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Miniaturized extraction techniques coupled to chromatography and tandem mass spectrometry and high resolution mass spectrometry for the determination of organic compounds of agricultural and environmental interest

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MINIATURIZED EXTRACTION TECHNIQUES COUPLED TO CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY AND HIGH RESOLUTION MASS SPECTROMETRY FOR THE DETERMINATION OF ORGANIC COMPOUNDS OF AGRICULTURAL AND ENVIRONMENTAL INTEREST

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ABBREVIATIONS

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_____#

µMSPD: micro-matrix solid-phase dispersion

2EHMC: 2-ethylhexylmethoxycinnamate

4MBC: 4-methoxybenylidene camphor

_____A

A: absorbance

AA: antioxidant activity

ACN: acetonitrile

ANOVA: analysis of variance

AOPs: advanced oxidation processes

APCI: atmospheric pressure chemical ionization

Azi: azithromycin

_____B

bbCID: broadbant collision-induced dissociation

BBP: benzylbutylphthalate

BHA: butylhydroxyanisole

BHT: butylhydroxytoluene

BMDM: butyl methoxy dibenzoyl methane

BP3: benzophenone-3

BS: benzyl salicylate

BuP: butylparaben

BzP: benzylparaben

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C

CAS: Chemical Abstracts Service

CE: collision energy

CECs: contaminants of emerging concern

Ceph: cephalexin

Cipro: ciprofloxacin

Clar: clarithromycin

Clar-D3: clarithromycin-D3

D

DBP: dibutyl phthalate

DCHP: dicyclohexyl phthalate

DEA: diethyl adipate

DEHA: di-2-(ethylhexyl) adipate

DEHP: di-2(ethylhexyl) phthalate

DEP: diethyl phthalate

DHHB: diethylaminohydroxy benzoyl hexyl benzoate

DIA: data independent acquisition

DIBP: diisobutyl phthalate

DIHP: diisoheptyl phthalate

DIPP: diisophentyl phthalate

DMA: dimethyl adipate

DMEP: dimethoxyethyl phthalate

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DMP: dimethylphthalate

DMPE: 1-(2,3-dimethylphenyl)ethenone

DNOP: di-n-octylphthalate

DOE: design of experiments

DPhP: diphenylphthalate

DPP: dipentylphthalate

DPPH: 1,1-diphenyl-2-picrylhydrazyl

DRT: drometrizole trisiloxane

E

EFSA: European Food Safety Authority

EHPABA: ethylhexyl dimethyl p-aminobenzoic acid

EHS: ethylhexyl salicylate

EI: electron impact

Enro: enrofloxacin

Ery: erythromycin

ESI: electrospray ionization

EtAc: ethyl acetate

ETO: etocrylene

EtP: ethylparaben

F

FPSE: fabric phase sorptive extraction

FRAC: Fungicide Resistance Action Committee

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FRAP: ferric reducing antioxidant power

