CENTRO INTERNACIONAL DE ESTUDOS
DE DOUTORAMENTO E AVANZADOS
DA USC (CIEDUS)

TESE DE DOUTORAMENTO

NEW APPLICATIONS OF MASS SPECTROMETRY TO THE IDENTIFICATION AND QUANTIFICATION OF EMERGING POLLUTANTS AND THEIR TRANSFORMATION PRODUCTS

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ESCOLA DE DOUTORAMENTO INTERNACIONAL
PROGRAMA DE DOUTORAMENTO EN CIENCIA E TECNOLOXÍA QUÍMICA

Santiago de Compostela
2020

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NEW APPLICATIONS OF MASS SPECTROMETRY TO THE IDENTIFICATION AND QUANTIFICATION OF EMERGING POLLUTANTS AND THEIR TRANSFORMATION PRODUCTS

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Mi más sincero agradecimiento…

A mis directores, **Isaac** y **María**, por darme esta oportunidad, por creer en mí y guiarme durante estos cinco años. Esta tesis jamás hubiera sido posible sin vosotros.
Isaac, gracias por todo el tiempo que me has dedicado, tu confianza y paciencia, y por todos los conocimientos que me has regalado, no sólo de química, sino de la vida. Tu pasión por la investigación y tu perseverancia han sido una lección de vida. **María**, gracias por acompañarnos en este camino y por enseñarme que además de investigadores, somos humanos con sentimientos. Gracias por tu empatía y tu cariño en los momentos complicados.

Al Instituto de Investigación y Análisis Alimentario (IIAA), en especial a **Rafael**, por haberme acogido en el grupo de investigación y poner en mis manos los medios necesarios para llevar a cabo esta tesis. Por toda su experiencia y sabiduría, sobre todo en esta recta final.

A mis compañeros, los que siguen en el laboratorio y los que ya se han ido. A **Jorge** y a **Tamara**, por contagiarme la ilusión por la investigación y animarme a empezar esta tesis. A **Rosita,** por poner banda sonora a mis horas de laboratorio y que junto con **Iria** e **Inma**, han actuado como voz de la experiencia, preocupándose y solucionando mis dudas- *"Repite, repite, repite y cuando salga, paras"*-. A **Sara**, **Vero**, **Andrea** y **Miguel**, por llenar los descansos para café con las conversaciones más extrañas y divertidas. A **Javiere**, porque mola. A todos, gracias por los buenos momentos que hemos compartido, la química subterránea y las risas. Mención muy especial a **Leti**, por compartir conmigo mucho más que mesa de trabajo y proyecto.

Y por supuesto, a **Beni**, por ser mi apoyo incondicional desde el primer momento y haberse convertido en mi fiel compañero de fatigas las 24 horas, los 7 días de la semana, durante todos estos años, y, en contra de todo pronóstico, en mi mejor amigo. Gracias por ser familia.

A mi gente de Santiago, porque sin ellos todos estos años no hubieran sido lo mismo, ni la mitad de especiales y divertidos. A mi dulce **Aimé**, por compartir conmigo la etapa más importante de mi vida y llenarla de luz. Todos necesitamos a una persona que preserve nuestra salud mental, y esa es ella. Gracias, por tanto. A **Vir**, por estar siempre dispuesta a todo y por alegrar mis días, aunque a veces estuviéramos a miles de km de distancia. A **Javi**, por su apoyo incondicional durante todos estos años y creer en mí incluso cuando yo no lo hice. A mis **Marus**, que tampoco me olvido de vosotras.

A todas las personas que tuve la suerte de conocer en Amberes. To **Adrian**, for welcoming me with his kind arms open. A **Noelia**, por ayudarme y enseñarme tanto, incluso antes de conocernos personalmente. And of course, to **Yunsun**, for opening the doors of her house and her life to me, my Korean soul in Antwerp.

A mis amigos de toda la vida, gracias por seguir ahí haciendo magia. A **Alexiña**, por ser hogar y encontrar siempre el momento perfecto para desconectar, escucharme y aconsejarme y, sobre todo, por regalarme tan buenos momentos y hacerme reír tantísimo. A **Leli**, por hacerme la vida mucho más fácil y por todo su cariño, que no es poco. Si existen los abrazos reparadores, esos son sin duda, los suyos.

A mi familia, a la que tanto quiero, es imposible agradeceros todo. Gracias a **mi madre** y a **mi hermano**, por animarme siempre a hacer lo que me hace feliz. Por aguantar en primera línea todas mis quejas y preocupaciones acerca de esta tesis, y llenarme siempre de palabras de ánimo y buenos consejos. A **Xabi**, por ser un modelo a seguir, aunque él no lo sepa. A **Anxo**, por estar siempre ahí, y junto con **Álex**, haber sido mis cómplices y creadores de sonrisas desde 1991. A **mis tíos**, por su infinita paciencia y todo su amor. A **Yaguitus** y **Yery**, porque su hermosa inocencia me hace muy feliz. Y, por último, a **mis abuelos**, que aunque a veces no entiendan lo que hago, siempre me han animado a seguir adelante. Sin vosotros no hubiera sido posible llegar hasta aquí.

Gracias.

Agradezco a la Xunta de Galicia y a los fondos FEDER (proyectos GRC-ED431C 2017/36 y CTQ2015-68660-P, PGC2018-094613-B-I00) por la financiación recibida para el desarrollo de las actividades descritas en esta memoria de tesis.

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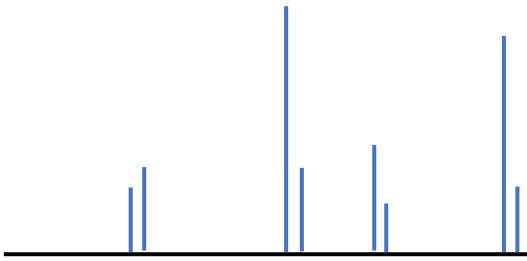

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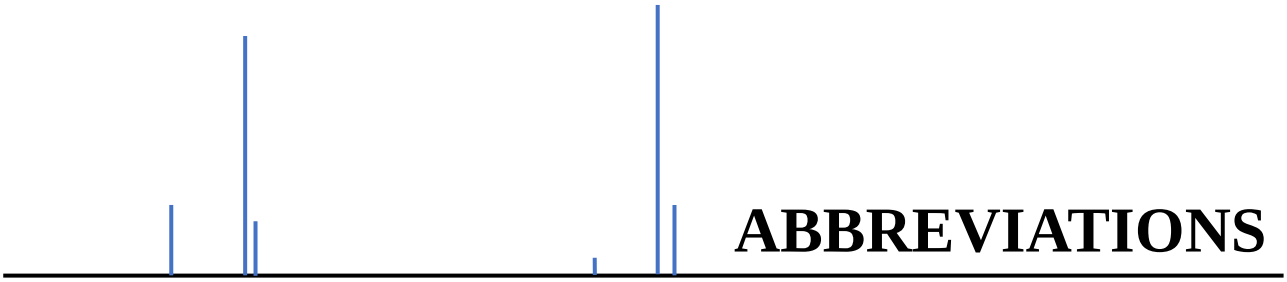

ABBREVIATIONS

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Abbreviations

A

AEMPS · *Spanish Agency for Medicines and Health Products*
AMI · *Amiodarone*
AOPs · *Advance oxidation processes*
ASE · *Accelerated solvent extraction*

B

BPA · *Bisphenol A*
BPADA · *Bisphenol A diacetilated*
BPB · *Bisphenol B*
BPE · *Bisphenol E*
BPF · *Bisphenol F*

C

CAN · *Candesartan*
CBA · *Carboxy methylsilane*
CBZ · *Climbazol*
CHMP · *Committee for Medicinal Products for Human Use*
CN · *Cyanopropylsilane*
CTZ · *Clotrimazole*

D

DART · *Direct analysis in real time*
DBA · *Diethylamino propylsilane*
DDA · *Data dependent acquisition mode*
DDD · *Defined daily dose*
DIA · *Data independent acquisition mode*
DLLME · *Dispersive liquid–liquid microextraction*
DOM · *Dissolved organic matter*
DPs · *Disinfection products*
DRO · *Dronedarone*
DWTPs · *Drinking water treatment plants*

E

ECDC · *European Centre for Disease Prevention and Control*
ECHA · *European Chemicals Agency*
ECZ · *Econazole*

EI · *Electron ionization*
EPRO · *Eprosartan*
EPs · *Emerging pollutants*
ESAC-Net · *European Countries through the European Surveillance of Antimicrobial Consumption Network*
ESI · *Electrospray ionization*
ETZ · *Etaconazole*

F

FCZ · *Fluconazole*
FLE · *Flecainide*
FR · *Flame retardants*
FTZ · *Fenticonazole*

G

GC · *Gas chromatography*

H

HLB · *Hydrophilic-lipophilic balanced sorbent - poly (divinylbenzene-co-N-vinylpirrolidone) polymer*
HPLC · *High-performance liquid chromatography*
HRMS · *High resolution mass spectrometry*

I

IMZ · *Imazalil*
IRB · *Irbesartan*
IT · *Ion trap*
ITZ · *Itraconazole*
IUPAC · *International Union of Pure and Applied Chemistry*

K

KTZ · *Ketoconazole*

L

LC · *Liquid chromatography*
LOD · *Limit of detection*
LOQ · *Limit of quantification*
LOS · *Losartan*

M

MAX · *Mixed-mode strong anion exchanger*
MCX · *Mixed-mode strong cation exchanger*
MCZ · *Miconazole*
MFE · *Molecular features extraction*
mP · *Methylparaben*
MRM · *Multiple reaction monitoring mode*
MS · *Mass spectrometry*
MS/MS · *Mass spectrometry in tandem*

N

ND · *Not detected*
N-DES · *N-Desethylamiodarone*
NORMAN · *European Commission network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances*

O

OLM · *Olmesartan*
OPP · *Orthophenylphenol*

P

PCPs · *Personal care compounds*
PCDL · *Personal Compound Database and Library*
PFAS · *Perfluoroalkyl substances*
PLE · *Pressurized liquid extraction*
pP · *Propylparaben*
PRO · *Propranolol*
PSA · *N-propylethylene diaminosilane*
PS-DVB · *Poly (styrene-divinylbenzene) copolymers*

Q

Q · *Quadrupole*
QA/QC · *Quality assurance and quality control*
QqQ · *Triple quadrupole*
Q-Rai · Q*uadrupole-resolved all ions*

R

RAM · *Restricted access materials*

S

SAX · *Strong anion exchanger (trimethylamine propylsilane)*
SCX · *Strong cation exchanger (benzene sulphonyl propylsilane)*
SIM · *Selected ion monitoring*
SPE · *Solid-phase extraction*
SPME · *Solid-phase microextraction*
STPs · *Sewage Treatment Plants*
STZ · *Sertaconazole*

T

TCS · *Triclosan*
TCZ · *Tioconazole*
TELM · *Telmisartan*
TOF · *Time of flight*
TRB · *Terbinafine*

U

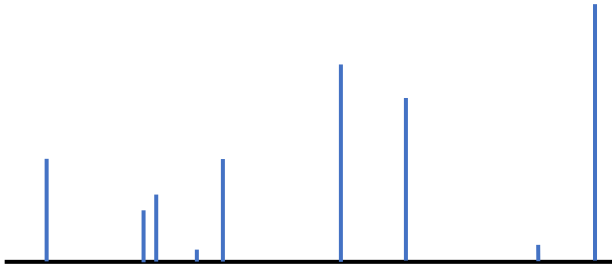
UHPLC · *Ultra-high-performance liquid chromatography*

V

VAL · *Valsartan*
VALA · *Valsartan acid*

W

WAX · *Mixed-mode weak anion exchanger*
WCX · *Mixed-mode weak cation exchanger*
WHO · *World Health Organization*



ABSTRACT

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Abstract

The presence of emerging pollutants in the environment constitutes a serious problem in many countries, as they present a new challenge to human and environmental health. It is considered that the urban wastewater is the main route of entry of these pollutants into the environment. However, the environmental fate of some of these compounds in urban wastewater and sludge remains still unknown. Even more, very likely the range of anthropogenic chemicals existing in the aquatic environment is larger than those so far recognized as emerging pollutants. Their behaviour and toxicity, as well as their potential transformation products, have become a problem of increasing social and scientific concern.

For this reason, one of the main objectives of this doctoral thesis is to develop screening analytical strategies for the detection and identification of new emerging pollutants in different environmental samples, such as wastewater, sludge and dust. The aim of these screening strategies is two-fold, on the one hand, to investigate the presence of pollutants and on the other hand, to develop new analytical techniques focused on the quantification of different families of novel pollutants. Obviously, the availability of reliable analytical methodologies is a basic requirement to understand the environmental fate and the effects of novel detected compounds.

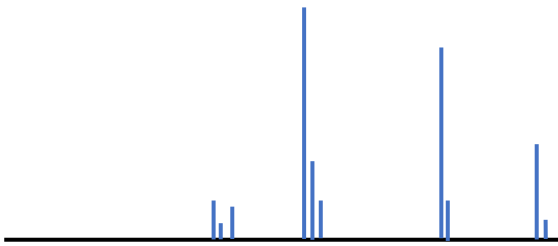
Along this thesis several screening strategies are proposed, based on the combination of different sample preparation protocols and accurate mass spectrometry. The sample preparation techniques developed consisted mainly on matrix solid-phase dispersion (MSPD) and pressurized liquid extraction (PLE) for solid samples, such as sludge and dust, and solid-phase extraction (SPE) for liquid samples. During screening studies, generic extraction conditions were employed in order to transfer most of the chemical information existing in the sample to the extract. Furthermore, optimization of selective extraction procedures was a major issue when addressing the determination of particular families of pollutants. Regarding the determination techniques, different strategies were followed, from liquid chromatography (LC) or gas chromatography (GC) for separation, to non-chromatographic techniques, such as direct analysis in real time (DART). All of them have in common the use of mass spectrometry (MS), either using triple quadrupole (QqQ) instruments, or hybrid quadrupole-time-of-flight (QTOF) ones.

As a result of these screening analysis, valuable information about the presence and concentrations of different families of pharmaceuticals with high consumption rate, such as antimycotic and cardiovascular, was obtained. These substances together with phenolic related compounds, are deeply studied along this thesis.

Once the analytical methodologies for the determination of these pollutants were optimized and their concentrations measured, their behaviour under simulated environmental conditions, and/or oxidative treatments, was assessed. The purpose of these studies was to evaluate the efficiency of such treatments to improve their removal efficiencies at Sewage Treatment Plants (STPs), and to identify potential transformation products.

Research activities performed through the development of this thesis are compiled in this memory and presented in four chapters.

I. Justification and objectives.
II. Introduction.
III. Results and discussion.
IV. Conclusions.



I. JUSTIFICATION AND OBJECTIVES

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I. JUSTIFICATION AND OBJECTIVES

Emerging contaminants are continuously released into the environment due to their use (or misuse in some cases) as pharmaceuticals, personal care products, household cleaning and compounds with industrial applications, among other products. Some of these pollutants are not effectively removed at Sewage Treatment Plants (STPs), which is considered as the most important route of entry of pollutants into the environment. Thus, conventional wastewater treatment strategies, mainly based on physical and biological treatments, need to be improved with additional treatments (usually oxidative processes) capable of reducing the levels of emerging pollutants in the outlet stream of STP. The presence of emerging pollutants in solid wastes from STPs has been scarcely investigated considering that, so far, dried sludge has been destroyed mostly through incineration. However, incineration is not a sustainable practise. In addition, sludge is regarded as a source of nutrients (nitrogen, phosphorous and carbon) with potential application in intensive agriculture. Additionally, the basic pH of sludge stabilized with calcium oxide might help to correct acidic conditions of fields in some areas, such as the Community of Galicia.

The anthropogenic chemicals concentrated in sludge and stable during the sludge stabilization at STPs, might be absorbed by crops and/or by invertebrates entering the trophic chain, and reaching human beings. Obviously, the transformation of these pollutants in agriculture soils, through microbiological processes and/or photochemical reactions, might generate novel species, whose toxicity is totally unknown. For that reason, the study of the levels of emerging pollutants in sludge for agricultural use, as well as the identification of their transformation and disposal pathways in the STPs are the most important aspects to investigate the use of these residues, the modernization of the agricultural sector and, above all, the production of safe food.

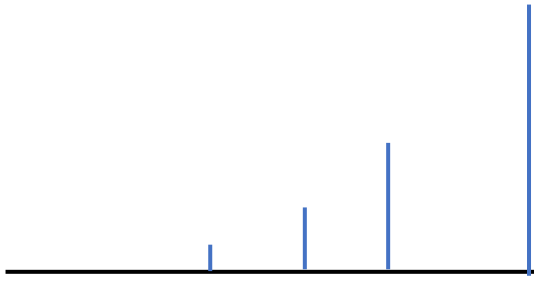
However, emerging pollutants are not only discharged into the environment through STPs, but household and personal care products, together with high production volume chemicals employed in building materials, furniture and upholstery are directly released into the environment of confined areas, such as homes, commercial centres, working places and vehicles. After releasing from host materials, they are distributed between dust and air depending on properties such as vapour pressure, air-octanol partition coefficient and environmental temperature. Emerging pollutants in gas phase and those concentrated in dust particles directly affect the health of individuals spending most of their lives in closed areas. Despite efforts devoted to investigating the occurrence of emerging pollutants, their presence in indoor areas have received much less attention than in the aquatic environment.

The determination of emerging pollutants in complex matrices is commonly based on classical analytical methodologies, which combine a chromatographic separation step followed by tandem mass spectrometry for a pre-selection of target compounds. Along these lines, the use of triple quadrupole (QqQ) mass spectrometers, working in MRM mode, results in high selectivity combined to extremely low quantification limits. However, the above strategy is unsuitable for detecting new species or understanding

their transformation routes, either in experiments at laboratory scale or under real conditions. As an alternative to QqQ mass analyzers, high resolution/accurate mass spectrometry (HRMS) combined with liquid chromatography (LC) and, more recently, gas chromatography (GC) has become popular in recent years. HRMS systems, are based on the use of time-of-flight (TOF) and Orbitrap mass analyzers. In both cases, it is possible to obtain accurate mass records (scan mode) along the entire chromatographic separation process. Thus, the combination of HRMS with mild ionization sources, such as electrospray ionization (ESI), allows obtaining pseudo-molecular ions ($M+H^+$ or $M-H^-$) of any species present in the sample, which survives the preparation and ionization steps. Fortunately, this latent information offers the possibility of detecting new pollutants in environmental matrices and results very attractive for searching for new contaminants in complex matrices such as wastewater, sludge and biosolids. Obviously, data mining strategies employed in LC-ESI-QTOF-MS are unsuitable when compounds are submitted to ionization techniques, which do not render a pseudo-molecular ion as base signal in MS spectra, as happens with GC-EI-TOF, where new strategies of search should be developed.

The main objective of this doctoral thesis is to assess the possibilities offered by accurate mass spectrometry, derived from the use of TOF instruments, for the identification and determination of new pollutants in high complexity matrices, particularly wastewater and sewage sludge from STPs, as well as dust from indoor areas. Accurate mass spectrometry will be also applied to the detection and structural elucidation of the transformation products derived from emerging pollutants, generated during tertiary treatments and under experimental conditions attempting to mimic those existing in the environment, both in terrestrial and aquatic systems (wastewater from the STPs and surface and runoff waters). To achieve this end, different studies have been developed in connection with activities reported below.

1. Non-target screening of emerging contaminants in solid samples, such as sludge, biosolids and dust, using TOF mass analyzers, either combined with LC or GC separation techniques.
2. Development of quantitative methods for the determination of specific groups of contaminants identified in screening studies, particularly for those non reported before in literature.
3. Studies of transformation of emerging pollutants during real conditions and under oxidizing processes (performed at laboratory scale), such as photolysis or chlorination, were also developed. In addition, the identification of the transformation products generated, and toxicity assessment were carried out.
4. Assessment of a new ionization source based on ambient ionization (direct analysis in real time- DART) to the direct analysis of emerging pollutants.



II. INTRODUCTION

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II. INTRODUCTION

II.A. Studied compounds

1. Emerging pollutants

1.1. Definition

According to the definition given by the European Commission network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances (NORMAN), emerging pollutants (EPs) can be defined as "chemical substances that have been detected in the environment, but which are currently not included in the routine monitoring programs at the European level, however they should be considered as candidate for future regulations, due to their (eco)toxicity, potential health effects and public perception" [1].

Hence, this type of compounds has raised significant interest by the scientific community in the last decades and their occurrence has been reported worldwide in a range of different environmental matrices, such as air, soils, sediments and waters [2–5]. The number of publications reporting the concentrations of these compounds was so vast that, in 2015, the NORMAN network decided to elaborate a list including the most frequently found EPs, based on the number of citations in the scientific literature [1]. This list includes some pharmaceuticals, drugs of abuse, surfactants, steroids and hormones, personal care compounds (PCPs), perfluoroalkyl substances (PFAS), flame retardants (FR), industrial additives, pesticides, as well as different transformation products (TPs). In recent years, three new groups have been incorporated to the list: nanomaterials, 1,4-dioxane and swimming pool disinfection products (DPs) [6,7]. In the same year, the European Union published the first version of a *watch list* of substances for the Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council [8].

1.2. Environmental distribution

Nowadays, all of us use, at least, one emerging contaminant daily; either personal care products, pharmaceuticals, household cleaning products or even during another type of actions such as agriculture or aquaculture. Regardless of where they come from, the fact is that high amounts of these pollutants are continuously emitted into the environment.

According to the World Health Organization (WHO), more than 100,000 chemicals are released into the environment every year [9] due to their high production, use and disposal. Although there are so many studies about the occurrence, fate and environmental distribution of EPs in the environment [10–12], the pathway of these contaminants from the source is still diffuse and depends mostly on their physicochemical properties, but also on their usage and application mode, such as pharmaceutical treatments in human and vets, agricultural treatments, industrial activities, municipal disposal or even accidental spills. Once released into the environment, these substances

can be widely distributed until their elimination. Since the principal contamination sources and pathways of the EPs are related to industrial, agricultural and municipal activities, the main collector of these substances is the wastewater, therefore the study of the presence of EPs in sewage treatment plants (STPs) is highly recommended [4].

Once in the STPs, the EPs are submitted to different treatments, such as biological, chemical and photochemical degradation, until their elimination or complete mineralization. However, some of these substances are resistant to those degradation processes, remaining in the effluents at the output of STPs [13], which is the case of the most polar compounds; whereas non-polar EPs might remain adsorbed to sludge particles, being further transferred to agriculture fields, where stabilized sludge is disposed as fertilizer.

Through the discharges of the STPs, these compounds are widely distributed into the environment. On the one hand, they can reach superficial waters, as lakes and rivers, and even, in the worst case, tap water [14,15] and on the other hand, they can arrive to agricultural soils by the application of wastewater as irrigation water, or through the use of stabilized sludge as fertilizer [16]. Once in soil, these compounds can be filtrated to groundwater, producing the contamination of springs or reaching again superficial waters. Thus, one of the biggest problems of the presence of these compounds in environment is the feasibility to be incorporated into the trophic chain, producing resistance and/or endocrine alterations, among others. The distribution of EPs into the environment is summarized in **Figure 1**.

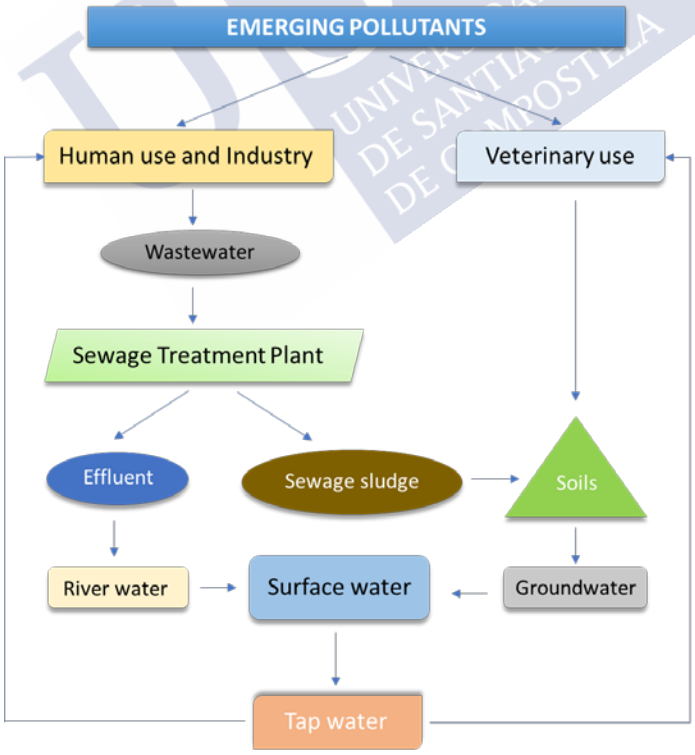

Figure 1. Environmental distribution of emerging pollutants

2. Antimycotic compounds

2.1. Definition

Antimycotic compounds are a family of pharmaceuticals widely applied in the treatment of infections caused by fungi or mycosis, due to their capacity of eliminating or preventing the growth of fungi potentially harmful to living beings. Healthy individuals are susceptible to get superficial cutaneous and subcutaneous infections, or even systemic infections [17]. These types of infections are usually treated with pharmaceuticals, based on azolic substances. These compounds are sterol demethylation inhibitors; thus, their mechanism of action is based on the inhibition of the synthesis of ergosterol, which is an important compound of the fungal cytoplasmatic membrane, inducing thereby the cell membrane disorganization and decreasing the fungal growth [18].

One of the main drawbacks of these substances is their endocrine disruptor activity, due to the inhibition of cytochrome P450, so they could cause non-desire effects in patients and produce the elimination of other substances [17,19]. The high toxicity of first generation antimycotic pharmaceuticals (mainly of nyastin, polyenes and amphotericin B), or the appearance of microbiological resistance made necessary the development of new antimycotics which provided advantages to the existing ones. As a result, a second generation of antimycotic compounds based on triazole structures has been synthetized [20].

In last years, these compounds have generated much interest from an analytical and ecotoxicological point of view, due to their potential environmental impact, their low elimination rates in the STPs and their high bioaccumulation.

2.2. Structure and physicochemical properties

There are several families of antimycotic drugs employed in medicine. Among them, the most often employed group corresponds to compounds containing an azolic ring in their structure.

Azolic compounds have a simple structure based on an azolic ring formed by 5 atoms, of which 2 or 3 correspond to nitrogen atoms, depending on whether they are imidazoles or triazoles, respectively. A lateral complex chain is conjugated to one of the nitrogen atoms, which establishes the classification of the different types of azolic antimycotic compounds. In many cases, the presence of benzene rings with halogenated substituents, such as chlorine or fluorine close to the imidazole or triazole ring, helps to improve the biological response of the molecule, since it confers lipophilic character and increases its efficiency against fungal infections.

Table 1 shows the list of antimycotic pharmaceuticals studied in this doctoral thesis, together with their CAS number, empirical formula, molecular weight and some relevant physicochemical properties to understand their behaviour during the different steps involved in their analytical determination. As we can see in the table, in general, these drugs are slightly basic, being able to be partially protonated at the pH of surface waters. The structures of the studied antimycotics are shown in **Figure 2.**

The discovery of these azole compounds constitutes an important fact in the history of medicine and it is considered that systemic-use antimycotics derived from imidazole and triazole constitute the most important advance in the treatment of systemic mycosis in the last decades, allowing a considerable decrease in the toxicity of previously employed treatments.

Table 1. Name, abbreviature, CAS, formula and some physicochemical properties of the studied antimycotic pharmaceuticals (ChemSpider).

Name	Abbreviature	CAS	Formula	Monoisotopic Mass	log K_{ow}	pK_a
Climbazole	CBZ	38083-17-9	$C_{15}H_{17}ClN_2O_2$	292.0979	4.34	6.49
Clotrimazole	CTZ	23593-75-1	$C_{22}H_{17}ClN_2$	344.1080	5.84	6.26
Econazole	ECZ	27220-47-9	$C_{18}H_{15}Cl_3N_2O$	380.0250	5.35	6.48
Etaconazole	ETZ	60207-93-4	$C_{14}H_{15}Cl_2N_3O_2$	311.0592	3.88	1.95
Fenticonazole	FTZ	72479-26-6	$C_{24}H_{20}Cl_2N_2OS$	454.0673	6.94	6.48
Fluconazole	FCZ	86386-73-4	$C_{13}H_{12}N_6F_2O$	306.1041	0.56	2.3[a] 12.68[b]
[c]**Imazalil**	IMZ	35554-44-0	$C_{14}H_{14}Cl_2N_2O$	296.0483	3.76	6.48
Itraconazole	ITZ	84625-61-6	$C_{35}H_{38}Cl_2N_8O_4$	704.2393	7.31	3.91
Ketoconazole	KTZ	65277-42-1	$C_{26}H_{28}Cl_2N_4O_4$	530.1488	4.19	6.42
Miconazole	MCZ	22916-47-8	$C_{18}H_{14}Cl_4N_2O$	413.9860	5.96	6.48
Sertaconazole	STZ	99592-32-2	$C_{20}H_{15}Cl_3N_2OS$	435.9971	6.23	6.48
[d]**Terbinafine**	TRB	91161-71-6	$C_{21}H_{25}N$	291.1987	5.53	8.86
Tioconazole	TCZ	65899-73-2	$C_{16}H_{13}Cl_3N_2OS$	385.9814	5.30	6.48

[a] Strongest basic pK_a
[b] Strongest acidic pK_a
[c] Imazalil is employed as azolic fungicide in agriculture, not in medicine.
[d] This antimycotic compound does not belong to the azolic family.

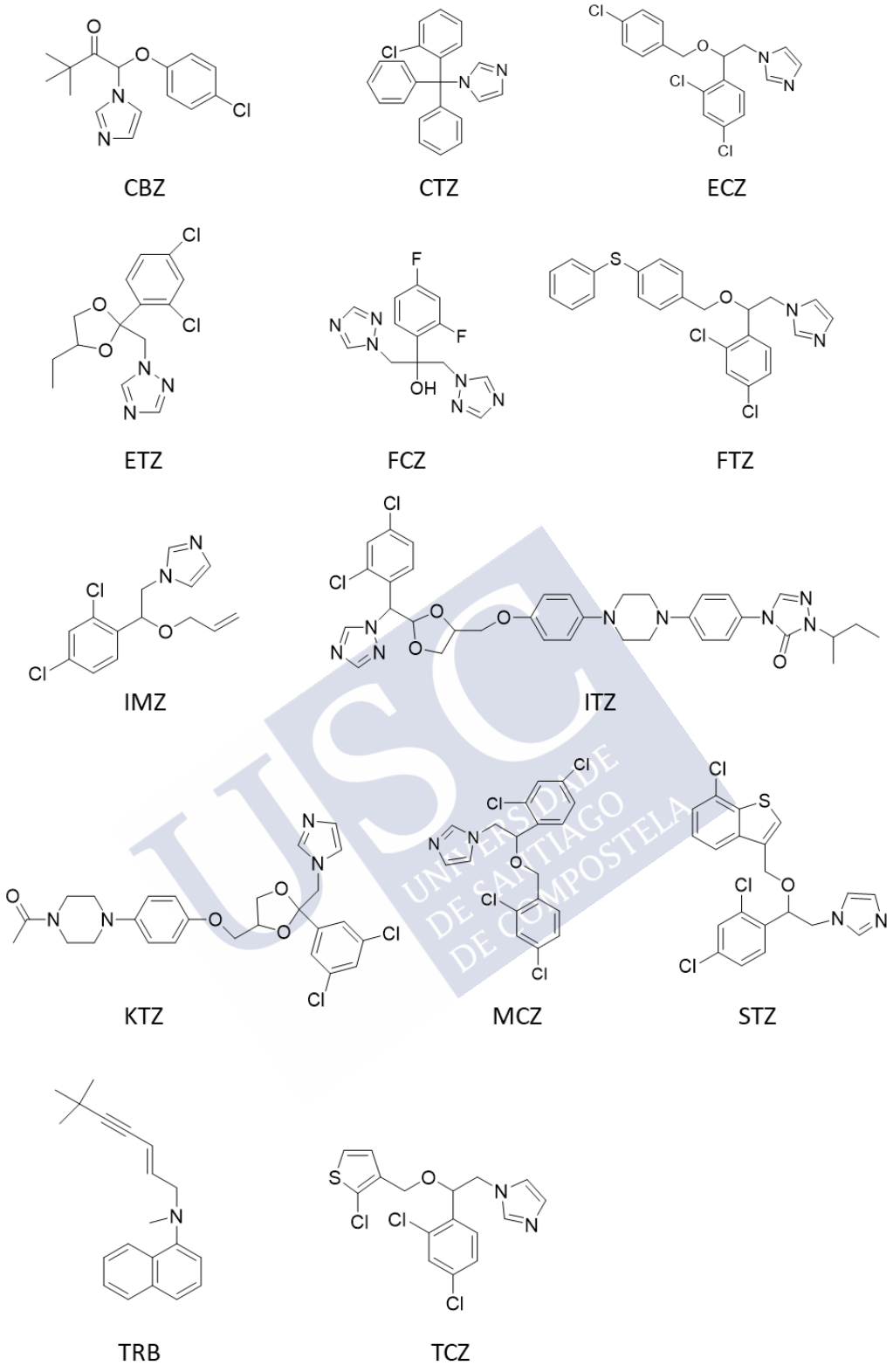

Figure 2. Structures of the studied antimycotic compounds

2.3. Consumption

The available data regarding the consumption of antimycotic compounds in medical applications is still limited. In 2012, the European Centre for Disease Prevention and Control (ECDC) collected data on consumption of antimycotic and antifungal compounds for systemic treatments reported by 29 European Countries through the European Surveillance of Antimicrobial Consumption Network (ESAC-Net) [21]. According to the data presented by the Network, the average consumption by the population is 1.24 DDD (defined daily dose) per 1000 inhabitants and per day, being TRB, KTZ, FCZ and ITZ the most highly consumed, and representing the 98 % of the total consumption in all countries. **Figure 3** shows the consumption data for these compounds in the community (primary care) in 2012, expressed as DDD per 1000 inhabitants and per day [21]. TRB represents the 50 % of the total consumption in 18 out of the 29 countries participating in the study, followed in most cases by KTZ.

