the analyte itself. Standard extraction methods for pharmaceuticals in sediment do not exist and conditions for extraction and analytical methods and clean up need to be optimized. Consequently, because of sediment complexity as an analysis matrix and co extracted interferences, sample clean-up is important to improve analysis in chromatography. SPE was the most reviewed clean up method in use for pharmaceuticals in sediment.

In sediment, the potential of pharmaceuticals to contaminate and adversely impact the aquatic environment can be conventionally estimated using information on partitioning and persistence. Only a small number of studies have investigated the sorption of pharmaceuticals in sediment and even fewer have explored dissipation behaviour. The studies that have been done indicate that sediment specific properties such as pH, surface charge and organic content of receiving sediment play an important role in sorption process. In terms of the pharmaceutical, the physicochemical properties and characteristics of functional group also affect sorption behaviour. Many of the pharmaceuticals were found to have the potential to accumulate, persist and even resist degradation. The general trend of pharmaceuticals dissipation in sediment is

believed to be via microbial degradation. Therefore, better understanding of those factors and processes affecting the sorption and persistence of pharmaceuticals in sediment is highly warranted.

Uptake and toxicity tests at different concentrations of contaminants in sediments can be used to establish relationships between pharmaceuticals and biological responses. The uptake of pharmaceuticals in sediment is less considered than other environmental compartments. Studies performed in sediment have reported BSAF of pharmaceuticals into organisms and found that the accumulation is even directly via ingestion of contaminated sediment or through sediment pore water and both routes depend on Log Koc and/or Log Kow values. On the other hand, only a few compounds including carbamazepine and fluoxetine were frequently studied and found to cause wide variety of effects. Further work to understand the uptake mechanisms of pharmaceutical into dwelling organisms and their ecotoxicity is therefore highly needed.

In the following chapters, we try to address many of the knowledge gaps. Data would allow a better understanding of ionisable pharmaceuticals behaviour in sediment environment, and how would these data useful for risk assessment purposes.

Chapter 3

Risk-based prioritisation of pharmaceuticals in the natural environment in Iraq

3.1 Introduction

It is estimated that more than 1500 active pharmaceutical ingredients (APIs) are currently in use. Following use, these compounds can be emitted into the natural environment e.g. *via* wastewater collection and treatment networks (Boxall et al., 2012; Ginebreda et al., 2010). The ongoing use of many of these APIs by society means that the active substances and their major metabolites will occur in the environment continuously (Monteiro and Boxall, 2010).

For most pharmaceuticals in use, the evidence that they have deleterious effects on the natural environment is still limited and our knowledge of the fate of these pharmaceuticals in the environment is still deficient (Roos et al., 2012). This is partly due to the fact that the number of APIs in use is large and that experimental data on the environmental levels, fate and effects are available for only a small proportion of these substances. For example, the knowledge of environmental exposure to antibiotics which may lead to possible evolution and dissemination of antibiotic-resistant pathogens in bacteria is limited (Bengtsson-Palme and Larsson, 2016). To experimentally assess the environmental risk of all APIs in use would be a challenge (Perazzolo et al., 2010). One solution is to use formalized prioritisation procedures that identify those substances in use that pose the greatest risks towards the natural environment (Boxall et al., 2012). By using these approaches, experimental testing resources can then be focused on those substances that are likely to have the greatest impact.

Several studies have been recently performed different approaches for ranking and assessing the risk posed by APIs to the environment. Most have focused on surface or drinking water and the risks to aquatic organisms or human health. These approaches have been applied in Switzerland (Perazzolo et al., 2010), USA (Dong et al., 2013; Kostich and Lazorchak, 2008), France (Besse

and Garric, 2008), the UK (Boxall et al., 2003; Guo et al., 2016), South Korea (Kim et al., 2008) and Sweden (Roos et al., 2012). Many of these approaches use exposure and toxicological predictions so they can be readily applied to large numbers of compounds with limited data (Boxall et al., 2012).

Most prioritisation studies have focused on North America and Western Europe, so our knowledge of priorities in other geographical areas such as Eastern Europe, Africa, Asia and South America is limited. This can be partly explained by the challenges in obtaining information on API usage in these regions. Moreover, although there are strong incentives to introduce the evaluation of an antibiotic to select for resistance into environmental risk assessment guidelines (Bengtsson-Palme and Larsson, 2016), none of the previous prioritisation approaches has attempted to assess the risk of antibiotics in the environment in terms of their potential to select for antimicrobial resistance.

In Iraq, there is no specific management guideline for pharmaceuticals in the environment. Pharmaceuticals are freely available to everyone without any restriction and regulation or even without prescription. There are many routes by which these substances are distributed to the population. One route is the public health sector represented by the Ministry of Health (MOH) *via* the state company for importation and distribution of drug and medical appliances (KIMADIA). The second source is the private sector (licensed and unlicensed low value manufacturers) which includes 23 manufacturing plants, importers and dispensers who supply the local markets with unknown quantities of pharmaceuticals. Additionally, all the locally produced and imported finished pharmaceuticals are not subjected to taxes in order to make them affordable for most of the population (USAID; 2007; EMRO WHO 2011; MOH 2011).

With a highly urbanized population, Iraq still has insufficient environmental management and suffers from poor and old water distribution systems and contaminated main water resources (UNEP 2003). Due to the absence of water quality regulations and the continuous discharges from industry and households via insufficient wastewater treatment plants (WWTPs), up to 19 % of the Iraqi population is exposed to unsafe water (UNEP 2003; USAID 2007). In addition,

only 32 % of the population is served with wastewater treatment works, meaning that a significant amount of untreated wastewater is released to the environment without treatment (COSIT 2012). Few studies to evaluate the quality of environmental systems in Iraq have been performed, and most that have been performed have focused on monitoring the occurrence of trace metals, polycyclic aromatic hydrocarbons (PAHs) and non-polar lipids in the aquatic environment (Abaychi and DouAbul, 1985; Al-Saad, 1987; Rushdi et al., 2014). The risks of emerging contaminants such as pharmaceuticals have been neglected.

The aim of this chapter is therefore to establish the importance of API exposure as a pressure on the natural environment in Iraq and to identify APIs of most concern in local aquatic and terrestrial environments of the three main cities in the country (Baghdad, Mosul and Basrah), where only little is currently known about the exposure and effects of these substances. The prioritisation approaches used to achieve this were based on the potential for APIs to enter the aquatic and terrestrial environments and their potential toxic effects on the ecosystems, bacterial community and human health.

3.2 Methods

3.2.1 Prioritisation Approach

The prioritisation approach is illustrated in Figure 3.1 and involved the use of predicted environmental concentrations (PECs) and concentrations relating to different effect endpoints (i.e. predicted no-effect concentrations (PNECs)), human therapeutic plasma concentrations (H_TPCs), minimal inhibitory concentrations (MICs) and minimal selective concentrations (MSCs)) for each of the pharmaceuticals in aquatic and terrestrial systems. PECs and PNECs were then used to calculate risk characterization ratios (RCRs) for apical endpoints, secondary poisoning, toxicity to humans and antimicrobial resistance selection. Pharmaceuticals were then ranked based on their RCRs where compounds with the highest RCRs were considered the highest priority.

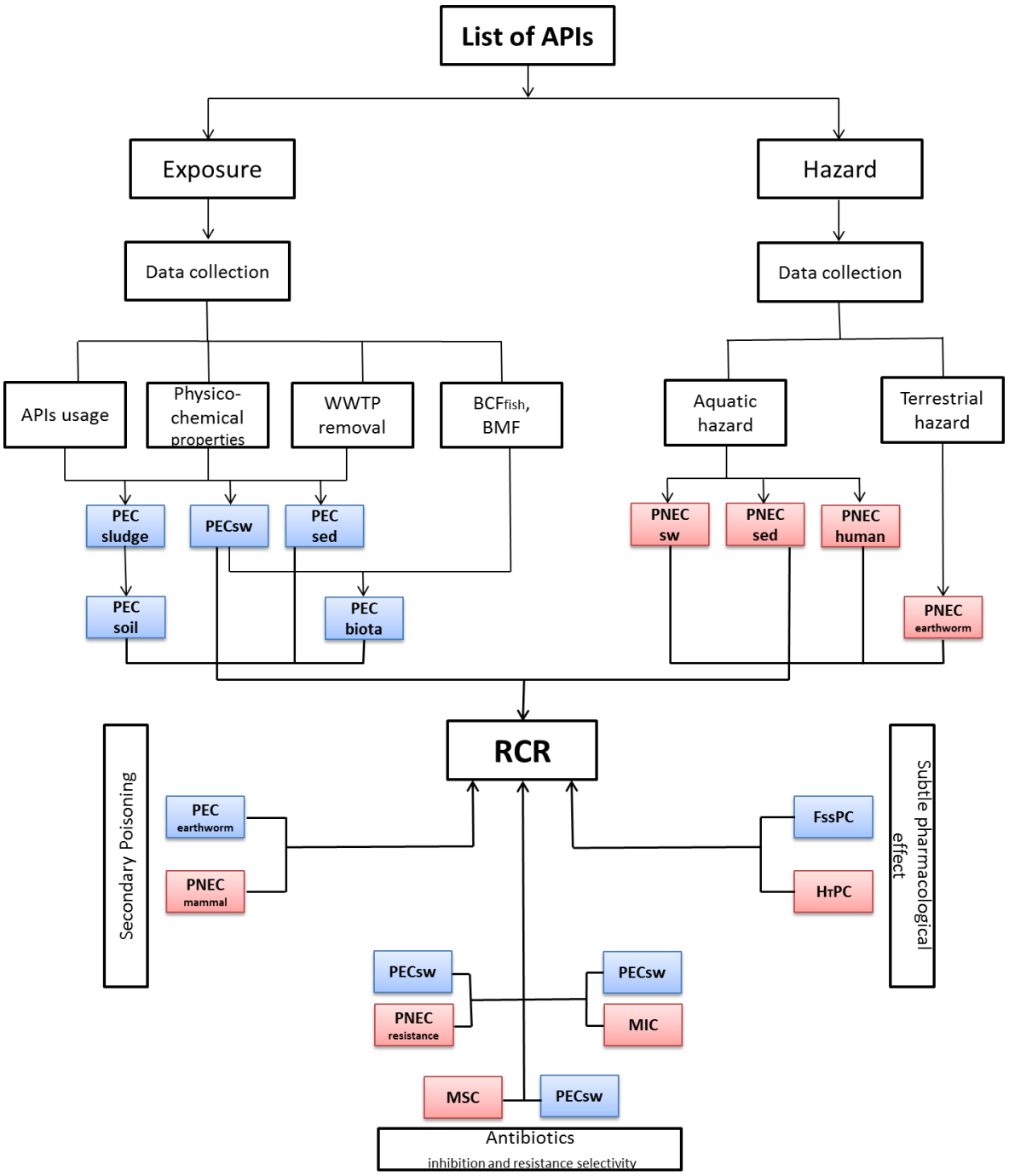


Figure 3.1The developments of prioritisation approach of pharmaceuticals in the environment in Iraq

RCR: risk characterization ratios; PECsw, PNECsw: predicted environmental concentration and predicted no-effect concentration in surfacewater; PECsludge: predicted environmental concentration in sludge compartment; PECsed, PNECsed: predicted environmental concentration and predicted no-effect concentration in sediment compartment; WWTP:wastewater treatment plan; BCF: fish bioconcentration factor; BMF: biomagnification factor; PECbiota: predicted environmental concentration in biota (e.g. fish); PNEChuman: predicted no-effect concentration in humans from drinking water and fishery products consumption; PECsoil: predicted environmental concentration in soil; FSSPC: fish steady state plasma concentration; H_TPC: human therapeutic plasma concentration; PECearthworm: predicted environmental concentration in earthworm; PNECearthworm: predicted no effect concentration in earthworm; PNECmammal: predicted no effect concentration in mammal. MIC: minimal inhibitory concentration; PNECresistance: predicted no effect concentration for antibiotics resistance selection; MSC: minimal selective concentration.

3.2.2 Data Collection

3.2.2.1 Usage Data

Data on the consumption of pharmaceuticals for hospitals and primary care centres in Iraq in 2014 were obtained from the state company KIMADIA (Kimadia, access 2014). To obtain the total amount of pharmaceuticals consumed, concentrations of active ingredient in packaging units (i.e. blister, bottle, etc.) were converted into mass units. Vitamins, medical supplements, electrolytes and vaccines were excluded which reduced the list of APIs to 99 compounds. In the case of combined medicines, only individual active ingredients were considered and summed up to calculate the weight of pharmaceutical compound.

Information is scarce on the use of over-the-counter pharmaceuticals in Iraq. However, research by the Centre of Market Research and Consumer Protection at the University of Baghdad (Mohammed et al., 2009) indicates that over-the counter usage can contribute 68 % of the total usage of pharmaceuticals in Iraq. Therefore, to obtain a total pharmaceutical usage in Iraq (for both hospitals and primary care centres and over the counter), the results of the analysis of the KIMADIA data were multiplied by a factor of 3.125. Some APIs, such as cancer treatments or those used in surgical procedures in hospitals, were not corrected (multiplied by the factor) as they would not be distributed over the counter. The final usage data are provided in the appendices (Table A.A1).

3.2.2.2 Effects data and Physicochemical properties

To estimate the environmental risk posed by the pharmaceuticals to aquatic and terrestrial ecosystems in Iraq, data on toxicity of the APIs to algae, daphnia, fish and earthworms were used. The data collection included acute and chronic ecotoxicity endpoints (typically the most sensitive LC/EC50 value). These data were obtained from the peer-reviewed literature, grey literature and available online databases (e.g. Swedish voluntary environmental classification of pharmaceuticals at www.fass.se). As experimental ecotoxicity data were not available for a large number of the pharmaceuticals, estimation tools, such as Quantitative Structure-Activity

Relationships (QSAR) used in the Organisation for Economic Co-operation and Development (OECD QSAR, 2013) Toolbox and the Ecological Structure Activity Relationship ECOSAR (USEPI 4.1) software, were used to fill data gaps. The database present in the QSAR Toolbox was used to identify experimental data for molecules deemed 'similar' to each of the individual pharmaceutical with no data. Then, within the software, a relationship was built to allow an estimation of the ecotoxicological endpoint for the query molecule. Regarding human and mammalian toxicity effects from oral exposure, endpoints such as the acceptable daily intake (ADI) values and the median lethal dose (LD50) for rat/mouse were used (Carvalho et al., 2015; EC, 2011; Guo et al., 2016). The H_TPCs available in peer reviewed publications were used in the fish plasma model. Finally, for terrestrial toxicity, earthworm acute toxicity (14- day LC50 in mM kg^{-1} dry soil) was predicted using the QSAR available in ECOSAR for compounds with no experimentally determined earthworm ecotoxicity data. Due to the absence of experimentally determined effects of antibiotics in complex microbial communities, the theoretical MICs, MSCs and PNECs for resistance selection that were proposed by Bengtsson-Palme and Larsson, (2016) were used.

Physicochemical properties required for predicting the fate and behaviour of pharmaceuticals in the environment were collated from published articles and open resources. DrugBank, NCCOS (2014) was used to obtain acid dissociation constants (pKa), and the CNC-CODATA (2014) database was used to obtain octanol-water partition coefficients (Kow). As there was a lack of experimental data on organic carbon partition coefficients (Kocs) for the APIs, for compounds where experimental Koc data were not available, we used the estimation model developed by Franco and Trapp, (2008). Excretion profiles for pharmaceuticals were obtained from the peer-reviewed literature, databases or pharmaceutical safety data sheets (i.e. MEDSAFE, Pfizer).

3.2.2.3 Wastewater Generation and Dilution factor

Information on wastewater disposal for the main highly urbanized cities in Iraq (Baghdad, Mosul and Basrah) was collected. The daily generated wastewater discharges are 1.6 million m^3

day^{-1} in Baghdad, 0.5 million m^3 day^{-1} in Mosul and 0.331 million m^3 day^{-1} in Basrah (COSIT 2014). These data were used to calculate the wastewater generated per inhabitant (Appendix A, Equation A.A1).

It is difficult to determine the dilution factor (DF) in countries, like Iraq, with none or very scarce hydrological information. For this purpose, we therefore used two dilution factors of 10 and 40 which had been estimated based on a national scale for Iraq by Keller et al., (2014). The percentage of wastewater treatment efficiency will also be important for the calculation of exposure concentrations in surface water so information was also collected on the percentage connectivity to wastewater treatment plants for the three cities. Data on the population, wastewater per capita, wastewater treatment percentage and dilution factors for the cities under study is provided in Table A.A2 in the appendix A.

3.2.3 Exposure Assessment

Predicted environmental concentrations (PECs) of APIs on the usage list were calculated in aquatic systems (surface water and sediment) and terrestrial systems according to the Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use (EMEA, 2006) and the Technical Guidance Document on Risk Assessment (EC, 2003) with some modifications to be fitted to the case of Iraq. In surface water, PECsw values for APIs were calculated using the following equation (3.1):

$$\mathbf{PECsw} = \frac{\mathbf{Subinhab} \times \mathbf{Fexc}}{\mathbf{WasteWinhab} \times \mathbf{Dilution}} \times \left(\mathbf{1} - \frac{\mathbf{Sludge\ inhab} \times \mathbf{Koc} \times \mathbf{focsludge}}{\mathbf{WasteWinhab} + (\mathbf{Sludge\ inhab} \times \mathbf{Koc} \times \mathbf{focsludge})}\right) \quad \textbf{(3.1)}$$

Where: PEC_{sw} is the predicted concentration of an API in surface water. Subinhab is the consumed amount of pharmaceuticals per inhabitant in Iraq per day (mg/inh/d) and was calculated based on annual pharmaceutical consumption (kg yr^{-1}) and using the population of

Iraq (34.2 million), (Equation Table A.A2, Appendix); DF is the dilution factor of 10 or 40 and WW_{inhab} is the daily amount of wastewater per inhabitant in either Baghdad, Mosul and Basrah. F_{exc} is the fraction of parent ingredients excreted unchanged *via* human metabolism. $Sludge_{inhab}$ [kg $inhd^{-1}$] is the mass of waste sludge per inhabitant per day, 0.074 (EC 2001); Koc is the organic carbon partitioning coefficient obtained either experimentally or estimated according to the method of Franco and Trap (2008) for ionisable chemicals using Kow and pKa; foc_{sludge} is the fraction of sludge organic carbon, 0.326 (Struijs et al., 1991).

The assumption of removal by adsorption was just used in the case of Baghdad because of the absence of wastewater treatment in both Mosul and Basrah. For Mosul there are no wastewater treatment plants in the city while for Basrah the removal efficiency in existing WWTPs is zero since they were out of working order and influents just pass through the plant without any treatment (COSIT 2014).

For sediment, the standard algorithms (Equation 3.2) in the Technical Guidance Document, TGD, (EC, 2003) was used to estimate concentrations of the APIs in terms of wet weight (ww) (PEC_{sed_ww}) and since the final PEC_{sed} was calculated in terms of dry weight, a conversion step was applied to determine PEC_{sed} on a dry weight base (Carvalho et al., 2015) (Equations A.A4 and A.A5 in Appendix A).

$$\mathbf{PECsed\_ww} = \frac{\mathbf{K\,susp-water}}{\mathbf{RHOsusp}} \times \mathbf{PECwater} \times \mathbf{1000} \qquad \mathbf{(3.2)}$$

Where: Ksusp-water is the suspended matter-water partitioning coefficient (Equation A.A3); and RHO is bulk density of suspended matter (Kg m^{-3}) (EC, 2003).

For the calculation of the PEC_{biota} the following equation was used:

$$\mathbf{PEC_{biota} = PEC_{sw} \times BCF_{biota} \times BMF} \qquad \mathbf{(3.3)}$$

Where: BCF_{biota}: Bioconcentration factor for biota (e.g. Fish). Default Biomagnification factor BMF values were retrieved from technical guidance document (EC, 2011).

Data on measured levels of pharmaceuticals in fish plasma following exposure *via* water are still scarce (Fick et al., 2010). As an indicator of a specific drugs' potential to cause adverse pharmacological effects at certain concentrations, the fish steady state plasma concentration (FSSPC) resulting from exposure via surface water was calculated (Equation 3.4). Prediction was based on the estimating the partitioning of an API between the aqueous phase and arterial blood in the fish ($P_{blood:\ water}$) (Equations 3.5, 3.6). This partition coefficient was initially estimated based on the Log Kow of the API, and this was subsequently combined with the PEC_{sw} to estimate the FSSPC.

$$\mathbf{FSSPC} = \mathbf{P}\text{blood: water} \times \mathbf{PECsw} \quad \textbf{(3.4)}$$

Where: FSSPC is the fish steady state plasma concentration [mg L^{-1}]; and PECSW is the Predicted environmental concentration for surface water [mg L^{-1}]; $P_{blood:\ water}$ is the aqueous phase and fish arterial blood partition coefficient.

When Log Kow <3:

$$\textbf{Log P}_{blood:\ water} \textbf{ = 0.73 * log Kow – 0.88} \quad \textbf{(3.5)}$$

When Log Kow >3:

$$\textbf{Log P}_{blood:\ water} \textbf{ = log [(100.73 * log Kow * 0.16) + 0.84]} \quad \textbf{(3.6)}$$

PEC_{soil} was derived from the PEC_{sludge} which was calculated using algorithms described in the TGD (EC, 2003) (Equations 3.7 and 3.8). To estimate the concentration of an API in earthworms ($PEC_{earthworm}$), the concentration in the earthworms on a wet weight basis (C earthworm) was calculated using an estimate of the concentration in pore water ($C_{porewater}$) from PEC_{soil} by considering the partitioning behaviour of substances between the soil and aqueous phase (Equations 3.9 and 3.10). The BCF for earthworms was calculated according to the approach in the TGD (EC, 2003), Equation 3.10.

$$\mathbf{PECsludge} = \frac{\mathbf{Koc} \times \mathbf{focsludge} \times \mathbf{Subinhab}}{\mathbf{WasteWinhab}} \quad \mathbf{(3.7)}$$

Where PECsludge is Predicted environmental concentration for sludge [mg kg^{-1}], $f$oc is organic carbon fraction (0.326).

$$\mathbf{PECsoil} = \frac{\mathbf{PECsludge} \times \mathbf{Asludge}}{\mathbf{Dsoil} \times \mathbf{RHOsoil}} \quad \mathbf{(3.8)}$$

Where: PEC_{soil} is the Predicted environmental concentration for soil [mg kg-1]; A sludge is the Sludge application rate to land, i.e. 0.5, [kg m-2 yr^{-1}]; D_{soil} is the Soil mixing depth, i.e. 0.2, [m]; RHO_{soil} is the Bulk density of soil, i.e. 1700, [kg m^{-3}]; and foc_{soil} is the Fraction of soil organic carbon, i.e. 0.02.

$$\mathbf{C_{earthworm}} = \frac{\mathbf{BCFearthworm} * \mathbf{Cporewater} + \mathbf{Csoil} * \mathbf{Fgut} * \mathbf{CONVsoil}}{\mathbf{1 + Fgut} * \mathbf{CONV\ soil}} \quad \mathbf{(3.9)}$$

Where: $C_{earthworm}$ ($PEC_{earthworm}$) is the Concentration in earthworm on a wet weight basis [mg kg-1]; $C_{porewater}$ is the Concentration in pore water [mg L^{-1}]; C_{soil} is the Concentration in soil [mg kg-1]; F_{gut} is the Fraction of gut loading in worm, i.e. 0.1; $CONV_{soil}$ is the Conversion factor for soil concentration wet to dry weight soil, i.e. 1.133, calculated from TGD (EC, 2003). The $BCF_{earthworm}$ was calculated according to the TGD (EC, 2003) approach.

$$\mathbf{PECporewater} = \frac{\mathbf{PECsoil}}{\mathbf{focsoil} \times \mathbf{Koc}} \quad \mathbf{(3.10)}$$

$PEC_{porewater}$ is concentration in pore water [mg L^{-1}]; foc_{soil} is fraction of soil organic carbon, 0.02.

$$\mathbf{BCFearthworm} = \frac{\mathbf{0.84} * \mathbf{0.012\ Log\ Kow}}{\mathbf{RHOearthworm}} \quad \mathbf{(3.11)}$$

Where $BCF_{earthworm}$ is the Bioconcentration factor for earthworms [L kg^{-1}]; and $RHO_{earthworm}$ is the Density of earthworms (default of 1) [kg L^{-1}].

3.2.4 Hazard Characterisation

In order to calculate PNECs for toxicity to surface water organisms, effects data were divided by a relevant assessment factor (AF), i.e. acute QSAR data=1000; acute experimental data= 100; chronic QSAR data=100, and chronic experimental data =10, (EC, 2003). The most sensitive endpoint was used for the generation of the PNEC where more than one ecotoxicological value was found. PNECs for earthworms were obtained by dividing the 14 d LC50 value by an AF of 1000. PNECs for mammals were obtained by dividing median lethal doses for mouse or rat by an AF of 100. PNECs for resistance were obtained from MSCs using an AF of 10. AFs were not used for the estimation of concentrations causing mode of action-based effects (using the human plasma therapeutic concentrations (H_TPC)) or for the MICs for microbes. Specific equations are provided in the Appendix A (Equations A.A7 – A.A12).

3.3 Results and Discussion

3.3.1 Experimental Data Availability

Experimental acute ecotoxicological data for only 51 of the 99 APIs under consideration were found in the literature. Chronic ecotoxicity endpoints were only available for 21 compounds so the ecotoxicity values of the others were estimated using the QSAR Toolbox and the ECOSAR software. In terms of data on mammalian safety, data were available on the toxicity of 72 compounds, 87 had an ADI and 88 had a H_TPC. Experimental bioconcentration factors in fish (BCF_{fish}) were only available for two compounds (diclofenac and naproxen). Experimental organic carbon partition coefficient (Koc) values were only available for 21 pharmaceuticals (Table 3.1).

Table 3.1 summary of experimental data available for the APIs under consideration

Parameter	Number of compounds
Excretion profile (Fex)	81
Log Kow	90
pKa	94
Experimental Koc	21
Experimental end point (acute LC(EC) 50)	51
Experimental end point (chronic LC(EC) 50)	21
Experimental Bioconcentration factor in fish	2
Acceptable daily intake (ADI)	87
Mammalian toxicity (LD50) for rat/mouse	72
Human therapeutic plasma concentration (H_TPC)	88

Fex: fraction of parent ingredients excreted unchanged via human metabolism; Log Kow: octanol-water partitioning coefficient; pKa: dissociation coefficient; Koc: organic carbon partition coefficient; EC50: 50% effective concentration; LC50: 50% lethal concentration; LD50: median lethal dose for rat/mouse; BCF: bioconcentration factor in fish; ADI: acceptable daily intake; H_TPC: human therapeutic plasma concentration.

3.3.2 RCR lists of APIs in different systems

The top ranked APIs with an RCR >0.1, derived from the different prioritisations for the aquatic environments in the three cities under consideration and at two dilution factors, are presented in Tables 3.2 and 3.3 for surface water and Table 3.4 for sediment. The compounds on the top of the prioritisation list with an RCR ≥1 according to PEC_{sw} and acute ecotoxicological endpoint were amoxicillin, azithromycin, cefalexine, valproic acid, erythromycin, paracetamol and clarithromycin in Mosul and Basrah. In Baghdad, only five compounds had an RCR ≥1 (amoxicillin, clarithromycin, azithromycin, valproic acid and paracetamol). This difference between the cities is due to the absence of wastewater treatment processes in Mosul and Basrah and hence that no removal of APIs by adsorption on sludge will occur in these cities. When chronic effects were considered, at the lower dilution factor, six compounds had RCR values ≥1 for all cities i.e. amoxicillin, clarithromycin, diclofenac, miconazole nitrate and mefenamic acid. At the higher dilution rate, only two compounds (amoxicillin and clarithromycin) had an RCR ≥1 (Table 3.3). All other pharmaceuticals had a risk score <0.1 (Table A.A3, Appendix A). When the potential impact of subtle pharmacological effects were considered by comparing the

human therapeutic concentration in plasma to estimated levels in fish plasma, using a dilution factor of 10, phenylephrine, atorvastatin and mebeverine showed RCR values >1 in all three cities. Additionally, amitriptyline and mefenamic acid had an RCR ≥1 In Mosul and Basrah. Using the higher dilution factor, only phenylephrine showed RCR >1 in Baghdad and Mosul whereas phenylephrine and atorvastatin exceeded an RCR of 1 in Basrah (Table 3.3).

Assessment of human exposure from consumption of fish products showed that phenylephrine and atorvastatin had an RCR >1 in all cities when a DF of 10 was used and only phenylephrine (RCR >1) when the DF of 40 was used. For human exposure via drinking water, tramadol HCL was the highest ranked compound (with an RCR between 0.1 and 1) while for the rest of pharmaceuticals the RCR was below 0.1.

The predicted concentrations for amoxicillin in all cities when DF= 10 were close to the MICs, and the resulting RCRs were between 1 to 10, suggesting that concentration could be high enough to inhibit growth of or kill bacteria. Amoxicillin and metronidazole were on the top list of antibiotics identified as a risk for selection for antimicrobial resistance (RCR >10), with a further seven APIs having RCR values between 1 and 10 (Tables 3.2 and 3.3).

The highest ranked APIs based on acute effect in sediment organisms were amoxicillin, erythromycin, azithromycin, ciprofloxacin, valproic acid and paracetamol in all cities with RCR >1 (Table 3.4). Ciprofloxacin dropped off the top priority list when a DF of 40 was applied in Mosul and Basrah, and paracetamol in Baghdad. The highest ranked compounds based on chronic endpoints were amoxicillin, clarithromycin, diclofenac, miconazole nitrate and mefenamic acid at DF= 10 and only amoxicillin showed RCR >10 in Basrah at DF= 40.

In soil, theophylline was ranked the highest priority based on the effect on lower trophic level organisms (earthworm). Based on the potential for secondary poisoning in the aquatic environment (i.e. risk to mammalian predators), only phenylephrine had an RCR >1 for all the city scenarios. For secondary poisoning in the terrestrial environments (i.e. earthworm eating birds and mammals), the highest ranked compound was atropine with an RCR between 0.1 and 1 (Table 3.5).

Table 3.2 Top ranked APIs with RCR>0.1 from each prioritisation approach for exposure via surface water at D=10

Location	RCR	Low levels trophic		Subtle effects on fish	Mammalian predator	Human (uptake from Fishery products)	Human (uptake from drinking water)	Effect of antibiotics on bacteria		
		Acute aquatic (PECsw: PNEC)	Chronic aquatic (PECsw: PNEC)	FSSPC: H_TPC	PECfish: PNEC mammal	PECfish:PNEC biota, hh	PECsw: PNEC dw, hh	PECsw:MIC	PECSW:MSC	PEC sw:PNEC resistance selection
		D10	D10	D10	D10	D10	D10/D40	D10	D10	D10
Baghdad	>10	Amoxicillin Clarithromycin	Amoxicillin Clarithromycin	Phenylephrine Atorvastatin Mebeverine Mefenamic	Phenylephrine	Phenylephrine				Amoxicillin Metronidazole
	1-≤10	Azithromycin Valproic acid Paracetamol	Diclofenac Miconazole nitrate Mefenamic acid		Mefenamic acid Miconazole nitrate	Atorvastatin		Amoxicillin	Amoxicillin Ceftriaxone Metronidazole	Trimethoprim Ceftriaxone Ampicillin Clarithromycin Cefalexine
	0.1-<1	Cefalexine Ciprofloxacin Miconazole nitrate Mefenamic acid Erythromycin Ibuprofen	Erythromycin Paracetamol Naproxen Azithromycin Mesalazine Mebeverine	Amitriptyline Metformin Miconazole nitrate	Valproic acid Diazepam Atorvastatin	Mefenamic acid Valproic acid Miconazole nitrate		Ceftriaxone Sodium Metronidazole Ampicillin	Clarithromycin Trimethoprim Cefalexine Ampicillin	Ciprofloxacin Azithromycin
Mosul	>10	Amoxicillin Azithromycin	Amoxicillin Clarithromycin	Phenylephrine Atorvastatin Mebeverine	Phenylephrine	Phenylephrine				Amoxicillin Metronidazole
	1-≤10	Ciprofloxacin Valproic acid Erythromycin Paracetamol Clarithromycin Cefalexine	Erythromycin Diclofenac Miconazole nitrate Mefenamic acid	Amitriptyline Mefenamic acid		Atorvastatin		Amoxicillin	Amoxicillin Ceftriaxone Metronidazole	Ciprofloxacin Trimethoprim Ceftriaxone Ampicillin Clarithromycin Cefalexine Erythromycin

Table 3.2 (Continue)

Location	RCR	Low levels trophic		Subtle effects on fish	Mammalian predator	Human (uptake from Fishery products)	Human (uptake from drinking water)	Effect of antibiotics on bacteria		
		Acute aquatic (PECsw: PNEC)	Chronic aquatic (PECsw: PNEC)	FSSPC: H_TPC	PECfish: PNEC mammal	PECfish:PNEC biota, hh	PECsw: PNEC dw, hh	PECsw:MIC	PECsw:MSC	PECsw:PNEC resistance selection
		D10	D10	D10	D10	D10	D10/D40	D10	D10	D10
	0.1-<1	Miconazole nitrate Mefenamic acid Ibuprofen Tetracycline Metronidazole Trimethoprim	Paracetamol Azithromycin Naproxen Mesalazine Mebeverine	Metformin Miconazole nitrate	Diazepam Atorvastatin Octreotide	Octreotide	Tramadol	Ceftriaxone Sodium Metronidazole Ciprofloxacin Ampicillin Trimethoprim	Ciprofloxacin Clarithromycin Trimethoprim Cefalexine Erythromycin Ampicillin	Azithromycin
Basrah	>10	Amoxicillin Azithromycin	Amoxicillin Clarithromycin Erythromycin	Phenylephrine Atorvastatin Mebeverine	Phenylephrine	Phenylephrine				Amoxicillin Metronidazole
	1-≤10	Ciprofloxacin Valproic acid Erythromycin Paracetamol Clarithromycin Cefalexine	Diclofenac Miconazole nitrate Mefenamic acid	Amitriptyline Mefenamic acid		Atorvastatin		Amoxicillin	Amoxicillin Ceftriaxone Metronidazole	Ciprofloxacin Trimethoprim Ceftriaxone Ampicillin Clarithromycin Cefalexine Erythromycin
	0.1-<1	Miconazole nitrate Mefenamic acid Ibuprofen Tetracycline Metronidazole Trimethoprim Atorvastatin	Paracetamol Azithromycin Naproxen Mesalazine Mebeverine	Metformin Miconazole nitrate Glibenclamide	Diazepam Atorvastatin Octreotide Miconazole nitrate Captopril	Octreotide Captopril	Tramadol	Ceftriaxone Metronidazole Ciprofloxacin Ampicillin Trimethoprim Erythromycin	Ciprofloxacin Clarithromycin Trimethoprim Cefalexine Erythromycin Ampicillin	Azithromycin

PECsw: predicted environmental concentration in surface water; FSSPC: fish steady-state plasma concentration; H_TPC: human plasma therapeutic concentration; PECFISH: predicted environmental concentration in fish; PNEC dw: predicted no-effect concentrations in drinking water; PNECaquatic/PNECmammal: predicted no-effect concentrations in aquatic and mammalian organisms; MIC: minimal inhibitory oncentration; MSC: minimal selective concentration; PNEC resistance selection: Predicted no effect concentrations for antimicrobial resistance; D: dilution factor.

Table 3.3 Top ranked APIs with RCR>0.1 from each prioritisation approach for exposure via surface water at D=40

		Low levels trophic		Subtle effects on fish	Mammalian predator	Human (uptake from Fishery products)	Human (uptake from drinking water)	Effect of antibiotics on bacteria		
		Acute aquatic	Chronic aquatic	FSSPC: H_TPC	PECfish: PNEC mammal	PECfish:PNEC biota, hh	(PECsw: PNEC dw, hh)	PECsw:MIC	PECsw:MSC	PECsw:PNEC resistance selection
Location	**RCR**	(PECsw: PNEC)	(PECsw: PNEC)							
		D40	**D40**	**D40**	**D40**	**D40**	**D10/D40**	**D40**	**D40**	**D40**
Baghdad	>10	Amoxicillin	Amoxicillin	Phenylephrine	Phenylephrine					Amoxicillin Metronidazole
	1-≤10	Clarithromycin Azithromycin Valproic acid	Clarithromycin	Atorvastatin Mebeverine		Phenylephrine			Amoxicillin	Trimethoprim Ceftriaxone Sodium Ampicillin Clarithromycin
	0.1-<1	Paracetamol Cefalexine Ciprofloxacin Miconazole nitrate Mefenamic acid	Diclofenac Miconazole nitrate Mefenamic acid Erythromycin Paracetamol	Mefenamic acid Amitriptyline	Mefenamic acid Miconazole nitrate	Atorvastatin		Amoxicillin	Ceftriaxone Metronidazole	Cefalexine Ciprofloxacin Azithromycin Ciprofloxacin
Mosul	>10	Amoxicillin	Amoxicillin	Phenylephrine	Phenylephrine	Phenylephrine				Amoxicillin Metronidazole
	1-≤10	Azithromycin Ciprofloxacin Valproic acid Erythromycin	Clarithromycin Erythromycin	Atorvastatin Mebeverine					Amoxicillin	Ciprofloxacin Trimethoprim Ceftriaxone Ampicillin Clarithromycin Cefalexine Erythromycin
	0.1-<1	Paracetamol Clarithromycin Cefalexine Miconazole nitrate Mefenamic acid	Diclofenac Miconazole nitrate Mefenamic acid Paracetamol Azithromycin Naproxen	Amitriptyline Mefenamic acid		Atorvastatin Octreotide	Tramadol	Amoxicillin Ceftriaxone	Ceftriaxone Metronidazole Ciprofloxacin	Azithromycin

Table 3.3 (Continue)

Location	RCR	Low levels trophic		Subtle effects on fish	Mammalian predator	Human (uptake from Fishery products)	Human (uptake from drinking water)	Effect of antibiotics on bacteria		
		Acute aquatic (PECsw: PNEC)	Chronic aquatic (PECsw: PNEC)	FSSPC: H_TPC	PECfish: PNEC mammal	PECfish:PNEC biota, hh	(PECsw: PNEC dw, hh)	PECsw:MIC	PECsw:MSC	PECsw:PNEC resistance selection
		D40	**D40**	**D40**	**D40**	**D40**	**D10/D40**	**D40**	**D40**	**D40**
Basrah	>10	Amoxicillin	Amoxicillin	Phenylephrine Atorvastatin	Phenylephrine	Phenylephrine				Amoxicillin
	1-≤10	Azithromycin Ciprofloxacin Valproic acid Erythromycin	Clarithromycin Erythromycin	Mebeverine				Amoxicillin	Amoxicillin	Metronidazole Ciprofloxacin Trimethoprim
	0.1-<1	Paracetamol Clarithromycin Miconazole nitrate Cefalexine Mefenamic acid Tetracycline Ibuprofen Diphenhydramine	Diclofenac Miconazole nitrate Mefenamic acid Paracetamol Azithromycin Naproxen	Amitriptyline Mefenamic acid Metformin	Diazepam	Atorvastatin Octreotide	Tramadol	Ceftriaxone Sodium Metronidazole	Ceftriaxone Metronidazole Ciprofloxacin	Ceftriaxone Ampicillin Clarithromycin Cefalexine Erythromycin Azithromycin

PECsw: predicted environmental concentration in surface water; FSSPC: fish steady-state plasma concentration; H_TPC: human plasma therapeutic concentration; PECFISH: predicted environmental concentration in fish; PNEC dw: predicted no-effect concentrations in drinking water; PNECaquatic/PNECmammal: predicted no-effect concentrations in aquatic and mammalian organisms; MIC: minimal inhibitory concentration; MSC: minimal selective concentration; PNEC resistance selection: Predicted no effect concentrations for antimicrobial resistance; D: dilution factor.

Table 3.4 Top ranked APIs with RCR>0.1 in the three cities (Baghdad, Mosul, Basrah) according to the predicted concentrations in sediment (PECsed) and at 10 and 40 dilution factors

	Baghdad				Mosul				Basrah			
	Acute aquatic (PECsed: acute PNECsed)		Chronic aquatic (PECsed: chronic PNECsed)		Acute aquatic (PECsed: acute PNECsed)		Chronic aquatic (PECsed: chronic PNECsed)		Acute aquatic (PECsed: acute PNECsed)		Chronic aquatic (PECsed: chronic PNECsed)	
RCR	**D10**	**D40**	**D10**	**D40**	**D10**	**D40**	**D10**	**D40**	**D10**	**D40**	**D10**	**D40**
>10	Amoxicillin				Amoxicillin Erythromycin Azithromycin Ciprofloxacin	Amoxicillin	Amoxicillin Clarithromycin		Amoxicillin Erythromycin Azithromycin Ciprofloxacin	Amoxicillin Erythromycin	Amoxicillin Clarithromycin	Amoxicillin
1-10	Erythromycin Azithromycin Valproic acid Paracetamol Ciprofloxacin	Amoxicillin Erythromycin Azithromycin Valproic acid	Amoxicillin Clarithromycin Diclofenac Miconazole nitrate Mefenamic acid	Amoxicillin Clarithromycin	Valproic acid Paracetamol Cefalexine	Azithromycin Erythromycin Ciprofloxacin Valproic acid	Erythromycin Diclofenac Miconazole nitrate Mefenamic acid	Amoxicillin Clarithromycin Erythromycin Diclofenac	Valproic acid Paracetamol Clarithromycin	Azithromycin Ciprofloxacin Valproic acid	Erythromycin Diclofenac Miconazole nitrate Mefenamic acid	Clarithromycin Erythromycin

Table 3.4 (Continued)

	Baghdad				Mosul				Basrah			
	Acute aquatic (PECsed: PNECsed)		Chronic aquatic (PECsed: chronic PNECsed)		Acute aquatic (PECsed: acute PNECsed)		Chronic aquatic (PECsed: chronic PNECsed)		Acute aquatic (PECsed: acute PNECsed)		Chronic aquatic (PECsed: chronic PNECsed)	
RCR	**D10**	**D40**	**D10**	**D40**	**D10**	**D40**	**D10**	**D40**	**D10**	**D40**	**D10**	**D40**
0.1-<1	Cefalexine Miconazole nitrate Clarithromycin Mefenamic acid Ibuprofen Metronidazole Erythromycin	Paracetamol Ciprofloxacin Cefalexine Miconazole nitrate Clarithromycin Mefenamic acid	Paracetamol Naproxen Erythromycin Azithromycin Mesalazine Mebeverine	Diclofenac Miconazole nitrate Mefenamic acid Paracetamol	Miconazole nitrate Clarithromycin Mefenamic acid Ibuprofen Tetracycline Metronidazole Trimethoprim	Paracetamol Cefalexine Miconazole nitrate Clarithromycin Mefenamic acid	Paracetamol Azithromycin Naproxen Mesalazine Mebeverine	Miconazole nitrate Mefenamic acid Paracetamol Azithromycin Naproxen	Cefalexine Miconazole nitrate Mefenamic acid Ibuprofen Metronidazole	Paracetamol Clarithromycin Cefalexine Miconazole nitrate Mefenamic acid	Paracetamol Naproxen Azithromycin Mesalazine Mebeverine	Diclofenac Miconazole nitrate Mefenamic acid Paracetamol

PECsed: predicted environmental concentration in sediment; PNECsed: Predicted no effect concentrations in sediment; D: Dilution factor. The PECsed and PNECsed were calculated with the Equilibrium Partitioning method from the PECsw and PNECsw respectively

Table 3.5 Top 20 compounds from each prioritisation approach considered (Baghdad only), according to the predicted concentrations in soil (PECsoil)

RCR	Low levels trophic	Higher trophic levels Mammalian predator	
	PEC_{soil}: $PNEC_{worm}$	**$PEC_{earthworm}$: $PNEC_{mammal}$**	**$PEC_{earthworm}$: ADI**
>10			
1-10	1 Theophylline 2 Omeprazole 3 Olanzapine		
0.1-<1	4 Fluoxetine 5 Atropine sulphate 6 Guaifenesin 7 Ciprofloxacin 8 Phenylephrine 9 Metoprolol 10 Mefenamic acid 11 Octreotide 12 Procyclidine 13 Valproic acid 14 Dextromethorphan Hydrobromide 15 Pethidine	1 Atropine sulphate	

Table 3.5 (Continued)

RCR	Low levels trophic	Higher trophic levels Mammalian predator	
	PECsoil: PNECworm	**PECearthworm: PNECmammal**	**PECearthworm: ADI**
<0.1	16 Diphenhydramine	2 Procyclidine	1 Atropine sulphate
	17 Sitagliptin	3 Olanzapine	2 Olanzapine
	18 Flutamide	4 Diazepam	3 Omeprazole
	19 Trifluoperazine	5 Metoclopramide	4 Octreotide
	20 Fluovastatin	6 Octreotide	5 Procyclidine
		7 Omeprazole	6 Metoprolol
		8 Sitagliptin	7 Escitalopram oxalate
		9 Guaifenesin	8 Sitagliptin
		10 Dextromethorphan Hydrobromide	9 Thyroxine sodium
		11 Diphenhydramine	10 Ranitidine
		12 Metoprolol	11 Guaifenesin
		13 Ranitidine	12 Dextromethorphan Hydrobromide
		14 Chlorphenamine Maleate	13 Trifluoperazine
		15 Ciprofloxacin	14 Ketotifen
		16 Theophylline	15 Letrozole
		17 Pseudoephedrine	16 Midazolam
		18 Pethidine	17 Metoclopramide
		19 Neostigmine	18 Infliximab
		20 Escitalopram oxalate	19 Pseudoephedrine
			20 Bromhexine

PECsoil, PECearthworm: predicted environmental concentrations in in soil and earthworm; ADI: acceptable daily intake; PNECmammal, PNECworm: predicted no-effect concentrations in mammals and in worm.

3.3.3 Comparison of ranking outcomes

Generally, the outcome of the risk-based prioritisation showed that the majority of the top ranked pharmaceuticals were antibiotics. Based on all risk comparisons, a final list of 23 compounds (amoxicillin, amitriptyline, ampicillin, atorvastatin, azithromycin, cefalexine, ceftriaxone sodium, ciprofloxacin, clarithromycin, diclofenac, erythromycin, ibuprofen, valproic acid, mebeverine, mefenamic acid, metronidazole, miconazole nitrate, olanzapine, omeprazole paracetamol, phenylephrine, theophylline, trimethoprim) which had an RCR >1 for at least one endpoint or compartment was generated. Interestingly, the results of the current prioritisation approach agreed with previously published prioritisation studies from other countries. Amoxicillin, the compound with the highest score in this study, was also ranked the top veterinary medicine with high hazard to aquatic organisms in the UK and Korea (Boxall et al., 2003; Kim et al., 2008). Clarithromycin and azithromycin were found alongside amoxicillin on the top priority list in France (Besse and Garric, 2008). Paracetamol, amoxicillin and azithromycin were ranked as highly prescribed pharmaceuticals of concern in the USA whereas ciprofloxacin was identified as posing a risk toward aquatic organisms and humans (Dong et al., 2013). Paracetamol, mefenamic acid, amoxicillin, ciprofloxacin, erythromycin and valproic acid were prioritised as highest environmental risk in Switzerland (Perazzolo et al., 2010). A prioritisation study performed by Roos et al., (2012) showed amitriptyline, paracetamol, diclofenac and valproic acid to be the highest ranked compounds in one or more comparison studies in Sweden while no antibiotics from this study were found in the ranking lists. Paracetamol ranked the 2nd in terms of usage volume in Sweden while in Iraq it was found to be the 1st on the prioritisation list. Diclofenac showed a risk score of 0.013 which is equal to the one reported in the UK by Ashton et al., (2004). On the other hand, this compound showed a higher risk score (1–10) in Iraq when chronic ecotoxicological endpoints were used. A recent risk-based prioritisation study in the UK has shown most of the antibiotics in our list (amoxicillin, azithromycin, ciprofloxacin, clarithromycin and atorvastatin) to have risk scores greater than 1 in one or more of the risk comparisons proposed (Guo et al., 2016). Amitriptyline was ranked as a high priority compound when the potential impact of subtle pharmacological

effects was considered by comparing the H_TPC to estimated levels in fish in the same study. Miconazole was ranked as one of the priority substances used as herd treatment that is moderately used and metabolized (Boxall et al., 2003). It was also found on the top ranking list of pharmaceuticals according to the fish plasma model (Roos et al., 2012). Theophylline showed low risk score in aquatic systems, and this agrees with a ranking score of 0.015 in surface water reported by Huschek et al., (2004); while in this study the RCR of theophylline toward terrestrial lower trophic levels was >1 followed by omeprazole and olanzapine. Omeprazole was ranked the 19th and 22nd in terms of number of prescribed pharmaceuticals in the prioritisation studies in the USA and Sweden (Dong et al., 2013; Roos et al., 2012). No previous prioritisation study has ranked phenylephrine as a compound of concern. To our knowledge and after reviewing the literature, antibiotics have not been previously prioritised in surface water in terms of their impacts on bacterial community or the susceptibility to pose bacterial resistance.

3.3.4 Pharmaceuticals of Concern on the Top of Priority lists

Antibiotics are often ranked as the highest priority compounds in risk characterization exercises. Recently, the awareness of the risks of antibiotics in the environment has been raised. For example, the European Environmental Quality Directive has added four antibiotics to the watch list of the Water Framework Directive (Carvalho et al., 2015). All of the added antibiotics (azithromycin, erythromycin, ciprofloxacin and clarithromycin) are ranked as high risk compounds in our priority list. Antibiotics are structurally diverse and do not share a common mode of action (Sanderson et al., 2004), and very low concentrations of antibiotics can be considered extremely harmful to organisms and high concentrations of antibiotics in sediment inhibit the growth of bacteria (Kümmerer, 2009b, 2009c).

The occurrence and diverse effects of some of the highly ranked APIs have been reported. Although ciprofloxacin, a fluoroquinolone antibiotic, is highly removed in WWTPs, a concentration of 3.8 μg L^{-1} was detected in wastewater effluent in Australia (Watkinson et al.,

2007) and much higher concentrations of 6.5 and 14.0 mg L^{-1} from two lakes and pharmaceutical production effluent in India, respectively. Ciprofloxacin showed luminescence inhibition to *Vibrio fisheri* at 5 mg L^{-1} of 30-min EC50 (Hernando et al., 2007) and shows high toxicity toward cyanobacteria (*Microcystis aeruginosa*) with an EC50 of 0.005 mg L^{-1} and growth inhibition as the endpoint (Halling-Sørensen et al., 2000). In a recent study, ciprofloxacin exposure resulted in growth inhibition of algae (*Pseudokirchneriella subcapitata*) at a 96-h EC50 of 4.83 mg L^{-1} (Martins et al., 2012). Erythromycin is frequently detected in waterbodies around the world with concentrations between 0.13 and 0.89 μg L^{-1} (Hernando et al., 2006; Meinertz et al., 2011). It was found to be toxic to algae using chronic tests with a reported EC50 between 0.01 and 0.1 mg L^{-1} while ecotoxicological results showed that acute toxicity values were in the range of 10–30 mg L^{-1} for algae, daphnia and bacteria (Isidori et al., 2005). Clarithromycin, a derivative of erythromycin, was detected in concentrations between 0.01 and 0.54 μg L^{-1} in different countries and has been shown to inhibit the growth of algae and cyanobacteria with EC50 values of 0.0371 and 0.0121 mg L^{-1}, respectively (Baumann et al., 2015). The PEC_{sw} of amoxicillin, a β-lactam antibiotic, in Iraqi cities was very high and ranged from 0.6 to 24.0 μg L^{-1}. This concentration is extremely high in comparison to levels <0.001 μg L^{-1} detected in other countries such as in Italy (Castiglioni et al., 2004). It shows high toxicity to blue-green algae (cyanobacteria) with a reported 96-h EC50 of 0.00222 mg L^{-1} (Fass.se) and is known to cause hepatocyte cytotoxicity as a side effect in rainbow trout with a 24-h EC50 >182.7 mg L^{-1} (Laville et al., 2004).

In addition to direct toxicological risks, the occurrence of antibiotics raises concerns in terms of the promotion of antibiotic resistance in bacteria in environment, which could subsequently make antibiotics ineffective in terms of treatment for both humans and animals since aquatic ecosystems are a recognized reservoir for antibiotic-resistant bacteria (Ågerstrand et al., 2015; Kostich et al., 2014; Santos et al., 2010). Interestingly, the occurrence of antibiotic resistance in the environment is not on the main list of priorities that should be addressed by guidelines for the environmental risk assessment of medicinal products for both human and veterinary use in the European Union [European Medicines Agency (EMEA) 2006; 2008]. Studies from different

parts of the world have highlighted the fact that resistant strains of bacteria occur in the environment. For example, in Slovakia, the occurrence of resistance to different antibiotics (erythromycin, clarithromycin, azithromycin, ciprofloxacin, trimethoprim) in coliforms and streptococci from WWTPs sludge was studied (Birošová et al., 2014). In Canada, isolated *Escherichia coli* retrieved from different sites and aquatic ecosystem compartments (biofilms, sediment and water) showed high frequency of resistance to ampicillin and ciprofloxacin (Maal-Bared et al., 2013). In Brazil, three strains of *Salmonella* from water samples of a shrimp farm exhibited multiresistance to ampicillin, tetracycline, oxytetracycline and nitrofurantoin (Carvalho et al., 2013). Recently, a study of tetracyclines, sulfonamides and (fluoro)quinolones in sediment and water samples in Guangdong, China, indicated that fish ponds are reservoirs of antimicrobial resistance genes and the presence of potential resistant and pathogen-associated taxonomic groups in fish ponds might imply the potential risk to human health (Xiong et al., 2015).