FS: full scan

G

GC: gas chromatography

H

HESI: heated electrospray ionization

HMS: homosalate

HMF: 5-hydroxymethylfurfural

HPLC: high-performance liquid chromatography

HRMS: high-resolution mass spectrometry

I

IAMC: isoamyl methoxycinnamate

iBuP: isobutylparaben

IPBC: iodopropynylbutyl carbamate

iPrP: isopropylparaben

IUPAC: International Union of Pure and Applied Chemistry

L

LC: liquid chromatography

LLE: liquid-liquid extraction

LOD: limit of detection

LOQ: limit of quantification

LSD: least significant difference

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M

MA: menthyl anthranilate

MAE: microwave assisted extraction

MeOH: methanol

MeP: methylparaben

MOA: mode or mechanism of action

MRLs: maximum residue limits

MS: mass spectrometry

MS/MS: tandem mass spectrometry

MSPD: matrix solid-phase dispersion

MTMA: megastigmatrienone A

MTMB: megastigmatrienone B

MTMC: megastigmatrienone C

MW: molecular weight

m/z: mass-to-charge ratio

N

Nor: norfloxacin

Nor-D5: norfloxacin-D5

O

Oflo: ofloxacin

ORAC: oxygen radical absorbance capacity

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P

PCA: principal component analysis

PCPs: personal care products

PDO: Protected Designation of Origin

PGI: Protected Geographical Identification

PhEtOH: phenoxyethanol

PrP: propylparaben

PPCPs: pharmaceutical and personal care products

Q

Q: quadrupole

QqQ: triple quadrupole mass spectrometer

QqToF: triple quadrupole time-of-flight mass spectrometry

QToF: quadrupole time-of-flight mass spectrometry

R

ROS: reactive oxidizing species

Rox: roxithromycin

RSD: relative standard deviation

S

SCCS: Scientific Committee on Consumer Safety

SDMD: sulfadimidine

SDZ: sulfadiazine

SIM: selected ion monitoring

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SMX: sulfamethoxazole

SMX-D4: sulfamethoxazole-D4

SMZ: sulfamerazine

SPE: solid-phase extraction

SPME: solid-phase microextraction

SRM: selective reaction monitoring

SWATH™: Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra

T

T: transmittance

TCS: triclosan

TDN: 1,1,4-trimethyl-1,2-dihydronaphthalene

TMP: trimethoprim

TOF: time-of-flight

TPC: total phenolic content

U

UAE: ultrasound assisted extraction

UHPLC: ultra-high performance liquid chromatography

UV: ultraviolet

UWW: urban wastewater

UV-Vis: ultraviolet-visible

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V

VALLME: vortex-assisted liquid-liquid microextraction

VE: vortex extraction

W

WHO: World Health Organization

WWTP: wastewater treatment plant

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ABSTRACT

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The present thesis is focused on the development and optimization of analytical methodologies for the determination of organic compounds of agronomic and environmental interest, as well as pharmaceuticals and personal care products. For this purpose, different miniaturized extraction techniques followed by analysis using different types of chromatography and mass spectrometry, including tandem mass spectrometry and high-resolution mass spectrometry, have been used.

The presence of different volatile, semi-volatile and phenolic compounds in honey samples, responsible for organoleptic and nutritional properties, has been studied. In the environmental and health fields, fungicides have been determined in different environmental compartments and crops, as well as antibiotics in wastewaters and natural waters. The degradation of antibiotics employing different approaches, including photodegradation and advanced oxidation processes (AOPs), have been also assessed. On the other hand, the study of personal care products (PCPs), also considered to be emerging pollutants in the environment, has been carried out in cosmetic products and hydroalcoholic gels.

The report is divided in six sections. The first is Section I JUSTIFICATION AND OBJECTIVES, which highlights the relevance of the selected analytes and samples, as well as the importance of developing new analytical methodologies for their determination. Section II INTRODUCTION describes the studied substances and matrices, followed by an explanation of the different analytical and extraction techniques employed. Section III METHODOLOGY includes the description of the sample preparation and analytical techniques for the different analytes and matrices. Section IV RESULTS AND DISCUSSION contains the experimental work carried out during this thesis and the obtained results. Nine different works, of which seven are already published, one is under review for publication and other is prepared for submission, are summarized in four chapters, and presented in this section:

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Chapter 1. DETERMINATION OF FUNGICIDES IN AGRICULTURAL AND ENVIRONMENTAL SAMPLES

This chapter comprises three publications dealing with the analysis of seventeen fungicides by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). The first two address the development of two analytical methods for the determination of these compounds in water. In the first one, a method based on fabric phase sorptive extraction (FPSE) is presented and in the second one, the solid-phase extraction (SPE) technique is optimized using cork by-products as a green sorbent. In the third article, the development of an ultrasound assisted extraction (UAE) method for the analysis of fungicides in grape leaves is carried out. In addition, the presence of these compounds in grapes and in the related environmental compartments (soil and water) is also assessed. In all cases, the developed methodologies are extensively validated and applied to real samples, and the observed concentration levels are compared with those included in the legislation in force.

Chapter 2. CHARACTERIZATION OF HONEY: VOLATILE, SEMI-VOLATILE AND PHENOLIC COMPOUNDS

This chapter includes two articles. The first one is based on the miniaturization of solid-phase microextraction (SPME) to determine volatile and semi-volatile compounds in honey samples from Galicia and foreign countries by gas chromatography-mass spectrometry (GC-MS). Regarding the second one, a characterization of the content of phenolic compounds in Galician honeys of different botanical origin is carried out, with the aim of identifying possible chemical markers. All honeys fell under the umbrella of the Protected Geographical Identification (PGI) of Galicia, the only Spanish PGI that currently exists for honey. Analytical determinations are conducted by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). In addition, indexes such as total phenolic content (TPC) and antioxidant activity (AA) are evaluated using ultraviolet-visible (UV-Vis) spectrophotometry.

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Chapter 3. DETERMINATION OF PERSONAL CARE PRODUCTS (PCPs) IN COSMETIC FORMULATIONS

This chapter includes two publications. In the first one, a miniaturized method based on micro-matrix solid-phase dispersion (μMSPD) is developed to determine UV filters in samples of different nature. The method is validated and extended to other families of cosmetic ingredients such as fragrance allergens, preservatives, plasticizers and synthetic musks. The second article deals with the development of a SPME method for the simultaneous extraction of personal care products (PCPs), including fragrance allergens, synthetic musks, preservatives and plasticizers, in hydroalcoholic gels, a cosmetic product of great importance in the current context of COVID-19 pandemic, and which demand has exponentially increased worldwide. In the two publications, analyses are carried out by GC-MS/MS.

Chapter 4. DETERMINATION OF ANTIBIOTICS IN WATER AND DEGRADATION STUDIES

This chapter includes two works. The first one is an article (under review) based on the optimization of a SPE method for the extraction of antibiotics in wastewater (urban and hospital) and natural water (river and well water) using ultra-high performance liquid chromatography (UHPLC) coupled to quadrupole time-of-flight high-resolution mass spectrometry (QToF-HRMS). The target analysis includes fourteen compounds belonging to different families of antibiotics such as macrolides, cephalosporins, fluoroquinolones, sulfonamides and diaminopyrimidines. In addition, a non-target analysis is carried out and other drugs, pesticides, personal care products and metabolites can be identified in the samples. On the other hand, a preliminary photodegradation study using UVC radiation is carried out. In the second work the operational conditions of a tube-in-tube membrane reactor are optimized for the removal of the fourteen antibiotics in urban wastewater. This reactor is based on AOPs, characterized by the generation of highly reactive oxidizing species. In both investigations, a detailed search of possible by-products formed after degradation processes are included.