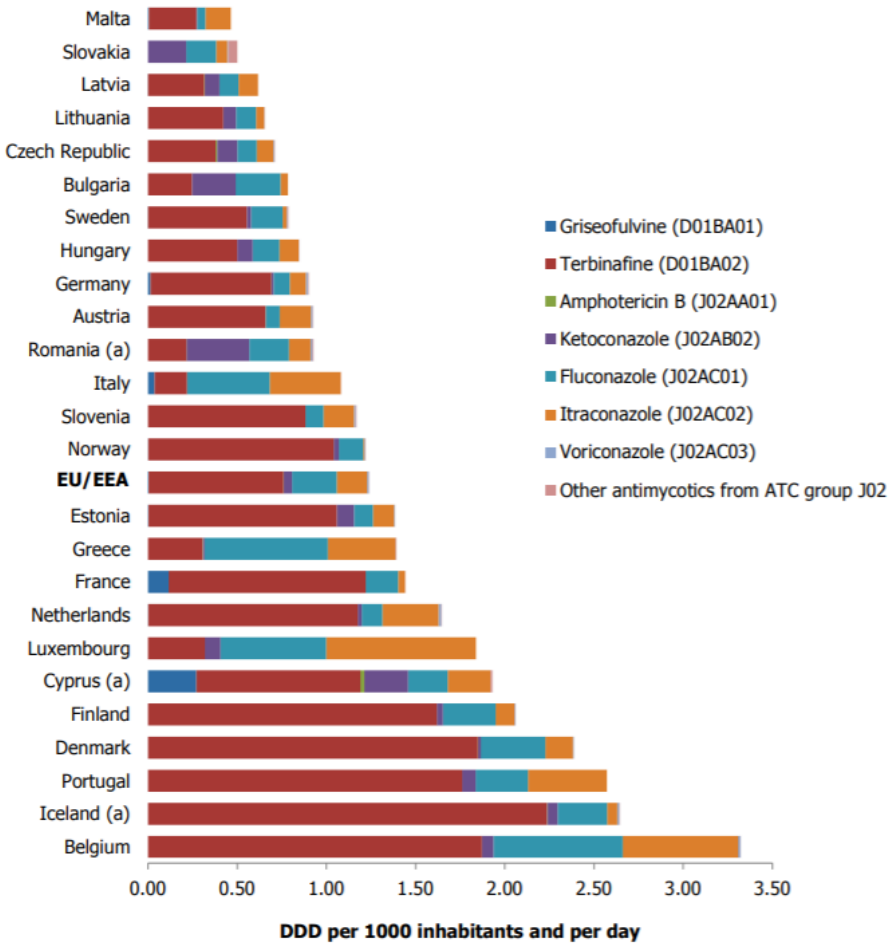

Figure 3. Consumption of antimycotic and antifungals in Europe

Focused on Spain, data of consumed amounts is even more limited [22]. According to L. Alou et al. [22], the antimycotics for topic treatment are the most used ones in Spain. The percentual consumption of antimycotics in Spain attending to their administration routes is compiled in **Figure 4**.

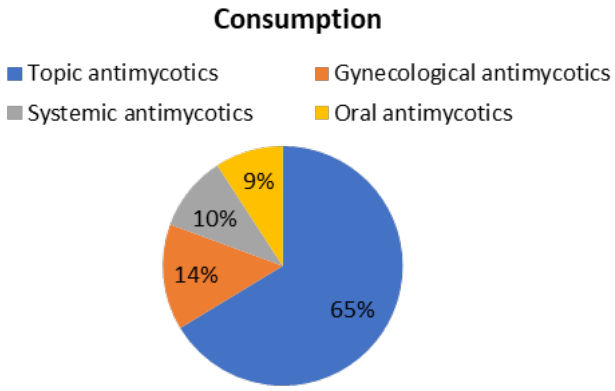

Figure 4. Consumption of the different families of antimycotics in Spain

Although the systemic antimycotics represent only the 10 % of the total consumption, the ECDC has presented a report with the consumption data since 2015 until 2018, where TRB, ITZ, FCZ and KTZ presented again the highest average number of consumed doses per habitant [23]. **Figure 5** compiles the DDD per 1000 inhabitants for the most consumed antimycotic in Spain in the last years.

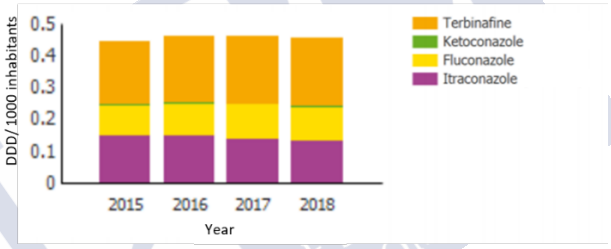

Figure 5. Consumption of antimycotics in Spain

2.4. Toxicity

Antimycotic pharmaceuticals are considered as EPs, so it is impossible to know their toxic effects in the immediate future, and there is not a legislation that limits the maximum permitted amounts of these compounds in the environment. However, their indiscriminate use and their cumulative tendency might cause changes and/or undesired effects on the environment and living beings.

Ecotoxicity studies during medium to long periods are required, since the emissions of this type of compounds from STPs release low, but continuous amounts of antimycotics to the aquatic environment. The low concentrations expected in environmental matrices can cause the appearance of fungal strains and families resistant to these compounds, since the expected levels in environmental compartments are lower than the lethal ones for target microorganisms, affecting to aquatic organisms and their corresponding predators.

The resistance to antimycotics depends mainly on the contact that fungi have previously had with this type of substances. Thus, the use of inappropriate products, inappropriate doses, or treatments during a too short period of time, can facilitate the change in the characteristics of the fungi and stop being sensitive to the drug and become resistant to it.

These pharmaceuticals can cause non desired reactions in various physiological systems. Among the azolic substances, the toxicity depends on the selectivity, being triazolic compounds more toxic than imidazolic ones. On the one hand, imidazolic compounds (CBZ, CTZ, ECZ, FTZ, IMZ, KTZ, MCZ, STZ and TCZ) can cause gastrointestinal intolerance, hepatitis [17], liver failure or even cirrhosis, being KTZ the most toxic one. For this reason, in 2013, the Committee for Medicinal Products for Human Use (CHMP) has recommended the suspension of pharmaceuticals containing KTZ for systemic treatments [24]. On the other hand, triazolic compounds (ETZ, FCZ and ITZ) produce teratogenic effects in ascidians and rats [25,26].

Some studies revealed the potential risk of these pharmaceuticals for the environment. According to their environmental distribution these compounds can be incorporated into superficial waters, as rivers and lakes; or be adsorbed into the sludge and be applied as fertilizers in agriculture. Thus, they can be easily incorporated into the plants [27]. In order to know the toxic effect of these pharmaceuticals in the environment, some authors have studied their toxicity in *Daphnia Magna*, fishes and plants.

Daphnia Magna is one of the most important planktonic herbivores in the pelagic trophic chain. *Daphnia* constitutes food for fish and invertebrates and is commonly used to assess the toxicity of EPs in the aquatic media, due to its reproductive activity changes when the environmental conditions are not optimal. The high bioconcentration factor of certain antimycotic pharmaceuticals, such as CTZ, affects its subsequent development. Since the species of *Daphnia* are at the base of food chain, the decrease in this population is closely related to problems of eutrophication of lakes, reduction of water quality and even the death of some species of living beings [28].

In addition, the phytotoxicity of CBZ, FCZ and KTZ was studied in terrestrial (*Brassica napus*) and aquatic (*Lemna minor*) plants at concentration levels similar to those present in sludge. The obtained results confirmed the high phytotoxicity of these compounds to plants, being CBZ the most toxic one for aquatic and similar to FCZ for terrestrial plants [27]. Toxicity towards aquatic plants, such as *Lemna minor*, is not considered as a standard endpoint required for the environmental risk assessment of pharmaceuticals, but in case of CBZ and other antimycotics, *Lemna minor* was more sensitive than the standard test organisms: algae, *Daphnia Magna* or fish [27,29].

2.5. Levels of antimycotics in the environment

Regarding their environmental distribution, antimycotic pharmaceuticals arrive to the STPs through urban sewers following two different pathways. Compounds of topic or local use can be incorporated directly into wastewater by dragging during daily hygiene, whereas antimycotics of systemic use, after their administration, are adsorbed and metabolized by the organism and finally excreted through urine and faeces.

Most of the studied antimycotic pharmaceuticals present a lipophilic character, with log K_{ow} between 3.76 and 6.94, apart from FCZ, which has a log K_{ow} of 0.56; therefore, these compounds have a tendency to adsorb into the sludge, widely used as fertilizer in agriculture, whereas in case of polar compounds, they remain mostly dissolved in water, arriving to superficial waters, rivers and even tap water. Some previous studies have revealed the presence of antimycotic pharmaceuticals in wastewater, rivers, sludge and soils. Data regarding their apparent removal rates at urban STPs are also available in the literature. **Table 2** summarizes the maximum concentration of these pharmaceuticals in different environment samples, collected from the literature.

Table 2. Maximum concentrations of antimycotic pharmaceuticals measured in influent, effluent and surface water (data in ng L^{-1}) and in sludge from STPs (values in ng g^{-1}).

Analyte	Sample	Country	Year	Influent (ng L^{-1})	Effluent (ng L^{-1})	Surface water (ng L^{-1})	Sludge (ng g^{-1})	Ref
CBZ	Guangzhou STP	China	2012	282	66,4	ND	152	[30]
	Schwarzbach STP/ Rhine River	Germany	2010	475	312	ND	1160	[31]
	Santiago de Compostela STP	Spain	2014	-	-	-	34	[32]
	Bangkok STP	Thailand	2019	204	120	154	2	[33]
CTZ	Guangzhou STP/Pearl River	China	2010	33	8	4	1442	[34]
	Madrid STP	Spain	2011	-	-	-	987	[35]
	Guangzhou STP	China	2012	6,7	3	ND	426	[30]
	Santiago de Compostela STP	Spain	2014	80	11	9	-	[36]
	Santiago de Compostela STP	Spain	2014	-	-	-	417	[32]
	Swedish STP	Sweden	2017	67	1	-	590	[37]
	Bangkok STP	Thailand	2019	41	6	11	73	[33]
ECZ	Guangzhou STP/Pearl River	China	2010	< LOD	< LOD	< LOD	140	[34]
	Madrid STP	Spain	2011	-	-	-	28	[35]
	Swedish STP	Sweden	2017	89	< LOD	-	335	[37]
FCZ	Santiago de Compostela STP	Spain	2014	93	95	32	-	[36]
	Dresden STP	Germany	2018	167	153	-	-	[38]
	Madrid STP	Spain	2011	-	-	-	58	[35]
	Guangzhou STP	China	2012	65	61	ND	8	[30]
	Swedish STP	Sweden	2017	333	170	-	< LOD	[37]
	Bangkok STP	Thailand	2019	102	102	75	1	[33]
IMZ	Schwarzbach STP/ Rhine River	Germany	2010	< LOD	< LOD	ND	23	[31]
ITZ	Guangzhou STP	China	2012	ND	ND	ND	ND	[30]
	Bangkok STP	Thailand	2019	ND	ND	ND	18	[33]

KTZ	Santiago de Compostela STP	Spain	2014	191	36	11	-	[36]
	Guangzhou STP/Pearl River	China	2010	16	2	1	437	[34]
	Guangzhou STP	China	2012	ND	ND	ND	ND	[30]
	Schwarzbach STP/ Rhine River	Germany	2010	< LOQ	< LOQ	ND	328	[31]
	Madrid STP	Spain	2011	-	-	-	4449	[35]
	Santiago de Compostela STP	Spain	2014	-	-	-	185	[32]
	Swedish STP	Sweden	2017	526	41	-	10000	[37]
	Dresden STP	Germany	2018	348	95	-	-	[38]
	Bangkok STP	Thailand	2019	52.9	4	10	40	[33]
MCZ	Santiago de Compostela STP	Spain	2014	55	15	5	-	[36]
	Guangzhou STP/Pearl River	China	2010	32	3	2	1405	[34]
	Guangzhou STP	China	2012	1.1	0.5	ND	150	[30]
	Santiago de Compostela STP	Spain	2014	-	-	-	141	[32]
	Swedish STP	Sweden	2017	60	< LOD	-	700	[37]
	Bangkok STP	Thailand	2019	20	3	5	18	[33]
TCZ	Guangzhou STP/Pearl River	China	2010	< LOQ	2	3	ND	[34]
TRB	Swedish STP	Sweden	2017	27	5	-	300	[37]

ND: not detected
-: not studied

According to data summarized in the above table, similar concentrations of FCZ have been observed in the influent and the effluent of several STPs [30,33,36,38], indicating that this compound is resistant to degradation in these facilities, reaching easily surface waters, where levels greater than 32 ng L^{-1} have been found. In addition, several authors point out to the possibility that this compound reaches the STPs not only in the free form, but also as a conjugate, undergoing a deconjugation reaction during the biological treatment [39].

In case of the most lipophilic compounds, such as CTZ, FTZ, ITZ, MCZ and STZ, the concentrations found in influent and effluent water samples are negligible in comparison with those found in sludge (73-1442 ng g^{-1}). These latter data demonstrate that mass balances performed on the basis of dissolved concentrations are not suitable to understand the efficiency of STPs for the removal of lipophilic EPs.

Finally, compounds from medium to low polarity, such as CBZ, ETZ, ECZ, IMZ, KTZ, TRB and TCZ, can remain in water matrices or be adsorbed into the sludge, thus concentrations of these compounds can be found in influent, effluent, surface water and sludge. For this reason, the study of the occurrence and fate of these compounds in several environmental compartments is required.

3. Cardiovascular compounds

3.1. Definition

Cardiovascular compounds are a group of pharmaceuticals applied in the treatment of different diseases related to the circulatory system and heart, such as arterial hypertension, arrhythmias and heart failure.

Nowadays, there is a plethora of useful pharmaceuticals to alleviate the above medical problems. These drugs are grouped into a few families, or categories, depending on their mode of action. Drugs belonging to the same family, usually present similar chemical structures and thus, close properties and environmental behaviour. The structures of the cardiovascular drugs studied along this doctoral thesis are compiled in **Figure 6**.

A basic classification of the different families of cardiovascular pharmaceuticals is shown below [40]:

- Lipid-lowering drugs
- *β*-blockers
- Calcium channels *β*-blockers
- Angiotensin II receptor antagonists (ARA II)
- Diuretics
- Antiarrhythmic drugs
- Anticoagulants
- Platelet anti-aggregants

3.2. Structure and physicochemical properties

As it was mentioned in section 3.1., there are different families of cardiovascular pharmaceuticals with different properties and chemical structures. In general terms, from a chemical point of view, antiarrhythmic compounds have in common in their structure a benzo[*b*]furan group and, in the same way of the *β*-blocker propranolol, they are positively charged at neutral pH. By contrast, most ARA II pharmaceuticals present in their structure, a biphenyl group attached to a tetrazole ring, which lends the compounds an anionic character. Additionally, in some of these compounds, a carboxylic group is also present in their structures and at neutral pH, it is negatively charged.

Table 3 compiles the cardiovascular pharmaceuticals considered in this doctoral thesis including their CAS number, empirical formulae, molecular weight and their most relevant physicochemical properties from an analytical point of view.

Table 3. Name, abbreviation, CAS number, formula and some physicochemical properties of the studied cardiovascular pharmaceuticals (ChemSpider).

Family	Name	Abbreviature	CAS	Formula	Monoisotopic Mass	log K_{ow}	pK_a
ß-blocker	Propranolol	PRO	13013-17-7	$C_{16}H_{21}NO_2$	259.1572	2.58	9.67[a] 14.09[b]
Antiarrhythmic	Amiodarone	AMI	1951-25-3	$C_{25}H_{29}I_2NO_3$	645.0237	7.64	8.47
	N-Desethylamiodarone	N-DES	96027-74-6	$C_{23}H_{25}I_2NO_3$	616.9924	7.86	9.40
	Dronedarone	DRO	141626-36-0	$C_{31}H_{44}N_2O_5S$	556.2971	5.18	10.31[a] 9.18[b]
	Flecainide	FLE	54143-55-4	$C_{17}H_{20}F_6N_2O_3$	414.1378	3.19	9.62[a] 3.68[b]
Angiotensin II receptor antagonist	Candesartan	CAN	139481-59-7	$C_{24}H_{20}N_6O_3$	440.1597	4.68	1.50[a] 3.44[b]
	Eprosartan	EPRO	133040-01-4	$C_{23}H_{24}N_2O_4S$	424.1457	3.75	6.67[a] 3.47[b]
	Irbesartan	IRB	138402-11-6	$C_{25}H_{28}N_6O$	428.2325	5.39	4.12[a] 5.85[b]
	Losartan	LOS	114798-26-4	$C_{22}H_{23}ClN_6O$	422.1622	4.06	3.82[a] 4.26[b]
	Olmesartan	OLM	144689-24-7	$C_{24}H_{26}N_6O_3$	446.2066	2.16	5.33[a] 0.89[b]
	Telmisartan	TELM	144701-48-4	$C_{33}H_{30}N_4O_2$	514.2367	6.13	5.86[a] 3.62[b]
	Valsartan	VAL	137862-53-4	$C_{24}H_{29}N_5O_3$	435.2270	4.59	- 0.52[a] 4.00[b]
	Valsartan Acid	VALA	164265-78-5	$C_{14}H_{10}N_4O_2$	266.0804	-	-

[a] Strongest basic pK_a
[b] Strongest acidic pK_a

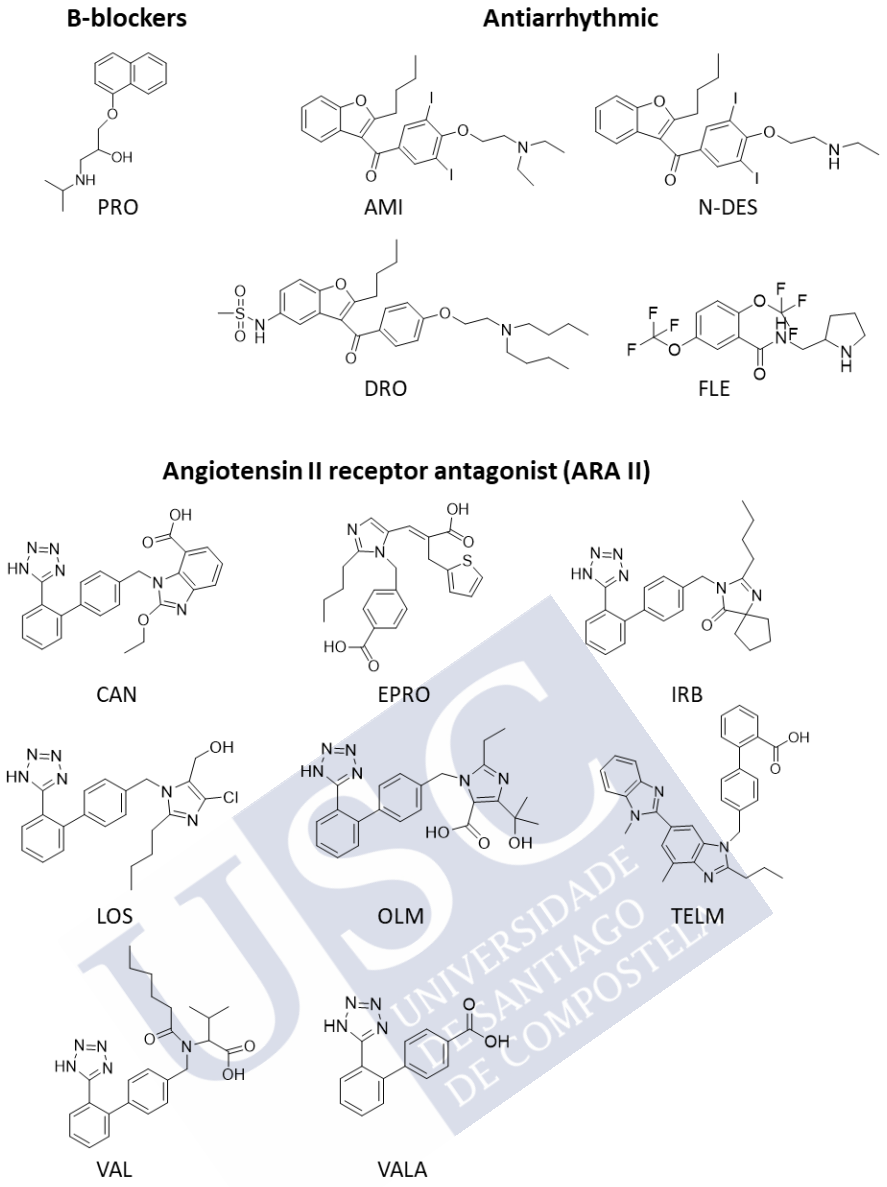

Figure 6. Structure of the cardiovascular pharmaceuticals studied

3.3. Consumption

Despite all the improvements in the medical treatments, cardiovascular diseases remain the most common cause of death in the world. Heart failure and stroke will cause an estimated of 24 million of deaths per year by 2030, representing the leading cause of mortality among the most prevalent chronic diseases [41]. Considering the increasement in the prevalence of cardiovascular diseases, it is not difficult to imagine that the consumption of this type of drugs has increased steady during the last decades.

Figure 7 compiles the consumption data of different active compounds of cardiovascular drugs in Spain between 2013 and 2018, expressed as diary dose per 1000 inhabitants [42]. According to the Spanish Agency for Medicines and Health Products (AEMPS), the most consumed cardiovascular pharmaceuticals are VAL, LOS and CAN. These data are referred to the number of doses, and since the administrated amount per

dose varies depending on each compound, the mass consumption could change depending on the dose associated.

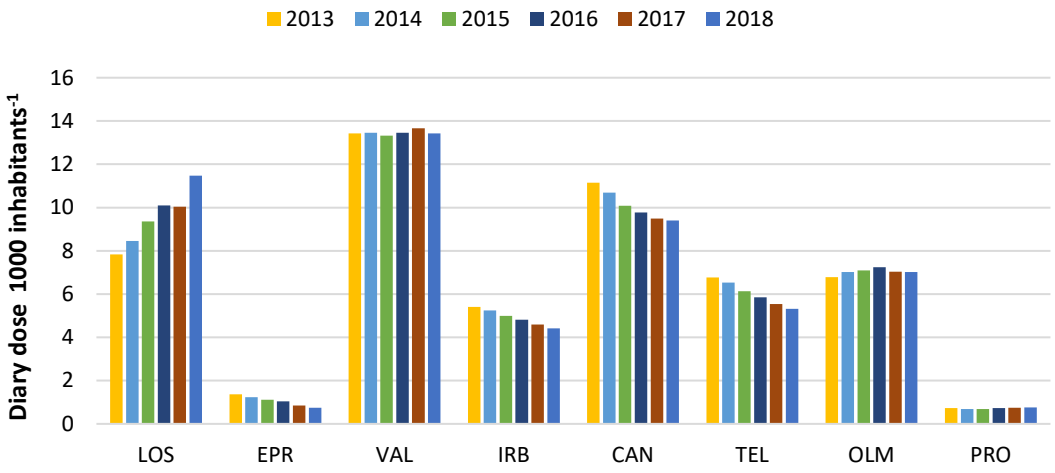

Figure 7. Consumption data of cardiovascular drugs in Spain

3.4. Toxicity

Although these substances constitute the main active ingredient in many pharmaceuticals and are highly consumed, there is still a lack of toxicological data regarding their effects in the environment and living beings. A few authors have evaluated the toxicity for some compounds *in vitro*, in *Daphnia Magna* [43,44] and *Lemna Minor* [45], and *in silico* in daphnid, algae and fish, using ECOSAR software [46]; and all studies reveal the same premise, that cardiovascular pharmaceuticals constitute a risk for aquatic species.

The toxicity of these compounds at the levels found in real samples, such as influent and effluent, was evaluated and revealed that OLM and VAL, which are within the most consumed compounds, do not show any acute toxic effect, but chronic effects for *Daphnia Magna* at those concentrations [43]. A. Villejas-Navarro studied the effect of cardiovascular drugs on heart using *Daphnia Magna.* The results reveal that all studied compounds were toxic with 48h-LC_{50} and in case of metoprolol, causes a significant acceleration in the heart rate with amplitude of contraction at 10^{-8} M and a significant decrease at higher concentrations (>10^{-3} M) [44]; a similar effect was previously reported in rats [47].

On the other hand, ecotoxicological studies were developed for LOS and PRO in *Lemna Minor* [45]. *Lemna Minor* is an aquatic macrophyte commonly used in phytotoxicity test, due to its structural simplicity, small size and rapid growth. Although both pharmaceutical products are considered as hazardous to the aquatic environment, LOS is slightly more toxic than PRO, and can produce chronic effects on some species such as algae, fish and benthic macroinvertebrates. In addition, *in silico* studies have compared the toxicity of antibiotics, sex hormones, antineoplastic and cardiovascular

pharmaceuticals. For cardiovascular drugs, approximately 25 % of the studied compounds were predicted to be very toxic to all test species [46].

3.5. Levels of cardiovascular pharmaceuticals in the environment

The increase in the consumption of cardiovascular pharmaceuticals in the last years has triggered the presence of these compounds in the urban wastewater, considered as their main route of entry into the environment.

In the same way as antimycotic pharmaceuticals (section 2.5.), cardiovascular drugs and their metabolites reach the environmental matrices through urban sewers, after human excretion. Although a variable percentage of these substances is eliminated during water treatments at STPs, the rest is emitted with the effluents to surface waters [48,49], reaching in some cases, the tap water [50,51]. In addition, the hydrophobic character of some compounds, as AMI, N-DES, IRB and TELM, favours the bioaccumulation of these compounds on sediments, in contaminated waters, and the adsorption in sludge at STPs [52,53], being subsequently distributed in soils as a result of the use of biosolids as fertilizers in agriculture.

Table 4 shows the levels of cardiovascular drugs detected in aquatic matrices and sludge collected from the literature.

Table 4. Maximum concentrations of cardiovascular active compounds measured in influent, effluent and surface water (river or lakes), tap water (values in ng L^{-1}) and in sludge samples (ng g^{-1}).

Analyte	Sample	Country	Influent (ng L^{-1})	Effluent (ng L^{-1})	Surface water (ng L^{-1})	Tap water (ng L^{-1})	Sludge (ng g^{-1})	Ref
PRO	Mar Menor Lagoon	Spain	-	-	0.5	-	-	[54]
	Llobregat River	Spain	-	-	270	ND	-	[50]
	Vistula River	Poland	-	-	69	-	-	[51]
	Hudson River Estuary	USA	-	-	134.1	-	-	[55]
	New York Harbor	USA	-	-	1.2	-	-	[55]
AMI	Markaryd STP	Sweden	-	< LOD	-	-	-	[56]
	French STP	France	-	-	-	-	1200	[57]
	Santiago Compostela STP	Spain	-	-	-	-	362	[52]
	Vistula River	Poland	-	-	< LOD	-	-	[51]
N-DES	Santiago Compostela STP	Spain	-	-	-	-	285	[52]
FLE	Markaryd STP	Sweden	-	32	-	-	-	[56]
CAN	Bavaria STP	Germany	1000	820	1100	-	-	[43]
EPRO	Bavaria STP	Germany	4510	6500	280	-	-	[43]
IRB	Markaryd STP	Sweden	-	25	-	-	-	[56]
	Llobregat River	Spain	-	-	830	28	-	[50]
	Bavaria STP	Germany	4520	2600	800	-	-	[43]
	Mar Menor Lagoon	Spain	-	-	17	-	-	[54]
	Grab samples	Greece	-	-	28	-	-	[58]

LOS	Antwerp-North STP	Belgium	32	-	-	-	-	[59]
	Brussels STP	Belgium	360	-	-	-	-	[59]
	Bavaria STP	Germany	2040	450	120	-	-	[43]
	Mar Menor Lagoon	Spain	-	-	104	-	-	[54]
	Llobregat River	Spain	-	-	620	ND	-	[50]
	Vistula River	Poland	-	-	610	-	-	[51]
	Hudson River Estuary	USA	-	-	1699.8	-	-	[55]
	New York Harbor	USA	-	-	48.6	-	-	[55]
OLM	Bavaria STP	Germany	1080	1200	2200	-	-	[43]
TELM	Antwerp-North STP	Belgium	73	-	-	-	-	[59]
	Brussels STP	Belgium	424	-	-	-	-	[59]
	Markaryd STP	Sweden	-	< LOD	-	-	-	[56]
	Bavaria STP	Germany	1570	1400	700	-	-	[43]
	Vistula River	Poland	-	-	1130	> 20	-	[51]
VAL	Antwerp-North STP	Belgium	84	-	-	-	-	[59]
	Brussels STP	Belgium	1809	-	-	-	-	[59]
	Grab samples	Greece	-	-	146	-	-	[58]
	Bavaria STP	Germany	8100	6000	770	-	-	[43]
	Mar Menor Lagoon	Spain	-	-	38	-	-	[54]
	Llobregat River	Spain	-	-	1300	70	-	[50]
	Vistula River	Poland	-	-	5260	> 20	-	[51]
	Hudson River Estuary	USA	-	-	3811.9	-	-	[55]
	New York Harbor	USA	-	-	117.4	-	-	[55]
VALA	Grab samples	Greece	-	-	16	-	-	[58]
	Göotingen STP	Germany	-	320	2119	72	-	[60]

ND: not detected
-: not studied

According to data summarized in **Table 4,** it can be concluded that cardiovascular pharmaceuticals are ubiquitous in different environmental compartments, from wastewater and other aquatic samples, to sludge due to the wide variety in their structures. In general terms, these compounds have octanol-water partition coefficients (log K_{ow}) that point out medium to very low polarities (2.16-7.86 for their neutral forms); however, the presence of acidic moieties in their structures, as carboxylic acid or tetraazolic rings, increases significantly their mobility to the aquatic media.

Among ARA II pharmaceuticals, the most studied are IRB, LOS, TELM and VAL, probably due to their high consumption and detection rate; and they are usually included in multi-analyte studies applied to the screening of emerging pollutants in the aquatic environment [61], or in methodologies focused on the determination of cardiovascular compounds. The average concentrations of these compounds in wastewater are usually above 1000 ng L^{-1}, with variable elimination percentages at STPs: 17 % OLM, 19 % CAN, 29 % IRB, 43 % EPR and 96 % VAL [43]. The main problem is that, in most cases, these compounds can be biodegraded to other substances, such as VALA. This species is usually formed by other compounds as VAL, OLM, LOS, CAN and IRB, during the treatment in the STPs. Thus, the estimated percentages of elimination should be considered with caution.

From municipal wastewaters, the most polar compounds that are not eliminated, can reach the surface waters. Most of the studied ARA II compounds were found in different groundwater samples, lakes and rivers, all over the world, in concentrations usually over 100 ng L^{-1}. In case of VALA, which is a very polar and stable species, is ubiquitous in different types of water samples, including tap water, at concentrations above 20 ng L^{-1}.

The presence of some compounds, such as AMI, N-DES, FLE and DRO, is not deeply studied, and the concentration data in environmental samples is still limited. In case of AMI and its metabolite, N-DES, there is some information about concentrations in sludge. Both are highly lipophilic, so they have tendency to be accumulated in the sludge. The maximum concentrations of these species (1200 ng g^{-1}) were reported by W. Peysson et al [57], in France.

4. Phenolic compounds

4.1. Definition

4.1.1. Phenolic bactericides

Those substances capable of reducing or eliminating bacterial development and unicellular microorganisms are considered as bactericides. These compounds can be classified as disinfectants, antiseptics or antibiotics. Some phenolic compounds constitute the base of the current biocides, due to their ability to inhibit the growth of gram positive and negative bacteria and some fungi. This type of compounds is widely used as additives in cosmetics, personal care products and food.

The phenolic compounds studied along this doctoral thesis are described below:

Parabens are a family of esters derived from 4-hydroxybenzoic acid. These compounds constitute the most common preservatives used in cosmetic, pharmaceutical and industrial products, due to their bactericide activity, high stability in a broad range of pH values and low toxicity and cost. Their antimicrobial activity increases with the length of the alkyl chain, being more effective against gram positive bacteria than gram negative, so the enhancement of antimicrobial coverage is only achieved by combination of different paraben compounds. For these reasons, it is common to find them in numerous daily products, such as personal care products (PCPs), pharmaceuticals, drinks and processed foods. Among parabens, the most consumed are methylparaben and propylparaben and often they are present together.