Two non-steroidal anti-inflammatory drugs (NSAIDs) were identified as high priority APIs i.e. diclofenac and mefenamic acid. In 2013, the European Directive identified diclofenac, alongside two synthetic hormones, as pollutants that should be included in the Water Framework Directive Watch List (Carvalho et al., 2015). van den Brandhof and Montforts, (2010) reported the effect of diclofenac on growth retardation in zebrafish after exposure to concentrations >1.5 mg L^{-1}. Hoeger et al., (2005) and Schwaiger et al., (2004) documented that diclofenac has the potential to cause histopathological damage to tissues (kidney) in fish at concentrations close to those regularly found in surface waters. Mefenamic acid showed a maximum PEC_{sw} (1.2 µg L^{-1}) which is higher than the reported levels (0.20-0.34 µg L^{-1}) in the UK by Roberts and Thomas, (2006). Ecotoxicological effect of mefenamic acid in chronic toxicity tests to *Daphnia magna* and *Moina macrocopa* showed significant changes in reproduction (number of young per adult) after exposure to 1.0 and 0.25 mg L^{-1} of mefenamic acid, respectively (Jung Collard et al., 2013). The top used compound in Iraq is paracetamol. In our study, the maximum PEC_{sw} for paracetamol in Iraqi cities was 23.99 µg L^{-1} in Basrah which is two times higher than the concentration obtained from a study by Jones et al., (2002) who found the maximum PEC in

English rivers to be 11.96 μg L^{-1} and more than two orders of magnitude higher than the concentration of 0.11 μg L^{-1} which was detected in 24 % of the rivers in the USA (Kolpin et al., 2002). In terms of ecotoxicological effect, Galus et al., (2013) found that embryonic mortality of zebrafish was raised after exposure to paracetamol at the level of ≥ 0.5 μg L^{-1}. Very limited studies have been performed on ecotoxicity of valproic acid toward environmental organisms. Herrmann, (1993) carried out a pre-screen test to investigate the possible hazard posed to humans using zebra fish exposure to valproic acid and revealed that exposure caused retardation and interruption of development. The cholesterol lowering agent atorvastatin was reported to affect lemna (*Lemna gibba*) by decreasing pigment content at EC50 of 0.17 mg L^{-1} (Brain et al. 2004). It was also found to inhibit growth of *Hyalella azteca* with LC50 values ranging from 1.30 to 3.56 mg L^{-1} and *Chironomus tentans* with LC50 values ranging from 3.94 to 16.42 mg L^{-1} (Dussault et al., 2008). Amitriptyline was identified as a high priority list due to its potential to elicit subtle effect in fish in the current study. It has previously been reported to pose a risk to surface waters and shows toxicity to fish and daphnia, EC50= 0.78 mg L^{-1} (Kasprzyk-Hordern, 2010). In lower trophic groups, amitriptyline was reported to inhibit the growth of the macrophyte *Lemna minor* with a 7-day EC50 of 1.69 mg L^{-1} (Ågerstrand and Rudén, 2010).

Ibuprofen is predicted to occur in Iraqi surface water at concentrations of 0.13–0.8 μg L^{-1} and sediment at concentrations of 3.0–20 μg Kg^{-1}. The log Kow of 3.73 and low solubility suggest the low mobility of ibuprofen in water and affinity to adsorb to sediment (Bouissou-Schurtz et al., 2014). Ibuprofen was detected at a concentration of 1.3 μg L^{-1} in surface water in Switzerland (Tixier et al., 2003) and 0.15–3.96 μg L^{-1} in the influent and effluent wastewater in Sweden (Bendz et al., 2005). It was found that exposure to chronic low levels of ibuprofen alters the pattern of reproduction of Japanese medaka, *Oryzias latipes*, and may produce sex-specific responses in *teleosts* (Flippin et al., 2007). Ibuprofen, at a concentration slightly higher than 0.2 μg L^{-1}, is able to significantly increase both genetic and cellular damage in freshwater bivalve *Dreissena polymorpha* (Parolini et al., 2011).

3.3.5 Limitation of the Methods

Knowledge about usage data is essential to establish a priority list of pharmaceuticals of most concern. In Iraq, it was difficult to obtain the consumption amount of all pharmaceuticals from the ministry of health list due to absence of a governmental statistical data and it is sometimes considered confidential. Moreover, it was not possible to quantify the usage data of over-the-counter (OTC) pharmaceuticals. Therefore, an accurate quantification approach of OTC usage should be a future priority. The project did not consider veterinary pharmaceuticals, but this use pattern could also be an important contributor to the environment, particularly for antibiotic compounds.

Another restraint which increases the uncertainty is the limited availability of ecotoxicological endpoints and the high dependence on the prediction of effects and properties. For example, the practice of using ECOSAR to extrapolate ecotoxicity data may not be appropriate since this software was developed to assess toxicity of compounds other than pharmaceuticals. Physicochemical properties were also limited; for instance, Koc, which was used to estimate adsorption during wastewater treatment, was calculated by an empirical estimation model (Franco and Trapp, 2008) due to absence of experimental values for all the pharmaceuticals on the list. Moreover, bioconcentration factors for worm (BCF_{worm}) were predicted according to the TGD (EC, 2003) to allow the secondary poisoning assessment of pharmaceuticals in the terrestrial compartment due to limited availability of experimental data. This estimation is usually conservative. Therefore, an improvement in the accuracy of BCF_{worm} estimation in soil warrants further consideration.

3.5 Conclusion

An approach has been developed for prioritising substances that may pose a risk to the aquatic and terrestrial systems in Iraq. Pharmaceutical usage data has been used together with information on the physicochemical properties, patient metabolism and wastewater treatment removal in this practice to predict API concentrations. The ranking has been performed by comparing these concentrations to a range of experimental and estimated ecotoxicological

endpoints including nonstandard endpoints such as the potential for subtle pharmacological effects, secondary poisoning and the impact on human *via* consuming fishery products and drinking water. Dilution factor was found to play an important role to reduce the risk suspected to be posed toward environmental organisms by pharmaceuticals, and results of this study showed that the release of pharmaceuticals to the aquatic environment represents a significant environmental threat, especially when DF is low.

Twenty-three APIs including antibiotics, analgesics, antiepileptics, anti-hypercholesterolemia and anti-asthma have been identified as high priority substances. The study indicates that antibiotics are the pharmaceutical class of most concern with annual consumption of these molecules in Iraq up to 420 t $year^{-1}$. Risks of pharmaceutical compounds in drinking water to human health are low with the exception of tramadol when no WWTP connectivity exists. Large numbers of pharmaceuticals considered in this study could be removed during wastewater treatment, and their risk towards environment will be highly reduced when a proper removal mechanism is used, but in our study case, the removal by this method is neglected due to the absence or inefficient operation of WWTPs in some regions of Iraq. Further evaluation is recommended to assess whether these compounds could indeed pose a risk to the environment as individuals or in a mixture since a broad range of different substances is used simultaneously by humans in any given area.

The results from this risk-based prioritisation study and those in the literature regarding the risk of pharmaceuticals in environment in UK (Guo et al., 2016) were used to select compounds for further study in the following chapters. Exposure predicted concentration in aquatic phase and sediment, potential risk to sediment-dwelling organisms in addition to physicochemical properties were the main selection criteria. Antibiotics fate and occurrence in sediment environment were heavily investigated (Chapter 2); therefore, they were excluded from the list of pharmaceuticals for further investigations except trimethoprim.

Chapter 4

Determination of pharmaceuticals in freshwater sediments using ultrasonic-assisted extraction with SPE clean-up and HPLC–DAD or LC-ESI-MS/MS detection

4.1 Introduction

In the last decade, the analysis of pharmaceutical residues in the environment has attracted significant scientific attention due to the potential risks that these compounds pose to ecosystems and human health (Buchberger, 2007; Carvalho et al., 2013; Darwano et al., 2014). Pharmaceuticals can enter wastewater systems and pass through wastewater treatment plants (WWTPs) into the natural environment where they can reach detectable concentrations (Bossio et al., 2008). Some of these compounds have the ability to partition to environmental solid phases (e.g. sediment and soil) (Minten et al., 2011). In order to understand the occurrence, fate and effects of these trace level contaminants, multi-residue, accurate, sensitive and powerful techniques such as liquid chromatography (LC) and gas chromatography (GC) are needed (Batt et al., 2008; Hao et al., 2007). However, most of the available analytical techniques have been developed for the determination of these trace of contaminants in the dissolved phase (Minten et al., 2011); and most monitoring studies have focused on detecting pharmaceuticals in surface water (Cahill et al., 2004; Madureira et al., 2010; Patrolecco et al., 2013) and wastewater (Benito-Peña et al., 2006; Paíga et al., 2017; Yuan et al., 2013). Fewer analytical methods are available for soil (Aznar et al., 2014; Blackwell et al., 2004; Xu et al., 2008) and sediment (Azzouz and Ballesteros, 2012; Löffler and Ternes, 2003).

The complexity of an environmental matrix can stifle the analysis of a pharmaceutical. Up to 90% of the analysis time can be spent on sample preparation and thus, great effort goes into the development of reliable sample preparation procedures which are as simple as possible in terms of operation and which minimise the number of steps in the analytical process (Zuloaga et al.,

2012). For the analysis of pharmaceuticals in environmental solids, sample pre-treatment steps typically include extraction and analyte enrichment and clean-up, as these processes are essential and provide the opportunity to quantify many pharmaceutical compounds down to ng Kg^{-1} concentrations (Díaz-Cruz et al., 2003; Kostopoulou and Nikolaou, 2008).

A variety of extraction procedures has been reported for organic pollutants, including pharmaceuticals, from solid environmental matrices such as sediment including methods based on microwave assisted extraction (MAE) (Azzouz and Ballesteros, 2012; Varga et al., 2010) and pressurized liquid extraction (PLE - which is also commonly known as accelerated solvent extraction (ASE)) (Zuloaga et al., 2012). For example, the use of ASE for extraction of pharmaceuticals from sediment results in recoveries greater than 116% (Dussault et al., 2009b; Langford et al., 2011; Li et al., 2012). Although these methods are comprehensive, use minimal amounts of solvent and reduce the processing time (Pérez-Carrera et al., 2010), they are considered less popular because the instruments themselves may be complicated to use and expensive (Blackwell et al., 2004; Chen et al., 2015). Therefore, ultrasonic-assisted extraction (UAE) is a frequently applied alternative technique for the extraction of pharmaceuticals from sediment (Zuloaga et al., 2012). The short extraction time and low solvent consumption of UAE, as well as its robustness, lower cost and ease of use, are some of the advantages of this extraction technique (Aznar et al., 2014; Chen et al., 2015; Darwano et al., 2014; Duan et al., 2013; Zhou et al., 2011).

The United States Environmental Agency (USEPA) published an analytical reference method (1694) based on UAE, involving two different extraction methods under acidic and basic conditions, for the determination of pharmaceuticals in environmental compartment including sediment (Method 1694 : 2007). Recently, Chen and Zhou, (2014) applied the UAE technique prior to UHPLC–MS/MS to investigate the occurrence and behaviour of 20 antibiotics from five classes in sediments from the Huangpu River, China. The method produced recoveries ranging from 44% to 141% for the targeted compounds. Lei et al., (2009) determined the concentrations of six estrogens in sediment and generally showed recoveries higher than 79%, the exception being estriol (E3) which showed a recovery of 66%. Martín et al., (2010) investigated the

occurrence of pharmaceuticals in sediment and sludge using UAE prior to HPLC–DAD and fluorescence (Fl) analysis. The pharmaceutical compounds evaluated were nonsteroidal anti-inflammatory drugs, antibiotics, an anti-epileptic drug, a β-blocker, a nervous system stimulant, estrogens and lipid regulators with recoveries ranging from 58.4 to 103% except for acetaminophen which showed a recovery <15%. More recently, de Sousa et al., (2015) developed a UAE method for the simultaneous determination of hormones and pharmaceuticals from different therapeutic classes in sediment. The highest recovery was 120% for 17-β-estradiol at a concentration of 5 ng g^{-1} while the lowest recovery was 54% for propranolol at a concentration of 50 ng g^{-1}.

The use of solid-phase extraction (SPE) as a clean-up and analyte enrichment step prior to analysis also has a positive influence on the recovery of targeted compounds since the extraction steps described above are not selective (Chen et al., 2015; Zuloaga et al., 2012). SPE cartridges such as hydrophilic–lipophilic balance (HLB) and Strong anion exchange (SAX) cartridges have been used extensively in the clean-up of extracts from sediment samples. For instance, Zhou et al., (2012) and Vazquez-Roig et al., (2010) used tandem SAX–HLB cartridges to reduce the matrix effects of complex co-extracted components for pharmaceutical determination in sediment samples. The SAX column retained humic material and the HLB column retained the pharmaceuticals. SPE using HLB cartridges was employed by Chen and Zhou, (2014) when they studied the occurrence and behaviour of pharmaceuticals in sediment. HLB cartridges were also used by de Sousa et al., (2015) for the clean-up and pre-concentration of pharmaceuticals extracted from sediment. Maximum obtained recoveries (54.1-156.0%) were seen at pH=9 and using 2 × 3 mL of methanol (MeOH) and 3mL of acetone for elution. The use of a tandem moderate anion exchange cartridge (MAX) and HLB was found to be the optimum method for pre-concentration and purification of 32 pharmaceuticals in sediment extracts (Pérez-Carrera et al., 2010). Highest recoveries were obtained with ethyl acetate, MeOH and MeOH containing 2% acetic acid as elution solvent for MAX cartridges while ethyl acetate and MeOH were found to be the most effective eluents for the HLB cartridges.

It is noteworthy that the published methods for analysis of pharmaceuticals in sediments have dealt with compounds from only a limited number of classes with a limited range of physicochemical properties. Moreover, individual methods have tended to focus on only a few sediment types so the applicability of the methods to sediments more generally is unknown. The development of a robust, low cost and easy to use method like UAE capable of simultaneously extracting pharmaceuticals from different classes from sediments with varying characteristics is therefore highly warranted.

The aim of the work described in this chapter was to develop a rapid and simple method to simultaneously extract pharmaceuticals from different pharmaceutical classes (anti-depressants, anti-ulcer medicines, β-blockers, calcium channel blockers and nonsteroidal anti-inflammatory drugs (NSAID)) with different physicochemical characteristics (such as polarity and pKa) from a range of sediments. The developed methods combine the simplicity of UAE and the efficiency of clean-up and sample enrichment of SPE followed by detection and quantification using either the highly readily HPLC–DAD technique or the highly sensitive LC-MS/MS method. The influences of sonication time, shaking time, solvent type and pH on extraction efficiency were evaluated, as was the type of solvent used in the SPE/clean-up steps (conditioning, washing and sample elution). We believe that this method will be invaluable for use in future experimental fate studies and environmental monitoring programmes.

4.2 Materials and Methods

4.2.1 Chemicals and Reagents

Pharmaceutical standards (amitriptyline hydrochloride, atenolol, cimetidine, diltiazem hydrochloride, mefenamic acid and ranitidine) were purchased from Sigma–Aldrich (UK). All pharmaceutical standards were 98–99% pure. CAS registry numbers, therapeutic class and physicochemical properties of the study compounds are detailed in Chapter 1 in Table 1.1. The solvents used (acetonitrile (ACN), MeOH and acetone) were of high-performance liquid chromatography (HPLC) grade and were obtained from Fisher Scientific (UK). Ammonium

hydroxide solution (NH_4OH, 35%), ammonium acetate and citric acid were also obtained from Fisher Scientific. Formic acid (96 %) was obtained from Sigma-Aldrich (UK). Nitrogen, 99.9% pure, used for drying, was supplied by the University of Leeds (UK). Ultrapure water (18.2 MΩ.cm) was obtained from a Milli-Q device manufactured by ELGALabWater (UK). Stock solutions of 1000 mg L^{-1} were prepared in MeOH for each pharmaceutical. Working standards solutions were then prepared from the stock solution by serial dilution with MeOH and water (20:80) and kept in the dark at 4 °C until use.

4.2.2 Sediment Collection and Characterisation

Samples of river sediment were collected from different sampling sites: two from Iraq (Baghdad and Karbala) and eight from around the Yorkshire and Leicestershire regions in England, UK. The sediments had different textures and organic carbon content (OC) and were selected in order to provide real environmental matrices for method development and validation and for the use in the fate studies which are described in Chapters 5 and 6 (Table 4.1). Sediments were collected from remote and sparsely urbanized areas expecting to be less affected by pharmaceutical contamination sources (e.g. discharges of WWTPs and hospitals). Sediments were sampled from the top 0-5 cm surface layer using a pre-cleaned stainless steel spade and placed along with overlying water into 1 L amber glass bottles, which had been cleaned with acetone, deionized water and then dried. Following collection, sediments were transferred to the laboratory, where plant residues and debris were removed manually. The wet slurry was then sieved to 2 mm, homogenized and stored at 4^{o}C for less than a month prior to the study. For characterisation, the sediments were subjected to granulometric analysis to determine the texture using a Malvern laser granulometer (Hydro 2000MU, UK); the OC in the sediments was measured according to the ISO10694 protocol using a total carbon content analyser (Viro Macro Elemental (CN) Analyser, Germany). Sediment pH values in 0.01 M $CaCl_2$ were determined using a sediment to solution ratio of 1:5. Cation exchange capacity and exchangeable metals were analysed by Forest Research UK following the ISO 11260 & 14254 protocols using a dual view ICP-OES (Thermo iCAP 6500 duo).

Table 4.1 Measured properties of the study sediments used in the analytical method development studies

Sediment	Coordinate	Texture	Silt %	Clay %	Sand%	OC %	pH $CaCl_2$	CEC	Total AL^{3+} mg/Kg	Total Fe^{2+} mg/Kg	Total Ca^{2+} mg/Kg	Total K^{+} mg/Kg	Total Mg^{2+} mg/Kg	Total Na^{+} mg/Kg
									Ex. AL^{3+} (cmol+/kg)	Ex. Fe^{2+} (cmol+/kg)	Ex. Ca^{2+} (cmol+/kg)	Ex. K^{+} (cmol+/kg)	Ex. Mg^{2+} (cmol+/kg)	Ex. Na^{+} (cmol+/kg)
Buttercrambe (BTC), YO, UK	54.017012, -0.881074	Sandy loam	35.48	34.25	62.92	2.83	6.88	13.45	405.7	697.3	423.6	44.1	86.5	0.07
									0.016	0.001	12.72	0.140	0.413	0.112
Bishop Wilton (BW), YO, UK	53.982712, -0.790092	Loam	45.92	4.73	49.35	9.9	8.1	35.58	979.2	1130.4	2227.4	196.8	662.9	10.7
									0.025	0.001	32.49	0.658	1.847	0.200
Millington (MIL), YO, UK	53.964920, -0.719305	Sandy clay	0.88	37.25	61.87	8.02	7.15	37.08	972.1	1400.6	975.3	88.6	134.2	3.0
									0.026	0.002	34.53	0.379	1.592	0.376
German beck (GER),YO, UK	53.935850, -1.054470	Sandy clay loam	1.22	30.97	67.81	5.69	7.1	24.26	635.3	1252.0	825.6	86.3	306.9	8.4
									0.020	0.002	19.90	0.283	2.446	0.336
Helmsley (HLM), YO, UK	54.242978, -1.055166	Sandy	10.08	0.12	89.8	0.98	6.65	5.85	299.6	1307.5	215.2	27.1	40.7	0.0
									0.013	0.001	5.05	0.079	0.303	0.067
Moors (MOR), YO, UK	54.371324, -0.965524	Loamy sand	21.05	0.35	78.6	3.52	6.35	11.26	510.5	1367.6	490.1	32.5	101.2	2.2
									0.017	0.002	8.89	0.173	1.181	0.160
Harborough (HAB), LT, UK	52.626226, -0.890155	Loamy sand	26.7	1.12	72.18	1.12	7.45	11.34	753.9	3706.1	682.1	62.9	116.3	1.9
									0.015	0.001	10.58	0.170	0.422	0.146
Skeffington (SKF), LT, UK	52.620847, -0.905779	Sandy clay loam	0.38	36.52	63.1	7.92	7.02	28.39	662.8	827.3	2113.5	79.2	365.9	5.2
									0.123	0.021	27.18	0.195	0.595	0.123
Tigris River (BGD), Baghdad, Iraq	33.361904, 44.370943	Silt loam	58.15	2.04	39.81	3.42	7.1	12.99	973.5	1204.1	2374.4	94.3	923.4	9.7
									0.015	0.001	10.34	0.262	2.006	0.355
Alhussainya River (HUS), Karbala, Iraq	32.623024, 44.027632	Silt loam	71.15	2.91	25.94	3.51	7.3	19.07	1270.3	1884.5	2245.5	116.8	1170.6	34.5
									0.018	0.001	13.46	0.430	3.768	1.389

4.2.3 Extraction of Pharmaceuticals from Sediment

Ultrasonic extraction was used for the extraction of the study pharmaceuticals from sediment using a Grant XUBA3 ultrasonic water bath (65 W, 35 kHz) using three extraction cycles. A mass of 5 g of sediment (dry weight) was weighed into a 50 mL centrifuge tube. In the first cycle, 10 mL of 2% NH_4OH in MeOH was added and the mixture was then vortexed for 15 seconds. The slurry was then ultra-sonicated for 15 min and then agitated at 250 rpm for 10 minutes (Grant bio PSU-20i, UK). Afterward, the slurry sample was centrifuged at 4500 rpm for 10 min. The supernatant was filtered through a 0.45 μm nylon filter and then decanted into a 500-mL Erlenmeyer flask. The sediment residue was then further extracted with 10 mL of 2% formic acid in MeOH in the second cycle and then with only 5 mL of MeOH in the final cycle. The supernatants from the three steps were then combined. The MeOH was allowed to evaporate overnight, after which the extracts were filtered through GF/F glass microfiber filters from Whatman Int. (Maidstone, UK) by suction into Erlenmeyer flasks and diluted with Milli-Q water to give a total volume of 400 mL (MeOH < 5%).

4.2.4 SPE/Clean-up

The diluted sediment extracts were adjusted to pH=10 using NH_4OH solution prior to solid phase extraction (SPE). The SPE was conducted on 6-mL (200 mg) Oasis HLB SPE cartridges (Waters, UK). The SPE cartridges were preconditioned using 5 mL of MeOH followed by 10 mL of Milli-Q water. Diluted aqueous extract samples were loaded onto the SPE cartridge at a rate of 10-20 mL min^{-1} and passed through the cartridges under vacuum (Supelco VisiprepTM, UK). Cartridges were then rinsed with 10 mL of 5% MeOH in Milli-Q water and then dried under air for 30 minutes. Finally, cartridges were eluted with 2×2.5 mL MeOH followed by 1 mL of 2% NH_4OH in MeOH. The eluates were dried under a gentle nitrogen stream using a DB-3A, TECHNE (UK) concentrator at 30 ^oC. The extract was reconstituted into 1.0 mL of water: MeOH (20:80) and then sonicated for 1 minute and filtered through a 0.22 μm nylon filter. The samples were then stored in a freezer at -20 ^oC prior to HPLC-DAD or LC/MS/MS analysis.

4.2.5 Instrumental Analysis

4.2.5.1 HPLC-DAD Analysis

HPLC, coupled with diode array detector (DAD), analysis of cleaned up extracts was performed using a Perkin Elmer, Flexar system. A reversed phase C18 analytical column of 150 mm × 4.6 mm, 5.0 μm (Zorbax Eclipse XDB-C18) was used for separation and quantification. A Zorbax Eclipse XDB-C18 4.6 mm × 12.5 mm, 5.0 μm guard column was also used. The column temperature was maintained at 35 °C and the injected sample volume was 10 μL. Two mobile phases were used (A and B) comprising 10 mM ammonium acetate/acetic acid buffer (pH 4.8) and ACN respectively and the flow rate was 1.0 mL min^{-1}. The gradient elution program was as follows: 90% of A for the equilibration and sample holding steps which lasted for 1 min each, mobile phase B then increased to 25% from 1-11 min and then rapidly increased to 90 % from 11-13 min. This composition was held for a further 5 min before the mobile phase composition then returned to the initial condition. The column was then re-equilibrated for 6 min at the initial mobile phase composition. The use of a step function rather than a smooth gradient reduced the retention times of the more strongly retained compounds so that all analytes were eluted in less than 25 min, which was also the total run time. The detection wavelength was 225 nm. Quantification was achieved based on peak area using calibration curves developed from known standards.

4.2.5.2 LC-ESI-MS/MS Analysis

LC-MS/MS was performed using the same chromatographic conditions as the HPLC-DAD using an Applied Biosystems/MDS Sciex API 3000 triple quadrupole mass spectrometer interfaced with a Dionex UltiMate® 3000 LCi. The tandem MS was performed using a triple quadrupole (TQD) mass spectrometer equipped with an electro spray ionization (ESI) source. All compounds were analysed in positive ionization mode. For MS detection, the instrument was operated in Multiple Reaction Monitoring (MRM) mode and identifications were made by

comparing retention times and substance specific mass spectra. All data were processed using Analyst 1.4.2 software. Instrumental conditions are listed in Table A.B2 (Appendix B).

4.2.6 Method Characterisation

Before the validation of the extraction methods, analytical methods were validated in terms of instrumental linearity, sensitivity (instrumental detection limit IDL and instrumental quantification limit IQL) and precision using standard solutions of the pharmaceuticals. The calibration curves were constructed by analysing at least five concentration levels (in triplicate) in the ranges of 0.1- 10.0 μg mL^{-1} for HPLC-DAD method and from 0.01 to 2.0 μg mL^{-1} for LC-MS/MS method to confirm linearity. IDLs and IQLs were calculated by using the signal-to-noise ratio of 3 and 10, respectively. The precision of the method was determined by repeated analysis of samples at three concentrations. The precision of the methods was expressed as the relative standard deviation (% RSD).

Matrix-matched calibration curves (6 points) were prepared by spiking the target pharmaceuticals into an extract of 5.0 g of sediment. The extraction recoveries of the different pharmaceuticals for the entire procedure (RECtotal), SPE/clean-up step (REC_{SPE}) were determined using BTC sediment. Triplicate samples of sediment (5g) were spiked with 0.2, 0.5 and 1.0 μg g^{-1} of a mixture of the study pharmaceuticals and were then extracted using the methods described above. Extracts were analysed in duplicate to allow calculation of method uncertainty. Validation of the method was performed for different parameters such as linearity, accuracy, precision and sensitivity. Limit of detection (LOD) and limit of quantification (LOQ) were estimated at a signal to noise (S/N) ratio of 3 and 10, respectively using the lowest spiked concentration into the sediment. Blank samples were used to determine the specificity and selectivity of the method.

Recoveries for the SPE/clean-up step (REC_{SPE}) were determined by spiking extract samples (400 mL) containing <5% of MeOH with a mixture of the pharmaceutical analytes. In another tube, sediment samples were extracted without spiking. Target compounds were added just in

the reconstitution step. All recoveries were calculated in comparison to a standard sample. The differences in recoveries between spiked samples in the extraction step, prior to clean-up and the standard was helpful to distinguish between recoveries for each step. The detailed validation procedure used and equations to calculate each extraction step recoveries are provided in the Appendix B (Section A.B1). The matrix effects were studied by the evaluation of signal suppression or enhancement for each pharmaceutical when LC-ESI-MS/MS analysis was used and was calculated according to equation A.B2 (more details in section A.B2, Appendix B).

4.3 Result and Discussion

4.3.1 Development of Chromatographic Methodology

The main objective of the chromatographic method was to analyse six pharmaceuticals with different physicochemical characteristics. Preliminary experiments were carried out to optimize the instrumental conditions for the detection of target compounds. Parameters, such as column type, mobile phase, optimum pH, flow rate, and column oven temperature were carefully studied. First, a Supelco 516 C-18-DB reverse-phase (150 x 4.6 mm, 5 μm) column was tested with a variety of mobile phases but was found to be inadequate. Analyte peaks showed significant tailing and reproducibility and resolution was not acceptable. These problems were overcome when a Zorbax Eclipse XDB-C18 reverse-phase column (150x 4.6 mm i.d., 5μm) was used.

A variety of mobile phases was investigated for optimization of the chromatographic conditions. The challenge was to optimize the mobile phase conditions for a series of compounds with a wide range of retention factors while providing an acceptable analysis time. The use of mobile phases consisting of formic acid and ammonium acetate with MeOH and/ or ACN were explored. The suitability of each mobile phase was determined on the basis of the sensitivity, stability and run time required for the analysis. The pH adjustment of the mobile phase played an important role in optimizing the chromatographic separation of ionisable chemicals. Different pH values were tested and the highest resolution with good retention times was

achieved with 10 mM ammonium acetate at a pH adjusted to 4.8 as the aqueous mobile phase. At this pH value, all compounds were in the protonated form and retention was at maximum and constant. Lower pH of the mobile phase resulted in peak tailing. The best wavelength obtained to show best peak shapes and higher response was at 225 nm for the HPLC-DAD method. Several gradient elution programs were tested to achieve the optimal separation of the analytes. For example, one and two segment linear gradient programmes were tested to improve the resolution for gradient separations. The first segment was optimized to achieve the desired separation for poor retention pharmaceuticals (atenolol, cimetidine and ranitidine) by the column. This segment was slow due to the narrow range of elution which found to be affected by a rapid increase of solvent B and consequently resulted in poor resolution. On the other hand, a scouting gradient method was used to optimize parameters such as initial and final % of mobile phase B.

In the MS/MS analysis, a standard solution (10 μg mL^{-1}) of each pharmaceutical was directly infused along with the mobile phase into the mass spectrometer with ESI, as the ionization source. The mass spectrometer was tuned in positive ionization mode and full scan mode was used for the identification of precursor ions. MRM mode was used for monitoring and ESI source temperature, capillary and cone voltage and flow rate of desolvation gas were optimized to obtain the highest intensity of precursor molecules of the six analytes. The collision gas pressure and energy of collision were optimized for maximum response of the fragment ions obtained. Precursor ions and product ions for MS detection and their respective collision energies are listed in the Appendix B (Table A.B2) together with typical retention times of all target analytes. MRM and UV chromatograms of standard solutions are shown in Figure 4.1.

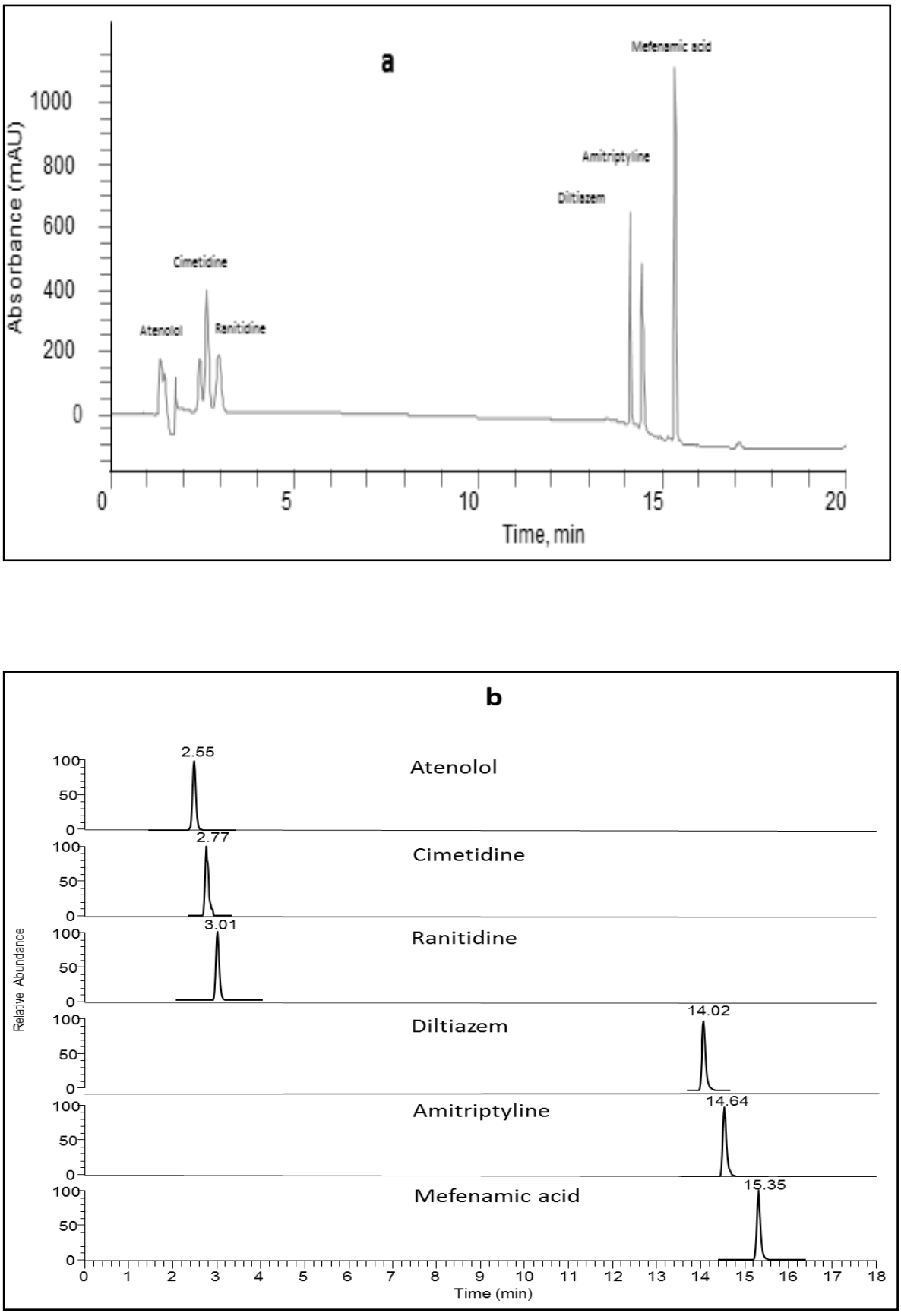


Figure 4.1 Chromatogram of a mixture of the pharmaceuticals in methanol. Chromatographic conditions: Zorbax Eclipse XDB-C18 reverse-phase column (250 mm × 4 mm i.d., 5 µm); Mobile phase of 10mM ammonium acetate with acetic acid and ACN at pH= 4.8; flow rate 1.0 mL

4.3.2 Optimization of Sediment Extraction Method

The study pharmaceuticals covered a diverse range of classes which were considerably different in polarity and acid–base properties. To extract the selected pharmaceuticals, it was important to consider the likely degree of binding of the compounds to sediment and organic matter and how these factors affect the efficiency of UAE of the organic contaminants (Bossio et al., 2008; Minten et al., 2011). Partitioning data for sediment/water systems are presented in the next Chapter. Diltiazem and amitriptyline exhibit moderately strong adsorption to sediment while atenolol, mefenamic and cimetidine show weak affinity to sediment. No partitioning coefficient values were available in the literature for ranitidine in sediment or soil. The optimization of extraction parameters were performed on BTC sediment. The variables optimized were solvent type, pH and sonication and shaking time.

Method optimisation was done using the BTC sediment spiked with the study pharmaceuticals at a concentration of 1 μg g^{-1}. A number of extraction solvents were tested in order to identify the optimum solvent (Table 4.2). Test solvents (ACN, MeOH and acetone) were chosen according to literature data as these solvents have commonly been used in previous studies (Paíga et al., 2017; Riemenschneider et al., 2017; Xu et al., 2008). Two extraction cycles involving 15 min sonication using 10 mL of solvent were used in the solvent optimization procedures. An initial experiment using only ACN was conducted and resulted in mean recoveries of around 65% for amitriptyline and less than 40% for the other compounds. Better recoveries were achieved for atenolol, ranitidine and mefenamic acid when MeOH was used as the extraction solvent while amitriptyline and diltiazem showed a slight decline in recoveries when MeOH was used. Overall, acetone showed poor extraction recoveries for all pharmaceuticals (Table 4.2). These finding indicated the need for a combination of solvents since the compounds have different physicochemical properties and using a single solvent resulted in low extraction efficiencies.

Several reported studies have indicated that the use of a mixture of polar organic solvents in water results in superior extraction of pharmaceuticals from solid environmental samples (Ding

et al., 2011; Lillenberg et al., 2009; Zhou et al., 2012). Mixtures of ACN: 0.2M citric acid (50:50) and MeOH: 0.2M citric acid (50:50) were therefore tested. Significant improvements in recovery were observed for diltiazem and mefenamic acid compared to the single solvent evaluations with mean recovery percentages of >50% been obtained when citric acid was combined with ACN or MeOH. However, the recoveries of cimetidine and ranitidine were poor (<20%) while atenolol showed lower recovery when a MeOH: citric acid mixture was used (Table 4.2). The method proposed by Li et al., (2013) using ACN with 2% NH_4OH was used and resulted in very good recoveries for amitriptyline, atenolol, mefenamic acid with mean recoveries of just over 50% being seen for diltiazem. However, the presence of interfering compounds in the chromatograms was found to be more significant when this method was used. When 2% NH_4OH in MeOH was tested, higher recoveries were found compared to those obtained using ACN except for amitriptyline which showed a lower mean recovery (86.3%).

The obtained results led us to incorporate another extraction step instead of the second extraction cycle using 2% NH_4OH in MeOH, to improve the recovery of acidic compounds by using 2% formic acid in MeOH. The acidification of the extraction solvent protonates the acidic functional groups (e.g. carboxylic acid, phenol groups) in the organic fractions of solid matrix (Ding et al., 2011). This step improved the overall recoveries of pharmaceuticals even though the obtained recovery of amitriptyline was lower than seen in the earlier work but still greater than 90%. A final step, using 5 mL of pure MeOH, was then added and showed improvement in the recoveries (>50%) for ranitidine and cimetidine without significantly affecting the recoveries of other pharmaceuticals in the mixture.

Table 4.2 Recovery (± 1S.D) of selected pharmaceuticals using single solvent and mixtures (2 cycles) and ultrasonic extraction of sediment spiked at 1µg g^{-1} (BTC sediment)

Pharmaceutical	Extraction solvent						
	ACN	MeOH	Acetone	ACN:0.2Mcitric acid	MeOH:0.2Mcitric acid	ACN:2%NH_4OH	MeOH:2%NH_4OH
Amitriptyline	65.0±4.2	51.0±7.6	18.0±4.8	68.0±5.2	59.0±5.5	105.2±11.4	86.3±5.2
Atenolol	10.5±1.2	44.3±3.5	12.2±1.2	19.5±1.2	49.0±6.2	80.1±9.6	88±4.6
Cimetidine	14.3±2.3	12.5±4.6	8.1±1.5	14.3±2.3	12.5±4.6	43.1±2.4	46.2±2.0
Diltiazem	39.0±5.3	30.0±4.1	22.0±2.5	57.0±2.9	55.5±3.5	54.3±2.1	78.2±3.1
Mefenamic acid	29.5±4.9	41.9±4.9	22.3±6.2	52.5±2.2	56.0±4.1	74.2±7.9	75.12±2.5
Ranitidine	10.8±1.0	18.2±2.5	11.0±1.7	10.8±0.9	18.2±2.5	31.2±4.3	35.2±1.2

The effects of the sonication time and shaking step were also examined. Short sonication times (5 and 10 min) were tested. Using a 5 min sonication time, a significant decrease in recovery of pharmaceuticals was observed while cimetidine and ranitidine were not detected. Slightly better recoveries were observed at 10 minutes sonication for all pharmaceuticals except diltiazem which showed a lower recovery percentage (74.2%). More efficient extraction was achieved at 15 min so this was selected as the final sonication time (Figure 4.2). Although UAE efficiency increases with time, sonication for more than 15 minutes showed no improvements in the recoveries of selected pharmaceuticals. Using an extraction slurry shaking step at 250 rpm for 10 min was found to increase pharmaceutical recoveries in sediment by up to 8.5% compared to no shaking. Recoveries obtained after shaking extraction slurry for 5 min showed no significant increase in recoveries from optimized shaking time (10 min). Longer shaking (15min) showed no improvements in recoveries (data not shown). Therefore, a 10 min shaking time was chosen to decrease the total extraction time (Figure 4.2).

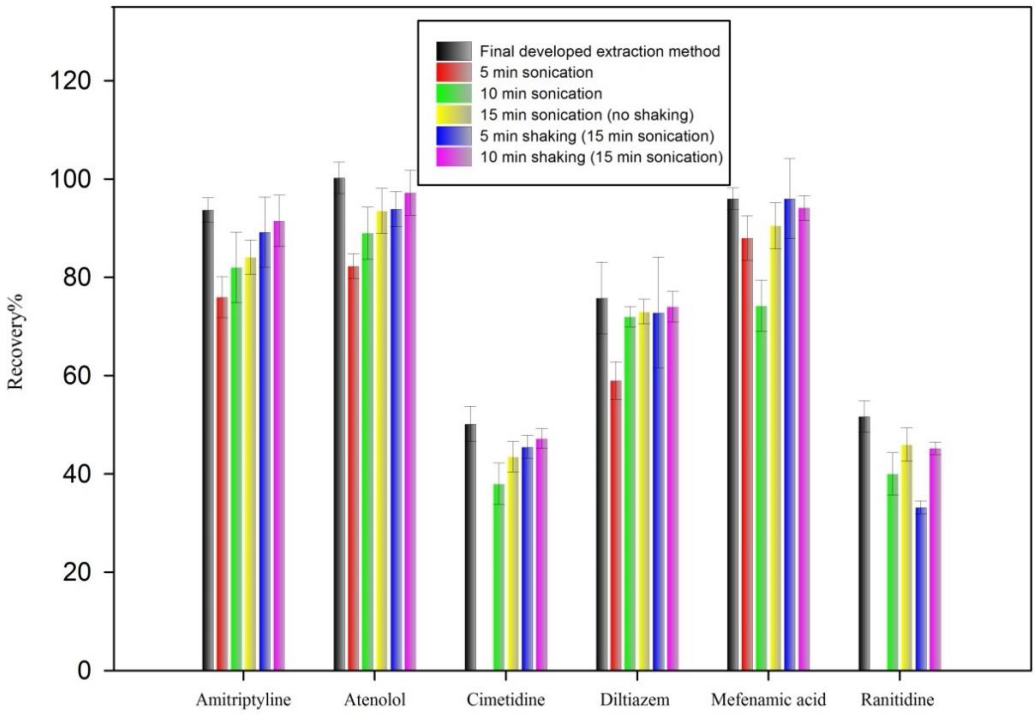


Figure 4.2 Recovery (± 1S.D) of selected pharmaceuticals using the optimized method at different sonication and shaking times (BTC sediment)

4.3.3 Optimization of SPE/Clean-up

Due to the complex nature of sediment components, the analytes can be masked in the chromatographic separation and in the final detection. Thus, the use of an SPE step was necessary for purification and reducing the effects of the matrix interfering substances, resulting in sample enrichment (Berlioz-Barbier et al., 2014; Gómez et al., 2006; Pérez-Carrera et al., 2010). The effect of pH manipulation of the diluted UAE extract on SPE recoveries using the optimal elution solvents was tested by adjusting the pH to 2, 4.6 and 10 (Table 4.3). The acidification of extracts prior to SPE clean-up increased the recoveries for all pharmaceuticals except cimetidine which showed a low SPE recovery (60.8%) and total recovery of 46.2%. At pH 4.6, amitriptyline showed better SPE mean recovery (110.5%) than at pH 2.0 and a significant improvement was seen in the SPE recovery of cimetidine (75.2%) while total recoveries showed a slight decrease. On the other hand, significant improvements in recoveries were observed at pH 10 with overall SPE recoveries > 88% and total recoveries > 50%. Based

on the obtained SPE results, the loss of analytes during the clean-up step appeared to be minimal and the low recoveries of the overall method for some pharmaceuticals (e.g. cimetidine and ranitidine) could be attributed to the inefficient extraction from sediment during the UAE step (Ding et al., 2011) or the presence of organic matter in sediment which may affect the sensitivity of the analysis (Jelić et al., 2009).

The elution solvent type is frequently the most important and frequently studied variable in the optimisation of an analytical method (Xu et al., 2008). MeOH (2x2.5 mL) was selected as the best choice for the elution of all the analytes. To improve the elution of pharmaceuticals, a third step has been added by using MeOH and 2% formic acid in MeOH and MeOH and 2% NH_4OH in MeOH. The use of 1.0 mL of 2% formic acid in MeOH slightly enhanced the total recovery for most of the pharmaceuticals except atenolol and ranitidine (Figure 4.3). Consequently, 1 mL of 2% NH_4OH in MeOH was used.

Table 4. 3 Recoveries of the optimized SPE (± 1S.D) and corresponding total recoveries (± SD) and of pharmaceuticals at different pH values (n=3)

Compound	Total recovery at pH=2	REC_{SPE} pH=2	Total recovery at pH=4.6	REC_{SPE} pH= 4.6	Total recovery at pH=10	REC_{SPE} pH=10
Amitriptyline	86.3±5.2	95.5 ± 4.6	93.3±3.3	110.5 ± 4.2	93.7±2.5	102.0 ± 3.8
Atenolol	88.0±4.6	90.8 ± 5.2	91.0±6.0	90.0 ± 3.4	100.24±3.2	110.0 ± 7.5
Cimetidine	46.2±2.0	60.8 ± 6.3	44.2±4.0	75.2 ± 7.0	50.18±3.6	88.5 ± 4.2
Diltiazem	78.2±3.1	95.5 ± 8.1	76.2±3.1	80.1 ± 5.8	75.8±7.3	90.8 ± 6.2
Mefenamic acid	86.1±2.5	110.4 ± 6.7	88.1±3.5	104.2 ± 3.8	96.0±2.2	99.8 ± 4.7
Ranitidine	35.2±1.2	78.5 ± 8.2	43.2±2.2	85.5 ± 4.2	51.7±3.2	90.5 ± 5.5

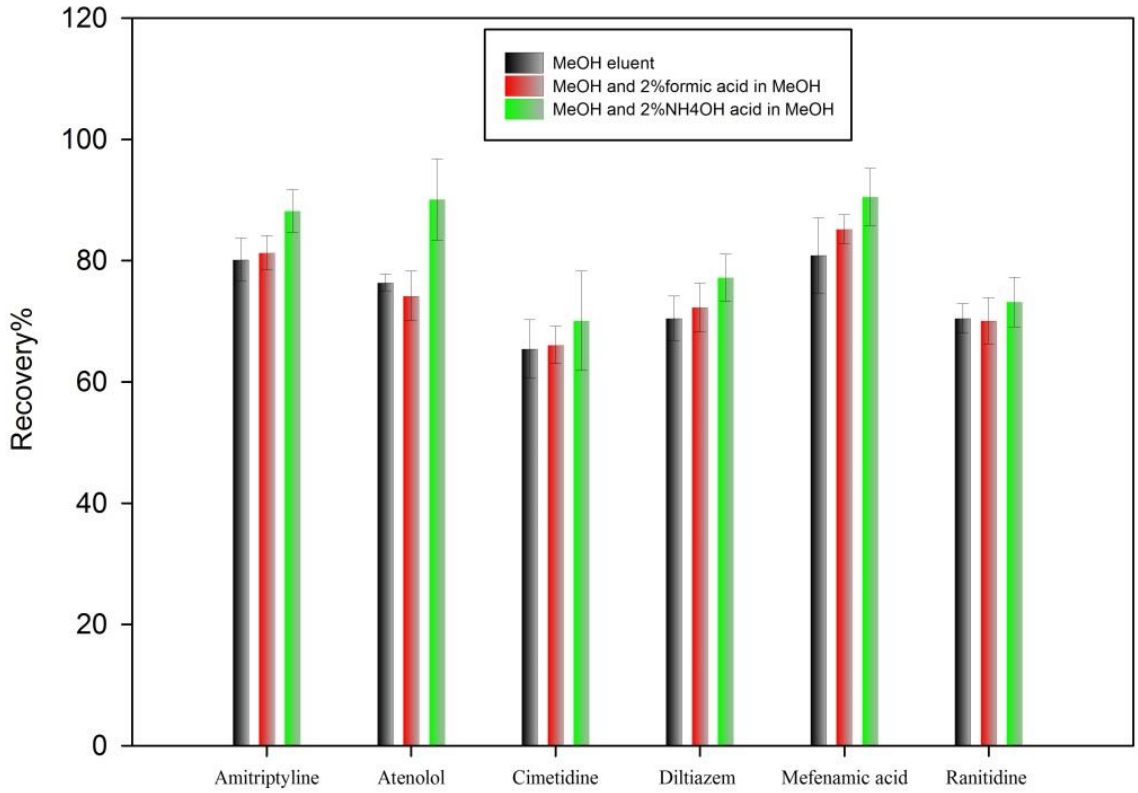


Figure 4.3 Total recoveries (± 1S.D) of selected pharmaceuticals using different SPE elution solvents (BTC sediment)

4.4 Validation and Method Performance

The HPLC-DAD and LC-ESI-MS/MS methods were validated in terms of linearity, recovery, precision and potential for matrix effects. Chromatograms of blank sample extracts showed no interferences from the method. Table 4.4 and Figure 4.4 show the method validation data (Tables A.B3 and A.B4). The extraction method performance was validated using the optimized method in ten types of sediment in terms of recovery for three spiking levels of 0.2, 0.5 and 1.0 μg g^{-1} and 0.1, 0.2, 0.5 μg g^{-1} for the HPLC-DAD and LC-MS/MS, respectively. For all sediments, relative standard deviation (RSD%) values of the analytical method ranged from 1.6 to 15.8%. These RSD values demonstrate good precision since values up to 20.0% are accepted for pharmaceutical analysis in environmental samples considering the complexity of the matrix and the number of steps involved (Garcia-Rodríguez et al., 2014; Martín et al., 2010; Silveira et al., 2013).

The obtained recoveries at the three concentration levels indicate the applicability of method to determine a wide range of concentrations. The performance of the methods for the studied pharmaceuticals in different sediments at different concentrations is shown in Tables A.B3 and A.B4 (Appendix B). Generally, the results showed better recovery results for pharmaceuticals in sediments with low organic content (HLM and HAB) and lower recoveries in the sediments with higher organic matter content (BW and GER sediments). This behaviour might be explained by the presence of naturally occurring organic matter in these samples which may mask the analytes and diminish their recovery since some pharmaceuticals have a relatively higher bonding affinity to sediment with high organic carbon content (e.g. amitriptyline, diltiazem) and may therefore have affected the efficiency of UAE of pharmaceuticals from the sediment (Bossio et al., 2008).

For amitriptyline, the recoveries from all sediments using the proposed extraction method were good and ranged from 70.4 to 111.8% for the lowest spiked concentration (200 ng g^{-1}) using HPLC-DAD. The LODs ranged from 17.3 ng g^{-1} (GER sediment) to 56.9 ng g^{-1} (BW sediment) while the RSDs ranged from 3.7 to 12.1%. Using LC-MS/MS analysis, amitriptyline showed recoveries ranging from 96.0% (GER sediment) to 106.6% (BGD sediment) at the lowest concentration of 100 ng g^{-1} and showed a very low LOD (0.07 ng g^{-1}) (Table 4.4). For atenolol, recoveries were within the same range for amitriptyline and ranged from 75.0 (BW sediment) to 113.2% (HLM sediment) using HPLC-DAD while this compound showed lower recoveries at the lower concentrations determined by LC-MS/MS. Mefenamic acid showed recoveries for all pharmaceuticals ranging from 76.5 to 102.5% while the LODs were relatively low and ranged from 14.0 ng g^{-1} (SKF sediment) to 24.0 ng g^{-1} (HLM sediment) using HPLC-DAD. The highest recovery obtained for diltiazem was 99.35% (HAB) while the lowest was 58.8% (BW sediment). The LODs ranged from 12.6 ng g^{-1} (HLM sediment) to 45.2 ng g^{-1} (GER sediment) and 0.03 ng g^{-1} (MIL sediment) to 0.1 ng g^{-1} (BTC sediment) using UV and tandem MS detectors respectively. The efficiencies of recovery for both cimetidine and ranitidine were low and ranged from 40.5 (BW sediment) to 67.3% (HAB sediment) and from 29.5 (MIL sediment) to 52.3% (MOR sediment) respectively. Ranitidine showed a low LOD of 0.2 ng g^{-1} (HLM

sediment) using LC-MS/MS. Overall, the optimized method provided acceptable recoveries and sensitivities for most of the target compounds.

When the impact of potential matrix effects was evaluated, most of pharmaceuticals were subjected to ion suppression at least in one sediment type. Atenolol exhibited signal suppression of up to 42.5% followed by cimetidine with signal suppression of 38.0% in the high organic content sediment (BW). On the other hand, amitriptyline and diltiazem showed signal enhancement of 12.4% in HLM and BGD sediments respectively (Table A.B5, Appendix B). Many strategies to reduce matrix effects are suggested in the literature including dilution, clean-up steps after extraction, the use of isotopically labeled standards, preparation of a matrix-matched standard curve and single-point standard addition where the actual samples (hydrophilic/polar pharmaceuticals) are used to create a calibration plot individually (Chambers et al., 2007; Gergov et al., 2015; Panuwet et al., 2015; Stahnke et al., 2012; Vazquez-Roig et al., 2013). In the current study, due to the clear effects of the co-eluting interferences during analysis by the mass spectrometry detector with electrospray interfaces and the different polarity of analytes, matrix-matched calibration was selected as an appropriate approach to compensate for the matrix effects during analysis (Huerta et al., 2015; Panuwet et al., 2015; Vazquez-Roig et al.,2013).

A number of studies in the literature have reported methods for the successful extraction of the study pharmaceuticals from sediment although these studies used different solvents, clean-up steps, and vary in the complexity of the matrix tested and the detection technique. Our results are in line with other work for atenolol analysis in sediment using ASE extraction, MeOH as a solvent and UHPLC-MS for detection where recoveries ranged from 118-135% (Langford et al., 2011); and higher than those reported (65.7-74.8%) using a UAE method, two cycles of MeOH and MeOH water (50:50) as solvents and using UHPLC-MS/MS analysis (de Sousa et al., 2015). Diltiazem recoveries using UAE were comparable to a PLE method using 0.1 M ammonium solution and MeOH (50:50) as solvent while cimetidine showed recoveries similar to or better than what was obtained in this study ranging from 50to > 80% (Pérez-Carrera et al., 2010). Amitriptyline and mefenamic acid showed better recoveries than results (39.3% and

28%, respectively) obtained by pressurized hot water extraction–stir bar sorptive extraction–derivatization (Pintado-Herrera et al., 2013).

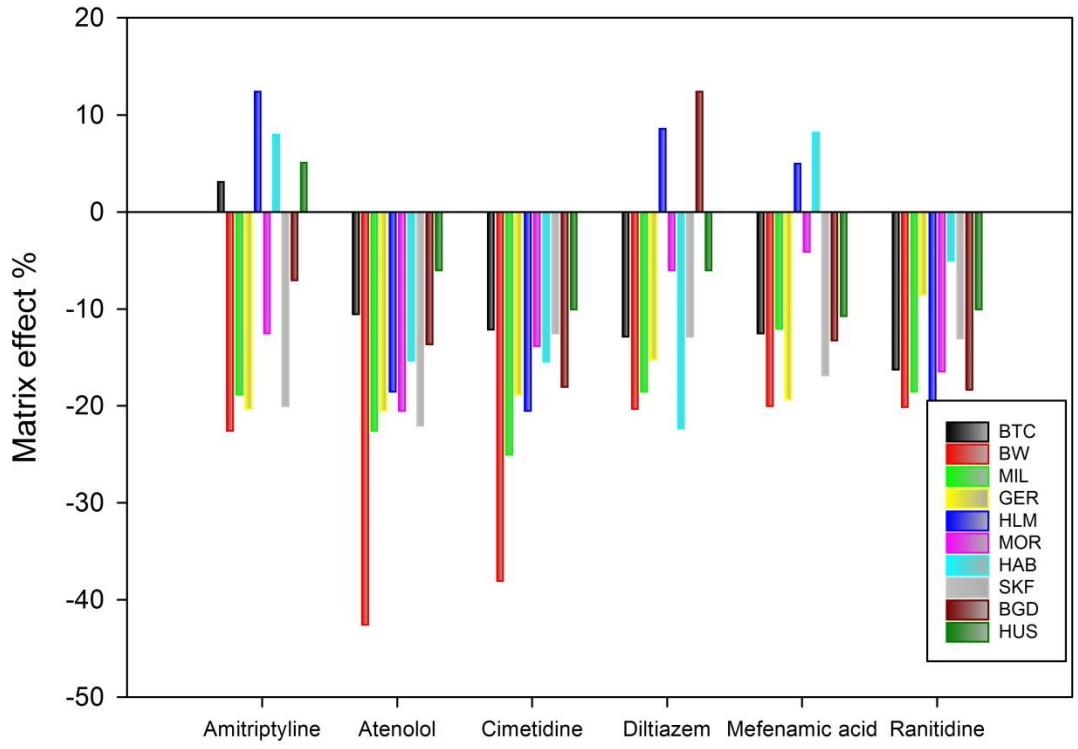


Figure 4.4 Matrix effect on pharmaceuticals analysis in LC-ESI-MS/MS analysis at 100 ng g^{-1}

4.5 Conclusion

Sample preparation is a key prerequisite for the analysis of pharmaceuticals at trace levels in environmental media, and it is often the most labour-intensive, time-consuming and least sophisticated step of the analytical procedure. In this study, a simple, inexpensive, low solvent consumption and ambient temperature UAE method was developed and validated for a range of pharmaceuticals in sediment samples with different physicochemical properties and produced reasonable recoveries and precisions. The obtained recoveries demonstrate that UAE is an attractive, affordable, and effective alternative to existing extraction methods (i.e. PLE, MAE) for organic contaminants from sediment. This work also provides evidence about the employability of UAE to extract pharmaceuticals or other organic contaminants with different properties. The more widely available analytical (HPLC-DAD) and the highly sensitive LC-ESI-MS/MS techniques were used to determine the analytes after SPE to clean-up the samples. The data on the performance of the proposed method demonstrate its suitability for use in environmental fate and monitoring studies described in Chapters 6 and 7.