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Section V GENERAL DISCUSSION comprises a joint discussion of all the works included in the present thesis and, finally, Section VI CONCLUSIONS contains the general conclusions drawn from all the studies carried out.

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I. JUSTIFICATION AND OBJECTIVES

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Sample preparation is one of the most important steps to ensure an efficient extraction of analytes of interest, as well as to facilitate the analysis of complex matrices. Nowadays, to achieve sustainable features, the development of miniaturized procedures that are simple, low-cost, fast and of easy implementation in routine laboratories is of great importance.

In this context, the main objective of the present thesis is the development of analytical methodologies to determine a wide variety of substances in different matrices employing chromatography and mass spectrometry (low and high resolution) for the analysis.

Fungicides are a type of pesticides usually used in viticulture to avoid fungi infection. Their continuous application on crops induces their persistent entry into the natural environment since they easily reach different aquatic systems due to their leaching from soils, which poses an environmental and health risk.

Therefore, the determination of fungicides was developed according to the following objectives:

I. Optimization of miniaturized extraction techniques for the determination of fungicides in environmental samples, such as water, in which they are present at very low concentration levels (trace level), and in crops.

A method based on fabric phase sorptive extraction (FPSE) and a second one based on solid-phase extraction (SPE) employing cork by-products as sorbent, are proposed to analyse seventeen fungicides in water (**Section IV, Chapter 1, *1.1* and *1.2)***.

In addition, an ultrasound assisted extraction (UAE) method is developed to analyse these seventeen fungicides in vine leaves and other type of leaves such as tea, in grapes and in the related environmental compartments (**Section IV, Chapter 1, *1.3***).

To achieve high selectivity and sensitivity the analysis is carried out by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS)

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II. Relate the presence of fungicides in real samples with the applicable legislation since their contents are regulated in fruits and vegetables by means of maximum residue limits. Although there is still no specific guidelines for fungicides in water, the European Commission regulates the presence of pesticides in groundwater and surface water intended for human consumption. (**Section IV, Chapter 1, *1.1*, *1.2* and *1.3***).

On the other hand, food samples are complex matrices which need sample preparation prior to their analysis. In Galicia (NW Spain), honey is an important and highly consumed product, and a high proportion of the produced honey falls under the umbrella of a Protected Geographical Identification (PGI). For these reasons, robust and reliable analytical tools deemed important for the identification of possible chemical markers in these honeys.

Hence, the aims of the studies conducted with honey are described below:

III. Development of miniaturized and simple methods for the characterization of honeys with the aim of consuming the less amount of honey and solvents as possible.

The miniaturization of solid-phase microextraction (SPME) is proposed to determine volatile and semi-volatile compounds in Galician honey samples by gas chromatography-mass spectrometry (GC-MS) (**Section IV, Chapter 2, *2.1***).

Regarding the determination of phenolic compounds, simple methods using combining vortex extraction (VE) and ultrasound assisted extraction (UAE) followed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is optimized (**Section IV, Chapter 2, *2.2***).

IV. Evaluation of total phenolic compounds (TPC) and antioxidant activity (AA) indexes as well as application of advanced statistical tools, such as analysis of variance (ANOVA)

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and principal component analysis (PCA), to allow the classification of honeys according to their botanical origin (**Section IV, Chapter 2, *2.2***).

Finally, pharmaceutical and personal care products (PPCPs), one of the most important groups of organic emerging contaminants, were analysed. Furthermore, their presence in cosmetic formulations is also a matter of concern due to their adverse health effects and propensity to be a source of environmental pollution. Thus, the objectives regarding the analysis of PPCPs can be listed as follows:

V. Development of methods to determine personal care products (PCPs) such as fragrance allergens, synthetic musks, preservatives, plasticizers and UV filters in cosmetic formulations by gas chromatography-tandem mass spectrometry (GC-MS/MS) and discussion of their compliance with the current legislation.

Micro-matrix solid-phase dispersion (μMSPD) is proposed to assess the presence of seventy-eight compounds belonging to all the mentioned families in cosmetic samples of different nature (**Section IV, Chapter 3, *3.1***).

In the context of the COVID-19 pandemic, the analysis of hydroalcoholic gels deems necessary. Therefore, a method based on solid-phase microextraction (SPME) is optimized for the determination of sixty PCPs including fragrance allergens, synthetic musks, preservatives and plasticizers (**Section IV, Chapter 3, *3.2***).

VI. Determination and degradation of antibiotics in water and non-target analyses by ultra-high-performance liquid chromatography coupled to quadrupole time-of-flight high resolution mass spectrometry (UHPLC-QToF).

Optimization of a method based on solid-phase extraction (SPE) to determine fourteen antibiotics, including macrolides, cephalosporins, fluoroquinolones, sulfonamides and

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diaminopyrimidines in wastewater and natural waters and implementation of non-target analyses by high resolution mass spectrometry (HRMS) (**Section IV, Chapter 4, *4.1***).