Despite all their advantages, these compounds have been the object of study in recent years, because their indiscriminate use and their high persistence in the environment results in the presence of high levels of these compounds in environmental compartments. In addition, some studies *in vivo* and *in vitro* have revealed that they exert endocrine disruption activity. Thus, since 1995, the presence of these compounds in cosmetic is regulated in Europe [62], establishing a maximum concentration of 0.4 % (as p-hydroxybenzoic acid) for a single ester, and 0.8 % (as acid) for a mixture of esters. In case of food, the maximum concentration is lower, 0.1 % (as acid), according to the Directive 95/2/CE of 20 February of 1995 from de European Union [63].

Triclosan (5-chloro-2-(2,4-dichloro-phenoxy)-phenol), commercially known as Irgasan DP300, is a powerful bactericide and fungicide, usually applied in the domestic and personal hygiene industry as antiseptic and disinfectant in toothpastes, deodorants, surgery cleaners, shampoos, etc. Its mechanism of action is based on the inhibition of the transporter protein enoyl-acyl reductase, blocking the biosynthesis of fatty acids required for the formation of the cell wall and reproduction. The concentration of this substance in cosmetic is also regulated, establishing a maximum value of 0.3 % [62].

Orthophenylphenol, or 2-phenylphenol, is a bactericide used in diverse products as glues, leather, concrete additives, preservative and surface disinfectant agent for commercial purposes. Another relevant application of this compound is as additive in the food industry, protecting the stored fruit, and as a fungistatic wax for coating vegetables. The presence of this compound in fresh fruit or treated fruit must be lower than 0.012 %, while in cosmetic, the maximum concentration allowable is 0.2 % (as phenol) [62].

4.1.2. Bisphenol compounds

Bisphenols are a family of compounds with two hydroxyl-phenyl functionalities, whose main application worldwide is the plastic elaboration. The first bisphenol synthetized by the industry was bisphenol A (BPA), which is an organic compound commonly used as monomer during the preparation of polycarbonate plastic material and epoxy resins since 1950. In addition, this compound is further used in the production of epoxy resins employed in many consumer products as drinking bottles, dental sealant, coating in food tins, thermal paper, and so on.

The main disadvantage of this compound is its high capacity to interact with the estrogenic receptors, constituting a potential endocrine disruptor, which can lead to a feminization in many animal species, and a wide range of health issues in animals and humans [64], as breast cancer and hormonal imbalance, as well as some difficulties in reproduction and development behaviour [65]. Therefore, in order to replace the BPA in many fields, several bisphenol analogues with similar properties have been synthetized. The most common analogues of BPA are bisphenol AF (BPAF), bisphenol B (BPB), bisphenol C (BPC), bisphenol E (BPE), bisphenol F (BPF), bisphenol S (BPS), … [66]. Despite the hardly efforts to replace the BPA with safer alternatives, some studies outlined the occurrence and fate of this compound and their analogues in environmental samples, and therefore, restrictions on the use of these substances have been applied. As concerns about BPA, the maximum intake has been established at 4 μg kg^{-1} body weight day^{-1} according to the Commission Regulation (EU) 2018/213 of 12 February 2018 [67]. In addition, in case of thermal paper, which is the main topic to deal with along in this doctoral thesis, the EU proposed a restriction to maximum concentration of BPA of 0.02 % by weight (equivalent to 0.2 mg g^{-1}) for thermal paper commercialized after 2020 [68].

4.2. Structure and physicochemical properties

4.2.1. Phenolic bactericides

Phenolic bactericides considered in this doctoral thesis are compiled in **Table 5**, as well as their CAS number, empirical formulae, molecular weight and the most relevant physicochemical properties of these compounds from an analytical point of view: log K_{ow} and pK_a. Their structures are shown in **Figure 8** and **Figure 10**.

Parabens are alkyl esters of *p*-hydroxybenzoic acid that differ in the *para* position of the benzene ring occupied by different chemical substituents, which provide each paraben ester with a different solubility and antimicrobial activity. Usually, the longer the alkyl chain is, the lower the water solubility. The parabens studied in this thesis are methylparaben (mP) and propylparaben (pP), that are soluble in water and present high stability in an extensive range of pH values, being stable in acidic aqueous solutions and hydrolysed to *p*-hydroxybenzoic acid and the corresponding alcohol in alkaline media. At the pH of natural waters, these compounds are in neutral form.

Triclosan is a chlorinated biphenyl ether. This compound is resistant to the hydrolysis, but no to thermal and photochemical degradation. Regarding its environmental distribution, it is practically insoluble in water (solubility 10^{-6} g mL^{-1}),

although its solubility increases when the pH becomes more alkaline. Its octanol-water coefficient (log K_{ow} 4.98) suggests that this compound is lipophilic, so it tends to be adsorbed into solid materials.

Orthophenylphenol, also known as 2-hydroxybiphenyl, is a chemical compound with a medium polarity (log K_{ow} 3.32) and pK_a of 9.69, so, according to its physicochemical properties, this compound can remain dissolved in natural waters.

Table 5. Name, abbreviature, CAS, formula and some physicochemical properties of the studied phenolic bactericides (ChemSpider).

Name	Abbreviature	CAS	Formula	Monoisotopic Mass	log K_{ow}	pK_a
Methylparaben	mP	99-76-3	$C_8H_8O_3$	152.0473	1.67	8.5
Propylparaben	pP	94-13-3	$C_{10}H_{12}O_3$	180.0786	2.55	8.5
Triclosan	TCS	3380-34-5	$C_{12}H_7Cl_3O_2$	287.9512	4.98	7.68
Ortho-phenylphenol	OPP	90-43-7	$C_{12}H_{10}O$	170.0732	3.32	9.69
Bisphenol A	BPA	80-05-7	$C_{15}H_{16}O_2$	228.1150	4.04	9.78
Bisphenol A diacetilated	BPADA	10192-62-8	$C_{19}H_{20}O_4$	312.1362	3.87	*
Bisphenol B	BPB	77-40-7	$C_{16}H_{18}O_2$	242.1307	4.49	9.77
Bisphenol E	BPE	2081-08-5	$C_{14}H_{14}O_2$	214.0994	3.74	9.81
Bisphenol F	BPF	620-92-8	$C_{13}H_{12}O_2$	200.0837	3.46	9.84

*No ionizable atoms found.

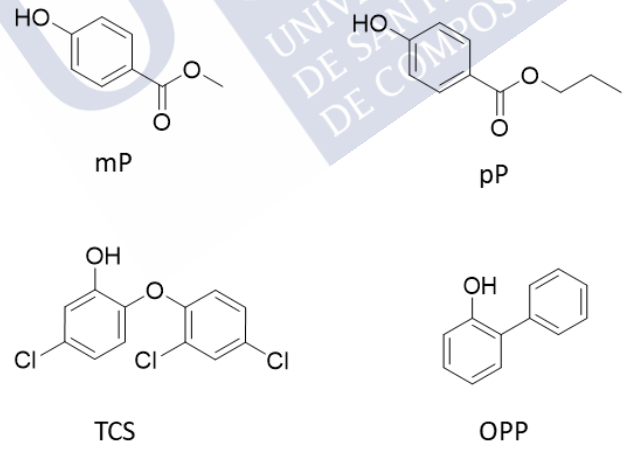

Figure 8. Structures of the studied phenolic bactericides

4.2.2. Bisphenol compounds

Bisphenols are aromatic compounds constituted by two phenolic rings bonded through a bridge group. The general formula is $X(C_6H_5O)_2$, where X is the bridge group, is showed in **Figure 9**.

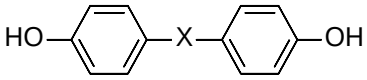

Figure 9. General structure of bisphenol compounds

In general terms, BPA and their analogues are highly reactive compounds. These compounds show a similar reactivity to *para*-substituted phenols, being able to suffer modifications in any of the aromatic rings, the hydroxyl group, or even in the central carbon between both rings. The solubility in water of these substances is quite low, being greater at alkaline conditions ($pK_a \approx 9.77$-9.84). Most of these compounds are usually described as lipophilic substances, so they can be adsorbed in soils or sediments, or even through skin and mucous membranes, being accumulated in fish and mammals [69].

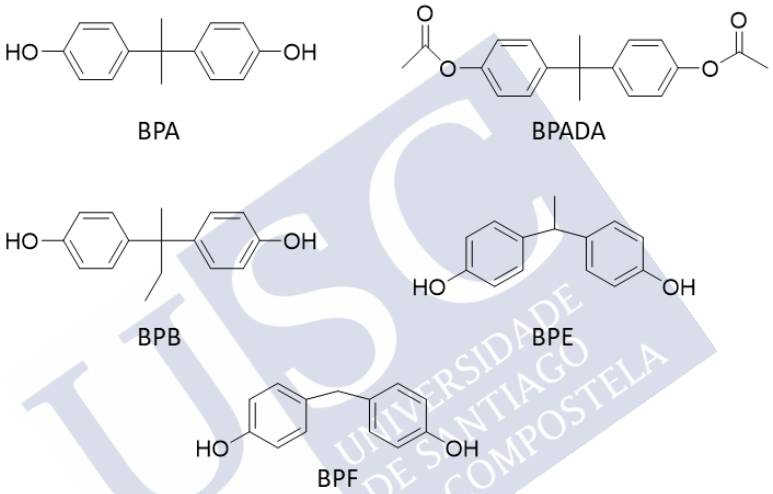

Figure 10. Structures of BPA and their analogues considered in this thesis

4.3. Global production of phenolic compounds

The production data per year of these phenolic compounds, obtained from the European Chemicals Agency (ECHA), are presented in **Table 6**.

Table 6. Volume of phenolic compounds production in tons year^{-1} in Europe.

	Tons year^{-1}
mP	$1x10^3$ -$10x10^3$
pP	100-$1x10^3$
TCS	$15x10^3$-$227x10^3$
OPP	10 - 100
BPA	$1x10^5$-$1x10^6$

The data provided by ECHA are an overview of the calculated volume of each substance which is manufactured and/or imported in the European Economic Area. However, there are not available data for BPA analogues in the ECHA database.

4.4. Toxicity

Phenolic compounds are a group of substances with similar chemical structure considered as potential endocrine disruptors. These substances produce different biological activities with adverse and beneficial associations related to metabolic health outcomes. There are some authors who describe the toxicological effects of these compounds in environment and humans, which are described in the following paragraphs.

4.4.1. Phenolic bactericides

Parabens had been considered as environmental and human health safer preservatives, until 2004, when Darbre et al. [70] revealed that parabens may be related to breast cancer, probably due to their use in antiperspirant and deodorant products. This statement raised broad the attention on the toxicity of parabens, and several authors published that the exposure to these compounds may change, or disrupt, endocrine systems, causing harmful consequences on human health [70–72]. *In vitro* and *in vivo* studies have shown that parabens and their metabolites have estrogenic activity, which may lead to breast tumours [70], skin cancer [73], and infertility [72,73] among other diseases.

Regarding ecotoxicity, according to the classification provided by companies to ECHA in REACH registrations, parabens are considered as harmful to aquatic life with long lasting effects, due to their readily bioaccumulation in aquatic species. The toxicity of parabens in bacteria is closely related to the chain length, the longer, the more toxic. mP appears to be the least acutely toxic and pP one of the most toxic ones, together with benzylparaben [74]. The information about chronic toxicity of this family of compounds is still limited; however; there are some authors that studied the toxicity of these compounds in aquatic organism as *Daphnia Magna* and *Pimephales promelas* [75]. The obtained results were in concordance with the acute toxicity data: mP is less toxic than propyl or benzylparaben.

Triclosan. Direct applications of triclosan-based products as deodorants or toothpastes results in the absorption of this compound through skin, mucous membranes of the oral cavity or gastrointestinal tract, reaching the circulatory system. This exposure of TCS to humans, although at the low levels, can promote genotoxic, teratogenic, mutagenic or carcinogenic disorders [76]. This compound is also considered an endocrine disruptor, since it produces estrogenic and androgenic activity in humans and animals [77]. In addition, it causes the perturbation of the thyroid homeostasis and reduces the levels of hypothyroxinaemia hormone in blood [78], which can reduce the production of oestrogens and androgens and affect the normal function of placenta and the implantation of embryo [77].

In the matter of ecotoxicological effects, several authors report the toxicity of this substance to aquatic organisms, particularly fish, crustaceans and microalgae [72,74,79]. D. Orvos et al. [79] studied the toxicological effects of TCS to wastewater microorganisms, algae, daphnids and fish, and they found that this substance had no toxic effect to wastewater microorganisms at concentrations lower than aqueous solubility,

however, the rest of models were highly sensitive to the exposure of TCS, being more toxic in the early life-stage.

Orthophenylphenol and its sodium salt are widely used as antimicrobial agents for consumer purposes. This huge utilization in a variety of applications suggests that exists a potential risk for animals and humans; and some authors have reported that this substance is a powerful carcinogenic and genotoxic agent, closely related to bladder cancer [80,81].

Ecotoxicological information of OPP is quite limited, however, toxicological effects to *Daphnia Magna*, *Poecilia Reticulata* and *Lymnaea Stagnalis* have been reported [82], confirming the high toxicity of this compound for the studied microorganism and its potential bioaccumulation.

4.4.2. Bisphenol compounds

The main human exposure to BPA is the consumption of food that has been stored in packages coated with BPA related resins. The toxicological risk of this compound has been investigated in depth, and, nowadays, BPA is recognized to be an endocrine disruptor, with the capacity to alter functions and systems in the organism. BPA is rapidly assimilated by the organism without bioaccumulation, but its daily exposure can lead to different issues, as impairment of liver, kidney, reproductive and hormonal system. Some authors indicate that BPA is closely related with [64,83,84]:

1. Cell proliferation and cancer, especially breast, liver and prostate cancer.
2. Alterations in the development and maturation of cells.
3. Oxidative stress and damage to the genetic material.
4. Metabolism alterations.
5. Alterations of the reproductive system.
6. Neuronal disfunction.
7. Cardiovascular diseases.
8. Obesity.
9. Diabetes.

Regarding the toxicological effects of the analogues of BPA, they are still under investigation, however, some studies reveals that some of them, as BPF, BPAF and BPB are even more toxic than BPA, thus, the use of these compounds as alternatives to BPA should be carefully investigated [85–88].

4.5. Levels of phenolic compounds in the environment

In the same way as many other EPs, phenolic compounds are continuously released into the environment, due to their huge daily consumption worldwide.

Phenolic compounds can reach the environment through urban sewage, similarly as pharmaceuticals (described in section 2.5. and 3.5.), but in this case, the high concentrations in the environment are originated from household wastewater, due to dragging action during daily hygiene; from hospital effluents, and from food and pharmaceutical industry.

Basically, the molecular properties of contaminants determine the fate and distribution in the environment. In general terms, phenolic compounds present medium to high polarities, thus, effluents, surface waters, soils and sludge, and even indoor air are susceptible to contamination. **Table 7** compiles the levels of the different phenolic compounds measured in aquatic matrices and sludge samples obtained from previous literature studies.

Table 7. Maximum concentrations (ng L^{-1}) measured in influent, effluent and surface water (river or lakes), sediments and sludge samples (ng g^{-1}).

Analyte	Sample	Country	Year	Influent (ng L^{-1})	Effluent (ng L^{-1})	Surface water (ng L^{-1})	Sediment (ng g^{-1})	Sludge (ng g^{-1})	Ref
mP	Ontario	Canada	2005	1470	30	-	-	-	[89]
	Guanzhou STP	China	2008	10002	8	-	-	-	[90]
	Madrid STP	Spain	2010	-	-	-	-	26	[91]
	Santiago Compostela STP	Spain	2010	10000	50	-	-	-	[92]
	Melbourne	Australia	2011	-	-	4	5	-	[93]
	Guanzhou	China	2012	372	8	ND	-	31	[30]
	Bohai Sea	China	2012	-	-	-	2	-	[94]
	Antartic	Antartic	2013	-	ND	33	-	-	[95]
	Yellow Sea	China	2016	-	-	-	3	-	[94]
	Xiamen STP	China	2016	154	94	-	-	222	[96]
	Jilin Sonshua River	China	2017	-	30	10	40	-	[97]
	Harbin STP	China	2017	1310	-	-	-	-	[98]
	China STP	China	2018	-	-	-	-	263	[99]
pP	Ontario	Canada	2005	2430	40	-	-	-	[89]
	Guanzhou STP	China	2008	579	11	-	-	-	[90]
	Madrid STP	Spain	2010	-	-	-	-	44	[91]
	Santiago Compostela STP	Spain	2010	28000	21	-	-	-	[92]
	Melbourne	Australia	2011	-	-	< LOD	< LOD	-	[93]
	Guanzhou	China	2012	69	1	ND	-	ND	[30]
	Bohai Sea	China	2012	-	-	-	1	-	[94]
	Antartic	Antartic	2013	-	ND	3	-	-	[95]
	Yellow Sea	China	2016	-	-	-	1	-	[94]
	Xiamen STP	China	2016	380	115	-	-	13	[96]
	Harbin STP	China	2017	496	-	-	-	-	[98]
	China STP	China	2018	-	-	-	-	57	[99]

TCS	Ontario	Canada	2005	1830	360	-	-	-	[89]
	Guanzhou STP	China	2008	712	81	-	-	-	[90]
	Guanzhou	China	2012	113	19	ND	-	189	[30]
	Jundiaí River	Brazil	2013	-	-	< LOD	-	-	[100]
	Pirai Creek	Brazil	2013	-	-	44	-	-	[100]
	Antartic	Antartic	2013	-	ND	248	-	-	[95]
	California	USA	2013	4400	160	-	-	-	[101]
	Dourado River	Brazil	2015	-	-	9	-	-	[102]
	Brilhante River	Brazil	2015	-	-	< LOD	-	-	[102]
	Xiamen STP	China	2016	66	89	-	-	659	[96]
	Jilin Sonshua River	China	2017	-	2	2	2	-	[97]
	China STP	China	2018	-	-	-	-	3890	[99]
OPP	Ontario	Canada	2005	5320	140	-	-	-	[89]
	Guanzhou STP	China	2008	191	26	-	-	-	[90]
	Santiago Compostela	Spain	2014	-	123	ND	-	-	[103]
	Castellón	Spain	2015	1750	ND	-	-	-	[104]
BPA	Ontario	Canada	2005	2400	450	-	-	-	[89]
	35 states	USA	2006-2007	-	-	-	-	459	[105]
	Guanzhou STP	China	2008	13808	132	-	-	-	[90]
	Bohai Sea	China	2012	-	-	-	2	-	[94]
	Indian STP	India	2012	61	5	-	-	6	[106]
	Dourado River	Brazil	2015	-	-	19	-	-	[102]
	Brilhante River	Brazil	2015	-	-	49	-	-	[102]
	Liao River	China	2015	-	-	11131	58	-	[107]
	Yellow Sea	China	2016	-	-	-	1	-	[94]
	Dalian STP	China	2018	412	30	-	-	64	[108]
	Henan STP	China	2018	-	-	-	-	1539	[109]
	China STP	China	2018	-	-	-	-	1210	[99]

BPB	35 states	USA	2006-2007	-	-	-	-	1	[105]
	Indian STP	India	2012	3	1	-	-	ND	[106]
	Bohai Sea	China	2012	-	-	-	0.3	-	[94]
	Domzale-Kamik STP	Slovenia	2015	9	1	-	-	-	[110]
	Yellow Sea	China	2016	-	-	-	0.4	-	[94]
	Henan STP	China	2018	-	-	-	-	5	[109]
	Dalian STP	China	2018	< LOD	ND	-	-	ND	[108]
BPE	Domzale-Kamik STP	Slovenia	2015	84	2	-	-	-	[110]
	Dalian STP	China	2018	9	ND	-	-	ND	[108]
BPF	Bohai Sea	China	2012	-	-	-	1	-	[94]
	Indian STP	India	2012	10	1	-	-	8	[106]
	Domzale-Kamik STP	Slovenia	2015	16	< LOD	-	-	-	[110]
	Yellow Sea	China	2016	-	-	-	1	-	[94]
	Dalian STP	China	2018	66	ND	-	-	ND	[108]
	Henan STP	China	2018	-	-	-	-	40	[109]
	China STP	China	2018	-	-	-	-	245	[99]

ND: not detected
-: not studied

Concentration and occurrence of EPs in environment is seasonal dependent. During warm seasons, the water flow is lower, so the loads of the released compounds are dissolved in smaller volume of water, which results in an increase of their concentrations, and viceversa. Paraben compounds present high solubility in water; thus, their concentrations are usually higher in water than in sludge, which is just the opposite behaviour to TCS, that has tendency to be deposited in sediments and sludge from STPs. In addition, some authors reported the presence of these compounds in indoor dust, where mP and pP were the parabens found in highest average concentrations, between 226-1670 ng g^{-1} for mP and 123-761 ng g^{-1} for pP [111,112]. Surprisingly, those concentrations were even higher than those detected in sludge. The levels of parabens detected in dust showed high variability, in terms of location, probably due to the different consumption of personal care products per capita. TCS was also detected in dust at concentrations over 1018 ng g^{-1} [111].

In case of OPP, actual information in the environment is quite limited, but in recent years, some authors had reported removal percentages in the STPs and all of them were over 86 % [89,90].

There are many authors that describe the presence of BPA in the environment, but the analogues of BPA are scarcely explored. These compounds are mostly lipophilic; thus, they are expected to be adsorbed in sediments and sludge.

During this doctoral thesis, one of the concerned topics was the quantification of bisphenol-related compounds in thermal paper. The concentrations of BPA and its analogues in thermal paper reported in the literature are compiled in **Table 8**.

Table 8. Summary of maximum concentrations (mg g^{-1}) of BPA, and its analogues, in thermal paper samples described in the literature.

Analyte	Country	Year	Concentration (mg g^{-1})	Ref
BPA	Switzerland	2014	30.4	[113]
	Denmark	2014	17.6	[114]
	Brazil	2017	16.9	[115]
	France	2017	20.3	[115]
	Spain	2017	19.3	[115]
	China	2017	18.7	[116]
BPS	Denmark	2014	8.1	[114]
	Switzerland	2014	12.6	[113]
	Brazil	2017	8.9	[115]
	France	2017	12.6	[115]
	Spain	2017	13.3	[115]
	China	2017	16.8	[116]
BPE	Switzerland	2014	< LOD	[114]
BPB	Switzerland	2014	< LOD	[114]
BPF	Switzerland	2014	< LOD	[114]

Thermal papers from different countries were analysed in last years, reporting concentrations over 8 mg g^{-1}; thus, none of the analysed samples fulfil the legislation established from 2020 [66], which limits the concentration of BPA at a maximum of 0.02

% (w/w, equivalent to 0.2 mg g^{-1}). Considering the toxicological effects of this family of compounds and the high concentration levels recorded in thermal paper, it is essential to reduce the use of BPA and its analogues in order to avoid future health problems.

II.B. Sample preparation

1. Solid-phase extraction

1.1. Fundaments

Solid-phase extraction (SPE) is a simple and versatile sample preparation technique applied to the extraction of liquid samples in contact with a solid sorbent. SPE was described for the first time in the late forties [117]. In the continuous, and most popular mode, the sample solution is passed through the sorbent and the analytes are strongly retained, whilst the interferences flow out without any kind of interaction. Once analytes have been retained, they are recovered by elution with an appropriate elution solvent for analysis. This way, SPE achieves very selective extractions providing that the selected sorbent retains the analytes, but not the interferences existing in the liquid sample.

SPE consists on the following steps [118], represented in **Figure 11**.

- Conditioning: The first step in SPE consists on washing the sorbent material in order to remove impurities and to solvate the functional groups to improve the interaction between the polymer and the analytes. Usually, the solvents applied in the conditioning step are the same applied in the further elution of the analytes. Excess of conditioning solvent must be removed with a weak elution strength solvent, which shows a good miscibility with the conditioning solvent and with the liquid sample to concentrate. Very often, ultrapure water is used in the 2nd step of sorbent conditioning.

- Sample loading: the sample is passed through the sorbent and the analytes are retained in the solid phase. The sample flowrate through the sorbent should be slow in order to facilitate the interaction analyte-sorbent. This step can be developed by gravity; however, when the volume of sample to concentrate exceeds several mL, the use of vacuum is the most successful approach to permit a constant flow of liquid passing through the bed of the SPE sorbent.

- Washing: Once the sample is loaded into the SPE cartridge, it is necessary to clean the solid phase with an appropriate solvent to eliminate interferences.

- Dryness of the solid phase: The sorbent should be dried in order to eliminate the rest of sample or washing solvent. This step usually takes place under a nitrogen flow, or by the application of slight vacuum.

- Elution of the analytes: The analytes of interest are eluted with an adequate solvent, which can break the interaction sorbent-analyte, whilst the interferences remain adsorbed. Whenever possible, the elution solvent should be compatible with the determination technique.

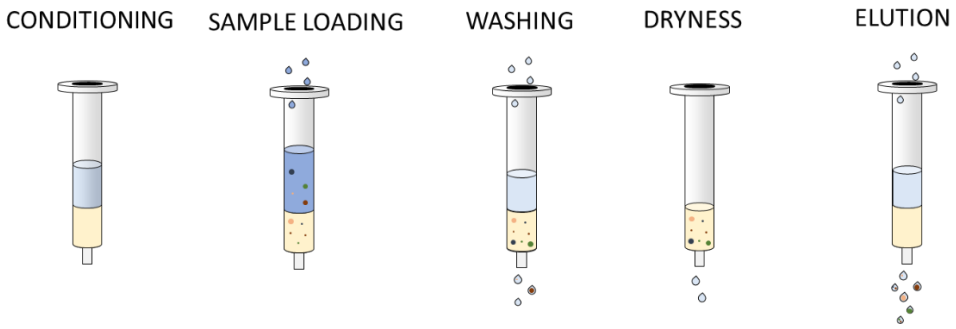

Figure 11. Scheme of the common steps of SPE methods

Theoretically, SPE parameters should be managed to achieve selective interactions of target compounds with the sorbent-solvent system during SPE sample extraction. In SPE, both extraction and elution steps are dependent on the interactions between the solid phase and the analyte, therefore, the parameters that should be taken into consideration during the optimization of this technique are the type of sample, the nature of the analytes, the sorbent applied and the elution solvent. In addition, it is necessary to consider other factors, such as sample pH and ionic strength.

1.2. Sorbent materials

The following paragraphs show a possible classification of main SPE sorbents sorted in three large groups using the criteria proposed by Poole [119]:

- *Inorganic oxides:* The most commonly used materials are silica gel, Florisil (synthetic magnesium silicate), alumina and diatomaceous earth. The strong retention depends on the sorbent properties, larger surface area and high activity, as well as the properties of the analyte, such as the number and type of functional groups. For example, compounds containing hydrogen-bonding functional groups (i.e. carboxylic acid, phenol and hydroxyl groups) are strongly retained by these sorbents; those with high dipole character (ketone, ester and nitro groups) are less retained; and finally, polarizable functional groups (aromatic rings and alkene groups), are the least retained.

  The main applications of this type of materials are the extraction of polar compounds from non-polar samples. Some typical examples are the extraction of pesticides from oils and fats, and the clean-up step of organic extracts obtained in the SPE of water samples using reversed-phase sorbents.

- *Low specificity sorbents:* This type of sorbents includes chemically bonded silicas, carbon and porous polymers. The most common application of these sorbents is the extraction and concentration of organic compounds from aqueous samples.

Bonded silicas are generally synthesized by reaction of mono- or tri-functional organosilanes with silica-gel followed by endcapping of residual

silanol groups. Following this scheme, materials displaying three different types of interactions can be prepared.

- Reversed-phase: This type of sorbents isolates relative non-polar analytes from polar samples. The isolating mechanism consists on Van Deer Waals forces and secondary interactions. The elution of the analytes of interest is achieved with a small volume of organic solvent. Typical examples of common reversed-phase materials are silica functionalized with octyl (C_8) or octadecyl (C_{18}) groups.
- Normal phase: The extraction of polar compounds from non-polar samples takes places in this case. The interaction mechanism is based on hydrogen bond, dipole-dipole or induced dipole-dipole interactions. The elution of the analytes is carried out with polar solvents. Materials such as N-propylethylene diaminosilane (PSA) or cyanopropylsilane (CN) display this interaction mechanism.
- Ionic exchange: Ionic components are extracted from the sample. This kind of materials contains anionic or cationic exchange groups. Thus, the interaction mechanism is based on electrostatic interactions between the sorbent and the analyte. Anionic exchangers can be bonded to strong basic groups, as quaternary amines; or weak, as primary, secondary and tertiary amines. The most common anionic exchangers are, quaternary amines, as those included in (trimethylamine) propylsilane (SAX) or secondary amines, present in (diethylamino) propylsilane phases (DBA). In case of cationic exchangers, they present acidic groups, such as sulphonates, i.e. (benzene sulphonyl) propylsilane (SCX); or carboxylates, as (carboxy) methylsilane (CBA).

  The elution can be carried out in two different ways: by neutralization (retained ions are converted to their neutral form using an organic solvent with a suitable additive) or displacement (using a solvent with relatively high concentration of the displacing ion, which can bind to active sites more strongly than analytes, displacing it).

Graphitized carbon black is a non-porous sorbent with moderate surface areas. It displays a high affinity towards planar molecules with several polar groups and for species rich in polarizable electrons, establishing π-π interactions. This way, this sorbent is often applied to isolate polar compounds with high water solubility. The elution of the analytes takes place with conventional organic solvents, as methanol, toluene or acetonitrile. Carbon materials have a limited acceptation as reversed-phase sorbents due to non-reversible sorption of polar and acidic compounds on the positively charged sites of the sorbent.

Porous polymeric sorbents are usually copolymers of styrene and divinylbenzene processed to improve their characteristics for SPE. This type of sorbents constitutes an alternative to bonded silicas, due to their large surface area results in a higher retention, presenting an elevate stability throughout the pH range. The most common porous polymer sorbents are those formed by poly (styrene-divinylbenzene) copolymers (PS-DVB), applied in SPE in reversed-phase. Because of the low capacity of the PS-DVB polymers to retain polar compounds, alternative sorbents based on polymers with larger surface area have

been developed by combination of hydrophilic monomers, or by the chemical modification of the PS-DVB skeleton, adding a polar group. One of the most popular of these new porous sorbents is the macroporous poly (divinylbenzene-co-N-vinylpyrrolidone) polymer, a hydrophilic-lipophilic balanced sorbent, known under the commercial name of Oasis HLB from Waters.

- *Compound-specific and class-specific sorbents:* In recent years, the lack of specific sorbents has promoted the design of new polymers that improve the selectivity during the extraction step. This group of specific sorbents includes the mixed-mode polymers, the restricted access materials (RAM), the immunosorbents and the molecularly imprinted polymers (MIPs) [120].

Mixed-mode sorbents were presented for the first time in the late nineties. These sorbents were created as an alternative to reversed-phase polymers (e.g. PS-DVB and C_{18} functionalized silicas) to improve the selectivity during SPE. The combination of a polymer skeleton with ionic groups, confers them the possibility of establishing two types of interactions: reversed-phase and ionic exchange. The main application of these sorbents is the extraction of analytes (charged or not) from complex samples. Interferences and analytes are eluted separately during the cleaning and elution steps, respectively, selecting properly the sample pH and the type of elution solvent.

Some of these sorbents are based on the above mentioned HLB polymer, chemically modified with strong cationic exchange groups such as sulphonic groups, OASIS® MCX materials (Mixed-Mode Cation Exchanger); with weak groups as the carboxylic ones, OASIS® WCX (Mixed-Mode Weak Cation Exchanger), or with strong anion exchange groups such as quaternary amines in the OASIS® MAX (Mixed-Mode Anion Exchanger) and weak materials such as piperazine for OASIS® WAX (Mixed-Mode Weak Anion Exchanger), **Figure 12**.

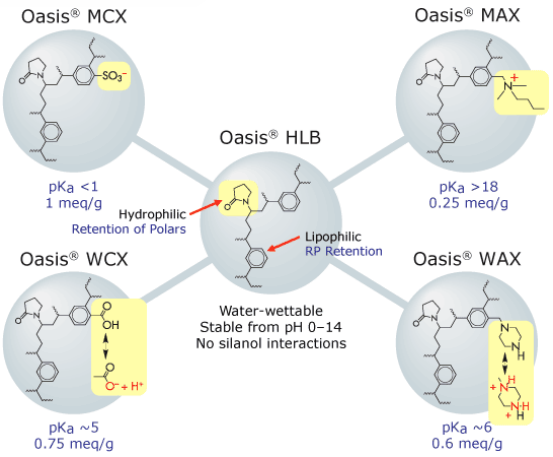

Figure 12. Chemical structures of reversed-phase and mixed-mode sorbents sharing the divinylbenzene-co-N-vinylpyrrolidone skeleton

Restricted access materials (RAM) were developed for the isolation of low-molecular-weight compounds from biological fluids. The isolation of analytes

with this type of materials takes place in terms of affinity and size. Thus, only those compounds with a specific size will be retained by the sorbent, since they are the only ones penetrating into the functionalized pores of the material, while the non-compatible ones will be directly eluted, passing through without any chance to reach the functionalized areas [119]. The most often used RAM sorbents are the semipermeable surface (SPS), shielded hydrophobic packings (SHP) and dual-zone phase (DZP).

Immunosorbents were the first materials considered as specific sorbents. They are formed by a base of silica with an immobilized antibody, which presents exclusive selectivity for the molecules of a given analyte behaving as an antigen [120].

Molecularly imprinted polymers (MIPs) were developed due to the limitations of the immunosorbents, such as long preparation times, irreproducibility, poor stability… These sorbents present a cavity with specific shape and structure to recognize a certain analyte; thus, in principle, they are able to retain a single compound (sometimes a chemical family) in a complex sample.

