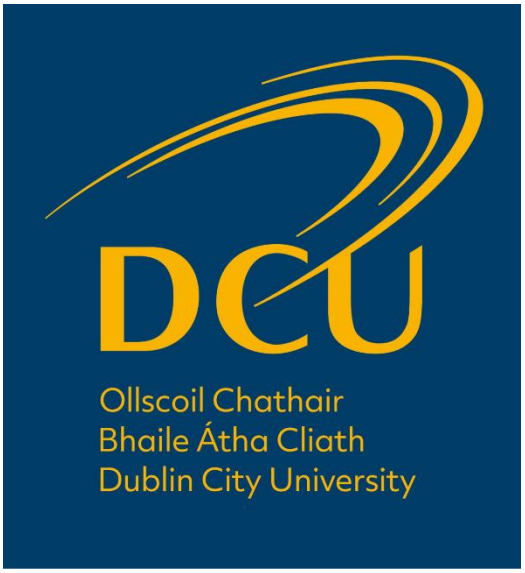

Novel detection and risk assessment of contaminants of emerging concern in a range of aquatic matrices in Ireland

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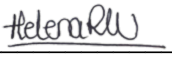
A thesis submitted for consideration for the award of:

Doctor of Philosophy

September 2021

Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of **Doctor of Philosophy** is entirely my own work, and that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed:  ID No.: 18212189

Date: 09 September 2021

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List of Abbreviations

ACN	Acetonitrile
AF	Assessment factor
ANOVA	Analysis of variance
APCI	Atmospheric pressure chemical ionisation
APPI	Atmospheric-pressure photoionisation
BCF	Bioconcentration factor
BeP	Benzylparaben
BHT	Butylated hydroxytoluene
BP-3	Benzophenone-3
BP-4	Benzophenone-4
BPs	Bisphenols
BPA	Bisphenol A
BPAF	Bisphenol AF
BPB	Bisphenol B
BPF	Bisphenol F
BPS	Bisphenol S
BT	Benzotriazole
CCL	Contaminant candidate list
CEC	Contaminant of emerging concern
CO	Carbon monoxide
COD	Chemical Oxygen Demand
CV	Coefficient of variation
DCM	Dichloromethane
DI	Direct injection
DMSO	Dimethyl sulfoxide
dMRM	Dynamic MRM
DVB	Divinylbenzene

E1	Estrone
E1-3S	Estrone 3-sulfate
E2	17-β-estradiol
EE2	17-α-ethynylestradiol
E3	Estriol
E3-3S	Estriol 3-sulfate
EC50	Median effective concentration
ECOSAR	Ecological Structure Activity Relationships
EDCs	Endocrine disruptor compounds
EMEA	European Medicines Evaluation Agency
EMV	Electron multiplier voltage
EPA	Environmental Protection Agency
EQS	Environmental Quality Standards
ERA	Environmental risk assessment
ESI	Electrospray ionisation
EtP	Ethylparaben
EU	European Union
FDA	Food and drug administration
GC	Gas chromatography
GMS	General Medical Service
GWRC	Global Water Research Coalition
HPLC	High performance liquid chromatography
HSE	Health Service Executive
K_{ow}	Octanol-water partition coefficient
LC	Liquid chromatography
LC50	Median lethal concentration
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LOD	Limit of detection
LOQ	Limit of quantification

MADL	Maximum acceptable method detection limit
MDMA	3,4-methylenedioxy-methamphetamine
MEC	Measured environmental concentration
MeOH	Methanol
MeP	Methylparaben
MRM	Multiple reaction monitoring
MS	Mass spectrometer
n	Sample size
n.a.	Not available
n.d.	Not detected
NOEC	No observed effect concentration
NP	Nonylphenol
NSAID	Non-steroidal anti-inflammatory
OP	Octylphenol
PBT	Persistent, bioaccumulation and toxic
PCPs	Personal care products
PE	Population Equivalent
PEC	Predicted environmental concentration
pK_a	Acid dissociation constant
PNEC	Predicted no effect concentration
PPCPs	Pharmaceutical and personal care products
PrP	Propylparaben
QqQ	Triple Quadrupole
QSAR	Quantitative structural activity relationship
RBMP	River basin management plan
RQ	Risk Quotient
RSD	Relative standard deviation

SPE	Solid-phase extraction
TBBPA	Tetrabromobisphenol A
TBEP	Tris(2-butoxyethyl) phosphate
TCCP	Tris(2-chloroisopropyl) phosphate
TCEP	Tris(2-chlorethyl) phosphate
THF	Tetrahydrofuran
UHPLC	Ultra High Performance Liquid Chromatography
UK	United Kingdom
UV	Ultra-Violet
UWWTD	Urban Waste Water Treatment Directive
VOC	Volatile Organic Compounds
WFD	Water Framework Directive
WL	Watch List
WWTP	Wastewater Treatment Plant

Abstract

Novel detection and risk assessment of contaminants of emerging concern in a range of aquatic matrices in Ireland

Helena Rapp Wright

Water pollution is one of the biggest concerns as a potential hazard for the environment and consequently for human health. Contaminants of emerging concern (CECs) are increasingly being shown to occur in water samples across the world, present at ng/L to µg/L concentrations, and their risks require further investigation. The breadth of such compounds is increasing and therefore there is a constant need for reliable analytical methods for identification and their determination in order to detect them due to their presence at low levels. Limited previous studies have explored the spatial occurrence and relative distribution of pharmaceutical residues in the Irish environment and demonstrated their presence, however, Irish studies evaluating more comprehensively these pollutants and matrices are required. In this study a broad range of CECs including pharmaceuticals, pesticides, and personal care products were detected, monitored and risk assessed in the environment from a rural and an urban influenced area in Ireland for surface waters and wastewater samples (both influent and effluent). Quantitative analysis has been carried out for target compounds; this will allow contaminants which are not efficiently removed during treatment to be highlighted and provide critical information for tools to evaluate their potential risks to human health. Occurrence frequency was established and interpreted to inform prioritisation of these compounds. This will support wastewater treatment plants to develop and optimise strategies for the efficient removal of identified CECs and to minimise the potential risk posed by them. Additionally, an international case study has been carried out comparing occurrence in Irish, UK and Spanish rivers. The monitoring data collected is used to demonstrate the application of a decision support system for monitoring these pollutants as a risk assessment tool for Irish water catchments. This research provides comprehensive insight into the occurrence, fate and impact of CECs in Irish waters.

1.0 Introduction to contaminants of emerging concern

1.1 Contaminants of emerging concern

Water pollution is one of the biggest concerns as a potential hazard for the environment and consequently for human health. Biological agents and toxic chemicals in water have been present for a long time, however, methods have only recently been developed in order to detect them due to their presence at low levels of concentration (pg/L to µg/L).[1,2] New synthesis routes and methods to dispose these chemicals have made new potential sources of contamination; however, these have not been studied yet, and as such the consequences are unknown. Moreover, there is a lack of research exploring their potential impact on the aquatic environment. Additionally, concern has been raised due to the toxic and secondary effects in organisms that can lead to human exposure via ingestion of contaminated food.

These emerging substances which are potentially hazardous are referred to as contaminants of emerging concern (CECs) and organizations such as The United States Geological Survey (USGS) define them as "*any synthetic or naturally occurring chemical or any microorganism that is not commonly monitored in the environment but has the potential to enter the environment and cause known or suspected adverse ecological and/or human health effects*".[3] The wide number of chemistry compounds found in surface and groundwater belong to different categories due to the numerous and diverse types of chemicals. The most common classes are pharmaceuticals and hormones, personal care products, illicit drugs, artificial sweeteners, disinfection by-products, UV filters and other organic pollutants arising from industry or agriculture such as pesticides[4] and as the population grows their use increases rapidly.

1.1.1 CEC degradation pathways

CECs are commonly found in the environment, due to human excretion (via urine and faeces), household waste, sewage, improper direct disposal, agricultural, manufacturing and wastewater.[1] Due to their widespread use in many products of everyday use, the increase in consumption of CECs continuously releases them into the environment ending up in the water or soil where they can be accumulated over time, leading to unknown effects on the ecosystem and human health. These products interact differently in the environment depending on their physiochemical properties and matrix effects within the environment (the biological, physicochemical and hydrological parameters of the river).[5] They can be broken down or degraded but many of them can persist creating hazards to the environment and therefore in human life. Their elimination in water samples is difficult as there is insufficient knowledge of what happens during treatment processes of the compounds' life cycle in the environment; hydrolysis, biodegradation, volatilization, oxidation, dilution, conversion to metabolites and desorption from particulate matter are some examples of potential degradation pathways.[6]

1.1.1.1 Factors influencing degradation pathways

There are different factors to consider that will influence potential degradation pathways of these contaminants. Concentrations of CECs in receiving waters can be influenced by spatial and/or temporal variations giving different results during the year. Examples of lower concentrations due to dilutions can be seen when coming from manufactures inputs, in-stream degradation process such as photolysis, and temporal variations such as rain, and higher concentrations when occasional events like music festivals and sports games take place.[7] An example of this dilution factor was seen in Morogoro, Tanzania, where the removal of ten steroid hormones (including 17-β-estradiol (E2) and 17-α-ethinylestradiol (EE2)) in two different wastewater treatment plants (WWTPs) varied

between 70±21% and 97±3% in dry season and 52±32% and 94±8% during rainy season.[8] Differences over the seasons are also taken into account as concentrations can be quite variable during summer and winter. Sunscreens and medicines to treat allergies are used more frequently in summer and spring respectively, increasing their concentrations, however, during winter, pharmaceutical compounds used in medicines to treat colds will typically be higher. Benzophenone-4 is a UV-filter commonly used in sunscreens and it has been observed at concentrations ranging from 3,597 to 5,790 ng/L in effluent samples depending on the season.[7] In the Alpine rivers, 36 out of 80 pharmaceutical and personal care products (PPCPs) compounds studied were detected in winter with diclofenac and furosemide having the highest concentrations (>300 ng/L).[1] Concentrations can also vary throughout the day as seen in some antibiotics, where their concentrations were higher during the morning because of their excretion on the first toilet flush. They can vary inter-day as well, MDMA (3,4-methylenedioxy-methamphetamine) was quantified with higher concentrations during the weekends due to recreational purposes; in addition to these fluctuations being observed in touristic areas, manufacturing outputs can also vary as industries will have higher flow during the week.

In addition to variation in concentration as a result of the physiochemical and temporal parameters discussed above, sampling regimes can also significantly impact on concentrations of CECs detected. There are different types of sampling where grab samples are the most common, however, they only give a concentration for a specific time. Composite samples can be taken to solve this issue, where individual samples are collected at regular intervals on the same container over a period of time, typically 24 hours. This way it reduces the possibility of missing analytes if only grab samples are collected, as the composition changes throughout the day.[9] But composite samples are not always available and they need additional devices such as automatic water samplers,

so grab samples are used in those cases, and even in these cases, this would not address inter-day variations. Due to these reasons there is a limited understanding in spatial and temporal variations of CEC concentrations.[7]

1.1.2 Introduction to WWTPs

The pathways of CEC contaminants entering the surface waters are well known; with effluents and WWTPs recognised as the more common entry routes compared to groundwater.[10] WWTPs are not designed to remove CECs and are instead characterised by dilution, so this is one of the reasons why CECs are not completely removed,[11] and are present at very low levels of concentration, pg/L to µg/L. As WWTPs are designed to perform partial purification, substances at these levels cannot be treated. The potential transport and fate of these contaminants from household to the WWTP into drinking water is described in Figure 1.1.

a) b)

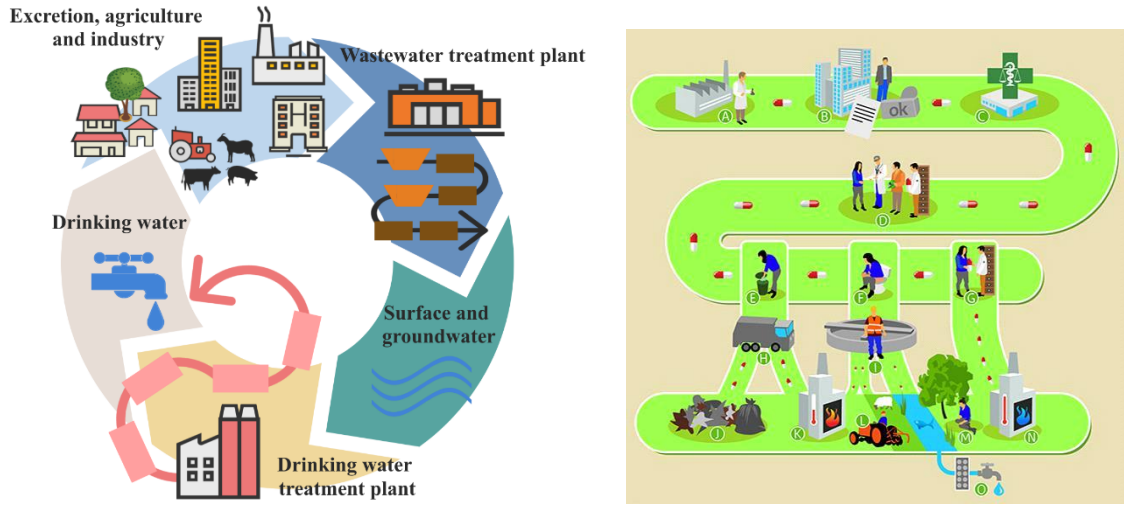


Figure 1.1 a) Transport of CECs from WWTP to drinking water reproduced with permission from K. Sewoon et al. 2018[12] and b) the pharmaceutical product chain from the development and production of pharmaceutical products to delivering drinking water.[13]

1.1.2.1 Treatment stages in WWTPs

There are different stages of the treatment of water in WWTPs. First, a preliminary physical treatment is performed where materials that can damage the equipment are removed, such as plastics, papers or fat. After that, water passes to a sedimentary tank for primary treatment, where, by gravity the solids settle at the bottom and are transferred to sludge treatment facilities.[14] The wastewater contains lots of different types of impurities such as suspended solids, dissolved solids and inorganic and organic particles among others. Their removal brings technologies where coagulation/flocculation is one of the most used methods for the separation process for solid-liquid. Coagulants and/or flocculants are added to the wastewater forming large particles that will settle helping their removal from the system.[15]

In the secondary treatment the biological content is degraded using large amounts of air and a tertiary treatment is only used in some plants to further improve the quality of the water.[14] The properties of the compounds, the type of treatment used and having a tertiary treatment applied, all impact the removal of CECs, so the understanding of how WWTPs work are essential to assess the fate of these contaminants.

Several processes can be used in normal European plants, with biological degradation and the incineration of the sewage sludge (the residual semi-solid by-product of wastewater treatment) among the most prevalent techniques that reduce these contaminants. Photochemical processes are sometimes being utilised too, depending on the fate of the treated sludge, but they pose a minor part.[16] Technologies are divided in physical removal such as precipitation and sedimentation, chemical oxidation/disinfection such as chlorination, and biological transformation like activated sludge. Due to the complexity of the matrix and despite the higher cost, several technologies can be coupled in order to get higher quality results.[17]

- Sorption to sludge: there are two mechanisms by which compounds can sorb, absorption (hydrophobic interactions of the aliphatic and aromatic groups of a compound with the lipophilic cell membrane of the microorganisms and with the lipid fraction of the sludge) and adsorption (electrostatic interactions of positively charged groups of the chemicals with the negatively charged surfaces of the biomass).[16]
- Stripping: this takes place in the aerobic sludge components and it depends on aeration intensity. This does not have good rate of removals as most of the pharmaceutical compounds have a molecular mass above 250 g/mol and are hydrophobic.[16]
- Physical removal: in membrane bioreactors (MBR) the second clarifies are substituted by membranes with a pore size between 100 and 1000 times bigger than the size of the compounds, so no retention can be expected if it is not due to sorption or biological degradation.[16]
- Chemical oxidation: some concentrations of PPCPs decrease with doses of ozone. However, the demand of energy required is 40-50% more in the WWTP so not all of them uses this process.[16]
- Biological degradation or transformation: the mechanisms that each individual chemical will undergo during biological degradation are only fully understood for a very limited number of compounds. Current research explores the impact of parameters such as sludge age (solids retention time), substrate availability (substrate inhibition), redox conditions (aerobic, anaerobic or anoxic), sorption (as competitive reaction), and reactor configuration (sand filtration).[16]

1.1.2.2 CECs in WWTPs

When CECs enter a WWTP, they retain partially in the sludge or are metabolised to more hydrophilic compounds to pass through the WWTP ending up in receiving waters and land via discharge of wastewater and sludge disposal. The first report published about the partial elimination of CECs (steroid hormones) from wastewater treatment was in 1965,[18] since then many investigations have been carried out in order to remove PPCPs, microorganisms and organic matter from it. Efficient wastewater treatments have the potential to be one of the most effective ways of trying to solve this issue and in Europe, WWTPs are regulated by 91/271/ECC and 98/15/ECC for organic matter and suspended solids, but there is no regulation for CECs.[19] There are several ways of significantly reducing these trace contaminants: optimising the actual technology at the WWTPs, upgrading WWTPs with newest (and frequently most expensive) technologies, source control and source separation,[16] such as the separation of the treatment wastewater in hospitals to reduce the pharmaceutical load into the WWTPs.[20] The capacity for elimination of the CECs depends on the physico-chemical properties, biological persistence of the compound and the technology and process conditions so removals vary greatly depending on the treatment used.[21]

As mentioned before, there are different removal pathways, however, it must be remembered that WWTPs have not been designed in order to reduce trace pollutants but to remove pathogens and loads of organic pollutants such as microorganisms and toxic minerals like heavy metals. As a result, new technologies have been tested for CEC elimination. Studies are focused on the analysis of influent and effluent samples in order to calculate the removal rate of CECs after the treatments performed in the WWTPs. Removal levels are variable and examples show variation between 12.5 to 100 % for some compounds in 14 countries/regions.[22] Removal is dependent on the techniques used

during the treatment process and it also depends on the quantity of wastewater that enters the plant apart from the weather conditions such as temperature and/or seasonal variation.[2,8] With 10^6 compounds possible in WWTP influent, compound-specific variation between the contaminants raises a huge challenge. Compounds with high water solubility and low biodegradability are the most difficult to remove during treatment in WWTPs. Physiochemical properties can be used to predict the removal rate such as the octanol-water partition coefficient (K_{ow}), n-octanol-water partition coefficient (D_{ow}), solid-water distribution coefficient (K_d), half-life and the biodegradation constant (K_{bio}).[23]

As stated before, there are a number of different technologies developed for WWTP use; one alternative presented is a membrane technology that has been studied for organic and inorganic CECs depending on their properties.[12] A study compared the membrane bioreactor (MBR) technology against the conventional activated-sludge (CAS) process for different pharmaceuticals and they all varied, however, for most compounds their elimination was higher with the MBR; nevertheless, some substances were not removed by either of them raising the concern.[24] Alternative treatments also take into consideration operating parameters, such as a requirement for a relatively low energy consumption, greenhouse gas emission and life costs.[25] A microalgae technology system was compared to a conventional method in a WWTP in Spain, where 81 pharmaceutical compounds were tested under real operational conditions using High Rate Algae Pond (HRAP) as secondary and tertiary treatments. All removals varied depending on the compounds, but 64 were detected in influent and 55 and 54 were detected in the effluents, respectively. Average removal efficiencies were 94 vs 92% for both treatments, obtaining just a 2% removal difference between them. However, it really depends on the type of chemical analysed, though HRAP could be an alternative or added as a tertiary

treatment.[19] There is a need to find solutions to improve treatments in WWTPs in order to reduce the levels at what these contaminants are found in the environment delivering higher quality and safer water. For these reasons, further technology will need to be optimised and developed to improve elimination rates at trace levels.

Moreover, removals are usually calculated analysing the aqueous phase only, however, particulate phase of the influent samples is not normally analysed because it requires a minimum weight of the dry solids and more extraction is needed, which can end up being time consuming. After the treatments in the WWTP, there is usually not enough suspended matter in the final effluent to carry out the analysis. Few research examines the solids for CECs: solid particulate matter (SPM), sludge, sediments and soil.[26] Domestic/municipal wastewater involves approximately 99.9 % water and 0.1 % dissolved and suspended solid material (such as organic matter, microorganisms and inorganic compounds).[27] However, some compounds will have more affinity to the particulate matter than the aqueous phase being released into the environment without being monitored.[7] The sludge generated after anaerobic digestion treatment in a WWTP is sometimes used for agricultural purposes as fertilisers but some CEC compounds will still be present, thereby transferring them to land. Some countries such as Switzerland have banned their use and the sludge is incinerated. However, over 70% of European countries treat it by incineration or reuse it for fertiliser.[28] Ireland, reuses a big quantity as a soil enhancer or fertiliser on agricultural land due to their nutrients such as nitrogen and phosphorus[29] (Table 1.1) and all compost sludge is also reused in soil/agriculture.[30]

Table 1.1 Sewage sludge reuse and disposal routes measured in tonnes of dry solids.

Year	Agriculture	Compost	Landfill	Other[a]	Total	Reference
2014	42,483	9,266	361	1,433	53,543	31
2015	46,697	10,946	94	650	58,387	32
2016	45,344	9,610	102	962	56,018	33
2017	46,487	10,065	87	2,134	58,773	34
2018	44,003	10,605	91	527	55,226	29
2019	52,139	6,099	155	277	58,630	30

[a] Use of sludge in anaerobic digestion, cement production and in storage awaiting landspreading.

An example of a compound in the sludge is triclosan, an antimicrobial compound that is hydrophobic (log K_{ow} = 4.2) which means that it will be retained in the particular matter instead of the aqueous phase. In one study it was measured in three different soils receiving sludge and an 80% decrease was observed after 12 months. However, the majority was recovered as methyl-triclosan - a known metabolite which is more environmentally persistent and has also been detected in effluent wastewater samples.[35] A more recent study, stated removal rates of 97.6 – 98.8% in the wastewater treatment plant, however, there was an enrichment in the sewage sludge from 36.4 – 49%.[36] Another example are the estrogens E2 and EE2; they are weakly soluble in water so they are more likely to be removed during treatment by sorption and/or biodegradation.[23] Therefore, there is a need to also study sludge samples before their reuse. An alternative is the compost of the sludge before their use in land suggesting a way of degradation of some compounds such as triclosan.[37]

A major review studied 115 international research papers and 2 research reports from 1997 to 2007, covering 184 compounds in total. Removals were obtained with activated sludge processes (ASP), with high sludge retention time configuration for nitrogen removal, low sludge retention time configuration for carbon removal, phosphorus treatment, membrane bioreactors with nitrogen treatment among others from the dissolved phase. Data was collected from influent and effluent samples for ASP

combined with a pre-treatment which depended on the WWTP (primary sedimentation tank, treatment of nitrogen/phosphorous or tertiary treatment) where only 15 publications took into account the sludge and suspended solid concentrations. However, removals did not take into account both liquid and solid phases.[38] The different physical properties of the compounds will give different mobilities in the solids so particular matter extractions should also be considered when possible.[35]

1.1.3 CEC classes studied within this thesis

1.1.3.1 Pharmaceuticals

Pharmaceuticals belong to one of the most important classes of CECs. This is due to thousands of tons that are used every year by humans, such as prescription and over-the-counter, and by animals for veterinary medicine.[11] In Ireland the number of compounds licensed by the Health Products Registration Authority (previously known as the Irish Medicines Board, until 2014) for human consumption increased by 942 to approximately 6,000 in 2005.[39] Only during 2017, a total number of 684 new human medicines were authorised excluding 152 for veterinary purposes, compared to the 637 in 2016. This does not count other illegal medicines, which in 2017 had an increase of 40% compared to 2016 (948,15 dosage units were detained including tablets, capsules and vials). Just in this operation the value of the detained medicines was in excess of €2 million.[40] Across Europe, there are approximately 4,000 different active compounds used[41] and the most common pharmaceuticals are analgesics/anti-inflammatories, antibiotics, cardiovascular (β-blockers/diuretics), psycho-stimulants, estrogens and hormonal compounds, and antiepileptic drugs. It is predicted that by 2030 the consumption of antibiotics will increase from 63.2 to 105.3 thousand tons by the World Health Organization (WHO)[42] and in Ireland, some pharmaceuticals from the most commonly prescribed products contain this class such as amoxicillin. Other most prescribed pharmaceuticals are:

salbutamol, citalopram, clopidogrel, tramadol, warfarin, diclofenac, venlafaxine, amitriptyline, and atorvastatin, according to the Statistical Analysis of Claims and Payments in 2019 realised by the Health Service Executive (HSE).[43] Table 1.2 contains the rank order of CECs of some potential concern for Ireland (detected throughout this thesis) within the top 100 prescribed pharmaceutical compounds from 2011 to 2017 yearly[40,44–50] and monthly for the sampling campaign performed within the thesis (2018-2019).[43,51]

Table 1.2 Rank of detected analytes throughout the study on the top 100 most commonly prescribed products in the order of their prescribing frequency (no additional information provided) based on General Medical Services (GMS). Yearly data is shown for previous years to the performed study, where monthly data has been collected for the sampling campaign period performed from October 2018 to September 2019 for the identified pharmaceutical compounds in all three matrices tested (influent, effluent and receiving waters).

Compound name	**Rank**																		
								2018							**2019**				
	2011[44]	**2012**[45]	**2013**[46]	**2014**[47]	**2015**[48]	**2016**[49]	**2017**[50]	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**
17-α-ethinylestradiol (EE2)	-	-	-	-	-	68	90	76	74	74	77	80	78	79	75	73	76	78	79
Amitriptyline	64	62	61	52	47	46	40	38	38	37	38	38	38	38	38	38	37	37	38
Amlodipine	9	9	9	9	10	12	12	13	10	10	11	10	10	10	12	11	11	11	12
Amoxicillin	30	28	30	28	28	26	27	27	26	17	18	25	29	29	32	35	43	46	26
Atorvastatin	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Bisoprolol	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Citalopram	52	55	54	56	52	51	54	56	56	58	59	58	57	58	58	59	59	58	58
Clarithromycin	53	50	53	59	56	55	65	69	71	54	48	65	76	71	87	92	-	-	88
Clopidogrel	45	49	51	54	58	61	57	58	57	57	58	57	56	57	59	56	56	55	56
Diclofenac	22	24	29	34	36	38	39	43	45	48	45	43	43	42	42	44	42	42	43
Estrogen	76	59	57	66	63	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fluoxetine	95	94	88	86	82	77	79	80	78	80	82	83	81	80	80	79	80	79	80
Lidocaine	-	-	-	83	74	58	59	-	-	-	-	-	-	-	-	-	-	-	-
Mefenamic Acid	91	93	98	97	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Memantine	-	-	-	-	-	99	98	94	95	95	95	96	96	98	98	97	98	98	99
Salbutamol	7	7	7	7	7	7	7	7	7	7	7	7	7	7	8	8	8	8	7
Tamsulosin	67	68	70	70	67	69	69	68	67	67	68	70	70	69	68	67	67	67	67
Temazepam	83	96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Timolol	-	-	-	-	-	-	-	100	100	99	100	100	99	100	100	-	97	96	97
Tramadol	42	47	46	46	45	45	46	52	52	56	57	55	54	53	55	57	55	57	57
Valsartan	62	63	63	63	60	62	56	61	59	60	61	60	59	59	62	68	71	70	71
Venlafaxine	39	37	35	31	29	29	26	26	27	27	28	29	28	28	27	26	26	25	27

- : Not on the list.

As mentioned previously, the use of antibiotics has increased in recent years and researchers have explored the damage and threat to human health[3] and their potential to induce resistance bacterial strains in the environment.[52] They are widely used for veterinary and human medicine and has an annual production of 100,000 to 200,000 tonnes in the world.[53] The consumption of macrolide antibiotics in Europe in the primary care and hospital sector has increased in 2019 as seen in Figure 1.2, where Ireland is the fifth highest country of Europe in terms of per capita consumption. Data is indicated as "defined daily doses (DDD) per 1000 inhabitants per day", an international unit that provides an estimation of the population treated daily with antibiotics taking into consideration the dose; the amount of antibiotics consumed in a country. This data is obtained from sales of antibiotics in the country and/or reimbursement data and the country population.[54] Moreover, the number of DDDs might not reflect the number of prescriptions, the number of patients, and the doses used in practice in certain countries. Therefore, certain limitations arise to the established method where data should be treated as a rough estimation of consumption.[55,56]

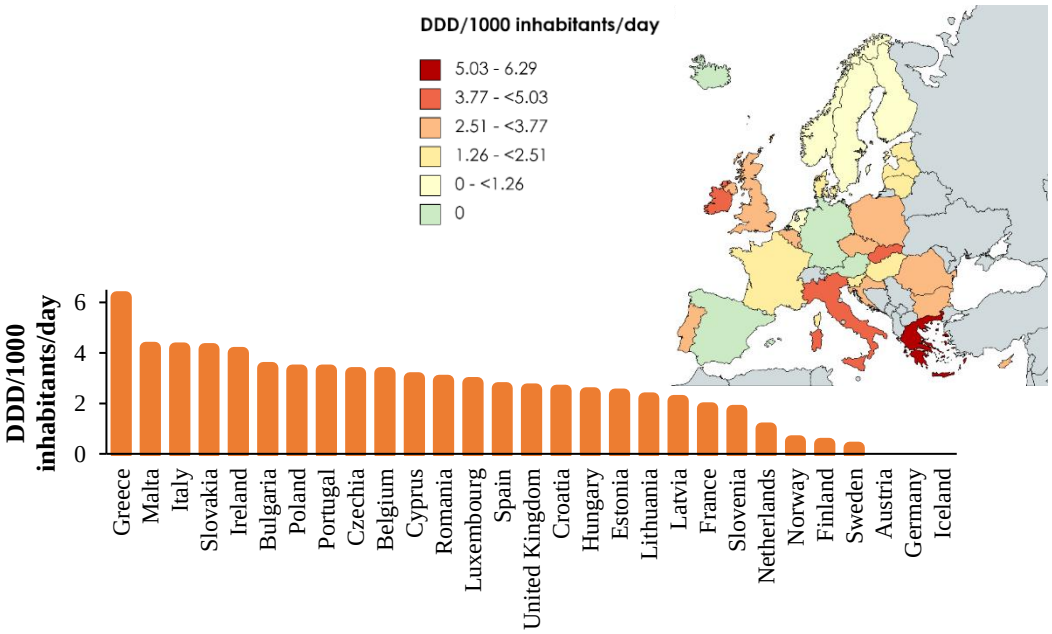


Figure 1.2 Consumption of macrolides antibiotics in the community (primary care sector) and hospital sector in Europe in 2019 indicated as "defined daily doses (DDD) per 1000 inhabitants per day" and reproduced with permission from the European Centre or Disease Prevention and Control.[54]

Ireland's trend for the consumption of macrolides in the community and hospital sector from 1998 to 2019 can be seen in Figure 1.3, where it seems that consumption has plateau in the last ten years.[54]

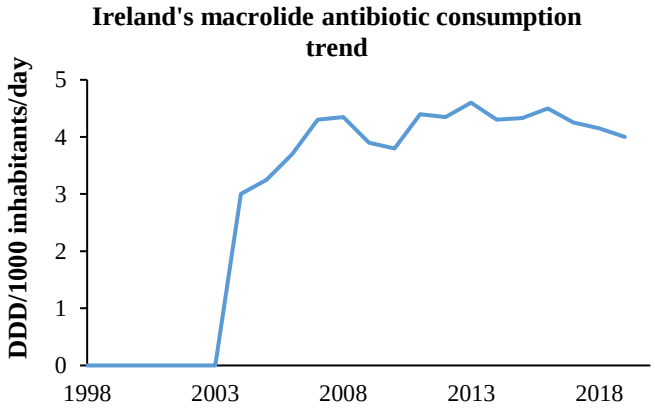


Figure 1.3 Consumption of macrolides antibiotics in the community (primary care sector) and hospital sector in Ireland from 1998 to 2019 indicated as "defined daily doses (DDD) per 1000 inhabitants per day"and reproduced with permission from the European Centre or Disease Prevention and Control.[54]

1.1.3.1.1 Pharmaceuticals in water samples

CECs have been investigated in water samples since 1970 but only now studies can be performed due to the development and improvement of recent analytical techniques for their identification and quantitation at trace levels.[57] Pharmaceuticals are continuously released into the environment from excretion, household waste, hospitals, industrial, sewage, direct disposal, agriculture (animal waste or landfill) and wastewater. They are produced to liberate the active ingredient at a specific spot to provide the required pharmacological effect. They contain thousands of different chemical compounds and as a result of human and animal consumption, they can be excreted as a mixture of the parent drug (unchanged form) and metabolites (conjugated with an inactive compound attached), mainly transformation products and conjugated glucuronides.[5] The excreted individuals or mixtures are then passed onto the sewage and therefore arriving to the WWTPs as mentioned before. These type of compounds contain polar functional groups

making them quite water soluble and consequently being discharged into the receiving waters in the effluent.[58] The effluents from WWTPs are therefore one of the main points sources for these contaminants[6] and potential sources of contamination are most likely to be from hospitals and agriculture, as well as manufacturing (pharmaceutical and chemical industry). Some studies select pharmaceutical analytes based in hospitals lists, which contains the most used ones recently. An example was seen in a WWTP in Dublin where 15 of 20 pharmaceutical compounds studied were detected in the effluent samples. Salicylic acid and ibuprofen had maximum concentrations, 9.17 and 3.20 μg/L respectively. In this case, salicylic acid is a predominant metabolite from aspirin, which had the second/third position on the most frequently prescribed product in Ireland, depending on the month, during 2019.[43] The remaining 14 compounds were not detected in the influent samples but were detected in effluent samples and this may be due to compounds being present as conjugated metabolites in influent and then released as parents after the treatment process in the WWTP. Some of these compounds were diclofenac, sulfamethoxazole, mefenamic acid and carbamazepine.[39] In the case of azithromycin it has been seen that its level of concentration increased from the influent to the effluent after the treatment, this could be due to the present form of metabolites in raw wastewater that can be de-conjugated to the parent compound during the treatment or from desorption from particulate matter increasing its concentration during the treatment.[42]

1.1.3.2 Personal Care Products

Personal care products (PCPs) is an umbrella term typically used to describe non-medicinal products which aim to improve the quality of daily life[59] and it includes any products used in personal healthcare and cosmetics containing several compounds such as preservatives (parabens), fragrances (musk xylol, mux ketone, galaxolide, tonalide,

celestolide), bactericides/disinfectants (triclosan), UV screens (benzophenone-3, homosalate, 4-methyl-benzylidene camphor, octyl-methoxycinnamte, octyl-dimethyl-PABA), deodorants, shampoos, laundry, cleaning products and lotions. PCPs are classified based on their removal after their application, they can be rinse-off or leave-on products. They are placed in contact with the epidermis and/or the hair such as skin creams or hair conditioners, containing a portion of polymers and poorly soluble ingredients so their biodegradation is slow or does not happen at all.[60] They typically include one ingredient that contains a specific property that will influence the performance of the product.[61] An example is fragrances, there are more than 3,000 chemical substances, natural or synthetic, responsible for odorous properties, and a mixture of 20 to over 200 construct the fragrance compounds.[61] There are differences between cosmetics and household products depending on the chemical properties and resulting in them behaving in different forms in the environment. Approximately 80% of the mass of organic product ingredient is biodegradable in most cases, such as rinse-off products like shower gels or shampoos, which have the highest consumption rate.[62] In Ireland alone, 174 cosmetics free sale certificates were issued[40] and a recent review paper examined and composed a dataset based on the occurrence of 72 PCPs in water sources from 30 countries using 141 articles, where fragrances, antiseptics and sunscreens were the most reported groups. Spain and United States were the countries with the higher number of PCPs reported.[63]

UV filters are extensively used in cosmetics and toiletries in the last few decades[64] and in the European Union, certain compounds such as benzophenone-4 and benzophenone-3, have been approved to be used in sunscreens at a maximum individual concentration of 5 and 10% respectively. Due to their water solubility they have been found in water systems including wastewater samples,[65] in both influent and effluent in

the range of µg/L in countries such as Korea and Spain.[22] Also, high quantities are typically used in sunscreens as recommendations for an average sized adult which is around 6 full teaspoons and needs to be applied repeatedly at a minimum of every two hours.[66] This application will be directly in contact with bathing water.

1.1.3.2.1 PCPs in water samples

After their use, PCPs such as shampoos, make-up and hair-care products among others, are removed from the body by processes as bathing or swimming which then go down the drain entering the wastewater sewage at low levels of concentration. The source which contributes the most to their route into the environment are the sewage effluents from WWTPs.[63] Once they are in the WWTP they are not easily removed by conventional treatments because of their different properties, particularly their medium to high polarity,[67,68] they cannot be completely degraded by treatment processes.[63] Additionally, their low removal in secondary treatment can be due to their transformation into metabolites or by-products and the conjugation of PCPs.[69] While not all PCPs are persistent they are continuously being released into the environment and they can end up in surface or drinking waters in concentrations ranging from ng/L to µg/L. They have been detected in algae, raising the concern of their impact in the environment, as algae are the biggest abundance of plant biomass in the aquatic system.[59] There is a potential for negative effects in human and wildlife raising the concern, however, there is not a high quantity of studies using these contaminants in the aquatic systems.[63] The most investigated PCPs in wastewater treatment plants are galaxolide and tonalide, that can be found in fragrances, and triclosan, found in disinfectants and antiseptics.[38] PCPs concentration can also be impacted by seasonality, for example DEET, the most common ingredient in insect repellents, is reduced in winter, as there is a decrease in its usage. The same happens to most UV-filters where their concentration increases in summer

periods.[69] However, their relation between concentration and impact in the environment still needs to be addressed and for this reason new analytical techniques, more sensitive and robust, need to be developed in order to perform these investigations.

In wastewater, 64 PCPs were reported between 1996 and 2016 in influent and effluent samples worldwide. And in over 10 countries, 26 fragrances were detected in the effluent samples in the ng/L range, so complete removal of PCPs has not been achieved after treatments. Therefore, their presence in surface waters has been proven across the world as seen in Figure 1.4. This study describes their presence and not their concentration or loads, however, there have been multiple studies which detail concentration of specific compounds, including triclosan as one of the most frequently found compounds in this matrix (up to 13,920 ng/L).[63] Again, it should be noted that this is not of itself indicative of a failing of water treatment, as current processes are typically not designed to remove CECs such as PCPs.

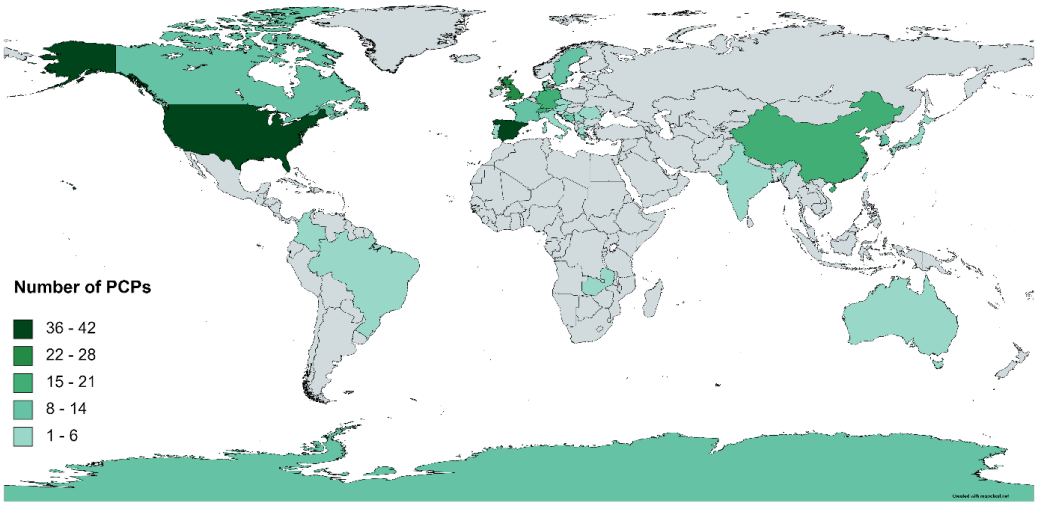


Figure 1.4 PCPs reported in water systems in countries around the world reproduced with permission from D. Montes-Grajales et al. 2017.[63]

The European regulation on chemicals (REACH) has an impact on the risk assessment for cosmetics so the attention on PCPs is increasing. Human safety of PCPs is regulated by EU primarily in the EC Cosmetics Directive, however, as the consumption of these compounds is growing, their environmental impact also needs to be regulated.

For this reason, REACH requires a minimum of data for each chemical with a production tonnage over one ton per year.[62] There is a lack of published research for just the identification and quantitation of PCP compounds by its own in wastewater samples, however, there are studies that combine them with pharmaceuticals. PCPs can be detected and quantified at low concentration, μg/L, in order to assess the risk. Most effects have not been studied yet and they are unknown, however, there are examples such as benzophenone-4 (BP-4), the most used UV filter, that has been demonstrated to be a serious hazard due to its endocrine disrupting effects, which are adverse in development, reproductive, neurological and immune systems in both humans and animals. It has been shown that when zebrafish are exposed to this compound at concentrations between 30 to 3,000 μg/L it results in alterations of gene expression in hormonal pathways.[70]

There is a lack of knowledge of the fate of PCPs in the environment, though it has been shown that they are not entirely removed after treatment in WWTPs. For this reason the EU has highlighted the need to investigate their detection and quantification in order to evaluate the extent to which WWTPs can affect their reduction in the environment decreasing their possible toxic effect in the environment, especially countries in continents such as South America, Asia and Africa, where there is limited data collected.[63]

1.1.3.3 Pesticides

Pesticides compromise chemical mixtures that prevent, destroy, repel or mitigate organisms accounting agricultural problems.[71] They are one of the main CEC classes studied and includes herbicides, insecticides, fungicides, repellents, etc.[72] While agriculture keeps increasing due to growing population (vital for human survival), these chemicals are widely used in order to control pests and weeds, enhancing food production around the world, however, their use can affect the environment contaminating the

ecosystem.[71] They have been documented as relevant contaminants by the Water Framework Directive (WFD) in the European Union,[73] priority substances by the Directive 2013/39/EU and dangerous substances by the Directive 2006/11/EC.[72] These chemicals are considered persistent organic pollutants (POPs) characterized by the persistence, bioaccumulation, toxicity and long environmental transport properties, even at low concentrations. Pesticides are introduced in the trophic chain increasing the concern for human and animals. Examples of health human risks are cancer, infertility, and diseases (such as Parkinson and Alzheimer's). An example is the herbicide atrazine which causes cancer, cardiovascular problems, infertility, etc. in humans. However, aquatic organisms can also be affected by this chemical, as the proof of *Oryzias latipes* (fish) decrease of fertility after exposure.[71] This chemical has been banned since 2003 in Europe due to its toxicity, however, it still has been detected in different water bodies.[71,74] Mixtures of pesticides can also be more toxic, magnifying the effects, and therefore the need to be considered unique, where research needs to be performed.[71]

In 2019, there were 1,331 types of pesticides recorded by the European Commission.[71] Pesticides contain various chemical and physical characteristics giving multiple possibilities for their environmental fates.[75] Parameters such as the climate, the field characteristics or the soil properties, etc. interfere directly with their mobility and transport.[71] Agriculture can lead to decreasing water quality due to the pass of pesticides into the water, usually by agricultural industries, rainfall events or mishandling during dry periods.[76] In Ireland, agriculture plays a major role, where 71.6% of the land is used for this purpose while just 11% belong to forests, from a total area of 69,798 km^2. Just in 2019, €14.5 billion were estimated for the exportation of Ireland's agricultural food.[77] Therefore, there is a need to investigate the potential risk of these type of chemicals in the environment as there is very limited data on this particular class of compounds.

1.1.3.3.1 Pesticides in water samples

Pesticides production and application produce approximately 150 million tons of wastewater every year.[78] They pose a complex biodegradation and therefore there is a need for their removal from water matrices.[71] Removal of pesticides is very complex due to their variability in physical structures and the pH of the contaminated water (ranging from 0.5 – 14),[75] leading to their detection throughout different water matrices worldwide (e.g. surface water as seen in Figure 1.5). The selection of incorrect treatments has resulted in the production of more toxic by-products.[75] The Drinking Water Directive (Irish S.I. No. 122/2014) has established that the threshold limits for concentrations of individual pesticides (or its metabolites) must be below 0.1 µg/L, and the total amount, must be under 0.5 µg/L per sample.[79] In a recent study around seven sites in Ireland, more metabolites were present than the actual parent compounds in groundwater samples, exceeding the 0.1 µg/L EU threshold limit.[74] Some pesticides have been banned in this country, mainly the ones that pose a 0.03 µg/L limit. However, some compounds have been detected above their thresholds as the case of the herbicide MCPA (4-chloro-o-tolyxyacetic acid).[80] Other non-approved pesticides are also detected, such as atrazine, mentioned before, which is one of the most detected pesticides in surface waters across the world. This has been related to the symmetry of its molecular structure, causing high hydrophobicity leading to low solubility and therefore its persistence in the aquatic media.[71]

The concentrations in which they are found in the aquatic environment are usually affected by different factors, but seasonal variation is one of the most important ones. An example is rain, which could have two opposite behaviours in terms of the levels found on aquatic matrices, compounds could be either diluted or on the opposite hand, they could runoff from the soils ending up at higher concentrations in lakes and rivers.

Therefore, certain pesticides can have expected seasonal occurrence patterns,[71] however, research needs to be carried out empathising compounds with higher risk.

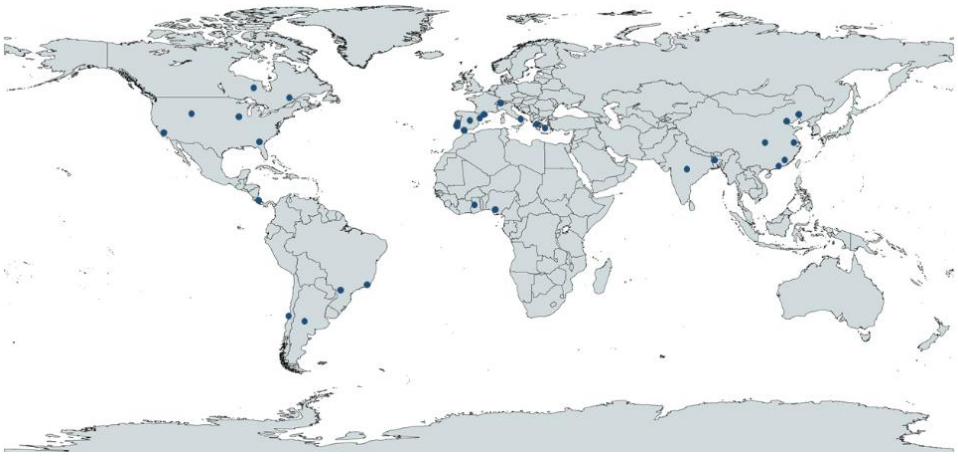


Figure 1.5 Pesticides reported in surface waters around the world (2012 – 2019); reproduced with permission from R. M. de Souza et al. 2020.[71]

1.1.4 Environmental fate based on the physiochemical properties of the compounds

Environmental fate and therefore human toxicity and/or ecotoxicity are directly dependant on the physicochemical properties of the compound such as physical properties (e.g. freezing point, viscosity and density), solvation properties (interactions with different phases and its partitioning between them, such as log *D* and pK_a) and molecular attributes related to molecular shape and size related to chemical reactivity (such as polarizability) as seen in Figure 1.6. Data can be collected from experiments, however, this is not always available and it is time consuming to do it for every single compound. Recently, predictions can be obtained using *in silico* methods, low-cost approaches which are increasingly reliability over time.[81] Table A.2 (Appendix A) presents some of the most used physicochemical properties for the analytes studied within this thesis, obtained by prediction using an *in silico* method using ACD/Labs with Percepta software and SMILES formulas (Table A.1, Appendix A).

CECs pose a wide range of chemistries, giving different physicochemical properties. However, we could estimate based on certain properties if they will partition to the aqueous or the organic phase by adsorption onto organic carbons (remaining in soils and sediments) or moving into lipid phases (moving to biota and have potential to bioaccumulate). Compounds could also escape from soil and water ending up in the air, this is the case of analytes presenting high volatilities (i.e. high vapour pressure). This would be the starting point of understanding their transport. The most used parameters for the understanding of their fate is log K_{ow} (or *log P*), inside the organism and also in the environment, determining bioaccumulation and toxicity for example.[82] Normally, compounds obtaining values lower than 10 will have higher solubility and therefore stay in the aqueous phase with small adsorption. In the case of our compounds all of them are lower than 10 (Table A.2, Appendix A). Some studies have attributed poor removals in WWTPs for compounds with values <3,[83] nevertheless it depends on the type of treatment performed because certain hydrophilic compounds (e.g. caffeine) were shown to have a higher removal efficiency when log K_{ow} values were decreasing as biodegradation treatments were considered (sorption processes would unlikely have an impact on their removal).[84] There are many compounds studied within in this thesis that carry this specific characteristic, including pesticides such as acetamiprid, clothianidin and fenuron, and pharmaceuticals such as amoxicillin, ciprofloxacin, trimethoprim and sulfamethoxazole. Nevertheless, PCPs present log K_{ow} values between 4 – 7, apart from benzophenone-4. Ionisable compounds, express a variety of different species at a certain pH, therefore pH-dependant and log *D* will be used for them. pH of the treatment process can alter the removal of the contaminants, higher pH would result in acidic compounds remaining in the treated water and basic compounds moving into the solid phase. This

can be predicted by the use of pK_a, acid dissociation constant, which will indicate the relative strength of an acid or a base during the reaction.

In terms of aquatic biota, compounds not easily degradable (pseudo persistent) that are absorbed by biota (log K_{ow} >3) can bioaccumulate even present at low environmental levels, as seen before in different aquatic organisms. Bioaccumulation is considered low when values are <2. Very lipophilic compounds will be more likely to cross and be retained by biota (>5), examples could be most PCPs studied (e.g. triclosan and octinoxate). However, compounds can be converted into metabolites or they could degrade, and bioaccumulation might not happen apart from exceptions such as large molecules.

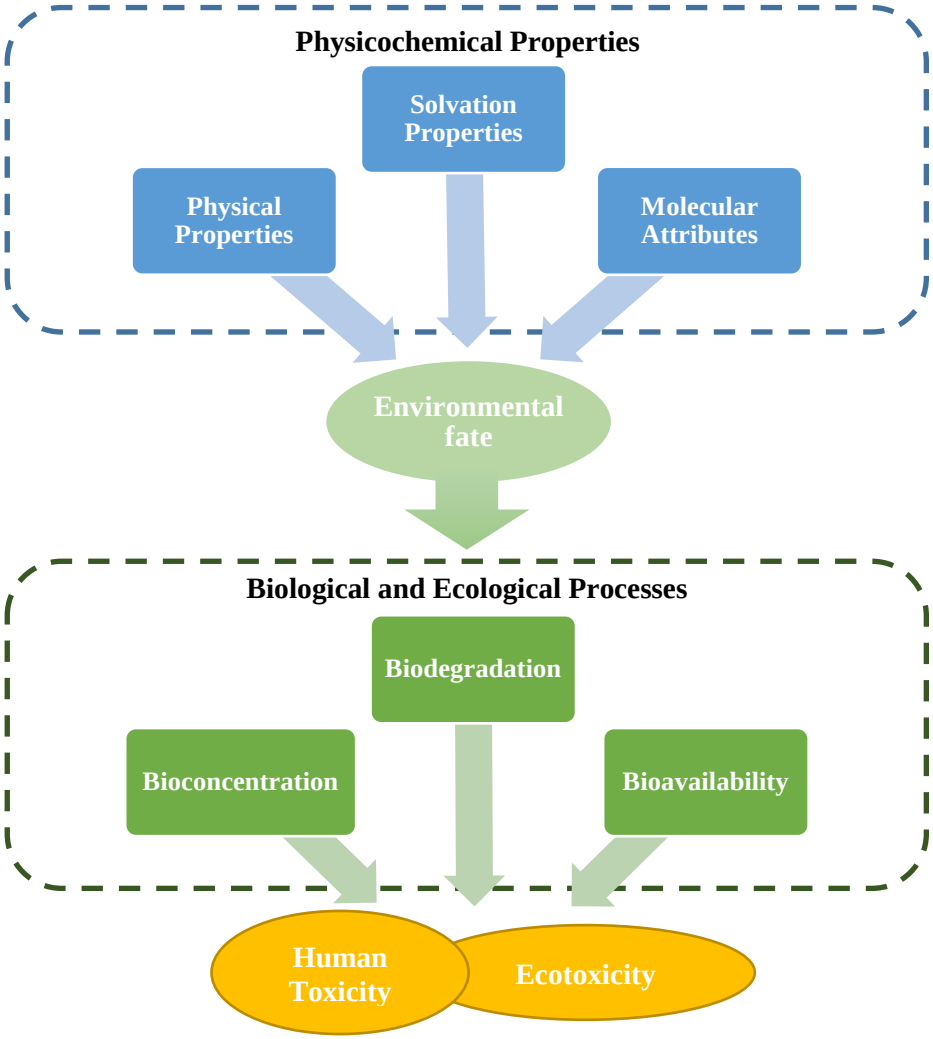


Figure 1.6 Physicochemical properties relationship with environmental fate, biological and ecological processes, and toxicity.[81]

1.1.5 CECs risks in the environment

While the impact is not yet fully known, examples across Europe and the United States that have studied the evaluation of the negative risks have typically concluded that their effects are chronic rather than acute depending on the exposure and concentration.[24] The exposure to a mixture of these compounds can have an impact on human health, which can potentially be even lethal. Typically many of these compounds remain bioactive at low concentrations and can be accumulated in the food chain, having a potentially negative impact in different species, as demonstrated for example by the decline of the vulture population due to treated diclofenac livestock (a non-steroidal anti-inflammatory drug (NSAID) widely used) in India subcontinent[11,85] and Pakistan.[86] Diclofenac has also been highlighted in the alteration of behaviour and reproduction as well as a delay in the development in fish and frogs[57] and steroid hormones have also been demonstrated to result in changes in reproduction and development to fish and wildlife at low concentrations in the range of ng/L.[8] In a recent study, six pharmaceutical compounds were quantified in the invertebrates, sourced from eight different sites in the River Thames (UK), in the order of ng/g.[87] The study was performed using Gammarus pulex, a freshwater amphipod crustacean found across much Europe that is typically food for other invertebrates, fish and birds, in order to biomonitor several pharmaceuticals residues in water samples. Antibiotics have also been observed in molluscs species around the world[88] but also animals. A hormone (medroxyprogesterone acetate) was detected in pig feed in Holland resulting in their infertility after contamination from some industrial waste from a pharmaceutical company based in Ireland.[89] This type of pharmaceuticals can also lead to resistance to bacteria in humans. As a result of antibiotics being typically present at low concentrations that cannot kill bacteria after biological treatments processes in WWTPs are carried out, bacteria can mutate

developing genes protecting them against these antibiotics, with these bacteria subsequently entering the soil, surface water, drinking water, groundwater, etc.[90] The resistant genes can be transferred to humans, via ingestion of contaminated food for example, resulting in an antibiotic-resistant infection. WHO has predicted that by 2050, 10 million people per year can be killed by antibiotic resistance[17] as infections will be impossible to treat. In Ireland, during the European Antibiotics Awareness Day by the Irish Pharmacy Union (IPU), the use of antibiotics was identified as one of the most significant threats to long term public health due to the antibiotic resistance explained above. Health services are experiencing an increase in antibiotic resistant infections due to antibiotic misuse and the lack of new antibiotics raising the concern, when 7% more antibiotics are used in Ireland compared to 15 years ago.[91]

In terms of potential CEC risks, it should be recognised that there is a risk not only for individual pharmaceuticals but also as a mixture of pharmaceuticals, as they can accumulate over time and have a synergistic effect. They are also considered "pseudopersistent compounds"[92] and the Funnel Hypothesis argues that if there is a mixture of pharmaceutical compounds where each chemical contributes to the same toxicity, this toxicity will dominate the compounds with specific modes of toxic action. Also, they are concentrating addition so in non-target aquatic life as wastewater, they can act as baseline toxicants creating hazard to all kind of species.[20] Furthermore, it is important to note the impact of the number of studies performed on specific areas, as this does not mean that there are no contaminants detected in areas where there are no available reports. In fact, this could be due to a potential lack of research or limitations regarding analytical techniques. This could then lead to an artificially high contamination frequency and therefore data cannot be used to extrapolate and draw conclusions in a global perspective.

1.2 Regulation of CECs in water

In order to provide safe water for long-term sustainable use and control pollution, some countries have performed studies to avoid the discharge of substances into groundwater; however, CECs are by definition outside the scope of these studies as they are not regulated around the world as there is not sufficient data to set thresholds (either a minimum of removal or maximum concentration of CECs in water). The use of a legislative system can help to control pollutant levels and for that reason CECs need to be studied and monitored. In 2013, the European Union (EU) Water Framework Directive's (WFD) developed a first Watch List (WL) under the Priority Substances Directive 2008/105/EC, a piece of legislation which contains priority pollutants and other substances that need to be monitored in all European wastewaters due to insufficient information to assess the exposure of these substances, and the need to evaluate if further regulation is required.[93] The European Communities Environmental Objectives (Surface Water) Regulations 2009 and the European Communities Environmental Objectives (Groundwater) Regulations 2010 established the framework needed to implement the objectives of the WFD.[94] The list is updated every two years and allows Environmental Quality Standards (EQS) to be set, regulating the maximum concentration allowed in water, across either a particular geographic area or nationwide at EU level,[95] for a maximum of 14 groups of substances.[96] Within Europe, these substances must be monitored at least once annually in each country, and they can remain or be removed from the WL depending on the risk at EU-level. The dataset of the first WL is mainly from river (98.3%), lakes (1.2%) and coastal/transitional waters (0.5%) from 25 Member States in the EU. Some countries experienced difficulties in reaching the low limits of detection (LODs) below the maximum acceptable method detection limit (MADL) of

2015 for 5 of the 17 substances, which included 17-α-ethinylestradiol (EE2), 17-β-estradiol (E2) and azithromycin as seen in Table 1.3. Exceedances of the 2015 MADLs were observed mainly for compounds such as EE2, E2, diclofenac, azithromycin and clarithromycin.

A review was performed in 2018, where it was observed that in some countries, LOQs were not sufficient for the low EQS required for EE2, E2 and estrone (E1), so it was decided for them to remain in the WL until sufficient information could be collected to allow for an informed decision. Addition of new compounds were performed such as antibiotics, including amoxicillin and ciprofloxacin. However, diclofenac was removed as sufficient information was collected to allow a decision to be made about whether it needed to be further regulated (and it is not controlled via further regulation). For butylated hydroxytoluene (BHT) and octinoxate, almost all LOQs were below the MADL so sufficient information was collected to allow for these compounds to also be removed from the WL (in this case it was decided that there was no need to further monitor their presence in water bodies), but octinoxate was going to be considered for re-inclusion in 2019 for sediment monitoring, to allow sufficient information to be collected to facilitate decision making. For the macrolide antibiotics, just azithromycin had a lower MADL level proposed which requires lower LOQs in half of the tested laboratories. For this reason it remains in the WL and therefore the other two macrolides were decided to also remain in order to monitor them together, even when all LOQs were below MADL.[95]

A recent report, 2020, has been done in order to propose new candidate substances for a 3rd WL (Table 1.3). Other compounds studied within this thesis were considered for their inclusion such as octinoxate and norethisterone. The first one was not considered at the end due to the matrix, this compound has a log $K_{ow} > 5$, therefore it is preferable to be analysed more in sediments or suspended particulate matter (SPM). On the other hand,

norethisterone was not continued for the final inclusion as it needs further investigation and could be proposed for a next list with levonorgestrel (another synthetic hormone).[96]

Table 1.3 Information of Watch List substances of interest within this thesis.[95–97]

Substance	Class	MADL WL 2015 (µg/L)	MADL WL 2018 (µg/L)	2018 JRC's recommendation	MADL WL 2020 (µg/L)	2020 JRC's recommendation
17-α-ethinylestradiol	Synthetic hormone	0.000035	-	Inclusion in 2nd WL	-	Removal from the WL
17-β-estradiol	Natural hormone	0.0004	-	Inclusion in 2nd WL	-	Removal from the WL
Estrone	Hormone	0.0036	-	Inclusion in 2nd WL	-	Removal from the WL
Diclofenac	Non-steroidal anti-inflammatory drug (NSAID)	0.1	0.05	Removal from WL	-	-
Erythromycin	Macrolide antibiotic	0.2	-	Fulfils removal criteria but recommended for 2nd WL	-	Removal from the WL
Clarithromycin	Macrolide antibiotic	0.13	0.12	Fulfils removal criteria but recommended for 2nd WL	-	Removal from the WL
Azithromycin	Macrolide antibiotic	0.09	0.019	Inclusion 2nd WL	-	Removal from the WL
2,6-di-tert-butyl-4-methylphenol (BHT)	Antioxidant	3.16	-	Removal from the WL	-	-
2-ethylhexyl-4-methoxycinnamate (Octinoxate)	Sunscreen ingredient/UV filter	6.0	-	Removal from the WL	-	Inclusion 3rd WL (sediment/SPM)
Amoxicillin	Antibiotic	-	0.078	Inclusion 2nd WL	-	Remains in 3rd WL
Ciprofloxacin	Antibiotic	-	0.089	Inclusion 2nd WL	-	Remains in 3rd WL
Sulfamethoxazole	Antibiotic	-	-	-	0.1	Inclusion 3rd WL
Acetamiprid	Neonicotinoid insecticide	0.5	-	Fulfils removal criteria but recommended for 2nd WL	-	Removal from the WL
Thiamethoxam	Neonicotinoid insecticide	0.14	0.042	Inclusion 2nd WL	-	Removal from the WL
Clothianidin	Neonicotinoid	0.13	-	Fulfils removal criteria but recommended for 2nd WL	-	Removal from the WL
Imidacloprid	Neonicotinoid insecticide	0.009	0.0083	Inclusion 2nd WL	-	Removal from the WL
Trimethoprim	Antibiotic	-	-	-	0.1	Inclusion 3rd WL
Venlafaxine	Antidepressant	-	-	-	0.006	Inclusion 3rd WL
Famoxadone	Fungicide	-	-	-	0.0085	Inclusion 3rd WL

Examples of CECs detected have not only been observed in Europe but extended worldwide in different types of water samples such as seawater in the Western Mediterranean,[98] surface water and drinking water in Brazil,[6] wastewater samples from Canada,[17] Switzerland,[20] or the United States.[99] In all of the studies, CECs have been detected in the range of ng/L to µg/L giving examples such as Almadinah Almunawarah (Saudi Arabia) where 5 of 19 compounds were detected in influent and effluent[57] from hospital wastewater samples. Therefore, there are other regulations outside the EU. In the States, The United States Environmental Protection Agency (USEPA) developed a list named Drinking Water Contaminant Candidate List (CCL) with contaminants that are not proposed or in any national primary drinking water regulation. They can require future regulation under the Safe Drinking Water Act (SDWA) and requires the US EPA to report the list every five years. CCL 1 was developed in 1998 and the final report for CCL4 was published in 2016, including 97 chemicals or groups and 12 microbial contaminants, which will be monitored to allow for decision making in the future. The list contains antibiotics and synthetic hormones such as erythromycin, E2 and EE2.[100] Another regulatory list is The Global Water Research Coalition (GWRC), an organisation for water research formed officially in 2002 with members around the world such as Canadian Water Network (Canada), PUB (Singapore), Suez (France), Water Research Australia (Australia) or DVGW TZW-German Water Centre (Germany) among others. They consolidated a list of compounds with risk to the water cycle including antibiotics, anti-inflammatories and psychoactive drugs. The antibacterial triclosan and natural hormones E2 and E1 were subsequently deleted from the list, but on the grounds that this one only covers pharmaceuticals and metabolites, and so should not be interpreted as an assessment of their safety. A total of 153 pharmaceutical compounds were reviewed and ranked in three classes in order to prioritise future research, monitoring, risks

assessments, etc. Class I (high priority) contains 10 compounds (including diclofenac, ciprofloxacin and erythromycin), Class II (medium priority) with 18 compounds (including clarithromycin and amoxicillin) and 16 compounds in Class III (lower priority).[101]

1.2.1 CECs in Ireland

Ireland has a surface water extending to 70,000 km of river channel, 12,000 lakes, 850 km^2 of estuaries and 13,000 km^2 of coastal waters, supplying approximately 75% of the country's drinking water (as 20 to 25% comes from groundwater).[102] There are approximately 1,100 WWTPs[23] around the country as seen in Figure 1.7, where more than a billion litres are collected daily.[30] However, limited studies about the impact of CECs have been carried out in an Irish context. Typical studies have included only a minimum number of substances, e.g. only three CECs tested from wastewater samples (diclofenac, E2 and EE2),[103] or a few sampling locations, as the identification of 15 out of 20 pharmaceutical compounds from three different waste water treatment plants (WWTPs) in the greater Dublin area. These had an equivalent population of 60,000, 90,000 and 1.7 million and discharged to an estuary, river and a bay[39] raising the concern. Not only PPCPs are a concern in Ireland; illicit drugs have increased in consumption and cocaine and its respective metabolites were detected in treated wastewater and receiving waters from Dublin area (Ringsend, Swords, Shanganagh, Leixlip and Navan).[104]

A 2010 – 2011 study performed in Galway and Cork revealed that 72% of the individuals surveyed (398 individuals, 207 in Galway and 191 in Cork) disposed their medicines in the past in an improper way, either through general waste or sewage system.[105] Only 16 WWTPs have monitored at least one CEC in the Republic of Ireland, with two of the compounds, salicylic acid and ibuprofen, quantified in the influent but

not detected in effluent, indicating they may have been removed during treatment. Estrogen levels were not reported to have a negative threat to the environment in the River Lee at the time, though the only evidence reported was in fish from the River Liffey, close to the Osberstown WWTP. However, all three compounds (diclofenac, E2 and EE2) were shown to be distributed throughout the country with high levels in large cities (Galway, Dublin and Cork).[39] Based on the limited data on sources of pollution, notwithstanding that they not exceed the EQS values of diclofenac, E2 and EE2 in Irish surface waters, this could lead to occasionally hotspots that can exceed the limits from the European Union. Five of the sixteen WWTPs (Leixlip, Osberstown, Kilkenny, Killarney and Longford) had a significant positive impact in terms of these three analytes. They vary in each location but they were all large WWTP-generated load, with no tertiary treatment, the primary discharge point is near/at a sensitive area and the discharge flow rates into the receiving waters is low 95 percentile.[103]

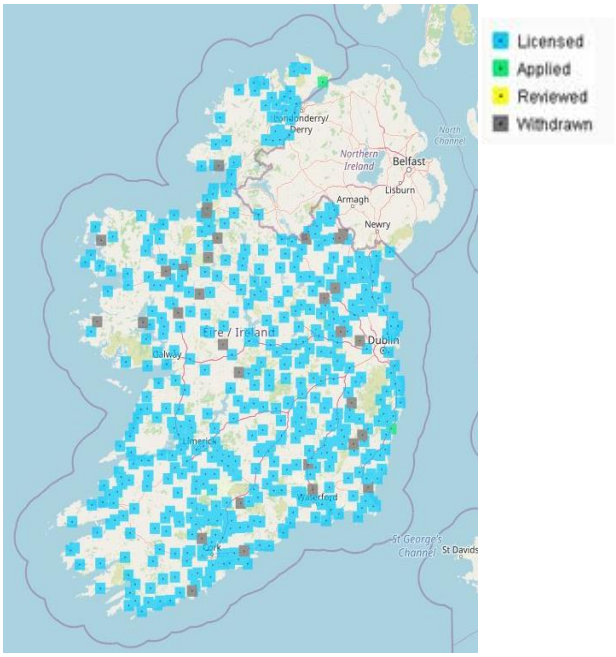


Figure 1.7 WWTPs in towns/cities with a population equivalent of over 500 during 2006, 2007 and 2008, that were on compliance under The Urban Waste Water Treatment Regulations, 2001 (S.I. No. 254 of 2001) and 2004 (S.I. 440 of 2004; free data from "©OpenStreetMap contributors". [106]

Only 21 publications used in the EPA report for Emerging Standards on Pharmaceuticals in receiving waters were from Ireland, highlighting a need for monitoring of Irish receiving waters for a future risk assessment.[23] The lack of research is a challenge to the Ireland's ability to accurately assess the requirements needed to assess the risk of these contaminants in an Irish context.

1.2.2 Regulation in Ireland

In Ireland, CECs contamination in waters is a real concern and it has been highlighted in the Department of the Environment, Community and Local Government document "Significant Water Management Issues in Ireland" (DECLG, 2015). The Irish river basin management plans (RBMPs) and the WFD (2000/60/EC) set frameworks to protect Irish waters in a long-term scenario.[23] The first RBMP was from 2009 to 2015 with different plans developed to cover each individual river basin district, but in 2018 a second RBMP was published covering the period 2018 to 2021.[102] It outlines what Ireland is currently doing to protect and improve their waters including investment in waste water projects at 255 urban areas.[34] In total, 46 catchment management units, including 583 sub-catchments with 4,832 water bodies, are covered in the Republic of Ireland.[102] The Minister for Housing, Planning and Local Government in Ireland under the WFD develops policies to protect the water for an estimated population of 4,757,976. This has led to the creation of the Water Forum, the Local Authority Water and Communities Offices among others in order to help with this issue. Ireland is currently trying to monitor and assess the presence and concentration of contaminants of emerging concern in the aquatic environment by a variety of state agencies and departments with a range of statutory responsibilities relating to water quality. However, only a very limited subset of potentially concerning substances such as pesticides has been studied and in a country where the key export sectors include medicinal and pharmaceutical products (€30 billion)

and organic chemicals (€21 billion),[94] there is a need and a concern to monitor substances such as PPCPs.

The Waste Water Discharge (Authorisation) Regulations 2007 gives the requirements to the Urban Waste Water Treatment Directive (UWWTD) and the WFD in Ireland. The UWWTD sets the requirements for collection, treatment and discharge of urban wastewater before it is released into the environment.[94] EPA is responsible for licensing and regulating the urban wastewater discharges and it has issued over 1,060 waste water discharge authorisations[34] (Figure 1.7), where urban areas with a population equivalent or greater than 500 needs a license, if it is below this population then they require a Certificate of Authorisation.

A total of 7% of water bodies were identified as At Risk due to industry, where 20 IPC (Integral Pollution Control) and 26 IE (Industrial Emissions) facilities were licensed by EPA and 43 industries with Section 4 Discharge to Water licenses by local authorities.[94] However, EPA does not require a minimum threshold of CEC emissions into the environment at the moment; licenses provided only account for Volatile Organic Compounds (VOC) and Carbon monoxide (CO) in general. Water discharges are monitored for pH and determine parameter as Chemical Oxygen Demand (COD), total particulate matter, hydrogen chloride, hydrogen fluoride, sulphur oxides, nitrogen oxides and metals such as mercury, cadmium, lead, arsenic, copper, cobalt, chromium, tin, antimony, manganese, nickel, vanadium, thallium and their compounds. An example of monitoring frequency and parameters in a chemical company in Kilkenny are described in Table 1.4.

Table 1.4 Parameters and their frequency of monitoring required for an ICP license from EPA in Ireland.[107]

Type of water	Parameter	Monitoring frequency	Analysis method/technique
Surface water	pH	Continuously	pH electrode/meter
	COD	Weekly	Standard method
	Visual inspection	Weekly	Not applicable
	Organohalogens[a]	Quarterly	GC-MS
	Organic solvents[b]	Quarterly	GC-MS
Groundwater	pH	Quarterly	pH electrode/meter
	Redox Potential	Quarterly	Redox meter
	DO	Quarterly	DO meter
	Temperature	Quarterly	Thermometer
	COD	Quarterly	Standard method
	Nitrate	Quarterly	Standard method
	Nitrite	Quarterly	Standard method
	Total ammonia	Quarterly	Standard method
	Iron	Quarterly	Standard method
	Manganese	Quarterly	Standard method
	Conductivity	Quarterly	Standard method
	Chloride	Quarterly	Standard method
	Fluoride	Quarterly	Standard method
	Organohalogens[a]	Quarterly	GC-MS
	Organic solvents[b]	Quarterly	GC-MS

[a]Screening of priority pollutant substances (such as perchloroethylene, trichloroethylene, dichloroethylene and vinyl chloride).
[b]Includes toluene, aliphatic alcohols, etc.

1.2.3 Compliance with legislation

Ireland's compliance with relevant legislation is evaluated by the EPA in their annual report named Urban Waste Water Treatment (UWWT) (yearly reports can be found here: https://www.epa.ie/pubs/reports/water/wastewater/), in which it can be seen that certain locations from the Republic of Ireland lead to water bodies 'at risk of pollution'. Pollution of water can originate from discharges of WWTPs, leaks, spills, and overflows from sewers and pump stations. Deficiencies in many WWTPs and public sewers around the country, still discharge with no adequate treatment into the receiving waters. In the latest years, the European Commission has taken Ireland to the Court of Justice for failure to comply with the obligations under the UWWT Directive[29,30,33,34] facing substantial fines; Irish Water is currently working towards the improvement of infrastructures and deficiencies of WWTPs to ensure 90% of wastewater from large urban areas is treated before their release by the end of 2021. However, delays in works have already moved the target date for the next few years, and some areas are not in the provided investment plan for 2020-2024, meaning that it is unlikely they will undergo any changes before

2025.[30] Nevertheless, the number of areas identified for non-compliance with the European Union's legally binding standards for waste water treatment are getting slowly reduced over time as seen in Figure 1.8. In 2016, 50 of 185 large urban areas, including Dublin and Cork, did not meet the European Union's criteria. These areas related to 64% of the national wastewater load collected in all large urban areas, therefore the extent of non-compliance is expected to have an impact in the environment.[33] In 2017, the number was reduced to 28 (out of 179) where a 47% of the wastewater load collected did not meet the secondary treatment requirements.[34] For 2018, 21 plants still failed (out of 169), meaning that 42% of wastewater from large urban areas was treated and passed the criteria.[29] In the most recent report, 2019, the number was further reduced to 19, 92% of Ireland's urban wastewater, leading to 113 areas to prioritise in order to improve treatments. Therefore, only 44% of the water was treated in a plant where requirements were met, far away from the European average of 81%. Dublin's plant Ringsend contributes to the majority of the failure, as it supplies a 44% of the country's urban wastewater, the plant is not big enough to treat all the water collected.[30]

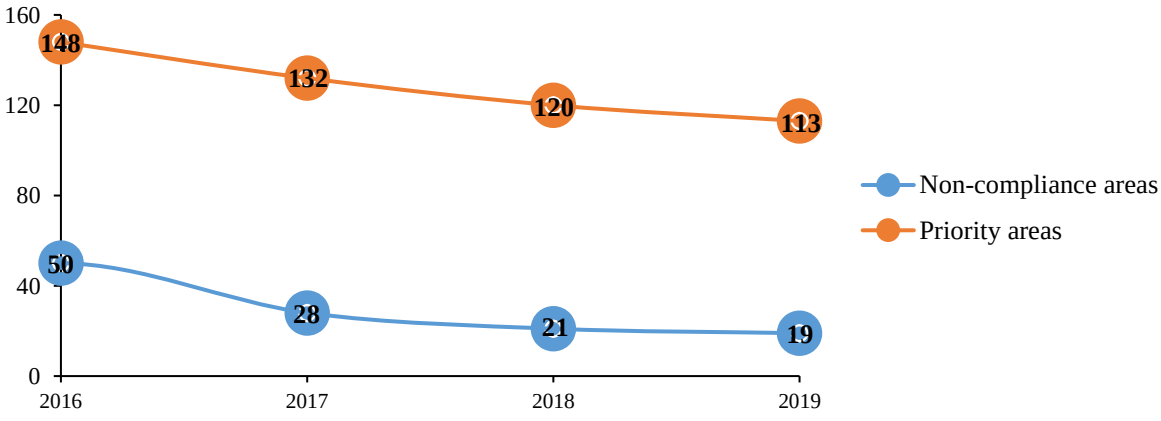


Figure 1.8 Number of areas with non-compliance with European Union legislation and the prioritise areas listed by the EPA in order to have improvements since 2017.[29,30,33,34]

More than one billion litres of wastewater are collected every day in approximately 30,000 km of sewers[33] from 1.1 million homes throughout the country but 44 areas discharged raw sewage, untreated water, to the environment in 2016 including large urban areas in Clare, Cork, Donegal and Dublin. This number was reduced to 38 in 2017 and it has been predicted that 33 of them will perform primary and secondary treatment by 2021. In 2017, raw sewage of 88,000 people in 38 towns and villages flowed into the environment per day and just 93% bathing water in Ireland met the minimum standards. This lead to poor quality bathing waters in places such as Rush South Beach (Dublin) and Ballyloughane Beach (Galway).[34] In 2018, it was further reduced to 36 towns and villages equivalent to 77,000 people[29] but this is still a huge concern. At the end of 2019, 34 areas are still continuing to release untreated wastewater (1.4% of collected water goes directly into the environment), an equivalent to 78,000 people, increasing from the previous year. It includes counties such as Dublin, where an area around Howth discharges into Doldrum Bay with an approximately population of 130, Cork, and Donegal (containing 8 areas in total), and there are still three large areas with no WWTPs (Arklow, Cobh and Moville). New timeframes have been given to all areas in order to provide treatment in the next few years.[30]

Treatment infrastructures can determine the effluent quality, and in Ireland the trends in treatment are positive, with the levels of treatment provided to the national wastewater load decreasing for no treatment performed and increasing for the application of secondary treatment and nutrient removal for the last past years as seen in Figure 1.9. There has been progress at some areas but concerns have been raised about leaks or spills from 13 collection systems (Cavan, Kildare, Wexford, etc.),[34] with recommendations focusing reducing the leakage in order to achieve compliance.[102] Overflows lead to poor bathing quality again in 2019, repeating Ballyloughane in 2019, apart from 27 short

incidents mostly from storm water overflows. In the latest report (2019), EPA identified 48 areas at risk of pollution, reduced from 57 from the previous year. EPA has required action for these specific areas, however, the investment needed to overcome all issues is quite high. The investment in infrastructure is nonetheless increasing annually, with €172 million invested in 2016, €215 million in 2017[34] and €230 million in 2018.[29] It has been estimated that €500 million will be invested between 2017 and 2021,[34] which should significantly support Ireland's efforts to ensure that water complies the requirements for a better quality water.[102] Due to Covid-19 restrictions, works have been delayed in the most prioritised areas of improvement such as Ringsend (Dublin), where works for the first phase are expected to be finished by the end of 2021.[30]

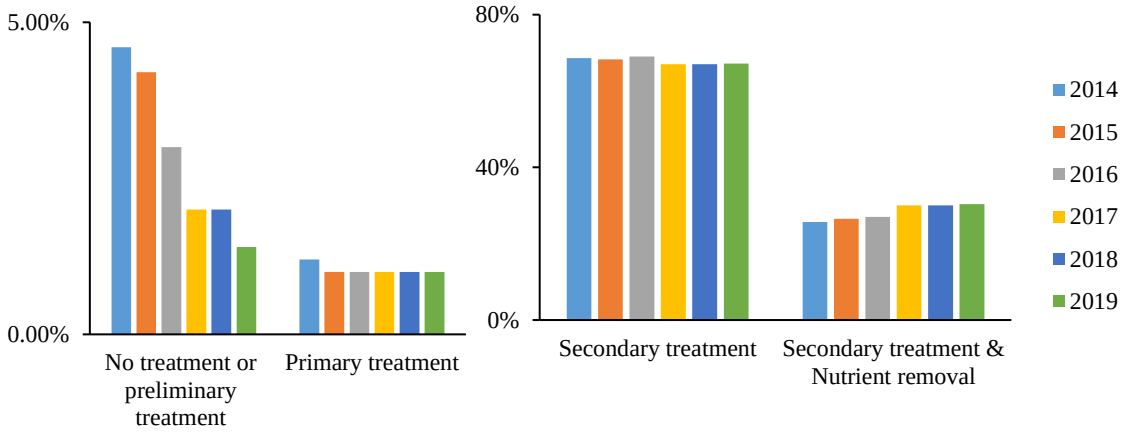


Figure 1.9 Level of treatment provided for Ireland's wastewater load in the last years.[29,31–34]

In terms of the Irish River Basin District, the compliance with the EU standards in 2015 can be seen in Table 1.5, where only 57% meets compliance for river waters, and the next one will be by the end of 2021.

Table 1.5 The Irish River Basin District (RBD) in relation to compliance with EU standards in 2015.[94]

	Area	Compliance with EU standards (2015)
Irish River Basin District	Rivers	57%
	Lakes	46%
	Coastal waters	79%
	Groundwater bodies	91%
Protected Areas	140 Designated bathing waters	93%
	64 Shellfish waters	75%
	358 Water-dependent Special Areas of Conservation (SACs)	60%

1.3 CEC analysis of environmental aquatic samples

1.3.1 Sample pre-treatment and collection

Wastewater is typically sampled using grab or composite samples, taken on different locations depending on what the study requires. Examples are the collection in the inlet of the plant after initial screening (influents) and from the outlet after chlorination (tertiary treatment, effluent) but can also include post-primary treatment and final effluent samples.[11] Composite samples are typically taken during 12h[11] or 24h,[24] however, 24h collection is recommended as it seems the most representative period, if instrumentation is not available one sample every hour during a certain period of time to create the composite sample can also be performed.[42] Most samples have been collected in amber glass bottles[11,24,39] or polypropylene bottles[57] and all the glassware used have been normally pre-treated. One case pre-rinsed it with just ultra-pure water[24] but most studies tend to silanise glassware in order to avoid loss of analytes that can bind into the glass.[108] Different methods for silanisation have been used across studies, rinsing the glassware with reagents like 10% dichlorodimethylsilane in toluene,[39] dimethyldichlorosilane (DMDCS)[57] or alternatively soaking all glass and plastic overnight in nitric acid.

After sampling, if possible, samples collected are shipped on ice[108] and they can be stored short-term in a fridge (+4°C)[39] and long term in a freezer (-18°C, -20°C)[4] in the dark until analysis.[67] In one case, a time-proportional method was used to sample, where every 15 minutes a 50 mL aliquot was taken using a rack of bottles that were surrounded by ice cubes. This was done to prevent the degradation of the compounds at high temperatures.[109]

Before analysis, samples are filtered in order to remove suspended particulate matter throughout vacuum filtration generally using filters such as 0.2-1.2 µm PVDF,[110]

glass microfiber,[39,110–114] cellulose,[9,115] and nylon membrane filters.[24,114,116–118] A pre-treatment is usually performed such as adjusting the pH. When acids are added to the wastewater samples, it stops bacteria from growing and enhanced sample stability, preventing degradation of the compounds. Many studies use this approach where they adjust the pH to 2 or 4 using hydrochloric acid[11] or sulphuric acid, respectively. It has been demonstrated that acidic pHs give higher recoveries for some analytes used[39] as it promotes interactions with the sorbent of the SPE cartridge. As part of the pre-treatment there is the addition of reagents such as ethylenediaminetetraacetic acid disodium salt dehydrate (Na_2EDTA) solution or ascorbic acid. In this case, Na_2EDTA is added as a chelating agent mainly for antibiotic compounds, as EDTA will bind to the present metals in the sample so they will not interfere in the extraction;[119] and ascorbic acid is to remove any chlorine residues present in samples after the treatment.[57,108] A solution of internal standard has also been added before storage but this is not common[108] as they usually are added before performing extraction. Most studies do not take into account the solid matter developed after filtration, however, one study extracted it with diethyl ether; where it did not show any presence of analytes.[11]

1.3.2 Extraction methods

Previous studies have shown that depending on the properties of the analytes selected, extraction analytical methods included SPME (solid-phase microextraction) and LPME (liquid-phase microextraction).[120] However, solid-phase extraction (SPE) is the most common method used as different sorbents can be purchased in order to extract the different analytes required. Target analytes are isolated and pre-concentrated while interferences from the matrix are reduced. This leads to an increase of the sensitivity achieving better results compared to traditional techniques such as liquid-liquid extraction (LLE). There are two ways of performing SPE, on-line (sorbent is packed on

a pre-column on the HPLC system) or off-line (sorbent comes in disposable cartridges). Off-line is more time consuming, however, on-line SPE reuses the sorbent increasing the risk of contamination of the samples, carryover and there is also a need of specific instrumentation.

There are different types of sorbents that have been used in literature such as Oasis HLB,[24,98] Strata-X,[39] and Oasis MCX.[57,108] Most methods were developed for a wide range of pharmaceutical compounds with different chemistries so the selection of the sorbent will inevitably compromise certain analytes recoveries. The most used one is Oasis HLB, a copolymer of divinylbenzene and vinylpyrrolidone, as it covers a wide range of different compound polarities as seen in Table 1.6. Once the sorbent is selected, conditioning steps are carried followed by loading the sample. After passing the sample, the sorbent is normally washed and some methods dry the cartridges under vacuum for a period between 30 minutes to an hour before continuing.[11,39] Then, elution is performed and some articles describe how they perform two elutions, one for acidic and neutral compounds and one for basic analytes, where they normally add ammonia to the elution solvent used.[57] After, in order to increase the concentration, as pharmaceutical compounds are present at low levels of concentration, they are evaporated using nitrogen gas and reconstituted in a mixture of organic and aqueous solvent to have a final extract compatible for the analysis conditions or a derivatisation can be performed if gas chromatography is selected as the analytical instrument. Another strategy is leaving the analytes on SPE sorbents as they will remain stable until analysis if stored at -20°C conditions, even up to a month, and then eluted on the day of analysis.[109,110,121] For PCPs, the most common extraction techniques are as follows: solid-phase extraction (SPE), liquid-liquid extraction (LLE), liquid-liquid micro-extraction (LLME) and solid-phase

micro-extraction (SPME). But the most used widely is SPE with Oasis HLB sorbent, similarly to pharmaceuticals.

Nevertheless, SPE is known as a time consuming process which can lead to operational errors due to their multi-step process. Also, the chemistry of the sorbent can limit the amount of diverse compounds under the same method. Alternatively, new research has led to more sensitive mass spectrometers where direct injection of the sample is possible, reaching the low levels required without the need of a pre-concentration step[115] decreasing the cost of the entire analysis.[122] Different injection volumes can be tested in order to further increase sensitivity if needed, however, the use of large volumes of injection can affect the source of the mass spectrometer when using complex matrices such as influent wastewaters.[123] Therefore, the source of the instrument should be taken into account with these type of matrices and also if a high number of samples are injected, performing the instrument to drift alongside the run.

Table 1.6 Comparison of SPE methods used for water samples.

Matching compounds	SPE Sorbent	pH	Pre-treatment	Volume of sample loaded	Reference
Diclofenac, erythromycin, sulfamethoxazole, trimethoprim, carbamazepine	Oasis MCX (150 mg)	-	Na_2EDTA Ascorbic acid	500 mL	[57]
Amoxicillin, ciprofloxacin, lincomycin, spiramycin, sulfamethoxazole, salbutamol, clarithromycin, erythromycin, diclofenac, benzophenone-4, 17-β-estradiol, 17-α-ethinylestradiol, triclosan, carbamazepine, enalapril, hydrochlorothiazide, atorvastatin, bezafibrate, clofibric acid	Oasis MCX (60 mg) Oasis HLB (60 mg)	-	-	500 mL	4
Azithromycin, erythromycin, diclofenac, carbamazepine, trimethoprim, sulfamethoxazole, propranolol, clofibric acid, mefenamic acid, bezafibrate, hydrochlorothiazide, metoprolol, fluoxentine	Oasis HLB (60 mg)	-	-	100 mL influent 200 mL effluent	24
Diclofenac, bezafibrate, carbamazepine, clofibric acid, flurbiprofen, mefenamic acid, metoptolol, propranolol, salbutamol, sulfamethoxazole, trimethoprim	Strata-X (200 mg)	4	-	500 mL	39
Diclofenac, clofibric acid, mefenamic acid	ENVI-18 reverse phase packed tube	2	-	250 mL	11
Diclofenac, triclosan, carbamazepine, ciprofloxacin, clofibric acid, hydrochlorothiazide, sulfamethoxazole, atrazine, fenoxaprop-ethyl, simazine	Oasis HLB (1 g)	2	-	2.5 L	[98]
Alprazolam, trimethoprim, sulfamethoxazole, amitriptyline, benzotropine, fluoxentine, metropolol, enalipril, propranolol, verapamil, amlodipine, carbamazepine, valsartan, atorvastatin, hydrochlorothiazide	Oasis MCX (150 mg)	-	Na_2EDTA Ascorbic acid Internal Standard	500 mL	[108]
-	Oasis HLB (150 mg)	-	Internal Standard	100 mL	[109]
Atorvastatin, bezafibrate, bisoprolol, bupropion, carbamazepine, chloramphenicol, cilazapril, citalopram, ciprofloxacin, clarithromycin, diclofenac, azithromycin, diphenhydramine, flutamide, isradipine, memantine, metoprolol, risperidone, roxithromycin, orphenadrine, BP-4, erythromycin, triclosan, sulfapyridine, sulfadimethoxine, sulfamerazine,	On-line SPE Hypersil GOLD C18 column	-	Internal Standard	1.1 mL	[67]

Matching compounds	SPE Sorbent	pH	Pre-treatment	Volume of sample loaded	Reference
sulfamethazine, sulfathiazole, tramadol, trimethoprim, valsartan, venlafaxine, verapamil					
Azithromycin, clarithromycin, roxithromycin, spiramycin, lincomycin, amoxicillin, ciprofloxacin, sulfamethoxazole, sulfathiazole, sulfapyridine, sulfamerazine, sulfisoxazole, trimethoprim	Oasis HLB (60 mg)	2.5	Na_2EDTA	25 mL influent 50 mL effluent	42
Fluoxentine, venlafaxine, bupropion, citalopram	Oasis HLB (500 mg)	Acidified (0.1% formic acid)	-	1 L (unfiltered)	[124]
Amoxicillin, rimethoprim, sulfamethoxazole, fluoxentine, enalapril, nifedipine	Oasis HLB (500 mg)	-	Internal Standard	1 L	[111]
Diclofenac, bezafibrate, erythromycin, azithromycin, clarithromycin, atorvastatin, carbamazepine, citalopram, ciprofloxacin, venlafaxine, fluoxetine, lorazepam, alprazolam, propranolol, metoprolol, nadolol, valsartan, clopidogrel, amlodipine, tamsulosin, salbutamol, levamisole, hydrochlorothiazide, sulfamethoxazole, trimethoprim, ronidazole, verapamil	Oasis HLB (60 mg sea waters and 200 mg for the other matrices)	-	Ascorbic acid (tap water only) Na_2EDTA	25 mL influent 50 mL effluent 100 mL river 200 mL reservoir waters	[125]
Diclofenac, triclosan, hydrochlorothiazide, sulfapyridine, sulfamethoxazole, nadolol, sulfamethazine, antipyrin, trimethoprim, clofibric acid, timolol, metoprolol, tramadol, methylphenidate, chloramphenicol, bezafibrate, bisoprolol, propranolol, betaxolol, carbamazepine, mefenamic acid, nortriptyline, temazepam, fluoxetine, amitryptiline, nifidepine	HyperSep Retain PEP (200 mg)	2	-	100 mL	[126]
Diclofenac, octocrylene, antipyrine, mefenamic acid, sulfamethoxazole, sulfamethazine, sulfadimethoxine, ciprofloxacin, metoprolol, propranolol, carbamazepine, clofibric acid, pirenzepine	Oasis HLB (500 mg)	2	Na_2EDTA	500 mL	[127]
Tramadol, temazepam, amitriptyline, nordiazepam, nortriptyline, fluoxentine, venlafaxine	Oasis MCX (60 mg)	1.8 – 1.9	-	500 mL river 100 mL influent 100 mL effluent	[113]
Diclofenac, erythromycin, clarithromycin, triclosan, tramadol, roxithromycin, sulfamethoxazole, sulfameraine,	Direct Injection of sample	Acidified with formic acid	Internal Standard	60 mL	[128]

Matching compounds	SPE Sorbent	pH	Pre-treatment	Volume of sample loaded	Reference
sulfamethazine, trimethoprim, chloramphenicol, metoprolol, hydrochlorothiazide, carbamazepine, sulfapyridine, bezafibrate					
17-β-estradiol, ethinylestradiol, triclosan, carbamazepine, sulfadimethoxine, sulfamethazine, sulfamethoxazole, trimethoprim, estrone	Oasis HLB (200 mg)	-	Internal Standard	100 mL	[129]
Clarithromycn, erythromycin, diclofenac, 17-β-estradiol, 17-α-ethinylestradiol, amoxicillin, ciprofloxacin, lincomycin, sulfamethoxazole, enalapril, valsartan, salbutamol, carbamazepine, hydrochlorotiazide, atorvastatine, bezafibrate, clofibric acid	Oasis MCX	2	Internal Standard	200 mL	[130]
Diclofenac, erythromycin, sulfamethoxazole, carbamazepine, fluoxetine, metoptolol, bezafibrate, clofibric acid, amitriptyline	Oasis HLB (200 mg)	-	Ascorbic acid Na_2EDTA	500 mL	[131]
Azithromycin, clarithromycin, diclofenac, alprazolam, amiodrarone, amitriptyline, atorvastatin, bezafibrate, bisoprolol, bupropion, carbamazepine, ciprofloxacin, diphenhydramine, fluoxentine, ketoconazole, medroxyprogesterone, memantine, metoprolol, orphenadrine, risperidone, roxithromycin, sulfamethoxazole, tramadol, trimethoprim, venlafaxine, verapamil	Oasis HLB (200 mg)	3	-	100 mL	[132]
17-β-estradiol, azithromycin, benzophenone-4, clarithromycin, diclofenac, erythromycin, triclosan, acetamiprid, alprazolam, atorvastatin, atrazine, azoxystrobin, bezafibrate, carbamazepine, chloramphenicol, ciprofloxacin, enalapril, estrone, lincomycin, lorazepam, roxithromycin, sulfamethazine, sulfamethiazole, sulfamethoxazole, trimethoprim, valsartan, venlafaxine	Oasis HLB (60 mg)	-	-	100 mL	[133]
Sulfapyridine, sulfamethoxazole, metoprolol, sulfamethazine, ciprofloxacin, risperidone, erythromycin	Oasis HLB (200 mg)	3	-	100 mL	[134]
Diclofenac, mefenamic acid, sulfamethoxazole, trimethoprim, enalapril, salbutamol, betaxolol, nadolol, propranolol,	Oasis HLB (60 mg)	-	-	500 mL surface water 200 mL effluent	[114]

Matching compounds	SPE Sorbent	pH	Pre-treatment	Volume of sample loaded	Reference
timolol, hydrochlorothiazide, bezafibrate, clofibric acid, carbamazepine, fluoxentine				100 mL influent	
Diclofenac	Oasis HLB (200 mg)	-	-	500 mL	[135]
Roxithromycin, erythromycin, azithromycin, josamicyn, clarithromycin	Oasis HLB (30 mg)	2 (readjusted to 6 prior to analysis)	Internal standard	250 mL	[116]
Erythromycin, clarithromycin, roxithromycin, triclosan, diclofenac, mefenamic acid, clofibric acid, carbamazepine, sulfamethoxazole, sulfamethazine, sulfadimethoxine, sulfamonomethoxine, sulfapyridine, sulfisoxazole, sulfathiazole, ciprofloxacin, trimethoprim, lincomycin, chloramphenicol, ketoconazole	Several methods for the different classes HR-X (500 mg) (Ass, ICMs and caffeine) Oasis HLB (500 mg) (antibiotics, biocides, corrosion inhibitors, acidic and neutral pharmaceuticals)	-	Na_2EDTA	1 L	[136]

1.3.3 Analysis

Due to the wide scale of CEC compounds, hyphenated techniques, such as liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS), are required in order to determine the low concentrations in complex matrices. Moreover, the nature of water is quite complex as it interacts with all the components of the natural environment during its hydrological cycle and it is also influenced by human activities. Therefore, it has a high variability on chemical composition presenting minerals and organic compounds as an example.[137] The components of the water can interact with the target compounds such as their possible sorption to the organic matter already present in the sample resulting in a decrease of concentration of the free dissolved compound and therefore increasing the analytical challenges. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is ideal for the analysis of environmental samples, used frequently as detection due to higher sensitivity achieved (ng/L) as well as specificity when using complex matrices. Gas chromatography with tandem mass spectrometry (GC-MS/MS) can also be used but only volatile and thermally stable analytes can be determined by this technique and most times a derivatisation needs to be performed ending in time consuming methods with costly extractions and increasing variability and error of the method. An example is benzophenone-4, which contains a sulfonic group attached to the aromatic ring in addition to the phenolic group limiting the GC analysis.[65,138]

In a single analytical instrument, multi-class detection of CECs from environmental samples can be analysed at the same time. For this reason, reverse-phase (RV) LC is the most common approach, where mobile phases involve an aqueous (water) and the organic (generally methanol or acetonitrile) phase. Addition of additives are usually performed on the aqueous phase in order to help the ionisation of compounds,

examples are formic acid (proton donors) and ammonium additives (proton acceptors).[139] Conventional RV high performance liquid chromatography (HPLC) can separate complex mixtures with a range of properties, normally they contain silica particles with a size ranging from 3 to 5 µm. However, when reducing the size of the particles (<2 µm), column efficacy and resolution increases.[138,140] Nevertheless, this reduction leads to increments on pressure in order to move the mobile phases through the stationary phase.[141,142] In 2004, the company Waters developed the first system which allowed to work at those high pressures. The commercial company registered the system as ultra-pressure liquid chromatography (UPLC®).[143] Nowadays, not to refer to this company, the term ultra-high pressure liquid chromatography (UHPLC) has been established, which combines hybrid materials with particles size lower than 2 µm with the capacity of the system to move mobile phases at high pressures. Narrower chromatographic peaks are therefore obtained in faster analysis times. Most recent studies for the detection of CECs of several compounds with different chemical properties use this analytical technique (UHPLC) with C_{18} columns for their detection;[4,11,24,108,138,144] using simple sample preparation techniques such as SPE or no pre-treatment (e.g. direct injection) leading to high sensitivity detection in order to quantify these trace pollutants. Column selection is one of the most important steps when developing an LC method and it is chemistry-compound dependant. There is a wide range of commercial columns available, including UHPLC columns (<2 µm porous particles), solid core particle columns (<3 µm particles with a solid inner core and a porous outer core), monolith columns and hydrophilic interaction liquid chromatography (HILIC). Solid core particles have been proved to obtain higher resolution and sensitivity for multi-class detection methods improving the time of analysis. The combination of the solid core with a porous outer layer results in an increase of surface area favouring the chromatographic separation and

lower pressures in UHPLC. On the other hand, HILIC columns are used for the analysis of polar contaminants as they improved their retention time (poorly retained when using RV chromatography techniques), this could help analytes with low K_{ow}. Due to the complexity of the environmental matrix samples, guard columns are typically used in these analysis, where normally come from the same stationary phase, in order to prevent the decrease of the column life. Matrix effects are an issue when using LC as it decreases ionisation efficiencies, to overcome this problem, internal standards are used but it is an expensive approach, especially for multi-compound analysis.

For detection, most methods are developed initially using detectors such as UV, DAD and fluorescence as it has less costs, but because of the need of high sensitivity in real samples and not all compounds are UV detected (do not possess a chromophore group), mass spectrometry is the best detector available achieving detection limits of ng/L. Analytical methods for this type of compounds are widely based around SPE LC-MS/MS techniques. In terms of ionisation sources, for environmental matrices, electrospray ionisation (ESI) is the most common technique compared to atmospheric pressure chemical ionisation (APCI). ESI is a soft ionisation technique which converts ions into the gas-phase for analysis whilst retaining intact molecular structure. However, environmental samples are known by the matrix effects, these alter the ionisation efficiency because of the co-elution of substances present on the extract, leading to signal enhancement or suppression for the analyte signal, loosing repeatability and increasing detection limits of the method therefore affecting quantification.[139] Analytical techniques need improving in order to reduce matrix effects in these complex samples improving the quality of the method. Usually, investigation of injection volume, SPE concentration factor, dilution of samples, 'matrix-matched' calibration standards, internal standard addition, etc. help reducing matrix effects. However, not only matrix effects should be

considered when developing a method, simple things as developing the sampling can have a large impact. Sampling is also one of the most important parts of the process, as it can change the stability of the compounds altering the final concentrations of the samples, making results unreliable.[145]

Mass analysers are the most important component of the MS as it is where the separations of ions occur. There are different types that can be used for the analysis of aquatic environmental samples offering different resolutions and mass accuracies depending on the requirements of the analysis which will directly impact the type and quality of data.[146] The most common types are triple quadrupole (QqQ), quadrupole ion trap (QIT), and Orbitrap among others. Moreover, hybrid instruments, a combination of two mass analysers like triple quadrupole linear ion trap (QqQLIT), quadrupole time of flight (QTOF) and linear ion trap orbitrap (LTQ-Orbitrap), can also be possible in order to obtain a higher performance. All of them have their unique properties such as resolution, analysis speed and sensitivity, and their selection will be based on the application required. Depending on the mass analyser used, analysis can be carried out using target or non-target approaches, where target is the most frequent one. Traditional target methods are developed based on a list of analytes selected which is limited by the maximum number of transitions that can be monitored. Low resolution instruments such as quadrupole and ion traps are generally used where QqQ is the most common one due to its high sensitivity and selectivity when using selected reaction monitoring (SRM). On the other hand, non-target approaches analyse the sample using full scan, where all components between a certain m/z scan ratio will be measured by the MS. Thanks to the full-scan product-ion spectrum and high resolution exact mass measurement of precursor and product ions, compounds can be identified in these complex matrices. This technique allows to produce more data from unlimited unknown compounds, qualitative and

quantitative data can be obtained from accurate masses and the extraction from total ion chromatograms (TIC) using specific softwares. Therefore, certain criteria needs to be applied for the confirmation of the "unknown" compounds in order to avoid their unequivocal identification and the use of reference standards will aid for their confimation. Furthermore, to avoid reporting false positives the European Union Commission Decision 2002/657/EC requires the detection of at least four identification points for LC-MS/MS analysis such as one precursor ion and two daughter ions or two precursor ions each with one daughter ion.[89] For accurate mass screening (non-target) high resolution is needed and time of flight (TOF) and Orbitrap mass analysers are used, achieving high sensitivity and high mass accuracy (<5 ppm), with the possibility of detection of unlimited number of compounds, however, due to its elevated costs these are not common in literature.[139] Nevertheless, as previously mentioned, the most used instrument for these type of samples and contaminats would be triple quadrupoles (QqQ) due to their selectivity via multiple reaction monitoring (MRM) and sensitivity reaching ng/L levels of detection and quantification, allowing the monitoring of certain compounds with reliable detection and quantification.

1.4 Conclusions

Contaminants of emerging concern in environmental aquatic matrices have been detected worldwide increasing public concern due to their unknown effects and possible toxicity. Regulations around Europe provide monitoring data for certain substances, however, this only includes a narrow number of compounds which do not cover a great extent of the unlimited emerging compounds that contain a possibility of ending up in the environment. Additionally, only limited data is available for certain countries such as Ireland and therefore required. The objective of this study was to develop and optimise analytical techniques in order to risk assess CECs in Ireland, including the three main types of contaminants: pharmaceuticals, PCPs and pesticides. The research carried out in this thesis provides comprehensive insight into the occurrence, fate and impact of over >100 compounds in Irish waters. This will enable to support wastewater treatment plants to develop and optimise strategies for the efficient removal of identified CECs and to minimise the potential risk posed by them.

1.5 Aims and objectives

The aim of this work is to investigate the occurrence and frequency of CECs in Ireland and perform a comparison to different countries.

The main objectives are to:

- Develop and validate a SPE LC-MS/MS method for selected pharmaceuticals and personal care products of concern in Ireland.
- Optimise and validate a previously developed direct injection LC-MS/MS method for >100 CECs.
- Apply both methods to Irish influent and effluent wastewater and surface water samples, risk assess these analytes and make a prioritisation list of contaminants of emerging concern.
- Carry out an international comparison between Spain, UK and Ireland.