Due to the presence of not degraded antibiotics after wastewater treatment, a preliminary study of photodegradation by UVC radiation is carried out. In addition, the use of a new approach based on tube-in-tube membrane reactor as an advanced oxidation process (AOPs) is proposed for antibiotics removal. The identification of possible by-products after degradation processes is also performed (**Section IV, Chapter 4, *4.1 and 4.2***).

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II. INTRODUCTION

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1. FUNGICIDES

1.1 INTRODUCTION

Pesticide is defined by the European Commission as “something that prevents, destroys, or controls a harmful organism ('pest') or disease, or protects plants or plant products during production, storage and transport”. There is a wide variety of pesticides, and different families can be distinguished according to the organism on which they act, such as herbicides, insecticides and fungicides, among many others [1].

Fungicides prevent or eradicate fungal infections on plants or seeds. They have become indispensable for food safety preventing economic damage caused by fungi in different fields as agriculture, horticulture or viticulture. Despite their widespread use, there is public concern about the damage they can cause due to their toxicity both to the environment and to animal and human health [2].

1.2 CLASSIFICATION

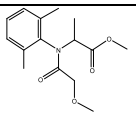
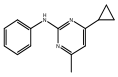
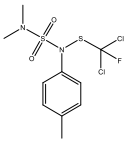
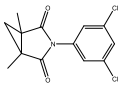
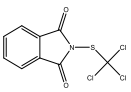
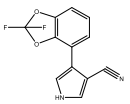
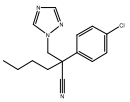
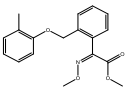
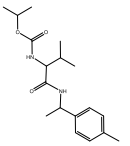
Fungicides can be classified according to their active ingredients, including amides, anilinopyrimidines, cloroalkylthiodicarboximides triazoles, morpholines, strobilurins, halogenated substituted monocyclic aromatics, benzimidazoles, among others [2,3].

In addition, the World Health Organization (WHO) also classifies pesticides by their hazard, and the classification is based on the acute oral LD50 in the rat, although dermal toxicity should also be taken into account in some cases. The LD_{50} is a statistical estimate of the number of mg of toxicant per kg body weight required to kill 50% of a large population of experimental animals. **Table 1** includes the studied fungicides in the present thesis, their CAS number, molecular weight

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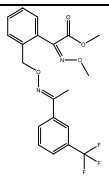
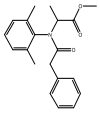
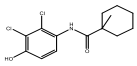
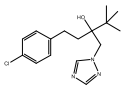
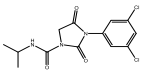
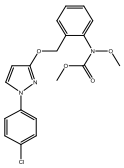
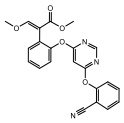
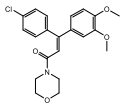
(MW), structure, and their classification according to their chemical group and their hazard [4]. The data was obtained from ChemSpider (Predicted data is generated using the US Environmental Protection Agency's EPISuite™) and chemical structures were made by ChemDraw 20.1.1.

Table 1. Fungicides name, CAS number, molecular weight (MW, g mol^{-1}), chemical class, class hazard according to WHO [4] and chemical structure.

Name	CAS number	MW	Chemical class	Class hazard*	Chemical structure
Metalaxyl	57837-19-1	279.3	Amide	II	
Cyprodinil	121552-61-2	225.3	Anilinopyrimidine	-	
Tolylfluanid	731-27-1	347.3	Amide	U	
Procymidone	32809-16-8	284.1	Dicarboxamide	U	
Folpet	133-07-3	296.6	Chloroalkylthio-dicarboximides	U	
Fludioxonil	131341-86-1	248.2	Phenylpyrrole	U	
Myclobutanil	88671-89-0	288.8	Triazole	II	
Kresoxim methyl	143390-89-0	313.3	Strobilurin	III	
Iprovalicarb	140923-17-7	320.4	Amide, carbamate	U	

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Table 1. (cont.) Fungicides name, CAS number, molecular weight (MW, g mol^{-1}), chemical class, class hazard according to WHO [4] and chemical structure.

Name	CAS number	MW	Chemical class	Class hazard *	Chemical structure
Trifoxystrobin	141517-21-7	408.4	Strobilurin	U	
Benalaxyl	71626-11-4	325.4	Amide	III	
Fenhexamid	126833-17-8	302.2	Amide	U	
Tebuconazole	80443-41-0	307.8	Triazole	II	
Iprodione	36734-19-7	330.2	Dicarboximide	III	
Pyraclostrobin	175013-18-0	387.8	Strobilurin	-	
Azoxystrobin	131860-33-8	403.4	Strobilurin	U	
Dimethomorph	113210-97-2	387.9	Morpholine	III	

*U: Unlikely to present acute hazard in normal use (LD_{50} > 5000 mg kg^{-1}); II: Moderately hazardous (LD_{50} 2000-5000 mg kg^{-1}); III: Slightly hazardous (LD_{50}: 50-2000 mg kg^{-1})

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However, other parameters can be taken into account to classify fungicides. The mobility is one factor which can determine the type of substance. Non-penetrant fungicides are not absorbed by the plant tissues, so they remain on the surface as a protective barrier. By contrast, penetrant fungicides are deposited on the plant surface and absorbed by leaf tissues. Among penetrant fungicides can be differentiated the systemic (they are transported within the plant) or mesostemic ones (they adhere strongly to the leaf surface and a part of them passes from one side of the leaf to the other) [5].