1.3. Advantages of mixed-mode sorbents

In this doctoral thesis, the strategy of SPE carried out for the extraction of cardiovascular drugs from wastewater samples, was based on mixed-mode sorbents. For this reason, it is important to focus on the advantages offered by this type of sorbents over those of reversed-phase.

As mentioned above, the mixed-mode sorbent is based on the combination of a low polarity polymer skeleton with ionic groups, which confers them the ability to establish reversed-phase and ionic exchange interactions. Given that cardiovascular drugs are strong acidic compounds, the selectivity of their extraction from environmental water samples was improved using reversed-phase and weak-anionic exchange sorbents.

In addition, this strategy was also applied during the extraction of azolic pharmaceuticals from sludge samples, where cationic exchange-type sorbents were used to improve the selectivity of the process. Given that compounds were first recovered from the solid matrix using methanol, the mixed-mode (reversed-phase and cationic exchange) material did not permit the selective retention and isolation of these species from neutrals and acids. Instead, a silica based strong cationic-exchange cartridge (SCX) was used.

1.4. Revision of SPE applications to the extraction of antimycotic and cardiovascular pharmaceuticals

B. Kasprzyk-Horden et al. [121] have employed mixed-mode SPE as sample preparation technique to investigate the presence of a large range of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water samples. An Oasis MCX cartridge was applied to the extraction of the analytes, which were eluted with 5 mL of methanol and 5 mL of methanol (5 % NH_4OH). As regards the set of

compounds involved in this PhD dissertation, the proposed method provided recoveries of 111 % for VAL, which was recovered in the methanolic fraction together with species which do not display basic functionalities (neutrals and acids).

I. Carpinteiro et al. [122] have developed the determination of azolic compounds in wine samples, by mixed-mode SPE. In this case, an Oasis MAX (150 mg) cartridge was used to isolate charged analytes together with neutral species, whilst polar acidic wine interferences (mostly compounds presenting acidic groups in their structures) remained strongly retained in the anionic exchange sorbent through electrostatic interactions. The elution was carried out with 1 mL of methanol. This fraction contains neutrals and basic species, including azolic fungicides. Absolute recoveries over 72 % were achieved. In comparison with pure reversed-phase SPE protocol, lower complexity extracts were obtained.

J. Casado et al. [36] presented a selective method for the determination of antimycotic drugs in environmental samples by mixed-mode SPE. Under optimized conditions, wastewater samples were adjusted to pH 3 and mixed with a 5 % of methanol. The obtained solution was passed through an Oasis MCX (150 mg) cartridge. After cleaning and drying the sorbent, neutral and acidic species (potential interferences) were removed with 2.5 mL of methanol (0.1 % formic acid). In a second step, antimycotic drugs were recovered with 2 mL of methanol (2 % NH_3). Recoveries ranged between 75 and 117% and limits of quantification (LOQs) were comprised between 2 and 15 ng L^{-1}.

M. Papageorgiou et al. [123] have developed an analytical methodology for the determination of pharmaceutical and personal care products (PPCPs) in wastewater using LC-QqQ-MS. Sample extraction was carried out by conventional SPE, with Oasis HLB (200 mg) cartridges. The elution of the target analytes was performed with 2 x 5 mL of methanol, which were evaporated to dryness and reconstituted with 0.5 mL of methanol and injected in the LC-MS/MS system. Average accuracy was in the range between 70 to 120 %. A total of 138 compounds were studied and among them, around 20, including VAL, IRB and TELM were the most frequently detected in the samples at concentrations between 35 to 70 ng L^{-1}.

2. Matrix solid-phase dispersion

2.1. Fundaments

Matrix solid-phase dispersion (MSPD) is a sample preparation technique proposed by Barker et al. [124] in 1989. This technique combines different processes with the aim to extract and to fractionate compounds from a complex matrix, through the complete disruption and dispersion of the sample in a solid support (sorbent), producing an appropriate chromatographic material for the extraction of target compounds. MSPD consists on the following steps [125,126]:

- Dispersion of the sample matrix: The sample is placed in a mortar containing a sorbent material, and the disruption and dispersion is carried out with the aid of a pestle until a homogeneous mixture is obtained. The mortar and pestle

should be of glass or agate, since porous materials lead to analyte and sample losses. Typically, the amount of sample ranges from 0.5 to 1 g, and the sorbent to sample ratio varies between 2 and 4.

- Packaging: Once the sample is completely homogenised, the obtaining blend is transferred to the MSPD cartridge (usually an empty polypropylene SPE-type cartridge), which contains a polypropylene frit at the bottom, in order to avoid sample losses. A second frit is placed on the top of the sample, helping to compact the blend.

  Sometimes, in order to improve the selectivity of the extraction, a co-sorbent is used as clean-up material. This co-sorbent does not participate in the dispersion; it only serves to fractionate the analytes or to retain different interferences. The clean-up material can be placed at the bottom of the MSPD cartridge, before loading the sample, or integrated in a second cartridge connected on-line with the MSPD one.

- Elution: The last step of MSPD is the elution of the analytes. An appropriate solvent is passed through the dispersed sample, and the clean-up sorbent, either by gravity flow or by the application of moderate vacuum. The solvent interacts with the dispersed sample and dissolves the analytes, producing their elution. In most applications the volume of extraction solvent stays around 8 mL [125]. Any parameter potentially affecting the solubility of the analytes in the elution solvent, such as pH, temperature or addition of complexing agents, might impact the yield and the selectivity of the MSPD process.

  There are two different elution strategies to improve the selectivity of the MSPD:

  - Removal of possible interferences in a washing step, followed by the analyte elution with the appropriate solvent.
  - Direct elution of the analytes whilst interferences are retained in the clean-up material.

Finally, the obtained extract could be directly analysed, or it might require a further purification step. The main MSPD steps are presented in **Figure 13**.

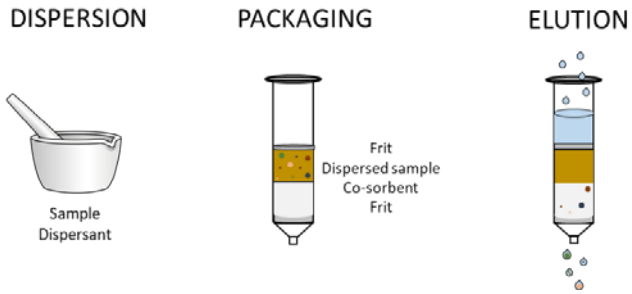

Figure 13. Main steps of the MSPD protocol

The most important factors that affect the yield and the selectivity of the MSPD process are the dispersant and the clean-up sorbent, as well as the elution solvent.

Therefore, these are the most relevant parameters to be optimized during the development of MSPD extraction methods [126,127]:

a. Sorbent selection:

The sample-sorbent ratio is an important parameter that affects the MSPD performance. The amount of sorbent should be large enough to ensure the proper disruption of the sample and the homogeneous dispersion of all the components present in the sample into the sorbent. The most commonly used sample-sorbent ratios are in the range from 1:1 to 1:4 [127–129].

Up to now, most applications of MSPD use silica-based materials as dispersant sorbents. Usually, they contain free silanol groups capable of forming hydrogen bonds with water molecules, so they serve as dispersant sorbent and drying agent at the same time.

Within the silica group, the most often used sorbents are reversed-phase materials, such as silica bonded to octyl (C_8) or octadecyl groups (C_{18}) [52,53,130], whose lipophilic character improves the disrupting, dispersion and retention of non-polar compounds present in the sample. On the other hand, in case of polar compounds, normal-phase materials are applied. Normal-phase sorbents are constituted by silica functionalized with polar groups, or inorganic oxides, such as Florisil or alumina [91,131]. Another type of sorbent commonly used in MSPD are the weak ionic exchangers, including silica bonded to ethylenediamine-N-propyl groups (PSA).

An alternative to active sorbents is the use of inert and abrasive materials, such as sand, *Celite* and diatomaceous earth [132]. These materials are relatively cheap; however, their main disadvantage is the low selectivity of the extraction. The obvious reason is that they do not show any interaction with the different sample components. Thus, the elution profile of analytes is determined just by their relative solubility in the elution solvent.

The nature of the co-sorbent depends on the type of sorbent applied. The basic idea is that both materials present different interactions with the analytes and the rest of sample components. As example, in case of matrix samples previously dispersed in reversed-phase materials (C_8 or C_{18}), normal phase co-sorbents (silica, Florisil or alumina) are used in order to retain polar interferences during the elution step.

b. Elution solvent selection:

The second parameter to optimize in MSPD methods is the nature of elution solvent. It must develop an efficient elution of the analytes, while the interferences remain retained in the MSPD cartridge. The relation between sorbent and solvent applied in MSPD depends on the polarity of the analytes and the type of matrix in which they are present. Polar solvents such as acetonitrile, ethyl acetate or methanol have been applied to the extraction of medium or high polarity analytes, whereas, nonpolar solvents, such as hexane, dichloromethane or mixtures of both, have been used for non-polar compounds.

2.2. Applications of MSPD to the extraction of organic compounds from sludge samples

MSPD was initially designed for the handling of semi-solid food and biota samples. Nowadays, its field of application has been extended to the extraction of EPs from environmental samples. The main advantages of MSPD over other extraction techniques are its low cost, relative selectivity derived from the use of mild extraction conditions, often combined with on-line clean-up strategies, low consumption of organic solvents and the absence of cross contamination due to the use of disposable material (polypropylene cartridge and sorbents/co-sorbents).

The following paragraphs show an overview of some applications of MSPD to the extraction of organic pollutants from sludge samples.

R. Celano et al. [133] have determined the presence of organophosphorus compound residues in sludge by MSPD. For the extraction, 0.5 g of sludge were dispersed in 2 g of C_{18} in a glass mortar and transferred to a syringe containing 1 g of PSA. The analytes were eluted with 15 mL of acetonitrile. The extract was concentrated to a final volume of 1 mL, diluted with ultrapure water (1:1) and injected in a LC-QTOF-MS system. In order to optimize the sample preparation protocol, authors studied the use of different materials as clean-up sorbents, packed at the bottom of the MSPD syringe; however, differences in polarity for the range of selected compounds prevented the success of the clean-up step.

M. Li et al. [53] have optimized the extraction of 45 pharmaceuticals and personal care products from sludge using MSPD as sample preparation technique. To this end, 0.1 g of sludge were put into an agate mortar and dispersed in 0.4 g of C_{18}. The obtained blend was loaded into a polypropylene cartridge and eluted with 6 mL of methanol followed by 10 mL of acetonitrile/5 % oxalic acid (8:2, v/v). Extracts were evaporated to dryness and then dissolved in 1 mL of acetonitrile/water (1:1). Final extracts were injected in a LC-QqQ-MS instrument. Obtained recoveries ranged from 50 to 107 % with relative standard deviation (RSD) lower than 15 %.

K. L. Soares et al. [134] describes a methodology for the extraction of 15 pesticides from sludge samples. For the optimized method, 1.5 g of lyophilized sludge were dispersed on 0.5 g of chitin. Elution of analytes was performed with 5 mL of ethyl acetate and the extract was submitted to a clean-up step (a mixture of 0.5 g of alumina and 0.5 g of Florisil). Finally, 1 mL of extract was collected for chromatographic analysis in GC-MS. Recoveries of the above methodology ranged from 70 to 120 % with RSD below 20 % for all studied compounds.

J. Casado et al. [32] have applied the MSPD technique for the extraction of antimycotic drugs from sludge samples. For that purpose, they dispersed 0.5 g of freeze-dried sludge with 2 g of C_{18}. The obtained blend was loaded into a MSPD syringe, which was connected on-line to a SCX cartridge, used as clean-up material. Then, 10 mL of methanol were passed through the on-line system, retaining the compounds in the SCX sorbent while the neutral interferences were removed in the extraction solvent. Finally, after removing the MSPD packed cartridge, antimycotics were recovered from the SCX cartridge with 10 mL of methanol (0.5 % NH_3). The final extract was concentrated to 5 mL and injected in LC-MS/MS. The obtained recoveries were between 70 and 118 %.

3. Pressurized liquid extraction

3.1. Fundaments

Pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE), is an exhaustive solid-liquid extraction (SLE) technique, that employs a solvent, or mixture of solvents, at elevate temperature and pressure to isolate compounds from solid samples [135]. The extraction of the analytes from the matrix is strongly influenced by their physical properties and the polarity of the solvent. High temperature improves the solubility of the analytes in the extractant, breaking matrix-analyte interactions achieving their desorption and increasing the diffusion rate, while the application of high pressure maintain the solvent below its boiling point. Surface tension and viscosity of the solvent decrease at elevated temperature and pressure, facilitating the extraction of analytes trapped in matrix pores and reducing the volume of solvent required to extract the whole number of compounds embedded in the sample matrix.

PLE is a simple and versatile technique that provides quantitative extractions in relatively short time and with a moderate solvent consumption. This technique presents two extraction modes, static and dynamic. The main difference between static and dynamic modes is that, in the first one, the extraction cell is filled and pressurized with the solvent and maintained at a defined temperature, for a given time. Then, a fresh volume of solvent, usually referred as flush volume, is introduced into the cell in order to repeat the static extraction. On the other hand, in the dynamic mode, the extraction solvent is continuously being pumped through the pressurized extraction cell. Commercial PLE extractors are designed to operate in the static mode since this way reduces the consumption of solvents and produces more concentrated extracts.

The main steps and the instrumentation of PLE [136] are shown in **Figure 14**:

- Introduction of the sample into the cell: Usually samples are dried before PLE extraction, in order to facilitate the solvent-matrix interaction, which improves the extraction efficiency of organic compounds. The weighed sample is loaded into the stainless-steel cell. The amount of sample depends on the cell volume (i.e. 0.5-2 g for cells of 11 mL volume), and the extra space is filled with an inert material in order to reduce the volume of extract.
- Heating and pressurizing the cell: The system heats the cell (between 50 to 200 ºC) maintaining the pressure (from 1500 to a maximum 3000 psi) at pre-defined values.
- Introduction of the solvent and development of static or dynamic extractions, usually in several cycles.
- Flushing the cell with a stream of nitrogen to recover solvent drops in sample pores.

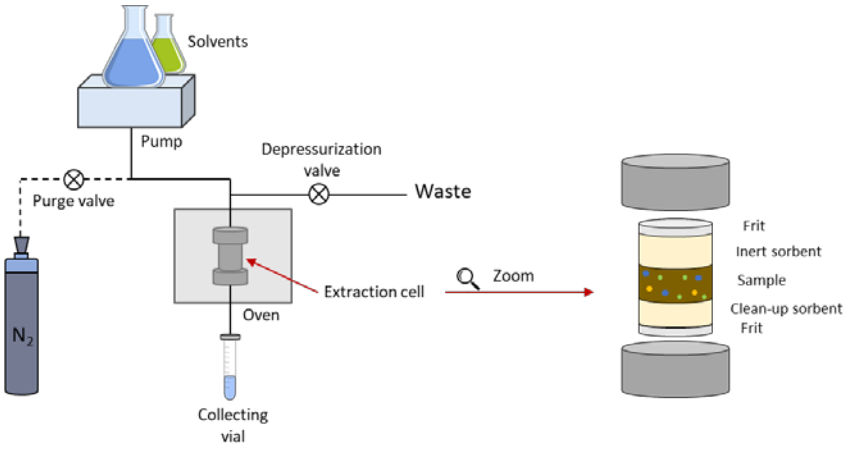

Figure 14. Instrumentation and main steps of the PLE technique

During PLE method optimization, it is necessary to test several parameters that affect to the extraction efficiency, such as temperature, pressure, extraction mode (static or dynamic), extraction time, flush volume, number of extraction cycles and finally, the type of solvents.

There is the possibility to improve the selectivity of the extraction by using different sorbents within the PLE cell. In general lines, extraction conditions inside the cell are rather hard; so, the feasibility to improve the extraction selectivity considering the different interaction of target analytes and interferences is more limited than in the case of MSPD. Other possibilities explored in PLE consist on rinsing the samples with a solvent displaying a low affinity by target compounds previously to a harder extraction cycle; or, the use of oxidative treatments (i.e. sulphuric acid impregnated silica) to remove organic compounds during extraction of recalcitrant halogenated species.

3.2. Applications of PLE to the extraction of dust samples

I. Carpinteiro et al. [137] have applied PLE as sample preparation technique to the determination of benzotriazole light stabilizers in dust samples. Under optimized conditions, 0.5 g of dust were submitted to a set of 3 static extraction cycles of 10 min. Analytes were recovered with a mixture of hexane:dichloromethane (7:3) at 90 ºC. In this application, 1 g of silica was placed in the bottom of the extraction cell, as clean-up sorbent, in order to retain highly polar compounds which might damage the performance of the GC column during the determination step. Obtained recoveries ranged between 82 to 112 %, with standard deviation below 13 %.

P. Vázquez-Roig et al. [138] presented an analytical methodology for the determination of pharmaceutical residues in soils and sediments using PLE as sample preparation technique. For that purpose, 3 g of sample were mixed with 25 g of Na_2–EDTA washed sea sand in the mortar. The resulting mixture was loaded into the extraction cell (22 mL) and extracted with 3 cycles of 7 min at 90 ºC using water as elution solvent. As clean-up technique, an off-line reversed-phase SPE of the obtained extracts was carried out. Absolute recoveries varied between 50 % and 105 %.

F. Mercier et al. [139] have developed a multi-residue methodology for the analysis of semi-volatile organic compounds in indoor dust samples by PLE. In this case, 200 mg of sample were mixed with *Celite* and submitted to 1 static cycle of 5 min. Dichloromethane was used as extraction solvent at 100 ºC. The obtained extracts were divided in two fractions, 0.5 mL were directly injected in GC-MS, in order to quantify compounds in highest concentrations and the others 9.5 mL were concentrated to 1 mL under a nitrogen stream, and then submitted to a clean-up step based on a Chromabond® NH_2 glass columns. Compounds were eluted with 5 mL of dichloromethane and concentrated to 0.5 mL. Final extracts were injected in GC-MS. LOQs ranged from 26 to 1579 ng g^{-1}.

In general, particularly during analysis of EPs by LC-ESI-MS based methods, PLE extractions are carried out using water miscible solvents, such as methanol or acetonitrile, with different modifiers. Primary extracts are diluted with ultrapure water, and then processed following SPE protocols previously optimized during analysis of wastewater samples [140].

II.C. Determination techniques

The analysis of environmental samples, such as wastewater and sludge samples, for trace organic compounds determination, represents one of the most typical challenges which face analytical chemists. Such kind of problems require the combination of dedicated sample preparation methodologies with powerful determination techniques. As regards the latter step, chromatographic techniques (particularly liquid chromatography, LC, and to a lesser extent gas chromatography, GC) combined with mass spectrometry are the logical choice.

In this doctoral thesis, both chromatographic techniques have been used coupled to mass spectrometry (MS) and mass spectrometry in tandem (MS/MS) using different ionization sources, types of mass analyzers and data acquisition modes. The most important characteristics of these techniques are described below, including a brief bibliographic review of their applications to the determination of the different families of compounds involved in this thesis.

1. Liquid chromatography (LC)

Liquid chromatography (LC) is the most widely used analytical separation technique, due to its high versatility, easy adaptation to quantitative determinations, automatization capabilities and, especially, because of its wide field of application.

This separation technique is based on the distribution of the different components present in a sample, between a stationary phase and a liquid mobile phase. LC constitutes the technique chosen par excellence to separate polar and/or thermally unstable compounds, or compounds with low volatility [141]. The main components of a liquid chromatograph are compiled in **Figure 15** [142].

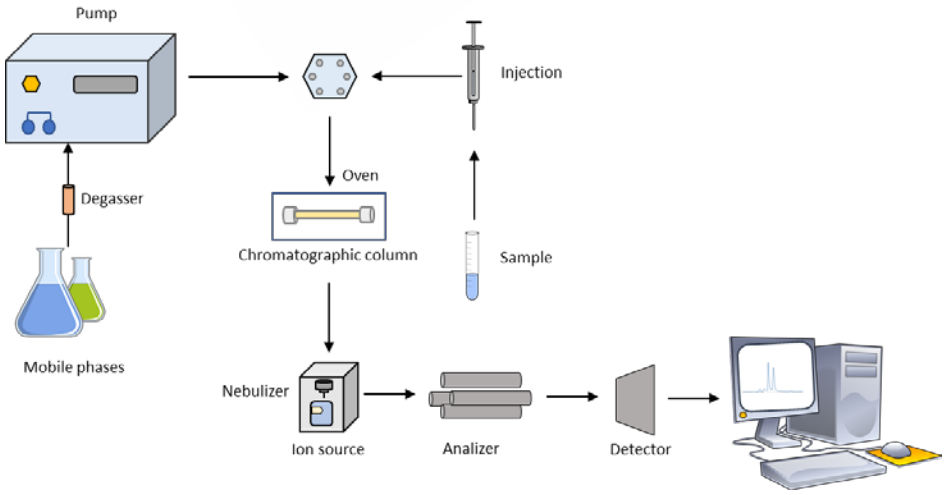

Figure 15. Scheme of the main components of a liquid chromatograph

There are several types of LC classified according to the separation mechanism or the type of stationary phase. Among these types are included the distribution chromatography, adsorption, ion exchange, size exclusion, affinity, and chiral chromatography. The modality most commonly used for the separation of organic compounds is, undoubtedly, the first one, which is normally termed as reversed-phase LC.

In the late 1970s, conventional LC was upgraded, creating the so-knowing high-performance liquid chromatography (HPLC). The chromatographic columns most used for the separation of organic compounds are the reversed phase columns, C_{18}, with particle size between 2 and 5 µm. The technology employed in HPLC columns evolved very quickly to faster methods, based on columns with a smaller particle size (usually below 2 µm), which improved the sensitivity, the separation efficiency, the resolution and reduce the time of analysis to less than 10 minutes. This new technology is called ultra-high-performance liquid chromatography (UHPLC).

1.1. Liquid chromatography coupled to mass spectrometry (LC-MS)

Liquid chromatography coupled to mass spectrometry (LC-MS) can be considered as one of the most important analytical techniques of the last decades. This technique combines the separation power and the versatility of LC with the excellent selectivity and sensitivity of mass spectrometry, providing qualitative and quantitative information of time-resolved species after their ionization.

The most challenging issue of the combination between LC and MS was the necessity to transform the molecules in solution into ions in gas phase, avoiding their thermal degradation and removing the excess of solvent before entering the high vacuum region of the mass spectrometer. Several ionization sources have been developed, but the interfaces most commonly used are those based on the atmospheric-pressure ionization (API) processes, such as *atmospheric-pressure chemical ionization (APCI)*, *atmospheric-pressure photoionization (APPI)* and *electrospray ionization (ESI)*. The latter is the one used in the LC-MS instruments employed in this thesis.

The ESI source was designed by Horton et al. in 1975. The main feature of the ESI source is that compounds separated in the LC column are volatilized and ionized simultaneously. To this end, the sample in solution is nebulized at the outlet of the chromatographic column, by a capillary that applies an electric potential of several kV, creating a cloud of charged micro-drops. By applying a high-temperature gas, the solvent is evaporated, and the electrostatic charge is concentrated in smaller drops. When the repulsive forces between charges are bigger than the cohesion forces, the ions are desorbed from aerosol particles to the gas phase and guided to the mass analyzer.

In this doctoral thesis several LC-ESI-MS systems have been used. A LC-MS system equipped with a triple quadrupole (QqQ) mass analyzer was employed in the quantification of residues of target pharmaceuticals in environmental samples. Moreover, the search and characterization of transformation products in laboratory degradation experiments was carried out in a LC system equipped with a time-of-flight (QTOF) mass analyzer.

1.2. Applications of LC-MS to the determination of emerging pollutants in environmental samples

Giebułtowicz et al. [51] have determined the presence of 30 pharmaceutical compounds and some of their metabolites, in river and in tap water samples from the Warsaw region. The analysis of samples was performed using SPE and LC-ESI-MS/MS as sample preparation and determination techniques, respectively. In their study, they found 26 out of 30 studied compounds in the Vistula river, while in the case of tap water, 20 out of 30 target cardiovascular compounds were detected, with average concentrations over 15 ng L^{-1}.

J. Aceña et al. [143] presented a review about the main advances of LC-HRMS achieved during the last decade in the simultaneous qualitative and quantitative analysis of emerging pollutants in environmental samples.

M. Li et al. [53] carried out the determination of 45 pharmaceuticals and personal care products (PPCPs) in sludge samples, using MSPD as sample preparation technique and LC-MS/MS as determination technique. Among the 45 studied PPCPs, 13 were detected in the studied samples with average concentrations over 1000 μg kg^{-1}.

2. Gas chromatography (GC)

Gas chromatography (GC) is a separation technique that permits the separation of the different components of a sample in terms of volatility and interaction with the stationary phase.

The analytes in gas phase are passed through a chromatographic column and carried by a gaseous mobile phase, called carrier gas. The stationary phase is usually a non-volatile liquid, which covers the inside wall of the column or the particles of a solid support. This technique is usually chosen for the separation of volatile or semi-volatile, and thermally stable compounds. Liquid samples (usually extracts prepared in an organic solvent) are volatilized maintaining the temperature of the GC injector at high values. The carrier gas flow transfers the volatilized species to the head of the column. In order to expand the application field of GC, some compounds are usually derivatized to improve their volatility and thermal stability [141]. In fact, the use of derivatization reactions was very popular in those times when LC-MS was still not available in most laboratories. The main components of a gas chromatograph are shown in **Figure 16** [142].

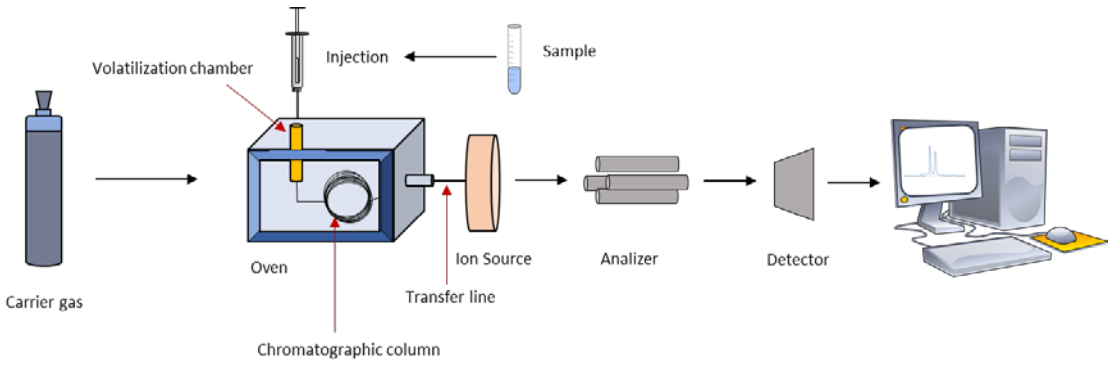

Figure 16. Scheme of the main components of a gas chromatograph

The liquid sample is introduced into the gas chromatograph by a syringe. There are 4 modes of injection of liquid samples in GC: volatilization at constant temperature with or without flow division, termed as split or splitless, respectively, injection on-column and volatilization with a temperature program (PTV).

- Split and splitless injection: these injection modes are usually employed for trace analysis, where analytes constitute less than 0.1 and 0.01 % of the sample, for split and splitless, respectively. 1-2 µL of liquid sample are introduced into the injector of the gas chromatograph, which is maintained at a temperature of 50 ºC higher than the boiling temperature of the less volatile component. During split injection, only the 0.2-2 % of the volatilized sample enter into the column and the rest is removed through the venting outlet, whereas during splitless injection most of the volatilized sample enters the column.
- Injection on-column: this injection mode is usually applied for samples which experiment decomposition over the boiling point of the solvent. It constitutes the best choice to transfer the whole sample components into the column; however, on the contrary, it is poor compatible with complex extracts, containing non-volatile co-extracted species which tend to be accumulated in the first part of the GC column.
- Temperature program volatilization (PTV) injection: this mode is similar to split/splitless, but with the possibility of establishing a temperature program and venting most of the injected solvent. It requires high volumes of sample.

2.1. Gas chromatography coupled to mass spectrometry (GC-MS)

In the same way as LC-MS, the combination of gas chromatography and mass spectrometry (GC-MS), joins the best advantages of both instrumentations, achieving a high-resolution separation of components with selective and sensitive mass detection. Since a relative small range of compounds can be analysed by GC-MS, compared to LC-MS, this latter technology is growing very fast [144]. However, GC-EI-MS retains some advantages over LC-MS, such as the higher spectral information of the electron ionization MS spectra versus that contained in ESI-MS ones, which provide only information about the molecular mass of each compound, and the availability of spectral libraries highly useful during screening studies.

The GC-MS coupling consists on a gas chromatograph, furnished with a capillary column, directly connected to a mass spectrometer, which has an ion source, a mass analyzer and finally a detector. The most common source applied in gas chromatography is the *electron ionization (EI)* source, which was the one used in all the analysis developed in GC-MS along this thesis. In this source, neutral molecules (M) that elute from the chromatographic column are bombarded with electrons, at typically 70 eV of energy from a wolfram filament, creating a radical cation $[M]^{+}\cdot$, known as the molecular ion. Usually, the molecular ion undergoes further fragmentation reactions, and in some cases, it is absent in the EI-MS spectrum.

$$[M] + e^{-} \rightarrow [M]^{+}\cdot + 2\ e^{-}$$

The resulting ions are directed to the analyzer, where they are separated and sent to the detector, which generates an electrical signal that is processed and recorded, producing a chromatographic peak. **Figure 16** shows the main components of a GC-MS instrument.

Among the mass analyzer usually coupled to GC, the quadrupoles (Q) and the ion trap (IT) are the most commonly used. During this doctoral thesis, the mass analyzer coupled to GC, was the quadrupole, as reference technique for the analysis of bisphenol compounds in thermal paper extracts, and the QTOF hybrid system for the screening of EPs in the extracts from dust samples.

2.2. Applications of GC-MS to the determination of emerging pollutants in environmental samples

X. Fan et al. [145] studied the presence of bisphenol A, alkylphenols and alkylphenol ethoxylates in indoor dust samples using GC-MS/MS. Authors employed sonication extraction followed by SPE as extraction and clean-up techniques. Extracts were analysed by GC-QqQ-MS. The developed methodology was very sensitive, with detection limits ranging from 0.05 to 5.1 μg g^{-1}, and average recoveries between 82 and 115 %. All target analytes were quantified in all studied samples, being NP_3EO and NP_4EO reported for the first time in this type of matrix.

J. Casado et al. [146] determined the presence of benzotriazoles in water samples using the acetylation–dispersive liquid–liquid microextraction (DLLME) combined with GC–MS, selected as sample preparation and analysis technique, respectively. Under final conditions, the methodology presented LOQs from 0.007 to 0.080 ng mL^{-1} and quantitative recoveries between 86 and 112 %. Authors found 3 out of 5 selected benzotriazoles in environmental water samples at concentrations up to 1.9 ng mL^{-1}.

G. Álvarez-Rivera et. al. [147] developed the identification of halogenated photoproducts of parabens and benzoates in water using solid-phase microextraction (SPME) as extraction technique and GC-MS/MS as determination technique. Degradation experiments of parent compounds were developed under UV-light and the transformation by-products created in the photolysis were concentrated by SPME. Under final conditions the monobrominated, dibrominated and bromochlorinated hydroxybenzoates were identified, and the transformation of benzoates into halogenated parabens was also confirmed.

Y. Wang et al. [148] developed a suspect screening analysis to investigate the occurrence and the removal of micropollutants during wastewater treatment. Samples were pre-treated by SPE and the obtained extract was analysed by GC-QTOF-MS. The screening results show the identification of 117 compounds using the *Unknowns Analysis* software.

3. Direct analysis in real time (DART)

Direct analysis in real time (DART) is an ambient pressure ionization source presented by Cody et al. [149] in 2005. This technique has been developed for rapid and noncontact analysis of materials at ambient pressure and at ground potential, succeeding in the analysis of hundreds of chemicals adsorbed on to several surfaces from different natures.

The ion formation during DART ionization is based on the ambient air-chemical reactivity activated by a gas stream of species generated in an excited metastable state. The ionization mechanism depends mainly on the nature of the carrier gas, the concentration of analyte and the polarity of the ions. In general, compounds are first volatilized and then ionized, mostly through charge transfer reactions with reactive ions and, in a minor extent, through *Penning* ionization.

The main components of the DART ionization source are illustrated in **Figure 17**. In this source, the ionization process begins when a gas stream containing neutral atoms, or molecules of an inert gas, usually of helium (less often argon and nitrogen have been investigated as ionization gases), is introduced in a discharge zone delimited by a needle electrode, where the molecules are excited into a metastable state. The gas flows through the ceramic heater in order to raise the gas to a desired temperature and pass through the exit grid, which has a charge applied to filter only the metastable species and discarding the other charged ions. Finally, the heated gas containing metastable ions reacts with the moist ambient air made of nitrogen (N_2), oxygen (O_2), and water (H_2O), which is the surrounding atmosphere of ionization, generating protonated water clusters that further ionize analytes through a series of sequential reactions [150,151].

$$M^* + S \rightarrow S^{+\cdot} + M + e^-$$

Where M is a gas capable of forming metastable species (M*), such as helium, nitrogen or argon, and S is the solvent. Regarding the gas, the most widely used is helium, since the internal energy of its metastable state is higher than those corresponding to nitrogen or argon.