Table 4.4 Method validation data for the complete UAE-SPE HPLC-DAD at 200 ng g^{-1} and UAE-SPE LC-MS/MS at 100 ng g^{-1} for the studied pharmaceuticals in different sediment

Sediment	Analysis method	Atenolol		Cimetidine		Amitriptyline		Diltiazem		Mefenamic acid		Ranitidine	
		Recovery% (± RSD%)	LOD (LOQ) ng g^{-1}	Recovery% (± RSD%)	LOD (LOQ) ng g^{-1}	Recovery % (± RSD%)	LOD (LOQ) ng g^{-1}	Recovery % (± RSD%)	LOD (LOQ) ng g^{-1}	Recovery% (± RSD%)	LOD (LOQ) ng g^{-1}	Recovery% (± RSD%)	LOD (LOQ) ng g^{-1}
***BTC**	HPLC-DAD	95.2 (6.2)	37.1 (122.5)	48.3 (10.4)	31.3 (103.3)	85.5 (6.3)	34.0 (112.2)	75.9 (4.3)	20.4 (67.3)	90.4 (4.1)	23.3 (76.9)	50.3 (6.4)	20.2 (66.7)
	LC-MS/MS	93.1 (5.9)	1.9 (6.0)	50.3 (2.1)	0.7 (2.3)	99.6 (10.3)	0.3 (1.0)	80.2 (10.2)	0.1 (0.4)	82.5 (2.3)	2.3 (8.0)	45.2 (7.8)	0.3 (1.0)
BGD	HPLC-DAD	79.2 (11.0)	55.6 (183.5)	45.22 (5.4)	15.4 (50.8)	72.5 (4.5)	20.5 (67.7)	77.30 (7.5)	36.8 (121.5)	99.2 (2.4)	15.0 (49.5)	33.4 (6.5)	13.2 (43.6)
	LC-MS/MS	75.2 (6.2)	1.5 (5.0)	42.5 (8.1)	0.5 (1.7)	106.6 (9.8)	0.2 (0.7)	75.6 (2.8)	0.07 (0.2)	75.8 (4.1)	0.1 (0.4)	50.3 (2.3)	0.8 (2.4)
HUS	HPLC-DAD	79.6 (6.2)	32.2 (106.0)	40.5 (9.9)	26.7 (88.1)	80.0 (5.5)	28.2 (93.1)	63.21 (8.3)	34.1 (112.5)	102.5 (3.2)	21.3 (70.3)	30.4 (15.8)	31.4 (103.6)
	LC-MS/MS	72.2 (10.5)	2.7 (9.0)	52.1 (3.6)	0.7 (2.4)	70.0 (9.3)	0.13 (0.5)	77.8 (7.2)	0.04 (0.13)	80.0 (3.6)	0.15 (0.5)	40.3 (7.2)	0.4 (1.3)
SKF1	HPLC-DAD	81.5 (4.5)	23.0 (76.0)	42.6 (5.7)	15.4 (50.8)	111.8 (3.7)	25.5 (84.2)	60.2 (9.3)	35.2 (116.2)	85.3 (2.5)	14.0 (46.2)	32.1 (6.3)	12.4 (40.9)
	LC-MS/MS	83.0 (9.8)	3.5 (12.0)	49.6 (2.8)	0.5 (1.7)	95.5 (5.7)	0.18 (0.6)	60.3 (5.3)	0.04 (0.13)	90.1 (5.3)	2.5 (8.0)	45.5 (4.5)	0.6 (1.8)
HAB2	HPLC-DAD	94.4 (4.7)	28.0 (92.5)	67.3 (4.5)	19.0 (62.7)	80.5 (4.1)	20.5 (67.7)	99.4 (4.9)	30.8 (101.6)	101.3 (3.0)	19.5 (64.4)	48.5 (9.7)	30.2 (99.7)
	LC-MS/MS	73.9 (5.2)	2.2 (7.0)	51.9 (5.5)	0.7 (2.3)	102 (7.3)	0.14 (0.5)	92.2 (9.1)	0.06 (0.2)	74.1 (8.0)	2.0 (6.0)	42.3 (6.6)	0.6 (1.9)
MIL3	HPLC-DAD	97.7 (5.6)	34.6 (114.2)	52.4 (5.4)	17.8 (58.7)	70.45 (5.6)	25.3 (84.0)	69.3 (9.5)	39.1 (129.0)	87.5 (3.7)	20.8 (68.6)	29.5 (10.8)	21.3 (70.3)
	LC-MS/MS	70.5 (6.4)	1.3 (5.0)	44.2 (8.2)	0.6 (1.9)	81.8 (5.6)	0.07 (0.25)	65.1 (5.2)	0.03 (0.1)	70.2 (5.1)	0.3 (1.0)	45.2 (8.1)	0.6 (1.9)
HLM	HPLC-DAD	113.2 (3.6)	25.5 (84.2)	49.3 (9.6)	30.2 (100.0)	101.7 (6.0)	38.0 (125.5)	69.5 (2.5)	12.6 (42.0)	96.5 (4.0)	24.0 (79.2)	45.5 (9.8)	27.8 (91.7)
	LC-MS/MS	73.2 (6.2)	1.8 (6.0)	48 .1 (14.3)	0.6 (1.9)	80.0 (5.9)	0.09 (0.3)	91.5 (8.6)	0.04 (0.16)	87.8 (5.9)	0.1 (0.3)	44.3 (5.1)	0.2 (0.6)
MOOR	HPLC-DAD	85.4 (4.9)	25.8 (85.1)	55.5 (8.2)	28.7 (94.7)	99.3 (4.1)	25.3 (83.5)	77.3 (4.1)	20.5 (67.7)	89.5 (4.1)	23.2 (106.3)	52.1 (9.5)	32.1 (105.9)
	LC-MS/MS	75.6 (12.3)	2.5 (8.0)	41.2 (6.5)	0.8 (2.5)	83.6 (9.1)	0.13 (0.5)	65.3 (7.7)	0.05 (0.17)	70.1 (5.1)	0.2 (0.6)	48.0 (5.8)	0.3 (0.9)
GER	HPLC-DAD	88.6 (5.1)	28.3 (93.3)	50.9 (6.1)	19.7 (65.0)	88.7 (3.1)	17.3 (57.1)	71.8 (11.6)	45.2 (149.1)	80.8 (3.5)	18.2 (60.1)	52.3 (7.5)	24.8 (81.8)
	LC-MS/MS	80.2 (6.8)	2.5 (8.0)	50.4 (15.5)	1.2 (4.0)	69.1 (10.4)	0.2 (0.7)	72.1 (3.4)	0.02 (0.07)	83.1 (5.8)	2.0 (6.0)	52.5 (6.9)	0.5 (1.6)
BW	HPLC-DAD	75.0 (12.4)	58.5 (193.1)	40.5 (12.4)	31.2 (103.0)	90.3 (10.1)	56.9 (187.8)	58.2 (5.9)	29.6 (97.7)	76.4 (3.4)	16.2 (53.5)	36.4 (12.4)	28.5 (94.1)
	LC-MS/MS	77.1 (6.1)	1.9 (6.0)	48.0 (4.6)	0.6 (1.8)	70.3 (8.1)	0.14 (0.5)	60.2 (6.3)	0.05 (0.17)	70.0 (4.4)	0.1 (0.4)	40.1 (9.1)	0.7 (2.2)

* Sediment used for method development

Chapter 5

Impacts of compound properties and sediment characteristics on the sorption behaviour of pharmaceuticals in aquatic systems

5.1 Introduction

As discussed in Chapter 2, once pharmaceuticals are introduced into surface water, they may undergo biodegradation, hydrolysis or photodegradation, as well as partition to natural solid matter such as suspended solids and bed sediments or to the dissolved colloidal matter, in pore water (Lees et al., 2016; Liang et al., 2013; Yamamoto et al., 2009). The fate of a pharmaceutical is thought to depend on factors such as the compounds lipophilicity, water solubility, chemical functionality as well as the ambient conditions of the receiving environment (Aga, 2007; Boxall, 2007; Packer et al., 2003). Sorption is one of the major factors determining the persistence and attenuation of pharmaceuticals in the natural environment (Schaffer et al., 2012b; Zhou and Broodbank, 2014). Unlike neutral organic compounds, where differences in partitioning typically occurs through van der Waals interaction with soil organic carbon and is correlated to the hydrophobicity of the chemical (e.g. the octanol–water partitioning coefficients (Kow)), the sorption of pharmaceuticals, which are typically ionisable compounds, to environmental solids is thought to be through a combination of interactions e.g. hydrogen bonds, electrostatic interactions, ionic exchange and hydrophobic interactions (Martínez-Hernández et al., 2014; Niedbala et al., 2013; Rowney et al., 2009; Stein et al., 2008; Tolls, 2001). Moreover, while the organic carbon content (OC) of sediments is known to be important in explaining the differences in the sorption behaviour of a neutral organic chemical across different soil or sediment types, factors such as the solid phase component (clay and metal content), surface exchangeable cations and pH probably play an important role in determining sorption of ionisable compounds (Calvet, 1989; Dubus et al., 2001; Niedbala et al., 2013).

While research into the sorption of pharmaceuticals in water-sediment systems has recently increased (Jones et al., 2006; S. Kim and Carlson, 2007; Y. Li et al., 2014; Loffler et al., 2005; Stein et al., 2008; Yamamoto et al., 2009, 2005), data are still only available for a few active ingredients so our understanding of the factors and processes affecting sorption of pharmaceuticals is limited. A number of studies have also proposed predictive models for estimating the sorption behaviour of pharmaceuticals in sewage sludge and soil (Barron et al., 2009; Franco and Trapp, 2008). For example, Franco and Trapp (2008) showed that predictors such as log Kow and pKa could be used successfully to predict the sorption of cationic dissociating groups to organic content in soils while failing to predict sorption for anionic groups. A major reason for uncertainty in the model predictions is the variability of soil pH, which influences speciation equilibria as well as the soil surface chemistry. Sorption of organic acids is greater at lower pH. Probably due to the local acidity near the organic colloid-water interface. Low pH enhances lipophilic sorption of the neutral molecule; at the same time, anions are less repulsed from the sorbent surface, thus anion exchange (and sorption of anions) increases. Barron et al., (2009) used a non-linear correlation modelling techniques (artificial neural networks) to predict the value of the distribution coefficient (Kd) in sewage sludge and found good agreement between the model predictions and experimental observations (R = 0.88). Log Kow was found to be the largest contributor to Kd with approximately 11% deviation while pKa was the second most important descriptor. It is not surprising since only molecular descriptors such as log Kow, pKa, molar refractivity, aromatic ratio, hydrophilic factor and topological surface area were included in the model. However, models for predicting sorption behaviour of pharmaceuticals in the sediment compartment are still lacking. The development of these models would be invaluable in supporting the assessment of environmental risks of pharmaceuticals released to surface waters and, in particular, characterizing likely impacts on benthic organisms.

Given the paucity of information on sorption of pharmaceuticals in the sediment compartment, the objective of this chapter was to develop a better understanding of the sorption behaviour of pharmaceuticals in sediment-water systems and of how sediment and pharmaceutical

physicochemical properties influence this behaviour. The specific objectives were to: (1) explore the effects of sediment type on the sorption behaviour of range of pharmaceuticals with different properties; (2) evaluate the suitability of existing predictive models for ionisable compounds in sediments; and (3) develop improved models for estimating the sorption behaviour of pharmaceuticals in different sediment types. The study was performed using five pharmaceuticals (amitriptyline, atenolol, cimetidine, diltiazem and mefenamic acid).

5.2 Materials and Methods

5.2.1 Chemicals and Solvents

Amitriptyline hydrochloride (≥98% purity), atenolol (≥98%), cimetidine (≥98%), diltiazem hydrochloride (≥99%) and mefenamic acid (≥98%) were all purchased from Sigma-Aldrich (UK), (Table 1.1; Chapter 1). The solvents used, including methanol (high performance liquid chromatography (HPLC) gradient grade), acetonitrile (gradient grade) and HPLC grade water were purchased from Fisher scientific (UK). Calcium chloride, hydrogen peroxide, potassium dihydrogen orthophosphate, nitric acid and hydrochloric acid were purchased from Fisher scientific (UK); formic acid was obtained from Sigma-Aldrich (UK).

5.2.2 Sediment Collection and Characterisation

Sediments used in this study were the same sediment used in the analytical development work described in Chapter 4. These were collected from Iraq and England and details of the individual sediment characteristics are provided in Chapter 4 (Section 4.2.2, Table 4.1). Sorption studies were performed within three months of sediment collection.

5.2.3 Sorption Studies

Sorption studies were conducted based on the OECD test (106) guideline ‘Adsorption-Desorption Using a Batch Equilibrium Method’ (OECD, 2000). The study was performed in two phases. Initial experiments were done to identify the optimum sediment: solution ratio for

each pharmaceutical. A definitive study was then done to develop the sorption isotherm. In the initial experiments, 1 g of sediment (dry weight equivalent) was weighed into 50 ml centrifuge tubes (centrifugation tube, Fisher scientific, Mexico) and either mixed with 10, 25 or 30 ml of 0.01 M $CaCl_2$ over 24 h prior to spiking of the test pharmaceuticals. Triplicate tubes were prepared for the sediment: solution ratio at time point and pharmaceutical. Aluminium foil was used to wrap the centrifuge tubes to prevent photochemical reactions during mixing. The pharmaceuticals were then spiked into the aqueous phase to give a concentration of 100 mg L^{-1}. Tubes were then agitated at 120 oscillation min^{-1} at room temperature (20 $\pm 2^{\circ}C$) for 2, 4, 6, 8 and 24 h. At the end of mixing, samples were centrifuged at 4500 rpm for 10 min and the supernatant solution was filtered through a 0.22 μm nylon filter to remove the suspended solids and particulate matter. Finally, 2 ml of the supernatant was taken for determination of pharmaceuticals concentrations. A control treatment with the same test conditions but without sediment was set up to determine possible degradation or adsorption of the pharmaceuticals to vessels. In the main study, a sediment to solution ratio of 1:10 was used for atenolol, cimetidine and mefenamic acid while ratios of 1:25 and 1:30 were used for diltiazem and amitriptyline respectively (as determined in the preliminary experiments). In order to create sorption isotherms, pharmaceuticals were spiked into vessels to give concentrations between 10 and 100 mg L^{-1}.

5.2.4 Analytical Method

As the difference between the amount of test pharmaceutical initially present in solution and the amount remaining at the end of the experiment represent the amount adsorbed to sediment (OECD 106); and since high pharmaceutical concentration used and the long run time of the developed analytical method in Chapter 4, analytical methods with short run time were developed. Concentrations of the study compounds in supernatant from the sorption experiments were determined using an HPLC (Perkin Elmer, Flexar) coupled with photodiode array detection and equipped with an automated injection system. An isocratic elution method

was used for all compounds. Separation was achieved using a Supelco 516C-18-DB reverse-phase column (5 μm, 4.6 × 150 mm). For atenolol and cimetidine, the mobile phase comprised 1% formic acid [v/v], pH 2.7 (± 0.05) and acetonitrile (65:35 v/v), the column was kept at 30 °C and the detection wavelength was 227 nm. The flow rate of the mobile phase was 1.0 ml min^{-1} into which 10 μL of sample was injected. For amitriptyline and diltiazem, the mobile phase comprised 30 mM potassium dihydrogen orthophosphate (KH_2PO_4) and acetonitrile (35:65 v/v), pH 3.65 (±0.05). The flow rate was 1 ml min^{-1}, the injection volume was 20 μL and the detection wave-length was 210 nm. The column was kept at 35◦C. For mefenamic acid, the mobile phase consisted of 0.05% formic acid in HPLC water [v/v], pH 2.7 (±0.05) and methanol (20:80 v/v), and the flow rate was 1 ml min^{-1}. The sample injection volume was 20 μL and the detection wavelength was 227 nm. The column temperature was 30°C. Analytical method details are shown in Table A.C.1 and Figure AC.1 (Appendix C).

5.2.5 Sorption Isotherm Modelling

The mass difference between the initial (C_i) and residual concentration (C_e) were used to determine the sorbed amount (Q_e) in the sediment [mg kg^{-1}], Eq. (5.1).

$$\mathbf{Q_e=(C_i-C_e)\times V_w/\ m_s} \qquad \mathbf{(5.1)}$$

where V_w is the solute volume [L]; and m_s is the sediment mass [Kg], respectively. Sorption isotherms were then modelled using the linear, Freundlich, and Langmuir isotherm models and Kd, Kf and KL values were derived. Sorption modelling was done by SigmaPlot12.0 (Systat Software, Inc). The organic carbon-normalised sorption coefficient was then estimated from the Kd value and the total organic carbon content of the soil Eq. (5.2).

$$\mathbf{K_{OC}=Kd\ /\mathit{f}oc\times 100} \qquad \mathbf{(5.2)}$$

Statistical analyses were conducted on the resulting sorption coefficients, using the SPSS 22.0 statistical software package, to evaluate differences in a compound behaviour across sediment types. One-way ANOVA was performed to explore the effect of sediment type on sorption of individual pharmaceuticals. Post Hoc ANOVA test was used to show the difference of sorption from sediment to another. Kruskal Wallis non-parametric analysis of variance was used when normality test failed.

5.2.6 Evaluation of Existing Models for Estimating the Sorption Behaviour of Pharmaceuticals

The Koc values were calculated for each pharmaceutical and each sediment type using models proposed by (Franco and Trapp, 2008) for acidic (Equation 5.3) and basic electrolytes (Equation 5.4).

$$\text{Log Koc} = \log(\phi_n \cdot 10^{0.54 \cdot \log \text{Kow} + 1.11} + \phi_{ion} \cdot 10^{0.11 \cdot \log \text{Kow} + 1.54}) \quad (5.3)$$

$$\text{Log Koc} = \log(\phi_n \cdot 10^{0.37 \cdot \log \text{Kow} + 1.70} + \phi_{ion} \cdot 10^{\text{pKa}^{0.65} \cdot f^{0.14}}) \quad (5.4)$$

Where: Kow is the octanol-water partition coefficient; pKa is the acid dissociation constant; *f* is a parameter expresses a diffusion limiting factor and equal to Kow/(Kow+ 1). While, ϕ_n and ϕ_i are neutral and ion fractions, respectively; and were determined using Equations 5.5 and 5.6.

$$\phi_n = 1/1 + 10^{a\,(pH-pKa)} \quad (5.5)$$

$$\phi_{ion} = 1 - \phi_n \quad (5.6)$$

Where a = 1 for acids and -1 for bases.

Estimates of Koc were then compared to measured values to assess the performance of the models.

5.2.7 Development of New Models for Estimating the Sorption Behaviour of the Study Pharmaceuticals across Sediment types

The stepwise multiple-linear regression (MLR) function in SPSS 22.0 was employed to try to develop relationships between Kd as the dependent variable and combinations of sediment physical-chemical property parameters as the explanatory variables. MLR is widely used, and produces linear models in which descriptors are weighted by coefficients found by minimizing the sum of squared residuals between experimental and predicted responses (Kennicutt et al., 2016). The Dow, which is a measure of the pH-corrected hydrophobicity of an ionisable compound in a particular environment, was also estimated (using Equations 5.7 and 5.8) and used in the analyses as this parameter has previously been shown to explain differences in the sorption behaviour of ionisable compounds (Kah and Brown, 2007; Schaffer et al., 2012b). The Pearson correlation coefficient (R and P-value) was used to show the significance and the degree of the linear relationship between Kd and single sediment or pharmaceutical properties (Table A.C2, Appendix C).

$$\mathbf{LogDow_{acid} = logKow - log\ (1+10^{(pH-pKa)})} \qquad \mathbf{(5.7)}$$

$$\mathbf{LogDow_{base} = logKow - log\ (1+10^{(pKa-pH)})} \qquad \mathbf{(5.8)}$$

5.3 Results and Discussion

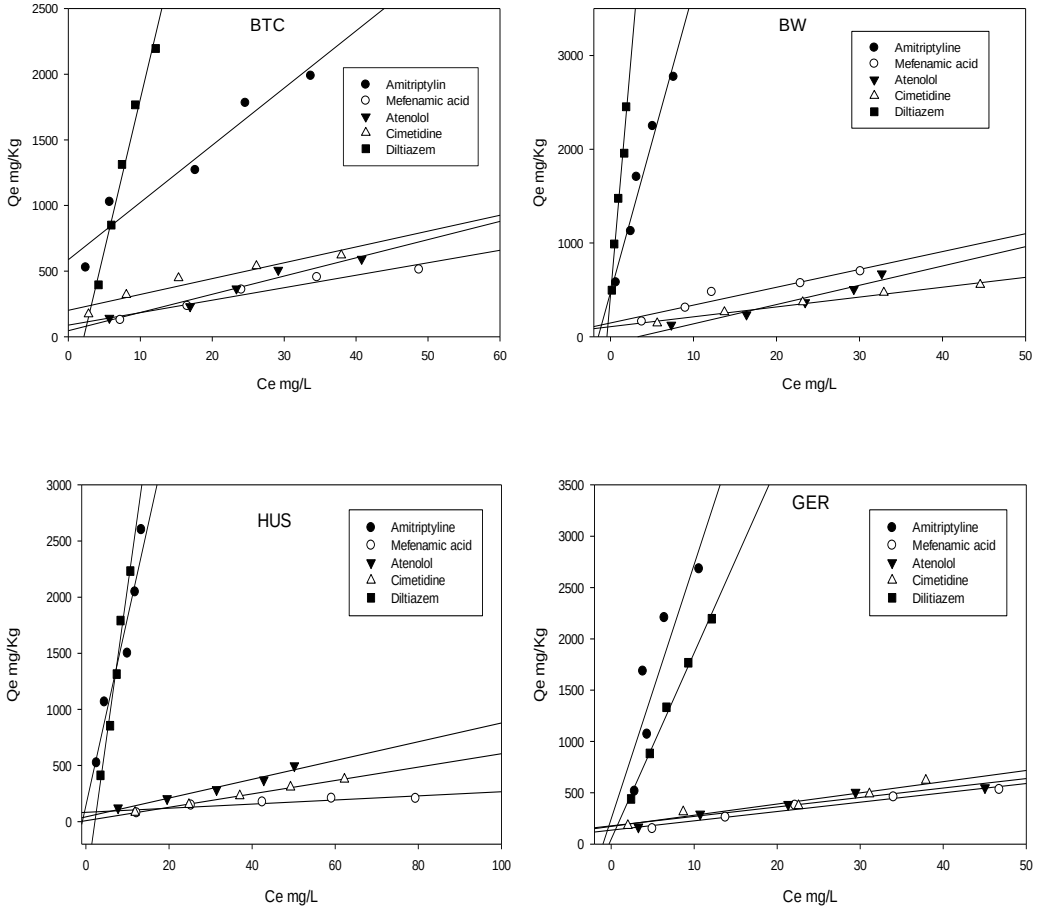
5.3.1 Partitioning of Pharmaceuticals between Water and Sediment

In the control treatments, for all pharmaceuticals, at least 95% of the initial concentrations remained after 24 h suggesting no significant degradation or adsorption onto centrifuge tubes. The linear (R^2 = 0.540–0.999), Freundlich (R^2 = 0.571–0.999) and Langmuir (R^2 = 0.283–0.998) models all appropriately described the sorption of the investigated pharmaceuticals over the

range of test concentrations (see Appendix C, Table A.C2). The sorption coefficients obtained using the linear model were selected for use in the model evaluation and development studies and are discussed more fully below. Linear sorption isotherms for the five study compounds across the ten sediment types are shown in Figure 5.1. Sorption coefficients for the compounds increased in the order mefenamic acid (Kd 1.83–19.04; Koc 75.86–331.13) < cimetidine (Kd 2.28–15.88; Koc 102.33–426.78) < atenolol (Kd 2.22–20.56; Koc 85.11–489.78) < amitriptyline (Kd 8.79–247.97320.8; Koc 912.01–12589.25) < diltiazem (Kd 22.03–1022.6; Koc 799.24–13182.57) (Figure 5.2; Table A.C2).

Variability in pharmaceuticals sorption behaviour across sediments is likely due to several factors including total organic content, sediment texture, pH, salinity, the duration of incubation, particle size, degree of sediment–water interactions or the heterogeneity of the organic carbon in the sediments (Chen and Zhou, 2014; Hyland et al., 2012; Karapanagioti et al., 2001; Kwon and Armbrust, 2008; Liang et al., 2013; Petrie et al., 2014; Tolls, 2001; Zhou and Broodbank, 2014). The patterns of sorption across the different test sediments were different for each study pharmaceutical. For amitriptyline, greatest sorption was seen for the BW sediment which had a high organic carbon content and CEC (9.9%, 35.58 cmol+/kg) while the lowest Kd value was obtained for the HLM sediment which had a low organic carbon content and CEC (0.98%, 5.85 cmol+/kg). Based on the hydrophobicity of amitriptyline (log Kow 4.92), a higher sorption was expected than seen in the current study. No previous data are available on sorption of amitriptyline in sediments but our Kd values are at the lower end of the range of Kd values reported for soils and sludge for this compound (Franco and Trapp, 2008; Hyland et al., 2012). Significant differences in sorption across sediment types were also seen for atenolol (excluding MIL and SKF; $p < 0.05$), cimetidine (excluding MIL and SKF and BW and BTC sediments; $p < 0.01$), diltiazem (excluding GER and HUS; $p < 0.05$), and mefenamic acid (excluding HUS and SKF and HLM and SKF sediments; $p < 0.001$). For atenolol, the highest and lowest Kd values were seen for BW and HLM sediments respectively. Diltiazem sorption was the most variable amongst the studied pharmaceuticals across the sediment types with Kd values ranging from 22.03 to 1022.6 L kg^{-1} with the greatest sorption being seen in the BW sediment and lowest

sorption being observed in the HLM sediment. For mefenamic acid, the greatest Kd was obtained for BW sediment whereas the lowest Kd was for sediment HUS from Iraq. For cimetidine, highest sorption was seen in the SKF (OC% 7.92, clay% 36.52) sediment and lowest in the HAB (OC%= 1.12, clay%= 1.12) sediment. For atenolol, diltiazem and mefenamic acid where sediment sorption data are available in the literature, Kd ranges that we observed are not dissimilar from literature values (Table 5.1).



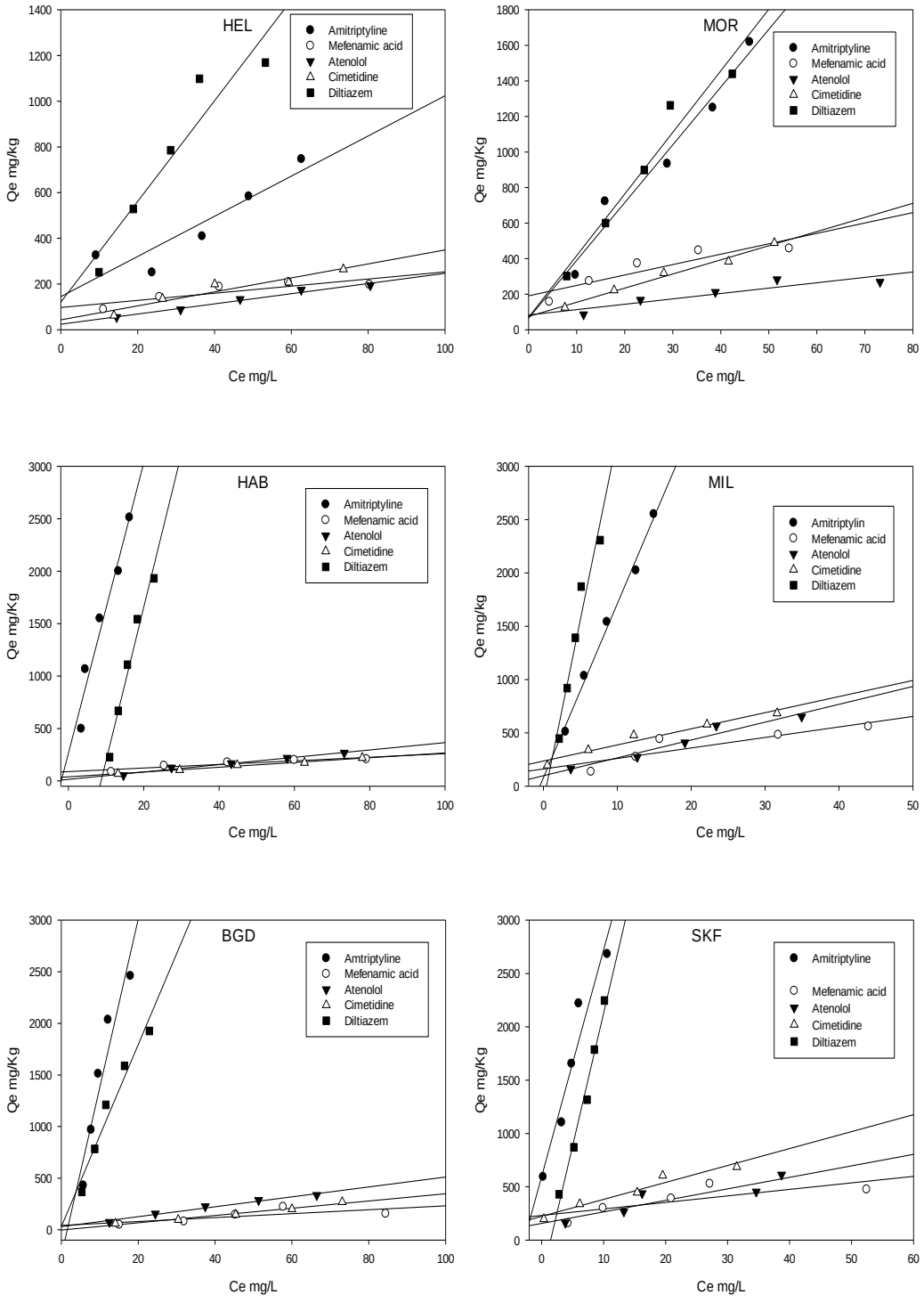


Figure 5.1Adsorption isotherms of selected pharmaceuticals in sediments at 20 ± 2°C. Initial concentrations ranged from 20 to 100 mg L^{-1}. Points represent means of three replicates

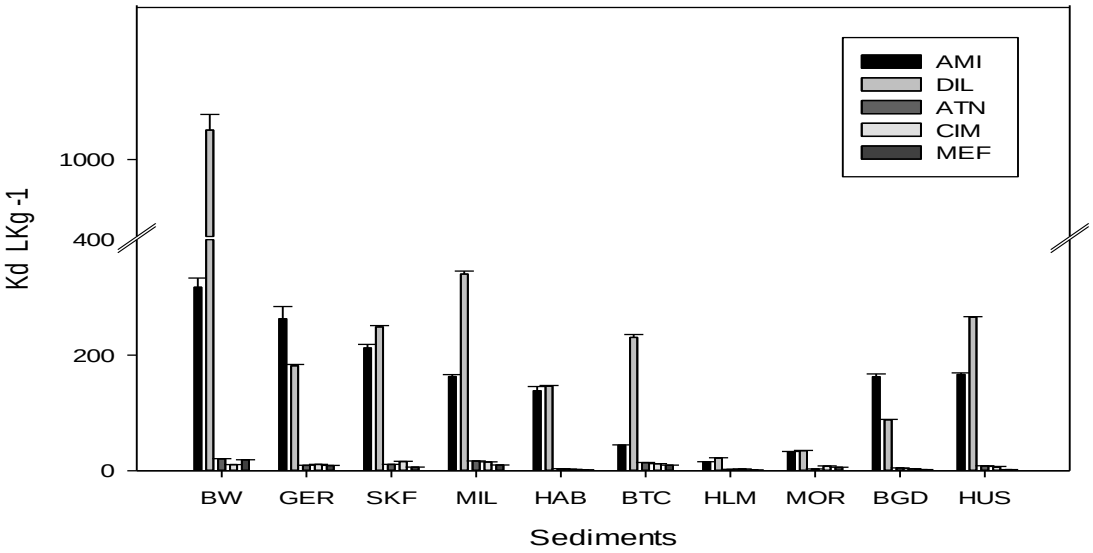


Figure 5.2 Kd values (± 1S.D) for the study pharmaceuticals in the ten different study sediment

Table 5.1 Comparison of Kd and Koc values measured for pharmaceuticals in sediments in the current study with predictions using the model of Franco and Trapp (2008) and other experimental data on sorption to environmental matrices reported in the literature

Compound	Measured		Predicted [a]		Literature		Matrix	Reference
	Kd	Koc	Kd	Koc	Kd	Koc		
Amitriptyline	147.9 (8.79-247.97)	2818.38 (912.01-12589.25)	905.71 191.13-1857.02	19382.42 18757.7-19517.3	2343±292-5694±684	6025.6-11481.5	sludge	(Stevens-Garmon et al., 2011)
					346.7-1318.3	1621.8	sludge	(Hyland et al., 2012)
					138	3630.8	soil	(Franco and Trapp, 2008)
					1049	3388.4	sludge	(Franco and Trapp, 2008)
					4100, 2800	-		(Hörsing et al., 2011) (Lajeunesse et al., 2012)
					2600-26000	-		
Atenolol	9.31 (2.22-20.56)	197.51 (85.11-489.78)	1040.03 219.08-2148.2	22249.68 21699.05-22367.65	<30-46	77.6-91.2	sludge	(Stevens-Garmon et al., 2011)
					15	398.1	soil	(Franco and Trapp, 2008)
					8.1±0.6	1000	sediment	(Yamamoto et al., 2005)
					1.3±0.3-8.1±0.6	310±60-1700±400	sediment	(Yamamoto et al., 2009)
					7.93	0.56-12.68	sediment	(Yamamoto et al., 2009)
					0.85-4.08	-	sediment	(Schaffer et al., 2012b)
					460-1900	-	sludge	(Hörsing et al., 2011)
Cimetidine	8.73 (2.28-15.88)	199.07 (102.33-426.78)	45.63 6.78-92.67	1123.62 210.05-2229.1	199.5-616.6	724.4	sludge	(Hyland et al., 2012)

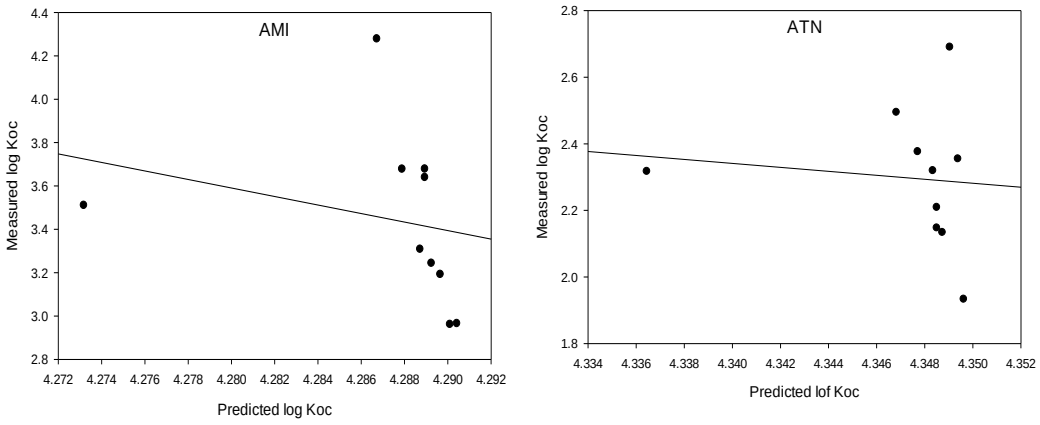
Table 5.1 (Continued)

Compound	Measured		Predicted [a]		Literature		Matrix	Reference
	Kd	Koc	Kd	Koc	Kd	Koc		
					11	301.1	soil	(Franco and Trapp, 2008)
					22	-	sediment	(Furlong et al, 2004)
					142-188	-	sediment	(Williams et al., 2006)
					17	-	soil	
Diltiazem	258.19 (22.03-1022.6)	4265.79 (799.24-13182.57)	16.64 3.98-30.86	370.41 236.61-412.13	53	-	sediment	(Furlong et al, 2004)
					190-869	-	sediment	(Williams et al., 2006)
					140	-	soil	
					440	-	sludge	(Hörsing et al., 2011)
					125.9-501.2		sludge	(Narumiya et al., 2013)
Mefenamic acid	6.64 (1.83-19.04)	149.04 (75.86-331.13)	3.0 0.63-6.33	64.06 64.0-64.11	294±379-434 ± 304	-	sludge	(Radjenović et al., 2009)
					12±2-20±5	580±60-27000±7000	sediment	(Yamamoto et al., 2009)
					21	-	Soil	(Franco and Trapp, 2008)
					17	-	soil	(Narumiya et al., 2013)
					630.9-5011.9		sludge	

[a] Franco and Trapp (2008)

5.3.2 Evaluation of Existing Predictive Model for Sorption

Generally, for each study pharmaceutical, the variability in predicted Koc across sediments obtained using the model of Franco and Trapp (2008) was lower than the variability observed in the experiments (Table 5.1). Experimentally obtained log Koc values varied by more than one log unit for all tested pharmaceuticals across the different sediment. On the other hand, the narrow range in predicted log Koc values may be related to the assumption that the tendency of a molecule to penetrate into the organic matter is proportional to its Kow. Moreover, the model does not consider the variability of soil pH, which may in some case limit the accuracy of estimates. The model tended to over-predict the sorption of the basic compounds and under-predict the sorption of the acids. No correlation between predicted and measured Koc was observed except for cimetidine (Figure 5.3). This is unsurprising as the properties of the sediments investigated in this study fall outside the applicability domain specified by Franco and Trapp for their model in terms of the relationship between soil organic carbon content and clay%. It is important to also recognize that this is a model for soils so may not be directly transferrable to sediments (Boxall and Ericson, 2012). Therefore, sorption models that consider specific properties of the sorbate and sorbent are probably needed to describe the partitioning of ionisable chemicals in the environment (Franco and Trapp, 2010).



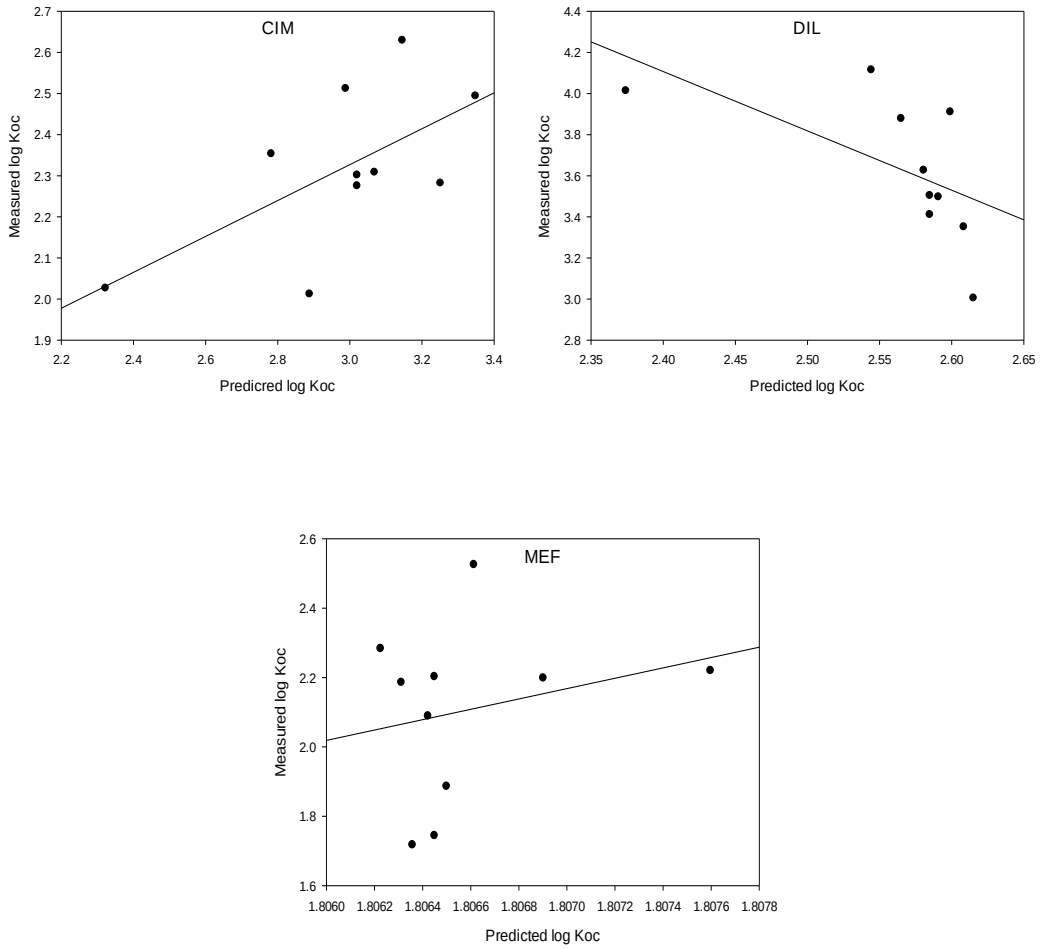


Figure 5.3 Correlation between experimentally obtained log Koc values and log Koc values predicted using the Franco and Trapp (2008) model for the study pharmaceuticals in the ten study sediments

5.3.3 Multiple Linear Regressions for Kd Prediction

As the Franco and Tapp, (2008) model did not perform well for the study pharmaceuticals and sediment systems being investigated, studies were done to explore whether it is possible to model the sorption behavior of each study pharmaceutical based on sediment properties. This approach has been used for other ionisable compounds in different environmental matrices (Kah and Brown, 2007; Kodešová et al., 2015). Multiple linear regression analysis was performed to explore relationships between sediment and pharmaceutical properties and sorption coefficients (Kd) for each individual pharmaceuticals. The best performing regression models for each study

compound are shown in Table 5.2. Combinations of only significantly correlated properties (sediment and pharmaceutical) were selected by the software package. In the case of cimetidine (clay %, OC %) and diltiazem (log Dow, Ex.Ca^{2+}), a combination of properties resulted in the best prediction of Kd with R^2 values of 0.922 ($p < 0.001$) and 0.956 ($p < 0.001$), respectively. The regression equations for amitriptyline, atenolol and mefenamic acid only included a single descriptor with Log D being found to be one of the strongest predictors of sorption behaviour chosen by the software.

To evaluate the developed regression equations, we applied them to sediment types that have been used for the study compounds elsewhere in the literature (Figure 5.4). For cimetidine and mefenamic acid, there were limited data in the literature for this evaluation. For atenolol, there was enough data to allow comparison, while amitriptyline has no previous adsorption study in sediment and exchangeable calcium cation (EX Ca^{2+}) in sediment have not been listed in literature when sorption of diltiazem was studied. The equation based on CEC for atenolol sorption resulted in a close match to Kd values for atenolol reported by Yamamoto et al. (2009), Martínez-Hernández et al. (2014) and Schaffer et al. (2012) with $R^2= 0.72$ and $p < 0.001$. For mefenamic acid and cimetidine, the regression equation failed to predict the literature Kd values for both compounds with ($R^2= 0.07$, $p <0.05$) and ($R^2= 0.3$, $p < 0.05$). The wider applicability of some of the regression equations is uncertain and further experimental evaluation is needed before strong conclusions can be made as to the predictive power of the relationships.

Table 5.2 Multiple-linear regression equations for predicting Kd values from sediment properties and sediment-specific physicochemical properties of a pharmaceutical

Compound	Predictor	R^2	Regression equation [a]
Amitriptyline	Log Dow	0.793**	Kd= -349.2+ 190.06 (log Dow)
Atenolol	CEC	0.731**	Kd= -0.445+ 0.49 (CEC)
Cimetidine	Clay, OC	0.922***	Kd= 2.4+ 0.198(%clay) +0.744 (%OC)
Diltiazem	Log Dow, $Ex.Ca^{2+}$	0.956***	Kd= -902.75+ 543.4 (log Dow)+8.018 ($Ex.Ca^{2+}$)
Mefenamic acid	OC%	0.621**	Kd= -0.044+ 1.425 (%OC)

[a] Regression equation only for significantly correlated properties. *p<0.05, **p<0.01, ***p<0.001.

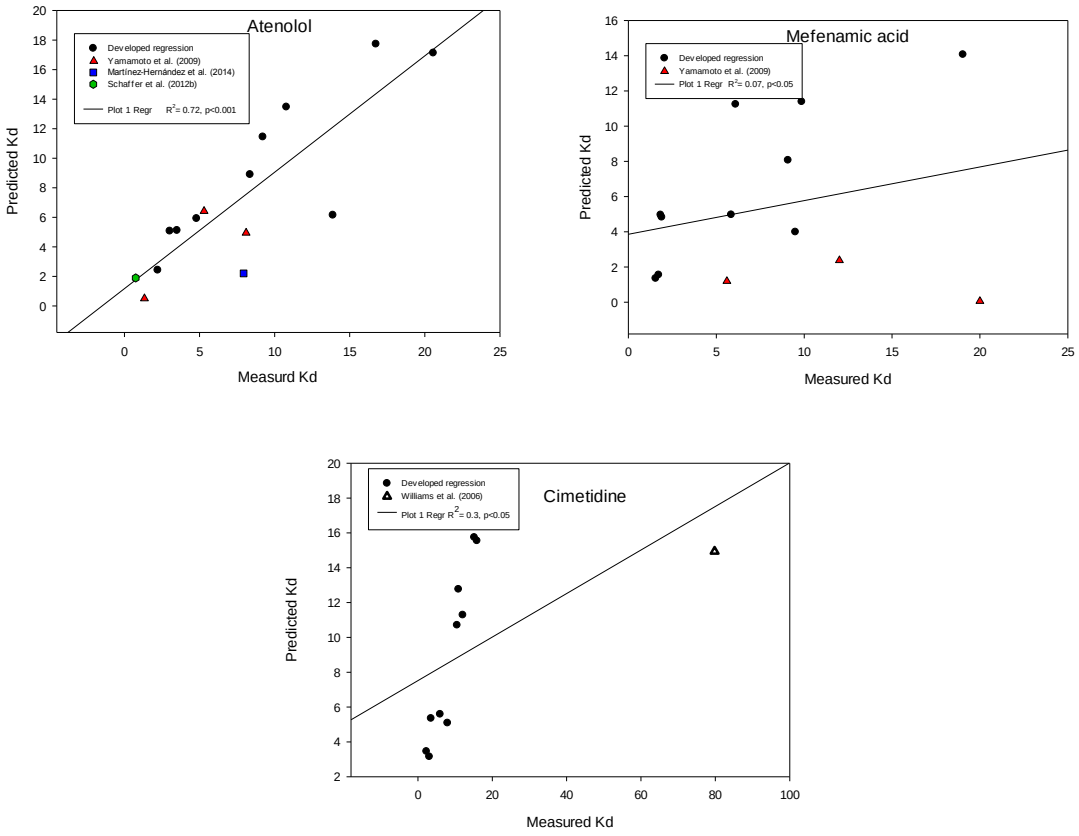


Figure 5.4 Correlation between Kd predicted by developed method and measured values from this study and literature

5.3.4 Suggested Mechanisms of Interaction

Potential Mechanisms for the adsorption of selected pharmaceuticals, and how they are influenced by properties of the compound and the sediment properties are shown in Table 5.3 and Figure 5.5. For amitriptyline, the only property extrapolated from multiple regression models to best explain the variability in sorption across sediment types was the log Dow. This suggests that the hydrophobic interaction of the non-ionised form of this cationic pharmaceutical is the dominant sorption mechanism for amitriptyline. Sorption was also correlated with CEC and concentrations of selected cations (Table A.C3, Appendix C); so these properties may also be contributing to sorption and additional mechanism such as electrostatic interactions between sorbent and substance is also possible (Hyland et al., 2012; Stevens-Garmon et al., 2011). The sorption of mefenamic acid and atenolol across sediment types appeared to be dependent on OC% and CEC respectively. Mefenamic acid is highly dissociated at natural pH values; and when the carboxylic group deprotonates, the negatively charged species become dominant (Narumiya et al., 2013). This may lead to electrostatic repulsion between mefenamic acid molecules and the negatively charged sediment particles which might explain why this compound is not highly adsorbed by sediments (Araujo et al., 2011). The bonding mechanism seems to be much more complex than simple hydrophobicity and hydrogen bonding though suggesting another interaction mechanism such as bridging between COOH group and exchangeable cations on clay or organic matter (Araujo et al., 2011; Nowara et al., 1997; Tolls, 2001). The extent and strength of this coordination depends on the nature of the cation that saturated the clays (Kah and Brown, 2006).

With a pKa of 9.6, atenolol is predominantly positively charged at environmental pH values. The main suggested sorption mechanisms of atenolol in the literature are electrochemical interaction and ion exchange (Martínez-Hernández et al., 2014; Rakić et al., 2013; Ramil et al., 2010; Schaffer et al., 2012a), and could be via charge transfer interaction due to the structure of the molecule, with its electron donor atoms (two nitrogen atoms and one oxygen form OH group) or hydrogen bonding interaction (Rakić et al., 2013; Silva et al., 2011). Schaffer et al., (2012a) found that 99% of the total sorption of atenolol was by cation exchange interaction. On

the other hand, Williams et al., (2009) found that atenolol sorption is concentration dependent due to 1/n value <1 which is similar to the adsorption behaviour on sediments in this study except for HAB sediment. Despite the significant correlation to different sediment properties, CEC in this study seemed to be the most important driver of sorption.

For diltiazem, sorption was found to depend on log Dow and sediment exchangeable Ca^{2+}. The relationships with Dow is likely explained by hydrophobic interactions of the neutral species with sediment organic matter (Hyland et al., 2012; Wegst-Uhrich et al., 2014). Additionally, higher concentration of exchangeable divalent cations (e.g. Ca^{2+}) adhering to the surface of sorbent increase the sorption of pharmaceuticals greater than monovalent cations (K^{+}) via ion-exchange interaction (Bui and Choi, 2010; Tolls, 2001; Wang and Wang, 2015).

The Kd of cimetidine is positively impacted by clay% and OC%. Hydrophobic interaction with organic matter and hydrogen bonding probably play a greater role in the sorption process due to the presence of a greater neutral form fraction. In addition, basic ionisable compounds are known to interact to clay fraction via electrostatic interaction to surface particles (Delle Site, 2001; Kah and Brown, 2007). However, the high surface area of clay leads to an increase in the number of available sorption sites (Kodešová et al., 2015).