The mode or mechanism of action (MOA) is the way in which a fungicide molecule inhibits the growth or kills a particular fungus. The Fungicide Resistance Action Committee (FRAC) describes the different MOAs by which fungicides are classified (nucleic acids metabolism, melanin synthesis…) [6].

They can also be differentiated according to the sub-stage of the infectious process in which they are applied, classified as preventive (acting as protectants by preventing infection before fungal spores are deposited), curative (inhibiting fungal growth within plant tissues before symptoms are observed) and eradicating (inhibiting disease progression when symptoms are present) [5].

1.3 OCCURRENCE IN VINEYARDS AND THE ENVIRONMENT

The fungicides studied in the present thesis are usually used in viticulture, since the work is based on vineyards from Galicia (NW Spain), because it is a region with a high wine production in a large part of its territory. Hence, the continuous application of fungicides in vineyards is a matter of public concern, due to the resulting presence of pesticide residues in the environment and in wine products used for human consumption [7].

When fungicides are applied, much of them are not deposited on crops, indicating low application efficacy and high exposure to soil organisms.

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Fungicides that reach soils, as well as their possible degradation products, can be persistent, remaining in soils for several years, or even decades, long after their use, causing a high exposure to soil organisms. On the other hand, fungicides can be transported in the environment, entering directly into the food chain, leaching or reaching water resources, making this a global environmental problem [8–10].

Fungicides have been found in waters near vineyard areas, such as rivers, springs or reservoirs, which make these ecosystems vulnerable to fungicide contamination. These substances have been already found in fish samples, although long-term toxicity effects at low concentration exposures, which are those normally found in waters, are largely unknown. However, exposure to high concentrations has led to their accumulation and subsequent mortality [8,11–13].

The European Food Safety Authority (EFSA) published peer review reports of different fungicides evaluating, persistence in soils, toxicological activity in surface and ground waters, among other characteristics [14].

1.4 LEGISLATION

Fungicides, which are regularly applied in vineyards against vine diseases, can pose a health risk. Their intensive use leads to their presence in crops and consumer products, and their residues can reach soils and aquatic environments through leaching.

Due to the potential adverse effects of fungicides, the European Commission established the Directive 2009/128/EC in order to achieve the sustainable use of pesticides [15]. In fact, the European Commission also establishes maximum residue limits (MRLs) in food and feed of plant and animal origin by Regulation EC No 396/2005 and its subsequent amendments. In viticulture, these MRLs concern vine leaves as well as grapes, both table and wine grapes [16]. The MRLs of the target fungicides for these matrices are included in **Table 2**.

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Table 2. Permitted MRLs for vine leaves, table grapes and wine grapes (mg kg^{-1}) [16].

Fungicides	Vine leaves	Table grapes	Wine grapes
Metalaxyl	0.01	2	1
Cyprodinil	0.02	3	3
Tolylfluanid	0.02	0.02	0.02
Procymidone	0.01	0.01	0.01
Folpet	0.03	6	20
Fludioxonil	0.01	5	4
Myclobutanil	0.05	1.5	1.5
Kresoxim methyl	15	1.5	1.5
Iprovalicarb	0.01	2	2
Trifoxystrobin	0.01	3	3
Benalaxyl	0.05	0.3	0.3
Fenhexamid	0.01	15	15
Tebuconazole	0.02	0.5	1
Iprodione	0.01	0.01	0.01
Pyraclostrobin	0.02	0.3	2
Azoxystrobin	0.01	3	3
Dimethomorph	0.01	3	3

As far as the environment is concerned, in order to guarantee the quality of water, although there is still no specific regulation for fungicides, the European Commission controls the presence of pesticides (0.1 µg L^{-1} for individual pesticide and 0.5 µg L^{-1} for the sum of pesticides) in groundwater and water intended for human consumption [17,18]. On the other hand, there is a watch list of substances for Union-wide monitoring in the field of water policy, in which one of the fungicides under study in the present thesis, tebuconazole, is currently included [19].

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2. HONEY

2.1 INTRODUCTION

Honey is defined in the standard for honey adopted by the Codex Alimentarius Commission as "*Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature*" [20]. The European Union in Directive 2001/110/EC, following the Codex recommendations, defines honey in the same way but specifying that the bee species is *Apis mellifera* [21].

Honey is mainly composed by sugars, especially fructose and glucose, and water, but other minor component as minerals, proteins, enzymes, vitamins and other numerous valuable substances are also present. It is a product highly consumed all over the world in diets, medicine and even in cosmetic industry due to its properties as an antioxidant, antibacterial and anti-inflammatory, among others [22,23].

The main criterion of classification of honey is according to its botanical origin. Blossom honey or nectar honey is obtained from the nectar of plants, and honeydew honey is mainly obtained from the excretions of plant-sucking insects. Moreover, honey can also be differentiated by its geographical origin. Honeys can thus be characterized on the basis of organoleptic properties (odour, flavor, etc.), physicochemical analysis (moisture, viscosity, colour, sugars, chemical substances) and pollen spectra (honeydew, multifloral, monofloral as eucalyptus, heather, blackberry, etc), parameters which clearly depend on their origin [21,24–26].