Penning ionization reaction undergoes between the gas in its excited electronic state, in case of helium, He($2s^3$), and the air compounds, following the next reactions [150]:

$$\mathbf{He}(2s^3) + H_2O \rightarrow H_2O^{+\cdot} + \mathbf{He}(1s^1) + e^-$$

$$H_2O^{+\cdot} + H_2O \rightarrow H_3O^+ + OH^\cdot$$

$$H_3O^+ + nH_2O \rightarrow [(H_2O)_nH]^+$$

$$[(H_2O)_nH]^+ + \mathbf{S} \rightarrow \mathbf{S}H^+ + nH_2O$$

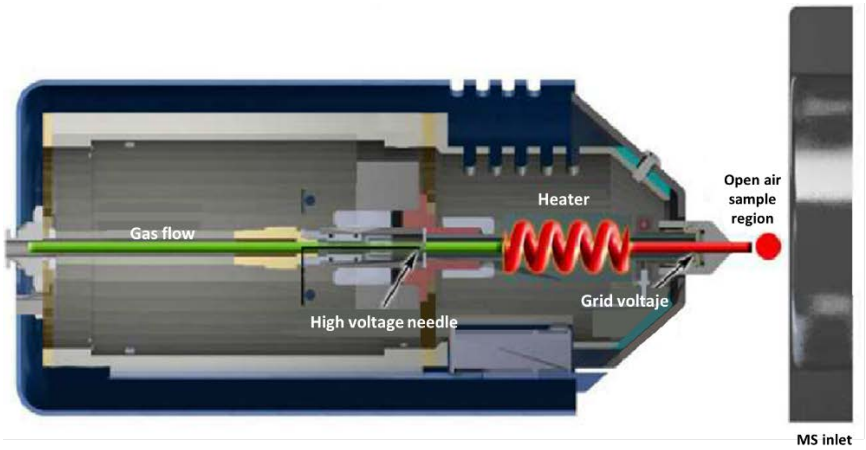

Figure 17. Components of DART source (IonSense)

3.1. Direct analysis in real time coupled to mass spectrometry (DART-MS)

The novel DART ionization source has been coupled to different kinds of mass spectrometers and applied to the analysis of a wide range of materials in different states (solid, liquid and gas), demonstrating that DART-MS is a powerful technique for sensitive and rapid qualitative determination of target analytes in different matrices [150,152].

The main advantage of this technique is that a huge number of compounds can be analysed directly on a variety of surfaces without (or with a minimum) sample treatment, or previous chromatographic separation. For that reason, this technique has emerged as an appealing alternative to solve several analytical problems.

In the same way as other detection methods, the parameters of both DART and MS need to be optimized in order to achieve the higher sensitivity, detectability and reproducibility. There are some critical parameters that have a significant effect in the signal intensity and in the signal-to-noise (S/N) ratio, due to the quality and the intensity of the signal depend on the production of the excited metastable atoms and molecules, the generation of protonated water clusters and the desorption and desolvation of analytes [152]. Therefore, the most relevant parameters during optimization of DART-MS methodologies are:

- Ionization mode
- Gas type and flow rate
- Gas heater temperature
- Grid voltage
- Sampling speed
- Distance between the exit of the DART gun and the MS inlet

3.2. Applications of DART-MS to the determination of emerging pollutants

DART-MS has become a technique highly recommended for the rapid analysis of a wide variety of samples, succeeding in the sampling of hundreds of polar and nonpolar compounds on different surfaces. Several applications of DART-MS have been developed in the areas of environment [153,154], food [155–158], forensic [159,160] and manufactured goods analysis [161], mainly focused on qualitative approaches.

In this doctoral thesis, DART-MS was applied to the determination of bisphenol compounds in thermal printing paper samples. To the best of our knowledge, previous applications of DART-MS to bisphenol-related compounds determination are still limited. They have been mainly focussed on the qualitative identification of BPA in epoxy coatings used in food packaging [162], and on the evaluation of its relative ionization efficiency comparing DART with other ambient desorption ionization sources [163].

J. Adams et al. [161] have developed a rapid and direct methodology for the identification of organic components present in printing paper and writing papers using DART-MS. Under optimal conditions, they succeed to characterize and differentiate several printings and writing papers. To that aim, they analysed 16 reference papers with a QTOF mass spectrometer, obtaining the mass spectra of the main components of the samples in real time without extraction, derivatization and chromatographic separation.

4. Target and non-target analysis by mass spectrometry

Nowadays, different kinds of MS instruments are employed in combination with several separation techniques (LC, GC and even supercritical fluid chromatography, SFC) to determine the amount and/or the nature of compounds eluting from the column and reaching the ionization source. The obtained information is conditioned by the features of the ionization technique, the characteristics of the mass analyzer and the data acquisition mode.

The *quadrupole (Q)* analyzer is one of the most popular instruments either combined with LC, or with GC. This mass analyzer consists on a set of four cylindrical, ideally hyperbolic, and parallel bars of metal that serve as electrodes. The ions generated in the source are accelerated into the space among the four bars by applying a small potential (from 5 to 15 eV). The opposite bars are electrically connected, a pair to a positive pole of a source of DC and the other pair to the negative. In addition, a variable radiofrequency potential is applied to the bars of the quadrupole, differenced in 180° [142]. A scheme of this analyzer is shown in **Figure 18**.

The quadrupole can work in two different modes. One of them is the selected ion monitoring (SIM), where the potential applied remains constant and the ions with an adequate *m/z* value follow a stable trajectory through the potentials applied in the quadrupole along the space defined by the four electrodes, reaching the detector. In the SCAN mode the potentials are increased from 0 to a maximum value, performing a continuous filtering of ions with increasing *m/z* values. As a result, conventional quadrupole mass spectrometers can easily resolve ions that differ in a unit of *m/z* values, reaching up to 3000 or 4000 *m/z* units.

The *time of flight (TOF)* analyzer is also shown in **Figure 18**. The ions are accelerated and expelled from the ion source by the application of a potential from 10^3 to 10^4 V, and then introduced into the drift region, which is a tube of 1 m of length, approximately, without any electric or magnetic field for further acceleration. Theoretically, all ions that enter into the tube have the same kinetic energy, thus the square value of their velocity is inversely proportional to their masses and the lighter ones travel faster than the heavier ones [141]. Typical times of flight stay around 1 µs.

In the past, the sensitivity and resolution of time of flight mass analyzers were lower than those provided by quadrupole ones. However, the new generation instruments have overcome the previous limitations and their high resolving power and mass accuracy permit to establish molecular formulas. In addition, their elevated sensitivity in scan mode makes possible to evaluate the presence of non-preselected compounds. In that way, they can detect an unlimited number of species without the necessity to inject standard compounds. For all those reasons, TOF analyzers offer a wide range of possibilities.

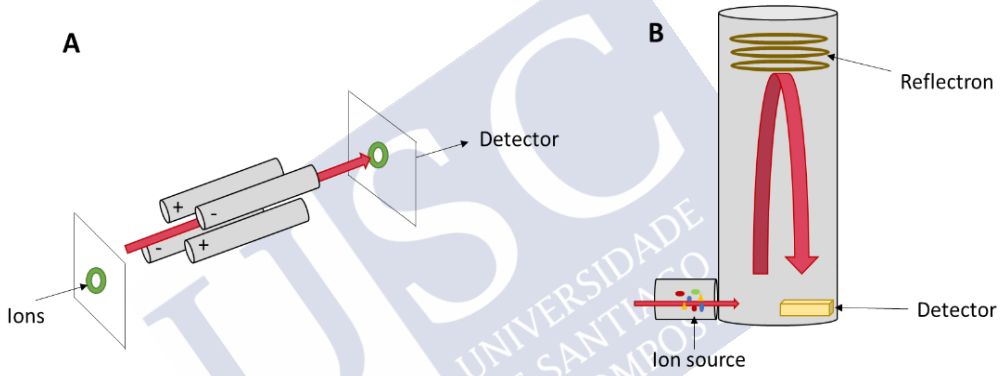

Figure 18. Basic scheme of different mass analyzers: A, quadrupole mass analyzer (Q) and B, time of flight analyzer (TOF)

4.1. Tandem mass spectrometry

4.1.1. Triple quadrupole (QqQ)

The most widespread MS/MS instrument is the *triple quadrupole (QqQ)*, whose scheme is shown in **Figure 19**. Whatever the chromatographic technique, serving as time-resolved sample introduction device and the ionization source, in mass spectrometry in tandem (MS/MS), using the triple quadrupole (QqQ) analyzer, the first (Q1) and the third (Q3) quadrupoles work as ion mass filters, while the second one (Q2) acts as collision cell, producing the collision-induced dissociation (CID) of the precursor ions previously filtered by Q1, and finally the resulting fragments are filtered by Q3 [164]. The MS/MS modes for QqQ are described below (**Figure 19**):

A) *Product ion scan:* Q1 operates in SIM mode, selecting an ion with known *m/z*, which is fragmented in Q2 and finally filtered in Q3 in scan mode, generating a full-product ion spectrum of the previous ion.

B) *Precursor ion scanning:* Q3 selects a fixed product ion and Q1 develop a scan of its precursor ions.

C) *Neutral loss scanning:* Q1 and Q3 operate in full scan. This mode is especially helpful in the selective recognition of the ions that fragment in Q2 producing the same neutral fragment (as NH_3, CO_2, H_2O).

D) *Multiple ion monitoring:* Q1 and Q3 operate in SIM mode, selecting a precursor ion and after its fragmentation in Q2, Q3 filters a selection of product ions (usually 2-3 ions per precursor). This mode minimizes the interferences, reduces the chemical noise in the chromatograms and provides an excellent selectivity and sensitivity.

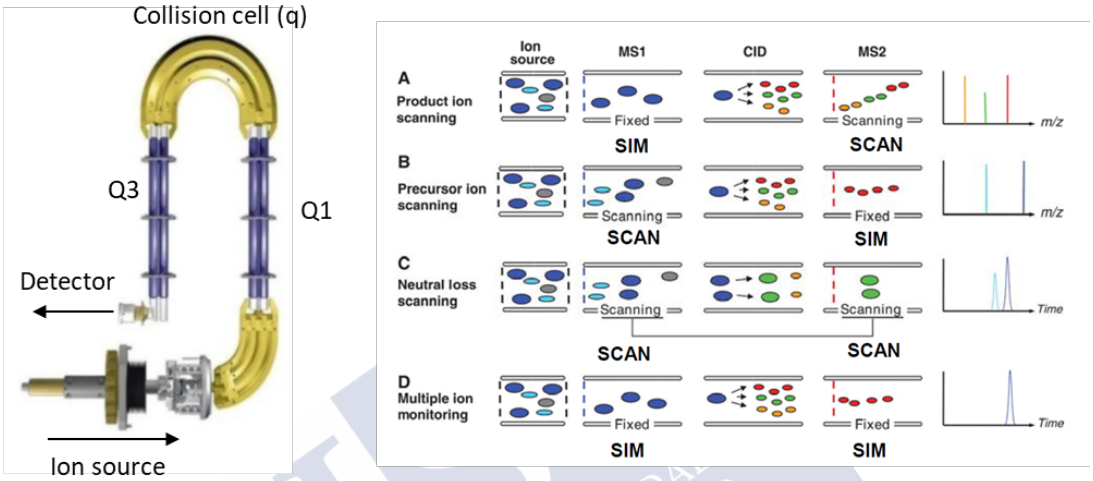

Figure 19. Scheme of the triple quadrupole (QqQ) instrument and a summary of its 4 MS/MS operation modes

4.1.2. Quadrupole-time of flight (QTOF)

One of the MS/MS instruments used during this thesis is the *quadrupole-time of flight mass analyzer (QTOF)* presented in **Figure 20**. This hybrid system was widely applied in the screening studies developed, either using LC or GC as separation techniques, due to the fast obtention of complete and accurate spectral data. This instrument combines the capacity of ion selection and filtration of the quadrupoles with the power of simultaneous analysis of all the fragments generated in the collision cell, typical of time of flight mass analyzers.

In combination with a soft ionization source, as it is the case of ESI or APCI, QTOF systems provide two types of information in a single experiment: data about the empirical formula of the analysed compounds (MS mode), and information about their structure inferred from accurate *m/z* values for product ions observed in MS/MS spectra. Thus, QTOF systems are especially useful for the analysis of unknown compounds (non-target analysis) and for removing interferences derived from isobaric compounds, with the same nominal *m/z* ratios but different empirical formulae, in the analysis of certain analytes (target analysis).

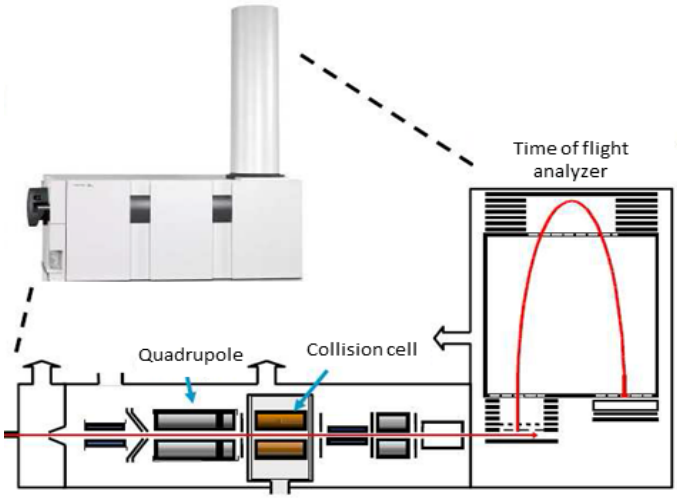

Figure 20. Scheme of a quadrupole-time of flight system (Agilent Technologies)

4.2. Accurate mass spectrometry

Due to the increased concern about the occurrence and the effects of EPs in the environment, the number of studies dealing with the screening and the quantification of different families of compounds, and their transformation products, has steadily increased during the last two decades.

Under this scenario, and from an analytical point of view, there are two main ways to obtain the required information [165,166]:

- *Target analysis* of specific compounds or families, by the application of optimized methodologies (including sample preparation and determination steps), specially developed for the compounds of interest. Under this concept, target screening is referred to the screening of substances for which reference standards are available, while those analysis where the identification of substances is based on a list of suspect compounds, but reference standards are not available, are known as suspect screening.
- *Non-target analysis*. A rapid and efficient screening methodology is applied in order to discover the 'unknown compounds' present in a sample, for which neither prior information nor reference standards are available.

Both approaches require high technology to provide selective and sensitive methods, capable of generating accurate information on the identification, confirmation and quantification of EPs.

The development of high-resolution mass spectrometry (HRMS), or more correctly accurate mass spectrometry, is considered as one of the most relevant achievements for the identification of unknown compounds, being the QTOF and the Orbitrap the most commonly used mass analyzers nowadays. Even when operated in the single MS mode, both instruments are able to record *m/z* ratios for the whole set of ions generated into the ionization source within a range of pre-defined *m/z* values. As example, in the default

operational mode, TOF-MS instruments record ions in the range from 40 to 3200 *m/z* units. Thus, at difference to the MRM acquisition mode, data files can be further explored for additional compounds. The information contained in these records is mainly conditioned by the acquisition mode (accurate MS and accurate MS with simultaneous product ion scan) and the ionization source installed in the MS instrument.

4.3. Acquisition modes in LC-ESI-QTOF-MS

The technology referred to the instrumentation of mass spectrometry, has improved significantly in the recent years, particularly in terms of selectivity, sensitivity and specificity. At the same time, software developments have permitted to combine, in the same injection, MS and product-ion scan acquisition functions, with or without a previous selection of precursor ions. Obviously, the latter option is the most interesting one during screening studies. According to the literature, functions used to record product ion scan spectra with QTOF-MS systems can be divided into two categories: Data dependent acquisition mode (DDA), which is the most commonly used methodology, and data independent acquisition mode (DIA) [167,168].

4.3.1. Data dependent acquisition modes: *Target MS/MS* and *auto MS/MS*

In target analysis, the DDA mode is recommended. In this mode, the instrument performs a first MS scan of the eluting parent ion. This first scan searches the *m/z* and intensity of the current parent ions and generates a list. Thereafter, it selects different ions under predetermined rules, and transfer them to the collision cell to generate the fragment ions. The instrument is capable of recording the full-scan MS and MS/MS spectra of a high number of compounds in the same chromatographic run. The switching between MS and MS/MS modes is automatically controlled by the intensity of the precursor ions observed, or even can be defined by a list of inclusion/exclusion *m/z* values and different criteria, such as charge state or isotopic pattern [165,168].

In order to facilitate the screening studies, there are two different strategies that can be followed depending on the required information:

- *Target MS/MS* experiments: a list of target parent ions, including *m/z* values and retention time (RT) for each compound is created in the method. Thereby, the instrument is registering full-scan MS spectra through the entire chromatographic analysis. In addition, the MS/MS spectra of pre-defined compounds are recorded within set retention time windows. So, it is possible to obtain the information related to the cluster of signals corresponding to the molecular ion (MS function) and the accurate product ion scan spectra using one or several collision energies, in a single chromatographic run.

- *Auto MS/MS* experiments: a list of excluded or preferred ions could be included in the same way as *Target MS/MS* or, the instrument could select the precursor ions by its own, according to their intensities, isotopic profile and/or

charge (known as 'Decision engine, DE' in Agilent Technologies). Even when a list of precursor ions is defined, no retention time data are provided in the method. Usually, *auto MS/MS* methods include a pre-selection of preferred and/or excluded ions, as well as those selected by the DE. So, the QTOF instrument is 'automatically' changing from MS to MS/MS mode. The following figure, **Figure 21**, shows an outline of the DE decision flow [169].

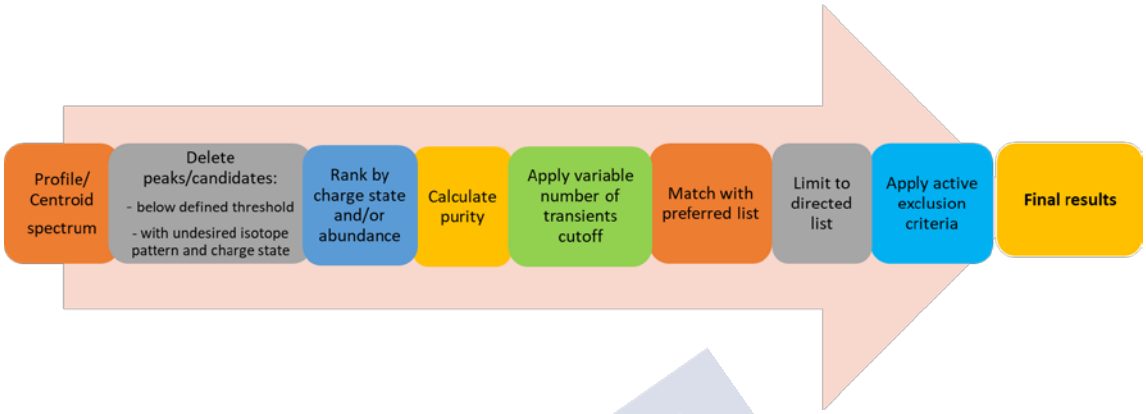

Figure 21. Decision flow of DE

The main limitations of DDA mode is the loss of MS/MS information for the ions with lower abundance, due to the fact that the acquisition of MS/MS data is triggered by the detection of ions above a defined threshold in the MS mode. Additionally, the number of precursors per cycle and the use of an exclusion list, can affect negatively to the acquisition of MS/MS spectra when compounds with similar retention time are present. Thus, additional *target MS/MS* can be required for a correct identification of compounds.

In addition, DDA usually presents a loss in the sensitivity of the QTOF instrument, due to the simultaneous combination of different acquisition functions in the same time window. When following narrow peaks, as those provided by UHPLC columns, the simultaneous fragmentation of several precursor ions requires reducing the number of transients accumulated per MS and product ion scan spectra. This fact has a significant, negative effect in the LOQs of QTOF-MS systems. That is, the lower the number of transients per spectrum, the higher the LOQs.

I. Ferrer et al. [170] presented a technical overview about *auto MS/MS* and identification of unknowns in water samples, in collaboration with Agilent Technologies, in which they discussed the different parameters to be considered during the optimization of a screening workflow of complex water samples. They performed the non-target screening obtaining the MS and MS/MS spectra using *auto MS/MS* experiments. The identification was carried out by comparison against a PCDL library, which contained MS/MS spectra, but they found that even with the appropriate settings to *auto MS/MS* experiments, important compounds with low intensity in regions of high chromatographic coelution were lost, so, those type of analysis would have better accomplished with *all ions* experiments. The main conclusion of the overview is the selection of the appropriate acquisition mode is critical to obtain the best results.

4.3.2. Data independent acquisition modes: MS^E

In the so-called MS^E mode (also known as *all ions*), the QTOF instrument is always operating in the scan mode, recording data without any pre-selection or discrimination in the Q analyzer, losing in that way, the specificity of the DDA mode. Data acquisition can be performed at different energies applied in the collision cell, low, high or even with a collision energy (CE) ramp, during the same chromatographic run, with the aim to obtain as much information as possible about the MS/MS spectra [168,171].

MS^E is also known as pseudo MS/MS mode, because it does not render authentic product ion spectra, but product ion spectra coming from all ions which achieve the collision cell at a given time. Obviously, in case of single mixture of standards, well separated in the LC column, these MS^E spectra are equivalent to authentic MS/MS spectra, obtained after the previous isolation of the precursor ion by the Q mass analyzer [165]. The lower CE applied provides spectra with information about the precursor ion, while higher CE channel gives information about the fragment ions. The main strength of MS^E is the high MS/MS acquisition rate. By contrast, the main weakness of *all ions* is the high amount of interfere nces, since any type of pre-selection is done. For that reason, an innovative solution, which overcome the mentioned issues, was recently presented. The *quadrupole-resolved all ions (Q-Rai)* is a DIA mode capable of reducing the complexity of MS/MS spectra while maximizing the accuracy and quality of the data acquired. The main difference with *all ions* is that *Q-Rai* develops an ion pre-selection, creating different *m/z* windows, filtered by the Q MS analyzer before reaching the collision cell, with the aim of reducing the possible interferences in the chromatograms. The narrower the *m/z* window, the larger the number of acquisition functions are necessary to cover a pre-fixed range of masses (i.e. 100-1000 Da). Obviously, the duration of each acquisition cycle (comprising MS and pseudo MS/MS experiments for each window within the range of acquired *m/z* data) must remain short enough to record a minimum of 10-12 spectral points per chromatographic peak. Depending on vendors, *Q-Rai* mode is also referred as SWATH (sequential window acquisition of all theoretical fragment ion spectra) [172].

DIA mode present significant advantages over DDA methods, since it allows the comprehensive fragmentation of all ions generated in the ionization source and the acquisition of the corresponding MS/MS spectrum without defining any *m/z* or threshold before the analysis. In combination with a database of accurate product ion spectra, the *all ions* mode provides valuable information to detect the presence of database compounds in complex sample extracts.

Figure 22 summarizes the operational schemes of DDA and DIA acquisition modes in in QTOF-MS system.

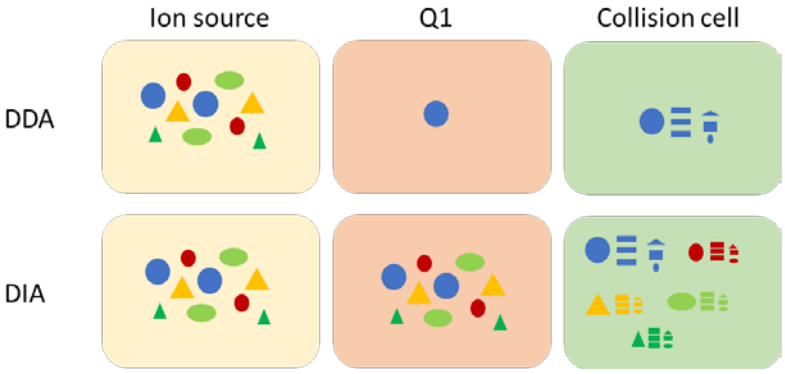

Figure 22. Operating scheme of DDA and DIA.

4.4. Acquisition modes in GC-EI-QTOF-MS

Since some emerging contaminants are not amenable to LC-ESI-MS determination, GC-MS is a necessary methodology for the analysis of these non-polar compounds. Quite often, the amount of information contained in the EI-accurate-MS spectra is enough for the unambiguous identification of a given compound, or at least, to assign the compound to a chemical family. Moreover, as far as we know, software controlling the GC-EI-QTOF-MS instruments does not offer the possibility to operate in the above described *auto MS/MS* and MS^E modes. So, in most cases, data are just acquired in the scan MS mode and product ion scan spectra are limited to a pre-selection of target compounds.

4.5. HR-MS/MS libraries. PCDLs and on-line libraries

Regardless the chromatographic technique applied, LC or GC, once the MS and MS/MS spectra have been recorded, it is necessary to carry out the data processing for the identification of EPs in the analysed samples. The number of compounds that can be detected by sample is enormous, so manual inspection of each extracted ion chromatogram represents a non-feasible choice during comprehensive screening studies. For that reason, the extraction of compounds from the raw data can be performed using different software tools. Data mining approaches are completely different for LC-ESI-QTOF-MS and GC-EI-QTOF-MS records, which are based in several algorithms often employed in combination with data generated by the first technique.

In general, the workflow for target and non-target screening involves three main steps and it is compiled in **Figure 23**:

1. *Data acquisition* (described in section 4.3 and 4.4).

2. *Extraction of molecular features*. One of the simplest algorithms of search is the molecular features extraction (MFE), which operates on data obtained through the single MS function. This algorithm extracts a list of possible compounds with accurate masses, retention times and ion abundances from the entire chromatogram. For each compound, MFE presents a list of possible molecular formulae ranked

according to a weighted score calculated considering the matching of masses and isotopic profiles between the experimental and the predicted spectrum for each molecular formula.

The main disadvantage of MFE is the limited reliability of the provided information, due to the high number of false-positives and false-negatives for a given empirical formula, where several candidates are possible. So, a further step to compare the obtained product ion scan spectra for each molecular feature with those contained in a database is required. However, the MFE is normally enough to detect the potential formation of transformation products from a given precursor during modelization of degradation experiments using simple matrices (e.g. spiked ultrapure water) under controlled laboratory conditions. In this case, a comparison of the list of molecular features with their relative intensities, present in control and degradation experiments permits to identify the formation of the derivatives.

3. *Data processing* from MS and MS/MS spectra for the structure elucidation and identification.

When data are acquired in *auto MS/MS* mode, the information contained in MS and MSMS functions can be automatically extracted. Thus, once a relevant peak is detected, a molecular formula is generated based on the accurate mass and isotopic pattern obtained from MS data. The molecular formulae proposed can be searched in different databases in order to assign a tentative identity to each molecular feature. There are several molecular databases, which also include MS/MS spectra for data comparison, so the experimental product ion spectra can be compared with those contained in one of these databases.

In a similar way, different algorithms can associate the data corresponding to precursor and fragment ions recorded in *all ions* and compared with an accurate database. Usually, these algorithms search for pseudo-molecular ions of compounds included in the database through LC-ESI-MS records obtained with the collision cell inactive. Tentative identifications are confirmed checking whether any of the fragment ions in the database spectrum appear at the same retention time as parent ions in the LC-ESI-MS channel, for the same collision energy.

There are several high-resolution/accurate MS spectral libraries containing data obtained using QTOF and Orbitrap instruments in combination with soft ionization techniques, mainly ESI. Some of the databases are of free access, such as MassBank [173], *m/z* cloud [174] or METLIN [175], which is a database for metabolites; and others are commercials, as Personal Compound Database and Library (PCDL) from Agilent Technologies. Comparison of experimental spectra (MS and product ion scan) with those existing in the libraries increases the probabilities to identify the detected unknowns during screening of real samples and/or in laboratory degradation experiments.

Nowadays, most of the instruments incorporate some of these databases in their software in order to make easier the identification and MS/MS interpretation. The MS/MS spectra contained in the database are automatically compared with the experimental one and ranked by a confidence score, based on the concordance of *m/z* values, retention times (when are available) and abundance of precursor and fragment ions between experimental spectra and those contained in the database.

In case of GC-EI-QTOF-MS data, the data mining strategy is completely different to that defined in the paragraphs above. The main advantage is that the acquired MS channel usually contains enough information for the identification of a given peak. On the other hand, the molecular ion is very often absent from EI-MS spectra and also, fragment ions with similar retention times, belong to co-eluted species. For that reasons, the first step in screening studies based on this technique involves a de-convolution of the entire chromatogram to obtain a list of chromatogram components with the accurate EI-MS spectrum of each component, not interfered by those of nearby eluting species. The next step is the spectral search against an EI-MS spectra database. As the fragmentation pattern of EI is highly reproducible between instruments, reliable unit mass library spectra have been developed for over 15,000 compounds (NIST v17) [176]. Unfortunately, at this moment, there are not accurate EI-MS spectra libraries, apart from small collections of accurate spectra for relatively short families of compounds (e.g. pesticides). So, in most cases, the spectral search is performed using the low resolution NIST EI-MS database. Further comparison of experimental *m/z* values with calculated ratios for fragment ions with known composition in the NIST database is sometimes useful to discriminate candidates with different empirical formulae.

Although all these workflows are very useful for the identification of unknowns, they cannot avoid the manual revision for each identified compound, and when the number of positive compounds is quite vast, the process can be tedious and time-consuming. In order to facilitate this work, some software packages incorporate automated workflows for searching several molecular formulas in several data records, using different databases in the same workflow. This solution can be very useful and saves a lot of time, but manual inspection of the results is always required.

4.6. Reliability of accurate mass spectrometry identifications

Despite all the possibilities provided by the software, and the vast amount of information compiled in the accurate MS and product ion spectra, there is still a possibility to make wrong identifications of unknowns (molecular feature entities or deconvoluted components) detected in the chromatographic records obtained by LC-ESI-HRMS or GC-EI-HRMS. Schymanski and co-workers have defined a scale, from 1 to 5, to calculate the confidence of these identifications [177]. In this scale, level 5 corresponds to compound identification derived just from the empirical formula; so, it represents the lowest confidence level. On the other hand, level 1 presents the highest reliability when accurate MS, product ion scan spectra and retention times have been confirmed with those obtained for a standard of the candidate compound.

- Level 1: *Confirmed structure* against standard through MS, MS/MS and retention time matching.
- Level 2: *Probable structure.* There is an exact structure proposed using two different evidences:
    - Level 2a: Library, where the proposed structure matches with the library or literature spectrum, and the spectrum-structure match is unambiguous.

    - Level 2b: Diagnostic, when no other structure fits with the experimental information, but no standard or literature information is available for confirmation.
- Level 3: *Tentative candidate.* There is a structure proposed, but insufficient information for only one exact structure.
- Level 4: *Unequivocal molecular formula* is presented, but insufficient evidences exist to propose an adequate structure.
- Level 5: *Exact mass (m/z)* is presented, due to the possible interest in investigation; however, there is insufficient information to propose a molecular formula.

During the screening studies is important to keep in mind some basic rules of any analytical determination, in order to be able to state that the identified compounds really exist on samples and they are not artefacts of the analytical procedure. There are several quality assurance and quality control (QA/QC) measures to take into consideration during the screening analysis, but non harmonized protocols with minimum quality requirements have been established yet [178]. The Schymanski scale is widely accepted by the scientific community; however, some requisites are required to achieve those levels of confidence. The principal one is the analysis of at least one procedural blank for each batch of samples, to establish the number of fragments required to reach an unambiguous identification and the minimum accepted score, the analysis of duplicates and the required level above a blank signal needed for confirming occurrence. Based on the above considerations, **Figure 23** shows a possible workflow to follow during data mining of accurate MS (MS/MS) records obtained for sample extracts.

The number of publications following these workflows have been increased during the last decade, demonstrating the high interest of laboratories to incorporate these techniques for the target and non-target analysis. To sum up, HRMS combined with accurate mass libraries, constitutes a fast, simple and efficient methodology, being its potential of high-resolution/accurate mass in the selectivity and confirmation capability of the analysis, the main strength of these techniques. Despite all these advantages, some improvements in the sensitivity of the technique, and in the data mining algorithms in order to avoid false-negatives and false-positives (particularly at low concentrations where the matrix effects can lead to a false identification) are still required.