Table 5.3 Potential mechanisms for the adsorption of pharmaceuticals and how they are influenced by properties of the compound and the sediment

Compound	Potential Mechanisms	Type of interaction	Pharmaceutical properties	Sediment properties	Ranking according to sorption affinity
Amitriptyline	Hydrophobic interaction	Partitioning	Hydrophobicity	High OC%	2
Atenolol	Cation exchange	Nonspecific electrostatic interaction	Basicity	Concentration of exchangeable cations	3
Cimetidine	Hydrophobic interaction	Partitioning	Hydrophobicity	High OC%	4
	Cation exchange	Nonspecific electrostatic interaction	Basicity	Concentration of exchangeable cations	
Diltiazem	Hydrophobic interaction	Partitioning	Hydrophobicity	High OC%	1
	Cation exchange	Nonspecific electrostatic interaction	Basicity	Concentration of exchangeable cations	
Mefenamic acid	Cation bridging	Inner-sphere complex	Anionic, low valence functional group	High-valence exchangeable cations	5

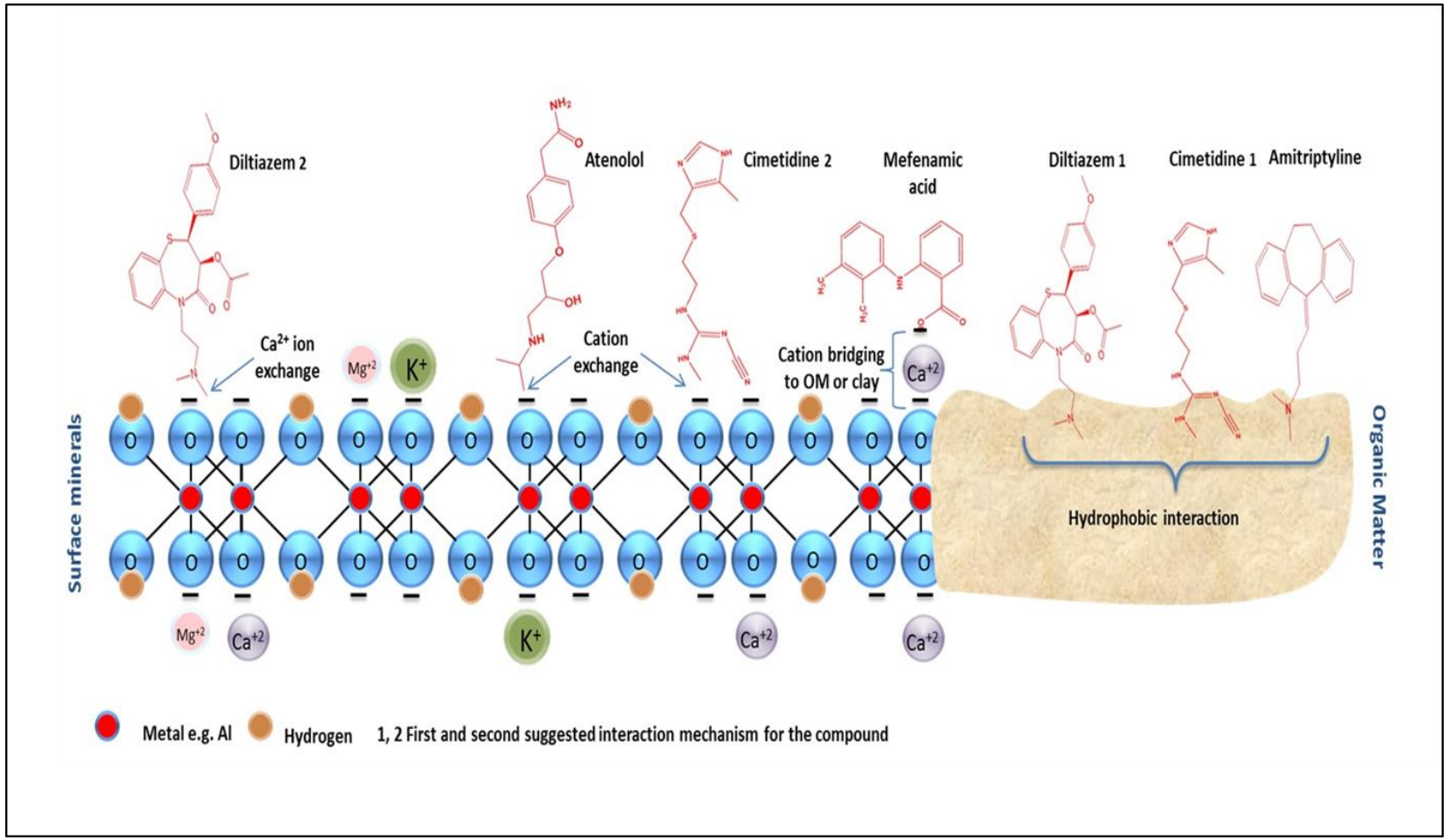


Figure 5.5 Suggested mechanisms of interaction between pharmaceuticals and sediment at environmental pH

5.4 Conclusion

This study investigated the sorption of five pharmaceuticals with different physicochemical properties onto ten different sediments. The study showed that organic carbon content is not the only predominant factor controlling the sorption behaviour in sediments with high variability in CEC and texture content. Multiple linear regressions showed that the Kd prediction using proposed models depended on a combination of OC% and clay% in the case of cimetidine and Log Dow and exchangeable cations (Ca^{2+}) for diltiazem. Single predictors were chosen to predict the sorption of amitriptyline, atenolol and mefenamic acid respectively across sediment types. The validity of the proposed regression equations was tested using independent data and gave good results for atenolol. The model evaluation indicated that the models performed poorly for mefenamic acid and cimetidine. Overall, the results demonstrate how complex the processes driving the sorption of pharmaceuticals in sediments are. Much more work of this type is needed before we can fully understand the interplays between pharmaceutical and sediment properties and sorption. In the future, we recommend that work is done using a wide range of pharmaceuticals and sediments that are well characterized in terms of the properties of the sediment solids and pore water chemistry. Such work could lead to the development of new models that would allow the prediction of partitioning of a wide range of pharmaceuticals at high spatial resolutions. These models will be invaluable for better characterizing the environmental risks of pharmaceuticals in natural systems.

Chapter 6

Effects of Sediment Properties on the Dissipation of Pharmaceuticals in Freshwater Sediments

6.1 Introduction

Alongside sorption, degradation is one of the key processes governing the fate and impacts of organic compounds in the environment. Consequently, an assessment of the persistence of a pharmaceutical is required as part of the environmental risk assessment process during the marketing authorisation of new pharmaceutical active ingredients (EMEA, 2006). Many studies have explored the degradation of pharmaceuticals in wastewaters (e.g. Quintana et al. 2005; Joss et al. 2006), sludge (e.g. Carballa et al. 2007; Radjenović et al. 2009; Li and Zhang 2010) and soils (Lin et al., 2011; Monteiro and Boxall, 2009; Xu et al., 2009). The most important dissipation pathway for pharmaceuticals in the environment is microbial degradation (Fang et al., 2012). The rate and degree of degradation of pharmaceuticals are affected by the environmental conditions such as pH, temperature, the abundance of microorganisms and the presence of biosolids as well as the physicochemical properties of the compound such as the degree of dissociation and lipophilicity (Monteiro and Boxall, 2009). For example, soil texture was found to affect the degradation rates of caffeine, with faster degradation being observed in loam and sandy loam soils compared to a silt loam soil (Topp et al., 2006). Amitriptyline also showed faster dissipation in a loam soil compared to a clay loam soil (Li et al., 2013). The reason may due to the higher sorption affinity of amitriptyline as a cation to the negatively charged surface of clay and thus affect the bioavailability of the compound to the microorganisms. Benotti and Brownawell (2009) reported variations in degradation rates for 19 pharmaceuticals in estuarine and coastal surface water samples and suggested that faster rates of pharmaceutical degradation occurred in waters with a greater abundance of total bacteria or the presence of microbial communities that are better able to transform these compounds. While

data are available on the persistence of pharmaceuticals in soils and surface waters, few studies have investigated the behaviour and degradation of pharmaceuticals in sediments, even though this compartment is considered as a sink for many pharmaceuticals, especially cationic pharmaceuticals (Boxall and Ericson, 2012; Loffler et al., 2005; Löffler and Ternes, 2003).

The studies that have been done on sediments have focused on anti-inflammatory compounds, antibiotics, and lipid regulators. Conkle et al. (2012) focused on the degradation of selected pharmaceuticals under aerobic and anaerobic conditions in wetland sediments. Carbamazepine was found to be highly persistent with half-lives between 165-264 d under aerobic conditions, and these were increased by factors of 1.5-2.5 under anaerobic conditions. Ibuprofen and gemfibrozil showed relatively short half-lives of around 20 d under aerobic conditions and these increased by factors of 11-34 under anaerobic conditions. Ibuprofen showed low half-lives ranging from 1.2 to 2.5 d depending on the flow rate of overlying water (Kunkel and Radke, 2008). These findings show that hydraulic conditions can drive the rate of pharmaceutical degradation in sediment. Thuy and Loan (2014) studied the fate of antibiotics (ciprofloxacin, griseofulvin, and rifampicin) in water only and a water-sediment system. The half-lives of the antibiotics in the water system (8-20 d) were shorter than in the water-sediment system (23-39 d).

Although previous studies have reported the degradation of selected pharmaceuticals in sediments, limited information is available on the effects of sediment characteristics on the persistence of these molecules. Therefore, the aim of the work described in this chapter was to characterise the dissipation of six human-use pharmaceuticals with different chemical properties and therapeutic usages in ten freshwater sediments with a wide range of physicochemical properties. The resulting data were then used to explore relationships between degradation rates and physicochemical properties, which can offer a better understanding of the factors that influence dissipation of pharmaceuticals in sediment. This could help to better inform the environmental risk assessment of pharmaceutical active ingredients.

6.2 Materials and Methods

6.2.1 Chemicals and Solvents

Analytical grade (≥98% purity) amitriptyline hydrochloride, atenolol, cimetidine, diltiazem hydrochloride, mefenamic acid and ranitidine were purchased from Sigma-Aldrich (UK). Chemical structures, physicochemical properties and therapeutic uses of the selected pharmaceuticals are given in Chapter 1 (Table 1.1). Solvents (acetonitrile, methanol, acetone, ethyl acetate and water) were of high-performance liquid chromatography (HPLC) grade (Fisher Scientific). Ammonium hydroxide solution (35%) was purchased from Fisher Scientific. Formic acid (96 %), 2,3,5-Triphenyl-tetrazolium chloride (TTC) solution, tris (hydroxymethyl) ammoniomethane and triphenylformazane (TPF) were obtained from Sigma-Aldrich (UK). Stock solutions of the reference compounds were prepared in methanol and stored at −20 ^{o}C until use.

6.2.2 Sediment Collection and Characterisation

Sediments used in this study were the same as those used in Chapters 4 and 5 (full details are provided in Chapter 4, Section 4.2.2, Table 4.1). Degradation studies were performed within one to three months of sediment collection.

6.2.3 Degradation of Pharmaceuticals

Aerobic degradation studies were performed using sterilised and non-sterilised sediments following the method of Ying and Kookana (2003). The persistence of the pharmaceuticals in all sediment types was investigated under non-sterile conditions, while four sediments, selected to give a range of extremes of sediment characteristics, were used for the sterile studies. In brief, 5 g (dry weight equivalent) of sediment were weighed into 40-mL screw capped amber glass vials. The ratio of sediment to solution was 1:1. Samples were pre-incubated for 6 d, in the dark. The samples (three replicates) were then spiked with 50 µL of the standard solution (containing

20 mg L^{-1} of each pharmaceutical) to give a nominal concentration of 200 ng g^{-1} (dry weight) for each pharmaceutical. Vials were thoroughly shaken for 30 seconds, and placed in an incubator in the dark at 20 ± 2 ^{o}C. Vials were loosely capped to avoid contamination while allowing air exchange. A headspace of about 60% of the vials height was used to provide sufficient headspace to ensure aerobic conditions.

Sterilisation of the sediments was achieved by autoclaving (Prestige medical, UK), at 120 ^{o}C under 300 kPa for 30 min three times over three consecutive days. To confirm the sterility of the autoclaved sediments, the microbial activity of the sediments was tested following the method described in the next section. Due to possible water loss during the incubation, sample vials were regularly weighed to monitor the water content of the water-sediment system and, if necessary, water content was adjusted with HPLC grade water. After each addition, samples were gently shaken (without resuspension of sediment fines to avoid the disturbance of sediment-borne microorganisms (Abia et al., 2017)) to ensure aerobic conditions. Triplicate subsamples of the sterilised and non-sterilised sediment were withdrawn at 0, 3, 7, 14, 28, 56 and 90 d following the start of the study and immediately stored in a freezer at – 22 ^{o}C until analysis (analysis occurred within one week).

6.2.4 Sediment Bioactivity

The bioactivity of sediments in the degradation experiments was measured by using TTC solution (0.1 g, distilled water: 10 ml) to measure dehydrogenase activity in living organisms which is an indicator of sediment microbial activity (Monteiro and Boxall, 2009). Subsamples of each sediment type (from day 90 of the incubation) were incubated with 5 ml (0.5% by weight) of colourless TTC at 30 ^{o}C in 0.1M tris buffer (tris (hydroxymethyl) ammoniomethane) adjusted to pH 7.6. The colourless TTC is reduced to red water-insoluble TPF by the dehydrogenase enzyme in bacteria. After incubation for 24 hours, the TPF was extracted with 25 mL of acetone. The samples were then agitated for 1 hour at 250 oscillations min^{-1} and centrifuged at 2500 g for 10 min. The absorbance of the supernatant was then measured at 485

nm using an Ultraviolet-visible (UV-Vis) spectrophotometer (160 Spectrophotometer, Shimadzu, Japan). The absorbance measurements were converted to bioactivity (mg TPF kg^{-1}) based on a calibration curve developed from a set of TPF standards. Microbial bioactivities measured in sediment samples are listed in Table 6.1

Table 6.1 Microbial activity in study sediment (mg TPF /kg sediment) using UV-Vis at 485 nm

Sediment	BTC	BW	MIL	GER	HLM	MOR	HAB	SKF	BGD	HUS
Bioactivity (day 90, non-sterile sediments)	5161.1	5521.0	280.2	2630.8	825.3	4767.2	404.1	4256.0	258.1	428.4
Bioactivity (sterile sediments at day 0)	<LOD	-	-	<LOD	<LOD	-	-	-	<LOD	-

Sediments from England were collected from Buttercrambe (BTC), Bishop Wilton (BW), Millington (MIL), German beck (GER), Helmsley (HLM) and North Yorkshire Moors National Park (MOR), all in North Yorkshire; and Harborough (HAB) and Skeffington (SKF) in Leicestershire. The sediments from Iraq were collected from the Tigris River in Baghdad (BGD) and the Alhussainya River (HUS) in Karbala city.

6.2.5 Extraction of Pharmaceuticals and SPE/Clean-up

The study pharmaceuticals were extracted from the test sediments using sonication-assisted extraction and extracts were then cleaned-up using solid phase extraction (SPE) according to the procedures previously presented in Chapter 4.

The performance of the analytical method (precision, accuracy, LOD and LOQs) which employed liquid chromatography tandem mass spectrometry (LC-MS/MS) using an Applied Biosystems/MDS Sciex API 3000 triple quadrupole mass spectrometer interfaced with a Dionex UltiMate1 3000 HPLC is described in Chapter 4 (see Table A.B3 in the Appendix). The stability of the study pharmaceuticals during storage was also checked. Recoveries from freshly spiked high microbial activity sediment (BTC sediment) were compared to equivalent spiked sediments at day 0 that had been stored in a freezer for 90 days. The recoveries obtained for all pharmaceuticals from the frozen samples were approximately the same as those of the freshly spiked samples showing negligible concentration changes (Table A.D3, Appendix D).

6.2.7 Data Analysis

The concentrations of pharmaceuticals in the sediment systems were plotted against time of incubation using Microsoft Excel 2010 software. The degradation rate constant k (per day) was then estimated by fitting a first-order exponential decay model to the data. The times for 50% (DT50) and 90% (DT90) dissipation were then estimated.

6.2.8 Statistical Analysis

Statistical analyses (ANOVA and Multiple linear regression (MLR)) were performed using the SPSS 23.0 statistical software package with the significance level being $p<0.05$. Prior to the statistical analyses, the normality of the data was first evaluated using the Kolmogorov-Smirnov and Shapiro-Wilk methods. All variables were found to be normally distributed ($p>0.05$), except microbial activity and sorption coefficients (Kds) for diltiazem and mefenamic acid in the MLR analysis, these were therefore normalized using logarithmic transformations. Two way-ANOVA was used for each sediment and pharmaceutical to explore differences between concentrations in sediment over time. Stepwise MLR analysis was employed to find relationships between degradation rate as the dependent variable and combinations of sediment physical-chemical property parameters as the explanatory variables. The general form of the regression equations is described in Equation 1:

$$Y=b_0 + b_1X_1 + b_2X_2+ \ldots.. + b_5X_5 + \ldots.. +b_nX_n \quad (1)$$

Where Y is the dependent variable representing degradation rate (k), b_0 is the intercept, $b_1 \ldots b_n$ are regression coefficients, and $X_1–X_n$ are independent variables referring to the chosen predictors.

6.3 Results and Discussion

6.3.1 Degradation of Pharmaceuticals in Sediment

The dissipation of pharmaceutical concentrations in sediments over time is plotted in Figure 6.1 for the non-sterile treatments and the sterile treatments. Calculated times for half of the compound to be removed (DT50) for sterile and non-sterile treatments are summarised in Table 6.2. Associated first-order degradation rate constants (k) and DT90 values are given in the Appendix (Tables A.D3 and A.D4). In the non-sterile sediments, no lag phase was observed for the pharmaceuticals, and the degradation of pharmaceuticals in the ten sediments was well described by the first-order exponential decay model. Relatively poor fits of the dissipation curves were seen for amitriptyline and atenolol in SKF sediment and for mefenamic acid in BGD sediment. This may be related to the rapid dissipation during the first 7-14 days of incubation. There were some marked differences between sediments in their ability to degrade different pharmaceuticals even where test sediments had similar characteristics. For example, BGD and HUS sediments both have a silt loam texture and sediments MOR and HAB have a loamy sand texture. All six pharmaceuticals showed moderate persistence, with DT50 values ranging from 9.5 (atenolol) to 78.8 (amitriptyline) days. In general, the degradation half-lives of pharmaceuticals decreased in the order amitriptyline > mefenamic acid > diltiazem > cimetidine > ranitidine > atenolol in sediments. The dissipation of all six compounds in the four sediments tested under sterilised conditions was also found to follow the first-order exponential decay kinetics (Figure 6.1). Generally, the dissipation of pharmaceuticals in the sterile sediments was slower than in the non-sterile systems indicating that biodegradation was mainly responsible for the observed dissipation of the study pharmaceuticals. Findings for the individual pharmaceuticals are discussed below.

Table 6.2 Degradation rate constants (k, day^{-1}) and calculated half-lives (in days) for the study pharmaceuticals in sediments under non-sterilized (based on ten sediments) and sterilised (based on four sediments) conditions

Compound		Non-sterilised				Sterilised			
	Kinetics	k, d^{-1}	Median	Min.	Max.	k, d^{-1}	Median	Min.	Max.
Amitriptyline	First order	0.0088-0.0156	62.2	44.4	78.8	0.0065-0.0104	90.6	76.2	106.6
Atenolol	First order	0.0398-0.073	13.0	9.5	17.4	0.024-0.0337	23.5	20.5	28.8
Cimetidine	First order	0.019-0.0638	27.6	10.9	36.5	0.0128-0.0246	42.5	28.2	54.1
Diltiazem	First order	0.0196-0.032	26.7	21.7	35.4	0.0088-0.012	65.2	57.7	78.7
Mefenamic acid	First order	0.0198-0.0351	29.3	19.7	35.0	0.0114-0.0155	54.7	44.7	60.9
Ranitidine	First order	0.0185-0.0683	16.7	10.1	37.5	0.0148-0.0193	40.9	35.9	46.8

Amitriptyline

Under non-sterilised conditions, the dissipation of amitriptyline was described well by the first order kinetic model and the dissipation was slow compared to the other pharmaceuticals (Figure 6.1). DT50 values for the compound ranged from 44.4 to 78.8 d and DT90 values from 147.6 to 261.4 d (Table A.D3, Appendix). Significant differences in dissipation were observed across the sediment types ($F= 45.3$; $P<0.001$). Under sterile conditions, half-lives for amitriptyline were higher (Table A.D4, Appendix D). The half-lives of amitriptyline in BGD and HLM sediment increased from 66.6 to 106.6 d and from 78.8 to 105.0 d, respectively, indicating the importance of microorganisms for amitriptyline dissipation in these two sediments. While, to the best of our knowledge, no literature data are available on the degradation of amitriptyline in sediment, the persistence of the compound has been explored in soil. Our half-lives are similar to those reported in soils with different textures where DT50s ranging from 34.1 to 85.3 d were observed (Li et al. 2013). In this study, the dissipation of amitriptyline was suggested to result from the formation of non-extractable residues and the transformation of the parent compound to nortriptyline (N-desmethyl amitriptyline) and amitriptyline-N-oxide.

Atenolol

Under non-sterilised conditions, atenolol degraded more quickly than the other pharmaceuticals in all sediments over time. Dissipation differed significantly between sediment types ($F= 4.2$ and $P<0.001$). In sterilised treatments, atenolol showed DT50s almost 2-3 times higher than the non-sterile treatments in BTC and GER sediments while in BGD and HLM sediment atenolol exhibited only a small increase in DT50 values (Table A.D4, Appendix D). This behaviour suggests that microbial activity may have contributed less to the observed dissipation in these sediments and that the observed loss was caused by abiotic processes. The observed half-lives for atenolol are greater than the DT50s of between 2.8 and 10.3 d observed by Kodešová et al., (2016) in soil. They showed that dissipation of the compound is slow in soil with a higher adsorption affinity to atenolol. For many of the compounds, data from other studies suggest that the observed dissipation is due to conversion into transformation products. It is important to

recognise that these transformation products could be more stable than the parent compound and also may pose a risk to the environment (Boxall et al., 2004). For atenolol, based on previous work, the observed dissipation may be explained by the conversion of the parent molecule to metoprolol acid which comparatively more stable in water-sediment systems than atenolol (Svan et al., 2016).

Cimetidine

Under non-sterile conditions, sediments showed significant differences in their ability to degrade cimetidine ($F = 9.6$; $p<0.001$) with half-lives ranging from 18.5 d in the HUS sediment to 36.5 d for both the MOR and MIL sediments. With the exception of the HUS sediment, cimetidine was found at measurable concentrations after 90 d of incubation. Dissipation half times under sterile conditions ranged from 28.2 to 54.1 d. The differences in half-lives between sterilised and non-sterilised conditions indicate that microorganisms play a role in the dissipation of the molecule. The degradation of the cimetidine in solid phase environment has not been studied and reported half-lives are only available for seawater with DT50 values ranging from 9.8 to >100 d (Benotti and Brownawell, 2009). Degradation of cimetidine in aqueous solutions is believed to be via photo oxidation or chlorination (Buth et al., 2007; Latch et al., 2003).

Diltiazem

Diltiazem showed moderate dissipation in the study sediments with DT50 values of up to 35.4 d being obtained. Significant differences in diltiazem dissipation were seen across sediment types ($F= 16.0$; $p<0.01$). Slow dissipation was observed for diltiazem under sterilised conditions with half-lives ranging from 57.7 to 78.7 d. Previously reported half-lives of diltiazem were only found for surface water and soil. Benotti and Brownawell, (2009) reported half-lives of diltiazem ranging from 5.5 to 36 d in coastal seawater under non-sterile conditions. Wu et al.

(2010) reported half-lives for diltiazem of 11- 44 and 14- 84 d in soils amended with biosolids under aerobic and anaerobic conditions, respectively. In this same study, biodegradation and soil texture were reported as the main drivers for the observed dissipation of diltiazem.

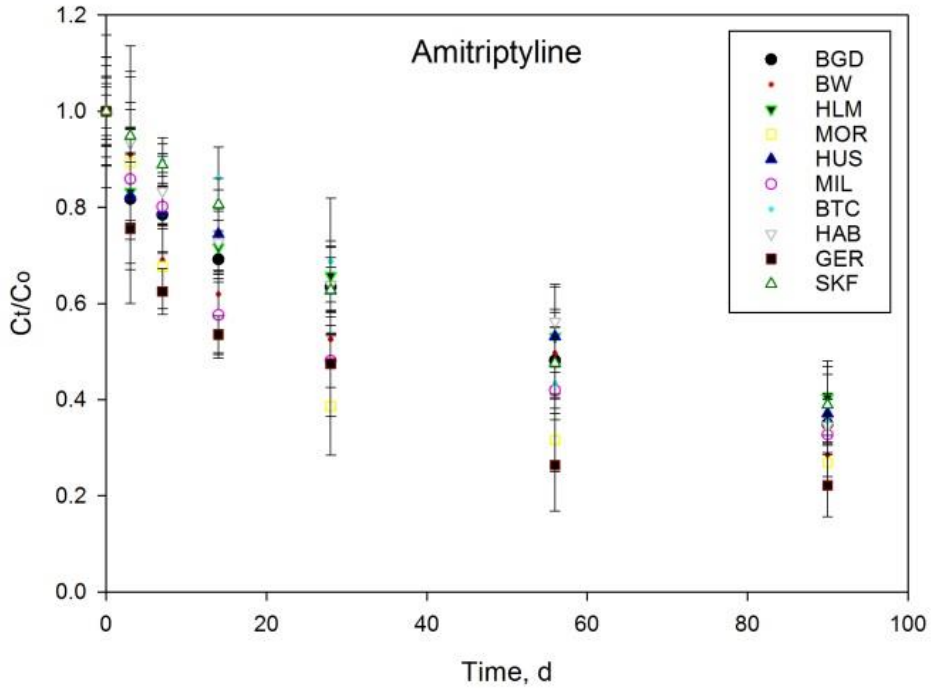
Mefenamic acid

Mefenamic acid displayed significant differences in dissipation between the non-sterile sediments ($F= 11.5$; $p<0.001$) with DT50 values ranging from 19.7 to 35.0 d. Unlike in the non-sterilised studies, degradation curves for the mefenamic acid were characterized by an initial lag phase (day 0–7) in BTC sediment (Figure 6.1). This would most likely be attributed to adaptation of the microbial population. Nevertheless, this lag phase was not observed for the other sediments. Half-lives obtained here agree well with those obtained in lake water under different experimental conditions (filtered and non-filtered water, sunlight and dark) which ranged from 15.5 to 66.6 d (Araujo et al., 2011) and are to the lower end of the range (12.5 to 104 d) found by Yamamoto et al. (2009) in river water sampled from two different urban streams. These higher DT50s, previously observed, are probably explained by the lower abundance of microbial activity found in surface water in comparison to sediment (Boxall and Ericson, 2012).

Ranitidine

For ranitidine, the maximum half-life observed in non-sterilised treatments was in the HAB sediment (37.5 d) and the minimum was 10.1 d in the BW sediment. Significant differences were seen in dissipation across the sediment types ($F=5.8$ and $p<0.001$). In sterilised sediments, with the exception of the BGD sediment, an initial lag phase in the degradation of ranitidine was observed. Half-lives were found to be 1.6 (HLM sediment) to 3.1 (GER sediment) times greater than the non-sterilised treatments (Tables A.D3 and 4, Appendix D) suggesting that biodegradation is the main dissipation mechanism. Half-lives ranging from 15 to 100 d for

ranitidine have been reported in the literature for seawater (Benotti and Brownawell, 2009). Ranitidine has also been characterized as not readily biodegradable in a Zahn–Wellens inherent biodegradability test (OECD 302 B) (Bergheim et al., 2012).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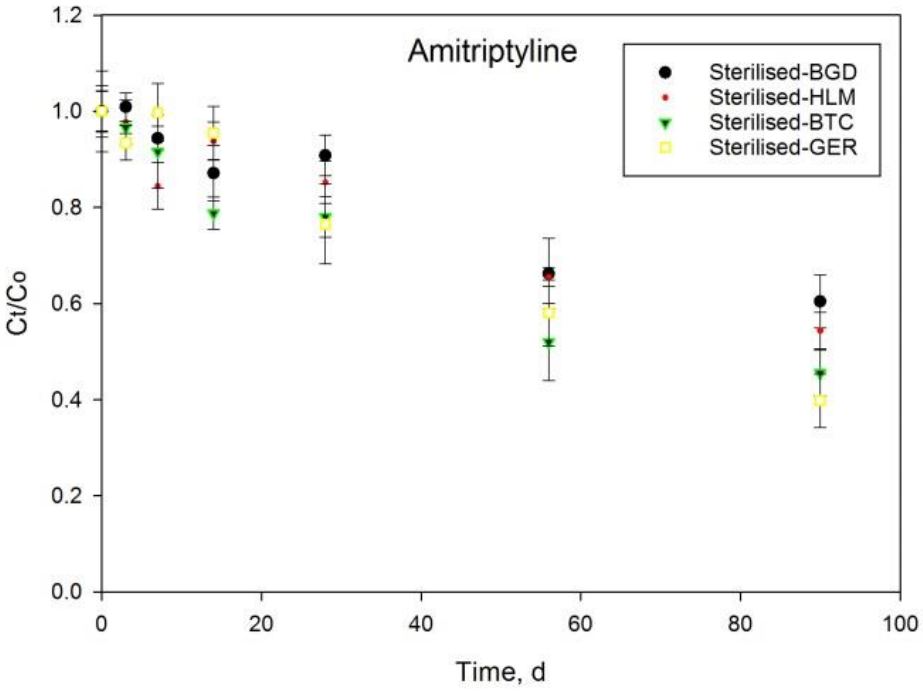
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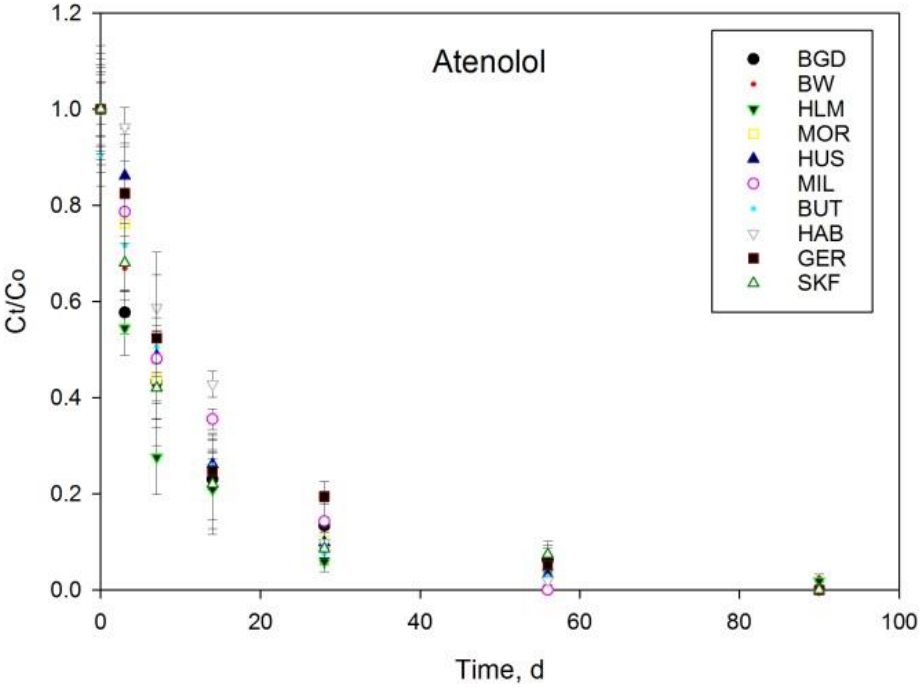
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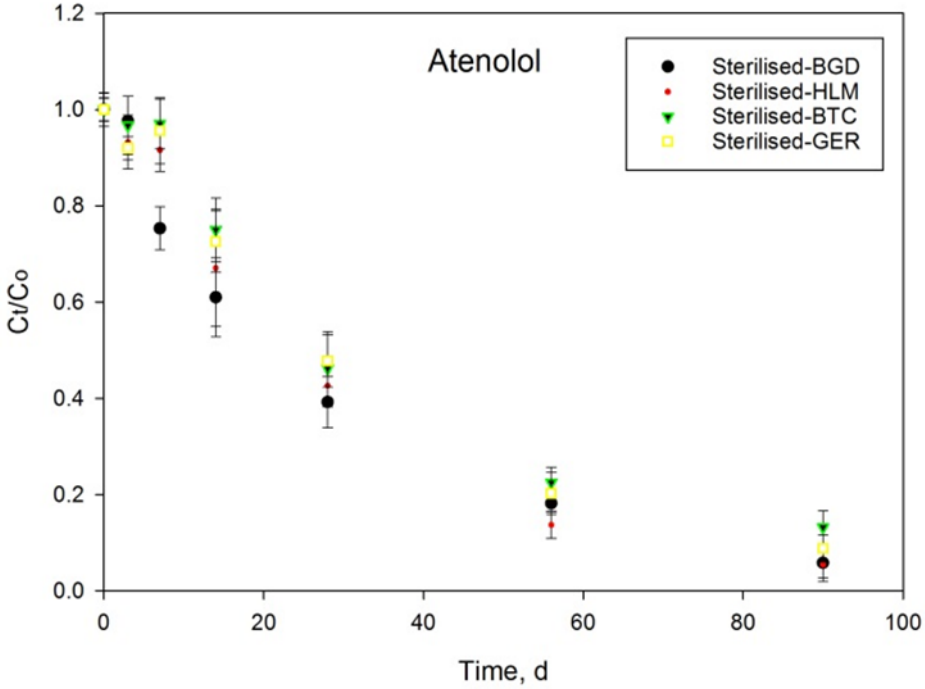
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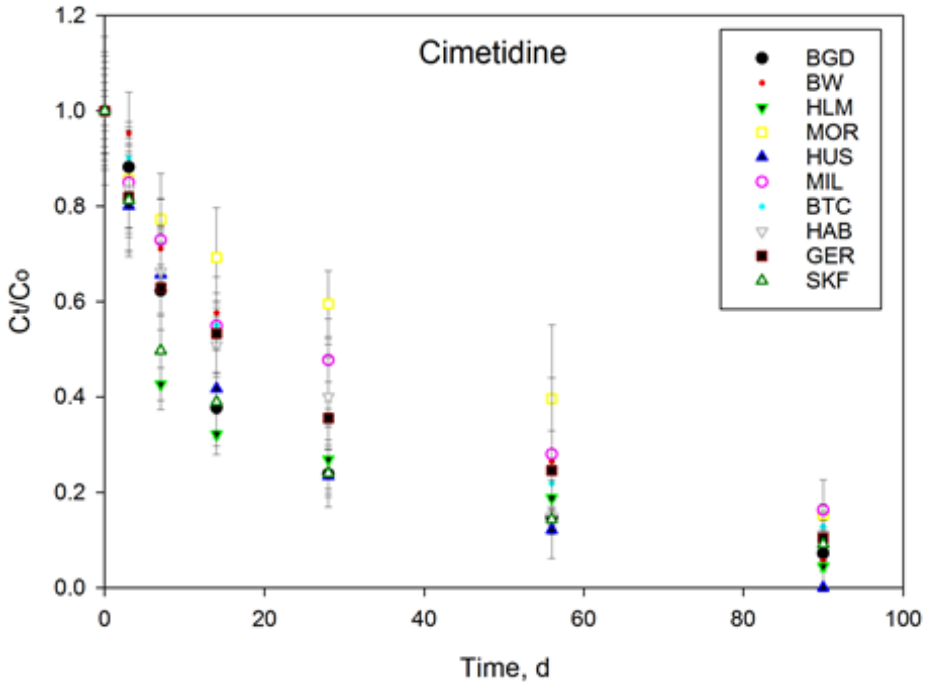
Cimetidine
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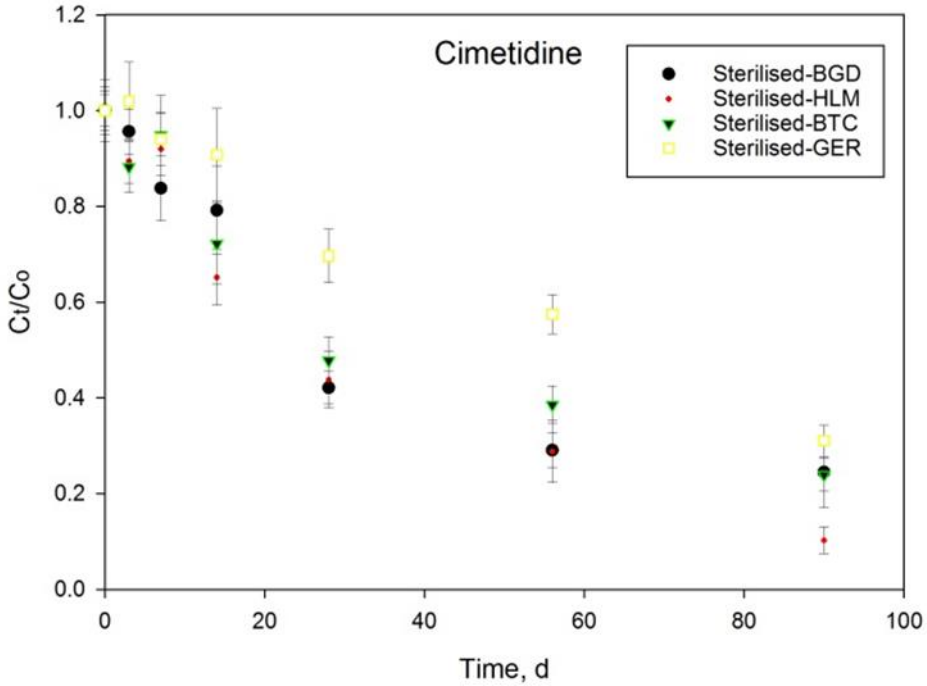
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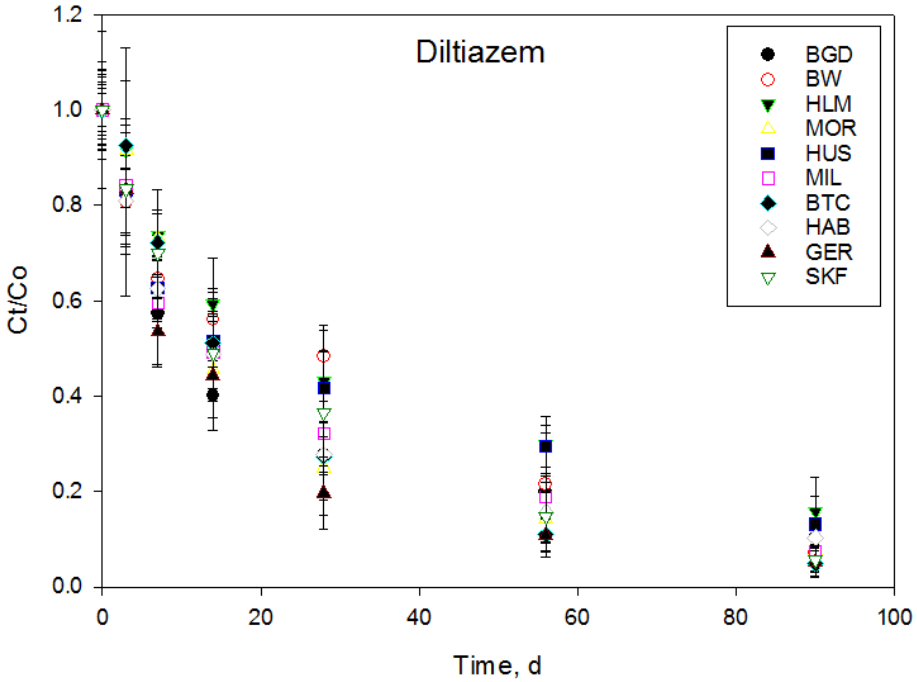
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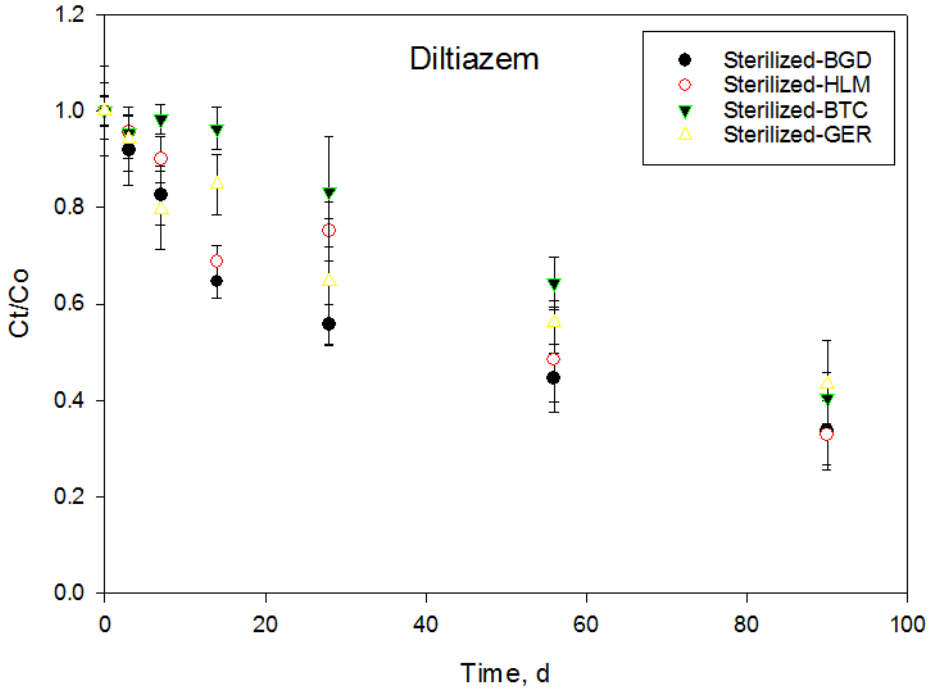
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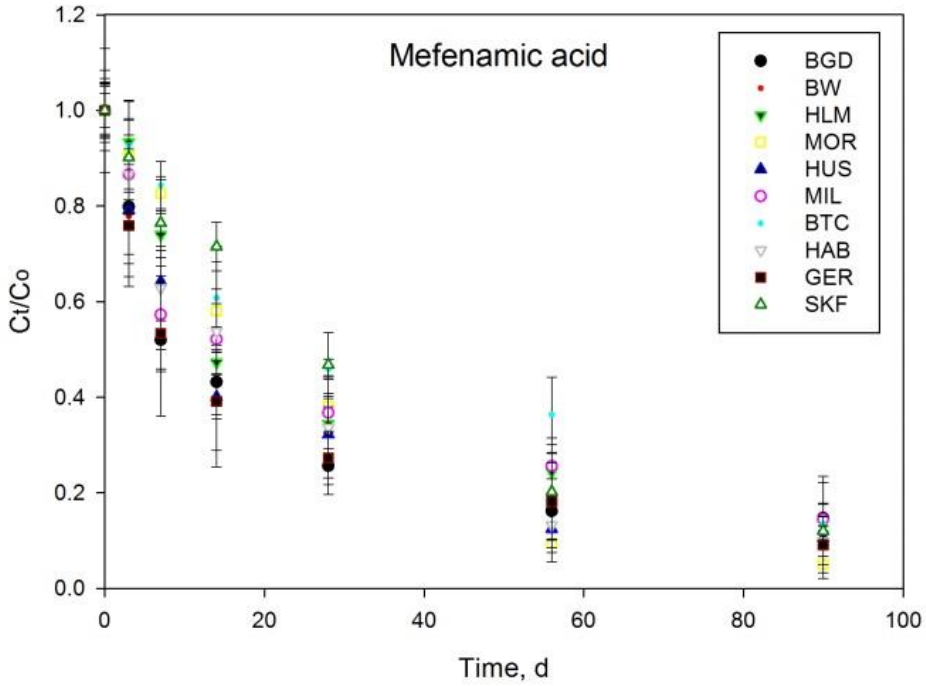
Mefenamic acid
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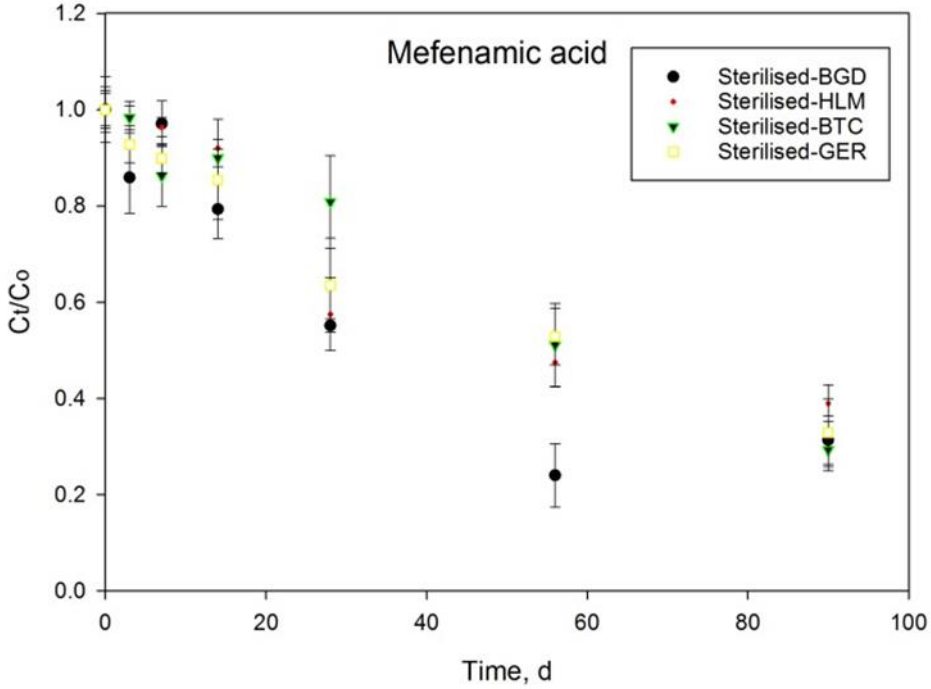
Mefenamic acid
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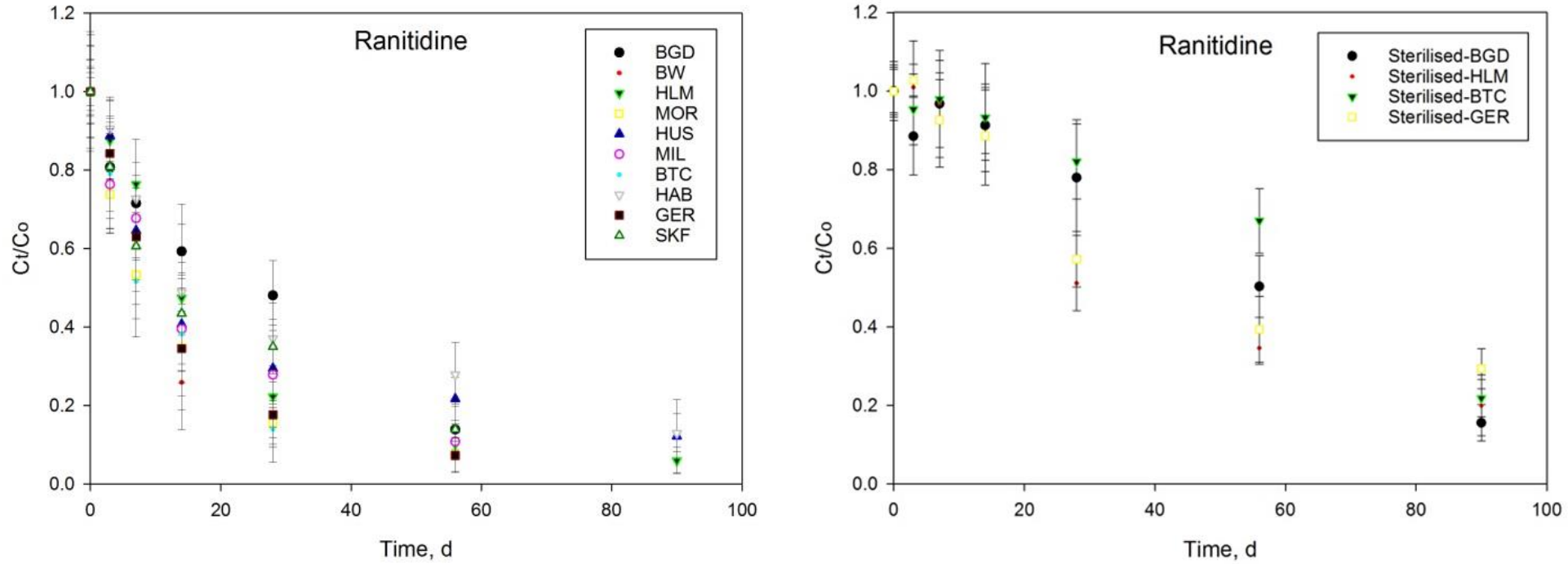


Figure 6.1 Mean concentration (± 1S.D) for pharmaceuticals in the test sediments (four sterilised versus ten non-sterilised sediments) over time, corrected for day 0 concentrations

6.3.2 Multiple Linear Regression Analysis

To better understand the drivers of the observed degradation of pharmaceuticals across sediments, relationships between sediment physicochemical properties, microbial activity and adsorption coefficients and degradation rate were explored using multiple linear regression analysis. Lipophilicity of the pharmaceutical corrected for the sediment pH (Log Dow) was also included. Factors such as the OC content of the matrix, pH and the level of microbial activity have previously been shown to be important parameters determining degradation rates of ionisable compounds (Kah et al., 2007; Xu et al., 2009). The adsorption coefficient (Kd) was also included (obtained from Chapter 5) since adsorption may modify the bioavailability of chemicals (Maqueda et al., 2009). Each pharmaceutical and sediment was considered individually. The best performing regression models for each study compound are shown in Table 6.3.

For diltiazem, the first proposed model only included clay % as the main variable describing degradation (R^2 =0.534; $p<0.05$). The inclusion of the sediment microbial activity (in log form) in the equation improved the fit (R^2 = 0.812; $p<0.01$; Table 6.3). This suggests that biodegradation is a key process in diltiazem dissipation in the tested sediments. The decreasing DT50 of diltiazem with increasing clay content is supported by findings of degradation studies of pesticides and pharmaceuticals in other matrices like soil (Ghafoor et al., 2011; Wu et al., 2012; Xu et al., 2009). Silt % (R2= 0.461, $p<0.05$) was selected as the only descriptor for cimetidine. The result observed in the present study for diltiazem and cimetidine regarding the involvement of clay and silt in the final regression models is expected since in our previous study (Chapter 5) we found that the sorption affinity of the compounds is highly dependent on the log Dow (diltiazem) and OC% and clay% (cimetidine) so the identification of these parameters may be a reflection of the fact that they provide information on the bioavailability of the molecules to the microbes. For ranitidine the first descriptor chosen by the model was microbial activity (R^2= 0.631; $p<0.01$) but when OC% was included, the fit improved (R^2 of 0.869; $p<0.001$). These two descriptors are normally found to dominate the degradation of chemicals since microbial activity would be higher in an OC rich matrix (Maqueda et al., 2009;

Villaverde et al., 2008). None of the sediment parameters was identified by the model to clearly describe the degradation of amitriptyline, atenolol and mefenamic acid. This may be explained by the fact that degradation of these molecules is driven by factors other than those evaluated in this study. Factors such as the diversity structure of the microbial communities in the different sediments and the chemistry of the sediment pore water could be important in determining rates of degradation of the molecules (Boxall and Ericson, 2012). For example, in a study of microbial communities from ten full-scale treatment systems, a positive association between taxonomic and functional biodiversity and the rates of degradation of some compounds was observed (Johnson et al., 2015). Moreover, a study of the environmental fate and transport of wastewater effluent derived organic contaminants (including pharmaceuticals), suggested that indigenous microbial communities may be altered through community-level adaptation to prolonged wastewater discharge, and thereby altering the microbial transformation of these compounds (Blunt et al., 2017).

Table 6.3 Multiple linear regression equations for predicting degradation rates of the study pharmaceuticals based on sediment properties. No relationships were obtained for amitriptyline, atenolol and mefenamic acid

Compound	Predictor	R^2	Multiple regression function
Diltiazem	Clay %Microbial activity	0.821**	k= 0.01+ 0.00017 (clay %)+ 0.005 log (microbial activity)
Ranitidine	Microbial activity OC %	0.869 **	k= 0.16 log (microbial activity)+ 0.02 (OC%)- 0.021
Cimetidine	Silt %	0.461 *	k= 0.022 + 0.00015 (silt %)

** $p < 0.01$, *$p<0.05$.

6.4 Conclusion

The study in this Chapter focused on primary degradation of the study compounds. Results showed some marked differences between the sediments in their ability to degrade different pharmaceuticals. The most persistent compound amongst the pharmaceuticals studied was amitriptyline while atenolol was found to degrade the most quickly. The present study also investigated the effects of a range of variables on the dissipation of targeted pharmaceuticals in environmental freshwater sediment. Results indicated that some pharmaceuticals are amenable to microbial degradation while for others, the dissipation was probably driven by abiotic processes or the formation of nonextractable residues. MLR demonstrated that degradation of pharmaceuticals in sediment is a very complex process and cannot be explained by a single mechanism due to different interactions between different processes that influence the breakdown of pharmaceuticals. Pharmaceuticals with similar structures may also behave differently as shown in the current study for the two antihistamines. Microbial degradation appeared to dominate the dissipation of diltiazem and ranitidine. The factors governing the other pharmaceuticals in sediments were unclear. In the future, we recommend that work is done using a wider range of well characterized pharmaceuticals and sediments. Such work could lead to the development of new models that would allow the prediction of degradation of pharmaceuticals at high spatial resolutions. These models will be invaluable for better characterizing the environmental fate of pharmaceuticals in natural systems.

Chapter 7

Temporal and Spatial Distribution of Pharmaceuticals in Urban River Environments

7.1 Introduction

As discussed in Chapter 2, a large body of literature is available on the occurrence of pharmaceuticals in environmental matrices (e.g. see reviews by Li, 2014; Luo et al., 2014; Pal et al., 2010; Sarmah et al., 2006; and Verlicchi et al., 2012). The significant increase in monitoring studies for pharmaceuticals over the past 20 years is attributable to advances in analytical techniques (Berlioz-Barbier et al., 2014; Jelić et al., 2009). Most of the studies have investigated the occurrence of pharmaceuticals in surface waters, including rivers, estuaries and coastal regions, and wastewater treatment plant (WWTP) effluents; suggesting that contamination may be widespread in aquatic systems (Cizmas et al., 2015). However, information on the occurrence and distribution of pharmaceuticals in the sediment is still scarce (Chen and Zhou, 2014; Kim and Carlson, 2007; Lara-Martín et al., 2014; Vazquez-Roig et al., 2013). This is despite the fact that sediments are the natural repositories of many anthropogenic chemicals that are released into the water column (Monteiro and Boxall, 2010).

Rather than the snapshot obtained with water samples analysis, monitoring of the sediment phase may provide an understanding of the longer-term occurrence and storage of the pharmaceuticals, which may provide a direct source of exposure to benthic organisms (Antonić and Heath, 2007; Chen and Zhou, 2014b). Sediment can serve as a record of historical consumption of pharmaceuticals through the detecting of old discharges of pharmaceuticals in sediment profiles. This can be evaluated by comparing rates of sediment accumulation, pharmaceuticals consumption, and their detected concentrations (Lahti and Oikari, 2012). The earliest study into the occurrence of natural and synthetic hormones in riverine sediment was

done in Germany by Ternes et al., (2002). Antibiotics are the most monitored pharmaceutical class in sediment. The highest levels have been recorded for the sulfonamides (sulfadiazine) with concentrations up to 12300 ng g^{-1} (Hu et al., 2012) followed by tetracyclines (oxytetracycline) with reported concentration of 9287.5 ng g^{-1} (Yong-shan, 2011). Silva et al., (2011) reported a comprehensive monitoring study of 43 pharmaceuticals in surface water, suspended solids and sediments in the Ebro River in Spain. Amongst the studied compounds, 30 pharmaceuticals were reported to be detectable in sediment. The highest concentration was reported for acetaminophen with concentrations up to 222.0 ng g^{-1}. Generally, the number of pharmaceuticals reported in sediments is limited and reflects many factors e.g. the number of pharmaceuticals determined by the analytical methods. For example, some analytical methods have been developed to study the occurrence of only a limited number pharmaceuticals in sediment while other methods have been developed for multi-residues analysis adding to the demanding efforts required and the cost of the analysis in this matrix (e.g. Pérez-Carrera et al., 2010; Silva et al., 2011). Moreover, the majority of existed monitoring studies suffer from lacking sufficient sampling designs that consider seasonal fluctuation, hydrologic conditions, or spatiotemporal variability (Ort et al., 2010).

The emission of pharmaceuticals into the environment is believed to be related to different variables such as proximity to WWTPs, the density of agricultural feed operations, higher effluent outflows, hydrology, size of the urban area and population, demographics and usage patterns that vary by region and season (Blair et al., 2013; Gaw et al., 2014; Kim and Carlson, 2005). Moreover, pharmaceuticals may undergo specific interaction (e.g. sorption processes) with sediment particles. Sorption of pharmaceuticals in sediment is one of the key mechanisms controlling their mobility in the aquatic environment (Martínez-Hernández et al., 2014). Thus, there is a need to understand such distribution of pharmaceuticals between sediments and water in a dynamic aquatic environment (Liang et al., 2013).

Given the highlighted research gaps, the aim of the work reported in this Chapter was to perform a field monitoring study to establish the temporal and spatial variations in the concentrations of a range of pharmaceuticals in water and sediment in an urban system. We use

the data to determine whether observed differences in concentrations can be explained by likely drivers of water and sediment exposure including usage, water flow, sediment physicochemical properties and the difference in partitioning between sites.