2.0 Analytical techniques for CECs identification from water samples

Abstract

Analysis techniques for contaminants of emerging concern (CECs) are broad and compound dependent. Due to the limited data available for this type of compounds in Ireland, a selection of CECs was performed from a literature review in order to develop a method to detect and quantify them. This selection included anti-inflammatories, antioxidants, antibiotics, hormones, etc. which are quite changeling due to their presence in the environment at low concentrations. Therefore, these compounds were extracted and analysed using solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The analytical method was validated for three types of water matrices, surface waters, influent and effluent wastewater. Limits of detection (LODs) and quantification (LOQs) ≤2 and ≤7 ng/L for surface waters, ≤5 and ≤16 ng/L for influent, and ≤2 and ≤6 ng/L for effluent, respectively, were achieved. Linearities were good overall showing coefficients of determination of $R^2 \geq 0.90$, however, some estrogens hormones did not meet this criterion in certain matrices tested; E2 and EE2 for surface waters, E2 and E1 for influent, and EE2 for effluent. Consequently, the method was considered qualitative for them. In order to increase the number of compounds to be determined, a direct injection (DI) method coupled to LC-MS/MS for detection, was optimised and validated for all water matrices. DI methods are known to have certain advantages over SPE methods, including the decrease on time of analysis due to no extraction or long pre-treatment needed. Hence, a total of 135 compounds were validated obtaining LOQs ≤50, ≤500 and ≤72 ng/L for surface waters, influent and effluent, respectively. Method linearity of $R^2 \geq 0.90$ were obtained overall, except for cyromazine in influent wastewater, which is only reported qualitatively. This work has demonstrated the combination of different analytical techniques in order to generate robust occurrence data for low level detection of CECs in different aquatic matrices.

Aims and Objectives

- To select a representative range of contaminants of emerging concern (CECs) for their future monitoring in water samples. These compounds included a non-steroidal anti-inflammatory (diclofenac), endocrine-disrupting chemicals (estrone, 17-β-estradiol and 17-α-ethinylestradiol), antibiotics (erythromycin, clarithromycin, azithromycin, amoxicillin and ciprofloxacin), an antibacterial (triclosan), UV-filters (octinoxate, octocrylene and benzophenone-4) and a preservative (BHT).
- To develop an analytical method using solid-phase extraction (SPE) with liquid chromatography-tandem mass spectrometry (LC-MS/MS) for quantification of selected CECs in different water matrices.
- To optimise a direct injection LC-MS/MS method for different classes of CECs selected for this study.
- To validate optimised methods in different water matrices including surface waters, influent and effluent wastewater.
- To design and carry out an experiment for compound stability to investigate their possible degradation during international sample transportation, between Ireland and UK, for their analysis.

2.1 Introduction

Contaminants of emerging concern (CECs) have entered the environment for decades but only now they have been investigated. More than 100 million chemical substances are registered in the Chemical Abstracts Service (CAS) where 30,000 – 50,000 are found in daily-use products, suspected to end up in the environment.[147] Waste water treatment plants (WWTPs) are major points of release of CECs into the environment.[148] Once water arrives at the WWTP, some substances are not completely removed from the influent and will pass to effluents. Then, they are finally released into the aquatic environment where concentrations are expected to decrease upon dilution, and they are found at the pg/L-ng/L level. This is due to the different treatments performed along the cycle and the dilution they experience once entering the surface waters. Nevertheless, their distribution pathway and fate knowledge are still limited and also depends on the physicochemical properties of the compound. Thousands of chemical substances have been detected in the environment in the past few decades having evidence of occurrence of 160 different drugs in different water bodies such as effluent wastewater and surface waters.[149] Some CECs degrade quickly and not all of them are bioactive[149] (not all CECs are pharmaceuticals), however, even that single compounds may not pose a risk, they are usually found as complex mixtures and metabolites or transformations products (TPs), which could show new or concentration addition risks.[20] Due to their widespread use and their increased in consumption they can be quantified at even concentrations of µg/L,[150] therefore raising concern for the environment and human risk. Regardless of the expected dilution and treatments performed, some CECs have been detected in drinking water systems and in package seafood at concentrations that alarms the health safety for consumers. Their effects are not fully known yet and even at low concentrations, chronic or long-term

exposure[114] can lead to toxic effects being a threat to humans and the environment.[9] To investigate these potential threats, new emerging analytical techniques are being developed to detect and quantify these compounds at these low levels.[150] Consequently, scientific publications have increased in the past few years to approximately a 1000 per year in 2017[149] on this topic as observed in Figure 2.1.

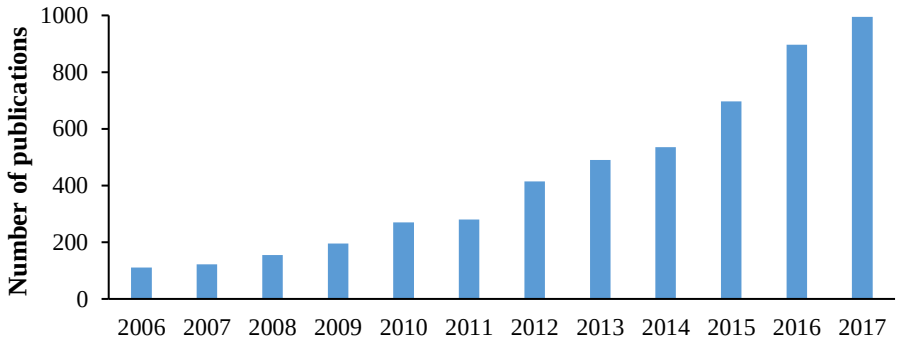


Figure 2.1 Number of CEC publications per year from 2006 to 2017, search in Web of Science for TOPIC: micropollut, OR "emerging pollut", OR "emerging contamina*" AND TITLE: "Review"); reproduced with permission from G. Oberg and A. Leopold 2019.*[149]

Regarding analysis, there is a wide range of analytical methods described throughout the literature. However, the choice of technique varies depending on the compounds selected and the goals of the study (i.e. sensitivity, multi-class determination, etc.). Target analysis for quantification of CECs by SPE LC-MS/MS is the most common technique along literature review.[139] In the majority of cases, an extraction method to pre-concentrate and clean samples is included, with SPE the most common one,[9] so target LODs can be achieved. The use of other hyphenated techniques such as GC require a derivatisation process[79] incrementing not only the cost but the time of analysis. For their separation, reverse-phase chromatography using HPLC and UPLC columns are typically used, where triple quadrupole (QQQ) analysers in ESI mode are the most common ways of analysis for these types of substances, reaching LODs in the range of ng/L.[139] As a high sensitivity is needed for these type of studies, usually just a limited amount of

compounds, or in this case multiple reaction monitoring (MRM) transitions, can be analysed at the same time. Also, a certain polarity at a time (either positive or negative mode) is established throughout the run in order to increase sensitivity of the method.[126]

Many detected CECs have been reported as acutely toxic to the aquatic organisms and also health risks have been associated with them.[59,92] Unfortunately, as previously stated, there is not enough knowledge to overcome this problem.[151] Regular monitoring to provide real-time information of contaminants would be ideal, however, common and current methods are usually costly and time-consuming making it almost an impossible task. When using target analysis, methods use an established and pre-selected number of compounds, depending on the sensitivity of the instrument. This number is further limited when using several transitions; including for the same analyte such as the quantitative and qualitative transitions. High resolution mass spectrometry (HRMS) has overcome this issue in some cases, where non-target approaches offer a larger range of compounds to be analysed from a range of m/z selected, however, these instruments are costly. Limitations are also observed from the extraction process. An example is SPE, which is compound specific, the chemistry of the sorbent will determine the compounds that will be analysed, resulting in the compromise of mixtures of compounds such as CECs. It also needs a large amount of sample, as examples seen in Table 1.6, where it can take up to a litre,[136] resulting in a laborious multi-step method leading to reproducibility errors, enormous overall preparation time and expensive analysis. In order to overcome these, different methods can be combined on the same sample in order to maximise the number of compounds. For example, one study used six methods in order to get data for a total of 174 compounds from drinking water.[99] To account for these problems, recently, new more sensitive instruments have led to the increase of MRMs per run simultaneously, leading to multi-class detection of even >100 compounds[73] in a short amount of time,

usually refer as rapid or fast methods. Investigation of alternatives to extraction methods such as direct injection (DI) has also been accomplished, where the sample is analysed with minimal or no previous preparation steps.[152] The sample is usually just filtered, diluted or centrifuged before the injection.[73] This reduces the overall time of sample preparation and the risk of contamination while increasing reproducibility in the final results,[153] reducing the overall cost of analysis.[152] DI methods have been applied successfully to a wide variety of matrices including wastewater,[73,152–154] surface water[123,154,155] and groundwater.[115] There are two types of DI methods depending on the injection volume, large volume injection (LVI) (100 – 5000 µL) and small sample volume (10 – 20 µL); both methods present limitations. Injecting large volumes of samples will need the use of complex LC systems in order to handle such volumes. Matrix effects are also increased, however, addition of internal standard, the use of 'matrix-matched' calibration lines and/or sample dilution can help to overcome these issues. There are not many approaches for CEC analysis that combine a great number of compounds[153] without the need of these volumes (e.g. 100 µL).[154] On the other hand, small volumes could led to insufficient sensitivity decreasing the number of compounds on the method, usually less than 50.[73] Combining DI and rapid LC-MS methods has been studied resulting in a faster and more affordable process, giving a great amount of useful data in a short period of time.[144] However, in order to get sufficient sensitivity for all compounds, if both polarities are used at once the run time increases (e.g. 27 min).[123] If the study is performed for a large volume of compounds, runs are split into separate positive and negative modes, and high injection volumes are applied in order to reach sensitivities (50 and 100 µL were injected respectively as an example).[156] A direct rapid LC-MS/MS approach managed to reduce these long run times to just 5 min for 135 compounds including different classes and chemistries of CECs at the same time. This was achieved

due to fast polarity switching allowing 261 injections per day.[144] This method was applied using influent wastewater samples across different cities around the world proving its precision, accuracy, sensitivity and reproducibility among other parameters. In this chapter, based on the promising results achieved in that study, the same method was optimised and validated for different water matrices for its future application in Irish samples. Also, development of conventional SPE LC-MS/MS methods were carried out in order to expand the number of contaminants measured for this study after a selection of target compounds based on literature review. Method performance was investigated for all methods in three matrices including influent and effluent wastewater and surface waters.

2.2 Selection of target CECs for method development

The chemical structures of the CECs chosen for future monitoring in different water matrices can be found in Table A.3 (Appendix B). Different categories of pharmaceuticals were considered for the selection of the target substances for the method, to encompass the likely classes of compounds of potential concern in Ireland. These included classes such as anti-inflammatories, endocrine-disrupting chemicals (EDCs), and antibiotics, whilst oxidants, UV-filters, and antibacterial agents were considered within personal care products (PCPs). The selection of the compounds was mainly based on the Water Framework Directive regulations and the Irish Environmental Protection Agency advice, and further compound specific information is given below.

2.2.1 Anti-inflammatories

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) used for the acute and chronic treatment of osteoarthritis and rheumatic arthritis, back pain, toothache, migraine, etc. Several common medicines such as Solaraze, Zorvolex, Voltaren and, Cambia, contain this compound. It is considered as a high priority pollutant (Class I) in the Global Water Research Coalition (GWRC) list[101] and it was part of the first Watch List (WL) of the EU Water Framework Directive. It has since been removed from the WL, but it is important to note that removal from WL does not automatically mean a compound is no longer of concern, and in the case of diclofenac it has been removed simply as sufficient data has been collected about it. Diclofenac is still considered a priority CEC and it has always been quantified with one of the highest concentrations;[1] up to one order of magnitude higher compared to other compounds in the same study.[157] This could be due to its low removal, limited and incomplete in conventional treatments in WWTPs,[158] though depending on the treatment performed removal rates can vary. In a steady-state

removal study performed over a two-year monitoring period, low values were presented, 49 – 59%, when compared to >87% for other compounds.[21] Nevertheless, other studies show that the compound is not removed at all, in fact, its concentration increases in treated wastewater resulting in up to -40% of removal rates.[159,160] This pattern has also been observed in Ireland, where three WWTPs in the greater Dublin area presented the compound in the effluent but not influent wastewater. Deconjugation of conjugated metabolites during the treatment process or concentrations below the LOD of the methods could explain the results. Concentrations of diclofenac were therefore reported in the environment, exceeding 100 ng/L and 1,000 ng/L for surface waters and effluents, respectively;[103] these levels of concentration were as high as other European cities suggesting its necessary monitoring.

Furthermore, environmental risks associated to the compound vary depending on the location of the samples taken as seen in Table 2.1, where risk quotient (RQ) values varied from 0 (negligible risk) to 18,740 (very high risk). This compound was ranked in the second position with the highest RQ obtained in European surface waters indicating its high chance of causing adverse effects in the aquatic environment.[161] Following short and long term exposures on mollusc and fish, diclofenac can affect biochemical processes and/or physiological functions at concentrations as low as 0.5 µg/L. The lowest observed effect concentration (LOEC) for fish is just 1 µg/L for alterations to kidney, liver, gills, etc.;[162] and median effective concentration (EC50) values ranged from 7.5-72 mg/L in algae and 0.07-68 mg/L in crustacean (Table 2.1). Due to the possible threat to the aquatic environment and the high concentrations seen across Ireland, diclofenac was selected in the study.

2.2.2 Steroid hormones

Endocrine-disrupting chemicals (EDCs) such as hormones generate large awareness due to their possible impact in humans or wildlife. Human population and livestock are the two main sources of release into the environment, discharging 30,000 and 83,000 kg/year respectively.[163] Even at low ng/L concentrations, they have been proven to be endocrine disruptors, posing an elevated risk for human health related to the decrease in fertility, birth defects, and breast[8] and testicular cancer.[103,112] They can also cause damage to living aquatic organisms and adverse effects at these trace concentrations (<1 μg/L)[164] such as hormonal imbalance, intersex, egg mortality and infertility.[165] Studies show that these hormones are not completely removed after treatment in the WWTP, and therefore released into the environment,[103,138] where they seem to be extremely persistent.[166] Therefore, they are the most investigated compounds in a compilation of studies between 1997 and 2007[38] all around the world.

Three hormones, including both natural and synthetic, have been selected for this study: estrone (E1), 17-β-estradiol (E2), and 17-α-ethinylestradiol (EE2). E1 can be used as a medication for menopausal hormone therapy,[167,168] and based on the physicochemical properties it possesses a mean removal efficiency of -25%.[169] This can be due to its resistance to biodegradation, where sometimes its concentration is higher in the effluent due to degradation of E2 and/or deconjugation of estrone sulfonide.[164] E2 is produced by both men and women, however there are higher volumes in women, particularly when pregnant.[23] It is indicated for different treatments regarding menopause and has high variable removal efficiencies between -1 and 98%.[169,170] On the other hand, EE2 is a synthetic hormone, a metabolite of mestranol. This compound is an active ingredient in many of the oral contraceptives and hormone replacement therapy drugs.[23] Its removals are also very variable ranging from -100 to 100% depending on the

treatment.[165] The three hormones have been listed in the in the most recent candidate list, CCL4, from the US EPA.[100] They are also included in the first WL, remaining into the second one due to the difficulty of achieving the predicted no effect concentration (PNEC) levels required.[95] The lowest PNEC value is for EE2, $3.5 \cdot 10^{-8}$ mg/L, and no observed effect concentrations (NOEC) in fish can be as low as 0.3 ng/L as seen in Table 2.1. This compound was used to expose two different species of fish at only 3 and 4 ng/L, and both species suffered sex gender reveal from male to female.[171] Concentrations of EE2 in effluents have been reported from non-detected up to 549 ng/L with an average concentration of 12.3 ng/L,[165] achieving the highest RQ (28,500) in a European ranking for surface waters.[161] Consequently, it is evident that these compounds will all have a high potential of adverse effects on fish as well as other wildlife.[165] Their presence has been confirmed in effluent wastewater and lake water in Ireland, unfortunately results were limited due to their low levels of quantification. However, estrogenic levels were investigated using yeast estrogen screen (YES) assays in eight WWTPs across the country where enough levels were observed in effluents (1.1-16 ng/L) and surface waters (0.9-2.9 ng/L). Even that the compounds cannot be identified, authors attributed the results of one of the WWTPs (Osberstown, Kildare) to mainly EE2 due to the collection of the sample coming from a contraceptive pill manufacture. The concentration was reported as high enough to have an impact on wild fish[103] and therefore the need of monitoring these compounds.

2.2.3 Antibiotics

Antibiotics are biologically active and can cause non-target toxicity to aquatic organisms. They are increasing concern due to their continuous exposure threatening human health through diet and environmental ecosystems. Even at low concentrations they can produce antibiotic resistant bacteria, which has been detected in sludge, ultimately used as a

fertilizer on agricultural fields,[172] and antibiotic resistant genes apart from genotoxic and histopathological changes in different aquatic organisms. Moreover, it has been reported that microplastics can increase the accumulation of these analytes in fish and algae.[173] Therefore, two groups of the most frequently found antibiotics in the environment have been selected in this study: macrolides and β-lactams.

Macrolide antibiotics are effective in treating a wide range of bacterial infections such as ear, nose and throat, chest, skin, mouth, and sexually transmitted infections.[27] Compared to other antibiotics, they have reported being more persistent in wastewater and have been detected and quantified across the world in ranges of ng/L. For this reason, erythromycin, clarithromycin, and azithromycin have been selected for this study. They have been previously reported in river waters at concentrations of 40-200[116], 5-360[175], and 40[116] ng/L, respectively; and azithromycin and clarithromycin have been detected in final effluents in Ireland with concentrations up to 267 and 204 ng/L, respectivey.[110] The three of them can be found in the first and second WL, as they were kept on the list to be monitored altogether.[95] Erythromycin also belongs to the most recent candidate list from US EPA, CCL4,[100] and to the Class I (high priority pharmaceuticals) in the GWRC list with clarithromycin in Class II (medium priority).[101] Regarding aquatic toxicity, clarithromycin and erythromycin showed more toxic sensitivity in algae, which plays a key role in the ecosystem such as primary production (food chain),[176] and EC_{50} values range from 0.002-0.03 mg/L and 0.02-33.8 mg/L respectively for them (Table 2.1). However, there was no data found for azithromycin for this trophic level but half lethal concentration (LC_{50}) values were reported as low as 0.33 mg/L in fish. Therefore, RQ values achieved can suggest very high risks and they have been reported at high levels, 42.5, 120 and 10.5 for erythromycin, clarithromycin, and azithromycin respectively; raising their concern.

β-lactam antibiotics are the most widely used and consumed antibiotics across the world, which is why they are the most found in aquatic environments.[177] For this reason, amoxicillin was selected for the study. Because of its structure, it is known as the main persistence contaminant in water,[178] where is being discharged in the same form due to its low metabolic rate in humans,[53,178] and quantified at concentrations such as 82.7 mg/L in effluent wastewater and 48 ng/L in surface waters.[177] Ciprofloxacin was also considered, it is used to treat microbial infections, and belongs to the fluoroquinolones group. It is widely prescribed and frequently found in sewage due to its incomplete uptake and metabolism in patients[179] at concentrations between 0.7 and 125 µg/L in hospital effluents.[177] Both antibiotics have been found at high levels in effluent waters around Europe,[53] and are in the latest surface waters Watch List updated in August 2020 and therefore selected. Very environmental high risks have been reported for both analytes in European surface waters, 136 and 17, for ciprofloxacin and amoxicillin respectively.[161] This could be due to their low PNEC values reported in the WL of just 0.078 and 0.089 µg/L for amoxicillin and ciprofloxacin respectively; mainly due to their sensitive toxicity to algae.[95]

2.2.4 Antioxidants

Antioxidants protect substances from deterioration caused by oxidation prolonging their life. Synthetic antioxidants have longer stability in the product, and one of the most used is butylated hydroxytoluene (BHT), an organic chemical, which inhibits autoxidation of unsaturated organic compounds. It is used in food, food additive (E321) permitted by the FDA and EU,[180] cosmetics, and industrial fluids to prevent oxidation.[181] However, some studies have linked this compound with cancer, though this is still uncertain[180] and more research is needed for confirmation. Some metabolites have resulted in DNA damage in mice and rats.[182] BHT has been removed from the second WL. Nevertheless, the literature

suggests monitoring it in WWTPs to understand their environmental occurrence and fate,[180] as it has been detected in the order of 10-2,000 ng/L depending on the sample nature (e.g. river, ground and wastewater);[183] when posing a PNEC value of 5.3 µg/L (Table 2.1). However, limited data has been reported in literature review and only one study was found to report an environmental risk assessment of the compound, where a low risk was attributed to it. Therefore, more research is needed and BHT was included in this study.

2.2.5 UV-filters

UV-filters are used in personal care products (PCPs) in order to minimize DNA photodamage, protecting from the effects of sunrays. Octinoxate, octocrylene and benzophenone-4 are common ingredients in sunscreens and other skincare products such as lip balm.[1] However, they can also be used as a UV stabilizer in the manufacture of unplasticized polyvinyl chloride (PVC) and polyethylene terephthalate (PET) polymers. Moreover, they are added in materials, textiles and paints reducing photodegradation of the products.[184–186] Due to their wide application they have large annual production quantities and they have been detected across different environmental samples including wastewater and surface waters.[184] Due to their high lipophilicity, stability, and photo-degradation resistance, they are considered as persistant contaminants and therefore a particular concern.[1,184,185] UV filters and their metabolites can interfere with endocrine function by acting as environmental estrogens, and this has been proven both *in vitro* and *in vivo*,[187] causing cellular and oxidative stress responses, reproduction impairments, neurotoxicity, etc.[188] Furthermore, compounds with high K_{ow} values, as UV-filters (Table A.2 from Appendix A), can partition rapidly from the aquatic to the hydrophobic phase such as fish tissue, resulting in bioaccumulation and leading to a faster mortality.[187] Analytes such as octinoxate have been found in fish, cormorants and marine mussels in

rivers at concentrations up to 3,400,[188] 701 and 256 ng/g lw (lipid weight)[184] respectively. Octocrylene was also quantified at 11,875 ng/g lw in fish[188] and at 7,112 ng/g dry weight (dw) in marine mussels.[184] Their accumulation transmitted into the food chain has become a concern for not only environmental but human health, and they have not only been found in mussels, fish, corals, shrimp, and squids but also human breast milk.[185,189] Effluent concentrations in Europe have been reported up to 6.3 mg/L,[190] 100 ng/L[191] and 300 ng/L[192] for benzophenone-4, octinoxate and octocrylene respectively. Considering their low PNEC values reported due to their possible toxicity of 5.4, 6 and 0.023 µg/L respectively (Table 2.1), all three compounds have been selected for this study.

2.2.6 Antibacterial agents

Triclosan is an antimicrobial frequently used in toothpaste, cosmetics, clothes, toys, and other products, with an estimation of 15,000 tons produced annually worldwide.[193] There are recent reports that indicate its health effects,[194] and it is also considered as an endocrine disruptor compound (observed on amphibians and mammals)[36]. Triclosan has removal rates of approximately 90% in WWTPs[195] but depending on the treatment performed they can vary from 0 and 98%,[196] however, due to its high sorption to biosolids, significant amounts of the compound persist and transfer to effluent and sludge.[195] Consequently, it has been found in wastewater and surface water samples worldwide.[197] However, even its biodegradability and photo-instability, it has a half-life of approximately 11 days in surface waters,[198] where it has been reported at concentrations as high as 6.75 µg/L.[199] Moreover, it is one of the top 10 most frequently detected compounds and also for its high levels of concentration. Its lipophilicity, log K_{ow} >4, suggests bioaccumulation raising toxicity concern and in comparison with other disinfectants, it appears to be highly toxic, independently of the organism tested (e.g. alae, fish, crustacean, etc.).[200] Bioaccumulation has been observed in snails, fish, algae,

etc. and it has been reported to be more sensitive to aquatic bacteria and biofilm algae, LC_{50} values as low as 0.53 µg/L for algae (Table 2.1), causing an increase in mortality,[63] where its by-products are even more hazardous. Furthermore, this analyte has shown antimicrobial strains of resistant bacteria and could result in major human health and aquatic ecosystems risks.[199] Its detection in aquatic biota and in human urine, breast milk, nails, blood, etc.[193,201] alongside its continuous release from effluent wastewaters, propose its high ecological and human risks and how reducing its concentrations in treated water should be considered a priority.[202]

Based on the information presented in Section 2.2 and previous results from different monitoring studies around Europe, these nine pharmaceuticals and five PCPs were chosen for their analysis in different water samples from Ireland, resulting in a final analysis list of 14 prioritised compounds: diclofenac, E1, E2, EE2, erythromycin, clarithromycin, azithromycin, amoxicillin, ciprofloxacin, BHT, octinoxate, benzophenone-4, octocrylene and triclosan.

Table 2.1 Toxicity data, PNEC and risk quotient (RQ) values in aquatic systems for compounds selected.

Compound	Taxon	Specie	Toxicological endpoint	Ecotoxicity data (µg/L)	Ref	PNEC (µg/L)	RQ
Amoxicillin	Fish	Juvenile goldfish (Tilapia nilotica)	LC50	35.72	[187]	0.078[a,b]	0.001-0.8,[20] 17,[161] 62.3[203]
	Microalgae	M. aeruginosa	EC50	3.7	[204]		
		S. capricornutum	NOEC	250,000	[204]		
		S. leopoliensis	EC50	2.22	[204]		
			NOEC	0.78	[204]		
	Bacteria	V. fischeri	EC50	3,597	[204]		
Azithromycin	Fish	Oreochromis niloticus (Tilapia)	LC50	>100,000	[205]		0-10.5,[206] 0.001-0.11,[20] 0-0.58[110]
		Discentrarchus labrax (sea bass)	LC50	31,000	[207]		
		Fathead minnow	LC50	330	[208]		
	Crustacean	Daphnia magna	LC50	120,000	[207]		
		Branchio poda	LC50	3,340	[208]		
		Daphnia magna	LC50	80,840	[208]		
Benzophenone-4	Microalgae	Pseudokirch neriella subcapitata	EC50	670	[209]	5.4[a]	<0.01,[185] 0.01–0.30,[190] 0.001-0.19,[184] 2.7[210]
		chlorella vulgaris	EC50	2,980	[209]		
		Desmodesmus subspicatus	EC50	960	[209]		
	Crustacean	Daphnia magna	EC50	1,900	[209]		
			LC50	1,100	[209]		
			LC50	50,000	[199]		
	Fish	Oryzias latipes	LC50	3,800	[209]		
		Brachydanio rerio	LC50	3,900	[209]		
Ciprofloxacin	Cyanobacterium	Anabaena flos-aquae	NOEC	5.65	[211]	0.089[a,b]	0.001-92,[206] 2.18,[212] 0-0.9,[110] 136[161]
			EC50	10.2	[211]		
	Microalgae	Desmodesmus subspicatus	NOEC	≥8.04	[211]		
			EC50	>8.04	[211]		

		Lemna minor	NOEC	10	[211]		
			EC50	62.5	[211]		
		Myriophyllum spicatum	NOEC	980	[211]		
Clarithromycin	Fish	Danio rerio	EC50	>2,000	[175]	0.12[a,b]	0-92,[206] 0.0005-0.044,[20] 0.06-1.04,[172] 0.25,[212] 0-0.06,[110] 120,[161] 0.3[203]
		Oryzias latipes	LC50	>100,000	[204]		
	Crustacean	Daphnia magna	EC50	>2,000	[175]		
			NOEC	2,100	[175]		
		T. platyurus	LC50	94,230	[204]		
		C. Dubia	EC50	8,160	[204]		
	Microalgae	Lemna minor	NOEC	800 (dry weight)	[175]		
		Desmodesmus subspicatus	EC50	32.1	[175]		
		Pseudokirch neriella subcapitata	EC50	2	[204]		
			NOEC	3.1	[204]		
	Cyanobacterium	Anabaena flos-aquae	EC50	5.6	[175]		
	Rotifer	B. calyciflorus	LC50	35,460	[204]		
			EC50	12,210	[204]		
Diclofenac	Crustacean	Daphnia simils	EC50	46,000	[166]	0.05[a,b,c,d]	0,[213] ≤0.1,[128] 0.0063-0.7,[20] 5.58-39.5,[214] 0.26,[212] 28-44,[114] 18740,[161] 0.25,[203] ≤6.8[95]
		Daphnia magna	EC50	67	[166]		
			NOEC	10	[166]		
			EC50	68,000	[204]		
		C. dubia	EC50	22,704	[204]		
			NOEC	1,000	[204]		
	Fish	Danio rerio	NOEC	1,131	[166]		
			NOEC	4,000	[204]		
		Oncorhynchus mykiss	LOEC	1	[204]		
		Salmo trout f. fario	NOEC	0.5	[204]		
	Microalgae	Desmodesmus subspicatus	EC50	72,000	[204]		
		Lemna minor	EC50	7,500	[204]		
		Dunaliella tertiolecta	EC50	18,5690	[204]		

		Pseudokirch neriella subcapitata	NOEC	10,000	[204]		
	Bacteria	V. fischeri	EC50	11,454	[204]		
E1	Microalgae	Green algae	EC50	355	[171]	0.0036[a]	0.43-5.52,[214] 4.45,[161] 0.79-8.9[215]
	Invertebrates	Dugesia japónic	LC50	>50,000	[171]		
		Daphnid	LC50	3,160	[171]		
	Fish	Oryzias latipes	NOEC	0.2	[171]		
		Fathead minnow	LOEC	0.03	[171]		
		Cyprinodon variegatus	NOEC	0.04	[171]		
		Gabiocypris rarus	NOEC	0.05	[171]		
E2	Microalgae	Green algae	EC50	162	[171]	0.0001[a]	0.033-9.8,[214] 1.39,[212] 75,[161] 1.1-74.3[203]
	Invertebrates	Dugesia japónic	LC50	>5,000	[171]		
	Fish	Oryzias latipes	NOEC	0.03	[204]		
		Danio rerio	NOEC	0.025	[171]		
		Melanotaenia fluviatilis	NOEC	0.1	[171]		
		Oryziaz javanicus	NOEC	0.016	[171]		
		Poecilia reticulate	NOEC	0.1	[171]		
		Pomatoschistus minutus	NOEC	0.016	[171]		
	Crustacean	Daphnia magna	LC50	2,870	[171]		
EE2	Crustacean	Daphnia similis	EC50	1,630	[166]	0.000035[a,b]	0.41,[214] 0.32,[212] 28500,[161] 3.9-3662[203]
		Daphnia magna	NOEC	500	[166]		
			LOEC	1	[166]		
		Ceriodaphnia reticulata	EC50	1,814	[171]		
		Sida crystallina	EC50	>4,100	[171]		
		Hyalella azteca	NOEC	0.1	[171]		
	Microalgae	Pseudokirchneriel la subcapitata	EC50	800	[171]		
		Desmodesmus subspicatus	EC50	730	[171]		
	Fish	Fundulus heteroclitus	LC50	0.05-0.25	[166]		
		Pimephales promelas	LOEC	0.001	[204]		
		Danio rerio	NOEC	0.0003	[171]		

		Gobiocypris rarus	NOEC	0.001	[171]		
		Oncorhynchus Mykiss	NOEC	0.008	[171]		
Erythromycin	Fish	Juvenile goldfish (Tilapia nilotica)	LC50	242.7	[187]	0.2[a,b]	0-1.02,[206] 0.0001-0.011,[20] 0.02-0.27,[172] 0.38,[212] 42.5[161]
		Oryzias latipes	LC50	>100,000	[204]		
	Crustacean	Daphnia magna	LC50	135,500	[208]		
		Branchio poda	LC50	8600	[208]		
		T. platyurus	LC50	>100,000	[204]		
		C. Dubia	EC50	220	[204]		
	Microalgae	Lemna minor	EC50	5,620	[204]		
		Pseudokirch neriella subcapitata	EC50	20	[204]		
		S. capricornutum	EC50	36.6	[204]		
		C. vulgaris	EC50	33,800	[204]		
	Rotifer	B. calyciflorus	LC50	27,530	[204]		
			EC50	940	[204]		
Triclosan	Invertebrates	Palaemonetes pugio (adult, larvae, embryo)	LC50	305, 154, 651	[216]	0.02[a]	0.032-19.2,[199] 11,[161] >1,[202] 1-5813[217]
		Americamysis bahia	LC50	74.3	[216]		
		Ampelisca abdita	LC50	73.4	[216]		
		Chironomus plumosus	LC50	2890	[218]		
		Baetis sp.	LC50	72	[218]		
		Branchinella thailandensis	LC50	100	[218]		
		Tubifex tubifex	LC50	259	[218]		
		Leptocerus sp.	LC50	760	[218]		
		Macrobrachium lanchesteri	LC50	962	[218]		
		Ceriodaphnia dubia	LC50	115	[218]		
		Plationus patulus	LC50	320	[218]		
		Thamnocephalus platyurus	LC50	470	[218]		
		Neocaridina denticulara sinensis	LC50	772	[218]		
		Limnodrilus hoffmeisteri	LC50	2046	[218]		

	Fish	Pimephales promelas	LC50	260-360	[199]		
		Lepomis macrochirus	LC50	370-440	[199]		
		Oryzias latipes (larvae, embryos)	LC50	602, 399	[199]		
	Microalgae	Chlorella ellipsoidea	LC50	4.3-28.9	[218]		
		Pseudokirch-neriella subcapitata	LC50	0.53	[199]		
	Crustacean	Daphnia magna	LC50	363	[218]		
			LC50	390	[199]		
	Amphibian	Xenopus laevis	LC50	259	[199]		
		Acris blanchardii	LC50	367	[199]		
		Bufo woodhousii		152	[199]		
		Rana sphenocephala	LC50	562	[199]		
BHT	Microalgae	Scenedesmus subspicatus	NOEC	400	[219]	5.3[220]	0.15[220]
		Pseudokirchneriella subcapitata	EC50	>240	[220]		
	Crustacean	Daphnia magna	EC0	≥170	[219]		
			EC50	480	[220]		
	Fish	Brachydanio rerio	LC0	≥570	[219]		
		Oryzias latipes	LC50	1,100	[220]		
Octocrylene	Crustacean	Daphnia magna	EC50	3,180	[221]	0.5[189], 0.023[a]	0.04,[189] 0.27,[222] >1[223]
	Invertebrates	Artemia salina	LC50	600	[224]		
		Mytilus galloprovincialis	EC50	>650	[222]		
		Paracentrotus lividus	EC50	737	[222]		
	Microalgae	Isochrysis galbana	EC50	>150	[222]		
Octinoxate	Crustacean	Daphnia magna	LC50	290	[199]	6[a]	1.14-1.76,[225] 3.29,[226] 0.4-18.9[227]
			EC50	2,730	[221]		

[a]NORMAN Ecotoxicology Database.
[b]Watch List.
[c] Mendoza et al., 2015.[228]
[d]Rivera-Jaimes et al., 2018.[114]
Risk quotient classification: RQ < 0.01: "Unlikely to pose risk"; 0.01 < RQ < 0.1: "Low risk"; 0.1 < RQ < 1: "Medium risk" and RQ > 1: "High risk".

2.3 Experimental

2.3.1 Reagents and chemicals

LC-MS grade methanol (Dorset, UK) and acetonitrile (Rehovot, Israel), and analytical grade acetonitrile, methanol, dicholomethane, dichlorodimethysilane and ammonium hydroxide (Steinheim, Germany) were purchased from Sigma Aldrich. Hydrochloric acid (37% v/v) and formic acid were obtained from Fisher Scientific (Loughborough, UK). Solids of ammonium acetate and ammonium fluoride were purchased from Merck (Darmstadt, Germany) and Sigma Aldrich (Steinheim, Germany) respectively. Ultrapure water was supplied from a Millipore Milli-Q water purification system at 18.3 MΩ (Millipore, Bedford, MA, USA).

All reference standards materials and isotope labelled internal standards (SIL-IS), with ≥98% purity, are listed in Appendix C. Stocks were prepared at a concentration of 1 mg/L or 0.1 mg/mL in methanol or acetonitrile, and formic acid was added to ciprofloxacin stocks in order to dissolve the compound. They were stored at -20°C in silanised amber vials in the dark for stability purposes. Multi-compound working solutions were prepared weekly in methanol, or in the reconstitute solvent of acetonitrile: water (10:90, v/v) where appropriate, by dilution of the stocks and also stored at the same conditions.

2.3.2 Glassware preparation and silanisation

Glassware silanisation was performed on all glassware used during experimental procedures in order to avoid binding of the analytes to the surface of the glass. Glassware was washed in triplicate with the following solutions and solvents in the following order respectively: methanol: water (50:50, v/v), dichloromethane, dimethyldichlorosilane:

dichloromethane (10:90, v/v), dichloromethane, methanol: water (50:50, v/v) and finalised with ultrapure water.

2.3.3 Sample collection and pre-treatment

Grab samples of one litre of influent, effluent and surface waters were collected in duplicate using amber Nalgene bottles, previously washed in triplicate with methanol and water, in two different locations (a rural and an urban area); samples were transported chilled on ice. Grab samples were used due to composite samples not being available at the time of the study and therefore diurnal variation has not being considered. Upon arrival in the laboratory one set of samples was not pre-treated and did not follow any pH treatment. However, the other set was acidified to pH 2, using hydrochloric acid (HCl) (37% v/v), where under these acidic condtions weak acids could adsorb onto solids underestimating the concentrations of the compounds. Therefore, all samples were stored in the freezer at -20°C until further treatment (Dublin, Ireland) in order to minimise possible chemical reactions that could alter the compounds and reduce biological activity.

In order to make a representative matrix, two sets of three pooled composite samples were prepared, leading to three types of pooled water samples: surface waters, influent and effluent wastewater; one pH treated and one without pre-treatment. Fixed volumes of every sample collected throughout the year in both areas were mixed into a silanised amber Winchester glass (2.5 L) or Nalgene bottles. Composite samples were prepared for method performance and calibration lines purposes.

2.3.4 Method 1: SPE LC-MS/MS

2.3.4.1 Sample preparation

Before extraction, samples were first defrosted and filtered under vacuum using nalgene sterile disposable filters with 0.45 µm nylon membranes (Thermo Fisher Scientific, UK).

Samples were spiked with standards were appropriate after filtering, including stable isotope labelled internal standards (IS-SIL) where required, prior to extraction. Matrix-matched calibration lines were also prepared following the same approach using the pooled samples, ranging from 0 to 1,000 ng/L (n≤5) and an internal standard at a constant concentration of 100 ng/L and 500 ng/L for hormones and rest of analytes, respectively.

2.3.4.2 Extraction and clean-up

Solid phase extraction was carried out using a vacuum manifold (Phenomenex, Cheshire, UK) and Oasis HLB (200 mg, 6 mL barrel, 30 µm, Hertfordshire, UK) cartridges. They were conditioned with 4 mL of methanol and 4 mL of ultrapure water, then 100 mL of sample was loaded at a flow rate of 1 mL/min in order to avoid causing break-through of the analytes. Next, a washing step was performed using 4 mL of methanol: water (5:95, v/v) followed by drying the cartridge under vacuum for 20 min to remove excess water. Cartridges were either stored at -20°C in the freezer until elution or eluted straight after the drying step. Elution was completed with 4 mL of 5 mM ammonium acetate in acetonitrile: methanol (25:75, v/v) into a pre-silanised container. Later, they were evaporated under nitrogen gas flow at room temperature and reconstituted to a final 1 mL of acetonitrile: water (10:90, v/v). Extracts were vortex mixed for 30 seconds, sonicated for 10 min, and lastly followed by vortex mix again. They were further filtered using syringe filters of 0.20 µm nylon membranes (VWR, Dublin, Ireland) before their transfer the into capped amber silanised 1.5 mL LC-MS vials (Agilent Technologies, Cork, Ireland) and storing them at -20°C until analysis.

2.3.4.3 Instrumental conditions

2.3.4.3.1 Method 1.1: LC-UV

LC-UV separations were performed using a Thermo Accucore C_{18} column (150 x 2.1 mm, 2.6 µm) with an Accucore C_{18} guard column (10 x 2.1 mm, 2.6 µm) (Thermo Fisher Scientific, Hertfordshire, UK) at room temperature. Mobile phases of 10 mM ammonium Acetate in water (A) and acetonitrile (B) were used with a flow rate of 0.3 mL/min in a gradient separation. At starting conditions, B was set at 10%; 0-6 min: B increased to 50%; 6-8 min: another linear ramp of B increased to 90%; 8-10 min: B further increased to 100%; 10-13 min: B stayed at 100%; from 13-18 min: linear ramp decrease to 10%of B. A re-equilibration time of 7 min gave a total time of 25 min. Injection volumes were set at 10 µL using a Shimadzu LC-20AD XR system coupled to a SPD-20A UV-Vis detector diode array detector set at 254 nm (Shimadzu, Kyoto, Japan) for detection.

2.3.4.3.2 Method 1.2: LC-MS/MS

LC-MS/MS was carried out using a 1290 Infinity II LC system, consisting of a binary solvent manager, an Agilent Infinity II 1290, a 1290 high-speed pump, and a 1290 multicolumn thermostat compartment. An InfinityLab Poroshell 120 EC-C18 (2.1 x 150 mm, 1.9 µm) LC column and an UHPLC InfinityLab Poroshell 120 EC-C18 guard column (2.1 mm, 1.9 um) were used at 30°C. All was purchased from Agilent Technologies (Cork, Ireland). Two different methods were established: one for the hormone compounds (E1, E2, EE2, β-estradiol-d_2, and estrone-d_4) and one for the rest of the analytes selected in the study (diclofenac, diclofenac-d_4, erythromycin, clarithromycin, azithromycin, azithromycin-d_3, amoxicillin, ciprofloxacin, triclosan, triclosan-d_3, octinoxate, octocrylene, and benzophenone-4); using injection volumes of 100 µL and 20 µL respectively.

For the hormones method (Method 1.2.1) 1 mM ammonium fluoride in water (A) and acetonitrile (B) were used as mobile phases with a flow rate of 0.35 mL/min and a gradient as follows: 0-2.2 min: B was set at 70%; 2.2-2.7 min: linear ramp of B from 70-100%; 2.7-3.7 min: B stayed constant at 100%; 3.7-4 min: it decreased to 70% of B; from 4-5 min stayed at 70% of B. A final re-equilibration time of 0.5 min was provided, having a total time of analysis of 5.50 min.

Mobile phases of 0.1% formic acid in water (A) and acetonitrile (B) were used for the second method developed, including the rest of the analytes (Method 1.2.2). A flow rate of 0.4 mL/min and a gradient were set as follows: 0-4 min: B increased from 10% to 50%; 4-5.5 min: B increased further to 90%; 5.5-6 min: B remained constant at 90%; 6-6.5 min: B raised to 100% until 10 min; finally, 10-10.5 min decreased to 10% of B. A re-equilibration time of 1 min was added at the end for a total time of 11.5 min.

The MS detector employed was an Agilent 6470A Triple Quadrupole mass spectrometer with the Agilent Jet Stream ion source (Agilent Technologies, Cork, Ireland). Nitrogen was applied as a nebulising and desolvation gas (high purity nitrogen generator through tap) while helium was the collision gas. MassHunter Data Acquisition software from Agilent Technologies was used to control the LC-MS/MS system. The analysis was carried out in scan type "dynamic MRM" (dMRM) mode for both LC methods, and parameters of the method can be observed in Table A.4 and A.5 from Appendix D. Using dMRM allows longer dwell times by selecting the elution time with a delta retention time window, so it does not scan continuously throughout the chromatogram increasing sensitivity. Two transitions were selected for ion confirmation and the most intense one was used for quantification. dMRM cycle times of 500 ms were used with dwell times set between 20 – 50 ms. Wide mode was set for both Q1 and Q3 resolution to acquire the data. Optimum conditions were achieved by direct infusion of

each analyte in methanol onto the source. A cell accelerator voltage of 4 V was set for both methods, and the delta of electron multiplier voltage (EMV) was set at 200 V for the negative mode in the hormones method and 200 V for the negative and positive for the rest of the compounds method.

2.3.5 Method 2: Direct injection LC-MS/MS

2.3.5.1 Sample preparation

Sample analysis took place at King's College London (London, UK). Samples of 15 mL in centrifuge tubes were shipped frozen in a cool polystyrene box filled with icepacks to prevent compound degradation and matrix alterations. Upon their arrival to the laboratory, approximately 30 hours later, they were kept in the freezer (-20°C) until further treatment and analysis.

For their preparation, PTFE membrane syringe filters of 4 mm, 0.2 µm (Whatman, UK) and 1 mL BD Plastipak™ syringes (Becton Dickinson S.A., Madrid, Spain) were used for filtering. LC silanised amber vials (Agilent, UK) were used to store and prepare the samples, where100 µL of a standard solution in methanol was added (including IS-SIL where needed) to a 900 µL fixed volume of sample in order to get a final volume of 1 mL using positive displacement pipettes; therefore the standards added in all samples related to just 10% of the final volume.[156] The same dilution procedure was applied across all samples, including method performance purposes and matrix-match calibration points, where the standards prepared in methanol were added to filtered pooled matrix samples. A matrix-matched calibration line was also prepared, using pooled filtered matrix as per Section 2.3.3, following the same approach, ranging from 0 to 5,000 ng/L (n=13) and an IS solution was added at a constant concentration of 500 ng/L where required.

2.3.5.2 Instrumental conditions

The analytical LC-MS/MS conditions were previously optimised by the Emerging Chemical Contaminants team at King's College London for 135 compounds.[144] Separations were achieved using a Shimadzu Nexera™ X2 ultra-high pressure LC (Shimadzu Corporation, Kyoto, Japan) for liquid chromatography. Direct analysis was performed so a Raptor™ biphenyl cartridge of 5.0 x 3.0 mm, 2.7 µm particle size (Thames Restek, Saunderton, UK) was utilised with an EXP® Direct Connect Holder as a column. Detection was performed with mass spectrometry using an LCMS- 8060 (Shimadzu Corporation, Kyoto, Japan) and electrospray ionisation (ESI), +/- polarity switching, as the source where the column was bypassed using a short piece of narrow bore polyether ether ketone (PEEK) tubing to the source. For this method 0.1% formic acid in water (A) and 0.1% formic acid in methanol: acetonitrile (50:50, v/v) (B) were used as mobile phases with a flow rate of 0.5 mL/min and a gradient as follows: until 0.20 min B was set at 10%; 0.20-3.0 min B was increased to 60%; B was increased to 100% at 3 mins, then held for 1 min to clean. A re-equilibration time of 2.5 min was performed giving a total time of 6.50 min. Acetonitrile was used as autosampler needle wash solvent and injection volumes of 10 µL was optimised for the method.

For collision-induced dissociation gas Pureshield argon was used (BOC Gases, Guildford, UK) and nitrogen and dry air were produced using Genius 1051 gas generator (Peak Scientific, Inchinnan, UK). ESI source conditions were as follows: gas flows were set up at 3 L/min for nebulising, 10 L/min for heating gas, and 10 L/min for drying gas. Temperatures were: 300ºC for interface, 250ºC for DL, and lastly 400ºC for the heat block. Dwell times were set at a maximum of 20 ms and minimum of 1 ms with maximum events of 49 and maximum loop time of 0.572 s; giving ≥20 datapoints per peak for reliable peak definition. Analysis was carried out in MRM scan type switching between

positive and negative ionisation polarity. The quadrupoles Q1 and Q3 were set to unit resolution; source parameters and transitions used during the experiment can be found in Table A.6 in Appendix D. Where possible two MRMs were selected, one for quantification and one as a qualifier. Only one transition was selected for internal standards. LabSolutionsTM (version 5.93, Shimadzu) was used to acquire chromatographic data and LabSolutions Insight (version 3.2, Shimadzu, Kyoto, Japan) to process it.

2.3.5.3 Filter recovery investigation

PTFE membrane syringe filters were used for sample preparation in order to remove unwanted particulates. A test was performed in order to assess any compound loss during this process, where a standard solution prepared at 1,000 ng/L in methanol was spiked before and after (n=3) the process of filtering. Pooled samples of every type of matrix (surface waters, effluent and influent wastewater) were used as well as DI water for their comparison. Percentages of recovery were calculated as the ratio of the peak areas measured before the filtering process and the areas of the samples spiked after following Equation 2.1. Standard deviations of obtained recoveries were also performed for n=3 replicates tested.

$$\text{Recovery } (\%) = \left(\frac{\text{Pre-spike matrix-matched standard}}{\text{Post-spiked matrix-matched standard}}\right) \text{x } 100$$

Equation 2.1 Recovery calculation for filtration process.

2.3.5.4 Transport storage stability

Stability experiments were carried out in order to see whether compounds were stable during transportation of samples from Dublin to London. Pooled matrix samples of each type were spiked with a medium concentration of 500 ng/L (including SIL-IS). Six aliquots were prepared and stored in the freezer (-20°C) for 24 hours to simulate real

samples. Once they were completely frozen, n=3 replicates were taken out and left at room temperature for 48 hours in the same polystyrene box used for transportation. This period of time simulates the worst case scenario regarding temperature stability (room temperature tested instead of the cool box temperature) and maximum time of transportation. Samples were analysed and a comparison was performed after these hours. The relative % instability data (Equation 2.2) was calculated as the ratio of the peak areas measured in the room temperature and frozen matrix samples respectively as follows:

$$\text{Instability (\%)} = 100 - \left(\frac{\text{RT matrix-matched standard}}{\text{Freezer matrix-matched standard}}\right) \times 100$$

where: RT standard: standard (matrix) left at room temperature for 48h

Freezer standard: standard (matrix) kept in freezer (-20°C)

Equation 2.2 Compound instability (%) calculation for transportation stability storage investigation.

Data was presented with standard deviation of the replicates (SD).

2.3.6 Chromatography

Several chromatographic parameters can be established to assess chromatography methods. In order to evaluate retention and profile of the peaks, tailing, asymmetry and retention factor were determined following the next equations (Equation 2.3 – Equation 2.8):

$$k = \frac{t_R - t_0}{t_0}$$

where: k: retention factor

t_R: retention time of the compound (min)

t_0: column void time (min)

Equation 2.3 Retention factor equation, which relates the equilibrium of distribution of analytes on the column.

$$T_f = 0.5 \cdot \frac{(t_b - t_a)}{(t_R - t_a)}$$

where: T_f: tailing factor

t_b: retention time at tailing edge (min)

t_a: retention time at front of the peak (min)

t_R: retention time of the compound (min)

Equation 2.4 Tailing factor studies the symmetry of the peak and it is measured at 5% of the total height; in order to be symmetric T_f needs to be equal to 1.

$$A_s = \frac{t_b}{t_a}$$

where: A_s: asymmetry factor

t_b: retention time at tailing edge (min)

t_a: retention time at front of the peak (min)

Equation 2.5 Asymmetry factor studies the symmetry of the peak and it is measured at 10% of peak height. If A_s>1 the peak tails while if A_s<1 the peak is fronting.

$$\alpha = \frac{t_{R2} - t_0}{t_{R1} - t_0}$$

where: α: selectivity

t_{R2} and t_{R1}: retention times for corresponded analytes

t_0: void time

Equation 2.6 Selectivity factor measures the ability of the method to distinguish between compounds. Optimum values varied from 2 – 5.

$$R_s = 1.18 \text{ x } \frac{t_{R2} - t_{R1}}{w_{0.5h1} - w_{0.5h2}}$$

where: R_s: resolution

t_{R2} and t_{R1}: retention times for corresponded analytes

w: width of peaks

Equation 2.7 Resolution optimum value is <1.5.

a) PV = Surface area (m^2/g) x PD / 40,000

where: PV: pore volume (0.39 for the column selected)

PD: Pore diameter (Å)

b) $v = PV \times \pi \times (\frac{\text{column ID}}{2})^2 \times L$

where: v: column void volume (µL) (0.20 for the column selected)
PV: pore volume
ID: Internal diameter
L: column's length

c) $t_0 = v/F$

where: t_0: column void time (min)
v: column void volume (mL)
F: flow rate (mL/min)

Equation 2.8 Different equations for the mathematical approach of the column void time using an Infinity Lab Poroshell 120 EC-C18 (2.1 x 150 mm, 1.9 µm) LC column; where a) calculates the pore volume in order to calculate the void volume (b). With the void volume, the column void time (c) can be generated to give the final value of retention factor.[229,230]

Column void time was also determined injecting uracil marker standards prepared in the reconstitution solvent at a concentration of 500 ng/L (n=6) for the developed methods in order to obtain experimental values.

2.3.7 Quantification of CECs

For quantification purposes, matrix-matched calibration standards were prepared per matrix type following the same dilutions previously detailed for every method. Peak area ratios were used to perform calculations for analytes that had a correspondent internal standard available. If that was not the case, only peak area was used for calculations, however, in order to assure the accuracy of the results matrix-match calibrants (>10 points) were used and sufficient blank samples were analysed. Moreover, different parameters were checked across the samples such as retention time drifts and quality of peak shapes on different MRM transitions (e.g. qualitative ion). Compounds where peak

area ratio were used has been clearly stated throughout the validation tables in the text of this thesis. When matrix-match was used, background subtraction was applied to analytes already present at the composite blanks for each matrix. In order to do this, three replicates of 0 ng/L were prepared and quantified. This allowed the calibration line to be set at zero for quantification purposes in subsequent samples.

2.3.8 Method performance

The analytical performance of the method was assessed for three different types of water matrices: surface waters and influent and effluent wastewater samples, according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines[231] for the target ions permitting confirmation of compounds. Peak areas and/or peak area ratios were used for quantification. Pooled composite samples were used to obtain the validation parameters of linearity, range, limits of detection and quantification (LOD and LOQ, respectively), precision, accuracy, recovery, and matrix effects, where required. As pooled matrices could have compounds existing, for analytes that were already present in the sample, a subtraction of the blank (n=3) was performed.

Linearity was determined using linear (Method 1.2) and logarithm linear regression (Method 2) analysis with a minimum of $n \geq 5$ matrix-match calibration points. It was assessed through coefficients of determinations for every compound (R^2). LODs and LOQs were determined from a matrix-match calibration line as 3.3 and 10 times, respectively, the standard deviation of the response by the slope as follows in Equation 2.9:

$$LOD = \frac{3.3\ \sigma}{S} \qquad LOQ = \frac{10\ \sigma}{S}$$

where: σ = the standard deviation of the response

S = the slope of the matrix-match calibration line

Equation 2.9 LOD and LOQ equations.

Accuracy was measured using n≥3 replicates of a matrix-matched standard at different concentrations depending on the method: 50 ng/L for method 1.2.1, 100 ng/L for method 1.2.2, and 100, 250, 750 and 1,000 ng/L for method 2; expressed as inaccuracy percentage of coefficient of variation (±%CV). Precision of the method was performed at different levels of concentration: method 1.2.1 at 50 ng/L, method 1.2.2 at 100 ng/L, and method 2 at 100 and 1,000 ng/L, for n≥3, and expressed as percentage relative standard deviation (%RSD). Matrix effect was also studied in order to see the ion suppression/enhancement of the analytes. Fortified matrix (n=3) was subtracted and compared to final fortified standards prepared at the same concentration using DI water for n=3 replicates. Recovery was determined for the conventional SPE method by comparison of matrix-match standards (n≥3 replicates) via fortification of the reconstituted extract at the end, at 100 ng/L for the mix of analytes and 50 ng/L for the hormones, to pre-fortified samples, following Equation 2.1.

2.3.9 Data analysis

All data analysis was completed using IBM® SPSS Statistics 27 software and Microsoft® Office Excel (WA, USA), for method development, performance and quantification purposes.

2.4 Results and Discussion

2.4.1 Method 1: SPE LC-MS/MS

2.4.1.1 Method 1.1: Development of an LC-UV method for initial SPE development

In order to develop an SPE method for the target compounds, an LC-UV method was established previously for their detection. LC-UV was chosen as a preliminary analysis due to its low cost and the lack of availability of LC-MS for initial part of the project. It is the most common universal instrument,[232] easily accessible in most laboratories and cheaper compare to other detectors. The method was developed for eight analytes including benzophenone-4, diclofenac, E2, EE2, triclosan, BHT, octocrylene and octinoxate. E1, amoxicillin and ciprofloxacin were added later on to the study. Given the challenges with detecting macrolide antibiotics (e.g. azithromycin and clarithromycin) with methods using UV detection,[233] due to a lack of a suitable chromophore and non-selective low-UV wavelengths,[232] it was decided not to include them for the initial SPE method development. Moreover, previous extensive studies of SPE extraction using different types of sorbents have obtained high recoveries,[116] including the cartridges selected after optimisation of the method.[116,234,235] Macrolides have medium polarity (log *P* between 3.12 – 3.29) and Oasis HLB sorbents have been applied successfully over a wide range of compounds with different physicochemical properties[236] (e.g. polarities from log *P* = -0.5, caffeine, to log *P* = 6.26, clotrimazole).[237,238]

Ammonium acetate was chosen as the mobile phase, based on previous studies[126] and after optimisation of multiple gradients and low flow rates the separation in Figure 2.2 was achieved based on parameters in Section 2.3.4.3.1 (method 1.1).

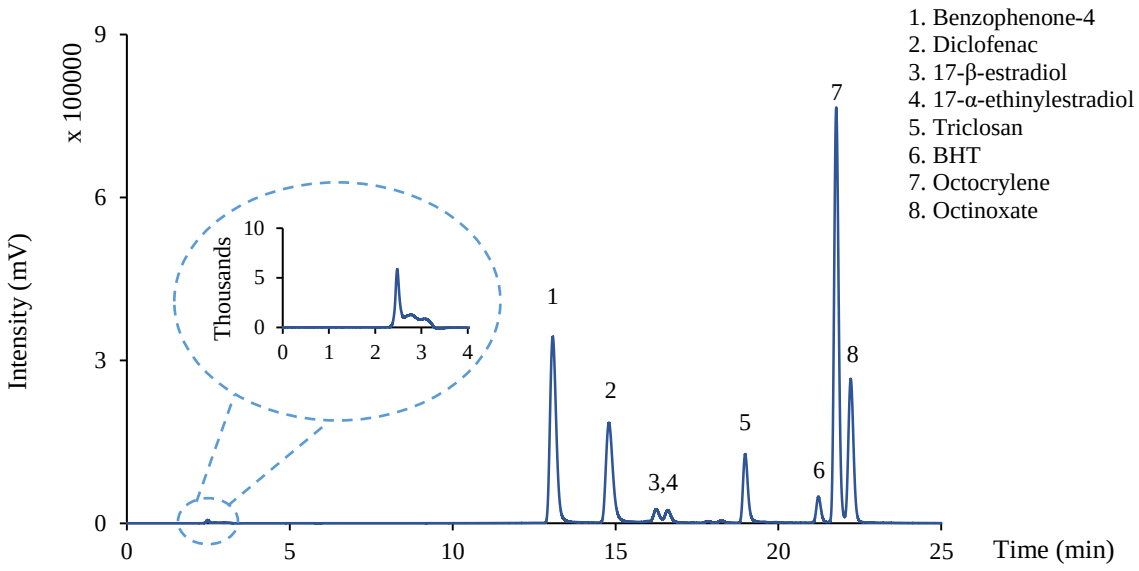


Figure 2.2 Chromatogram using LC-UV method at 254 nm including void time.

The minor disturbance method was used to determine the void time, t_0, where the mobile phase was injected on the column. The components of the phase reached the detector forming a peak on the chromatogram due to the disturbance of the mobile phase adsorbed on the stationary phase.[239] Therefore, as seen in Figure 2.2, t_0 had a value of 2.67 min approximately. Retention, tailing and asymmetry factor values can be observed on Table 2.2 for every compound of the method. Overall, retention values ranged from 3.85 – 7.29, meaning that the chromatographic run time was not too long. The study of peak symmetry was performed with tailing and asymmetry factors. Tailing factors varied from 1.04 – 1.35, for EE2 and diclofenac respectively. Asymmetry varied from 1.0 (EE2) – 1.5 (diclofenac), meaning that peaks were tailing apart from EE2, which was considered symmetrical. The rest of the compounds obtained values lower than 2, therefore results were considered acceptable and fit for purpose.

Table 2.2 Retention and asymmetry factors for chromatographic peaks using a C_{18} column.

Compound	k	Tf	As
Benzophenone-4	3.85	1.28	1.4
Diclofenac	4.56	1.35	1.5
E2	5.08	1.13	1.4
EE2	5.21	1.04	1.0
Triclosan	6.09	1.31	1.4
BHT	6.93	1.19	1.3
Octocrylene	7.13	1.15	1.2
Octinoxate	7.29	1.11	1.2

During development of the method, the separation of octocrylene and octinoxate became a challenge. Both compounds have similar log K_{ow} values and structure (Table A.3 from Appendix B), and they were expected to elute at similar retention times. Unfortunately, the literature review for the determination of UV-filters using UV-Vis detectors is quite limited as most methods use MS.[240–242] If UV-Vis detection was employed, it typically only included one of these two problematic compounds at the same time.[243,244] Only a few approaches contain both of them, and co-elutions are avoided by using long run times, 30 minutes approximately. However, the two peaks still elute quite close to each other,[245] leading to problems when applied in real samples because of matrix. If UV-filter compounds with close retention times are present in high concentrations, peaks will not resolve appropriately, and quantification will not be able to be achieved.[246] However, the purpose of the method was only to develop an extraction method, and the quantification of real samples was performed with MS as a detector, where two different MRM transitions were used for their total separation.

Resolution and selectivity parameters were studied for all compounds (Table 2.3) in order to examine the separation dimensions using the method developed. This was extremely important for compounds with similar properties, which elute at similar retention times, such as octocrylene and octinoxate, and E2 and EE2. Selectivity values

ranged from 1.02 – 1.18, where the higher the value the more separated the compounds were. Almost all compound pairs had an optimum value of α >1.1, except the following separations: as E2 – EE2, BHT – Octocrylene, and octocrylene – octinoxate. However, this factor only characterizes the separation based on retention times but not in terms of lack of overlap, as it does not take width of the peak into account. Because of this, it can happen that selectivity values are similar but resolution will be different. Therefore, resolution was calculated and values ranged from 1.17 – 8.34. Optimum values are >1.5, of the three separation pairs that had a α >1.1, only one compound pair did not meet this criteria, E2 and EE2. Both hormones have similar property values and their separation would need further investigation, however, the proposed separation was considered acceptable for the purpose of the study.

Table 2.3 Selectivity and resolution factors for targeted compounds for chromatographic separation using a C_{18} stationary phase.

Compound	**α**	**Rs**
Benzophenone-4		
	1.18	4.56
Diclofenac		
	1.11	4.12
E2		
	1.03	1.17
EE2		
	1.17	8.23
Triclosan		
	1.14	8.34
BHT		
	1.03	2.08
Octocrylene		
	1.02	1.60
Octinoxate		

2.4.1.2 Method 1.2: Extraction

The CECs in our study were expected to be detectable at really low concentrations, therefore, SPE was decided as the extraction method, due to its pre-concentration ability. During method development of the extraction method, recoveries were obtained for n=3 replicates so standard deviation could be calculated.

2.4.1.2.1 Format and sorbent selection

Selecting the correct SPE format was first investigated. As water samples are normally extracted with high volumes, up to a litre,[111,136] plates were discarded giving the cartridge format as the best option available. Sample volume was set to 100 mL (as will be discussed below), so the syringe-barrel cartridges of 6 mL volume was utilised with 200 mg as complex samples can be load up to 200 mL with this amount of sorbent by the recommendation on the specification sheet.

Oasis HLB cartridges are the most recommended sorbent in literature reviews for different PPCPs multi-analyte analysis, and are been proven to yield high recoveries in a wide range of PPCPs. This is due to its sorbent chemistry, a copolymer of divinylbenzene and vinylpyrrolidone, binding very polar (hydrophilic part) and non-polar compounds (lipophilic side). Therefore, high recoveries are achieved in lots of different matrices such as water samples including antibiotics[140] and even for one of the most unstable compounds from our method, hormones, reaching recoveries between 94 – 107%.[112] Consequently, two sorbents were selected from the literature review based on the analytes selected for the study, Oasis HLB and HyperSep Retain PEP (highly porous polystyrene divinylbenzene material with N-vinylpyrrolidone groups) (Thermo Fisher Scientific, Hertfordshire, UK). Both contain the same type of sorbent but are manufactured from different brands. The main differences between them are the particle size, 30 and 40 – 60

µm, the pore size, 80 and 55 – 90 Å, and the surface area, 800 and 550 – 750 m^2/g, for Oasis HLB and HyperSep Retain PEP respectively.

Both sorbents were initially tested using a generic preliminary method suggested for 166 pharmaceutical compounds.[126] It consisted of conditioning with 4 mL of methanol and 4 mL of water; after which 100 mL of sample was loaded and washed with 4 mL of methanol: water (5:95, v/v). However, in this case, elution was carried out with 2 mL of methanol and injected straight away into the LC-UV in order to calculate recoveries.

An independent-samples t-test was conducted to compare recoveries between both sorbents. There was a significant difference in the scores for benzophenone-4 ($t(4)=2.90$, $p=0.044$) and BHT ($t(4)=3.73$, $p=0.020$), where Oasis HLB achieved higher recoveries, though there was no significant effect for diclofenac ($t(4)=2.56$, $p=0.063$), E2 ($t(2.11)=0.267$, $p=0.814$), EE2 ($t(2.01)=-.030$, $p=0.979$), triclosan ($t(4)=0.811$, $p=0.463$) and octinoxate ($t(4)=-0.354$, $p=0.741$). Octocrylene resulted in non-parametric results, so a non-parametric test was run (Independent-samples Mann-Whitney U test) where the distribution of recoveries is the same across categories of sorbent type, having no significant effect ($U=5$, $p=1.00$). Overall, Oasis HLB cartridges provided higher values compared to HyperSep Retain PEP (average of 54% ± 7 and 47 % ± 4, respectively). As they both contain the same chemistry, physical characteristics such as the particle size, porosity, pore volume, or surface area, could explain the differences.[247] Interactions between the sorbent and the analytes are higher with smaller particle sizes and higher surface areas, such as the Oasis HLB, enhancing the retention and elution properties resulting in better recoveries.[236,248] Even though the variation of the repeats was lower with HyperSep sorbents, Oasis HLB was selected for further optimisation due to higher recoveries obtained, Figure 2.3, considering the LODs required for the estrogen hormones (Table 1.3).

Furthermore, it should be noted that low recoveries were obtained overall for the more non-polar compounds (e.g. octocrylene and octinoxate). Oasis HLB has been reported to yield higher recoveries for high polarity compounds with strong hydrogen-bond properties.[248] This can be observed in Figure 2.3 (b), where higher recoveries are obtained for medium log *P* values. Non-polar compounds can retain longer in the sorbent and are more difficult to elute, so different solvents were tested for optimisation. However, benzophenone-4, highly polar compound (log *P* = 0.88), obtained low recoveries (<50%) for both cartridges as well. This is due to low affinity to the solvent methanol,[248] and therefore not being eluted. As mentioned previously, different elution solvents were experimented for the optimisation of the method and are discussed later on.

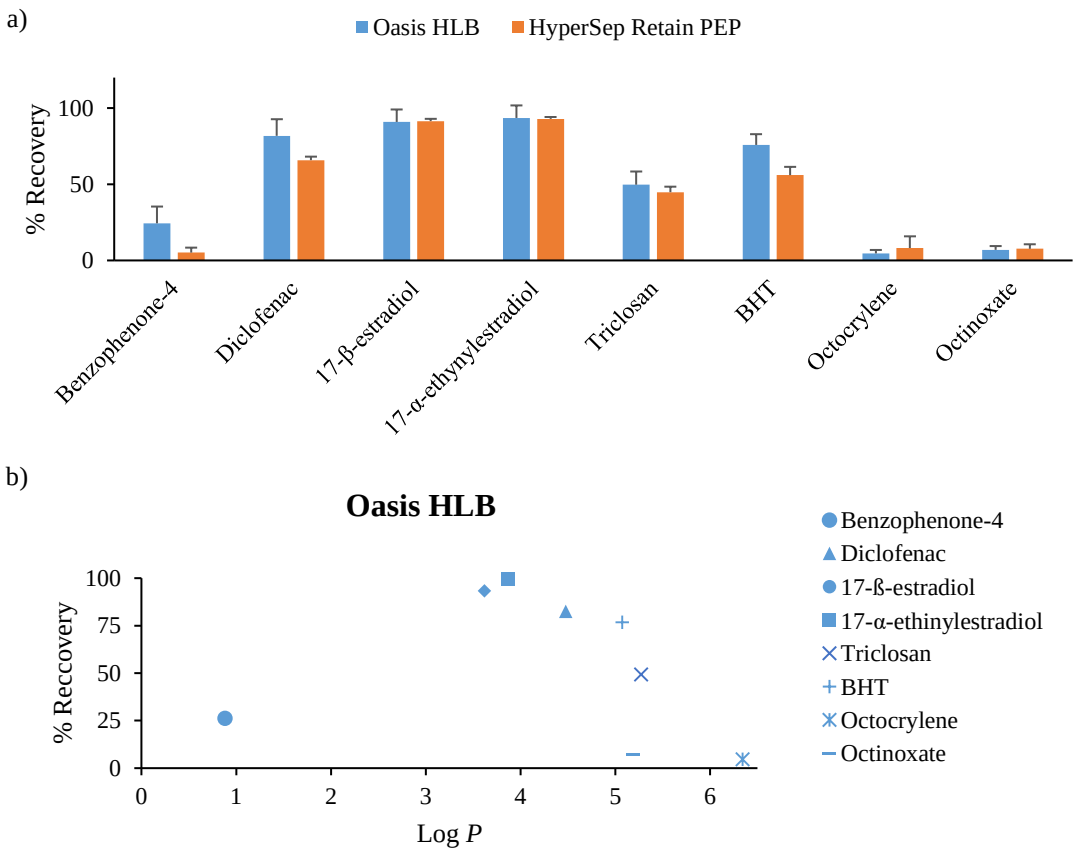


Figure 2.3 a) Average recoveries ± standard deviation for compounds (n=3) in the LC-UV method for the selection of sorbent type comparing Oasis HLB and Hypersep Retain PEP (6 mL, 200 mg) after elution with 2 mL of MeOH; and b) recoveries using Oasis HLB sorbent selected vs log P data for n=3 replicates.

Once the sorbent was selected, further optimisation was carried out where different parameters were tested including the wash step, pH of the sample, evaporation step, and elution solvent and volume.

2.4.1.2.2 pH of samples

Acidification of water samples is normally used to help to minimise the growth of bacteria in the sample,[249] bacteria cells will lyse due to the acidic conditions minimising the biological activity of samples. Therefore, their stability is often preserved as bacteria could also digest compounds. Also, the effect of pH is one of the most important factors for interactions between the analytes and the sorbents.[250] Normally the use of acids such as HCl or H_2SO_4 are used and it has also been proven to contribute higher recoveries for some compounds, as increases the log *P* of acidic compounds.[9,240,251] For compounds such as octocrylene and octinoxate, which presented low recoveries (Figure 2.3), it has been observed that when samples are adjusted to pH 2, SPE recoveries were 3-5 times higher for this type of compounds.[240] Therefore, for pH adjustment of the samples, two different pHs, 2 and 4 (n=3 each), were selected from literature review for further optimisation (Table 1.6).

Recoveries were achieved at those two pHs (Figure 2.4) and compared with samples that had no pH adjustment. A one-way ANOVA test between subjects was conducted to compare the effect of pH on the sample for no adjusted pH, pH 2 and pH 4 conditions. There was no significant effect of pH on the sample at the $p<0.05$ level for the three conditions for diclofenac [$F(2,6)=3.83$, $p=0.085$], E2 [$F(2,6)=1.75$, $p=0.252$], EE2 [$F(2,6)=3.43$, $p=0.101$] and octinoxate [$F(2,6)=0.001$, $p=0.999$]. However, there was for benzophenone-4 [$F(6,2)=27.7$, $p=0.001$], triclosan [$F(2,6)=5.72$, $p=0.041$], BHT [$F(2,6)=13.83$, $p=0.006$] and octocrylene [$F(2,6)=34.71$, $p=0.001$]. Post Hoc comparisons using the Tukey HSD test were further tested for these analytes, and indicated that for

triclosan there were no significant effects, benzophenone-4 had a significant difference for pH 4 with pH 2 and no pH adjusted, and for BHT and octocrylene there was a difference between no adjusted pH and pH 4 and pH 2.

Decreasing the pH resulted in lower recovery values, however, there was no significant difference between them apart from benzophenone-4, BHT, and octocrylene as illustrated by the ANOVA test performed. BHT recoveries decreased and results are comparable to previous studies.[180] At pH 2, some compounds may hydrolyse becoming less stable. This could explain why yielded recoveries are better at greater pHs. Moreover, compounds can also become more hydrophobic and will also stay more in the sorbent, therefore stronger solvents are further required for elution. As mentioned before, octocrylene was expected to obtain higher recoveries compared to neutral pH. Acidification below the compound's pK_a allows complete deionisation of the analyte becoming a neutral molecule. Compounds can now bind strongly to the sorbent, where Oasis HLB performs reverse-phase retention.[240] On the other hand, benzophenone-4 obtained low recoveries at pH 2, this is because it has a pK_a of 7.6, so in acidic pH it will accept proton ions resulting in an overall positive charge, obtaining low recoveries. However, at higher pH, the analyte is easily displaced on the adsorbent, so little or no adsorption occurs.[252] Previous studies have also reported breakthrough volume when using neutral pHs (6 – 8), decreasing its recovery.[65] These results are in accordance with our study, where pH 4 presents the highest recoveries overall for this compound. Nevertheless, it presents solubility issues with the solvents tested[248] and further optimisation was performed and is discussed next. As a result, pH 2 was considered for the optimised method, and in order to prevent/slow destabilisation processes, as soon as samples were acidified, they were frozen.

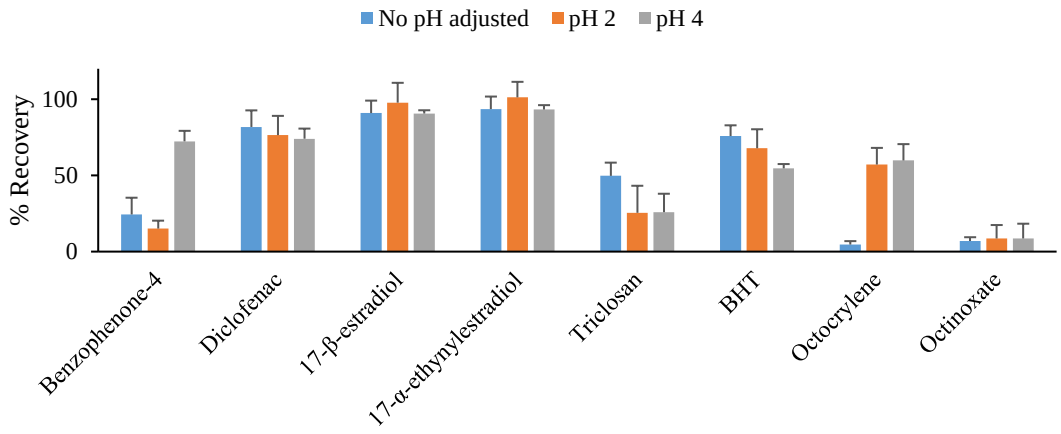


Figure 2.4 Average recoveries ± standard deviation (n=3) for compounds in the LC-UV method for the selection of pH.

2.4.1.2.3 Washing step

After loading the sample, a wash step is normally conducted in order to remove all the unwanted contaminants from the sorbent and reduce matrix effects. Wastewater samples can be very complex, so this step was optimised where the wash step from the generic method, methanol: water (5:95, v/v), was tested alongside just water to see possible analytes losses, elution can happen during this step (Figure 2.5).

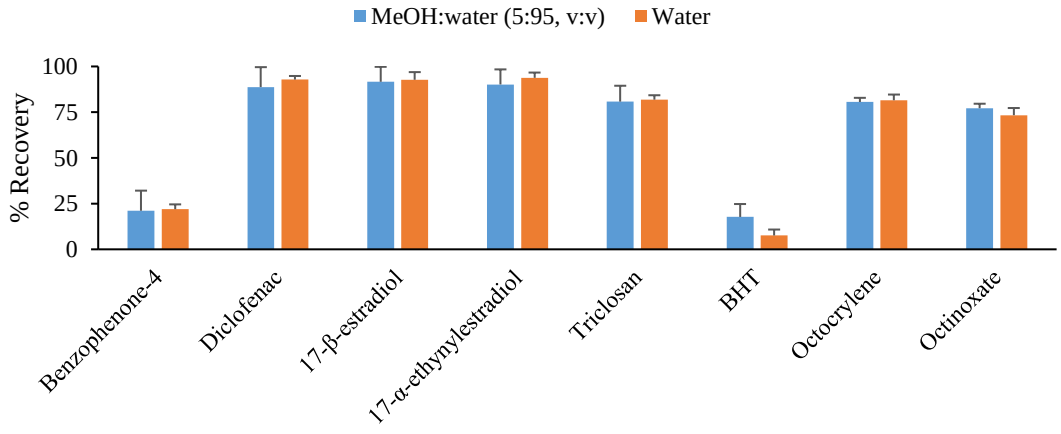


Figure 2.5 Average recoveries ± standard deviation (n=3) for compounds in the LC-UV method for washing solvent selection using pH 2 samples with 4 mL of acetonitrile: methanol (25:75, v/v) as elution followed by an evaporation step.

Overall recoveries were lower with the percentage of protic solvent apart from BHT and octinoxate, however, the differences between them were minimal. An independent-samples t-test was conducted to compare recoveries with water and methanol: water (5:95, v/v) wash steps, where there was no significant effect in the scores for any of the analytes; benzophenone-4 ($t(4)=0.146$, $p=0.891$), diclofenac ($t(4)=2.299$, $p=0.083$), E2 ($t(4)=0.361$, $p=0.736$), EE2 ($t(4)=-1.432$, $p=0.225$), triclosan ($t(4)=0.612$, $p=0.573$), BHT ($t(4)=-1.624$, $p=0.180$), octocrylene ($t(4)=0.413$, $p=0.701$) and octinoxate ($t(4)=-1.590$, $p=0.187$). Therefore, methanol: water (5:95, v/v) was preferred for the optimised method because organic solvent could help reducing unwanted contaminants bound on the sorbent.

2.4.1.2.4 Elution volume and evaporation

Another factor investigated to potentially improve the analytes recoveries was the elution volume. In case compounds were still bounded into the sorbent, the volume was doubled, to try to elute the full analyte from the cartridge. A volume of 4 mL of methanol was evaluated, and an increase in recoveries was achieved (Figure 2.6). An independent-samples t-test was conducted to compare recoveries with 2 mL and 4 mL elution volumes using the generic method. There was no significant effect in the scores for the following analytes; benzophenone-4 ($t(4)=-1.078$, $p=0.342$), diclofenac ($t(4)=-1.468$, $p=0.216$), E2 ($t(4)=-0.198$, $p=0.853$) and EE2 ($t(4)=0.718$, $p=0.513$); however, there was a significant difference for triclosan ($t(4)=-6.061$, $p=0.004$), BHT ($t(2.138)=4.483$, $p=0.041$) and octinoxate ($t(2.238)=-6.655$, $p=0.016$); confirming their strong retention to the sorbent. Octocrylene resulted in non-parametric values so an independent-samples Mann-Whitney U test was completed. The distribution of recoveries presented was the same across categories of elution volume having no significant effect ($U=9$, $p=0.100$) possibly

due to the high standard deviation of the replicates. Therefore, 4 mL was optimised as the elution volume.

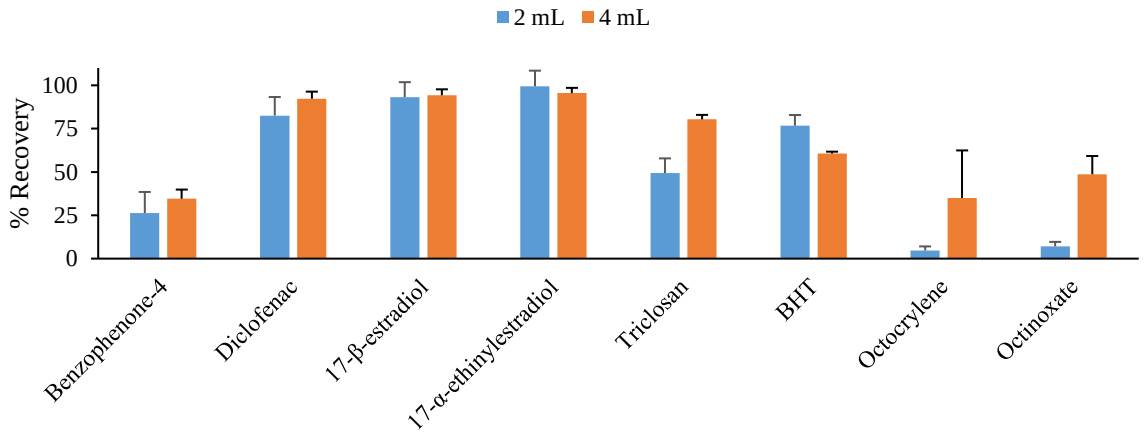


Figure 2.6 Average recoveries ± standard deviation (n=3) for compounds in the LC-UV method for elution volume selection using generic method with no evaporation step included.

Evaporation steps were needed in order to increase the concentration factor of the compounds and reach the low concentrations required for those types of samples. At the beginning of the development, evaporation was performed using the generic method to see how the analytes would behave with the addition of this step. Experiments were performed using nitrogen gas to dry the samples down completely at room temperature. Because of BHT being thermally unstable (high vapour pressure),[181] all evaporations were carried out at room temperature. An independent-samples t-test was conducted to compare recoveries with no evaporation and evaporation step where there was no significant effect for benzophenone-4 ($t(4)=1.141$, $p=0.318$), diclofenac ($t(4)=1.995$, $p=0.117$), E2 ($t(4)=1.039$, $p=0.357$), EE2 ($t(4)=1.328$, $p=0.255$), octocrylene ($t(4)=-2.123$, $p=0.101$) and octinoxate ($t(4)=-2.097$, $p=0.104$). A significant effect was shown for triclosan ($t(4)=4.605$, $p<0.01$) and BHT ($t(4)=13.74$, $p=0.000$). Most of the analytes presented lower recovery values except for octinoxate and octocrylene (Figure 2.7), however, this step was needed to achieve the desire LODs as sample volume was set to

100 mL. Moreover, the elution volume of the method was 4 mL of organic solvent, which is needed to reconstitute in an aqueous solvent for mass spectrometry analysis.

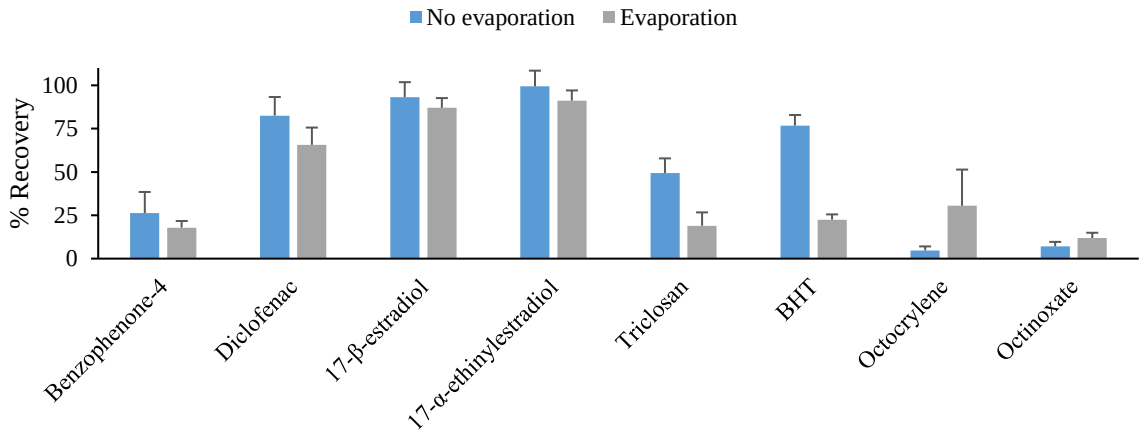


Figure 2.7 Average recoveries ± standard deviation for compounds in the LC-UV method for evaporation step using initial generic method.

The biggest decreases in recoveries were for triclosan and BHT, therefore two different types of evaporation were tested to try to improve the recoveries obtained. Two solutions prepared in the elution volume were prepared, where one was evaporated to complete dryness and another one until half volume using appropriated volumetric glassware, avoiding drying completely the extract. Both sets of solutions achieved similar recoveries, apart from BHT as expected. The more solvent evaporated, the more loss of analyte was observed (Figure 2.8). BHT has shown low thermal resistance due to its low boiling point.[181] BHT belongs to volatile organic chemicals (VOCs) categories[253] and has a vapour pressure of 0.3 x 10^{-3} hpa[254] (0.00624 torr),[180] therefore a decrease in recoveries after evaporations are predicted. Consequently, a compromise needs to be decided due to the low limits of detection required, and a full evaporation process was deemed necessary and as a result was performed in subsequent samples. Previous studies of BHT extracted by Oasis HLB also investigated the two types of evaporations. After evaporating directly to the required volume, recoveries of the analyte increased. Nevertheless, they were still

quite low (35%), and poor repeatability was accomplished. This led to no evaporation step for the method although a decrease in elution volume was carried out.[180] Extracts were injected right after elution into the instrument[180,183] to avoid losses, however, there are still limited studies performing evaporation until dryness.[254] Direct injection of the extracts is possible when GC-MS is the analysis technique selected. Unfortunately, these organic solvents (e.g. ethyl acetate) cannot be used in LC-MS, so this was not considered for the method. Other possible losses were attributed to the degradation of the compound or its adsorption to glassware as an example, although these were not confirmed.[180]

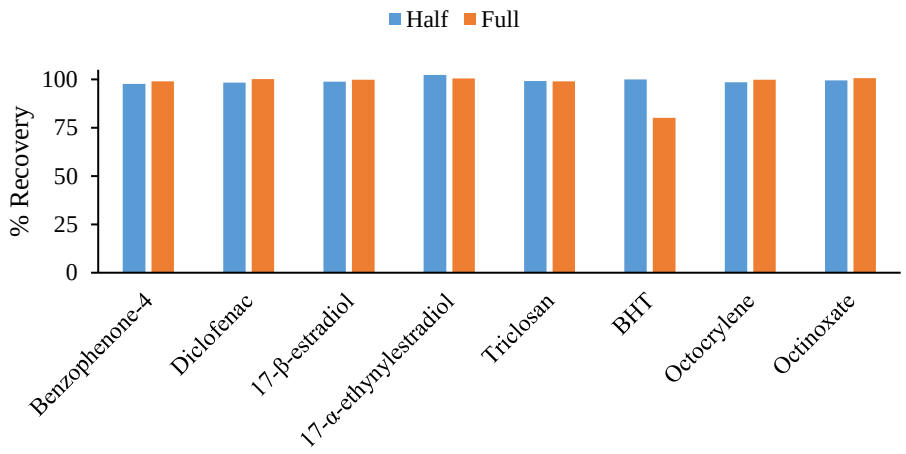


Figure 2.8 Percentage recoveries for compounds in the LC-UV method for optimisation of the evaporation step until dryness (full) and half of the volume of evaporation (half).

A number of protic solvents were investigated for extraction in this step. Different solvents were tested including methanol, acetonitrile, ethyl acetate, tetrahydrofuran (THF), dichloromethane, and hexane (in order of increasing polarity). Poor peak shapes were presented when using ethyl acetate, dichloromethane, and hexane hence no recoveries could be calculated due to the non-integration of peaks, and when these solvents were evaporated, no peaks were detected at all. In general, UV-filters were recovered to a lesser extent, and this could be due to high log K_{ow} values (e.g. octocrylene); they can retain more on the sorbent. In order to elute them, a stronger

organic solvent, THF, was also investigated. However, a white residue was left after the evaporation step, which was not soluble in the reconstitution solvent, probably due to instability of the sorbent in THF. Filtration was carried out before its injection onto the instrument, possibly losing high part of the compound. This was confirmed when lower recoveries were obtained. An independent-samples t-test was conducted to compare recoveries with methanol and acetonitrile where there was no significant effect for benzophenone-4 ($t(4)=2.682$, $p=0.055$) due to solubility issues in both solvents,[248] however, there was a very significant effect for diclofenac ($t(4)=11.080$, $p<0.01$), E2 ($t(4)=11.406$, $p<0.01$), EE2 ($t(4)=10.278$, $p<0.01$), triclosan ($t(2)=4.561$, $p=0.045$, BHT ($t(4)=3.662$, $p=0.022$), octocrylene ($t(4)=-10.929$, $p<0.01$) and octinoxate ($t(4)=-9.049$, $p<0.01$), as seen in Figure 2.9. Acetonitrile presented lower recoveries overall and the most polar compounds yielded higher recoveries with methanol. However, higher recoveries were obtained for octocrylene and octinoxate, which raised from 5 to 85 and 7 to 72%, respectively (Figure 2.9). This is due to their very non-polar characteristics (highest log K_{ow}), increasing the solvent strength (methanol to acetonitrile) helped eluting the compounds, which were bound strongly in the sorbent. Retention mechanisms in reverse-phase involves interactions such as hydrophobic, electrostatic and π-π interactions. However, the hydrophobic ones are the major governing forces where polar compounds will retain less on the sorbent.[250] Highly hydrophobic compounds have been reported to bind to the polystyrene divinylbenzene materials of the Oasis HLB by hydrophobic and polar interactions. As polar interactions are needed, a more polar eluent will achieve better elutions and therefore recoveries.[255] For this reason, acetonitrile solvent was introduced as part of the elution solvent, acetonitrile: methanol (25:75, v/v) where recoveries were higher including the evaporation step.

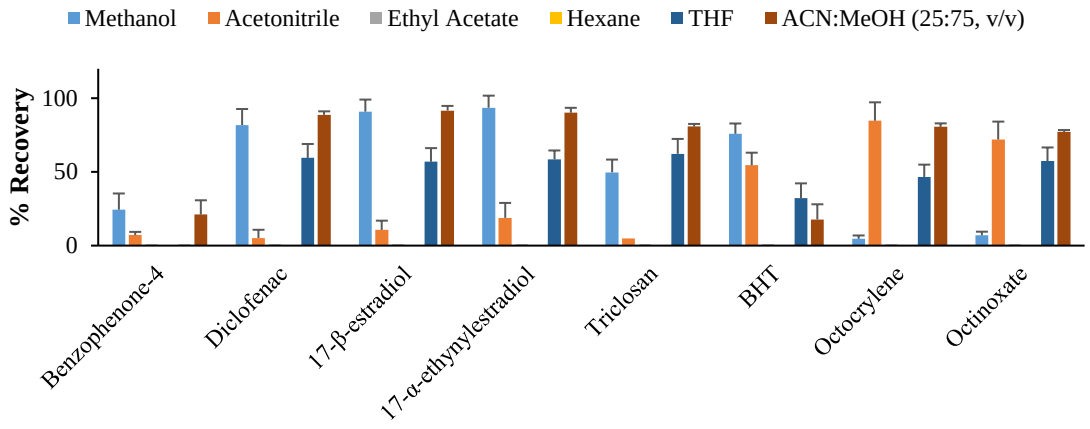


Figure 2.9 Average recoveries ± standard deviation for compounds in the LC-UV method for solvent selection where THF and ACN:MeOH (25:75, v/v) had an evaporation and reconstituted in MeOH step performed.

After different solvents were tested, benzophenone-4 still resulted in low recoveries. Therefore, an extended literature review was carried out where the use of large volumes of elution (e.g. 3x10 mL of methanol[242] or 3x2 mL of ethyl acetate)[241] or the introduction of 5 mM ammonium acetate in solvents as acetonitrile (online-SPE)[190] and methanol (using only 3 mL)[65] were suggested. The addition of 5 mM ammonium acetate was therefore evaluated, it was added to methanol and to the mixture of acetonitrile: methanol (25:75, v/v) including evaporation step (Figure 2.10). Comparing the recoveries achieved to previous results obtained, significant increases were observed for the compound. This is due to its low affinity for solvents such as methanol and acetonitrile, requiring much larger volumes. However, when ammonium acetate is introduced, the compound forms a salt which is more soluble enhancing its elution.[65] On the other hand, recoveries for BHT decreased, though, this could occur due to its volatility problems after evaporation as explained before. In terms of macrolides antibiotics, the use of mixtures of ammonia and organic solvents was demonstrated in literature for helping their elution,[233] apart from the conventional organic solvents. Regarding the selection between methanol and acetonitrile: methanol (25:75, v/v), an independent-samples t-test was

conducted where there was no significant effect for benzophenone-4 (t(4)=-1.760, p=0.153), E2 (t(4)=-1.732, p=0.158), EE2 (t(4)=-0.982, p=0.382), triclosan (t(4)=0.315, p=0.769), BHT (t(4)=-0.471, p=0.662). Nonetheless, there was a very significant effect for octocrylene (t(4)=-3.300, p=0.030) and octinoxate (t(4)=-13.000, p<0.01). Diclofenac resulted non-parametric values so an independent-samples Mann-Whitney U test was performed. The distribution of recoveries was the same across categories of solvent having no significant effect (U=6, p=0.700). Consequently, 5 mM ammonium acetate in acetonitrile: methanol (25:75, v/v) was preferred for the rest of the optimisation of the final method.

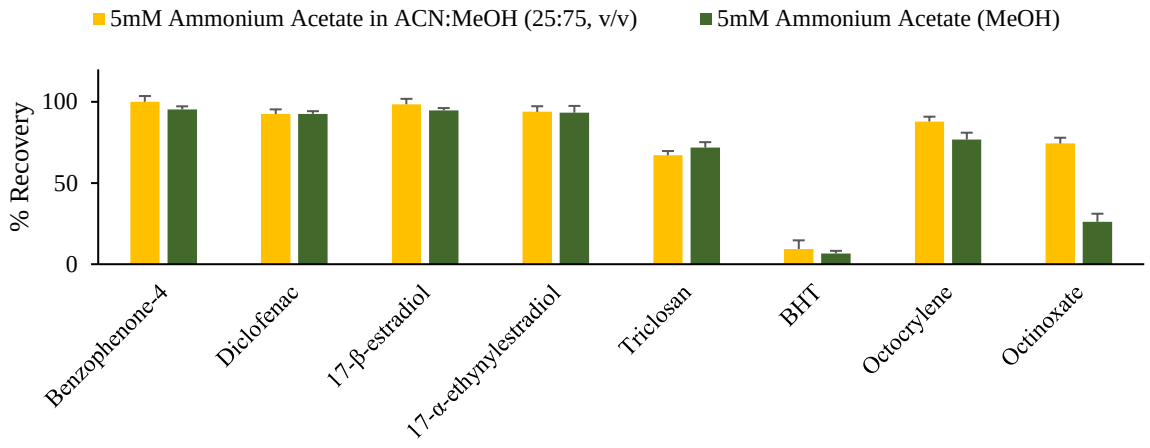


Figure 2.10 Average recoveries ± standard deviation for compounds in the LC-UV method for solvent selection introducing ammonium acetate after evaporation.

The solvent selected yielded higher recoveries, therefore the need of 4 mL for the elution step might not be necessary. Two volumes were tested again with the chosen solvent (Figure 2.11), and an independent-samples t-test was conducted where there was no significant effect for diclofenac (t(4)=0.134, p=0.900), E2 (t(4)=-0.056, p=0.958), EE2 (t(4)=-0.120, p=0.910) and octocrylene (t(4)=-1.150, p=0.314); however, significant difference was observed for triclosan (t(4)=-4.128, p=0.015), BHT (t(4)=-3.502, p=0.025) and octinoxate (t(4)=-4.177, p=0.014) which recoveries obtained were higher.

Benzophenone-4 resulted in non-parametric results, an independent-samples Mann-Whitney U test was performed where the distribution of recoveries is the same across categories having no significant effect (U=4.5, p=1.000). The volume of 4 mL had a significant difference achieving higher recoveries for 3 compounds so this was the final volume selected.

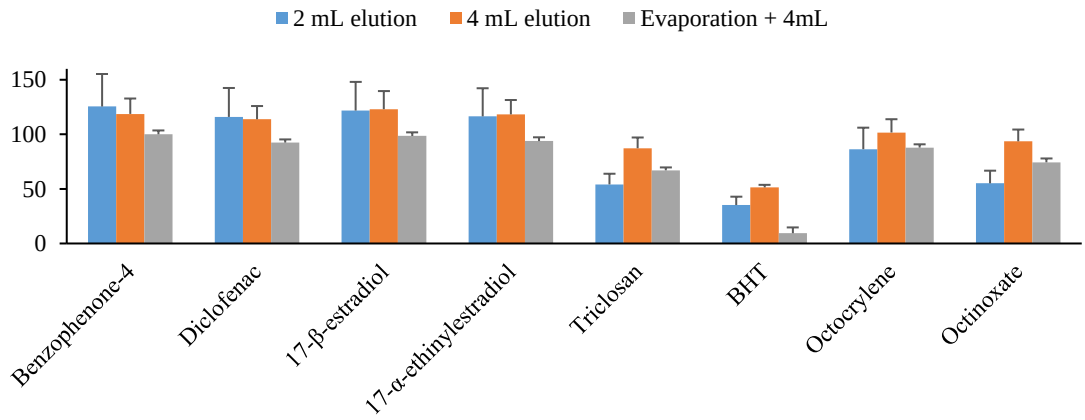


Figure 2.11 Average recoveries ± standard deviation for compounds in the LC-UV method for elution volume and evaporation step using the elution solvent selected of 5 mM ammonium acetate in ACN:MeOH, (25:75, v/v).

2.4.1.2.5 Finalised method

The finalised method using all optimised steps, including evaporation, can be seen in Figure 2.11. The method was as follows:

- Conditioning: 4 mL of methanol and 4 mL of water
- Loading: 100 mL of pH 2 sample
- Washing: 4 mL of methanol: water (5:95, v/v)
- Drying: 20 min under vacuum
- Elution: 4 mL of 5 mM ammonium acetate in acetonitrile: methanol (25:75, v/v)
- Evaporation to dryness: N_2 gas under room temperature
- Reconstitution: 1 mL of methanol (LC-UV) or acetonitrile: water (10:90, v/v) (LC-MS/MS)

2.4.1.3 Method 1.2: LC-MS/MS

Due to the polar nature and mostly non-volatility of the analytes selected (e.g. hormones are non-volatile),[138] LC-MS/MS was preferred for the method of analysis. In order to use GC-MS, derivatisation processes would need to be added making methods more time-consuming. As suggested by many previous studies for pharmaceuticals, LC-MS/MS using reversed-phase HPLC is one of the most common techniques. All analytes were directly infused in negative and/or positive mode in order to get the transitions for product and precursor ions for confirmation of analytes using methanol, where the most intense was used for quantification, and the different conditions such as collision energy. Selected parameters for both methods are shown in Table A.5 (Appendix D), using dMRM conditions. dMRM mode is generally used for multianalyte analysis, and the study contains nineteen compounds in total, therefore selected. This mode permits cycle times to define the maximum dwell time for each transition,[256] increasing sensitivity. MRM transitions are performed around the elution time of the compound and not throughout the entire chromatogram, allowing longer dwell times.[257] Nevertheless, BHT was unsuccessfully detected, and it was concluded that higher concentrations would be needed in order to get the transitions. LC-MS is not sensitive enough for this compound, and after performing research in the literature it was concluded that this compound would need to be analysed by a suitable technique such as GC-MS,[181] therefore no further investigation was carried out from this point for this compound.

In order to achieve lower limits of detection for hormones, it was decided to develop a method separately. Therefore, two methods were developed: one for the hormones and other one for the remaining compounds.

2.4.1.3.1 Method 1.2.1: hormones

Hormones are known for their problematic assay sensitivities during their detection in analytical techniques.[258] Their structural properties lead to poor ionisation efficiency compromising the method sensitivity. The estrogens selected have a minimum of a phenolic hydroxyl group, which is then ionised with the use of negative-mode ESI.[259] Different mobile phases were tested in order to increase the sensitivity of these compounds suggested by literature review including: formic acid, ammonium acetate, ammonium hydroxide and ammonium fluoride. A chromatogram of a standard at 50 ng/L comparing the four mobile phases can be seen in Figure 2.12; where it can be observed the difference not just in intensity but also in peak shape and retention time. The basification of mobile phases in negative mode is quite common, as it helps analyte deprotonation depending on the analyte.[9] After a comparison between the signals, it was observed that that ammonium fluoride significantly increased the sensitivity for this type of analytes.[260] This could be due to the fluoride ion's strong basicity in the gas phase, resulting in HF and forming $[M+F]^-$ and $[M+FHF]^-$.[261] For their separation, an isocratic elution was designated, analytes have similar log K_{ow} values and elute close to each other (Table A.3 from Appendix B). This type of gradient helps to get the highest separation between the peaks in order to get the best values for dwell times possible, increasing sensitivity. Other parameters were also established in order to increase sensitivity further. Injection volume was set to the maximum of a 100 µL, the electron multiplier voltage (EMV)[262] mode was set to 200 V (-), and wide mode was selected for all hormones. A chromatogram obtained with the final method is presented for a standard prepared in reconstitute solvent at 500 ng/L for the analytes and 100 ng/L for the IS compounds (Figure 2.13).

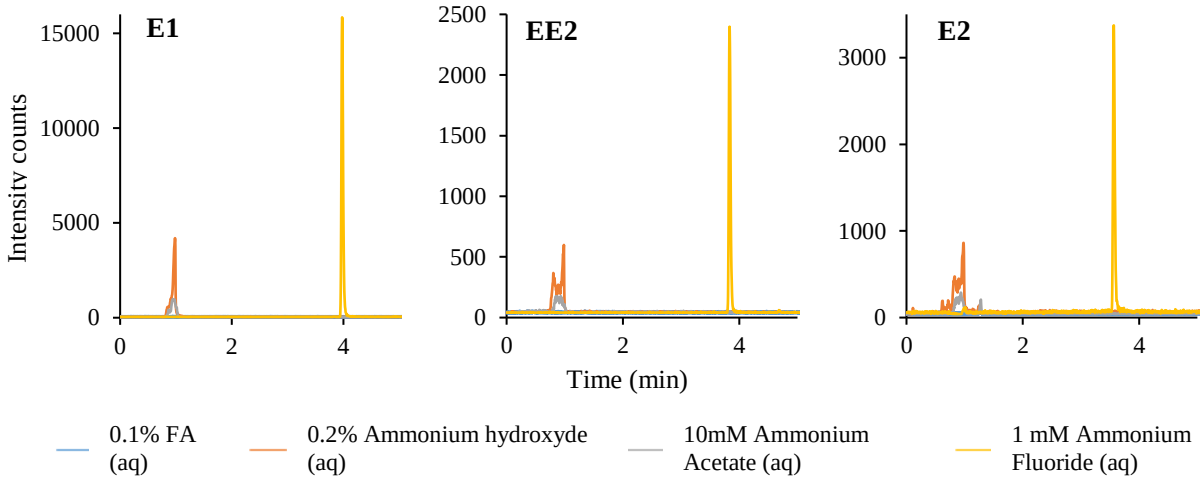


Figure 2.12 Chromatograms comparison of different additives added to an aqueous phase for a standard containing estrogens analytes (quantification ions) prepared in acetonitrile:water (10:90, v:v) at a concentration of 50 ng/L using an injection volume of 10 µL and same chromatographic conditions.

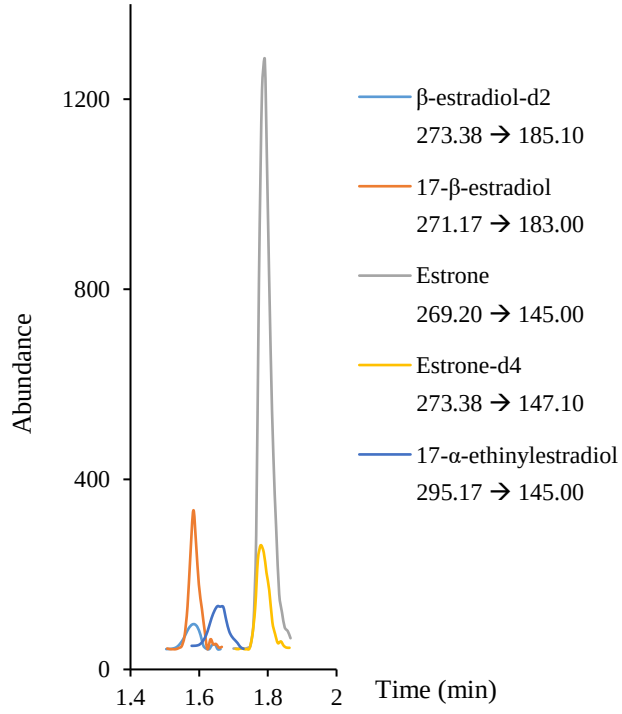


Figure 2.13 Separation of a standard solution at 500 ng/L for estrogens hormones and 100 ng/L for the SIL-IS using the final LC-MS/MS method using an Infinity Lab Poroshell 120 EC-C18 column with a gradient elution and 100 µL injection volume and just showing quantification ion transitions.