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In addition, honey is a food that undergoes many changes in its composition during storage, due to possible chemical reactions such as oxidation, fermentation or thermal processing [27]. For this reason, the treatment of honey or its presentation (comb honey, chunk honey or honeycomb cut into honey, drained, extracted, pressed or filtered honey), can be a way of classifying honey in terms of quality according to Directive 2001/110/EC [21].

Due to high labour costs and low profits in the honey business, honey production is declining. Therefore, in order to obtain higher production quantities, pure honey is often adulterated with water or chemical compounds [28]. The European Parliament report adopted in 2014 on "The food crisis, fraud in the food chain and its control" considered honey as one of the ten food products with the highest risk of fraud [29].

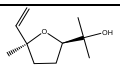
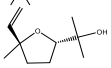
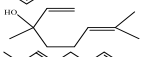
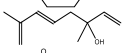
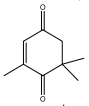
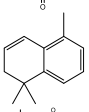
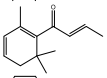
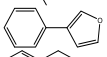
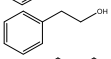
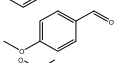
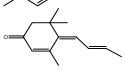
2.2 VOLATILE AND SEMI-VOLATILE COMPOUNDS

Volatile and semi-volatile compounds in honey highly influence on the organoleptic profile and provide an additional tool in its authentication. Although complex mixtures of volatile substances are present in honey, some of them seem to be specific to certain types of honey, giving a particular fingerprint especially in the case of monofloral honeys. Therefore, the volatile profile can be a way to characterize the product [27,30,31].

Numerous families of volatile and semi-volatile compounds are present in honeys, such as terpenes, ketones, aldehydes, alcohols esters, carotenoids or fatty acids among many others [32]. In the present thesis, an initial screening was carried out to select the compounds to further perform method optimization. **Table 3** includes the selected chemical compounds, their CAS number, the molecular weight (MW) and the chemical structure. The data was obtained from ChemSpider (Predicted data is generated using the US Environmental Protection Agency‘s EPISuite™) and chemical structures were made by ChemDraw 20.1.1.

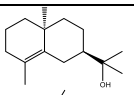
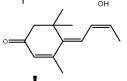
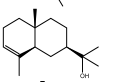
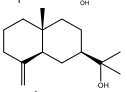
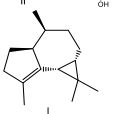
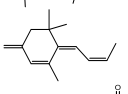
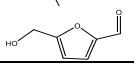
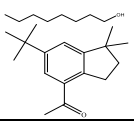
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Table 3. Volatile and semi-volatiles, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Volatile and semi-volatiles	CAS Number	MW	Chemical structure
Cis-Linalool oxide	1365-19-1	170.2	
Furfural	98-01-1	96.1	
α-Ionene	475-03-6	174.3	
Trans-Linalool oxide	34995-77-2	170.2	
Benzaldehyde	100-52-7	106.1	
Β-Linalool	78-70-6	154.3	
Hotrienol	20053-88-7	152.2	
Isophorone	78-59-1	138.2	
4-Oxoisophorone	1125-21-9	152.2	
1,1,5-Trimethyl-1,2-dihydronaphthalene (TDN)	30364-38-6	172.3	
Β-Damascenone	23696-85-7	190.3	
Phenylfurane	13679-41-9	144.2	
Benzyl alcohol	100-51-6	108.1	
Phenylethyl Alcohol	60-12-8	122.2	
Anisic aldehyde	123-11-5	136.1	
1-(2,3-Dimethylphenyl)ethenone (DMPE)	2142-71-4	148.2	
Megastigmatrienone A (MTMA)	5492-79-5	190.3	

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Table 3. (cont.) Volatile and semi-volatiles, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Volatile and semi-volatiles	CAS Number	MW	Chemical structure
γ-Eudesmol	15051-81-7	220.4	
Megastigmatrienone B (MTMB)	5298-13-5	190.3	
α -Eudesmol	473-16-5	220.4	
ß-Eudesmol	473-15-4	220.4	
α-Gurjunene	489-40-7	204.4	
Megastigmatrienone C (MTMC)	13215-88-8	190.3	
5-Hydroxymethylfurfural (HMF)	67-47-0	126.1	
Internal standards			
1-Octanol	111-87-5	130.2	
Celestolide	33704-61-9	244.4	

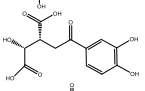
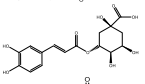
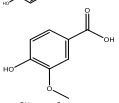
2.3 PHENOLIC COMPOUNDS

The antioxidant properties of honey are mainly attributed to its phenolic compound content, since they can eliminate free radicals and inhibit lipid oxidation. The different types of these compounds that may be present in honey, as well as their amount, depend mainly on the botanical origin, but also on other factors such as the climate or the processing. Phenolic compounds are deemed potentially useful markers for the characterization of honey, the determination of its botanical and/or geographical origin, the quality of honey and the nutraceutical value [25,27,33–35].