7.2 Materials and Methods

7.2.1 Chemicals and Materials

The study explored ten pharmaceuticals: amitriptyline, atenolol, cimetidine, diclofenac, diltiazem, mefenamic acid, ibuprofen, trimethoprim, naproxen, and ranitidine. All pharmaceuticals were purchased from Sigma–Aldrich (UK) with a purity ≥ 98%. A summary of the physicochemical properties and therapeutic uses for the study pharmaceuticals is provided in in Table 1.1 (Chapter 1). HPLC gradient grade methanol, HPLC-grade water and ammonium hydroxide solution (35%) were purchased from Fisher Scientific, UK. Formic acid (96 %) was purchased from Sigma-Aldrich (UK). Stock solutions for each individual pharmaceutical and a mixture of all pharmaceuticals were prepared in methanol–water (20:80, v/v) and stored at −20 ^{o}C in the dark.

7.2.2 Sampling Sites and Sample Collection

Water and sediment samples were obtained from seven locations (R1-R7) along the Rivers Foss (R1-R3) and Ouse (R4-R7) near and in the City of York, North Yorkshire, UK, on four occasions between November 2015 and July 2016. An overview of the location of sampling sites is given in Figure 7.1. Details for the sampling sites are given in Table A.E1 of the Appendix E. The River Ouse is hydrologically the continuation of the River Ure. Together they form the fourth and the sixth longest river in England and the UK, respectively. The River Ouse is 82 km long with a basin area of 10,704 km^2 and flows with an average water flow of 51.1 m^3 s^{-1}. The River Ouse catchment includes agricultural areas (31.4 %), grass areas (44.0%) and urban extents (1.5%). The River Foss is a tributary of the River Ouse and has a length 31 km, a basin area of 118 Km^2 and an average flow rate of 0.85 m^3 s^{-1}. The land description along the

River Foss is variable with about 54.5% agricultural areas, 28% grassland, and 3.4% urban areas.

Two WWTPs with different treatment technologies serve the population in the study catchment. The first one on the river Foss serves a population of 18600 and employs trickling filter technology as secondary treatment paired with biological aerated filtration for tertiary treatment. The second (on the river Ouse), uses conventional activated sludge (CAS) as secondary treatment and nitrifying filters as a tertiary treatment process and serves a population of 27900. Sample sites were chosen based on their proximity to WWTP outfalls discharging into the rivers with a maximum distance to the treatment plant of 12.3 Km. These particular sampling sites also allow capturing the spatio-temporal changes in concentrations of pharmaceuticals in water and sediment during their journey downstream the river Foss (R1-R3) and Ouse (R4 to R7). Due to temporary site closures, it was not possible to take samples of sediment from sites R3 and R4 in July 2016. Moreover, several factors were taken into consideration including ease of access and various anthropogenic activities.

Three individual 1 L samples of river water were collected from each sampling site. A 10 mL aliquot was taken from each 1 L replicate and immediately filtered through a 0.7 µm glass microfiber (GF/F) disposable filter (Whatman Inc.) and subsequently frozen with dry ice and stored at -20 ^{o}C until further analysis. To determine field cross contamination during filtering or collection, high- performance liquid chromatography (HPLC)-grade water was also filtered and prepared in the field during each field visit. Water samples were characterized (see Table A.E2 in the Appendix E) on site using an AP- 2000 advanced portable multi-parameter Aquaprobe (Aquaread, USA).

Sediment samples (approx. 500 g, composite) were collected from the sediment surface (0-10 cm) using a Van Veen grab sampler. Plant residues and debris were removed and sediment was wet sieved (2 mm), using river water, in the field. Sediment samples were then stored at -20 ^{o}C until they were characterized (see Table A.E3 in the Appendix E) and analysed for the study pharmaceuticals.

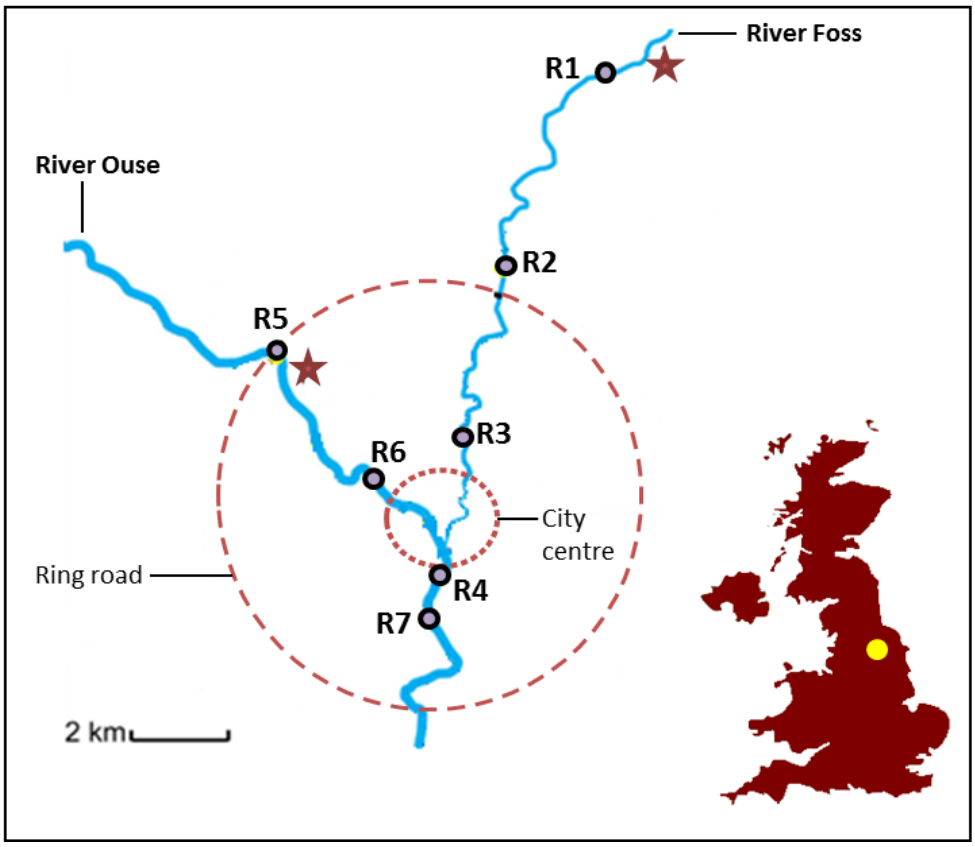


Figure 7.1 Sampling map representing sites along the River Foss (R1-R3) and the River Ouse (R4-R7), York, UK. Stars represent WWTPs

7.2.3 Sample Preparation

Whilst sediment samples were subject to extraction and clean-up-methods prior to chemical analysis, water samples were directly analysed for the pharmaceuticals. Preparation of sediment samples (3 replicates) for instrumental analysis was carried out using the extraction and clean-up methodologies described in Chapter 4 (sections 4.2.3 and 4.2.4).

7.2.4 Pharmaceutical Analysis

The analytical method used in this study was an adaption with some modification of the method previously developed in Chapter 4, to include more target pharmaceuticals. A sample volume of 20 µL (sediment extract or water samples) was analysed with a Thermo Scientific Ultimate 3000 HPLC system connected to a TSQ Endura™ Triple Quadrupole Mass Spectrometer with

an electrospray ionisation (ESI) source (Thermo Fisher, USA) equipped with two Zorbax Eclipse XDB-C18 columns (guard column: 4.6 mm x 12.5 mm 5.0 µm; analytical column: reversed phase, 150 mm × 4.6 mm and 5.0 µm) at a constant column temperature (30 ^{o}C) and a flow rate of 1.0 mL/min. Pharmaceuticals were separated using a gradient mobile phase. Phase A consisted of 10 mM ammonium acetate/acetic acid buffer (pH 4.8) and phase B consisted of acetonitrile, methanol and formic acid (75:25:0.01%). Initial column conditioning (1min, 90% of solvent A) was followed by a steady decrease to 75% of solvent A within 8 min. After 15 min solvent A was further decreased to 10% which was maintained for 2 min and then followed by a rapid increase of solvent A to 90 % (1 min). Column re-equilibration to the initial mobile phase composition took 3 min. The MS/MS was operated in selective reaction monitoring (SRM) mode where quantification was based on the major transition (see Table A.E4 in the Appendix E).

7.2.5 Validation of Analytical Method

Retention times and the characteristic ions (precursor ion and product ion transitions in SRM mode) were used as the main criteria for pharmaceutical identification. The method was validated considering the following parameters: linearity, recovery, precision, limit of detection (LOD), limit of quantification (LOQ) and the effect of sample storage and matrix effect. Linearity was studied using external and matrix-matched (river water for water samples and SPE extract for sediments) calibrations by analyzing six concentrations (n=3) in the range of 0.5 to 500 ng L^{-1} and 2 ng g^{-1} to 500 ng g^{-1} for water and sediment samples respectively. The linearity was qualified by the linear correlation coefficient, R^2. Recoveries of the ten pharmaceuticals for the direct injection for water samples or the entire UAE–SPE–LC–ESI-MS/MS procedures for sediment samples were calculated. The precision of the method, which was expressed as the relative standard deviation of the result (RSD%), was determined by the repeated analysis of samples of sediments spiked at low concentration. Blank samples were used to correct for background residues during analysis. The limits of detection (LOD) and limits of quantification (LOQ) were calculated based on the standard deviation of the 6

calibration curve intercepts divided by the slope with a signal to noise (S/N) ratio of 3 and 10, respectively. For quality control, the loss during storage due to degradation was determined by measuring the pharmaceuticals concentrations before and after 3 months at -20 ^{o}C.

The matrix effects were studied by the evaluation of signal suppression or enhancement for each pharmaceutical. To assess the influence of matrix components, signals of final sediment extracts spiked with analytes were compared with signals observed from solvent dissolved pharmaceuticals. A value of greater or less than zero indicates signal enhancement or suppression; respectively. The equation used for the signal suppression is given in Equation 7.1:

$$\textbf{Matrix effect}\ \% = \left(\frac{(\textbf{Area sediment} - \textbf{Area blank})}{\textbf{Area solvent}} - \mathbf{1}\right) \times \mathbf{100} \qquad \textbf{(7.1)}$$

7.2.6 Usage Data and river Flow

The prescription data for the study active pharmaceuticals ingredient in England from 2015 and 2016 were derived from prescription cost analysis data available for England (www.nhsbsa.nhs.uk/prescription-data). Usage data for each active ingredient were grouped for all therapeutic forms to derive the final usage in Kg for each month of sampling (Table A.E5, Appendix E). The obtained usage data were then divided by the population of England (55.4 million) and multiplied by the population of study catchment (207000 inhabitants) to obtain the final monthly usage amount for the city of York.

Sampling month and annual average flow data for the nearest gauging station to the sampling network were obtained from the National River Flow Archive (NRFA) (www.nrfa.ceh.ac.uk), Table A.E2 (Appendix E). Generally, recorded flow rates were 30-50% below the archived mean flow rates recorded for the period between 1987 and 2016. Moreover, the approximate recorded dilution factor was of 540 and 17.8 for the River Ouse and the River Foss, respectively (Burns et al., 2017).

7.2.7 Statistical Analysis

Statistical analysis was performed using SPSS 22.0 (IBM, USA). Comparisons of concentrations were performed using Pearson's correlation tests and a level of $p < 0.05$ was considered statistically significant. In the event that the data were normally distributed, the differences in mean concentration between seasons or sampling locations were determined using Two-way ANOVA. For non-normally distributed data, a non-parametric analysis of variance Kruskal-Wallis test was used. Graphical representations of the results were carried out with Microsoft Excel 2010 software. Both censored (statistically treated dataset where one or more measurements are partially unknown (e.g. when environmentally observed concentrations are less than the field LOD or LOQ)) and non-censored data were used in subsequent data analyses. In instances where data were censored, the substitution method of $\sqrt{2}/2$ times the LOD or LOQ (Antweiler, 2015; Zeghnoun et al., 2007) was used to derive a value for use in the data analysis.

7.3. Results and Discussion

7.3.1 Performance of Analytical Method

The quantifications were based on analyte peak area and retention time. The total ion chromatograms of the pharmaceuticals showed distinct peaks, except for ibuprofen which showed an isomeric peak $[M-H]^-$ at low concentrations (Figure 7.2). Due to the clear effects of the co-eluting interferences during analysis by the mass spectrometry detector with electrospray interfaces, matrix-matched calibration was selected as the most suitable approach to compensate for matrix effects during analysis (Huerta et al., 2015; Vazquez-Roig et al., 2013). For water samples, no field cross contamination during filtering or collection was detected in the field blanks. Recoveries from quality control matrix spike samples ranged from 86.5% (cimetidine) to 115.8 (Trimethoprim) as shown in Table A.E6 (Appendix E). Limits of detection (LOD) for water samples ranged from 0.5 ng L^{-1} (amitriptyline and diltiazem) to 5.3 ng L^{-1} (naproxen). The RSD% values ranged from 0.8 to 4.6.

Extraction recoveries, LOD, LOQ, RSD% and matrix effects were evaluated for the target pharmaceuticals in all sediments at low concentration as shown in Table A.E6. Recoveries of the target pharmaceuticals in sediment ranged over the entire method from 55.2% (ranitidine) to 148% (mefenamic acid) at the lower spiking level of 5.0 ng g^{-1}. At the same spiking concentration, the method was most sensitive to ranitidine with LOD (LOQ) of 0.01 (0.03) ng g^{-1} and least sensitive for diclofenac with LOD (LOQ) values of 0.92 (3.0) g g^{-1}. The RSD% values ranged from 0.6 to 12.1. For evaluating the effect of the matrix, a highly complex sediment matrix (R3) was chosen. Atenolol showed the greatest suppression when the effect of the matrix was studied with -16.2% while naproxen showed the highest enhancement of 7.1%.

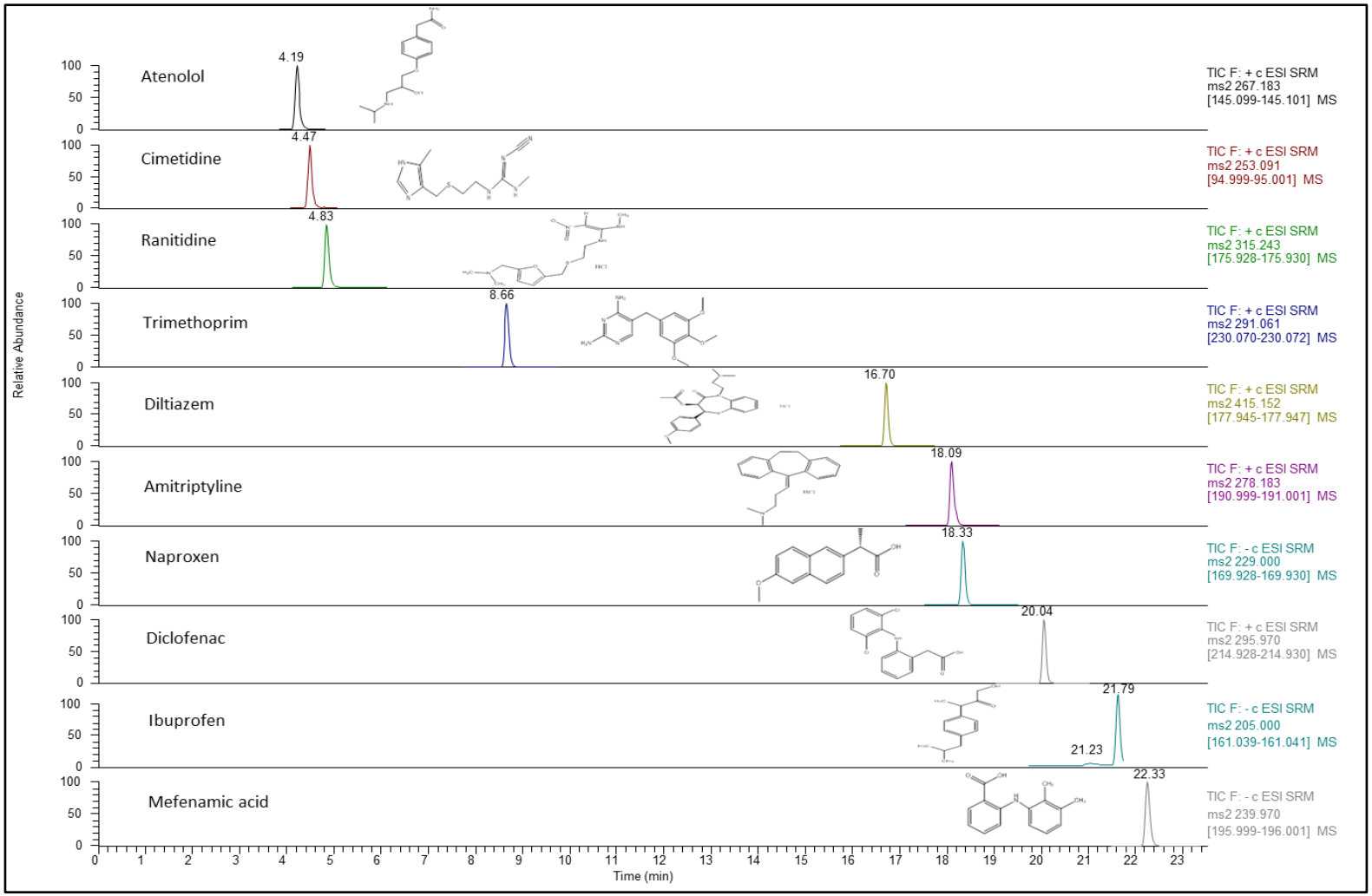


Figure 7.2 SRM chromatograms of targeted pharmaceuticals from an extract of sediment spiked at 20 ng g^{-1}

7.3.2 Occurrence of Pharmaceuticals in Water and Sediment Samples

All 10 pharmaceuticals were detected at least once during sampling events in water or sediment demonstrating their widespread presence in the river systems and pseudo persistence. Overall, the maximum concentrations detected in the water phase was highest for atenolol followed by trimethoprim > cimetidine > naproxen > ranitidine > diclofenac > diltiazem > ibuprofen > amitriptyline and the lowest maximum detection was for mefenamic acid. The concentrations and frequency of detection of target pharmaceuticals in water and sediment samples are summarized in Table 7.1.

The antibiotic (trimethoprim) and the antidepressant (amitriptyline) were the most frequently detected pharmaceuticals in all of the collected water samples. The frequent detection of trimethoprim is likely due to the relatively high excretion from the human body (80%) (Kasprzyk-Hordern et al., 2008) and the the weak biodegradability through conventional activated sludge processes in WWTPs (<26.4%) (Li and Zhang, 2010). The average detected concentrations of trimethoprim (17.7 ng L^{-1}) in water is higher than those found in small streams (<LOQ) in France (Feitosa-Felizzola and Chiron, 2009), similar to the concentration range reported in Spain (Moreno-González et al., 2015), and within the range of those reported in Wales, UK (Kasprzyk-Hordern et al., 2008). The Anti-depressant amitriptyline was also detected frequently in the samples from both rivers with concentrations ranging from 0.8 to 17.7 ng L^{-1}. Concentrations detected were generally higher than those reported by Klosterhaus et al., (2013) and similar to concentrations reported by Kasprzyk-Hordern et al., (2008) in river waters in Wales (UK).

For the non-steroidal anti-inflammatory drugs ibuprofen, diclofenac and mefenamic acid, detected concentrations varied in water samples. Whilst ibuprofen was not frequently detected on most sampling occasions, diclofenac, mefenamic acid and naproxen were more frequently detected at peak concentrations of 29.8, 13.9 and 36.6 ng L^{-1}, respectively. Concentration ranges observed in the current study were within the ranges reported elsewhere (Kasprzyk-Hordern et al., 2008; Lara-Martín et al., 2014; Silva et al., 2011). For anti-histamines, cimetidine showed the lowest frequency of detection in the water phase with detected concentrations ranging from nd to 37.1 ng L^{-1}. Ranitidine was more frequently detected (89%) with a mean concentration of

11.5 ng L^{-1}. Anti-histamines concentrations were higher than those reported in Korea (Choi et al., 2014) and lower than those reported in Spain (Pérez-Carrera et al., 2010; Silva et al., 2011). The β- blocker, atenolol, was detected at an average concentration of 25.4 ng L^{-1} in water samples and highest detected concentration of 57.9 ng L^{-1} among all targeted pharmaceuticals in water samples. Detected concentrations are comparable to those recently reported in water for the US (Klosterhaus et al., 2013). Diltiazem was frequently detected in water samples (86%) with a mean concentration of 7.8 ng L^{-1}. This detected mean is similar to those of below 10 ng L^{-1} recently reported in the USA (Cantwell et al., 2017).

The order of the maximum measured pharmaceutical concentrations differed for the sediment analysis, with trimethoprim being detected at the highest concentration followed by amitriptyline, concentrations of the other drugs then declined in the order ibuprofen > cimetidine > naproxen > diclofenac > atenolol > mefenamic acid > ranitidine and then diltiazem. Trimethoprim and amitriptyline were also the most widely detected (100%) pharmaceuticals. Atenolol, cimetidine, diltiazem, mefenamic acid and ranitidine showed a higher frequency of detection in sediment than the water phase. Trimethoprim was detected at concentrations ranging from 0.2 to 18.4 ng g^{-1} and peak concentrations were mainly detected in samples collected from the river Foss. Observed concentrations are similar to those reported by Klosterhaus et al., (2013) from San Francisco Bay, CA, USA; and greater than concentrations found in stream sediment from northern New Jersey, USA which was suggested to be related to the dilution from groundwater discharge and flow from tributaries (Gibs et al., 2013) and South Africa when human activities and recreational facilities around sampling sites are highly affecting the detected concentrations (Matongo et al., 2015).

Amitriptyline was detected at concentrations ranging from 0.25 to 12.9 ng g^{-1}. Concentrations detected were generally higher than those previously reported by Klosterhaus et al., (2013) in sediment samples in the USA. The NSAIDs were detected less frequently except for mefenamic acid which was detected in all samples. The highest concentration among all those detected NSAIDs was for ibuprofen (8.1 ng g^{-1}) followed by diclofenac (4.6 ng g^{-1}). These maximum concentrations were only reported occasionally. The detected concentrations in the current study were consistent with the ranges reported in sediment by Silva et al., (2011) in Spain and Varga

et al., (2010) in Hungary. Atenolol exhibited concentrations <LOQ on most occasions except for samples collected in November 2015 with a maximum concentration of 4.1 ng g^{-1}. The mean concentrations of atenolol in this study were similar to those reports for sediment in Brazil (Beretta et al., 2014b). Diltiazem exhibited concentrations higher than LOD in 100% of the sediment samples with mean detected concentrations of 0.4 ng g^{-1}. Generally, concentrations detected in the current study were higher than those reported in lake sediment (<LOD) by Blair et al., (2013).

Table 7.1 Occurrence data (±1 SD) for the water and sediment samples collected from the sampling network (the River Ouse and Foss)

Compound	Water				Sediment			
	Frequency of detection %	Mean (ng L^{-1})	Max. (ng L^{-1})	Min. (ng L^{-1})	Frequency of detection %	Mean (ng g^{-1})	Max. (ng g^{-1})	Min. (ng g^{-1})
Amitriptyline	100	4.4	17.7	0.8	100	2.6	12.9	0.25
Atenolol	77	25.4	59.7	<LOQ	97	1.1	4.1	<LOQ
Cimetidine	61	15.7	37.1	Nd	89	1.7	5.9	nd
Diclofenac	100	14.0	29.8	7.2	30	4.6	4.6	<LOD
Diltiazem	85	7.8	25.2	Nd	100	0.4	1.23	<LOQ
Ibuprofen	73	16.1	22.5	Nd	23	8.1	8.1	nd
Mefenamic acid	81	7.6	13.9	Nd	100	0.45	1.7	0.13
Naproxen	73	25.0	36.6	Nd	73	2.4	5.0	nd
Ranitidine	89	11.5	32.8	Nd	100	0.6	2.3	0.4
Trimethoprim	100	17.7	48.4	4.4	100	1.3	18.4	0.2

<LOD and <LOQ = lower than limit of detection and quantification, respectively. nd = not detected.

7.3.3 Seasonal Occurrence of Pharmaceuticals

More in depth analysis indicates seasonal variation in concentrations detected in water and sediment samples, Table 7.2 (more details in Tables A.E7 and A.E8, Appendix). For water samples, all pharmaceuticals were quantified in all seasons on more than one occasion showing their continuous occurrence in the catchment area. Whilst low photo- and/or bio-degradation due to low temperatures and short days in winter and increased degradation and other attenuation processes during summer (Cheng et al., 2014b; Lin and Reinhard, 2005; Lin et al., 2010; Moreno-González et al., 2015; Sun et al., 2014); and higher treatment efficiencies in WWTPs (Kay et al., 2017) would suggest that peak concentrations would be observed in the samples from November and February, the opposite was the case. For example, the median concentration of cimetidine detected in water samples in summer (July) was three times higher than what was observed in the winter (February). This trend may be attributed to increases in the use of antihistamines to treat allergies (e.g. hay fever) which will peak in spring and summer when pollen production is greatest (Petrie et al., 2014); or increased river flows (ultimately increased dilution factor) in November and February, which consequently increased the dilution of the pharmaceuticals (Papageorgiou et al., 2016). Amitriptyline showed significant differences between seasons ($F= 3.83$; $p<0.05$) with median concentrations ranging from 2.1 to 4.1 ng L^{-1} and peak concentrations in the winter samples (November 2015) and summer samples (July 2016). A recent study by Moreno-González et al., (2015) reported a weak relationship between psychiatric pharmaceuticals consumption and their seasonal occurrence in the environment.

Although it is expected that the high consumption of antibiotics in the flu season will increase exposure levels in the environment (Kim and Carlson, 2007; Yan et al., 2013), trimethoprim was detected at lower concentration ($p<0.01$) in the Winter samples (November and February) than in Spring and Summer samples (May and July) with median concentrations ranging from 6.2 to 17 ng L^{-1}. No statistical differences ($F< 3.0$; $p>0.05$) in detected concentrations were observed for the NSAID pharmaceuticals between seasons. Maximum detected concentrations for NSAIDs were seen in samples collected in July except ibuprofen where peak concentrations of 22.5 ng L^{-1} were seen for samples obtained in February. Similar seasonal patterns were

reported for NSAIDs in water samples from the Pearl river, China (Hao et al., 2010). Atenolol concentrations were similar ($F= 2.2$; $p>0.05$) across the seasons except for May 2016 where concentrations were slightly elevated. Diltiazem and ranitidine showed significant differences between seasons ($F= 4.9$; $p<0.05$ and $F=3.3$; $p<0.05$, respectively).

In sediment, selected pharmaceuticals were detected across all seasons suggesting their abundant use and potential persistence. Amitriptyline showed a significant difference in concentrations ($F= 4.3$; $p<0.05$) between seasons with median concentrations ranging from 0.9 to 2.8 ng g^{-1} and peak concentrations being recorded in November. Trimethoprim exhibited no significant differences ($p>0.05$) in occurrence levels between seasons with median concentrations ranging from 0.4 to 0.8 ng g^{-1} and maximum concentration of 18.4 ng g^{-1} being recorded in November 2015. Statistical analysis also indicated no significant ($F= 2.7$; $p>0.05$) seasonal variations in concentrations of mefenamic acid. Diclofenac and atenolol varied little over the study period with median concentrations of <LOQ except in spring and summer samples where atenolol showed median concentration of 1.28 and 1.25 ng g^{-1}, respectively. Diclofenac, with log Kow of 4.51, was expected to occur at highest concentrations in sediment but according to distribution coefficient (Kd) levels obtained in this study, it seems to show low adsorption potential to sediment. However, the general trend was towards samples collected in November 2015 which showed the greatest occurrence of pharmaceuticals in sediment than samples taken later.

The peak detected concentration in November 2015 can be explained due to the expected high concentrations of pharmaceuticals can be accumulated on sediment over long period of low flow hence higher concentrations are expected in water column. The latter depletion (February 2016) in concentration in contrast to the expectations was most likely related to a major flooding event in this area which may have transported sediment and sorbed chemicals further downstream than the usual river flow would have. This is confirmed by changes in the sediment texture of study sites. For example, texture of site R1 changed from sandy loam in samples from November 2015 (before the flood event one month later) to loam sand in February 2016 and bed sediments became less embedded due to the reduction in fine particles as likely to be found after floods (Eaton and Lapointe, 2001; Karimaee Tabarestani and Zarrati, 2014; Mürle et al., 2003).

This may change the sediment from a long-term sink into a secondary contamination source with the possibility of contaminants to be reversibly distributed to the water phase (Mazurová et al., 2008). In addition, increasing dilution factors, due to increased precipitation levels, can affect the concentration of pharmaceuticals subjected to sorption processes in the water column. After the flood event, concentrations were more constant and most of the pharmaceuticals showed higher concentrations in spring and summer (May to July 2016) than winter (February 2016), but still lower than concentration observed in November 2015. Generally, seasonal variations in concentrations of pharmaceuticals in sediments could be affected by many factors, such as their concentrations in water, sediment properties, water flows and usage in the study catchment (Zhou et al., 2011). Moreover, the current results may indicate that pharmaceutical occurrence in water and sediment is more intensively influenced by seasonal changes in environmental conditions than being subject to short-term changes between seasons, due to the frequent discharge from WWTPs.

Table 7.2 Seasonal measured concentration (±1 SD) of pharmaceutical residues in the surface water (ng L^{-1}) and sediment (ng g^{-1}, dw) samples over the duration of the river monitoring study

Compound	Matrix	Season											
		November-2015			February-2016			May-2016			July-2016		
		Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.
Amitriptyline	Water	2.6	11.7	2.5	2.1	5.2	1.5	2.7	8.0	0.8	4.1	17.7	2.2
	Sediment	2.36	12.9	0.76	0.9	2.82	0.24	1.5	2.1	0.5	1.99	4.92	1.31
Atenolol	Water	30.4	37.5	<LOQ	21.7	35.9	<LOQ	13.9	36.7	10.4	41.2	59.7	<LOQ
	Sediment	2.67	4.1	<LOQ	<LOQ	<LOQ	<LOQ	1.28	1.28	<LOQ	1.25	1.43	<LOQ
Cimetidine	Water	14.8	17.4	nd	7.4	12.3	nd	14.5	14.5	Nd	24.8	37.1	<LOD
	Sediment	1.09	5.9	<LOQ	1.525	2.35	<LOQ	0.99	2.7	Nd	1.09	1.17	nd
Diclofenac	Water	10.5	18.2	8.9	12.1	24.7	7.2	11.7	21.6	8.7	10.8	29.8	10
	Sediment	4.6	4.6	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOQ
Diltiazem	Water	5.05	7	<LOQ	5.5	6.6	<LOQ	9	9.1	Nd	6.1	25.2	6.1
	Sediment	0.48	1.23	<LOQ	0.16	0.16	<LOQ	0.39	0.42	<LOQ	0.42	0.48	0.39
Ibuprofen	Water	15.5	18.7	nd	18.85	22.5	nd	13.8	19.6	Nd	15.4	18	<LOQ
	Sediment	8.1	8.1	nd	nd	<LOQ	nd	Nd	<LOQ	Nd	nd	<LOD	nd
Mefenamic acid	Water	9.0	10.7	nd	6.9	13.9	nd	8.4	9.6	<LOD	8.6	11.8	<LOQ
	Sediment	0.41	1.7	0.26	0.58	1.14	0.17	0.17	0.54	0.13	0.17	0.3	0.14
Naproxen	Water	29.7	31.5	nd	24.3	27.8	nd	21.75	24.8	<LOQ	25.6	36.6	<LOQ
	Sediment	2.42	4.26	nd	2.03	2.23	nd	2.26	5.03	<LOQ	2.405	2.86	nd
Ranitidine	Water	13.8	32.8	<LOQ	6.9	10.0	nd	9.9	13.5	<LOQ	9.15	20.2	<LOQ
	Sediment	0.43	0.8	0.4	0.58	0.81	0.43	0.48	2.26	0.44	0.45	0.72	0.42
Trimethoprim	Water	11.4	14.5	5.4	6.2	26.2	4.4	17.0	48.4	14.0	16.4	42.9	8.9
	Sediment	0.8	18.4	0.25	0.53	1.11	0.2	0.4	1.15	0.23	0.4	0.64	0.26

LOD= limit of detection; LOQ = limit of quantification, nd = not detected

7.3.4 Spatial Distribution of Pharmaceuticals in Water and Sediment

Spatial distributions of the pharmaceuticals detected in the current study were also compared among the sampling network on the two rivers. Peak concentrations and more frequent detection of pharmaceuticals occurred in samples collected from the River Foss, particularly in samples collected at the site (R1) close to the urban areas and the WWTP effluent discharge. The river Ouse had the lowest mean concentrations for all pharmaceuticals. Across sites, pharmaceuticals were detected in >86% and >81% of the water and sediment samples, respectively. Concentrations in water and sediment over the sampling sites and seasons are provided in Figures 7.3 and 7.4 (more details in Tables A.E7 and A.E8, Appendix E).

Trimethoprim showed significant differences ($F= 7.3$; $p<0.001$) in detected concentrations at all water sampling sites with concentrations ranged from 4.4 to 48.4 ng L^{-1}. Peak concentrations were found to be at sampling points on the river Foss just after a discharge of the WWTP effluent into river water and were found to decrease significantly downstream. Previous studies showed that the major pathway for release of antibiotics is wastewater treatment facilities whilst concentrations correlated with water-flow conditions (Göbel et al., 2004; Hirsch et al., 1999; Kolpin et al., 2004). Similarly to trimethoprim, the maximum concentrations for amitriptyline were detected in samples collected from the River Foss and concentrations decreased downstream the WWTP. Amitriptyline showed a significant decrease ($F=7.4$; $p<0.001$) at water samples sites downstream the WWTPs on both rivers except at site R3 in November 2015 where showed increased detected concentration. The β- blocker, atenolol, was found to be present in all water samples from the river Foss at levels exceeding 30 ng L^{-1} and the maximum detected concentration (59.7 ng L^{-1}) in water samples at site R1 on the River Foss. In contrast to other detected pharmaceuticals, atenolol showed very low (<50 %) but significant ($F= 10.4$; $p<0.05$) decrease downstream the discharge point confirming that atenolol is persistent in the aqueous environment.

For the non-steroidal anti-inflammatory drugs, their presence in the River Foss is again strongly related with the discharge of WWTP. The detection concentrations in water samples from the river Foss were significantly ($p<0.05$) higher than those found in the Ouse. They showed a decrease in concentration by less than 40% downstream the WWTP indicating their widespread

in the Foss, and persistence nature of the targeted pharmaceuticals in the water phase (Kasprzyk-Hordern et al., 2008). The pattern was similar for diclofenac and ibuprofen on the river Ouse except for mefenamic acid and naproxen where higher concentrations were reported upstream the WWTP (R5) with infrequent occurrence downstream. This might be due to a run-off or an uncontrolled discharge of untreated sewage from surrounding housing estates. Ranitidine showed significant differences in detected concentration between sampling sites on the both rivers ($F= 9.5$; $p<0.001$). The concentrations at sites on the river Foss (R1-R3) were found to decrease downstream the river while fluctuated and less frequently detected in samples from the river Ouse. Cimetidine showed more occurrences in samples from the river Foss than the Ouse with peak concentration up to 37.1 ng L^{-1} in samples collected after WWTP discharge point at site R1. These concentrations were decreased downstream by 50% for cimetidine and <LOQ for ranitidine. The calcium channel blocker, diltiazem, was frequently detected at relatively stable concentrations ($F= 2.5$; $p>0.05$) downstream the WWTP in water samples from the River Foss while showed variability from samples along the Ouse.

The spatial distributions of targeted pharmaceuticals in sediment samples in the river Ouse and Foss were also analysed showing less significant variability between sites. Trimethoprim showed no significant differences ($F= 0.9$; $p>0.05$) in detected concentrations across sampling sites with a mean concentration of 1.3 ng g^{-1}, and maximum concentrations of 18.4 ng g^{-1} reported for sediment samples at site R1 on the river Foss. Amitriptyline concentrations were found significantly different between rivers ($F= 2.9$; $p<0.05$). Concentrations of amitriptyline were found to continuously decrease with increasing distance from the WWTP but were found to be higher again in samples at the furthest distance from the WWTP (R3). No significant changes ($p>0.05$) in concentrations were observed in sediment at sampling sites R4 to R7 on the river Ouse with detected concentrations of the lower ng g^{-1}.

Atenolol showed no significant differences ($F= 0.97$; $p>0.05$) even when censored detected concentrations were combined with the non-censored. Maximum detected concentration was found at site R3 from November 2015 sampling campaign. The detection of both compounds (amitriptyline and atenolol) peaking in sediments at site R3 can be attributed to the high organic carbon content (OC) and the high cation exchange capacity (CEC) of the sediment at this

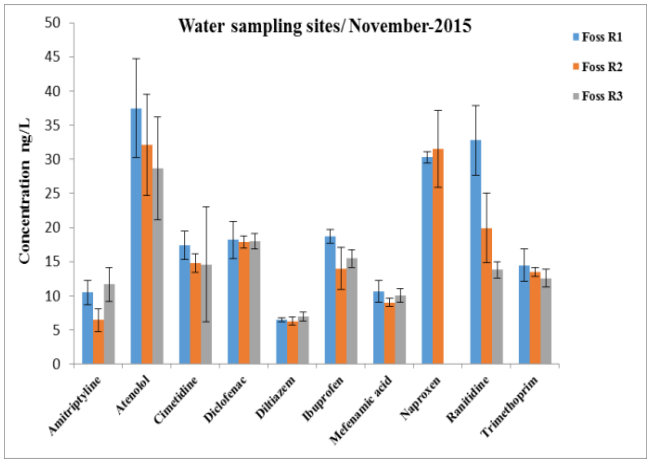
location (see Table A.E3, Appendix). The OC and CEC were previously shown to be the predominant factors affecting the sorption of amitriptyline in sediment and CEC cation exchange was suggested to be the predominant sorption mechanism for atenolol (Chapter 5; Schaffer et al., 2012a).

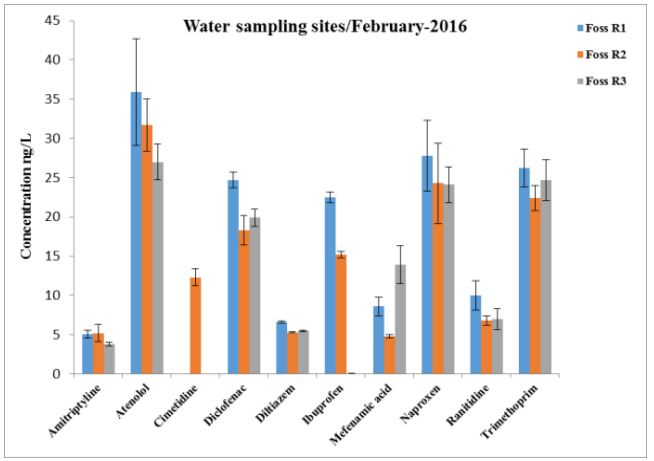
In sediment, ibuprofen and diclofenac showed detection concentrations ranging from nd-<LOQ except in two occasions where quantifiable concentrations recorded at sampling sites R4 and R7 on the river Ouse downstream the high-density populated area of the city centre. This was expected since ibuprofen and diclofenac are highly soluble in the water phase and have low affinity to partition to sedimentary phase. For mefenamic acid even downstream of the two rivers, the recorded concentrations were not significantly variable ($F= 0.7$; $p>0.05$) with maximum concentrations of 1.14 and 1.7 ng g^{-1} at sites R3 and R7, respectively. Cimetidine showed significant differences ($F= 3.3$; $p<0.05$) in concentration across the sediment sampling sites with peak concentration at site R3 on the river Foss and site R5 (upstream the WWTP) on the river Ouse. This pattern was different in for ranitidine which showed a steady state ($F= 2.4$; $p>0.05$) with <1 ng g^{-1} occurrence levels. Generally, the increased detection of pharmaceuticals near the effluent of the River Foss is most likely associated with the lower water flow (the reduced dilution derived from the smaller basin area) and the less removal efficacy of the WWTP (uses trickling filter technology as secondary treatment paired with biological aerated filtration for tertiary treatment) in comparison to the River Ouse (uses conventional activated sludge (CAS) as secondary treatment and nitrifying filters as a tertiary treatment process). Finally, the significant differences in pharmaceuticals detected concentrations between river sediments and the directly overlying water indicating the importance of sorption as an attenuation mechanism in the aquatic environments. Moreover, the occurrence of the pharmaceuticals in sediments believed to be related to their potential to accumulate onto sediment particles.

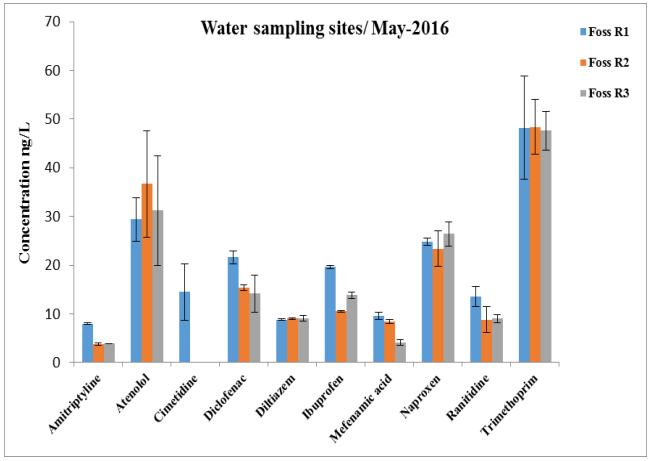
A

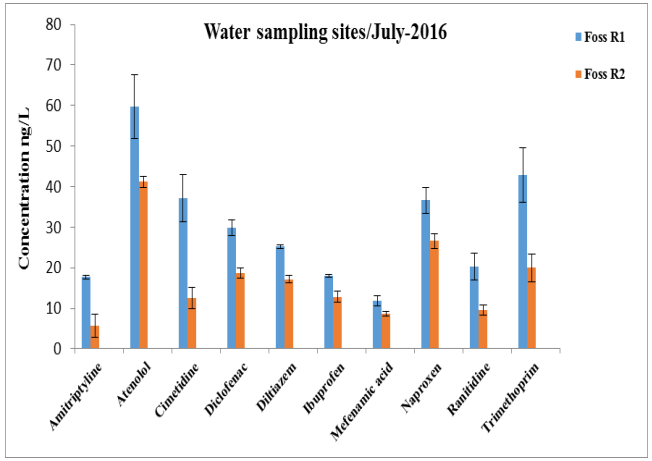
Sampling Site

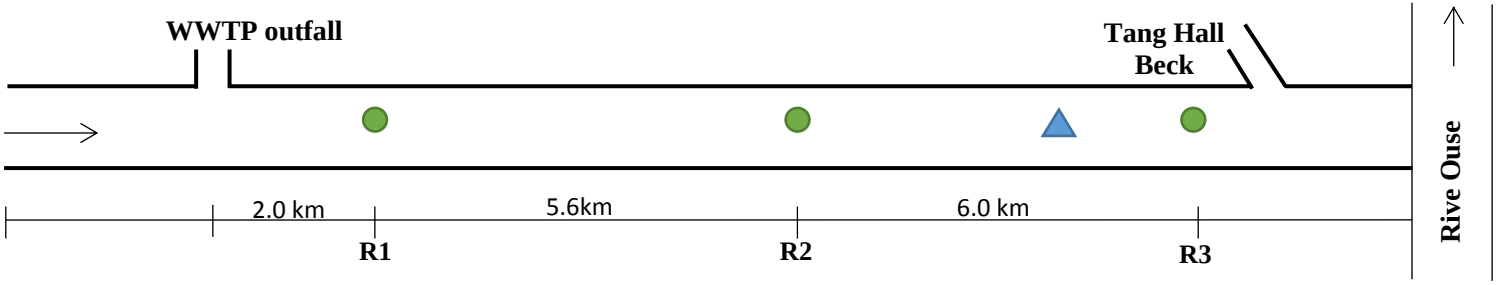
Flow Gauge











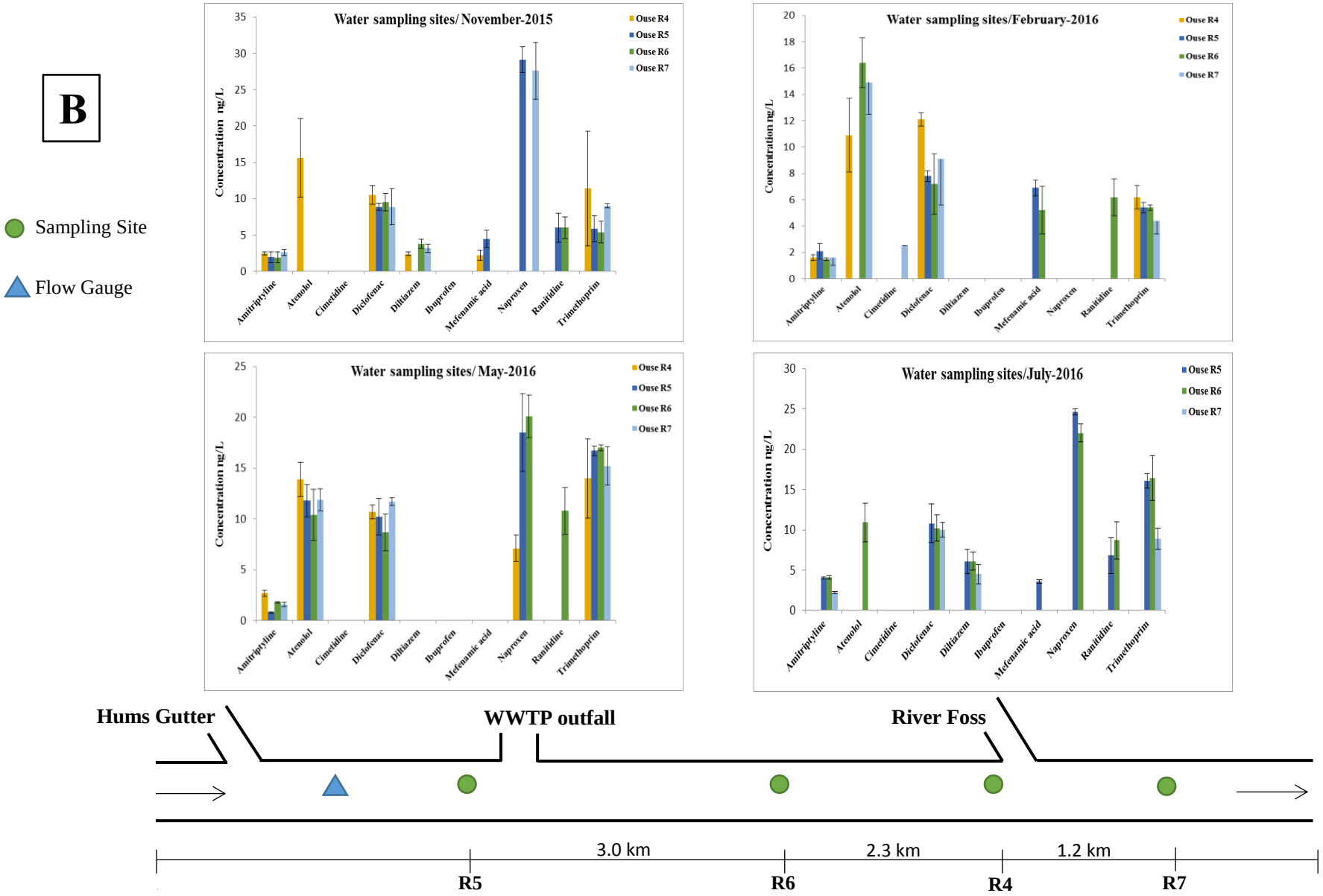
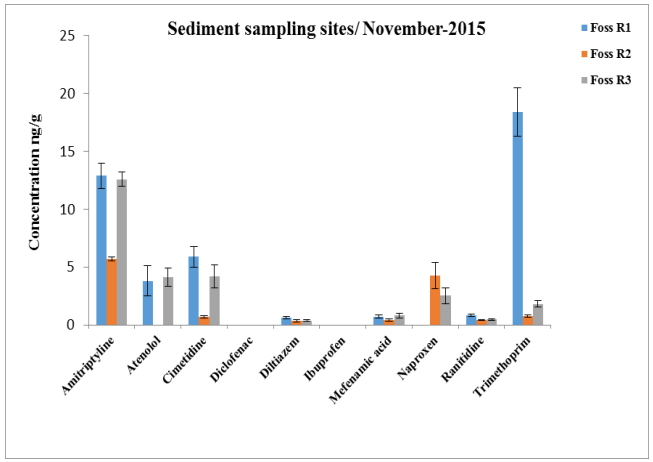


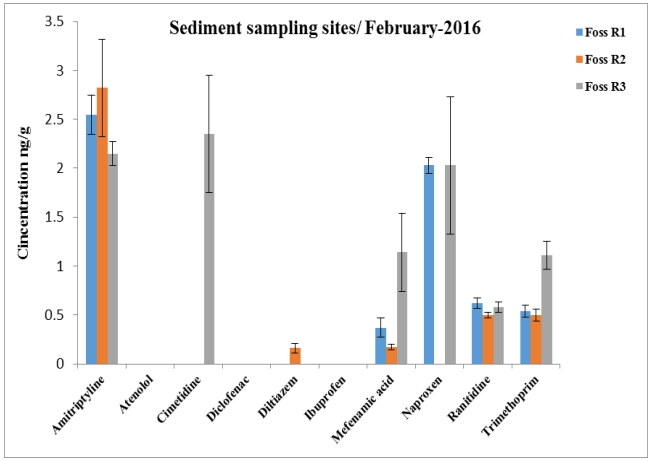
Figure 7.3 Spatial and temporal variations of pharmaceuticals concentrations (±1 SD) in water (A) River Foss, (River Foss). No sampling events at sites R3 and R4 on July- 2016 due to sites closure

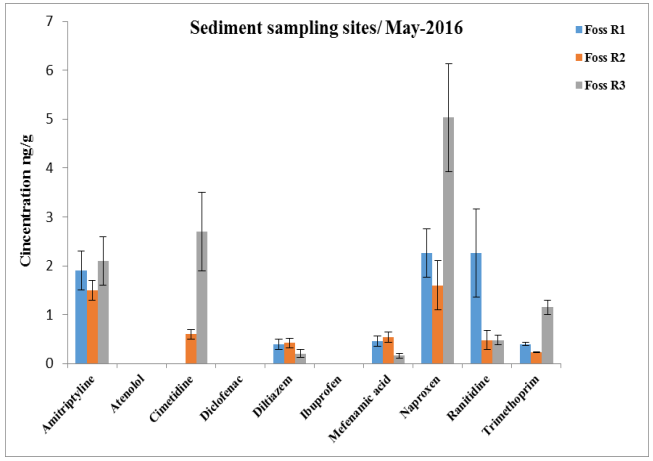
A

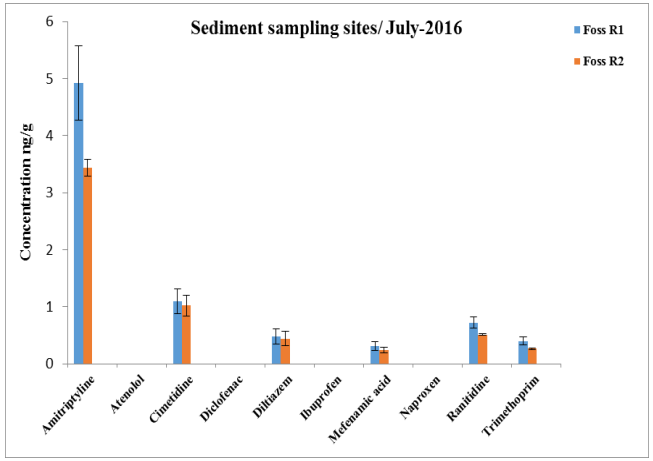
Sampling Site

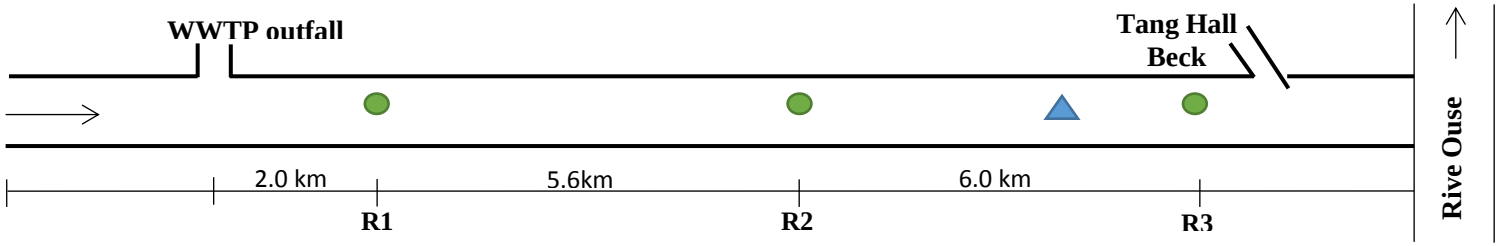
Flow Gauge











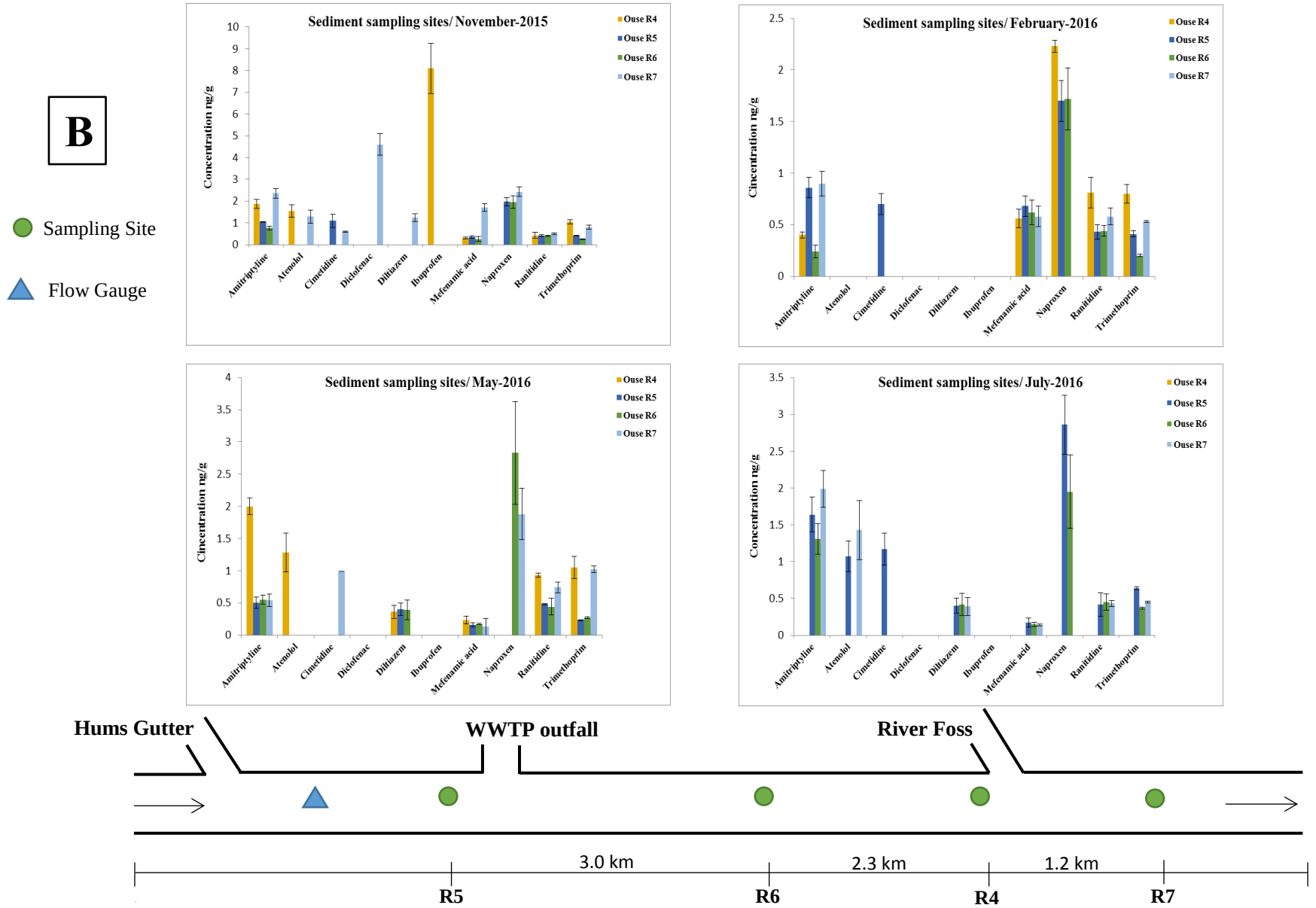


Figure 7.4 Spatial and temporal variations of pharmaceuticals concentrations (±1 SD) in sediment (A) River Foss, (B) River Ouse. No sampling events at sires R3 and R4 on July- 2016 due to sites closure

7.3.5 Effect of Flow Conditions on Pharmaceuticals Distribution

The results obtained for the 10-month sampling period on the River Foss and Ouse revealed that concentrations of pharmaceuticals vary in both water and sediment samples and found to be considerably higher during summer. Studied rivers flow rates were found to substantially vary over the year (Table A.E2, Appendix E). In water samples, lower pharmaceutical concentrations were generally seen at higher flow rates indicating that river flow rate is an important factor in determining pharmaceutical concentrations but this was not similar for sediment samples, which indicates that variation, cannot be explained by this factor. In order to explore whether differences in concentrations over time could be explained by differences in rivers flow rates, relationships between flow data and concentrations of pharmaceuticals in water and sediment samples were explored by normalizing the detected concentration to the corresponding seasonal river flows (Figure A.E1 and A.E2, Appendix E). Normalization of the results to flow support the hypothesis that the concentration of pharmaceuticals in water and sediment samples in the river Ouse is a function of dilution while river flow alone does not explain all the variation in observed concentrations from the river Foss. Further normalization using other drivers is therefore needed.