Chromatographic parameters were assessed and are presented in Table 2.4, where retention values were calculated by two methods: theoretical and experimental using uracil, where theoretical values were higher when compared to experimental ones. This could be related to the use of salts in the mobile phase selected, as higher values are usually obtained when salts are injected.[263] Experimental retention factor values ranged from 0.84 – 1.09, leading to a short run time for the method. Tailing factors varied from 0.92 – 1.68, for EE2 and estrone-d_4, respectively. Values between 0.7 – 2.0 were obtained for asymmetry (EE2 and estrone-d_4). Values obtained for E2 and EE2 under the LC-UV method developed were 1.13 and 1.04, respectively. Therefore, the UV method obtained narrower peaks with less tailing. This could be associated to the difference in pH of the mobile phases or the shorter runtime in the MS method. However, the difference between them is minimal, 0.16 and 0.12 for E2 and EE2 respectively. All peaks were tailing apart from EE2, which had a value <1 resulting in fronting. This could be due to the low flow set for the method, however, as a good separation needs to be achieved in order to increase sensitivity, flow rate cannot be increased and an isocratic elution is already used. Acceptable results were therefore obtained, even that a 100 µL injections volume were used.

Table 2.4 Retention and asymmetry factors for chromatographic peaks using a C_{18} column.

Compound	**k**		**Tf**	**As**
	a	**b**		
E2	1.73	0.85	1.29	1.6
EE2	1.84	0.92	0.92	0.7
E1	2.09	1.09	1.66	1.7
17-β-estradiol-d_2	1.72	0.84	0.99	1.0
Estrone-d_4	2.07	1.07	1.68	2.0

a: Calculated using theoretical method.
b: Calculated using experimental, uracil, method.

Preliminary calibration lines in reconstitution solvent were prepared for the three analytes (≥5 points) in order the investigate their linearity previous to validation experiments. An average of coefficients of determination of R^2=0.9934 (±0.0024) for the three compounds using analyte peak area ratio with their respective standards was carried out (Table 2.5). Only an R^2<0.99 was achieved when using peak area ratios between E2 and estrone-d_4 as the internal standard. Sensitivities were also good overall, and from all hormones tested, EE2 was best. If several MRM transitions are scanned in the same window, the sensitivity decreases considerably. Therefore, the IS was not purchased for this compound, and calibration lines were tested with the two already available ones. Both of them reached R^2>0.99, hence any of them could be selected for future method performance experiments.

Table 2.5 Linearity values for the hormones LC-MS/MS method developed using reconstitute solvent standards.

Analyte	**IS compound**	**Range (ng/L)**	**R^2**
E1	Estrone-d_4	3 - 1000	0.9907
	β-estradiol-d_2	3 - 1000	0.9899
E2	β-estradiol-d_2	25 - 1000	0.9942
	Estrone-d_4	25 - 1000	0.9692
EE2	β-estradiol-d_2	5 - 1000	0.9954
	Estrone-d_4	5 - 1000	0.9959

2.4.1.3.2 Method 1.2.2: mix analytes

The LC-UV method developed in Section 2.4.1.1 was modified to enhance ionisation during MS detection, with formic acid selected as the mobile phase modifier for the method. To enable the reconstituted samples to be analysed by both the hormones method and the mix analytes method, a reconstitution solvent suitable for both was required, and hence, acetonitrile: water (10:90, v/v) was designated as final reconstitution solvent where no modifiers were added.

After the mobile phase and reconstitution solvent selection was completed, optimisation of LC separation was started. A good initial separation of the remaining

compounds was achieved overall (Figure 2.14) with retention factors varying from 0.60 – 9.39, tailing factors from 0.57 – 2.04 and asymmetry ranging from -1.0 – 3.1 (Table 2.6).

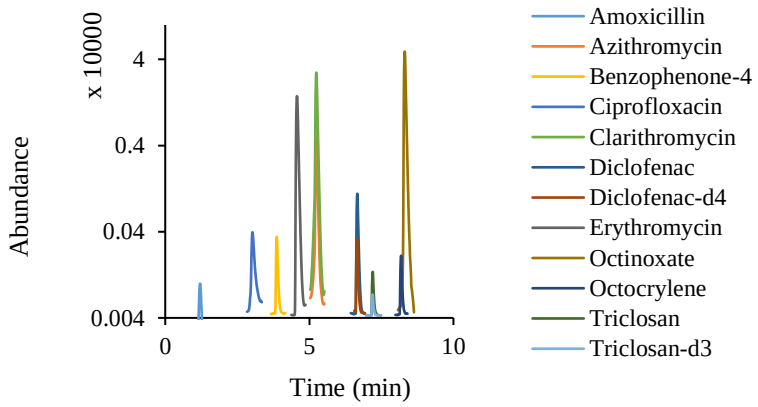


Figure 2.14 Chromatogram showing quantification ions of a log scale separation of a standard solution at 500 ng/L for target compounds and 100 ng/L for SIL-IS using the final LC-MS/MS. An Infinity Lab Poroshell 120 EC-C18 column is used with a gradient elution and 20 µL injection volume in order to get the separation.

Table 2.6 Retention, tailing and asymmetry factors for chromatographic peaks using a C_{18} column.

Compound	**k**		**Tf**	**As**
	a	**b**		
Amoxicillin	1.51	0.60	0.57	-1.0
Azithromycin	9.36	5.56	1.50	1.8
Azithromycin-d_3	9.35	5.55	1.62	2.0
Benzophenone-4	6.65	3.84	1.69	2.3
Ciprofloxacin	4.97	2.78	2.04	3.1
Clarithromycin	9.35	5.55	1.73	2.5
Diclofenac	12.16	7.33	2.02	2.3
Diclofenac-d_4	12.15	7.32	1.49	1.6
Erythromycin	8.02	4.71	1.80	2.5
Octinoxate	15.41	9.39	2.03	2.3
Octocrylene	15.17	9.23	1.62	1.9
Triclosan	13.22	8.00	1.60	2.3
Triclosan-d_3	13.21	8.00	1.60	1.8

a: Calculated using theoretical method.
b: Calculated using uracil method.

Linearity was assessed with coefficients of determination (R^2) by preparing calibration lines in reconstitution solvent (≥5 points). R^2 average values were 0.9937 (±0.0061) for all compounds using analyte peak area ratios with both internal standards (Table 2.7). All compounds presented R^2>0.98 therefore linearity was excellent overall. Notwithstanding that amoxicillin eluted quite close to t_0, it had a good linearity and so it was decided that the retention time obtained was acceptable to fit for purpose, therefore enough interaction was considered between the compound and the column. Further investigation was performed during method performance experiments.

Table 2.7 Linearity values for the compounds LC-MS/MS method developed using reconstitute solvent standards.

Analyte	**IS compound**	**Range (ng/L)**	**R^2**
Amoxicillin	-	3 - 1000	0.9995
	Diclofenac-d_4	3 - 1000	0.9985
	Triclosan-d_3	3 - 1000	0.9996
Ciprofloxacin	-	1 - 1000	0.9807
	Diclofenac-d_4	1 - 1000	0.9902
	Triclosan-d_3	1 - 1000	0.9857
Benzophenone-4	-	3 - 1000	0.9881
	Triclosan-d_3	3 - 1000	0.9817
	Diclofenac-d_4	3 - 1000	0.9851
Erythromycin	-	1 - 1000	0.9982
	Diclofenac-d_4	1 - 1000	0.9962
	Triclosan-d_3	1 - 1000	0.9955
Azithromycin	-	1 - 1000	0.9977
	Diclofenac-d_4	1 - 1000	0.9977
	Triclosan-d_3	1 - 1000	0.9976
Clarithromycin	-	1 - 1000	0.9978
	Diclofenac-d_4	1 - 1000	0.9969
	Triclosan-d_3	1 - 1000	0.9966
Diclofenac	-	3 - 1000	0.9989
	Diclofenac-d_4	3 - 1000	0.9946
	Triclosan-d_3	3 - 1000	0.9986
Triclosan	-	1 - 1000	0.9985
	Triclosan-d_3	1 - 1000	0.9962
	Diclofenac-d_4	1 - 1000	0.9982
Octocrylene	-	1 - 750[a]	0.9810
	Triclosan-d_3	1 - 750[a]	0. 9945
	Diclofenac-d_4	1 - 750[a]	0.9860
Octinoxate	-	1 - 1000	0.9893
	Triclosan-d_3	1 - 1000	0.9963
	Diclofenac-d_4	1 - 1000	0.9954

[a]Analyte starts going quadratic after 750 ng/L.

2.4.2 Method 2: Direct injection method optimisation

More than 200 pharmaceuticals have been detected in surface waters worldwide,[7] not counting with other types of contaminants such as pesticides, PCPs, etc. Production of CECs has been estimated to increase from 1 to 500 million tons per year across the world.[151] Therefore, the use of new, reliable and fast analytical techniques enable their monitoring in order to investigate their environmental fate and risk. SPE LC-MS/MS is the most common analytical procedure for water samples and therefore selected for this study. However, this method is known for being time consuming, costly and compound specific, limiting the number of compounds that can be analysed. As the numbers of different classes and chemistries of CECs are increasing in the environment, the selected contaminants in this thesis were quite limited for a future investigation and risk assessment. Consequently, a previously developed DI method for the quantification of 135 CECs was established for its application in influent wastewater sample analysis.[144] This method was optimised here for three different matrices (surface waters, influent and effluent) in order to broaden the number of compounds analysed for future samples within this thesis. For optimisation purposes, two parameters were investigated, flow rate and injection sample volume. Additionally, a filter recovery test and a sample stability experiment were performed.

2.4.2.1 Flow rate

In HPLC, efficiency of chromatographic peaks is directly affected by the flow rate or linear velocity. The use of a low or high flow rate could translate to poor chromatography, so an investigation of the effect of the ratio between mobile phase (flow rate) and injection volumes was performed. Different flow rates were considered using a constant injection volume (10 µL) and a gradient that changed proportionally to time to keep it

constant. Flow rates were kept constant to maintain the linear velocity of the mobile phase through the column. They were tested ranging from 0.1 to 2 mL/min from a matrix-match internal standard sample at a concentration of 500 ng/L. Lower peak height values were achieved when using low flow rates, this is due to band broadening on the peak and probably ion suppression from matrix as a low flow translates in a low dilution factor. With the addition of higher flow rates, peaks become narrower and shorter times are obtained. However, narrow peaks require a higher set of data points per second (Hz) in order to measure the peak. Due to the quick elution of the compounds, the analyte spends less time in the detector cell, so the data system has less time to detect the peak. Reducing peak heights will result in difficulties detecting very small quantities of each analytes, losing some compounds (as the case of the last two rates tested, n=19). This can be seen in Figure 2.15, where once the maximum height value is reached, it starts decreasing again. In terms of peak asymmetry (Figure 2.16), average values were calculated for the compounds selected, were 0.4 and 0.5 mL/min were the closest ones to the optimum asymmetry result. Nevertheless, peaks present a more “Gaussian” shape when the height is maximum at 0.5 mL/min, so this flow rate was therefore selected for the final method.

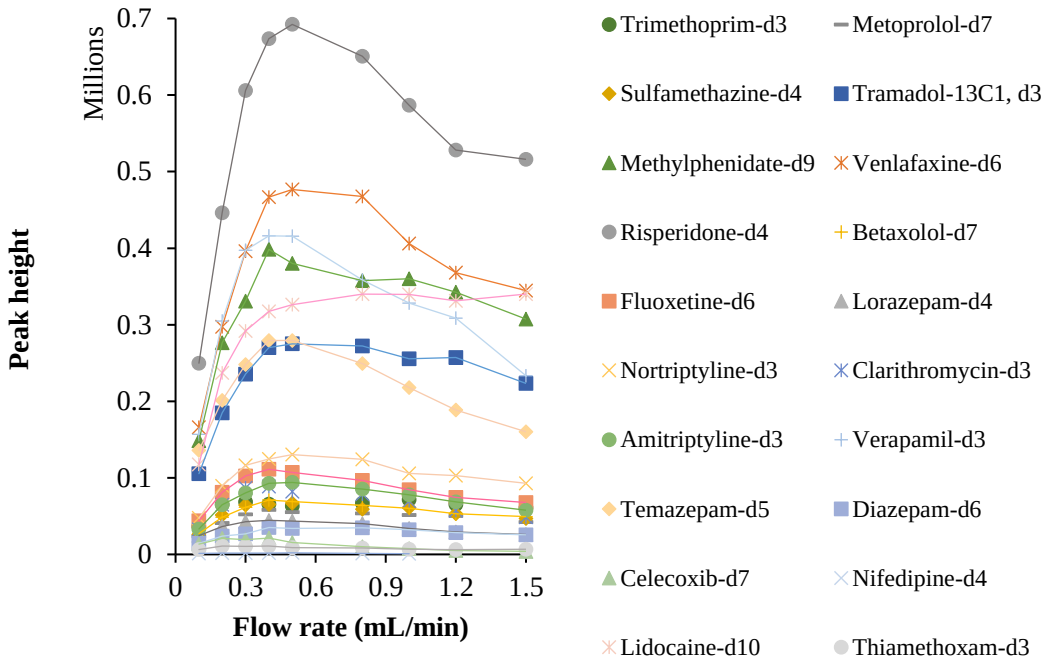


Figure 2.15 Peak height intensities obtained at different flow rates tested for its optimisation in a matrix-match standard at 500 ng/L of SIL-IS using an injection volume of 10 µL.

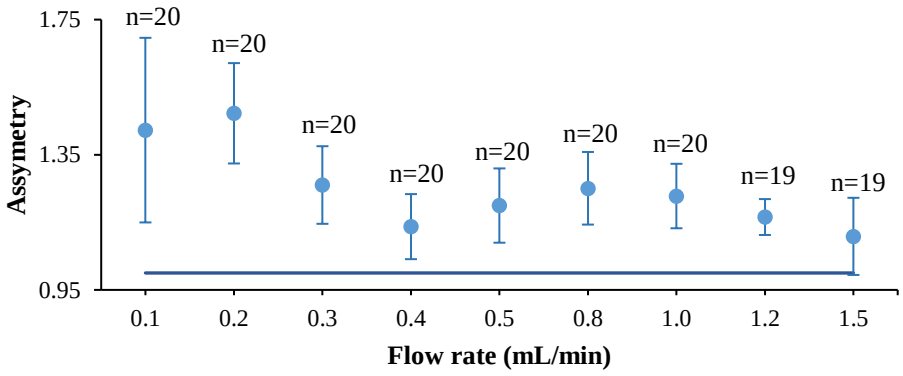


Figure 2.16 Average asymmetry values (±SD) obtained at different flow rates tested for its optimisation in a matrix-match standard at 500 ng/L of SIL-IS using an injection volume of 10 µL. Optimum asymmetry values represented as a solid line on the graph.

2.4.2.2 Injection volume

Injection volume can have a big impact on the chromatography of an analyte peak. If it is too low, not enough sensitivity could be reached for some compounds present at low concentrations when real samples are applied. However, if the value is set too high, there

are different negative aspects to consider. Examples are: regression lines turning quadratic due to saturation of the detector, more matrix is sprayed onto the source which will become dirtier affecting chromatography over time, the increase of pressures on the system, and analyte peaks can result in poor shapes (e.g. splitting, broadening, etc.). In order to get the optimised volume, an influent wastewater sample spiked with internal standards at a concentration of 500 ng/L was used. This matrix was considered as it presented the most complex matrix from the ones used in the study. A total of seven different volumes were investigated ranging from 0.5 – 20 µL in triplicate. The % RSD values between replicate injections were calculated and are presented in Figure 2.17, where lower volumes achieved higher percentages resulting in poorer reproducibility. Volumes above 10 µL caused deviations of signal intensity from linearity (Figure 2.18), and split peaks and unsatisfactory shapes were found[144] for some compounds such as thiamethoxam; therefore 10 µL volume was selected.

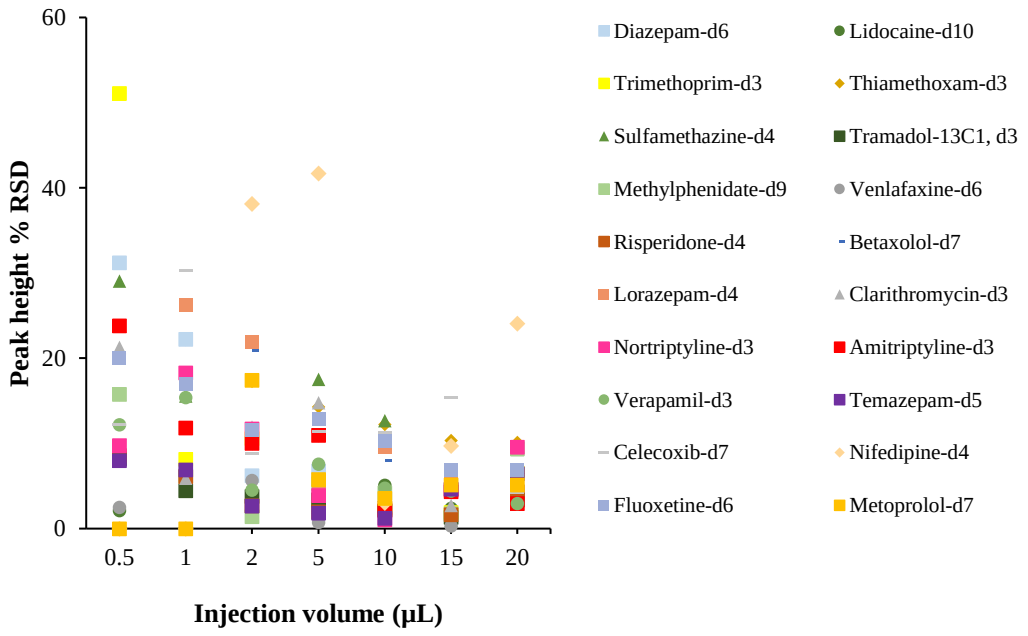


Figure 2.17 Peak height intensities obtained at different injection volumes tested (n=3 injections) for its optimisation in a matrix-match standard at 500 ng/L of SIL-IS.

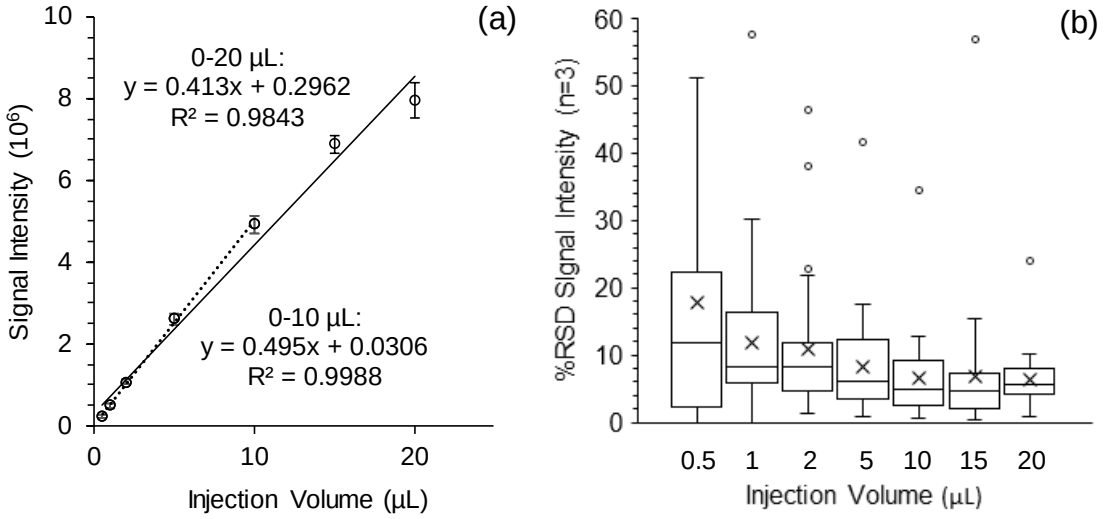


Figure 2.18 Injection volume optimisation (n=3) where a) presents the signal intensity for 135 compounds using a 500 ng/L spiked matrix-match standard; and b) shows box plot of %RSD of signal intensities for all 135 compounds (where the line represents the median and the X the mean). Both plots have been used with permission from K. T. Ng et al 2020.[144]

This small volume allowed more injections from the same vial, needing less sample collected. Also, after a sample analysis batch was run (n=167), no change was seen in chromatography suggesting that this injection volume fit for this method application. Precision of internal standards through sample batches of 167 injections, obtained good %RSD peak area/peak area ratio values overall for n=119: 17 (±10) % for surface waters, 18 (±8) % for effluent, and 20 (±6) % for influent.

2.4.2.3 Filter recovery test

This multi-analyte method included a broad variety of chemistries, therefore a recovery investigation after the use of PTFE filters was completed for all types of matrices. This was done in a non-matrix (ultrapure water) and matrix-matched standards prepared at the medium concentration of the calibration line, 500 ng/L, including internal standards. Individual recoveries can be found Table A.7 from Appendix E. Overall, average percentages of recovery results for all compounds were high for every matrix tested: 93

(±21), 92 (±16), 95 (±24) and 105 (±25) %, for surface waters, influent, effluent and ultrapure (UP) water, respectively. This could be due to the hydrophilic PTFE filters, resulting in lower interactions with various analytes. This leads to lower compound binding, therefore recoveries are increased. However, certain compounds obtained low recoveries such as macrolide antibiotics which obtained the lowest values, except for influent wastewater matrices. Log *D* values of all compounds were predicted using an *in silico* (ACD/Labs) method at the measured pH of every matrix tested. These values were plotted against the recoveries obtained (Figure 2.19). This relates the chemistry of the compound with the recovery value obtained and it can be observed that macrolide antibiotics which contain similar chemistries between them were at the low range of the charts, except for influent wastewater. This could be because influent composite had already analyte in the samples at high concentrations, saturating the membranes of the filter, and/or competitive binding of matrix from wastewater. Once the filter is saturated, no more binding can happen, as these filters contain small surface areas, it will mainly depend on the concentration of the sample. The lower the concentration, the higher the saturation needs to be for the surface. Consequently, a full volume of 1 mL (<2 mL recommended by the manufacture) was always used using the same filter in order to get that full saturation to avoid possible losses.

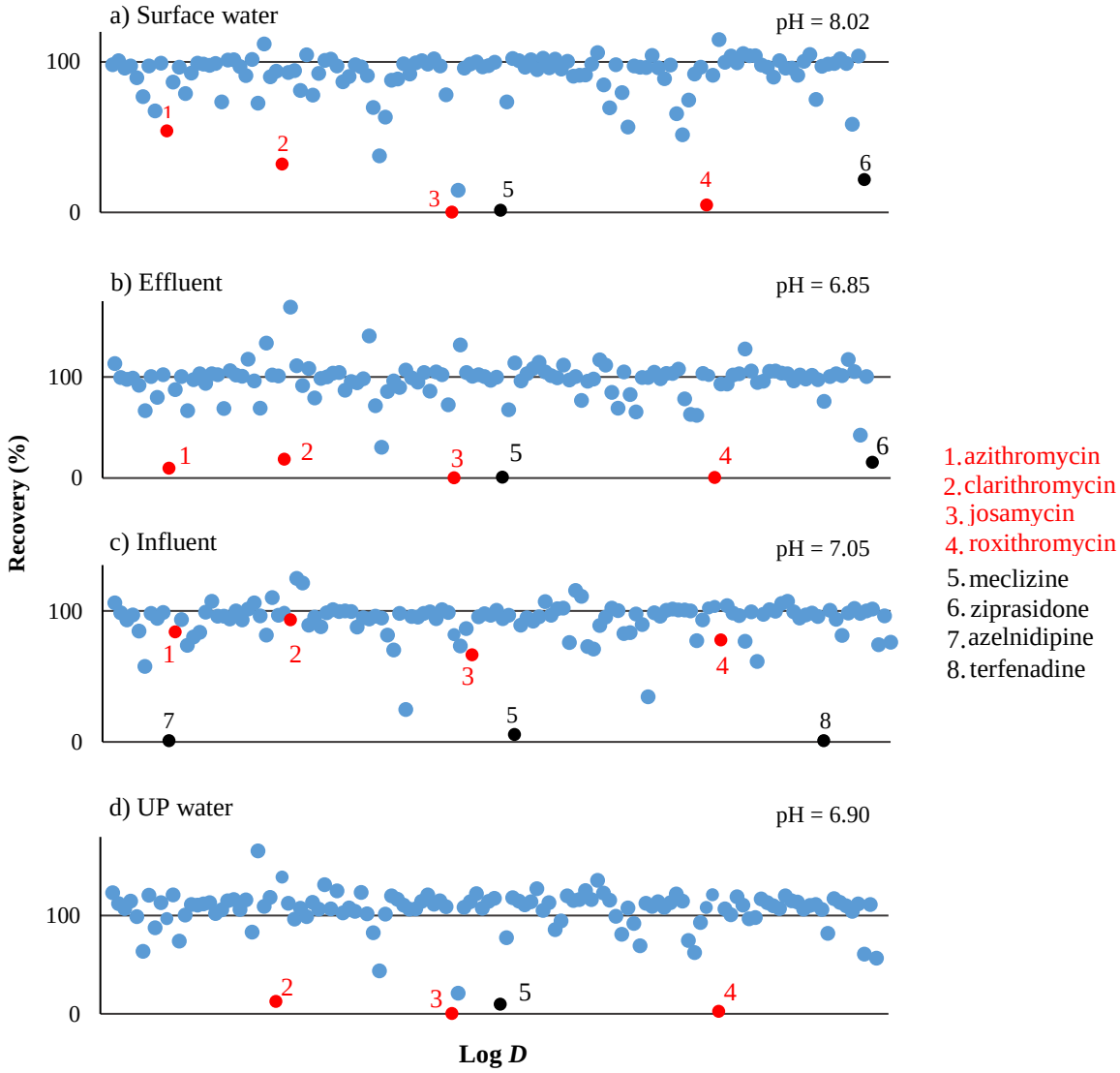


Figure 2.19 Recoveries vs log D data at a specific pH of a 1000 ng/L sample prepared in matrix (a) surface waters, (b) effluent, (c) influent and (d) UP water.

2.4.2.4 Stability experiments

All samples were shipped from Dublin to London in order to perform a broader screening analysis by LC-MS/MS. They were sent in a frozen state to prevent degradation/ transformation of the compounds. On arrival to the laboratory, samples were still ice cold. However, the stability was assessed as the worst case scenario for a period of 48 hours (maximum transport time between the two laboratories), at room temperature instead of fridge temperature (+4°C). The absolute mean instability across all compounds was 2

(±14) % for surface waters, 1 (±14) % for influent wastewater and 2 (±12) % for effluent wastewater, all of them for the n=3 replicates prepared. These values show great stability across all the compounds tested. However, some analytes presented values >±20% of instability (Table A.8, Appendix E). The highest instability found relates to amiodarone (-106%), azelnidipine (85%) and azelnidipine again (-104%) for surface waters, influent and effluent wastewaters, respectively. The change in matrix could have resulted in ion suppression or that these compounds transformed rapidly over time.[144] Moreover, both analytes are slightly soluble in water[264,265] and rapid photodegradation of azelnidipine has been reported previously when the drug was exposed to sunlight.[266] All these compounds were reported when detected, but considering that there was no possible way to take accurate stability into account form every sample received. SIL-IS compounds were tested for stability too and included in the mean calculations but they were added during preparation of the samples just before their analysis. This means that all of SIL-IS compounds have been taken into account.

2.4.3 Method performance

Method performance was carried out in all the matrices used in the study, surface waters, influent and effluent wastewater for all methods, in order to ensure their fitness for purpose for application to real samples.

2.4.3.1 Method 1.2: SPE LC-MS/MS

All method performance data obtained can be observed in Tables 2.8, 2.9 and 2.10 for every type of matrix. Linearities obtained >0.90 coefficients of determination (R^2) values all compounds except for hormones. For surface waters R^2 values of 0.8454 and 0.8891 were determined for E2 and EE2 respectively. Effluent just showed one hormone, EE2, with 0.8505; influent wastewater matrix had E2 with 0.8509 and E1 with 0.8601. These

type of compounds are known by their poor stability and inaccurate analysis,[267] they are normally kept in the dark to avoid photolysis and in cool conditions.[138] The extraction procedure was not possible to be carried out in light-sensitive conditions and the autosampler did not have a temperature controlled system. This could lead to further instability for these specific compounds achieving lower linearities apart of possible matrix interferences. Previous studies have reported their quantification using regression lines prepared in solvent without the matrix getting $R^2 > 0.99$,[268] however, matrix effects are usually quite high in these matrices and quantification results will lose reliability. Therefore, compounds obtaining $R^2<0.90$, will not be reported with quantification data but qualitative. Clarithromycin peak shape presented split peaks due to a matrix interference peak close to the elution time in wastewater, both effluent and influent. The use of peak height can be used in these situations, however, clarithromycin was included on the DI method optimised (method 2) with no peak adjacent to it, therefore the matrix interference was not further investigated and data for this compound will be reported by the use of DI method, where no interferences were observed.

Precision of the peak areas of the analytes were good overall considering SPE extraction, imprecisions obtained were 30 (±16), 24 (±19) and 28 (±11) % across all samples, for surface waters, influent and effluent, respectively. These values are higher than the ones obtained by the DI method due to the extra procedural steps (e.g. SPE). Inaccuracy was determined with average values of inaccuracy of 9 (±35), 27 (±21), and 22 (±29) % across all compounds for surface waters, influent and effluent respectively. It can be seen that the more complex the sample is; the average inaccuracy is higher (surface waters < effluent < influent). Matrix effects (ME) are common when using ESI mode in LC-MS/MS and they were calculated resulting in average values of 14 (±119), 30 (±72), and 27 (±50) % for surface waters, influent and effluent respectively. For

surface waters the highest value was amoxicillin for enhancement (335%) and erythromycin (-92%) for suppression. For influent, values ranged from 94% of diclofenac for enhancement, to -97% for suppression for both octocrylene and triclosan. Effluent samples also suffered from enhancement, with highest value for ciprofloxacin (57%) and suppression erythromycin (-92%). All compounds in all matrices had considerable matrix effects, this could be caused by dissolved organic and inorganic matter, pH, turbidity and sample source.[269] ß-lactam antibiotics such as amoxicillin and ciprofloxacin have been reported as instable and easily degradable in aqueous solutions,[270] and previous studies in surface waters using SPE obtained values of matrix effects between 297 – 501% of enhancement for this type of compounds. Diclofenac, azithromycin and E1 were also reported with an enhancement of 196, 237 and 152% respectively.[270] Ion suppression of erythromycin and clarithromycin have been previously reported as -81 and -68% respectively.[270,271] Triclosan ion suppression values have been observed to increase depending on the matrix, where raw influent showed the lowest highest values in a previous study with approximately -90%,[196] close to the value obtained within this thesis (-97%). Overall, values are comparable with our study, where they are either similar or lower, however, the difference in sample volume should be noted, as one litre or 500 mL of sample were used in reported methods compared to our 100 mL, with the possibility of increasing matrix effects. Accounting that they were obtained using surface waters, matrix effects in this study have been more successfully removed. Moreover, the use of internal standards[272] and three different matrix-matched calibration standards,[273] one per matrix, were used in order to compensate these matrix effects. Matrix effects were also determined using ultrapure water for comparison, and some studies recommend the use of synthetic wastewater in order to obtain more accurate results, ensuring that no analytes are present on the matrix itself but adding complexity to the matrix.[172] Additionally,

matrix effects can affect recoveries significantly,[274] however, these were considered acceptable as discussed as follows. Recoveries obtained varied depending on the matrix, for surface waters they ranged from 77 (clarithromycin) to 173% (triclosan). For influent samples ranged from 58 (octinoxate) to 140% (EE2). The low value obtained for octinoxate could be due to the ion suppression measured by the ME obtained of -96%. Effluent wastewater showed recoveries from 85 (E2) to 178% (BP-4). This was the highest recovery obtained throughout the SPE method and could be associated to the 56% ME enhancement achieved. Acceptable recovery ranges are usually reported from 50 to 150%;[270] only triclosan (surface waters and effluent) and benzophenone-4 (effluent) did not meet this criteria, however, as mentioned previously could be due to their high matrix effects.

LODs were determined for all compounds acquired where, as expected, lower values were obtained for surface water matrix, ranging from 0.045 (triclosan) to 2.21 (clarithromycin) ng/L. Influent wastewater presented values ranging from 0.174 (octocrylene) to 5.3 (E1) ng/L and for effluent they varied from 0.144 (diclofenac) to 1.95 (EE2) ng/L. For LOQs they varied from 0.137 (triclosan) to 6.69 (clarithromycin) ng/L; 0.526 (octocrylene) to 16.1 (E1) ng/L; and 0.436 (diclofenac) to 5.9 (EE2) ng/L, for surface waters, influent and effluent matrices respectively. Even that low values were obtained overall, estrogen hormones did not achieved the target LODs presented in Table 1.3. However, a study performed by Z. D. Jauković *et al*.[112] reported LOQs of 9.7, 22.7 and 8.7 ng/L for surface waters, and 10, 10.7 and 29.3 for effluent for E1, E2 and EE2, respectively. All these limits were significantly higher than the ones obtained within this study, where they loaded a sample volume of 200 mL and used an APCI source for their detection, both parameters are known to enhance hormones sensitivity further;[112] APCI sources have shown reduced matrix effects compared to ESI.[275] Other studies have also

reported higher values than the ones stipulated by the WL,[117] even 25, 62 and 49 ng/L for river, effluent and influent for EE2 respectively,[267] and the difficulty of reaching the low LODs and LOQs for these compounds is common across literature.[276] As mentioned before, these compounds are really challenging when it comes to analysis due to their rapid degradation and low expected concentrations in the environment (<10 ng/L).[260] Sensitivity of the method depends on different parameters such as the extraction technique, the source used for analysis, etc. Every effort was made when developing the SPE method in order to increase their sensitivity. However, there is one suggestion that was not possible that could decrease LODs, the volume of sample loaded onto the SPE cartridge. This will increase the concentration factor overall, however, matrix effects might also escalate compromising other compounds in the method and an investigation would need to be assessed. Moreover, "self-elution" of the compounds as well as blockages on the cartridges can occur. Nevertheless, the LODs obtained for hormones compounds, fit for the purpose of the study.

Table 2.8 Summary of method performance for the SPE LC-MS/MS method in surface water matrix based on ICH guidelines.[231]

Analyte	Range (ng/L)	Recovery ± SD (%,n≥3)	Linearity (n≥5) R^2	Peak area precision (%RSD, n≥3)	Matrix Effect (%CV, n≥3)	Inaccuracy (%CV, n≥3)	LOD (ng/L)	LOQ (ng/L)
E2*	5 - 500	128 ± 14	0.8454	14	-55	-18	0.69	2.09
EE2*	3 - 500	87 ± 14	0.8891	16	-55	59	0.080	0.24
E1*	3 - 1000	147 ± 7	0.9832	11	-46	-19	1.63	4.94
Amoxicillin*	3 - 500	84 ± 9	0.9703	51	335	21	0.941	2.85
Azithromycin*	3 - 500	137 ± 32	0.9768	35	71	-37	0.362	1.10
Benzophenone-4*	10 - 500	131 ± 20	0.9953	57	11	20	0.555	1.68
Ciprofloxacin*	3 - 500	134 ± 29	0.9511	29	168	26	0.646	1.96
Clarithromycin*	3.5 - 500	77 ± 55	0.9287	54	-70	53	2.21	6.69
Diclofenac*	3 - 500	100 ± 20	0.9848	20	-25	56	0.334	1.01
Erythromycin*	10 - 500	99 ± 38	0.9792	35	-92	-12	1.14	3.47
Octinoxate*	3 - 500	109 ± 17	0.9772	17	5	-34	1.20	3.65
Octocrylene*	10 - 500	118 ± 22	0.9957	22	-0.2	26	0.514	1.56
Triclosan*	3 - 500	173 ± 26	0.9836	26	-65	-28	0.0453	0.137

* SIL-IS used for peak area ratio linearity.

Table 2.9 Summary of method performance for the SPE LC-MS/MS method in influent wastewater matrix based on ICH guidelines.[231]

Analyte	Range (ng/L)	Recovery ± SD (%,n≥3)	Linearity (n≥5) R^2	Peak area precision (%RSD, n≥3)	Matrix Effect (%CV, n≥3)	Inaccuracy (%CV, n≥3)	LOD (ng/L)	LOQ (ng/L)
E2*	5 - 100	90 ± 30	0.8509	57	-69	45	0.342	1.04
EE2*	5 - 100	140 ± 32	0.9116	32	-4	30	0.473	1.43
E1*	5 - 1000	88 ± 23	0.8601	1	-98	4	5.30	16.1
Amoxicillin*	3 - 500	79 ± 17	0.9768	15	-88	5	1.12	3.39
Azithromycin*	3 - 100	111 ± 12	0.9843	12	23	58	0.198	0.600
Benzophenone-4*	3 - 500	97 ± 15	0.9730	15	66	15	1.22	3.70

Ciprofloxacin*	3 - 100	110 ± 12	0.9442	12	-36	34	0.381	1.16
Clarithromycin*	-	-	-	-	-	-	-	-
Diclofenac*	3 - 500	87 ± 13	0.9888	13	94	16	0.875	2.65
Erythromycin*	3 - 500	92 ± 9	0.9804	9	43	-5	1.01	3.06
Octinoxate*	3 - 100	58 ± 40	0.9872	57	-96	56	0.184	0.558
Octocrylene*	3 - 500	75 ± 22	0.9886	27	-97	48	0.174	0.526
Triclosan*	5 - 100	60 ± 38	0.9743	38	-97	20	0.247	0.748

* SIL-IS used for peak area ratio linearity.

Table 2.10 Summary of method performance for the SPE LC-MS/MS method in effluent wastewater matrix based on ICH guidelines.[231]

Analyte	**Range (ng/L)**	**Recovery ± SD (%,n≥3)**	**Linearity (n≥5) R^2**	**Peak area precision (%RSD, n≥3)**	**Matrix Effect (%CV, n≥3)**	**Inaccuracy (%CV, n≥3)**	**LOD (ng/L)**	**LOQ (ng/L)**
E2*	3.5 - 1000	85 ± 60	0.9589	37	-86	-31	1.63	4.93
EE2*	3 - 250	87 ± 34	0.8505	23	-28	34	1.95	5.90
E1*	3 - 1000	91 ± 18	0.9787	16	-77	10	1.20	3.63
Amoxicillin*	3.5 - 500	104 ± 49	0.9702	41	-33	51	1.42	4.31
Azithromycin*	3.5 - 500	89 ± 18	0.9954	18	-59	38	0.202	0.611
Benzophenone-4*	5 - 500	178 ± 15	0.9073	15	51	56	1.26	3.81
Ciprofloxacin*	3 - 500	115 ± 12	0.9607	12	57	51	1.61	4.87
Clarithromycin*	-	-	-	-	-	-	-	-
Diclofenac*	3 - 100	114 ± 26	0.9918	26	-22	35	0.144	0.436
Erythromycin*	3 - 500	93 ± 43	0.9773	43	-92	-15	1.01	3.05
Octinoxate	3 - 100	155 ± 48	0.9872	28	-4	21	0.178	0.539
Octocrylene*	3 - 100	119 ± 62	0.9845	39	-47	28	0.177	0.535
Triclosan*	3.5 - 100	183 ± 54	0.9870	39	16	-14	0.180	0.547

* SIL-IS used for peak area ratio linearity.

2.4.3.2 Method 2: Direct injection LC-MS/MS

In order to simplify the data obtained for this method, only a summary of method performance data from the compounds detected on the samples has been presented in Tables 2.11, 2.12 and 2.13. Results are discussed by type of matrix and data for individual analytes are found in Table A.9 – A.11, Appendix E.

As previously mentioned, LODs and LOQs can be challenging when using complex matrices as they can be already present in the sample. When samples did not present the compound already in the sample, all LODs and LOQs were checked against the standards run on the instrument and therefore replaced by the lowest calibrant detected with a signal to noise (S/N) of 5 and 10, respectively. Therefore, instrumental LODs and LOQs were considered for them.

2.4.3.2.1 Surface waters

For surface waters, linearity presented an average of coefficients of determination of R^2=0.9950 (± 0.0010) for 15 compounds found on the samples tested including 5 IS compounds, where values ranged from 0.9928 to 0.9967. Therefore, all compounds obtained $R^2 \geq 0.99$ using ≥5 points of the calibration line (5 – 12 points). Precision of the peak areas of the analytes were good overall as imprecision at two levels tested, 100 and 1000 ng/L, were 7 (±4) and 5 (±2) % on average. Only hydrochlorothiazide had >15% RSD at 100 ng/L, however, at 1,000 ng/L all compounds were below this threshold. Accuracy at four levels was assessed and was considered acceptable with average values of inaccuracy of 3 (±4), 1 (±3), 4 (±1) and 1 (±1) %, for 100, 250, 750 and 1,000 ng/L respectively. At all concentrations all compounds had inaccuracies below ±20%. Matrix effects absolute averages were 8 (±21) and 31 (±17) % at both concentrations for 100 and 1,000 ng/L, respectively. The highest value achieved for enhancement was for clozapine with 69 and 67% for 100 and 1,000 ng/L, respectively. These results are in accordance

with previous studies as clozapine has been reported with ion enhancement for river water samples when using SPE, 88%,[277] and direct injection, 59%.[278] Ion suppression was only observed at 100 ng/L with a minimum value of only -15% for salbutamol. LODs were determined for all compounds acquired 4 (±0) ng/L and LOQs ranged from 11 to 50 (hydrochlorothiazide) ng/L where the average LOQ was 14 (±10) ng/L. Hydrochlorothiazide LOQ was replaced with the lowest calibrant having a 10 S/N ratio with the background noise of the chromatograms, therefore limited sensitivity was achieved for this analyte in terms of quantification with the highest LOQ reported. Hydrochlorothiazide is prescribed for long-term treatments[279] and it is not metabolised by the human body where a minimum of 61% of the oral dose is eliminated by the kidney unchanged within 24 hours.[280] This results in high occurrence, however, concentrations depend on the consumption factor[279] and surface water concentrations have been previously reported between 12 – 8,700 ng/L.[281] A method performed using SPE for pre-concentration of this analyte reported an LOQ of 35 ng/L when using 500 mL of sample, therefore, the LOQ achieved, close to the lower concentrations reported, fits for purpose demonstrating the overall sensitivity achieved when using the direct injection method developed. As overall all compounds succeeded validation parameters, quantification values can be reported on all samples for this type of matrix.

2.4.3.2.2 Influent wastewater

Influent wastewater corresponded to the most complex matrix tested, however, an average of coefficients of determination of R^2=0.9832 (±0.0198) was obtained for linearity, this value was acquired from the 54 compounds found on the samples. R^2 ranged from 0.8798 to 0.9958 and all compounds used ≥5 points of the calibration line (5 – 12 points), where 61% obtained $R^2 \geq 0.98$ values. However, cyromazine obtained an $R^2 < 0.90$, 0.8798, therefore this compound is reported qualitatively. Peak areas precision

of the analytes were tested as imprecision at 100 and 1,000 ng/L and values obtained were 8 (±5) and 8 (±3) % on average. Only 7% of these compounds had ≥15% RSD at 100 ng/L corresponding to three pharmaceuticals and one pesticide: amitriptyline, amlodipine, nordiazepam and cyromazine. Nevertheless, only two compounds amlodipine and ketoconazole, at 1,000 ng/L were above this threshold; therefore, precision values are good overall. Inaccuracy average percentage values of 8 (±17), 3 (±10), 3 (±5) and 4 (±4) % were obtained for 100, 250, 750 and 1,000 ng/L respectively. Consequently, accuracy was also considered acceptable. Matrix effects achieved the highest values from all matrices as expected, due its complexity. They were performed at two different concentrations, 100 and 1000 ng/L, where absolute percentage average values were 37 (±138) and 37 (±87) respectively. The highest value corresponded for azithromycin for enhancement (936 and 590%), and cyromazine (-87 and -82%) for ion suppression, for both concentrations respectively. Macrolide antibiotics have been previously reported with high matrix effects, even severe.[140,144,282] Higher values are usually obtained in influent wastewater compared to effluent, as per results obtained, especially for azithromycin and clarithromycin.[282] This is due to the complexity of the matrix containing interferences and organic matter as well as the higher dissolved organic carbon (DOC) content.[283] Azithromycin has been reported with high percentages of ion enhancement even in surface waters (237%) where matrix interferences are usually lower.[270] These results were achieved using SPE which is declared to reduce matrix effects by removing interferances,[206] however, still high enhancement or suppression issues are observed for these analytes (e.g. erythromycin in influent with 743%).[268] Matrix effects could also explain the low R^2 and high imprecision obtained for cyromazine probably caused by the ion suppression. Even that higher matrix effects were observed for some compounds, LODs acquired 4 (±1) ng/L for the majority of the

compounds and LOQs ranged from 10 (diclofenac and clarithromycin) to 500 (cymoxanil) ng/L. The average LOQ was 14 (±74) as several analytes had values replaced with the lowest calibrant having a 10 S/N ratio with the background, due to poorer sensitivities. Cymoxanil presented the highest LOQ value and this could explain the limited number of studies reporting this analyte. Kiefer *et al.* mentioned its instability during analysis leading to low sensitivities when using a biphenyl column.[284] Overall, almost all compounds had acceptable method performance results, however, cyromazine did not meet linearity criteria (≥0.90) and as mentioned before, qualitative data is only reported.

2.4.3.2.3 Effluent wastewater

An average of coefficients of determination of R^2=0.9793 (±0.0212) was determined for linearity for a total of 39 compounds and 13 correspondents SIL-IS compounds using ≥5 points of the calibration line (5 – 12 points) for all of them. R^2 values ranged from 0.9197 to 0.9968, however, the majority of the compounds (62%) had values of R^2≥0.98. Peak area imprecision from the replicates at 100 and 1,000 ng/L were on average 8 (±5) and 10 (±4) % respectively. Therefore, analytes showed good precision achieved for this type of matrix using the DI method. Compounds presenting >15% RSD resulted only on 8% and 10% of the total compounds for 100 and 1,000 ng/L respectively. Accuracy at four levels was evaluated as well and was considered suitable. Average values of inaccuracy obtained were 11 (±11), 3 (±8), 2 (±3) and 3 (±3) %, for the following respectively concentrations 100, 250, 750 and 1,000 ng/L. For matrix effects, absolute average values were 16 (±36) and 10 (±12) % at both concentrations respectively. The highest value at 100 ng/L was determined for fluoxetine (120%) showing ion enhancement and hydrochlorothiazide (-88%) for ion suppression. At 1,000 ng/L, fluoxetine showed an enhancement of 20% and salbutamol showed the higher ion suppression achieved, -37%.

Ion suppression values for hydrochlorothiazide and salbutamol have been reported before at -40 and -90% respectively for SPE methods in this matrix,[271] however, fluoxetine has been reported with ion suppression instead of enhancement across literature.[272,285,286] This is probably due to the different matrix properties (e.g. treatment used, organic matter, etc.). Regarding method sensitivity, the average LOD and LOQ are 5 (±2) and 19 (±16) ng/L. The lowest LOD and LOQ acquired corresponds to 3 and 6 ng/L (hydrochlorothiazole) and maximum to 14 and 72 ng/L (trimethoprim), respectively. Overall, all compounds had acceptable method performance results and are quantitatively reported.

To summarise the above section (2.4.3.2, method 2), accuracies and precisions were good overall in all matrices tested, this could be due to no deviations or drifts in peak areas or retention times; facilitated by the low injection volume used of 10 µL, improving chromatographic and mass spectrometry responses enabling long batch sequences. The low injection volume selected probably minimised the matrix effects, playing an important role as dilution as usually the higher the concentrations the higher the matrix effects.[268] The mechanisms of matrix effects probably initiate in competition between the compound and interference substances which co-elute at the same time, however, it is still not clear but highly dependent on the sample and the compound.[283] The matrix can act as a dopant ionising sample components, non-volatiles, resulting in signal enhancements or suppressions. In order to compensate matrix effects, calibrations were performed using matrix-matched standards[273] and internal standards were used to compensate when possible.[272] As matrix effects varied widely between the aquatic matrices tested, three different calibrations were performed (one per matrix). Moreover,

to avoid false positive detections, two fragment ions per analyte are monitored, adding an extra layer of selectivity.

Regarding sensitivity, LOQs achieved within this study were compared to other studies from literature using direct injection for all matrices tested, however, not many studies pose the same common analytes. Results can be observed in Table 2.14, where overall some methods are similar or slightly more sensitive than the one proposed. Examples include the study by Boix *et al.*[154], Oliveria *et al*.[156], Hermes *et al*., [287] and Hao *et al*.,[288] however, all these methods used higher injection volumes ranging from 50 to 100 µL, resulting in up to a ten-fold increase compared to our method. Moreover, other studies not only presented higher injection volumes but also separate runs for positive and negative ESI modes with run times of up to 30 minutes.[73,156] Nevertheless, a full comparison cannot be achieved as there are only few compounds in common. Overall, even that sensitivities were lower in some cases, the small volume injected resulted in less matrix effects[272] and longer analysis batches were able to run obtaining excellent precision and accuracy results as mentioned before.

Table 2.11 Summary of method performance of compounds detected for surface water matrix based on ICH guidelines.[231]

15 analytes (5 IS)	Range (ng/L)	Linearity (n≥5) R^2	Peak area/height precision (%RSD, n=6)		Matrix Effect (%CV, n=6)		Inaccuracy (%CV, n≥3)				LOD (ng/L)	LOQ (ng/L)
			100 ng/L	1000 ng/L	100 ng/L	1000 ng/L	100 ng/L[a]	250 ng/L[a]	750 ng/L[a]	1000 ng/L[a]		
Bisoprolol	5 - 5000	0.9955	5	5	2	33	-2	0	4	2	4	11
Carbamazepine	10 - 5000	0.9949	7	5	-10	19	-1	-2	3	1	4	12
Citalopram	25 - 5000	0.9947	5	5	28	48	-2	2	4	1	4	11
Clozapine	5 - 5000	0.9957	7	3	69	67	-2	1	4	2	4	11
Diphenhydramine	5 - 5000	0.9961	6	5	18	36	-3	1	5	2	4	12
Fenuron	10 - 5000	0.9928	9	3	-9	4	-11	-5	2	1	4	11
Hydrochlorothiazide	75 - 5000	0.9941	17	6	10	13	-3	-2	3	2	4	50[b]
Lidocaine*	5 - 5000	0.9967	3	4	3	29	0	-1	5	2	4	12
Propamocarb	25 - 5000	0.9941	3	5	-9	21	-15	-7	2	0	4	12
Propranolol	25 - 5000	0.9951	5	7	3	30	-1	2	4	1	4	13
Salbutamol	10 - 5000	0.9954	4	4	-15	3	1	-1	4	2	4	14
Tramadol*	5 - 5000	0.9960	3	4	-1	30	-4	-3	3	0	4	13
Trimethoprim*	5 - 5000	0.9938	8	7	7	41	-2	-2	4	2	4	11
Venlafaxine*	5 - 5000	0.9955	8	7	5	33	-2	-1	5	2	4	12
Verapamil*	5 - 5000	0.9950	11	10	21	51	-1	1	6	1	4	12
Minimum		0.9928	3	3	-15	3	-15	-7	2	0	4	11
Maximum		0.9967	17	10	69	67	1	2	6	2	4	50
Absolute Median		0.9951	6	5	3	30	2	1	4	2	4	12
Absolute Mean (±SD)		0.9950 ± (0.0010)	7 ± (4)	5 ± (2)	8 ± (21)	31 ± (17)	3 ± (4)	1 ± (3)	4 ± (1)	1 ± (1)	4 ± (0)	14 ± (10)

*SIL-IS used for peak area ratio linearity assessment.
[a]for 250 and 750 ng/L n=3 and for 100 and 1000 ng/L n=6.
[b]LOQ taken as the lowest matrix-match calibration standards with 10 S/N signal.

Table 2.12 Summary of method performance of compounds detected for influent wastewater matrix based on ICH guidelines.[231]

54 analytes	Range (ng/L)	Linearity (n≥5) R^2	Peak area/height precision (%RSD, n=6)		Matrix Effect (%CV, n=6)		Inaccuracy (%CV, n≥3)				LOD (ng/L)	LOQ (ng/L)
			100 ng/L	1000 ng/L	100 ng/L	1000 ng/L	100 ng/L[a]	250 ng/L[a]	750 ng/L[a]	1000 ng/L[a]		
Acetamiprid	75 - 5000	0.9843	4	7	-14	-2	-2	-2	4	5	4	50[b]
Ametryn	10 - 5000	0.9939	4	9	4	18	0	0	5	5	4	12
Amitriptyline*	100 - 5000	0.9735	25	7	86	56	-7	-1	3	6	6	100[b]
Amlodipine	100 - 5000	0.9785	23	15	189	164	-20	-3	-2	2	3	50[b]
Antipyrine	25 - 5000	0.9806	10	11	6	10	2	1	6	6	5	19
Atorvastatin	5 - 5000	0.9757	9	8	29	33	-11	-2	2	3	6	20
Atrazine	5 - 5000	0.9891	6	7	-2	16	2	1	5	5	5	16
Azithromycin	5 - 5000	0.9563	9	5	936	590	-29	-12	1	2	4	11
Antipyrine	25 - 5000	0.9806	10	11	6	10	2	1	6	6	5	19
Benztropine	25 - 5000	0.9866	7	12	120	77	-2	6	3	5	4	13
Bisoprolol	10 - 5000	0.9698	5	7	31	41	-18	-8	2	3	4	12
Carbamazepine	25 - 5000	0.9831	6	7	9	13	-35	-19	-3	-2	5	15
Carbamazepine epoxide	10 - 5000	0.9941	10	7	9	25	-8	-4	3	4	4	13
Carboxin	25 - 5000	0.9907	11	6	3	4	-2	3	4	4	4	13
Citalopram	75 - 5000	0.9902	14	3	46	29	-28	-13	-3	0	4	12
Clarithromycin*	250 - 5000	0.9698	4	2	-64	35	-50	-28	-10	-7	4	10
Clopidogrel	5 - 5000	0.9912	8	8	8	-5	-6	-1	4	4	4	14
Clozapine	5 - 5000	0.9958	9	10	65	18	-7	-1	4	4	4	12
Cymoxanil	500 - 5000	0.9651	n.d.	16	n.d.	35	n.d.	n.d.	2	3	5	500[b]
Cyromazine	75 - 5000	0.8798	18	6	-87	-82	-51	-30	-10	-8	6	18
Diclofenac	25 - 5000	0.9753	7	4	-21	4	-41	-22	-5	-3	4	10

Diphenhydramine	10 - 5000	0.9928	11	8	30	24	-15	-6	2	3	4	13
Fenuron	5 - 5000	0.9934	8	9	-3	14	-14	-6	2	2	4	13
Fluoxetine*	25 - 5000	0.9874	10	14	-7	15	-9	3	0	3	4	11
Flurochloridone	500 - 5000	0.9442	n.d.	11	n.d.	56	n.d.	n.d.	2	4	5	100[b]
Hydrochlorothiazide	75 - 5000	0.9664	6	13	38	8	-32	-15	-3	0	4	12
Ketoconazole	250 - 5000	0.9596	n.d.	21	n.d.	23	n.d.	2	2	3	3	250[b]
Lidocaine*	5 - 5000	0.9875	2	7	-21	-8	-14	-7	2	3	4	14
Lincomycin	25 - 5000	0.9840	8	7	185	181	1	1	5	5	5	16
Mefenamic acid	5 - 5000	0.9821	4	9	18	37	-34	-15	-3	-2	5	17
Memantine	25 - 5000	0.9919	6	9	-3	15	-10	-5	3	3	4	11
Methylphenidate*	5 - 5000	0.9950	8	9	16	43	-1	-1	5	5	4	12
Metoprolol*	5 - 5000	0.9902	10	8	19	4	-8	-4	3	4	4	13
Nordiazepam	100 - 5000	0.9793	15	9	0	44	-2	-3	5	5	4	50[b]
Nortriptyline*	5 - 5000	0.9832	11	13	40	53	-5	3	4	4	4	13
Prometryn	25 - 5000	0.9909	4	6	2	18	0	0	5	5	4	13
Propamocarb	25 - 5000	0.9637	5	11	28	-4	2	2	8	7	7	27
Propranolol	10 - 5000	0.9753	9	10	-12	17	-13	-6	3	4	4	12
Pyracarbolid	25 - 5000	0.9900	8	9	-28	-16	1	-3	5	5	4	14
Risperidone*	5 - 5000	0.9923	7	8	84	87	0	0	4	5	4	14
Ronidazole	50 - 5000	0.9500	9	6	-7	-10	-2	5	7	8	8	41
Salbutamol	5 - 5000	0.9808	5	7	-31	-24	-12	-6	3	2	6	21
Simazine	25 - 5000	0.9910	10	7	0	11	-2	0	5	4	4	13
Sulfamethoxazole	50 - 5000	0.9529	11	13	17	20	-24	-10	-1	2	4	11
Sulfapyridine	25 - 5000	0.9890	4	7	0	18	-32	-16	-3	-1	4	15
Tamsulosin	10 - 5000	0.9936	10	7	39	56	1	0	4	5	4	13
Temazepam*	10 - 5000	0.9795	11	6	24	32	-17	-7	3	3	4	12

Terbutryn	5 - 5000	0.9943	3	8	1	10	-2	0	4	5	4	13
Timolol	5 - 5000	0.9931	5	7	-16	-3	2	0	5	5	4	15
Tramadol*	25 - 5000	0.9916	3	5	-12	1	-38	-20	-5	-3	4	13
Trimethoprim*	10 - 5000	0.9814	3	5	7	32	-36	-19	-4	-3	5	16
Valsartan	250 - 5000	0.9622	7	8	-28	39	-54	-32	-13	-9	4	12
Venlafaxine*	10 - 5000	0.9716	1	7	14	33	-49	-28	-10	-7	5	16
Verapamil*	10 - 5000	0.9917	3	12	104	101	1	1	5	5	4	14
Minimum		0.8798	1	2	-87	-82	54	32	13	9	3	10
Maximum		0.9958	25	21	936	590	2	6	8	8	8	500
Absolute Median		0.9792	8	8	7	18	14	6	1	2	4	33
Absolute Mean (±SD)		0.9836	8	8	37	37	8	3	3	4	4	14
		±	±	±	±	±	±	±	±	±	±	±
		(0.0188)	(5)	(3)	(138)	(87)	(17)	(10)	(5)	(4)	(1)	(74)

*SIL-IS not use in this type of matrix.
[a]for 250 and 750 ng/L n=3 and for 100 and 1000 ng/L n=6.
[b]LOQ taken as the lowest matrix-match calibration standards with 10 S/N signal.
n.d.: not detected or signal < 5 S/N.

Table 2.13 Summary of method performance of compounds detected for effluent wastewater matrix based on ICH guidelines.[231]

39 analytes (13 IS)	**Range (ng/L)**	**Linearity (n≥5) R^2**	**Peak area/height precision (%RSD, n=6)**		**Matrix Effect (%CV, n=6)**		**Inaccuracy (%CV, n≥3)**				**LOD (ng/L)**	**LOQ (ng/L)**
			100 ng/L	**1000 ng/L**	**100 ng/L**	**1000 ng/L**	**100 ng/L[a]**	**250 ng/L[a]**	**750 ng/L[a]**	**1000 ng/L[a]**		
Acetamiprid	10 - 5000	0.9707	3	8	1	-19	-18	-6	2	3	4	11
Amitriptyline*	5 - 5000	0.9709	14	21	77	10	-4	6	0	6	5	16
Atorvastatin	50 - 5000	0.9822	15	11	4	-18	-4	2	6	4	4	50[b]
Bisoprolol	5 - 5000	0.9197	7	8	11	-9	-15	-4	3	4	5	13
Carbamazepine	5 - 5000	0.9936	5	9	27	-15	-25	-10	0	1	4	15

Carbamazepine epoxide	10 - 5000	0.9892	12	5	19	-16	-12	-5	3	3	4	12
Citalopram	5 - 5000	0.9539	6	8	40	8	-19	-5	1	2	5	16
Clarithromycin*	10 - 5000	0.9913	15	16	35	-4	2	0	3	6	4	14
Clopidogrel	5 - 5000	0.9934	5	10	2	-15	-2	1	4	4	4	13
Clozapine	5 - 5000	0.9929	3	8	92	19	-5	1	4	5	4	11
Diclofenac	50 - 5000	0.9563	14	9	-10	-14	-34	-16	-3	-1	4	12
Diphenhydramine	5 - 5000	0.9352	3	7	25	-3	-16	-4	3	4	6	16
Fenuron	10 - 5000	0.9841	5	7	1	-18	-15	-5	3	3	4	10
Fluoxetine*	5 - 5000	0.9851	16	20	120	20	-2	8	1	6	4	13
Hydrochlorothiazide	100 - 5000	0.9968	10	14	-88	-30	-45	-24	-7	-4	3	6
Lidocaine*	5 - 5000	0.9866	7	9	4	-6	-7	-6	1	4	5	18
Lincomycin	5 - 5000	0.9940	4	7	20	-1	-1	2	5	5	4	14
Mefenamic acid	10 - 5000	0.9796	18	12	-36	-20	-21	-9	1	1	4	13
Memantine	5 - 5000	0.9944	8	8	6	-14	-9	-1	3	4	4	13
Methylphenidate*	5 - 5000	0.9955	5	9	11	-13	0	-1	3	6	4	12
Metoprolol*	5 - 5000	0.9716	9	9	6	-13	-7	-5	3	5	4	12
Nordiazepam	50 - 5000	0.9765	12	12	12	-10	1	3	6	5	5	50[b]
Nortriptyline*	5 - 5000	0.9823	10	17	95	11	-3	6	1	7	4	12
Prometryn	5 - 5000	0.9925	5	10	-2	-16	-3	3	4	5	4	12
Propamocarb	25 - 5000	0.9912	3	10	-19	-20	-12	-3	3	3	4	11
Propranolol	10 - 5000	0.9940	4	5	2	-12	-11	-2	2	2	4	12
Risperidone*	10 - 5000	0.9921	8	12	34	1	2	0	4	6	4	14
Salbutamol	25 - 5000	0.9903	5	9	-23	-37	-7	0	4	4	4	13
Simazine	5 - 5000	0.9942	9	9	6	-18	-1	2	5	5	4	15
Sulfamethoxazole	10 - 5000	0.9921	10	10	10	-15	-10	-2	4	4	4	13
Sulfapyridine	5 - 5000	0.9907	7	7	14	-17	-13	-3	2	3	5	16
Tamsulosin	5 - 5000	0.9928	5	7	13	-12	-2	2	5	4	4	13

Temazepam*	5 - 5000	0.9728	4	7	12	-15	-7	-5	2	4	6	25
Terbutryn	5 - 5000	0.9922	4	11	-3	-16	-3	3	5	4	4	13
Tramadol*	5 - 5000	0.9246	3	6	5	-16	-32	-24	-9	-5	12	67
Trimethoprim*	5 - 5000	0.9878	6	8	25	-5	-21	-16	-3	-1	14	72
Valsartan	25 - 5000	0.9743	26	14	47	-5	-19	6	4	3	4	14
Venlafaxine*	5 - 5000	0.9224	10	10	-3	-17	-29	-19	-6	-2	10	47
Verapamil*	5 - 5000	0.9918	6	13	39	5	0	1	4	6	4	13
Minimum		0.9197	3	5	-88	-37	45	24	9	5	3	6
Maximum		0.9968	26	21	120	20	2	8	6	7	14	72
Absolute Median		0.9892	7	9	11	14	7	2	3	4	4	13
Absolute Mean (±SD)		0.9793	8	10	16	10	11	3	2	3	5	19
		±	±	±	±	±	±	±	±	±	±	±
		(0.0212)	(5)	(4)	(36)	(12)	(11)	(8)	(3)	(3)	(2)	(16)

*SIL-IS used for peak area ratio linearity assessment.
[a]for 250 and 750 ng/L n=3 and for 100 and 1000 ng/L n=6.
[b]LOQ taken as the lowest matrix-match calibration standards with 10 S/N signal.

Table 2.14 LOQ comparison with direct injection LC-MS/MS methods for compounds detected within this thesis in all matrices tested.

Analyte	**$LOQ_{Surface\ water}$ (ng/L)**		**$LOQ_{Influent}$ (ng/L)**		**$LOQ_{Effluent}$ (ng/L)**	
	Method 2	**Literature**	**Method 2**	**Literature**	**Method 2**	**Literature**
Acetamiprid	14	63 (LOD)[288]	50	-	11	20[73]
Amitriptyline	14	-	100	10[156]	16	10[156], 10[73]
Atorvastatin	12	0.8[154]	20	500[285], 25[156]	50	0.8[154], 500[285], 25[156]
Atrazine	14	-	16	-	14	10[73]
Azithromycin	8	-	11	-	11	20[73]
Antipyrine	15	-	19	-	75	30[73]
Carbamazepine	12	0.2[154],1[287]	15	5[287], 50[285], 10[156]	15	1.1[154], 5[287], 50[285], 10[156], 2[153], 10[73]

Carbamazepine epoxide	13	-	13	100^{285}, 10^{156}	12	100^{285}, 10^{156}, 10^{73}
Citalopram	11	10^{287}	12	15^{287}	16	15^{287}, 10^{73}
Clarithromycin	11	2.9^{154}, 5^{287}	10	10^{287}, 10^{156}	14	4.1^{154}, 2^{287}, 10^{156}, 10^{73}
Clopidogrel	12	0.5^{287}	14	0.5^{287}	13	0.5^{287}
Diclofenac	75	6.8^{154}, 2^{287}	10	5^{287}, 50^{285}, 50^{156}	12	7.2^{154}, 2^{287}, 50^{285}, 50^{156}, 10^{153}
Diphenhydramine	12	5^{287}	13	10^{287}, 10^{156}	16	10^{287}, 10^{156}, 5^{153}
Fluoxetine	11	-	11	50^{285}, 10^{156}	13	50^{285}, 10^{156}, 20^{73}
Hydrochlorothiazide	50	20^{287}	12	15^{287}, 250^{285}, 10^{156}	6	20^{287}, 250^{285}, 10^{156}, 10^{153}, 10^{73}
Lidocaine	12	2^{287}	14	10^{287}, 10^{156}	18	5^{287}, 10^{156}
Lincomycin	12	0.1^{154}	16	10^{156}	14	0.4^{154}, 10^{156}, 10^{73}
Mefenamic acid	250	-	17	10^{156}	13	10^{156}, 10^{73}
Methylphenidate	12	-	12	10^{156}	12	10^{156}
Metoprolol	11	5^{287}	13	20^{287}, 500^{285}, 10^{156}	12	15^{287}, 500^{285}, 10^{156}, 20^{73}
Propamocarb	12	-	27	-	11	10^{73}
Propranolol	13	-	12	50^{285}, 10^{156}	12	50^{285}, 10^{156}, 20^{73}
Salbutamol	14	-	21	-	13	20^{73}
Simazine	14	-	13	-	15	20^{73}
Sulfamethoxazole	14	0.5^{154}, 15^{287}	11	20^{287}, 250^{285}, 10^{156}	13	0.8^{154}, 35^{287}, 250^{285}, 10^{156}, 5^{153}, 20^{73}
Sulfapyridine	14	-	15	-	16	20^{73}
Temazepam	13	-	12	25^{156}	25	25^{156}
Terbutryn	11	1^{287}	13	1^{287}	13	1^{287}, 20^{73}
Tramadol	13	15^{287}	13	15^{287}, 50^{285}, 10^{156}	67	15^{287}, 50^{285}, 10^{156}
Trimethoprim	11	1.8^{154}, 10^{287}	16	10^{287}, 10^{156}	72	2.3^{154}, 10^{287}, 10^{156}, 5^{153}, 20^{73}
Valsartan	14	3.8^{154}, 5^{287}	12	10^{287}, 100^{285}, 10^{156}	14	4.2^{154}, 10^{287}, 100^{285}, 10^{156}
Venlafaxine	12	0.2^{154}, 2^{287}	16	5^{287}, 50^{285}, 10^{156}	47	1^{154}, 2^{287}, 50^{285}, 10^{156}, 20^{73}
Verapamil	12	-	14	10^{156}	13	10^{156}

-: compound not found in literature review for the matrix tested using a direct injection LC-MS/MS analysis method.

2.5 Conclusions

An analytical SPE extraction technique for the determination of 13 CECs, selected from literature review, in water matrices was developed. LC-MS/MS analysis for the targeted compounds was also developed. The final method was subjected to method performance investigation in three different matrices, surface waters, effluent and influent. Recoveries ranged from 84 – 173%, 58 – 140%, 85 – 183%, for surfaces water, influent and effluent respectively. Overall method performance was considered good, except for some compounds which obtained linearities with coefficient of determination R^2<0.90. These anlaytes included E2 and EE2 for surface waters, E1 and E2 for influent, and EE2 for effluent wastewater. Therefore, they are reported only with qualitative data throughout this thesis.

On the other hand, the selection of CECs was still quite limited compared to the amount of pollutants that have been previously reported in the environment with potential risks. Therefore, it was decided to widely broaden the number of compounds analysed. As a consequence, a DI LC-MS/MS method for the determination of different classes of CECs was implemented and optimised in different aquatic matrices (surface waters, influent and effluent wastewater). This resulted in a quantitative method for >100 compounds, being less time consuming than the SPE method and reduced cost of the total analysis. For its optimisation, the selection of flow rate and injection volume, 0.5 mL/min and 10 µL respectively, were selected and a filter recovery and stability investigations were performed. Results depend on the type of compound and matrix; therefore, they are taken into account when interpreting data in samples. Across all matrices, method performance experiments showed good values overall. Linearities for all compounds were R^2>0.90, except for cyromazine in influent wastewater, which did not meet the

criteria, as the linearity obtained was below this number. Consequently, it was not reported quantitatively within this thesis.

This study has confirmed the possibility for combination of different analytical methods in order to cover a wide number of compounds with different physicochemical properties; enabling their detection and quantification in water samples from Ireland in order to risk assess their impact in the environment.

3.0 A year-long monitoring Irish WWTPs study from a rural and an urban area

Abstract

Knowledge of the nature and extent of contamination of water bodies with contaminants of emerging concern (CECs) in Ireland is limited. In order to determine the possible risk of CECs in the aquatic environment, it is necessary to investigate their presence, concentration and fate during the treatment in wastewater treatment plants (WWTPs) and their entry in the natural environment. Therefore, in this study, all methods previously developed and optimised were used in order to quantify, when possible, >100 CECs in influent, effluent and surface waters from two different locations in Ireland. Selected CECs included pharmaceuticals, personal care products (PCPs) and pesticides. Investigation of occurrence and frequency data across all matrices are presented, where highest concentrations were obtained by pharmaceuticals, specifically propranolol for surface waters (134 ng/L), hydrochlorothiazide for effluent (1,067 ng/L) and venlafaxine for influent (8,273 ng/L). This chapter also demonstrates seasonal and geographical variations using statistics analysis, where no high significant differences were obtained in most of the categories investigated. Fate of compounds detected was examined by the percentage of removal from the influent and effluent concentrations quantified, and the surface waters concentrations once contaminants leave the effluent and enter the natural aquatic environment. Removals were variable across sites, compounds and samples, due to their dependence on the treatments performed, physicochemical properties and weather conditions, respectively. Surface waters concentrations downstream the WWTP output showed that for most contaminants, effluents were the main point source. This work has demonstrated the presence of a variety of contaminants across different water matrices and locations in Ireland which will allow to risk assess their possible ecological risk in the natural environment.

Aims and objectives

- Perform a sampling campaign for a year in two different locations, an urban and a rural area, collecting monthly surface waters and influent and effluent wastewater samples.
- Apply methods developed to monitor and determine the persistent of these contaminants of emerging concern (CECs) in the samples collected.
- Investigation of occurrence and frequency of different classes of CECs detected through all samples; pharmaceuticals, personal care products (PCPs) and pesticides, including an EU-watch list chemicals analysis section.
- Examination of seasonal, geographical and spatial (fate of contaminants) variations, including data of removal rates of both WWTPs studied.

3.1 Introduction

CECs in the environment have been studied for decades[149] where compounds such as most PPCPs have high polarity and low volatility properties so after their released into the environment, due to mainly human use and excretion, among others, they are transported into the aquatic environment.[289] The final concentrations observed depend on the physicochemical properties of the compound, season, geographical variation, etc. ranging from ng/L to µg/L.[290] WWTPs as a point source, are an identifiable discharge point, once they enter to them they normally end up in receiving waters.[24] This is because their removal depends on the properties of the compounds and because it is not the purpose of a WWTP to remove them. Due to their hydrophilic characteristics, polar and non-volatile drugs are more likely to be preserved in the aqueous phase. However, they can have effects on living systems at even these trace levels.[98] At the beginning it was thought that when they arrive to rivers or coastal waters they would be diluted, but hundreds of them have been detected still at relatively high concentrations.[291] Also, the fate of contaminants is dependent on many factors as river flows and tides, which control directly the concentration of the compounds by dilution.[292] There are not that many studies for spatial and temporal factors to allow us to fully understand the source and fate of these contaminants in the environment as they provide information that can be related to concentrations and compounds found.[293] They are performed generally only including one type of matrix, freshwater or wastewater, and for a certain period of time, for example just a week, presenting a lack of research. A study measured the occurrence of several compounds alongside the river Thames (London, UK), from source to sea, where lower concentrations were found towards the source of the river due to fewer WWTPs in proximity and higher flow rates.[150] Therefore, WWTP inputs increase concentrations and

compounds are being continuously released into the environment.[291] This was also observed in Humber Estuary (Yorkshire, UK) where peak concentrations were found due to an outlet of a WWTP directly onto the stream while concentrations decreased downstream by dilution. Compounds such as ibuprofen, paracetamol and trimethoprim were found up to 10 km downstream from a WWTP.[291] In terms of seasonal variations, compounds such as trimethoprim showed differences with almost double concentrations in winter than in summer, however, daily variations like rainfall and temperature also influence the fate of compounds.[291] Water quality differs depending on the season as well, mainly due to differences in temperature but also pH and turbidity. Highest temperatures were reached during summer and lowest values of pH were recorded during winter and concerning turbidity, this was higher in spring and summer maybe because of high precipitations;[294] these will have a direct effect on CECs fate. Differences in water quality have been also seen between urban and rural areas, e.g. a spatial discrimination was observed between an urban and rural river in Texas, based primarily on differences of dissolved ion concentrations (salinity and hardness), where water in the city is derived almost entirety of twice-reclaimed wastewater and urban runoff.[294] In relation to CECs detected, they are expected to be different in both of them as well. Population has been shown to have a direct relation to detected CECs concentrations in wastewater and surface waters, as they are affected by urbanization and industrialization.[295] However, four different types of WWTPs were studied in China, where highest concentrations were found in livestock-WWTP, pharmaceutical manufacture-WWTP, hospital-WWTP and municipal-WWTP for influent samples, respectively; but effluent samples results were not the same due to the different treatments performed at each WWTP, so there is a diverse fate and removals of these compounds. Nevertheless, they showed similar loads of pharmaceuticals.[296] Higher concentrations found in rural areas could be due to farms

not having any facilities for the treatment and disposal of wastewater or that they use simple treatment technologies. In China there are two examples of simple technologies used alongside the farms, lagoon and anaerobic digester, and 25 antibiotics were detected in the range of ng/L in two swine farms effluents[297] meaning that compounds are not fully removed by these treatments.

There is a need in monitoring campaigns for seasonal or year-long sampling at a reasonable spatial resolution.[293] Even though the analysis of CECs has attracted attention over the last years across the world, limited research has been carried out in Ireland. There is a special need of performing a monitoring campaign for a range of compounds in the country to have a better understanding overall. Therefore, in this study, >100 compounds including pharmaceuticals, PCPs and pesticides were monitored for a year after a monthly sampling for occurrence and frequency analysis. A spatial distribution and removal ratios were also investigated over two locations, one rural and one urban. Highest concentrations were obtained for pharmaceutical compounds across all matrices tested, where venlafaxine achieved the highest value overall for influent wastewater at 8,273 ng/L. Seasonal variations were only observed for urban surface waters and effluent samples for specific categories such as heart disease/hypertension and PCPs, respectively. Geographical variations were also not significant for the majority of categories, not being able to differentiate fully both areas using multivariate analysis. Moreover, removal rates were variable across compounds, sites and months. However, most concentrations decreased after treatment and after the entrance in the environment due to dilution, suggesting WWTPs as a point of source.

3.2 Experimental

3.2.1 Reagents and chemicals

All reagents and chemicals used were previously stated in Section 2.3.1.

3.2.2 Glassware preparation and silanisation

All glassware used was pre-cleaned and silanised using the method described in Section 2.3.2.

3.2.3 Sample collection and pre-treatment

Grab samples of surface water, influent and effluent wastewater were collected monthly from October 2018 to September 2019 from two WWTPs, one located in a rural area and another one in an urban area. Locations and details of both WWTPs are not mentioned due to confidentiality agreements.

A standard operational procedure (SOP) was developed and given to the staff in the WWTPs in order to collect the required influent and effluent samples. Surface water samples were collected using the same procedure but not with staff members. Amber Nalgene bottles of 1 litre volume were used, they were washed in triplicate with methanol and water separately prior to sampling. Once on location, they were rinsed twice with sample before samples were taken in duplicate. Bottles were filled to the top and no headspace was present during transportation. Bottles were placed in a cool box as soon as possible and transported to Dublin City University (DCU). Once the bottles arrived to the laboratory, one set of samples was acidified to pH 2 using hydrochloric acid (37% v/v) and stored in the freezer at -20°C until further analysis as described in Section 2.3.4.1. The other set of samples did not proceed any treatment and samples were shipped frozen to King's College London (KCL, UK) for analysis as per Section 2.3.5.1.

3.2.4 Extraction and analysis

Analysis was performed using two different analytical methods which were validated after they were previously developed and optimised as per Chapter 2.0; a solid-phase extraction (SPE), method 1.2, and a direct injection (DI), method 2, both using liquid chromatography coupled to mass spectrometer detection (LC-MS/MS). Consequently, samples were prepared differently for both techniques and description on sample preparation and analysis are recorded in Sections 2.3.4.2 and 2.3.4.3.2, and Section 2.3.5, for methods 1.2 (estrogens hormones and rest of compounds) and method 2, respectively. Therefore, a total of 135 compounds were investigated for the analysis of samples collected.

3.2.5 Data and statistical analysis

Data and statistical analysis was accomplished by descriptive statistics (mean, range, standard deviation and frequency of detection, etc.) using Microsoft® Office Excel (WA, USA), IBM® SPSS Statistics v27 (New York, USA), R v4.0.5, RStudio v1.4.1106 (Boston, USA), Python v3.7.9 and Flourish Studio (London, UK).

Data throughout this thesis has not been converted to daily mass loads (g/day) nor normalised due to the lack of flow rate and population data provided from the wastewater treatment plants. The importance of normalised data has been highlited in previous studies due to the limitations regarding just analyte concentrations and their possible under or over estimation of the levels quantified.[298] Furthermore, calculations of removal rates during wastewater treatment are just indicative due to the lack of information on the type of treatment, hydraulic retention times and loads. Additionally, grab samples were only available at the time of the study, so diurnal changes on the concentrations of the compounds detected cannot be extrapolated.

3.2.5.1 Frequency

Compound presence was assessed through the calculation of the frequency of percentage of quantification.[299] Samples detected below the limit of detection (<LOD) were not considered, therefore cases ≥LOQ were compared to the total number of samples analysed (n=12 months) as shows Equation 3.1:

$$\text{Frequency } (\%) = \left(\frac{\text{nº of samples} \geq \text{LOQ}}{\text{nº of samples analysed}}\right) \times 100$$

Equation 3.1 Percentage of frequency of detection.[299]

3.2.5.2 Removal rates

The removal rates of the contaminants detected in influent and effluent wastewater were calculated as the percentage of concentrations found in the effluent deducted from the ones on the influent (Equation 3.2).[300] Average removals and standard deviations of compounds are reported for n=12 months.

$$\text{Removal } (\%) = \left(\frac{C_{influent} - C_{effluent}}{C_{influent}}\right) \times 100$$

Equation 3.2 Percentage of overall removal.[300]

Certain compounds were detected in the influent samples but not in the effluent; in order to assume the "worst" case scenario and not obtain a removal rate of 100% as it might not be accurate, LOD values were used as the effluent concentration.[300] Also, if compounds were detected in the effluent and not in the influent, in order to calculate the % of removal in this occasion, again LOD values of the analytes were used as the influent concentration. This factor is to correct the possibility of the analyte being present but not being able to be detected due to methodology limitations. For the compounds obtained at LOD and LOQ concentrations, half the values of their limits have been used for their removal calculations.

3.2.5.3 Statistical analysis

Statistical analysis was performed in order to assess possible differences for temporal and geographical locations variations. Mean values by categories of contaminants were used where normality of data was tested by Shapiro-Wilk W test applying $p<0.005$ significance level. Analysis of variance (ANOVA) with the post hoc Tuckey's test ($p<0.05$) and independent t-test were used for parametric data where necessary. Kruskal-Wallis ANOVA by ranks and independent-samples Mann-Whitney U tests were used for non-parametric data. Concentrations reported below the LOQ were assumed as half of the value of the LOQ of the specific compound for the specific matrix in order to perform the data analysis. Results obtained below LOD were set to zero.[110] Data was represented as box and whisker box plots, where lines in the box showed the lower (25%) and upper (75%) quartiles of the corresponded values for the category of compounds or compound itself. Lines extending from the boxes indicates the variability outside the upper and lower quartiles, where the line inside the box represents the median concentration.[118] Outlier and far outliers values were also marked with symbols (° and * respectively) when numbers were outside the 1.5 times interquartile range (IQR).

Due to the wide data set obtained within this study, principal component analysis (PCA) is used in order to explore variability and trends of the data. PCA transforms the data into uncorrelated variables (axes) reducing the correlation matrix to a minimum number of factors, simplifying the data.[301] Relationship between compounds detected, their concentration and the locations are investigated performing this analysis. Data was previously normalised by subtracting the mean and diving it by the standard deviation previous to their analysis.[118]

3.3 Results and Discussion

3.3.1 Occurrence and frequency of compounds of emerging concern

Occurrence of CECs tested in all aquatic matrices are present in Figures 3.1 – 3.3 for both areas across all matrices investigated. Of 135 compounds analysed, 52 and 51 were detected for influent samples, and 16 and 23 for surface waters, in the rural and urban area respectively. The same number of compounds, 47, were detected at both areas for effluent samples. The majority of the contaminants detected across all samples were detected with a 100% frequency for influent and effluent wastewater as seen in Figure 3.4. However, rural surface water samples were mostly presented at 0% frequency due to quantifications at <LODs (LODs are not taken into account for frequency data calculations); for the urban area, the majority presented 17% of frequency, meaning two samples out of twelve detected. Compound specific frequencies and individual quantifications values can be observed in Table A.12-A.13 from Appendix F. The different classes of CECs presented were quantified at different levels with concentrations ranging in surface waters from <LOD to 99 (propranolol) and 134 (propranolol) ng/L for rural and urban area respectively. This matrix showed the lowest number of compounds detected overall where only five were detected at quantifiable concentrations (>LOQ) in rural areas (such as fenuron, octocrylene and propamocarb). Nine compounds were quantified for urban areas including carbamazepine, fenuron and tramadol among others. All compounds detected in surface waters samples were also identified in the effluent wastewater samples. For these samples, a total of 35 and 34 compounds were quantified (>LOQ) for rural and urban areas respectively, including compounds such as temazepam and venlafaxine. Concentrations varied from <LOD to 1,067 ng/L (hydrochlorothiazide) for rural areas and <LOD to 976 ng/L (mefenamic acid)

for urban areas. Several compounds detected in effluent samples were not detected in influent. These compounds were acetamiprid, E2, lincomycin, methylphenidate, promertyn and verapamil for rural areas. For urban areas they were octocrylene, risperidone and simazine. As they were detected in the effluent, it is noted that they could have resulted from treatment processes and this is discussed more detailed in Section 3.3.4. Influent wastewaters showed a total of 48 and 42 compounds quantified where concentrations ranged from <LOD to 8,273 (venlafaxine) ng/L and <LOD to 3,476 ng/L (valsartan) for rural and urban areas respectively. Occurrence data is next discussed by classification of the different types of CECs studied.

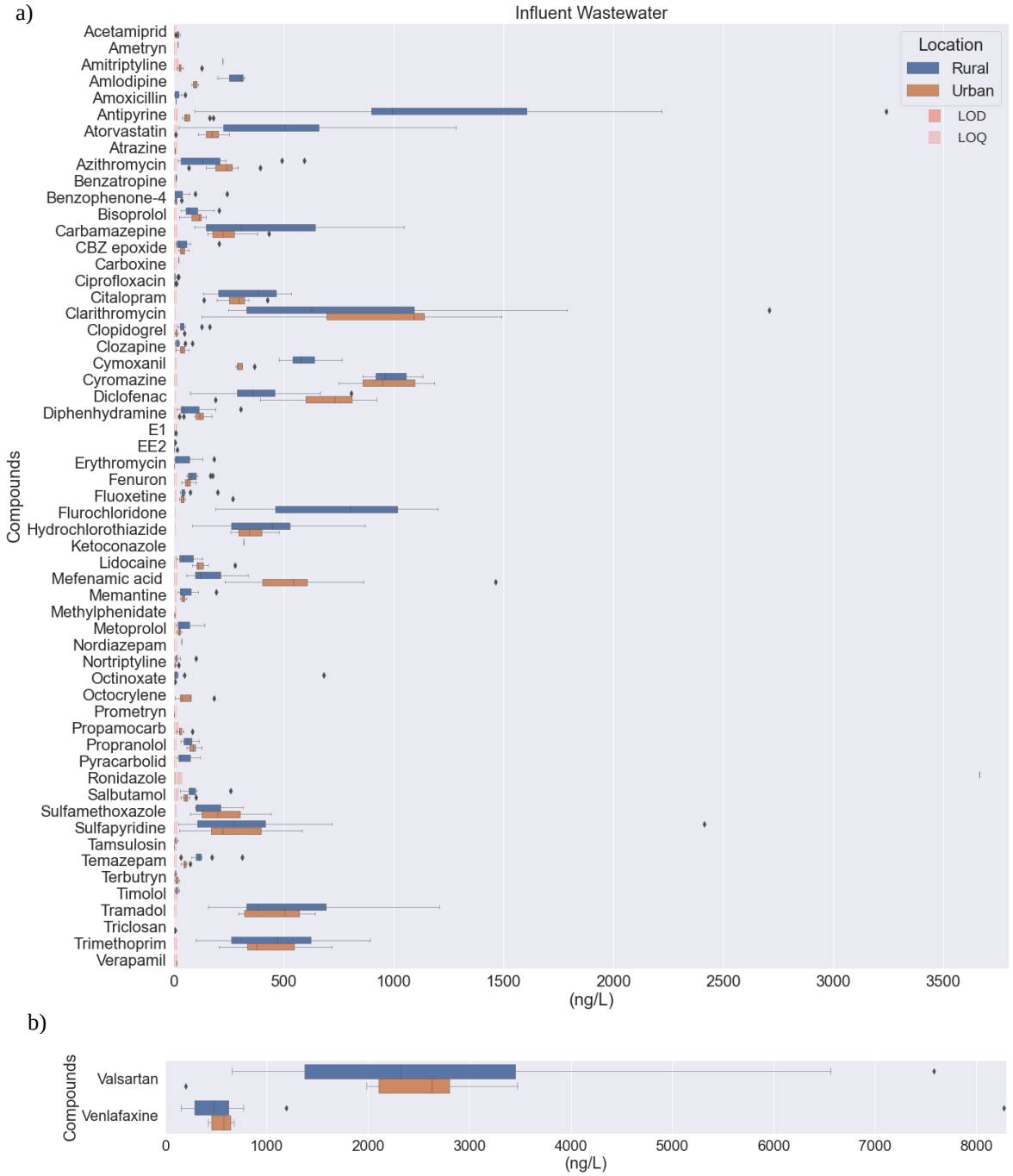


Figure 3.1 Concentration of selected CECs in all samples for influent wastewater a) for all compounds quantified up to 3500 ng/L and b) up to 8300 ng/L; for both areas investigated rural (blue) and urban (orange), where solid bars show the median and the box represents the 0.25 and 0.75 percentiles. Error bars presents minimum to maximum for n=12 (months analysed) and LODs and LOQs are represented by chart bars.

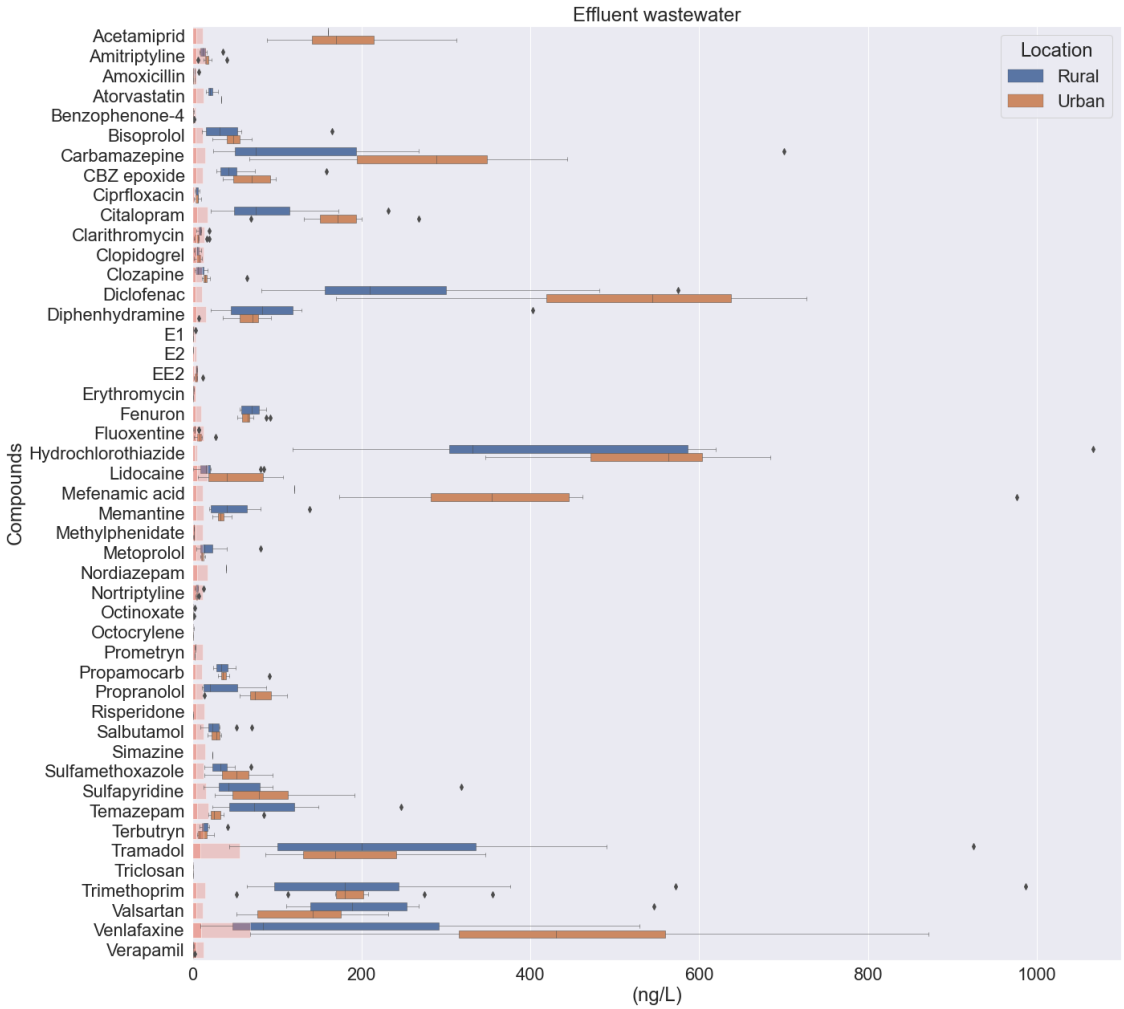


Figure 3.2 Concentration of selected CECs in effluent wastewater for all compounds detected for both areas investigated rural (blue) and urban (orange), where error bars present minimum to maximum (n=12, months analysed) and LODs and LOQs are represented by chart bars.

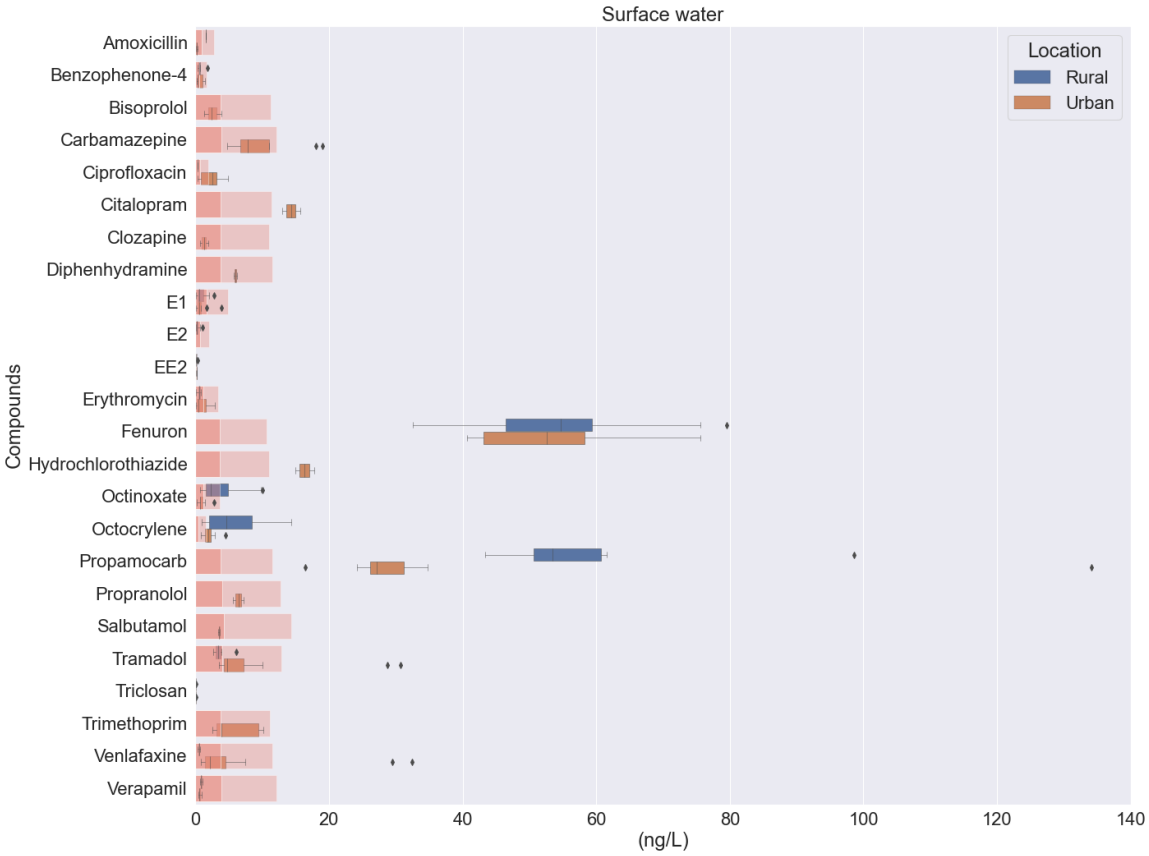


Figure 3.3 Concentration of selected CECs in surface waters for all compounds detected for both areas investigated rural (blue) and urban (orange), where error bars present minimum to maximum (n=12, months analysed) and LODs and LOQs are represented by chart bars.

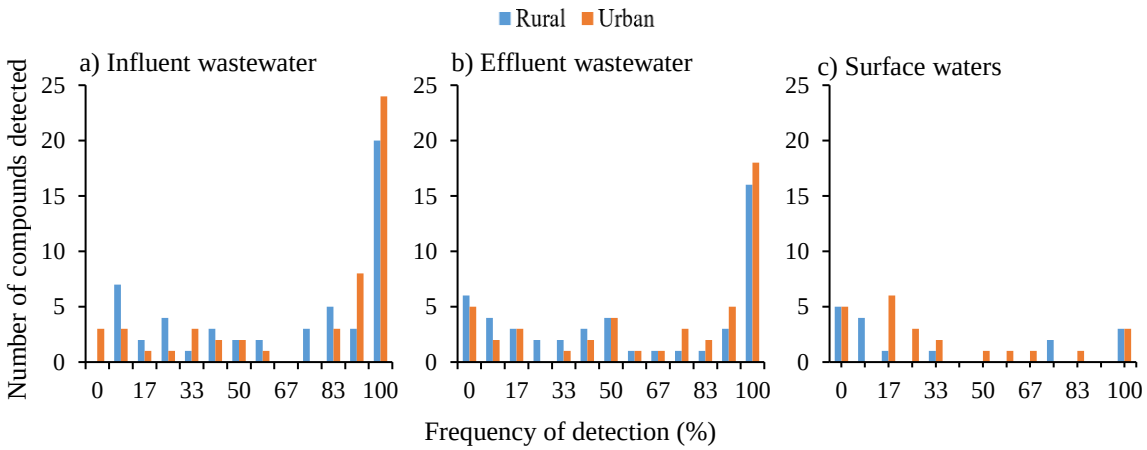


Figure 3.4 Number of compounds per frequency of detection in influent (a), effluent (b) and surface waters (c) for both rural and urban areas for the sampling campaign of n=12.

3.3.1.1 Pharmaceutical compounds

Pharmaceuticals are the main group investigated in this study, this is due to their widespread use and their frequent presence in the environment. Compound classification showed that the majority of identified compounds overall were pharmaceuticals, mostly belonging to the psychiatric or psychotropic (including antidepressants and antipsychotics) and heart disease/hypertension category as seen in Figure 3.5. From all 47 pharmaceuticals detected across all samples, 17 were present on the top 100 most prescribed drugs by the General Medical Service (GMS) when the sample period campaign was carried out, including atorvastatin in position two and bisoprolol in position six on the rank (see Table 1.2). Some of them are also included in the preferred list of drugs by The Medicines Management Programme (MMP) such as amlodipine, bisoprolol, citalopram, and venlafaxine.[43] Pharmaceuticals were also found at higher concentrations than other type of CECs throughout the influent wastewater samples as observed in Figure 3.6. The antidepressant venlafaxine presented the highest value quantified, 8,273 (±1) ng/L, for the month of October in the rural area; average concentrations of the compound across the year were 1,133 (±2267) and 553 (±101) ng/L for rural and urban area respectively. This compound was ranked between position 26 – 29 during the sample period on the top 100 pharmaceuticals most prescribed (Table 1.2) and it is widely used to treat anxiety, panic attacks, etc. Therefore, it has been found in the environment at concentrations higher than other antidepressants such as fluoxetine,[121,302] this can also be observed within the samples studied. In the UK, venlafaxine has been quantified at higher concentrations in wastewater than the ones expected by prescription data, this antidepressant is not available over the counter (only prescription), however, it is available online, has a low cost and has been considered as an abused compound before.[121] Daily intake (consumption) of venlafaxine could provide

a more insight view of the trend of this compound in order to see whether the high concentrations obtained could be related to just prescription data or its potential to abuse, however, it was not possible to perform calculations at the time.

Valsartan followed with the highest concentrations detected across both sites showing average concentrations of 2,894 (±2283) and 2,423 (±821) ng/L for rural and urban respectively. This compound is used to treat high blood pressure, and it is usually detected at high concentrations in surface waters and effluents, quantified up to 4.6 µg/L in effluent samples.[154] In Ireland, valsartan was ranked with positions between 59 – 71 for most prescribed pharmaceutical during the sample campaign. Consequently, lower concentrations in comparison with venlafaxine were expected in accordance to prescription data. Additionally, valsartan is usually prescribed with hydrochlorothiazide, and both appeared together on the top 100 products by ingredient cost in Ireland at the time of sampling.[43,51] The combination medicine, which brand name is Co-Diovan (Novartis Pharmaceuticals Ltd.), is approved by the European Medicines Agency (EMEA)[303] and usually has tablet contents of 80/12.5, 160/12.5 or 160/25 mg of valsartan/hydrochlorothiazide mixture. These results are in accordance with the wastewater concentrations obtained within this thesis as valsartan concentrations were up to approximately 25 times higher than hydrochlorothiazide across all influent samples.

Other compounds such as antipyrine, an analgesic, were detected at high concentrations (average of 1,302 (±826) for rural area) but were not in the rank of the top 100 most prescribed drugs during the sampling campaign. This pharmaceutical is commonly detected in the aquatic environment[304] due to its widely consumption, however, it has a main use in veterinary purposes,[305] explaining its absence in the list of most prescribed pharmaceuticals and high concentrations observed in the rural area.

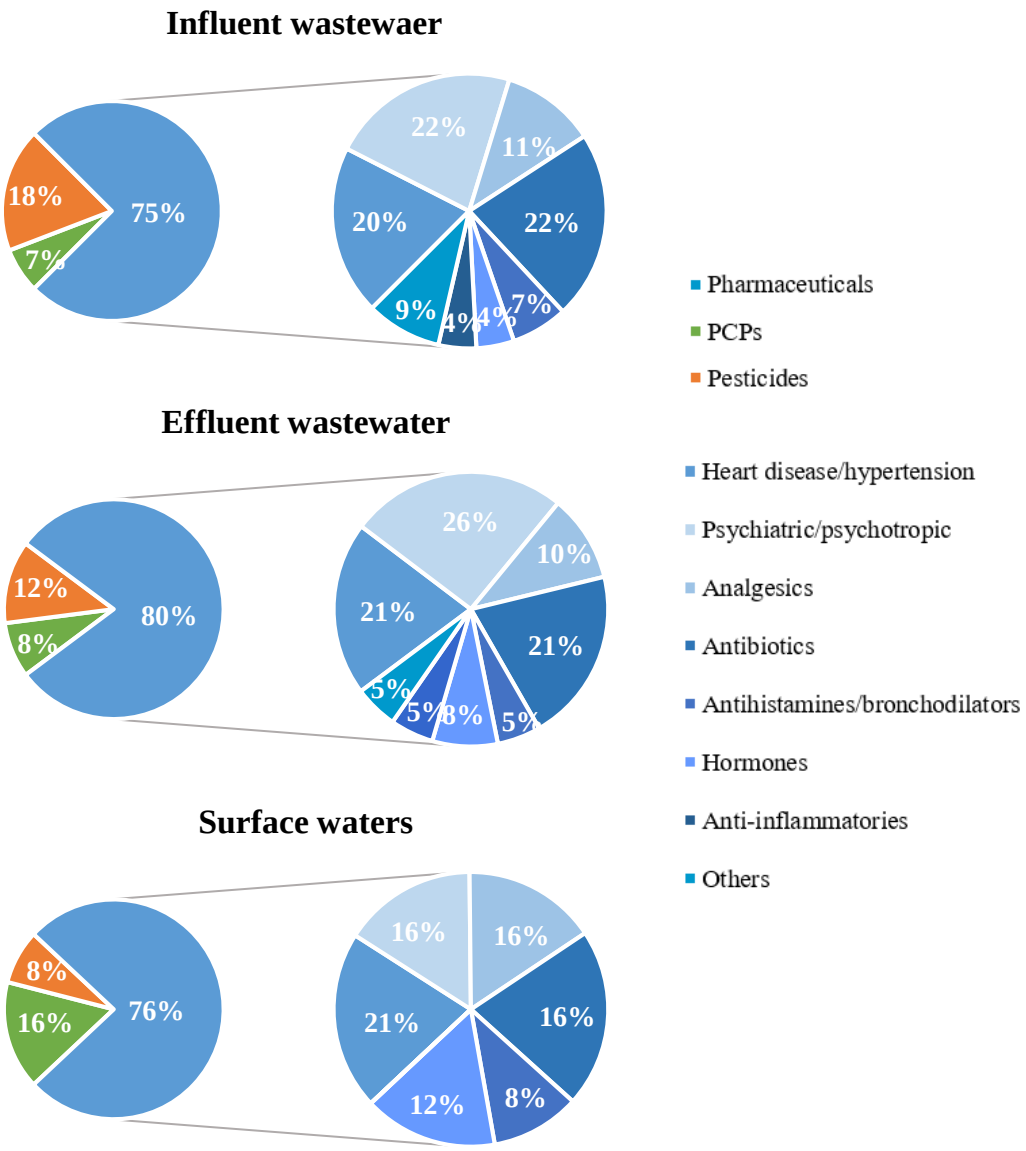


Figure 3.5 Compound classification of identified analytes in all matrices investigated: influent, effluent and surface waters, showing further classification of the different pharmaceutical classes.