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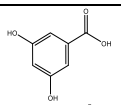
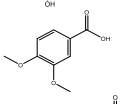
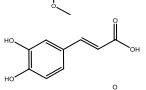
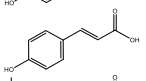
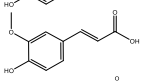
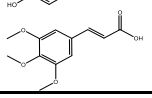
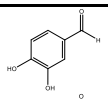
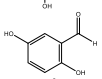
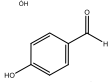
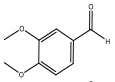
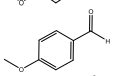
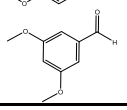
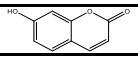
A wide variety of phenolic compounds can be present in honey or other food matrices, and they can be classified as phenolic acids, flavonoids (flavonols, flavones, proanthocyanidins…), tannins, phenolic aldehydes, and others. **Table 4** includes the phenolic compounds assessed in the present thesis, their CAS number, molecular weight (MW) and chemical structure. The data was obtained from ChemSpider (Predicted data is generated using the US Environmental Protection Agency's EPISuite™) and chemical structures were made by ChemDraw 20.1.1.

Table 4. Phenolic compounds, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Phenolic compounds	CAS number	MW	Chemical structure
Phenolic acids			
Gallic acid	149-91-7	170.1	
Phloroglucinic acid	71989-93-0	188.1	
ß-resorcylic acid	89-86-1	154.1	
Protocatechuic acid	99-50-3	154.1	
Caftaric acid	67879-58-7	312.2	
p-hydroxybenzoic acid	99-96-7	138.1	
Gentisic acid	490-79-9	117.1	
3-hydroxyphenylacetic acid	621-37-4	152.2	
Chlorogenic acid	327-97-9	354.3	
Vanillic acid	121-34-6	168.2	
γ-resorcylic acid	303-07-1	154.1	

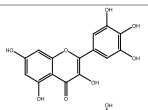
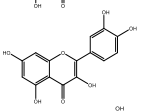
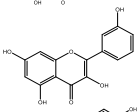
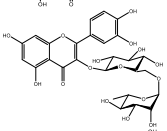
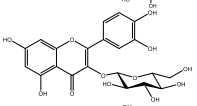
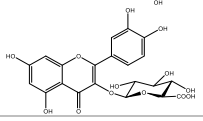
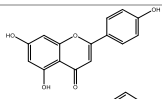
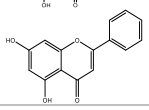
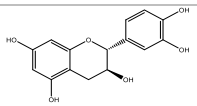
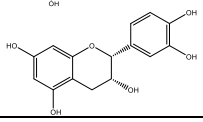
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Table 4. (cont.) Phenolic compounds, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Phenolic compounds	CAS number	MW	Chemical structure
α-resorcylic acid	99-10-5	154.1	
Veratric acid	93-07-2	182.2	
Caffeic acid	331-39-5	180.2	
p-coumaric acid	501-98-4	164.2	
trans-ferulic acid	537-98-4	194.2	
3,4,5-trimethoxycinnamic acid	90-50-6	238.2	
Phenolic aldehydes			
Protocatechualdehyde	139-85-5	138.1	
Gentisaldehyde	1194-98-5	138.1	
3-hydroxybenzaldehyde	100-83-4	122.1	
4-hydroxybenzaldehyde	123-08-0	122.1	
Veratraldehyde	120-14-9	166.2	
4-anisaldehyde	123-11-5	136.1	
3,5-dimethoxybenzaldehyde	7311-34-4	166.2	
Coumarins			
Umbelliferone	93-35-6	162.1	

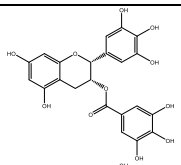
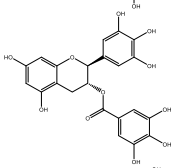
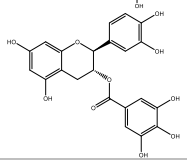
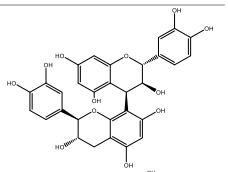
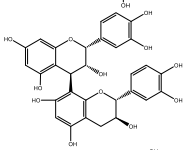
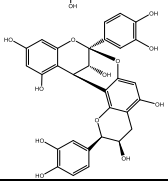
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Table 4. (cont.) Phenolic compounds, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Phenolic compounds	CAS number	MW	Chemical structure
Flavonoids			
Flavonols			
Myricetin	529-44-2	318.2	
Quercetin	117-39-5	302.2	
Kaempferol	520-18-3	286.2	
Rutin	153-18-4	610.5	
isoquercitrin	482-35-9	463.4	
Miquelianin	22688-79-5	478.4	
Flavones			
Apigenin	520-36-5	270.2	
Chrysin	480-40-0	254.2	
Flavanols			
Catechin	18829-70-4	290.3	
Epicatechin	35323-91-2	290.3	