7.3.6 Effect of Usage on Pharmaceuticals Distribution

Next to variations in pharmaceutical occurrence being influenced by river flows, the therapeutic use is expected to have an influence on temporal observation pattern, as usage itself is seasonal. Whilst antibiotics and anti-histamines are generally prescribed more frequently in some seasons and are therefore detectable more frequently and at higher concentrations following these events (Kim and Carlson, 2007), 2007; Yan et al., 2013) other pharmaceuticals show less strong (NSAIDs) or absent (anti-depressants) seasonal variation in frequency and level of detection in both water and sediment samples (Moreno-González et al., 2015). Our analysis of seasonal prescription data for the river catchment area of the Foss and Ouse showed a little variation in the usage of the different pharmaceutical studied with the exception of the cimetidine, where the use in October 2015 and June 2016 was around 13 and 29% greater than the average monthly

use, respectively. Even though, normalization of detected concentrations in both water and sediment samples to usage data showed that variations in concentration are caused by variations in use of a particular compound (Figure A.E1 and A.E2, Appendix E). The effect of usage on the detected concentrations compared to the original values was clearer for samples from the river Ouse than the Foss. On the other hand, the inconsistency between usage and detection data is likely a relic of not accounting for the use of over the counter medicines and many other occasional factors influencing both the actual seasonal anthropogenic excretion in the catchment area e.g. tourist activity, University breaks and exam periods and sport and festival events (Petrie et al., 2014), and the variable actual release into surface waters (e.g. variation in removal rates in the WWTPs).

7.3.7 Influence of Sediment properties on Pharmaceuticals Distribution

The sediments in the study catchment area are characterized by low to moderate OC and CEC and high sand contents (Table A.E3, Appendix E). Statistical analysis using stepwise multiple linear regression (MLR) was performed to identify the predominant sediment properties (OC, texture, CEC, pH) affecting the distribution trend of pharmaceuticals in sediment. MLRs for pharmaceuticals in all seasons combined (Table A.E9, Appendix E) showed OC as the main factors controlling the distribution of amitriptyline and atenolol in sediment. Cimetidine and diclofenac found to be affected by the CEC and pH of the receiving sediment, respectively. MLRs for the rest of pharmaceuticals failed to determine any sediment properties that may affect the distribution.

More in depth, when sites were independently analysed over seasons, different properties were identified as factors influencing pharmaceuticals distribution. In November 2015, only sand % (R^2= 0.66; $p<0.05$) was found as driving factor affects the occurrence of atenolol in sediment. No sediment properties were found for the rest of pharmaceuticals in this month. Similarly to November samples, only OC was chosen by the MLR as a factor influences the distribution of trimethoprim in sediments from February 2016. Sediment properties were found to have more influence on the detected concentration of pharmaceuticals in sediment samples in May 2016;

where CEC (R^2= 0.97; p<0.001) and OC (R^2= 0.72; p<0.01), OC (R^2=0.68; p<0.05) and silt % (R^2= 0.79; p<0.01) were identified as controlling factors for cimetidine, diltiazem and trimethoprim, respectively. In samples collected in July 2016 only clay (R^2= 0.92; p<0.01) and CEC (R^2= 0.88; p<0.05) were identified by the MLR as influencing properties for cimetidine and trimethoprim, respectively.

7.3.8 Water-Sediment Partitioning

To understand the partitioning behaviours of pharmaceuticals between water and sediments, the partitioning coefficient, so called pseudo-partitioning coefficient (*p*-Kd), was calculated by normalizing pharmaceutical concentration in the sediments (Cs, μg Kg^{-1}) to their corresponding concentrations in the water (Cw, ng L^{-1}) using the following equation: *p*-Kd = Cs/Cw (Kim and Carlson, 2007). Only pharmaceuticals that occurred at measurable concentrations in both the water and sediment were used in this analysis. Calculated *p*-Kd under field conditions showed variability along the water: sediment sampling sites and seasons (Figure 7.5, more details in Table A.E10). For example, *p*-Kd values ranging from 158.7 to 1241.0 L Kg^{-1} were observed for amitriptyline and from 5.0 to 1270 for trimethoprim; which likely contributed to the relatively low observed aqueous concentrations (Luo et al., 2011). The obtained *p*-Kd values for amitriptyline are within the same order of those previously reported from laboratory-based sorption experiments described in Chapter 5. Generally, lab-based Kds for those pharmaceuticals investigated in Chapter 5 were within the range or at the lower end of the range values reported for field- based *p*-Kds. Only atenolol showed *p*-Kd values higher than those found in batch sorption experiment ranging from 99.1-132.2 L Kg^{-1}. The NSAIDs (diclofenac, ibuprofen, mefenamic acid) showed Kd values higher than those obtained somewhere else in sediments (Martínez-Hernández et al., 2014; Scheytt et al., 2005; Yamamoto et al., 2009; Zhou and Broodbank, 2014). To our knowledge, the only compounds found to have field-based *p*-Kd values in the literature are trimethoprim, atenolol, ranitidine and mefenamic acid. The *p*-Kd values reported in the current study are in consistency with values ranging from 6.4 to 688.8 L Kg^{-1} reported by Xue et al., (2013) and in most occasions similar to values of <100 L Kg^{-1}

reported by Gibs et al., (2013) for trimethoprim in sediment. Lara-Martín et al., (2014) recently reported very high Kd values for trimethoprim and mefenamic acid in suspended sediment of 7169 and 59,924 L Kg^{-1}, respectively. Recently, Cantwell et al., (2017) have reported *p*-Kds highly similar to those determined for trimethoprim, atenolol and ranitidine in the current study when investigated the spatiotemporal distribution between water and sediment in the USA. This variability shows that pharmaceuticals had variable tendencies to accumulate in the sediments. It has been suggested that the sorption affinity is most likely related to the physicochemical properties of sediment, the concentration of compounds in the water phase and pH of water (Cheng et al., 2014a; Luo et al., 2011; Zhou and Broodbank, 2014). Our previous work that is discussed in Chapter 5 suggests that physicochemical properties, such as OC, clay content, exchangeable Ca ions and organic carbon played significant roles in governing the sorption between basic pharmaceuticals and sediment. A large part of the difference in the sorption of the acidic compounds could be explained by deprotonation with increasing pH which makes the molecule more polar. For example, the sorption of neutral species of naproxen found to be higher than anion forms by a factor of 63 (Tülp et al., 2009). Moreover, in field, colloidal phase in sediment combined water and particulate matter were found to increase the association of naproxen and diclofenac by 8–26% and 22–33%, respectively (Duan et al., 2013).

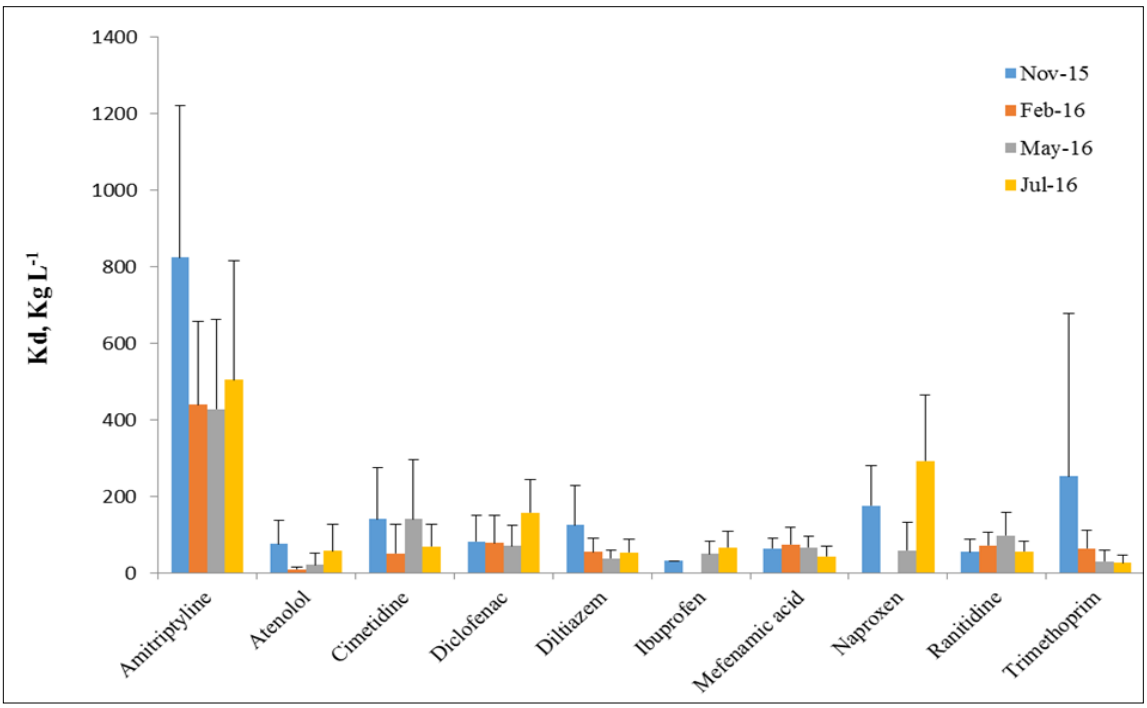


Figure 7.5 Sediment–water pseudo-partitioning coefficients (*p*-Kd) of pharmaceuticals over the study period

7.4 Conclusion

This study provides the first systematic seasonal data on the occurrence and partitioning of pharmaceuticals between the water column and underlying sediment over a 10 month period in two contrasting English rivers: the River Ouse, one of major rivers in the UK and its tributary the River Foss. To achieve this goal, analytical methods were adapted and validated to target pharmaceuticals in water and sediment samples. The pharmaceuticals showed a spatial and seasonal variability in concentrations in the water and sediment compartments. Amitriptyline, trimethoprim, ranitidine and mefenamic acid were the most frequently detected pharmaceuticals in water and sediment. WWTP effluents input to the surface water and dilution factor were the major factors affecting the pharmaceuticals occurrence. Basic pharmaceuticals showed expected high sorption affinity to sediment under field conditions but also acidic pharmaceuticals were found to have occasional high sorption affinity, therefore this behaviour needs further investigation. Finally, the question that needs to be asked, however, is whether *p*-Kd values

derivable from monitoring studies by detecting pharmaceutical concentrations in water and sediment may interchangeably be used with Kd-values obtained from standard laboratory based experiments using the same set of sediment samples.

Chapter 8

General discussion and Conclusion

8.1 Introduction

Research regarding the occurrence and fate of pharmaceuticals in the environment has increased remarkably in recent decades (Boxall et al., 2012; Kolpin et al., 2002). The majority of the studies to date into the occurrence, fate and effects of pharmaceuticals have focused on water and terrestrial compartments (Li et al., 2013; Monteiro and Boxall, 2009; Petrovic et al., 2009; Yamamoto et al., 2009). Fewer work has been done to investigate the occurrence and fate of pharmaceuticals in sediment (aus der Beek et al., 2016; Boxall and Ericson, 2012); even though, sediment has been shown to be a sink for many chemicals including pharmaceuticals (Loffler et al., 2005). The work that has been conducted in sediments has tended to focus on only a few compound classes and sediment types so the wider applicability of findings from these studies is unclear (Chapter 2). There is also the added complexity caused by difficulties in developing reliable analytical methods for many of the compounds within this environmental phase. The aim of the work presented in this thesis was therefore to develop a better understanding of the environmental behaviour of pharmaceuticals in the sediment compartment and of the factors affecting this behaviour. This Chapter gives a brief summary of the findings and implications of the different components of the thesis. The recommendations for future research based on each data chapter are presented.

8.2 Summary and Implications of Findings

A series of studies were conducted to assess the sorption, dissipation and distribution of pharmaceuticals in sediment. Based on the outcomes from risk-based prioritisation study for

pharmaceuticals, as well as an understanding of the current concerns highlighted in literature, ten pharmaceuticals from different therapeutic classes covering a wide range of physicochemical properties were selected for laboratory studies. The results demonstrated that the study pharmaceuticals did behave differently across sediment types. An overview of the results obtained for the study compounds is provided in Table 8.1.

Table 8.1 Synthesis of data obtained from the research chapters

Compound	Rank Chapter 3 and literature	Kd (L Kg^{-1}) Chapter 5	Koc Chapter 5	DT50 (day) Chapter 6	Detected concentration in sediment (ng g^{-1}) Chapter 7	Pseudo Kd L Kg^{-1} Chapter 7
Amitriptyline	4 (subtle effects on fish), 19 (acute/ sediment), 13 (acute/ water), 47 (usage) Exposure (PEC/water)*	8.79-247.97	912.01-12589.25	44.3-78.8	0.24-12.9	158.7-1241.0
Atenolol	13 (chronic/ sediment), 21 (PEC/ sediment), 13 (chronic/ water), 24 (usage) Exposure (PEC/water)*	2.22-20.56	85.11-489.78	9.49-17.4	<LOQ-4.1	99.1-132.2
Cimetidine	Exposure (PEC/water)*	2.28-15.88	102.33-426.78	18.5-36.5	<LOQ-5.9	29.3-338.2
Diclofenac	4 (chronic/ water), 3 (chronic/ sediment), 25 (usage) Exposure (PEC/water)*	-	-	-	<LOD-4.6	534.9
Diltiazem	25 (acute/ water), 22 (chronic/ sediment), 37 (usage) Exposure (PEC/water)*	22.03-1022.6	799.24-13182.57	21.57-35.4	<LOQ-1.23	19.0-396.4
Ibuprofen	11 (acute/ water), 11 (acute/ sediment), 7 (usage) Exposure (PEC/water)*	-	-	-	nd-8.1	NE
Mefenamic acid	3 (uptake from fishery products), 5 (chronic water), 10 (soil), 2 (mammalian predator), 6 (usage) Exposure (PEC/water)*	1.83-19.04	75.86-331.13	19.74-35.0	0.13-1.7	26.0-163.1
Naproxen	7 (chronic water), 6 (chronic/ sediment), 21 (usage) Exposure (PEC/water)*	-	-	-	nd-4.26	67.6-139.0
Ranitidine	10 (mammalian predator/ soil) 18 (chronic/ water), 19 (usage) Exposure (PEC/water)*	-	-	10.14-37.5	0.4-2.26	22.2-163.1
Trimethoprim	14 (acute/ water), 6 (growth inhibition/ bacteria), 4 (resistance selection), 13 (acute/ sediment),9 (usage) Exposure (PEC/water)*	-	-	-	0.2-18.4	5.0-1270.0

***Guo et al., (2016)**

The research in this PhD thesis initially prioritised pharmaceutical active ingredients (API) in the environment in Iraq based on their potential risk. A comprehensive approach was developed for prioritising pharmaceuticals in the environment in terms of risk to aquatic and soil organisms, mammalian wildlife and humans. The top ranked APIs derived from the different prioritisations belonged to the antibiotic class with risk characterization ratios (RCR) > 1. The high ranking of the antibiotics resulted from their high potency to algal or bacterial species or due to their potential to accelerate the evolution and dissemination of antibiotic resistance genes in environmental bacteria. Amoxicillin and metronidazole were on the top list of antibiotics, prioritised based on their potential to select for bacterial resistance, with RCR values of >10. Interestingly, the potential for selection of antibiotic resistance in the environment is not one of the endpoints that should be addressed when assessing the environmental risk of a new pharmaceutical as part of the marketing authorisation process [European Medicines Agency (EMEA) 2006; 2008]. Antibiotics were absent from the top priority list of compounds in terrestrial systems due to different exposure estimation approaches.

Although most prioritisation studies have focused on North America and Western Europe (Dong et al., 2013; Kostich et al., 2014; Perazzolo et al., 2010; Roos et al., 2012), the prioritisation study results show that these approaches are transferable to other countries for setting priorities and developing environmental risk management plans (Boxall et al., 2003; Guo et al., 2016). Many of highly prioritised APIs identified in this study were also found as compounds of high priority in other countries (Boxall et al., 2003; Guo et al., 2016; Kim et al., 2008). The limitations of these prioritisation approaches and recommended solutions are discussed in the recommendations and future work section. The study compounds investigated in this thesis were identified as priorities based on different endpoints. Amitriptyline, ibuprofen and trimethoprim were ranked the 19th, 11th, and 13th to cause acute effects in sediment. For prioritisation based on long term effects, diclofenac was ranked the 3rd followed by naproxen and atenolol which were ranked the 6th and 13th, respectively. For other toxicity end points, amitriptyline was ranked the 4th for subtle effects on fish; mefenamic acid was the 4th for uptake

from fishery products and trimethoprim was ranked the 4th for antimicrobial resistance selection (Table 8.1).

After the selection of the study pharmaceuticals, a robust and sensitive analytical method was developed in the laboratory through a series of optimization studies in which six pharmaceuticals were simultaneously extracted and determined in fresh water sediment using ultrasonic-assisted extraction (UAE) followed by solid phase extraction (SPE) clean up (Chapter 4). A variety of extraction procedures has been reported to extract pharmaceuticals from sediment including UAE (Chapter 2). Some of the modern (novel) extraction methods reviewed in the literature have been shown to be less popular and suffer from limitations. For example, microwave-assisted extraction technique (MAE) is limited to those solvents that absorb microwaves in addition this method has limited availability and high operation cost. Thus, the short extraction time and low solvent consumption, low cost and easy handling are some of the advantages of choosing the UAE technique (Aznar et al., 2014; Chen et al., 2015; Darwano et al., 2014). Among the various sole and combined solvents tested, 2% NH_4OH in methanol, 2% formic acid in methanol and methanol were chosen as the best solvents combinations to be used in the extraction processes. The use of solid-phase extraction (SPE) (hydrophilic–lipophilic balance cartridge (HLB)) as a clean-up and analyte enrichment step prior to analysis also has a positive influence on the recovery of the targeted pharmaceuticals; since the used extraction method is not selective (Chen et al., 2015; Zuloaga et al., 2012). The recoveries for fortified samples in all studied sediments for the entire analytical method ranged from 74.5 to 114.6% for atenolol, 72.3 to 124.9% for amitriptyline, 76.5 to 105% for mefenamic acid and 70.1 to 102% for diltiazem. Cimetidine and ranitidine showed lower recoveries ranging from 40.2 to 68.4% and 30.4 to 55.2% respectively. This variability in recovery is likely to be explained due to naturally occurring organic matter in a sample which may mask the analytes and diminish their signal (Bossio et al., 2008). Overall, the recoveries obtained by our method are similar to those reported elsewhere in the literature (de Sousa et al., 2015; Langford et al., 2011; Pérez-Carrera et al., 2010). A major contributor to extraction and clean-up recoveries of the pharmaceuticals was pH, which typically has been shown as one of the most important parameters that controls

the degree of dissociation of pharmaceuticals and consequently affects the UAE and SPE extraction efficiencies (Ding et al., 2011; Santos et al., 2005).

Analytes were detected using the widely available HPLC-DAD method, and the highly sensitive LC-ESI-MS/MS technique. Both methods were found to be suitable for the determination of pharmaceuticals in sediment samples. The detection limits in sediments for the six analytes varied from 15 to 58.5 ng g^{-1} and 0.03 to 3.5 ng g^{-1}, dry weight, for HPLC-DAD and LC-ESI-MS/MS respectively. Only LC-ESI-MS/MS technique was used in the degradation and monitoring studies.

A series of studies were then performed to understand that fate (adsorption and degradation) and occurrence of a range of pharmaceuticals in sediment systems. Sorption is a key factor in determining the persistence, transportation and bioavailability of sediment-associated contaminants (Schaffer et al., 2012b; Zhou and Broodbank, 2014). While research into the sorption of pharmaceuticals in water-sediment systems has recently increased (Y. Li et al., 2014; Loffler et al., 2005; Stein et al., 2008; Yamamoto et al., 2009, 2005), data are only available for a few active ingredients (Chapter 2) and our understanding of the factors and processes affecting sorption of most pharmaceuticals is limited.

The experimental research in this thesis investigated the sorption behaviour of five pharmaceuticals using a batch equilibrium method in a range of sediment types (Chapter 5). The selected pharmaceuticals had a diverse range of physicochemical properties and this was reflected in their extent of sorption to the test sediments. Sorption was found to be variable and increased in the order: mefenamic acid (Kd= 1.83-19.04) < cimetidine (Kd= 2.28-15.88) < atenolol (Kd= 2.22-20.56) < amitriptyline (Kd= 8.79-247.97) < diltiazem (Kd= 22.03-1022.6). Sorption of cations tended to be higher than that of anions, possibly due to the variety of ionic interactions that occurred between the pharmaceutical molecule with the negatively charged sediment particles (Williams et al., 2009).

To experimentally assess the partitioning behaviour of all pharmaceuticals for sediment risk assessment would be a mammoth task. Prediction of the partition coefficient (Kd) by statistical

models has therefore been an area of interest for the scientific society over the last decades. Initially, models were applied to pesticides in a soil matrix, but since the presence of pharmaceuticals in the environment has grown, models have also been developed for this class of compounds. Therefore, the use of predictive mathematical models to estimate sorption may provide a faster and lower-cost alternative to laboratory-based methods (Berthod et al., 2017). Many quantitative structure activity relationship (QSAR) models for estimating sorption of chemicals in soil have been published in the literature (EC, 2003). These models typically assume that the sorption is determined by the organic carbon content of the solid matrix and predict sorption base on the octanol-water partition coefficient of a chemical. In most cases, these models fail to accurately estimate sorption behaviour for ionisable pharmaceuticals. The reason is that these models have been developed for non-polar organic chemicals by using compounds Log Kow as the main input descriptor (Doucette, 2003; Tolls, 2001). In Chapter 5, the sorption measurements for the study compounds were used to evaluate available predictive model developed by Franco and Trapp, (2008) specifically for neutral and ionised substances for estimating the sorption behaviour of organic compounds in sediments. This comparison of these models highlighted the limitation of the Koc/Kow theory, in which hydrophobicity is assumed to be the main mechanism of interaction for pharmaceuticals in sediment. The mismatch between model predictions and sorption measurements probably reflects the complexity of the interactions that occur between ionisable pharmaceuticals and sediment particles and other descriptors besides Kow are needed to understand and model adequately these ion classes. It is also important to recognize that this is a model for soil so may not be directly transferrable to sediment (Boxall and Ericson, 2012).

The results from this study showed a wide range of Koc values across sediment indicating that the role of hydrophobic interactions in adsorption is probably not dominant and more investigations are needed to determine what other processes affecting sorption behaviour. The selection and application of the most appropriate model for predicting distribution depends on several factors, including the availability of required input, the appropriateness of model to chemical of interest, and the methodology for calculating the necessary physicochemical or

structural information (Doucette, 2003). Generally, each new generation of QSARs shows an incremental improvement over previous approaches because of the availability of new (and sometimes higher quality) experimental data, the increased statistical rigor associated with model validation, or through improved chemical descriptors generated via new computational techniques. The most important future developments in estimating sorption coefficients likely will come through a better understanding of the sorption mechanism and improvements in our ability to characterize the differences in organic matter properties and quantify the magnitude of nonorganic matter contributions to the sorption process. Multiple linear regression (MLR) modelling was therefore used in an attempt to identify the best combination of properties that describes the variation in adsorption of a pharmaceutical across sediment types. Properties such as lipophilicity corrected for the sediment pH (Log Dow) of a compound in the sediment, cation exchange capacity, clay %, organic carbon content and exchangeable Ca^{2+} were chosen by the model. The modelling outcomes confirmed the hypothesis that many sorption mechanisms, including e.g. hydrophobic interaction, hydrogen bonding and complexation to sorbent minerals occur for ionisable pharmaceuticals in sediments (Boxall and Ericson, 2012). As discussed above, quite a number of different factors and interaction mechanisms appear to be involved in pharmaceutical sorption. Pharmaceutical risk assessment in sediment could be better achieved by developing improved QSARs for estimating the sorption behaviour based on molecular properties and sediment characteristics.

Degradation is one of the key processes governing the fate and impact of organic compounds in the environment. Most degradation studies of pharmaceuticals that have been reported in the literature have focused on water, soil and sludge (Li and Zhang, 2010; Li et al., 2013; Quintana et al., 2005; Radjenović et al., 2009). However, little is known about their persistence in freshwater sediments.

Laboratory based experiments were therefore performed to determine the aerobic dissipation of six pharmaceuticals in sediment under sterile and non-sterile conditions. Results showed marked differences between sediments in their ability to degrade different pharmaceuticals with DT50s ranging from 9.5 to 78.8 d (Table 8.1). Dissipation of pharmaceuticals was found to

follow first-order exponential decay and to decrease in the order: amitriptyline (DT50= 44.3-78.8 d) > mefenamic acid (DT50=19.74-35.0 d) > diltiazem (DT50=21.57-35.4 d) > cimetidine (DT50=18.5-36.5 d) > ranitidine (DT50=10.14-37.5 d) > atenolol (DT50=9.49-17.4 d). Under sterile conditions, the persistence of pharmaceuticals was considerably longer. The differences in DT50 for pharmaceuticals between sterile and non-sterile conditions indicate the role of microorganisms in the dissipation of pharmaceuticals indicating that biodegradation is one of the main dissipation mechanisms (Yang et al., 2009).

Previously reported degradation rates for pharmaceuticals differ within the same pharmaceutical class or even for the same pharmaceutical in different matrices with half-lives ranging from days to years (Boxall and Ericson, 2012). Our findings are similar and show that DT50 values for individual compound differ significantly ($p<0.05$) across sediment types.

Usually, factors such as the OC content of the matrix, pH and the level of microbial activity are hypothesised to be important parameters for determining degradation rates of ionisable compounds. For example, the increase of OC and pH influence the microbial activity ((Monteiro and Boxall, 2009; Xu et al., 2009). MLR modelling of our data suggested that clay %, organic carbon content, microbial activity and silt % were the predominant factors determining the degradation rates of diltiazem, cimetidine and ranitidine in sediment. MLR analysis failed to highlight a key property which may be responsible for the differences in the degradation of the other pharmaceuticals. Sorption processes may also affect biodegradation mainly by modifying a compounds bioavailability (Kah et al., 2007). However, in our study no significant statistical relationship was found between sorption behaviour and rate of degradation for all compounds. Overall, the dissipation data suggest that the degradations are driven by a variety of factors and processes and do not rely on a single sediment parameter.

Field studies into the occurrence and spatiotemporal distribution of pharmaceuticals in the aquatic systems of the River Ouse and River Foss, York, UK were performed between November 2015 and July 2016. The developed extraction method and modified LC-MS/MS technique from Chapter 4 was validated for four additional pharmaceuticals (diclofenac,

ibuprofen, naproxen and trimethoprim). Recoveries ranged from 72.1 to 88.0 for diclofenac, 59.4 to 77.3 for ibuprofen, 95.5 to 140.4 for naproxen and 77.2 to 95.5 for trimethoprim. A total of 10 pharmaceuticals were therefore determined in the field samples and found at concentrations up to 59.7 ng L^{-1} (atenolol) and 18.4 ng g^{-1} (trimethoprim) in water and sediment samples, respectively. Detected concentrations in sediment and water were comparable to those reported in the literature (Beretta et al., 2014b; Blair et al., 2013; Silva et al., 2011). Amitriptyline and trimethoprim were found to be the most frequently detected pharmaceuticals (100%) in both matrices. High frequencies of detections are likely due to the use pattern, flow rates and/or low efficiency removals in WWTPs.

Concentrations of the study compounds varied across seasons and sites for both water and sediment samples. There was however no clear distribution pattern for all pharmaceuticals and only samples collected from site R1 had consistently higher concentrations than the other sites for all seasons. Spatial and seasonal distribution profiles were driven by the proximity of a site to a WWTP, river hydrological characteristics, climate conditions and the consumption profile of each active ingredient. MLR results showed that the distribution of pharmaceuticals between the water column and sediment is affected by sorbents physicochemical properties (e.g. OC %, CEC, texture). It was also noticed that those factors controlling the distribution changes between seasons for the same compound which may be explained by the effect of sediment transportation in the catchment, which results in textural and property changes in the bed sediment.

Pseudo-partitioning coefficients (*p*-Kds), which is the measure of the extent of accumulation or mobility of pharmaceuticals between sediment and water column, were calculated. Highest *p*-Kd values of 158.7-1241.0 were obtained for amitriptyline. For pharmaceuticals previously investigated for sorption behaviour in this thesis (Chapter 5), lab-based Kds and field- based *p*-Kds were generally comparable although there were some exceptions where field observed values were higher than the laboratory-derived values (Table 8.1). The high *p*-Kd values reported in current study were found to be similar or lower than those reported in the literature. For example, mefenamic acid showed *p*-Kd values that were consistent with the value of 100 L

Kg^{-1} reported by Gibs et al., (2013). The greater observed *p*-Kds in some sites may be related to a long-term accumulative effect or the impact of a point or non-point pharmaceuticals source or of possible impact of biogeochemistry features that favour the sequestration of these pharmaceuticals into the sediment phase (Agunbiade and Moodley, 2015).

Comparison of the exposure predictions from previous studies with measured concentrations from the current study showed that measured concentrations in waters are lower than concentrations predicted in UK by Guo et al., (2016). Pharmaceuticals showed PEC values 2 to 132 times higher than measured concentrations (MEC) except for cimetidine (Table 8.2). The differences between the predicted and measured concentrations may be related to variability in WWTPs removal values in catchment area to those associated with studies in the literature and used in the prioritisation approach (Burns et al., 2017); or differences in dilution factors that are normally applied in such approaches to what is found in real environment. Pharmaceutical usage data employed in the previous studies was taken from 2012 so it might possible that pharmaceutical usage has changed. No prioritisation study for pharmaceuticals in sediment phase has been conducted in the UK and therefore comparison between MECs from this studies and reported $PEC_{sediment}$ values is not possible. In the future, comparisons between models that evaluate PEC and monitoring data on a catchment scale are needed. Such comparisons may reduce the uncertainty of prioritisation approaches and ensure the correct identification of pharmaceuticals for further investigations.

Table 8.2 Comparison between predictive environmental concentrations (PEC) used in prioritisation model by Guo et al., (2016) and this thesis monitoring data in water phase

Exposure concentration	Compound									
	Amitriptyline	**Atenolol**	**Cimetidine**	**Diclofenac**	**Diltiazem**	**Ibuprofen**	**Mefenamic acid**	**Naproxen**	**Ranitidine**	**Trimethoprim**
MECmax Water (ng L^{-1})	17.7	59.7	37.5	29.8	25.2	22.5	13.9	36.6	32.8	48.4
PEC* Water (ng L^{-1})	77.5	122.1	53.3	123.4	555.3	2779.8	30.0	1212.2	681.4	136.9

* Guo et al., (2016)

8.3 Recommendations and Future Work

The work performed in this thesis has produced novel information on the fate and occurrence of a range of pharmaceuticals in the water-sediment environment. Below we provide recommendations on future work that is needed to build on the findings in the thesis.

8.3.1 Recommendations based on the research in this thesis

1. Further development of the prioritisation approach – The main restraint in the risk based prioritisation approach used in the study is the limited availability of ecotoxicological endpoints and the high dependence on the prediction of effects and properties for the filling of data gaps. A number of compounds will therefore have been identified as a priority based on data generated from predictive approaches. Therefore, it is recommended that monitoring studies at small scale (e.g. a few WWTP) be undertaken to identify whether these high priority compounds do occur in the environment or not.
2. Although the results from prioritisation study show antibiotics could be at concentrations in the environment that select for antimicrobial resistance, current regulatory systems on pharmaceutical pollution do not account for antimicrobial resistance (AMR). Therefore, to limit the environmental impacts of antibiotics, there is a need for refining current environmental risk assessments for these substances which take into consideration the risks to microbial communities and for promoting AMR. To do this, risk assessment for antibiotics must take into account recent developments in the scientific understanding of the effects of antibiotics on microbial communities and on the selection for AMR, to ensure discharge levels for antibiotics are protective of the environment.
3. Degradation of pharmaceuticals during wastewater treatment has not been considered during the prioritisation yet we know that some pharmaceuticals are susceptible to degradation so we will have overestimated environmental risk. In the future, work should focus on further developing and validating prioritisation approaches to reduce these uncertainties. This can be done by applying more complex models that consider

fluctuations in pharmaceutical use, hydrology and the potential for a compound to dissipate in different environmental compartments within the catchment of interest.

4. This research clearly showed the limitation of hydrophobicity as a predictor of sorption of pharmaceuticals. Results from the sorption experiment and sorption prediction models indicate that the degree of partitioning of pharmaceuticals into sediment is affected by both pharmaceutical and sediment properties. The most important future developments in the predictive models currently used in environmental risk assessment of pharmaceuticals likely will come through a better understanding of the sorption mechanisms, improvements in our ability to characterize the differences in organic matter properties (POM and DOM) and through quantifying the magnitude of nonorganic matter contributions to the sorption process. In parallel, a literature search on available sediment Kd data could be launched in order to evaluate and develop more appropriate models for pharmaceuticals in sediment.
5. The degradation studies in this thesis were performed to determine the dissipation of pharmaceuticals in sediment under aerobic conditions. In the future, we recommend that work be done under both aerobic and anaerobic conditions to identify the role of microorganisms in the degradation process. Moreover, the potential adaptation of microorganisms to degrade pharmaceuticals should also be explored by using repeat application studies.
6. A comparison of pharmaceutical concentrations in surface waters and sediment up- and down-stream of the final effluent discharge point from WWTPs confirm these compounds are not completely removed during wastewater treatment. Therefore surface waters are vulnerable to pharmaceutical contamination from point sources. This is a concern, as the monitoring of these compounds in natural waters is not enforced. In the future, pharmaceuticals levels in water-sediment system should be routinely monitored as a precautionary measure due to their potential threats.

8.3.2 General recommendations

1. It is important that transformation products are included during any risk assessment activities for pharmaceuticals as they can add significantly to the overall impact. Moreover, as pharmaceuticals will not occur on their own in the environment but rather mixtures of parent compound and some of its transformation products, it can be important to consider the impact of overall mixture.
2. Although analytical methods developed in this study and elsewhere in the literature have been validated, and shown to be reliable and sensitive to determine pharmaceuticals in solid environmental matrices, the area of sample preparation needs to be further developed in order to obtain more selective approaches with higher analyte recovery. Moreover, the inclusion of pharmaceutical metabolites in any newly developed analytical method is also needed.
3. The formation of non-extractable residues of chemicals in solid compartment during degradation studies is known to reduce their availability for microorganisms. However changes in environmental conditions may cause non bioavailable residues to become more available. Further investigations are needed to understand factors driving the non-extractability of compounds and their re-release following a change in environmental conditions.
4. Data on the occurrence and fate of pharmaceuticals in the real environment are needed since the current risk assessment regulations (e.g. EMEA, 2006) are using predictive exposure data to identify pharmaceuticals of priority. Those data alongside the growing dataset on ecotoxicity of pharmaceuticals might be synthesised to perform a more realistic risk assessment of pharmaceuticals in environment.
5. Sediments as environmental matrices are different to sewage sludge and soil and cross-over of data between the compartments should be avoided.
6. Due to the limitations of previously used sampling methods, sampling modes and strategies need to be re-evaluated and should move towards more robust and comprehensive

approaches. For example, sampling time and the maintenance of analyte stability are essential to obtaining representative data in monitoring studies. This can be achieved by collecting an intraday composite sample representative of a system over a longer period (e.g. 24 hours); or inter day samples including week and weekend days, and flows. To maintain the stability of the analytes a suitable preservation technique (i.e., acidification) to minimize samples biodegradation during the carry-over are needed. Therefore, passive sampling method and in situ real-time sensors should be considered as alternative sampling methods.

7. Determining pharmaceutical exposures in environmental matrices has become an important area of research since the 1990s. The presence of pharmaceuticals in freshwater systems has now been documented worldwide, with research especially focused in Europe and North America. In the future, monitoring studies of APIs are needed in lower-middle income countries to assess the contemporary understanding of their occurrence and fate in order to contribute to the development of risk assessments in these countries.

Appendices

Appendix A

$$\mathbf{WWinhab} = \frac{\mathbf{Total\ daily\ wastewater\ discharges}}{\mathbf{population\ of\ catchment}} \qquad \mathbf{(A.A1)}$$

Where: Total daily wastewater discharges is the waste water discharge in each of the three cities under study; population of the catchment is the population in each city (Baghdad, Mosul, Basrah).

$$\mathbf{Subinhab} = \frac{\mathbf{AP{*}10\hat{}6}}{\mathbf{Iqpop{*}365}} \qquad \mathbf{(A.A2)}$$

Where: Subinhab is the amount of substance consumed per inhabitant per day for the Iraqi population [mg inh d^{-1}]; AP is the Annual pharmaceutical usage [kg $year^{-1}$]; and Iq_{POP} is Iraq population, which is 34.2 million (COSIT 2012).

$$\mathbf{Ksed-water} = \mathbf{Fair\_sed} \times \mathbf{Kair-water} + \mathbf{Fwater\_sed} + \mathbf{Fsolid\_sed} \times \frac{\mathbf{KpSed}}{\mathbf{1000}} \times \mathbf{RHOsolid} \qquad \mathbf{(A.A3)}$$

Default values for Fair_sed, RHOsolid, Fwater_sed and Fsolid_sed were taken from the TGD (EC, 2003).

Since the final PECsed was calculated in terms of dry weight, a conversion step was required, using the equations S5 and S6 (Carvalho et al. 2015).

$$\mathbf{CONVsed} = \frac{\mathbf{RHOsed}}{\mathbf{FsolidSed \times RHOsolid}} \qquad \mathbf{(A.A\ 4)}$$

$$\mathbf{PECsed} = \mathbf{CONVsed} \times \mathbf{PECsed\_ww} \qquad \mathbf{(A.A\ 5)}$$

Default values for RHOsolid, Fsolid_sed and RHOsed (bulk density of sediment (Kg m^{-3})) were taken from the 2003.

$$\mathbf{PNECSW} = \frac{\mathbf{LC50\ or\ EC50}}{\mathbf{AF}} \qquad \mathbf{(A.A\ 6)}$$

Where: AF is an assessment factor, (acute QSAR data 1000, acute experimental data 100, chronic QSAR data 100, and chronic experimental data, 10 (EC, 2003).

Sediment water partition coefficient Ksusp-water were derived separately based on Koc and the fraction of organic carbon in sediment (0.05; EC, 2003). Equation (18) was applied to calculate PNECsediment ($mgKg^{-1}$):

$$\mathbf{PNECsediment} = \frac{\mathbf{K\ susp-water}}{\mathbf{RHOsusp}} \times \mathbf{PNECwater} \times \mathbf{1000} \qquad \textbf{(A.A 7)}$$

Since the final PNECsediment was calculated in terms of dry weight, a conversion step was also required.

For compounds with no experimentally determined earthworm ecotoxicity data, the terrestrial toxicity (14-d LC50 in mM/kg dry soil) was predicted using the quantitative structure–activity relationship (QSAR) available in ECOSAR using the following equation.

$$\mathbf{LogLC50earthworm=1.405–0.308LogKOW} \qquad \textbf{(A.A 8)}$$

Where: $LC_{50\ EARTHWORM}$: Acute earthworm ecotoxicity, [mM/kg dry soil]

$$\mathbf{PNECmammal} = \frac{\mathbf{LD50\ (rat\ or\ mouse)}}{\mathbf{AF}} \qquad \textbf{(A.A 9)}$$

$$\mathbf{PNEC\ biota, hh} = \frac{\mathbf{0.1\ TL \times Bw}}{\mathbf{0.115}} \qquad \textbf{(A.A 10)}$$

Where: The PNECbiota, hh is the predicted no effect concentration in biota expressed in mg kg^{-1}, and uses a default value of human body weight (Bw) of 70 kg, and a daily consumption of fishery products of 0.115 kg. TL, hh, is the acceptable daily intake (ADI). In addition, it is assumed that fishery products make up no more than 10% of the threshold level value (0.1x TL), (EC 2011).

The predicted no effect concentration in drinking water for human (PNECdw, hh) was calculated according to the following equation, retrieved from technical guidance document (EC 2011).

$$\mathbf{PNECdw} = \frac{\mathbf{0.1\ TL \times Bw}}{\mathbf{uptake\ dw}} \qquad \textbf{(A.A 11)}$$

A fraction of 0.1 of the human toxicological standard was used, TL hh, the acceptable daily intake (ADI). A human body weight (bw) of 70 kg and a daily uptake of drinking water (uptake dw) of 2 litres were used.

Table A.A1 Usage quantities in public health sector and over the counter in Iraq, therapeutic class and ecotoxicological information of pharmaceuticals

compound	Therapeutic class	Amount (Kg) over-the-counter	Amount (Kg) public health sector	Organism	Endpoint	Statistic	Value mg/L
Paracetamol	Analgesic	456254.7	214708.04	Daphnia	Immobility	48h EC50	9.2
Amoxicillin	Antibiotic	285607.3	134403.395	Algae	Growth inhibition	72h EC50	0.002
Metformin Hydrochloride	Antidiabetic	85810.24	40381.284	Daphnia		EC50	130
Cefalexine	Antibiotic	84515.47	39771.98	Daphnia		EC50	5.8*
Metronidazole	Antiprotozoal	83020.64	39068.53	Algae	Growth inhibition	72h EC50	39.8
Mefenamic acid	Anti- inflammatory	42946.6	20210.16	Daphnia	Mortality	24h EC50	3.95
Ibuprofen	NSAID	30819.52	14503.3	Daphnia	Immobilisation	48h EC50	10.0
Erythromycin	Antibiotic	30339.16	14277.25	Algae	Growth inhibition	72h EC50	0.02
Trimethoprim	Antibiotic	27277.55	12836.49	Algae	Growth inhibition	72h EC50	16.0
Carbamazepine	Anti-epileptic	16617.08	7819.8	Fish	Mortality	96h LC50	45.8
Ceftriaxone Sodium	Antibiotic	15204.06	7154.85	Algae	Growth inhibition	72h EC50	100**
Guaifenesin	Expectorant	13922.79	6551.9	Algae	Growth inhibition	72h EC50	398.6*
Aspirin	Analgesic	12312.44	5794.09	Algae	Growth inhibition	72h EC50	106.7
Clarithromycin	Macrolide antibiotic	12262.31	5770.5	Daphnia	Reproduction	EC50	0.04
Ciprofloxacin	Fluoroquinolone antibiotic	9740.959	4583.98	Algae	Growth inhibition	EC50	0.005
Ampicillin	Beta-lactam antibiotic	8941.874	4207.94	Algae	Growth inhibition	EC50	145.0*
Valproic acid	Anti-epileptic	8862.909	4170.78	Daphnia		EC50	0.05*
Theophylline	Respiratory diseases	7933.731	3733.52	Daphnia	Immobility	EC50	155.0*
Ranitidine HCL	Anti-ulcer	5878.388	2766.3	Daphnia		EC50	650
Phenylephrine HCL	α1-adrenergic receptor agonist	4581.182	2155.85	Algae		96h EC50	739.0*
Naproxen	NSAID	3990.007	1877.65	Algae	Growth inhibition	72 EC50	30.0
Mebeverine HCL	Antispasmodic	3888.134	1829.71	Algae		96h EC50	6.74*
Captopril	Anti-hypertension	3769.687	1773.97	Daphnia	Immobilization	48h EC50	100
Atenolol	β-blockers	3584.557	1686.85	Fish	Mortality	96h LC50	100
Diclofenac	NSAID	3308.009	1556.71	Daphnia	Immobilization	48h EC50	22.4
Pseudoephedrine	Sympathomimetic drug	3162.851	1488.4	Algae		96EC50	204.7*
Azithromycin	Macrolide antibiotic	2510.752	1181.53	Algae		72EC50	0.001
Ceftazidime	Antibiotic	2471.078	1162.86	Algae		EC50	12,378*
Diphenhydramine Hydrochloride	Antihistamine	2260.15	1063.6	Daphnia	Survival	48h LC50	374.00
Dextromethorphan Hydrobromide	Antitussive	1878.585	884.04	Algae		96h EC50	3.2*
Methyldopa	antihypertensive	1835.575	863.8	Algae		96h EC50	8703*
Losartan Potassium	Anti-hypertension	1701.807	800.85	Algae		EC50	9.14**

Miconazole nitrate	Antifungal	1583.423	745.14	Fish		96h LC50	0.08*
Procyclidine HCL	Anticholinergic	1517.42	714.08	Daphnia		48h EC50	0.79*
Amikacin sulphate	Aminoglycoside antibiotic	1145.481	539.05	Algae		96h EC50	$4.8x10^{-8}$*
Cyclosporine	Immunosuppressant	1035.3	487.2	Daphnia		EC50	20.0
Diltiazem HCL	Calcium channel blocker	1022.061	480.97	Daphnia		96h EC50	8.2
Tramadol HCL	Opioid analgesic	957.6314	450.65	Daphnia		EC50	73.0
Hydrochlorothiazide	Diuretic	925.4376	435.5	Daphnia		EC50	100
Mesalazine	Anti-inflammatory	818.8689	385.35	Fish	Mortality	24h EC50	10.0*
Fluovastatin	Anti-hypercholesterolemia	816.9776	384.46	Daphnia		48h EC50	1.0*
Tetracycline	Antibiotic	797.3214	375.21	Algae	Growth rate	EC50	0.09
Hyoscine Butylbromide	Antispasmodic	636.7627	299.653	Algae		LC50	80**
Tranexamic acid	Anti-fibrinolytic	618.5876	291.1				104
Amitriptyline	Anti-depressant	575.8538	270.99	Daphnia		EC50	7.8
Glibenclamide	Antidiabetic	563.0188	264.95	Daphnia		48h LC50	1.34
Lidocaine	Antiarrhythmic	544.7651	256.36				106
Salbutamol	β2-receptor agonist	460.3388	216.63	Algae		96h EC50	316.8*
Acyclovir	Anti-viral	388.5563	182.85				
Omeprazole	Anti-ulcer	365.8188	172.15	Daphnia		EC50	88.0
Atorvastatin	Anti-hypercholesterolemia	330.7563	155.65	Fish		96h EC50	0.1
Bromhexine HCL	Expectorant	322.1501	151.6	Daphnia		48h EC50	0.87*
Vancomycin HCL	Antibiotic	283.69	133.5	Algae		96h EC50	15,592.0*
Chlorphenamine Maleate	Antihistamine	236.385	111.24	Algae		96h EC50	4.0*
Levamisole	Anti- cancer	231.3488	108.87	Algae		96h EC50	12.06*
Metoclopramide Hydrochloride	Antiemetic	204.1488	96.07	Algae		96h EC50	0.8*
Flutamide	Non-steroidal antiandrogen	184.365	86.76	Algae		96h EC50	3.56*
Amiodarone HCL	antiarrhythmic agent	168.3213	79.21				-
Sitagliptin	Antidiabetic	155.55	73.2	Algae		96h EC50	185.25*
Nitrofurantoin	Antibiotic	144.0113	67.77	Algae		96h EC50	0.0000446*
Dexamethasone	Anti-inflammatory and immunosuppressant	139.1875	65.5	Fish		96h LC50	82.1*
Mycophenolic acid	Immunosuppressant	119.7225	56.34	Algae		96h EC50	2.48*
Diazepam	Antianxiety	115.7488	54.47	Daphnia		24h EC50	4.27
Furosemide	Diuretic	95.67814	45.025				40.56
Ifosfamide	Nitrogen mustard	-	42.65	Algae		96h EC50	214.3*

	alkylating agent (cancer treatment)						
Fluconazole	Anti-fungal	88.82501	41.8	Algae		EC50	100
Danazol	Sex hormone	87.23126	41.05	Algae		96h EC50	2.53*
Azathioprine	Immunosuppressive	-	38.81	Algae		96h EC50	1,027.0*
Phenobarbiton	Anti-epileptic	76.86126	36.17	Algae		96h EC50	115.0*
Ketotifen	Antihistamine	73.58876	34.63	Algae		96h EC50	4.33*
Suxamethonium chloride	Depolarizing neuromuscular blocker	70.33751	33.1	Algae		96h EC50	$7.6x10^{10}$*
Bisacodyl	laxative	67.66639	31.843	Algae		96h EC50	9.91 *
Mesna	Cancer chemotherapy	-	26.82	Algae		96h EC50	$1.7x10^{6}$*
Rosuvastatin Calcium	Anti-hypercholesterolemia	51.94563	24.445	Algae		96h EC50	7.82*
Cyclophosphamide	Alkylating agent (chemotherapy)	-	24.19	Algae		96h EC50	214.31*
Chlorpromazine Hydrochloride	Anti-psychotic	45.45376	21.39	Algae		96h EC50	0.41*
Teicoplanin	Antibiotic	43.15876	20.31				
Pethidine HCL	Opioid analgesic	37.23001	17.52	Algae		96h EC50	11.04*
Fluoxetine HCL	Anti-depressant	28.7725	13.54	Algae		48h EC50	0.027
Olanzapine	Anti-psychotic	21.52625	10.13	Algae		96h EC50	0.25*
Escitalopram oxalate	Anti-depressant	11.39	5.36				1.6
Dactinomycin	Antibiotic	7.437501	3.5	Algae		96h EC50	14,923.0*
Ganciclovir	Antiviral	5.822501	2.74	Algae		96h EC50	5.57*
Doxorubicin HCL	Chemotherapy	-	2.69	Algae		96h EC50	128.77*
Metoprolol	β- blocker	5.631251	2.65	Algae	Growth inhibition	EC50	7.3
Cerezyme	Antibiotic antineoplastic	4.250001	2				
Ondansetron HCL	Gastrointestinal Agent	3.570001	1.68	Daphnia		EC50	88.0**
Trifluoperazine HCL	Anti-psychotic	3.400001	1.6	Daphnia		48h EC50	0.62*
Neostigmine	Anesthetic	-	1.33	Algae		96h EC50	874.89*
Midazolam	Anesthetic	-	1.31	Daphnia		EC50	0.2**
Bosentan	Antihypertensive	2.61375	1.23	Algae		96h EC50	23.55*
Letrozole	Anti-cancer	-	0.93	Algae		96h EC50	39.93*
Atropine sulphate	Antisialogogue	-	0.7	Algae		96h EC50	63.10*
Infliximab	Antibody	-	0.65				
Memantine HCL	Anti-Alzheimer's	1.275	0.6	Algae		96h EC50	5.2*
Thyroxine sodium	Thyroid Supplement	0.74375	0.35	Algae		96h EC50	7.14*
Epinephrine	Hormone	-	0.27	Algae		96h EC50	1,600.36*

Octreotide	Growth hormone	-	0.215				
Atosiban	Tocolytic agent	0.2125	0.1	Algae		96h EC50	$1{,}9x10^{5}$*

Table A.A2 Population, wastewater per capita, wastewater treatment percentage and dilution factors for Baghdad, Mosul and Basrah

Catchment (city)	**Number of population (million)[a]**	**WW_{inhab} L/day[b]**	**DF[c]**	**Wastewater treatment %[d]**
Baghdad	7.255	192	10/40	50
Mosul	3.354	149	10/40	0.0
Basrah	2.602	127	10/40	0.0

a: COSIT, 2012; b,d: COSIT, 2014, c: Keller et al. (2014); WWinhab L/day: daily amount of wastewater per inhabitant; DF: dilution factor; Wastewater treatment %: percentage of wastewater treatment plant efficiency

Table A.A3 Top 20 compounds from each prioritization approach for exposure via surface water in Basrah at 10 and 40 dilution factors

RCR	Low trophic levels				Subtle effects on fish		Mammalian predator		Human (uptake from Fishery products)		Human (uptake from drinking water)
	Acute aquatic (PECSW: acute PNECAQUATIC)		Chronic aquatic (PECSW: chronic PNECAQUATIC)		FSSPC: H_TPC ratio		PECFISH: PNECmammal		PECFISH:PNEC biota, hh		(PECSW: PNECdw, hh)
	D10	D40	D10	D40	D10	D40	D10	D40	D10	D40	D10/D40
>10	Amoxicillin Azithromycin	Amoxicillin	Amoxicillin Clarithromycin Erythromycin	Amoxicillin	Phenylephrine Atorvastatin Mebeverine	Phenylephrine Atorvastatin	Phenylephrine	Phenylephrine	Phenylephrine	Phenylephrine	
1-10	Ciprofloxacin Valproic acid Erythromycin Paracetamol Clarithromycin Cefalexine	Azithromycin Ciprofloxacin Valproic acid Erythromycin	Diclofenac Miconazole nitrate Mefenamic acid	Clarithromycin Erythromycin	Amitriptyline Mefenamic acid	Mebeverine			Atorvastatin		
0.1-1	Miconazole nitrate Mefenamic acid Ibuprofen Tetracycline Metronidazole	Paracetamol Clarithromycin Miconazole nitrate Cefalexine Mefenamic acid Tetracycline Ibuprofen Diphenhydramine	Paracetamol Azithromycin Naproxen Mesalazine Mebeverine	Diclofenac Miconazole nitrate Mefenamic acid Paracetamol Azithromycin Naproxen	Metformin Hydrochloride Miconazole nitrate Glibenclamide	Amitriptyline Mefenamic acid Metformin Hydrochloride	Diazepam Atorvastatin Octreotide	Diazepam	Octreotide Captopril	Atorvastatin Octreotide	Tramadol
<0.1	Trimethoprim Atorvastatin Metformin Hydrochloride Mebeverine Glibenclamide Amitriptyline Aspirin	Hydrochloride Atorvastatin Metronidazole Procyclidine Trimethoprim Amiodarone Fluoxetine Diclofenac	Valproic acid Atenolol Ranitidine Ceftazidime Flutamide Aspirin Diazepam Diltiazem Nitrofurantoin	Mesalazine Mebeverine Valproic acid Atenolol Ranitidine Ceftazidime Flutamide Aspirin Diazepam Diltiazem Nitrofurantoin	Amiodarone Chlorpromazine Hydrochloride Dextromethorphan Hydrobromide Ibuprofen Valproic acid Diphenhydramine Hydrochloride Diltiazem Aspirin Captopril Ceftazidime Diazepam Metoclopramide Hydrochloride	Miconazole nitrate Glibenclamide Amiodarone Chlorpromazine Hydrochloride Dextromethorphan Hydrobromide Ibuprofen Valproic acid Diphenhydramine Hydrochloride Diltiazem Aspirin Captopril Ceftazidime Diazepam Metoclopramide Hydrochloride	Miconazole nitrate Captopril Ibuprofen Paracetamol Azithromycin Metoclopramide Hydrochloride Amiodarone Chlorpromazine Hydrochloride Mefenamic acid Amoxicillin Trimethoprim Metronidazole Amitriptyline Ceftazidime Dextromethorphan Hydrobromide Diphenhydramine Hydrochloride	Atorvastatin Octreotide Miconazole nitrate Captopril Ibuprofen Paracetamol Azithromycin Metoclopramide Hydrochloride Amiodarone HCl Chlorpromazine Hydrochloride Mefenamic acid Amoxicillin Trimethoprim Metronidazole Amitriptyline Ceftazidime Dextromethorphan Hydrobromide Diphenhydramine	Amiodarone Ibuprofen Azithromycin Miconazole nitrate Mefenamic acid Metoclopramide Hydrochloride Chlorpromazine Hydrochloride Ranitidine Ceftazidime Dextromethorphan Hydrobromide Amitriptyline Trimethoprim Diltiazem Diazepam Metronidazole Amoxicillin	Captopril Amiodarone Ibuprofen Azithromycin Miconazole nitrate Mefenamic acid Metoclopramide Hydrochloride Chlorpromazine Hydrochloride Ranitidine Ceftazidime Dextromethorphan Hydrobromide Amitriptyline Trimethoprim Diltiazem Diazepam Metronidazole Amoxicillin	Ranitidine Amoxicillin Phenylephrine Paracetamol Metformin Trimethoprim Captopril Metronidazole Cefalexine Atenolol Valproic acid Mefenamic acid Erythromycin Pseudoephedrine Theophylline Ibuprofen Mebeverine Clarithromycin

PECsw: predicted environmental concentration in surface water; FSSPC: fish steady-state plasma concentration; H_TPC: human plasma therapeutic concentration; PECFISH: predicted environmental concentration in fish; PNEC dw: predicted no-effect concentrations in drinking water; PNECaquatic/PNECmammal: predicted no-effect concentrations in aquatic and mammalian organisms; D: dilution factor.