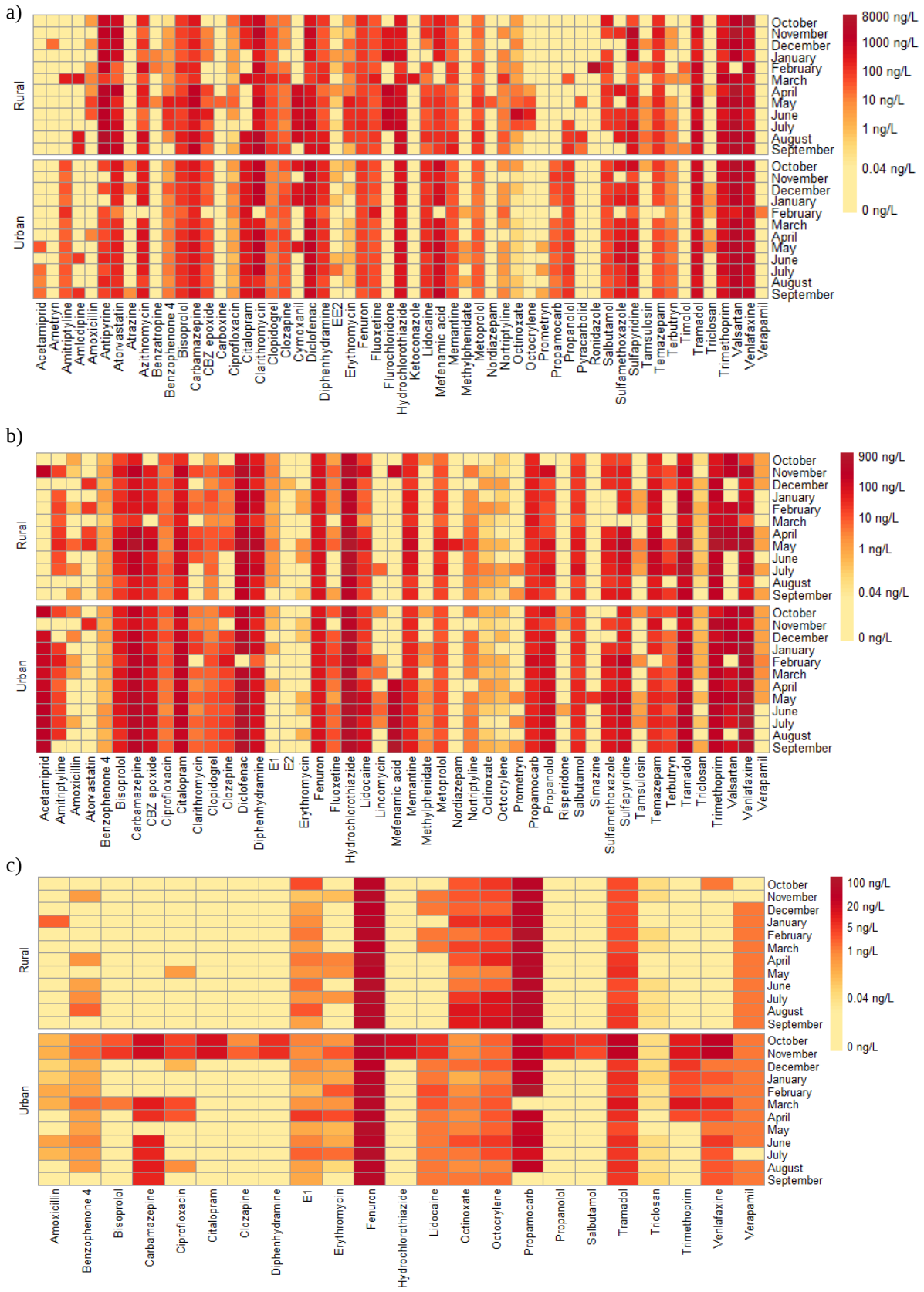


Figure 3.6 Heatmaps of compounds determined in the influent, effluent and surface water samples for both areas showing the ranges in concentrations (ng/L), where darker colours mean higher concentrations obtained.

For effluent samples, maximum concentrations were quantified for hydrochlorothiazide with an average of 444 (±251) and 547 (±99) ng/L for rural and urban areas respectively. The following compounds also obtained higher quantification mean concentrations (ng/L): diclofenac at 253 (±152) and 519 (±173), carbamazepine at 159 (±188) and 276 (±116), tramadol at 267 (±249) and 187 (±78), trimethoprim at 266 (±269) and 190 (±75), valsartan at 239 (±161) and 136 (±64), and venlafaxine at 163 (±170) and 448 (±226) for rural and urban sites respectively. These concentrations are in agreement with other studies, such as effluent wastewater samples from Castellon and Valencia (Spain), where diclofenac, carbamazepine, valsartan and venlafaxine concentrations ranged from 158–884, 2–149, not detected–4,575, not detected–414, ng/L respectively.[154] However, trimethoprim concentrations were lower, ranging from not detected–86 ng/L. This could be due to the bias of effluent wastewater matrices due to different treatments performed, weather conditions, etc. From the compounds detected at higher concentrations, mentioned above, only venlafaxine belongs to the psychiatric/ psychotropic class, the most detected class from all pharmaceuticals, accounting for 26% of all pharmaceuticals detected. Antibiotics followed with a 21% of the total pharmaceuticals, but most detections were under <LODs and <LOQs, except for sulfamethoxazole, sulfapyridine, trimethoprim and ciprofloxacin with average concentrations of 44 (±23), 81 (±67), 228 (±197), and 6 (±2) ng/L respectively.

Regarding surface waters, the majority of pharmaceuticals were detected at <LOD and <LOQ levels. Of the pharmaceuticals, antibiotics and heart disease/hypertension drugs were obtained at higher percentages (21% each of the total pharmaceuticals). However, all antibiotics were detected at concentrations below the LODs and LOQs, except for ciprofloxacin in urban areas, where it was quantified up to 5 (±26) ng/L. The same trend was observed for the heart disease/hypertension category,

except for hydrochlorothiazide which was presented at a maximum concentration of 18 (±25) ng/L for the urban area, clearly reduced concentrations from the ones obtained previously for influent wastewaters. Tramadol and carbamazepine achieved the maximum concentrations of the category for the urban area with 31 (±6) and 19 (±10) ng/L respectively. This is not a surprise as carbamazepine is one of the most frequently reported compounds in environmental samples[98] and possible high concentrations could be due to low removal rates during treatments in the WWTPs.[306] On the other hand, tramadol is usually observed in several types of water samples, with high concentrations in effluents and surface waters,[307] as per observed within this study where concentrations were higher in influent>effluent and then surface waters, reporting the highest concentration for urban surface waters.

3.3.1.2 Pharmaceutical temporal variations in Ireland

Limited data is available for occurrence of CECs in Ireland. However, three studies were found during literature review which contain common pharmaceutical compounds within this thesis enabling to perform a temporal comparison; this will help to understand changes in occurrence of selected compounds.

A study published in 2008 by Lacey *et al.*[39] contained several common compounds investigated in this work. Samples were collected in the Dublin area before 2008 (referenced as pre-2008) for effluent and influent wastewater samples from three WWTPs. Regarding influent samples, diclofenac, sulfamethoxazole, mefenamic acid, metoprolol, propranolol, carbamazepine and salbutamol, were not detected in the study, however, quantification values have been achieved for this thesis. This could be due to the high LODs reported for this type of matrix, which were 855, 72, 20, 633, 7, 10 and 8 ng/L respectively, limiting the detection of these analytes.[39] On the other hand, all LODs obtained within this thesis were significantly lower, even that SPE was not used as pre-

concentration step when using the direct injection method (Method 2, Section 2.3.5). Compounds such as diclofenac were quantified at average concentrations of 382 (±212) and 673 (±212) ng/L for rural and urban areas respectively within this thesis. These concentrations are lower than the LODs obtained by the reported study suggesting that they could have been present at the samples at the time but not been able to be detected due to method sensitivity limitations. Nevertheless, other compounds such as carbamazepine were now quantified at concentrations ranging from 95–1,048 ng/L in this study, higher concentrations than their LOD, 10 ng/L, so this was not a limitation at the moment for the study carried out. This could mean an increase on concentrations of the compounds over time (10-year gap between both studies) or that their occurrence depends on sample location. Then again, trimethoprim was also detected in the pre-2008 study ranging from <171–<570 ng/L and they have been obtained at concentrations between 100–891 ng/L across all samples in this study, therefore a slightly increase can be observed. The same group published a subsequent article in 2012 based on a sampling campaign in 2007-2008[308] (referenced as 2008) where diclofenac, mefenamic acid and propranolol were not detected; these findings are consistent with the data reported in the pre-2008 campaign.[39] However, carbamazepine was detected with maximum concentrations of 720 ng/L for the campaign suggesting an increase throughout the studies (not detected in the pre-2008 study, maximum concentration of 720 ng/L in 2008 and maximum concentration of 1,048 ng/L in the 2018-2019 samples within this study). Metoprolol was detected at a maximum of 2,570 ng/L in 2008 compared to the maximum concentration of 141 ng/L observed within this thesis. The same can be observed with trimethoprim detected at a maximum of 15,700 ng/L in 2008 and just up to 891 ng/L within this thesis, as previously stated. All concentrations of both studies for the same compounds studied within this thesis are stated in Table 3.1.

For effluent wastewater, several compounds were detected within this thesis that were not previously observed in the pre-2008 campaign, including metoprolol, propranolol and salbutamol. However, the LODs achieved at that time were in the µg/L range with propranolol presenting the lowest LOD with 17 ng/L.[39] All limits were higher than the ones achieved within this thesis for effluent samples. Nevertheless, within this thesis, propranolol was obtained with average concentrations of 67 (±35) and 88(±20) ng/L for the rural and urban area, concentrations higher than the LOD reported in pre-2008. In the 2008 campaign, metoprolol and propranolol were then quantified up to 4,340 and 310 ng/L respectively.[308] These concentrations were higher than the ones obtained for our study, maximum concentrations (80 and 112 ng/L respectively). Also, bezafibrate was quantified in effluent in 2008 at a maximum concentration of 120 ng/L[308] and it was not detected in within this thesis samples. There are other compounds previously quantified at high concentrations in Ireland which presented lower concentrations in the samples analysed in this thesis. An example is carbamazepine that ranged from 25 to 701 ng/L here, and has previously been quantified at high concentrations up to 881 ng/L (pre-2008)[39] and 6500 ng/L (2008).[308] For the anti-inflammatory mefenamic acid, concentrations in this study ranged from 120 to 976 ng/L, lower than previously reported in Ireland at 540–1,050 ng/L (pre-2008) [39] and LOQ–9,100 (2008).[308] Several antibiotics were also detected where trimethoprim seems to increase over time as it has been quantified at concentrations from 52 to 987 ng/L for all samples, higher concentrations than previously reported in effluent from <67 to 360 ng/L in pre-2008[39] and <67–850 ng/L in 2008.[308] On the other hand, sulfamethoxazole ranged from 14 to 77 ng/L lower than previously reported ranges of <166 to <553ng/L (not detected in influent)[39] in the pre-2008 study, however, in subsequent 2008 sampling study samples collected were below the LODs of 166 ng/L for both influent and effluent samples.[308] From macrolide

antibiotics, only clarithromycin was observed in the effluent samples at mean concentrations of 57 (±6) ng/L, lower than other quantitation values obtained at two locations in Ireland, 204 and 189 ng/L respectively, in a different study published in 2020 by Rodriguez-Mozaz *et al*,[110] where samples were collected in 2015-2016. As mentioned before, this could be due to location dependent as variations of concentrations depend on the type of treatment performed in the WWTP, the population equivalent of the WWTP and the consumption pattern of the area. The three WWTPs tested by Rodriguez-Mozaz *et al.* had a population equivalent to 50,000, 90,000 and 1.7 million people in the Dublin area, however, the ones sampled for this study were significantly lower, with a population equivalent (PE) of 50,000-100,000 for the urban site and <2,000 PE for the rural site. Estimation consumption data in loads (i.e. g/day) would therefore be a more appropriate way of comparing the occurrence of these compounds between different matrices and sites.[26] Also, the gap between the collection of samples in the last article (2008) and the collection of samples within this thesis (2018) needs to be considered. Consequently, there is not enough data available in order to estimate trends of the detected pharmaceuticals.

Table 3.1 Concentrations of compounds previously reported for Ireland compared to the ones obtained within this study for influent and effluent wastewaters.

Analyte	**Sampling campaign**							
	Pre 2008[39]		**2007-2008**[308]		**2015**[110]	**2016**[110]	**2018-2019**	
	Influent	Effluent	Influent	Effluent	Effluent		Influent	Effluent
Amoxicillin	-	-	-	-	n.d.	n.d.	3-52	<1.4-7
Azithromycin	-	-	-	-	108-635	111-212	19-594	n.d.
Carbamazepine	n.d.	163-881	<34-720	<13-6500	-	-	95-1048	25-701
Ciprofloxacin	-	-	-	-	186-483	134-223	<0.4-22	<1.3-11
Clarithromycin	-	-	-	-	<27-264	216-338	128-2709	<4-20
Bezafibrate	n.d.	n.d.	<33-7250	<50-120	-	-	n.d.	n.d.
Diclofenac	n.d.	<743-<2478	n.d.	<743-2950	-	-	75-922	81-727

Lincomicyn	-	-	-	-	n.d.	n.d.	*	<4
Mefenamic acid	n.d.	<32-330	n.d.	<13-9100	-	-	59-1463	120-976
Metoptolol	n.d.	n.d.	<633-2570	<97-4340	-	-	<13-141	<12-80
Propranolol	n.d.	n.d.	n.d.	<17-310	-	-	32-126	<12-112
Roxythromycin	-	-	-	-	n.d.	n.d.	n.d.	n.d.
Salbutamol	n.d.	n.d.	<8	<155	-	-	29-257	<13-70
Spiramycin	-	-	-	-	n.d.	n.d.	n.d.	n.d.
Sulfadimethoxine	-	-	-	-	n.d.	n.d.	n.d.	n.d.
Sulfamerazine	-	-	-	-	n.d.	n.d.	n.d.	n.d.
Sulfamethoxazole	n.d.	<166-<553	<72	<166	<33	<38-175	74-443	14-95
Sulfapyridine	-	-	-	-	<10-60	61-272	22-2414	<5-318
Sulfathiazole	-	-	-	-	n.d.	n.d.	n.d.	n.d.
Sulfisoxazole	-	-	-	-	n.d.	n.d.	n.d.	n.d.
Trimethoprim	<171-<570	<67-360	<171-15700	<67-850	<10-162	156-184	100-891	52-987

n.d.: not detected
*compound detected but quantification not able to be performed due to a peak on the matrix

In terms of surface waters, a previous report by Loos *et al.* for the European Commission stated concentrations of compounds studied within this thesis for the river Liffey (Dublin) in a campaign performed in autumn 2007.[309] Maximum concentrations for carbamazepine, diclofenac, sulfamethoxazole and naproxen were 55, 9, 7 and 17 ng/L respectively. Compounds such as atrazine, ketoprofen, bezafibrate, simazine and E1 were reported as <LOD which were 1, 3, 1, 1 and 2 ng/L respectively.[309] Within the samples analysed for this thesis, only carbamazepine and E1 were detected, with maximum concentrations of 19 (±10) ng/L and <LOQ (5 ng/L) respectively. Overall, these substances were detected in lower concentrations, apart from E1 and carbamazepine, for our samples than the previously reported, therefore suggesting a decrease. However, this is unsurprising due to temporal variability of grab sampling[278] and/or, as mentioned before, due to the differences in sampling sites and prescribing patterns.[301]

3.3.1.3 EU-watch list

Several compounds analysed by this method belong to the most recent EU-watch list (2020) by the WFD, including amoxicillin, ciprofloxacin, sulfamethoxazole, trimethoprim, venlafaxine and famoxadone in the liquid phase and octinoxate in the solid phase (solid particle matter, SPM). However, other compounds such as E1, E2, EE2, erythromycin, clarithromycin and azithromycin were removed from the Watch List, as seen in Table 1.3. Most data that informs the watch list come from rivers (98.3%) and in the surface waters investigated in this study, the antibiotics amoxicillin and trimethoprim were detected for all samples ranging from <LOD – <LOQ concentrations. Ciprofloxacin and venlafaxine were only quantified at the urban area with a maximum concentration of 5 (±26) and 32 (±7) ng/L respectively, while sulfamethoxazole and famoxadone were not detected in any sample. Given that octinoxate preferentially partitions into the solid phase, that this compound was detected at concentrations ranging from <LOD – 10 (±32) ng/L suggests that higher concentrations could be found on the SPM due to its physicochemical characteristics.[26] Therefore, amoxicillin, ciprofloxacin, trimethoprim and octinoxate were all detected below the maximum acceptable method detection limits (MADL) stipulated by the WFD (78, 89, 100 and 6,000 ng/L) suggesting a possibility of presenting a lower risk, however, even at low concentrations these compounds have potential to affect the environment and a risk assessment will be performed. Nevertheless, venlafaxine maximum concentration was 32 ng/L, above the 6 ng/L limit, therefore as mentioned before, risk assessment will be performed.

3.3.1.4 PCPs

Four PCP compounds were investigated in this study and they all were detected across all matrices. Benzophenone-4 was mainly detected at concentrations <LOD, therefore

decreasing their frequencies as per effluent rural samples, where peaks were observed at all samples but they were quantified at <LODs resulting in a frequency of detection of 0%. Its maximum concentration was in influent rural at 242 (±95) ng/L, however, majority of samples are <2 ng/L in surface waters and effluents. Triclosan was also quantified at low concentrations with a maximum of 7 (±46) ng/L for urban influent. Influent samples showed maximum concentrations for octocrylene and octinoxate as well, up to 182 (±66) and 682 (±153) ng/L for urban and rural areas respectively. Consequently, concentrations levels for PCPs were observed as follows for the majority of compounds: influents>effluents>surface waters; where maximum concentrations for environmental potential risks matrices, effluent and surface waters, did not exceed ≤14 ng/L.

3.3.1.5 Pesticides

One of the most major contaminants in the environment are pesticides, as they are widely use in agriculture and are transported into the environment after their application[310] contributing to water quality degradation.[311] In this study, herbicides, fungicides and insecticides are among the pesticide compounds investigated. Across all samples analysed 11 pesticides were detected for influent, 6 for effluent and 2 for surface waters. Only two compounds, fenuron and propamocarb, were found at all matrices investigated, however, the latter was not detected in influent rural samples. Therefore, they were the only compounds detected at surface waters suggesting that the main point source is WWTP effluents. Propamocarb is used to treat different diseases like seedings, white tip, downy mildew, pythium, etc. and it is used for a variety of vegetables such as for lettuce, onions, spinach and tomatoes confirming its widely use. Maximum frequencies (100%) were obtained for this analyte, except for urban surface waters (83%) and influents (92%) which were still quite high. On the other hand, fenuron was detected with a 100%

frequency across all matrices. However, it is also used in industry as formulation or repacking being part of adhesives and sealants, plastics, vehicles, coating products, fillers, etc. It is manufactured and/or imported in Europe in 10 to 100 tonnes per year.[312] Their manufacture and use in industry could contribute to their release into the environment in addition to pesticide contamination routes used. Fenuron is marked as essential use and has a default maximum residue levels (MRLs) of 0.01 mg/kg according to Art 18(1)(b) Reg 396 / 2005.[313] This compound is an example of the 26 out of total 51 pesticides investigated in this study that are not approved by the EU Commission, E, 2009 for pesticide use.[313,314] The rest of pesticides are approved with exception of benoxacor (not assessed yet at EU level) and dimethametryn, piperophos, prodiamine, prometon, pyracarbolid, spinosyn A, and spinosyn D which were not found on the EU pesticides database, however, none of them were detected in this study. Nevertheless, ametryn, atrazine, cyromazine, prometryn and terbutryn are also non-approved compounds which were detected only in influent samples, suggesting their removal during treatment. Only prometryn and terbutryn were further detected in effluents but not in surface waters, so they could have been diluted once released from the WWTP. However, simazine, non-approved, was just detected in effluent samples so it could be a product of treatment processes and then diluted once entering the surface waters. Its removal rate will be further discussed under Section 3.3.4.

Neonicotinoids are among most widely used classes of insecticides in agriculture, though recent rulings have restricted their use amongst flowering crops, and they have been found at higher concentrations than the limits by European Union so they were included in EU first and second Watch List.[315] From this group, acetamiprid was detected only in urban influent samples with maximum concentrations of 27 (±14) ng/L. It has potential toxicity to several organisms but it is not proposed that its use be prohibited

(European commission, 2018). Studies have showed its relation to reduced sperm density in birds, and it is claimed that agriculture contributes to the decline in farmland bird populations.[316] This pesticide is also associated with detrimental impacts for pollinators and ecosystem services. Acetamiprid was not found in surface waters which is consistent with other studies, where it has been infrequently detected and if so, with quantification values below LOQ as well as in biota.[317]

In summary, all pesticides seemed to be removed or diluted up to their arrival to the natural environment apart from propamocarb and fenuron. Therefore, WWTP effluents are their main route of entry, however, maximum concentrations were 134 (urban) and 80 (rural) ng/L respectively in surface waters. Even that fenuron is not authorized by the EU for pesticide use its detection could be attributed to its widely use in industry as mentioned before.

3.3.2 Seasonal variation

Samples were taken monthly over a period of a year for both sites, therefore temporal occurrence was investigated by comparing data compiled into the different seasons: spring (March – May), summer (June – August), autumn (September – November) and winter (December – February). Frequency of compounds during the seasons campaign for surface waters can be observed in Figure 3.7 for both areas, rural and urban, where most compounds were detected in autumn (15) and summer/winter (6), respectively. Examples of frequencies for the rest of matrices are located in Appendix F (Figures A.1–A.4), where effluent wastewaters had a maximum of 36 compounds per season for both areas in spring (rural) and autumn (urban). For the influent samples, rural areas obtained the maximum of compounds overall, 48, in spring and the urban area had 39 for spring, summer and autumn.

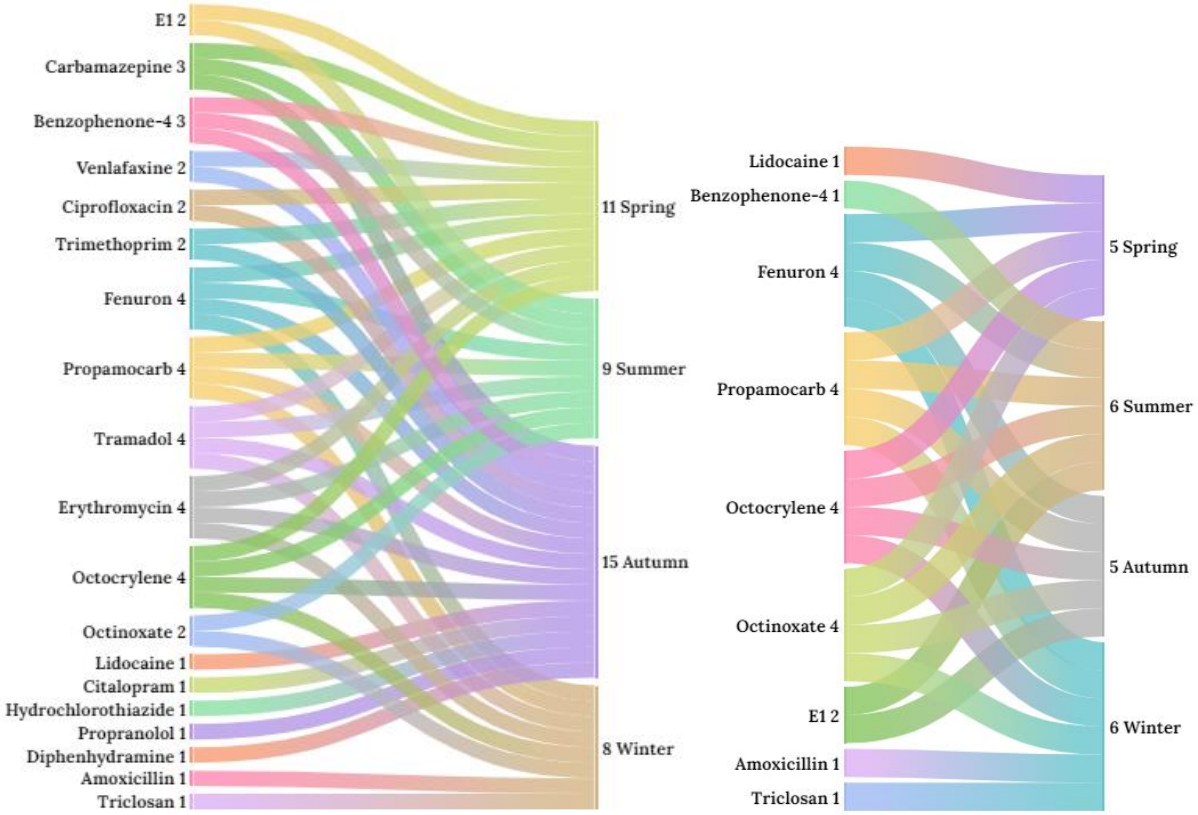


Figure 3.7 Sankey diagrams for surface water samples investigated over a period of a year and distributed by seasons for the urban (left) and the rural (right) locations. Compounds quantified <LOD and with coefficients of regression of R^2 <0.90 have not been included in the season comparison.

Due to the high amount of compounds studied, cumulative values were based on the type of class (i.e. pesticides, PCPs and pharmaceuticals), where pharmaceuticals were grouped by class (i.e. hormones, analgesics, psychiatric/psychotropic, etc.) for statistical analysis purposes. In rural surface waters, all categories presented values of $p<0.05$ when performing the test of normality (Shapiro-Wilk) resulting in the requirement that non-parametric tests (data not normally distributed) be performed. Therefore, Kruskal-Wallis tests were performed and no significant effects were observed for any category detected: pesticides ($\chi^2(3) = 4.715$, $p = 0.194$), analgesics ($\chi^2(3) = 2.4$, $p = 0.494$), hormones ($\chi^2(3) = 0.791$, $p = 0.852$), antibiotics ($\chi^2(3) = 5$, $p = 0.172$) and PCPs ($\chi^2(3) = 0.179$, $p = 0.981$).

Urban surface waters also presented non-parametric datasets and non-significant effects were found for pesticides ($\chi^2(3) = 1.193$, $p = 0.755$), hormones ($\chi^2(3) = 1.459$, $p = 0.692$), antihistamines or bronchodilators ($\chi^2(3) = 6.273$, $p = 0.099$), analgesics ($\chi^2(3) = 7.604$, $p = 0.055$) and PCPs ($\chi^2(3) = 0.217$, $p = 0.975$). However, significant differences were presented for the rest of the categories. The psychiatric/psychotropic category had significant effect ($\chi^2(3) = 7.826$, $p = 0.05$) with a mean rank season score of 6.67 for spring, 5.00 for summer, 11.00 for autumn and 5.00 for winter. Therefore, as seen in Figure 3.8, summer-autumn and winter-autumn had significant differences between them. The heart disease/hypertension category also showed significant differences, ($\chi^2(3) = 8.050$, $p = 0.045$), between spring-autumn, summer-autumn, and winter-autumn with a mean rank season score of 11.00 for spring, 11.00 for summer, 16.56 for autumn and 11.00 for winter. Lastly, antibiotics indicated significant difference between groups ($\chi^2(3) = 8.562$, $p = 0.036$), winter-autumn and summer-autumn, with a mean rank season score of 19.19 for spring, 1.83 for summer, 21.81 for autumn and 12.30 for winter. Consequently, only urban surface waters presented significant differences between seasons for certain pharmaceutical categories. Venlafaxine and citalopram were responsible for the significant change in autumn (compared to the rest of seasons) for the psychiatric/psychotropic category. These two compounds were quantified above <LOQ for October and November compared to the lower concentrations (<LODs or not detected) obtained for the rest of months. For citalopram, this could be due its higher prescription over those two months; it was ranked in a higher position in the prescription data from Table 1.2 for these months (position 56) compared to the rest of months of sampling campaign (57-59). However, venlafaxine presented the same position than other months where the compound was not quantified. Relatively consistent levels across the year have been reported previously in other studies since these compounds are used

in long term treatments, decreasing their relation to seasonal variation.[318] Nevertheless, higher concentrations have been attributed in winter time due to its use for treating depression, anxiety, panic disorder, etc. possibly explaining the higher concentrations found during the October-November period. The same two months (October and November) presented the only two compounds quantified for the heart disease/hypertension, propranolol and hydrochlorothiazide, and for antibiotics, mainly ciprofloxacin and trimethoprim. Higher concentrations of antibiotics during autumn could be related to sickness of the people during these periods,[319] however, only amoxicillin was in the most prescribed top 100 rank in Ireland in the months studied; which was at higher positions for these months than the rest of the year except December-February, where they were not detected even though it would be expected that they were more frequently prescribed. Antibiotic concentrations have been reported to change depending on the season, for example quinolones (e.g. ciprofloxacin) and macrolides (e.g. erythromycin) were obtained at higher levels during the dry season (March) in Huangpu surface waters (Shanghai, China). They concluded that urban rivers were rain-source resulting in different flows between dry and wet seasons. Also, weather influences such as high temperatures and sunlight in summer contributed to photo and bio degradation of the antibiotics resulting in lower concentrations in June (wet season).[320] Similar trends can be observed for the antibiotics in the urban Irish surface waters, where compounds were detected at <LOD, except erythromycin in July at <LOQ. Ireland usually lacks of extreme temperatures and has abundant rainfall which spreads uniformly across the year and a large number of days resulting in low rainfall intensities but a high total annual rainfall.[321] Nevertheless, there was a significant drought recorded in Ireland in 2018 (June),[322] however, the sampling campaign did not start until October. Therefore,

high significant differences between seasons for most categories of compounds are not expected matching results obtained in this study.

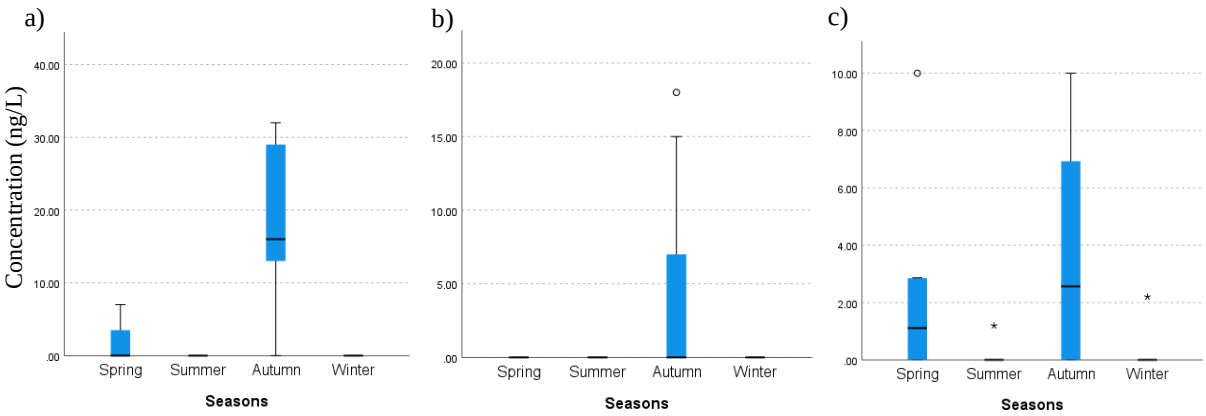


Figure 3.8 Box plot results from independent-samples Kruskal-Wallis test for different CEC categories in urban surface waters a) psychiatric/psychotropic, (b) heart disease/hypertension, and (c) antibiotics.

Effluent rural samples were also compared seasonally and all of them showed non-parametric data ($p<0.05$). Therefore, Kruskal-Wallis tests were performed where pesticides ($\chi^2(3) = 0.843$, $p = 0.839$), analgesics ($\chi^2(3) = 1.832$, $p = 0.608$), antibiotics ($\chi^2(3) = 1.346$, $p = 0.718$), anti-inflammatories ($\chi^2(3) = 0.140$, $p = 0.987$), heart disease/hypertension ($\chi^2(3) = 3.006$, $p = 0.391$), hormones ($\chi^2(3) = 4.536$, $p = 0.209$) and psychiatric/psychotropic ($\chi^2(3) = 2.679$, $p = 0.444$) categories presented no significant differences. Antihistamines/bronchodilators and the others categories presented parametric data and a one-way ANOVA test was performed where again, no significant differences were obtained, ($F(3,18) = 1.351$, $p = 0.289$) and ($F(3,8) = 1.299$, $p = 0.340$) respectively. Therefore, distribution was determined to be the same across categories of seasons, except for PCPs where the Kruskal-Wallis test showed significant differences between groups ($\chi^2(3) = 11.434$, $p = 0.010$) with a mean rank season score of 13.44 for spring, 21.67 for summer, 10.50 for autumn and 22.83 for winter. Significant differences were observed for autumn-summer ($p=0.013$), autumn-winter ($p=0.006$), spring-summer

(p=0.050), and spring-winter (p=0.025) as seen in Figure 3.9. This contribution is mainly from octinoxate, an organic UV filter which levels of detection depend on the seasons[185] and higher concentrations have been found in wet seasons (May-August) or summer.[225] This has been related to the high use of UV filter products during warmer months.[186] However, high amounts were also observed in winter and this could be due to their not restrictive use in sunscreen but also cosmetics, shampoos, and lotions as well as industrial products (e.g. insecticides, plastics, detergents, etc.).[185] All categories for urban effluent waters presented no significant differences between groups when analysed for both non-parametric and parametric data: pesticides ($\chi^2(3)= 0.463, p=0.927$), antibiotics ($\chi^2(3) = 0.570$, $p = 0.903$), anti-inflammatories ($\chi^2(3) = 5.480$, $p = 0.140$), heart disease/hypertension ($\chi^2(3) = 0.540$, $p = 0.910$), hormones ($\chi^2(3) = 3.730$, $p = 0.292$), psychiatric/psychotropic ($\chi^2(3) = 1.672$, $p = 0.643$), PCPs ($\chi^2(3) = 2.749$, $p = 0.432$), analgesics ($F(3,44) = 1.711$, $p = 0.178$), antihistamines/bronchodilators ($F(3,20) = 0.135$, $p = 0.938$), and others ($F(3,8) = 0.241$, $p = 0.865$). Consequently, all categories are spread across all year in this urban area.

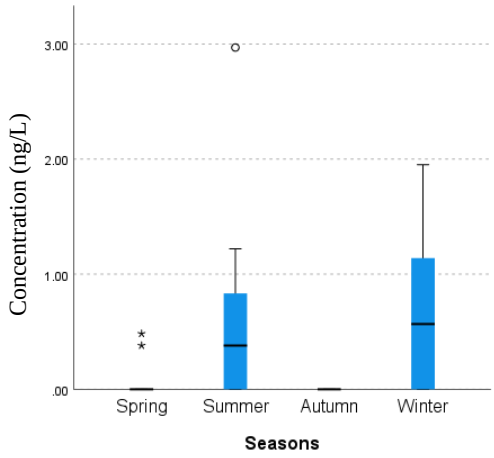


Figure 3.9 Significant difference between seasons for the PCPs category using ANOVA Kruskal-Wallis test for non-parametric distributed data ($p<0.05$) for rural effluent samples.

For influent rural samples, no significant differences were determined for pesticides ($\chi^2(3) = 2.461$, $p = 0.482$), analgesics ($\chi^2(3) = 1.392$, $p = 0.707$), antibiotics ($\chi^2(3) = 0.327$, $p = 0.955$), antihistamines/bronchodilators ($F(3,18) = 1.351$, $p = 0.289$), anti-inflammatories ($\chi^2(3) = 1.633$, $p = 0.652$), heart disease/hypertension ($\chi^2(3)= 3.564$,$p=0.313$), hormones ($F(3,5) = 0.732$, $p = 0.576$), psychiatric/psychotropic ($\chi^2(3) = 1.629$, $p = 0.653$), PCPs ($\chi^2(3)= 0.1.602$, $p=0.659$) and others ($\chi^2(3)= 0.562$, $p=0.905$).

For the urban area, pesticides ($\chi^2(3) = 0.517$, $p = 0.915$), analgesics ($\chi^2(3) = 0.728$, $p = 0.867$), antibiotics ($\chi^2(3) = 0.805$, $p = 0.848$), antihistamines/bronchodilators ($F(3,20) = 0.689$, $p = 0.569$), anti-inflammatories ($F(3,19) = 2.361$, $p = 0.104$), heart disease/hypertension ($\chi^2(3) = 2.880$, $p = 0.411$), hormones ($\chi^2(3) = 3.730$, $p = 0.292$), psychiatric/psychotropic ($\chi^2(3) = 0.646$, $p = 0.886$), PCPs ($\chi^2(3) = 0.721$, $p = 0.868$) and others ($F(3,8) = 0.279$, $p = 0.839$) also resulted in no significant differences. Therefore, no dramatic seasonal changes were found for any category in any influent sample. However, certain compounds were found at higher concentrations in certain months, for example octocrylene, which had no significant difference on its own between the different seasons in rural samples ($F(3,8) = 1.471$, $p = 0.294$) but as seen in Figure 3.10 it was detected at higher concentrations overall for spring and summer, ranging from not detected during winter and autumn to a maximum concentration of 182 (±66) ng/L in June. Similar trends were observed for octinoxate in the rural area as observed in Figure 3.10, no significant differences were obtained on its own ($\chi^2(2) = 2.833$, $p = 0.243$), however, concentrations ranged from <LOQ to 10 ng/L in autumn and winter and 2 to 682 ng/L in spring and summer. These results were similar to other reported studies where concentrations were almost two times higher in summer periods in influent and river samples for UV filters concentrations. This was attributed to the higher amount of UV filters used in summer due to higher temperatures and also the higher number of people

involved in recreational activities due to the holiday season.[225] However, octocrylene was not detected for the urban site and octinoxate concentrations were stable across the year; this could be related to the higher population in this location and the compound's use in skin and cosmetic products, etc. On the other hand, pesticides remained stable throughout the year, which is unexpected due to their application patterns from approximately May-August. Pesticides could also be used in winter for weed control in winter grains such as wheat, rye and barley,[319] however, this is not the case in Ireland, but as mentioned before, some pesticides are also used for manufacturing products which happens across the year.

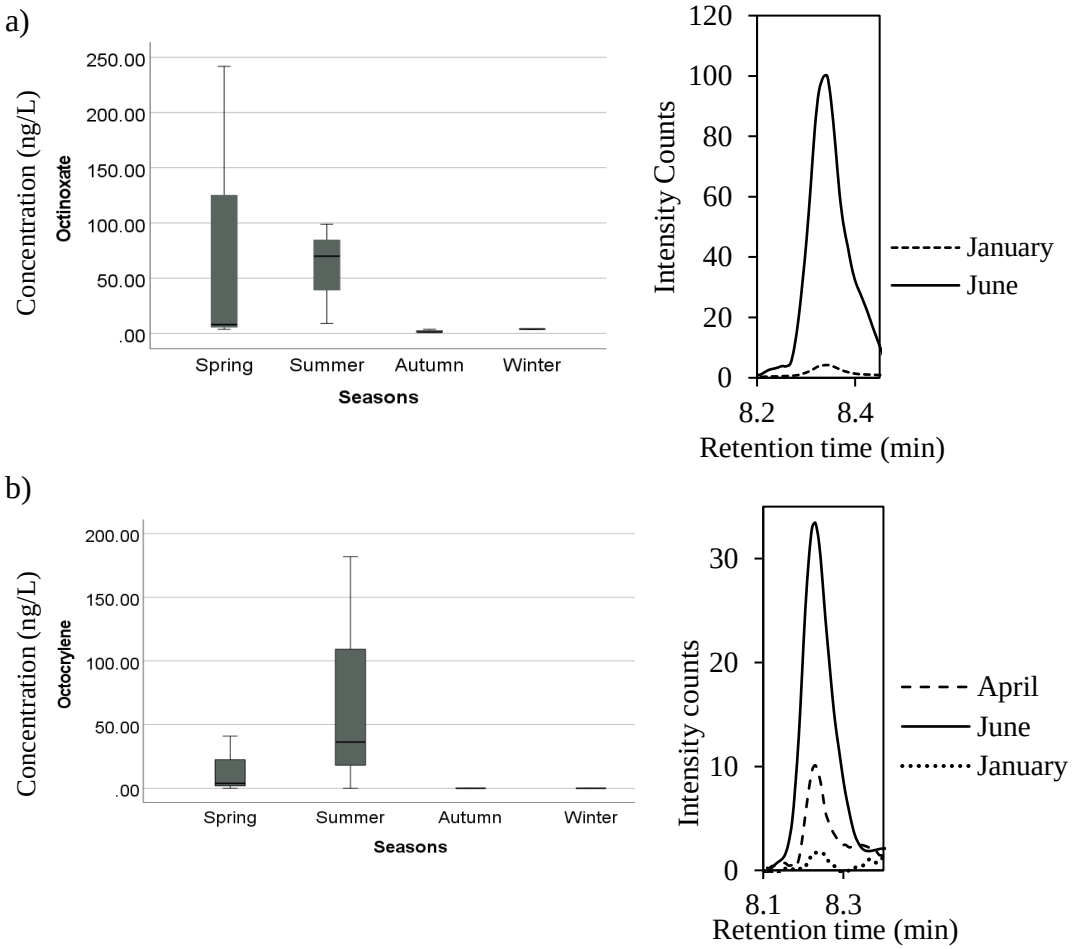


Figure 3.10 a) Octinoxate in rural influent wastewater box plot results from independent-samples Kruskal-Wallis test (left) and chromatograms showing contamination for two different months collected (June = 682 ng/L and January <LOQ) (right); b) Octocrylene in rural influent wastewater box plot results from independent-samples Kruskal-Wallis test (left) and chromatograms showing contamination for three different months collected (April = 4 ng/L, June = 182 ng/L and January = not detected) (right).

3.3.3 Geographical variation

Two different morphological areas were sampled in Ireland, one influenced predominantly by agriculture and one urbanized area. Both locations were investigated for all type of matrices using independent samples t-test for the two variables by categories of contaminants in order to see differences between both catchments. In surface waters, the rural area showed 16 compounds detected overall with 19% of them (i.e. 3 compounds) detected in every sample while the urban site obtained 24 compounds in total and 13% (i.e. 3 compounds) of them with 100% frequency. Two of them were in common, fenuron and octocrylene, however, the third compound was propamocarb for the rural area and octinoxate for the urban one. Carbamazepine, citalopram, clozapine, bisoprolol, hydrochlorothiazide, propranolol, trimethoprim, diphenhydramine and salbutamol were just detected for the urban location, and E2 just in the rural area. The different categories were compared and pesticides ($t(44) = 1.715$, $p = 0.093$), psychiatric/psychotropic ($U = 11$, $p = 0.549$), heart disease/hypertension ($U = 75$, $p = 0.377$), antibiotics ($U = 74.5$, $p = 0.399$) and hormones ($U = 72$, $p = 1.000$) categories did not present any significant difference between both areas, resulting in the same distribution of concentrations across both locations. However, analgesics and PCPs showed significant differences, ($U = 148$, $p = 0.001$) and ($U = 456$, $p = 0.012$) respectively. For the analgesics group, a clear example was tramadol which was quantified at a maximum concentration of 31 (±6) ng/L in the urban area while in the rural location ranged from LOD to LOQ concentrations with no possible quantification (Figure 3.11 (a)). This analyte has additional antidepressant and anxiolytic effects and illegal trades have been reported including global seizures of even 125 tons in 2017.[307] These factors and the higher population of the urban area could explain the difference for this type of compound. However, PCPs showed the opposite results, where

concentrations were higher for the rural area, as observed in Figure 3.11 (b). Lastly, it was not possible to carry out any statistical analysis for the category of antihistamines/bronchodilator as no compounds were detected in any month of the rural area and just two LOQ values in two months for the urban location (October and November).

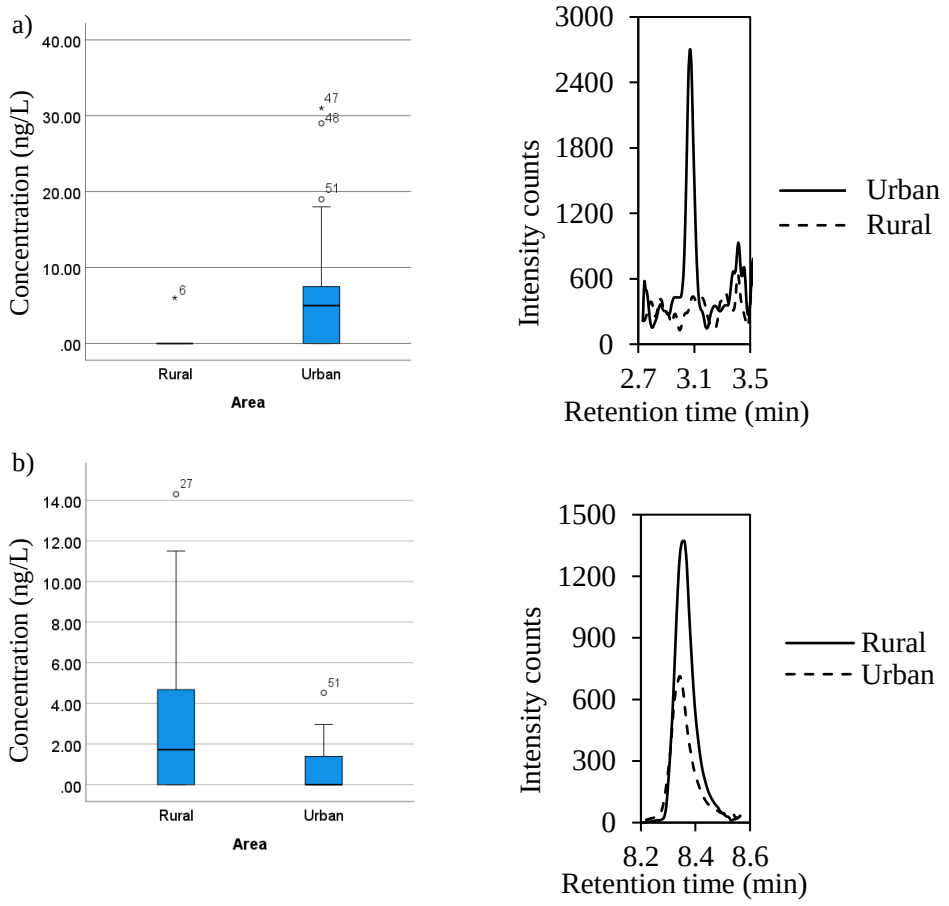


Figure 3.11 Box plot results from independent-samples t-test (Mann-Whitney U test) are presented on the right and on the left examples of MRM chromatograms for selected analytes in surface water samples are showed for (a) the category analgesics with the example of tramadol from October 2018 samples for both areas (rural <LOD and urban 31 (±6) ng/L); and (b) the category of PCPs showing octinoxate chromatograms from August 2019 for both areas (rural = 10 (±32) ng/L and urban <LOD).

For effluent samples, 47 compounds were detected in both areas, E2 and nordiazepam were only found in rural while risperidone and simazine were just in the urban location. From these compounds, attention was given to simazine, a non-approved

herbicide often used for crop protection in agriculture across the world (e.g. China and Australia),[323,324] therefore expected to be observed in the rural area instead of the urban one. However, it has been detected in rivers and runoff from factories in China,[325] suburban areas from WWTPs in Germany,[326,327] and urban road and roof runoff, as well as stormwaters across the world.[324] Even though simazine has been prohibited in Europe[325] it has been detected in several European countries. In Spain, simazine has been excluded from the active substances list,[328] however, it was still present in influents (0.63 –1.8 ng/L) with 100% frequency and effluents with a 62% frequency of samples investigated (not detected–0.54 ng/L) from a drinking water treatment plant (DWTP) during April-September 2019.[276] Additionally, in Germany, it was found at concentrations up to 25 ng/L in surface waters, 50 meters both upstream and downstream an effluent WWTP point source, in 2013 during April and July. However, its presence was irregular and had a frequency of only 4%.[327] In this thesis, its presence is irregular as well and it was only detected, and also quantified, in one effluent sample, for the month of May, from all matrices studied. This month is in agreement with previous stated studies and the use of herbicides around May (once a year) has been reported for road kerbstones and backwalls in the UK, however, this was prior to its removal for industrial use.[329] Nevertheless, the category of pesticides did not show any significant difference between the areas studied ($U = 757$, $p = 0.527$). The following categories also did not present significant difference between locations: antibiotics ($U = 2082$, $p = 0.527$), antihistamines/bronchodilators ($U = 295$, $p = 0.920$), hormones ($U = 22.5$, $p = 0.080$), psychiatric/psychotropic ($U = 2401$, $p = 0.249$), PCPs ($U = 753$, $p = 0.759$), and others ($U = 128.5$, $p = 0.586$). On the other hand, the heart disease/hypertension ($U = 2110$, $p = 0.030$), analgesics ($U = 819$, $p = 0.031$), and anti-inflammatories ($t(28) = -3.510$, $p = 0.002$) categories reported significant differences as seen in Figure 3.12. Higher mean

concentrations were overall achieved for the urban areas. This could be related to higher populations and number of hospitals in the urban area in respect to the rural location in terms of prescription and use of these pharmaceutical compounds.

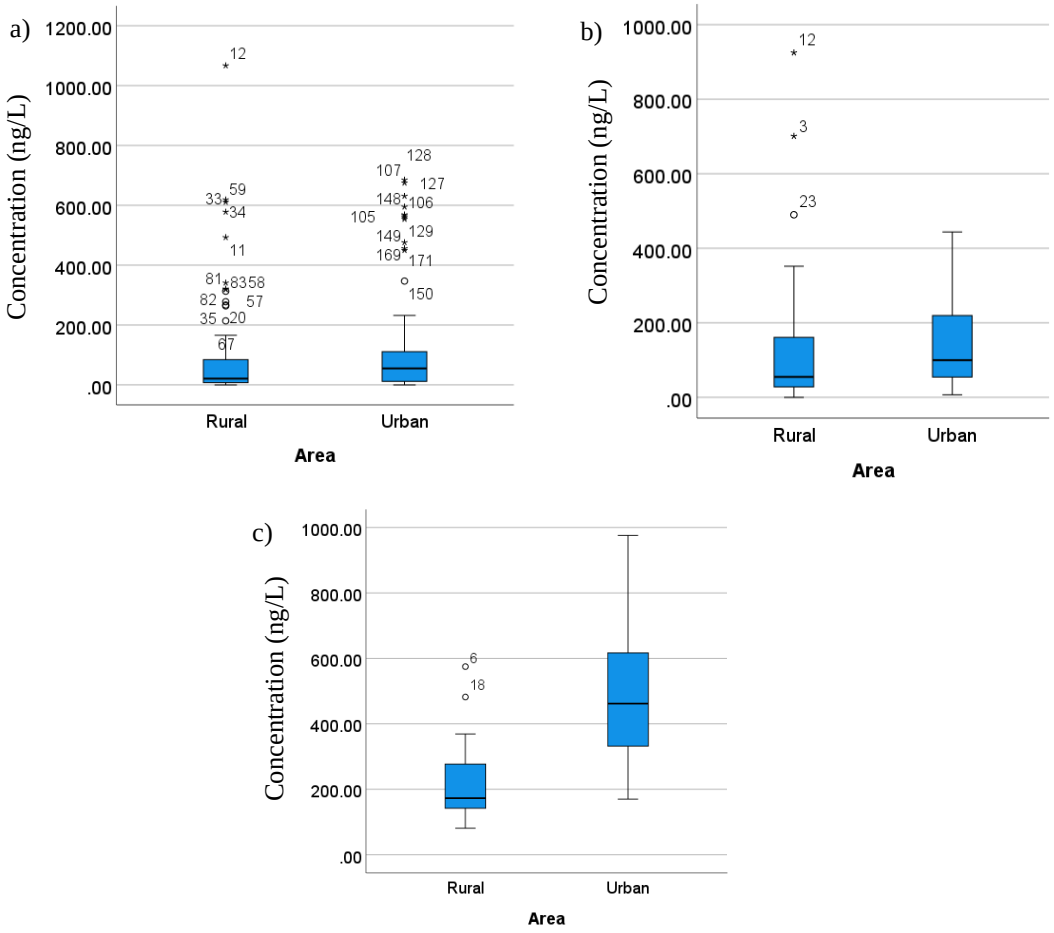


Figure 3.12 Box plot results for a) heart disease/hypertension and b) analgesics categories using independent-samples t-test (Mann-Whitney U test) and c) anti-inflammatories using independent-samples t-test; all showing significant differences between both locations, rural and urban.

Influent waters showed no significant difference for the following categories: antibiotics (U = 2448.5, p = 0.079), heart disease/hypertension (U = 2917.5, p = 0.121), psychiatric/psychotropic (U = 2679.5, p = 0.896), PCPs (U = 286.5, p = 0.056), antihistamines/bronchodilators (U = 228, p = 0.428), and hormones (U = 8, p = 0.060) between the areas investigated. This is not a surprise as compounds such as the antibiotic

sulfamethoxazole are also used for veterinary purposes as antibiotic in animals[330] and similar average concentrations have been obtained for both areas, 165 (±96) and 217 (±120) ng/L for rural and urban areas respectively. Analgesics (U = 1337, p = 0.103) also did not present significant differences, however, antipyrine was studied independently due to its higher concentrations on the rural area (Figure 3.6). After statistical analysis, significant differences were clearly obtained (U = 2, p = 0) as seen in Figure 3.13. This could be due to its widely veterinary medicine use in combination with diminazene diaceturate (DD).[331] Antipyrine contributes to 55% of the mixture in order to make it soluble,[332] as DD is unstable in water on its own, and it is used to treat babesiosis (parasits) in cats, cattle, goats, dogs, swine,[331] horses, etc.[305]

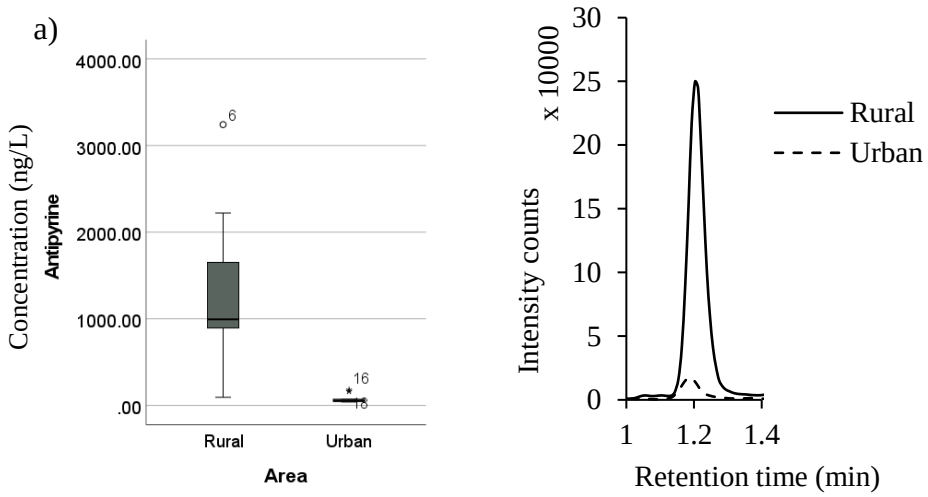


Figure 3.13 Antipyrine a) box plot results from independent-samples t-test (Mann-Whitney U test) presented on the right and on the left b) examples of MRM chromatograms for influent wastewater samples from August 2019 for both areas (rural = 3242 (±2) ng/L and urban = 162 (±1) ng/L).

On the other hand, the anti-inflammatories (U = 67, p = 0), pesticides (U = 516.5, p = 0.02), and others (U = 77, p = 0.034) had significant differences between the areas studied. For the anti-inflammatories group, mefenamic acid mainly contributed to the difference due to the different range of concentrations obtained, 59–336 ng/L and 231–1,463 ng/L for rural and urban area respectively. This compound obtained quite high

concentrations overall, however, it was not on the rank of most prescribed drugs in Ireland at the time of the study (Table 1.2) but it has been previously detected in effluents from hospitals such as Suffolk County (NY, USA)[156] and Negeri Sembilan (Malaysia).[333] This could explain the higher amounts found in the urban area, where there is a higher population and number of hospitals. In the pesticides category, seven compounds were detected in the rural area and six in the urban. Flurochloridone was only detected in the rural area and presented the maximum concentration for the group of compounds with 1,200 (±12) ng/L for the month of April, however, the maximum concentration obtained for the urban location was 367 (±18) ng/L for cymoxanil. Therefore, overall higher concentrations were found in the rural area (Figure 3.14) as expected due to agricultutal patterns. However, three pesticides, acetamiprid, propamocarb and atrazine, were only found in the urban location. This is not a surprise as they all have been detected in urban areas around the world. Acetamiprid has been seen in urban influenced locations such as Galindo in Spain which serves Bilbao city and all towns around (1.2 million population equivalents)[299] and propramocarb has been detected with 92% frequencies in urban samples in Sweden.[334] Atrazine is one of the most frequently detected herbicides in wastewater (e.g. Australia).[335] This compound is used as household and garden products and has been occasionally found in urban areas such as wastewaters from Haikou City (China)[336] and Australia.[335] However, this compound has been banned in Europe but it has been detected in other countries such as Sweden (31% frequency of samples tested) in 2013.[334] Terbutryn is also a non EU approved herbicide which was also detected in this study in both areas, ranging from <LOD to 26 (±10) ng/L. This has also been detected in other European countries such as Sweden at 38% of frequency in 2013 during October-November. Both herbicides detections were attributed to their persistence and also alternative uses such as terbutryn in roof paintings, which can be washed off by rain

ending up in urban wastewaters.[334] Fenuron was also obtained in both areas, but not only in influent samples but effluent and surface waters all detected with 100% of frequency across all matrices. This non-approved herbicide has also been detected in another countries such as the UK, where it was found in river Thames and even biota in a river in Suffolk at high frequency. However, it was not detected in influent wastewater suggesting alternative sources of entering the environment.[317] As mentioned before, fenuron is used in industry as formulation or repacking, being manufactured across Europe.

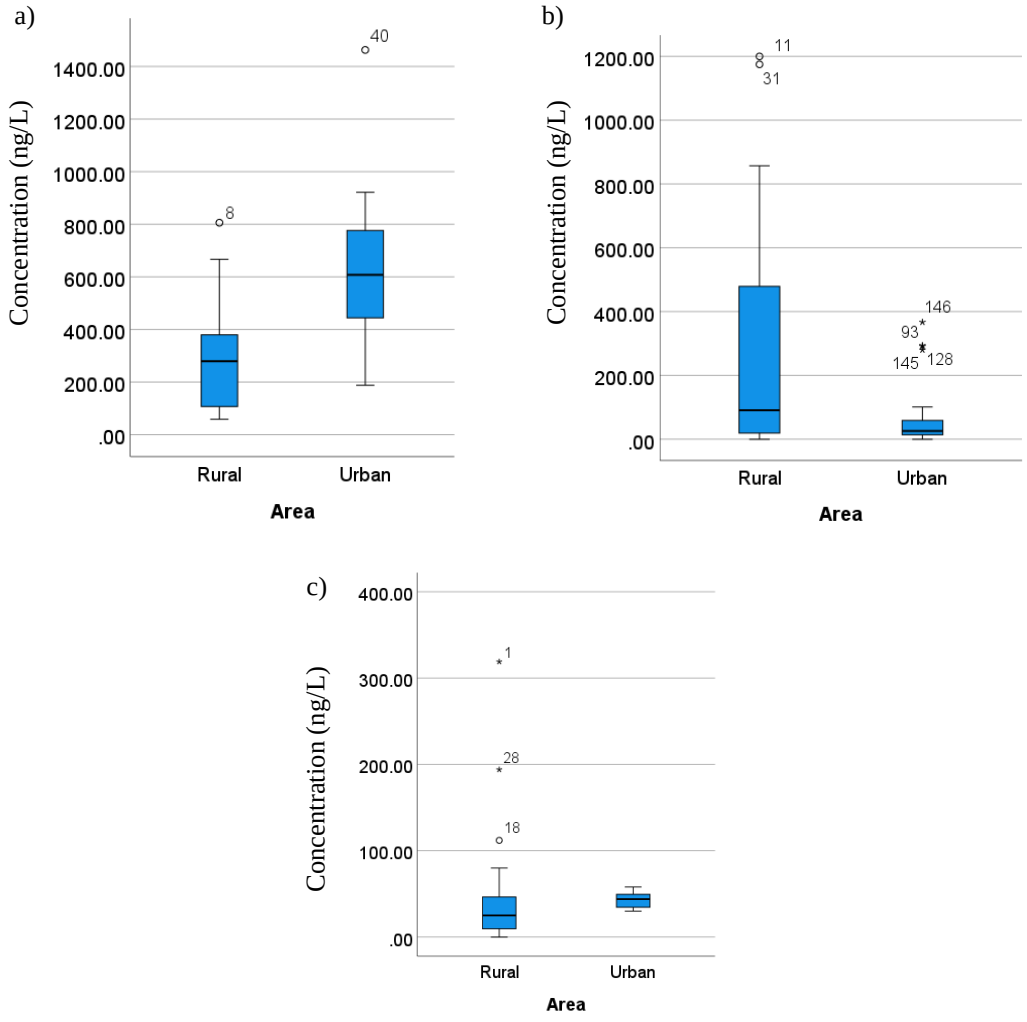


Figure 3.14 Box plot results from independent-samples t-test (Mann-Whitney U test) showing significant differences for both areas, rural and urban, for a) anti-inflammatories, b) pesticides and c) others categories in influent wastewater samples.

3.3.3.1 Urban and rural environments

WWTPs are affected by number of inhabitants, size of catchment, seasonal and diurnal water usage patterns, filtrations, industrial discharges, soil type, environmental patterns like rainfall, temperature, etc. influencing the concentration of CECs.[337] Many CECs such as pharmaceuticals and PCPs, lifestyle chemicals or anti-inflammatories, are freely available without prescription (over-the-counter) and are used widely therefore little variation between catchments can be observed.[26] This is the case of for example UV-filters and diclofenac, detected across both locations and in different matrices. Nevertheless, CECs in municipal sewage are mainly constituted by pharmaceuticals, PCPs, artificial sweeteners, flame retardants, hormones, insect repellents and plasticizers.[336] Pesticides could provide differences between contamination sources (mainly agriculture), however, there is a great number of pesticides available commercially and their application depends on identifying the various sources with specific pesticides within the location, concentration and species between wastewater and agriculture making it a difficult task.[335] In this study pesticides were found across both areas, and even though most pesticide contaminations have been attributed to the agricultural sector, urban pesticides are gaining attention due to their environmental and human health's risks[338] where limit exceedances of these compounds in streams (urban-impacted) have increased (53 to 90%).[324] Hydrological characteristics of urban surfaces are ideal for herbicides to be transported to sewer pipes ending up in surface waters, resulting in worry concentrations.[329] Urban pesticides sources have been related to the maintenance of urban green areas (e.g. public parks, gardens, riverside footpaths, children's play areas, etc.),[339] and to control unwanted vegetation by local and county authorities, for rail operators and airports.[329] Biocides are also used for building materials

and they have been found as contaminants in urban stormwater runoff with other contaminants such as heavy metals, polycyclic aromatic hydrocarbons, and PCPs.[339]

3.3.3.2 Multivariate analysis

Multivariate statistical analysis of both areas was performed using principal component analysis (PCA) to better understand the relationship between contaminants and locations. The PCA plots explained 53% and 9% of the variance on the first and second axes, respectively for surface waters (Figure 3.15). The PCA revealed two clusters for both areas, however, only two months are able to completely differentiate between locations (October and November), as previously mentioned. It shows a clear negative correlation between PCPs (e.g. octinoxate and octocryelene) for the rural area. Compounds in the urban area can be seen at higher concentrations and mainly driven by analgesics such as lidocaine. These results align with the ones obtained by the previous t-tests performed. For effluent wastewater, another two clusters can be observed where urban areas present a higher number of compounds detected. However, only a total of 52% of the variance is represented by PC1 and PC2. Examples of compounds driving towards the urban area are acetamiprid and methylphenidate; and tramadol, E1 and temazepam for the rural area. Influent PCA plot shows the highest concentrations and number of compounds detected towards both areas, making impossible their differentiation between them, as seen by the low variance representation of only 33%. Only two compounds, propranolol and clozapine, are observed to have the greatest discrimination pattern between the two areas; due to the higher concentrations quantified for the urban area.

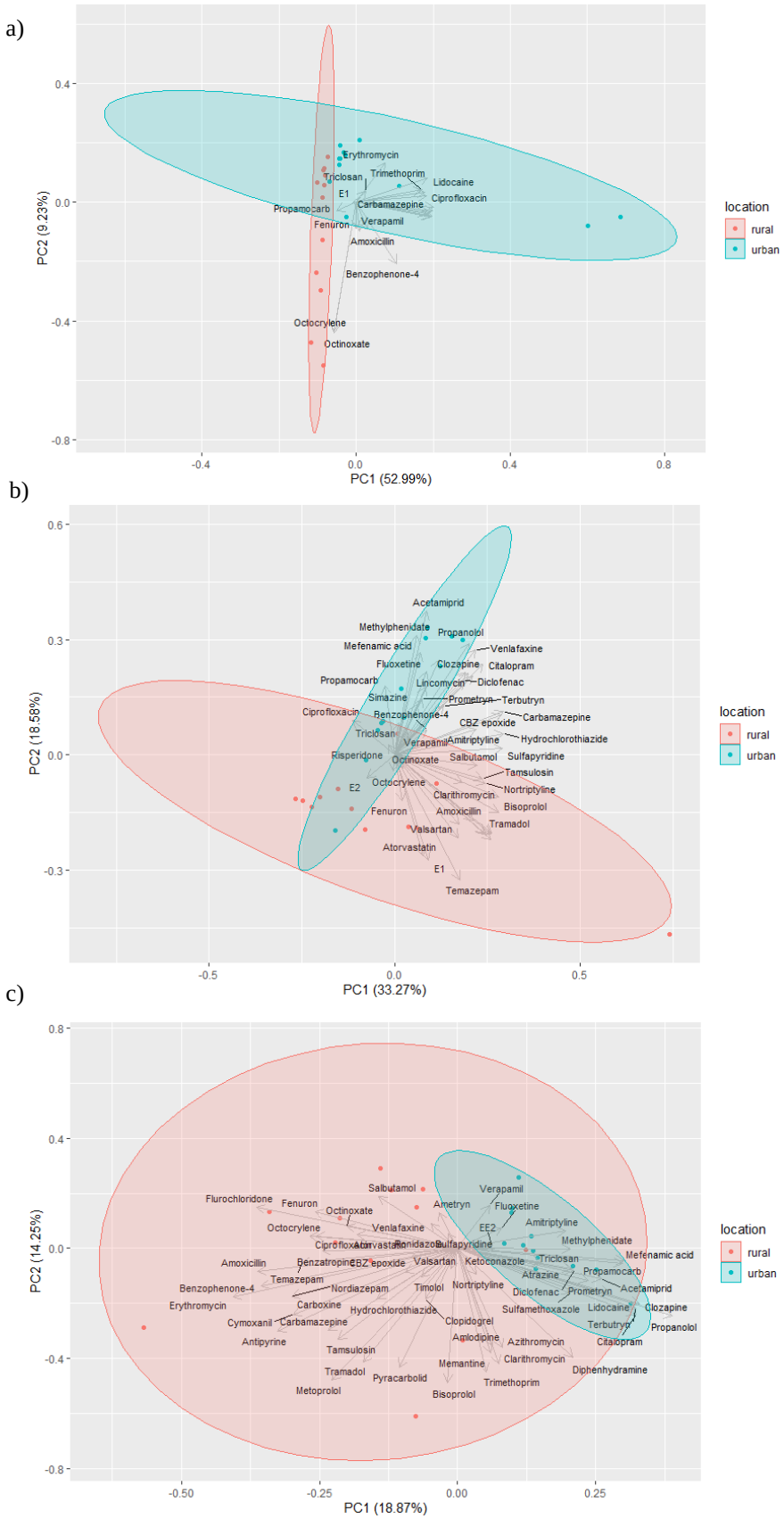


Figure 3.15 Principal component analysis (PCA) of the relationship between compounds detected in a) surface waters, b) effluent and c) influent wastewater in different locations rural (pink) and urban (blue). Percentage explained by the axes is shown in brackets.

3.3.4 Spatial variation (fate) and removal of CECs

CECs sources varied and depended on the type of chemicals, however, WWTPs are considered the main route into the aquatic environment due to its continuous input. There are also other pathways such as diffuse sources like agricultural land uses for animal and crop production or direct points like improper disposal.[291] Transport of detected contaminants can be studied beginning in the WWTP (influent) until entering the environment (surface water). Generally, a decrease in concentration from the input point source (effluent) until entering the environment (downstream) is expected as seen in previous studies. For both sites, the number of compounds detected decreased in surface waters and most of the concentrations did as well as observed in Figure 3.16. As discussed, this reduction was expected, and can be attributed to potential dilution, degradation and partitioning to sediment.[291] A clear example is temazepam, a prescribed pharmaceutical,[104] which was found in influents ranging from 32–311 ng/L, effluents at <LOQ–149 ng/L and finally not detected at any sample for surface waters.

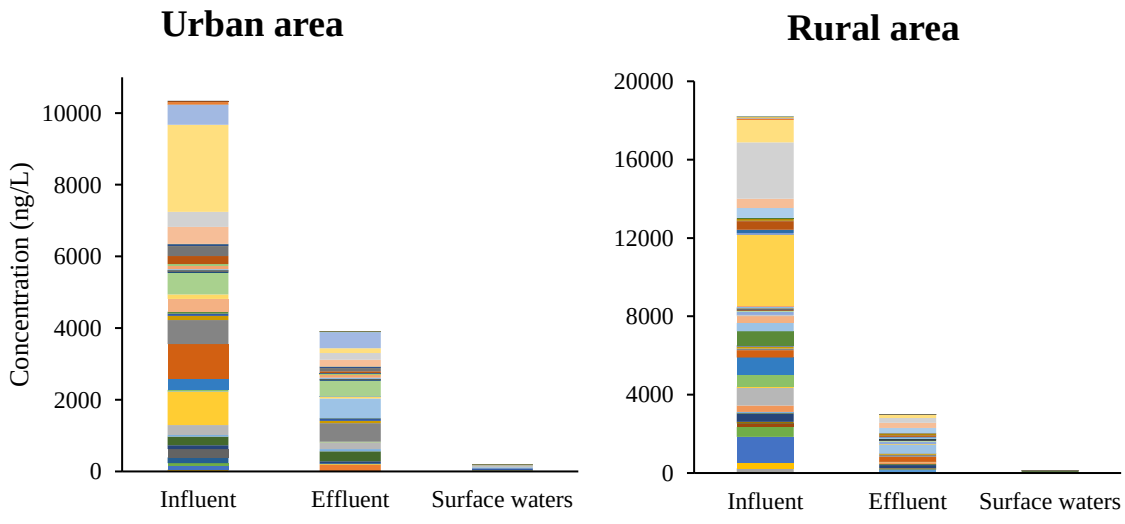


Figure 3.16 Cumulative results of average compound concentrations detected for the sampling campaign for the three matrices tested for the urban and rural areas. Each colour represents a different compound detected.

Even that WWTPs are not designed to remove CECs, it can be appreciated that approximately a 62% of the total concentrations were removed for the urban area, where 95% of the concentrations remaining in the effluent were further diluted in the surface waters. Higher results were even obtained for the rural area, where almost 84% of the concentrations were removed during treatment and 95% of the remaining in effluents were further diluted once entering the natural environment.

To fully understand CECs, it is essential to know their transport and fate but also removal in WWTPs, as they are the main source of entrance.[340] A characterisation of influent and effluent wastewater samples was performed to determine the removal efficiency of both WWTPs selected. Removals varied overall, as they depend on the physicochemical properties of the molecules and also the type of treatment performed,[293] possibly different in both areas investigated. For this reason, removals are treated separated depending on the location and they can be seen in Figure 3.17. Individual removals are presented in Appendix F in Table A.15 with the range and average percentage. Overall, 19%, urban area, and 24%, rural area, of the total compounds obtained ≥80% removal efficiencies. High removals were achieved for certain compounds such as amlodipine, 98 (±1) and 94 (±1) %; fluoxetine, 92 (±5) and 86 (±5) %; azithromycin, 92 (±8) and 98 (±1) %; antipyrine, 99 (±1) and 92 (±3) %; E1, 79 (±13) and 88 (±4) %; for the rural and urban respectively. These results are promising due to the endocrine disrupting characteristics of certain compounds such as E1 and the development of antibiotic resistance bacteria (ARB) and environmental presence of antibiotic resistance genes (ARGs), threatening human and animal life,[341] for compounds such as azithromycin. Nevertheless, these specific compounds have different chemical structures between them and this has been also previously reported where the removal variabilities have no evident correlation to the structure of the compound,[342] following

the results obtained within this study. Even that the compounds have different chemical structures and functional groups, log *P* values were in close proximity, 3.29–4.27 (Table A.2, Appendix A), with the exception of antipyrine (log *P* = 0.72). Antipyrine removals during conventional treatments usually are around 30%[304] due to its difficulty to biodegrade, however, other studies removals range from 0 to 100% based on the type of treatment. Nonetheless, high removals of this compound are usually achieved due to its photodegradation concluding that treatments such as ultraviolet disinfection show high effects for its removal,[343] however, the use of this specific treatment in both locations tested was not possible to confirm. Pharmaceutical compounds with high log *P* values (usually >5) and high molecular weights have been reported to removed easier from aqueous phases and to sorb easily to soils and sediments. On the other hand, compounds with log *P* values <2.5 tend to remain in the aqueous phase.[344] This is also in agreement with the low removals obtained for compounds such as bisoprolol and tamsulosin, with average removals of 46 (±33) and 49 (±21) %, and 45 (±32) and 40 (±22) %; for rural and urban areas respectively; where log *P* values were 2.21 and 2.14, respectively (Table A.2, Appendix A). However, as observed by their high standard deviations calculated, their removals vary widely between samples collected across the year. Moreover, negative removals (that is, an apparent increase in concentration or frequency) were also observed for certain compounds across both sites such as acetamiprid, carbamazepine, nordiazepam, sulfamethoxazole, etc. This is due to higher concentrations found in effluent samples or compounds not detected at all in influent samples. This could be attributed to analytes present as conjugated metabolites (transformation of some human or microbiological metabolites) in the influent which after the treatment in the WWTP deconjugate[300] or transform back to the parent compounds.[39] Previous reported examples are sulfamethoxazole, which is excreted as only 10% of the dose as the parent drug and

approximately 50% as the metabolite N_4-acetylsulfamethoxazole,[39] and carbamazepine, which could be excreted also as conjugates from urine, releasing the parent compound after enzymatic activity and therefore increasing effluent concentrations.[345] Another hypothesis that has been reported is the desorption of pharmaceuticals from biological materials for compounds such as macrolide antibiotics (e.g. azithromycin and clarithromycin), venlafaxine and trimethoprim. Possible desorbsion of parent compounds could happen in biological treatment processes increasing the effluent concentration and playing an important role in the fate of these compounds,[300] highlighting the variability of removals depending on the type of treatment. Venlafaxine and trimethoprim could follow this hypothesis for some months which obtained negative removals within this study. However, compounds such as azithromycin and clarithromycin were obtained with removals of >92% for both areas, however, these compounds pose really high molecular weights and, as mentioned previously, this characteristic helps with their removals. Another possible explanation could be the filtration process that occurs before sample analysis, explained before in Section 2.4.2.3, where these type of compounds are more retained in the filter and possibly underestimating the concentrations of effluent samples. However, in order to avoid this problem and overestimate the % of removal, LOD values were given for the effluent samples that were not detected. Nevertheless, further research would be needed to assess these specific compounds.

Consequently, removal rates are not consistent throughout the year sampled for the same compound on the same area resulting in negative and positive removals with wide ranges of removal. An example is memantine in the urban area, where only the month of January presented a negative percentage compared to the rest of samples. Dilutions due to increase in wastewater flows can occur[346] but weather conditions such as rainfall and temperatures also contribute to CECs removals, usually decreasing

concentrations. It has been reported that higher removal efficiencies have been obtained in warmer periods due to higher temperatures, enhancing the degradation of the compounds. However, this depends on the compound itself, as ketoprofen for example, which has been previously demonstrated to be unaffected by temperature or to be removed even more efficiently in winter periods.[128] Another example is atorvastatin which presented high removals in both areas across the year except for the month of February and this could be explained to the weather conditions as mentioned before. An additional factor to take into account are possible analytical errors. There are not standardized methods for CECs analysis, and due to their presence at low concentrations, analytical uncertainties are raised. The analysis usually consists of a multiple step process including filtration, extraction (e.g. SPE), chromatography and mass spectrometry, potentially leading to cumulative analytical errors. Interferences by particles and other substances in the samples can also cause matrix effects. High matrix effects were obtained for majority of compounds across all matrices investigated (Tables 2.8 – 2.10 and Tables 2.11 – 2.13) and this could result in ion suppression or enhancement which can decrease precisions and accuracies.[347] Matrix-match calibration lines were performed for every compound in this study in order to minimise possible errors of this type, as well as the use of internal standards when possible. Additionally, improper sampling of a non-constant flow of wastewater can also result in greater uncertainty in results obtained. The type of sample collection affects directly to the final concentrations obtained. Nitrogen compounds, mainly as ammonium, come from influent wastewater and it can also be generated during certain treatments and accumulate at high levels.[348] Variability of ammonium concentration also differs on the stages on the wastewater treatment and ammonia-nitrogen (NH_3) is a major constituent in the raw domestic effluent.[349] Its concentration is dependent on pH and temperature of wastewater.[350] Diurnal variations

of treatments affect the dissolved oxygen (DO) and pH during the day. Usually low pH and DO are obtained at night and high concentrations are observed for both during midday. These variations affect the removal efficiencies during the course of the day.[351] Ammonia concentrations have been reported to be related to high concentrations of certain contaminants such as PPCPs (including endocrine disrupting compounds like hormones). The elevated concentrations of NH_3 in effluent after treatment forms chloramines which are less effective when removing/transforming some of these compounds.[352] Diurnal variation of contaminants concentrations and wastewater hydraulic and mass flows can also impact the results. Variations in concentrations during the day have previously been reported, whereby for 83 different pharmaceutical compounds, higher concentrations were obtained between 15:00–22:00 pm compared to 23:00 pm–10:00 am, related to pharmaceutical consumption.[346] Calculation errors could also result from sampling without taking into account the hydraulic retention time. Therefore, flow proportional 24 hours-composite samples are a better option as they cover the diurnal concentration of the contaminant and hydraulic and mass flows.[342] As a result, in order to minimise possible errors, effluent and influent grab samples were taken always in the morning and at the same time when possible (11:00 am approximately) as composite samples were not possible to obtain. However, variations in the wastewater loads and the use of grab samples could have also lead to the increase of concentration in the effluent achieving negative results.[345]

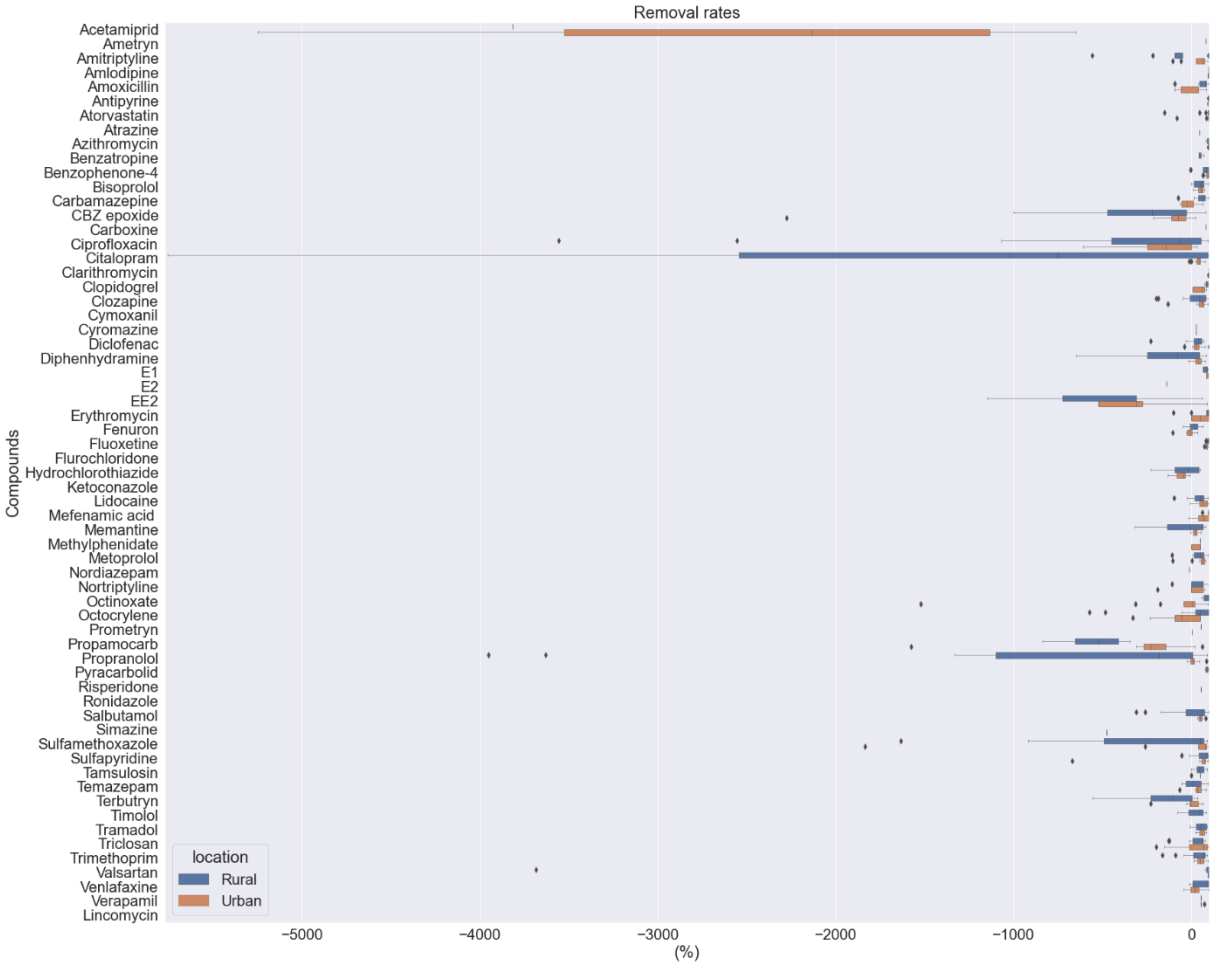


Figure 3.17 Removal rates of detected CECs a) for all compounds with negative values for both areas investigated rural (blue) and urban (orange), where error bars present minimum to maximum (n=12, months analysed).

Once effluents are discharged from the WWTP they enter the environment. All compounds detected in surface waters were detected in the effluent wastewaters suggesting the WWTP as their point source. For both areas, 47 compounds were detected in the effluent and 24 of them were further detected downstream the discharge point. The majority of the compounds presented lower values in the surface water samples, e.g. bisoprolol, hydrochlorothiazide and carbamazepine. As the distance between the sampling location and the effluent discharge is not too long, approximately 50 meters for the rural area and 1 km for the urban location, the opportunity for additional biological degradation and photodegradation between sampling locations is limited. Lower

concentrations have been reported for sampling locations further away from the wastewater outputs, as would be expected.[299] Compounds with high resistance to these variables were found to be diluted (e.g. carbamazepine) or not detected at all (e.g. diclofenac).[26] However, for certain months, some compounds presented higher values in the aquatic environment. An example is fenuron in the urban area, which was obtained at higher concentrations for the months of February, May and July. This suggests that the WWTP is not the only source for this compound (detected across all influent samples). Similar results were found in London (UK) where it was detected in the river Thames but not in any influent samples suggesting more spatial and temporal monitoring was required in order to locate its source.[144] Additional herbicides, such as simazine and atrazine, are characterised by their persistence in the environment due to their increased polarity and water solubility.[335] However, neither of these two were found either in effluent or surface waters, suggesting in this case their effective removal during treatment. Overall only two pesticides were found in surface waters, fenuron and propamocarb. This could be due to the pesticide properties and weather characteristics like rainfalls, such as the time between the pesticide application and rainfall.[335] In the rural area, propamocarb was also found at higher concentrations in all samples suggesting another source as well. On the other hand, only two months presented higher concentrations in the urban area. This could be related to their application in urban surfaces, where pesticides are lost by volatilization and photodegradation as they are directly exposed to sunlight.[335]

3.4 Conclusions

For the first time, the temporal and spatial occurrence of >100 contaminants of emerging concern were monitored in the aquatic environment and WWTPs over a period of a year (12 months) in Ireland. The analytical techniques from Chapter 2.0 were combined and applied to Irish surface water, influent and effluent wastewater samples in two different locations, an urban and a rural area, where ≥16 different compounds were obtained across all samples in the ng/L level. Maximum concentrations obtained were 134 (propranolol), 1,067 (hydrochlorothiazide) and 8,273 (venlafaxine) ng/L, resulting in pharmaceuticals being detected in highest concentrations across all matrices tested from all samples analysed. Seasonal variations (spring, summer, autumn and winter) showed no significant results for the majority of the categories studied. Only urban surface waters presented significant differences for psychiatric/psychotropic, heart disease/hypertension and antibiotics categories; and for effluent samples, PCPs showed a significant difference between the seasons. Geographical variations were also studied by categories (t-test) and within the whole dataset (PCA). Only analgesics and PCPs presented significant differences between both locations for surface waters. Effluent samples showed differences for heart disease/hypertension, analgesics and anti-inflammatories. Influent wastewaters showed differences for anti-inflammatories, pesticides and others. However, when datasets were analysed by multi-variable analysis (PCA), clusters were grouped close to each other representing low percentages of the total variance (≤62%), making difficult the difference between both locations for any of the matrix tested.

Contaminants showed a clear decrease in concentration once entering the aquatic environment, surface waters, possibly because of dilution or degradation; suggesting the WWTPs as the main point source. However, some compounds, particularly

agrochemicals, presented higher values than in effluent and more spatial investigation should be considered in order to determine the possible sources of these compounds. Removal efficiencies, on the other hand, were quite variable depending not only on the compound but on the month when samples were collected. This is attributed to not only weather conditions but also to calculations which contained increased uncertainties due to grab sampling inefficacy. Nevertheless, this study has confirmed the presence of contaminants of emerging concern from a wide number of types (e.g. pesticides, PCPs, pharmaceuticals) and categories in all matrices across both locations in Irish water samples. It also highlights the importance of the application of new analytical emerging techniques in order to reach the low concentrations of certain contaminants such as hormones (majority of samples detected as LOD and LOQs). These findings will enable an assessment of the risk posed by these contaminants of ecological risks in the aquatic environment. It will also support the development and optimisation of strategies for the efficient removal of identified CECs and to minimise their potential risk in surface waters.

4.0 Risk assessment of CECs in Irish waters

Abstract

With the increasing occurrence of contaminants of emerging concern in surface waters, it is essential to assess whether these compounds can affect the environment. In this study, two different morphological regions and two types of water matrix, surface waters and effluent wastewater, were selected for risk assessment. The European Medicines Evaluation Agency (EU EMEA) risk assessment tier approach was considered after a literature review in order to perform the environmental risk assessment (ERA). An initial PBT assessment was investigated for a total of 49 compounds detected in Irish samples. After its application, compounds determined to result in highest risk were pharmaceuticals such as atorvastatin, citalopram and E2. Compounds quantified at ≤10 ng/L but with a high PBT index score achieved were selected for further investigation. For these compounds, an ERA analysis was performed where 8 and 7% of compounds studied from a total of 24 and 15 posed a high risk for urban and rural surface waters respectively. However, in effluent samples the percentage increased to 14% for rural and 13% for the urban areas out of 45 compounds detected in total. This is due to higher concentrations quantified in this matrix, possibly because they are considered the output of the WWTP following a dilution in surface waters. Compounds with higher contribution factors at both sites belonged to the pharmaceutical category, raising the concern for these compounds at these levels detected. For surface waters, main contributions were achieved by E2 and propranolol while for effluents, sulfamethoxazole and propranolol acquired the higher percentages. This work has provided a prioritised list of contaminants following PBT and ERA assessments for two areas in Irish effluent and surface water samples.

Aims and Objectives

- Evaluate data obtained from Irish samples within this thesis in order to perform a hazard and a risk assessment for effluent wastewater and surface waters, following fate of contaminants into the environment.
- Perform a hazard assessment for contaminants determined within this thesis.
- After preliminary analysis of compounds detected, apply EU EMEA approach for Environmental Risk Assessment performance.
- Estimate PNEC values in order to calculate RQs.
- Following ERA, study the classification of risk for all the required compounds.
- Establish a comparison of sites depending on the accumulated potential risk calculated and estimate contribution per compound.

4.1 Introduction

Contaminants of emerging concern (CECs) are of concern as they have been linked to ecological effects[340] and hundreds of these compounds have been detected globally in the aquatic environment at concentrations in the range of ng/L to µg/L.[150] Once they are released into surface waters, CECs have the potential to accumulate in aquatic organisms, which is increasingly a cause for alarm for scientists. This process of bioaccumulation can occur not only with water exposure but also with food, sediment and air.[89] Certain pharmaceutical CECs have been shown to have negative impacts in biota at similar concentrations to those found in surface waters.[291] The interaction of CECs with living organisms after their absorption makes them a likely hazard, with ecotoxicological effects resulting from their biological activity.[353] Compounds such as venlafaxine, previously determined in this thesis to be present in water samples analysed, have been proven to exhibit hazardous effects such as disruption in early development in fish.[293] However, CECs are typically present as a cocktail of compounds with different chemistries and different physicochemical characteristics, making their individual determination challenging at low levels and providing additional challenges for simultaneous determination of a broad spectrum of compounds.[317] The research of ecotoxicity is a growing study area[150] as there is an urgent need to investigate the source and fate of contaminants to examine their risk in the environment.[293] As waste water treatment plants (WWTPs) are not designed to remove these compounds, and thus are a significant accumulation point source for these compounds,[340] WWTP continuous discharge could present a long-term exposure for potentially impacted organisms.[306] Monitoring CECs is crucial in order to help to better understand removal efficiencies, therefore improving exposure predictions. However, robust spatiotemporal studies are necessary to determine

this information. Reliable monitoring data is not easily achieved and in some countries such as Ireland only a limited dataset exists, making effective risk assessment quite difficult.[354]

The impact of pharmaceuticals on the environment can be investigated by performing an environmental risk assessment (ERA). This assesses the probability of the compound posing a risk to aquatic organisms in the specific investigated area.[114] Different models exist but in 1993 the European Medicines Evaluation Agency (EU EMEA) was established. Under Directive 2001/83/EC, any new drug needs an ERA before licensing but its impact will not be considered as a refusal criterion. This procedure follows a tiered approach[330] (Figure 4.1) and has been extended to other types of CECs such as pesticides,[355] however, the model is compound related so it does not consider compound combinations. It estimates the risk based on the comparison of a predicted environmental concentration (PEC), from WWTPs monitoring, with predicted no-effect concentrations (PNEC), generally calculated from acute toxicity tests.[354] This model has a preliminary phase which considers a Persistent, Bioaccumulation and Toxic (PBT) assessment[353] in the first instance, following from which the technique compromises two phases:

- Phase I consists of a pre-screening for compounds with a log K_{ow} > 4.5 or with potential risk, such as lipophilic compounds and potential endocrine disruptors (EDCs). It estimates the exposure using PEC values of the compound. These PEC values are derived from predicted amounts of compound used and data from the WWTP or surface waters (Equation 4.1).

$$PEC_{surface\ water} = \frac{DOSEai*Fpen}{WASTE_{Winhab}*Dilution}$$

where: DOSEai: daily maximum dose consumed per inhabitant (mg/inh·d)
Fpen: fraction of market penetration
$WASTE_{Winhab}$: amount of wastewater per inhabitant per day (L/inh·d)
Dilution: dilution factor

Equation 4.1 PEC of local surface water concentration calculation.[356]

If the value of PEC is below 10 ng/L it is concluded that there is no appreciable risk associated with the compound, on the basis of the small levels of concentration in the environment. However, it should be noted that this threshold limit might not be universally applicable as some substances even at these low levels could have repercussions on the biota and therefore still need to be evaluated in phase II of the assessment.[356] If the compound is suspected to result in ecotoxicological effects or the value is greater than 10 ng/L, further assessment is necessary, and so the assessment moves to the next phase.

- Phase II divides into two tiers, A and B. The first tier (A) is based on screening and evaluates the physicochemical and toxicological data of the compound. This is when the compound's PNEC forms part of the assessment and standard acute toxicity tests are performed (algae, daphnia and fish) in order to calculate it. To account for more realistic conditions, an assessment factor (AF) is considered in conjunction with this number. Due to limited toxicity data,[357] AFs are an expression of the degree of uncertainty in the extrapolation from the test data on a limited number of species to the actual environment. They account for inter-species (differences in sensitivity) and intra-species variabilities and laboratory data to field impact exptrapolation.[356] Applying this factor results in an exposure level considered safe for the entire ecosystem. They are usually resulting from professional experience, and are intended to ensure conservative outcomes in

tiered risk assessments approaches; meaning a PNEC from limited toxicity data. AFs improved when statistical methods were used to analyse toxicity data from different studies.[357] A risk quotient (RQ) is then calculated by dividing the PEC by the PNEC. If the ratio is below 1 and there is no suspicion of bioaccumulation risk, the assessment is concluded. However, if it is higher than 1 or potential accumulation in the environment is indicated (using log K_{ow}), the assessment of the compound should move to tier B. This tier is an extended stage where further considerations are taken including the incorporation of data such as analysis of consumption, metabolism, emissions, modelling environmental fate, WWTPs removals, effects of the compound and chronic toxicity test concentrations (fish, daphnia or algae).[330]

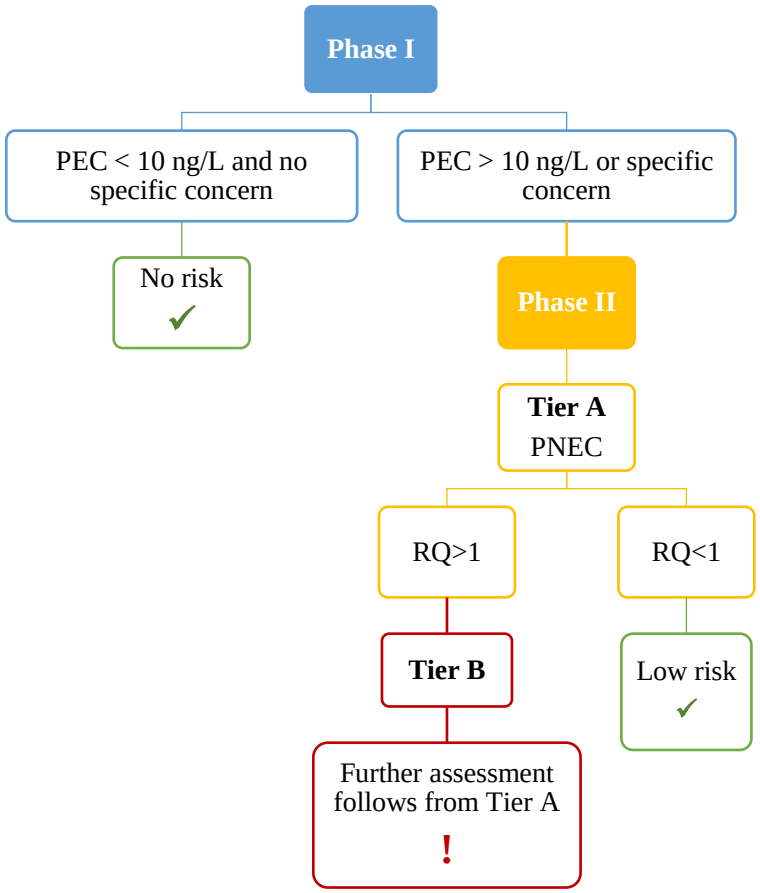


Figure 4.1 Schematic of the environmental risk assessment (ERA) tier approach of the European Medicines Evaluation Agency (EU EMEA) protocol.[330]

Risk assessment of CECs is a priority in order to analyse the risks for the environment and therefore biota and human health. The purpose of this work is to provide an ERA based on the samples analysed within this thesis by estimating the PNEC values of contaminants detected in Chapter 3.0. After evaluating their presence once entering the environment, an ERA will help prioritising contaminants for the investigated areas after the temporal collection period of a year. Compounds detected for effluent wastewater and surface waters will be examined for their potential hazard at the selected regions.

4.2 Experimental

4.2.1 Environmental hazard

A characterization and prioritisation of the potential hazard of the active substances detected was performed in order to see which compounds would have the highest potentiality to be PBTs. The first characteristic was persistence, assessed by the readily biodegradation of the compound. Bioaccumulation was based on the octanol/water partition coefficient (log K_{ow}), where log $K_{ow} \geq 3$ indicated likely bioaccumulation of the compound. These log K_{ow} values are presented in Table A.16 (Appendix G). The last characteristic, toxicity, was based on the ecotoxicity data collected (Table 4.1); as well as PNEC calculations, the lowest value was selected and if it was lower than 0.001 or 0.1 mg/L, for no observed effect concentrations (NOEC) or lethal or median effective concentration 50% (L(E)C_{50}) respectively, toxicity was considered for the substance.

PBT assessment is not based on PECs and therefore was achieved using a PTB index. This was obtained by the sum of every value designated to each characteristic (P, B and T). Values are 0 or 3, therefore PBT values could be 0, 3, 6 or 9, where the maximum values is 9 for the most potential risk and 0 for the lowest.[228]

4.2.2 ERA calculations and classification

The risk associated with the contaminants detected in both sites (rural and urban) was carried out by estimating risk quotients (RQs) at three trophic levels: algae, daphnia and fish;[114] these levels represent the aquatic system. In most cases PECs are higher than measured environmental concentrations (MECs) in order to account for errors - predicted value calculations cannot be accurate as they depend on the fate of the contaminants depending in many variables, limiting the predicted value if insufficient data is used.[358]

An example is diclofenac in France water resources, which was predicted to have an estimated risk of RQ=15, determining high risk, and the real risk obtained was 0, no risk assosiated.[213] Calculations using MEC values instead of PECs are considered acceptable due to more approximate results, however, predicted models can result in a good tool when data is not available.[355] Therefore, MECs were used instead of PECs in order to calculate RQs (Equation 4.2). The highest concentration quantified for the compound per site was used as the MEC value.[114] Nevertheless, if any compound was detected below the limit of detection (LOD) or quantification (LOQ), half of the method limit (LOD or LOQ, respectively) was used as the MEC, consequently considering the worst case scenario.[228]

$$RQ = \frac{MEC}{PNEC_{(EC50)} \text{ or } PNEC_{(NOEC)}}$$

where: MEC: measured environmental concentration
$PNEC_{(EC50)}$: predicted no-effect concentration produced using the lowest EC50
$PNEC_{(NOEC)}$: predicted no-effect concentration produced using the lowest NOEC
RQ: risk quotient

Equation 4.2 Risk Quotient calculation based on MEC concentrations.[330]

European guidelines suggest the use of chronic data over acute toxicity, as contaminants are more likely to induce chronic rather than acute toxic effects.[228] For this study, NOECs (no observed effect concentrations) values were taken from the literature and PNECs were calculated with them when possible. However, NOEC data is quite limited and not always available[228] so acute data, $L(E)C_{50}$ values, the median lethal or effective concentration (LC_{50} or EC_{50}, respectively) were used when necessary.[355] NORMAN Ecotoxicology database was used to obtain lowest PNEC values for different matrices including freshwater obtained experimentally, as this database has been verified. If experimental values were absent, $L(E)C_{50}$ values were predicted by quantitative

structure-activity relationship (QSAR) models and U.S. EPA Ecological Structure Activity Relationships (ECOSAR) predicted model (v 2.0) software. In order to get the predicted values using ECOSAR for the three trophic levels designated, canonical SMILES from the required compounds were used (Table A.1, Appendix A).

After obtaining L(E)C_{50} values, the lowest acute toxicity value (either EC_{50} or LC_{50}) was taken[114] for PNEC calculations as per Equation 4.3. Establishment of AFs depends on the data used and is inversely proportional to the amount of data.[330] If three NOECs were available, an AF of 10 was applied, a value of 50 when two NOECs were employed and a value of 100 when there was just one NOEC presented. A maximum value of 1000 was applied when there was at least one L(E)C_{50} taken into account.[355,228]

$$PNEC = \frac{EC_{50} \text{ or } LC_{50}}{AF}$$

where: EC_{50}: median effective concentration
LC_{50}: median lethal concentration
AF: assessment factor
PNEC: predicted no-effect concentrations

Equation 4.3 PNEC calculation equation.[330]

RQ values were used to determine the risk of the compound in the specific environment tested. The following classification was reflected for their arrangement. RQs below 0.1 were assessed to pose an insignificant effect, and between 0.1 and 1 were considered to carry a low or negligible risk for the chemical. If values were between 1 and 10, a medium risk was assigned. Finally if any compound present was determined to have a RQ higher or equal than 10, a high ecological risk was assigned.[114]

4.2.3 Site risk assessment

The earlier section detailed the process utilised to complete compound specific assessments. In order to see the potential risk of the entire site, rural and urban areas, ΣRQ_{site} were calculated according to Equation 4.4:

$$\sum RQ_{site} = \sum_{i=1}^{n} RQ_i$$

where: RQ_i: risk quotient for the compound
ΣRQ_{site}: risk of the area investigated

Equation 4.4 Potential risk site (ΣRQ_{site}) equation.[355]

In order to assess the risk of all compounds per site, the following rank was considered: if ΣRQ_{site} is below 0.01 no risk is associated. However, a low risk is determined for values ranging from 0.01 to 0.1; a medium risk is reflected when between 0.1 and 1; high risk with harmful effects is expected for values greater than 1; finally, if obtained values are greater than 10 a very high risk is assigned.[355]

The estimation of contribution of each compound to the site followed Equation 4.5:

$$\text{contribution } (\%) = \left(\frac{RQ_i}{\Sigma RQ_{site}}\right) \text{x } 100$$

where: RQ_i: the risk quotient for the compound
ΣRQ_{site}: risk of the area investigated

Equation 4.5 Compound contribution estimation of ΣRQ_{site} equation.[355]

4.2.4 MEC data

As MECs were being used for the risk assessment, concentrations obtained for surface waters and effluent wastewater samples were considered in this study. The highest values obtained for rural and urban areas throughout the year sampled were selected from Table A.12 and A.13 (Appendix F), after their monitoring in Chapter 3.0.

4.2.5 Statistical and data analysis

Microsoft® Office Excel (WA, USA), EPI Suite™ version 4.1 (US Environmental Protection Agency's Office of Pollution Prevention and Toxics and Syracuse Research Corporation (SRC)) and Python version 3.7.9 were utilised in this chapter.