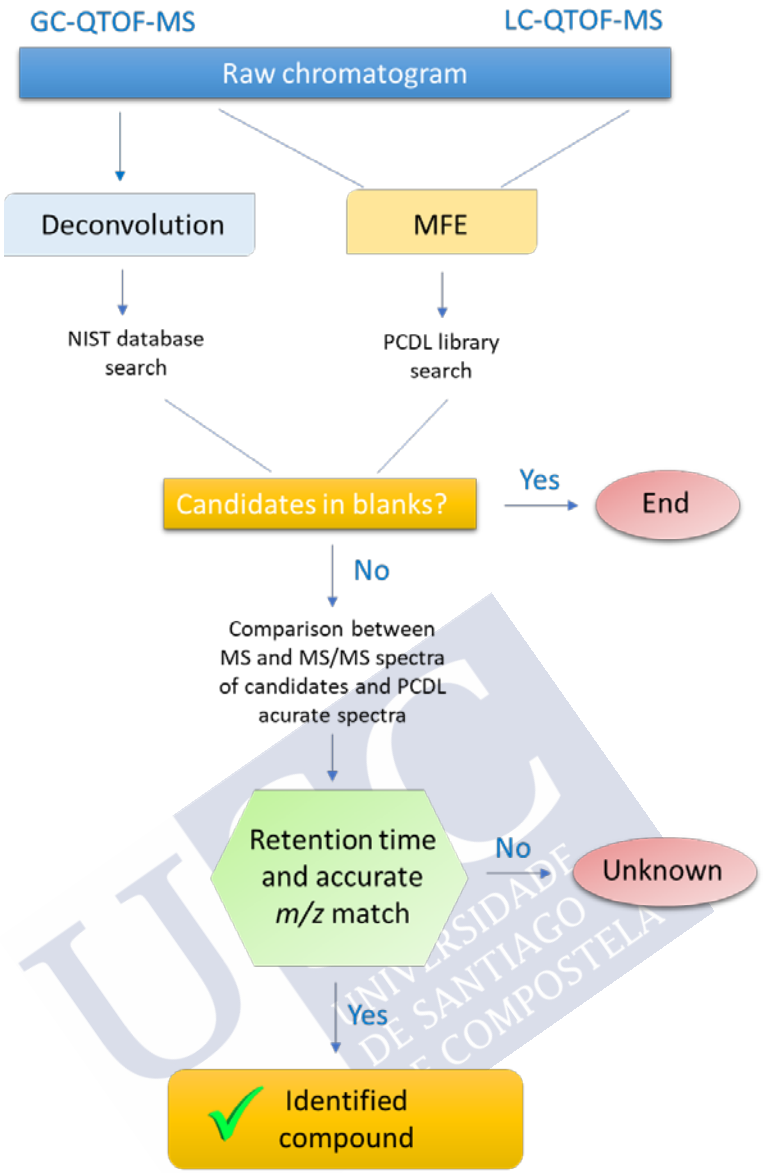

Figure 23. Analytical workflow for the identification of target and non-target compounds

II.D. Tertiary treatments applied in water treatment plants

The occurrence of organic micropollutants, and their transformation products (TPs), in water resources has been a matter of concern during the last decades. These emerging pollutants, natural or anthropogenic substances, usually belong to different chemical groups, such as pesticides, pharmaceuticals, personal care products (PCPs), drugs of abuse, industrial compounds, among others [179]. These compounds could be persistent or pseudo-persistent, due to their continuous introduction into the environment and their presence in aquatic and terrestrial matrices, as wastewaters, rivers, soils and even drinking water, which constitute a serious environmental problem.

The treatment of wastewaters at the STPs, might reduce the presence of micropollutants in the receiving waters and improve the quality of discharges. However, the common applied processes, as primary and biological treatments, often provide an incomplete removal of many EPs, resulting in the spread of these substances in surface waters, which are used as major sources of drinking water. Consequently, some contaminants have been identified and quantified at concentrations in the range of ng L^{-1} to µg L^{-1} [180] in tap water, due to their incomplete elimination during the disinfection treatment in the drinking water treatment plants (DWTPs). The main pathway of exposure of contaminants is summarized in **Figure 24**.

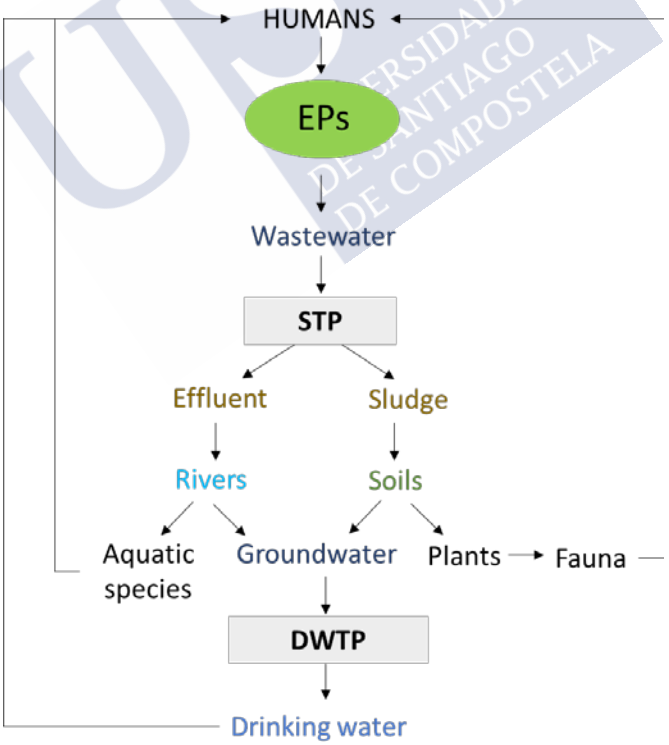

Figure 24. Distribution of contaminants in the environment

In order to solve this problem, the implementation of new technologies at STPs has been carried out in the recent years, constituting a significant improvement in the quality

of the effluents. These new technologies are based on chemical oxidation processes, usually referred as tertiary treatments, implemented after biological digestion units at STPs, which transform the organic pollutants into less complex compounds. Among oxidant agents employed in the treatment of wastewater, free chlorine and UV photolysis are the most often used ones.

Advance oxidation processes (AOPs) are based on the production of highly reactive radicals, which can attack the organic pollutants. The high efficiency of these processes is due to the generation of hydroxyl radicals ($HO^{\cdot}$), which are non-selective oxidizing species, capable of mineralizing micropollutants, producing CO_2, H_2O and inorganic ions as final products [181]. Some of the most commonly used AOPs are the Fenton process, photolysis, photolysis combined with H_2O_2 or free chlorine, ozonisation, etc. **Table 9** shows a summary of the most popular tertiary treatments considered in the removal of organic pollutants from water samples [181].

Table 9. Summary of tertiary treatments applied in water treatment plants.

NO PHOTOCHEMICAL PROCESSES	PHOTOCHEMICAL PROCESSES
Ozonisation	Direct photolysis
Ozonisation with H_2O_2/O_3	UV/H_2O_2
Fenton process (Fe^{2+}/ H_2O_2) and derivatives	UV/O_3
Electrochemical oxidation	UV/ H_2O_2/O_3
Radiolysis	UV/HClO
Gamma irradiation	Solar photodegradation
	Homogeneous: Photo-Fenton and derivatives
	Heterogeneous: Photodegradation with TiO_2

The photodegradation techniques used during this thesis are described in detail in the following pages.

1. Photodegradation processes

The International Union of Pure and Applied Chemistry (IUPAC) defines the process of photodegradation as the transformation of a molecule into smaller fragments, usually through an oxidation process. This term is widely applied for the degradation of emerging pollutants by UV-based processes [182].

Photodegradation can occur following two different pathways:

- Direct photodegradation, which consists on the direct absorption of light by the pollutant followed by a chemical reaction that causes its decomposition, without the participation of any other substance.
- Indirect photodegradation. Light is absorbed by other substances and transmitted to the pollutants, producing their degradation. In aqueous environments, light irradiation can generate highly reactive species such as hydroxyl radicals, capable of transforming the contaminants.

1.1. Kinetics of photodegradation processes

The direct photodegradation of EPs in environmental waters is typically described in the literature as a first-order kinetic process [183]. That is, the reaction rate is directly proportional to the concentration of the pollutant (*A*). Therefore, the reaction rate equation will be:

$$-\frac{d[A]}{dt} = k[A]$$

Where *A* is the concentration of the compound existing at time *t* and *k* is the value of the first order rate constant.

Integrating the previous equation, an expression that indicates the variation of *A* with time is obtained:

$$[A] = [A]_0\, e^{-kt}$$

Where $[A]_0$ is the initial concentration of *A* before the reaction occurs. From this equation it can be deduced that the concentration of a compound is an exponential function of irradiation time. The plot of the natural logarithmic concentration of the compound (*A*) versus time (*t*) produces a curve fitting to a linear regression, whose slope can be related to the value of first order rate constant (*k*) and the half-life time ($t_{1/2}$).

$$\ln[A] = ln[A]_0 - kt$$

$$t_{1/2} = ln2\ /k$$

Half-life time ($t_{1/2}$) is defined as the time needed to reduce the initial concentration of *A* to the half.

1.2. Photolysis

The transformation of any compound under photolysis is closely related to its ability to absorb radiation. For that reason, the most important element during photochemical processes is light, UV or visible light, which constitutes a selective reagent capable of producing chemical reactions, breaking bonds and forming new simpler compounds. The application of UV light to disinfect water has been widely used in the last decades [184].

Direct photolysis is the most common oxidation process, based on the interaction between UV radiation and the organic pollutants, producing their dissociation in different fragments.

$$\text{Compounds} + h\nu \rightarrow \text{Intermediates} \quad (1)$$

$$\text{Intermediates} + h\nu \rightarrow CO_2 + H_2O + \text{Inorganic ion} \quad (2)$$

This type of photolysis requires the use of solar simulators or UV lamps, among which the most common are the low-pressure mercury lamps emitting at 254 nm. Some studies of direct photolysis reveal an efficient degradation of endocrine disruptors, such as butyl paraben [185] and propyl paraben [147], using UV low-pressure mercury lamps. However, in many cases, direct photolysis does not lead to full mineralization of organic

pollutants, but generation of new organic substances, referred as transformation products (TPs).

Apart from direct photolysis, indirect photochemical reactions might enhance the removal of persistent pollutants in the aquatic environment. In these processes, light is absorbed by other substances whose transformation products, or unstable intermediates, react with the pollutants leading to their degradation [184]. This type of photolysis usually occurs in natural waters under solar irradiation, due to the presence of dissolved organic matter (DOM) in natural waters, such as humic and fulvic acids, and nitrate and bicarbonate anions [186], which are capable of absorbing light. Some of the radical species, generated from the interaction between light and the above substances, exhibit a high redox potential, such as chlorine, nitro and hydroxyl radicals, playing a significant role during natural photochemical reactions occurring in the surface of environmental waters, and also in the atmosphere. Thus, promoting the formation of such radicals emerges as appealing alternative to direct photolysis during treatment of waste and/or drinking water samples.

Some authors have investigated the indirect photodegradation of emerging contaminants in natural waters, such as pharmaceuticals [186–188] and fungicides [189], studying the influence of different reactive species during the photodegradation.

In general, photodegradation processes are affected by several parameters, such as the pH and temperature of the solution, the content of organic matter and the concentration of oxygen and finally, the type of radiation, direct or indirect UV and/or VIS irradiation. [184].

1.3. UV/H_2O_2

The combination of UV irradiation and an oxidant agent, such as H_2O_2, leads to a more efficient, faster and successful degradation of emerging contaminants in polluted waters than the single use of UV light.

This technique is based on the formation of $HO^{\cdot}$ radicals during the H_2O_2 photolysis and the subsequent propagation reactions:

$$H_2O_2 + h\nu \rightarrow 2\ HO^{\cdot} \quad (3)$$

$$HO^{\cdot} + \text{Organic compounds} \rightarrow \text{Products} \quad (4)$$

The photolysis reaction of H_2O_2 (reaction 3) has a quantum yield ($\varphi_{HO\cdot}$) of 0.5, producing almost one $HO^{\cdot}$ species per quantum of radiation absorbed in the range between 200 and 300 nm. However, the molar absorption of H_2O_2 at 254 nm is relatively low (ε= 18.6 M^{-1} cm^{-1}), so this degradation technique requires relatively high doses of H_2O_2, or instead of that, a long exposure time to UV radiation [190]. Despite this limitation, the combination of UV radiation and H_2O_2 is the most common AOP applied to the removal of pollutants in environmental waters.

One of the main advantages of UV/H_2O_2 is the fact that H_2O_2 is commercially very accessible, cheap and is thermally stable. Thus, this technology is totally commercialized nowadays, being the capital investment, minimal and the application, very simple.

UV/H_2O_2 was applied to the degradation of bisphenol A by M. Neamţu et al. [191]. They compared the degradation rate between direct photolysis versus UV/H_2O_2, and observed that an optimal concentration of H_2O_2 leads to an increase in the reaction rate. In addition, they observed the formation of three degradation products of bisphenol A: phenol, 1,4-dihydroxylbenzene and 1,4-benzoquinone.

H. Zhu et al. [192] developed a comparison between the effect of UV, H_2O_2 and the combination of both (UV/H_2O_2) in the degradation of trichloroanisoles in water. They observed that the combination of UV/H_2O_2 has the potential to eliminate these off-flavours from water, producing their total mineralization, while direct photolysis leads to the formation of toxic degradation products.

2. Reactions with free chlorine

Another tertiary treatment often evaluated for the degradation of EPs is the application of chemical oxidants, such as chlorine-containing species, being free chlorine ($HClO/ClO^-$) one of the cheapest and more used reagents [179].

Water chlorination is a treatment commonly used in the process of water disinfection to achieve the elimination of bacteria and pathogenic organisms, as well as, the degradation of contaminants. This treatment is usually applied in the last step of the disinfection treatment, desalination and purification, in order to eliminate contaminants and microorganisms that may have survived all the previous processes, guaranteeing the quality of the final water. Considering that in less than 50 % of STPs in Spain (in 2012) [193] no tertiary treatment is implemented yet, and due to the fact that the drinking water sources are affected by the discharges of those STPs, the chlorination treatments are highly recommended.

According to the European Federation of chlor-alkali industry, 98 % of the DWTPs in Europe use chlorination as the principal disinfection step [180], and it is also applied to treat wastewaters after passing through the biological reactor units.

The application of chlorine, as gaseous chlorine or hypochlorite, to polluted waters, renders two chemical reactions: hydrolysis and dissociation (Eq. 9 and 10).

$$Cl_2 + H_2O \rightarrow HClO + Cl^- + H^+ \quad (9)$$

$$HClO \leftrightarrow H^+ + ClO^- \quad (10)$$

Chlorine hydrolizes in presence of water, producing hypochlorous acid (HClO) (Eq. 9), which is a weak acid that dissociates in aqueous solutions (Eq. 10). The dissociation constant of HClO is $K_{HClO} = 2.9 \times 10^{-8}$ ($pK_a = 7.54$ at 25º C). Thus, at the pH of natural waters (between 6 to 9), the main species present are hypochlorous acid and hypochlorite [194].

The disinfection of drinking waters with free chlorine has been a matter of interest during the last decades, not only for its multiple advantages, including its low cost and its ability of reacting with numerous pharmaceuticals and other micropollutants leading to

their degradation and mineralisation, but mostly because it also leads to the formation of harmful disinfection by-products, as halogenated organic compounds, which exhibit a potential carcinogenic activity, such as the well-known trihalomethanes [194].

R. F. Lane et al. [195] studied the degradation of bisphenol compounds and some metabolites in drinking water and they observed the complete degradation of the pollutants in presence of 2 mg L^{-1} of free chlorine, with half-lifes ranged from 1 to 35 minutes at pH 7 and 25 ºC. In addition, they reported three disinfection by-products with greater and lesser toxicities than the precursors.

B. J. Sieira et al. [196] evaluated the chlorination of some polymer related chemicals, which have emerged as relevant water contaminants. They reported a rapid degradation of the pollutants at the pH of the natural waters and the formation of several transformation products, resulting from hydroxylation, halogenation and cyclization, whose toxicity was evaluated *in-silico*, resulting in transformation products more toxic than the original compounds.

The chlorination process can be also combined with UV radiation, achieving higher removal efficiencies and a lower energy consumption. UV/chlorine leads to the generation of powerful oxidizing species, as chlorine and hydroxyl radicals. In case of $Cl^{\cdot}$, this radical can react by abstracting a H atom from a C-H bond and reacts with alcohol functional groups to produce an alkoxyl radical; while in case of $HO^{\cdot}$ radicals, they abstract the H from a C-H bond, producing a carbon-centred radical [197]. The reactivities of $Cl^{\cdot}$ and $HO^{\cdot}$ radicals are similar, but the mechanism of their reactions is different, so the potential transformation products generated are very different.

Some studies show that many EPs can be effectively degraded by UV/chlorine process. W. Cai et al. [197] compared different AOPs for the degradation of the antimycotic CBZ in natural water. They observed that the combination of UV radiation with free chlorine dramatically enhanced the degradation of the pollutant when compared to the UV photolysis and chlorination alone. In addition, they proposed a degradation route of the pollutant, which includes isomerization, de-chlorination, hydroxylation, cleavage and chlorine substitution, reducing the toxicity of CBZ in solution through its transformation.

2.1. Parameters affecting the reaction rate of free chlorine treatments

The efficiency of chlorine disinfection treatments depends on the chemical structure of the contaminant, the pH and temperature of the water, as well as the DOM. Other factors that also might affect the degradation kinetics are the concentration of chlorine and the presence of salts that compete with chlorine for the contaminants.

Chlorination experiments applied to water disinfection treatments are usually developed in presence of an excess of chlorine (molar excess of 20 times). Under these conditions, contaminants are degraded following a pseudo-first-order kinetics.

For a given concentration of chlorine, the stability of the analytes may change as the temperature and pH of the water samples varies. On the one hand, temperature affects to any reaction, so the transformation reactions in STPs and DWTPs, where free chlorine is

applied as disinfection agent, might change between different seasons. Some studies reveal that degradation rates increases with the temperature [198]. In addition, hypochlorous acid (HClO), pK_a 7.5, is more reactive than the hypochlorite anion (ClO^-), so the degradation reaction is favoured when the acid-base equilibrium is shifted to the neutral form of the acid [194]. Obviously, the reactivity of pollutants is also affected by their acid-base equilibria, as example, phenolates are more reactive than their neutral forms.

Several authors have demonstrated that the degradation kinetics of chlorination treatments can be altered by the presence of other species in solution. This effect was observed in presence of low concentration of bromide, where an enhance of degradation kinetics was noticed [199]. In presence of an excess of free chlorine, bromide is converted to HBrO (Eq. 11), which can compete with HClO for the pollutants, leading a significant reduction of their half-lifes, particularly if halogenation processes are involved in the degradation of the investigated compound. In addition, brominated derivatives might be formed from reaction of HBrO and parent pollutants.

$$HOCl + Br^- \rightarrow HOBr + Cl^- \quad (11)$$

2.2. Most common reaction routes

Reaction routes of EPs with free chlorine depend on the functional groups presenting in their chemical structures. The main reactions of aromatic compounds with free chlorine-aqueous phase are summarized in **Figure 25** and described in the following paragraphs.

For aromatic compounds, the most common reaction is the *electrophilic substitution* of H atom by Cl (*electrophilic aromatic halogenation*). This reaction is strongly affected by the functional groups linked to the benzene ring. In case of compounds with donor groups, such as amino and hydroxyl, the substitution reaction in ortho- and para- positions is favoured; whereas, those aromatic compounds bonded to electron-withdrawing substituents, as carboxylic groups, or without the presence of donor groups, show negligible reaction rates in chlorinated water samples [197,200–202].

Another common reaction is the *nucleophilic aromatic substitution*, where a halide group, or simply hydrogen, is replaced by a nucleophile. In aqueous chlorination reactions, the nucleophile is usually the hydroxyl anion, forming different hydroxyl derivatives [200].

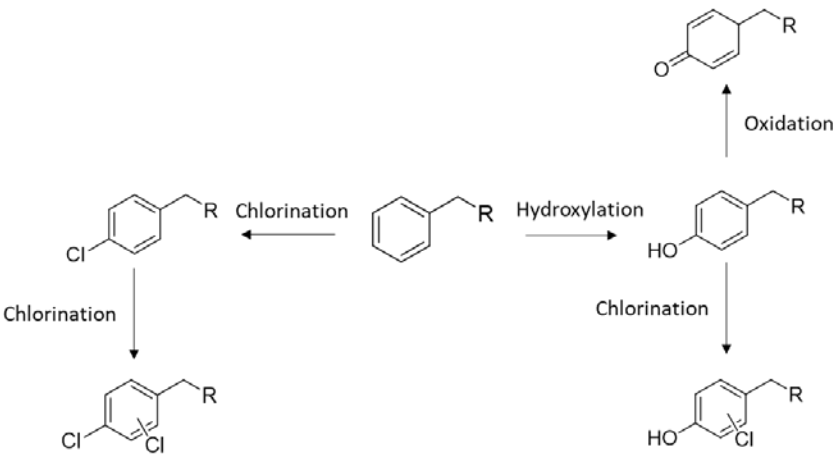

Figure 25. Summary of the main reactions between aromatic compounds and free chlorine in solution

3. Photo-nitration

The process of photo-nitration is not a disinfection treatment but is included in this section because this type of indirect photolysis reactions can occur naturally in the environment and constitutes a matter of study during this thesis.

Nitrate and nitrite ions are usually present in natural waters at concentrations below 10^{-3} mol L^{-1} [203]. Both species are chemically stable but photochemically unstable, and their excitation produces reactive species in solution, such as hydroxyl and nitrogen containing radicals, which can oxidize and react with most of the organic compounds.

The reactivity of these species in presence of UV radiation has been studied deeply in the last decades, due to the fact that nitration reactions are recognized as a potential source of mutagenic or carcinogenic TPs in different environmental samples. Analytical studies were mainly developed with aromatic pollutants, particularly phenolic compounds, because these compounds are prone to interact with nitrogen reactive species.

The main reactions during the excitation of nitrate ions in diluted solutions are oxidation, nitration and nitrosation. Oxidation reaction constitutes an important source of hydroxyl radicals in natural waters. However, under such excitation, nitrate ions can be transformed into nitrogen dioxide (Eq. 15), which disproportionate to produce nitrite and nitrate ions but, in some cases, can lead to nitration or nitrosation, generating highly toxic compounds.

$$NO_3^- + h\nu \rightarrow NO_2^- + O \quad (12)$$

$$NO_3^- + h\nu \rightarrow NO_2^{\cdot} + O^{\cdot -} \quad (13)$$

$$O^{\cdot -} + H_2O \rightarrow HO^{\cdot} + HO^- \quad (14)$$

$$2\ NO_2^{\cdot} \leftrightarrow N_2O_4\ (k_1)$$

$$N_2O_4 + H_2O \rightarrow NO_2^- + NO_3^- + 2\ H^+\ (k_2) \quad (15)$$

k_1= 4.5 x 10^8 L mol^{-1} s^{-1}; k_2= 1000 s^{-1}; equilibrium constant K= 1.53 x 10^{-5} mol L^{-1}

In case of nitrite ions, the reactivity mainly depends on their concentration: nitration and nitrosation are only favoured in presence of high concentrations of substrate and nitrite, since nitrite ions behave as a source of $HO^{\cdot}$, and as $HO^{\cdot}$ quenchers [203].

$$NO_2^- + h\nu \rightarrow NO^{\cdot} + O^{\cdot -} \quad (16)$$

$$O^{\cdot -} + H_2O \rightarrow HO^{\cdot} + HO^- \quad (17)$$

In natural waters, the concentration of nitrite compared to that of organic matter is very low, so the quenching effect of nitrite ions is negligible. The mechanisms of nitration and nitrosation of aromatic compounds are not completely understood, but it is clear that $NO_2^{\cdot}$ and $NO^{\cdot}$ species play an important role during the reactions. Both processes compete with the regeneration of nitrite and nitrate ions by oxidation of $NO^{\cdot}$, recombination and hydrolysis of nitrogen oxides. Therefore, the occurrence of these reactions is highly dependent on the environmental conditions, taking place in waters with high concentration of organic matter and nitrate ions (NO_3^- > ca. 50 ppm) or nitrite ions (NO_2^- > ca. 3 ppm), which is a very uncommon situation in natural waters.

Nitration and photo-nitration of aromatic compounds constitute a matter of concern and several authors studied their occurrence and pathways in different environmental matrices, such as aqueous samples [204,205], wastewater [206] and sludge [207,208]. Some of these authors propose that aromatic compounds in presence of HNO_3, suffer an electrophilic nitration through an electron-transfer nitration [203,204]:

$$ArH + NO_2^+ \rightarrow ArH^{\cdot +} + NO_2^{\cdot} \quad (18)$$

$$ArH^{\cdot +} + NO_2^{\cdot} \rightarrow H\text{-}Ar\text{-}NO_2^+ \quad (19)$$

$$H\text{-}Ar\text{-}NO_2^+ \leftrightarrow ArNO_2 + H^+ \quad (20)$$

This reaction of nitration of aromatic compounds usually leads to the formation of mutagenic or carcinogenic compounds, so the study of this type of reactions in natural waters and soils is required.

4. Study of transformation products of selected compounds during oxidative treatments

As it was explained along this manuscript, the occurrence of pharmaceuticals in the aquatic and terrestrial environment is closely related to different sources, such as industry emissions, direct disposal or human and animal applications. In the latter case, after administration, pharmaceuticals and their metabolites are excreted through faeces and/or urine and arrive to STPs through urban sewers. The different treatments applied in the STPs can achieve the complete mineralization of these EPs or lead to the formation of different TPs, which can be more or less toxic than the parent compound.

These compounds and their new TPs can remain in the aquatic media, reaching rivers and surface waters or even, drinking waters, or in case of the most lipophilic compounds, be adsorbed in the sludge, widely used as agricultural fertiliser.

The discharge into surface waters has a special importance, because it constitutes a possible source for drinking water. In DWTPs these compounds can be degraded by different treatments, but as it was described in the section 2 of this chapter, the principal disinfection technique is the application of chlorine, thereby the compounds that are not removed in the DWTPs and their transformation products can reach the consumer. The study of the presence of EPs in aquatic environment, as well as, in drinking water, has constituted an important field of study during the last decades.

K. Nödler et al. [60] studied the occurrence and fate of VALA, a transformation product of certain pharmaceuticals during biological treatment of wastewater, in the water cycle. They conclude that treated effluents from STPs constitute the main source of this compound in the environment and also, the treatments at the DWTPs are not effective removing this species. They have reported concentrations of VALA in tap waters from Berlin between 57 and 72 ng L^{-1}.

M. Huerta-Fontela et al. [50] investigated the occurrence of 55 pharmaceuticals and their metabolites during drinking water production in a DWTP located in the NE of Spain. A total of 35 out of the 55 selected pollutants were detected in the raw water at the facility intake with concentrations up to 1200 ng L^{-1}.

M. Cai et al. [209] studied the presence of 14 pharmaceuticals compounds in tap water and in a drinking water treatment plant from Beijing. They found 9 out of 14 studied compounds at concentrations up to 38 ng L^{-1} in the treated water.

P. Canosa et al. [198] have investigated the chemical transformation of paraben derivatives in chlorinated water samples. Experiments carried out at chlorine levels usually contained in tap water, demonstrated the formation of several halogenated by-products more stable than the paraben precursor, which are relatively labile.

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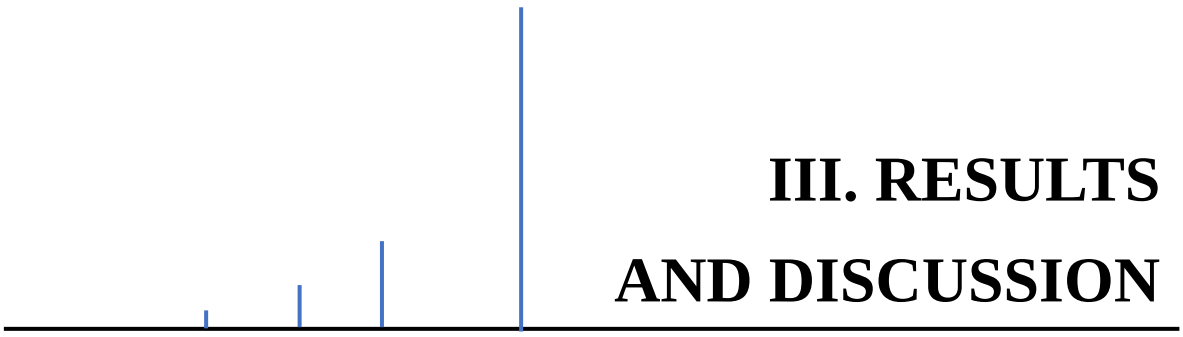

III. RESULTS AND DISCUSSION

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III. RESULTS AND DISCUSSION

In the following pages, the analytical methodologies applied along this doctoral thesis, as well as the resulting scientific publications are grouped in different chapters. The first chapter compiles the results obtained during the non-target screening of emerging pollutants in environmental samples. Depicted data corresponds to two different matrices (sludge from STPs and dust from indoor areas), employing accurate MS techniques. As it was mentioned along this memory, the sample preparation approaches used during screening studies involved generic and non-selective extraction conditions, with the aim of recovering as much species present in the sample as possible, but taking into account the potential limitations of the further determination technique. To this end, the sample preparation techniques developed consisted mainly on matrix solid-phase dispersion (MSPD) and pressurized liquid extraction (PLE) for solid samples.

Regarding the determination techniques, two different analytical platforms were employed. They are based on the combination of liquid chromatography (LC) or gas chromatography (GC) with accurate mass spectrometry, through different ionization sources. All of them have in common the use of accurate mass spectrometry (MS).

Once the compounds present in the environmental matrices were identified, the quantification of those pollutants with high potential environmental impact, and/or limited occurrence data, such as chlorinated azolic compounds and cardiovascular pharmaceuticals, was developed for sludge and water samples, where the QqQ instrument replaced the QTOF system. All these studies are presented in the second chapter, within the results and discussion section.

Thereafter, the possibility of removing some of the identified pollutants from the aquatic environment was evaluated using different degradation techniques, particularly oxidizing treatments, such as photolysis and chlorination. The performed methodologies, as well as the obtained results are presented in the third chapter of this section. In addition, the identification of possible transformation products originated during these processes and their predicted toxicity was also investigated. Additional transformation routes of some relevant pollutants under simulated conditions are also reported in the third chapter of this section.

Finally, a latter sub-section describes the use of a non-chromatographic technique, such as direct analysis in real time (DART) coupled to accurate mass spectrometry (MS) for the rapid determination of bisphenol A in thermal paper.

Chapter I. Screening and determination of emerging pollutants in environmental samples

This section of the PhD memory summarizes the results obtained applying accurate MS to the screening of emerging pollutants in different environmental samples.

a) LC-HRMS

Firstly, the use of LC-QTOF-MS for monitoring lipophilic species in sludge and biosolids samples from STPs is presented. Given the complexity of the investigated matrix, a sample preparation strategy based on the use of matrix solid-phase dispersion (MSPD) combined on-line with a fractionation step was developed. The analytes were separated in base on their polarities, following two different fractionation protocols, consisting on the use of cationic or anionic exchange cartridges, which fractionate compounds in a basic and neutrals plus acidic (cationic exchange sorbent) fractions; or acidic species separated from neutrals and basic substances (anionic exchanger). This sample preparation strategy is presented in **Figure 26.**

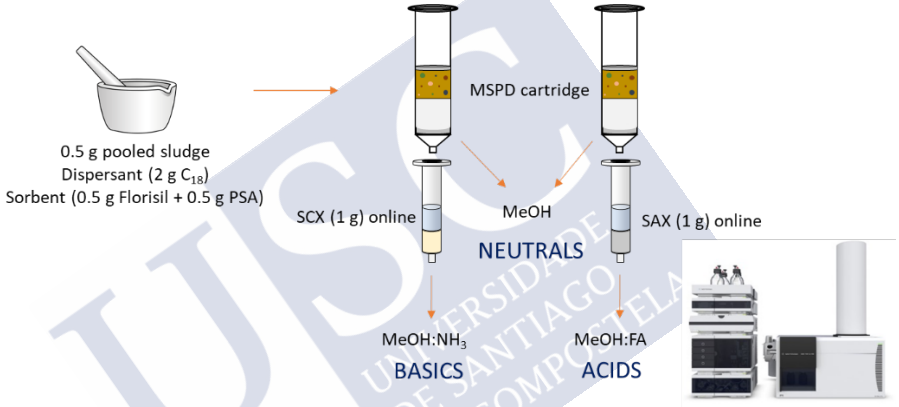

Figure 26. Sample preparation strategy applied to the screening of sludge

Even considering the above analytical fractionation strategy for sample preparation, the number of molecular features present in the sludge and biosolids LC chromatograms is massive, so the data analysis strategies applied to these spectra generate a large number of empirical formulas whose structures are difficult to establish in a systematic way. The main data analysis strategies applied for searching, identifying, and elucidating new contaminants in sludge and biosolids are explained as follow.

1. First strategy:

Natural chlorinated and brominated species are scarce in the environment. However, chlorine is a relatively common element in pharmaceuticals, phytochemicals and certain flame retardants products, in which bromine can be also used. Additionally, the spectra of chlorinated and/or brominated compounds have characteristic molecular clusters, which make them easily identifiable given the relative intensities of the M and M+2 species, and also the typical mass defect between ^{35}Cl and ^{37}Cl isotopes (- 3.0 mDa) and between ^{79}Br and ^{81}Br (- 2 mDa) [1]. In addition, automatic tools applied to peak search in LC-HRMS chromatograms, as

Find by molecular feature (included in *MassHunter Qualitative Analysis B.07.00* software), followed by a filtration of those molecular entities compatible with empirical formulas containing at least one atom of chorine or bromine, permit to identify and extract the peaks corresponding to both kinds of halogenated species and generate their empirical formulas with high reliability. Thus, using HRMS databases such as *Metlin* or *Massbank*, a preliminary search of candidates whose mass spectra are compatible with the corresponding halogenated species detected in the sludge extracts can be developed. Finally, compound confirmation was performed by comparison between experimental MS/MS spectra, acquired in a second injection, and those compiled in the database.

This first strategy, based on the use of the chlorine filter, permitted to detect several halogenated pollutants, including the azolic antimycotic compounds (compiled in **Table 1** of the Introduction section). Moreover, a series of chlorinated tri-aryl imidazole species, showing significant intensity responses were found in sludge from a single STP. The occurrence of some of these species was reported for the first time in this environmental compartment. Identities and levels of these substances are presented in the first two publications included in this section of the PhD memory.

2. Second strategy:

A general search, without filtering halogenated compounds, was also carried out. To this aim, different workflows were developed for the screening of contaminants in sludge and biosolids.