Table A.A4 Top 20 compounds from each prioritization approach for exposure via surface water in Mosul at 10 and 40 dilution factors.

RCR	Low trophic levels				Subtle effects on fish		Mammalian predator		Human (uptake Fishery products)		Human (uptake from drinking water)
	Acute aquatic (PECSW: acute PNECAQUATIC)		**Chronic aquatic (PECSW: chronic PNECAQUATIC)**		**FSSPC: H_TPC ratio**		**PECFISH: PNECmammal**		**PECFISH:PNEC biota, hh**		**(PECSW: PNECdw, hh)**
	D10	D40	D10	D40	D10	D40	D10	D40	D10	D40	D10/D40
>10	Amoxicillin Azithromycin	Amoxicillin	Amoxicillin Clarithromycin	Amoxicillin	Phenylephrine Atorvastatin Mebeverine	Phenylephrine	Phenylephrine	Phenylephrine HCL	Phenylephrine HCL	Phenylephrine HCL	
1-10	Ciprofloxacin Valproic acid Erythromycin Paracetamol Clarithromycin	Azithromycin Ciprofloxacin Valproic acid Erythromycin	Erythromycin Diclofenac Miconazole nitrate Mefenamic acid	Clarithromycin Erythromycin	Amitriptyline Mefenamic acid	Atorvastatin Mebeverine			Atorvastatin		
0.1-1	Cefalexine Miconazole nitrate Mefenamic acid Ibuprofen Tetracycline Metronidazole	Paracetamol Clarithromycin Cefalexine Miconazole nitrate Mefenamic acid	Paracetamol Azithromycin Naproxen Mesalazine Mebeverine	Diclofenac Miconazole nitrate Mefenamic acid Paracetamol	Metformin Hydrochloride Miconazole nitrate	Amitriptyline Mefenamic acid	Diazepam Atorvastatin Octreotide		Octreotide	Atorvastatin Octreotide	Tramadol
<0.1	Trimethoprim Atorvastatin Metformin Hydrochloride Mebeverine Glibenclamide Amitriptyline Aspirin	Ibuprofen Tetracycline Metronidazole Trimethoprim Atorvastatin Metformin Hydrochloride Mebeverine Glibenclamide Amitriptyline Aspirin	Valproic acid Atenolol Ranitidine HC Ceftazidime Flutamide Diazepam Diltiazem Nitrofurantoin Metformin Hydrochloride	Azithromycin Naproxen Mesalazine Mebeverine Valproic acid Atenolol Ranitidine Ceftazidime Flutamide Diazepam Diltiazem Nitrofurantoin Metformin Hydrochloride	Glibenclamide Amiodarone Chlorpromazine Hydrochloride Dextromethorphan Hydrobromide Ibuprofen Valproic acid Diphenhydramine Hydrochloride Diltiazem Aspirin Captopril Ceftazidime Diazepam Metoclopramide Hydrochloride	Metformin Hydrochloride Miconazole nitrate Glibenclamide Amiodarone Chlorpromazine Hydrochloride Dextromethorphan Hydrobromide Ibuprofen Valproic acid Diphenhydramine Hydrochloride Diltiazem Aspirin Captopril Ceftazidime Diazepam Metoclopramide Hydrochloride	Miconazole nitrate Captopril Ibuprofen Paracetamol Azithromycin Metoclopramide Hydrochloride Amiodarone Chlorpromazine Hydrochloride Mefenamic acid Amoxicillin Trimethoprim Metronidazole Amitriptyline Ceftazidime Dextromethorphan Hydrobromide Diphenhydramine Hydrochloride	Diazepam Atorvastatin Octreotide Miconazole nitrate Captopril Ibuprofen Paracetamol Azithromycin Metoclopramide Hydrochloride Amiodarone Chlorpromazine Hydrochloride Mefenamic acid Amoxicillin Trimethoprim Metronidazole Amitriptyline Ceftazidime Dextromethorphan Hydrobromide Diphenhydramine Hydrochloride	Captopril Amiodarone Ibuprofen Azithromycin Miconazole nitrate Mefenamic acid Metoclopramide Hydrochloride Chlorpromazine Hydrochloride Ranitidine Ceftazidime Dextromethorphan Hydrobromide Amitriptyline Trimethoprim Diltiazem Diazepam Metronidazole Amoxicillin	Captopril Amiodarone Ibuprofen Azithromycin Miconazole nitrate Mefenamic acid Metoclopramide Hydrochloride Chlorpromazine Hydrochloride Ranitidine HCL Ceftazidime Dextromethorphan Hydrobromide Amitriptyline Trimethoprim Diltiazem Diazepam Metronidazole Amoxicillin	Ranitidine Amoxicillin Phenylephrine Paracetamol Metformin Hydrochloride Trimethoprim Captopril Metronidazole Cefalexine Atenolol Valproic acid Mefenamic acid Erythromycin Pseudoephedrine Theophylline Ibuprofen Mebeverine Clarithromycin Amiodarone

PECsw: predicted environmental concentration in surface water; FSSPC: fish steady-state plasma concentration; H_TPC: human plasma therapeutic concentration; PECFISH: predicted environmental concentration in fish; PNEC dw: predicted no-effect concentrations in drinking water; PNECaquatic/PNECmammal: predicted no-effect concentrations in aquatic and mammalian organisms; D: dilution factor.

Table A.A5 Top 20 compounds from each prioritization approach for exposure via surface water in Basrah at 10 and 40 dilution factors.

RCR	Low trophic levels				Subtle effects on fish		Mammalian predator		Human (uptake Fishery products)		Human (uptake from drinking water)
	Acute aquatic (PECSW: acute PNECAQUATIC)		**Chronic aquatic (PECSW: chronic PNECAQUATIC)**		**FSSPC: H_TPC ratio**		**PECFISH: PNECmammal**		**PECFISH:PNEC biota, hh**		**(PECSW: PNECdw, hh)**
	D10	D40	D10	D40	D10	D40	D10	D40	D10	D40	D10/D40
>10	Amoxicillin Azithromycin	Amoxicillin	Amoxicillin Clarithromycin Erythromycin	Amoxicillin	Phenylephrine Atorvastatin Mebeverine	Phenylephrine	Phenylephrine	Phenylephrine	Phenylephrine HCL	Phenylephrine HCL	
1-10	Ciprofloxacin Valproic acid Erythromycin Paracetamol Clarithromycin Cefalexine	Azithromycin Ciprofloxacin Valproic acid Erythromycin Paracetamol	Diclofenac Miconazole nitrate Mefenamic acid	Clarithromycin Erythromycin	Amitriptyline Mefenamic acid	Atorvastatin Mebeverine			Atorvastatin		
0.1-1	Miconazole nitrate Mefenamic acid Ibuprofen Tetracycline Metronidazole Trimethoprim Atorvastatin	Clarithromycin Miconazole nitrate Cefalexine Mefenamic acid Tetracycline Ibuprofen Diphenhydramine	Paracetamol Azithromycin Naproxen Mesalazine Mebeverine	Diclofenac Miconazole nitrate Mefenamic acid Paracetamol Azithromycin Naproxen	Metformin Miconazole nitrate Glibenclamide	Amitriptyline Mefenamic acid Metformin	Diazepam Atorvastatin Octreotide Miconazole nitrate Captopril		Octreotide	Atorvastatin Octreotide	Tramadol
<0.1	Metformin Mebeverine Glibenclamide Amitriptyline Aspirin	Atorvastatin Metronidazole Procyclidine Trimethoprim Amiodarone Fluoxetine Diclofenac	Valproic acid Atenolol Ranitidine HC Ceftazidime Flutamide Diazepam Diltiazem Nitrofurantoin Metformin	Mesalazine Mebeverine Valproic acid Atenolol Ranitidine Ceftazidime Flutamide Diazepam Diltiazem Nitrofurantoin Metformin Hydrochloride	Amiodarone Chlorpromazine Hydrochloride Dextromethorphan Hydrobromide Ibuprofen Valproic acid Diphenhydramine Hydrochloride Diltiazem Aspirin Captopril Ceftazidime Diazepam Metoclopramide Hydrochloride	Miconazole nitrate Glibenclamide Amiodarone Chlorpromazine Hydrochloride Dextromethorphan Hydrobromide Ibuprofen Valproic acid Diphenhydramine Hydrochloride Diltiazem Aspirin Captopril Ceftazidime Diazepam Metoclopramide Hydrochloride	Ibuprofen Paracetamol Azithromycin Metoclopramide Hydrochloride Amiodarone Chlorpromazine Hydrochloride Mefenamic acid Amoxicillin Trimethoprim Metronidazole Amitriptyline Ceftazidime Dextromethorphan Hydrobromide Diphenhydramine Hydrochloride	Diazepam Atorvastatin Octreotide Miconazole nitrate Captopril Ibuprofen Paracetamol Azithromycin Metoclopramide Hydrochloride Amiodarone Chlorpromazine Hydrochloride Mefenamic acid Amoxicillin Trimethoprim Metronidazole Amitriptyline Ceftazidime Dextromethorphan Hydrobromide Diphenhydramine Hydrochloride	Captopril Amiodarone Ibuprofen Azithromycin Miconazole nitrate Mefenamic acid Metoclopramide Hydrochloride Chlorpromazine Hydrochloride Ranitidine Ceftazidime Dextromethorphan Hydrobromide Amitriptyline Trimethoprim Diltiazem Diazepam Metronidazole Amoxicillin	Captopril Amiodarone Ibuprofen Azithromycin Miconazole nitrate Mefenamic acid Metoclopramide Hydrochloride Chlorpromazine Hydrochloride Ranitidine HCL Ceftazidime Dextromethorphan Hydrobromide Amitriptyline Trimethoprim Diltiazem Diazepam Metronidazole Amoxicillin	Ranitidine Amoxicillin Phenylephrine Paracetamol Metformin Hydrochloride Trimethoprim Captopril Metronidazole Cefalexine Atenolol Valproic acid Mefenamic acid Erythromycin Pseudoephedrine Theophylline Ibuprofen Mebeverine Clarithromycin Amiodarone

PECsw: predicted environmental concentration in surface water; FSSPC: fish steady-state plasma concentration; H_TPC: human plasma therapeutic concentration; PECFISH: predicted environmental concentration in fish; PNEC dw: predicted no-effect concentrations in drinking water; PNECaquatic/PNECmammal: predicted no-effect concentrations in aquatic and mammalian organisms; D: dilution factor.

Table A.A6 Top 20 compounds in the three cities from each prioritization approach considered, according to the predicted concentrations in sediment (PECsed) and at 10 and 40 dilution factors.

RCR	Baghdad				Mosul				Basrah			
	Acute aquatic (PECsed: acute PNECsed)		Chronic aquatic (PECsed: chronic PNECsed)		Acute aquatic (PECsed: acute PNECsed)		Chronic aquatic (PECsed: chronic PNECsed)		Acute aquatic (PECsed: acute PNECsed)		Chronic aquatic (PECsed: chronic PNECsed)	
	D10	D40	D10	D40	D10	D40	D10	D40	D10	D40	D10	D40
>10	Amoxicillin				Amoxicillin Erythromycin Azithromycin Ciprofloxacin	Amoxicillin	Amoxicillin Clarithromycin		Amoxicillin Erythromycin Azithromycin Ciprofloxacin	Amoxicillin Erythromycin	Amoxicillin Clarithromycin	Amoxicillin
1-10	Erythromycin Azithromycin Valproic acid Paracetamol Ciprofloxacin	Amoxicillin Erythromycin Azithromycin Valproic acid	Amoxicillin Clarithromycin Diclofenac Miconazole nitrate Mefenamic acid	Amoxicillin Clarithromycin	Valproic acid Paracetamol	Azithromycin Erythromycin Ciprofloxacin Valproic acid	Erythromycin Diclofenac Miconazole nitrate Mefenamic acid	Amoxicillin Clarithromycin Erythromycin	Valproic acid Paracetamol	Azithromycin Ciprofloxacin Valproic acid	Erythromycin Diclofenac Miconazole nitrate Mefenamic acid	Clarithromycin Erythromycin
0.1-1	Cefalexine Miconazole nitrate Clarithromycin Mefenamic acid Ibuprofen	Paracetamol Ciprofloxacin Cefalexine Miconazole nitrate	Paracetamol Naproxen Erythromycin Azithromycin	Diclofenac Miconazole nitrate Mefenamic acid	Cefalexine Miconazole nitrate Clarithromycin Mefenamic acid Ibuprofen Tetracycline Metronidazole	Paracetamol Cefalexine Miconazole nitrate Clarithromycin Mefenamic acid	Paracetamol Azithromycin Naproxen Mesalazine Mebeverine	Diclofenac Miconazole nitrate Mefenamic acid Paracetamol	Clarithromycin Cefalexine Miconazole nitrate Mefenamic acid Ibuprofen	Paracetamol Clarithromycin Cefalexine Miconazole nitrate	Paracetamol Naproxen Azithromycin	Diclofenac Miconazole nitrate Mefenamic acid
<0.1	Metronidazole Tetracycline Trimethoprim Atorvastatin Metformin Mebeverine Glibenclamide Hydrochlorothiazide	Clarithromycin Mefenamic acid Ibuprofen Metronidazole Tetracycline Trimethoprim Atorvastatin Metformin Mebeverine Glibenclamide Bromhexine Hydrochlorothiazide	Mesalazine Mebeverine Valproic acid Atenolol Ceftazidime Flutamide Diazepam Ranitidine Nitrofurantoin Metformin Dexamethasone	Paracetamol Naproxen Erythromycin Azithromycin Mesalazine Mebeverine Valproic acid Atenolol Ceftazidime Flutamide Diazepam Ranitidine Nitrofurantoin Metformin Dexamethasone	Trimethoprim Atorvastatin Metformin Mebeverine Glibenclamide Amitriptyline Metoclopramide	Ibuprofen Tetracycline Metronidazole Trimethoprim Atorvastatin Metformin Mebeverine Glibenclamide Amitriptyline Metoclopramide	Valproic acid Atenolol Ranitidine Ceftazidime Flutamide Diazepam Nitrofurantoin Metformin Dexamethasone	Azithromycin Naproxen Mesalazine Mebeverine Valproic acid Atenolol Ranitidine Ceftazidime Flutamide Diazepam Nitrofurantoin Metformin Dexamethasone	Metronidazole Tetracycline Trimethoprim Atorvastatin Metformin Mebeverine Glibenclamide Bromhexine Hydrochlorothiazide	Mefenamic acid Ibuprofen Metronidazole Tetracycline Trimethoprim Atorvastatin Metformin Mebeverine Glibenclamide bromhexine Hydrochlorothiazide	Mesalazine Mebeverine Valproic acid Atenolol Ceftazidime Flutamide Aspirin Diazepam Ranitidine Nitrofurantoin Metformin	Paracetamol Naproxen Azithromycin Mesalazine Mebeverine Valproic acid Atenolol Ceftazidime Flutamide Aspirin Diazepam Ranitidine Nitrofurantoin Metformin

PECsed: predicted environmental concentration in sediment; PNECsed: Predicted no effect concentrations in sediment; D: Dilution factor. The PECsed and PNECsed were calculated with the Equilibrium Partitioning method from the PECsw and PNECsw respectively.

Appendix B

Table A.B2 Optimized HPLC-DAD and LC-MS/MS conditions: recovery %, instrumental limit of detection (quantification) IDL (IQL) and for the analysis of the target pharmaceuticals

Compound	Retention time (min)	LC-MS/MS						HPLC-DAD	
		Ionization Mode and acquisition	Precursor ion *(m/z)*	Product ion *(m/z)*	Collision energy (eV)	Recovery% (RSD%) at 20 ng mL^{-1} LC-MS/MS	IDL (IQL) ng L^{-1} LC-MS/MS	Recovery% (RSD%) at 2.0 µg mL^{-1} HPLC-DAD	IDL (IQL) ng mL^{-1} HPLC-DAD
Amitriptyline	14.35	+ve MRM	278.2	105.1	35	112.2 (2.7)	10 (30.0)	104.1 (1.1)	9 (30.0)
Atenolol	2.55	+ve MRM	267	145	40	98.5 (3.1)	22 (70.0)	99.2 (3.4)	14 (46.5)
Cimetidine	2.77	+ve MRM	253	95	35	100.1 (2.2)	15 (47.5)	101.5 (2.6)	10 (33.3)
Diltiazem	14.02	+ve MRM	415	178	35	102.2 (1.3)	6 (20.3)	100.1 (1.2)	7 (23.3)
Mefenamic acid	14.64	+ve MRM	242.1	224.1	20	106.3 (1.0)	6 (19.0)	99.9 (0.3)	6 (20.0)
Ranitidine	3.01	+ve MRM	315	176	30	99.6 (3.1)	9 (30.3)	101.5 (3.0)	12 (40)

Section A.B 1: Recoveries calculation

Recoveries were calculated based on the obtained response (concentration) of the analytes in the spiked sediment at different steps of the extraction process. For calculation of the total recovery (RECtotal) we calculated the ratio of the response of the analyte from the sediment sample spiked with the target compounds at 1µg g^{-1} (X) to the response obtained after spiking the reconstituted extract (Z) as shown in Equation (A.B1).

$$RECtotal = \left(\frac{X}{Z}\right) \times 100 \qquad \text{(A.B1)}$$

Recoveries of the SPE step (REC_{SPE}) were calculated as the ratio of the response of the analyte obtained from the extracted sample spiked with analyte (Y) to the post-extracted spiked sample (obtained from spiked reconstituted extract (Z) (Equation A.B2).

$$REC_{SPE} = \left(\frac{Y}{Z}\right) \times 100 \qquad \text{(A.B2)}$$

Table A.B3. Pharmaceuticals recoveries, limits of detection (LODs), and limits of quantitation (LOQs) in sediments. Standard deviation (±RSD %) is presented in parentheses for three replicates using HPLC-DAD

Sediment	Spiking level µg g^{-1}	Atenolol		Cimetidine		Amitriptyline		Diltiazem		Mefenamic acid		Ranitidine	
		Recovery% (±RSD%)	LOD (LOQ) (ng g^{-1})	Recovery% (±RSD%)	LOD (LOQ) (ng g^{-1})	Recovery% (±RSD%)	LOD (LOQ) (ng g^{-1})	Recovery% (±RSD%)	LOD (LOQ) (ng g^{-1})	Recovery% (±RSD%)	LOD (LOQ) (ng g^{-1})	Recovery% (±RSD%)	LOD (LOQ) (ng g^{-1})
BTC	1.0	100.24 (3.2)	37.1 (122.5)	50.18 (3.6)	31.3 (103.3)	93.97 (2.5)	34.0 (112.2)	75.81 (7.3)	20.4 (67.3)	96.94 (3.6)	23.3 (76.9)	51.72 (3.2)	20.2 (66.7)
	0.5	99.3 (9.9)		50.05 (7.8)		95.50 (5.4)		70.25 (5.6)		93.25 (2.8)		45.2 (4.5)	
	0.2	95.21 6.2)		48.32 (10.4)		85.52 (6.3)		75.88 (4.3)		90.41 (4.1)		50.3 (6.4)	
BGD	1.0	84.68 (5.1)	55.6 (183.5)	46.31 (4.2)	15.4 (50.8)	78.29 (5.8)	20.5 (67.7)	79.43 (5.9)	36.8 (121.5)	100.1 (2.8)	15.0 (49.5)	34.63 (5.6)	13.2 (43.6)
	0.5	86.32 (5.5)		42.65 (4.1)		72.36 (4.3)		80.52 (9.8)		92.35 (3.1)		38.25 (6.9)	
	0.2	79.22 (11.0)		45.22 (5.4)		72.52 (4.5)		77.30 (7.5)		99.25 (2.4)		33.4 (4.2)	
HUS	1.0	74.55 (2.6)	32.2 (106.0)	42.34 (4.7)	26.7 (88.1)	80.96 (6.6)	28.2 (93.1)	70.09 (3.7)	34.1 (112.5)	105.04 (6.1)	21.3 (70.3)	31.28 (1.6)	31.4 (103.6)
	0.5	75.22 (3.5)		42.38 (7.7)		81.65 (3.8)		69.47 (9.5)		105.8 (7.2)		31.14 (2.8)	
	0.2	79.64 (6.2)		40.52 (9.9)		80.02 (5.5)		63.21 (8.3)		102.52 (6.8)		30.4 (4.3)	
SKF	1.0	87.99 (5.3)	23.0 (76.0)	43.47 (3.2)	15.4 (50.8)	124.94 (9.2)	25.5 (84.2)	62.40 (4.3)	35.2 (116.2)	84.02 (1.3)	14.0 (46.2)	30.24 (4.4)	12.4 (40.9)
	0.5	80.35 (4.8)		40.62 (1.6)		111.20 (7.5)		62.58 (8.7)		79.32 (2.4)		33.25 (1.7)	
	0.2	81.46 (4.5)		42.56 (5.7)		111.85 (3.7)		60.21 (9.3)		85.28 (2.5)		32.1 (3.8)	
HAB	1.0	93.58 (2.5)	28.0 (92.5)	68.45 (4.9)	19.0 (62.7)	82.30 (1.8)	20.5 (67.7)	102.05 (4.1)	30.8 (101.6)	103.21 (4.1)	19.5 (64.4)	50.98 (7.5)	30.2 (99.7)
	0.5	99.25 (2.9)		67.50 (2.5)		80.52 (2.7)		101.10 (5.2)		101.20 (3.1)		44.6 (6.8)	
	0.2	94.4 (4.7)		67.33 (4.5)		80.46 (6.5)		99.35 (4.9)		101.32 (3.2)		48.5 (9.6)	
MIL	1.0	108.86 (6.1)	34.6 (114.2)	55.09 (2.9)	17.8 (58.7)	75.77 (8.8)	25.3 (84.0)	68.16 (5.3)	39.1 (129.0)	88.78 (3.5)	20.8 (68.6)	32.78 (4.1)	21.3 (70.3)
	0.5	107.62 (8.8)		50.42 (4.8)		76.65 (6.2)		66.52 (6.5)		85.25 (4.1)		30.2 (4.8)	
	0.2	97.7 (5.6)		52.38 (5.7)		70.45 (8.0)		69.25 (12.5)		87.46 (6.6)		29.5 (6.8)	
HLM	1.0	114.64 (6.7)	25.5 (84.2)	57.45 (8.2)	30.2 (100.0)	104.42 (5.1)	38.0 (125.5)	74.98 (2.9)	12.6 (42.0)	97.21 (3.2)	24.0 (79.2)	45.20 (3.2)	27.8 (91.7)
	0.5	107.32 (8.6)		56.55 (8.1)		103.50 (5.8)		70.52 (2.8)		92.35 (3.9)		41.2 (5.6)	
	0.2	113.2 (8.1)		49.32 (9.6)		101.65 (12.1)		69.54 (2.5)		96.52 (7.6)		45.5 (8.8)	
MOOR	1.0	94.17 (2.5)	25.8 (85.1)	56.81 (4.5)	28.7 (94.7)	123.79 (11.8)	25.3 (83.5)	80.14 (3.3)	20.5 (67.7)	90.65 (5.6)	23.2 (106.3)	54.87 (8.2)	32.1 (105.9)
	0.5	88.66 (5.1)		50.87 (3.8)		107.54 (9.8)		82.36 (2.8)		90.50 (4.5)		55.2 (9.5)	
	0.2	85.35 (8.1)		55.54 (8.2)		99.28 (7.9)		77.25 (4.1)		89.52 (4.1)		52.1 (6.3)	
GER	1.0	88.17 (5.8)	28.3 (93.3)	50.18 (2.5)	19.7 (56.0)	97.84 (3.7)	17.3 (57.1)	70.67 (6.6)	45.2 (149.1)	82.19 (3.1)	18.2 (60.1)	53.86 (7.5)	24.8 (81.1)
	0.5	82.12 (9.1)		54.50 (4.7)		95.85 (6.6)		65.35 (5.1)		75.32 (4.4)		50.1 (4.2)	
	0.2	88.62 (5.1)		50.88 (6.2)		88.74 (5.5)		71.85 (11.6)		80.84 (5.8)		52.3 (7.8)	
BW	1.0	77.85 (11.5)	58.5 (193.1)	42.61 (9.9)	31.2 (103.0)	100.82 (2.9)	56.9 (187.8)	60.32 (4.8)	29.6 (97.7)	76.51 (4.4)	16.2 (53.5)	37.49 (8.2)	28.5 (94.1)
	0.5	77.65 (9.8)		40.20 (7.2)		96.52 (2.8)		60.88 (6.2)		77.2 (2.3)		33.5 (5.3)	
	0.2	74.98 (12.4)		40.52 (15.8)		90.31 (10.1)		58.22 (5.7)		76.4 (5.1)		36.4 (9.1)	

Table A.B4. Recoveries and limits of detection (LODs) and limits of quantitation (LOQs) for each pharmaceutical in sediment. Relative standard deviation (RSD %) is presented in parentheses for three replicates using LC-MS/MS

		Amitriptyline		**Atenolol**		**Cimetidine**		**Diltiazem**		**Mefenamic acid**		**Ranitidine**	
Sediment	Conc. ngg-$^{-1}$ (d.w)	Recover% (± RSD%)	LOD (LOQ) (ng g^{-1})	Recover% (± RSD%)	LOD (LOQ) (ng g^{-1})	Recover% (± RSD%)	LOD (LOQ) (ng g^{-1})	Recover% (± RSD%)	LOD (LOQ) (ng g^{-1})	Recover% (± RSD%)	LOD (LOQ) (ng g^{-1})	Recover% (± RSD%)	LOD (LOQ) (n gg^{-1})
BTC	100	99.6 (10.3)	0.3 (1.0)	93.1 (5.9)	1.9 (6.0)	50.3 (2.1)	0.7 (2.3)	80.2 (10.2)	0.1 (0.4)	82.5 (2.3)	2.3 (8.0)	45.2 (7.8)	0.3 (1.0)
	200	110.5 (15.8)		94.7 (6.4)		57.6 (4.3)		75.8 (16.5)		83.8 (5.0)		43.9 (5.8)	
	500	105.4 (10.5)		98.2 (8.1)		58.5 (1.9)		80.1 (3.8)		90.5 (6.7)		51.0 (2.5)	
BW	100	70.3 (8.1)	0.14 (0.5)	77.1 (6.1)	1.9 (6.0)	48.0 (4.6)	0.6 (1.8)	60.2 (6.3)	0.05 (0.17)	70.0 (4.4)	0.1 (0.4)	40.1 (9.1)	0.7 (2.2)
	200	74.2 (7.3)		80.9 (7.4)		48.4 (3.1)		63.4 (8.5)		72.3 (5.7)		43.0 (14.4)	
	500	76.8 (5.2)		83.3(2.3)		51.4 (3.4)		70.8 (4.9)		72.8 (3.4)		49.3 (4.8)	
MIL	100	81.8 (5.6)	0.07 (0.25)	70.5 (6.4)	1.3 (5.0)	44.2 (8.2)	0.6 (1.9)	65.1 (5.2)	0.03 (0.1)	70.2 (5.1)	0.3 (1.0)	45.2 (8.1)	0.6 (1.9)
	200	84.8 (3.4)		76.4 (5.4)		48.7 (11.5)		69.0 (4.6)		77.1 (13.0)		47.0 (11.5)	
	500	87.3 (5.1)		77.1 (3.8)		49.0 (5.8)		72.0 (10.2)		79.5 (9.4)		47.2 (5.1)	
GER	100	69.1 (10.4)	0.2 (0.7)	80.2 (6.8)	2.5 (8.0)	50.4 (15.5)	1.2 (4.0)	72.1 (3.4)	0.02 (0.07)	83.1 (5.8)	2.0 (6.0)	52.5 (6.9)	0.5 (1.6)
	200	73.2 (11.3)		88.1 (9.2)		55.1 (15.8)		76.0 (3.5)		84.7 (5.4)		53.0 (8.4)	
	500	79.2 (3.8)		87.6 (4.2)		56.8 (9.2)		76.3 (4.8)		90.3 (11.2)		58.2 (4.3)	
HLM	100	80.0 (5.9)	0.09 (0.3)	73.2 (6.2)	1.8 (6.0)	48 .1 (14.3)	0.6 (1.9)	91.5 (8.6)	0.04 (0.16)	87.8 (5.9)	0.1 (0.3)	44.3 (5.1)	0.2 (0.6)
	200	80.7 (5.8)		73.0 (7.9)		48.8 (8.9)		96.2 (5.3)		89.0 (3.5)		46.7 (3.6)	
	500	85.3 (7.2)		77.8 (5.4)		45.3 (5.2)		99.0 (4.3)		92.3 (6.1)		50.2 (3.6)	
MOR	100	83.6 (9.1)	0.13 (0.5)	75.6 (12.3)	2.5 (8.0)	41.2 (6.5)	0.8 (2.5)	65.3 (7.7)	0.05 (0.17)	70.1 (5.1)	0.2 (0.6)	48.0 (5.8)	0.3 (0.9)
	200	90.0 (7.0)		76.4 (10.4)		46.1 (10.3)		76.2 (8.2)		73.6 (8.4)		48.2 (4.9)	
	500	92.4 (11.5)		80.2 (6.8)		55.1 (7.8)		77.0 (4.1)		77.0 (2.0)		55.6 (7.0)	
HAB	100	102 (7.3)	0.14 (0.5)	73.9 (5.2)	2.2 (7.0)	51.9 (5.5)	0.7 (2.3)	92.2 (9.1)	0.06 (0.2)	74.1 (8.0)	2.0 (6.0)	42.3 (6.6)	0.6 (1.9)
	200	120.6 (5.9)		78.7 (8.7)		55.4 (12.4)		97.3 (7.4)		76.5 (6.7)		45.0 (11.8)	
	500	115.7 (7.6)		78.2 (6.9)		57.8 (7.1)		96.3 (5.6)		76.8 (10.1)		49.6 (4.3)	
SKF	100	95.5 (5.7)	0.18 (0.6)	83.0 (9.8)	3.5 (12.0)	49.6 (2.8)	0.5 (1.7)	60.3 (5.3)	0.04 (0.13)	90.1 (5.3)	2.5 (8.0)	45.5 (4.5)	0.6 (1.8)
	200	100.7 (9.5)		82.5 (13.2)		53.0 (7.5)		67.4 (7.1)		92.5 (8.4)		48.2 (6.3)	
	500	102.2 (3.8)		88.5 (7.2)		60.2 (5.7)		70.1 (4.9)		100.1 (8.2)		49.3 (5.1)	
BGD	100	106.6 (9.8)	0.2 (0.7)	75.3 (6.2)	1.5 (5.0)	42.5 (8.1)	0.5 (1.7)	75.6 (2.8)	0.07 (0.2)	75.8 (4.1)	0.1 (0.4)	50.3 (2.3)	0.8 (2.4)
	200	120.0 (11.2)		80.1 (5.7)		42.7 (5.9)		78.3 (10.3)		76.8 (5.8)		43.6 (15.2)	
	500	109.8 (6.2)		82.2 (7.1)		45.3 (2.9)		77.8 (6.6)		82.3 (6.9)		46.2 (4.1)	
HUS	100	70.0 (9.3)	0.13 (0.5)	72.2 (10.5)	2.7 (9.0)	52.1 (3.6)	0.7 (2.4)	77.8 (7.2)	0.04 (0.13)	80.0 (3.6)	0.15 (0.5)	40.3 (7.2)	0.4 (1.3)
	200	68.8 (6.9)		73.3 (11.5)		50.0 (8.9)		80.1 (6.0)		82.5 (5.9)		45.3 (8.1)	
	500	74.5 (3.6)		73.5 (5.6)		50.3 (5.2)		88.1 (5.6)		85.5 (3.1)		46.3 (1.1)	

Section A.B2: Matrix effects

The matrix effects were studied by evaluating the signal suppression or enhancement for each pharmaceutical. To assess the influence of matrix components, signals of final sediment extracts spiked with analytes were compared with signals observed from solvent dissolved pharmaceuticals. A value of greater or less than zero indicates signal enhancement or suppression; respectively. The equation used for the signal suppression calculation was (Eq. A.B3):

$$\textbf{Matrix effect}\ \% = \left(\frac{(\textbf{Area sediment} - \textbf{Area blank})}{\textbf{Area standard}} - \mathbf{1}\right) \times \mathbf{100} \qquad \text{(A.B3)}$$

Where: Area $_{sediment}$ is the peak area of the analyte(s) recorded for the sediment spiked with the target compound (s) after extraction, Area $_{blank}$ is the peak area of analytes recorded for blank samples and Area $_{standard}$ is peak area of the analyte(s) recorded for the standard solution.

Table A.B5 Matrix effect of pharmaceuticals in LC-ESI-MS/MS analysis at concentration of 100 ng g^{-1} in all sediment samples

Compound	Matrix effect %									
	BTC	BW	MIL	GER	HLM	MOR	HAB	SKF	BGD	HUS
Amitriptyline	3.1	-22.5	-18.8	-20.3	12.4	-12.5	8.1	-20.1	-7.0	5.1
Atenolol	-10.5	-42.5	-22.5	-20.5	-18.5	-20.5	-15.3	-22.0	-13.6	-6.1
Cimetidine	-12.1	-38.0	-25.1	-18.8	-20.5	-13.8	-15.4	-12.5	-18	-10.0
Diltiazem	-12.8	-20.3	-18.5	-15.2	8.6	-6.0	-22.3	-12.8	12.4	-6.0
Mefenamic acid	-12.5	-20.1	-12.0	-19.3	5.0	-4.1	8.2	-16.8	-13.2	-10.7
Ranitidine	-16.2	-20.1	-18.5	-8.5	-20	-16.4	-5.0	-13.1	-18.3	-10.0

(-) for signal suppression, (+) for signal enhancement

Appendix C

A.C1 Analytical method

Chromatographic methods were developed using HPLC (Perkin Elmer, Flexar) coupled with photodiode array detection and a Supelco 516 C-18-DB column (5µm, 4.6×150 mm) for pharmaceutical detection and quantification. The calibration curves were constructed using ranges of concentration (in triplicate) to confirm linearity. The precision of the methods (recovery) was determined by the repeated analysis of samples at concentrations of 1.0, 5.0, 10 µg mL^{-1}. Limits of detection and quantifications LODs and LOQs were calculated using the signal-to-noise ratio of 3 and 10, respectively.

Table A.C1 Details of the developed HPLC procedures for selected pharmaceuticals

Compound	Mobile phase	Wavelength (nm)	Flow rate (ml min^{-1})	Injection volume (µL)	Retention time (min)	LOD (µg mL^{-1})	LOQ (µg mL^{-1})	Recovery %
Amitriptyline	30mM KH_2PO_4: acetonitrile (35:65), pH 3.65	210	1.0	10	2.95	0.1	0.34	94.9-101.6
Atenolol	0.1% formic acid: acetonitrile (65:35), pH 2.7	227	1.0	20	3.45	0.25	0.8	96.8-108.1
Cimetidine	0.1% formic acid: acetonitrile (65:35), pH 2.7	227	1.0	20	4.42	0.08	0.26	97.8-109.6
Diltiazem	30mM KH_2PO_4: acetonitrile (35:65), pH 3.65	210	1.0	10	3.80	0.14	0.42	95.1-109.5
Mefenamic acid	0.05% formic acid: methanol (20:80), pH 2.7	230	1.0	10	5.48	0.05	0.15	96.8-108.6

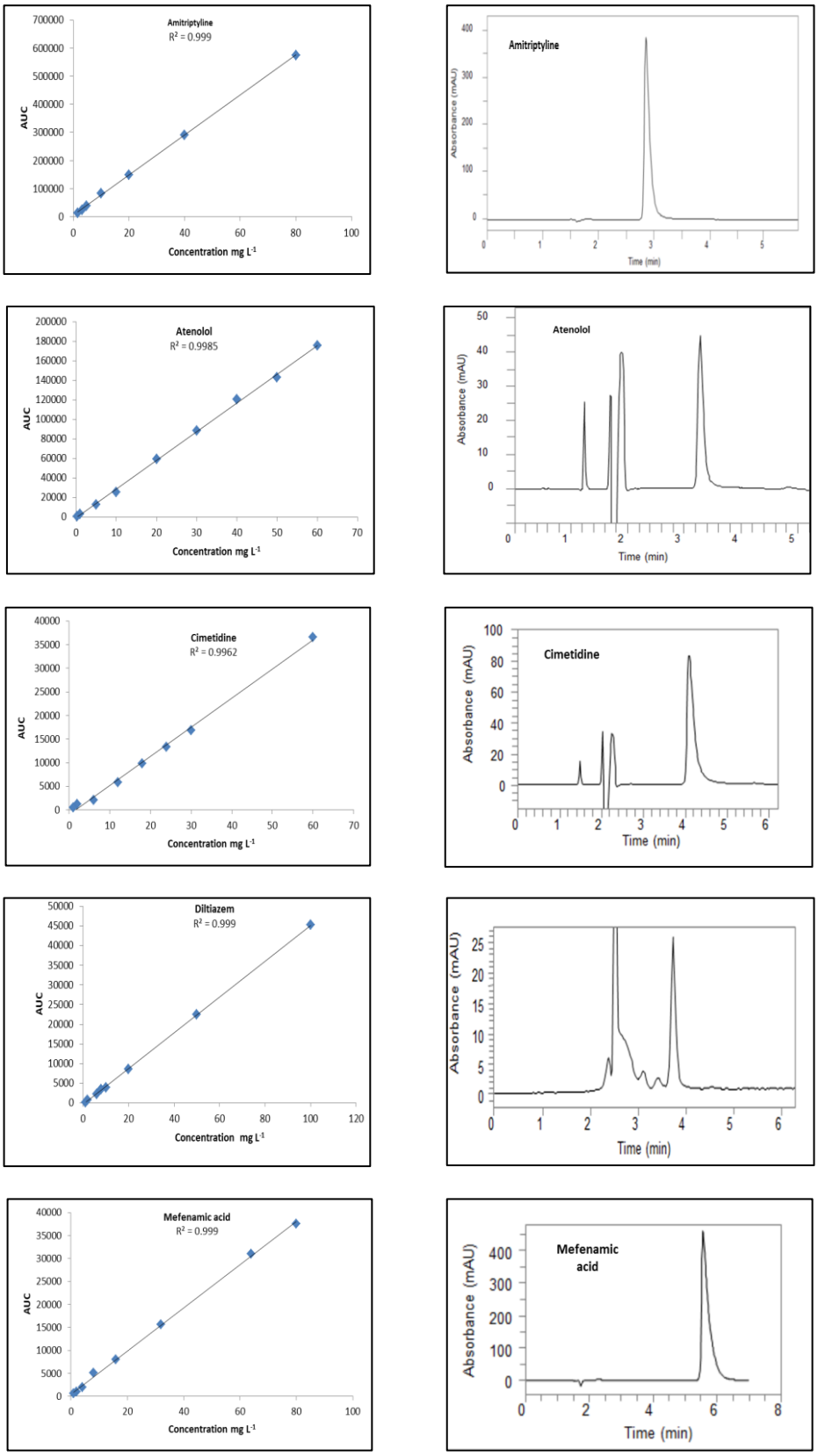


Figure A.C1 HPLC chromatograms and calibration curves for studied pharmaceuticals

Table A.C2 fitting results of linear, Freundlich and Langmuir models

Sediment	Compound	Linear			Freundlich			Langmuir		
		Kd L kg^{-1} (±SD)	Koc (±SD)	R^2	K_F $(mg/kg)•(mg/L)^{-n}$	1/n	R^2	K_L (L Kg^{-1})	Qmax $mg.g^{-1}$	R^2
BTC	Amitriptyline	44.07(±7.80)	1557.24(±275.62)	0.886	380.19	0.476	0.936	0.12	2.50	0.975
	Atenolol	13.88(±0.42)	490.54(±14.84)	0.953	35.24	0.750	0.935	0.04	0.77	0.919
	Cimetidine	12.06(±1.10)	426.15(±38.87)	0.920	107.15	0.491	0.989	1.12	0.71	0.995
	Diltiazem	230.59(±5.18)	8148.56(±183.04)	0.980	42.95	1.633	0.965	0.07	1.11	0.946
	Mefenamic acid	9.5(±0.6)	335.69(±21.20)	0.942	5.77	0.684	0.983	0.02	1.11	0.993
BW	Amitriptyline	320.8(±15.83)	3240.3(±159.90)	0.960	741.31	0.655	0.975	0.33	3.33	0.962
	Atenolol	20.56(±0.25)	207.67(±2.52)	0.942	13.80	1.069	0.963	0.002	10.00	0.979
	Cimetidine	10.53(±0.9)	106.36(±9.0)	0.984	45.71	0.657	0.999	0.04	0.83	0.993
	Diltiazem	1022.6(±16.11)	10329.29(±162.72)	0.973	1556.68	0.616	0.992	1.33	2.5	0.998
	Mefenamic acid	19.04(±0.56)	192.32(±5.0)	0.926	9.31	0.544	0.962	0.034	1.43	0.989
MIL3	Amitriptyline	163.1(±3.42)	2033.66(±42.64)	0.992	186.21	0.968	0.993	0.002	111.1	0.883
	Atenolol	16.75(±1.67)	208.85(±20.82)	0.948	65.61	0.638	0.946	0.08	0.71	0.926
	Cimetidine	15.14(±0.15)	188.78(±1.87)	0.955	223.87	0.301	0.974	0.02	0.53	0.898
	Diltiazem	340.36(±5.25)	4243.89(±65.46)	0.942	187.50	1.315	0.953	0.1	2.00	0.959
	Mefenamic acid	9.86(±0.5)	122.94(±6.23)	0.785	5.01	0.604	0.858	0.01	2.5	0.946
GER	Amitriptyline	247.97(±21.04)	4157.55(±369.77)	0.778	234.42	1.11	0.741	0.07	2.5	0.749
	Atenolol	9.21(±0.31)	160.28(±5.45)	0.927	95.9	0.467	0.99	0.12	0.59	0.976
	Cimetidine	10.9(±0.7)	191.56(±12.3)	0.947	134.90	0.379	0.945	0.27	0.05	0.935
	Diltiazem	181.61(±2.17)	3191.74(±38.14)	0.994	187.50	1.004	0.996	0.007	25.00	0.998
	Mefenamic acid	9.08(±1.24)	159.58(±24.59)	0.96	3.76	0.562	0.96	0.06	0.67	0.985
HLM	Amitriptyline	8.97(±0.31)	915.31(±31.63)	0.834	96.80	0.438	0.571	0.031	0.53	0.283
	Atenolol	2.22(±0.60)	226.53(±61.22)	0.980	6.76	0.771	0.988	0.01	0.4	0.981
	Cimetidine	3.06(±0.63)	312.24(±64.28)	0.910	8.07	0.823	0.943	0.003	1.67	0.975
	Diltiazem	22.03(±2.89)	2247.96(±304.08)	29.92	9.17	0.962	0.963	0.002	1.43	0.989
	Mefenamic acid	1.55(±0.01)	158.16(±1.0)	0.751	2.99	0.653	0.919	0.043	0.28	0.988
MOR	Amitriptyline	32.5(±1.71)	923.29(±33.24)	0.963	39.81	0.963	0.947	0.002	11.11	0.943
	Atenolol	3.02(±0.04)	85.79(±1.14)	0.826	19.5	0.646	0.935	0.015	0.59	0.984
	Cimetidine	7.95(±0.24)	225.85(±6.82)	0.986	30.20	0.691	0.995	0.025	0.77	0.992
	Diltiazem	35.64(±5.03)	1012.5(±142.9)	0.952	40.83	0.975	0.984	0.0004	100.00	0.996
	Mefenamic acid	5.85(±0.89)	158.53(±25.28)	0.826	2.78	0.516	0.969	0.1	0.56	0.993
HAB2	Amitriptyline	138.74(±7.10)	12387.5(±633.93)	0.952	213.8	0.894	0.908	0.007	25.00	0.867
	Atenolol	3.50(±0.05)	312.5(±4.46)	0.985	9.27	1.555	0.977	0.022	2.5	0.979
	Cimetidine	2.28(±0.53)	203.57(±47.32)	0.985	12.46	0.647	0.986	0.02	0.31	0.973
	Diltiazem	146.24(±11.38)	13057.14(±1016.1)	0.972	3.00	2.860	0.899	0.05	0.28	0.801
	Mefenamic acid	1.72(±0.04)	153.57(±3.57)	0.870	2.88	0.675	0.959	0.38	0.28	0.997
SKF1	Amitriptyline	212.73(±6.23)	2685.98(±105.23)	0.933	950.9	0.39	0.903	1.7	2.00	0.883
	Atenolol	10.78(±0.08)	182.1(±1.35)	0.892	95.5	0.534	0.902	0.09	0.62	0.925
	Cimetidine	15.88(±0.33)	268.42(±5.57)	0.946	244.90	0.267	0.929	1.82	0.50	0.869
	Diltiazem	249.04(±24.03)	4206.75(±405.91)	0.979	110.92	1.285	0.922	0.04	3.33	0.999
	Mefenamic acid	6.10(±0.50)	103.04(±7.31)	0.605	2.93	0.509	0.873	0.07	0.71	0.983
BGD	Amitriptyline	162.99(±4.93)	4765.79(±144.15)	0.906	40.66	1.508	0.891	0.05	1.25	0.877
	Atenolol	4.80(±0.11)	140.35(±3.22)	0.981	8.11	0.903	0.989	0.002	3.33	0.996
	Cimetidine	3.52(±0.07)	102.92(±2.0)	0.977	244.34	0.267	0.971	0.008	0.59	0.967
	Diltiazem	88.18(±5.73)	2578.36(±167.54)	0.954	61.66	1.146	0.957	0.03	2.5	0.965
	Mefenamic acid	1.9(±0.04)	55.55(±1.17)	0.540	6.62	1.326	0.808	0.005	0.67	0.934
HUS	Amitriptyline	167.03(±2.67)	4785.69(±76.07)	0.929	254.10	0.853	0.949	0.023	10.00	0.970
	Atenolol	8.36(±0.15)	238.18(±4.27)	0.97	26.85	0.709	0.967	0.03	0.62	0.963
	Cimetidine	5.96(±1.37)	169.8(±39.03)	0.999	8.00	0.931	0.998	0.004	1.67	0.996
	Diltiazem	265.67(±8.93)	7568.95(±254.41)	0.977	56.88	1.573	0.988	0.07	12.5	0.993
	Mefenamic acid	1.833(±0.1)	52.22(±2.85)	0.823	3.36	0.730	0.924	0.025	0.34	0.981

Table A.C3 correlation coefficient (R) between Kd and sediment properties, exchangeable metals correlation in parentheses

Compound	R (%OC)	R pH (CaCl2)	R Clay	R Sand	LogDow	CEC cmol+/kg	Total AL mg/Kg Ex. AL (cmol+/kg)	Total Fe mg/Kg Ex. Fe (cmol+/kg)	Total Ca mg/Kg Ex. Ca (cmol+/kg)	Total K mg/Kg Ex. K (cmol+/kg)	Total Mg mg/Kg Ex. Mg (cmol+/kg)	Total Na mg/Kg Ex. Na (cmol+/kg)
Amitriptyline	0.584	0.890***	0.028	0.471	0.890***	0.646*	0.638*	0.248	0.553*	0.848**	0.435	0.352
							(0.585)	(0.0142)	(0.600)	(0.771**)	(0.467)	(0.197)
Atenolol	0.821**	0.613	0.538	0.348	0.613	0.855**	0.337	0.404	0.324	0.628*	0.112	0.085
							(0.844**)	(0.0390)	(0.864***)	(0.715*)	(-0.197)	(0.012)
Cimetidine	0.769**	0.0546	0.871***	0.124	0.0898	0.756*	0.05	0.58	0.103	0.193	-0.035	-0.144
							(0.759*)	(0.363)	0.785**	(0.243)	(0.0355)	(-0.138)
Diltiazem	0.752*	0.832**	0.056	0.374	0.939***	0.728*	0.432	0.165	0.471	0.888***	0.288	0.213
							(0.722*)	(0.174)	(0.723*)	(0.874***)	(0.224)	(0.036)
Mefenamic acid	0.788**	0.521	0.354	0.072	- 0.522	0.728*	0.083	0.136	0.419	0.626	-0.072	-0.124
							(0.764**)	(0.202)	(0.742*)	(0.663*)	(0.048)	(-0.248)

*p<0.05, **p<0.01, ***p<0.001.

Appendix D

Table A.D1 LC-MS/MS conditions, recovery %, instrumental LOD (LOQ) and correlation coefficient (R^2) for the analysis of the target pharmaceuticals

Compound	Retention time (min)	Ionization Mode and acquisition	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
Amitriptyline	14.35	+ve MRM	278.2	105.1	35
Atenolol	2.55	+ve MRM	267	145	40
Cimetidine	2.77	+ve MRM	253	95	35
Diltiazem	14.02	+ve MRM	415	178	35
Mefenamic acid	14.64	+ve MRM	242.1	224.1	20
Ranitidine	3.01	+ve MRM	315	176	30

A.D1 Stability of frozen samples

All sediments samples were stored in a freezer prior to extraction. Therefore, the stability of pharmaceuticals in frozen samples was evaluated by spiking fresh samples of BTC sediment (in triplicate) with the same concentration of pharmaceuticals spiked at day 0. All pharmaceuticals were shown to be stable in frozen sediments with recoveries similar to those obtained in day 0 samples (Table A.D2).

Table A.D2 Recoveries of pharmaceuticals in frozen samples and freshly spiked sediment (BTC sediment). Standard deviation presented in parentheses.

Compound	Recovery% (SD) Freshly spiked samples	Recovery% (SD) Frozen samples (day 0)
Amitriptyline	108.6 (2.2)	110.4 (7.5)
Atenolol	96.3 (4.1)	90.7 (6.1)
Cimetidine	55.3 (2.0)	57.5 (2.5)
Diltiazem	78.9 (5.2)	69.5 (12.5)
Mefenamic acid	80.6 (1.4)	78.7 (4.2)
Ranitidine	49.4 (4.3)	43.9 (2.6)

Table A.D3 Degradation rate constant (k, d^{-1}), DT50 (±1S.D.) and DT90 (±1S.D.) of pharmaceuticals in sediment under non-sterilised conditions.