4.3 Results and Discussion

4.3.1 Environmental hazard

The hazard of the detected compounds was evaluated with the calculation of their PBT indexes, and individual values are presented in Table A.16 (Appendix G). In terms of toxicity calculation, when the data was obtained from the NORMAN database, there was an AF value and a final PNEC, so that the NOEC or L(E)C_{50} value could be investigated to see whether was less than 0.01 or 0.1 mg/L respectively. Nine compounds presented the highest score for the PBT index, namely atorvastatin, citalopram, clarithromycin, clozapine, diclofenac, E2, EE2, nortriptyline and venlafaxine, corresponding to 18% of all 49 compounds investigated (Figure 4.2). They all belong to the pharmaceuticals category and were designated as being of high potential of danger to the environment, after obtaining maximum values for each component of the index. The majority of compounds scored a value of 6, corresponding to 37% of the total attainable score, indicating a medium potential of danger. Low hazard potential was achieved by 29% of compounds, however, only 6 of them (12%), reached a nul value, with no danger assigned to them. It should be noted that this could be due to the absence of data for persistence in the literature, lowering the final risk for those compounds, in particular for pesticides and PCPs, as usually this is just performed for pharmaceutical compounds. Therefore, while pharmaceuticals presented more risk values overall using this method of classification, it should be noted that this method does not take any concentrations into consideration, and consequently a full risk assessment was performed next.

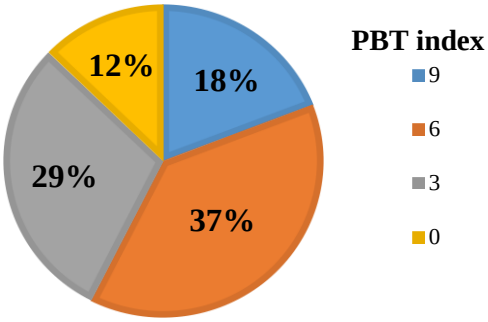


Figure 4.2 Percentage of compounds after PBT index classification.

4.3.2 Environmental Risk Assessment (ERA)

4.3.2.1 Phase I for ERA classification

All compounds detected in surface waters and effluent wastewater samples were considered for the first approach of the risk assessment. Effluent concentrations will determine the worst case scenario in terms of higher concentrations as they are the ones directly discharged into the environment.[359] Notwithstanding, surface waters are considered a more realistic scenario due to dilution of concentrations from the output of the WWTP into the river, as seen before in Figure 3.16 (Chapter 3.0).

Following the EU EMEA protocol, the first objective was to see if compounds were quantified at higher concentrations than the threshold limit of 10 ng/L established by the method. Following data analysis in effluent samples, certain compounds did not result in a MEC above this limit. These compounds included: amoxicillin (both areas), benzophenone-4 (both areas), ciprofloxacin (rural area), clopidrogel (both areas), erythromycin (both areas), E1 (both areas), E2, EE2 (only qualitative data reported but detected in both areas), fluoxetine (rural), lincomycin (both areas), methylphenidate (both areas), nortriptyline (urban area), octinoxate (both areas), octocrylene (both areas), prometryn (both areas), risperidone, tamsulosin (both areas), triclosan (both areas), and verapamil (both areas) (Table A.13, Appendix F). All these analytes were not considered to pose a risk and therefore were not required to pass to phase II of our model (Figure

4.1). However, it was decided to progress some of these compounds to the next step due to the results obtained after the PBT hazard assessment performed in Section 4.3.1. Compounds declared to have a high (E2, EE2 and nortriptyline) and moderate risk (amoxicillin, ciprofloxacin, clopidrogel, erythromycin, E1, fluoxentine, methylphenidate, octocrylene, prometryn, risperidone, triclosan and verapamil) were further assessed on the next phase. Moreover, hormones are endocrine disruptors (EDCs) and they are further assessed independently of their quantity measured.[356] Benzophenone-4 and octinoxate were associated with low risks, however, these PCPs compounds PBT values were only calculated and not measured, as they do not belong to the pharmaceuticals category, so PBT values could have been underestimated due to the persistence category data being unavailable. Therefore, both were also included for further assessment. Some of the compounds were detected below the limits of LOD and LOQs (Tables 2.8 – 2.10 and Tables 2.11 – 2.13), including: amoxicillin (urban), benzophenone-4 (both areas), clopidrogel, E1 (both areas), E2, erythromycin (both areas), fluoxentine (rural area), methylphenidate (both areas), nortriptyline (urban area), prometryn, risperidone and verapamil. As described in the experimental section, the concentrations of these compounds were included at half of their LOQs as worst case scenario, where values of 2, 1 and 2 (rural and urban), 7, 1 and 2 (urban and rural), 1, 2 and 1 (rural and urban), 6, 2, 6, 2, 2, and 2 ng/L were utilised for them, respectively. As EE2 was reported as qualitative data only, similarly, half of the LOQ value, 3 ng/L, was selected for both areas. Even though these calculated values are still below the 10 ng/L threshold of the first tier, a further investigation was continued for them due to either PBT score values obtained or their EDC properties. Consequently, only two compounds detected in the effluent samples, lincomycin and tamsulosin, were not further analysed,

and it was concluded that they do not pose a risk at the MECs obtained for this specific sampling campaign of a period of a year.

In terms of surface water samples, amoxicillin (both areas), benzophenone-4 (urban), bisoprolol, ciprofloxacin (both areas), clozapine, diphenhydramine, E1 (both areas), erythromycin (both areas), lidocaine (both areas), octinoxate (urban), octocrylene (urban), propranolol, salbutamol, tramadol (rural), triclosan (both areas), trimethoprim, venlafaxine (rural) and verapamil (both areas), were all obtained below 10 ng/L (Table A.12, Appendix F). For the compounds E2 and EE2 (both areas) only qualitative data was achieved after method performance in this matrix. Hence, due to their high risk associated with them after the PBT assessment they were further assessed utilising concentrations at half of their LOQ values, 1 and 0.12 ng/L. For the rest of the compounds, based on previous results obtained for their hazard assessments, clozapine and venlafaxine were assessed as being of high risk and compounds associated with moderate risks were amoxicillin, bisoprolol, ciprofloxacin, E1, erythromycin, octocrylene, propranolol, salbutamol, triclosan, trimethoprim and verapamil. Due to this classification, they were moved into the next phase for further assessment to determine their impact in the investigated areas. Benzophenone-4, octinoxate, tramadol and diphenhydramine were also added; as mentioned previously, these compounds only had PBT values calculated as no accurate value was available in the literature. As this could lead to underestimation of their values due to the persistence characteristic, a further investigation was therefore performed. Amoxicillin, benzophenone-4 (urban), bisoprolol, ciprofloxacin (rural), clozapine, diphenhydramine, E1, erythromycin, octinoxate (urban), propranolol, salbutamol, tramadol (rural), triclosan (both areas), trimethoprim, venlafaxine (rural) and verapamil were detected at concentrations <LOD and <LOQ (Tables 2.8 – 2.10 and Tables 2.11 – 2.13). Therefore, half the limits values were selected

for their investigation, resulting in: 0.5 and 1 for urban and rural area, 1, 2, 0.32, 2, 6, 2, 1 and 2 for rural and urban area, 2, 6, 2, 6, 0.1, 6, 2 and 2 ng/L, respectively. Notwithstanding that these new values were below the 10 ng/L threshold limit, they are also considered after their PBT assessment and/or ECD properties. Accordingly, only one substance, lidocaine, was not taken for further analysis, as it was determined that it was no associated risk at concentrations detected.

4.3.2.2 Phase II for ERA classification

4.3.2.2.1 Calculation of PNEC values

In order to perform phase II of the ERA and continue with the assessment, PNEC values were calculated and summarised in Table 4.1. This table includes the ecotoxicity data (mg/L) obtained from published literature, the ecotoxicological NORMAN database and ECOSAR software. Different PNEC values were achieved and therefore one of them needed to be selected to proceed with calculations. As suggested by other studies[228] and EU guidelines,[360] NOECs were used if available, however, after extensive literature review, NOECs were only identified for a few compounds. This is a weakness in determining an effective risk assessment, as chronic effects are more likely to occur than just acute toxic effects.[359] Consequently, L(E)C_{50} values were designated and the lowest value (reported in bold in Table 4.1) considered for calculations. Once all PNEC values were calculated, the ones in bold were further selected for RQ calculations, based on the detailed rationale below. Following previous studies, this was the order of selection taken into consideration: chronic data, experimental values, QSAR data from Norman database and finally ECOSAR.[228,355] QSAR predictions were first considered as the majority have been verified by experts. ECOSAR predictions depend on the chemistry of the compounds, depending on the different parts of the structure, functional groups, different

values are calculated. When ECOSAR predictions were used, the main functional group and/or the lowest value was taken in order to consider the worst case scenario.

Table 4.1 Summary of ecotoxicity data and PNEC values for detected compounds in effluent and surface water Irish samples.

Analytes		Ecotoxicity data (mg/L)			Data	AF	PNEC (µg/L)
		Green Algae	Daphnia	Fish			
Acetamiprid		-	-	-	IC_{50}	1000	3.74[a]
		>98.3	**0.005**	19.2	NOEC	10	**0.5**[b]
	(aliphatic amines)	**1.73**[c]	2.38[c]	18.7[c]	EC_{50}	1000	1.73
	(halopyridines)		**1.12**[c]	1.58[c]	LC_{50}	1000	1.12
	(neonicotinoids)	**4.34**[c]	8.11[c]	16.6[c]	EC_{50}	1000	4.34
Amitriptyline		-	-	-	IC_{50}	1000	**0.14**[a]
	(aliphatic amines)	**0.043**[c]	0.103[c]	0.616[c]	EC_{50}	1000	0.043
Amoxicillin		-	-	-	-	0	**0.078**[a,b] (AA-EQS)
	(aliphatic amines)	367[c]	**351**[c]	3320[c]	LC_{50}	1000	351
	(phenols)	**214**[c]	689[c]	2530[c]	EC_{50}	1000	214
	(amides)	**539**[c]	7330[c]	6450[c]	EC_{50}	1000	539
	(phenol amines)	30.3[c]	**15.3**[c]	184[c]	LC_{50}	1000	15.3
Atorvastatin		**-**	-	-	IC_{50}	1000	**0.01**[a]
	(amides)	0.369[c]	**0.312**[c]	0.621[c]	LC_{50}	1000	0.312
	(pyrroles/diazoles)	0.27[c]	0.871[c]	**0.015**[c]	LC_{50}	1000	0.015
Bisoprolol		**8.01**[c]	9.35[c]	79.9[c]	EC_{50}	1000	**8.01**
Benzophenone-4		**-**	-	-	NOEC	100	**5.4**[a]
	(phenols)	**462**[c]	1160[c]	5580[c]	EC_{50}	1000	462
Carbamazepine		-	-	-	NOEC	10	0.05[a]
		6.4[d,e]	**0.02**[d,e]	25[d,e]	NOEC	10	**0.02**
	(substituted ureas)	**0.26**[c]	14.1[c]	40.9[c]	EC_{50}	1000	0.26
CBZ epoxide	(epoxides, mono)	**94.8**[c]	327[c]	464[c]	EC_{50}	1000	**94.8**
	(substituted ureas)	**-**	**302**	514	LC_{50}	1000	302
Ciprofloxacin		-	**-**	-	-	0	**0.089**[a,b] (EQS proposal)
	(aliphatic amines)	1620[c]	**1240**[c]	1310[c]	LC_{50}	1000	1240
	(vinyl/allyl/propargyl keones)	**55600**[c]	140000[c]	113000[c]	EC_{50}	1000	55600

Citalopram	-	-	-	NOEC	1000	**10[a]**
(aliphatic amines)	**0.36[c]**	0.652[c]	4.47[c]	EC_{50}	1000	0.36
Clarithromycin	2.6	3.1	>100,000	EC_{10}	20	**0.12[a,b]**
	0.002[e]	0.0031[e]	>100[e]	EC_{50}	100	0.02
(aliphatic amines)	**2.08[c]**	3.31[c]	24.2[c]	EC_{50}	1000	2.08
(esters)	**13.3[c]**	37.6[c]	20.5[c]	EC_{50}	1000	13.3
(ketone alcohol)	**1.4[c]**	4.2[c]	4.99[c]	EC_{50}	1000	1.4
Clopidogrel	-	-	-	IC_{50}	1000	**0.62[a]**
(aliphatic amines)	**0.315[c]**	0.579[c]	3.93[c]	EC_{50}	1000	0.315
(esters)	**2.03[c]**	6.36[c]	3.72[c]	EC_{50}	1000	2.03
(thiophenes)	4.51[c]	**2.54[c]**	3.32[c]	LC_{50}	1000	2.54
Clozapine	-	**-**	-	NOEC	100	**0.18[a]**
(aliphatic amines)	**1.58[c]**	2.32[c]	17.7[c]	EC_{50}	1000	1.58
Diclofenac	10	10	**0.0005**	NOEC	10	**0.05[a,b,d,e]**
(neutral organics)	41.4[c]	**25.8[c]**	37.7[c]	EC_{50}	1000	25.8
Diphenhydramine	-	-	-	-	1000	**0.99[a]***
(aliphatic amines)	**0.798[c]**	1.25[c]	9.2[c]	EC_{50}	1000	0.798
E1	**-**	-	-	NOEC	10	**0.0036[a]** (EQS proposal)
(phenols)	**0.355[c]**	3.16[c]	3.82[c]	EC_{50}	1000	0.355
E2	**-**	-	-	-	0	**0.0001[a]** (AA-EQS)
(phenols)	**0.160[c]**	1.78[c]	1.71[c]	EC_{50}	1000	0.16
EE2	**-**	-	-	-	2	**0.000035[a,b]** (AA-EQS)
(phenols)	**0.135[c]**	1.60[c]	1.42[c]	EC_{50}	1000	0.135
(vinyl/allyl/propargyl alcohols-hindered)	**5.15[c]**	8.93[c]	7.45[c]	EC_{50}	1000	5.15
Erythromycin	**-**	-	-	-	0	**0.2[a,b]** (EQS proposal)
(aliphatic amines)	**6.37[c]**	8.62[c]	68.4[c]	EC_{50}	1000	6.37
(esters)	**40.2[c]**	102[c]	51.6[c]	EC_{50}	1000	40.2
(ketone alcohols)	**3.45[c]**	11.3[c]	15.2[c]	EC_{50}	1000	3.45
Fenuron	-	-	-	NOEC	1000	**1.45[a]**
(substituted ureas)	**0.387[c]**	71.8[c]	146[c]	EC_{50}	1000	0.387

Fluoxetine		-	-	-	NOEC	10	**0.1[a]**
	(aliphatic amines)	**0.079[c,d]**	0.175[c,d]	1.08[c,d]	EC_{50}	1000	0.079[c,d]
Hydrochlorothiazide		-	-	-	IC_{50}	1000	**8.38[a]**
		34.35[d]	477[d]	2428.57[d]	EC_{50}	1000	34.35[d]
	(amides)	**203[c]**	4810[c]	3600[c]	EC_{50}	1000	203
Lidocaine		-	-	-	IC_{50}	1000	**4.67[a]**
	(aliphatic amines)	**7.71[c]**	8.64[c]	75.4[c]	EC_{50}	1000	7.71
	(amides)	**13[c]**	124[c]	121[c]	EC_{50}	1000	13
Mefenamic acid		-	-	-	IC_{50}	1000	0.2[a] (not verified)
		2.09[d]	**1.72[d]**	2.25[d]	LC_{50}	1000	**1.72[d]**
	(neutral organics)	4.5[c]	**1.73[c]**	2.25[c]	LC_{50}	1000	1.73
Memantine		-	-	-	EC_{50}	1000	**1.84[a]** (not verified)
	(aliphatic amines)	**0.386[c]**	0.636[c]	4.57[c]	EC_{50}	1000	0.386
Methylphenidate		**-**	-	-	$L(E)C_{50}$	1000	11.6[a] (predicted)
	(aliphatic amines)	**1.24[c]**	1.80[c]	13.80[c]	EC_{50}	1000	**1.24**
	(esters)	**7.85[c]**	20.9[c]	10.9[c]	EC_{50}	1000	7.85
Metoprolol		**-**	-	-	-	50	**8.6[a]** (AA-EQS)
	(aliphatic amines)	**8.31[c]**	9.38[c]	81.6[c]	EC_{50}	1000	8.31
Nordiazepam (amides)		**2.63[c]**	13.4[c]	15.8[c]	EC_{50}	1000	**2.63**
Nortriptyline		-	-	-	IC_{50}	1000	**0.19[a]**
	(aliphatic amines)	**0.058[c]**	0.132[c]	0.805[c]	EC_{50}	1000	0.058
Octinoxate		**-**	-	-	$L(E)C_{50}$	1000	**6[a]** (EQS proposal)
	(esters)	**0.075[c]**	0.323[c]	0.234[c]	EC_{50}	1000	0.075
Octocrylene		**-**	-	-	LC_{50}	1000	**0.023[a]**
	(esters)	**0.016[c]**	0.084[c]	0.068[c]	EC_{50}	1000	0.016
	(vinyl/allyl/propargyl nitriles)	**0.068[c]**	0.228[c]	0.206[c]	EC_{50}	1000	0.068
Prometryn	(triazines, aromatic)	**0.037[c]**	4.81[c]	3.9[c]	EC_{50}	1000	**0.037**
	(aliphatic amines)	14.7[c]	**14.6[c]**	136[c]	LC_{50}	1000	14.6
	(carbamate esters)	**0.395[c]**	98[c]	114[c]	EC_{50}	1000	0.395
Propamocarb		**-**	-	-	-	0	**710[a]**

	(aliphatic amines)	14.7[c]	**14.6[c]**	136[c]	LC_{50}	1000	14.6
	(carbamate esters)	**0.395[c]**	98[c]	114[c]	EC_{50}	1000	0.395
Propranolol		-	-	-	NOEC	10	0.41[a]
		0.1[d,e]	0.001[d,e]	**0.0005[d,e]**	NOEC	10	**5.00E+01[d,e]**
	(aliphatic amines)	**1.85[c]**	2.58[c]	20.2[c]	EC_{50}	1000	1.85
Risperidone		-	-	-	IC_{50}	1000	**75.1[a]**
	(aliphatic amines)	**0.688[c]**	1.18[c]	8.29[c]	EC_{50}	1000	0.688
Salbutamol	(aliphatic amines)	41.6[c,d]	**36.8[c,d]**	362[c,d]	LC_{50}	1000	**36.8[d]**
	(phenols)	**23.6[c]**	66.1[c]	283[c]	EC_{50}	1000	23.6
	(benzyl alcohols)	**213[c]**	822[c]	1150[c]	EC_{50}	1000	213
	(phenol amines)	2.61[c]	**1.17[c]**	16.9[c]	LC_{50}	1000	1.17
Simazine		**-**	-	-	NOEC	0	**1[a]** (AA-EQS)
	(triazines, aromatic)	**0.166[c]**	26.4[c]	42.1[c]	EC_{50}	1000	0.166
Sulfamethoxazole		**0.02[d]**	25.2[d]	562.5[d]	EC_{50}	1000	**2.70E+01[d]**
		-	-	-	NOEC	0	0.6[a] (AA-EQS)
	(anilines, unhindered)	21.8[c]	**6.43[c]**	267[c]	LC_{50}	1000	6.43
	(amides)	**75.3[c]**	1320[c]	1080[c]	EC_{50}	1000	75.3
Sulfapyridine		-	-	-	IC_{50}	1000	**1.83[a]** (not verified)
	(anilines, unhindered)	20.8[c]	**6.17[c]**	246[c]	LC_{50}	1000	6.17
	(amides)	**69.8[c]**	1200[c]	983[c]	EC_{50}	1000	69.8
Temazepam		**-**	-	-	NOEC	100	**0.07[a]**
	(amides)	**8.23[c]**	61.1[c]	64.2[c]	EC_{50}	1000	8.23
Terbutryn	(triazines, aromatic)	**0.036[c]**	4.57[c]	3.64[c]	EC_{50}	1000	**0.036**
Tramadol		**-**	-	-	IC_{50}	1000	**8.65[a]**
	(aliphatic amines)	**0.959[c]**	1.47[c]	10.9[c]	EC_{50}	1000	0.959
	(benzyl alcohols)	**5.67[c]**	8.8[c]	10.2[c]	EC_{50}	1000	5.67
Triclosan		**-**	-	-	NOEC	10	**0.02[a]**
	(phenols)	**0.057[c]**	0.839[c]	0.582[c]	EC_{50}	1000	0.057
		0.0014[f]	0.39[f]	0.26[f]	EC_{50}	1000	0.0014
		0.0045[f]	0.13[f]	0.37[f]	EC_{50}	1000	0.0045

		0.00069[f]	-	0.034[f]	NOEC	1000	0.00069
Trimethoprim		**0.0016[d,e]**	3.12[d,e]	25[d,e]	NOEC	10	**0.16[d,e]**
		-	-	-	-	0	120[a] (AA-EQS)
	(anilines, unhindered)	20.7[c]	**6.38[c]**	212[c]	LC_{50}	1000	6.38
	(anilines, hindered)	**2.68[c]**	4.53[c]	800[c]	EC_{50}	1000	2.68
	(anilines, amino-meta)	**2.68[c]**	4.53[c]	800[c]	EC_{50}	1000	2.68
Valsartan		**-**	-	-	-	10	**560[a]** (AA-EQS)
	(amides)	**13.9[c]**	47.6[c]	62.8[c]	EC_{50}	1000	13.9
Venlafaxine		**-**	-	-	-	100	**0.038[a]** (EQS proposal)
	(aliphatic amines)	**0.653[c]**	1.06[c]	7.68[c]	EC_{50}	1000	0.653
Verapamil		-	-	-	NOEC	100	**2.53[a]**
	(aliphatic amines)	**0.091[c]**	0.21[c]	1.27[c]	EC_{50}	1000	0.091
	(benzyl nitriles)	**0.321[c]**	0.881[c]	0.774[c]	EC_{50}	1000	0.321

Values in bold represent the ones selected for PNEC and RQ calculations.
[a]NORMAN Ecotoxicology Database.
[b]Watch List.
[c]ECOSAR.
[d]Rivera-Jaimes *et al*., 2018.[114]
[e]Mendoza *et al*., 2015.[228]
[f]Kosma *et al*., 2014.[359]
*Experimental value.
IC50: half maximal inhibitory concentration.
AA-EQS: annual average EQS.
MTR: Maximum Toelaatbaar Risiconiveau.

4.3.2.2.2 Risk Quotients

All compounds detected had potential to pose a hazard to the aquatic environment and RQs were calculated following European guidelines, after preliminary Phase I analysis. Table 4.2 shows all compound values and Figure 4.3 shows all RQs obtained; where higher risks are more often seen in effluent wastewater samples. This is because WWTPs are one of the main points of input of these contaminants into the environment and effluent corresponds to the discharge point. Effluent points are considered the worst case scenario as it releases the highest concentrations before their dilution into surface waters. A value of ≤58% of compounds presented insignificant risk for all the matrices and sites tested (Figure 4.4). Higher risks in surface waters were associated with one compound, E2, for both areas, and only one compound gave medium risk for both areas, EE2. Overall the rural area was determined to have a lower overall risk, with more compounds assessed as posing a low risk. This is due to compounds either not detected or being quantified at lower concentrations. E1 and octocrylene were the only two compounds found at this level (low) in the rural area; they were also obtained as low risk for the urban area with the addition of three more at the same level (carbamazepine, fenuron, propranolol and venlafaxine). Consequently, the urban area overall presented more compounds at higher concentrations resulting in an increased number of compounds in the low risk category. The majority of compounds presented insignificant risks with values ≤0.055 resulting in 73% and 67% for the rural and urban areas respectively. This means that the urban area overall presented more compounds at higher concentrations resulting in an increased number of compounds in the high category.

Table 4.2 MEC and PNEC selected values for environmental risk classification of Phase II compounds.

Analytes	PNEC (ng/L)	Rural						Urban					
		Surface water			Effluent			Surface water			Effluent		
		MEC (ng/L)	RQ	Risk	MEC (ng/L)	RQ	Risk	MEC (ng/L)	RQ	Risk	MEC (ng/L)	RQ	Risk
Acetamiprid	500	n.d.	-	-	160	0.321	Low	n.d.	-	-	312	0.625	Low
Amitriptyline	140	n.d.	-	-	36	0.255	Low	n.d.	-	-	41	0.289	Low
Amoxicillin	78	1[a]	0.0183	Ins	7	0.0907	Ins	0.5[a]	0.006	Ins	2[a]	0.0276	Ins
Atorvastatin	10	n.d.	-	-	31	3.1	Med	n.d.	-	-	34	3.41	Med
Benzophenone-4	5400	1.85	0.0003	Ins	1[a]	0.0001	Ins	1[a]	0.0002	Ins	2[a]	0.0004	Ins
Bisoprolol	8010	n.d.	-	-	166	0.0207	Ins	2[a]	0.0002	Ins	70	0.00877	Ins
Carbamazepine	20	n.d.	-	-	701	35.0	High	19	0.948	Low	444	22.2	High
CBZ epoxide	94800	n.d.	-	-	158	0.00167	Ins	n.d.	-	-	99	0.00104	Ins
Ciprofloxacin	89	0.32[a]	0.0036	Ins	8	0.0878	Ins	5	0.0546	Ins	11	0.119	Low
Citalopram	10000	n.d.	-	-	232	0.0232	Ins	16	0.00157	Ins	268	0.0268	Ins
Clarithromycin	120	n.d.	-	-	20	0.165	Low	n.d.	-	-	20	0.168	Low
Clopidogrel	620	n.d.	-	-	7[a]	0.011	Ins	n.d.	-	-	7[a]	0.0107	Ins
Clozapine	180	n.d.	-	-	17	0.097	Ins	2[a]	0.0103	Ins	65	0.360	Low
Diclofenac	50	n.d.	-	-	575	11.5	High	n.d.	-	-	727	14.5	High
Diphenhydramine	990	n.d.	-	-	403	0.407	Low	6[a]	0.00583	Ins	93	0.0936	Ins
E1	3.6	2[a]	0.687	Low	2[a]	0.504	Low	2[a]	0.687	Low	1[a]	0.166	Low
E2	0.1	1[a]	10.5	High	1	24.7	High	1[a]	10.5	High	-	-	-
EE2	0.035	0.12[a]	3.46	Med	3[a]	84.4	High	0.12[a]	3.46	Med	3[a]	84.4	High
Erythromycin	200	1[a]	0.00286	Ins	2[a]	0.00764	Ins	2[a]	0.00866	Ins	1[a]	0.00252	Ins
Fenuron	1450	80	0.0548	Ins	87	0.0601	Ins	76	0.0521	Low	92	0.0633	Ins
Fluoxetine	100	n.d.	-	-	6[a]	0.065	Ins	n.d.	-	-	27	0.271	Low
Hydrochlorothiazide	8380	n.d.	-	-	1067	0.127	Low	18	0.00212	Ins	685	0.0817	Ins
Lidocaine	4670	NC	-	-	84	0.0180	Ins	NC	-	-	107	0.0230	Ins
Mefenamic acid	1720	n.d.	-	-	120	0.070	Ins	n.d.	-	-	976	0.567	Low
Memantine	1840	n.d.	-	-	139	0.075	Ins	n.d.	-	-	46	0.0250	Ins

Methylphenidate	1240	n.d.	-	-	2[a]	0.00159	Ins	n.d.	-	-	2[a]	0.00159	Ins
Metoprolol	8600	n.d.	-	-	80	0.0093	Ins	n.d.	-	-	15	0.00172	Ins
Nordiazepam	2630	n.d.	-	-	40	0.0151	Ins	n.d.	-	-	-	-	-
Nortriptyline	190	n.d.	-	-	13	0.070	Ins	n.d.	-	-	6[a]	0.0328	Ins
Octinoxate	6000	10	0.00168	Ins	2.97	0.000495	Ins	2[a]	0.000304	Ins	1.49	0.000249	Ins
Octocrylene	23	14	0.622	Low	1.17	0.0529	Ins	5	0.197	Low	0.75	0.0326	Ins
Prometryn	37	n.d.	-	-	2[a]	0.05	Ins	n.d.	-	-	2[a]	0.0539	Ins
Propamocarb	710000	99	0.00014	Ins	51	0.000072	Ins	134	0.000189	Ins	91	0.000129	Ins
Propranolol	50	n.d.	-	-	88	1.76	Med	6[a]	0.12	low	112	2.24	Med
Risperidone	75100	n.d.	-	-	n.d.	-	-	n.d.	-	-	2[a]	0.0000281	Ins
Salbutamol	36800	n.d.	-	-	70	0.00190	Ins	2[a]	0.0000580	Ins	34	0.000930	Ins
Simazine	1000	n.d.	-	-	n.d.	-	-	n.d.	-	-	23	0.0234	Ins
Sulfamethoxazole	30	n.d.	-	-	69	2.30	Med	n.d.	-	-	95	3.20	Med
Sulfapyridine	1830	n.d.	-	-	318	0.174	Low	n.d.	-	-	192	0.105	Low
Temazepam	70	n.d.	-	-	247	3.52	Med	n.d.	-	-	84	1.21	Med
Terbutryn	36	n.d.	-	-	42	1.15	Med	n.d.	-	-	25	0.693	Low
Tramadol	8650	6[a]	0.000743	Ins	925	0.107	Low	31	0.00355	Ins	347	0.0401	Ins
Triclosan	20	0.1a	0.00343	Ins	0.57	0.0283	Ins	0.1a	0.00343	Ins	0.74	0.0370	Ins
Trimethoprim	160	n.d.	-	-	987	6.17	Med	6[a]	0.0350	Ins	355	2.22	Med
Valsartan	560000	n.d.	-	-	546	0.00098	Ins	n.d.	-	-	232	0.000414	Ins
Venlafaxine	38	2[a]	0.0503	Ins	529	13.9	High	32	0.853	Low	872	22.9	High
Verapamil	2530	2[a]	0.0008	Ins	2[a]	0.0008	Ins	2[a]	0.000779	Ins	2[a]	0.000779	Ins

n.d.: not detected.
[a] Half of the method LOD or LOQ.
NC: not considered - analyte assessed in Phase I.
Ins: insignificant risk.
Low: low risk.
Med: medium risk.
High: high risk.

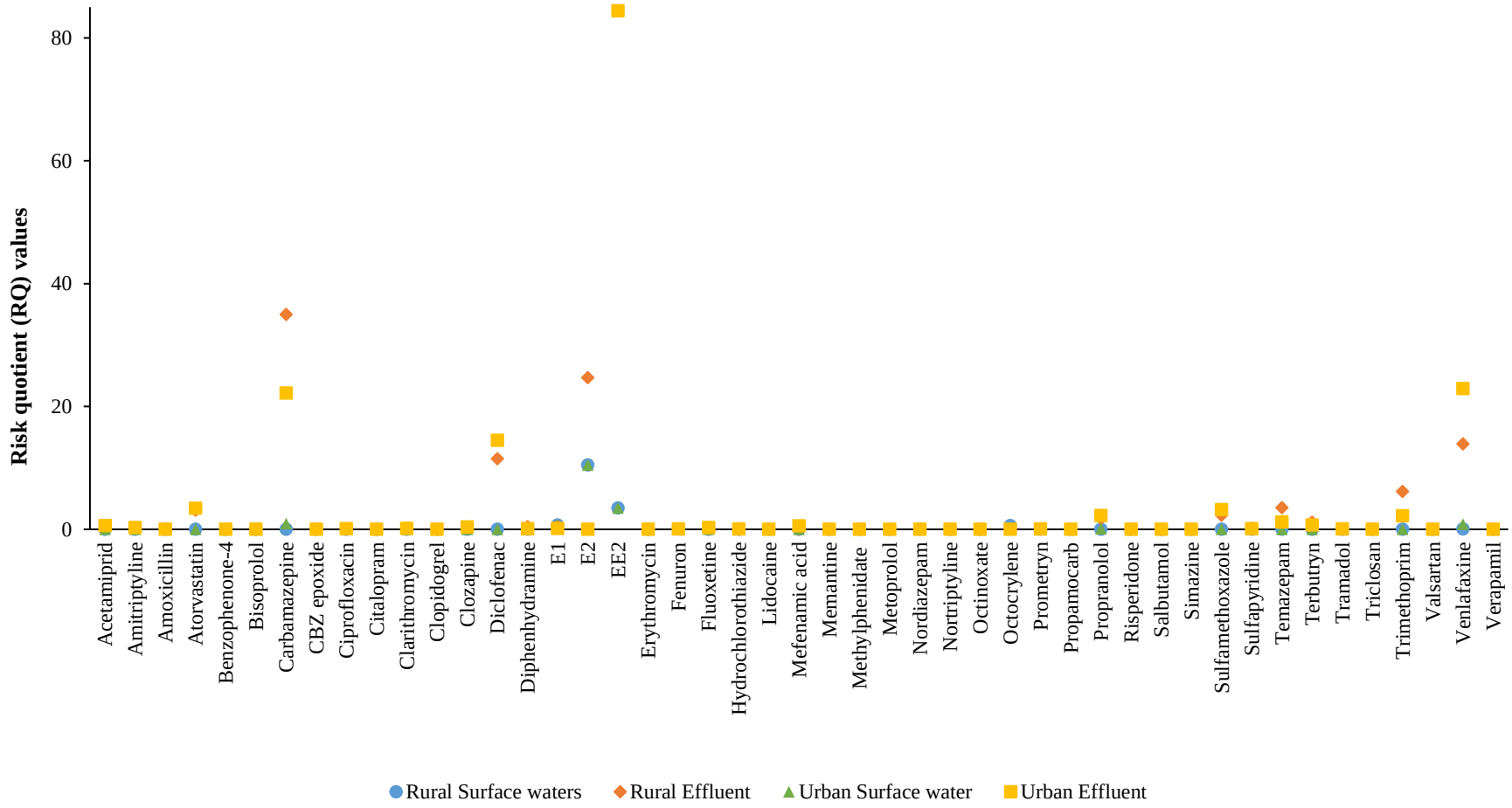


Figure 4.3 Risk quotients of CECs in wastewater effluent and surface water for both areas (rural and urban).

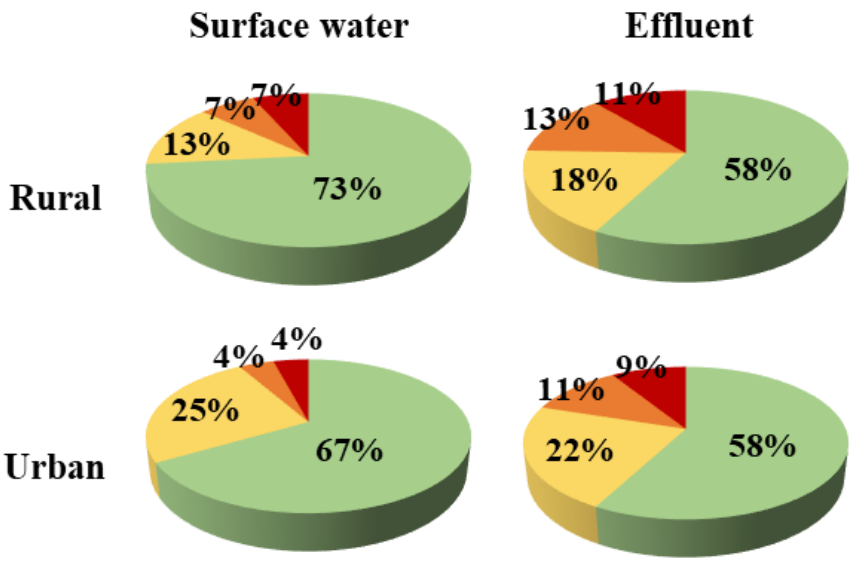


Figure 4.4 Risk classification for compounds detected at both areas tested for surface and effluent samples.

For effluent samples, an insignificant level of risk was also achieved for the majority of the compounds (≤58%), resulting in the same number of compounds posing insignificant level of risk for both areas, i.e. 26. The same compounds were obtained with the same levels throughout both areas except the following: ciprofloxacin, clozapine, fluoxentine and mefenamic acid which posed a low risk in the urban area; and diphenhydramine, hydrochlorothiazide and tramadol that had a low risk associated to them in the rural area. Low risks were obtained for 22% and 18% with 10 and 8 compounds for the urban and rural area respectively, with difference in compounds between them mentioned previously. Medium risks were found for the same five compounds for both areas, with the addition of terbutryn, a herbicide, in the rural area due to a higher MEC obtained. In terms of high risks, both areas presented similar levels. This is due to almost the same amount of compounds within the category for both areas. The same four compounds (all pharmaceuticals: diclofenac, carbamazepine, EE2 and venlafaxine) were observed within both areas, while the rural area had an extra substance, E2. Some of these compounds have been demonstrated in the literature to result in

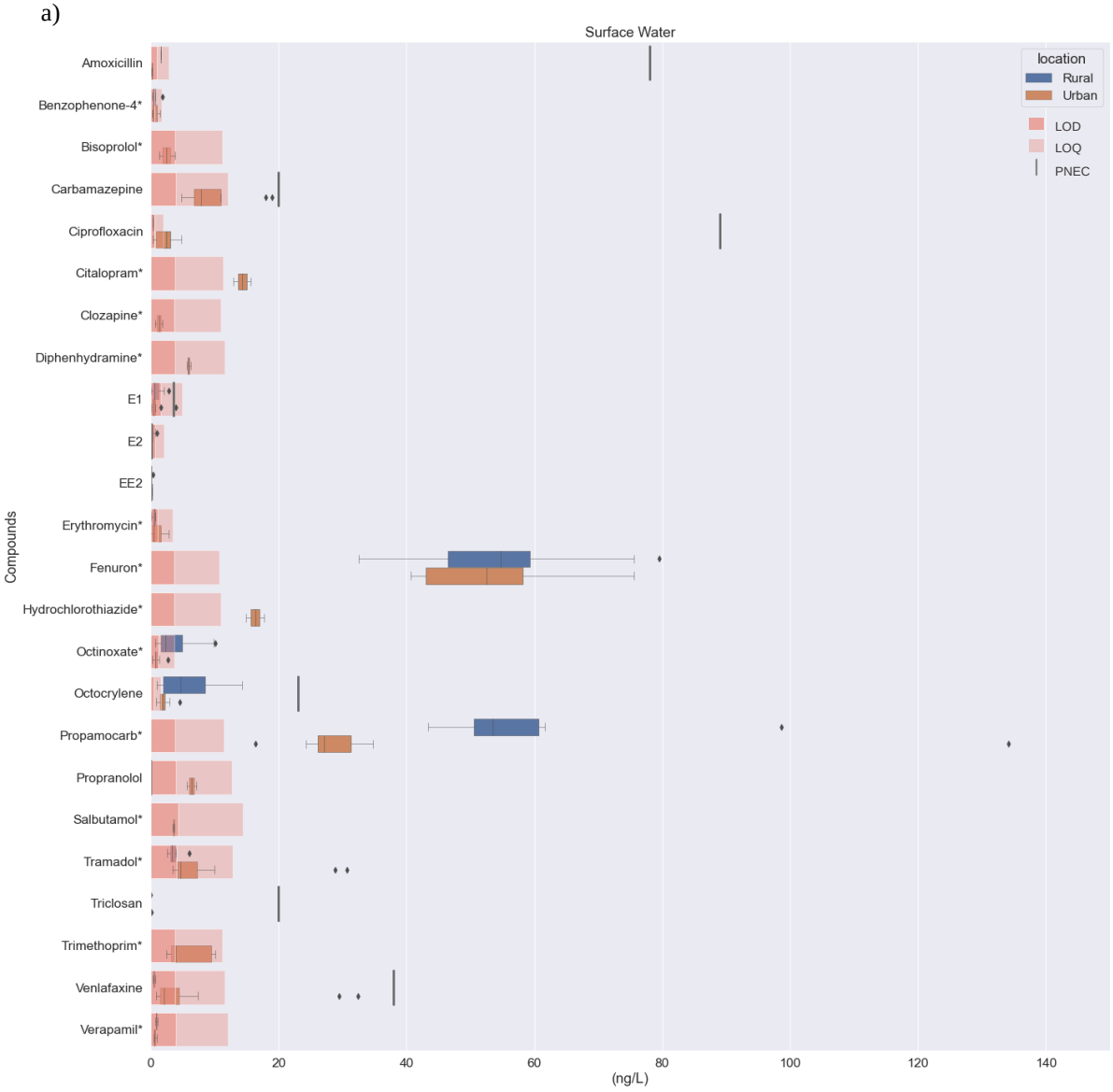
negative effects on human health, e.g. venlafaxine, which has been indicated to decrease brain serotonin concentrations, however, more research is needed in this area.[302] This number of compounds resulted in 11% and 9% of the total for rural and urban area, respectively. The highest RQs were obtained for EE2. This is due to its low PNEC value used for the RQ calculation as the MEC values considered were not higher in comparison with the rest of the compounds. Carbamazepine followed within the second position for the rural area and venlafaxine for the urban one. All compounds showed dilution from the effluent to the surface waters, which was expected, and this reduced the RQ value, as they were either not detected or quantified at a lower concentration in surface waters. An example was diclofenac which was not detected in surface waters in any area after a high risk was obtained in effluent samples. Carbamazepine was also diluted as it was found in surface waters just in urban areas where the risk dropped from high to low category. However, it belongs to one of the most frequent pharmaceuticals[98] and several studies have investigated its ecotoxicological effects, where it has been found to be the most dangerous compound when tested with diclofenac and clofibric acid at the same time.[306] It has also been classified as "R52/53 Harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment".[361] This demonstrates the importance of reduction of CECs before they arrive at WWTPs; as these plants are not designed to reduce the risk of these compounds. Notwithstanding this, there are multiple compounds where it was shown that WWTPs reduced their concentrations, indicating that if influent wastewater was discharged without treatment, higher risks would have been obtained due to possible higher MECs quantified.

It is worth mentioning that in this study, not many metabolites or transformation/degradation products from WWTPs were considered, though they could also have an impact. An example is carbamazepine, where it has previously been

indicated that its degradation products can be more toxic than the compound itself;[362] however, in this case, carbamazepine epoxide was not detected at any surface water sample tested after its analysis and insignificant risks were associated for both effluents. Attention is given to some of the compounds that were obtained <10 ng/L but were decided to continue their assessment because of PBT assessment results achieved and/or ECD properties. An example was the hormone E2 which was characterised with high risk in every matrix detected even that concentrations used as MEC were <1 ng/L. As a result, performing a hazard assessment previous to ERA analysis seems completely necessary.

In order to investigate the range of concentrations detected over the PNEC values calculated, box-whisker plots were performed (Figure 4.5) containing LODs and LOQs as well. Only PNECs below 150 and 1,000 have been added to the graph for surface water and effluent respectively. Compounds with higher PNEC values are marked with an asterisk. For surface waters, most compounds concentrations are below these values and concentrations lay in the LOD-LOQ range. However, PNECs for compounds such as propranolol, or hormones (E1, E2 and EE2) lay around the LOD/LOQ value, meaning that any concentration detected and/or quantified, using the methods developed/optimised within this thesis, will present a risk for the environment, matching the high risk associated from the RQs calculated previously. For effluent samples, compounds such as carbamazepine and diclofenac had all concentrations quantified for both areas above PNEC levels.

a)


Surface Water
location
Rural
Urban
LOD
LOQ
PNEC
Amoxicillin
Benzophenone-4*
Bisoprolol*
Carbamazepine
Ciprofloxacin
Citalopram*
Clozapine*
Diphenhydramine*
E1
E2
EE2
Erythromycin*
Fenuron*
Hydrochlorothiazide*
Octinoxate*
Octocrylene
Propamocarb*
Propranolol
Salbutamol*
Tramadol*
Triclosan
Trimethoprim*
Venlafaxine
Verapamil*
Compounds
0
20
40
60
80
100
120
140
(ng/L)

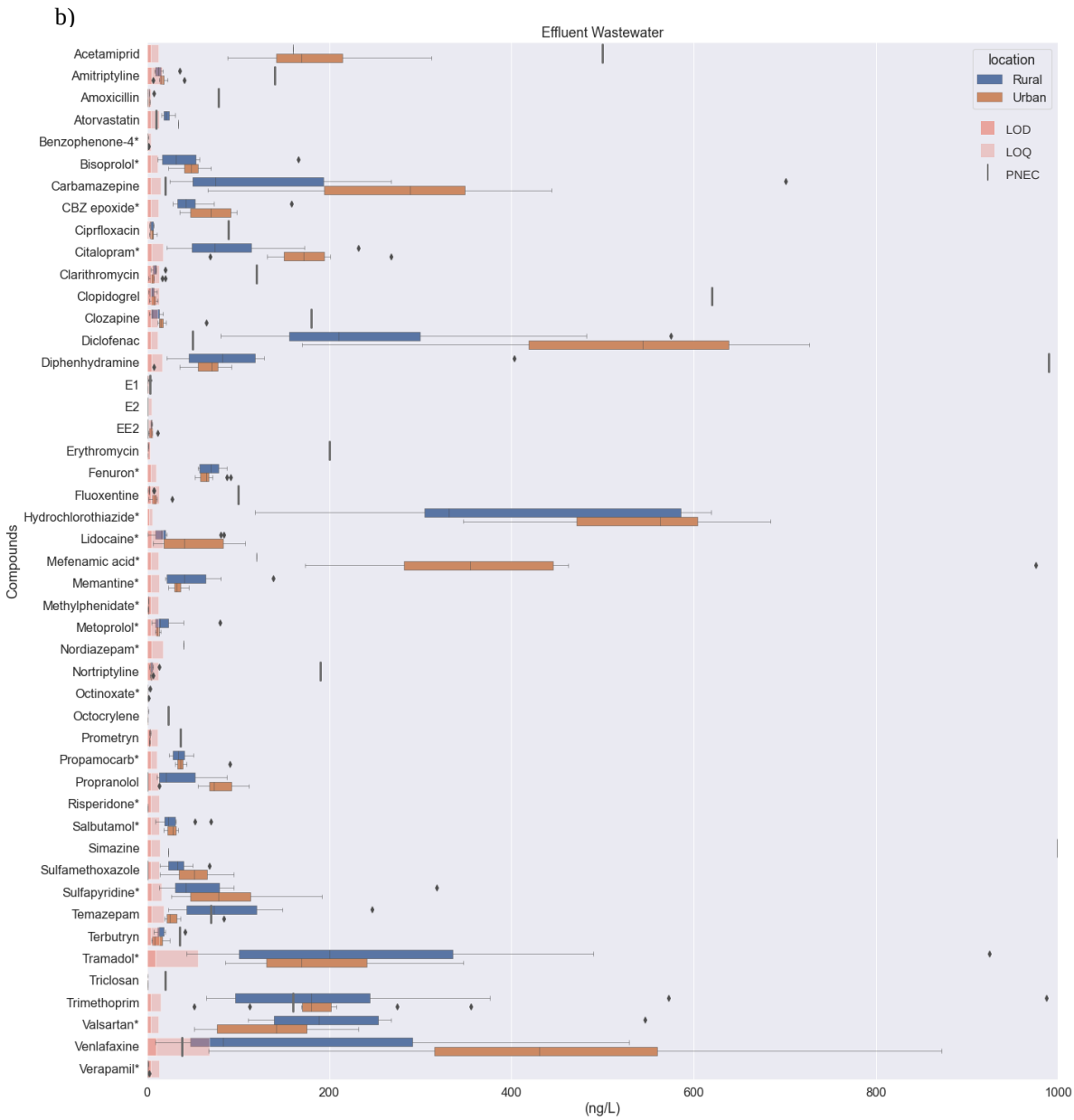


Figure 4.5 Box-whisker plots for compounds detected for rural (blue) and urban (orange) areas for surface waters (a) and effluent wastewater (b). Limits of detection and quantification (LODs and LOQs respectively) are represented as two colours bar chart. PNEC values over 150 and 1000 have not been plotted for surface water and effluent wastewater respectively; corresponding compounds are marked with an asterisk (*).

4.3.2.2.3 International comparison based on Risk Quotients

CEC concentrations pose an unknown risk associated for ecological species. Therefore, it is essential to see whether or not environmental concentrations carry a threat to exposed biota. Ecological risk assessments have been performed around the world in order to assess the possible ecotoxicological risks. Compounds such as diclofenac have been detected posing high risks in other countries such as Pakistan wastewater effluent[295] or in surface waters such as Morelos in Mexico[114] or Europe, where it was on the top of the rank of 33 countries studied with an RQ of 154.[161] This value is much higher than the one obtained within our study; it was not detected at any surface water samples and the maximum risk achieved was 14.5 for urban effluents. Another examples with similar outcomes are compounds such as metoprolol and fluoxetine, which were obtained as high risks in wastewater effluents in Europe[161] but insignificant or low within this study, respectively. In this study, sulfamethoxazole presented a medium risk, with RQs of 2.3 (rural) and 3.2 (urban) obtained in effluent samples, but it was not detected in surface waters. This is consistent with studies in several surface waters European countries where RQs ranged from 0 (e.g. Ireland, Portugal, Germany, Finland and Norway) to 0.02 (Cyprus)[110] as observed in Table 4.3. However, high risks have been reported in other countries such as China (RQ = 1,955). Usually not many antibiotics obtain high RQs due to their hydrophilicity which leads to less toxicity.[363] This case has been observed in different countries around Europe for compounds such as trimethoprim, clarithromycin and sulfapyridine with no or low risk; and ciprofloxacin and azithromycin with moderate risks. Dilution of the compound concentrations when reaching the natural aquatic environment leading to lower risks have also been reported in other studies. Compounds such as mefenamic acid and sulfamethoxazole had high or medium risks in wastewaters in Europe and minimal in surface waters.[295] These results agree with this study, where

any of them were detected in surface waters but had greater risks in effluents (however still determined as low or insignificant).

As observed in Table 4.3, RQs varied between country and matrix. RQs can be obtained using predicted concentrations, PEC (estimated risk), or measured concentrations, MEC (real risk).[213] Consequently, the final RQ varies depending not only on the concentration used for their calculation (season, weather, WWTP treatment used, etc.)[214] but also the country (population, drug use patterns, etc.) and the exact geographical location. Moreover, countries reporting lower number of compounds presenting risks could not be due to only low risks but also limited or no data available from monitoring campaigns.[161] Therefore, variations between not only countries but locations are expected as mentioned before.

Table 4.3 Examples of RQs obtained from literature review in different countries for some of the compounds studied in the risk assessment.

Analytes	**RQ_{EFF}**	**Risk**	**RQ_{SW}**	**Risk**	**Country**	**Ref**
Amoxicillin	-	-	17	High	33 EU	161
	≤0.06	Insignificant	-	-	Switzerland	20
Atorvastatin	-	-	492	High	33 EU	161
	≤6.13	Medium	-	-	Switzerland	20
Benzophenone-4	-	-	≤0.01	Insignificant	-	185
	≤0.3	Low	-	-	Spain	190
Carbamazepine	≤0.001	Insignificant	-	-	Pakistan	295
	-	-	3,132	High	33 EU	161
	-	-	0	Insignificant	France	213
	-	-	0.008	Insignificant	Hungary	214
	≤0.3	Low	-	-	Switzerland	20
Citalopram	-	-	6	Medium	33 EU	161
	-	-	0.24	Low	Hungary	214
	≤0.03	Insignificant	-	-	Switzerland	20
Ciprofloxacin	-	-	≤0.16	Low	Portugal	110
	-	-	0.13	Low	Spain	110
	-	-	≤0.90	Low	Cyprus	110
	-	-	0.02	Insignificant	Ireland	110
	-	-	≤0.12	Low	Germany	110
	-	-	0	Insignificant	Finland	110

	-	-	0	Insignificant	Norway	110
	-	-	136	High	33 EU	161
	≤27.0	High	≤6.24	Medium	Pakistan	295
Clarithromycin	-	-	≤0.06	Insignificant	Portugal	110
	-	-	0.05	Insignificant	Spain	110
	-	-	≤0.03	Insignificant	Cyprus	110
	-	-	0.01	Insignificant	Ireland	110
	-	-	≤0.05	Insignificant	Germany	110
	-	-	0	Insignificant	Finland	110
	-	-	0	Insignificant	Norway	110
	-	-	120	High	33 EU	161
	≤0.035	Insignificant	-	-	Switzerland	20
Clopidogrel	≤1.09	Medium	-	-	Switzerland	20
Clozapine	-	-	0.002	Insignificant	Hungary	214
	≤0.06	Insignificant	-	-	Switzerland	20
	<0.05	Insignificant	-	-	Greece	318
Diclofenac	≤30	High	≤0.14	Low	Pakistan	295
	-	-	18,740	High	33 EU	161
	-	-	0	Insignificant	France	213
	-	-	39.5	High	Hungary	214
	<0.05	Insignificant	-	-	Czech Republic	128
	≤14.6	High	-	-	Switzerland	20
E1	-	-	4	Medium	33 EU	161
	-	-	5.52	Medium	Hungary	214
	<11	High	<2.5	Medium	Tanzania	8
E2	-	-	75	High	33 EU	161
	-	-	9.8	Medium	Hungary	214
	<30	High	<3	Medium	Tanzania	8
EE2	-	-	28,500	High	33 EU	161
	-	-	0.41	Low	Hungary	214
	<37	High	<24	High	Tanzania	8
Erythromycin	-	-	43	High	33 EU	161
	≤0.008	Insignificant	-	-	Switzerland	20
Fluoxetine	≤4.28	Medium	≤2.39	Medium	Pakistan	295
	-	-	15	High	33 EU	161
	-	-	0.002	Insignificant	Hungary	214
	≤0.05	Insignificant	-	-	Switzerland	20
	≤0.5	Low	-	-	Greece	318
Hydrochlorothiazide	-	-	0	Insignificant	France	213
Mefenamic acid	≤5.09	Medium	≤0.04	Insignificant	Pakistan	295
	≤4.33	Medium	-	-	Switzerland	20
Metoprolol	≤3.76	Medium	≤0.02	Insignificant	Pakistan	295
	-	-	8.3E-05	Insignificant	Hungary	214
	<0.03	Insignificant	-	-	Czech Republic	128
Octinoxate	-	-	≤1.60	Medium	-	185
Octocrylene	-	-	≤156	High	-	185

Propranolol	≤0.03	Insignificant	-	-	Pakistan	295
	-	-	1.48	Medium	33 EU	161
Simazine	-	-	≤0.24	Low	China	325
Sulfamethoxazole	-	-	0	Insignificant	Portugal	110
	-	-	0.01	Insignificant	Spain	110
	-	-	≤0.02	Insignificant	Cyprus	110
	-	-	0	Insignificant	Ireland	110
	-	-	0	Insignificant	Germany	110
	-	-	0	Insignificant	Finland	110
	-	-	0	Insignificant	Norway	110
	-	-	5	Medium	33 EU	161
	≤3.70	Medium	≤0.04	Insignificant	Pakistan	295
Sulfapyridine	-	-	0	Insignificant	Portugal	110
	-	-	0.01	Insignificant	Spain	110
	-	-	≤0.02	Insignificant	Cyprus	110
	-	-	0	Insignificant	Ireland	110
	-	-	0	Insignificant	Germany	110
	-	-	0	Insignificant	Finland	110
	-	-	0	Insignificant	Norway	110
	-	-	7	Medium	33 EU	161
Terbutryn	-	-	>1	Medium	Spain	72
Tramadol	-	-	8	Medium	33 EU	161
	≤0.04	Insignificant	-	-	Switzerland	20
Triclosan	-	-	11	High	33 EU	161
Trimethoprim	-	-	≤0.01	Insignificant	Portugal	110
	-	-	0.01	Insignificant	Spain	110
	-	-	≤0.03	Insignificant	Cyprus	110
	-	-	0	Insignificant	Ireland	110
	-	-	≤0.01	Insignificant	Germany	110
	-	-	0	Insignificant	Finland	110
	-	-	0	Insignificant	Norway	110
	<0.2	Low	-	-	Czech Republic	128
Valsartan	-	-	1.94	Medium	33 EU	161
	≤0.011	Insignificant	-	-	Switzerland	20
Venlafaxine	-	-	94	High	33 EU	161
	≤0.69	Low	-	-	Switzerland	20
	≤0.1	Low	-	-	Greece	318

RQ_{EFF}: RQ for effluent samples
RQ_{SW}: RQ for surface water samples

4.3.2.2.4 Potential risk per site

When all compounds are taken into account an overall potential risk can be determined for the area investigated. This is important as contaminants are present as mixtures in the environment not as a single compound. Also, pharmaceuticals are manufactured and/or prescribed as mixtures that in the environment can be present as multi-component mixture from parent drugs, metabolites and transformation products. Most research conducted has been previously performed for a single compound at a time, however, these are not isolated in the environment and cumulative CECs could have a higher impact.[364] The EMEA guidelines followed only risk assessment for individual compounds but cumulative values can be studied to estimate the overall potential risk of the site. This was calculated for a particular sampling site by ΣRQ_{site}, as discussed in Section 4.2.3. All sites and matrices presented a very high risk due to values ≤10, where the rural site showed ΣRQ_{rural} values of 15 and 190 for surface water and effluent, respectively. Different analytes contributed to their total risk depending on the matrix; for surface waters, main contributions came from E2 followed by EE2 with 68% and 22%, while for effluent samples 44% and 18% were obtained for EE2 and carbamazepine, of the total contribution respectively. On the other hand, ΣRQ_{urban} values of 17 and 160 were obtained for surface waters and effluent respectively. Two compounds were the main contributors for surface waters, namely E2, which accounted for 62% of the total risk, as EE2 followed with 20% of the total risk assessed. However, effluent sample risk assessments were characterised by EE2, 53%, and carbamazepine and venlafaxine, both with 14%. Main contributions can be appreciated in Figure 4.6 while all individual contributions are reported in Table A.17 from Appendix G.

Even though higher risks were obtained for effluent samples, as mentioned before these samples are pre-dilution samples, and therefore ΣRQ_s for surface waters mixtures

can provide a more accurate potential risk assessment of which compound pose a risk to the environment and need to be prioritised for remediate measures to be taken. Attention is focused on the high percentages of contribution achieved for hormones in surface waters based on concentrations <10 ng/L. These compounds have endocrine disrupting properties that cause negatives effects on the hormonal functions decreasing fertility as an example.[112]

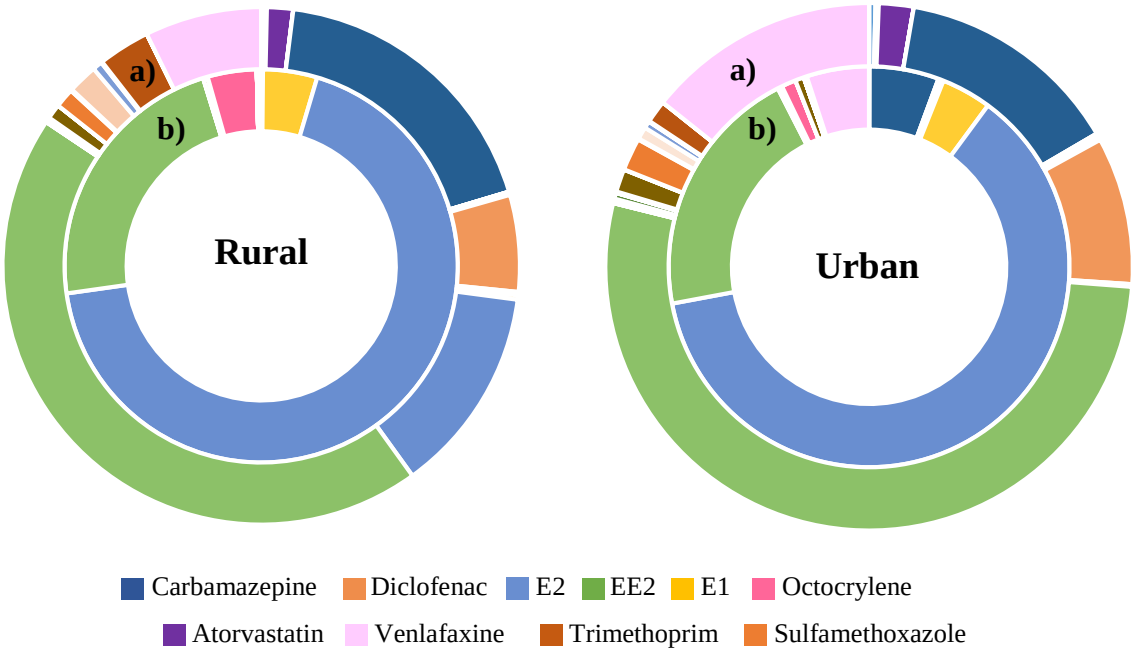


Figure 4.6 Doughnut plots for both rural and urban areas showing the contribution of individual compounds of the risk per site; where outer layers (a) belong to effluent samples and inside layers to surface waters (b).

4.4 Conclusions

CECs can be linked to negative effects in the environment, therefore a tiered assessment can be utilised to determine whether compounds pose an environmental risk. Currently, Ireland has limited data in monitoring water matrices for a high number of substances. Within this thesis, quantification values and qualification data have been achieved for 49 compounds in effluent and surface waters from an initial analysis of 135 CECs, including pharmaceuticals, PCPs and pesticides. A risk assessment has been performed for substances of emerging concern detected in collected samples at two different locations in Ireland, one rural and one urban. In each location, samples were collected monthly for a full calendar year, and two different types of matrices were analysed, surface waters and effluent wastewater. An initial PBT hazard assessment was performed where nine compounds presented the highest index value, 9, all of which were pharmaceuticals, including atorvastatin, clarithromycin, diclofenac, E2 and venlafaxine among others. They correspond to 18% of substances of the total 49 compounds studied, revealing the danger that they could potentially have in the environment. This number could have been possibly higher due to the absence of data for the persistence category found in the literature leading to an underestimation of their PBT values. Nevertheless, PBT assessments do not depend on concentrations found in the environment, unlike RQs, just on the compound itself so under or overestimation of the risk could happen. Therefore, an ERA assessment was implemented where effluent most risk values were from the following compounds: carbamazepine, diclofenac, E2, EE2, atorvastatin, trimethoprim and venlafaxine. Compounds with higher risk for surface waters included E2, EE2 and E1. Their surface water RQ values obtained were lower than their effluent values, but this is expected, due to their dilution once they entered the environment from the output

of the WWTP. The risk assessment performed here however, determines that even after dilution in the river, E2 still pose a high potential risk to the environment, which raises concern. Regarding the different locations investigated, two different morphological regions, urban and rural, were examined. However, findings between them did not show a difference of classified risk for effluent wastewater as both posed a very high risk. For surface waters, risk assessments also determined very high risks for both areas, however, the rural area had a ΣRQ_{rural} of 15 compared to 17 for the urban site. This higher risk calculated is because the urban area presented a greater amount of compounds detected, 24, with most of them at higher concentrations, possibly for the reason that a bigger population is expected to impact the waters in comparison to the rural area.

Concerning CEC classes, pharmaceuticals were determined to result in higher potential risk classification at both areas and matrices, which was also confirmed when using the initial PBT index calculations. However, lower amount of PCPs and pesticides were tested in comparison, and underestimation of their PBT and/or PENC values could have occurred due to the limited data available for these compounds, particularly relative to pharmaceuticals. Nonetheless, all PCPs were further assessed in the ERA assessment in order to avoid their underestimation, though their day to day use should suffice as a trigger for further investigation of ecotoxicological effects and potential risks of this type of compounds.

The assessments performed only accounted the risk for water measures, aqueous phase, it does not take into account any soil sediments or the possible retention of the target compounds on the solid particulate matter (SPM). Therefore, future assessments could include them as the risk could have been underestimated for some compounds as it does not represent the environment as a whole.[213] Also, antimicrobial resistance risks have not been considered in this study and compounds such as sulfamethoxazole were

obtained posing a medium risk. These considerations are lacking due to limited research available and should be added in future investigations.[358] Overall, a potential risk has been determined for both sites and certain substances on the samples analysed over the period of a year enabling a prioritisation list for future investigations on those areas. For effluent wastewaters, the prioritisation list includes carbamazepine, diclofenac, E2, EE2, and venlafaxine. However, attention should be focused on surface waters specifically on the endocrine disruptor E2. However, the outcomes of the study are preliminary as they depend on frequency, concentration or geographical location.[213]

5.0 International comparison of endocrine disrupting compounds in river waters

Abstract

Contaminants of emerging concern (CECs) pose a wide variety of chemistries resulting in extensive physicochemical properties. These properties will result in different effects on reaching the environment and could result in potential hazards to wildlife and humans. Certain CECs pose endocrine disrupting properties, disrupting hormonal balance of people and animals exposed to them. Therefore, these endocrine disrupting compounds (EDCs) are a major concern and consequently selected for their monitoring in this study. A total of 26 analytes (including steroids, flame retardants, plasticizers, preservatives, etc.) were monitored in three major rivers, Liffey (Ireland), Thames (UK) and Ter (Spain). Occurrence and frequency were investigated across all locations, where the highest concentration overall was obtained for the flame retardant TCEP (4,767 ng/L) in the river Thames, attributed to the high population of the central catchment in London. While most compounds detected at <LOD concentrations, geographical variations were observed obtaining significant differences for plasticizers, caffeine, flame retardants and benzotriazole, with overall higher concentrations for the river Thames. This was further confirmed by PCA analysis, which showed more discrimination patterns between the rivers Ter and Thames based on flame retardants, benzotriazole and steroids. However, clusters were overlapping and PCA explained up to 38% of the variance. Moreover, even though most EDCs were obtained <LOD or in the low ng/L range, at extremely low concentrations these compounds can pose harmful effects. Therefore, a risk assessment was performed resulting in 14% of compounds obtaining a high risk classification for the rivers Liffey and Thames and 7% for the Ter river. The highest RQ achieved overall was for caffeine (RQ=705) in the Thames river. Moreover, the potential risks of the entire location were calculated and the following ΣRQ_{river} values were obtained: 361, 455, 723 for the rivers Liffey, Thames and Ter respectively. Higher contributions of caffeine and

BPA were observed across the three matrices for the site risk, and therefore these compounds should be prioritised.

Aims and Objectives

- To perform a sampling campaign for an international comparison between UK, Spain and Ireland for surface waters over a 10-week period.
- Select compounds for monitoring in three different countries in surface waters.
- Evaluate the data obtained in order to perform occurrence and frequency investigation over the three sampling locations.
- Examine all compounds analysed for a geographical comparison between the three rivers depending on the compounds detected.
- Perform principal component analysis (PCA) in order to investigate the possibility of characterization of the different rivers tested.
- Examine the quantification values of the analytes to perform an environmental risk assessment (ERA), utilising calculated PNEC vales and RQs.
- Following ERA assessment, to classify the risks determined for all compounds and determine the full site risk estimating the contributions per compound.

5.1 Introduction

Endocrine disrupting compounds (EDCs) have been defined by the U.S. Environmental Protection Agency (EPA) as *"an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process."*[365] In recent decades, special attention has been given to EDCs due to adverse effects in intact organisms or its progeny.[366] These compounds can interfere with endocrine and hormone systems disrupting the body's normal functions and the group of molecules is very varied, having both natural (e.g. steroid hormones) or synthetic (e.g. plastics, pesticides, fungicides, etc.) origins. They are widely used in industry (e.g. plasticizers) and domestic activities (e.g. personal care products (PCPs), detergents, surfactants, etc.), therefore increasing their consumption, such as estrogens because of their content in oral contraceptives.[367] Since the 1990s, concern about these compounds has been growing[368] due to the hundreds of environmental contaminants reported which are known to have or be potential endocrine disruptors which can cause effects in biological systems at very low concentrations. A challenge has been generated due to their high compound diversity resulting in almost no structural similarities, making difficult to predict if they pose any ED properties. Moreover, due to their industry use, some of these compounds have been projected to have long half-lives meaning that they do not deteriorate or they do it slowly, they may not metabolised or if they do, transformation products could be even more toxic than the parent compounds. Even compounds that are not persistent, their use is so high that they are widespread in the environment.[365,369]

Different effects have been observed including reduction of fertility, reproductive organ anomalies and changes in the sexual behaviour in aquatic organisms such as fish, frogs, etc.[370] However, effects have also been observed in humans and it has been shown to be related to the increase of particular metabolic disorders (e.g. obesity, type 2 diabetes and cardiovascular disease) to even cancer.[367,371,372] Moreover, their toxicity depends on the affected organism and hence the lowest observable effect concentrations (LOEL) depend on the specific contaminant. Additionally, some of these compounds are persistent due to their physicochemical properties (e.g. log K_{ow}) and can bioacummulate[367] (e.g. triclosan) increasing their concern.[373] An example is the bioaccumulation of bisphenol A (BPA) in microalgae (0.16 pg BPA $cell^{-1}$) and the potential transport of this contaminant into other organisms such as clams entering the food chain becoming a threat for humans.[373] Consequently, EDCs are able to accumulate in tissues for years and with their continuous release a "cocktail" effect could be produced carrying cumulative, additive and/or synergic effects.[374] These effects have been highlighted by the scientific community but there is no specific regulation for EDCs and their regulation has been considered poor due to the lack of scientific data.[369,370] Furthermore, EDCs have potential to be toxic at extremely low levels which becomes a challenge for their investigation and accurate thresholds for the detection of these analytes have therefore not been established. However, some pieces of legislation cover some compounds and the European Union has introduced an evaluation system as an endocrine disruptor assessment under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) by the European Chemicals Agency (ECHA) in order to minimise their overall exposure.[375] From 533 compounds in total, 194 were classified as category 1 and 125 as category 2. Category 1 shows compounds presenting evidence of disrupting effects (such as nonylphenol, octylphenol, ethylparaben, propylparaben,

and BPA) and category 2 the ones with potential effects (such as nonylphenol monocarboxylate).[370] Building on this, some compounds are stated as priority substances in the Watch List (WL) from the Water Framework Directive (WFD), such as estrogen hormones, for their monitoring in surface waters.[376] Moreover, a challenge arises from the difficulty to control the vast number of EDCs, which increases every day, due to their wide and different chemical properties as previously mentioned.[377] The absence of an effective legislation and regulation for these compounds has been stated as a contribution to their exposure and therefore significant threat to wildlife and human health.[369]

EDCs occurrence has been confirmed in different water ecosystems including wastewaters, natural waters, oceans, and even at trace levels in drinking waters,[367,370] in the ng/L-µg/L level.[378,379] Their release into the environment is mainly attributed to industrial manufacturing, the human use of materials such as plastics, pesticides, etc. and also their incomplete removal during treatment in wastewater and drinking treatment plants (WWTPs and DWTPs, respectively).[369] Their presence is usually higher in rivers where industrial effluents discharge and/or flow through highly densely populated areas,[367] reaching high µg/L concentrations. Nevertheless, their source of exposure is constantly changing as some compounds were banned years ago and others more recently, resulting in different occurrence and frequency depending on the country.[365] Therefore, concentrations vary depending on different factors such as the geographical location, treatment performed in the WWTP, weather conditions, etc.[380] Consequently, the investigation of these compounds is necessary in order to evaluate their fate and their high potential risk.

In this study, the analysis of 26 compounds presenting or suspected to have endocrine disrupting activities properties was performed and they can be found in Table 5.1. The main objective was to examine the occurrence and frequency of these

compounds in different surface waters to evaluate their contamination. For this purpose, three different locations were selected for an international comparison over a 10-week period (October 2020-January 2021). An environmental risk assessment (ERA) was also performed resulting in a priority list of substances regarding high potential hazards to the aquatic ecosystem.

Table 5.1 Classification of endocrine-disrupting (EDCs)and related compounds analysed in this study[370,371] with their respective CAS number and canonical SMILES (from PubChem).

Family	Compounds	CAS number	SMILES
Steroids (Natural and synthetic human estrogens and conjugates)	17-β-estradiol (E2)	50-28-2	CC12CCC3C(C1CCC2O)CCC4=C3C=CC(=C4)O
	Estriol (E3)	50-27-1	CC12CCC3C(C1CC(C2O)O)CCC4=C3C=CC(=C4)O
	17-α-ethinylestradiol (EE2)	57-63-6	CC12CCC3C(C1CCC2(C#C)O)CCC4=C3C=CC(=C4)O
	Estriol 3-sufate (E3-3S)	481-95-8	CC12CCC3C(C1CC(C2O)O)CCC4=C3C=CC(=C4)OS(=O)(=O)O
	Estrone 3-sulfate (E1-3S)	438-67-5	CC12CCC3C(C1CCC2=O)CCC4=C3C=CC(=C4)OS(=O)(=O)O
	Estrone (E1)	53-16-7	CC12CCC3C(C1CCC2=O)CCC4=C3C=CC(=C4)O
	Testosterone	58-22-0	CC12CCC3C(C1CCC2O)CCC4=CC(=O)CCC34C
	Progesterone	57-83-0	CC(=O)C1CCC2C1(CCC3C2CCC4=CC(=O)CCC34C)C
Antimicrobials/disinfectants	Triclosan	3380-34-5	C1=CC(=C(C=C1Cl)O)OC2=C(C=C(C=C2)Cl)Cl
Preservatives	Methylparaben (MeP)	99-76-3	COC(=O)C1=CC=C(C=C1)O
	Ethyllparaben (EtP)	120-47-8	CCOC(=O)C1=CC=C(C=C1)O
	Propylparaben (PrP)	94-13-3	CCCOC(=O)C1=CC=C(C=C1)O
	Benzylparaben (BeP)	94-18-8	C1=CC=C(C=C1)COC(=O)C2=CC=C(C=C2)O
Plasticizer (Industrial production of polycarbonates and epoxy resins)	Bisphenol A (BPA)	80-05-7	CC(C)(C1=CC=C(C=C1)O)C2=CC=C(C=C2)O
	Bisphenol B (BPB)	77-40-7	CCC(C)(C1=CC=C(C=C1)O)C2=CC=C(C=C2)O
	Bisphenol F (BPF)	620-92-8	C1=CC(=CC=C1CC2=CC=C(C=C2)O)O
	Bisphenol S (BPS)	80-09-1	C1=CC(=CC=C1O)S(=O)(=O)C2=CC=C(C=C2)O
	Bisphenol AF (BPAF)	1478-61-1	C1=CC(=CC=C1C(C2=CC=C(C=C2)O)(C(F)(F)F)C(F)(F)F)O
Alkylphenols (Manufacture of household and industrial products)	Nonylphenol (NP)	25154-52-3	CCCCCCCCCC1=CC=C(C=C1)O
	Octylphenol (OP)	140-66-9	CCCCCCCCC1=CC=CC=C1O
Anticorrosive	1H-Benzotriazole (BT)	95-14-7	C1=CC2=NNN=C2C=C1
Organo-phosphorus and brominated- based flame retardants	Tris(butoxyethyl) phosphate (TBEP)	78-51-3	CCCCOCCOP(=O)(OCCOCCCC)OCCOCCCC
	Tris(chloroisopropyl) phosphate (TCPP)	13674-87-8	CC(CCl)OP(=O)(OC(C)CCl)OC(C)CCl
	Tris(2-chloroethyl) phosphate (TCEP)	115-96-8	C(CCl)OP(=O)(OCCCl)OCCCl
	Tetrabromobisphenol A (TBBPA)	79-94-7	CC(C)(C1=CC(=C(C(=C1)Br)O)Br)C2=CC(=C(C(=C2)Br)O)Br
Chemical marker	Caffeine	58-08-2	CN1C=NC2=C1C(=O)N(C(=O)N2C)C

5.2 Experimental

5.2.1 Reagents, chemicals and consumables

LC-MS optima grade methanol and water were acquired from Fisher Scientific (Loughborough, UK). Ultrapure water (resistance of 18.3 MΩ cm) was generated from a Millipore Milli-Q water purification system (Millipore, Bedford, MA, USA).

Reference standards for estrone (E1, >99%), 17-α-ethinyl-estradiol (EE2, >96%), estriol (E3, >97%), progesterone (>99%), testosterone (>99%), and tris-(2-chloroisopropyl) phosphate (TCCP, >99%) were acquired from LGC Standards Ltd. (Teddington, UK). Bisphenol A (BPA, 100%), bisphenol B (BPB, >98%), bisphenol S (BPS, 99%), bisphenol F (>98%), bisphenol AF (BPAF, 100%), triclosan (>99%), methylparaben (MeP, >99%), benzotriazole (>99%), caffeine (100%), tris(2-chloroethyl) phosphate (TCEP, 97%), tris(2-butoxyethyl) phosphate (TBEP, 95%), estrone-3-sulfate potassium salt (E3-3S, 99%), benzyl 4-hydroxybenzoate (BeP, 99%), propylparaben (PrP, 100%), estriol-3-sulfate (E1-3S, 99%), (>99%), 3,3',5,5'-tetrabromobisphenol A (TBBPA, >99%) were purchased from Sigma-Aldrich (Steinheim, Germany). Ethylparaben (EtP, >99%), 17-β-estradiol (E2, >98%), 4-nonylphenol (NP, >98%), 4-octylphenol (OP, 99%) and 17-α-ethinyl-estradiol (EE2, 100%) were obtained from Santa Cruz Biotechnology (Dallas, Texas, USA).

For internal standard reference materials, 17-α-ethinyl-estradiol-d_4 (>98%), 17-β-estradiol-d_2 (99%), estrone-d_4 (99%), methyl 4-hydroxybenzoate-d_4 (99%), 4-nonylphenol-d_4 (>98%), 4-octylphenol-d_{17} (>98%), benzotriazole-d_4 (99%), bisphenol A-d_4 (99%) and caffeine-d_3 (99%) were supplied by CDN Isotopes (Qmx Laboratories, Essex, UK). Triphenyl phosphate-d_{15} (>99%) was obtained from Sigma-Aldrich (Steinheim, Germany), ethyl 4-hydroxybenzoate-ring-$^{13}C_6$ solution from Fluka (Sigma-

Aldrich, Steinheim, Germany) and progesterone-d_9 from LGC Standards Ltd. (Teddington, UK).

Stock standard solutions, isotopically labelled internal standards and surrogate standards solutions were prepared at a concentration of 1 mg/L in methanol and stored at -20ºC. Further diluted solutions were prepared by mixing standards prepared in a mixture of methanol:water (15:85, v/v).

5.2.2 Sample collection and preparation

River grab samples were collected weekly in three different locations (Spain, UK and Ireland) during a 10-week period from October 2020 to January 2021 for an international comparison. All participants collected the samples following the same protocol where all samples were collected in 500 mL Nalgene bottles (Fisher Scientific, UK) during the morning between 9:00-11:00 am. Bottles were pre-rinsed twice with methanol and then ultrapure water separately prior to sampling. Then, bottles were further rinsed with river water before the collection of grab samples in duplicate, where bottles were filled to the top (no headspace present).

River samples were collected from three European cities for the international comparison purposes. The river Liffey was selected from Dublin (Ireland) which accounts for 25% of the country's population (approximately 4,761,865 people, where Dublin city has 1,173,179 in 2016, as per last census).[381,382] River Thames was chosen in London, being the largest city of the country, UK (population of approximately 9,176,530 people).[383] Central catchments were selected for both locations, Gabriel's Pier (51°30′30.3″N; 0°06′36.7″W) in London and O'Connell Bridge (53°20′49.2″N; 6°15′39.8″W) in Dublin. However, in Spain, water samples were collected from the river Ter, approximately 1250 meters downstream of a WWTP discharge (42°1′41.4″N;

2°50′53.5″E), which serves the entire city of Girona and surrounding villages (approximately 151,076 inhabitants).[384]

Once samples were collected, they were transported to the respective laboratories in a cool box. On arrival, samples were filtered using a 0.7 µm glass microfiber filter (Whatman®, Grade GF/F, Fisher Scientific Ltd., Loughborough, UK) followed by a 0.45 µm polyvinylidene fluoride (PVDF) membrane filter (Millipore; Billerica, MA, USA). After the filtering process, samples were stored under -20ºC freezing conditions prior to transportation or analysis. Samples collected in Dublin and London were shipped frozen to the Girona laboratory for analysis, however, samples were stored chilled for approximately three days at customs. Once they were released they were kept frozen until analysis.

5.2.3 Extraction

Analysis of the samples was carried out at the Catalan Institute for Water Research (ICRA) at the Sciemce and Technological Park of the University of Girona (Parc Científic I Tecnologic de la Universitat de Girona, Spain). Samples were extracted following the previously published protocol by Becker *et al.* (2017).[385] Solid-phase extraction (SPE) was carried out using a vacuum manifold (Phenomenex, Cheshire, UK) and Strata™-X cartridges (200 mg, 6 mL barrel, 33 µm, Phenomenex, Aliso Viejo, CA, USA). Conditioning was performed using 5 mL of methanol followed by 5 mL of ultrapure water at a pH of 1.5. After, a 100 mL of sample (pre-spiked with surrogate standards at a concentration of 500 ng/L where appropriate) was loaded at approximately 1 mL/min. Then, cartridges were washed using 6 mL of ultrapure water and dried under vacuum for 5 minutes. Elution was performed with 7 mL of a mixture of dichloromethane:methanol (50:50, v/v). Extracts were then evaporated to near dryness under a nitrogen stream and reconstituted in a mixture of methanol:water (15:85, v/v) to a final volume of 1 mL.

Finally, a standard mixture of isotopically labelled standards (ISL-IS) were added in the extract as internal standards at a final concentration of 50 µg/L.

5.2.4 Instrumental analysis

Analysis was determined following the previously published protocol by Becker *et al.*[385] where liquid chromatography was applied for the analysis of the final extracts using a Luna Omega C_{18} analytical column (100 x 2.1 mm, 1.6 µm) from Phenomenex (Torrance, CA, USA). The chromatographic system comprised of an Accela 4 Open AS autosampler and a quaternary pump from Thermo Fisher Scientific (San Jose, CA). Chromatographic separations were achieved using mobile phases of HPLC grade methanol (A) and water (B) with two different gradients for the negative and positive ionisation modes. The negative mode was utilized applying 20 µL sample injection volume, where mobile phases were at a constant flow rate of 0.4 mL/min for a total run time of 10.5 minutes. At starting conditions, A was set at 20% for a minute; 1-2.75 min: another linear ramp of A increased to 50%; 2.75-6.50 min: A further increased to 100%; 6.50-8: A stayed at 100%; 8-9.50: A returned to the initial conditions of 20% and maintained there for 1 extra minute. On the other hand, the positive mode method was performed using an injection volume of 10 µL applying the same mobile phases at a constant flow rate set at 0.3 mL/min. The gradient elution was as follows: A was set at 10% for a minute; from 1-2.75 min: A increased to 100%; 2.75-5.50: A stayed at 100%; 5.50-6.50: A returned to the initial conditions of 10% and re-equilibrated for 1 extra minute, resulting in a total run time of 7.5 minutes.

Analytical determination was carried out using mass spectrometry as detector with a TSQ Vantage triple quadrupole mass spectrometer equipped with an electrospray ionisation (ESI) source (Thermo Fisher Scientific, San Jose, CA). Final conditions of the MS can be found in Becker *et al.*[385] protocol. The acquisition of the selected compounds

was achieved in multiple reaction monitoring (MRM) mode where two transitions were selected for ion confirmation; the most abundant one was used for quantification and the other one for qualification/confirmation purposes. MRM transitions can be observed in Table A.18 (Appendix H) for both negative and positive mode. Data acquisition was performed through Xcalibur v2.2 software and was processed using TraceFinder v3.1 (both from Thermo Fisher Scientific, San Jose, CA).

5.2.5 Method performance

The utilised method was previously validated by Becker *et al.*,[385] however, method detection and quantification limits (LOD and LOQ, respectively) were determined as the minimum instrumental detectable amount of analyte with a signal-to-noise ratio of 3 and 10 respectively for every type of matrix used (i.e. Ireland, Spain and the UK) for higher quantification accuracy. Values are presented in Table A.19 from Appendix H for every river matrix investigated. Calibration lines were prepared at the following concentrations: 0.5, 1, 5, 10, 25, 50, 100 and 200 ppb in a final 1 mL of methanol:water (15:85, v/v) from stocks prepared at a concentration of 1 mg/L in methanol. Internal standards were added for a final concentration of 50 ppb in the vial. Moreover, for a more accurate quantification, recoveries were also calculated and used to correct calculations of final analyte concentrations due to possible different matrix effects between the different locations. Therefore, recovery experiments were performed for all three different location samples (Ter river in Spain, Liffey river in Ireland and Thames river in UK) and are presented in Table A.19 (Appendix H). Samples were prepared in triplicate for every water type by spiking the standard solution at 500 ng/L in the water samples. Internal calibration lines using isotopically labelled standards were prepared for the quantification of the compounds selected.

5.2.6 Statistical and data analysis

Microsoft® Office Excel (WA, USA), IBM® SPSS Statistics v27 (New York, USA), R v4.0.5, RStudio v1.4.1106 (Boston, USA) and Python v3.7.9 were employed for data and statistical analysis purposes.

5.2.6.1 Frequency

Compound frequency, the rate of presence of the analytes in the samples, was assessed as per Section 3.2.5.1 in Chapter 3.0, following Equation 3.1.

5.2.6.2 Statistical analysis

Statistical analysis was performed to evaluate if geographical variations were significant between the three rivers selected. For this purpose, the analysis was performed comparing the concentrations of the compounds classifying them by categories due to the high number of analytes selected. Categories of EDCs data were tested first for normality using the Shapiro-Wilk W test ($p<0.005$ significance level). Then, analysis of variance (ANOVA) was used for the mean values comparison using the post hoc Tuckey's test ($p<0.05$) for normal data and Kruskal-Wallis ANOVA by ranks ($p<0.05$) for non-parametric data. For samples where compounds were detected below the LOD, values of zero were set. If values were below LOQ, half of the specific limit (i.e. Spain, Ireland and UK) was designated. For ANOVA tests results, whisker box plots were used to represent the results as per Section 3.2.5.3 (Chapter 3.0), where the lower (25%) and upper (75%) quartiles of the corresponded values were shown by lines for the EDCs category or compound itself, if investigated on its own. The variability outside the upper and lower quartiles were represented by lines extended from the boxes. The median was denoted by the line inside the box and outliers and far outliers by symbols (° and * respectively) when numbers were outside the 1.5 times interquartile range (IQR).

Multivariate analysis was achieved using principal component analysis (PCA) in order to investigate the possible characterization of the rivers based on the EDCs quantified across the three locations, exploring the variability and trends of the data obtained. Data was normalised as previously detailed in Section 3.2.5.3 of Chapter 3.0.

5.2.7 Environmental risk assessment

The environmental risk calculations for the EDCs investigated in this study were performed following the European guidelines for an environmental risk assessment (ERA) by the European Medicines Agency (EU EMEA).[356] Accordingly, risk quotient values (RQ) were calculated using the maximum environmental compound concentrations (MEC) quantified per river that can be found in Tables A.20-A.22 (Appendix H). If any compound was detected below the LOD or LOQ (Table A.19, Appendix H), half their limits were used instead of the MEC. Calculations of predicted no-effect concentrations (PNEC) were calculated using no observed effect concentrations (NOEC), or the median lethal or effective concentration (LC_{50} or EC_{50}, respectively). Details of calculations, equations and procedures are specified in Section 4.2.2 from Chapter 4.0, where if RQs were below 0.1 an insignificant effect was determined; between 0.1 and 1 a low or negligible risk was associated; between 1 and 10, a medium risk was assigned; and if higher or equal than 10, a high ecological risk was allocated.

A location risk assessment was also performed in order to evaluate the potential risk of all EDCs detected per river. This was achieved following Section 4.2.3 from Chapter 4.0, where if ΣRQ_{river} values were below 0.01 no risk was designated to the river; values between 0.01 to 0.1 posed a low risk; between 0.1 and 1 a medium risk was associated; for values greater than 1 a high risk was expected; if values are greater than 10 a very high risk was finally determined. Moreover, the contribution of every

compound to the whole risk per location was calculated following Equation 4.5 from the same section (Section 4.2.3, Chapter 4.0).

5.3 Ressults and Discussion

5.3.1 Occurrence and frequency

Occurrence of the EDCs detected in the three locations are presented in Figure 5.1, with their respected LODs and LOQs. Of a total of 26 compounds analysed, 14 were detected for the river Liffey and Thames, and 15 for the river Ter. The majority of the compounds were detected with a 0% frequency across the three sites, due to concentrations below the limit of detection (LODs were not taken into account for frequency data calculations) as observed in Figure 5.2. Nevertheless, five (caffeine, ethylparaben (EtP), benzotriazole, tris-(2-chloroisopropyl) phosphate (TCPP) and tris(2-butoxyethyl) phosphate (TBEP)), six (caffeine, bisphenol S (BPS), benzotriazole, tris(2-chloroethyl) phosphate (TCEP), TCPP and TBEP) and four (caffeine, benzotriazole, TCPP and TBEP) compounds were detected with a 100% frequency for the rivers Liffey, Thames and Ter respectively. The compound individual frequencies and occurrence data is available in Tables A.20-A.22 (Appendix H). The detected EDCs were quantified at different levels with concentrations ranging from <LOD to 524 ng/L (TCCP), <LOD to 4,767 ng/L (TCEP) and <LOD to 705 ng/L (caffeine), for the Liffey, Thames and Ter respectively. Overall, of the 26 compounds analysed, only eight, nine and ten compounds were quantified for the rivers Liffey, Thames and Ter respectively. Whilst compounds such as propylparaben (PrP), benzyl 4-hydroxybenzoate (BeP) and bisphenol B (BPB) were not detected at any location throughout the sampling campaign, in contrast, compounds such as caffeine and triclosan were detected at all sites at similar frequency values. Overall, cumulative values showed that the river Thames presented the higher concentrations due to higher levels detected for the flame retardants class, resulting in up to almost 20,000 ng/L of all compounds detected (Figure 5.3). Lower cumulative values were obtained for the river

Liffey, 5,410 ng/L, and lastly for the river Ter, 4,356 ng/L. These values follow the trend of high-density populated area, i.e. London, Dublin and Girona.[386]

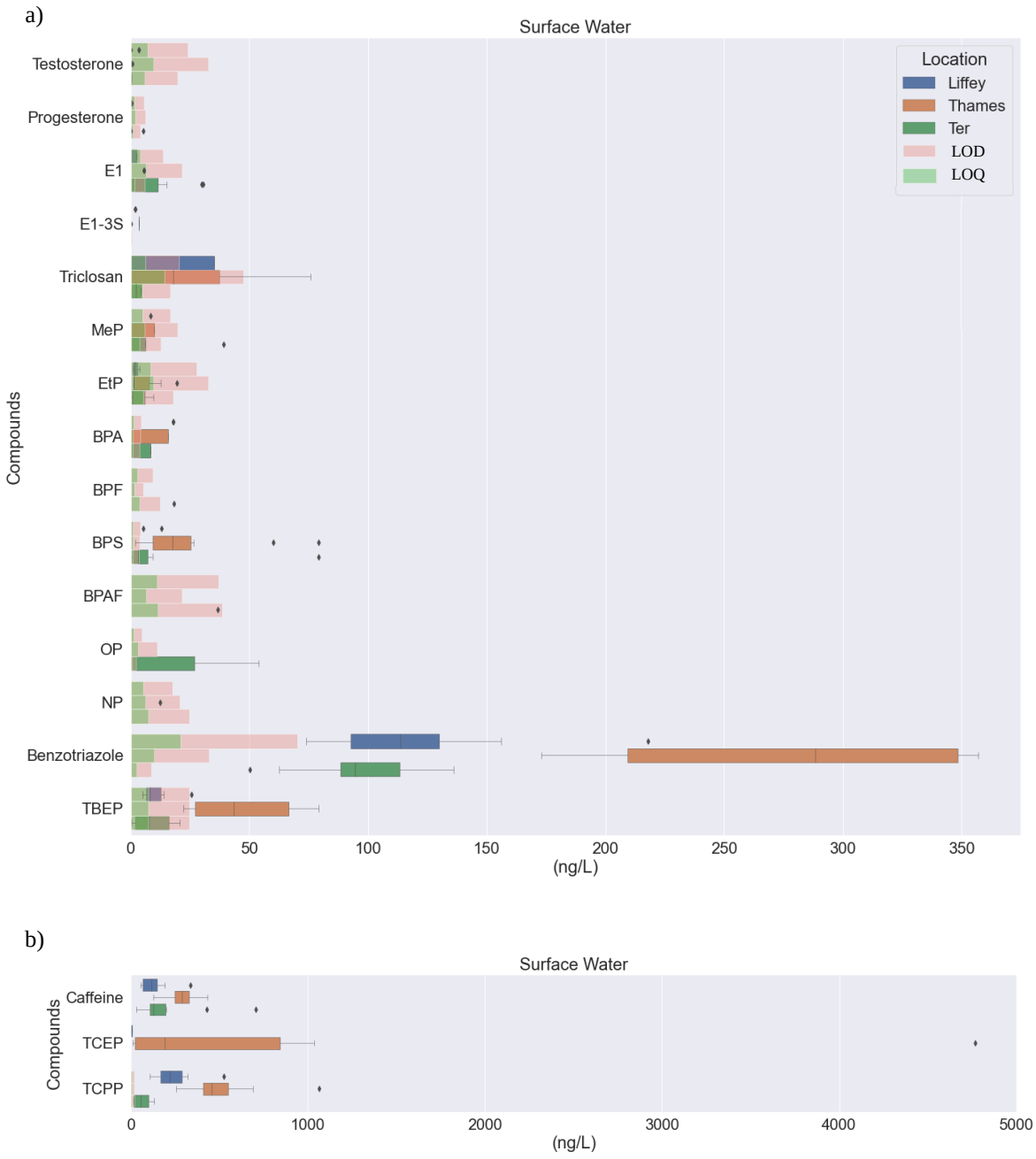


Figure 5.1 Concentration of selected EDCs in surface waters for all compounds detected for the three areas investigated: Liffey (blue), Thames (orange) and Ter (green), where error bars represent minimum to maximum (n=10, weeks analysed) and LODs and LOQs are represented by chart bars in light green and light pink respectively, for every type of matrix. a) for concentrations up to 375 ng/L and b) for concentrations detected up to 5000 ng/L.

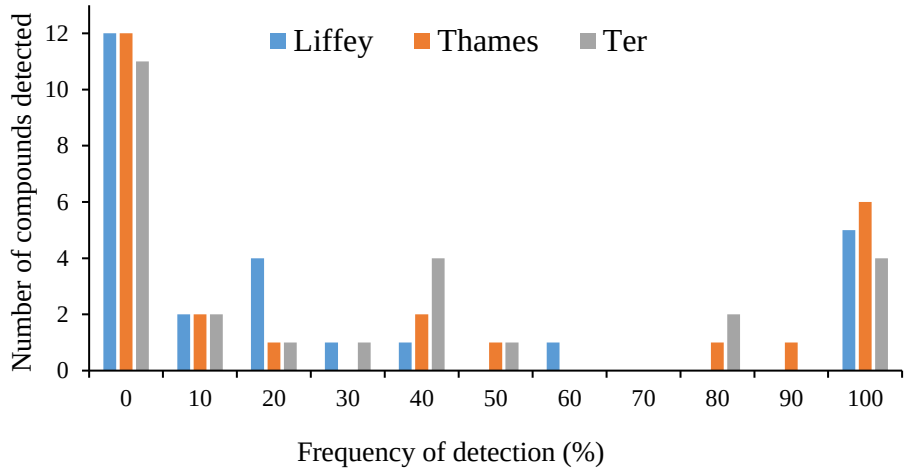


Figure 5.2 Number of compounds per frequency of detection for the three rivers tested for the10-week sampling campaign (n=10).

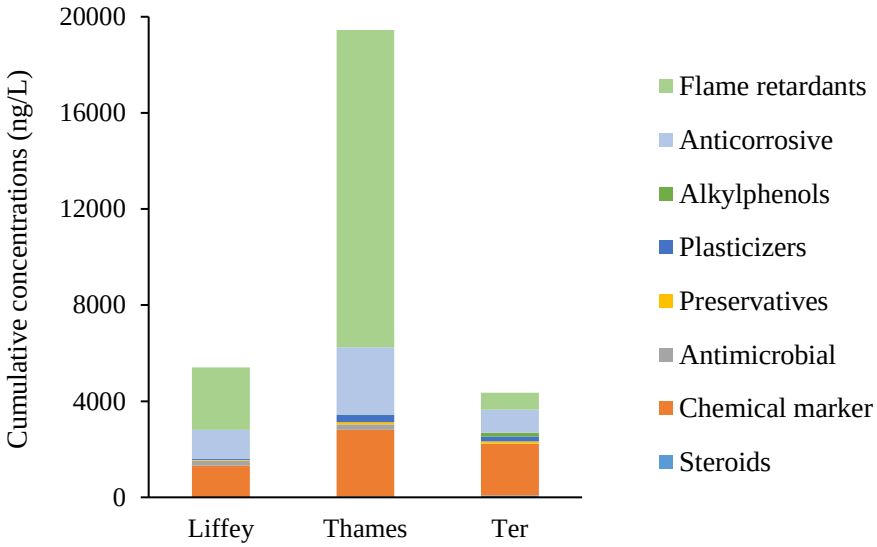


Figure 5.3 Cumulative concentrations of all EDCs detected for the 10-week sampling campaign for the three rivers investigated. Each colour represents a different class of EDCs detected.

5.3.1.1 Steroid hormones

Steroid hormones are one of the classes of EDCs which has caused abundant environmental concern over the last decades. Only three hormones (testosterone, progesterone and E1) were detected at quantifiable concentrations across all sites from the total eight studied. E1-3S was also detected but concentrations were always below

the limit of quantification (<LOQ). This is unsurprising due to the expected low concentrations at which are usually found in the environment (low ng/L range).[387] However, the low detection limits stipulated for water by regulations such as the Directive 2015/495/EU in the WL (WFD) have not been achieved in this study (e.g. 0.035 ng/L for EE2 (Table 1.3, Chapter 1.0)). This is not unexpected, as the low detection limits are analytically challenging to achieve and are therefore not frequently met by different reported studies, and this has limited the number of studies for this type of compounds in literature.[388] It cannot be discounted therefore that the hormones investigated could have been present at samples, but were not quantifiable due to the high limits of detection achieved.

The natural estrogen E1 was quantified with the highest concentration from all compounds of the category, detected at 31 ng/L in the river Ter. Overall, a contribution of only 2% of the total concentrations of all detected compounds was attributed to the steroids category in the Ter samples as observed in Figure 5.4. The higher concentrations in respect to the other locations could be due to the geographically close WWTP, located upstream the sampling point. WWTPs have been demonstrated to be point sources for this type of compounds, as they are not designed to remove CECs[385] with, e.g. compounds such as E1 remaining in concentrations of up to 45% when using conventional treatments.[389] On the other hand, no compounds quantified belonged to the category of steroids for the Thames samples, where E1-3S presented the highest concentration, <7.2 (<LOQ) ng/L. The same compound presented the highest concentration of all steroids in the river Liffey, <3.9 (<LOQ) ng/L. Only limited number of studies include the analysis of conjugate steroids in aquatic matrices, however, this compound has been previously detected at concentrations between 12-170 ng/L in influent and 7.5-34 ng/L in effluent,[389] suggesting significant removal during treatment. Sulphate steroid conjugates have low

log K_{ow} and high aqueous solubility values indicating hydrophilic characteristics leading to their occurrence in water samples. Nevertheless, low concentrations, <10.4 pg/L, have been reported in surface waters[390] upon dilution once entering the natural environment, lower than the ones determined in this study.

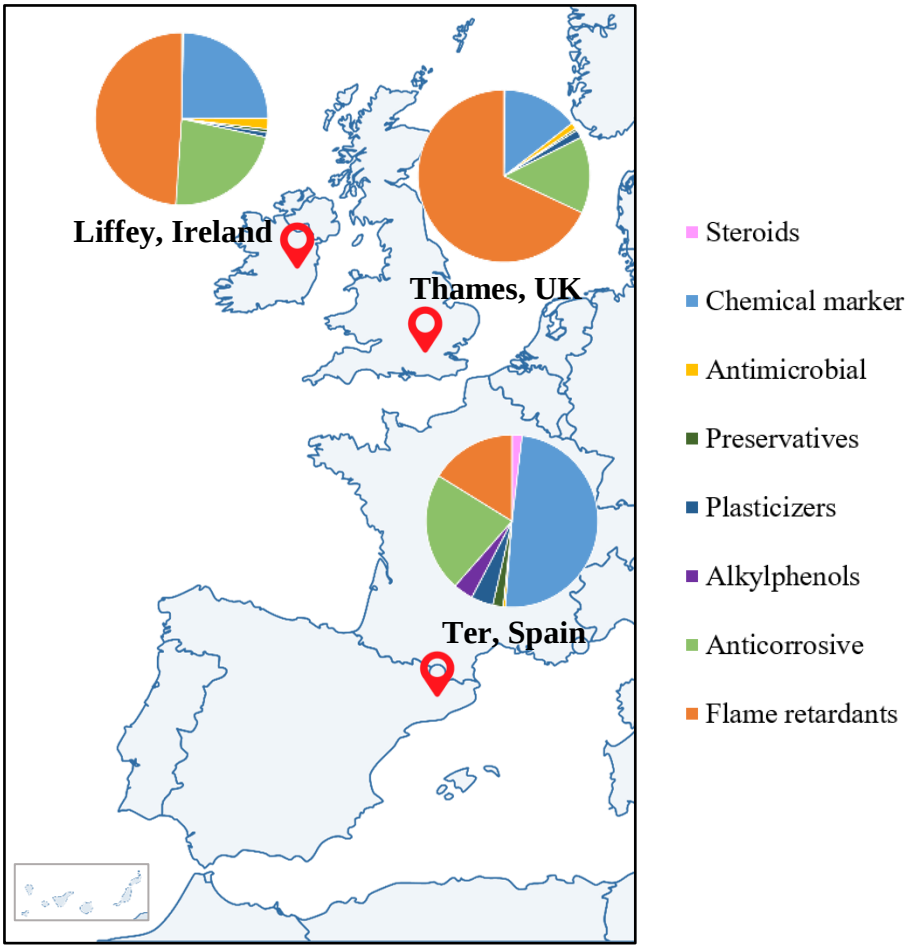


Figure 5.4 Compound classification of identified EDCs in all three locations: Liffey, Thames and Ter river waters.

5.3.1.2 Chemical markers

Several sources of CECs entering the environment have been confirmed in literature depending on the type of contaminant (e.g. agriculture runoff, road runoff, sewage and treated wastewater, etc.).[391] Therefore, to identify their source in surface waters has become a challenge and extensive research has been performed over the last two decades. To track pollution by domestic and private activities, many studies utilise chemical and

microbial markers or a combination of both, and recently studies have successfully used CECs such as pharmaceuticals, PCPs, artificial sweeteners, etc. This is due to their faster and reliable detection compared to microbial markers, however, chemical markers have several disadvantages such as the lack of specificity due to factors such as degradation and/or sorption.[392] An effective marker needs to be consumed regularly and constantly, so consumption habits should not change over the years. Moreover, concentrations must be sufficiently high after treatments performed in the WWTPs to be quantifiable in the environment (e.g. surface waters).[393] Consequently, there is no single marker that could be used in all sites precisely but certain pharmaceuticals and PCPs have been extensively found at mg/L concentrations across the world and new analytical techniques achieve the trace quantification levels required with a high level of specificity.[392] Caffeine has been previously used as a chemical marker by many studies meeting these requirements. It is found in a variety of food and beverages (e.g. tea, coffee, chocolate, etc.), is the most consumed psychoactive substance in the world[394] and has a global average consumption estimated to be between 80 and 400 mg/person/day, although there is a high variation between countries.[393,394] Due to the improper disposal of food, medicines, etc., containing this compound and its excretion in human urine (approximately 5% of ingested caffeine) it reaches the sewage systems. Caffeine rate removals during treatment (WWTPs) are variable depending on the system. Moreover, higher concentrations have been quantified in rivers than in effluent samples due to possible untreated effluents, overflows, etc.[395] resulting in its detection at concentrations such as 865 ng/L in river (China) which presented high correlations with total nitrogen and ammonia.[392] This compound has high solubility in water, it is non-volatile and presents a half-live of approximately 10 years,[396] showing its presence in even remote areas.[309] Accordingly, caffeine has been identified as a good marker to track pollution.