Firstly, a DDA methodology was carried out. *Auto MS/MS* was selected as acquisition methodology, using three different collision energies (0, 20 and 40 eV). The feature extraction and identification were developed using the compound discovery workflow, *Find by Auto MS/MS*, available in *MassHunter Qualitative Analysis Workflows B.08.00* software from Agilent Technologies. In addition, a second workflow, based on DIA analysis, was developed. Three experiments at three different collision energies (again 0, 20 and 40 eV) were carried out in a single *All Ions* run. The data analysis in this case, consisted on a target/suspect screening workflow, *Find by formula* (with fragment confirmation), also available in *MassHunter Qualitative Analysis Workflows B.08.00* software. No matter the acquisition strategy used, the identification of candidates is conducted with a database containing spectra for interesting compounds recorded in ESI positive and negative mode. This database, known as *ForTox* PCDL from Agilent Technologies, contains spectra for active ingredients present in pharmaceuticals, personal care and household cleaning products, additives used in paints and textiles, and other compounds with a high volume of production. After the generation of molecular feature on LC-QTOF-MS chromatogram, the empirical mass spectra were compared with those containing in the database. MS/MS of the entities that have the best match with the HRMS spectra of any of the species in the database, are considered as positives.

Following the second strategy, cardiovascular pharmaceuticals, among other different families of compounds, were systematically found in all the sludge samples

studied. These compounds have an acidic character (**Table 3** of the Introduction section), for that reason, they were mainly noticed in the acidic extract obtained from the MSPD cartridge. Target quantitative methods for the analysis of several cardiovascular drugs in sludge, and also in water samples, are shown in the second chapter (**section III.II**) of this section. Selection of target compounds was driven by findings obtained during non-target screening of LC-QTOF-MS records of sludge extracts.

Publication III.I.1. describes the strategy followed during the screening of halogenated species in sludge samples, where several antimycotic compounds, which had not been reported yet in the bibliography, are stated. The second part of this article describes a selective procedure for the quantitative determination of this family of compounds in sludge. To this end, 0.5 g of sample were dispersed with 2 g of C_{18} and loaded into a polypropylene cartridge, which contained 0.5 g of PSA and 0.5 g of Florisil. MSPD cartridge was connected on-line with a SCX cartridge (1g) in order to reduce the complexity of the extract. Analytes were eluted with 20 mL of a mixture of MeOH:NH_3 (2 %) and finally injected in a LC-QqQ-MS instrument. Quantitative recoveries were obtained for all studied compounds with negligible matrix effects, achieving limits of quantification (LOQs) of 2 ng g^{-1}, referred to the freeze-dried sludge matrix. Analysis of sludge and biosolid samples from different STPs from several points of Galicia (Spain) demonstrated the presence of antimycotic compounds in all studied samples, in average concentrations above 31 ng g^{-1}.

Alternatively, **Publication III.I.2.** submit an analytical methodology for the determination of aryl chloro imidazoles. Despite these compounds are also basic species, they turned unstable in contact with cationic exchange materials used in the previously reported MSPD approach. As a consequence, a series of related species, which turned to be analytical artefacts, not really existing in the processed samples were generated. So, an alternative MSPD methodology was proposed. Under final working conditions, it was confirmed for the first time the occurrence of 2-chloro-triaryl imidazole, and its dimer, is sludge and biosolids from a given STP, at concentrations between 0.02 to 14 μg g^{-1}.

a) GC-HRMS

The use of GC-QTOF-MS for the screening of semi-volatile compounds in indoor dust samples is also evaluated in this thesis. This type of matrix contains a complex mixture of anthropogenic and synthetic compounds closely related to dermal and respiratory diseases, usually grouped under the so-called sick building syndrome [2]. Target methods have been developed for the quantification of different groups of substances in dust, mainly based on the combination of well-tuned sample preparation with selective analytical techniques, such as liquid and gas chromatography tandem mass spectrometry (MS/MS). These procedures permit the sensitive determination of pre-selected compounds [3]; however, they are totally blind to any other compound not included in the list of MS/MS transitions. For that reason, the comprehensive characterization of the different species existing in dust remains a challenging issue.

In this doctoral thesis a new screening strategy for the analysis of dust samples was developed. This methodology was based on the pressurized liquid extraction (PLE) and gas chromatography coupled to quadrupole time-of flight mass spectrometry, using electron ionization (GC-EI-TOF-MS), as sample preparation and determination technique, respectively. The analysis strategy is showed in **Figure 27.** The selection of GC-TOF-MS is derived from the semi-volatile character of potential indoor pollutants, the low ionization efficiency expected for most of these compounds in ESI sources, and the paramount information derived from EI spectra. In contrast with LC-ESI-QTOF-MS, in the former case it was expected that data obtained using a single acquisition function (the GC-EI-MS scan channel) would suffice to the unambiguous identification of detected chromatogram compounds.

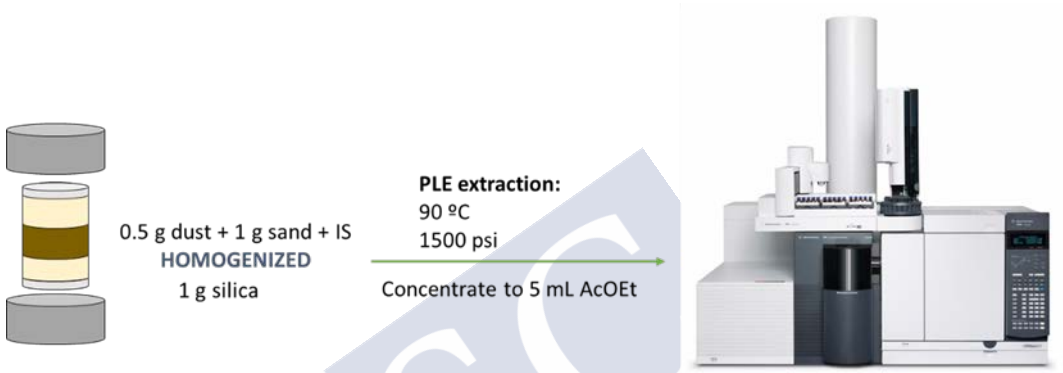

Figure 27. Analytical methodology for the screening of dust samples

A data mining workflow consisting on the use of *Unknowns Analysis* algorithm for spectral deconvolution of compounds in the GC-MS chromatograms was proposed in **Publication III.I.3.** For tentative/confirmed identification of the detected compounds, a preliminary comparison was developed with nominal resolution EI-MS spectra in the NIST17 library. In addition to experimental *m/z* values compiled in this library, the accurate ratios calculated for fragment ions with known structures were also considered. The reliability of the preliminary identifications, derived from direct comparison with NIST database, was further investigated considering calculated *m/z* values for fragment ions in the spectra compiled in the NIST17 library, acquisition of experimental EI-accurate MS spectra for standards, and construction of personal compound database (PCDL) of accurate EI-MS spectra. Identities of more than 75 species in dust from indoor environments were reported thereby. Some species, such as certain dyes and their degradation products, isocyanates, phthalimide, some UV absorbers and the bactericide octyl isothiazolinone were reported in indoor dust for the first time. Finally, a semi-quantitative determination of concentrations for a group of pollutants was carried out, demonstrating the ubiquity of selected compounds in dust in median concentrations between 0.09 to 17.7 µg g^{-1}.

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Publication III.I.1. Identification and determination of chlorinated azoles in sludge using liquid chromatography quadrupole time-of-flight and triple quadrupole mass spectrometry platforms

G. Castro, M. Roca, I. Rodríguez, M. Ramil, R. Cela

Journal of Chromatography A, 1476 (2016) 69-76
DOI: 10.1016/j.chroma.2016.11.020

Publication III.I.2. Liquid chromatography quadrupole time-of-flight mass spectrometry identification and determination of tri- and hexaaryl chloro imidazoles in sewage sludge

J. Casado, G. Castro, I. Rodríguez, M. Ramil, R. Montes, R. Cela

Journal of Mass Spectrometry, 52 (2017) 69-77
DOI: 10.1002/jms.3903

Publication III.I.3. Assessment of gas chromatography time-of-flight mass spectrometry for the screening of semi-volatile compounds in indoor dust

G. Castro, I. Rodríguez, M. Ramil, R. Cela

Science of the Total Environment 688 (2019) 162-173
DOI: 10.1016/j.scitotenv.2019.06.192

Chapter II. Determination of cardiovascular pharmaceuticals in sludge and water samples

The incidence of cardiovascular diseases has increased in developed societies, constituting the most common cause of death worldwide in the last decades. In order to prevent and to treat this kind of diseases, a large number of pharmaceuticals have been developed and commercialized. Attending to their mode of action, these compounds are classified in different families, which also have different physicochemical properties as it was explained along the introduction of this dissertation. It is worth noting again that, depending on these properties, cardiovascular drugs are distributed in different environmental compartments, such as sludge and aquatic samples (wastewater, rivers and tap water) [4-6]. Previous publications of the research group have demonstrated the existence of relevant concentration of certain cardiovascular drugs (i.e. the iodinated specie amiodarone) in sewage sludge [7]. Also, some unpublished data derived from the second strategy of the screening analysis, suggested the existence of certain cardiovascular drug residues, such as irbesartan in sludge.

During this doctoral thesis different analytical methodologies were developed for the determination of cardiovascular pharmaceuticals in environmental matrices. The sample preparation protocols were proposed based on the type of sample, which are described in the following paragraphs:

1. Sludge

An analytical methodology based on matrix solid-phase dispersion (MSPD) and ultra-performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS), selected as sample preparation and determination technique, respectively, was proposed for the analysis of a set of cardiovascular drugs, from different chemical groups, in sludge samples. This methodology is presented in the **Publication III.II.1**. Under optimal conditions, 0.5 g of freeze-dried sludge were dispersed with 2 g of C_{18}. The dispersed sample was loaded into a MSPD syringe containing 1 g of diatomaceous earth. Analytes were eluted with 10 mL of a mixture of MeOH:ACN:FA (30:69:1) and then injected in a UPLC-QqQ-MS instrument. **Figure 28** shows the scheme of the optimized methodology.

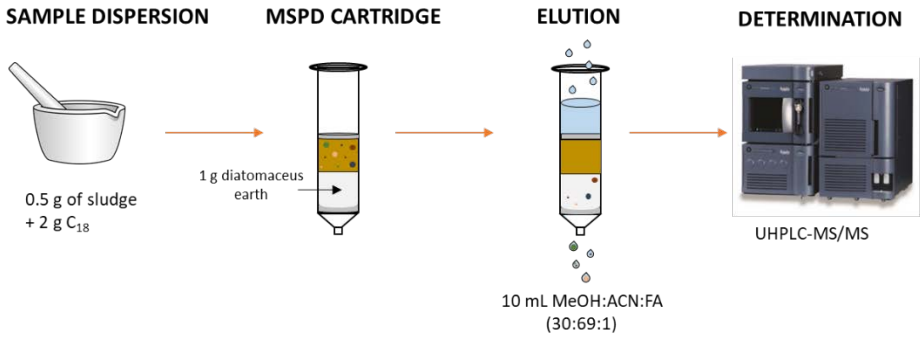

Figure 28. Scheme of the optimized methodology for the determination of cardiovascular pharmaceuticals in sludge samples

The overall recoveries of the optimized methodology varied from 82 to 124 %, with standard deviations between 2 and 16 %. Obtained LOQs stay below 10 ng g^{-1} for all studied compounds. The analysis of sludge from 14 STPs demonstrated the presence of significant residues of most of the investigated compounds.

2. Water samples

A totally different methodology, based on solid-phase extraction (SPE) and ultra-performance liquid chromatography (UPLC), was proposed for the selective analysis of a group of cardiovascular pharmaceuticals, all of them included in the family of sartans, in aquatic samples (wastewater, river and tap water). This methodology is presented in **Publication III.II.2.** and it consisted on the use of a mixed-mode SPE as sample preparation technique and again, UPLC-QqQ-MS as determination technique. Under optimized conditions, samples at neutral pH (6-8 units) were concentrated using mixed-mode (reversed-phase and anionic exchange) cartridges (OASIS WAX 150 mg). Thereafter, the sorbent was washed with 5 mL of a MeOH:ultrapure water (1:1) solution, dried under a nitrogen stream and compounds were eluted with 2 mL of MeOH:NH_3 (98:2) (**Figure 29**). This way, the positively charged groups in the SPE sorbent were neutralized and the strong acidic drugs released into the elution solvent.

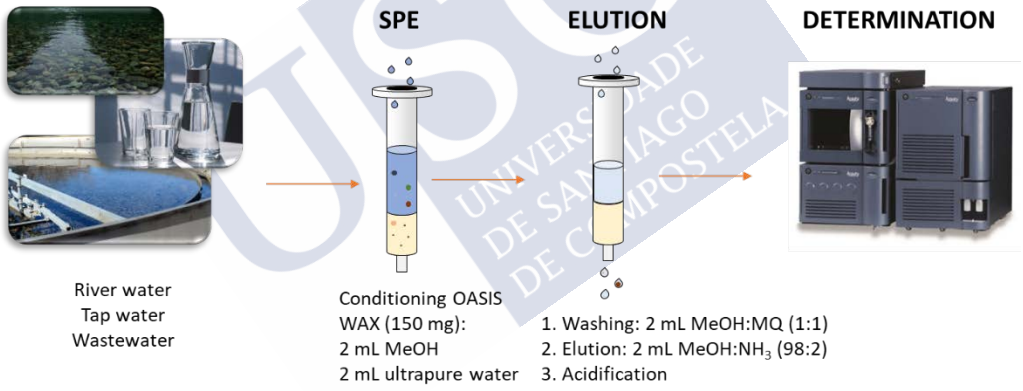

Figure 29. Optimal conditions of the analytical methodology applied in the determination of sartan drugs in water samples

The accuracy of the methodology (accounting for SPE efficiency and matrix effects during electrospray ionization) was tested using solvent-based calibration standards. Global recoveries, investigated in different types of water samples (influent, effluent, river and tap water), varied between 82 and 134 % with standard deviations from 2 to 18 %. In this case the achieved LOQs ranged from 2 to 50 ng L^{-1}. The analysis of these environmental samples demonstrated:

- The formation of a stable biodegradation product, VALA, during the municipal water treatment and its systematic presence in tap water.
- Incomplete removal of sartan drugs at the STPs.
- E-Z isomerization of EPR in environmental samples.

- Occurrence of hydroxylated derivatives of the sartan drugs IRB and LOS, generated during their human metabolism, in wastewater.

Publication III.II.1. Determination of cardiovascular drugs in sewage sludge by matrix solid-phase dispersion and ultra-performance liquid chromatography tandem mass spectrometry

G. Castro, I. Carpinteiro, I. Rodríguez, R. Cela

Analytical Bioanalytical Chemistry 410 (2018) 6807-6817
DOI: 10.1007/s00216-018-1268-3

Publication III.II.2. Selective determination of sartan drugs in environmental water samples by mixed-mode solid-phase extraction and liquid chromatography tandem mass spectrometry

G. Castro, I. Rodríguez, M. Ramil, R. Cela

Chemosphere 224 (2019) 562-571
DOI: 10.1016/j.chemosphere.2019.02.137

Chapter III. Photolysis and chlorination reactions of selected emerging pollutants

The main objective of this chapter of the doctoral thesis is two-fold. On the one hand, to study the applicability of different tertiary treatments, such as photolysis and chlorination, to the removal of those emerging pollutants that are resistant to the biodegradation processes in the STPs (certain antimycotics and cardiovascular pharmaceuticals, as well as some personal care products and plasticizers), determining their half-lifes and identifying their transformation products. On the other hand, the second objective is to evaluate the possibility to apply photolysis processes (using UV irradiation) and advanced oxidative treatments (UV radiation combined with H_2O_2) for the complete removal of these contaminants in the STPs, minimizing their discharge into the aquatic media.

Whatever the degradation technique followed, the methodology proposed for the identification of transformation products involves, in the first place, the systematic comparison of the LC-ESI-HRMS records for control experiments and sample aliquots obtained from degradation assays carried out at laboratory scale, at different reaction times, using the *Mass Profiler* software for the rapid detection of molecular entities present in both groups of LC-QTOF-MS records, detected by using the *Find by Molecular Feature* algorithm. Elucidation of the chemical structures of the species responsible for these chromatographic peaks was based on the interpretation of their accurate product ion scan spectra. Once the transformation products were identified, the reaction pathways that relate them to their precursors, are proposed. In some cases, the occurrence of described transformation products is further investigated in environmental water samples.

The different degradation treatments studied along this doctoral thesis are explained in the following paragraphs:

1. UV and UV/H_2O_2

The efficiency of direct UV irradiation and the combination of UV/H_2O_2 processes, for the removal of the antimycotic pharmaceuticals, fluconazole (FCZ) and climbazole (CBZ), from water samples, is presented in **Publication III.III.1**.

Mass balances performed at the STPs point out to a low elimination percentages of these pharmaceuticals, which show similar concentrations in the inlet and outlet of the STPs, resulting in the presence of these pollutants in the aquatic media and in the sludge [8-10]. Although several authors studied the stability of FCZ under advance oxidative treatments, no data regarding transformation products have been reported yet and also, to the best of our knowledge, the stability of CBZ under any oxidative treatment is still unexplored.

Degradation experiments, at laboratory scale, were carried out with spiked aliquots of ultrapure water solutions and treated wastewater samples using low pressure mercury lamps, emitting at 254 nm, to irradiate spiked samples. Time-course of precursor pollutants and identification of arising transformation products (TPs) was performed by injection of different reaction time aliquots in a LC-QTOF-MS system. The protocol followed during the degradation processes is presented in **Figure 30.** The presence of

possible transformation products resulting from the degradation treatments was also studied. Under optimal conditions, results revealed that UV/H_2O_2 render a more efficient removal of the parent pollutants than direct UV photolysis; moreover, no TPs were detected when applying the advanced photochemical process.

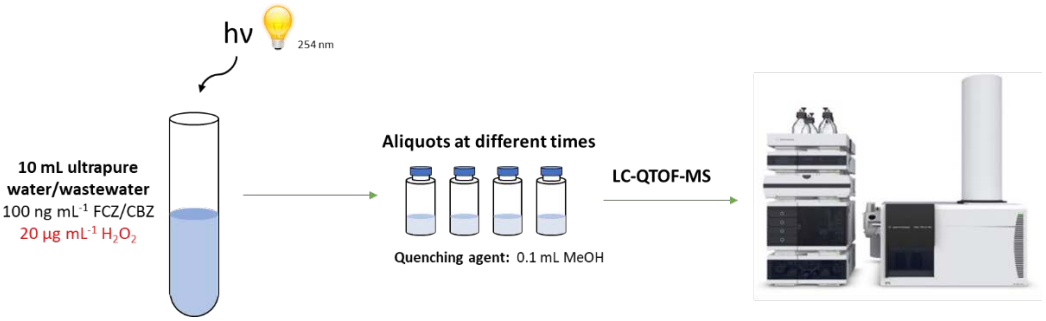

Figure 30. Scheme of the degradation studies with H_2O_2

2. Nitration

The effect of certain inorganic anions, such as nitrates, in the photodegradation kinetics of selected emerging pollutants and in the formation of nitro derivatives was also studied along this thesis. As it was explained in section II.D. of the introduction chapter, nitration reactions can be promoted by light; thus, nitrate anions existing in waters are decomposed by light (visible and UV radiation) leading to the formation of highly reactive nitro and hydroxyl radicals, which might be further involved in indirect photochemical transformation reactions. The generated transformation products, depending on the precursor, could be even more toxic than parent pollutants.

$$NO_3^- + H^+ + h\nu \rightarrow NO_2^{\cdot} + HO^{\cdot}$$

Aromatic pollutants, particularly phenolic-type compounds are prone to interact with nitrogen reactive species, leading to the formation of nitrophenols, which are highly toxic and stable compounds [11,12].

Publication III.III.2 investigates the effect of nitrate, at concentrations in the range of the values expected in river and wastewater samples, in the photodegradation kinetics of selected phenolic compounds, recognized as ubiquitous pollutants in the aquatic environment. Half-life values and formation of TPs have been evaluated considering two different cases: wastewater exposed to UV radiation (attempting to mimic the use of UV light during tertiary treatment of wastewater) and river water exposed to solar light, which simulates the scenario that would exist under environmental conditions. LC-QTOF-MS was employed as analytical technique to follow the dissipation of precursor pollutants and to investigate their photodegradation routes in presence of nitrate anions (**Figure 31**).

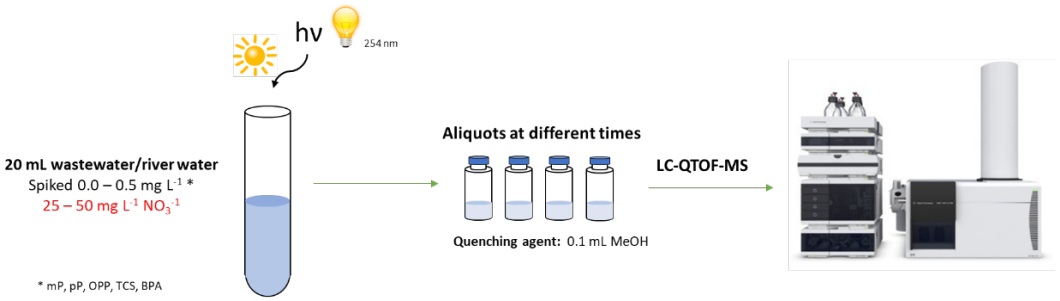

Figure 31. Scheme of the protocol followed during the study of the nitrate effect during the photodegradation of emerging pollutants

Nitrate ions, at environmental concentrations, enhanced the aqueous degradability of the investigated phenolic compounds (methyl and propyl paraben, o-phenylphenol, triclosan and bisphenol A) under solar and UV light. In general terms, the reactions involved in the degradation of pollutants are promoted by $HO^{\cdot}$ and $NO_2^{\cdot}$ radicals, derived from the photolysis of nitrate. Thus, the transformation routes of the studied bactericides, led to the formation of nitro-derivatives, sometimes even more toxic than the parent compound. Within the group of selected pollutants, OPP displayed the highest potential to generate photochemically stable mono- and di-nitro derivatives. This compound also led to the formation of 2-dibenzofuranol under solar light, which is considered to be more toxic than the precursor pollutant.

3. Chlorination

Free chlorine is one of the most popular oxidants employed during the production of drinking water. This species is added at the beginning and/or the end of the disinfection treatment, during the tap water production process.

Along this thesis, one of the families of compounds determined in the aquatic environment was the cardiovascular pharmaceuticals, particularly, the angiotensin II receptor antagonists (ARAs). These drugs are used in the treatment of the arterial hypertension and in the prevention of heart failure. Some compounds of this family, commonly known as sartans, have been detected in surface water samples and even in tap water [13-15]. For that reason, the reactivity of four commonly prescribed sartans in chlorinated water samples was investigated. The effect of sample pH and bromide concentration in their half-lifes was discussed, and their transformation products described in **Publication III.III.3**. Finally, the observed reaction pathways were correlated with the different functionalities existing in the chemical structures of parent pollutants. **Figure 32** compiles the complete protocol followed during the degradation study.

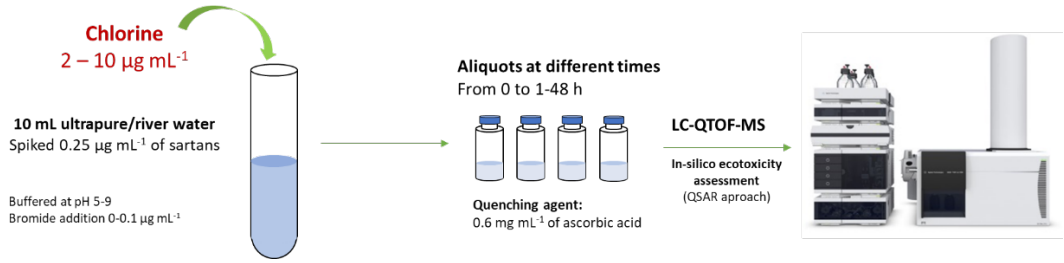

Figure 32. Methodology applied to the chlorination process of sartan pharmaceuticals in water

Degradation experiments were carried out using ultrapure and river water samples at different pHs (from 5 to 9 units) and considering different concentrations of bromide. Obtained results showed that, at pH values between 5 and 6, the degradation was faster than at higher values, due to the acid-base equilibrium of HClO, and the highest reactivity of the acid compared to that of the hypochlorite anion. Under investigated conditions telmisartan (TELM) and valsartan (VAL) remained stable when exposed to relatively high concentrations of chlorine, whilst irbesartan (IRB) and losartan (LOS) were removed following a pseudo-first order kinetics model. The addition of bromide to the solution had effect only in case of LOS, accelerating the degradation reaction.

The reactivity of IRB and LOS is mainly related to the presence of an imidazolic-like cycle in their structure. Several TPs were found for each compound, which in case of IRB are predicted to be less toxic than the parent compound. In contrast, some LOS derivatives (di-halogenated species) are expected to be more toxic.

Publication III.III.1. Time-of-flight mass spectrometry assessment of fluconazole and climbazole UV and UV/H_2O_2 degradability: Kinetics study and transformation products elucidation

G. Castro, J. Casado, I. Rodríguez, M. Ramil, A. Ferradás, R. Cela

Water Research 88 (2016) 681-690
DOI: 10.1016/j.watres.2015.10.053

Publication III.III.2. Evaluation of nitrate effects in the aqueous photodegradability of selected phenolic pollutants

G. Castro, I. Rodríguez, M. Ramil, R. Cela

Chemosphere 185 (2017) 127-136
DOI: 10.1016/j.chemosphere.2017.07.005

Publication III.III.3. Free chlorine reactions of angiotensin II receptor antagonists: Kinetics study, transformation products elucidation and in-silico ecotoxicity assessment

I. Carpinteiro, G. Castro, I. Rodríguez, R. Cela

Science of the Total Environment 647 (2019) 1000-1010
DOI: 10.1016/j.scitotenv.2018.08.082

Chapter IV. Other publications

The last publication included in this doctoral thesis, **Publication III.IV.1**. discusses the suitability of an ambient ionization source, (direct analysis in real time, DART) combined with accurate mass spectrometry (QTOF-MS), for the rapid and quantitative determination of bisphenol-related compounds in thermal paper samples. This topic has been a matter of concern in the last years, considering the endocrine disrupting activity of these compounds and the risk assessment data published for cashiers and individuals in daily dermal contact with thermal printing paper [16,17].

One of the main issues of this study was the assessment of compound structures in the yield of the ionization process and thus, in the detectability of target compounds. To this aim, acetylation of target compounds was necessary to improve the ionization efficiency at the DART source. **Figure 33** shows a simple scheme of the optimized methodology.

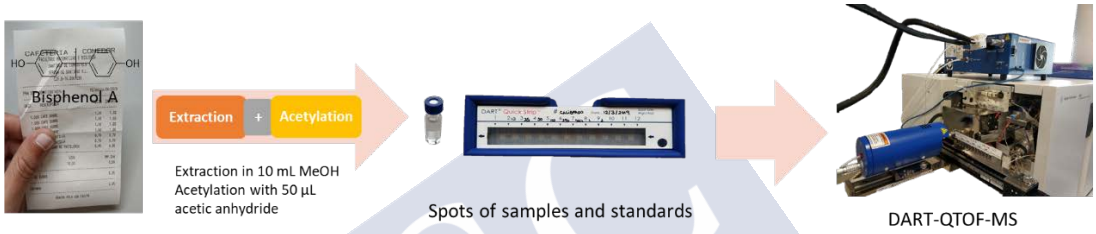

Figure 33. Application of DART-QTOF-MS to the quantitative determination of bisphenol-related compounds

Under optimal conditions, bisphenolic compounds were extracted from the thermal paper with MeOH, under sonication, and then acetylated with acetic anhydride. One drop of the final extract was deposited on the stainless-steel mesh of the Quick Strip sample cards and exposed to excited helium atoms generated in the DART source. Ions produced from acetylated bisphenols were determined by QTOF-MS. The acetylation reaction enhanced the ionization yield of the studied compounds, versus direct determination, in the DART source. Analysis of different thermal paper samples showed that bisphenol compounds were ubiquitous in this matrix, particularly BPA, whose concentrations were above of the maximum permitted by the EU regulation [18]. Data measured for thermal printing paper samples were in good concordance with those obtained using an independent analytical technique, based on GC separation and MS determination.

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Publication III.IV.1. Direct analysis in real time accurate mass spectrometry determination of bisphenol A in thermal printing paper

G. Castro, I. Rodríguez, M. Ramil, R. Cela

Talanta 205 (2019) 120086
DOI: 10.1016/j.talanta.2019.06.086

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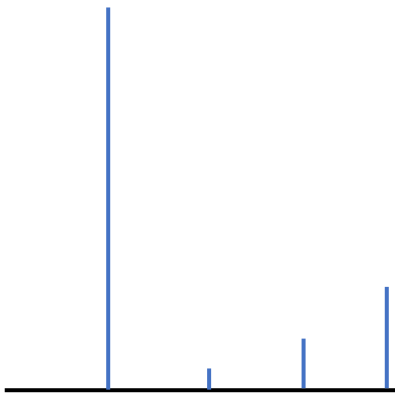

IV. CONCLUSIONS

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IV. CONCLUSIONS

Along this thesis, different target and non-target screening strategies are presented, as well as new analytical quantitative methodologies. All of them, in order to investigate the presence and concentration of different families of emerging pollutants and their fate and behaviour in the environment. In addition, several removal treatments are studied with the aim to eliminate these compounds from the environmental samples.

Studies of determination, distribution and transformation of bactericides and pharmaceuticals, as antimycotics and cardiovascular drugs, have been carried out. The main point in common in all those studies is the assessment of the large amount of possibilities offered by accurate mass spectrometry in the identification and inspection of new pollutants. The conclusions that can be obtained from each part of this thesis are summarized below.

1. Screening and determination of emerging pollutants in environmental samples

Three strategies for the screening of emerging pollutants in environmental samples, based on the use of high-resolution mass spectrometry (HRMS) have been proposed, either for LC or GC separation, both coupled to QTOF instruments.

Regarding the sample preparation technique, different protocols were developed depending on the type of matrix. These sample preparation approaches involved generic and non-selective conditions, with the aim of recovering as much species present in the sample as possible but considering the potential limitation of the selected determination technique.

The sample preparation technique chosen for the analysis of sludge samples was the MSPD, following different elution strategies, in order to recover analytes in a wide range of polarities. The combination of MSPD with LC-QTOF-MS permitted the confirmation of more than 30 chlorinated pollutants, belonging to different families, such as pharmaceuticals, PCPs, pesticides and flame retardants, among others. In a first step, chlorinated pollutants were screened in the LC-QTOF-MS records. To this aim, compound identification was performed following a semi-automated strategy involving: (1) *Find by molecular features*, (2) generation of empirical formulae of compounds containing, at least, one atom of chlorine in their structures and (3) recording their product ion accurate scan spectra with the LC-QTOF-MS instrument. Chemical structures of identified chlorinated compounds are inferred from the interpretation of their product ion scan spectra and the comparison of experimental spectra against those compiled in the high-resolution MS spectra database. Finally, the identities of compounds of interest were confirmed with authentic standards. This first strategy rendered to the identification of new members of an important family of emerging pollutants deeply studied along this thesis, that of antimycotic pharmaceuticals.

In a second step, sample preparation conditions were finely tuned to optimize the extraction recoveries and to minimize matrix effects during LC-ESI-MS/MS determination of several identified chlorinated pollutants, whose presence in sludge had not been previously reported. Finally, the presence of that compounds was confirmed in the analysed samples with measured concentrations ranging from 2 to 195 ng g^{-1}, in case of chlorinated antimycotic drugs. Moreover, higher concentrations (µg g^{-1} range) of chloro aryl imidazoles, likely from industrial origin, have been found in sludge samples obtained in different dates from the same STP.

Additionally, a general search without any filter of halogen substances, was also developed. This strategy consists on DDA and DIA analysis. On the one hand, DDA strategy was based on *auto MS/MS* acquisition methodology, combined with a data mining protocol consisted on *Find by Auto MS/MS*. On the other hand, *all ions* acquisition methodology was selected as DIA strategy. The data analysis developed consisted on the *Find by Formula* tool (with fragment confirmation) available in *MassHunter Qualitative Analysis Workflows B.08.00* software from Agilent Technologies. Regardless the acquisition technique followed, the identification was carried out by comparison between experimental spectra against those compiled in the database. This strategy, even though is not described in any publication (data are still pending of publication), produce some valuable information, the identification of more than 70 compounds and the following confirmation of 42 species, mostly pharmaceuticals, in sludge. One of the families found in the above-mentioned screening strategy was that of cardiovascular pharmaceuticals. Based on this information, the target determination of several pharmaceuticals from this group in sludge and water was addressed in the second chapter of the results section of this thesis.

On the other hand, the last proposed screening strategy was applied to the non-target identification of semi-volatile compounds present in indoor dust samples. To this aim, PLE and GC-EI-TOF-MS were selected as sample preparation and determination techniques, respectively. A new data mining workflow based on the *Unknown Analysis* software for the spectral deconvolution of compounds present in GC-MS chromatograms, in combination with a preliminary comparison against the NIST17 nominal spectral library, allowed the tentative identification of deconvoluted compounds. Thereby, the identities of more than 75 compounds were confirmed against authentic standards. Some species, such as indigo, phthalic anhydride, 2,4-toluene di-isocyanate, phthalimide, certain UV absorbers and octyl isothiazolinone were reported, with a confidence level 1, in this type of matrix for the first time. The accurate EI-MS experimental spectra of the identified compounds have been included in an in-house PCDL, implemented with linear retention index of the compounds. Finally, a semi-quantitative determination of the identified compounds demonstrates their ubiquity in dust samples from different indoor environments (27 different samples) at concentrations between 0.09 to 17.70 µg g^{-1}. These data might serve to perform a preliminary risk assessment study, based on known compounds toxicities and the estimated daily dust intake, which permits identifying those compounds for which accurate concentration determination is required.