Compound	Sediment	Non-sterilised			
		k (d-1)	R^2	DT50 (d)	DT90 (d)
Amitriptyline	BTC	0.0121	0.974	57.3 (0.9)	190.3 (3.1)
	BW	0.0119	0.893	58.24 (0.5)	193.52(1.6)
	MIL	0.0115	0.858	60.26 (9.7)	200.26 (32.4)
	GER	0.0156	0.907	44.3 (6.9)	147.6 (23.0)
	HLM	0.0088	0.951	78.8 (11.2)	261.7 (26.1)
	MOR	0.0138	0.821	50.22 (3.1)	166.88 (10.3)
	HAB	0.0107	0.959	64.77 (2.3)	215.63 (7.8)
	SKF	0.0108	0.963	64.18 (10.3)	213.24 (34.4)
	BGD	0.0104	0.961	66.6 (3.4)	221.4 (11.2)
	HUS	0.0097	0.959	71.45 (6.4)	237.42 (21.3)
Atenolol	BTC	0.0638	0.949	10.8 (0.5)	36.1 (1.7)
	BW	0.0398	0.943	17.41 (1.2)	57.86 (4.0)
	MIL	0.0678	0.982	10.23 (2.4)	33.98 (7.9)
	GER	0.0509	0.979	13.6 (0.7)	45.4 (2.2)
	HLM	0.0448	0.858	15.5 (0.0)	51.4 (0.1)
	MOR	0.0544	0.927	12.47 (1.0)	42.33 (3.2)
	HAB	0.0730	0.987	9.49 (0.6)_	31.54 (2.0)
	SKF	0.0484	0.822	14.32 (1.3)	47.85 (4.2)
	BGD	0.0488	0.961	14.3 (3.4)	47.4 (11.2)
	HUS	0.0662	0.959	10.47 (0.5)	34.79 (1.7)
Cimetidine	BTC	0.0638	0.948	10.8 (0.5)	36.1 (1.7)
	BW	0.0295	0.968	23.49 (8.9)	78.10 (29.5)
	MIL	0.019	0.974	36.47 (8.1)	121.21 (27.0)
	GER	0.0231	0.970	30.0 (4.7)	99.7 (15.5)
	HLM	0.0297	0.916	23.3 (1.6)	77.5 (5.3)
	MOR	0.019	0.973	36.48 (0.8)	121.21 (2.6)
	HAB	0.0247	0.949	28.06 (0.6)	93.24 (1.6)
	SKF	0.0251	0.898	27.61 (3.5)	91.72 (11.5)
	BGD	0.0282	0.930	27.6 (0.5)	81.7 (1.5)
	HUS	0.0374	0.956	18.53 (0.5)	61.57 (2.0)
Diltiazem	BTC	0.0343	0.981	27.9 (2.1)	67.1 (6.9)
	BW	0.0272	0.985	25.48 (3.0)	92.86 (10.1)
	MIL	0.0271	0.978	25.57 (2.8)	84.98 (9.4)
	GER	0.032	0.944	21.7 (3.5)	72.0 (11.5)
	HLM	0.0196	0.973	35.4 (7.4)	117.5 (24.7)
	MOR	0.0289	0.944	23.98 (5.0)	79.89 (16.7)
	HAB	0.0248	0.932	27.94 (2.3)	92.86 (7.7_
	SKF	0.0313	0.993	22.14 (1.9)	73.57 (6.3)
	BGD	0.0234	0.916	29.6 (3.7)	98.4 (12.3)
	HUS	0.0202	0.960	34.31 (5.1)	114.10 (16.8)
Mefenamic acid	BTC	0.0207	0.967	33.5 (1.2)	111.3 (3.9)
	BW	0.0233	0.958	29.75 (1.9)	98.84 (6.3)
	MIL	0.0198	0.935	35.0 (5.3)	116.31 (17.6)
	GER	0.0241	0.926	28.8 (3.6)	95.5 (12.1)
	HLM	0.0209	0.922	33.2 (3.1)	110.2 (10.4)
	MOR	0.0351	0.978	19.74 (3.0)	65.61 (9.9)
	HAB	0.0264	0.944	26.25 (1.0)	87.23 (2.7)
	SKF	0.0243	0.985	28.52 (1.6)	94.77 (5.0)
	BGD	0.0223	0.827	31.1 (7.0)	103.3 (23.1)
	HUS	0.0251	0.901	27.61 (5.1)	91.75 (16.9)
Ranitidine	BTC	0.0475	0.943	14.6 (2.4)	48.5 (8.1)
	BW	0.0683	0.945	10.14 (1.0)	33.72 (2.4)
	MIL	0.0383	0.973	18.10 (2.2)	60.13 (7.2)
	GER	0.0471	0.961	14.7 (2.6)	49.0 (3.5)
	HLM	0.0325	0.944	21.3 (3.0)	70.9 (10.0)
	MOR	0.0454	0.948	15.27 (0.3)	50.72 (9.9)
	HAB	0.0185	0.947	37.46 (4.2)	124.48 (13.9)
	SKF	0.0484	0.951	14.32 (5.7)	47.58 (29.2)
	BGD	0.0329	0.971	21.1 (4.0)	70.0 (13.1)
	HUS	0.022	0.900	31.50 (8.2)	104.68 (27.6)

Table A.D4 Degradation rate constant (k), DT50 (SD) and DT90 (SD) of the test compounds in four sediments under sterilised conditions.

Compound	Sediment	Sterilised			
		k (d-1)	R^2	DT50 (d)	DT90 (d)
Amitriptyline	BTC	0.0091	0.954	76.2 (7.3)	253.1 (24.4)
	GER	0.0104	0.984	66.6 (15.3)	221.4 (50.8)
	HLM	0.0066	0.946	105.0 (12.5)	348.9 (41.3)
	BGD	0.0065	0.935	106.6 (15.8)	354.3 (52.6)
Atenolol	BTC	0.024	0.982	28.8 (2.2)	95.9 (7.4)
	GER	0.0279	0.996	24.8 (2.7)	82.5 (8.9)
	HLM	0.0337	0.994	20.5 (2.5)	68.3 (8.2)
	BGD	0.0313	0.997	22.1 (1.2)	73.6 (3.4)
Cimetidine	BTC	0.0158	0.956	43.9 (1.3)	145.8 (4.3)
	GER	0.0128	0.978	54.1 (2.0)	179.9 (6.5)
	HLM	0.0246	0.986	28.2 (2.9)	93.6 (9.6)
	BGD	0.0169	0.905	41.0 (5.5)	136.3 (18.3)
Diltiazem	BTC	0.0099	0.971	70.0 (5.3)	104.2 (17.6)
	GER	0.0088	0.943	78.7 (12.4)	99.7 (41.2)
	HLM	0.012	0.961	57.7 (11.5)	93.6 (38.2)
	BGD	0.0115	0.927	60.3 (8.4)	136.3 (27.9)
Mefenamic acid	BTC	0.0134	0.973	51.7 (6.5)	171.8 (21.6)
	GER	0.012	0.985	57.7 (8.3)	191.9 (27.5)
	HLM	0.0114	0.917	60.9 (4.0)	202.0 (13.3)
	BGD	0.0155	0.912	44.7 (5.1)	148.6 (15.4)
Ranitidine	BTC	0.0153	0.874	45.3 (3.9)	150.5 (13.0)
	GER	0.0148	0.963	46.8 (4.1)	155.6 (13.6)
	HLM	0.019	0.973	36.5 (4.5)	121.2 (15.0)
	BGD	0.0193	0.92	35.9 (3.7)	119.3 (12.3)

Appendix E

Table A.E1: Sample identification number and location.

Sampling identification		
Sampling site	**Coordinate**	**Distance from WWTP (Km)**
R1	54.0408, -1.035	2.0
R2	54.007, -1.060	5.6
R3	53.968, -1.073	12.1
R4	53.954, 1.077	5.6
R5	53.983, 1.1295	0.2
R6	52.967, -1.103	3.0
R7	53.944, -1.082	6.5

Table A.E2: Water characteristics at the sampling sites at different seasons.

Water characteristics November 2015								
Sampling site	**pH**	**Temperature ^{o}C**	**Conductivity (µS)**	**DO (mg/L)**	**TDS (mg/L)**	**NH_3 (mg/L)**	**Flow Ouse (m^3/s)**	**Flow Foss (m^3/s)**
R1	7.64	7.8	686	10.7			155.14	2.28
R2	7.69	7.6	720	11.1				
R3	7.81	7.1	780	12.0				
R4	7.83	6.5	563	12.8				
R5	7.88	6.6	533	12.4				
R6	7.85	6.4	562	12.4				
R7	7.83	6.1	557	12.5				
Water characteristics February 2016								
Sampling site	**pH**	**Temperature ^{o}C**	**Conductivity (µS)**	**DO (mg/L)**	**TDS (mg/L)**	**NH_3 (mg/L)**	**Flow Ouse (m^3/s)**	**Flow Foss (m^3/s)**
R1	8.12	9.7	754	10.4			96.8	1.62
R2	8.13	9.6	734	11.6				
R3	8.2	9.7	737	10.7				
R4	8.14	8.5	213	11.1				
R5	8.1	9.85	230	8.1				
R6	8.11	9.0	216	8.1				
R7	8.2	8.1	217	12.0				
Water characteristics May 2016								
Sampling site	**pH**	**Temperature ^{o}C**	**Conductivity (µS)**	**DO (mg/L)**	**TDS (mg/L)**	**NH_3 (mg/L)**	**Flow Ouse (m^3/s)**	**Flow Foss (m^3/s)**
R1	8.05	15.0	767	10.4	495	1.8	19.8	0.34
R2	8.25	15.3	763	10.7	495	2.72		
R3	8.2	14.0	786	10.1	510	0.02		
R4	8.33	14.7	588	11.3	381	0.01		
R5	8.4	14.8	608	10.8	369	0.02		
R6	8.21	14.8	590	11.6	392	0.02		
R7	7.5	15.9	607	10.6	394	0.01		
Water characteristics July 2016								
Sampling site	**pH**	**Temperature ^{o}C**	**Conductivity (µS)**	**DO (mg/L)**	**TDS (mg/L)**	**NH_3 (mg/L)**	**Flow Ouse (m^3/s)**	**Flow Foss (m^3/s)**
R1	8.12	23.5	847	9.1	550	11.9	16.8	0.16
R2	7.85	23.1	717	8.1	465	4.0		
R5	7.7	24.0	407	7.9	264	6.0		
R6	7.6	22.0	400	7.5	260	3.3		
R7	7.65	22.1	406	8.0	263	1.23		

Table A.E3: Sediment characteristics at the sampling sites at different seasons.

Sediment characteristics November 2015							
Sampling site	**Texture**	**%clay**	**%sand**	**%silt**	**%OC**	**pH**	**CEC ($cmol_c$/Kg)**
R1	Sandy loam	0.79	69.7	29.5	1.49	7.02	8.9
R2	Loamy sand	0.72	82.8	16.5	1.09	7.0	5.6
R3	Sandy loam	1.40	54.3	44.3	7.83	6.95	34.2
R4	Sandy loam	0.82	61.8	37.4	2.37	6.85	12.5
R5	Loamy sand	0.57	78.7	20.7	1.12	7.05	6.3
R6	Sand	0.36	86.2	13.4	0.77	6.5	4.8
R7	Loamy sand	0.48	76.7	22.9	1.63	6.5	11.8
Sediment characteristics February 2016							
Sampling site	**Texture**	**%clay**	**%sand**	**%silt**	**%OC**	**pH**	**CEC ($cmol_c$/Kg)**
R1	Loamy sand	0.4	85.2	14.4	0.42	6.82	4.8
R2	Sand	0	97.08	2.92	0.38	7.10	4.1
R3	Sandy loam	0.5	66.5	33.0	3.0	7.23	17.6
R4	Sandy loam	0.2	75.2	24.6	6.1	6.98	26.7
R5	Sandy loam	0.055	61.5	38.5	1.72	6.85	10.7
R6	Sand	0.05	93.95	6.0	0.26	7.05	4.5
R7	Sandy loam	0.7	62.48	36.82	1.15	6.80	7.9
Sediment characteristics May 2016							
Sampling site	**Texture**	**%clay**	**%sand**	**%silt**	**%OC**	**pH**	**CEC ($cmol_c$/Kg)**
R1	Sand	0.23	96.15	3.62	0.22	6.7	2.6
R2	Sand	0.8	90.0	9.2	0.52	7.2	4.2
R3	Sandy loam	1.6	63.6	34.8	3.02	6.6	15.5
R4	Medium loam	4.1	48.57	47.33	0.57	7.0	5.0
R5	Loamy sand	2.18	83.82	14.0	0.36	6.7	3.7
R6	Loamy sand	3.21	80.79	16.0	1.48	6.92	11.8
R7	Silty loam	1.9	46.9	51.2	2.20	6.84	14.0
Sediment characteristics July 2016							
Sampling site	**Texture**	**%clay**	**%sand**	**%silt**	**%OC**	**pH**	**CEC ($cmol_c$/Kg)**
R1	Loamy sand	0.14	87.19	12.67	0.41	6.85	4.3
R2	Sand	0.21	92.69	7.1	0.39	6.65	4.1
R5	Loamy sand	0.30	88.2	11.5	0.89	7.05	7.5
R6	Loamy sand	1.25	77.05	21.7	0.55	6.42	4.7
R7	Loamy sand	2.15	81.35	16.5	0.67	6.5	5.0

Table A.E4: Identification of pharmaceuticals in SRM mode by LC–ESI–MS/MS

Compound	**Retention time (min)**	**Polarity (ESI)**	**Precursor ion**	**Product ion**	**Collision energy**
Amitriptyline	18.09	Positive	278.2	233	17.5
Atenolol	4.19	Positive	267.18	145.1	25
Cimetidine	4.47	Positive	253.1	160.0	14
diclofenac	20.04	Positive	296.0	215.0	20
Diltiazem	16.7	Positive	415.15	150.36	42
Ibuprofen	21.79	Negative	205	161.0	10.25
Mefenamic acid	22.33	Negative	240	196	17
Naproxen	18.33	Negative	229	169.9	15
Ranitidine	4.83	Positive	315.2	175.9	18
Trimethoprim	8.66	Positive	291.061	230.07	22.7

Table A.E5 Monthly pharmaceuticals consumption (Kg) in the study area (City of York)

Compound	Month									
	Oct-15	Nov-15	Dec-16	Jan-16	Feb-16	Mar-16	Apr-16	May-16	Jun-16	Jul-16
Amitriptyline	4.82	5.29	4.61	3.87	4.55	4.21	4.21	3.95	3.70	4.16
Atenolol	6.27	5.77	5.41	5.20	5.72	5.60	5.40	5.12	5.02	5.25
Cimetidine	0.71	1.20	1.02	1.09	1.12	1.05	0.98	1.14	0.87	1.13
diclofenac	72.70	69.42	62.10	67.60	65.20	65.45	76.01	68.45	79.26	72.53
Diltiazem	7.88	7.62	7.45	8.04	7.33	8.02	8.00	7.80	7.21	7.76
Ibuprofen	45.90	46.90	46.90	46.10	47.40	52.30	48.40	46.50	44.50	45.10
Mefenamic acid	2.50	2.41	2.75	2.69	2.61	2.40	2.49	2.72	2.48	2.63
Naproxen	51.66	48.90	44.32	48.20	49.20	49.80	52.04	48.77	51.95	51.80
Ranitidine	10.81	10.50	10.60	10.36	10.18	11.64	10.98	10.84	10.60	11.36
Trimethoprim	3.24	3.31	3.12	3.17	2.90	3.09	3.09	3.09	2.85	3.07

Table A.E6 Analytical performance of the entire UAE-SPE-LC-MS/MS method for the analysis of pharmaceuticals in sediments at 5 ng g^{-1}. Matrix effects were calculated for sediment collected in site R3. All from samples collected in November 2015,

		Sediment								Water	
Compound	LOD (LOQ) ng g^{-1}	Pharmaceuticals recovery% (± RSD %)							Matrix effect %	LOD (LOQ) ng L^{-1}	Pharmaceuticals recovery% (± RSD %)
		R1	R2	R3	R4	R5	R6	R7			
Amitriptyline	0.02 (0.03)	82.1 (3.1)	80.5 (2.8)	76.6 (0.6)	85.2 (0.8)	77.9 (3.4)	90.5 (4.0)	72.3 (3.6)	5.1	0.5 (1.6)	110.2 (±0.8)
Atenolol	0.07 (0.23)	104.5 (3.2)	94.1 (6.2)	95.9 (4.7)	105.2 (7.1)	87.5 (8.8)	99.6 (2.7)	94.5 (1.2)	-16.2	3.2 (10.6)	96.0 (±4.2)
Cimetidine	0.08 (0.26)	52.8 (3.0)	55.2 (3.1)	66.1 (12.2)	60.8 (2.7)	60.5 (6.4)	58.2 (2.8)	49.7 (7.2)	6.6	3.5 (11.5)	86.5 (±2.4)
Diclofenac	0.92 (3.0)	85.1 (6.3)	80.4 (3.4)	72.1 (4.6)	80.1 (6.2)	77.5 (3.8)	88.0 (10.2)	76.0 (6.6)	4.8	1.27 (4.2)	88.4 (±4.6)
Diltiazem	0.04 (0.15)	70.2 (3.3)	74.1 (2.4)	77.8 (5.1)	70.2 (5.1)	76.5 (4.8)	80.1 (5.4)	69.5 (4.2)	-10.6	0.5 (1.6)	100.4 (±2.8)
Ibuprofen	0.87 (2.87)	77.3 (5.1)	72.5 (4.4)	64.4 (12.1)	68.5 (3.2)	59.4 (7.7)	72.0 (3.2)	70.1 (3.1)	-12.3	3.06 (10.1)	93.5 (±4.5)
Mefenamic acid	0.03 (0.09)	115.5 (5.5)	118.3 (6.8)	148 (2.3)	100.8 (3.5)	125.1 (7.8)	140.4 (5.5)	105.5 (3.5)	-1.5	0.94 (3.1)	105.1 (±1.2)
Naproxen	0.09 (0.3)	95.5 (3.8)	97.8 (8.8)	122.0 (8.7)	120.1(10.5)	95.8 (3.5)	115.2 (4.6)	122.3 (5.5)	7.1	5.3 (17.5)	88.3 (±4.6)
Ranitidine	0.01 (0.03)	48.5 (4.4)	50.8 (3.0)	55.2 (2.0)	55.8 (4.5)	46.2 (3.1)	48.2 (3.4)	52.7 (4.0)	-1.12	1.76 (5.8)	90.8 (±2.5)
Trimethoprim	0.03 (0.09)	95.5 (2.4)	88.2 (6.3)	87.0 (4.6)	80.2 (5.0)	77.2 (8.1)	90.3 (5.2)	85.4 (2.5)	-10.3	0.6 (2.0)	115.8 (±3.8)

Table A.E7: Seasonal detected pharmaceutical concentrations (water samples) all study sites in the River Foss and Ouse downstream of WWTP

Water								
Compound	**Season**	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**
Amitriptyline	Nov-15	10.5 (1.8)	6.46 (1.65)	11.7 (2.5)	2.5 (0.21)	1.95 (0.76)	1.93 (0.73	2.6 (0.45)
	Feb-16	5.1 (0.5)	5.2 (1.1)	3.8 (0.2)	1.6 (0.22)	2.1 (0.6)	1.5 (0.1)	1.6 (0.6)
	May-16	8.0 (0.2)	3.8 (0.2)	3.9 (0.01)	2.7 (0.3)	0.8 (0.05)	1.8 (0.05)	1.6 (0.2)
	Jul-16	17.7 (0.5)	5.7 (2.9)	NE	NE	4.0 (0.12)	4.1 (0.2)	2.2 (0.13)
Atenolol	Nov-15	37.5 (7.2)	32.1 (7.4)	28.7 (7.5)	15.6 (5.4)	<LOQ	<LOQ	<LOQ
	Feb-16	35.9 (6.8)	31.7 (3.3)	27.0 (2.3)	10.9 (2.8)	<LOQ	16.4 (1.9)	14.9 (2.4)
	May-16	29.4 (4.5)	36.7 (10.9)	31.2 (11.3)	13.9 (1.7)	11.8 (1.6)	10.4 (2.5)	11.9 (1.1)
	Jul-16	59.7 (7.9)	41.2 (1.4)	NE	NE	<LOQ	10.9 (2.4)	<LOQ
Cimetidine	Nov-15	17.4 (2.1)	14.8 (1.4)	14.6 (8.4)	nd	nd	<LOD	nd
	Feb-16	<LOQ	12.3 (1.1)	<LOQ	<LOD	nd	nd	<LOD
	May-16	14.5 (2.0)	<LOQ	<LOQ	nd	<LOQ	<LOQ	nd
	Jul-16	37.1 (5.8)	12.5 (2.6)	NE	NE	<LOQ	<LOD	<LOD
Diclofenac	Nov-15	18.2 (2.7)	17.9 (0.9)	18.0 (1.1)	10.5	8.9 (0.5)	9.5 (2.1)	8.9 (2.5)
	Feb-16	24.7 (1.0)	18.3 (1.9)	19.9 (1.1)	12.1	7.8 (0.4)	7.2 (2.3)	9.1 (3.5)
	May-16	21.6 (1.3)	15.4 (0.6)	14.2 (3.8)	10.7	10.2 (1.8)	8.7 (1.8)	11.7 (0.4)
	Jul-16	29.8 (1.9)	18.7 (1.3)	NE	NE	10.8 (2.4)	10.2 (1.6)	10.0 (0.9)
Diltiazem	Nov-15	6.5 (0.35)	6.3 (0.62)	7.0 (0.66)	2.4 (0.24)	1.5 (0.12)	3.8 (0.6)	3.2 (0.6)
	Feb-16	6.6 (0.12)	5.3 (0.1)	5.5 (0.1	<LOQ	1.4 (0.03)	1.35 (0.013)	1.4 (0.1)
	May-16	8.8 (0.2)	9.0 (0.1)	9.1 (0.6)	nd	nd	nd	nd
	Jul-16	25.2 (0.4)	17.2 (0.9)	NE	NE	6.1 (1.5)	6.1 (1.1)	4.5 (1.2)
Ibuprofen	Nov-15	18.7 (1.0)	14.0 (3.1)	15.5 (1.6)	nd	nd	nd	nd
	Feb-16	22.5 (0.7)	15.2 (0.4)	10.8 (0.1)	nd	<LOQ	<LOQ	<LOQ
	May-16	19.6 (0.3)	10.5 (0.1)	13.8 (0.6)	nd	<LOQ	<LOQ	<LOQ
	Jul-16	18.0 (0.7)	12.8 (1.4)	NE	NE	<LOQ	<LOQ	<LOQ
Mefenamic acid	Nov-15	10.7 (1.6)	9.0 (0.6)	10.05 (1.0)	2.2	4.5 (1.2)	<LOQ	nd
	Feb-16	8.6 (1.2)	4.8 (0.2)	13.9 (2.4)	nd	6.9 (0.6)	5.2 (1.8)	nd
	May-16	9.6 (0.7)	8.4 (1.4)	4.1 (0.6)	<LOD	2.1 (0.2)	2.1 (0.07)	0.8 (0.02)
	Jul-16	11.8 (1.3)	8.6 (0.6)	NE	NE	3.6 (0.2)	2.9 (0.6)	2.0 (0.1)
Naproxen	Nov-15	30.3 (0.8)	31.5 (5.6)	nd	nd	29.1 (1.8)	nd	27.6 (3.9)
	Feb-16	27.8 (4.5)	24.3 (5.1)	24.1 (2.3)	nd	nd	nd	nd
	May-16	24.8 (0.8)	23.4 (3.7)	26.4 (2.5)	7.1	18.5 (3.8)	20.1 (2.05)	13.8 (0.6)
	Jul-16	36.6 (3.1)	26.6 (1.8)	NE	NE	24.6 (0.4)	22.0 (1.1)	11.7 (1.0)
Ranitidine	Nov-15	32.8 (5.1)	19.9 (5.1)	13.8 (1.2)	<LOQ	6.0 (2.0)	6.0 (1.5)	5.2 (1.1)
	Feb-16	10.0 (1.9)	6.8 (0.6)	7.0 (1.3)	nd	nd	6.2 (1.4)	nd
	May-16	13.5 (2.1)	8.8 (2.7)	9.0 (0.8)	<LOQ	<LOQ	10.8 (2.3)	<LOQ
	Jul-16	20.2 (3.3)	9.6 (1.3)	NE	NE	6.8 (2.2)	8.7 (2.3)	5.5 (0.6)
Trimethoprim	Nov-15	14.5 (1.8)	13.5 (0.7)	12.6 (1.3)	11.4 (7.9)	5.9 (1.8)	5.4 (1.5)	9.0 (0.3)
	Feb-16	26.2 (2.4)	22.4 (1.6)	24.7 (2.6)	6.2 (0.9)	5.4 (0.4)	5.4 (0.2)	4.4 (1.0)
	May-16	48.2 (10.6)	48.4 (5.6)	47.6 (4.0)	14.0 (3.9)	16.7 (0.5)	17.0 (0.3)	15.2 (1.9)
	Jul-16	42.9 (6.8)	20.0 (3.4)	NE	NE	16.1 (0.9)	16.4 (2.8)	8.9 (1.3)

nd = not detected, NE= not evaluated, <LOD and <LOQ= Lower than the detection and quantification limit, respectively

Table A.E8: Seasonal detected pharmaceutical concentrations (sediment samples) all study sites in the River Foss and Ouse downstream of WWTP

Sediment								
Compound	**Season**	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**
Amitriptyline	Nov-15	12.9 (1.1)	5.7 (0.2)	12.6 (0.6)	1.87 (0.2)	1.05 (0.02)	0.76 (0.09)	2.36 (0.22)
	Feb-16	2.55 (0.2)	2.82 (0.5)	2.15 (0.12)	0.4 (0.03)	0.86 (0.1)	0.24 (0.06)	0.9 (0.12)
	May-16	1.9 (0.4)	1.5 (0.2)	2.1 (0.5)	2.0 (0.13)	0.5 (0.09)	0.55 (0.07)	0.54 (0.1)
	Jul-16	4.92 (0.65)	3.44 (0.15)	-	-	1.64 (0.24)	1.31(0.21)	1.99(0.25)
Atenolol	Nov-15	3.8 (1.3)	<LOQ	4.1 (0.8)	1.54 (0.3)	<LOQ	<LOQ	1.28 (0.3)
	Feb-16	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	May-16	<LOQ	<LOQ	<LOQ	1.28 (0.3)	<LOQ	<LOQ	<LOQ
	Jul-16	<LOQ	<LOQ	-	-	1.07 (0.21)	<LOD	1.43 (0.4)
Cimetidine	Nov-15	5.9 (0.9)	0.7 (0.1)	4.2 (1.0)	<LOQ	1.09 (0.3)	<LOQ	0.6 (0.03)
	Feb-16	<LOQ	<LOQ	2.35 (0.6)	<LOQ	0.7 (0.1)	<LOQ	<LOQ
	May-16	nd	0.6 (0.1)	2.7 (0.8)	<LOQ	nd	<LOQ	0.99 (0.12)
	Jul-16	1.09 (0.22)	1.02 (0.18)	-	-	1.17 (0.22)	<LOQ	nd
Diclofenac	Nov-15	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.6 (0.5)
	Feb-16	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ
	May-16	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ
	Jul-16	<LOQ	<LOQ	-	-	<LOQ	<LOQ	<LOQ
Diltiazem	Nov-15	0.6 (0.11)	0.32 (0.1)	0.36 (0.08)	<LOQ	<LOQ	<LOQ	1.23 0.18)
	Feb-16	<LOQ	0.16 (0.05)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	May-16	0.39 (0.1)	0.42 (0.1)	0.2 (0.08)	0.36 (0.1)	0.4 (0.1)	0.39 (0.15)	<LOQ
	Jul-16	0.48 (0.13)	0.44 (0.13)	-	-	0.4 (0.1)	0.42 (0.15)	0.39 (0.12)
Ibuprofen	Nov-15	<LOD	nd	nd	8.1 (1.15)	nd	<LOQ	<LOQ
	Feb-16	nd	nd	<LOQ	<LOQ	nd	nd	nd
	May-16	<LOD	nd	nd	<LOQ	<LOD	nd	<LOD
	Jul-16	<LOD	nd	-	-	<LOD	nd	<LOD
Mefenamic acid	Nov-15	0.7 (0.14)	0.41 (0.1)	0.8 (0.2)	0.3 (0.05)	0.35 (0.06)	0.26 (0.1)	1.7 (0.18)
	Feb-16	0.37 (0.1)	0.17 (0.03)	1.14 (0.4)	0.56 (0.09)	0.68 (0.1)	0.62 (0.12	0.58 (0.1)
	May-16	0.46 0.11)	0.54 (0.1)	0.16 (0.05)	0.23 (0.06)	0.16 (0.03)	0.17 (0.01)	0.13
	Jul-16	0.31 (0.08)	0.24 (0.05)	-	-	0.17 (0.06)	0.15 (0.03)	0.14 (0.01)
Naproxen	Nov-15	nd	4.26 (1.1)	2.51 (0.7)	nd	1.97 (0.2)	1.95 (0.3)	2.42 (0.22)
	Feb-16	2.03 (0.08)	nd	2.03 (0.7)	2.23 (0.06)	1.7 (0.2)	1.72 (0.3)	nd
	May-16	2.26 (0.5)	1.6 (0.5)	5.03 (1.1)	<LOQ	<LOQ	2.83 (0.8)	1.88 (0.4)
	Jul-16	nd	nd	-	-	2.86 (0.4)	1.95 (0.5)	Nd
Ranitidine	Nov-15	0.8 (0.1)	0.43 (0.04)	0.45 (0.1)	0.42 (0.14)	0.41 (0.05)	0.4 (0.04)	0.5 (0.05)
	Feb-16	0.62 (0.05)	0.5 (0.03)	0.58 (0.05)	0.81 (0.15)	0.43 (0.07)	0.44 (0.05)	0.58 (0.08)
	May-16	2.26 (0.9)	0.48 (0.2)	0.48 (0.092)	0.93 (0.03)	0.48 (0.01)	0.44 (0.13)	0.74 (0.08)
	Jul-16	0.72 (0.1)	0.51 (0.001)	-	-	0.42 (0.16)	0.45 (0.11)	0.43 (0.04)
Trimethoprim	Nov-15	18.4 (2.1)	0.76 (0.1)	1.82 (0.25)	1.04 (0.1)	0.41 (0.02)	0.25 (0.01)	0.8 (0.1)
	Feb-16	0.54 (0.06)	0.5 (0.06)	1.11 (0.14)	0.8 (0.09)	0.41 (0.03)	0.2 (0.012)	0.53 (0.01)
	May-16	0.4 (0.03)	0.23 (0.01)	1.15 (0.15)	1.05 (0.17)	0.23 (0.01)	0.27 (0.015)	1.02 (0.05)
	Jul-16	0.4 (0.07)	0.26 (0.01)	-	-	0.64 (0.02)	0.37 (0.01)	0.45 (0.01)

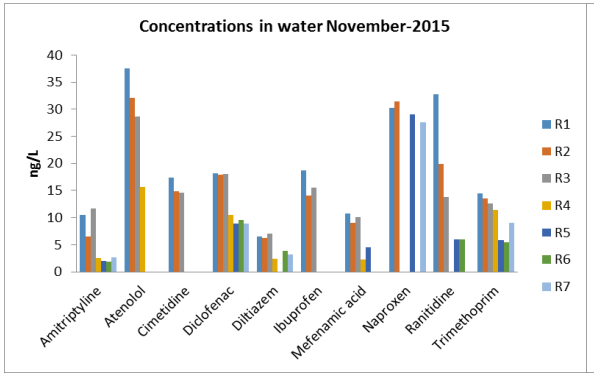
nd = not detected, NE= not evaluated, <LOD and <LOQ= Lower than the detection and quantification limit, respectively

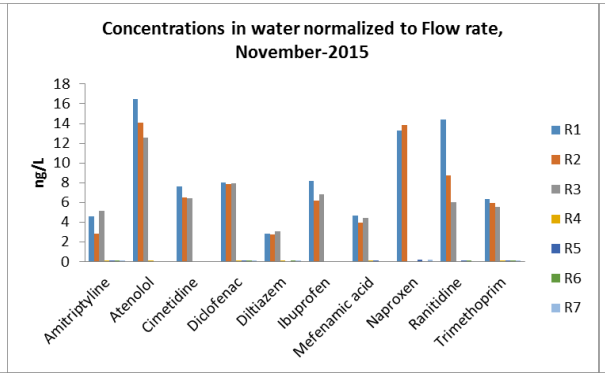
Table A.E9 Sediment properties influencing the distribution of pharmaceuticals in sediment selected by MLR

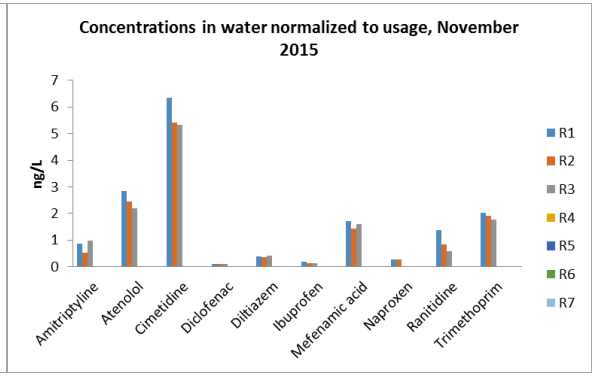
Compound	Properties	R^2	p value
Amitriptyline	OC%	0.39	<0.05
Atenolol	OC%	0.47	<0.05
Cimetidine	CEC	0.46	<0.01
Diclofenac	pH	0.49	<0.01

Table A.E10: Seasonal and spatial pseudo partitioning coefficient (Kd, L Kg^{-1}) retrieved from available water and sediment concentrations

Compound	Season	R1	R2	R3	R4	R5	R6	R7
Amitriptyline	Nov-15	1241 (109.6)	930 (230.1	1029.2 (86.3)	747.0 (17.3)	599.6 (241.2)	423.9(122.2)	916.3 (75.1)
	Feb-16	500.6 (9.9)	521 (25.0)	561.6 (8.7)	251.5 (16.0)	423.9 (76.6)	158.7 (29.5)	560.0 (161.8)
	May-16	236.8 (44.1)	393.6 (31.9)	558.0 (98.6)	743.3 (34.7)	621.9 (73.8)	318.4 (35.7)	335.8 (20.7)
	Jul-16	277.1 (28.9)	551 (116.7)	NE	NE	409.2(47.8).0)	318.9 (17.6)	902.2 (60.4)
Atenolol	Nov-15	99.1 (16.0)		132.2 (12.6)	102.6 (17.3)	NE	NE	NE
	Feb-16	NE	NE	NE	NE	NE	NE	NE
	May-16	NE	NE	NE	NE	NE	NE	NE
	Jul-16	NE	NE	NE	NE	NE	NE	NE
Cimetidine	Nov-15	338.2 (10.9)	47.9 (1.2)	249.7 (33.2)	NE	NE	NE	NE
	Feb-16	NE	NE	NE	NE	NE	NE	NE
	May-16	NE	NE	NE	NE	NE		NE
	Jul-16	29.3 (1.4)	95.5 (25.9)	NE	NE	NE		NE
Diclofenac	Nov-15	NE	NE	NE	NE	NE	NE	534.9 (97.9)
	Feb-16	NE	NE	NE	NE	NE	NE	NE
	May-16	NE	NE	NE	NE	NE	NE	NE
	Jul-16	NE	NE	NE	NE	NE	NE	NE
Diltiazem	Nov-15	92.0 (12.0)	50.0 (11.9)	49.8 (8.6)	NE	NE	NE	386.4 (16.5)
	Feb-16	NE	30.2 (9.1)	NE	NE	NE	NE	NE
	May-16	44.2 (10.4)	46.7 (10.4)	NE	NE	NE	NE	NE
	Jul-16	19.0 (4.9)	25.2 (6.6)	NE	NE	65.5 (0.3)	67.3 (12.7)	85.9 (3.9)
Ibuprofen	Nov-15	NE	NE	NE	NE	NE	NE	NE
	Feb-16	NE	NE	NE	NE	NE	NE	NE
	May-16	NE	NE	NE	NE	NE	NE	NE
	Jul-16	NE	NE	NE	NE	NE	NE	NE
Mefenamic acid	Nov-15	65.1 (3.4)	45.8 (5.7)	77.0 (14.9)	NE	79.2 (8.1)		NE
	Feb-16	42.5 (5.7)	38.1 (2.4)	77.1 (20.3)	NE	98.3 (11.60	124.0(21.1)	NE
	May-16	47.5 (8.0)	62.2 (4.5)	30.6 (7.3)	NE	NE	NE	NE
	Jul-16	26.0 (3.9)	28.5 (2.8)	NE	NE	46.7 (14.10	NE	NE
Naproxen	Nov-15	NE	126.7 (23.3)	NE	NE	67.7	NE	NE
	Feb-16	74.0 (9.2)	NE	81.5 (23.4)	NE	NE	NE	NE
	May-16	NE	NE	NE	NE	NE	139.0(25.7)	NE
	Jul-16	NE	NE	NE	NE	116.3 (14.3)	88.6 (18.6)	NE
Ranitidine	Nov-15	24.5 0.7)	22.2 (3.8)	31.6 (5.6)	NE	71.9 (16.6)	68.4 (10.8)	NE
	Feb-16	62.9 (7.1)	80.4 (13.5)	78.4 (3.9)	NE	NE	72.3 (8.5)	NE
	May-16	163.1 (41.8)	51.8 (9.0)	51.7 (7.5)	NE	NE	40.6 (3.5)	NE
	Jul-16	35.7 (1.0)	58.0 (6.2)		NE	NE	51.9 (1.1)	NE
Trimethoprim	Nov-15	1270.0 (12.9)	57.8 (2.4)	140.2 (11.5)	139.6 (112.8)	73.5 (20.0)	45.5 (12.1)	88.7 (8.2)
	Feb-16	20.6 (0.4)	22.6 (0.7)	43.3 (3.4)	129.5 (4.3)	75.9 (0.1)	37.0 (1.0)	124.5 (26.7)
	May-16	8.5 (1.3)	5.0 (0.7)	23.5 (2.1)	77.0 (11.2)	13.8 (0.1)	15.9 (0.6)	67.5 (5.2)
	Jul-16	9.3 (0.2)	14.5 (3.9)	NE	NE	39.8 (1.0)	22.9 (3.4)	51.2 (6.4)


Concentrations in water November-2015
ng/L
0
5
10
15
20
25
30
35
40
Amitriptyline
Atenolol
Cimetidine
Diclofenac
Diltiazem
Ibuprofen
Mefenamic acid
Naproxen
Ranitidine
Trimethoprim
R1
R2
R3
R4
R5
R6
R7


Concentrations in water normalized to Flow rate, November-2015
ng/L
0
2
4
6
8
10
12
14
16
18
Amitriptyline
Atenolol
Cimetidine
Diclofenac
Diltiazem
Ibuprofen
Mefenamic acid
Naproxen
Ranitidine
Trimethoprim
R1
R2
R3
R4
R5
R6
R7


Concentrations in water normalized to usage, November 2015
ng/L
0
1
2
3
4
5
6
7
Amitriptyline
Atenolol
Cimetidine
Diclofenac
Diltiazem
Ibuprofen
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Naproxen
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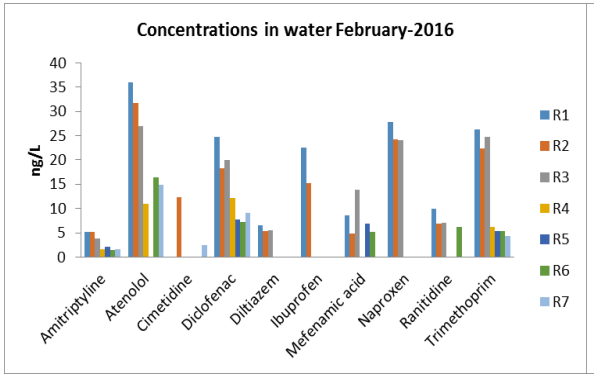
Trimethoprim
R1
R2
R3
R4
R5
R6
R7


Concentrations in water February-2016
ng/L
0
5
10
15
20
25
30
35
40
Amitriptyline
Atenolol
Cimetidine
Diclofenac
Diltiazem
Ibuprofen
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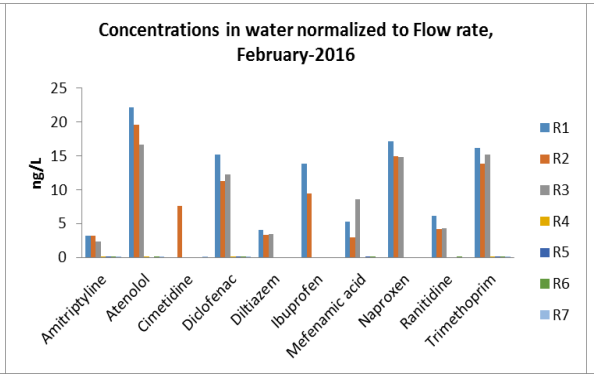
Trimethoprim
R1
R2
R3
R4
R5
R6
R7


Concentrations in water normalized to Flow rate, February-2016
ng/L
0
5
10
15
20
25
Amitriptyline
Atenolol
Cimetidine
Diclofenac
Diltiazem
Ibuprofen
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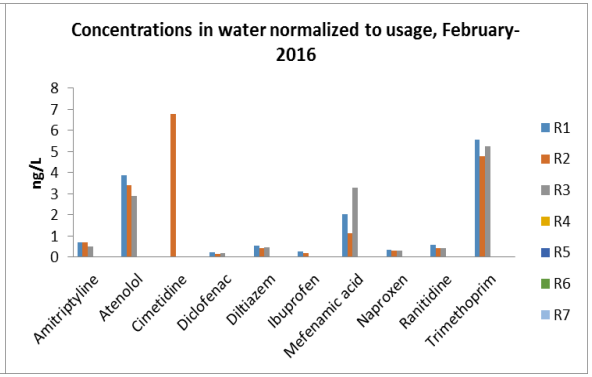
Naproxen
Ranitidine
Trimethoprim
R1
R2
R3
R4
R5
R6
R7


Concentrations in water normalized to usage, February-2016
ng/L
0
1
2
3
4
5
6
7
8
Amitriptyline
Atenolol
Cimetidine
Diclofenac
Diltiazem
Ibuprofen
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Naproxen
Ranitidine
Trimethoprim
R1
R2
R3
R4
R5
R6
R7

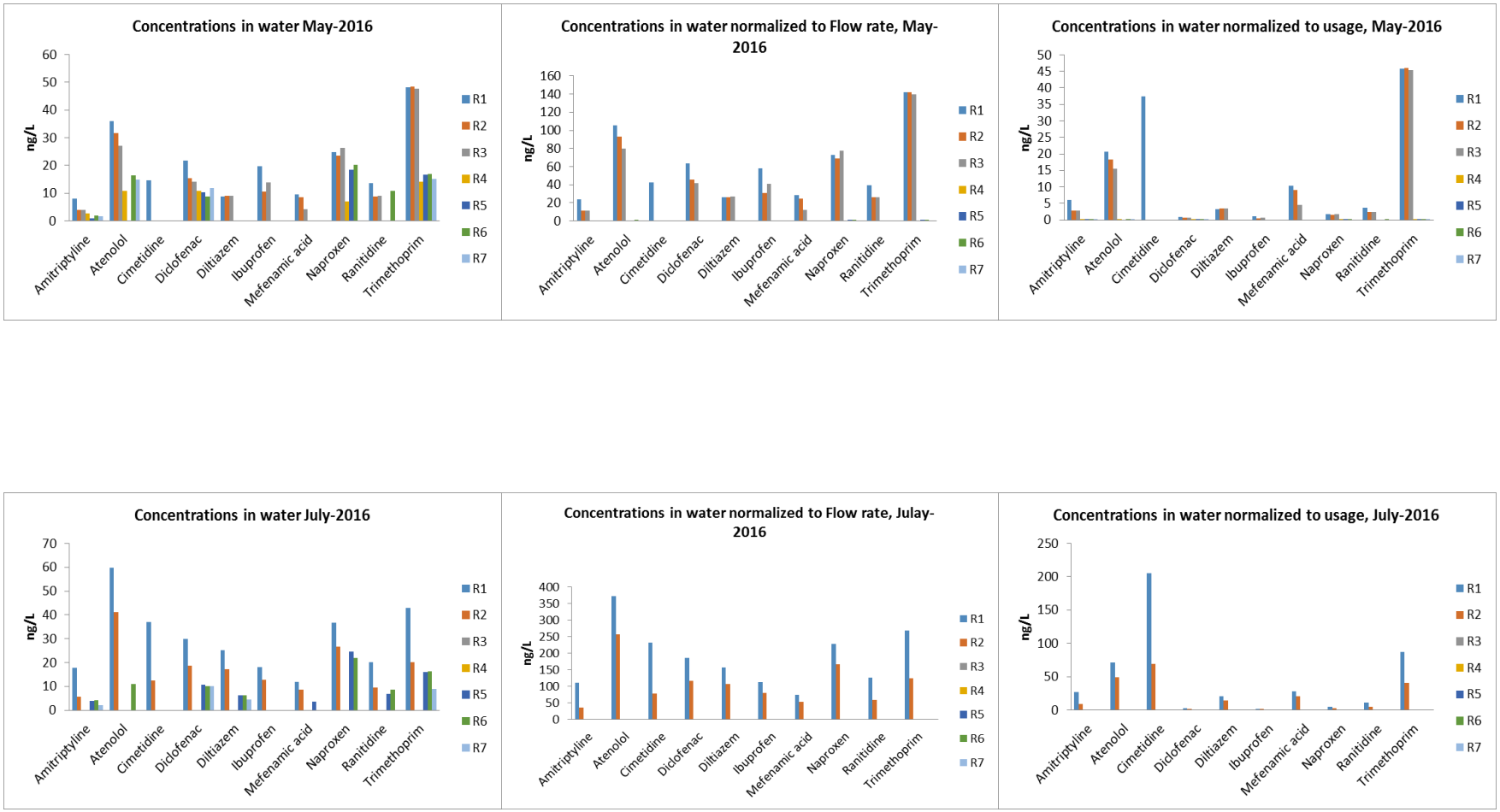
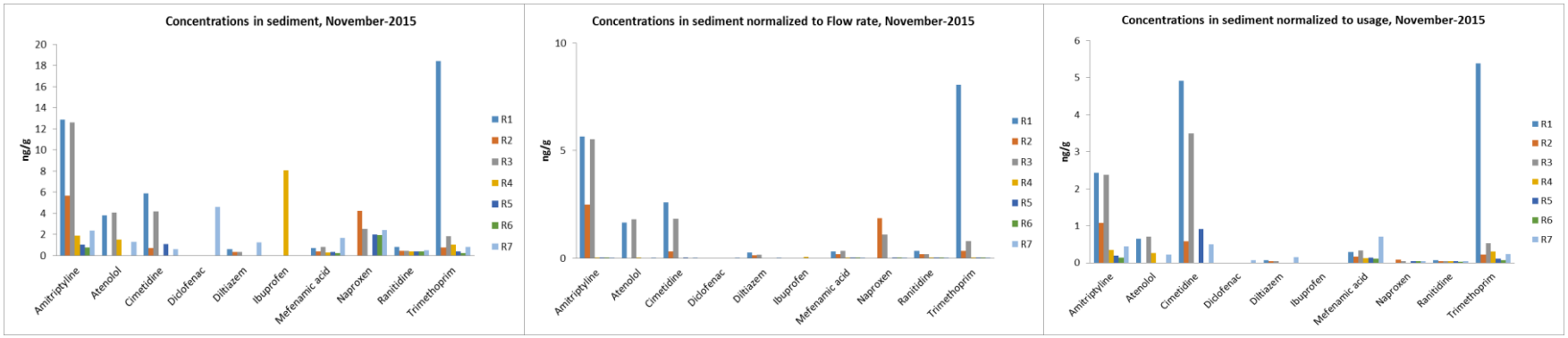
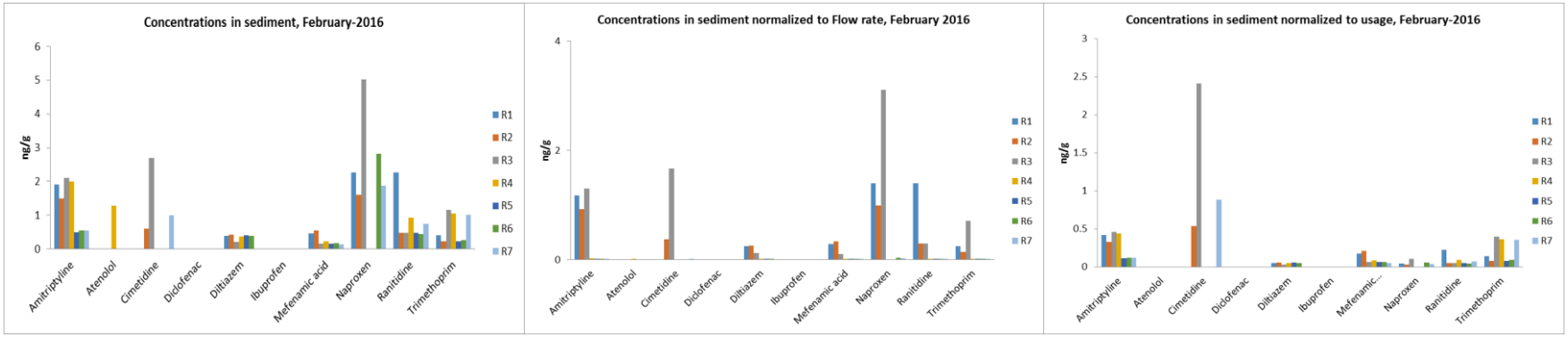


Figure A.E1 Average concentrations of pharmaceuticals across seasons and the effect of Flow rate and usage amounts on their distribution in the water phase


Concentrations in sediment, November-2015
ng/g
Amitriptyline
Atenolol
Cimetidine
Diclofenac
Diltiazem
Ibuprofen
Mefenamic acid
Naproxen
Ranitidine
Trimethoprim
R1
R2
R3
R4
R5
R6
R7
Concentrations in sediment normalized to Flow rate, November-2015
ng/g
Amitriptyline
Atenolol
Cimetidine
Diclofenac
Diltiazem
Ibuprofen
Mefenamic acid
Naproxen
Ranitidine
Trimethoprim
R1
R2
R3
R4
R5
R6
R7
Concentrations in sediment normalized to usage, November-2015
ng/g
Amitriptyline
Atenolol
Cimetidine
Diclofenac
Diltiazem
Ibuprofen
Mefenamic acid
Naproxen
Ranitidine
Trimethoprim
R1
R2
R3
R4
R5
R6
R7


Concentrations in sediment, February-2016
ng/g
Amitriptyline
Atenolol
Cimetidine
Diclofenac
Diltiazem
Ibuprofen
Mefenamic acid
Naproxen
Ranitidine
Trimethoprim
R1
R2
R3
R4
R5
R6
R7
Concentrations in sediment normalized to Flow rate, February 2016
ng/g
Amitriptyline
Atenolol
Cimetidine
Diclofenac
Diltiazem
Ibuprofen
Mefenamic acid
Naproxen
Ranitidine
Trimethoprim
R1
R2
R3
R4
R5
R6
R7
Concentrations in sediment normalized to usage, February-2016
ng/g
Amitriptyline
Atenolol
Cimetidine
Diclofenac
Diltiazem
Ibuprofen
Mefenamic...
Naproxen
Ranitidine
Trimethoprim
R1
R2
R3
R4
R5
R6
R7

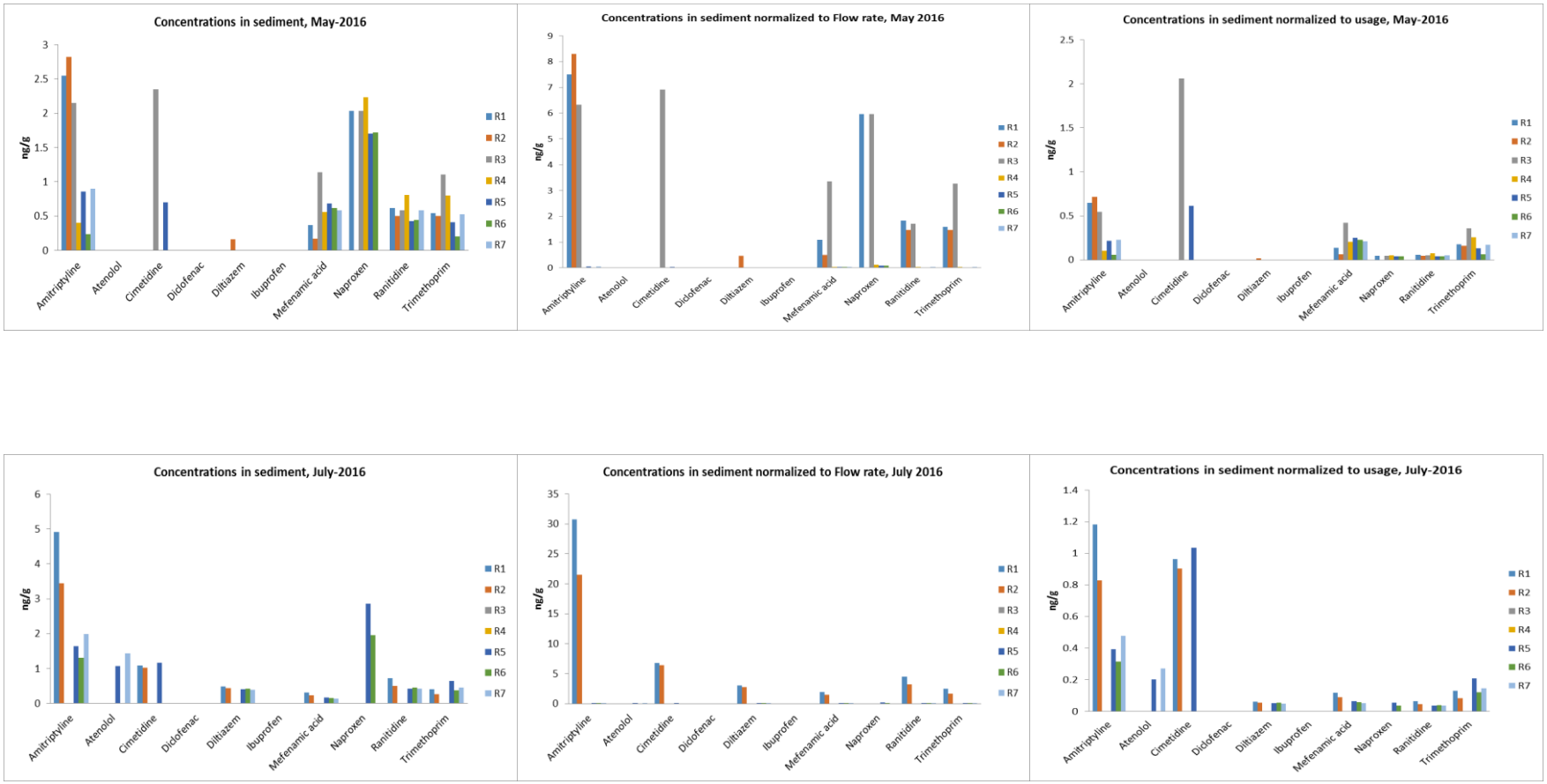


Figure A.E2 Average concentrations of pharmaceuticals across seasons and the effect of Flow rate and usage amounts on their distribution in the sediment phase

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