In this study, caffeine was detected constantly at >LOQ concentrations across all samples and locations, resulting in a 100% frequency as seen in Figure 5.2. Concentrations in rivers have been reported in Europe up to 880 ng/L in Germany, however, reported concentrations of caffeine downstream a WWTP have been reported up to 2,400 ng/L.[393] This trend was also observed in this study, as illustrated in Figure 5.5, where the river Ter was shown to have the highest concentrations overall for week two of the sampling campaign and similar concentrations to the other rivers, Thames and Liffey, for the rest of the weeks; which serve populations 60 and 8 times larger than the Ter respectively. It can be seen that caffeine concentrations in the Thames were in most cases higher than the Liffey. Previous reports have attributed high concentrations to larger populations, which is in agreement with what was observed here, though there was not a direct relationship observed between population size and caffeine concentration.[394] Caffeine has also been previously reported in the Thames at concentrations of 112 ng/L[397] and 389 ng/L in the river Liffey,[309] however, no concentrations were found for the river Ter. Furthermore, the total concentration of caffeine corresponded to 49% of all compounds detected for the river Ter, compared to just 25% and 14% for the Liffey and Thames, respectively (Figure 5.4). Overall, caffeine resulted in a good marker due to its high frequency of detection (100%) and also high and constant concentrations across all matrices (Figure 5.5).

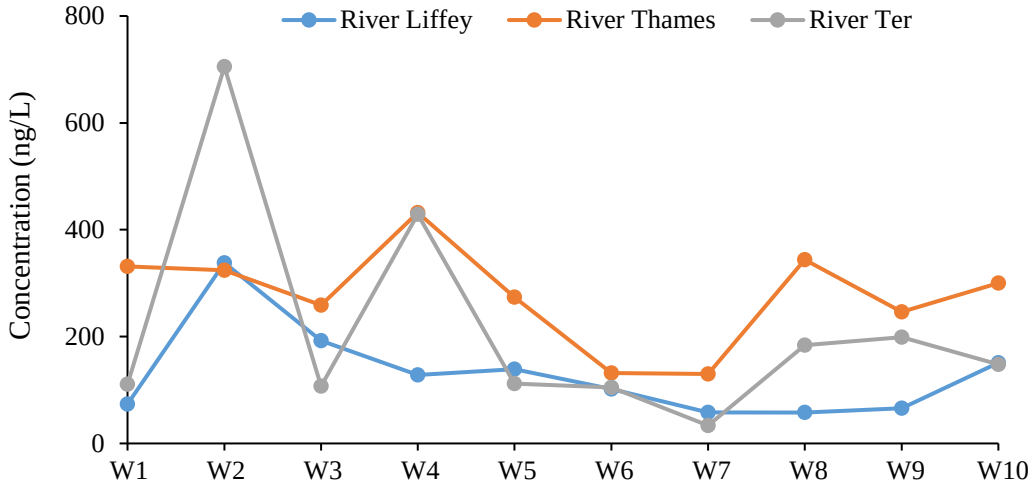


Figure 5.5 Caffeine concentrations (ng/L) across all samples analysed (10-week period) in all three locations: Liffey, Thames and Ter river waters, where W means week of sampling.

5.3.1.3 Antimicrobial/disinfectants

Recently, triclosan has gained popularity due to its possible bacterial resistance and its limited research in literature.[398] Triclosan is widely used as a preservative or antiseptic in medical products and it is also incorporated as biocide in footwear, carpet, plastics, etc.[196,398] Additionally, many household and approximately over 2,000 different personal care products (PCPs) contain this compound (e.g. toothpaste, mouthwash, deodorants, etc.)[217] and therefore its use has increased over the last 30 years, where in Europe alone for example, approximately 350 tons of triclosan are present annually, across a variety of products.[398] Concentrations in surface waters are usually in the ng/L level as this analyte normally is effectively removed by WWTP treatments, however, widely variable removals rates have been reported ranging from 0 to 98%[196] depending on the treatments performed, etc.[198] Once it enters the natural environment, it can stay for approximately 11 days[198] where it can result toxic to certain aquatic organisms such as *Scenedesmus subspicatus* algae at concentrations of only 500 ng/L.[398]

In this study, the maximum concentration of triclosan detected, 76 ng/L, was quantified for the river Thames. This concentration is similar to previous studies reported in river waters such as 72 ng/L in Cadiz (Spain),[399] up to 59 in Japan, up to 75 n/L in Australia,[400] up to 95 ng/L in South Wales (UK)[380] and up to 66 ng/L in Chang Jiang (China).[400] Triclosan could not be determined at comparable concentrations in the river Liffey, as a high LOQ, 70 ng/L, resulted from this analysis. However, in all samples, it was detected above the LOD (21 ng/L), indicating that this compound was also present in these samples. Triclosan concentrations were discernibly lower in the Ter relative to the Thames, with concentrations <LOQ of 9 ng/L obtained for samples analysed for the river Ter. These concentrations are lower than those previously reported in the literature, particularly considering the close discharge from the WWTP, where triclosan concentrations as high as 2,300 ng/L have been reported.[199] Previous studies have related low concentrations of this compound in surface waters to heavy rains due to the dilutions occurred in the natural environment, when comparing dry and wet seasons.[401] This could also be relevant to this study, where the sampling campaign was performed during the autumn-winter period (October-January months), the wet season in Spain. Nevertheless, in the Ebro basin (Spain), a sampling campaign was performed in 2010, where concentrations ranged from not detected to 2 ng/L,[117] more similar results to the ones obtained for this study. Consequently, a contribution of 0% of the total concentrations for the antimicrobial category was obtained for the Ter location, compared to the 2 and 1% for the rivers Liffey and Thames as observed in Figure 5.4.

Regarding frequency data, values obtained were similar across all locations, with results of 60, 50 and 50% determined for the rivers Liffey, Thames and Ter respectively. Frequency results are in accordance with previous data for 139 rivers in the USA, where frequency was calculated at 57.6%; lower values than the ones reported for China of

90%,[37] however, China has the largest production of PCPs in the world apart from the largest population, and triclosan has been quantified in the country with high values such as 1,023 ng/L in the Pearl river.[400] In summary, triclosan was present in at least half of the samples at the three locations where rivers Liffey and Thames presented similar sampling dates as illustrated in Figure 5.6.

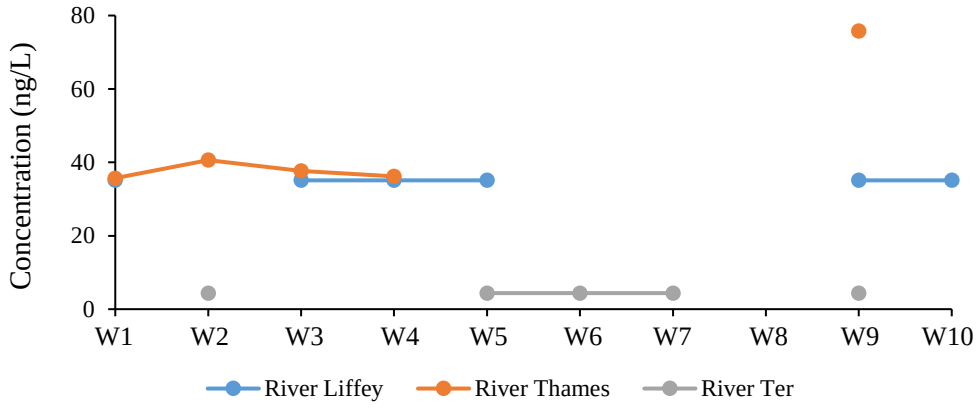


Figure 5.6 Triclosan concentrations (ng/L) across all samples analysed (10-week period) in all three locations: Liffey, Thames and Ter river waters, where W means week of sampling and the half LOQ values are represented for the samples quantified <LOQ for their visualisation.

5.3.1.4 Preservatives

Parabens are widely used as preservatives in a variety of products (e.g. cosmetics, pharmaceuticals, food, etc.).[402] In this study, only methylparaben (MeP) and EtP were detected across all locations, whilst no samples had PrP or BeP detected. MeP is one of the most used parabens across the world[117,199,403] due to its common use in cosmetics[404] and consistent with this, it was detected at the highest concentration of all compounds in this category, quantified at 39 ng/L in the river Ter, having a 40% detection frequency (detected between weeks 4 and 7, mid-November to early December). The concentrations of the other parabens studied were quantified at <LOQ concentrations, even though for the rest of matrices, LOQs obtained were 17 and 20 ng/L with 10 and 40% frequencies for the river Liffey and Thames respectively. Previously reported concentrations in rivers

in the Santiago de Compostela area (Spain) were up to 17 ng/L,[196] up to 27 ng/L in the Ebro basin (Spain)[117] and up to 14 ng/L in Boli river (Taiwan);[403] all lower than the concentration detected in the Thames. Consistent with the MeP findings, the maximum concentration detected of EtP, 20 ng/L, was found in the river Thames as well. It can be hypothesised that this is may be because the Thames serves the highest population of all three locations. In contrast, the maximum concentrations obtained were 4 and 10 ng/L for the rivers Liffey and Ter respectively. Moreover, no data has been reported for any paraben in the three locations. However, higher frequency values were obtained when compared to MeP; 100, 90 and 80% for the river Liffey, Thames and Ter respectively (Figure 5.2). Lower concentrations of EtP were also obtained in other studies when MeP and EtP were investigated together, having concentrations up to 3 ng/L in the Santiago de Compostela area (Spain),[196] up to 13 ng/L in the Ebro basin (Spain)[117] and not detected in Boli river (Taiwan),[403] also consistent with this study. In these reported studies, PrP was detected at concentrations up to 69 ng/L (Santiago de Compostela area, Spain),[196] up to 15 ng/L (Ebro basin, Spain)[117] and 9 ng/L (Boli river, Taiwan).[403] However, PrP removal rates in the WWTP in the Santiago de Compostela area were higher than 99.9% and concentrations were found to be higher in the river than the effluent, suggesting its presence to untreated wastewater discharges or leaks from the system.[196] On the other hand, BeP was detected <LOQ,[196] up to 1.1 ng/L[117] and not detected[403] respectively, in accordance to our study. In summary, total contributions of concentration for the preservative category was 1, 0 and 2% for the rivers Liffey, Thames and Ter as seen in Figure 5.4, due to the majority of samples detected at <LOD concentrations.

5.3.1.5 Plasticizers

Plasticizers such as BPA are usually added to artificial plastic or polymers in products such as epoxy resins, polycarbonate and polyvinyl chloride (PVC), being essential

components of plastics.[405] Consequently, their use has been increased by the plastic manufacture industry over the years where approximately 9.75 million tons are expected to be produced totally by 2024.[406] Several rivers have been demonstrated to be point sources for microplastics and macroplastics, where bisphenols (BPs) have been detected across the world in surface waters.[407] Five BP compounds in the plasticizers category were analysed, and of those, four were detected across all three locations. Of these compounds, two, BPA and BPF, were detected but could not be quantified as a result of the high LOQs achieved for both compounds in all locations (see Table A.19, Appendix H). These findings of detectable but not quantifiable concentrations, i.e. ≤36 ng/L (Liffey matrix) and ≤61 ng/L (Thames matrix) for BPA and BPF respectively, indicates that these compounds are likely present at similar concentrations that those detected in previous studies in Spain (not detected-61 ng/L for BPA)[117] and not detected in Poland (even with a lower limit of quantification, LOQ=5 ng/L),[408] however, BPA was not detected in the river Liffey previously (0 ng/L).[309] Whilst BPS and BPAF were detected at concentrations above their quantification limits (>LOQ), ≤0.2 and ≤0.9 ng/L (both for Liffey matrix) respectively, these limits are significantly lower when compared to the other two compounds. Maximum concentrations detected were 79 ng/L, for both river Ter and Thames, and 37 ng/L for the river Ter, for BPS and BPFA respectively. Previous reported concentrations of BPS in rivers ranged from 1.5-8.7 ng/L, not detected-42 ng/L, not detected-135 ng/L, and not detected-7,200 ng/L for river in Japan, Korea, China and India respectively.[409] BPAF has been previously detected at concentrations ranging between 1.5-16.2 ng/L in surface waters in China.[410] Therefore, concentrations within this study are similar or higher than others reported and potential risks could be attributed to the aquatic environment at these concentrations. It should be remembered too that BPA alternatives are primarily found in suspended particulate matter (SPM) due to their

physicochemical properties.[411] An example is the studied compound BPAF, which has a high lipophilicity due to its log K_{ow} of 4.47, increasing concern in possible bioaccumulation in aquatic organisms and high persistence in the environment.[412] This suggests that higher concentrations could be found in sediments and/or sludge (after treatment in WWTPs) for these compounds, raising the concern.

Regarding the total concentration contributions of all plasticizers detected, they were low for all three locations, 0, 0 and 4% for Liffey, Thames and Ter respectively, as seen in Figure 5.4. Frequencies varied depending on the specific compound and matrix, with BPS resulting in the higher presence of all of them, 100% frequency, for the river Thames, 80% for the river Ter and only 20% for the Liffey. BPFA and BPF were only detected once (10%) in the river Ter, while BPA had higher detection frequencies ranging from 20 (Liffey) to 40% (Thames and Ter). Therefore, irregular frequencies were obtained for this category with no clear pattern on the variation.

5.3.1.6 Alkylphenols

Alkylphenols are one of the most important categories of EDCs due to their high risks associated in wildlife and humans. Nonylphenol (NP) and octylphenol (OP) belong to category 1 of the priority list as mentioned before[370] and some alkylphenols have been suggested for their inclusion in the next WL chemicals from the WFD, to be classified as priority substances for their monitoring in surface waters.[96,370] Their wide use is mainly attributed to the manufacture of surfactants and also to degradation products of alkylphenol ethoxylates (APEOs) used in household detergents, pesticides, etc.[413] In this study, only NP was detected in the river Thames with a frequency of just 10% (i.e. one sample) with a concentration <LOQ (<21 ng/L), even that a previous report quantified NP at 75 ng/L in the river Liffey before.[309] OP was also only detected in one matrix, the river Ter, with concentrations ranging from 27-54 ng/L and a 40% frequency. Therefore,

the total contributions based on concentrations were 0% for the Thames and the Liffey and only 4% for the river Ter (Figure 5.4).

Usually, higher concentrations of NP are detected relative to OP as demonstrated in several European rivers[414] and other countries such as China, suggesting that OP is a minor component in APEOs.[413] These results are in line with the Thames sample, which was quantified at <LOQ for NP and not detected for OP. However, in the river Ter, NP was not detected in any sample. This could be associated to the WWTP discharge upstream of the collection point and the seasonal period when sampling (winter time). Previous reported studies have detected OP at concentrations up to 91 ng/L and 428 ng/L for NP (Hungary), higher than the ones obtained in this study. However, higher concentrations are usually reported in warmer months, summer periods, for these compounds when compared to winter. This was associated to higher production over summer (e.g. pesticides) and/or lower removal rates with higher temperatures.[414] Our study was performed between October-January months, therefore results cannot be extrapolated to the summer period.

5.3.1.7 Anticorrosives

Benzotriazole is widely used in applications such as corrosion inhibitors in aircraft and household dishwasher detergents, therefore considered as the second most frequent contaminant in water due to the difficulty of removal during treatments in WWTPs and high use.[415] It is usually resistant to biodegradation, resulting in a highly persistent compound in the environment once entering, where its presence has been confirmed globally in different aquatic matrices.[416] This compound was detected with 100% frequency in all three locations, with concentrations ranging between 74-218, 173-357 and 50-136 ng/L for the rivers Liffey, Thames and Ter respectively as observed in Figure 5.7. Previous concentrations in the river Liffey were quantified at 309 ng/L, higher than

the ones obtained in this study,[309] however, no data has been reported for this compound on the other two rivers. The concentrations detected in the river Ter could be attributed to the discharge of the WWTP. A previous study measured concentrations of this compound even up to one order of magnitude higher downstream of a WWTP in the river Leine (Germany).[416] Benzotriazole was reported with a 100% frequency, both upstream and downstream, and concentrations ranged between 34-176 and 248-733 ng/L, respectively. These concentrations are in line with the ones obtained within this study, where the maximum concentration was 357 ng/L (river Thames), lower than the maximum reported in Germany.[417] Nevertheless, concentrations in Europe for rivers have been reported up to 6,300 ng/L (Switzerland)[418] and always with a 100% frequency. This could be attributed to the wide use where only in Europe has a production of 1,000-10,000 tons per year.[419] Consequently, even though only one compound has been studied for this category, high contribution percentages were obtained in all rivers, 23, 14 and 22% for the rivers Liffey, Thames and Ter, respectively (Figure 5.4), raising the concern.

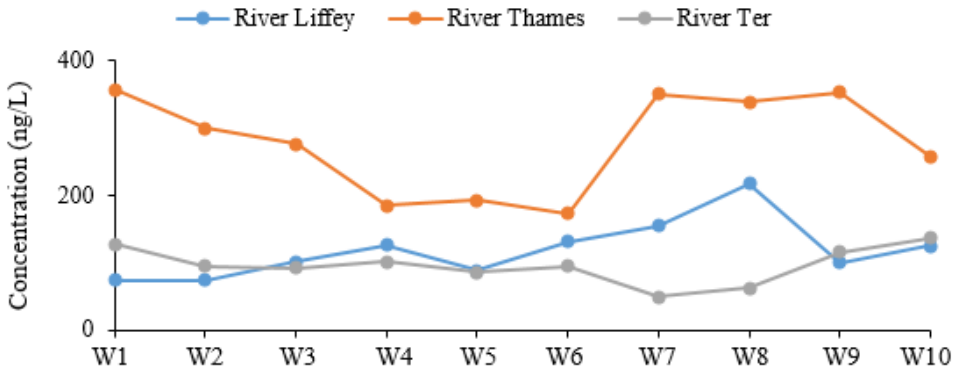


Figure 5.7 Benzotriazole concentrations (ng/L) across all samples analysed (10-week period) in all three locations: Liffey, Thames and Ter river waters, where W means week of sampling.

5.3.1.8 Flame retardants

Flame retardants are widely used in a variety of products such as plastics, textiles, furniture, etc. Compounds such as TCEP and TCPP are suspected carcinogens and recently their concern in the scientific community has increased due to their occurrence in the aquatic environment. Their detection in surface waters have been confirmed across the world (e.g. Germany, China, UK, etc.) at concentrations ranging from ng/L to µg/L due to their incomplete removal from industrial and domestic sewage discharges.[420] In this study, four compounds were studied in this category. Only three compounds were detected in the samples tested (i.e. TBBPA was not detected at any sample even though low LODs were achieved across all matrices, ≤0.3 ng/L (Thames matrix)). TCPP and TBEP were quantified in all detected samples (>LOQ). However, TCEP in the river Ter was not detected (frequency of 0%) and concentrations in the Liffey were below the limits (<LOQ). This category presented the higher contribution of the total EDC concentrations in Liffey and Thames locations as seen in Figure 5.4, with 49 and 68% respectively. A contribution of 16% was achieved for the Ter river, due to the lower concentrations of only two compounds detected. High frequency values were obtained for TCPP and TBEP (100%) in all locations, similar than previous studies which reported frequencies between 80-99% for this type of compounds.[420] This could be associated to their continuous release during manufacture.

TCEP obtained the maximum concentration throughout the study, 4,767 ng/L in the river Thames. This concentration is in accordance with previous reported ones for urban surface waters (e.g. 5,698 ng/L in Beijing, China) which has been related to the high city population.[420] In this case, Thames river has the highest population of all three locations as samples were collected in a central catchment area in London (capital of UK). TCPP also presented high concentrations in the river Thames, up to 1,065 ng/L,

similar to the ones reported as well for Beijing of 1,742 ng/L. However, in that study, TBEP was reported at concentrations up to 3,617 ng/L,[420] significantly higher than the ones obtained in this study (79 ng/L in the river Thames). Due to these high concentrations obtained, the river Thames presented the highest cumulative concentration values of all matrices investigated (Figure 5.3), which contained lower cumulative values in this category.

5.3.2 Geographical variation

Three different cities in Europe were chosen for the occurrence and frequency of 26 EDCs in a 10-week sampling campaign. Potential geographical variations were explored by comparing data compiled as cumulative values of concentrations by categories (those detailed above, e.g. steroids, plasticizers, etc.) for statistical analysis purposes.

For the majority of categories, when performing the test of normality (Shapiro-Wilk), values obtained were $p<0.05$ resulting in the requirement of non-parametric tests (data not normally distributed) needed to be performed. Therefore, Kruskal-Wallis tests were implemented and no significant effects were observed for steroids ($\chi^2(2) = 0.793$, $p = 0.673$), antimicrobial/disinfectant (triclosan) ($\chi^2(2) = 2.884$, $p = 0.236$), preservatives ($\chi^2(2) = 0.792$, $p = 0.673$) and alkylphenols ($\chi^2(2) = 5.827$, $p = 0.054$) categories. This could be due to most of the compounds not being detected (values set at zero) or only quantified at <LOQ concentrations. However, higher concentrations for E1 were found overall in the river Ter respect the other two locations, so it was decided to study this compound on its own, but no significant effect was also obtained ($\chi^2(2) = 0.560$, $p = 0.756$) as observed in Figure 5.8.

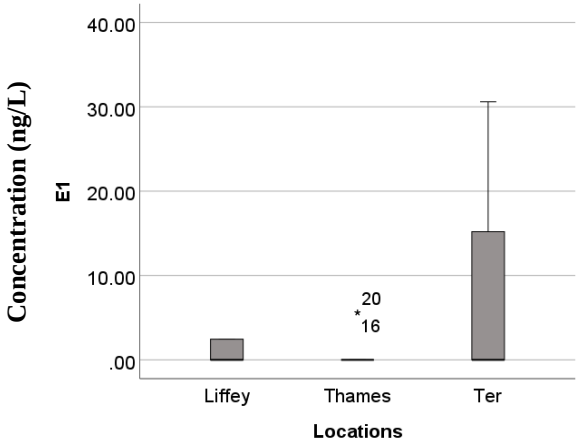


Figure 5.8 E1 box plot results from independent-samples Kruskal-Wallis test for different locations categories (Liffey, Thames and Ter rivers), showing no significant difference.

On the other hand, significant differences were obtained for caffeine (chemical marker) ($\chi^2(2) = 8.183$, $p = 0.017$) with a mean rank location score of 10.50 for Liffey, 21.60 for Thames and 14.40 for Ter; plasticizers ($\chi^2(2) = 7.681$, $p = 0.021$) with a mean rank location score of 65.60 for Liffey, 81.39 for Thames and 79.51 for Ter; and flame retardants ($\chi^2(2) = 17.231$, $p = 0.00$) with a mean rank location score of 55.78 for Liffey, 77.95 for Thames and 47.78 for Ter. Examples of the significant differences obtained can be observed in Figure 5.9 for some categories.

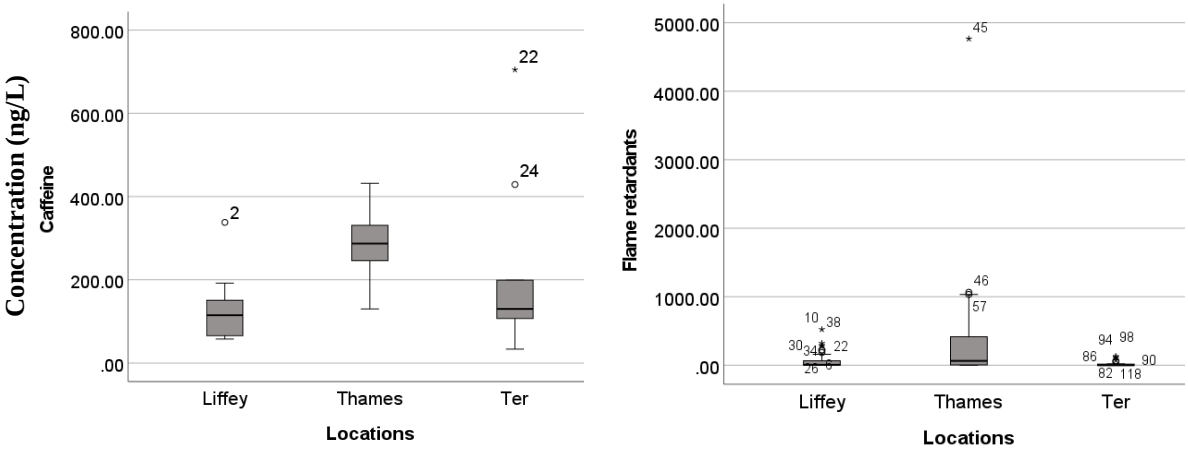


Figure 5.9 Significant difference between locations for the chemical marker (caffeine) on the left and the flame retardants category, on the right, using ANOVA Kruskal-Wallis test for non-parametric distributed data ($p<0.05$).

The anticorrosive benzotriazole was the only compound/category presenting parametric data when the normality test was performed. Consequently, a Tukey HSD test was performed which also indicated significant differences between the conditions [F (2, 27) = 36.89, p = 0.00]. Post hoc comparisons indicated that the mean score for the Thames location condition (M = 278.8, SD = 74) was significantly different than the Liffey (M = 119.86, SD = 43) and the Ter (M = 96.6, SD = 27) locations. Differences between Liffey-Thames and Thames-Ter can be observed in Figure 5.10.

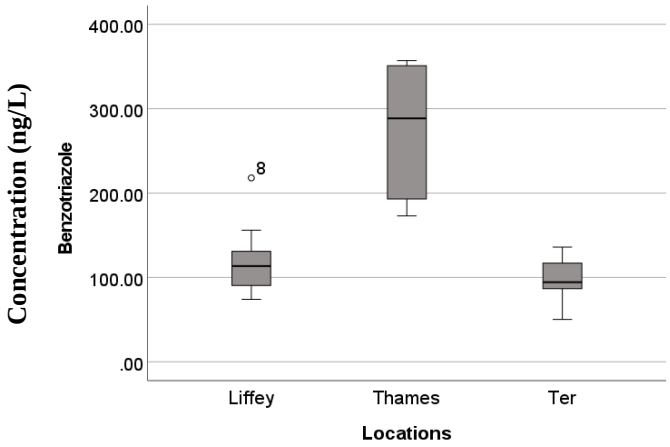


Figure 5.10 Significant difference between the three rivers investigated (Liffey, Thames and Ter) for the chemical benzotriazole (anticorrosive category) after performing the Post hoc Tuckey's test.

Overall, higher concentrations in the river Thames, which can be observed in heatmaps illustrated in Figure 5.11 for all locations, resulted in the significant difference for these compounds/categories. This is foreseeable due to the central-catchment location when collecting the Thames samples and also the high populated area, compared to the other two cities. Moreover, an average of approximately 39 million tonnes of sewage are discharged untreated into the Thames every year, increasing to 62 million tonnes during wet periods,[397] when the sampling campaign for this study occurred (October-January). As WWTPs are usually one of the main point sources for this type of pollutants, higher

concentrations in this matrix are not a surprise. Furthermore, treated effluents from several WWTPs in London (e.g. Beckton, Riverside and Crossness) discharge straight into the river Thames, which is approximately 25-30 km upstream the sampling site, serving a population of approximately 91% of Greater London.[397] Considering therefore, as previously mentioned, that caffeine is found in a wide variety of products (e.g. food and drugs) being the most used substance in the world[421] consequently, some studies have related the high levels detected to large populations due to higher consumption.[394] The high concentrations of plasticizers and flame retardants could also be attributed to the high population, as these are widely used in building materials, plastics, motor vehicles, furniture, textiles, electronics, etc.[420,422]

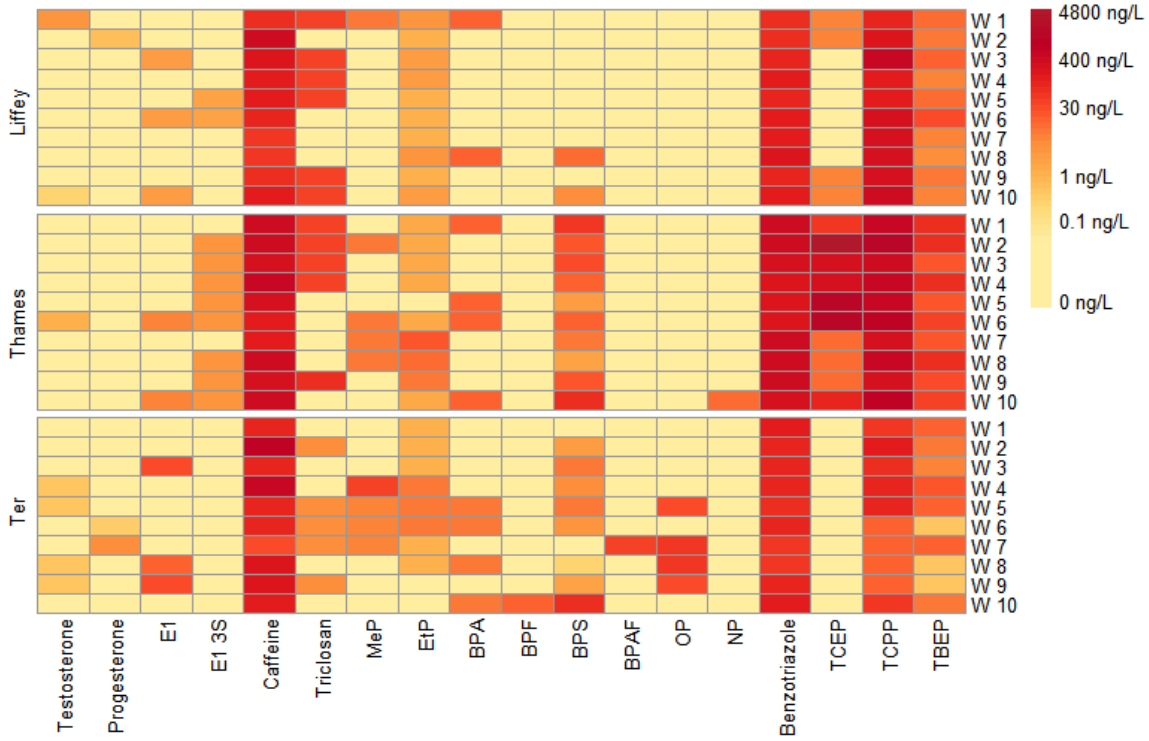


Figure 5.11 Heatmaps showing the EDCs detected in the rivers Liffey, Thames and Ter for all weeks sampled (W = week) and the ranges in concentrations (ng/L), where the darker the colour the higher the concentrations obtained.

5.3.3 Characterisation of the rivers by principal component analysis

Principal component analysis (PCA) was used for multivariate analysis of the three river locations based on the compounds detected and their quantification values. When the PCA was performed, the plot explained approximately 38% of the total variance as seen in Figure 5.12. The three clusters revealed by the PCA plot showed overlaps between them, however, differences can be observed for the river Ter, which contained higher concentrations of steroids. Moreover, the river Thames differences, with respect the other two clusters, are driven towards compounds such as flame retardants (e.g. TBEP, TCEP and TCPP) and the anticorrosive benzotriazole. This is due to the high concentrations obtained in this river for those compounds. On the other hand, main differences in the river Ter are attributed to hormone steroids (e.g. E1, progesterone and testosterone), as mentioned previously. Interestingly, there is no clear differentiation for the river Liffey and Ter, unless high concentrations for compounds such as E1 are obtained. These results aligned with the significant differences achieved by ANOVA during the geographical variation analysis, for the compounds caffeine and benzotriazole, and the flame retardants category, all showing higher differences towards the Thames matrix. Nevertheless, a complete characterisation of the rivers was not achieved, and no complete discrimination patterns were observed, except for the relationship between flame retardants and steroids, shown as well in Figure 5.4, where the characterisation was achieved by percentages of the total contribution based on the concentrations quantified by categories of EDCs.

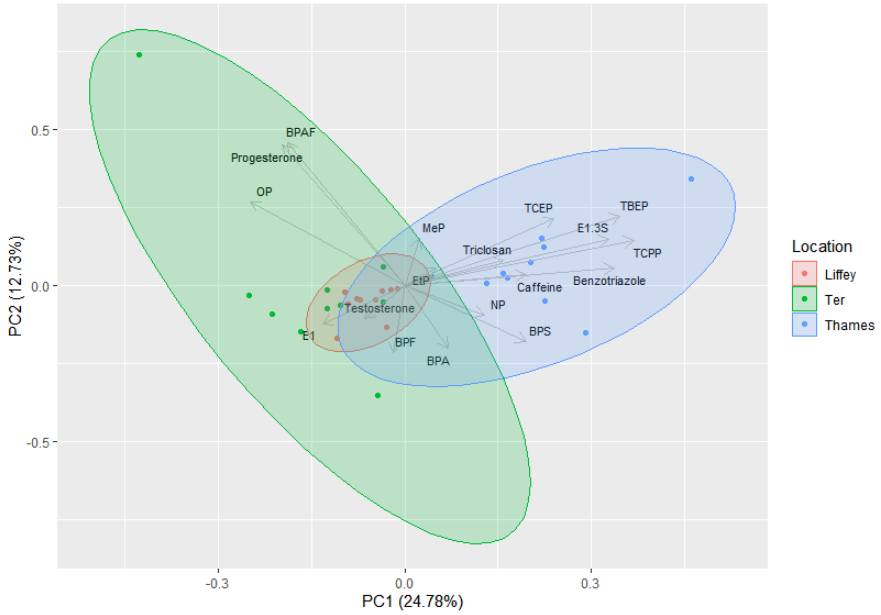


Figure 5.12 Principal component analysis (PCA) of the relationship between EDCs detected in the river Liffey (pink), Ter (green) and Thames (blue); where the percentage explained by the axes is presented in brackets and concentrations were normalised by compound.

5.3.4 Environmental risk assessment

Contaminants of emerging concern (CECs) are widely detected in different environmental matrices including surface waters, where they can pose a threat to the aquatic environment. In order to estimate the risks that these contaminants can induce, assessments can be carried out after the implementation of substances monitoring, to characterize the exposure and effects.[423] In this study, 26 compounds were selected due to their endocrine-disrupting properties, making them potentially of high risk to aquatic organisms. Therefore, after their monitoring in different rivers, an ERA was performed to investigate their potential impact. In this case, the majority of compounds were detected <LOD or 10 ng/L, which is the threshold for inclusion in the ERA as per guidelines,[356] however, they all pose endocrine disrupting properties. For this reason, all compounds detected were considered to move to phase II of the assessment (Figure 4.1, Chapter 4.0) in order to calculate RQ values, without the need to complete a PBT assessment beforehand.

5.3.4.1 ERA

In order to perform the second phase of the assessment, PNEC values were calculated and they can be found in Table A.23 (Appendix H), which includes the aquatic ecotoxicity data for different trophic levels obtained from literature review, NORMAN database and ECOSAR predictive software, as per Section 4.2.2 (Chapter 4.0). Subsequently, RQs were calculated for a total of 18 compounds detected across the three rivers as seen in Figure 5.13. Individual risks can also be observed in Table A.24 from Appendix H, where the highest RQ value was obtained for caffeine in the three matrices, with 705 as the maximum value obtained overall belonging to the river Thames. Therefore, this compound poses a very high risk in the environment, due to the highest concentrations obtained but also the low PNEC value selected; 1 ng/L was the NOEC concentration in the fish trophic level, according to previous ecotoxicity studies. Reassuringly, the majority of the compounds investigated were calculated to pose insignificant risks, resulting in 64, 57 and 53% of the risk being classed as "insignificant" for the rivers Liffey, Thames and Ter. This classification represented nine, eight and eight compounds respectively (Figure 5.14), including compounds such as progesterone, E1-3S, MeP and benzotriazole. Low risks were determined for 7% of the compounds (i.e. one compound) studied in the rivers Liffey and Thames, and 27% (i.e. four compounds) for the river Ter, however, these compounds varied between locations. For example, testosterone, which was classified as low risk for the rivers Thames and Ter, was classified as a medium risk in the Liffey due to a higher concentration quantified for this river. Moreover, medium risks were determined as 15, 22 and 13% respectively, which also varied across the sites. Finally, higher risks were associated with a minority of the compounds, resulting in only 14% of risk classification for both the Liffey and Thames rivers (i.e. two compounds), and 7% for the river Ter (i.e. one compound). This is due to

the different concentrations quantified across the sites, for example BPA, which was classified as a high risk for the Liffey and Thames but only a medium risk for the river Ter. However, caffeine presented high risks in all sites due to its high constant concentrations. Nevertheless, as mentioned before, this is unsurprising due to its high consumption globally.

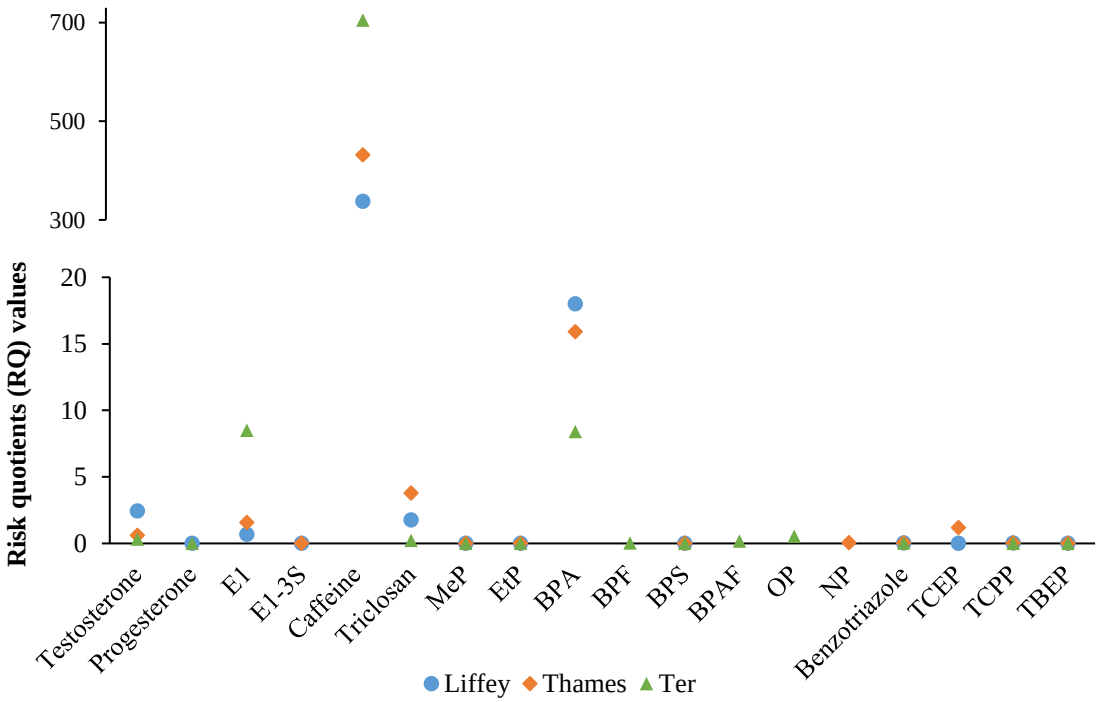


Figure 5.13 Risk quotients of EDCs detected in the rivers Liffey, Thames and Ter.

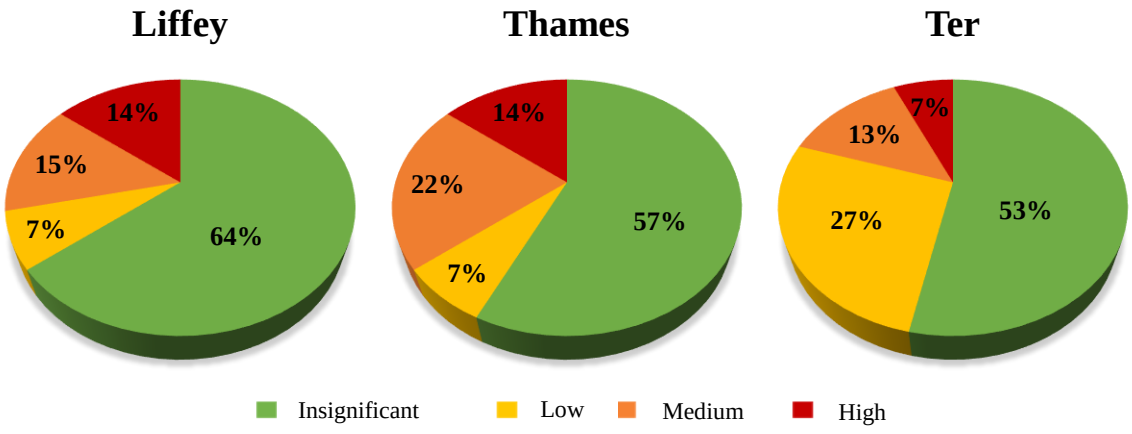


Figure 5.14 Risk classification for compounds detected in the rivers Liffey, Thames and Ter.

5.3.4.2 River location risk

Whilst the majority of compounds were classified as presenting insignificant risk levels for all three rivers, this risk is only determined for each chemical at an individual level. The risk assessment does not evaluate the combined risk as a result of multiple simultaneous exposures, even though organisms in contaminated environments are exposed to a mixture of CECs, and toxic effects can be observed even though the concentrations are lower than the NOEC levels.[424] This is the "cocktail effect", which arises due to a combination of contaminants that exist in the aquatic environment, rather than just one specific compound. Moreover, all the compounds studied in this study pose endocrine disrupting properties, giving rise to the potential for an enhancement of these effects even when individual compounds are present at extremely low concentrations. Therefore, the ERA assessment neglects the potential "cocktail effect" underestimating the results. Consequently, the site risk was calculated as per Section 4.2.3 (Chapter 4.0) for all compounds detected per river location. The following values of ΣRQ_{river} were obtained for the locations tested: 361, 455, 723 for the rivers Liffey, Thames and Ter respectively. All sites posed a very high risk overall, mainly associated with the high concentrations of caffeine in all rivers, contributing to 94, 95 and 97% of the total risk for the rivers Liffey, Thames and Ter respectively. This can be observed in Figure 5.15, where similar risks patterns were observed across all rivers. The second EDC contributing to the highest potential risk, again for all rivers, was BPA with 5, 3 and 1% for the rivers Liffey, Thames and Ter respectively. Lastly, 1% of contributions were obtained for testosterone, triclosan and E1 respectively. The remaining compounds had extremely low contributions to the total site risk.

These results highlighted that the following substances: caffeine, BPA and E1 for the river Ter; caffeine, BPA and triclosan for the river Thames; and caffeine, BPA and

testosterone for the river Liffey, contributed most to potential environmental risk. These substances should therefore be a main concern in terms of decision-making regarding prioritisation of chemicals for monitoring, emphasising caffeine and BPA for all locations, independently of the location investigated. Furthermore, it is worth hihlighting the ΣRQ_{river} obtained for the river Ter, the highest one acquired over the three locations. Even though the Ter presented the least highly populated area of the three rivers, the overall risk is highest. As mentioned previously, this could be due to the close proximity of the WWTP upstream of the sampling point, and again highlighting the importance of different treatments research for removals of these compounds.

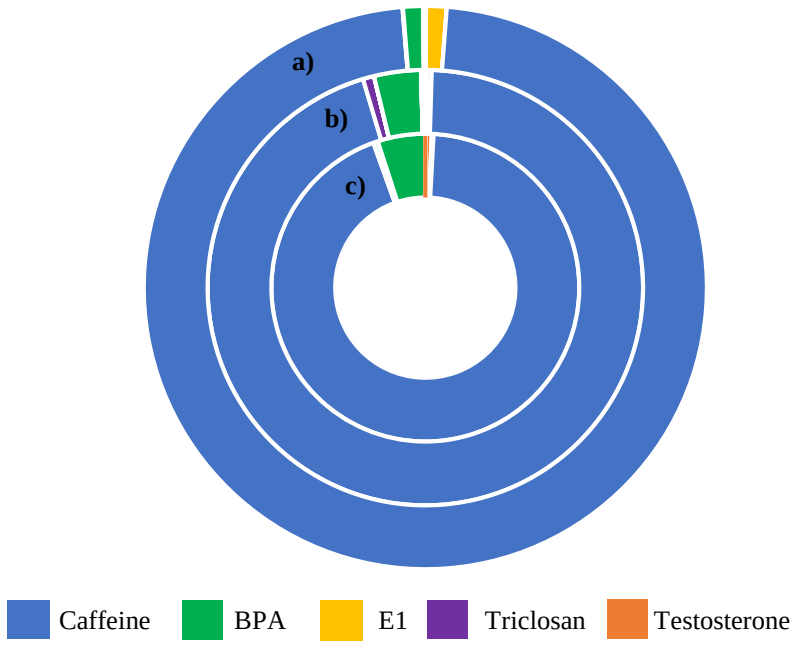


Figure 5.15 Doughnut plots for the rivers a) Ter, b) Thames and c) Liffey, showing the contribution (%) of individual EDCs detected of the total risk per site.

5.4 Conclusions

An international study of the occurrence and frequency of 26 endocrine disrupting compounds carried out across Ireland, the United Kingdom and Spain was presented in this chapter. EDCs were monitored over a 10-week period (October 2020-January 2021) in the rivers Liffey (Dublin, Ireland), Thames (London UK) and Ter (Girona, Spain). A total of 14 compounds were detected for the rivers Liffey and Thames and 15 for the Ter, where most of them were detected <LOD concentrations. Concentrations varied across the different matrices between <LOD–524 ng/L (TCCP), <LOD–4,767 ng/L (TCEP) and <LOD–705 ng/L (caffeine) for the rivers Liffey, Thames and Ter respectively. Overall, higher concentrations were achieved for the river Thames, where cumulative concentration values of up to 20,000 ng/L were detected. This has been attributed to the high dense population area and the central-catchment sampling point. Moreover, several WWTPs across London discharge effluents straight into the river increasing overall concentrations after poor removals during treatment.

Geographical location variations were studied using ANOVA tests for all compounds detected across the three sites, however, only four categories presented significant differences between locations: plasticizers, caffeine (chemical marker), flame retardants and benzotriazole (anticorrosive), as illustrated by PCA analysis. PCA was conducted in order to evaluate if it was possible to characterise the rivers by the type of compound detected and quantification levels achieved. Nevertheless, only a total of 38% of the variance was achieved but differences between the rivers Thames and Ter were observed, with steroids driving towards the Ter and flame retardants towards the river Thames cluster, having different “chemical fingerprints”. The Liffey cluster overlapped

with both rivers showing a less discriminate pattern and making it difficult to differentiate with the other two rivers.

A majority of concentrations were determined to be below <LOD or in the low ng/L but due to their known endocrine disrupting properties an environmental risk assessment was performed. Insignificant results were obtained for the majority of compounds (64, 57 and 53% for the rivers Liffey, Thames and Ter respectively), however, high risks were associated with 14% of the compounds for the rivers Liffey and Thames and 15% for the river Ter. Due to their ED properties, concern was prioritised for the highest RQ achieved, 705, for caffeine in the Ter, due to the high concentrations detected. Risks were also calculated by river where high risks were attributed to all locations due to the higher contributions of caffeine and BPA across all matrices tested. Therefore, a prioritisation substance list was determined by location with the following EDCs identified: caffeine, BPA and E1 for the river Ter; caffeine, BPA and triclosan for the river Thames; and caffeine, BPA and testosterone for the river Liffey; highlighting caffeine and BPA independently of the location. Consequently, these compounds should be prioritised in order to define future policy development to protect and enhance water quality across different geographical locations.

6.0 Conclusions and future work

6.1 Conclusions

Contaminants of emerging concern (CECs) are compounds that are not regulated, but that are being detected and quantified across the world in different environmental samples including aquatic ecosystems, and about which there are concerns of potential negative impacts. Their suspected negative effects in aquatic organisms raise concerns not only for animal but human health too. This is due to their potential to bioaccumulate and biomagnify in these organisms passing through the food chain; however, knowledge in this regard is limited. The extent of their potential impacts depends on their occurrence, frequency and fate. Once this is determined, risk assessments can be performed for these contaminants in the aquatic environment to evaluate their potential harm. Consequently, the main goal of this thesis was to investigate the occurrence and frequency of a range of selected CECs in different Irish aquatic matrices and environments and assess their potential risk, to create a list of priority CECs of concern. To enable the occurrence and frequency of compounds to be determined, analytical methods were developed, optimised and validated in three different matrices (surface waters, effluent and influent wastewaters). A sampling campaign was carried out over a full calendar year collecting samples from both urban and rural environments, to ensure a representative sample set for the evaluation of a range of CECs in an Irish context, in terms of population size, contaminants use patterns, etc. Furthermore, an international comparison based on selected CECs, which have endocrine disrupting properties, was completed in order to see the differences between three European countries in terms of occurrence, frequency and risks associated to them. The main outputs are described in Figure 6.1.

The research carried out in this thesis investigates the occurrence of CECs in the Irish aquatic environment and risks assesses these compounds providing a list of priority

pollutants. The current literature was assessed for the presence of these contaminants across different matrices, surface waters, effluent and influent wastewater, and their fate once entering the natural environment. Chapter 1.0 reviews the published literature evaluating the occurrence of CECs in Ireland and across the world, their pathway through WWTPs and their environmental fate based on their physicochemical properties. Moreover, regulation of CECs in the environment, specifically in Ireland, was also reviewed in order to see compliance with legislation and find potential compound candidates for their monitoring in this thesis. Additionally, recent analytical techniques were investigated for collection, preparation, extraction and analysis of water samples in order to select preferable techniques which allow enough sensitivity to detect these type of contaminants.

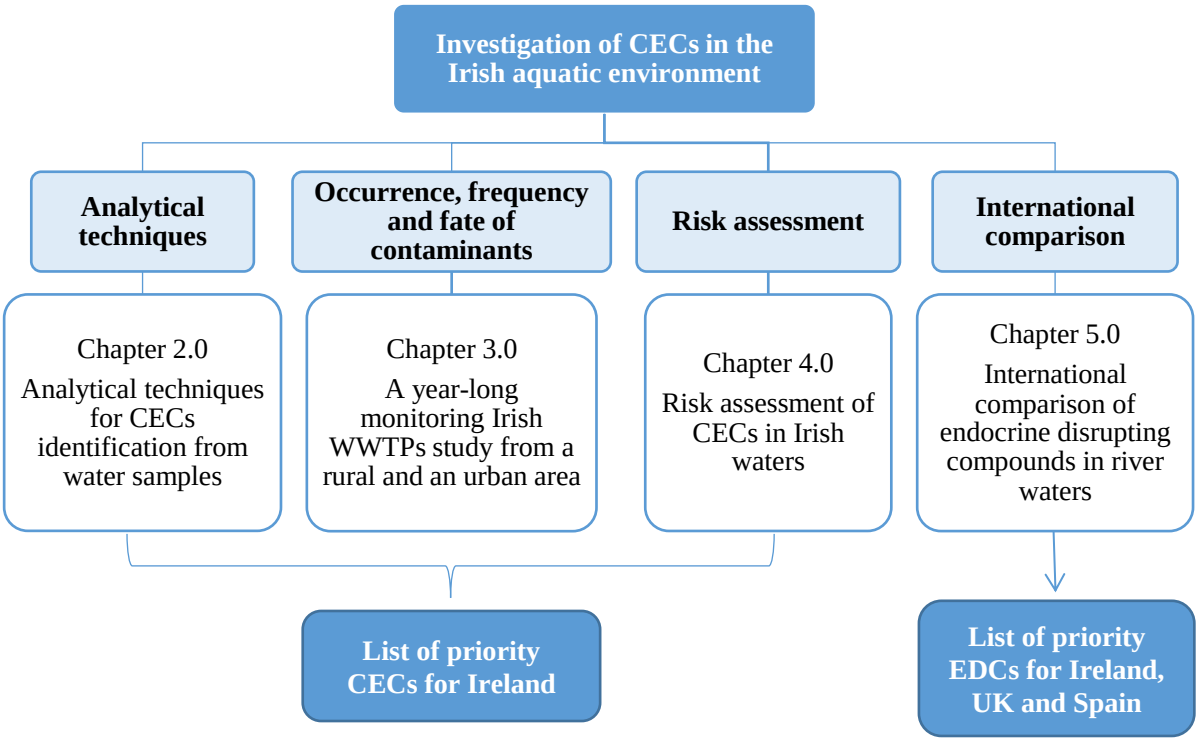


Figure 6.1 Schematic of main outputs of this thesis.

Once the analytical techniques were determined, a selection of different classes of contaminants was performed in Chapter 2.0, to ensure a relevant breadth of potential

CECs would be evaluated. This was followed by the development and optimisation of new analytical techniques, which were successfully performed for a total of 135 compounds. SPE LC-MS/MS and DI LC-MS/MS methods were validated in three different types of matrices: surface waters, effluent and influent wastewater.

A year-long monitoring study was performed in Chapter 3.0 for a total of 135 CECs using the previously validated analytical techniques (Chapter 2.0). Two different sites were sampled for influent and effluent wastewaters and surface waters downstream of the WWTPs. The presence of the selected CECs was investigated by evaluating their occurrence, at concentrations of ng/L, and frequencies in all matrices. Influent wastewaters were shown to contain 52 and 51 compounds in the rural and urban areas respectively. The number of contaminants detected reduced in effluent samples, with 47 compounds detected in both areas, and this number reduced further in surface waters with 16 and 23 compounds detected in the rural and urban areas respectively. The majority of compounds in influent and effluent were detected at 100% frequency, while in surface waters a decrease in frequency was observed. CECs were detected in surface waters in urban areas with a 17% frequency, whilst most compounds were not detected in the rural area, due to most compounds being detected at <LOD concentrations. Interestingly, there were no significant differences observed for the majority of the classes of CECs in terms of seasonal and geographical variation. The fate of contaminants was observed to result in a pattern of firstly a decrease of concentrations of contaminants after treatment in the WWTP, followed by a further dilution on entering the natural environments (influent>effluent>surface waters). Also, all compounds found in effluents were also determined in surface water samples suggesting WWTPs as a point source for CECs. However, removal rates were shown to be variable depending on the compound, month sampled and geographical location. Nevertheless, 62 and 84% of the total average

concentrations were removed from the influent for the urban and rural areas respectively. The remaining concentrations in effluents and therefore in surface waters were assessed for their potential risk to the environment (Chapter 4.0).

Hazard and environmental risk assessments were performed for a total of 49 contaminants detected in effluent and surface waters for the rural and urban areas in Chapter 4.0. After the assessment, 4 and 7% of the compounds were determined to present a high risk in surface waters for the urban and rural areas respectively, where the main contributions, and higher risks quotients obtained, were from E2 and EE2. These percentages increased to 9 and 11% for the urban and rural areas respectively in effluent wastewaters and main contributions were attributed to EE2, carbamazepine and venlafaxine. This is due to the higher concentrations obtained in effluent samples (Chapter 3.0), before their dilution once entering the environment (river). Consequently, these compounds were short-listed for a prioritisation list for CECs in both surface waters and effluent wastewaters respectively.

An international comparison was then performed for Ireland, UK and Spain in Chapter 5.0. For this purpose, a selection of CECs was completed where compounds posing endocrine disrupting compounds were nominated due to their high concern and potential harmful effect at extremely low concentrations. Therefore, 26 analytes were monitored in the surface waters of three main rivers: Liffey (Ireland), Thames (UK) and Ter (Spain) over a period of 10 weeks (October 2020 – January 2021). A total of 14 compounds were detected in the Liffey and Thames and 15 in the river Ter. Maximum concentrations quantified were 524 (TCCP), 4,764 (TCEP) and 705 (caffeine) ng/L for the rivers Liffey, Thames and Ter respectively. The majority of the compounds were not detected in any of the samples due to detections below <LODs. Geographical variations were shown to result in significant differences in occurrence and concentration of

plasticizers, chemical markers (i.e. caffeine), flame retardants and anticorrosive (i.e. benzotriazole) categories, with higher overall concentrations detected for the river Thames, which was attributed to the location being the most highly-populated area in the study (London). The majority of the compounds presented insignificant risks, but due to the "cocktail effect" of these compounds, site risks (ΣRQ_{river}) were calculated and determined to be 361, 455, 723 for the rivers Liffey, Thames and Ter respectively. Consequently, as these values were $\Sigma RQ_{river}>10$, high risks were determined for all matrices, and the main contributions were attributed to caffeine, BPA and E1 for the river Ter; caffeine, BPA and triclosan for the river Thames; and caffeine, BPA and testosterone for the river Liffey. These results also highlighted the prioritisation of caffeine and BPA, independently of the location tested.

6.2 Main contributions of study

This study has attempted to make a contribution to the field of environmental science for the occurrence, frequency and fate of CECs in Irish waters. The main research contributions arising from this study are as follows:

- The development of sensitive analytical techniques for the identification of different classes of CECs at low ng/L concentrations was accomplished for surface waters and effluent and influent wastewater matrices using SPE LC-MS/MS. Moreover, a new analytical technique approach, DI LC-MS/MS, was successfully applied to these complex matrices, which achieved low levels of detection without any pre-concentration step, reducing not only time of analysis but also cost.
- The occurrence and concentration of CECs were confirmed for two different geographical Irish aquatic environments using the above analytical methods. Concentrations ranged from low ng/L up to µg/L, mostly decreasing in concentration in the following order: influent > effluent > surface waters. However, concentrations found in surface waters may still negatively impact the aquatic environment. The maximum concentration achieved throughout the study was for the antidepressant venlafaxine, quantified at the influent rural area at 8.3 µg/L, which was at the top 100 most prescribed compounds in Ireland at the time of the study; results aligned with prescription data. Previous monitoring studies in Ireland showed an increased or decreased in CEC concentration over the years depending on the type of compound, however, different locations were sampled and no direct comparison can be made due to the different treatments in the WWTPs, population sizes, compound use patterns, etc.

- Fate of the CECs detected was also investigated, with some of the contaminants studied being shown to persist after treatments performed in the WWTPs, and as a result entering the natural environment, suggesting WWTPs as a point source for environmental pollution for these compounds. Furthermore, several compounds were detected in the effluent at higher concentrations than the influents, and one compound, simazine, was only detected in effluent suggesting its formation during the treatment process. This knowledge will enable WWTP operators to consider the development of new strategies to reduce these compounds entering the WWTP, or consider different types of treatments to improve removal rates of compounds identified, and as a result of either or both of these, to reduce the risk of the fate of CECs in the environment.
- Environmental risk assessments were performed at both sites for effluents and surface waters. High risks were associated with carbamazepine, diclofenac, E2, EE2, propranolol, sulfamethoxazole and venlafaxine for the effluent matrix, and E2 and propranolol for surface waters, enabling their prioritisation. When all compounds were investigated together, sites risks were calculated indicating very high risks for both matrices and locations. Consequently, special attention should be considered for these specific compounds, resulting in a priority list of substances: especially for E2, EE2 and propranolol as they were found in surface waters and were shown to contribute most to the total risks, even with concentrations of only 1, 0.12 and 6 ng/L respectively.
- Presence of endocrine disrupting compounds were confirmed for three European rivers over a period of 10-weeks. Significant differences were shown between rivers for the following categories: plasticizers, caffeine (chemical marker), flame retardants and benzotriazole (anticorrosive). Characterisation of the rivers was

presented using PCA, where the main discrimination patterns overall were attributed to flame retardants and steroids. Moreover, high risks were determined for all three locations and a priority substance list was generated, which highlighted the risk of BPA and caffeine across all locations.

6.3 Future work

Arising from what was achieved during this work, a number of areas have been identified for further research, as follows:

- During the selection of analytical techniques, low sensitivity techniques are necessary in order to achieve the low limits of detections required for these types of contaminants.[425] Consequently, the use of extraction methods is needed in order to pre-concentrate the samples but also remove interferences from these complex samples.[388] Nevertheless, high percentages of matrix effects were obtained even after performing extraction (SPE). These can contribute negatively in the accuracy of the analysis, making quantification processes unreliable.[347] Similar matrix effects were also obtained for the direct injection method, however, this step has no pre-treatment involved. Therefore, the use of SPE seems no longer mandatory in order to achieve these low levels of concentrations. However, the need of analytical techniques to remove interferences in these complex matrices remains. Further research to explore strategies to remove interferences when using the direct injection method will allow further accuracy in the quantification process making it more reliable.
- As the number of chemicals in the global market is constantly increasing, the need for monitoring and risk assessment of large numbers of contaminants also grows. Moreover, there is a need to include compounds such as metabolites and transformation products of these chemicals when considering the potential impact of these compounds on the environment.[425] However, limited number of CECs are usually monitored due to cost, availability and limitations on the analytical techniques involved.[426] Therefore, to overcome some of these issues, high

resolution mass spectrometry followed by suspect screening has been used in the latest years as there is no need of reference standard materials.[284] This has resulted in screening detection of over 400 compounds in wastewater in a single run.[126,427] This will also enable a wider risk assessment of the area based on qualitative data.

- The WWTPs investigated for this study did not remove all CECs completely as they are not designed for this purpose. Therefore, the presence of contaminants was confirmed in effluent samples across both locations studied. Moreover, rates of removals were variable not only depending on the compound but also the month when the sample was collected, suggesting possible untreated effluents, overflows, etc.[395] Notwithstanding that WWTPs are not designed for this purpose, the fact that they have been demonstrated to act as point sources for CEC introduction to the environment should drive evaluation of strategies to reduce the extent to which this is the case, either as a result of processes such as end-of-pipe initiatives to reduce their presence in WWTP influent, and/or the development and implementation of new and advanced technologies to reduce effluent concentrations further. Both strategies have the potential to reduce the possible effects caused by the release of CECs into the environment.
- Limited data has been reported for CECs in the Irish environment.[39] Consequently, temporal comparisons are difficult to perform. Moreover, the geographical location from where samples are collected will have a large impact on the final concentrations achieved due to the different treatments, population size, etc. Research on the same area (same WWTP) would allow the investigation of the occurrence of these contaminants over a temporal period.
- A comparison of trends in community consumption for pharmaceuticals could be investigated using the obtained influent wastewater data for both locations tested

in order to obtain information on the compounds use (e.g. illegal trade) and epidemiological data.[428] This falls under wastewater-based epidemiology (WBE) which allows the measurement of compounds consumption (e.g. pharmaceuticals) with high and temporal resolution.[429] A published approach suggests the use of estimation loads (g/day) to calculate consumption data.[121] Results could be compared to prescription data by the Health Service Executive (HSE) in Ireland in a more reliable way than just the occurrence data obtained within this thesis. This is due to the consideration of the flow rate (L/day) of influent, stability and adsorption to solids, population, etc.

- Aquatic environments can act as a natural reservoir of antimicrobial resistant (AMR) bacteria threatening both human and livestock. These compounds have been shown to bioacummulate in different aquatic organisms raising the concern.[90] In this study, a high risk was associated to sulfamethoxazole which contributed to ≤57% of the total risks to both effluent sites. A more detailed study should be performed for these type of compounds to determine their fate, and to evaluate whether their presence in aquatic systems likely contributes to increasing levels of AMR bacteria.
- The risks of effects from CECs in the environment were calculated using measured environmental concentrations (MEC).[213] However, final risk quotients (RQs) depend on the concentration measured and as concentrations are location dependent,[356] monitoring of a greater number of different sites across the country could help with the accuracy of RQs calculated. Moreover, the calculation of PNEC values were achieved by literature review, which is sometimes limited for certain compounds.[114] Prediction softwares can be used to obtained these values, however, this can result in over or under estimation of final RQs. Therefore, more

research on ecotoxicity is needed which would provide an expanded database resulting in more accurate results.

- Risk assessment has been performed using only the aqueous phase, however, this does not represent the whole environment. Compounds with log $P > 5$, such as octinoxate and triclosan, which were evaluated in this thesis, usually tend to retain in organic matter and the preferable environmental sample to be analysed would be sediments and/or suspended particulate matter (SPM) as suggested by the WFD.[7,95] That sediments and/or SPM was not evaluated in the work presented here could result in underestimation of the final concentrations found on the environment and therefore underestimation of their risk in the environment. Future research should consider sediments and/or SPM for compounds with these physicochemical properties.
- Regarding the field study, there are certain issues that limit the capabilities of the research area such as normalisation of the data, accurate population equivalence, stability of target compounds, etc. The main objective is to be able to analyse known targets accurately, to identify currently unknown pollutants and to facilitate inter-comparison of independent studies.

To conclude, this work has extended the knowledge of CECs in Irish environmental waters regarding their occurrence and frequency, fate and potential risks once they enter the natural aquatic ecosystem. These results offer significant gains of a better understanding of their fate and behaviour after their treatment (WWPT) in different locations. Additionally, it has proposed a list of priority substances which will contribute to policy decision making in order to obtain higher water quality.

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Appendix

Appendix A: CECs studied within this thesis

Table A.1 Compound classification and SMILES studied within this thesis.

Analyte	Class	SMILES[a]
2-(Thiocyano-methylthio) benzothiazole	Pesticide	C1=CC=C2C(=C1)N=C(S2)SCSC#N
Acetamiprid	Pesticide	CC(=NC#N)N(C)CC1=CN=C(C=C1)Cl
Alprazolam	Pharmaceutical	CC1=NN=C2N1C3=C(C=C(C=C3)Cl)C(=NC2)C4=CC=CC=C4
Ametryn	Pesticide	CCNC1=NC(=NC(=N1)SC)NC(C)C
Amiodarone	Pharmaceutical	CCCCC1=C(C2=CC=CC=C2O1)C(=O)C3=CC(=C(C(=C3)I)OCCN(CC)CC)I
Amitriptyline	Pharmaceutical	CN(C)CCC=C1C2=CC=CC=C2CCC3=CC=CC=C31
Amlodipine	Pharmaceutical	CCOC(=O)C1=C(NC(=C(C1C2=CC=CC=C2Cl)C(=O)OC)C)COCCN
Amoxicillin	Pharmaceutical	CC1(C(N2C(S1)C(C2=O)NC(=O)C(C3=CC=C(C=C3)O)N)C(=O)O)C
Antipyrine	Pharmaceutical	CC1=CC(=O)N(N1C)C2=CC=CC=C2
Atorvastatin	Pharmaceutical	CC(C)C1=C(C(=C(N1CCC(CC(CC(=O)O)O)O)O)C2=CC=C(C=C2)F)C3=CC=CC=C3)C(=O)NC4=CC=CC=C4
Atrazine	Pesticide	CCNC1=NC(=NC(=N1)Cl)NC(C)C
Azelnidipine	Pharmaceutical	CC1=C(C(C(=C(N1)N)C(=O)OC2CN(C2)C(C3=CC=CC=C3)C4=CC=CC=C4)C5=CC(=CC=C5)[N+](=O)[O-])C(=O)OC(C)C
Azithromycin	Pharmaceutical	CCC1C(C(C(N(CC(CC(C(C(C(C(C(=O)O1)C)OC2CC(C(C(O2)C)O)(C)OC)C)OC3C(C(CC(O3)C)N(C)C)O)(C)O)C)C)O)(C)O
Azoxystrobin	Pesticide	COC=C(C1=CC=CC=C1OC2=NC=NC(=C2)OC3=CC=CC=C3C#N)C(=O)OC
Benoxacor	Pesticide	CC1COC2=CC=CC=C2N1C(=O)C(Cl)Cl
Bensulide	Pesticide	CC(C)OP(=S)(OC(C)C)SCCNS(=O)(=O)C1=CC=CC=C1
Benzatropine	Pharmaceutical	CN1C2CCC1CC(C2)OC(C3=CC=CC=C3)C4=CC=CC=C4

Benzophenone-4	Personal Care Product	COC1=C(C=C(C(=C1)O)C(=O)C2=CC=CC=C2)S(=O)(=O)O
Betaxolol	Pharmaceutical	CC(C)NCC(COC1=CC=C(C=C1)CCOCC2CC2)O
Bezafibrate	Pharmaceutical	CC(C)(C(=O)O)OC1=CC=C(C=C1)CCNC(=O)C2=CC=C(C=C2)Cl
Bisoprolol	Pharmaceutical	CC(C)NCC(COC1=CC=C(C=C1)COCCOC(C)C)O
Bupropion	Pharmaceutical	CC(C(=O)C1=CC(=CC=C1)Cl)NC(C)(C)C
Buspirone	Pharmaceutical	C1CCC2(C1)CC(=O)N(C(=O)C2)CCCCN3CCN(CC3)C4=NC=CC=N4
Butylated hydroxytoluene	Personal Care Product	CC1=CC(=C(C(=C1)C(C)(C)C)O)C(C)(C)C
Carazolol	Pharmaceutical	CC(C)NCC(COC1=CC=CC2=C1C3=CC=CC=C3N2)O
Carbamazepine	Pharmaceutical	C1=CC=C2C(=C1)C=CC3=CC=CC=C3N2C(=O)N
Carboxine	Pharmaceutical	CC1=C(SCCO1)C(=O)NC2=CC=CC=C2
Carfentrazone-ethyl	Pesticide	CCOC(=O)C(CC1=CC(=C(C=C1Cl)F)N2C(=O)N(C(=N2)C)C(F)F)Cl
CBZ epoxide	Pharmaceutical	C1=CC=C2C(=C1)C3C(O3)C4=CC=CC=C4N2C(=O)N
Celecoxib	Pharmaceutical	CC1=CC=C(C=C1)C2=CC(=NN2C3=CC=C(C=C3)S(=O)(=O)N)C(F)(F)F
Chloramphenicol	Pharmaceutical	C1=CC(=CC=C1C(C(CO)NC(=O)C(Cl)Cl)O)[N+](=O)[O-]
Cilazapril	Pharmaceutical	CCOC(=O)C(CCC1=CC=CC=C1)NC2CCCN3CCCC(N3C2=O)C(=O)O
Ciprofloxacin	Pharmaceutical	C1CC1N2C=C(C(=O)C3=CC(=C(C=C32)N4CCNCC4)F)C(=O)O
Citalopram	Pharmaceutical	CN(C)CCCC1(C2=C(CO1)C=C(C=C2)C#N)C3=CC=C(C=C3)F
Clarithromycin	Pharmaceutical	CCC1C(C(C(C(=O)C(CC(C(C(C(C(C(=O)O1)C)OC2CC(C(C(O2)C)O)(C)OC)C)OC3C(C(CC(O3)C)N(C)C)O)(C)OC)C)C)O)(C)O
Clodinafop-propargyl	Pesticide	CC(C(=O)OCC#C)OC1=CC=C(C=C1)OC2=NC=C(C=C2F)Cl
Clofibric acid	Pharmaceutical	CC(C)(C(=O)O)OC1=CC=C(C=C1)Cl
Clopidogrel	Pharmaceutical	COC(=O)C(C1=CC=CC=C1Cl)N2CCC3=C(C2)C=CS3
Clothianidin	Pesticide	CN=C(NCC1=CN=C(S1)Cl)N[N+](=O)[O-]

Clozapine	Pharmaceutical	CN1CCN(CC1)C2=C3C=CC=CC3=NC4=C(N2)C=C(C=C4)Cl
Cyclouron	Pesticide	CN(C)C(=O)NC1CCCCCCC1
Cycloxyidim	Pesticide	CCCC(=NOCC)C1=C(CC(CC1=O)C2CCCSC2)O
Cymoxanil	Pesticide	CNC(=O)NC(=O)C(=NOC)C#N
Cyromazine	Pesticide	C1CC1NC2=NC(=NC(=N2)N)N
Diclofenac	Pharmaceutical	C1=CC=C(C(=C1)CC(=O)O)NC2=C(C=CC=C2Cl)Cl
Diflubenzuron	Pesticide	C1=CC(=C(C(=C1)F)C(=O)NC(=O)NC2=CC=C(C=C2)Cl)F
Dimethametryn	Pesticide	CCNC1=NC(=NC(=N1)SC)NC(C)C(C)C
Diphenhydramine	Pharmaceutical	CN(C)CCOC(C1=CC=CC=C1)C2=CC=CC=C2
Enalapril	Pharmaceutical	CCOC(=O)C(CCC1=CC=CC=C1)NC(C)C(=O)N2CCCC2C(=O)O
Erythromycin	Pharmaceutical	CCC1C(C(C(C(=O)C(CC(C(C(C(C(C(=O)O1)C)OC2CC(C(C(O2)C)O)(C)OC)C)OC3C(C(CC(O3)C)N(C)C)O)(C)O)C)C)O)(C)O
E1 (Estrone)	Pharmaceutical	CC12CCC3C(C1CCC2=O)CCC4=C3C=CC(=C4)O
E2 (17-ß-estradiol)	Pharmaceutical	CC12CCC3C(C1CCC2O)CCC4=C3C=CC(=C4)O
E22 (17-α-ethynylestradiol)	Pharmaceutical	CC12CCC3C(C1CCC2(C#C)O)CCC4=C3C=CC(=C4)O
Famoxadone	Pesticide	CC1(C(=O)N(C(=O)O1)NC2=CC=CC=C2)C3=CC=C(C=C3)OC4=CC=CC=C4
Fenoxaprop-ethyl	Pesticide	CCOC(=O)C(C)OC1=CC=C(C=C1)OC2=NC3=C(O2)C=C(C=C3)Cl
Fenuron	Pesticide	CN(C)C(=O)NC1=CC=CC=C1
Flufenoxuron	Pesticide	C1=CC(=C(C(=C1)F)C(=O)NC(=O)NC2=C(C=C(C=C2)OC3=C(C=C(C=C3)C(F)(F)F)Cl)F)F
Fluocinonide	Pharmaceutical	CC(=O)OCC(=O)C12C(CC3C1(CC(C4(C3CC(C5=CC(=O)C=CC54C)F)F)O)C)OC(O2)(C)C
Fluoxetine	Pharmaceutical	CNCCC(C1=CC=CC=C1)OC2=CC=C(C=C2)C(F)(F)F
Flurbiprofen	Pharmaceutical	CC(C1=CC(=C(C=C1)C2=CC=CC=C2)F)C(=O)O
Flurochloridone	Pesticide	C1C(C(C(=O)N1C2=CC=CC(=C2)C(F)(F)F)Cl)CCl
Flutamide	Pharmaceutical	CC(C)C(=O)NC1=CC(=C(C=C1)[N+](=O)[O-])C(F)(F)F
Flutolanil	Pharmaceutical	CC(C)OC1=CC=CC(=C1)NC(=O)C2=CC=CC=C2C(F)(F)F
Fuberidazole	Pesticide	C1=CC=C2C(=C1)NC(=N2)C3=CC=CO3
Hydrochlorothiazide	Pharmaceutical	C1NC2=CC(=C(C=C2S(=O)(=O)N1)S(=O)(=O)N)Cl

Imidacloprid	Pesticide	C1CN(C(=N1)N[N+](=O)[O-])CC2=CN=C(C=C2)Cl
Isocarbamid	Pesticide	CC(C)CNC(=O)N1CCNC1=O
Isradipine	Pharmaceutical	CC1=C(C(C(=C(N1)C)C(=O)OC(C)C)C2=CC=CC3=NON=C32)C(=O)OC
Josamycin	Pharmaceutical	CC1CC=CC=CC(C(CC(C(C(C(CC(=O)O1)OC(=O)C)OC)OC2C(C(C(C(O2)C)OC3CC(C(C(O3)C)OC(=O)CC(C)C)(C)O)N(C)C)O)CC=O)C)O
Ketoconazole	Pharmaceutical	CC(=O)N1CCN(CC1)C2=CC=C(C=C2)OCC3COC(O3)(CN4C=CN=C4)C5=C(C=C(C=C5)Cl)Cl
Ketotifen	Pharmaceutical	CN1CCC(=C2C3=C(C(=O)CC4=CC=CC=C42)SC=C3)CC1
Levamisole	Pharmaceutical	C1CSC2=NC(CN21)C3=CC=CC=C3
Levocabastine	Pharmaceutical	CC1CN(CCC1(C2=CC=CC=C2)C(=O)O)C3CCC(CC3)(C#N)C4=CC=C(C=C4)F
Lidocaine	Pharmaceutical	CCN(CC)CC(=O)NC1=C(C=CC=C1C)C
Lincomycin	Pharmaceutical	CCCC1CC(N(C1)C)C(=O)NC(C2C(C(C(C(O2)SC)O)O)O)C(C)O
Lorazepam	Pharmaceutical	C1=CC=C(C(=C1)C2=NC(C(=O)NC3=C2C=C(C=C3)Cl)O)Cl
Meclizine	Pharmaceutical	CC1=CC(=CC=C1)CN2CCN(CC2)C(C3=CC=CC=C3)C4=CC=C(C=C4)Cl
Medroxyprogesterone	Pharmaceutical	CC1CC2C(CCC3(C2CCC3(C(=O)C)O)C)C4(C1=CC(=O)CC4)C
Mefenamic acid	Pharmaceutical	CC1=C(C(=CC=C1)NC2=CC=CC=C2C(=O)O)C
Memantine	Pharmaceutical	CC12CC3CC(C1)(CC(C3)(C2)N)C
Mephosfolan	Pesticide	CCOP(=O)(N=C1SCC(S1)C)OCC
Methylphenidate	Pharmaceutical	COC(=O)C(C1CCCCN1)C2=CC=CC=C2
Metoprolol	Pharmaceutical	CC(C)NCC(COC1=CC=C(C=C1)CCOC)O
Nadolol	Pharmaceutical	CC(C)(C)NCC(COC1=CC=CC2=C1CC(C(C2)O)O)O
Nifedipine	Pharmaceutical	CC1=C(C(C(=C(N1)C)C(=O)OC)C2=CC=CC=C2[N+](=O)[O-])C(=O)OC
Nordiazepam	Pharmaceutical	C1C(=O)NC2=C(C=C(C=C2)Cl)C(=N1)C3=CC=CC=C3
Norethisterone	Pharmaceutical	CC12CCC3C(C1CCC2(C#C)O)CCC4=CC(=O)CCC34
Nortriptyline	Pharmaceutical	CNCCC=C1C2=CC=CC=C2CCC3=CC=CC=C31
Octinoxate	Personal Care Product	CCCCC(CC)COC(=O)C=CC1=CC=C(C=C1)OC
Octocrylene	Personal Care Product	CCCCC(CC)COC(=O)C(=C(C1=CC=CC=C1)C2=CC=CC=C2)C#N
Orphenadrine	Pharmaceutical	CC1=CC=CC=C1C(C2=CC=CC=C2)OCCN(C)C

Oxamyl	Pesticide	CNC(=O)ON=C(C(=O)N(C)C)SC
Oxycarboxin	Pesticide	CC1=C(S(=O)(=O)CCO1)C(=O)NC2=CC=CC=C2
Picoxystrobin	Pesticide	COC=C(C1=CC=CC=C1COC2=CC=CC(=N2)C(F)(F)F)C(=O)OC
Piperophos	Pesticide	CCCOP(=S)(OCCC)SCC(=O)N1CCCCC1C
Pirenzepine	Pharmaceutical	CN1CCN(CC1)CC(=O)N2C3=CC=CC=C3C(=O)NC4=C2N=CC=C4
Pretilachlor	Pesticide	CCCOCCN(C1=C(C=CC=C1CC)CC)C(=O)CCl
Prodiamine	Pesticide	CCCN(CCC)C1=C(C=C(C(=C1[N+](=O)[O-])N)C(F)(F)F)[N+](=O)[O-]
Prometon	Pesticide	CC(C)NC1=NC(=NC(=N1)OC)NC(C)C
Prometryn	Pesticide	CC(C)NC1=NC(=NC(=N1)SC)NC(C)C
Propamocarb	Pesticide	CCCOC(=O)NCCCN(C)C
Propanolol	Pharmaceutical	CC(C)NCC(COC1=CC=CC2=CC=CC=C21)O
Propazine	Pesticide	CC(C)NC1=NC(=NC(=N1)Cl)NC(C)C
Pymetrozine	Pesticide	CC1=NNC(=O)N(C1)N=CC2=CN=CC=C2
Pyracarbolid	Pesticide	CC1=C(CCCO1)C(=O)NC2=CC=CC=C2
Pyraclostrobin	Pesticide	COC(=O)N(C1=CC=CC=C1COC2=NN(C=C2)C3=CC=C(C=C3)Cl)OC
Pyraflufen-ethyl	Pesticide	CCOC(=O)COC1=C(C=C(C(=C1)C2=NN(C(=C2Cl)OC(F)F)C)F)Cl
Risperidone	Pharmaceutical	CC1=C(C(=O)N2CCCCC2=N1)CCN3CCC(CC3)C4=NOC5=C4C=CC(=C5)F
Rizatriptan	Pharmaceutical	CN(C)CCC1=CNC2=C1C=C(C=C2)CN3C=NC=N3
Ronidazole	Pharmaceutical	CN1C(=CN=C1COC(=O)N)[N+](=O)[O-]
Roxithromycin	Pharmaceutical	CCC1C(C(C(C(=NOCOCCOC)C(CC(C(C(C(C(C(=O)O1)C)OC2CC(C(C(O2)C)O)(C)OC)C)OC3C(C(CC(O3)C)N(C)C)O)(C)O)C)C)O)(C)O
Salbutamol	Pharmaceutical	CC(C)(C)NCC(C1=CC(=C(C=C1)O)CO)O
Simazine	Pesticide	CCNC1=NC(=NC(=N1)Cl)NCC
Spinosyn A	Pesticide	CCC1CCCC(C(C(=O)C2=CC3C4CC(CC4C=CC3C2CC(=O)O1)OC5C(C(C(C(O5)C)OC)OC)OC)C)OC6CCC(C(O6)C)N(C)C
Spinosyn D	Pesticide	CCC1CCCC(C(C(=O)C2=CC3C4CC(CC4C(=CC3C2CC(=O)O1)C)OC5C(C(C(C(O5)C)OC)OC)OC)C)OC6CCC(C(O6)C)N(C)C
Spiramycin	Pharmaceutical	CC1CC=CC=CC(C(CC(C(C(C(CC(=O)O1)O)OC)OC2C(C(C(C(O2)C)OC3CC(C(C(O3)C)O)(C)O)N(C)C)O)CC=O)C)OC4CCC(C(O4)C)N(C)C

Sulfadimethoxine	Pharmaceutical	COC1=NC(=NC(=C1)NS(=O)(=O)C2=CC=C(C=C2)N)OC
Sulfamerazine	Pharmaceutical	CC1=NC(=NC=C1)NS(=O)(=O)C2=CC=C(C=C2)N
Sulfamethazine	Pharmaceutical	CC1=CC(=NC(=N1)NS(=O)(=O)C2=CC=C(C=C2)N)C
Sulfamethoxazole	Pharmaceutical	CC1=CC(=NO1)NS(=O)(=O)C2=CC=C(C=C2)N
Sulfamonomethoxine	Pharmaceutical	COC1=NC=NC(=C1)NS(=O)(=O)C2=CC=C(C=C2)N
Sulfapyridine	Pharmaceutical	C1=CC=NC(=C1)NS(=O)(=O)C2=CC=C(C=C2)N
Sulfathiazole	Pharmaceutical	C1=CC(=CC=C1N)S(=O)(=O)NC2=NC=CS2
Sulfisoxazole	Pharmaceutical	CC1=C(ON=C1C)NS(=O)(=O)C2=CC=C(C=C2)N
Tacrine	Pharmaceutical	C1CCC2=NC3=CC=CC=C3C(=C2C1)N
Tamsulosin	Pharmaceutical	CCOC1=CC=CC=C1OCCNC(C)CC2=CC(=C(C=C2)OC)S(=O)(=O)N
Temazepam	Pharmaceutical	CN1C2=C(C=C(C=C2)Cl)C(=NC(C1=O)O)C3=CC=CC=C3
Terbutryn	Pesticide	CCNC1=NC(=NC(=N1)SC)NC(C)(C)C
Terfenadine	Pharmaceutical	CC(C)(C)C1=CC=C(C=C1)C(CCCN2CCC(CC2)C(C3=CC=CC=C3)(C4=CC=CC=C4)O)O
Thiacloprid	Pesticide	C1CSC(=NC#N)N1CC2=CN=C(C=C2)Cl
Thiamethoxam	Pesticide	CN1COCN(C1=N[N+](=O)[O-])CC2=CN=C(S2)Cl
Thiazopyr	Pesticide	CC(C)CC1=C(C(=NC(=C1C(=O)OC)C(F)F)C(F)(F)F)C2=NCCS2
Timolol	Pharmaceutical	CC(C)(C)NCC(COC1=NSN=C1N2CCOCC2)O
Tramadol	Pharmaceutical	CN(C)CC1CCCCC1(C2=CC(=CC=C2)OC)O
Triclosan	Personal Care Product	C1=CC(=C(C=C1Cl)O)OC2=C(C=C(C=C2)Cl)Cl
Trimethoprim	Pharmaceutical	COC1=CC(=CC(=C1OC)OC)CC2=CN=C(N=C2N)N
Valsartan	Pharmaceutical	CCCCC(=O)N(CC1=CC=C(C=C1)C2=CC=CC=C2C3=NNN=N3)C(C(C)C)C(=O)O
Venlafaxine	Pharmaceutical	CN(C)CC(C1=CC=C(C=C1)OC)C2(CCCCC2)O
Verapamil	Pharmaceutical	CC(C)C(CCCN(C)CCC1=CC(=C(C=C1)OC)OC)(C#N)C2=CC(=C(C=C2)OC)OC
Warfarin	Pharmaceutical	CC(=O)CC(C1=CC=CC=C1)C2=C(C3=CC=CC=C3OC2=O)O
Ziprasidone	Pharmaceutical	C1CN(CCN1CCC2=C(C=C3C(=C2)CC(=O)N3)Cl)C4=NSC5=CC=CC=C54

[a]SMILE formulas obtained from Pubchem

Table A.2 Physicochemical properties obtained from ACD/Labs Percepta software for compounds studied throughout the thesis.

Analyte	nHBD	nHBA	CR	NR	NOR	HR	HAR	nR	nArR	Log BCF	Log Koc	P	LogS (pH=7)	LogP	LogD (pH=2)	LogD (pH=10)	pKa Acid	pKa Base
2-(Thiocyano-methylthio) benzothiazole	0	2	0.64	0.14	0.14	0.36	0	2	2	2.14	3.07	25.96	-3.15	2.91	2.91	2.91		-0.09
Acetamiprid	0	4	0.67	0.27	0.27	0.27	0.07	1	1	0.24	1.71	24.69	-1.83	1.06	1.06	1.06		-0.44
Alprazolam	0	4	0.77	0.18	0.18	0.18	0.05	4	3	1.67	2.74	34.98	-4.2	2.63	1.79	2.63		-2.47 2.37 -0.47
Ametryn	2	5	0.6	0.33	0.33	0.4	0	1	1	2.12	3.06	25.06	-3.27	3.04	1.1	3.04		3.71 -2.09
Amiodarone	0	4	0.81	0.03	0.13	0.13	0.06	3	3	6.53	6.21	57.24	-5.44	7.36	4.26	7.27		9.37
Amitriptyline	0	1	0.95	0.05	0.05	0.05	0	3	2	3.51	4.05	36.28	-2.64	4.72	1.62	4.66		9.18
Amlodipine	3	7	0.71	0.07	0.25	0.25	0.04	2	1	2.93	3.64	41.79	-3.37	3.42	0.12	3.39		8.97 1.9
Amoxicillin	5	8	0.64	0.12	0.32	0.36	0	3	1	0.24	1.71	36.26	-0.87	0.48	-2.37	-3.7	2.44 9.76 13.25	7.14 -1.97 -3.79
Antipyrine	0	3	0.79	0.14	0.21	0.21	0	2	1	-0.03	1.52	21.63	-0.39	0.72	0.7	0.72		0.65 -9.58
Atorvastatin	4	7	0.8	0.05	0.17	0.17	0.02	4	4	2.91	3.62	61.53	-3.56	4.36	4.35	0.61	4.29 13.58 14.33 15	0.38 -9.19
Atrazine	2	5	0.57	0.36	0.36	0.36	0.07	1	1	1.77	2.81	23.19	-3.82	2.66	1.91	2.66		2.27 -2.56
Azelnidipine	3	10	0.77	0.09	0.23	0.23	0	5	3	2.97	3.67	63.93	-8.07	4.89	0.8	4.89		5.88 5.33
Azithromycin	5	14	0.73	0.04	0.27	0.27	0	3	0	2.3	3.19	78.32	-0.22	3.29	-0.81	3.27	13.28 13.5 13.55 14.35 14.53	-1.47 8.59 8.16

Azoxystrobin	0	8	0.73	0.1	0.27	0.27	0	3	3	3.67	4.17	42.12	-4.71	3.54	3.54	3.54	-0.93 -8.63
Benoxacor	0	3	0.69	0.06	0.19	0.19	0.13	2	1	2.2	3.11	24.8	-3.51	2.8	2.74	2.8	1.2
Bensulide	1	5	0.61	0.04	0.22	0.39	0	1	1	2.98	3.67	40.22	-4.54	4.12	4.12	3.92	10.21	-7.16
Benzatropine	0	2	0.91	0.04	0.09	0.09	0	4	2	3.54	4.08	37.5	-1.52	4.71	1.61	4.06	10.54
Benzophenone-4	2	6	0.67	0	0.29	0.33	0	2	2	0.45	1.86	29.51	0.51	0.88	-1.92	-3.62	-0.7 6.28
Betaxolol	2	4	0.82	0.05	0.18	0.18	0	2	1	1.81	2.84	35.25	0.21	2.87	-0.23	2.74	13.89	9.43
Bezafibrate	2	5	0.76	0.04	0.2	0.2	0.04	2	2	2.4	3.26	38.06	-1.62	3.48	3.46	-0.27	3.29 14.31	-2.09
Bisoprolol	2	5	0.78	0.04	0.22	0.22	0	1	1	1.4	2.54	36.74	0.49	2.21	-0.89	2.08	13.86	9.42
Bupropion	1	2	0.81	0.06	0.13	0.13	0.06	1	1	2.41	3.26	26.9	-2.53	3.08	-0.02	3.08	7.16
Buspirone	0	7	0.75	0.18	0.25	0.25	0	4	1	2.38	3.25	42.35	-1.86	2.95	-1.13	2.95	7.72 4.25
Butylated hydroxytoluene	1	1	0.94	0	0.06	0.06	0	1	1	3.81	4.27	27.64	-4.81	5.07	5.07	5.07	12.76	-1.5
Carazolol	3	4	0.82	0.09	0.18	0.18	0	3	3	2.5	3.33	36.18	-1.26	3.33	0.23	3.17	13.94 16.63	9.54 -3.69
Carbamazepine	2	3	0.83	0.11	0.17	0.17	0	3	2	1.8	2.83	27.63	-3.5	2.28	2.28	2.28	13.94	-0.49
Carboxine	1	3	0.75	0.06	0.19	0.25	0	2	1	2.05	3.01	26.27	-2.9	2.18	2.17	2.18	14.31	0.49
Carfentrazone-ethyl	0	6	0.58	0.12	0.23	0.23	0.19	2	1	2.21	3.12	35.31	-4.42	3.05	3.05	3.05	-2.26
CBZ epoxide	2	4	0.79	0.11	0.21	0.21	0	4	2	0.72	2.06	27.65	-2.89	1.31	1.31	1.31	13.91	-0.5
Celecoxib	2	5	0.65	0.12	0.19	0.23	0.12	3	3	2.97	3.67	36.43	-5.41	3.24	3.24	2.76	9.68	-3.81 -8.63
Chloramphenicol	3	7	0.55	0.1	0.35	0.35	0.1	1	1	0.54	1.93	28.76	-2.05	1.02	1.02	0.95	11.03 13.44	-1.73
Cilazapril	2	8	0.73	0.1	0.27	0.27	0	3	1	0.33	1.78	44.29	0.38	1	-2.36	-2.75	15.11 2.28	5.55 3.88
Ciprofloxacin	2	6	0.71	0.13	0.25	0.25	0.04	4	1	0.27	1.73	33.01	-3.13	-0.3	-3.4	-3.53	6.43	8.68 -0.33
Citalopram	0	3	0.83	0.08	0.13	0.13	0.04	3	2	1.68	2.74	36.53	-2.15	3.39	0.29	3.25	9.57
Clarithromycin	4	14	0.73	0.02	0.27	0.27	0	3	0	2.17	3.1	76.91	-2.22	3.12	0.02	3.11	13.08 13.49 13.55 14.03	8.16

Clodinafop-propargyl	0	5	0.71	0.04	0.21	0.21	0.08	2	2	1.54	2.64	33.73	-5.04	3.09	3.09	3.09		-1.54
Clofibric acid	1	3	0.71	0	0.21	0.21	0.07	1	1	1.84	2.86	21.11	0.65	2.74	2.71	-1.01	3.18	
Clopidogrel	0	3	0.76	0.05	0.14	0.19	0.05	3	2	2.99	3.68	33.89	-3.88	4.21	1.76	4.21		4.56
Clothianidin	2	7	0.4	0.33	0.47	0.53	0.07	1	1	0.07	1.59	22.89	-2	0.23	0.21	-1.65	7.47	0.51
Clozapine	1	4	0.78	0.17	0.17	0.17	0.04	4	1	1.84	2.86	37.16	-2.38	3.04	-1.51	1.52		11.33 8.38
Cyclouron	1	3	0.79	0.14	0.21	0.21	0	1	1	2.04	3	23.21	-2.34	2.93	2.93	2.93	13.97	-0.89 -2.65
Cycloxyidim	1	4	0.77	0.05	0.18	0.23	0	2	1	2.54	3.36	35.43	-0.78	3.87	3.86	0.37	3.76	
Cymoxanil	2	7	0.46	0.31	0.54	0.54	0	0	2	-0.12	1.45	17.44	0.11	0.42	0.41	-1.58	3.73 16.09	-1.54 -8.58
Cyromazine	5	6	0.5	0.5	0.5	0.5	0	2	1	-0.26	1.36	18.16	-1.78	0.24	-3.01	0.24		5.44 2.97
Diclofenac	2	3	0.74	0.05	0.16	0.16	0.11	2	2	2.85	3.58	30.34	-1.92	4.48	4.48	0.73	4.18	-4.7 -2.26
Diflubenzuron	2	4	0.67	0.1	0.19	0.19	0.14	2	2	2.56	3.38	29.32	-5.88	3.85	3.85	2.06	8.78 16.24	-0.49 -3.03
Dimethametryn	2	5	0.65	0.29	0.29	0.35	0	1	1	2.79	3.54	28.72	-3.83	3.73	1.74	3.73		3.69 -2.5
Diphenhydramine	0	2	0.89	0.05	0.11	0.11	0	2	2	2.55	3.37	31.54	-1.65	3.71	0.61	3.69		8.76
Enalapril	2	7	0.74	0.07	0.26	0.26	0	2	1	1.61	2.7	39.46	0.42	2	-0.82	-1.75	3.15 13.49 13.55 14.04 14.14	5.43 -3.01
E1 (Estrone)	1	2	0.9	0	0.1	0.1	0	4	1	2.57	3.38	30.94	-4.34	3.38	3.38	3.19	10.25	
E2 (17-ß-estradiol)	2	2	0.9	0	0.1	0.1	0	4	1	2.91	3.62	31.52	-4.56	3.62	3.62	3.44	10.27	
EE2 (17-α-ethynylestradiol)	2	2	0.91	0	0.09	0.09	0	4	1	3.2	3.84	34.2	-4.7	3.87	3.87	3.68	10.24	
Erythromycin	5	14	0.73	0.02	0.27	0.27	0	3	0	1.92	2.92	74.99	-1.55	2.44	-0.66	2.43	13.09	8.16
Famoxadone	1	6	0.79	0.07	0.21	0.21	0	4	3	3.39	3.97	41.23	-5.4	4.27	4.25	4.27		0.63 -6.73
Fenoxaprop-ethyl	0	6	0.72	0.04	0.24	0.24	0.04	3	3	3.4	3.98	36.78	-5.32	4.22	4.22	4.22		-0.08

Fenuron	1	3	0.75	0.17	0.25	0.25	0	1	1	0.51	1.91	19.38	-1.82	1.24	1.23	1.24	15.09	0.33
																		-2.37
Flufenoxuron	2	5	0.64	0.06	0.15	0.15	0.21	3	3	4.03	4.42	41.78	-8.4	5.88	5.88	4.04	8.68	-0.32
																	15.27	-3.04
Fluocinonide	1	7	0.74	0	0.2	0.2	0.06	5	0	2.32	3.2	47.21	-4.33	3.08	3.08	3.08	12.77	
Fluoxetine	1	2	0.77	0.05	0.09	0.09	0.14	2	2	2.88	3.6	31.67	-2.08	4.27	1.17	3.94		10.05
Flurbiprofen	1	2	0.83	0	0.11	0.11	0.06	2	2	2.9	3.62	26.4	-1.48	3.82	3.82	0.07	4.14	
Flurochloridone	0	2	0.63	0.05	0.11	0.11	0.26	2	1	1.65	2.72	26.35	-3.71	3.07	3.07	3.07		-3.59
Flutamide	1	5	0.58	0.11	0.26	0.26	0.16	1	1	2.59	3.4	24.29	-3.67	3.14	3.14	3.14	13.12	-4.58
Flutolanil	1	3	0.74	0.04	0.13	0.13	0.13	2	2	2.58	3.39	32.39	-4.85	4.06	4.06	4.06	12.44	-2.52
Fuberidazole	1	3	0.79	0.14	0.21	0.21	0	3	3	1.8	2.83	21.21	-3.48	2.54	0.1	2.51	11.2	5
Hydrochlorothiazide	4	7	0.41	0.18	0.41	0.53	0.06	2	1	-0.28	1.34	24.86	-2.54	0.01	0.01	-1.49	8.95 9.57	-4.08
Imidacloprid	1	7	0.53	0.29	0.41	0.41	0.06	2	1	-0.56	1.14	24.7	-2.29	0.2	-1.7	-1.75	7.16	4.8
																		-0.04
																	14.36	-0.49
Isocarbamid	2	5	0.62	0.23	0.38	0.38	0	1	0	-0.78	0.99	18.85	-1.46	0.65	0.65	0.65	16.1	-1.66
																		-3.57
Isradipine	1	8	0.7	0.11	0.3	0.3	0	3	2	2.5	3.33	38.42	-4.88	3.75	3.1	3.75		2.56
																		-1.39
																	13.06	
Josamycin	3	16	0.72	0.02	0.28	0.28	0	3	0	2.72	3.49	84.43	-3.07	3.11	0.01	3.11	13.26	7.4
																	13.97	
																		6.88
Ketoconazole	0	8	0.72	0.11	0.22	0.22	0.06	5	3	2.47	3.31	55.15	-4.15	3.61	0.16	3.61		3.58
																		-1.15
Ketotifen	0	2	0.86	0.05	0.09	0.14	0	4	2	3.48	4.03	35.88	-1.99	4.06	0.96	4.03		8.84
																		10
Levamisole	0	2	0.79	0.14	0.14	0.21	0	3	1	1.17	2.38	23.95	-1.23	2.15	0.15	1.86		-
																		10.11
Levocabastine	1	4	0.84	0.06	0.13	0.13	0.03	4	2	3.03	3.71	46.69	-3.89	4.48	1.42	1.29	3.97	9.38
																		7.96
Lidocaine	1	3	0.82	0.12	0.18	0.18	0	1	1	1.56	2.66	28.71	-1.01	2.33	-0.89	2.33	14.23	
																		1.62

Lincomycin	5	8	0.67	0.07	0.3	0.33	0	2	0	0.46	1.87	41.49	0.39	0.63	-2.47	0.62	12.91 13.58 14.32 14.83 15.25	8.78 -1.42
Lorazepam	2	4	0.71	0.1	0.19	0.19	0.1	3	2	1.64	2.72	32.13	-4.13	2.49	2.48	2.44	10.8 12.68	
Meclizine	0	2	0.89	0.07	0.07	0.07	0.04	4	3	3.56	4.09	46.79	-4.36	4.77	1.33	4.77		6.73
Medroxyprogesterone	1	3	0.88	0	0.12	0.12	0	4	0	2.34	3.22	38.5	-4.6	3.52	3.52	3.52	13.03	2.14
Mefenamic acid	2	3	0.83	0.06	0.17	0.17	0	2	2	3.82	4.28	28.63	-0.92	5	5	1.85	3.73	-1.31
Memantine	2	1	0.92	0.08	0.08	0.08	0	4	0	2.18	3.11	21.76	0.08	3.48	0.38	2.62		10.79
Mephosfolan	0	4	0.53	0.07	0.27	0.47	0	1	0	0.9	2.18	25.95	-2.66	1.57	1.57	1.57		-4.93
Methylphenidate	1	3	0.82	0.06	0.18	0.18	0	2	1	1.7	2.76	26.41	-0.02	2.34	-0.77	2.21		9.51
Metoprolol	2	4	0.79	0.05	0.21	0.21	0	1	1	1.13	2.35	30.55	0.57	1.85	-1.25	1.72	13.89	9.43
Nadolol	4	5	0.77	0.05	0.23	0.23	0	2	1	0.75	2.08	33.99	0.51	1.24	-1.86	1.11	13.91 14.24 15.08	9.54
Nifedipine	1	8	0.68	0.08	0.32	0.32	0	2	1	2.02	2.99	34.85	-4.7	3.45	2.7	3.45		2.69
Nordiazepam	1	3	0.79	0.11	0.16	0.16	0.05	3	2	2.17	3.09	29.97	-3.93	2.94	1.76	2.93	11.72	3.22 -4.58
Norethisterone	1	2	0.91	0	0.09	0.09	0	4	0	2.34	3.22	33.92	-4.76	2.98	2.98	2.98	13.09	
Nortriptyline	1	1	0.95	0.05	0.05	0.05	0	3	2	4.06	4.45	34.39	-1.93	4.76	1.66	4.46		10
Octinoxate	0	3	0.89	0.04	0.11	0.11	0	2	2	4.07	4.45	34.73	-4.87	5.19	5.19	5.19		
Octocrylene	0	2	0.9	0.05	0.1	0.1	0	2	2	5.49	5.47	42.96	-6.4	6.34	6.34	6.34		
Orphenadrine	1	6	0.5	0.21	0.43	0.5	0	0	0	2.9	3.62	33.46	-2.22	4.06	0.95	4.03		8.72
Oxamyl	1	5	0.67	0.06	0.28	0.33	0	2	1	-0.59	1.12	21.82	-0.56	-0.06	-0.06	-0.18	10.48	-1.93 -2.42
Oxycarboxin	0	5	0.69	0.04	0.19	0.19	0.12	2	2	0.64	2	26.27	-2.5	0.71	0.7	0.69	11.26	0.24
Picoxystrobin	0	4	0.67	0.05	0.19	0.33	0	1	0	3.17	3.81	34.81	-4.83	3.84	3.84	3.84		-1.09
Piperophos	1	7	0.73	0.19	0.27	0.27	0	4	2	3.04	3.72	37.25	-4.24	4.05	4.05	4.05		-0.87
Pirenzepine	0	3	0.81	0.05	0.14	0.14	0.05	1	1	-0.29	1.33	38.23	-1.42	0.31	-3.14	0.29	11.29	7.39 2.07 0.64
Pretilachlor	2	8	0.54	0.17	0.33	0.33	0.13	1	1	3	3.69	35.46	-3.87	4.04	3.94	4.04		-2.96 1.41
Prodiamine	2	6	0.63	0.31	0.38	0.38	0	1	1	5.03	5.14	32.27	-6.22	5.21	5.21	5.21		-1.4
Prometon	2	5	0.63	0.31	0.31	0.38	0	1	1	1.98	2.96	25.72	-2.31	2.79	0.55	2.79		-8.78 4.36

																	-1.52
Prometryn	1	4	0.69	0.15	0.31	0.31	0	0	0	2.38	3.25	26.88	-3.54	3.4	1.52	3.4	3.76
																	-1.58
Propamocarb	2	3	0.84	0.05	0.16	0.16	0	2	2	0.62	1.99	20.95	0.73	1.15	-1.95	1.08	12.73	9.53
																	-1.7
Propanolol	2	5	0.6	0.33	0.33	0.33	0.07	1	1	2.12	3.06	31.31	0.04	3.26	0.16	3.13	13.84	9.5
Propazine	1	6	0.63	0.31	0.38	0.38	0	2	1	2.03	3	25.01	-3.91	2.98	2.31	2.98		2.28
																		-2.06
																		3.71
Pymetrozine	1	3	0.81	0.06	0.19	0.19	0	2	1	-0.62	1.1	23.93	-2.49	0.3	-1.6	0.3	12.9	2.18
																		-3.8
Pyracarbolid	0	7	0.7	0.11	0.26	0.26	0.04	3	3	1.68	2.75	24.86	-3.16	2.03	2.02	2.03	14.41	0.49
Pyraclostrobin	0	6	0.58	0.08	0.23	0.23	0.19	2	2	3	3.69	40.71	-4.16	4.07	4.07	4.07		-0.23
Pyraflufen-ethyl	0	6	0.77	0.13	0.2	0.2	0.03	5	2	2.3	3.19	34.51	-5.11	3.68	3.68	3.68		-2.79
																		8.07
Risperidone	1	5	0.75	0.25	0.25	0.25	0	3	3	1.96	2.95	44.3	-2.92	2.6	-1.4	2.59		3.46
																		-2.66
																		-4.75
Rizatriptan	2	8	0.43	0.29	0.57	0.57	0	1	1	0.5	1.9	32	-0.32	1.97	-1.86	1.88	16.98	9.49
																		2.72
Ronidazole	5	17	0.71	0.03	0.29	0.29	0	3	0	-0.57	1.13	17.56	-1.33	-0.42	-0.48	-0.42	12.99	1.32
																		-1.66
																	13	
																	13.49	
Roxithromycin	4	4	0.76	0.06	0.24	0.24	0	1	1	2.6	3.41	82.26	-2.65	3.55	0.45	3.54	13.55	8.16
																	13.85	
																	14.06	
																	9.99	
Salbutamol	2	3	0.7	0	0.3	0.3	0	1	1	-0.22	1.38	26.86	0.62	0.61	-2.49	0.17	14.32	9.62
																	14.76	
Salicylic acid	2	5	0.54	0.38	0.38	0.38	0.08	1	1	1.34	2.5	13.9	0.86	2.37	2.15	-0.78	3.01	
																	13.06	
																		2.71
Simazine	0	11	0.79	0.02	0.21	0.21	0	6	0	1.51	2.62	21.37	-3.65	2.29	1.42	2.29		-3.56
Spinosyn A	0	11	0.79	0.02	0.21	0.21	0	6	0	3.42	3.99	78.06	-3.71	5.01	1.91	4.99		8.62

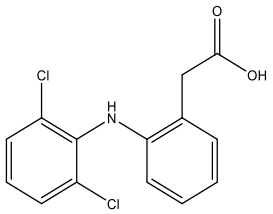
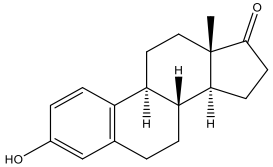
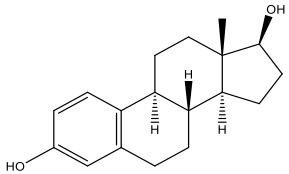
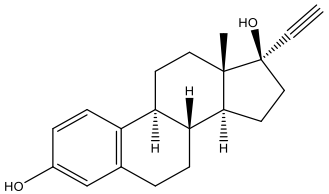
Spinosyn D	4	16	0.73	0.03	0.27	0.27	0	4	0	3.86	4.31	79.8	-4.61	5.6	2.5	5.58	8.62
Spiramycin	3	8	0.57	0.19	0.38	0.43	0	2	2	2.1	3.04	87.5	-0.61	1.94	-2.16	1.92	13.06 13.39 13.69 14.58	8.61 7.4
Sulfadimethoxine	3	6	0.61	0.22	0.33	0.39	0	2	2	0.9	2.18	30.08	-1.02	1.46	0.36	-0.54	6.21	3 0.37
Sulfamerazine	3	6	0.63	0.21	0.32	0.37	0	2	2	0.03	1.56	26.87	-2.69	0.4	0.25	-1.52	7.35	1.58 -0.07
Sulfamethazine	3	6	0.59	0.18	0.35	0.41	0	2	2	0.38	1.81	28.7	-2.85	0.44	0.4	-1.35	7.89	1.08 -1.74
Sulfamethoxazole	3	7	0.58	0.21	0.37	0.42	0	2	2	0.44	1.86	24.76	-1.81	0.65	0.46	-1.34	5.81	1.39 -4.25
Sulfamonomethoxine	3	5	0.65	0.18	0.29	0.35	0	2	2	-0.26	1.36	27.56	-1.23	0.56	-0.3	-1.44	6.67	2.81 0.47
Sulfapyridine	3	5	0.56	0.19	0.31	0.44	0	2	2	-0.2	1.4	25.91	-2.84	0.47	0.15	-1.12	8.54	2.13 0.78
Sulfathiazole	3	6	0.61	0.17	0.33	0.39	0	2	2	-0.19	1.4	25.17	-2.46	0.42	-0.04	-1.51	7.24	2.19 0.71
Sulfisoxazole	2	2	0.87	0.13	0.13	0.13	0	3	2	0.54	1.93	26.59	-1.36	0.85	0.62	-1.15	4.83	1.52 -2.43
Tacrine	3	7	0.71	0.07	0.25	0.29	0	2	2	2.29	3.18	25.06	-1.66	2.87	0.36	2.7		- 18.38 9.64 -3.57
Tamsulosin	1	4	0.76	0.1	0.19	0.19	0.05	3	2	1.47	2.59	43.52	-1.53	2.14	-0.96	1.85	10.08	8.78 -6.72
Temazepam	2	5	0.63	0.31	0.31	0.38	0	1	1	1.4	2.55	32.41	-3.58	2.11	1.92	2.1	11.66	1.58
Terbutryn	2	3	0.91	0.03	0.09	0.09	0	4	3	2.38	3.25	26.9	-3.68	3.35	1.21	3.35		4.03 -1.95
Terfenadine	0	4	0.63	0.25	0.25	0.31	0.06	2	1	4.72	4.92	57.21	-3.64	5.67	2.57	5.56	13.32 14.51	9.42
Thiacloprid	0	8	0.44	0.28	0.44	0.5	0.06	2	1	0.19	1.68	26.93	-2.86	1.22	1.22	1.22		0.01
Thiamethoxam	0	4	0.62	0.08	0.15	0.19	0.19	2	1	-1.11	0.75	26.8	-1.6	-0.33	-0.37	-0.33		0.99
Thiazopyr	2	7	0.62	0.19	0.33	0.38	0	2	1	2.44	3.29	34.36	-4.78	4.07	4.04	4.07		0.78

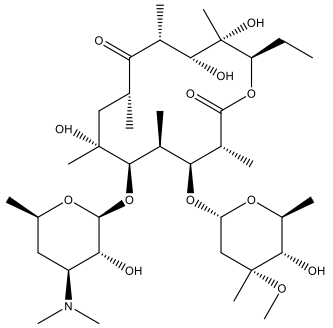
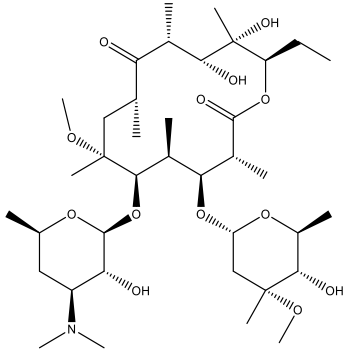
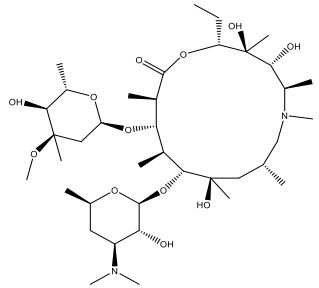
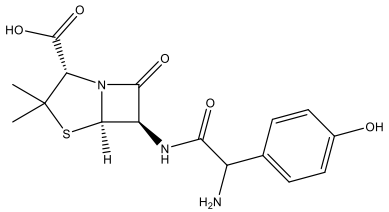
																	-9.7
Timolol	1	3	0.84	0.05	0.16	0.16	0	2	1	0.29	1.75	32.57	0.5	1.53	-1.59	1.45	13.38	9.35
																		0.57
Tramadol	1	2	0.71	0	0.12	0.12	0.18	2	2	1.68	2.74	30.91	0.29	2.54	-0.56	2.43	14.47	9.61
Triclosan	4	7	0.67	0.19	0.33	0.33	0	2	2	3.7	4.19	27.46	-4.87	5.27	5.27	3.11	7.8	
																		6.9
Trimethoprim	2	8	0.75	0.16	0.25	0.25	0	3	3	0.37	1.81	31.82	-2.3	1.12	-1.38	1.12		-0.32
																	3.56	0.6
Valsartan	1	3	0.85	0.05	0.15	0.15	0	2	1	3.38	3.96	47.82	0.36	3.87	3.81	-0.88	4.24	-0.98
Venlafaxine	0	6	0.82	0.06	0.18	0.18	0	2	2	1.98	2.96	32.76	-1.43	3.15	0.05	3.1	14.84	9.26
Verapamil	1	4	0.83	0	0.17	0.17	0	3	2	2.73	3.5	52.28	-3	3.95	0.85	3.91		8.97
Warfarin	1	5	0.75	0.14	0.18	0.21	0.04	5	3	2.37	3.24	33.48	-1.87	3.11	3.11	-0.39	4.5	
																		8.41
Ziprasidone										2.81	3.55	45.23	-3.73	4.08	-0.02	4.07	13.34	6.31
																		-0.08
																		-4.3

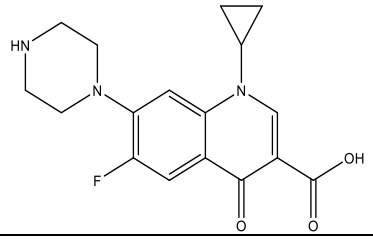
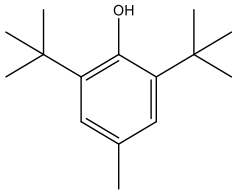
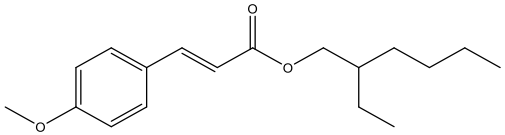
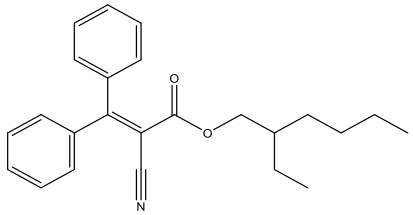
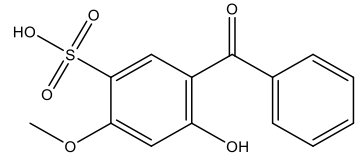
nHBD: No. of hydrogen bond donors.
nHBA: No. of hydrogens bond acceptors.
CR: Carbon ratio.
NR: Nitrogen ratio.
NOR: Nitro- ratio.
HR: Hetero ratio.
HAR: Halogen ratio.
nR: No. of rings.
nArR: No. of aromatic rings.
P: Polarizability.