The general conclusions that can be extracted for this first part of the thesis are explained below:

- The suitability of HRMS for screening analysis, since this type of instruments provides spectral information of any compound recovered in the sample preparation process which is amenable to the chromatographic separation and ionization steps, whereas tandem mass spectrometers, mainly based on QqQ spectrometers, are totally blind to any other compound not included in the MS/MS transition list. In practise, the feasibility of the screening and compound identification depends on the combination of HRMS and an optimized data analysis workflow, which includes an accurate database of product ion scan or EI-MS spectra.
- The necessity to propose new strategies for the screening analysis of pollutants with high reliability and less time-consuming.

Regarding to LC-ESI-QTOF-MS instruments, the number of MS/MS functions which can be simultaneous acquired is mainly limited by the number of scans accumulated per spectrum, due to their high impact on the instrumental limits of quantification of this technique. Improving the acquisition rate, without worsening the sensitivity, is particularly relevant when working with LC columns rendering peak widths below 6-7 s (sub 2-µm columns). Likely, DIA based on the use of isolation windows of precursor compounds would be a comprise solution between current DIA and DDA.

- The necessity to propose a harmonized protocol with minimum quality requirements for the non-target screening analysis of environmental samples, which includes the number of procedural blanks and duplicates needed for each batch of samples, as well as the number of fragments required to reach an unambiguous identification and the minimum accepted score, the analysis of duplicates and the required level above a blank signal needed for confirming occurrence.

2. Determination of cardiovascular pharmaceuticals in sludge and water samples

New analytical methodologies were developed for the determination of cardiovascular pharmaceutical in sludge and water samples. To that aim, an UPLC-QqQ-MS/MS instrument was used for the selective determination of these species in the obtained sample extract.

In case of sludge samples, the performance of MSPD was evaluated once again, in this occasion for the particular case of the extraction of cardiovascular pharmaceuticals, belonging to different chemical families. Despite the fact that the different chemical properties of the compounds prevented the development of a selective extraction strategy, mild conditions existing during MSPD extraction turned in moderately complex sample extracts. Finally, the optimized methodology demonstrated that this sample preparation technique is suitable for the extraction of compounds with different functionalities from sludge, and in combination with UPLC-QqQ-MS/MS, it constitutes a rapid and sensitive

methodology, achieving quantitative recoveries and negligible matrix effects. The application of this methodology to real sludge samples reveals that five out of nine studied cardiovascular drugs were present in every studied sample at concentrations above 100 ng g^{-1}.

Regarding water samples, a dedicated SPE procedure was optimized. The results obtained in this work demonstrate the suitability of mixed-mode cartridges (reversed-phase and anionic exchanger) for the selective extraction of a relevant family of cardiovascular pharmaceuticals, known as sartan drugs, from water samples.

The developed strategy was based on a SPE protocol using a mixed-mode cartridge (WAX 150 mg). The first step consisted on a pre-wash with a mixture of MeOH:ultrapure water (1:1) after the sample loading, in order to remove the interferences retained in the cartridge. The elution of the analytes was carried out with a mixture of MeOH:NH_3 (2 %) and the obtained extracts were analysed by UPLC-QqQ-MS. The proposed methodology was evaluated for the first time for the selective extraction of the whole group of currently authorized sartan drugs. Once applied to real samples, it revealed the presence of all investigated compounds in wastewater in concentrations above 50 ng L^{-1}. Moreover, the proposed methodology permitted the study of the stability of these compounds along the aquatic environment, where the biodegradation species, VALA (mainly formed at STPs), was systematically found in all studied surface water samples, even in tap water at concentrations above 20 ng L^{-1}.

Throughout both techniques, compounds were accurately quantified using solvent-based calibration standards, with a low consumption of organic solvents and without any type of solvent exchange step. Results obtained from both studies render to the following conclusions:

- The treatments applied in the STPs and DWTPs are inefficient and lead to an incomplete elimination of this family of compounds, favouring its spread and occurrence in different compartments of the aquatic environment. In addition, several transformation products, including degradation and isomerization, are generated during these treatments and finally released into the environment. Therefore, the disposal elimination rates at the STPs are actually lower than those calculated in previous publications, due to the high percentage of pollutants that are adsorbed into the sludge as well as the possibility of above transformations. Biodegradation of certain sartans was confirmed to lead to a stable, highly polar species, VALA, quantified in the processed samples of tap water. To date, no information is available regarding the toxicity of this compound.
- The necessity to implement new oxidative treatments at STPs in order to remove non-biodegradable polar compounds from effluent water samples, which eventually can reach the tap water sources.

3. Photolysis and chlorination reactions of selected emerging pollutants

Once the analytical methodologies for the determination of pollutants were optimized and the levels of the compounds measured, the stability of these families under different oxidizing treatments was investigated.

In case of antimycotic pharmaceuticals, fluconazole (FCZ) and climbazole (CBZ), which are known for their limited biodegradability at STPs and for which their behaviour during oxidative treatments remains unexplored, were submitted to different advance oxidative treatments in order to assess their stability under direct-photolysis, using UV light from low-pressure mercury lamps, and advanced oxidation in presence of H_2O_2. LC-QTOF-MS was applied to follow the time-course of both compounds and to identify the potential formation of TPs under the investigated experimental conditions.

Obtained results showed an effective removal of FCZ and CBZ under both treatments. The comparison between UV irradiation and the combination of UV irradiation and H_2O_2, reveals that despite both treatments lead to the degradation of parent pollutants, UV treatment generates different transformation products more toxic than the precursor. Those TPs were reported for the first time. Alternatively, the combination of UV irradiation and H_2O_2 leads to a faster elimination, avoiding the generation of noticeable TPs, when using LC-MS as determination technique.

The effect of nitrate anions in the photo-transformation of five phenolic pollutants was also studied. Experiments in different water samples (ultrapure water, surface water and wastewater) at natural concentrations of nitrates and exposed to UV irradiation and natural sunlight were carried out.

Results showed that nitrate anions, at environmental occurring levels, accelerate the photodegradation of the phenolic pollutants; however, under employed experimental conditions formation of concerning nitro and non-nitro derivatives was noticed. In the particular case of orthophenylphenol (OPP), formation of nitro and di-nitro species, in addition to a toxic dibenzofuranol, were recognized as relevant transformation routes. Given the systematic use of OPP as post-harvest fungicide in the food processing industry, such findings might have a particular relevance in aquatic environments affected by the wastewater discharges of food production and packaging areas.

The last oxidative treatment studied along this thesis was the chlorination. Free chlorine is the most popular oxidant during the production of drinking water. However, it results inefficient for the degradation of some pollutants, which is the case of some cardiovascular pharmaceuticals. Degradation experiments were developed in ultrapure and river water samples at different pHs (from 5 to 9 units) and using different concentrations of bromide (from 0.067 to 0.3 mg L^{-1}).

Obtained results revealed the stability of telmisartan (TELM) and valsartan (VAL) under such conditions, while irbesartan (IRB) and losartan (LOS) were removed following a pseudo-first order kinetics. Several TPs were found for each compound,

which in case of IRB TPs were predicted to be less toxic than the parent compound. In contrast, some LOS derivatives (di-halogenated species) are expected to be more toxic.

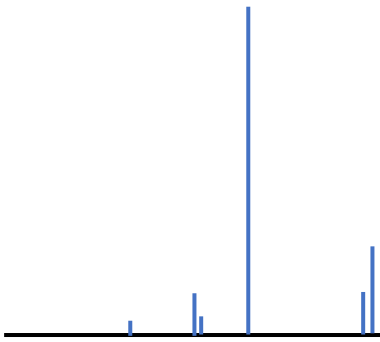
The main conclusions that can be extracted from this part are the following:

- The necessity of implement additional treatments to chlorination in water treatment plants, in order to remove residues of sartan pharmaceuticals from the aquatic environment, which guarantee the quality of drinking water.
- The suitability of HRMS for the detection and identification of potential TPs generated during the water treatments.
- Further toxicological studies should be carried out with the aim of knowing the hazard effect of the new TPs present in the environment, as well as, their stability
- Deeper research is required to assess the extent of nitration and chlorination reactions during UV treatment of wastewater (urban and/or industrial) at STPs, and in surface water reservoirs polluted with the investigated contaminants.

4. Direct analysis in real time accurate mass spectrometry

The last chapter of this thesis consisted on the study of the suitability of an ambient ionization source, the direct analysis on real time (DART) combined with accurate mass spectrometry (QTOF-MS), for the rapid and quantitative determination of bisphenol-related compounds in thermal paper.

To this aim a basic extraction protocol, followed by an acetylation step was proposed and a desorption-ionization process at the DART source, optimized. Finally, under optimal conditions, the reported method provided recoveries in the range from 90 to 110 % and limits of quantification of 0.004 % (w:w), demonstrating that DART-TOF-MS constitutes a fast alternative for the quantitative determination of bisphenol-type compounds in thermal paper. Efficiency of DART ionization was improved after compounds acetylation, without a significant reduction in the productivity of the analytical procedure. Further studies are required to evaluate whether the observed behaviour in this study can be extended to other phenolic species, and also to verify the impact of novel EU regulations in the content of bisphenol A in thermal printing paper.



ANNEX I:
Resumen en español

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ANNEX I: Resumen en español

En los últimos años, el elevado consumo de fármacos, productos de cuidado personal, limpieza del hogar y procesos industriales, ha suscitado una elevada preocupación social y científica. Este uso indiscriminado de los productos, combinado con una gestión ineficiente de los residuos en las estaciones depuradores de aguas residuales (EDARs), tiene como resultado una liberación continua de elevadas cantidades de contaminantes emergentes al medioambiente.

Las aguas residuales y los lodos de depuradora constituyen la vía principal de entrada de los contaminantes en el medioambiente, por lo que son un residuo que es necesario gestionar de la manera más eficiente posible. Las estrategias convencionales de tratamiento de aguas residuales, basadas principalmente en tratamientos físicos, biológicos y, ocasionalmente químicos; son ineficaces para la eliminación de gran parte de estos contaminantes emergentes. A su vez, la incineración de los lodos es una práctica poco sostenible desde el punto de vista energético y, sobre todo, medioambiental, constituyendo un riesgo importante, derivado de la generación de nuevos compuestos orgánicos persistentes. Como alternativa, se han desarrollado, e implementado, nuevos tratamientos terciarios con la finalidad de eliminar eficazmente los contaminantes emergentes y garantizar la calidad de las aguas, reduciendo así la descarga de estos compuestos en el medioambiente. En el caso de los lodos, éstos son sometidos a una etapa de estabilización previa a su reutilización como fertilizantes agrícolas. Durante esta estabilización, se utilizan óxidos de calcio, que confieren un pH básico al lodo estabilizado o biosólido, favorable en áreas agrícolas con suelos ácidos, como los que se encuentran en la Comunidad de Galicia. Sin embargo, aunque esta estabilización asegura la calidad microbiológica de los lodos, no garantiza la eliminación de los contaminantes emergentes acumulados.

Los nuevos tratamientos terciarios aplicados en las EDARs son procesos oxidativos que pueden mejorar la eficacia de los procedimientos convencionales, pero que a su vez pueden presentar ciertos riesgos, debido principalmente a la posibilidad de producir intermedios más polares que los compuestos de partida, los cuales podrían alcanzar fácilmente las aguas superficiales e incluso las aguas de consumo. Por otra parte, en el caso concreto de los contaminantes emergentes introducidos en los campos de cultivo mediante la aplicación de biosólidos como fertilizantes, pueden ser susceptibles de bio-concentración y/o degradación a través de procesos fotoquímicos o microbiológicos. Por todo esto, el estudio de los niveles de contaminantes emergentes en aguas residuales y en lodos de uso agrícola, así como la identificación de sus productos de transformación y eliminación en las EDARs son aspectos que es necesario evaluar mediante la obtención de información analítica cualitativa y cuantitativa.

A pesar de varias décadas de investigación sobre el papel de los contaminantes emergentes en el medioambiente, todavía se requiere de información adicional en relación con su distribución, toxicidad e impacto, tanto de estos compuestos como de sus derivados. Hasta el momento, la mayoría de los estudios publicados se han centrado únicamente en muestras de agua, mientras que la atención prestada a los lodos generados

en las EDARs o a otro tipo de compartimentos, a los que el ser humano está directamente expuesto, como las áreas interiores, es todavía escasa. Este mismo comentario es válido en relación con las rutas de transformación que sufren los contaminantes emergentes durante los procesos químicos aplicados en el tratamiento de aguas residuales y/o la producción de agua del grifo.

Durante estas décadas, los procedimientos de determinación de contaminantes emergentes desarrollados para el análisis de muestras de aguas y lodos se han basado en metodologías analíticas clásicas, que combinan una separación cromatográfica acoplada a la espectrometría de masas en tándem. En este sentido, el uso de espectrómetros de masas de triple cuadrupolo (QqQ), funcionando en modo MRM, proporcionan una elevada selectividad y unos límites de cuantificación extremadamente bajos. Sin embargo, esta estrategia dirigida no es adecuada para la detección de nuevas especies ni para el estudio de sus procesos de transformación, tanto en experimentos a escala de laboratorio, como en condiciones reales. Como alternativa a los métodos clásicos, se ha desarrollado la espectrometría de masas de alta resolución (HRMS), o hablando con más propiedad, la espectrometría de masas exactas, combinada con técnicas de separación cromatográficas, como la cromatografía líquida (LC) y, más recientemente, la cromatografía de gases (GC). Estos sistemas HRMS se basan en el uso de analizadores de masa de tiempo de vuelo (TOF) y Orbitrap, los cuales ofrecen la posibilidad de obtener registros de masas exactas (modo SCAN) a lo largo de todo el proceso de separación cromatográfica. Así pues, la combinación de HRMS con fuentes de ionización suaves, como la ionización por electrospray (ESI), permite la obtención de iones pseudomoleculares ($M+H^+$ o $M-H^-$) de cualquier especie presente en la muestra, que sobreviva a los pasos de preparación de muestra y ionización. Esta información combinada con una buena estrategia de análisis de datos ofrece la posibilidad de detectar nuevas especies en matrices ambientales complejas como las aguas residuales, los lodos y los biosólidos. Obviamente, las estrategias de procesado de datos empleadas en LC-ESI-QTOF-MS son completamente diferentes a las seguidas cuando los compuestos se someten a técnicas de ionización en las que, de forma sistemática, no se genera el ion pseudomolecular como pico base en los espectros de MS, como sucede en el caso de GC-EI-TOF.

Como consecuencia de toda esta problemática, en esta tesis se planteó estudiar en profundidad el abanico de posibilidades que brinda la espectrometría de masas exactas, centrada principalmente en el uso de instrumentos TOF, para la identificación y determinación de nuevos contaminantes en matrices de alta complejidad, concretamente en aguas residuales y lodos de EDARs, así como en muestras de ambientes interiores como el polvo. Con esta finalidad, se han propuesto las estrategias y metodologías analíticas que se describen a continuación:

1. Screening non-target de contaminantes emergentes en muestras ambientales

En primer lugar, se llevó a cabo una búsqueda no dirigida de contaminantes emergentes en matrices sólidas, como lodo y polvo, empleando técnicas de preparación de muestra genéricas y no selectivas, con el objetivo de extraer el máximo número de compuestos presentes en las muestras. Las técnicas consideradas fueron la dispersión de

matriz en fase sólida (MSPD), en el caso de las muestras de lodo, y la extracción con líquidos presurizados (PLE) en caso de las muestras de polvo. Los extractos obtenidos se analizaron utilizando espectrómetros de masas de tipo QTOF, empleando como técnicas de separación la cromatografía de líquidos (LC) o de gases (GC).

Para el *screening* de muestras de lodo se propone una técnica de preparación de muestra basada en el fraccionamiento de los analitos según su carácter ácido o básico. Los extractos obtenidos se inyectan en un sistema LC-QTOF-MS, siguiendo dos estrategias diferentes de análisis de datos, las cuales se describen a continuación.

a) Estrategia 1.

Las especies cloradas y bromadas de origen natural son escasas en el medioambiente. Sin embargo, el cloro es un elemento relativamente común en productos farmacéuticos, fitosanitarios y ciertos retardantes de llama, compuestos en los que también se puede utilizar bromo. Los espectros de los compuestos clorados y/o bromados presentan perfiles isotópicos característicos, lo que los hace fácilmente identificables en base a las intensidades relativas de las especies M y M+2, y también al defecto de masa típico entre los isótopos ^{35}Cl y ^{37}Cl (- 3.0 mDa), y ^{79}Br y ^{81}Br (- 2 mDa). Todo ello, sumado a la disponibilidad de herramientas automáticas de búsqueda, como *Find by molecular features*, incluida en el software de análisis de datos *MassHunter Qualitative Analysis B.07.00* (Agilent Technologies), ha permitido la identificación y la extracción de los picos correspondientes a este tipo de especies, a lo largo de todo el cromatograma obtenido mediante LC-QTOF-MS, generando las fórmulas empíricas correspondientes con elevada fiabilidad. Una vez obtenidas las fórmulas empíricas, la identificación se realiza con bases de datos de HRMS, como *Metlin* o *Massbank*, en las que se ha llevado a cabo una búsqueda preliminar de los candidatos cuyos espectros de masas sean compatibles con las especies halogenadas detectadas en los extractos de lodo. Finalmente, la confirmación se lleva a cabo mediante la comparación entre los espectros experimentales de MS/MS y los recopilados en la base de datos.

Esta estrategia de análisis, basada en la aplicación de un filtro de sustancias halogenadas, permitió la identificación de más de 30 contaminantes clorados, pertenecientes a diferentes familias, como productos farmacéuticos, productos de cuidado personal, pesticidas y retardantes de llama, entre otros, en los extractos de lodos. Esta estrategia de screening se resume en la **Publicación III.I.1**, donde se explica en profundidad la técnica de preparación de muestra propuesta y los resultados obtenidos.

b) Estrategia 2.

En esta segunda estrategia propuesta, se presentan dos flujos de trabajo diferentes, en los que se realiza una búsqueda general, sin utilizar el filtro de compuestos halogenados combinando en el mismo cromatograma datos de MS y MS/MS para la detección de contaminantes emergentes en lodos y biosólidos de EDARs.

En primer lugar, se presenta una metodología DDA (*Data dependent analysis*), en la que el método de adquisición seleccionado fue *auto MS/MS*, utilizando tres energías de colisión diferentes (0, 20 y 40 eV). La extracción e identificación de los compuestos se llevó a cabo utilizando la función *Find by auto MS/MS*, presente también en el software

MassHunter Qualitative Analysis Workflows B.08.00 de Agilent Technologies. Por otra parte, se presenta un segundo flujo de trabajo, basado en el análisis DIA (*Data independent analysis*). De nuevo se llevan a cabo tres experimentos de MS/MS, a tres energías de colisión diferentes (0, 20 y 40 eV), adquiridos en una única inyección. El análisis de datos, en este caso, consistió en la utilización de la herramienta *Find by formula* (con confirmación de fragmentos) disponible también en el software *MassHunter Qualitative*. Independientemente de la estrategia de adquisición utilizada, la identificación final de candidatos se realiza mediante la comparación de los espectros de MS/MS experimentales con los contenidos en una base de datos. La base de datos empleada fue la *ForTox PCDL* de Agilent Technologies, la cual contiene espectros para sustancias activas presentes en productos farmacéuticos, productos de cuidado personal y limpieza del hogar, aditivos utilizados en pinturas y textiles, así como otros compuestos con elevado volumen de producción, registrados tanto en modo positivo como negativo en la fuente de ionización (ESI).

Aunque esta segunda estrategia no está publicada todavía, permitió la identificación tentativa (nivel 2 en la escala Schymanski) de más de 70 compuestos, de los cuales 42 fueron confirmados utilizando patrones comerciales (nivel 1). Una de las familias de compuestos identificadas durante el análisis fue la de los fármacos cardiovasculares, estudiada en profundidad a lo largo de esta tesis.

La última estrategia de búsqueda no dirigida propuesta, consiste en una aproximación totalmente diferente, centrada en el *screening* de compuestos semi-volátiles en muestras de polvo. Esta metodología se basa en la utilización de PLE en combinación con GC-EI-TOF-MS, como técnicas de preparación de muestra y de análisis, respectivamente. El procesado de la información obtenida se ha realizado empleando el algoritmo de deconvolución presente en el *software* de Agilent Technologies, *Unknown Analysis*. Este algoritmo realiza una deconvolución espectral de los compuestos presentes en los cromatogramas obtenidos mediante GC-EI-TOF-MS. Para su identificación preliminar se compararon los espectros obtenidos mediante EI-MS contra los espectros de baja resolución (nominal) recopilados en la librería NIST17. Además, también se tuvieron en cuenta los valores de *m/z* calculados para los iones fragmentos con estructura conocida en la NIST17. Estas identificaciones preliminares se confirmaron mediante la inyección de los patrones de los candidatos, con los que se crea una base de datos personalizada.

Esta estrategia se presenta en la **Publicación III.I.3**. Los resultados obtenidos permitieron la identificación de más de 75 compuestos pertenecientes a diferentes familias químicas, como colorantes y sus productos de degradación, isocianatos, ftalimida, filtros UV y el bactericida, octilisotiazolinona, identificado por primera vez en este tipo de muestra.

2. Desarrollo de métodos cuantitativos para la determinación de contaminantes emergentes en muestras de lodo y aguas.

Una vez identificados los contaminantes presentes en las muestras ambientales, se optimizaron las metodologías de análisis para algunas de las familias de compuestos en las diferentes muestras. Los grupos de compuestos seleccionados para su determinación

cuantitativa comprenden especies de elevado interés medioambiental, que no habían sido estudiadas de forma exhaustiva en la bibliografía, como es el caso de los fármacos antimicóticos y cardiovasculares.

En el caso de los fármacos antimicóticos, se estudia su presencia en muestras de lodo y biosólidos. Para ello se proponen diferentes protocolos de preparación de muestra, todos ellos basados en la técnica de extracción MSPD, y optimizados en función de las características fisicoquímicas de los analitos a estudiar. En la **Publicación III.I.1**, además de presentarse una estrategia de análisis, basada en un filtro de compuestos halogenados, se lleva a cabo la optimización de una nueva metodología analítica para la cuantificación de fármacos antimicóticos. En esa misma línea, en la **Publicación III.I.2** se propone una estrategia analítica para la extracción, determinación y cuantificación de hexaaryl cloro imidazoles en muestras de lodo, utilizando como técnica de análisis LC-QTOF-MS.

Para el estudio de los fármacos cardiovasculares, cuyas características fisicoquímicas provocan que puedan distribuirse en diferentes compartimentos ambientales, como el medio acuático (agua residual, agua superficial e incluso agua de consumo) y los residuos sólidos generados en las EDARs, se han desarrollado diferentes aproximaciones analíticas para su cuantificación en ambas matrices. La metodología desarrollada para la cuantificación de fármacos cardiovasculares, de naturaleza lipofílica y pertenecientes a diferentes grupos químicos, en muestras de lodos, se presenta en la **Publicación III.II.1**. En el caso de los fármacos cardiovasculares más polares, como los sartanes, los cuales son responsables de la inhibición de los receptores de la angiotensina II, también se ha investigado su presencia en los diferentes compartimentos del medio acuático, desde agua residual, agua superficial y, por último, agua de grifo. Para ello, se optimizó un protocolo de extracción en fase sólida (SPE) utilizando un adsorbente de modo mixto (tipo WAX) para la concentración de estos compuestos de naturaleza ácida. El procedimiento analítico propuesto emplea, en ambos casos, como técnica de separación y determinación, la cromatografía de líquidos de ultra eficacia (UPLC) combinada con la espectrometría de masas exacta utilizando un analizador de masas de triple cuadrupolo (QqQ) y fuente de ionización por electrospray (ESI). Los resultados obtenidos revelan que las EDARs convencionales no resultan eficaces para la eliminación de estos compuestos en aguas residuales, a través de las cuales algunos de estos fármacos alcanzan las aguas superficiales e incluso las aguas de consumo. Todos estos datos están recopilados y explicados en profundidad en la **Publicación III.II.2**.

3. Estudios de transformación de contaminantes emergentes.

Una vez optimizadas las metodologías analíticas para la determinación de contaminantes emergentes y conocidos sus niveles en muestras ambientales, se estudió la estabilidad de algunos de estos compuestos bajo diferentes tratamientos oxidativos.

En primer lugar, se evaluó la eficacia de la fotólisis directa (UV), y la combinación de la fotólisis con un agente oxidante como el H_2O_2 (UV/ H_2O_2), para la eliminación en el medio acuático de los fármacos antimicóticos, climbazol (CBZ) y fluconazol (FCZ), los cuales presentan una escasa biodegradabilidad en las EDARs, y cuyo comportamiento durante los tratamientos oxidativos era desconocido en el momento de abordar estos

estudios. El seguimiento de la cinética de degradación de ambos compuestos y la evaluación de la formación de posibles productos de transformación (TPs), así como su posterior identificación, se llevó a cabo mediante LC-QTOF-MS. Los resultados obtenidos de esta comparativa están explicados en la **Publicación III.III.1**. Entre otros aspectos, se demuestra que ambos tratamientos conducen a una eliminación completa de los azoles. Sin embargo, en el caso de la fotólisis directa se generan TPs más tóxicos que los compuestos originales, mientras que, la combinación de radiación UV con H_2O_2, evita la formación de los mismos.

En la **Publicación III.III.2**, se presenta el estudio de otra de las reacciones investigadas durante esta tesis, los procesos de nitración. Este tipo de reacciones pueden ocurrir de forma natural en las aguas, generando derivados nitrados. Por esta razón, se ha evaluado el efecto de los iones nitrato en la estabilidad de cinco compuestos fenólicos bajo luz solar y bajo irradiación con luz UV. De nuevo, se utilizó el sistema LC-QTOF-MS para seguir la cinética de eliminación de los contaminantes, así como para investigar sus rutas de fotodegradación y la formación de posibles TPs. Los resultados obtenidos en este estudio confirman que las rutas de transformación seguidas por los cinco compuestos estudiados conducen a la formación de derivados nitrados más tóxicos que el compuesto original, siendo el ortofenilfenol (OPP), el compuesto que muestra mayor potencial para generar derivados mono- y di-nitro sustituidos que resultan fotoquímicamente estables.

El último tratamiento oxidativo estudiado a lo largo de esta tesis fue la cloración. El cloro libre es el oxidante más popular para el tratamiento de agua potable. Este tratamiento de desinfección ha suscitado elevado interés durante las últimas décadas, no solo por sus múltiples ventajas, sino también porque su aplicación puede conducir a la formación de subproductos de desinfección peligrosos, como compuestos orgánicos halogenados, que presentan una elevada actividad cancerígena.

En la **Publicación III.III.3**, se estudian las reacciones de cloración de varios miembros de la familia de fármacos cardiovasculares, sartanes, previamente cuantificados a concentraciones relativamente elevadas en el medio acuático (**Publicación III.II.2**). Los experimentos de degradación con cloro libre se llevaron a cabo en diferentes matrices acuosas (agua ultrapura y de río) a diferentes pH (de 5 a 9 unidades), en presencia de diferentes concentraciones de bromuro (de 0.067 a 0.3 mg L^{-1}). Los resultados obtenidos se presentan en la **Publicación III.III.3**.

4. Estudio de una nueva fuente de ionización para el análisis directo de compuestos bisfenólicos.

Durante la última parte de esta tesis se estudió de la posibilidad de utilizar una fuente de ionización directa, DART (análisis directo y en tiempo real) combinada con la espectrometría de masas exacta (QTOF-MS), para la determinación rápida y cuantitativa de compuestos fenólicos en muestras de papel térmico.

Con este objetivo, se propuso un protocolo de extracción por ultrasonidos, seguido de una etapa de acetilación y un proceso de desorción-ionización en la fuente DART. La metodología propuesta proporcionó recuperaciones entre 90 y 110 % y límites de cuantificación de 0.004 % (w:w), demostrando por primera vez la capacidad de la

metodología DART-QTOF-MS para la determinación cuantitativa de compuestos fenólicos, de la familia del bisfenol A, en este tipo de muestras. Una vez optimizada, esta metodología se aplicó en el análisis de muestras de papel térmico de diferentes procedencias. Los resultados obtenidos en el estudio, recogidos en la **Publicación III.IV.1**, revelan que el BPA estaba presente en todas las muestras estudiadas, en concentraciones superiores al máximo establecido por la regulación de la UE.

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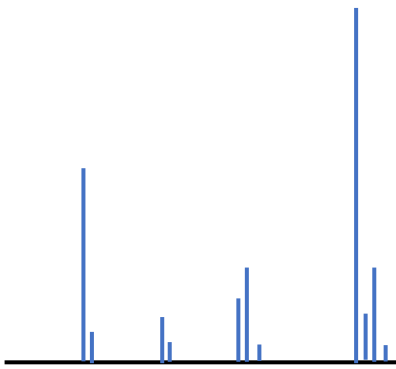

ANNEX II: List of publications

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ANNEX II: List of publications

Chapter I. Screening and determination of emerging pollutants in environmental samples

- Publication III.I.1.

G. Castro, M. Roca, I. Rodríguez, M. Ramil, R. Cela, Identification and determination of chlorinated azoles in sludge using liquid chromatography quadrupole time-of-flight and triple quadrupole mass spectrometry platforms, Journal of Chromatography A, 1476 (2016) 69-76.
DOI: : 10.1016/j.chroma.2016.11.020

- Publication III.I.2.

J. Casado, G. Castro, I. Rodríguez, M. Ramil, R. Montes, R. Cela, Liquid chromatography quadrupole time-of-flight mass spectrometry identification and determination of tri- and hexaaryl chloro imidazoles in sewage sludge, Journal of Mass Spectrometry, 52 (2017) 69-77.
DOI: 10.1002/jms.3903

- Publication III.I.3.

G. Castro, I. Rodríguez, M. Ramil, R. Cela, Assessment of gas chromatography time-of-flight mass spectrometry for the screening of semi-volatile compounds in indoor dust, Science of the Total Environment 688 (2019) 162-173.
DOI: 10.1016/j.scitotenv.2019.06.192

Chapter II. Determination of cardiovascular pharmaceuticals in water and sludge

- Publication III.II.1.

G. Castro, I. Carpinteiro, I. Rodríguez, R. Cela, Determination of cardiovascular drugs in sewage sludge by matrix solid-phase dispersion and ultra-performance liquid chromatography tandem mass spectrometry, Analytical Bioanalytical Chemistry 410 (2018) 6807-6817.
DOI: 10.1007/s00216-018-1268-3

- Publication III.II.2.

G. Castro, I. Rodríguez, M. Ramil, R. Cela, Selective determination of sartan drugs in environmental water samples by mixed-mode solid-phase extraction and liquid chromatography tandem mass spectrometry, Chemosphere 224 (2019) 562-571.
DOI: 10.1016/j.chemosphere.2019.02.137

Chapter III. Photolysis and chlorination reactions of selected emerging pollutants

- Publication III.III.1.

G. Castro, J. Casado, I. Rodríguez, M. Ramil, A. Ferradás, R. Cela, Time-of-flight mass spectrometry assessment of fluconazole and climbazole UV and UV/H_2O_2 degradability: Kinetics study and transformation products elucidation, Water Research 88 (2016) 681-690.
DOI: 10.1016/j.watres.2015.10.053

- Publication III.III.2.

G. Castro, I. Rodríguez, M. Ramil, R. Cela, Evaluation of nitrate effects in the aqueous photodegradability of selected phenolic pollutants, Chemosphere 185 (2017) 127-136.
DOI: 10.1016/j.chemosphere.2017.07.005

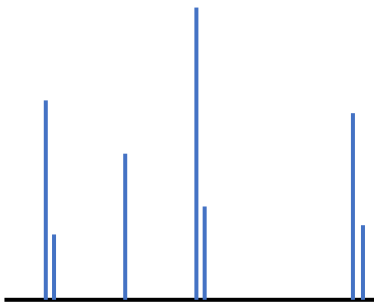
- Publication III.III.3.

I. Carpinteiro, G. Castro, I. Rodriguez, R. Cela, Free chlorine reactions of angiotensin II receptor antagonists: Kinetics study, transformation products elucidation and in-silico ecotoxicity assessment, Science of the Total Environment 647 (2019) 1000-1010.
DOI: 10.1016/j.scitotenv.2018.08.082

Chapter IV. Other publications

- Publication III.IV.1.

G. Castro, I. Rodríguez, M. Ramil, R. Cela, Direct analysis in real time accurate mass spectrometry determination of bisphenol A in thermal printing paper, Talanta 205 (2019) 120086.
DOI: 10.1016/j.talanta.2019.06.086



ANNEX III: Index of figures

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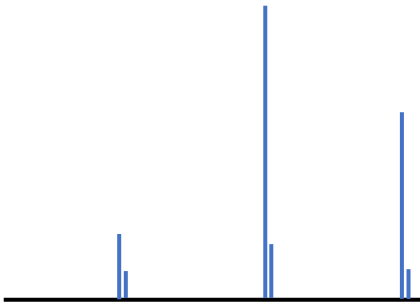

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