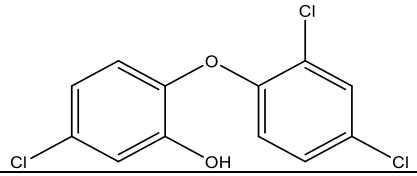
Appendix B: CECs details from SPE LC-MS/MS method

Table A.3 Target PPCPs compounds selected for the development of an SPE LC-MS/MS method.

Class Name	Structure	Formula[a]	Mw[a]	pK_a	Log K_{ow}	CAS Number
Pharmaceuticals						
Anti-inflammatory						
Diclofenac		$C_{14}H_{11}Cl_2NO_2$	296.15	4.15[a]	3.90[a]	15307-86-5
Estrogen steroid hormones						
Estrone (E1)		$C_{18}H_{22}O_2$	270.4	10.33 (strongest acidic)[b] -5.4 (strongest basic)[b]	3.13[a]	53-16-7
17-β-estradiol (E2)		$C_{18}H_{24}O_2$	272.39	10.33 (strongest acidic)[b] -0.88 (strongest basic)[b]	4.01[a]	50-28-2
17-α-ethinylestradiol (EE2)		$C_{20}H_{24}O_2$	296.41	10.33 (strongest acidic)[b] -1.7 (strongest basic)[b]	3.67[a]	57-63-6
Antibiotics						
Macrolides						

Erythromycin		$C_{37}H_{67}NO_{13}$	733.94	8.88[a]	3.06[a]	114-07-8
Clarithromycin		$C_{38}H_{69}NO_{13}$	747.96	8.99[a]	3.16[a]	81103-11-9
Azithromycin		$C_{38}H_{72}N_2O_{12}$	749.00	8.74[a]	4.02[a]	83905-01-5
Antibacterial						
Amoxicillin		$C_{16}H_{19}N_3O_5S$	365.40	3.23 (strongest acidic)[b] 7.43 (strongest basic)[b]	0.87[a]	26787-78-0

Ciprofloxacin		$C_{17}H_{18}FN_3O_3$	33.1.4	6.09 (carboxylic acid group)[b] 8.74 (nitrogen group)[b]	0.28[a]	85721-33-1
PCPs						
Antioxidant						
2,6-ditert-butyl-4-methylphenol (Butylated Hydroxytoluene)		$C_{15}H_{24}O$	220.36	11.6 (strogest acidic)[c] -4.6 (strongest basic)[c]	5.10[a]	128-37-0
UV stabilizer						
2-ethylhexyl-4-methoxycinnamate (Octinoxate)		$C_{18}H_{26}O_3$	290.40	Not available	6.10[a]	5466-77-3
Octocrylene		$C_{24}H_{27}NO_2$	361.49	Not available	6.88[c]	6197-30-4
Benzophenone-4 (Sulisobenzone)		$C_{14}H_{12}O_6S$	308.30	-2.4 (sulfonic acid)[d] 7.6 (hydroxyl)[b]	0.37[a]	4065-45-6
Antibacterial						

Triclosan		$C_{12}H_7Cl_3O_2$	289.54	7.9[a]	4.76[a]	3380-34-5

[a]Pubchem.
[b]Drugbank.
[c]ChemAxon.
[d]Silva *et al.*[241]

Appendix C: List of reference materials

Reference standards for the conventional SPE LC-MS/MS (method 2.1 and 2.2): 17-α-ethinylestradiol, 17-β-estradiol, estrone, butylated hydroxytoluene, diclofenac, erythromycin, clarithromycin, azithromycin, triclosan, octinoxate, octocrylene, benzophenone-4, amoxicillin, ciprofloxacin and β-estradiol-d_2 were obtained from Sigma-Aldrich (Steinheim, Germany). Diclofenac-d_4 was purchased from CDN Isotopes (Quebec, Canada), azithromycin-d_3 from Toronto Research Chemicals (ON, Canada) and estrone-d_4 and triclosan-d_3 were obtained from Santa Cruz Biotechnology (Heidelberg, Germany).

Reference standards for the direct injection LC-MS/MS (method 3): 2-(thiocyanomethylthio)benzothiazole, acetamiprid, alprazolam, ametryn, amiodarone-HCl, amitriptyline, amlodipine, antipyrine, atorvastatin, atrazine, azelnidipine, (±)azithromycin, azoxystrobin, benoxacor, bensulide, (±)benzatropine, (±)betaxolol, bezafibrate, (±)bisoprolol, bupropion, buspirone-HCl, carazolol, carbamazepine, carboxine, (±)carfentrazone-ethyl, carbamazepine-10,11-epoxide, celecoxib, (±)chloramphenicol, (±)cilazapril, (±)citalopram-HBr (total), (±)clarithromycin, (±)clodinafop-propargyl, clofibric acid, (±)clopidogrel-HSO_4^-, clothianidin, clozapine, cyclouron, cycloxyidim, cymoxanil, cyromazine, diazepam, diclofenac, diflubenzuron, (±)dimethametryn, dimethomorph, diphenhydramine-HCl, enalapril, (±)famoxadone, (±)fenoxaprop-p-ethyl, fenuron, flufenoxuron, (±)fluocinonide, (±)fluoxetine, flurbiprofen, flurochloridone, flutamide, flutolanil, fuberidazole, hydrochlorothiazide, imidacloprid, isocarbamid, isradipine, (±)josamycin, (±)ketoconazole, ketotifen, (±)levamisole-HCl, (±)levocabastine-HCl, lidocaine, lincomycin, lorazepam, (±)meclizine-HCl, (±)medroxyprogesterone, mefenamic acid, (±)memantine-HCl,

(±)mephosfolan, (±)methylphenidate-HCl, (±)metoprolol, (±)nadolol, nifedipine, nitenpyram, nordiazepam, (±)norethisterone, nortriptyline, (±)orphenadrine, oxamyl, oxycarboxin, picoxystrobin, (±)piperophos, pirenzepine-2HCl, pretilachlor, prodiamine, prometon, prometryn, propamocarb, (±)propranolol, propazine, pymetrozine, pyracarbolid, pyraclostrobin, pyraflufen-ethyl, risperidone, rizatriptan, ronidazole, (±)roxithromycin, salbutamol, simazine, (±)spinosyn A, (±)spinosyn D, (±)spiramycin, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine, sulfapyridine, sulfathiazole, sulfisoxazole, tacrine, (±)tamsulosin-HCl, (±)temazepam, terbutryn, (±)terfenadine, thiacloprid, thiamethoxam, thiazopyr, (±)timolol, (±)tramadol-HCl, (±)valsartan, (±)venlafaxine- HCl, (±)verapamil-HCl, (±)warfarin and ziprasidone-HCl were obtained from Sigma-Aldrich (Steinheim, Germany). Trimethoprim was acquired from Fluka (Buchs, Switzerland).

Stable isotope-labelled standards (SIL-IS) including amitriptyline-d_3-HCl, clothianidin-d_3, diazepam-d_6, (±)fluoxetine-d_6, (±)lorazepam-d_4, (±)methylphenidate-d_9, nortriptyline-d_3-HCl, risperidone-d_4, (±)temazepam-d_5, thiamethoxam-d_3, (±)tramadol-^{13}C,d_3-HCl, (±)venlafaxine-d_6-HCl, were purchased from Sigma Aldrich. (±)betaxolol-d_7-HCl, celecoxib-d_7, (±)clarithromycin-d_3, lidocaine-d_{10}-HCl, (±)metoprolol-d_7-HCl, sulfamethazine-d_4, trimethoprim-d_3 and (±)verapamil-d_3-HCl were ordered from QMX (Essex, UK).

All reference standards used within this thesis were ≥98% purity.

Appendix D: LC-MS/MS parameters

Table A.4 Source parameters for both SPE LC-MS/MS methods developed.

Electrospray ionisation and mass spectrometry conditions		
Gas temperature (°C)	340	
Gas flow (L/min)	8	
Nebulizer pressure (psi)	40	
Sheath gas temperature (°C)	350	
Sheath gas flow (L/min)	12	
Capillary (V)	3000 (+)	3000 (-)
Nozzle voltage (V)	500 (+)	1500 (-)

Table A.5 MRM transitions for both SPE LC-MS/MS methods (method 1.2.1 and 1.2.2) developed.

Compound	Precursor (m/z)	Product (m/z)		t_R (min)	Delta t_R (min)	Fragmentor	Collision Energy	Polarity
EE2	295.2	145.0	Quan	1.67 (±0.007)	0.6	185	29	-
		267.1	Qual		0.6		45	-
E2	271.2	183.0	Quan	1.58 (±0.008)	0.6	155	49	-
		145.0	Qual		0.6		45	-
β-estradiol-d_2	273.4	185.1	Quan	1.58 (±0.008)	0.6	205	49	-
		147.1	Qual		0.6		49	-
E1	269.2	145.0	Quan	1.79 (±0.036)	0.6	165	45	-
		143.0	Qual		0.6		60	-
Estrone-d_4	273.4	147.1	Quan	1.79 (±0.007)	0.6	190	41	-
		161.1	Qual		0.6		45	-
Amoxicillin	366.1	349.3	Quan	1.27 (±0.022)	0.5	80	5	+
		114.0	Qual		0.5		21	+
Azithromycin	749.5	591.4	Quan	5.25 (±0.023)	0.5	215	33	+
		158.1	Qual		0.5		45	+
Azithromycin-d_3	752.5	594.4	Quan	5.27 (±0.005)	0.5	205	33	+
		158.1	Qual		0.5		45	+
Benzophenone-4	307.0	227.0	Quan	3.86 (±0.005)	0.5	180	25	-
		211.0	Qual		0.5		53	-
Ciprofloxacin	332.1	298.4	Quan	3.00 (±0.004)	0.5	150	17	+
		231.2	Qual		0.5		41	+
Clarithromycin	748.5	590.4	Quan	5.27 (±0.003)	0.5	165	17	+
		158.1	Qual		0.5		33	+
Diclofenac	252.0	216.0	Quan	6.66 (±0.001)	0.5	160	33	+
		214.1	Qual		0.5		21	+
Diclofenac-d_4	256.0	220.1	Quan	6.65 (±0.003)	0.5	160	29	+
		218.8	Qual		0.5		9	+
Erythromycin	734.5	576.4	Quan	4.59 (±0.004)	0.5	145	17	+
		158.1	Qual		0.5		29	+
Octinoxate	291.2	178.9	Quan	8.29(±0.004)	0.5	65	5	+
		16.9	Qual		0.5		21	+
Octocrylene	362.2	250.0	Quan	8.16 (±0.001)	0.5	145	5	+
		232.0	Qual		0.5		21	+
Triclosan	288.9	37.2	Quan	7.20 (±0.003)	0.5	65	5	-
		35.2	Qual		0.5		5	-

Triclosan-d3	292.0	37.2	Quan	7.19 (±0.005)	0.5	65	13	-
		35.2	Qual		0.5		9	-

[a]n=6 replicates

Table A.6 MRM transitions used for the direct injection (DI) LC-MS/MS method (method 2).

Compounds	Precursor (m/z)	Transition (m/z)	Polarity	Pause Time (ms)	Dwell Time (ms)	Q1 Pre Bias (V)	Collision Energy (V)	Q3 Pre Bias (V)	Retention Time Window Range (min)	
2-(Thiocyanomethylthio) benzothiazole	238.7	180.1	+	2	2	-27	-14	-19	2.44	3.04
		136.1	+	2	2	-29	-26	-14		
Acetamiprid	233	126.1	+	2	13	-16	-21	-13	1.22	1.82
Alprazolam	309.1	281.1	+	2	2	-16	-26	-22	2.14	2.74
		205.1	+	2	2	-16	-40	-23		
Ametryn	228.1	186.1	+	2	4	-11	-20	-20	1.27	1.87
		96.1	+	2	4	-23	-28	-19		
Amiodarone	645.8	58.2	+	2	20	-32	-49	-27	3.07	3.67
		100.2	+	2	20	-32	-30	-10		
Amitriptyline	278.2	91.1	+	2	4	-13	-27	-19	1.97	2.57
		105.1	+	2	4	-13	-25	-21		
Amitriptyline-d3	281.1	233.2	+	2	4	-19	-17	-25	1.96	2.56
		105.2	+	2	4	-10	-23	-19		
Amlodipine	409.1	238.2	+	2	6	-15	-12	-24	1.94	2.54
Antipyrine	188.9	77.2	+	2	5	-13	-40	-30	0.83	1.43
		56.2	+	2	5	-13	-32	-23		
Atorvastatin	559.1	440.3	+	2	5	-20	-23	-16	2.88	3.48

		250.2	+	2	5	-20	-43	-27		
Atrazine	216.1	174.1	+	2	14	-11	-18	-18	1.48	2.08
Azelnidipine	583.2	167.2	+	2	2	-40	-27	-30	2.80	3.40
		165.2	+	2	2	-40	-55	-16		
Azithromycin	749.6	591.4	+	2	2	-28	-30	-22	1.30	1.90
		158.3	+	2	2	-28	-40	-16		
Azoxystrobin	404.2	372.1	+	2	2	-21	-16	-27	2.73	3.33
		344.1	+	2	2	-21	-26	-24		
Benoxacor	260	149.2	+	2	2	-30	-18	-27	2.20	2.80
		134.1	+	2	2	-30	-29	-25		
Bensulide	398.1	158.1	+	2	1	-20	-24	-16	3.00	3.60
		217.9	+	2	1	-20	-17	-23		
Benzatropine	308.1	167.2	+	2	5	-11	-30	-17	2.01	2.61
		265.2	+	2	5	-21	-52	-17		
Betaxolol	308.3	116.2	+	2	4	-15	-21	-21	1.38	1.98
		72.2	+	2	4	-21	-24	-27		
Betaxolol-d7	315.2	123.3	+	2	4	-23	-22	-22	1.37	1.97
		105.3	+	2	4	-23	-24	-11		
Bezafibrate	360.2	274	-	2	5	18	17	12	2.27	2.87
		154.1	-	2	5	20	29	15		
Bisoprolol	326.2	116.2	+	2	5	-23	-19	-21	1.15	1.75
		74.2	+	2	5	-12	-26	-29		
Bupropion	240.1	184.2	+	2	4	-29	-13	-12	0.98	1.58
		131.2	+	2	4	-27	-26	-13		
Buspirone	386.1	122.2	+	2	2	-26	-30	-23	1.49	2.09
		109.2	+	2	2	-25	-46	-11		
Carazolol	299.1	116.2	+	2	6	-21	-21	-20	1.32	1.92

		222.2	+	2	6	-21	-21	-24		
Carbamazepine	237.1	194	+	2	4	-12	-20	-23	1.71	2.31
		192.1	+	2	4	-12	-25	-22		
Carboxine	236	143.1	+	2	13	-26	-15	-14	1.77	2.37
Carfentrazone-ethyl	412.1	346	+	2	2	-19	-17	-24	2.81	3.41
		366	+	2	2	-19	-19	-21		
Carbamazepine epoxide	252.9	180.2	+	2	4	-17	-29	-18	1.40	2.00
		236.2	+	2	4	-17	-12	-16		
Celecoxib	382.1	362.1	+	2	4	-11	-28	-25	2.73	3.33
		300.2	+	2	4	-18	-28	-21		
Celecoxib-d7	389	369.1	+	2	2	-19	-29	-25	2.72	3.32
		289.2	+	2	2	-26	-37	-30		
Chloramphenicol	322.7	152.2	-	2	5	16	17	15	1.08	1.68
		257	-	2	5	17	11	25		
Cilazapril	418	211.2	+	2	5	-20	-20	-20	1.83	2.43
		70.2	+	2	5	-15	-46	-12		
Citalopram	325.1	109.2	+	2	4	-22	-26	-20	1.63	2.23
		262.2	+	2	4	-24	-20	-30		
Clarithromycin	748.2	158.2	+	2	2	-36	-34	-10	1.95	2.55
		290.4	+	2	2	-36	-20	-22		
Clarithromycin-d3	751.2	161.2	+	2	2	-38	-28	-16	1.95	2.55
		593.4	+	2	2	-38	-22	-22		
Clozapine	327.2	270.2	+	2	2	-16	-23	-20	1.18	1.78
		192.1	+	2	2	-16	-45	-14		
Cyclouron	199.1	72.2	+	2	3	-14	-25	-13	1.64	2.24
		89.2	+	2	3	-22	-14	-16		
Cycloxyidim	326.1	280.2	+	2	5	-16	-14	-19	2.97	3.57

		180.2	+	2	5	-12	-22	-19		
Cymoxanil	199.2	111.2	+	2	3	-23	-19	-18	0.93	1.53
		83	+	2	3	-23	-27	-17		
Diclofenac	296	215.1	+	2	6	-15	-20	-16	2.68	3.28
		214	+	2	6	-15	-40	-24		
Diflubenzuron	311	158.1	+	2	1	-16	-16	-29	2.60	3.20
		141.1	+	2	1	-16	-32	-24		
Dimethametryn	256.1	186.2	+	2	2	-28	-22	-30	1.79	2.39
		68.1	+	2	2	-13	-44	-11		
Diphenhydramine	256	167.1	+	2	2	-30	-11	-12	1.52	2.12
		152	+	2	2	-30	-40	-17		
Enalapril	375.10	114.0	-	2	10	27	24	21	1.34	2.14
Famoxadone	375.8	196.1	+	2	6	-25	-21	-20	3.07	3.67
Fenoxaprop-ethyl	362.1	288.1	+	2	2	-18	-18	-21	3.10	3.70
		121.1	+	2	2	-19	-33	-13		
Fenuron	165	72.2	+	2	5	-18	-22	-28	0.50	1.10
		46.1	+	2	5	-18	-14	-18		
Flufenoxuron	489.1	158.1	+	2	2	-15	-21	-17	3.14	3.74
		141	+	2	2	-24	-39	-15		
Fluocinonide	494.9	337.2	+	2	6	-23	-19	-25	2.69	3.29
Fluoxetine	310.2	44.2	+	2	2	-15	-16	-17	1.89	2.49
		148.2	+	2	2	-15	-10	-11		
Fluoxetine-d6	316.1	44.2	+	2	2	-20	-15	-20	1.88	2.48
		154.3	+	2	2	-15	-9	-16		
Flurbiprofen	245.2	188.7	+	2	10	-24	-15	-29	1.46	2.26
Flurochloridone	312.0	291.9	+	2	5	-16	-21	-20	2.52	3.32

		144.9	+	2	5	-16	-46	-29		
Flutamide	275	202	-	2	2	14	23	20	2.20	2.80
		205	-	2	2	13	21	23		
Flutolanil	324.3	242.2	+	2	2	-15	-26	-16	2.56	3.16
		262.2	+	2	2	-15	-19	-18		
Fuberidazole	184.9	157.2	+	2	5	-23	-21	-16	0.17	0.77
		156.2	+	2	5	-23	-27	-16		
Hydrochlorothiazide	296	269	-	2	10	11	19	11	0.14	0.74
Imidacloprid	256.1	175.1	+	2	5	-13	-19	-20	1.06	1.66
		209.1	+	2	5	-13	-18	-10		
Isocarbamid	186.1	87	+	2	2	-20	-16	-15	0.63	1.23
		44.2	+	2	2	-20	-32	-17		
Isradipine	370.2	119	-	2	2	14	16	11	2.52	3.12
		250	-	2	2	11	16	17		
Josamycin	828.5	109.3	+	2	6	-30	-47	-27	2.34	2.94
Ketoconazole	533	491.1	+	2	4	-36	-31	-24	2.18	2.78
		82.2	+	2	4	-36	-47	-14		
Ketotifen	310	96.2	+	2	10	-22	-24	-18	1.40	2.00
Levamisole	207.1	180	+	2	10	-10	-24	-20	0.24	0.84
		91.1	+	2	10	-10	-41	-17		
Levocabastine	421.4	174.2	+	2	6	-20	-32	-18	1.90	2.50
		70.2	+	2	6	-15	-37	-12		
Lidocaine	235	86.1	+	2	5	-26	-19	-18	0.21	0.81
		58.1	+	2	5	-26	-45	-12		
Lidocaine-d10	245.2	96.3	+	2	13	-29	-22	-17	0.21	0.81
Lincomycin	407.2	126.2	+	2	6	-22	-31	-13	0.30	0.71

		359.2	+	2	6	-23	-19	-27		
Lorazepam	321.1	302.9	+	2	4	-12	-10	-24	1.82	2.62
		274.9	+	2	4	-12	-22	-22		
Meclizine	391.1	201.1	+	2	1	-28	-20	-22	2.71	3.31
		165.1	+	2	1	-19	-55	-17		
Medroxyprogesterone	345.1	123.2	+	2	2	-23	-26	-21	2.71	3.31
		97.2	+	2	2	-23	-27	-10		
Mefenamic acid	240	196.2	-	2	12	17	18	12	2.82	3.42
Memantine	180.3	163.3	+	2	6	-19	-18	-17	0.95	1.55
		107.3	+	2	6	-20	-26	-20		
Mephosfolan	270.1	140	+	2	12	-13	-25	-14	1.50	2.10
Methylphenidate	234.2	84.1	+	2	6	-11	-20	-17	0.87	1.47
		56.1	+	2	6	-11	-45	-22		
Methylphenidate-d9	243.3	93.3	+	5	6	-28	-24	-17	0.85	1.45
		61.2	+	2	6	-27	-50	-23		
Metoprolol	268.2	116.2	+	2	5	-30	-21	-21	0.80	1.40
		159.1	+	2	5	-10	-22	-16		
Metoprolol-d7	275.2	123.2	+	2	5	-22	-21	-23	0.79	1.39
		105.2	+	2	5	-29	-22	-19		
Nadolol	310.3	254.2	+	2	3	-21	-17	-17	0.34	0.94
		236.2	+	2	3	-21	-21	-25		
Nifedipine	345.2	222.1	-	2	3	10	10	15	2.22	2.82
		122	-	2	3	13	12	27		
Nifedipine-d4	349	222.1	-	2	3	10	10	24	2.22	2.82
		126.1	-	2	3	13	12	28		
Nordiazepam	270.8	140.2	+	2	2	-18	-28	-14	1.86	2.46
		208.1	+	2	2	-10	-28	-14		

Norethisterone	299.1	109.2	+	2	2	-21	-26	-19	2.56	3.16
		231.2	+	2	2	-11	-20	-11		
Nortriptyline	264.2	233.1	+	2	4	-10	-15	-17	1.92	2.52
		91.1	+	2	4	-10	-25	-18		
Nortriptyline-d3	267	233.2	+	2	4	-18	-15	-16	1.92	2.52
		105.2	+	2	4	-18	-22	-19		
Orphenadrine	270.1	181.2	+	2	4	-29	-13	-29	1.71	2.31
		166.1	+	2	4	-10	-28	-30		
Oxamyl	237.1	72.1	+	2	4	-12	-12	-13	0.24	0.84
		90.2	+	2	4	-12	-10	-17		
Oxycarboxin	268.1	175	+	2	5	-13	-14	-18	1.20	1.80
		147	+	2	5	-13	-27	-30		
Oxycodone	316.2	298.2	+	2	20	-16	-19	-22	0.40	1.00
		241.1	+	2	20	-16	-30	-18		
Picoxystrobin	368	145.2	+	2	1	-25	-22	-15	2.92	3.52
		205.2	+	2	1	-25	-10	-14		
Piperophos	353.9	171.1	+	2	2	-23	-22	-18	3.07	3.67
		255.1	+	2	2	-22	-14	-12		
Pirenzipine	352.1	113.3	+	2	5	-26	-22	-22	0.50	1.10
		70.2	+	2	5	-10	-46	-12		
Pretilachlor	312.2	252.1	+	2	2	-16	-17	-17	2.97	3.57
		176.1	+	2	2	-16	-29	-18		
Prodiamine	349.2	232	-	2	2	10	24	10	3.10	3.70
		216	-	2	2	13	29	14		
Prometon	226.1	184.2	+	2	5	-11	-20	-12	1.08	1.68
		142.2	+	2	5	-11	-23	-14		
Prometryn	242.2	158.1	+	2	2	-12	-25	-16	1.52	2.12

		200	+	2	2	-12	-20	-22		
Propranolol	260.1	116.2	+	2	4	-30	-19	-21	1.39	1.99
		183.2	+	2	4	-10	-20	-18		
Propazine	230.1	188.2	+	2	4	-26	-19	-20	1.80	2.40
		146.1	+	2	4	-25	-24	-25		
Pymetrozine	218.1	105.1	+	2	6	-11	-21	-21	0.00	0.45
		79	+	2	6	-11	-45	-15		
Pyracarbolid	218.1	125.1	+	2	3	-24	-18	-24	1.56	2.16
		97.1	+	2	3	-24	-28	-17		
Pyraclostrobin	390.1	194.1	+	2	2	-19	-15	-21	3.09	3.69
		163.1	+	2	2	-19	-27	-17		
Pyraflufen-ethyl	413	339	+	2	2	-21	-20	-23	2.97	3.57
		253.1	+	2	2	-21	-35	-26		
Risperidone	411.2	191.1	+	2	10	-12	-31	-23	1.37	1.97
		69.1	+	2	10	-12	-50	-27		
Risperidone-d4	415.2	195.2	+	2	10	-29	-30	-21	1.36	1.96
Rizatriptan	270.1	201.2	+	2	5	-10	-14	-21	0.21	0.81
		158.2	+	2	5	-20	-21	-16		
Ronidazole	201	140.2	+	2	11	-14	-13	-14	0.14	0.74
Roxithromycin	837.3	679.4	+	2	4	-24	-22	-24	2.10	2.70
		158.1	+	2	4	-24	-34	-16		
Simazine	202.1	104	+	2	5	-23	-25	-19	1.14	1.74
		68.1	+	2	5	-23	-32	-27		
Spinosyn A	732.6	142.1	+	2	5	-22	-34	-29	2.79	3.39
		98	+	2	5	-22	-40	-19		
Spinosyn D	746.6	142.1	+	2	6	-22	-36	-15	2.93	3.53

		98	+	2	6	-22	-40	-19		
Spiramycin	843.5	174.2	+	2	10	-24	-36	-11	1.41	2.01
Sulfadimethoxine	311.1	156	+	1	10	-16	-20	-17	1.43	2.03
		92.1	+	1	10	-16	-32	-17		
Sulfamerazine	265.1	92.1	+	2	3	-13	-34	-17	1.92	2.52
		156	+	2	3	-13	-17	-16		
Sulfamethazine	278.9	186.1	+	2	6	-18	-17	-19	0.81	1.41
		124.2	+	2	6	-10	-24	-12		
Sulfamethazine-d4	282.8	186.2	+	2	3	-13	-20	-19	0.80	1.40
		124.2	+	2	3	-17	-25	-27		
Sulfamethoxazole	254.1	156	+	2	6	-12	-18	-16	1.04	1.64
		92.2	+	2	6	-12	-31	-16		
Sulfamonomethoxine	281.1	156.1	+	2	4	-14	-18	-17	0.94	1.54
		92.2	+	2	4	-14	-33	-18		
Sulfapyridine	250	156	+	2	4	-23	-17	-16	0.39	0.99
		92.1	+	2	4	-22	-32	-18		
Sulfathiazole	256	156	+	2	8	-24	-16	-16	0.42	1.02
		92.2	+	2	8	-24	-27	-17		
Sulfisoxazole	268	156.1	+	2	13	-30	-15	-16	1.14	1.74
		113.2	+	2	13	-10	-16	-11		
Tacrine	199	171.2	+	2	6	-23	-30	-17	0.73	1.33
		144.1	+	2	6	-14	-36	-28		
Tamsulosin	409.1	228.1	+	2	4	-28	-24	-24	1.43	2.03
		271.2	+	2	4	-28	-20	-13		
Temazepam	301.1	255.1	+	2	4	-11	-25	-30	2.17	2.77
		283.2	+	2	4	-11	-13	-23		

Temazepam-d5	306	260.1	+	2	10	-11	-24	-17	2.16	2.76
Terbutryn	242.1	186.1	+	2	2	-28	-20	-20	1.58	2.18
		158.2	+	2	2	-29	-24	-27		
Terfenadine	472.4	436.3	+	2	6	-24	-28	-22	2.69	3.29
		454.3	+	2	6	-13	-22	-22		
Thiacloprid	253.1	126.1	+	2	4	-13	-22	-25	1.53	2.13
		90.1	+	2	4	-13	-38	-18		
Thiamethoxam	292	211.1	+	2	2	-14	-13	-23	0.71	1.31
		181	+	2	2	-14	-24	-19		
Thiamethoxam-d3	296.6	214.1	+	2	3	-11	-12	-23	0.68	1.28
		184.1	+	2	3	-14	-24	-19		
Thiazopyr	397	377.1	+	2	6	-14	-23	-18	2.86	3.46
Timolol	317.1	261.1	+	2	13	-16	-17	-30	0.77	1.37
Tramadol	264.1	58.2	+	2	14	-19	-16	-22	0.82	1.42
Tramadol-13C1, d3	268.3	58.2	+	2	14	-13	-23	-24	0.81	1.41
Trimethoprim	291.1	230.1	+	2	5	-30	-25	-26	0.45	1.05
		123.2	+	2	5	-15	-28	-25		
Trimethoprim-d3	294.1	230.2	+	2	5	-20	-25	-25	0.44	1.04
		123.2	+	2	5	-11	-26	-12		
Valsartan	436.4	291.2	+	2	5	-15	-18	-14	2.50	3.10
		235.2	+	2	5	-12	-17	-16		
Venlafaxine	278.2	58.1	+	2	5	-13	-19	-22	1.13	1.73
		260.2	+	2	5	-13	-15	-20		
Venlafaxine-d6	284.2	64.2	+	2	5	-20	-23	-26	1.13	1.73
		260.2	+	2	5	-13	-15	-20		
Verapamil	455.2	165.2	+	2	2	-30	-29	-30	1.99	2.59

		414.4	+	2	2	-16	-16	-15		
Verapamil-d3	458.2	165.2	+	2	2	-30	-29	-29	1.99	2.59
		306.3	+	2	2	-30	-26	-15		
Warfarin	309.2	163.1	+	2	5	-15	-16	-19	2.36	2.96
		251.1	+	2	5	-15	-20	-29		
Ziprasidone	413.1	194.1	+	2	2	-20	-30	-20	1.75	2.35

Appendix E: DI method results for individual compounds

Table A.7 Recovery (%) of all compounds in matrix-match standards for n=3 replicates for filtration process using PTFE filters.

Compound	Surface waters Recovery ± SD (%)			Influent Recovery ± SD (%)			Effluent Recovery ± SD (%)			DI water Recovery ± SD (%)		
2-(Thiocyanomethylthio)benzothiazole	98	±	3	106	±	17	113	±	11	123	±	5
Acetamiprid	101	±	5	99	±	17	99	±	8	112	±	3
Alprazolam	96	±	12	93	±	8	98	±	1	107	±	8
Ametryn	97	±	5	97	±	16	99	±	5	115	±	7
Amiodarone		0		12	±	3		0			0	
Amitriptyline	89	±	4	85	±	12	92	±	10	99	±	3
Amitriptyline-d3	116	±	8	78	±	4	108	±	16	162	±	17
Amlodipine	77	±	14	58	±	11	66	±	8	63	±	24
Antipyrine	97	±	5	98	±	11	100	±	14	121	±	6
Atorvastatin	67	±	6	94	±	17	80	±	7	87	±	10
Atrazine	99	±	4	99	±	13	102	±	10	113	±	2
Azelnidipine		0		1	±	0		0			0	
Azithromycin	54	±	2	84	±	14	10	±	3		0	
Azoxystrobin	87	±	3	93	±	14	88	±	7	97	±	4
Benoxacor	96	±	30	74	±	16	100	±	20	121	±	21
Bensulide	79	±	11	80	±	9	67	±	17	74	±	6
Benzatropine	93	±	6	84	±	11	97	±	8	100	±	4
Betaxolol	99	±	19	99	±	3	103	±	15	111	±	10
Betaxolol-d7	112	±	4	84	±	5	100	±	7	104	±	3
Bezafibrate	99	±	12	108	±	23	94	±	9	110	±	8
Bisoprolol	98	±	8	96	±	13	103	±	14	112	±	4
Bupropion	99	±	9	96	±	14	102	±	6	113	±	2
Buspirone	73	±	4	94	±	12	69	±	6	102	±	6
Carazolol	101	±	4	100	±	19	106	±	12	106	±	7
Carbamazepine	101	±	5	93	±	12	102	±	11	115	±	7
Carbamazepine epoxide	97	±	8	102	±	9	101	±	6	116	±	15

Carboxine	91	±	24	106	±	14	117	±	13	106	±	12
Carfentrazone-ethyl	102	±	5	96	±	15	96	±	10	116	±	3
Celecoxib	72	±	6	82	±	11	69	±	13	83	±	8
Celecoxib-d7	80	±	12	64	±	7	71	±	10	72	±	5
Chloramphenicol	112	±	23	110	±	50	133	±	49	166	±	123
Cilazapril	90	±	3	97	±	16	102	±	8	109	±	2
Citalopram	94	±	9	99	±	16	101	±	5	118	±	8
Clarithromycin	32	±	6	93	±	7	18	±	3	13	±	10
Clarithromycin-d3	41	±	10	70	±	6	19	±	2	15	±	12
Clodinafop-propargyl	93	±	7	125	±	26	169	±	4	139	±	15
Clofibric acid	94	±	9	121	±	3	111	±	10	112	±	20
Clopidogrel	81	±	8	89	±	14	92	±	6	96	±	7
Clothianidin	105	±	16	95	±	2	108	±	17	107	±	22
Clozapine	78	±	9	88	±	12	79	±	3	99	±	7
Cyclouron	92	±	5	99	±	9	99	±	12	113	±	9
Cycloxyidim	101	±	4	101	±	14	100	±	16	106	±	4
Cyromazine				100	±	17	106	±	8	127	±	12
Diazepam	102	±	14	100	±	20	104	±	4	107	±	0
Diazepam-d6	113	±	14	88	±	6	98	±	11	104	±	10
Diclofenac	97	±	11	100	±	11	104	±	2	125	±	2
Diflubenzuron	87	±	9	88	±	13	87	±	10	102	±	1
Dimethametryn	90	±	4	95	±	16	95	±	6	108	±	9
Dimethomorph	98	±	9	94	±	13	94	±	8	104	±	5
Diphenhydramine	97	±	4	96	±	16	98	±	11	123	±	9
Enalapril	91	±	20	95	±	23	140	±	41	102	±	4
Famoxadone	70	±	5	82	±	14	71	±	6	82	±	8
Fenoxaprop-ethyl	37	±	7	70	±	11	30	±	10	44	±	7
Fenuron	96	±	3	102	±	16	97	±	9	115	±	3
Flufenoxuron		0			0			0			0	
Fluocinonide	63	±	7	98	±	3	86	±	17	101	±	24
Fluoxetine	88	±	10	25	±	6	96	±	15	120	±	9
Fluoxetine-d6	116	±	3	26	±	4	110	±	7	177	±	24
Flurbiprofen	89	±	43				89	±	9	116	±	33

Flurochloridone	99	±	16	96	±	10	107	±	13	110	±	35
Flutamide	92	±	4	95	±	21	99	±	13	106	±	8
Flutolanil	99	±	6	98	±	14	95	±	6	107	±	7
Fuberidazole	101	±	7	99	±	17	104	±	11	114	±	13
Hydrochlorothiazide	99	±	34	94	±	18	86	±	11	121	±	8
Imidacloprid	102	±	13	101	±	22	105	±	6	111	±	16
Isocarbamid	97	±	8	99	±	5	102	±	12	115	±	9
Isradipine	78	±	5	82	±	4	72	±	18	109	±	28
Josamycin	0			73	±	9	0			0		
Ketoconazole		0		87	±	13		0			0	
Ketotifen	96	±	4	96	±	11	104	±	8	108	±	3
Levamisole	98	±	5	98	±	25	101	±	22	114	±	7
Levocabastine	100	±	12	97	±	12	102	±	6	122	±	16
Lidocaine	97	±	6	101	±	11	100	±	7	107	±	7
Lidocaine-d10	109	±	1	90	±	7	100	±	5	103	±	1
Lincomycin	97	±	1	94	±	13	97	±	1	114	±	3
Lorazepam	100	±	7	97	±	11	100	±	12	117	±	14
Lorazepam-d4	109	±	3	88	±	5	98	±	6	102	±	11
Meclizine	1	±	0	6	±	0	1	±	0	10	±	4
Medroxyprogesterone	73	±	1	89	±	17	67	±	17	77	±	5
Mefenamic acid	102	±	6	95	±	5	114	±	13	118	±	13
Memantine	101	±	5	92	±	12	96	±	5	114	±	10
Mephosfolan	96	±	3	96	±	12	103	±	7	111	±	7
Methylone-d3	113	±	5	96	±	8	110	±	4	110	±	11
Methylphenidate	101	±	8	107	±	15	114	±	10	127	±	4
Methylphenidate-d9	110	±	5	94	±	9	116	±	7	124	±	5
Metoprolol	95	±	7	97	±	11	105	±	4	105	±	1
Metoprolol-d7	115	±	13	90	±	6	101	±	9	109	±	8
Nadolol	102	±	6	102	±	17	101	±	4	113	±	7
Nifedipine	102	±	22	76	±	31	111	±	21	94	±	31
Nifedipine-d4	146	±	93	111	±	20	117	±	45	93	±	45

Nitenpyram	95	±	6	116	±	21	97	±	8	120	±	7
Nordiazepam	100	±	10	111	±	22	100	±	4	115	±	9
Norethisterone	90	±	17	73	±	13	77	±	8	116	±	12
Nortriptyline	91	±	5	71	±	12	96	±	9	125	±	7
Nortriptyline-d3	118	±	6	67	±	5	111	±	7	162	±	12
Orphenadrine	91	±	5	89	±	13	98	±	4	116	±	4
Oxamyl	98	±	9	95	±	17	117	±	15	136	±	4
Oxycarboxin	106	±	5	102	±	16	112	±	10	123	±	15
Picoxystrobin	85	±	6	83	±	16	85	±	10	99	±	10
Piperophos	70	±	4	84	±	16	69	±	7	81	±	9
Pirenzipine	98	±	8	98	±	10	105	±	7	108	±	10
Pretilachlor	80	±	4	90	±	9	82	±	9	92	±	4
Prodiamine	57	±	7	35	±	9	65	±	16	69	±	16
Prometon	97	±	9	99	±	11	99	±	9	112	±	10
Prometryn	96	±	5	96	±	11	99	±	8	109	±	3
Propamocarb	96	±	13	101	±	15	104	±	11	114	±	3
Propanolol	104	±	3	102	±	12	98	±	4	108	±	7
Propazine	96	±	4	101	±	13	103	±	10	113	±	7
Pymetrozine	89	±	7	101	±	9	103	±	11	122	±	6
Pyracarbolid	98	±	6	100	±	17	108	±	10	114	±	2
Pyraclostrobin	66	±	2	77	±	8	78	±	5	74	±	3
Pyraflufen-ethyl	51	±	5				63	±	8	62	±	11
Risperidone	75	±	3	93	±	13	62	±	2	93	±	15
Risperidone-d4	88	±	1	87	±	2	66	±	1	91	±	6
Rizatriptan	92	±	6	102	±	13	103	±	2	108	±	8
Ronidazole	96	±	8	103	±	17	101	±	9	121	±	2
Roxithromycin	5	±	2	78	±	12	0	±	0	2	±	3
Salbutamol	91	±	3	104	±	14	93	±	3	107	±	7
Simazine	100	±	4	96	±	18	102	±	13	119	±	13
Spinosyn A		0			0			0			0	
Spinosyn D		0			0			0			0	
Spiramycin		0		77	±	8		0			0	
Sulfadimethoxine	104	±	9	99	±	12	103	±	14	110	±	4
Sulfamerazine	99	±	25	61	±	13	127	±	23	97	±	28

Sulfamethazine	105	±	14	97	±	16	106	±	9	98	±	2
Sulfamethazine-d4	117	±	7	86	±	6	105	±	11	95	±	6
Sulfamethoxazole	104	±	6	101	±	16	95	±	7	117	±	12
Sulfamonomethoxine	104	±	12	100	±	15	96	±	9	113	±	19
Sulfapyridine	98	±	6	106	±	12	105	±	9	110	±	4
Sulfathiazole	97	±	11	108	±	14	106	±	10	107	±	6
Sulfisoxazole	90	±	9	99	±	14	104	±	9	120	±	10
Tacrine	101	±	7	95	±	16	103	±	8	115	±	3
Tamsulosin	96	±	5	97	±	14	96	±	8	114	±	8
Temazepam	96	±	9	98	±	16	102	±	8	106	±	6
Temazepam-d5	109	±	2	89	±	5	102	±	4	102	±	3
Terbutryn	91	±	4	96	±	19	98	±	11	110	±	6
Terfenadine		0		1	±	0		0			0	
Thiacloprid	100	±	6	101	±	23	101	±	11	111	±	10
Thiamethoxam	105	±	4	94	±	12	97	±	6	106	±	4
Thiamethoxam-d3	126	±	30	121	±	35	99	±	28	91	±	25
Thiazopyr	75	±	2	81	±	15	76	±	7	82	±	6
Timolol	97	±	4	98	±	12	100	±	7	117	±	8
Tramadol	99	±	7	102	±	8	103	±	6	113	±	4
Tramadol-13C1, d3	108	±	6	91	±	4	102	±	3	107	±	3
Trimethoprim	99	±	8	98	±	6	101	±	6	110	±	8
Trimethoprim-d3	111	±	2	96	±	10	102	±	2	107	±	7
Valsartan	102	±	10	100	±	8	117	±	23	104	±	32
Venlafaxine	99	±	7	102	±	10	105	±	10	112	±	10
Venlafaxine-d6	111	±	7	95	±	6	105	±	4	106	±	10
Verapamil	58	±	5	74	±	7	42	±	3	61	±	8
Verapamil-d3	71	±	2	71	±	4	46	±	1	67	±	10
Warfarin	104	±	9	96	±	14	100	±	8	111	±	11
Ziprasidone	22	±	4	76	±	8	16	±	2	57	±	7

Table A.8 Relative instability (%) of all compounds in matrix-match standards thawing at room temperature for 48 hours for n=3 replicates.

	Surface waters			Influent			Effluent		
Compound	**Stability (%)**	**Instability (%)**	**SD (%)**	**Stability (%)**	**Instability (%)**	**SD (%)**	**Stability (%)**	**Instability (%)**	**SD (%)**
2-(Thiocyanomethylthio)benzothiazole	95	5	9	95	5	9	96	4	9
Acetamiprid	101	-1	8	99	1	4	99	1	4
Alprazolam	102	-2	7	100	0	7	101	-1	7
Ametryn	102	-2	6	101	-1	3	104	-4	4
Amiodarone	206	-106	187	117	-17	7	117	-17	63
Amitriptyline	116	-16	22	99	1	6	107	-7	5
Amitriptyline-d_3	106	-6	29	93	7	8	106	-6	6
Amlodipine	150	-50	87	89	11	8	102	-2	8
Antipyrine	100	0	13	63	37	4	103	-3	6
Atorvastatin	98	2	12	104	-4	4	114	-14	10
Atrazine	100	0	7	99	1	6	104	-4	2
Azelnidipine	44	56	16	15	85	2	204	-104	96
Azithromycin	113	-13	10	91	9	6	109	-9	10
Azoxystrobin	100	0	7	99	1	5	100	0	3
Benoxacor	109	-9	36	105	-5	30	101	-1	27
Bensulide	102	-2	8	104	-4	9	100	0	10
Benzatropine	123	-23	28	100	0	4	102	-2	7
Betaxolol	108	-8	7	101	-1	3	101	-1	5
Betaxolol-d_7	96	4	8	94	6	11	99	1	6
Bezafibrate	105	-5	28	103	-3	9	102	-2	16
Bisoprolol	100	0	10	101	-1	7	102	-2	8
Bupropion	95	5	9	95	5	4	99	1	7
Buspirone	98	2	7	99	1	4	96	4	5
Carazolol	103	-3	4	97	3	7	101	-1	6
Carbamazepine	101	-1	12	93	7	4	103	-3	9
Carbamazepine epoxide	101	-1	13	101	-1	4	100	0	3
Carboxine	101	-1	13	93	7	10	113	-13	6
Carfentrazone-ethyl	85	15	17	68	32	19	67	33	24
Celecoxib	99	1	12	95	5	16	107	-7	17
Celecoxib-d_7	94	6	14	92	8	10	96	4	14
Chloramphenicol	116	-16	86	128	-28	75	125	-25	40
Cilazapril	100	0	12	95	5	5	98	2	10
Citalopram	101	-1	8	99	1	6	102	-2	8
Clarithromycin	102	-2	10	101	-1	5	104	-4	9
Clarithromycin-d_3	102	-2	9	96	4	9	107	-7	3

Clodinafop-propargyl	78	22	9	0 [a]	100 [a]	0[a]	61	39	4
Clofibric acid	112	-12	27	106	-6	36	129	-29	52
Clopidogrel	102	-2	7	98	2	5	103	-3	4
Clothianidin	98	2	14	123	-23	22	97	3	10
Clothianidin-d_3	98	2	41	134	-34	102	103	-3	24
Clozapine	107	-7	12	101	-1	6	102	-2	6
Cyclouron	101	-1	13	98	2	8	100	0	7
Cycloxyidim	95	5	9	86	14	12	110	-10	7
Cymoxanil				155	-55	104			
Diazepam	101	-1	5	101	-1	4	103	-3	6
Diazepam-d6	96	4	8	96	4	6	95	5	7
Diclofenac	96	4	14	97	3	8	97	3	7
Diflubenzuron	97	3	9	99	1	6	102	-2	5
Dimethametryn	101	-1	9	107	-7	7	101	-1	5
Dimethomorph	103	-3	9	96	4	5	103	-3	11
Diphenhydramine	104	-4	5	99	1	4	103	-3	4
Enalapril	172	-72	149	99	1	26	106	-6	39
Famoxadone	95	5	4	104	-4	10	104	-4	7
Fenoxaprop-ethyl	107	-7	20	42	58	3	92	8	10
Fenuron	97	3	10	101	-1	5	102	-2	4
Flufenoxuron	81	19	45	109	-9	10	97	3	27
Fluocinonide	117	-17	22	95	5	16	100	0	56
Fluoxetine	119	-19	26	91	9	5	110	-10	9
Fluoxetine-d6	108	-8	24	98	2	14	111	-11	5
Flurbiprofen	104	-4	38	114	-14	68	109	-9	32
Flurochloridone	91	9	35	146	-46	73	128	-28	63
Flutamide	99	1	11	98	2	9	102	-2	10
Flutolanil	102	-2	5	96	4	3	106	-6	5
Fuberidazole	100	0	8	101	-1	4	102	-2	3
Hydrochlorothiazide	108	-8	11	90	10	6	103	-3	9
Imidacloprid	104	-4	12	111	-11	13	104	-4	10
Isocarbamid	98	2	8	99	1	12	107	-7	8
Isradipine	118	-18	27	101	-1	21	109	-9	20
Josamycin	88	12	5	100	0	5	103	-3	2
Ketamine	99	1	7	103	-3	4	104	-4	5
Ketamine-d_4	94	6	5	97	3	4	98	2	6
Ketoconazole	111	-11	16	26	74	14	110	-10	9
Ketotifen	107	-7	9	96	4	5	104	-4	4
Levamisole	98	2	18	95	5	18	112	-12	9
Levocabastine	98	2	15	105	-5	16	113	-13	12
Lidocaine	99	1	6	99	1	3	105	-5	3
Lidocaine-d_{10}	92	8	5	97	3	7	97	3	2
Lincomycin	101	-1	12	100	0	3	103	-3	3
Lorazepam	100	0	12	101	-1	7	101	-1	5

Lorazepam-d_4	100	0	13	93	7	7	102	-2	9
Meclizine	115	-15	41	102	-2	8	97	3	14
Medroxyprogesterone	104	-4	7	100	0	5	103	-3	12
Mefenamic acid	103	-3	11	92	8	7	104	-4	13
Memantine	106	-6	8	101	-1	7	102	-2	8
Mephosfolan	99	1	11	99	1	5	98	2	6
Methylphenidate	79	21	5	97	3	4	72	28	2
Methylphenidate-d_9	75	25	2	93	7	4	67	33	3
Metoprolol	98	2	8	97	3	5	104	-4	4
Metoprolol-d_7	93	7	7	94	6	6	96	4	5
Nadolol	99	1	6	99	1	3	103	-3	1
Nifedipine	104	-4	52	145	-45	126	103	-3	56
Nitenpyram	101	-1	7	102	-2	8	104	-4	5
Nordiazepam	102	-2	10	102	-2	12	111	-11	10
Norethisterone	95	5	21	99	1	4	108	-8	28
Nortriptyline	116	-16	21	100	0	10	99	1	6
Nortriptyline-d_3	112	-12	27	102	-2	8	100	0	8
Orphenadrine	107	-7	8	98	2	9	101	-1	6
Oxamyl	94	6	10	87	13	8	84	16	10
Oxycarboxin	101	-1	10	95	5	6	95	5	2
Picoxystrobin	103	-3	7	102	-2	4	106	-6	5
Piperophos	95	5	5	104	-4	4	104	-4	4
Pirenzipine	100	0	5	102	-2	3	103	-3	2
Pretilachlor	100	0	4	103	-3	4	100	0	6
Prodiamine	117	-17	57	81	19	16	98	2	31
Prometon	99	1	14	99	1	2	102	-2	7
Prometryn	100	0	9	99	1	5	111	-11	3
Propamocarb	102	-2	16	97	3	6	103	-3	6
Propanolol	105	-5	5	103	-3	7	96	4	6
Propazine	98	2	12	97	3	6	107	-7	7
Pymetrozine	100	0	5	100	0	3	105	-5	4
Pyracarbolid	97	3	5	97	3	5	98	2	3
Pyraclostrobin	102	-2	15	106	-6	13	108	-8	16
Pyraflufen-ethyl	92	8	10	101	-1	14	84	16	4
Risperidone	102	-2	7	96	4	3	103	-3	3
Risperidone-d_4	93	7	5	94	6	5	95	5	2
Rizatriptan	101	-1	7	98	2	3	103	-3	6
Ronidazole	98	2	4	97	3	9	103	-3	7
Roxithromycin	106	-6	23	94	6	7	104	-4	6
Salbutamol	98	2	7	98	2	10	102	-2	4
Simazine	99	1	7	108	-8	11	97	3	5
Spinosyn A	131	-31	77	93	7	7	105	-5	19
Spinosyn D	131	-31	108	95	5	10	110	-10	40
Spiramycin	108	-8	16	93	7	14	103	-3	16

Sulfadimethoxine	100	0	11	101	-1	5	109	-9	4
Sulfamerazine	133	-33	23	130	-30	51	108	-8	18
Sulfamethazine	100	0	6	95	5	8	106	-6	3
Sulfamethazine-d_4	96	4	12	94	6	8	97	3	5
Sulfamethoxazole	102	-2	11	100	0	10	96	4	7
Sulfamonomethoxine	106	-6	9	104	-4	8	104	-4	7
Sulfapyridine	99	1	8	103	-3	8	104	-4	8
Sulfathiazole	101	-1	9	97	3	8	105	-5	6
Sulfisoxazole	93	7	8	91	9	6	111	-11	11
Tacrine	98	2	5	100	0	6	101	-1	4
Tamsulosin	102	-2	3	98	2	4	102	-2	6
Temazepam	95	5	9	100	0	7	102	-2	5
Temazepam-d_5	94	6	5	93	7	7	98	2	3
Terbutryn	100	0	8	99	1	5	111	-11	5
Terfenadine	105	-5	18	101	-1	4	104	-4	7
Thiacloprid	101	-1	12	100	0	8	96	4	5
Thiamethoxam	101	-1	4	98	2	3	102	-2	7
Thiamethoxam-d_3	95	5	31	117	-17	36	96	4	18
Thiazopyr	100	0	4	99	1	7	104	-4	6
Timolol	99	1	5	100	0	2	102	-2	3
Tramadol	101	-1	7	99	1	3	102	-2	3
Tramadol-$^{13}C1$, d_3	95	5	6	95	5	7	98	2	2
Trimethoprim	100	0	5	100	0	5	101	-1	6
Trimethoprim-d_3	95	5	5	98	2	8	98	2	6
Valsartan	92	8	19	134	-34	8	99	1	23
Venlafaxine	102	-2	5	97	3	5	104	-4	6
Venlafaxine-d_6	93	7	8	95	5	8	97	3	7
Verapamil	107	-7	7	94	6	5	105	-5	5
Verapamil-d_3	99	1	5	94	6	8	99	1	4
Warfarin	122	-22	10	120	-20	6	131	-31	13
Ziprasidone	101	-1	7	91	9	18	101	-1	9

n.d. not detected at the prepared concentration of 500 ng/L.
[a] no peak detected at RT.

Table A.9 Method performance data of individual compounds for surface water matrix based on ICH guidelines.[231]

131 analytes (19 IS)	Range (ng/L)	Linearity (n≥5) R^2	Peak area/height precision (%RSD, n=6)		Matrix Effect (%CV, n=6)		Inaccuracy (%CV, n≤3)				LOD (ng/L)	LOQ (ng/L)
			100 ng/L	1000 ng/L	100 ng/L	1000 ng/L	100 ng/L[a]	250 ng/L[a]	750 ng/L[a]	1000 ng/L[a]		
2-(Thiocyanomethylthio) benzothiazole	100 - 5000	0.9866	25	3	-1	28	-5	0	5	1	4	100[b]
Acetamiprid	10 - 5000	0.9951	9	4	3	26	1	-1	4	1	4	14
Alprazolam	5 - 5000	0.9966	11	6	-9	28	0	-1	4	1	4	13
Ametryn	5 - 5000	0.9973	4	3	1	31	-1	-1	4	1	4	11
Amiodarone	25 - 5000	0.9277	17	5	39	246	-6	23	2	-2	6	17
Amitriptyline*	25 - 5000	0.9810	16	13	53	57	-2	5	5	1	4	14
Amlodipine	25 - 5000	0.9487	9	6	125	121	-2	20	3	-1	5	14
Antipyrine	25 - 5000	0.9908	23	4	-4	26	0	0	5	2	4	15
Atorvastatin	25 - 5000	0.9925	12	9	-4	33	-1	0	5	2	4	12
Atrazine	25 - 5000	0.9957	7	1	0	23	0	0	4	2	4	14
Azelnidipine	25 - 5000	0.9399	12	7	-4	30	-4	18	3	0	5	14
Azithromycin	25 - 5000	0.9905	17	4	819	444	-3	0	3	1	3	8
Azoxystrobin	25 - 5000	0.9959	5	2	-1	28	0	1	4	1	4	13
Benoxacor	100 - 5000	0.9813	n.d.	15	n.d.	30	n.d.	-2	5	2	4	250[b]
Bensulide	25 - 5000	0.9957	6	4	1	32	0	1	4	1	4	13
Benzatropine	5 - 5000	0.9909	5	4	64	60	-4	6	4	1	4	11
Betaxolol*	50 - 5000	0.9796	14	8	14	33	5	2	7	2	6	20
Bezafibrate	100 - 5000	0.9879	13	12	4	27	-3	-2	4	1	4	250[b]
Bisoprolol	5 - 5000	0.9955	5	5	2	33	-2	0	4	2	4	11
Bupropion	5 - 5000	0.9943	8	3	-3	27	-3	0	5	1	4	12
Buspirone	5 - 5000	0.9961	3	5	1	31	-1	0	4	2	4	12
Carazolol	5 - 5000	0.9876	9	3	10	34	-3	0	4	1	4	12

Carbamazepine	10 - 5000	0.9949	7	5	-10	19	-1	-2	3	1	4	12
Carbamazepine epoxide	10 - 5000	0.9956	9	3	3	24	-2	-1	4	1	4	13
Carboxine	10 - 5000	0.9964	9	4	9	28	-3	1	4	1	4	12
Carfentrazone-ethyl	500 - 5000	0.9752	n.d.	10	n.d.	35	n.d.	n.d.	3	1	4	500[b]
Celecoxib*	25 - 5000	0.9912	17	9	0	24	-1	0	5	2	4	11
Chloramphenicol	500 - 5000	0.9787	n.d.	39	n.d.	35	n.d.	n.d.	-2	0	3	500[b]
Cilazapril	10 - 5000	0.9970	10	9	5	41	0	-2	3	1	4	12
Citalopram	25 - 5000	0.9947	5	5	28	48	-2	2	4	1	4	11
Clarithromycin*	5 - 5000	0.9928	14	11	15	50	-3	0	7	1	4	11
Clodinafop-propargyl	25 - 5000	0.9952	7	3	11	25	-3	2	3	1	4	11
Clofibric acid	500 - 5000	0.9678	n.d.	13	n.d.	41	n.d.	n.d.	4	1	4	100[b]
Clopidogrel	5 - 5000	0.9976	3	5	-3	26	-1	0	4	1	4	12
Clothianidin	100 - 5000	0.9805	16	5	9	32	-1	1	5	2	4	100[b]
Clozapine	5 - 5000	0.9957	7	3	69	67	-2	1	4	2	4	11
Cyclouron	25 - 5000	0.9902	12	7	6	28	1	0	5	2	5	17
Cycloxyidim	75 - 5000	0.9874	11	5	-6	27	0	0	5	1	4	75[b]
Diazepam*	50 - 5000	0.9914	11	8	5	27	0	0	4	2	4	50[b]
Diclofenac	75 - 5000	0.9829	18	7	-13	23	0	-4	6	2	4	75[b]
Diflubenzuron	25 - 5000	0.9910	10	4	2	31	0	1	6	1	4	13
Dimethametryn	5 - 5000	0.9959	6	2	-3	26	-1	-1	5	2	4	13
Dimethomorph[c]	75 - 5000	0.9914	11	1	-12	25	-1	0	5	1	4	100[b]
Diphenhydramine	5 - 5000	0.9961	6	5	18	36	-3	1	5	2	4	12
Enalapril	500 - 5000	0.9781	n.d.	26	n.d.	13	n.d.	n.d.	2	1	3	500[b]
Famoxadone	5 - 5000	0.9972	7	8	3	32	-2	1	4	1	4	13
Fenoxaprop-ethyl	10 - 5000	0.9904	12	7	-5	26	2	2	5	2	4	15
Fenuron	10 - 5000	0.9928	9	3	-9	4	-11	-5	2	1	4	11

Flufenoxuron	25 - 5000	0.9830	24	12	-3	35	-5	10	3	-2	5	16
Fluocinonide	750 – 5000 (4 points)	0.9981	n.d.	6	n.d.	36	n.d.	n.d.	0	-26	3	1000[b]
Fluoxetine*	5 - 5000	0.9833	19	10	57	50	-5	6	3	1	4	11
Flurbiprofen	250 - 5000	0.9864	n.d.	12	n.d.	33	n.d.	n.d.	0	3	4	500[b]
Flurochloridone	250 - 5000	0.9781	n.d.	20	n.d.	22	n.d.	-3	4	2	4	250[b]
Flutamide	10 - 5000	0.9926	11	5	-5	30	-4	-1	3	1	4	11
Flutolanil	25 - 5000	0.9954	10	3	1	29	-1	0	4	1	4	13
Fuberidazole	5 - 5000	0.9973	2	3	-2	24	-1	0	4	2	4	12
Hydrochlorothiazide	75 - 5000	0.9941	17	6	10	13	-3	-2	3	2	4	50[b]
Imidacloprid	50 - 5000	0.9896	14	10	-6	27	-1	1	4	2	5	100[b]
Isocarbamid	50 - 5000	0.9934	9	4	-1	26	-2	-1	4	1	4	11
Isradipine	100 - 5000	0.9941	24	10	-4	62	1	1	3	0	3	75[b]
Josamycin	5 - 5000	0.9949	5	7	20	43	-4	0	4	2	4	11
Ketoconazole	50 - 5000	0.9811	18	6	38	45	1	8	5	1	4	14
Ketotifen	5 - 5000	0.9944	7	4	40	55	-3	2	4	1	4	11
Levamisole	50 - 5000	0.9861	34	10	-9	41	1	-2	3	1	4	10
Levocabastine	25 - 5000	0.9941	23	9	11	33	0	0	3	2	4	13
Lidocaine*	5 - 5000	0.9967	3	4	3	29	0	-1	5	2	4	12
Lincomycin	5 - 5000	0.9961	6	3	18	48	-2	-2	4	2	4	12
Lorazepam*	100 - 5000	0.9911	12	11	10	22	0	0	4	0	5	100[b]
Meclizine	5 - 5000	0.9885	10	4	54	60	-3	10	4	0	4	13
Medroxyprogesterone	75 - 5000	0.9871	12	9	17	25	-2	1	5	2	4	75[b]
Mefenamic acid	75 - 5000	0.9860	31	10	15	25	0	2	5	2	4	250[b]
Memantine	5 - 5000	0.9963	8	2	5	29	0	0	4	1	4	13
Mephosfolan	10 - 5000	0.9971	2	4	1	30	0	-1	4	1	4	13
Methylphenidate*	5 - 5000	0.9962	5	6	9	32	0	-1	5	2	4	12

Metoprolol*	10 - 5000	0.9921	6	4	-2	34	0	-2	5	1	4	11
Nadolol	5 - 5000	0.9967	4	3	-2	27	-1	-2	4	2	4	11
Nifedipine	500 - 5000	0.9368	n.d.	50	n.d.	93	n.d.	n.d.	5	-3	3	250[b]
Nitenpyram	75 - 5000	0.9883	15	5	-4	29	2	-1	5	2	4	75b
Nordiazepam	5 - 5000	0.9447	18	5	2	30	11	4	5	1	9	38
Norethisterone	250 - 5000	0.9687	n.d.	6	n.d.	36	n.d.	-4	5	2	5	500[b]
Nortriptyline*	10 - 5000	0.9804	12	12	53	48	-4	6	5	1	4	13
Orphenadrine	5 - 5000	0.9967	8	5	16	39	-2	1	4	1	4	11
Oxamyl	25 - 5000	0.9847	20	7	32	32	-7	1	5	1	4	12
Oxycarboxin	10 - 5000	0.9915	11	6	17	33	-5	2	4	1	4	12
Picoxystrobin	25 - 5000	0.9940	6	4	2	29	-1	1	4	1	4	13
Piperophos	25 - 5000	0.9951	4	5	-2	32	-1	1	4	1	4	13
Pirenzipine	5 - 5000	0.9974	4	3	15	42	-1	0	4	1	4	11
Pretilachlor	25 - 5000	0.9965	7	1	3	23	-1	0	3	2	4	12
Prodiamine	250 - 5000	0.9943	n.d.	32	n.d.	30	n.d.	3	0	2	4	250[b]
Prometon	10 - 5000	0.9947	6	2	-3	26	-1	-1	4	2	4	12
Prometryn	5 - 5000	0.9967	2	5	2	31	-2	0	4	2	4	12
Propamocarb	25 - 5000	0.9941	3	5	-9	21	-15	-7	2	0	4	12
Propranolol	25 - 5000	0.9951	5	7	3	30	-1	2	4	1	4	13
Propazine	10 - 5000	0.9953	8	4	-2	23	1	0	4	1	4	14
Pymetrozine	10 - 5000	0.9956	8	3	-3	16	1	0	4	2	4	14
Pyracarbolid	5 - 5000	0.9971	3	4	-4	22	0	-1	4	2	4	13
Pyraclostrobin	50 - 5000	0.9891	16	7	2	14	-1	3	4	1	4	14
Pyraflufen-ethyl	50 - 5000	0.9943	9	3	-4	26	-2	1	4	1	4	12
Risperidone*	10 - 5000	0.9943	6	3	28	56	0	0	6	2	4	13
Rizatriptan	25 - 5000	0.9956	10	3	18	55	-1	2	4	1	4	11
Ronidazole	50 - 5000	0.9937	8	3	-11	21	0	-1	4	2	4	13
Roxithromycin	5 - 5000	0.9926	17	7	92	89	-6	3	4	1	4	11

Salbutamol	10 - 5000	0.9954	4	4	-15	3	1	-1	4	2	4	14
Simazine	10 - 5000	0.9957	7	4	-1	25	2	-1	4	1	4	14
Spinosyn A	25 - 5000	0.9640	18	16	114	148	-4	15	3	1	4	11
Spinosyn D	50 - 5000	0.9189	28	12	32	105	-6	21	3	-1	5	12
Spiramycin	75 - 5000	0.9873	31	10	**	1899	-2	5	3	2	3	75[b]
Sulfadimethoxine	25 - 5000	0.9915	10	2	0	21	4	2	4	2	5	17
Sulfamerazine	250 - 5000	0.9937	n.d.	20	n.d.	42	n.d.	-1	2	0	4	750[b]
Sulfamethazine*	10 - 5000	0.9959	5	6	1	29	0	-1	4	2	4	12
Sulfamethoxazole	25 - 5000	0.9925	26	5	3	24	2	0	3	2	4	14
Sulfamonomethoxine	25 - 5000	0.9957	5	6	-20	31	1			1	4	11
Sulfapyridine	10 - 5000	0.9937	6	5	-2	26	2	1	4	1	4	14
Sulfathiazole	50 - 5000	0.9933	10	3	-1	22	-1	0	4	1	4	13
Sulfisoxazole	10 - 5000	0.9958	12	6	-13	21	-1	-1	4	1	4	12
Tacrine	10 - 5000	0.9970	5	5	6	30	-1	0	4	1	4	12
Tamsulosin	5 - 5000	0.9963	6	2	-1	35	-1	0	4	2	4	11
Temazepam*	10 - 5000	0.9961	8	5	7	30	0	-1	4	1	4	13
Terbutryn	5 - 5000	0.9916	4	4	1	32	-5	-1	4	1	4	11
Terfenadine	5 - 5000	0.9894	7	4	39	58	-4	9	3	1	4	13
Thiacloprid	25 - 5000	0.9952	6	6	2	31	-1	-1	4	1	4	14
Thiamethoxam*	50 - 5000	0.9874	23	12	5	24	1	-1	6	0	4	13
Thiazopyr	10 - 5000	0.9966	7	3	-2	28	-1	-1	4	2	4	12
Timolol	5 - 5000	0.9972	4	2	0	24	-2	-1	4	2	4	11
Tramadol*	5 - 5000	0.9960	3	4	-1	30	-4	-3	3	0	4	13
Trimethoprim*	5 - 5000	0.9938	8	7	7	41	-2	-2	4	2	4	11
Valsartan	50 - 5000	0.9812	35	14	47	16	-4	1	4	3	4	14
Venlafaxine*	5 - 5000	0.9955	8	7	5	33	-2	-1	5	2	4	12
Verapamil*	5 - 5000	0.9950	11	10	21	51	-1	1	6	1	4	12
Warfarin	10 - 5000	0.9392	22	7	6	21	6	0	3	2	7	25
Ziprasidone	10 - 5000	0.9907	10	7	9	27	-3	5	4	1	4	13

*SIL-IS used for peak area ratio linearity assessment.
[a]for 250 and 750 ng/L n=3 and for 100 and 1000 ng/L n=6.
[b]LOQ taken as the lowest matrix-match calibration standards with 10 S/N signal.
[c] Peak height used.
**No peak at ultrapure water sample detected.
n.d.: not detected or signal < 5 S/N.

Table A.10 Method performance of individual compounds for influent wastewater matrix based on ICH guidelines.[231]

130 analytes	Range (ng/L)	Linearity (n≥5) R^2	Peak area/height precision (%RSD, n=6)		Matrix Effect (%CV, n=6)		Inaccuracy (%CV, n≤3)				LOD (ng/L)	LOQ (ng/L)
			100 ng/L	1000 ng/L	100 ng/L	1000 ng/L	100 ng/L[a]	250 ng/L[a]	750 ng/L[a]	1000 ng/L[a]		
2-(Thiocyanomethylthio) benzothiazole	50 - 5000	0.9885	9	11	-6	18	1	2	4	5	4	50[b]
Acetamiprid	75 - 5000	0.9843	4	7	-14	-2	-2	-2	4	5	4	50[b]
Alprazolam	25 - 5000	0.9876	13	12	21	31	0	-3	6	5	4	14
Ametryn	10 - 5000	0.9939	4	9	4	18	0	0	5	5	4	12
Amiodarone	250 - 5000	0.9865	29	6	**	387	n.d.	2	-2	-1	3	250[b]
Amitriptyline*	100 - 5000	0.9854	25	7	86	56	-6	0	4	7	5	100[b]
Amlodipine	100 - 5000	0.9902	23	145	-9	93	-24	-6	-3	1	3	50[b]
Antipyrine	25 - 5000	0.9806	11	11	6	10	2	1	6	6	5	19
Atorvastatin	5 - 5000	0.9856	9	8	29	33	-12	-3	2	3	5	15
Atrazine	5 - 5000	0.9891	6	8	-2	16	2	1	5	5	5	16
Azelnidipine	50 - 5000	0.9586	7	20	**	**	-7	4	6	4	4	50[b]
Azithromycin	5 - 5000	0.9804	9	5	936	590	-27	-12	0	1	4	12
Azoxystrobin	25 - 5000	0.9907	4	7	27	48	0	-1	5	5	4	13
Benoxacor	250 - 5000	0.9851	15	12	26	29	-11	-2	3	4	4	250[b]

Bensulide	100 - 5000	0.9737	21	16	37	37	-3	0	5	5	4	100[b]
Benztropine	25 - 5000	0.9866	7	12	120	77	-2	6	3	5	4	13
Betaxolol*	25 - 5000	0.9879	15	10	39	47	0	0	4	5	4	14
Bezafibrate	50 - 5000	0.9848	16	13	-6	13	1	-2	6	5	4	13
Bisoprolol	10 - 5000	0.9881	5	7	31	41	-15	-7	2	3	4	13
Bupropion	10 - 5000	0.9911	8	10	-20	-11	1	2	4	5	4	14
Buspirone	5 - 5000	0.9957	3	10	-11	0	-1	0	4	5	4	13
Carazolol	50 - 5000	0.9939	7	10	13	36	-1	-1	3	4	4	11
Carbamazepine	25 - 5000	0.9831	6	7	9	13	-35	-19	-3	-2	5	15
Carbamazepine epoxide	10 - 5000	0.9941	10	7	48	32	-8	-4	3	4	4	13
Carboxin	25 - 5000	0.9907	11	6	3	4	-2	3	4	4	4	13
Carfentrazone-ethyl	250 - 5000	0.9461	n.d.	18	n.d.	-42	n.d.	-3	8	0	4	250[b]
Celecoxib*	5 - 5000	0.9813	18	12	4	20	4	3	6	4	6	20
Chloramphenicol	250 - 5000	0.9212	n.d.	17	n.d.	79	n.d.	-4	4	5	5	250[b]
Cilazapril	25 - 5000	0.9899	14	3	46	73	0	1	5	4	4	13
Citalopram	75 - 5000	0.9902	4	2	46	29	-28	-13	-3	0	4	12
Clarithromycin*	250 - 5000	0.9865	17	11	-64	35	-49	-27	-9	-7	3	10
Clofibric acid	100 - 5000	0.9684	8	8	2	27	-3	0	6	4	4	100[b]
Clopidogrel	5 - 5000	0.9912	14	3	8	-5	-6	-1	4	4	4	14
Clothianidin	250 - 5000	0.9599	n.d.	9	n.d.	33	n.d.	-5	4	5	4	250[b]
Clozapine	5 - 5000	0.9958	9	10	65	18	-7	-1	4	4	4	12
Cycloxydim	50 - 5000	0.9868	6	11	9	16	-1	1	5	5	4	14
Cycluron	75 - 5000	0.9728	18	11	-7	22	2	2	6	5	5	16
Cymoxanil	500 - 5000	0.9988	n.d.	16	n.d.	35	n.d.	n.d.	0	1	4	500[b]
Cyromazine	75 - 5000	0.8798	18	6	-87	-82	-49	-28	-9	-8	4	15

Diazepam*	50 - 5000	0.9869	18	6	29	-62	0	-2	4	5	4	50[b]
Diclofenac	25 - 5000	0.9841	17	11	-21	4	-40	-22	-5	-3	4	10
Diflubenzuron	25 - 5000	0.9869	7	4	-5	1	0	4	5	5	4	14
Dimethametryn	5 - 5000	0.9945	6	9	-10	6	0	0	5	5	4	12
Dimethomorph[c]	75 - 5000	0.9809	6	9	33	40	14	1	5	5	4	75[b]
Diphenhydramine	10 - 5000	0.9928	5	14	30	24	-15	-6	2	3	4	13
Enalapril	500 - 5000	0.9784	n.d.	24	n.d.	16	n.d.	n.d.	2	5	4	500[b]
Famoxadone	5 - 5000	0.9960	2	10	-35	-17	0	-1	4	5	4	13
Fenoxaprop-ethyl	250 - 5000	0.8235	n.d.	33	n.d.	-91	n.d.	-2	12	8	10	250[b]
Fenuron	5 - 5000	0.9934	8	9	-3	14	-14	-6	2	2	4	13
Flufenoxuron	25 - 5000	0.9305	13	29	52	64	-10	15	6	4	7	25
Fluocinonide	750 – 5000 (4 points)	0.9922	n.d.	10	n.d.	15	n.d.	n.d.	-1	1	3	750[b]
Fluoxetine*	25 - 5000	0.9874	10	14	-7	15	-9	3	0	3	4	11
Flurochloridone	500 - 5000	0.9952	n.d.	11	n.d.	56	n.d.	n.d.	-1	1	3	100[b]
Flutamide	5 - 5000	0.9953	9	9	15	36	-1	0	4	4	4	12
Flutolanil	25 - 5000	0.9899	6	10	12	28	0	-1	5	5	4	13
Fuberidazole	25 - 5000	0.9921	8	7	-29	-17	-1	-2	4	4	4	12
Hydrochlorothiazide	75 - 5000	0.9891	6	13	38	8	-35	-17	-4	-1	3	9
Imidacloprid	250 - 5000	0.9820	n.d.	16	n.d.	13	n.d.	-1	3	4	4	100[b]
Isocarbamid	25 - 5000	0.9904	11	10	-11	10	1	-3	5	5	4	13
Isradipine	100 - 5000	0.9894	n.d.	15	n.d.	40	n.d.	-1	4	3	4	10
Josamycin	5 - 5000	0.9954	4	7	38	49	-1	0	4	4	4	13
Ketoconazole	250 - 5000	0.9953	n.d.	21	n.d.	23	n.d.	-1	0	2	3	250[b]
Ketotifen	50 - 5000	0.9845	6	8	96	48	-1	0	5	5	4	15
Levamisole	250 - 5000	0.9696	n.d.	9	n.d.	69	n.d.	-2	5	3	4	250[b]

Levocabastine	100 - 5000	0.9784	8	9	38	50	-3	-4	4	4	4	75[b]
Lidocaine*	5 - 5000	0.9875	2	7	-21	-8	-14	-7	2	3	4	14
Lincomycin	25 - 5000	0.9840	8	7	185	181	1	1	5	5	5	16
Lorazepam*	250 - 5000	0.9586	n.d.	5	n.d.	25	n.d.	-4	4	5	4	250[b]
Meclizine	100 - 5000	0.9858	7	9	-24	90	-19	-4	-3	-1	3	7
Medroxyprogesterone	100 - 5000	0.9781	22	6	-6	22	-5	-2	4	5	4	100[b]
Mefenamic acid	5 - 5000	0.9821	4	9	18	37	-34	-15	-3	-2	5	17
Memantine	25 - 5000	0.9919	6	9	-3	15	-10	-5	3	3	4	11
Mephosfolan	5 - 5000	0.9944	4	8	-1	10	-1	-2	5	5	4	13
Methylphenidate*	5 - 5000	0.9950	8	9	16	43	-1	-1	5	5	4	12
Metoprolol*	5 - 5000	0.9902	10	8	19	4	-8	-4	3	4	4	13
Nadolol	10 - 5000	0.9928	9	6	33	58	-1	-1	5	5	4	12
Nifedipine*	250 - 5000	0.9865	n.d.	22	n.d.	19	n.d.	-2	0	5	3	100[b]
Nitenpyram	250 - 5000	0.9621	n.d.	7	n.d.	-24	n.d.	-2	3	4	5	500[b]
Nordiazepam	100 - 5000	0.9793	15	9	0	44	-2	-3	5	5	4	50[b]
Norethisterone	250 - 5000	0.9241	n.d.	27	n.d.	14	n.d.	-5	3	6	6	750[b]
Nortriptyline*	5 - 5000	0.9832	11	13	40	53	-5	3	4	4	4	13
Orphenadrine	10 - 5000	0.9921	7	11	14	11	0	1	4	5	4	13
Oxamyl	250 - 5000	0.9601	n.d.	11	n.d.	7	n.d.	-2	5	4	4	250[b]
Oxycarboxin	25 - 5000	0.9934	15	12	14	56	2	-1	4	4	4	12
Picoxystrobin	50 - 5000	0.9853	18	12	25	26	-2	-2	6	5	4	25[b]
Piperophos	25 - 5000	0.9928	4	8	4	17	-1	-1	3	4	4	12
Pirenzepine	25 - 5000	0.9910	8	8	56	56	-1	-2	5	4	4	13
Pretilachlor	25 - 5000	0.9930	5	9	1	26	0	0	4	5	4	12

Prodiamine	750 – 5000 (4 points)	0.9263	n.d.	27	n.d.	-13	n.d.	n.d.	4	-1	4	500[b]
Prometon	5 - 5000	0.9907	3	10	-1	9	2	0	6	5	5	15
Prometryn	25 - 5000	0.9909	4	6	2	18	0	0	5	5	4	13
Propamocarb	25 - 5000	0.9855	5	11	28	-4	3	4	10	10	5	21
Propranolol	10 - 5000	0.9853	9	10	-12	17	-11	-5	3	3	4	14
Propazine	10 - 5000	0.9920	11	10	-27	-4	1	-1	5	5	4	13
Pymetrozine	50 - 5000	0.9714	11	8	-3	-22	1	1	6	7	6	23
Pyracarbolid	25 - 5000	0.9900	8	9	-28	-16	1	-3	5	5	4	14
Pyraclostrobin	75 - 5000	0.9896	35	18	-30	-1	-4	1	4	3	4	12
Risperidone*	5 - 5000	0.9923	7	8	84	87	0	0	4	5	4	14
Rizatriptan	50 - 5000	0.9916	11	10	139	104	0	0	4	4	4	13
Ronidazole	50 - 5000	0.9631	9	6	-7	-10	-1	6	8	9	8	37
Roxithromycin	10 - 5000	0.9950	14	12	105	101	-2	0	4	4	4	10
Salbutamol	5 - 5000	0.9808	5	7	-31	-24	-12	-6	3	2	6	21
Simazine	25 - 5000	0.9910	10	7	0	11	-2	0	5	4	4	13
Spinosyn A	10 - 5000	0.9925	13	9	557	444	-3	6	1	5	4	11
Spinosyn D	25 - 5000	0.9865	21	13	844	328	-5	5	1	4	4	10
Spiramycin	50 - 5000	0.9913	13	11	**	2473	-1	0	3	4	4	11
Sulfadimethoxine	50 - 5000	0.9855	10	10	4	5	-1	2	4	5	4	14
Sulfamerazine	1000 – 5000 (3 points)	0.9659	n.d.	22	n.d.	75	n.d.	n.d.	-2	0	3	1000[b]
Sulfamethazine*	25 - 5000	0.9937	11	7	2	6	-3	0	3	4	4	13
Sulfamethoxazole	50 - 5000	0.9885	11	13	17	20	-20	-7	0	2	4	12
Sulfamonomethoxine	25 - 5000	0.9793	17	11	25	34	1	2	6	5	5	18
Sulfapyridine	25 - 5000	0.9890	4	7	0	18	-32	-16	-3	-1	4	15
Sulfathiazole	50 - 5000	0.9910	4	8	-21	-1	-1	2	3	4	4	13
Sulfisoxazole	50 - 5000	0.9814	19	10	4	0	-1	3	5	5	4	14
Tacrine	10 - 5000	0.9941	11	10	18	16	-1	-2	5	5	4	12

Tamsulosin	10 - 5000	0.9936	10	7	39	56	1	0	4	5	4	13
Temazepam*	10 - 5000	0.9886	11	6	24	32	-15	-7	3	3	4	13
Terbutryn	5 - 5000	0.9943	3	8	1	10	-2	0	4	5	4	13
Terfenadine	10 - 5000	0.9895	11	9	73	31	-2	4	3	4	4	13
Thiacloprid	75 - 5000	0.9890	20	13	34	24	-3	0	4	4	4	13
Thiamethoxam*	75 - 5000	0.9806	19	13	-24	-7	1	-1	6	5	5	16
Thiazopyr	5 - 5000	0.9944	6	8	10	25	1	0	5	5	4	13
Timolol	5 - 5000	0.9931	5	7	-16	-3	2	0	5	5	4	15
Tramadol*	25 - 5000	0.9916	3	5	-12	1	-38	-20	-5	-3	4	13
Trimethoprim*	10 - 5000	0.9814	3	5	7	32	-36	-19	-4	-3	5	16
Valsartan	250 - 5000	0.9813	7	8	-28	39	-56	-33	-13	-9	3	9
Venlafaxine*	10 - 5000	0.9801	1	7	14	33	-48	-28	-9	-7	5	17
Verapamil*	10 - 5000	0.9917	3	12	104	101	1	1	5	5	4	14
Warfarin	250 - 5000	0.9268	n.d.	9	n.d.	24	n.d.	-2	4	4	5	500[b]
Ziprasidone	75 - 5000	0.9773	23	13	45	-29	-6	5	4	4	4	13

*SIL-IS not used for peak area ratio linearity assessment.
[a]for 250 and 750 ng/L n=3 and for 100 and 1000 ng/L n=6.
[b]LOQ taken as the lowest matrix-match calibration standards with 10 S/N signal.
[c] Peak height used.
**No peak at ultrapure water sample detected.
n.d.: not detected or signal < 5 S/N.

Table A.11Method performance for effluent wastewater matrix based on ICH guidelines.[231]

132 analytes (19 IS)	**Range (ng/L)**	**Linearity (n≥5) R^2**	**Peak area/height precision (%RSD, n=6)**		**Matrix Effect (%CV, n=6)**		**Inaccuracy (%CV, n≤3)**				**LOD (ng/L)**	**LOQ (ng/L)**
			100 ng/L	**1000 ng/L**	**100 ng/L**	**1000 ng/L**	**100 ng/L[a]**	**250 ng/L[a]**	**750 ng/L[a]**	**1000 ng/L[a]**		
2-(Thiocyanomethylthio) benzothiazole	75 - 5000	0.9584	20	10	-2	-17	1	4	6	6	6	100[b]

Acetamiprid	25 - 5000	0.9865	3	8	1	-19	-16	-5	2	3	4	12
Alprazolam	10 - 5000	0.9941	13	9	12	-16	-3	0	4	4	4	12
Ametryn	5 - 5000	0.9937	4	9	3	-15	-4	1	5	5	4	12
Amiodarone	25 - 5000	0.9410	23	14	-33	-53	-4	18	-2	2	6	19
Amitriptyline*	10 - 5000	0.9872	14	21	77	10	0	9	1	7	5	16
Amlodipine	25 - 5000	0.9274	12	15	166	68	-1	23	3	2	6	19
Antipyrine	50 - 5000	0.9849	15	11	12	-13	-1	2	5	5	4	75[b]
Atorvastatin	50 - 5000	0.9822	15	11	4	-18	-4	2	6	4	4	50[b]
Atrazine	10 - 5000	0.9911	5	10	2	-20	-1	2	5	5	4	14
Azelnidipine	25 - 5000	0.9130	4	10	79	-51	-9	19	0	4	7	25
Azithromycin	50 - 5000	0.9833	14	10	615	329	-4	4	4	4	4	11
Azoxystrobin	25 - 5000	0.9888	5	9	-1	-17	-1	3	5	5	4	14
Benoxacor	250 - 5000	0.9659	n.d.	16	n.d.	-19	n.d.	1	3	4	4	500[b]
Bensulide	50 - 5000	0.9857	23	11	-3	-16	2	3	4	4	4	75[b]
Benzatropine	5 - 5000	0.9907	5	9	112	22	-4	5	3	4	4	11
Betaxolol*	50 - 5000	0.9830	10	7	7	-11	2	1	3	6	4	14
Bezafibrate	100 - 5000	0.9681	40	16	1	-17	-4	2	5	5	5	250[b]
Bisoprolol	10 - 5000	0.9928	7	8	11	-9	-10	-2	3	3	4	12
Bupropion	5 - 5000	0.9950	6	8	-4	-19	-3	1	5	4	4	12
Buspirone	5 - 5000	0.9949	4	7	8	-14	-2	1	4	5	4	12
Carazolol	5 - 5000	0.9935	9	12	8	-6	-3	2	4	5	4	11
Carbamazepine	5 - 5000	0.9936	5	9	27	-15	-25	-10	0	1	4	15
Carbamazepine epoxide	10 - 5000	0.9892	12	5	19	-16	-12	-5	3	3	4	12
Carboxine	25 - 5000	0.9910	7	8	9	-12	-3	4	4	4	4	13
Carfentrazone-ethyl	250 - 5000	0.9816	n.d.	25	n.d.	-21	n.d.	-1	3	4	3	500[b]
Celecoxib*	50 - 5000	0.9784	6	10	12	-20	1	0	5	7	4	13
Chloramphenicol	500 - 5000	0.9416	n.d.	31	n.d.	-23	n.d.	n.d.	6	1	4	250[b]

Cilazapril	10 - 5000	0.9878	13	8	18	-11	-2	2	5	5	4	13
Citalopram	10 - 5000	0.9811	6	8	40	8	-16	-4	1	2	5	18
Clarithromycin*	10 - 5000	0.9913	15	16	35	-4	2	0	3	6	4	14
Clodinafop-propargyl	50 - 5000	0.9855	8	9	0	-22	-2	3	4	5	4	75[b]
Clofibric acid	250 - 5000	0.9802	n.d.	17	n.d.	-13	n.d.	-1	3	4	4	250[b]
Clopidogrel	5 - 5000	0.9934	5	10	2	-15	-2	1	4	4	4	13
Clothianidin	75 - 5000	0.9779	23	15	21	-21	0	2	6	6	5	75b
Clozapine	5 - 5000	0.9929	3	8	92	19	-5	1	4	5	4	11
Cycloxydim	25 - 5000	0.9812	12	9	15	-17	-1	5	6	5	5	16
Cyclouron	50 - 5000	0.9861	23	7	-5	-15	-2	2	5	4	4	75[b]
Cyromazine	25 - 5000	0.9778	14	7	-18	-37	2	3	7	6	5	20
Diazepam*	50 - 5000	0.9868	10	12	-4	-16	1	0	4	5	4	50[b]
Diclofenac	50 - 5000	0.9838	14	9	-10	-14	-32	-14	-2	0	4	11
Diflubenzuron	50 - 5000	0.9896	14	10	8	-18	-4	0	4	4	4	50[b]
Dimethametryn	5 - 5000	0.9941	3	7	1	-16	-3	0	5	5	4	12
Dimethomorph[c]	50 - 5000	0.9853	10	9	8	-19	-2	3	5	4	4	100[b]
Diphenhydramine	5 - 5000	0.9850	3	7	25	-3	-12	-2	3	3	5	16
Enalapril	500 - 5000	0.9787	n.d.	23	n.d.	1	n.d.	n.d.	3	2	3	500[b]
Famoxadone	5 - 5000	0.9953	5	11	-1	-18	-2	2	4	5	4	13
Fenoxaprop-ethyl	10 - 5000	0.9904	12	11	-4	-10	1	3	5	4	4	14
Fenuron	10 - 5000	0.9841	5	7	1	-18	-15	-5	3	3	4	10
Flufenoxuron	25 - 5000	0.9823	23	15	8	-34	-5	10	3	1	4	14
Fluocinonide	500 - 5000	0.9771	n.d.	19	n.d.	3	n.d.	n.d.	2	2	4	750[b]
Fluoxetine*	5 - 5000	0.9851	16	20	120	20	-2	8	1	6	4	13
Flurbiprofen	500 - 5000	0.9490	53	26	75	-17	-23	-6	5	3	4	1000[b]
Flurochloridone	500 - 5000	0.9962	n.d.	27	n.d.	5	n.d.	n.d.	1	4	3	500[b]
Flutamide	25 - 5000	0.9893	4	8	-4	-17	-1	1	5	4	4	12

Flutolanil	25 - 5000	0.9892	9	10	-1	-16	0	2	5	5	4	14
Fuberidazole	5 - 5000	0.9951	4	9	1	-17	-3	1	5	4	4	12
Hydrochlorothiazide	250 - 5000	0.9968	10	14	-88	-30	-41	-21	-6	-4	3	6
Imidacloprid	75 - 5000	0.9786	15	11	27	-15	-3	2	6	5	5	100[b]
Isocarbamid	50 - 5000	0.9910	10	11	1	-19	-4	1	4	4	4	50[b]
Isradipine	250 - 5000	0.9680	n.d.	21	n.d.	-11	n.d.	0	4	5	4	100[b]
Josamycin	5 - 5000	0.9934	6	10	20	-4	-4	2	5	4	4	11
Ketoconazole	25 - 5000	0.9906	25	14	53	15	3	4	4	4	4	12
Ketotifen	5 - 5000	0.9907	5	10	50	10	-4	2	4	4	4	11
Levamisole	25 - 5000	0.9774	36	12	9	-9	5	7	5	5	5	16
Levocabastine	50 - 5000	0.9850	17	11	4	-18	-2	3	5	4	4	50[b]
Lidocaine*	5 - 5000	0.9866	7	9	4	-6	-7	-6	1	4	5	18
Lincomycin	5 - 5000	0.9940	4	7	20	-1	-1	2	5	5	4	14
Lorazepam*	75 - 5000	0.9831	17	11	5	-14	1	-3	2	5	6	75[b]
Meclizine	5 - 5000	0.9889	4	10	125	12	-4	6	2	3	4	12
Medroxyprogesterone	50 - 5000	0.9853	14	11	4	-13	-3	1	5	5	4	75[b]
Mefenamic acid	10 - 5000	0.9889	18	12	-36	-20	-19	-8	2	1	4	12
Memantine	5 - 5000	0.9944	8	8	6	-14	-9	-1	3	4	4	13
Mephosfolan	10 - 5000	0.9946	4	8	0	-14	-3	0	4	4	4	12
Methylphenidate*	5 - 5000	0.9955	5	9	11	-13	0	-1	3	6	4	12
Metoprolol*	10 - 5000	0.9916	9	9	6	-13	-4	-4	3	5	4	12
Nadolol	5 - 5000	0.9942	7	9	-4	-19	-2	2	5	4	4	12
Nifedipine	500 - 5000	0.9544	n.d.	44	n.d.	-20	n.d.	n.d.	5	1	3	250[b]
Nitenpyram	50 - 5000	0.9830	9	9	6	-12	-1	3	6	4	5	75[b]
Nordiazepam	50 - 5000	0.9809	12	12	12	-10	1	4	6	6	5	50[b]
Norethisterone	500 - 5000	0.9802	n.d.	13	n.d.	-14	n.d.	-1	2	4	4	500[b]
Nortriptyline*	5 - 5000	0.9823	10	17	95	11	-3	6	1	7	4	12

Orphenadrine	5 - 5000	0.9949	3	9	34	-2	-4	2	4	4	4	11
Oxamyl	25 - 5000	0.9888	19	8	11	-14	-7	-1	5	3	4	10
Oxycarboxin	10 - 5000	0.9942	6	8	2	-10	-2	2	4	4	4	12
Picoxystrobin	25 - 5000	0.9905	7	12	6	-16	-2	3	5	5	4	13
Piperophos	10 - 5000	0.9933	7	10	1	-15	-1	2	4	5	4	13
Pirenzepine	5 - 5000	0.9942	8	8	27	-7	-4	1	4	4	4	11
Pretilachlor	5 - 5000	0.9934	5	7	1	-15	-1	2	5	4	4	14
Prodiamine	250 - 5000	0.9738	n.d.	16	n.d.	-23	n.d.	1	2	3	4	250[b]
Prometon	10 - 5000	0.9918	5	7	0	-15	-2	1	5	5	4	12
Prometryn	5 - 5000	0.9925	5	10	-2	-16	-3	3	4	5	4	12
Propamocarb	25 - 5000	0.9912	3	10	-19	-20	-12	-3	3	3	4	11
Propanolol	10 - 5000	0.9940	4	5	2	-12	-11	-2	2	2	4	12
Propazine	25 - 5000	0.9883	6	9	-6	-14	1	3	5	4	4	15
Pymetrozine	10 - 5000	0.9865	7	9	-20	-40	-1	1	6	4	5	16
Pyracarbolid	5 - 5000	0.9960	6	8	-4	-20	-2	1	4	4	4	12
Pyraclostrobin	10 - 5000	0.9906	8	13	6	-18	-3	0	5	5	4	12
Pyraflufen-ethyl	50 - 5000	0.9893	14	10	-2	-14	-1	3	4	4	4	13
Risperidone*	10 - 5000	0.9921	8	12	34	1	2	0	4	6	4	14
Rizatriptan	25 - 5000	0.9894	7	8	44	8	0	5	4	4	4	13
Ronidazole	50 - 5000	0.9867	6	10	-12	-25	-3	2	5	5	4	13
Roxithromycin	25 - 5000	0.9867	13	10	92	14	-2	4	5	4	4	13
Salbutamol	25 - 5000	0.9903	5	9	-23	-37	-7	0	4	4	4	13
Simazine	5 - 5000	0.9942	9	9	6	-18	-1	2	5	5	4	15
Spinosyn A	25 - 5000	0.9774	4	12	242	85	-3	11	3	2	4	12
Spinosyn D	50 - 5000	0.9707	19	10	55	30	-2	12	2	1	4	25[b]
Spiramycin	75 - 5000	0.9863	32	8	**	1685	-4	4	3	4	4	50[b]
Sulfadimethoxine	25 - 5000	0.9868	11	10	-8	-21	0	4	5	4	5	16
Sulfamerazine	500 - 5000	0.9532	n.d.	22	n.d.	-1	n.d.	n.d.	4	5	4	500[b]
Sulfamethazine*	5 - 5000	0.9949	10	9	12	-14	-1	-1	3	6	4	13

Sulfamethoxazole	10 - 5000	0.9921	10	10	10	-15	-10	-2	4	4	4	13
Sulfamonomethoxine	10 - 5000	0.9947	12	9	8	-16	-1	1	4	4	4	12
Sulfapyridine	5 - 5000	0.9907	7	7	14	-17	-13	-3	2	3	5	16
Sulfathiazole	50 - 5000	0.9896	4	8	-4	-22	-3	1	4	4	4	50[b]
Sulfisoxazole	25 - 5000	0.9939	19	11	-5	-19	2	1	4	4	4	13
Tacrine	10 - 5000	0.9943	6	8	5	-15	-2	1	4	4	4	12
Tamsulosin	5 - 5000	0.9928	5	7	13	-12	-2	2	5	4	4	13
Temazepam*	10 - 5000	0.9831	4	7	12	-15	-9	-6	2	5	5	19
Terbutryn	5 - 5000	0.9922	4	11	-3	-16	-3	3	5	4	4	13
Terfenadine	5 - 5000	0.9936	6	9	105	22	-4	5	3	4	4	12
Thiacloprid	25 - 5000	0.9916	7	10	1	-17	-2	2	4	5	4	13
Thiamethoxam*	25 - 5000	0.9943	26	19	-1	-15	0	0	2	5	4	13
Thiazopyr	10 - 5000	0.9930	6	10	3	-16	-2	2	5	5	4	12
Timolol	5 - 5000	0.9946	5	8	1	-16	-2	2	4	4	4	13
Tramadol*	5 - 5000	0.9852	3	6	5	-16	-25	-18	-5	0	9	56
Trimethoprim*	5 - 5000	0.9878	6	8	25	-5	-25	-15	-1	2	4	15
Valsartan	25 - 5000	0.9847	26	14	47	-5	-18	5	5	4	4	12
Venlafaxine*	5 - 5000	0.9906	10	10	-3	-17	-33	-26	-13	-8	10	69
Verapamil*	5 - 5000	0.9918	6	13	39	5	0	1	4	6	4	13
Warfarin	25 - 5000	0.9894	19	10	1	-11	0	1	6	4	4	13
Ziprasidone	10 - 5000	0.9925	4	6	11	-12	-2	3	4	4	4	12

*SIL-IS used for peak area ratio linearity assessment.
[a]for 250 and 750 ng/L n=3 and for 100 and 1000 ng/L n=6.
[b]LOQ taken as the lowest matrix-match calibration standards with 10 S/N signal.
[c] Peak height used.
**No peak at ultrapure water sample detected.
n.d.: not detected or signal < 5 S/N.

Appendix F: CECs occurrence and frequency

Table A.12 Occurrence (average ± SD for n=3 replicates in ng/L) and frequency (%) of CECs in surface waters for both rural and urban area using SPE and DI with LC-MS/MS analysis.

Analyte	Rural												Frequency (%)
	October	November	December	January	February	March	April	May	June	July	August	September	
Amoxicillin	-	-	-	<LOQ	-	-	-	-	-	-	-	-	8
Benzophenone-4	-	<LOD	-	-	-	-	<LOD	-	<LOD	<LOQ	2 ± 15	-	17
Ciprofloxacin	-	-	-	-	-	-	-	<LOD	-	-	-	-	0
E1	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	8
E2	detected	-	-	detected	detected	-	detected	detected	detected	detected	detected	detected	75
EE2	-	-	-	detected	detected	detected	-	detected	-	-	-	-	33
Erythromycin	-	<LOD	-	-	-	-	<LOD	<LOD	-	<LOD	-	-	0
Fenuron	45 ± 27	57 ± 7	39 ± 7	47 ± 18	54 ± 11	33 ± 5	76 ± 3	52 ± 12	80 ± 10	67 ± 12	56 ± 28	56 ± 1	100
Lidocaine	-	<LOD	<LOD	-	<LOD	<LOD	<LOD	<LOD	-	-	-	-	0
Octinoxate	<LOQ	<LOQ	<LOQ	5 ± 27	<LOD	<LOQ	<LOQ	<LOD	<LOD	5 ± 55	10 ± 32	10 ± 4	75
Octocrylene	5 ± 67	3 ± 39	2 ± 17	6 ± 60	2 ± 56	5 ± 74	8 ± 77	<LOQ	2 ± 30	12 ± 40	11 ± 41	14 ± 23	100
Propamocarb	47 ± 9	51 ± 4	49 ± 7	51 ± 9	99 ± 6	62 ± 8	62 ± 7	60 ± 8	53 ± 7	55 ± 3	54 ± 8	43 ± 11	100
Tramadol	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	8
Triclosan	<LOD	<LOD			<LOQ	<LOD			<LOD	<LOD	<LOD	<LOD	8
Venlafaxine	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	0
Verapamil	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
Analyte	**Urban**												**Frequency (%)**
	October	**November**	**December**	**January**	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	
Amoxicillin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	<LOD	<LOD	-	-	0
Benzophenone-4	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	-	25
Bisoprolol	<LOD	<LOD	-	-	-	<LOD	-	-	-	-	-	-	0

Carbamazepine	18 ± 29	19 ± 10	-	-	-	<LOQ	<LOQ	-	<LOQ	<LOQ	<LOQ	<LOQ	67
Ciprofloxacin	3 ± 23	5 ± 26	<LOD	-	-	3 ± 18	2 ± 67	-	-	-	<LOD	-	33
Citalopram	16 ± 17	13 ± 28	-	-	-	-	-	-	-	-	-	-	17
Clozapine	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	0
Diphenhydramine	<LOQ	<LOQ	-	-	-	-	-	-	-	-	-	-	17
E1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	17
EE2	-	detected	detected	detected	-	detected	-	-	-	detected	-	detected	50
Erythromycin	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD	-	33
Fenuron	62 ± 6	49 ± 8	41 ± 15	42 ± 13	54 ± 9	53 ± 6	52 ± 8	76 ± 3	43 ± 17	66 ± 9	42 ± 9	57 ± 8	100
Hydrochlorothiazide	18 ± 25	15 ± 81	-	-	-	-	-	-	-	-	-	-	17
Lidocaine	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	17
Octinoxate	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	100
Octocrylene	2 ± 68	<LOQ	2 ± 33	<LOQ	2 ± 54	2 ± 22	2 ± 35	2 ± 27	5 ± 58	3 ± 76	<LOQ	<LOQ	100
Propamocarb	26 ± 14	32 ± 10	29 ± 2	35 ± 5	134 ± 13	-	27 ± 9	24 ± 9	27 ± 4	16 ± 17	26 ± 16	-	83
Propranolol	<LOQ	<LOQ	-	-	-	-	-	-	-	-	-	-	17
Salbutamol	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	0
Tramadol	31 ± 6	29 ± 7	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOD	<LOQ	<LOD	<LOQ	<LOD	58
Triclosan	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	-	-	<LOD	<LOD	<LOD	<LOD	17
Trimethoprim	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	-	-	-	-	-	25
Venlafaxine	29 ± 10	32 ± 7	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	25
Verapamil	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0

<LOD: concentration obtained lower than the limit of detection.
<LOQ: concentration obtained lower than the limit of quantification.
detected: compound that presents a peak but coefficient of regression (R^2) are <0.90.

Table A.13 Occurrence (average ± SD for n=3 replicates in ng/L) and frequency (%) of CECs in effluent wastewater for both rural and urban area using SPE and DI with LC-MS/MS analysis.

Analyte	Rural												Frequency (%)
	October	November	December	January	February	March	April	May	June	July	August	September	
Acetamiprid	-	160 ± 6	-	-	-	-	-	-	-	-	-	-	8
Amitriptyline	-	<LOQ	-	<LOQ	<LOQ	<LOQ	17 ± 29	36 ± 8	<LOQ	<LOQ	-	-	67
Amoxicillin	<LOD	<LOD	-	-	<LOD	-	-	7 ± 46	-	<LOQ	-	<LOD	17
Atorvastatin	-	-	22 ± 38	-	19 ± 20	-	16 ± 16	31 ± 45	-	-	-	-	33
Benzophenone-4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
Bisoprolol	14 ± 8	52 ± 12	21 ± 26	22 ± 8	50 ± 14	14 ± 8	58 ± 9	166 ± 4	57 ± 7	41 ± 5	<LOQ	17 ± 13	100
Carbamazepine	46 ± 3	207 ± 12	70 ± 1	80 ± 9	51 ± 5	25 ± 15	268 ± 3	701 ± 7	161 ± 3	189 ± 5	66 ± 9	43 ± 11	100
CBZ epoxide	-	50 ± 6	32 ± 20	30 ± 9	46 ± 11	-	74 ± 17	158 ± 15	39 ± 17	53 ± 7	37 ± 7	28 ± 26	83
Ciprofloxacin	8 ± 37	<LOQ	7 ± 36	4 ± 24	4 ± 41	7 ± 43	<LOQ	<LOQ	<LOQ	7 ± 65	5 ± 97	4 ± 12	100
Citalopram	22 ± 20	173 ± 6	62 ± 20	63 ± 14	86 ± 6	40 ± 14	105 ± 6	232 ± 11	105 ± 15	141 ± 10	50± 12	48 ± 16	100
Clarithromycin[a]	-	<LOQ	-	<LOD	<LOQ	-	<LOQ	20 ± 25	<LOQ	-	-	-	42
Clopidogrel	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	58
Clozapine	-	17 ± 11	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	17 ± 5	-	-	-	-	50
Diclofenac[a]	111 ± 10	482 ± 26	142 ± 15	173 ± 9	247 ± 20	81 ± 34	277 ± 8	575 ± 10	369 ± 8	259 ± 7	165 ± 33	161 ± 16	100
Diphenhydramine	50 ± 16	82 ± 4	35 ± 16	129 ± 2	89 ± 4	22 ± 24	115 ± 11	403 ± 5	83 ± 5	127 ± 5	46 ± 10	43 ± 7	100
E1	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	-	42
E2	-	-	<LOD	-	-	-	-	-	-	-	-	-	0
EE2	-	-	detected	-	-	detected	-	detected	-	-	-	-	25
Erythromycin	-	-	-	<LOD	<LOD	-	-	<LOQ	<LOQ	-	-	<LOD	17
Fenuron	58 ± 16	86 ± 12	68 ± 9	57 ± 11	85 ± 7	57 ± 7	71 ± 8	70 ± 2	75 ± 7	87 ± 13	56 ± 3	76 ± 10	100
Fluoxetine	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	-	-	-	<LOD	17
Hydrochlorothiazide	341 ± 20	611 ± 26	313 ± 5	321 ± 6	278 ± 11	119 ± 26	493 ± 7	1067 ± 5	578 ± 7	619 ± 4	264 ± 16	319 ± 5	100
Lidocaine	<LOQ	81 ± 15	<LOQ	<LOQ	<LOQ	<LOD	20 ± 16	84 ± 11	19 ± 3	22 ± 4	<LOQ	<LOQ	92
Lincomycin	-	-	-	-	-	-	-	-	-	<LOQ	-	-	8

Mefenamic acid	-	120 ± 27	-	-	-	-	-	-	-	-	-	-	8
Memantine	22 ± 14	38 ± 24	21 ± 9	44 ± 6	46 ± 7	22 ± 16	81 ± 4	139 ± 9	75 ± 18	61 ± 19	20 ± 3	34 ± 19	100
Methylphenidate	<LOD	-	-	-	-	-	-	-	-	-	-	-	0
Metoprolol	<LOQ	<LOQ	<LOQ	14 ± 44	21 ± 9	<LOQ	22 ± 28	80 ± 17	28 ± 31	40 ± 16	13 ± 3	14 ± 3	100
Nordiazepam	-	-	-	-	-	-	-	40 ± 35	-	-	-	-	8
Nortriptyline	-	<LOD	-	<LOD	<LOD	<LOD	<LOQ	13 ± 30	<LOQ	<LOQ	<LOD	<LOD	33
Octinoxate	<LOD	<LOD	1 ± 130	<LOD	2 ± 67	<LOD	<LOD	<LOQ	<LOQ	3 ± 131	1 ± 150	<LOD	50
Octocrylene	<LOD	<LOD	1 ± 55	<LOD	1 ± 76	<LOD	<LOD	<LOQ	<LOD	1 ± 81	<LOQ	<LOD	42
Prometryn	-	-	-	-	-	-	-	-	-	<LOD	-	<LOD	0
Propamocarb	37 ± 9	46 ± 12	40 ± 15	35 ± 4	43 ± 7	51 ± 9	34 ± 19	26 ± 5	29 ± 11	25 ± 14	24 ± 14	29 ± 12	100
Propranolol	-	88 ± 4	<LOQ	21 ± 16	<LOQ	<LOQ	15 ± 9	81 ± 21	31 ± 12	74 ± 13	30 ± 17	15 ± 30	92
Salbutamol	<LOQ	24 ± 11	20 ± 15	20 ± 13	27 ± 3	<LOQ	31 ± 9	52 ± 7	32 ± 24	70 ± 6	15 ± 20	23 ± 30	100
Sulfamethoxazole	14 ± 16	33 ± 42	24 ± 16	-	-	-	33 ± 46	69 ± 14	50 ± 17	34 ± 14	21 ± 19	40 ± 45	75
Sulfapyridine	<LOQ	42 ± 17	30 ± 16	31 ± 24	35 ± 19	-	88 ± 8	318 ± 4	95 ± 7	70 ± 7	31 ± 23	66 ± 6	92
Tamsulosin	-	-	-	<LOD	<LOD	-	-	<LOD	<LOD	<LOD	-	-	0
Temazepam	32 ± 23	23 ± 13	56 ± 19	97 ± 23	86 ± 10	45 ± 11	149 ± 5	247 ± 7	128 ± 4	118 ± 3	39 ± 1	61 ± 10	100
Terbutryn	-	<LOQ	<LOQ	-	-	-	-	<LOQ	<LOQ	42 ± 15	-	20 ± 22	50
Tramadol	57 ± 22	132 ± 26	<LOQ	208 ± 25	352 ± 0	59 ± 17	330 ± 23	925 ± 11	295 ± 4	490 ± 5	115 ± 14	193 ± 4	100
Triclosan	<LOD	-	<LOQ	<LOD	1 ± 70	<LOD	<LOD	<LOD	<LOD	1 ± 79	<LOD	-	25
Trimethoprim	65 ± 11	79 ± 8	169 ± 9	191 ± 9	91 ± 6	99 ± 14	572 ± 13	987 ± 10	200 ± 14	376 ± 6	200 ± 6	157 ± 0	100
Valsartan	214 ± 28	164 ± 45	-	-	131 ± 50	111 ± 42	267 ± 19	546 ± 2	-	-	-	-	50
Venlafaxine	<LOQ	357 ± 13	<LOQ	<LOQ	100 ± 11	<LOQ	274 ± 25	529 ± 14	117 ± 11	343 ± 15	<LOQ	<LOQ	100
Verapamil	<LOD	<LOD	<LOD	-	-	-	<LOD	<LOD	-	<LOD	-	<LOD	0

Analyte	**Urban**												**Frequency (%)**
	October	**November**	**December**	**January**	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	
Acetamiprid	170 ± 5	-	92 ± 12	137 ± 1	146 ± 2	88 ± 4	151 ± 5	187 ± 2	219 ± 6	305 ± 6	210 ± 1	312 ± 4	92
Amitriptyline	<LOQ	-	-	17 ± 71	41 ± 8	<LOQ	<LOQ	<LOQ	<LOQ	18 ± 37	22 ± 75	-	75
Amoxicillin	<LOQ	-	<LOQ	-	<LOD	<LOD	-	-	-	<LOD	-	-	17
Atorvastatin	-	34 ± 14	-	-	-	-	-	-	-	-	-	-	8
Benzophenone-4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	17

Bisoprolol	46 ± 7	33 ± 8	51 ± 7	52 ± 6	23 ± 21	58 ± 4	63 ± 9	70 ± 8	55 ± 5	43 ± 12	24 ± 12	45 ± 6	100
Carbamazepine	158 ± 6	67 ± 8	153 ± 11	207 ± 3	291 ± 5	258 ± 8	289 ± 2	412 ± 5	420 ± 11	444 ± 7	287 ± 7	328 ± 5	100
CBZ epoxide	39 ± 33	36 ± 31	41 ± 19	50 ± 18	99 ± 7	56 ± 20	54 ± 11	83 ± 6	93 ± 15	92 ± 7	87 ± 5	96 ± 15	100
Ciprofloxacin	9 ± 3	<LOQ	11 ± 47	6 ± 48	8 ± 4	6 ± 5	<LOQ	<LOQ	<LOQ	7 ± 8	4 ± 39	6 ± 18	100
Citalopram	160 ± 8	69 ± 2	132 ± 6	176 ± 5	268 ± 0	168 ± 1	149 ± 4	177 ± 9	201 ± 13	192 ± 6	151 ± 3	200 ± 7	100
Clarithromycin[a]	<LOD	<LOD	16 ± 27	20 ± 26	-	<LOQ	<LOD	<LOQ	<LOD	<LOQ	<LOD	<LOQ	50
Clopidogrel	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	75
Clozapine	13 ± 5	-	14 ± 6	17 ± 17	65 ± 3	15 ± 16	<LOQ	17 ± 9	12 ± 13	21 ± 15	17 ± 8	14 ± 13	92
Diclofenac[a]	332 ± 7	170 ± 4	380 ± 13	494 ± 11	-	459 ± 10	607 ± 4	718 ± 8	660 ± 6	727 ± 5	554 ± 13	617 ± 11	92
Diphenhydramine	77 ± 14	36 ± 8	57 ± 4	93 ± 10	<LOQ	70 ± 9	65 ± 8	80 ± 13	73 ± 16	71 ± 3	54 ± 3	92 ± 7	100
E1	<LOQ	<LOQ	-	<LOQ	-	-	<LOQ	-	-	-	-	-	33
EE2	detected	detected	-	detected	detected	-	detected	detected	-	-	-	-	50
Erythromycin	-	<LOQ	<LOQ	-	-	-	-	<LOQ	<LOQ	-	<LOQ	-	42
Fenuron	66 ± 14	92 ± 10	64 ± 1	66 ± 8	53 ± 2	64 ± 5	57 ± 10	66 ± 6	59 ± 11	55 ± 9	87 ± 3	72 ± 7	100
Fluoxetine	<LOQ	-	<LOQ	<LOQ	27 ± 13	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	75
Hydrochlorothiazide	450 ± 13	347 ± 16	476 ± 5	595 ± 1	456 ± 15	569 ± 4	566 ± 11	675 ± 7	631 ± 10	685 ± 15	554 ± 16	560 ± 11	100
Lidocaine	19 ± 3	22 ± 4	<LOQ	<LOQ	55 ± 9	<LOQ	34 ± 9	101 ± 49	98 ± 9	47 ± 4	78 ± 5	107 ± 12	100
Lincomycin	<LOD	-	-	-	<LOD	<LOQ	-	<LOD	<LOQ	<LOD	-	-	17
Mefenamic acid	-	-	-	-	-	-	174 ± 26	312 ± 25	272 ± 2	398 ± 4	462 ± 6	976 ± 9	50
Memantine	25 ± 2	32 ± 33	23 ± 3	31 ± 3	37 ± 11	29 ± 20	33 ± 7	38 ± 3	38 ± 13	34 ± 9	32 ± 18	46 ± 7	100
Methylphenidate	<LOD	-	-	<LOD	<LOD	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
Metoprolol	<LOQ	<LOQ	<LOQ	14 ± 59	14 ± 8	<LOQ	<LOQ	15 ± 25	<LOQ	13 ± 17	<LOQ	13 ± 26	100
Nortriptyline	<LOQ	-	<LOQ	<LOQ	<LOQ	<LOD	-	<LOD	<LOD	<LOQ	<LOD	<LOD	42
Octinoxate	<LOQ	<LOD	<LOD	<LOD	1 ± 148	<LOQ	1 ± 122	1 ± 24	<LOD	<LOD	<LOD	<LOQ	50
Octocrylene	1 ± 69	<LOD	<LOQ	<LOD	1 ± 62	<LOQ	1 ± 81	<LOQ	<LOD	<LOD	<LOD	<LOQ	58
Prometryn	-	-	-	-	-	-	-	<LOD	-	<LOD	-	<LOD	0
Propamocarb	34 ± 8	33 ± 14	40 ± 12	35 ± 25	91 ± 10	43 ± 12	31 ± 2	38 ± 19	38 ± 23	40 ± 7	31 ± 21	35 ± 16	100
Propranolol	69 ± 5	14 ± 22	56 ± 13	75 ± 6	70 ± 12	67 ± 2	80 ± 11	112 ± 9	92 ± 8	108 ± 8	72 ± 7	95 ± 12	100
Risperidone	<LOD	<LOD	-	-	-	-	-	-	<LOD	-	-	-	0
Salbutamol	24 ± 19	21 ± 18	26 ± 20	32 ± 19	27 ± 21	18 ± 42	34 ± 10	34 ± 7	30 ± 14	32 ± 14	18 ± 9	31 ± 5	100

Simazine	-	-	-	-	-	-	-	23 ± 22	-	-	-	-	8
Sulfamethoxazole	-	14 ± 6	-	22 ± 38	77 ± 28	39 ± 30	34 ± 9	95 ± 28	66 ± 11	66 ± 29	45 ± 21	58 ± 19	83
Sulfapyridine	48 ± 4	27 ± 43	47 ± 8	38 ± 12	192 ± 8	75 ± 8	53 ± 12	148 ± 11	121 ± 15	111 ± 11	81 ± 6	92 ± 10	100
Tamsulosin	<LOD	-	-	-	<LOD	-	-	<LOD	<LOD	<LOD	-	-	0
Temazepam	21 ± 26	84 ± 8	<LOQ	30 ± 42	-	21 ± 23	22 ± 6	34 ± 16	31 ± 31	37 ± 18	23 ± 64	25 ± 13	92
Terbutryn	<LOQ	-	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	22 ± 7	<LOQ	25 ± 13	<LOQ	19 ± 12	92
Tramadol	135 ± 10	86 ± 18	118 ± 6	232 ± 74	158 ± 9	126 ± 7	132 ± 3	267 ± 14	347 ± 28	275 ± 20	180 ± 49	192 ± 15	100
Triclosan	1 ± 36	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	-	<LOQ	-	1 ± 75	67
Trimethoprim	52 ± 4	355 ± 15	112 ± 10	200 ± 49	196 ± 4	169 ± 3	171 ± 5	208 ± 11	275 ± 22	180 ± 11	174 ± 32	181 ± 9	100
Valsartan	173 ± 5	208 ± 15	232 ± 18	174 ± 31	-	110 ± 38	74 ± 8	176 ± 10	86 ± 7	52 ± 54	-	71 ± 62	83
Venlafaxine	332 ± 4	<LOQ	205 ± 11	538 ± 72	429 ± 4	268 ± 10	366 ± 7	628 ± 30	730 ± 22	872 ± 24	432 ± 46	511 ± 17	100
Verapamil	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	-	-	-	0

[a] Compound quantification obtained using DI-LC-MS/MS analytical technique.
<LOD: concentration obtained lower than the limit of detection.
<LOQ: concentration obtained lower than the limit of quantification.
detected: compound that presents a peak but coefficient of regression (R^2) are <0.90.

Table A.14 Occurrence (average ± SD for n=3 replicates in ng/L) and frequency (%) of CECs in influent wastewater for both rural and urban area using SPE and DI with LC-MS/MS analysis.

Analyte	Rural												Frequency (%)
	October	November	December	January	February	March	April	May	June	July	August	September	
Ametryn	-	-	19 ± 15	-	-	-	-	-	-	-	-	-	8
Amitriptyline	-	-	-	-	-	222 ± 3	-	-	-	-	-	-	8
Amlodipine	-	-	-	-	-	305 ± 6	-	-	-	-	322 ± 9	198 ± 30	25
Amoxicillin	3 ± 84	-	4 ± 12	-	5 ± 13	9 ± 31	5 ± 47	52 ± 80	36 ± 88	-	-	-	58
Antipyrine	885 ± 3	904 ± 6	598 ± 2	933 ± 4	958 ± 4	95 ± 6	1740 ± 1	1562 ± 5	1450 ± 6	1030 ± 1	3242 ± 2	2221 ±4	100
Atorvastatin	638 ± 7	902 ± 7	657 ± 2	519 ± 1	<LOQ	<LOQ	1283 ± 5	170 ± 13	243 ± 9	490 ± 8	479 ± 4	662 ± 9	100
Azithromycin	150 ± 10	21 ±33	109 ± 31	19 ± 18	594 ± 7	37 ± 8	200 ± 9	170 ± 14	19 ± 27	36 ± 20	493 ± 9	235 ± 15	100
Benzatropine	-	-	-	<LOQ	13 ± 30	-	-	<LOQ	-	-	-	-	25
Benzophenone-4	<LOD	<LOQ	4 ± 5	<LOQ	4 ± 26	<LOQ	8 ± 55	242 ± 95	99 ± 49	70 ± 86	9 ± 24	<LOD	83
Bisoprolol	75 ± 22	63 ± 8	50 ± 7	31 ± 10	135 ± 11	32 ± 18	58 ± 13	182 ± 4	58 ± 8	87 ± 11	206 ± 2	97 ± 10	100
Carbamazepine	275± 5	118 ± 14	188 ± 5	95 ± 8	158 ± 12	102 ± 6	1048 ± 3	951 ± 4	463 ± 2	333 ± 13	544 ± 4	940 ± 9	100
CBZ epoxide	-	<LOQ	<LOQ	<LOQ	-	-	58 ± 9	26 ± 10	206 ± 5	26 ± 15	74 ± 21	22 ± 27	75
Carboxine	-	-	-	-	-	-	-	22 ± 13	-	-	-	-	8
Ciprofloxacin	18 ± 48	4 ± 32	2 ± 43	3 ± 11	2 ± 26	<LOQ	12 ± 24	22 ± 35	3 ± 76	<LOD	<LOD	1 ± 62	83
Citalopram	305 ± 5	134 ± 10	170 ± 13	-	-	462 ± 7	-	-	-	-	533 ± 11	467 ± 11	50
Clarithromycin[a]	852 ± 2	1746 ± 3	553 ± 6	248 ± 8	877 ± 6	265 ± 7	342 ± 6	698 ± 2	398 ± 9	301 ± 8	2709 ± 5	1789 ± 5	100
Clopidogrel	19 ± 12	40 ± 8	31 ± 22	24 ± 12	24 ± 27	162 ± 8	32 ± 9	41 ± 13	40 ± 16	36 ± 16	52 ± 18	127 ± 6	100
Clozapine	13 ± 16	<LOQ	<LOQ	-	<LOQ	83 ± 6	14 ± 45	<LOQ	19 ± 25	<LOQ	26 ± 12	53 ± 7	92
Cymoxanil	-	-	-	-	-	-	479 ± 9	766 ± 4	541 ± 8	638 ± 12	576 ± 34	-	42
Cyromazine	detected	detected	detected	detected	-	-	detected	detected	detected	detected	detected	-	75
Diclofenac[a]	260 ± 6	667 ± 4	380 ± 6	316 ± 3	75 ± 24	79 ± 19	338 ± 11	442 ± 5	504 ± 5	806 ± 5	299 ± 8	423 ± 11	100
Diphenhydramine	75 ± 10	32 ± 11	35 ± 12	30 ± 11	101 ± 13	154 ± 14	17 ± 13	54 ± 6	31 ± 12	39 ± 16	190 ± 10	303 ± 7	100

E1	-	-	-	-	-	detected	-	-	-	detected	-	-	17
EE2	-	<LOD	<LOD	3 ± 22	1 ± 20	-	5 ± 39	<LOD	-	<LOD	<LOD	-	25
Erythromycin	7 ± 28	<LOD	24 ± 13	5 ± 7	30 ± 13	7 ± 30	9 ± 25	182 ± 50	99 ± 57	130 ± 84	63 ± 19	<LOD	83
Fenuron	165 ± 8	71 ± 14	84 ± 6	91 ± 8	58 ± 4	78 ± 12	66 ± 9	101 ± 2	107 ± 2	178 ± 11	57 ± 10	63 ± 4	100
Fluoxetine	36 ± 36	44 ± 50	37 ± 6	30 ± 15	34 ± 31	200 ± 9	44 ± 38	34 ± 60	41 ± 17	53 ± 2	74 ± 16	48 ± 6	100
Fluochloridone	-	325 ± 15	191 ± 51	857 ± 26	-	-	1200 ± 12	599 ± 25	1175 ± 5	802 ± 5	-	-	58
Hydrochlorothiazide	280 ± 5	280 ± 1	166 ± 20	579 ± 15	85 ± 13	209 ± 22	400 ± 24	515 ± 9	496 ± 12	869 ± 0	504 ± 5	569 ± 10	100
Ketoconazole	-	-	-	-	-	319 ± 43	-	-	-	-	-	-	8
Lidocaine	95 ± 8	106 ± 7	<LOQ	-	46 ± 6	<LOQ	42 ± 1	82 ± 3	27 ± 9	43 ± 8	131 ± 2	25 ± 5	92
Mefenamic acid	197 ± 14	302 ± 12	336 ± 5	218 ± 10	-	-	59 ± 11	107 ± 12	113 ± 16	91 ± 19	126 ± 3	94 ± 6	83
Memantine	59 ± 22	26 ± 14	30 ± 22	19 ± 25	76 ± 8	80 ± 5	19 ± 29	34 ± 31	30 ± 22	32 ± 41	112 ± 7	194 ± 11	100
Metoprolol	19 ± 5	25 ± 15	<LOQ	<LOQ	37 ± 19	15 ± 34	47 ± 30	96 ± 5	33 ± 12	70 ± 3	141 ± 5	74 ± 21	100
Nordiazepam	-	-	-	-	-	-	-	35 ± 54	-	-	-	-	8
Nortriptyline	-	<LOQ	-	<LOQ	<LOQ	101 ± 2	<LOQ	<LOQ	<LOQ	<LOQ	29 ± 28	<LOQ	83
Octinoxate	4 ± 125	1 ± 86	10 ± 101	<LOQ	4 ± 132	3 ± 60	12 ± 86	20 ± 90	682 ± 153	48 ± 107	2 ± 18	<LOQ	100
Octocrylene	-	-	-	-	-	-	4 ± 70	41 ± 78	182 ± 66	36 ± 13	-	-	33
Propranolol	-	-	-	-	-	32 ± 21	-	-	-	51 ± 8	70 ± 24	114 ± 12	33
Pyracarbolid	-	-	-	-	-	-	-	27 ± 17	-	-	119 ± 11	16 ± 21	25
Ronidazole	-	-	-	-	3664 ± 2	-	-	-	-	-	-	-	8
Salbutamol	257 ± 12	61 ± 13	-	68 ± 12	104 ± 16	29 ± 42	99 ± 9	88 ± 8	70 ± 16	80 ± 17	-	-	75
Sulfamethoxazole	-	103 ± 7	-	-	-	-	313 ± 1	-	100 ± 6	98 ± 27	214 ± 12	-	42
Sulfapyridine	48 ± 19	2414 ± 6	414 ± 8	717 ± 8	22 ± 20	28 ± 21	159 ± 14	282 ± 7	127 ± 10	425 ± 7	330 ± 14	265 ± 7	100
Tamsulosin	-	-	<LOQ	-	18 ± 6	-	-	<LOQ	<LOD	<LOD	15 ± 14	<LOQ	42
Temazepam	128 ± 6	311 ± 5	116 ± 3	87 ± 11	105 ± 9	32 ± 22	114 ± 14	174 ± 6	81 ± 25	111 ± 16	108 ± 9	121 ± 10	100
Terbutryn	-	<LOQ	<LOD	-	-	<LOQ	-	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	50
Timolol	-	-	-	-	25 ± 19	<LOD	-	-	-	-	-	<LOQ	17

Tramadol	377 ± 4	350 ± 2	158 ± 5	248 ± 3	745 ± 2	381 ± 3	468 ± 3	1209 ± 3	273 ± 3	673 ± 3	858 ± 3	388 ± 1	100
Triclosan	-	-	-	-	-	-	<LOQ	-	-	-	-	-	8
Trimethoprim	233 ± 5	287 ± 8	509 ± 6	100 ± 7	437 ± 6	891 ± 5	218 ± 5	692 ± 3	271 ± 2	523 ± 4	880 ± 2	601 ± 4	100
Valsartan	1485 ± 8	7579 ± 4	1427 ± 5	1317 ± 13	-	657 ± 11	6564 ± 5	2318 ± 4	878 ± 9	3367 ± 10	2709 ± 5	3533 ± 0	92
Venlafaxine	8273 ± 1	395 ± 8	576 ± 3	155 ± 7	1191 ± 2	364 ± 2	251 ± 3	560 ± 2	204 ± 3	304 ± 6	774 ± 3	552 ± 3	100

Analyte	**Urban**												**Frequency (%)**
	October	**November**	**December**	**January**	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	
Acetamiprid	-	-	-	-	-	-	-	27 ± 14	-	19 ± 10	<LOQ	19 ± 19	33
Amitriptyline	43 ± 20	<LOQ	32 ± 26	<LOQ	128 ± 5	<LOQ	24 ± 22	28 ± 44	<LOQ	36 ± 16	<LOQ	31 ± 9	100
Amlodipine	-	-	-	-	-	-	-	-	82 ± 7	-	-	111 ± 23	17
Amoxicillin	-	-	-	-	-	-	9 ± 22	-	-	-	-	-	8
Antipyrine	65 ± 19	-	41 ± 4	-	50 ± 28	57 ± 10	40 ± 14	72 ± 5	180 ± 24	47 ± 10	162 ± 1	52 ± 24	83
Atorvastatin	252 ± 7	215 ± 9	199 ± 6	155 ± 6	<LOD	116 ± 14	178 ± 3	239 ± 7	158 ± 6	179 ± 7	112 ± 6	169 ± 6	92
Atrazine	<LOQ	-	<LOQ	-	-	-	-	-	-	-	-	<LOQ	25
Azithromycin	200 ± 13	194 ± 14	187 ± 26	290 ± 12	-	146 ± 9	393 ± 10	267 ± 12	261 ± 15	243 ± 14	68 ± 21	257 ± 7	92
Benzophenone-4	4 ± 66	<LOQ	35 ± 39	10 ± 13	12 ± 46	<LOQ	12 ± 8	10 ± 73	11 ± 11	13 ± 56	7 ± 45	11 ± 16	100
Bisoprolol	146 ± 8	116 ± 4	94 ± 2	76 ± 13	26 ± 16	68 ± 17	109 ± 2	132 ± 5	125 ± 3	127 ± 3	82 ± 8	116 ± 6	100
Carbamazepine	264 ± 2	198 ± 3	170 ± 6	155 ± 7	179 ± 7	166 ± 12	249 ± 5	433 ± 2	272 ± 9	280 ± 7	191 ± 8	381 ± 6	100
CBZ epoxide	50 ± 14	39 ± 11	22 ± 31	31 ± 6	-	29 ± 6	48 ± 13	46 ± 10	68 ± 7	29 ± 7	31 ± 11	57 ± 9	92
Ciprofloxacin	3 ± 82	<LOQ	13 ± 34	4 ± 13	8 ± 85	1 ± 74	2 ± 48	3 ± 81	<LOQ	2 ± 53	<LOQ	3 ±17	100
Citalopram	316 ± 12	284 ± 7	267 ± 14	306 ± 6	427 ± 5	211 ± 10	335 ± 3	273 ± 6	196 ± 3	341 ± 2	136 ± 2	304 ± 20	100
Clarithromycin[a]	1141 ± 6	1102 ± 3	1444 ± 21	1139 ± 3	-	182 ± 6	1063 ± 2	1490 ± 6	798 ± 8	1093 ± 2	128 ± 8	596 ± 13	92
Clopidogrel	<LOQ	<LOQ	<LOQ	<LOQ	18 ± 23	<LOQ	47 ± 7	16 ± 6	<LOQ	<LOQ	<LOQ	17 ± 16	100
Clozapine	45 ± 2	<LOQ	33 ± 11	53 ± 12	28 ± 12	21 ± 21	63 ± 11	68 ± 7	37 ± 28	46 ± 7	30 ± 9	41 ± 14	100
Cymoxanil	291 ± 16	-	280 ± 13	367 ± 18	-	-	-	294 ± 27	-	-	-	-	33
Cyromazine	detected	detected	detected	detected	-	detected	detected	detected	detected	detected	detected	-	83

Diclofenac[a]	874 ± 4	756 ± 1	574 ± 6	608 ± 7	188 ± 5	648 ± 5	797 ± 5	922 ± 11	708 ± 3	753 ± 10	394 ± 11	855 ± 7	100
Diphenhydramine	139 ± 6	166 ± 1	174 ± 5	117 ± 12	25 ± 22	93 ± 10	131 ± 11	125 ± 11	102 ± 3	112 ± 4	46 ± 9	117 ± 6	100
E1	detected	detected	detected	detected	detected	detected	detected	detected	detected	detected	detected	detected	100
EE2	<LOQ	<LOQ	-	<LOQ	-	-	-	-	<LOQ	16 ± 22	-	-	42
Erythromycin	<LOD	-	<LOD	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	0
Fenuron	101 ± 12	76 ± 4	71 ± 6	51 ± 1	51 ± 7	65 ± 10	59 ± 7	51 ± 9	48 ± 4	83 ± 5	74 ± 7	35 ± 47	100
Fluoxetine	46 ± 11	34 ± 44	45 ± 32	25 ± 39	267 ± 6	32 ± 23	33 ± 7	29 ± 35	42 ± 17	42 ± 10	42 ± 27	52 ± 14	100
Hydrochlorothiazide	389 ± 6	314 ± 2	346 ± 5	294 ± 13	257 ± 13	340 ± 12	390 ± 21	477 ± 21	458 ± 18	293 ± 9	258 ± 35	428 ± 19	100
Lidocaine	134 ± 2	135 ± 3	83 ± 9	106 ± 4	113 ± 2	156 ± 3	109 ± 3	277 ± 1	90 ± 2	123 ± 5	110 ± 7	105 ± 2	100
Lincomycin	NS	NS	-	NS	NS	NS	-	-	-	-	-	NS	50
Mefenamic acid	862 ± 12	231 ± 6	405 ± 8	562 ± 7	-	381 ± 3	545 ± 3	653 ± 5	484 ± 6	545 ± 12	400 ± 3	1463 ± 3	92
Memantine	36 ± 32	45 ± 15	48 ± 14	30 ± 13	37 ± 13	33 ± 17	43 ± 6	58 ± 17	51 ± 5	45 ± 5	33 ± 21	54 ± 21	100
Methylphenidate	-	-	-	-	<LOD	-	-	<LOD	<LOD	-	<LOD	-	0
Metoprolol	27 ± 18	23 ± 2	16 ± 35	14 ± 19	<LOQ	20 ± 10	30 ± 17	32 ± 19	19 ± 40	36 ± 13	27 ± 8	25 ± 17	100
Nortriptyline	<LOQ	-	<LOQ	<LOQ	23 ± 9	<LOQ	-	<LOQ	<LOD	<LOD	<LOD	<LOQ	58
Octinoxate	4 ± 155	<LOD	<LOQ	<LOD	-	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOQ	50
Prometryn	-	-	-	-	-	-	-	<LOD	-	<LOD	-	<LOD	0
Propamocarb	<LOQ	86 ± 3	37 ± 14	<LOQ	-	<LOQ	<LOQ	46 ± 8	<LOQ	<LOQ	<LOQ	<LOQ	92
Propranolol	126 ± 10	96 ± 25	71 ± 13	86 ± 26	67 ± 3	68 ± 12	91 ± 16	110 ± 16	81 ± 2	96 ± 4	58 ± 6	108 ± 12	100
Salbutamol	61 ± 33	101 ± 25	54 ± 17	71 ± 25	40 ± 4	43 ± 2	60 ± 14	50 ± 30	59 ± 13	65 ± 15	32 ± 24	45 ± 45	100
Sulfamethoxazole	329 ± 11	-	125 ± 4	191 ± 14	-	135 ± 11	217 ± 5	443 ± 14	349 ± 5	106 ± 38	74 ± 13	205 ± 4	83
Sulfapyridine	565 ± 4	50 ± 19	163 ± 10	186 ± 1	25 ± 29	173 ± 9	327 ± 11	584 ± 6	419 ± 5	387 ± 5	250 ± 3	196 ± 6	100
Tamsulosin	<LOD	-	-	-	-	-	-	-	-	-	-	-	8
Temazepam	76 ± 7	51 ± 14	59 ± 10	40 ± 26	-	32 ± 18	49 ± 5	59 ± 5	48 ± 20	48 ± 1	36 ± 6	53 ± 14	92
Terbutryn	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	17 ± 6	19 ± 12	14 ± 21	20 ± 10	19 ± 8	26 ± 10	92
Tramadol	644 ± 1	627 ± 2	533 ± 1	306 ± 4	295 ± 3	313 ± 2	423 ± 5	610 ± 4	504 ± 2	559 ± 1	322 ± 2	503 ± 0	100
Triclosan	-	-	1 ± 27	3 ± 32	1 ± 21	-	1 ± 70	7 ± 46	-	-	-	-	42

Trimethoprim	598 ± 3	532 ± 4	375 ± 7	342 ± 4	270 ± 11	304 ± 8	357 ± 4	720 ± 4	403 ± 9	625 ± 3	206 ± 7	378 ± 7	100
Valsartan	3476 ± 7	2627 ± 3	2634 ± 3	2017 ± 3	203 ± 20	2134 ± 0	2685 ± 7	2980 ± 2	3018 ± 3	2741 ± 5	1987 ± 3	2579 ± 5	100
Venlafaxine	680 ± 5	568 ± 3	461 ± 5	427 ± 6	474 ± 3	441 ± 3	583 ± 3	642 ± 7	661 ± 4	601 ± 7	425 ± 5	667 ± 4	100
Verapamil	-	-	-	-	<LOQ	-	-	-	-	-	-	-	8

[a] Compound quantification obtained using DI-LC-MS/MS analytical technique.
<LOD: concentration obtained lower than the limit of detection.
<LOQ: concentration obtained lower than the limit of quantification.
detected: compound that presents a peak but coefficient of regression (R^2) are <0.90.
NS: no separated peaks in sample due to peak coming from matrix.

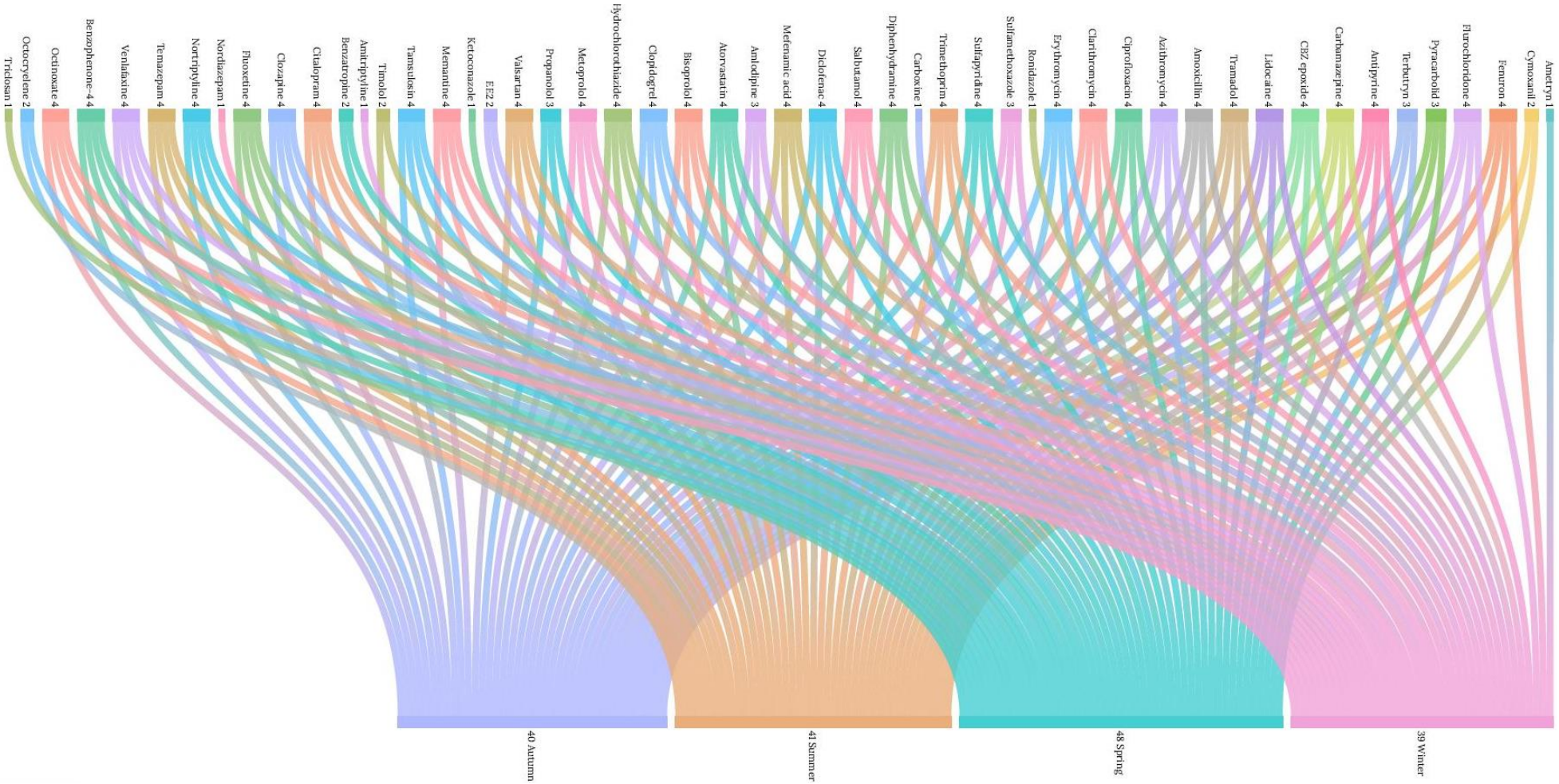


Figure A.1 Sankey diagram for influent wastewater samples investigated over a period of a year and distributed by seasons for the rural area. Compounds quantified <LOD and with coefficients of regression of R^2 <0.90 have not been included in the season comparison.

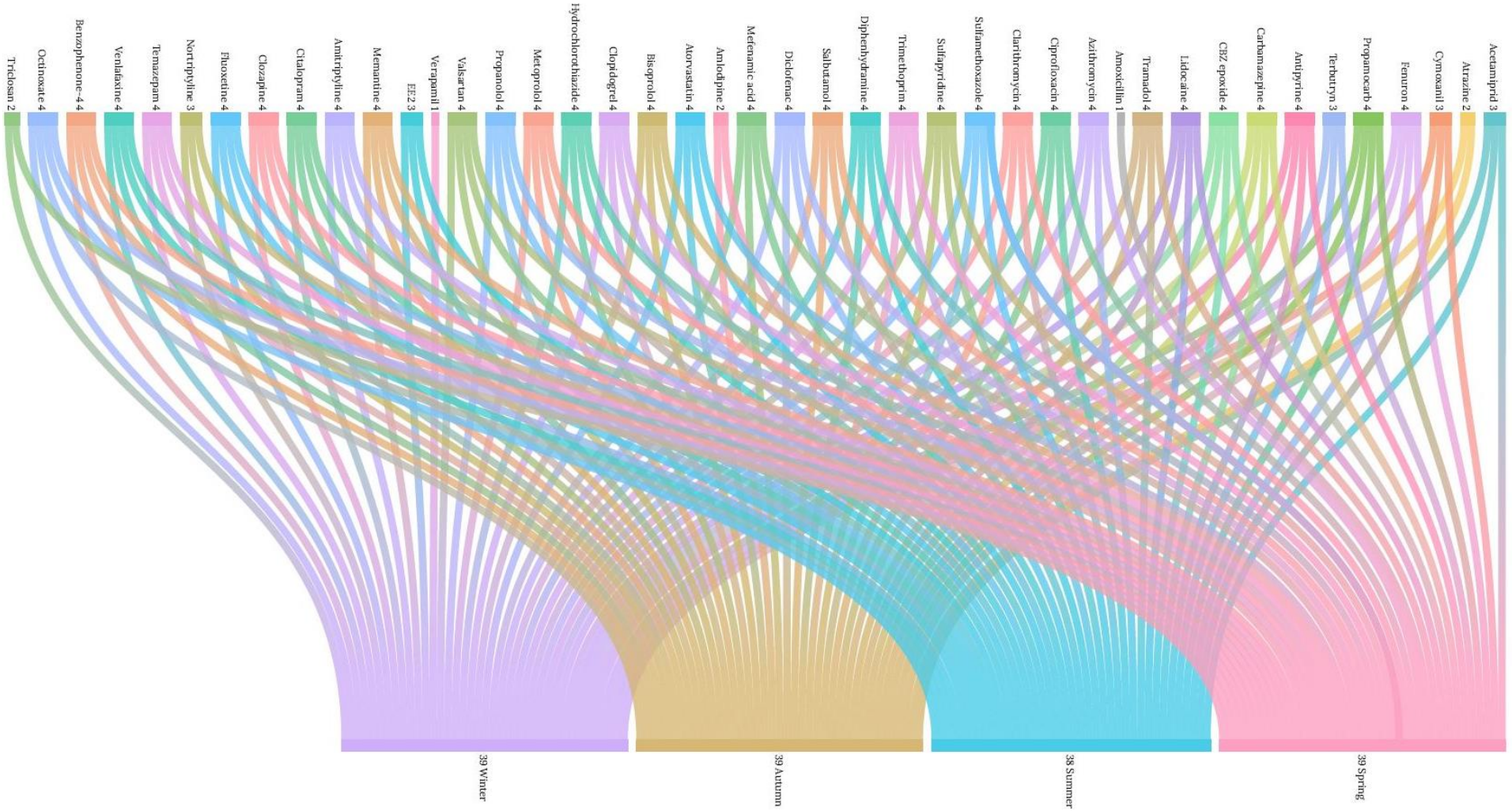


Figure A.2 Sankey diagram for influent wastewater samples investigated over a period of a year and distributed by seasons for the urban area. Compounds quantified <LOD and with coefficients of regression of R^2 <0.90 have not been included in the season comparison.

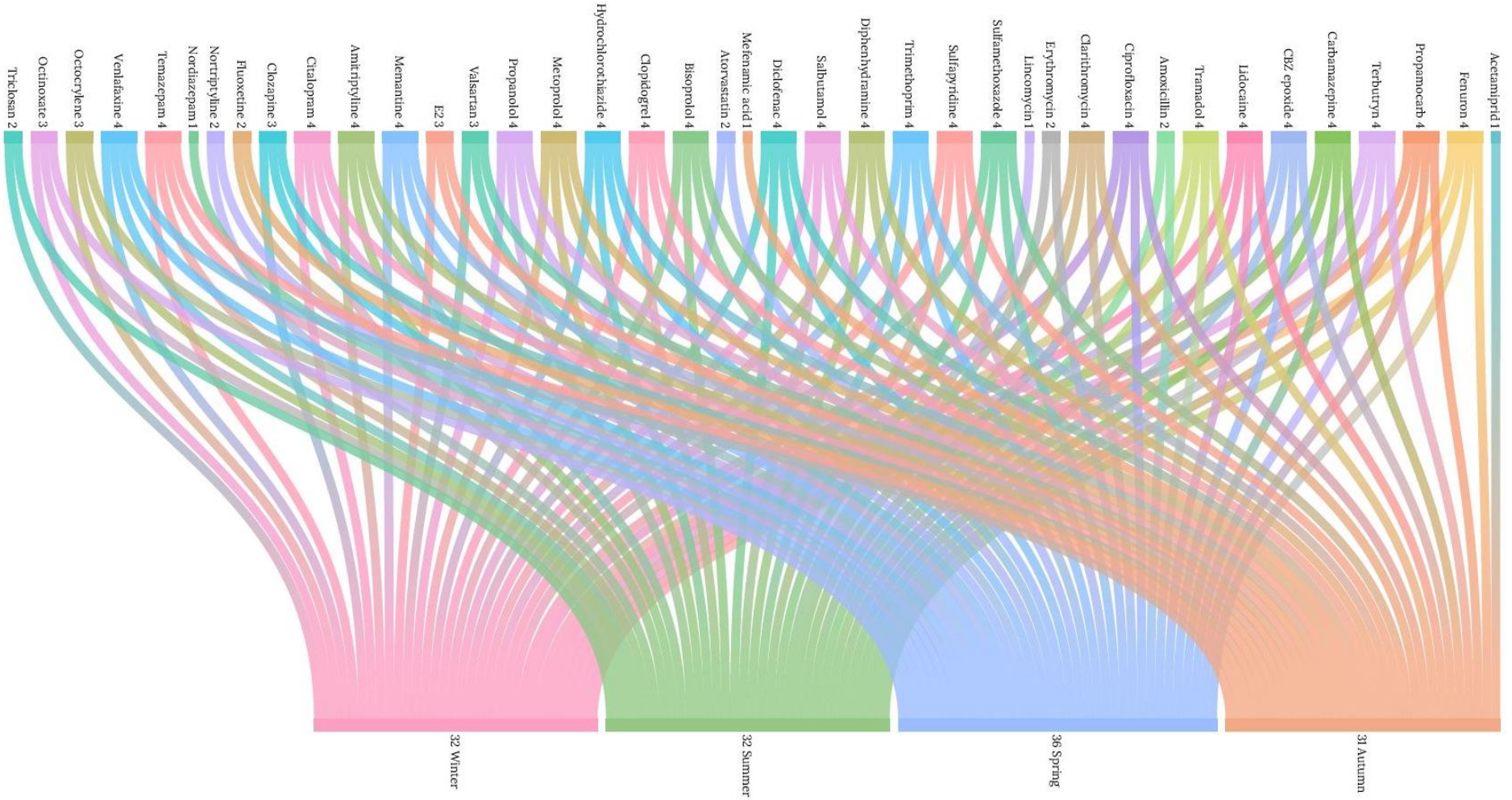


Figure A.3 Sankey diagram for effluent wastewater samples investigated over a period of a year and distributed by seasons for the rural area. Compounds quantified <LOD and with coefficients of regression of R^2 <0.90 have not been included in the season comparison.

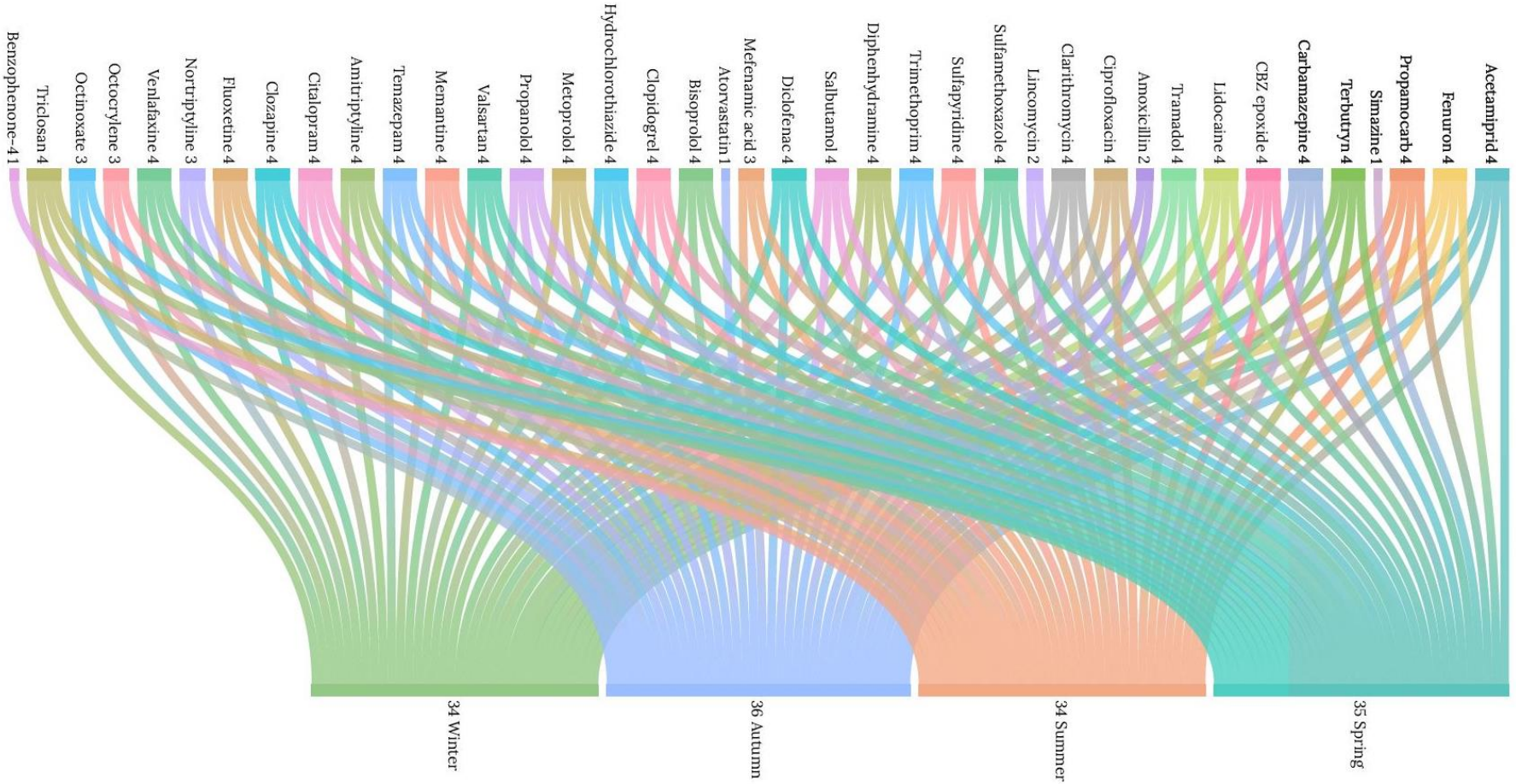


Figure A.4 Sankey diagram for effluent wastewater samples investigated over a period of a year and distributed by seasons for the urban area. Compounds quantified <LOD and with coefficients of regression of R^2 <0.90 have not been included in the season comparison.

Table A.15 Removal percentages for all samples detected across all samples.

Analyte	Rural													
	October	**November**	**December**	**January**	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**Average (±SD)**	**Range**
Acetamiprid	-	-3818	-	-	-	-	-	-	-	-	-	-	-3818	-
Ametryn	-	-	79.8	-	-	-	-	-	-	-	-	-	79.8	-
Amitriptilyine	-	-50.5	-	-50.5	-50.5	96.3	-216	-558	-50.5	-50.5	-	-	-116 (±197)	-558 – 96
Amlodipine	-	-	-	-	-	98.0	-	-	-	-	98.1	97.0	98 (±1)	97 – 98
Amoxicillin	79.6	36.5	66.7	-	86.0	85.0	72.6	86.4	96.0	-92.5	-	36.5	55 (±56)	-93 – 96
Antipyrine	99.5	99.5	99.2	99.5	99.5	95.3	99.7	99.7	99.7	99.6	99.9	99.8	99 (±1)	95 – 100
Atorvastatin	99.3	99.5	96.7	99.2	-151	44.7	98.8	82.0	98.3	99.1	99.1	99.4	72 (±72)	-151 – 100
Azithromycin	97.4	81.6	96.4	79.7	99.3	89.5	98.0	97.7	78.7	89.2	99.2	98.3	92 (±8)	79 – 99
Benzatropine	-	-	-	40.2	70.1	-	-	40.2	-	-	-	-	50 (±17)	40 – 70
Benzophenone-4	-2.93	66.0	84.5	66.0	85.5	66.0	92.3	99.7	99.4	99.1	93.2	-2.93	70 (±37)	-3 – 100
Bisoprolol	81.0	17.8	58.0	27.4	63.3	54.2	0.39	9.06	2.25	53.3	97.2	82.7	46 (±33)	0 – 97
Carbamazepine	83.3	-75.7	62.7	15.2	67.7	75.7	74.4	26.3	65.2	43.2	87.9	95.4	52 (±47)	-76 – 95
CBZ epoxide	-	-651	-371	-342	-999	-	-26.3	-506.4	81.1	-103	49.8	-26.8	-289 (±352)	-999 – 81
Carboxine	-	-	-	-	-	-	-	81.2	-	-	-	-	81	-
Ciprofloxacin	56.3	52.4	-207	-37.0	-96.1	-1070	83.9	91.3	40.5	-3559	-2555	-241	-620 (±1201)	-3559 – 91
Citalopram	92.9	-29.2	63.3	-1477	-2078.5	91.4	-2538	-5754	-2562	-3461	90.7	89.8	-1456 (±1888)	-5755 – 93
Clarithromycin	99.5	99.6	99.2	99.1	99.2	98.4	98.0	97.2	98.3	98.6	99.8	99.8	99 (±1)	97 – 100
Clopidogrel	88.7	83.5	93.3	72.8	72.3	98.7	79.0	83.9	83.5	81.5	96.0	98.4	86 (±9)	72 – 99
Clozapine	70.6	-199	2.37	-48.4	2.37	97.7	60.0	-188	79.6	33.6	85.2	92.7	7 (±104)	-199 – 98
Cymoxanil	-	-	-	-	-	-	100	100	100	100	100	-	100 (±1)	-
Cyromazine[a]	25.8	25.8	25.8	25.8	-	-	25.8	25.8	25.8	25.8	25.8	-	25.8 (±0)	-
Diclofenac	57.4	27.8	62.7	45.2	-230	-3.24	18.2	-30.2	26.8	67.9	44.6	61.9	12 (±82)	-229 – 68
Diphenhydramine	32.9	-158	1.44	-332	11.9	85.8	-572	-648	-170	-223	75.6	85.7	-151 (±254)	-648 – 86
E1[a]	65.7	65.7	65.7	65.7	88.7	92.5	88.7	65.7	88.7	92.5	88.7	-	79 (±13)	66 – 93

E2[a]	-	-	-139	-	-	-	-	-	-	-	-	-	-139	-
EE2[a]	-	-724	-1147	41.0	-309	-524	59.7	-1147	-	-724	-724	-	-578 (±444)	-1147 – 60
Erythromycin	86.1	-100	95.8	90.1	98.3	85.1	89.2	99.2	98.5	99.2	98.4	0.14	70 (±60)	-100 – 99
Fenuron	64.7	-20.8	19.0	37.1	-47.6	26.8	-7.23	31.0	30.0	51.1	0.76	-21.2	14 (±33)	-47 – 65
Fluoxetine	93.9	85.3	94.1	92.7	93.6	98.9	95.0	81.0	89.5	91.9	94.1	95.5	92 (±5)	81 – 99
Flurochloridone	-	99.2	98.6	99.7	-	-	99.8	99.6	99.8	99.7	-	-	99 (±0)	98 – 100
Hydrochlorothiazide	-21.9	-118	-88.3	44.5	-228	43.1	-23.4	-107	-16.6	28.7	47.6	43.9	-33 (±86)	-228 – 48
Ketoconazole	-	-	-	-	-	98.7	-	-	-	-	-	-	99	-
Lidocaine	90.6	23.9	-24.9	-98.4	80.5	64.5	52.6	-3.1	28.3	48.7	93.2	64.3	35 (±55)	-98 – 93
Mefenamic acid	97.9	60.1	98.8	98.1	-	-	93.1	96.2	96.4	95.5	96.8	95.6	93 (±12)	60 – 99
Memantine	62.5	-44.1	29.7	-134	39.9	73.1	-317	-312	-146	-92.9	82.3	82.4	-56 (±146)	-317 – 82
Methylphenidate	49.4	-	-	-	-	-	-	-	-	-	-	-	49	-
Metoprolol	66.6	75.4	6.46	-110	41.9	57.3	52.8	16.2	15.6	42.9	90.8	80.5	36 (±54)	-110 – 91
Nordiazepam	-	-	-	-	-	-	-	-13	-	-	-	-	-13	-
Nortriptyline	-	66.0	-	66.0	66.0	97.9	1.27	-111	1.27	1.27	92.5	66.0	35 (±63)	-111 – 98
Octinoxate	97.5	89.5	88.8	68.1	55.8	96.9	99.2	98.7	100	93.9	57.9	68.1	85 (±17)	56 – 100
Octocrylene	49.3	49.3	-575	49.3	-485	49.3	97.7	99.3	100	96.7	-53.7	49.3	-40 (±234)	-575 – 100
Prometryn	-	-	-	-	-	-	-	-	-	51.7	-	51.7	52 (±0)	52
Propamocarb	-577	-749	-642	-534	-694	-838	-516	-373	-431	-352	-345	-426	-540 (±164)	-838 – -345
Propranolol	-	-3952	-183	-867	-183	81.0	-595	-3631	-1332	-46.9	57.6	87.0	-961 (±1470)	-3952 – 87
Pyracarbolid	-	-	-	-	-	-	-	85.2	-	-	96.7	75.1	85.7 (±11)	75 – 97
Ronidazole	-	-	-	-	99.9	-	-	-	-	-	-	-	100	-
Salbutamol	97.5	61.1	-260	70.7	74.0	77.7	68.7	40.8	54.4	13.1	-173	-310	-15.4 (±145)	-310 – 98
Sulfamethoxazole	-249	68.3	-494	-	-	-	89.3	-1636	50.0	64.8	90.3	-919	-326 (±602)	-1636 – 90
Sulfapyridine	83.7	98.3	92.7	95.6	-56.7	83.7	44.6	-13.0	25.2	83.5	90.6	75.2	59 (±50)	-57 – 98
Tamsulosin	-	-	38.3	50.3	88.3	-	-	69.2	0.530	0.530	73.3	38.3	45 (±32)	0.5 – 88
Temazepam	75.4	92.5	51.8	-11.7	18.2	-41.4	-30.6	-41.4	-57.4	-5.90	64.1	49.8	14 (±52)	-57 – 93

Terbutryn	-	-5.47	-230	-	-	33.3	-	-230	-5.47	-555	33.3	-209	-146 (±203)	-555 – 33
Timolol	-	-	-	-	84.1	-80.2	-	-	-	-	-	46.6	16.8 (±86)	-80 – 84
Tramadol	85.0	62.4	82.3	16.2	52.8	84.5	29.3	23.5	-7.94	27.2	86.6	50.4	49 (±32)	-8 – 87
Triclosan	63.5	-	-10.7	63.5	-129	63.5	75.9	63.5	63.5	-123	63.5	-	19 (±80)	-129 – 76
Trimethoprim	72.2	72.5	66.8	-90.7	79.2	88.9	-162	-42.7	26.0	28.1	77.3	73.8	24 (±81)	-162 – 89
Valsartan	85.6	97.8	99.7	99.7	-3687	83.1	95.9	76.4	99.5	99.9	99.8	99.9	-221 (±1092)	-3687 – 100
Venlafaxine	99.6	9.48	94.0	77.9	91.6	90.6	-9.35	5.43	42.8	-12.8	95.6	93.8	57 (±46)	-13 – 100
Verapamil	51.8	51.8	51.8	-	-	-	51.8	51.8	-	51.8	-	51.8	52 (±0)	-

Analyte	**Urban**												**Average (±SD)**	**Range**
	October	**November**	**December**	**January**	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**		
Acetamiprid	-4043	-	-2138	-3245	-3467	-2049	-3586	-650	-5249	-1119	-741	-1149	-2494 (±1523)	-5249 – -650
Amitriptyline	81.0	55.8	85.1	-58.3	68.3	24.7	65.4	70.6	24.7	48.5	-106	100	38 (±61)	-106 – 100
Amlodipine	-	-	-	-	-	-	-	-	92.7	-	-	94.7	94 (±1)	93 – 95
Amoxicillin	-92.5	-	-92.5	-	36.5	36.5	84.5	-	-	36.5	-	-	2 (±75)	-93 – 85
Antipyrine	93.1	-	88.9	-	91.0	92.2	88.7	93.8	97.5	90.4	97.2	91.4	93 (±3)	89 – 98
Atorvastatin	98.3	84.1	97.9	97.3	-82.5	96.4	97.6	98.2	97.3	97.6	96.2	97.5	82 (±52)	-83 – 98
Atrazine	44.9	-	44.9	-	-	-	-	-	-	-	-	44.9	45 (±0)	-
Azithromycin	98.0	98.0	97.9	98.6	-	97.3	99.0	98.5	98.5	98.4	94.2	98.5	98 (±1)	94 – 99
Benzophenone-4	84.8	66.0	98.2	93.7	94.7	66.0	95.0	93.8	82.0	85.1	91.5	94.5	87 (±11)	66 – 98
Bisoprolol	68.5	71.3	45.4	32.1	10.2	14.6	41.9	46.7	55.9	66.3	70.8	60.9	49 (±21)	10 – 71
Carbamazepine	39.9	66.3	9.54	-33.3	-62.8	-55.9	-16.4	4.73	-54.7	-58.6	-50.3	13.9	-17 (±43)	-63 – 66
CBZ epoxide	21.9	9.37	-89.2	-63.0	-2278	-91.5	-12.9	-80.1	-36.5	-212	-177	-69.7	-257 (±640)	-2278 – 22
Ciprofloxacin	-216	-230	18.7	-45.5	-2.51	-313	0.330	31.5	-230	-297	-609	-72	-164 (±190)	-609 – 32
Citalopram	49.3	75.5	50.6	42.7	37.2	20.6	55.4	35.2	-2.35	43.7	-11.4	34.1	36 (±24)	-11 – 76
Clarithromycin	99.8	99.8	98.9	98.2	-	96.3	99.8	99.5	99.7	99.4	98.4	98.9	99 (±1)	96 – 100
Clopidogrel	70.9	70.9	70.9	7.59	63.3	7.59	85.9	59.3	7.59	7.59	7.59	60.0	43 (±32)	8 – 86
Clozapine	70.7	33.6	58	67.3	-132	29.8	91.0	74.8	68.2	55.0	43.0	65.7	44 (±58)	-132 – 91

Cymoxanil	100	-	100	100	-	-	-	100	-	-	-	-	100 (±0)	-
Cyromazine[a]	25.8	25.8	25.8	25.8	-	25.8	25.8	25.8	25.8	25.8	25.8	-	26 (±0)	-
Diclofenac	62.0	77.5	33.9	18.9	97.9	29.2	23.9	22.2	6.76	3.42	-38.1	27.8	30 (±36)	-38 – 98
Diphenhydramine	44.6	78.4	67.3	20.8	67.4	24.6	50.4	35.9	28.3	36.5	-17.5	21.1	38 (±26)	-18 – 78
E1	92.5	92.5	85.1	92.5	85.1	85.1	92.5	85.1	85.1	85.1	85.1	85.1	88 (±4)	85 – 93
EE2	-312	-312	-	-312	-524	-	-524	-524	-172	88	-	-	-324 (±212)	-524 – 88
Erythromycin	100	50.1	0.140	-	-	100	100	0.140	0.140	100	0.140	-	50 (±50)	0.1 – 100
Fenuron	34.5	-20.2	9.90	-27.4	-2.43	0.836	2.86	-28.6	-22.1	33.8	-17.6	-107	-12 (±37)	-107 – 35
Fluoxetine	85.9	87.2	85.5	74.2	89.8	80.0	93.4	92.5	84.4	84.4	84.7	87.5	86 (±5)	74 – 93
Hydrochlorothiazide	-15.6	-10.5	-37.7	-102.3	-77.8	-67.5	-45.4	-41.5	-37.7	-133	-115	-30.8	-60 (±40)	-133 – -11
Lidocaine	85.7	83.8	89.3	91.6	51.6	94.3	68.4	63.5	-9.30	61.8	28.8	-2.17	59 (±36)	-9 – 94
Mefenamic acid	99.5	98.2	99.0	99.3	-	98.9	68.1	52.3	43.7	27.0	-15.5	33.3	64 (±39)	-15 – 100
Memantine	29.9	28.3	51.6	-3.19	1.77	12.6	24.2	34.6	25.8	23.5	3.07	15.0	21 (±16)	-3 – 52
Methylphenidate	49.4	-	-	49.4	-1.13	-	49.4	-1.13	-1.13	49.4	-1.13	49.4	27 (±27)	-1 – 49
Metoprolol	76.8	72.5	60.6	4.01	-106	69.2	79.2	53.7	66.6	62.6	76.6	46.4	47 (±52)	-106 – 79
Nortriptyline	1.27	-	1.27	1.27	73.2	66.0	-	66.0	0.300	-190	0.300	66.0	9 (±77)	-190 – 73
Octinoxate	92.8	3.40	68.1	3.40	-314	3.40	-1522	-176	3.40	3.40	68.1	3.40	-147 (±448)	-1522 – 93
Octocrylene	-212	49.3	-53.7	49.3	-235	-53.7	-331	-53.7	49.3	49.3	49.3	-53.7	-62 (±130)	-331 – 49
Prometryn	-	-	-	-	-	-	-	3.31	-	3.31	-	3.31	3 (±0)	-
Propamocarb	-222	61.4	-5.87	-233	-1576	-312	-195	17.7	-265	-280	192	-236	-286 (±424)	-1575 – 61
Propranolol	45.1	86.0	20.8	12.5	-4.50	1.19	11.9	-1.35	-13.8	-12.4	-24.8	12.4	11 (±30)	-25 – 86
Risperidone	51.7	51.7	-	-	-	-	-	-	51.7	-	-	-	52 (±0)	-
Salbutamol	61.2	79.4	52.6	55.2	33.6	57.5	44.3	32.3	48.4	50.5	42.8	31.6	49 (±14)	32 – 79
Simazine	-	-	-	-	-	-	-	-477	-	-	-	-	-477	-
Sulfamethoxazole	98.7	-261	96.7	88.5	-1836	71.1	84.3	78.6	81.1	37.8	39.2	71.6	-112 (±552)	-1836 – 99
Sulfapyridine	91.5	46.7	71.1	79.8	-671	56.5	83.7	74.6	71.0	71.4	67.4	53.3	8 (±214)	-671 – 92
Tamsulosin	0.530	-	-	-	50.3	-	-	50.3	50.3	50.3	-	-	40 (±22)	1 – 50

Temazepam	72.6	-67.2	84.4	23.4	-	33.2	54.0	42.3	35.3	23.5	34.6	52.6	35 (±39)	-67 – 84
Terbutryn	-5.47	33.3	-5.47	-5.47	-230	-5.47	61.0	-16.4	52.9	-27.5	64.3	28.0	-5 (±78)	-230 – 64
Tramadol	79.0	86.3	77.8	24.2	46.4	59.7	68.7	56.3	31.2	50.7	44.1	61.9	54 (±19)	24 – 86
Triclosan	-200	63.5	78.4	91.6	77.4	-10.7	92.2	96.2	-	-10.7	-	-150	13 (±107)	-200 – 96
Trimethoprim	91.3	33.3	70.1	41.4	27.5	44.3	52.2	71.1	31.9	71.3	15.5	52.0	50 (±22)	15 – 91
Valsartan	95.0	92.1	91.2	91.4	98.0	94.8	97.2	94.1	97.1	98.1	99.8	97.3	96 (±3)	91 – 100
Venlafaxine	51.3	94.0	55.5	-25.9	9.51	39.2	37.2	2.33	-10.5	-45.0	-1.61	23.4	19 (±39)	-45 – 94
Verapamil	51.8	51.8	51.8	51.8	70.7	51.8	51.8	51.8	-	-	-	-	54 (±7)	52 – 71

[a]Compounds reported as qualitative data only, therefore half LOQs used as concentrations found.

Appendix G: Risk assessment results for individual compounds

Table A.16 PBT index for detected compounds and log K_{ow} values.

Analytes	log K_{ow}[a]	P	B	T	PBT Index
Acetamiprid[b]	2.55 (EST)	n.a.	0	3	3
Amitriptyline[c]	4.92 (EXP)	3	3	0	6
Amoxicillin[c]	0.97 (EST)	3	0	3	6
Atorvastatin	6.36 (EST)	3[c]	3[b]	3[b]	9
Bisoprolol[c]	1.87 (EXP)	3	0	3	6
Benzophenone-4[b]	0.37 (EST)	n.a.	0	3	3
Carbamazepine[d]	2.45 (EXP)	3	0	0	3
CBZ epoxide[b]	0.95 (EST)	n.a.	0	0	0
Ciprofloxacin[c]	0.28 (EXP)	3	0	3	6
Citalopram[e]	3.74 (EST)	3	3	3	9
Clarithromycin[d]	3.16 (EXP)	3	3	3	9
Clopidogrel[c]	3.82 (EST)	3	3	3	9
Clozapine[e]	3.23 (EXP)	3	3	3	9
Diclofenac[d]	4.51 (EXP)	3	3	3	9
Diphenhydramine[b]	3.27 (EXP)	n.a.	3	0	3
E1[b]	3.13 (EXP)	n.a.	3	3	6
E2[c]	4.01 (EXP)	3	3	3	9
EE2[c]	3.67 (EXP)	3	3	3	9
Erythromycin[c]	3.06 (EXP)	3	0	3	6
Fenuron[b]	0.98 (EXP)	n.a.	0	0	0
Fluoxetine[c]	4.05 (EXP)	3	0	3	6
Hydrochlorothiazide[d]	-0.07 (EXP)	3	0	0	3
Lidocaine[c]	2.44 (EXP)	3	0	0	3
Lincomycin[b]	0.20 (EXP)	n.a.	0	0	0
Mefenamic acid[b]	5.12 (EXP)	n.a.	3	0	3
Memantine[b]	3.28 (EXP)	n.a.	3	0	3
Methylphenidate[c]	0.20 (EXP)	3	0	3	6
Metoprolol[d]	1.88 (EXP)	3	0	0	3
Nordiazepam[b]	2.93 (EXP)	n.a.	0	0	0
Nortriptyline[c]	4.51 (EXP)	3	3	3	9
Octinoxate[b]	5.80 (EST)	n.a.	n.a.	3	0
Octocrylene[b]	6.88 (EST)	n.a.	n.a.	3	3
Prometryn[b]	3.51 (EXP)	n.a.	3	3	6
Propamocarb[b]	1.12 (EXP)	n.a.	0	3	3
Propranolol[d]	3.48 (EXP)	0	3	3	6
Risperidone[c]	3.49 (EST)	3	3	0	6
Salbutamol[c]	0.64 (EST)	3	0	3	6

Simazine[b]	2.18 (EXP)	n.a.	0	3	3
Sulfamethoxazole[c]	0.89 (EXP)	3	0	3	6
Sulfapyridine[b]	0.35 (EXP)	n.a.	0	0	0
Tamsulosin[b]	2.47 (EST)	n.a.	0	0	0
Temazepam[b]	2.19 (EXP)	n.a.	0	3	3
Terbutryn[b]	3.74 (EXP)	n.a.	3	3	6
Tramadol[b]	3.01 (EST)	n.a.	3	0	3
Triclosan[b]	4.76 (EXP)	n.a.	3	3	6
Trimethoprim[d]	0.91 (EXP)	3	0	3	6
Valsartan	3.65 (EST)	3[c]	3[b]	0[b]	6
Venlafaxine	3.28 (EST)	3[c]	3[b]	3[c]	9
Verapamil	3.79 (EXP)	3[c]	3[b]	0[b]	6

[a]log K_{ow} values predicted using EPI Suite™ version 4.1 software; where EST: estimate value and EXP: experimental value.
[b]calculated value using log K_{ow} and PNEC (Table 4.).
[c]Environmentally classified pharmaceuticals 2014-2015.[430]
[d]Medoza *et al*., 2015.[228]
[e]Environmentally classified pharmaceuticals, 2009.[431]
n.a.: not available.

Table A.17 Individual compound contribution to site risk at both matrices tested, surface water and effluent wastewater samples.

Analytes	**Contribution (%)**			
	Rural		**Urban**	
	Surface water	**Effluent**	**Surface water**	**Effluent**
Acetamiprid	n.d.	0.169	n.d.	0.390
Amitriptyline	n.d.	0.134	n.d.	0.180
Amoxicillin	0.119	0.0476	0.0354	1.72E-02
Atorvastatin	n.d.	1.63	n.d.	2.13
Benzophenone-4	1.95E-03	5.25E-05	1.18E-03	2.50E-04
Bisoprolol	n.d.	0.0109	1.18E-03	5.47E-03
Carbamazepine	n.d.	18.4	5.59	13.9
CBZ epoxide	n.d.	8.77E-04	n.d.	6.49E-04
Ciprofloxacin	2.34E-02	4.61E-02	0.322	7.43E-02
Citalopram	n.d.	1.22E-02	9.26E-03	1.67E-02
Clarithromycin	n.d.	8.67E-02	n.d.	0.105
Clopidogrel	n.d.	5.78E-03	n.d.	6.68E-03
Clozapine	n.d.	5.09E-02	6.08E-02	0.225
Diclofenac	n.d.	6.039	n.d.	9.05
Diphenhydramine	n.d.	0.214	3.44E-02	5.84E-02
E1	4.47	0.265	4.05	0.104
E2	68.0	13.0	61.9	n.d.
EE2	22.5	44.3	20.4	52.7
Erythromycin	1.86E-02	4.01E-03	5.11E-02	1.57E-03
Fenuron	0.357	3.16E-02	0.307	3.95E-02

Fluoxetine	n.d.	3.41E-02	n.d.	0.169
Hydrochlorothiazide	n.d.	6.67E-02	1.25E-02	5.10E-02
Lidocaine	n.d.	9.45E-03	n.d.	1.44E-02
Mefenamic acid	n.d.	3.68E-02	n.d.	0.354
Memantine	n.d.	3.94E-02	n.d.	1.56E-02
Methylphenidate	n.d.	8.35E-04	n.d.	9.92E-04
Metoprolol	n.d.	4.88E-03	n.d.	1.07E-03
Nordiazepam	n.d.	7.93E-03	n.d.	n.d.
Nortriptyline	n.d.	3.68E-02	n.d.	2.05E-02
Octinoxate	1.10E-02	2.60E-04	1.79E-03	1.55E-04
Octocrylene	4.05	2.78E-02	1.16	2.03E-02
Prometryn	n.d.	2.63E-02	n.d.	3.36E-02
Propamocarb	9.09E-04	3.78E-05	1.12E-03	8.05E-05
Propranolol	n.d.	0.924	0.708	1.40
Risperidone	n.d.	n.d.	n.d.	1.75E-05
Salbutamol	n.d.	9.98E-04	3.42E-04	5.80E-04
Simazine	n.d.	n.d.	n.d.	1.46E-02
Sulfamethoxazole	n.d.	1.21	n.d.	2.00
Sulfapyridine	n.d.	9.14E-02	n.d.	6.55E-02
Temazepam	n.d.	1.85	n.d.	0.755
Terbutryn	n.d.	0.604	n.d.	0.432
Tramadol	4.84E-03	5.62E-02	2.09E-02	2.50E-02
Triclosan	2.24E-02	1.49E-02	2.02E-02	2.31E-02
Trimethoprim	n.d.	3.24	2.06E-01	1.39
Valsartan	n.d.	5.15E-04	n.d.	2.58E-04
Venlafaxine	0.328	7.30	5.03	14.3
Verapamil	5.21E-03	4.20E-04	4.60E-03	4.86E-04

n.d.: Not detected.

Appendix H: International comparison of EDCs

Table A.18 Summary of MRM transitions for both negative and positive modes.

Analyte	Precursor (m/z)	Product (m/z)		t_R (min)	Polarity
Caffeine	195	138	Quan	3.76	+
		110	Qual		+
Caffeine-d_3	198	138	Quan	3.75	+
		110	Qual		+
Benzotriazole-1H	120	65	Quan	3.91	+
		92	Qual		+
Benzotriazole-1H-d_4	124	69	Quan	3.89	+
		96	Qual		+
Tris(2-chlorethyl)phosphate (TCEP)	287	98	Quan	4.34	+
		225	Qual		+
Tris(2-chloroisopropyl)phosphate (TCPP)	327	98	Quan	4.71	+
		80	Qual		+
Tris(2-butoxyethyl)phosphate (TBEP)	399	299	Quan	5.34	+
		199	Qual		+
Triphenylphosphate-d_{15}	342	160	Quan	4.97	+
		159	Qual		+
Testosterone	289	97	Quan	4.82	+
		109	Qual		+
Progesterone	315	97	Quan	5.10	+
		109	Qual		+
Progesterone-d_9	323	100	Quan	5.10	+
Caffeine $C^{13}{}_3$ (surrogate)	198	140	Quan	3.80	+
		112	Qual		+
Estrone (E1)	269	145	Quan	6.37	-
		143	Qual		-
Estrone-d_4	273	145	Quan	6.37	-
		147	Qual		-
17-β-estradiol (E2)	271	183	Quan		-
		145	Qual	6.39	-
17-β-estradiol-d_2	273	147	Quan	6.36	-
Estriol (E3)	287	171	Quan	5.18	-
		145	Qual		-
17-α-ethinylestradiol (EE2)	295	145	Quan	6.36	-
		159	Qual		-
17-α-ethinylestradiol-d_4	299	147	Quan	6.35	-
		161	Qual		-
Estriol-3-sufate	367	287	Quan	3.61	-
		171	Qual		-
Estrone-3-sulfate	349	269	Quan	4.69	-

		145	Qual		-
Methylparaben	151	92	Quan	4.63	-
		136	Qual		-
Methylparaben-d_4	154	96	Quan	4.60	-
Ethylparaben	165	92	Quan	5.29	-
		137	Qual		-
Propylparaben	179	92	Quan	5.86	-
		136	Qual		-
Benzylparaben	227	92	Quan	6.32	-
		136	Qual		-
Octylphenol	205	106	Quan	7.79	-
Octylphenol-d_{17}	222	108	Quan	7.76	-
Nonylphenol	219	106	Quan	8.01	-
Nonylphenol-d_4	223	110	Quan	8.00	-
Triclosan	286	35	Quan	7.33	-
Triclosan-d_3	289	35	Quan	7.33	-
Bisphenol A (BPA)	227	212	Quan	5.92	-
		133	Qual		-
Bisphenol A-d_4	231	216	Quan	5.91	-
Bisphenol B (BPB)	241	212	Quan	6.27	-
		211	Qual		-
Bisphenol B-d_8	249	219	Quan	6.24	-
		220	Qual		-
Bisphenol F (BPF)	199	93	Quan	5.33	-
		105	Qual		-
Bisphenol S (BPS)	249	108	Quan	4.33	-
		92	Qual		-
Bisphenol S-d_8	257	96	Quan	4.29	-
		112	Qual		-
Bisphenol AF (BPAF)	335	265	Quan	6.51	-
		197	Qual		-
Tetrabromobisphenol A (TBBPA)	542	418	Quan	7.38	-
		445	Qual		-
Ethylparaben $^{13}C_6$ (surrogate)	170	98	Quan	5.26	-
		143	Qual		-

Quan: quantification ion.
Qual: qualifier ion.

Table A.19 Recovery data (±%RSD) and limits of detection (LOD) and quantification (LOQ) for the three river matrices investigated in this study (rivers Liffey, Thames and Ter).

Analtes	Recovery ± %RSD			LOD (ng/L)			LOQ (ng/L)		
	Liffey	Thames	Ter	Liffey	Thames	Ter	Liffey	Thames	Ter
Caffeine	115 ± 6	150 ± 5	103 ± 3	7.3	9.8	5.9	24.2	32.8	19.7
Benzotriazole	101 ± 4	138 ± 8	117 ± 5	1.7	1.9	1.2	5.8	6.2	4.0
TCEP	26 ± 8	17 ± 19	19 ± 15	4.1	6.5	1.8	13.7	21.7	6.1
TCPP	79 ± 12	149 ± 14	64 ± 19	17.3	15.3	9.7	57.6	51.0	32.3
Testosterone	39 ± 12	48 ± 12	40 ± 9	0.1	0.1	0.3	0.4	0.4	0.9
Progesterone	57 ± 14	53 ± 21	68 ± 1	0.1	0.1	0.2	0.4	0.4	0.6
TBEP	78 ± 8	124 ± 19	111 ± 7	1.4	0.3	0.3	4.8	1.0	0.8
Estrone 3-sulfate	95,1*	95,1*	149 ± 0	1.2	2.2	1.0	3.9	7.2	3.3
BPS	146 ± 3	107 ± 5	138 ± 1	0.1	0.04	0.02	0.2	0.1	0.1
Estriol_3_sufate	n.r.	n.r.	124 ± 0	6.3	14.3	5.0	20.3	47.5	16.6
Methylparaben	90 ± 5	97 ± 7	62 ± 3	5.0	5.9	3.8	16.8	19.8	12.7
Estriol	74 ± 4	113 ± 10	63 ± 5	8.4	9.8	5.4	27.9	32.7	17.9
Ethylparaben	94 ± 3	96 ± 12	69 ± 4	0.6	0.8	0.5	2.1	2.6	1.8
BPF	93 ± 4	92 ± 7	78 ± 0	13.7	18.2	11.0	45.6	60.8	36.7
Propylparaben	91 ± 3	92 ± 13	61 ± 2	1.3	1.2	1.1	4.4	4.0	3.7
BPA	71 ± 4	75 ± 15	48 ± 1	10.8	9.5	5.0	36.0	31.8	16.8
BPB	106 ± 7	88 ± 7	85 ± 3	2.8	1.6	3.7	9.5	5.3	12.3
Benzylparaben	92 ± 4	80 ± 20	61 ± 3	1.2	1.2	1.1	4.1	3.9	3.6
Ethinylestradiol	98 ± 13	104,0*	78 ± 19	11.2	6,5*	11.6	37.2	21.8*	38.6
Estrone	78 ± 13	78 ± 15	72 ± 2	1.3	3.4	0.7	4.9	11.2	2.4
Estradiol	124 ± 8	103 ± 21	79 ± 4	5.3	6.3	7.4	17.7	20.9	24.6
Triclosan	75 ± 11	56 ± 0	48 ± 40	21.1	9.9	2.6	70.2	33.0	8.8
TBBPA	125*	125*	125*	0,1*	0,1*	0,1*	0,3*	0,3*	0,3*
Octylphenol	59*	59*	59*	6,0*	6,0*	6,0*	20*	20*	20*
Nonylphenol	24*	24*	24*	7.4*	7.4*	7.4*	25*	25*	25*
BPAF	108 ± 13	57 ± 20	67 ± 2	0.2	0.3	0.1	0.9	0.8	0.5

*from method development (Becker *et al*.,[385]).
n.r. = not recovered.

Table A.20 Occurrence (average for n=2 replicates in ng/L) and frequency (%) of EDCs in surface waters for the river Liffey (Ireland).

Analyte	River Liffey										Frequency (%)
	23/10/20	28/10/20	02/11/20	11/11/20	18/11/20	25/11/20	02/12/20	08/12/20	17/12/20	20/01/21	
Testosterone	3.63	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	20
Progesterone	<LOD	0.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	10
E1	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOQ	30
E2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
E1-3S	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	20
E3-3S	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
E3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
EE2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
Caffeine	73.7	338	192	128	139	102	58.2	57.8	65.9	151	100
Triclosan	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOQ	60
MeP	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	10
EtP	3.69	<LOQ	2.91	2.34	<LOQ	<LOQ	<LOQ	3.34	<LOQ	2.53	100
PrP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
BeP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
BPA	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	20
BPB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
BPF	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
BPS	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	13.0	<LOD	5.40	20
BPAF	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
OP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
NP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
Benzotriazole	75.0	74.1	102	127	90.5	131	156	218	100	125	100

TCEP	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	40
TCPP	107	187	524	150	161	277	221	293	222	322	100
TBEP	11.2	7.55	14.0	7.39	13.1	25.6	5.87	5.13	8.57	6.19	100
TBBPA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0

<LOD: concentration obtained lower than the limit of detection.
<LOQ: concentration obtained lower than the limit of quantification.

Table A.21 Occurrence (average for n=2 replicates in ng/L) and frequency (%) of EDCs in surface waters for the river Thames (UK).

Analyte	**River Thames**										**Frequency (%)**
	23/10/20	**28/10/20**	**02/11/20**	**11/11/20**	**18/11/20**	**25/11/20**	**02/12/20**	**09/12/20**	**16/12/20**	**20/01/21**	
Testosterone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	10
Progesterone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
E1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOQ	20
E2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
E1-3S	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	80
E3-3S	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
E3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
EE2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
Caffeine	331	324	259	432	274	132	130	344	246	300	100
Triclosan	35.7	40.6	37.7	36.2	<LOD	<LOD	<LOD	<LOD	75.8	<LOD	50
MeP	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOD	<LOD	40
EtP	<LOQ	<LOQ	<LOQ	<LOQ		<LOQ	19.5	12.8	7.82	<LOQ	90
PrP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
BeP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
BPA	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ	40
BPB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
BPF	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
BPS	60.0	19.2	26.7	14.0	2.87	16.4	7.73	1.87	20.8	79.3	100
BPAF	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0

OP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
NP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	10
Benzotriazole	357	301	276	186	193	173	351	340	353	258	100
TCEP	62.3	4767	268	274	1034	1033	<LOQ	<LOQ	<LOQ	116	100
TCPP	431	1065	402	445	523	692	257	469	298	559	100
TBEP	65.8	75.6	25.4	79.2	23.3	46.0	22.4	66.9	32.9	41.1	100
TBBPA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0

<LOD: concentration obtained lower than the limit of detection.
<LOQ: concentration obtained lower than the limit of quantification.

Table A.22 Occurrence (average for n=2 replicates in ng/L) and frequency (%) of EDCs in surface waters for the river Ter (Spain).

Analyte	**River Ter**										**Frequency (%)**
	21/10/20	**28/10/20**	**04/11/20**	**11/11/20**	**18/11/20**	**25/11/20**	**02/12/20**	**09/12/20**	**16/12/20**	**20/01/21**	
Testosterone	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOD	40
Progesterone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	5.4	<LOD	<LOD	<LOD	20
E1	<LOD	<LOD	30.6	<LOD	<LOD	<LOD	<LOD	15.2	30.0	<LOD	30
E2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
E1-3S	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
E3-3S	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
E3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
EE2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
Caffeine	111	705	107	429	112	105	33.6	184	199	148	100
Triclosan	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOD	50
MeP	<LOD	<LOD	<LOD	39.2	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	40
EtP	<LOQ	<LOQ	<LOQ	9.53	7.86	8.92	<LOQ	<LOQ	<LOD	<LOD	80
PrP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
BeP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
BPA	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOQ	<LOD	<LOQ	40
BPB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0

BPF	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	10
BPS	<LOD	2.61	9.31	4.68	8.24	3.62	<LOD	0.24	2.10	79.3	80
BPAF	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	36.9	<LOD	<LOD	<LOD	10
OP	<LOD	<LOD	<LOD	<LOD	26.9	<LOD	54.1	54.0	26.8	<LOD	40
NP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
Benzotriazole	129	94.7	93.5	102	86.7	94.2	50.3	62.7	117	136	100
TCEP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0
TCPP	63.9	132	71.2	117	111	<LOQ	<LOQ	<LOQ	<LOQ	47.6	100
TBEP	14.7	7.59	6.30	20.8	18.4	<LOQ	16.5	<LOQ	<LOQ	8.36	100
TBBPA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0

<LOD: concentration obtained lower than the limit of detection.
<LOQ: concentration obtained lower than the limit of quantification.

Table A.23 Aquatic ecotoxicity data and PNEC values for detected EDCs in rivers investigated, excluding E1 and triclosan which were previously calculated in Chapter 4.0.

Analytes		**Ecotoxicity data (mg/L)[b]**			**Data**	**AF**	**PNEC (ng/L)[b]**
		Green Algae	**Daphnia**	**Fish**			
Testosterone		-	-	-	-	20	**1.5**[363]
		10.7[c]	14.1[c]	31.6[c]	EC_{50}	1000	10,700
		-	-	-	-	1000	3,750[a]
Progesterone		-	-	-	-	1000	**1,000**[a]
		5.57[c]	6.78[c]	17.1[c]	EC_{50}	1000	5,570
E1-3S		**654**[c]	1310[c]	2550 [c]	EC_{50}	1000	654,000
		-	-	-	-	1000	**20,500**[a]
Caffeine		-	-	0.00001[363]	NOEC	10	**1**
		-	-	-	-	100	1,200[a]
	(pyrroles/diazoles)	**6.63**[c]	164[c]	694[c]	EC_{50}	1000	6630
	(carbonyl ureas)	**0.015**[c]	92.6[c]	329[c]	EC_{50}	1000	15
MeP		-	-	-	-	1000	**5,000**[a]
		9.6[199]	24.6[199]	-	LOEC	1000	9,600
		35.25[200]	41.23[200]	-	-	1000	35,250

	(esters)	**18.1**[c]	42.5[c]	20.4[c]	EC_{50}	1000	18,100
	(phenols)	**1.83**[c]	9.02[c]	20.8[c]	EC_{50}	1000	1,830
EtP		-	-	-	-	1000	13,300[a]
		2.5[199]	18.7[199]	34.3[199]	LOEC	1000	**2,500**
	(esters)	**8.96**[c]	22.7[c]	11.5[c]	EC_{50}	1000	8,960
	(phenols)	**0.938**[c]	5.65[c]	10.5[c]	EC_{50}	1000	938
PrP		-	-	-	-	1000	12,300[a]
		-	8.5[199]	17.5[199]	LOEC	1000	**8,500**
	(esters)	**4.41**[c]	12.21[c]	6.46[c]	EC_{50}	1000	4,410
	(phenols)	**0.476**[c]	3.51[c]	5.21[c]	EC_{50}	1000	476
BeP		-	-	-	-	1000	2,950[a]
		-	0.11[199]	-	LOEC	1000	**110**
	(esters)	**1.74**[c]	5.34[c]	3.08[c]	EC_{50}	1000	1,740
	(phenols)	**0.197**[c]	1.96[c]	2.09[c]	EC_{50}	1000	197
BPA				0.0001[363]	NOEC	10	10
		-	-	-	-	10	240[a]
		2.2[410]	3.9[410]	3.6[410]	-	1000	2,200[410]
		19.6[379]	5.00[379]	**2.00**[379]	-	1000	2,000
		-	-	-	-	-	**1**[406]
		-	-	-	EC_{50}	-	1,644,000[370]
		6.9[411]	3.9[411]	**3.6**[411]	-	1000	3,600[411]
		1.33[c]	5.24[c]	1.28[c]	EC_{50}	1000	1,330
BPB		-	-	-	-	1000	**1,350[a]**
		0.964[c]	2.33[c]	**0.695**[c]	LC_{50}	1000	695
BPF		-	-	-	-	1000	4,900[a]
		3[411]	**0.84**[411]	1.1[411]	LC_{50}	1000	**840**[411]
		22.1[410]	**0.84**[410]	1.1[410]	LC_{50}	1000	840
		1.85[c]	13.0[c]	2.52[c]	EC_{50}	1000	1,850
BPS		-	-	-	-	1000	12,900[a]
		6.9[410]	55[410]	155[410]	EC_{50}	1000	**6,900**[410]

		22.1[411]	55[411]	155[411]	EC_{50}	1000	22,100[411,379]
		6.90[c]	196[c]	21.8[c]	EC_{50}	1000	6,900
BPAF		-	-	-	-	1000	1,020[a]
		-	**0.23**[411]	0.92[411]	LC_{50}	1000	**230**[411]
		3.00[379]	**0.23**[379]	0.50[379]	LC_{50}	1000	**230**
		3.00[410]	**0.23**[410]	0.92[410]	LC_{50}	1000	**230**[410]
		1.03[c]	1.77[c]	0.605[c]	EC_{50}	1000	1,030
OP		-	-	-	-	0	**100**[a]
		-	-	-	EC_{50}	-	188,000[370]
		0.016[c]	0.299[c]	0.157[c]	EC_{50}	1000	16
NP		-	-	-	-	10	**300**[a]
		-	-	-	EC_{50}	-	79,200[370]
		0.0056[c]	0.142[c]	0.054[c]	EC_{50}	1000	5.6
Benzotriazole		-	-	-		1000	**7,770**[a]
		-	-	-	EC_{50}	-	5,490,000[370]
		8.19[c]	244[c]	40.7[c]	EC_{50}	1000	8,190
TCEP		-	-	-	-	50	**4,000**[a]
		51[420]	330[420]	90[420]	-	1000	51,000[420]
	(esters)	**60.9**[c]	135[c]	62.5[c]	EC_{50}	1000	60,900
	(esters, phosphates)	15200[c]	**0.016**[c]	8.33[c]	EC_{50}	1000	16
TCPP		-	-	-	-	50	120,000[a]
		3.59[c]	10.9[c]	6.28[c]	EC_{50}	1000	3,590
		45[420]	91[420]	**30**[420]	LC_{50}	1000	**30,000**[420]
	(esters)	**9.30**[c]	25.1[c]	13.3[c]	EC_{50}	1000	9,300
	(esters, phosphates)	89.7[c]	**0.0041**[c]	2.47[c]	LC_{50}	1000	4.1
TBEP		-	-	-	-	1000	24,000[a]
		-	75[420]	**13**[420]	LC_{50}	1000	**13,000**[420]
	(esters)	**9.49**[c]	26.1[c]	14.0[c]	EC_{50}	1000	9,490
	(esters, phosphates)	501[c]	**0.0067**[c]	3.88[c]	LC_{50}	1000	6.7

[a]NORMAN Ecotoxicology Database.

[b]Values in bold represent the ones selected for PNEC and RQ calculations.
[c]ECOSAR.

Table A.24 MEC and PNEC selected values for environmental risk classification of EDCs and their final contribution (%) to the total risk per site location.

Compounds	PNEC (ng/L)	Liffey			ΣRQ_{river} contribution (%)	Thames			ΣRQ_{river} contribution (%)	Ter			ΣRQ_{river} contribution (%)
		MEC (ng/L)	RQ	Risk		MEC (ng/L)	RQ	Risk		MEC (ng/L)	RQ	Risk	
Testosterone	1.5	3.63	2.42	Med	$6.71E^{-01}$	0.900	0.600	Low	$1.32E^{-01}$	0.459[a]	0.306	Low	$4.23E^{-02}$
Progesterone	1000	0.600	0.000600	Ins	$1.66E^{-04}$	-	-	-	-	5.40	0.00540	Ins	$7.47E^{-04}$
E1	3.6	2.46[a]	0.683	Low	$1.89E^{-01}$	5.60[a]	1.56	Med	$3.42E^{-01}$	30.6	8.50	Med	$1.18E^{+00}$
E1-3S	20500	1.95[a]	0.0000952	Ins	$2.64E^{-05}$	3.62[a]	0.000177	Ins	$3.88E^{-05}$	-	-	-	-
Caffeine	1	338	338	High	$9.37E^{+01}$	432	432	High	$9.49E^{+01}$	705	705	High	$9.75E^{+01}$
Triclosan	20	35.1[a]	1.75	Med	$4.86E^{-01}$	75.8	3.79	Med	$8.33E^{-01}$	4.38[a]	0.219	Low	$3.03E^{-02}$
MeP	5000	8.41[a]	0.00168	Ins	$4.66E^{-04}$	9.88[a]	0.00198	Ins	$4.34E^{-04}$	39.2	0.00784	Ins	$1.08E^{-03}$
EtP	2500	3.69	0.00148	Ins	$4.09E^{-04}$	19.5	0.00780	Ins	$1.71E^{-03}$	9.53	0.00381	Ins	$5.27E^{-04}$
BPA	1	18.0[a]	18.0	High	$4.99E^{+00}$	15.9[a]	15.9	High	$3.50E^{+00}$	8.41[a]	8.41	Med	$1.16E^{+00}$
BPF	840	-	-	-	-	-	-	-	-	18.3[a]	0.0218	Ins	$3.02E^{-03}$
BPS	6900	13.0	0.00188	Ins	$5.22E^{-04}$	79.3	0.0115	Ins	$2.52E^{-03}$	79.3	0.0115	Ins	$1.59E^{-03}$
BPAF	230	-	-	-	-	-	-	-	-	36.9	0.160	Low	$2.22E^{-02}$
OP	100	-	-	-	-	-	-	-	-	54.1	0.541	Low	$7.48E^{-02}$
NP	300	-	-	-	-	12.3[a]	0.0410	Ins	$9.01E^{-03}$	-	-	-	-
Benzotriazole	7770	218	0.0281	Ins	$7.77E^{-03}$	357	0.0459	Ins	$1.01E^{-02}$	136	0.0175	Ins	$2.42E^{-03}$
TCEP	4000	6.84[a]	0.00171	Ins	$4.74E^{-04}$	4767	1.19	Med	$2.62E^{-01}$	-	-	-	-
TCPP	30000	524	0.0175	Ins	$4.84E^{-03}$	1065	0.0355	Ins	$7.80E^{-03}$	132	0.00440	Ins	$6.08E^{-04}$
TBEP	13000	25.6	0.00197	Ins	$5.46E^{-04}$	79.2	0.00609	Ins	$1.34E^{-03}$	20.8	0.00160	Ins	$2.21E^{-04}$

-: not detected.
[a] Half of the method LOD or LOQ.

Ins: insignificant risk.
Low: low risk.
Med: medium risk.
High: high risk.