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Table 4. (cont.) Phenolic compounds, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Phenolic compounds	CAS number	MW	Chemical structure
Epigallocatechin gallate	989-51-5	458.4	
Gallocatechin gallate	84650-60-2	458.4	
Catechin gallate	130405-40-2	442.3	
Proanthocyanidin			
Procyanidin B1	20315-25-7	578.5	
Procyanidin B2	29106-49-8	578.5	
Procyanidin A2	41743-41-3	576.5	

2.4 LEGISLATION

The Codex Alimentarius Commission standard for honey and the Directive 2001/110/EC established required composition criteria for sugar content, moisture content, water-insoluble content, electrical conductivity, free acid, diastase activity, and hydroxymethylfurfural

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content. Regarding the botanical origin of the product, if the honey comes wholly or "mainly" from the source indicated and possesses the organoleptic, physico-chemical and microscopic characteristics, the product may be labelled according to its floral or vegetable origin. In addition, the label shall also indicate the country or countries of origin in which the honey was harvested. It is of great importance to note that everything indicated in Codex Alimentarius is voluntary, while the European directive must be implemented in the member states, although they can issue national provisions, decisions and guidelines [20,21].

According to Regulation No 2081/92, honey may have a designation of origin if it has characteristics exclusively related to a specific region or to the specific local environment without modification. Thus, honeys can be classified under the labels of Protected Designation of Origin (PDO) and Protected Geographical Identification (PGI). Currently, in Spain there are five PDO registered honeys and one PGI (Galician honey) [36,37].

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3. PERSONAL AND PHARMACEUTICAL CARE PRODUCTS

Personal and pharmaceutical care products (PPCPs) involve many chemical compounds used in many fields such as medicine, industry, livestock farming, aquaculture and people's daily life. Pharmaceuticals include all the drugs used for the treatment of diseases (antibiotics, anti-inflammatory, antidepressants, β-blockers, etc.) and personal care products (PCPs) are typically utilized in cosmetics, detergents or other household products (fragrances, preservatives, siloxanes, etc). In addition to their presence in products, these substances are considered contaminants of emerging concern (CECs) [38].

In the course of this thesis, the presence of several PCPs in different cosmetic products was evaluated. Regarding pharmaceuticals, the occurrence of antibiotics and non-target compounds was carried out, as well as the evaluation of degradation under photolysis or advanced oxidation processes (AOPs).

3.1 COSMETICS

3.1.1 Introduction

Cosmetics products are defined by the Regulation EC No 1223/2009 as "*any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours*" [39].

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Cosmetic formulations can be classified according to their application e.g. make-up, hair product, cream, etc. or the area of the body on which they are applied. Furthermore, it is also important to differentiate between leave-on products, which remain on the contact surface for a period, and rinse-off products, which are removed after application.

A wide variety of chemical compounds can be found in cosmetics, known as personal care products (PCPs). PCPs include different families of compounds of different nature, such as fragrance allergens, synthetic musks, preservatives, siloxanes, ultraviolet (UV) filters and plasticizers, among others.

Additionally, PCPs enter the environment through domestic and industrial discharges. Another important source is effluents from waste treatment plants, because they only partially degrade these compounds. Several studied have demonstrated their presence in different environmental compartments, revealing their persistence in the environment or bioaccumulation, among other undesirable effects [40–43].

3.1.2 Studied PCPs

Along this thesis, four different families of PCPs were determined: fragrances (allergens and synthetic musks), preservatives, UV filters and plasticizers.

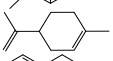
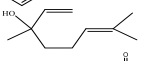
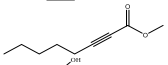
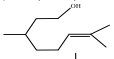
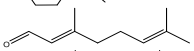
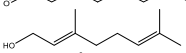
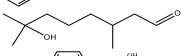
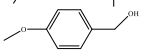
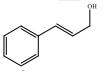
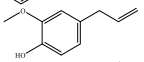
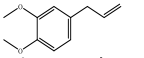
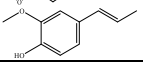
- Fragrances: they are often added to cosmetics in order to provide a pleasant scent and thus mask the unpleasant odours of the raw materials [44]. In this thesis two main groups can be differentiated:

    - Allergens: they provoke skin irritations or allergic reactions, such as skin sensitivity, rashes, dermatitis, coughing or asthma attacks. The Scientific Committee on Consumer Safety (SCCS) revealed that the frequency of contact allergy due to these

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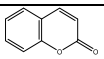
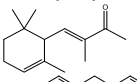
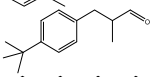
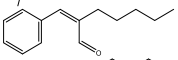
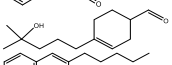
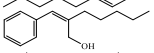
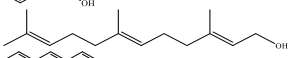
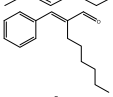
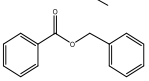
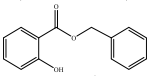
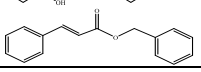
substances in the population in Europe is around 1-3%. There are 26 allergenic fragrances considered by the European Union, of which 24 are included in this thesis together with two other compounds (pinene and methyleugenol). The remaining two substances are natural extracts (oak moss and arborvitae extracts) [45]. The target fragrance allergens are included in **Table 5**, with their CAS number, molecular weight (MW) and chemical structure. The data was obtained from ChemSpider (Predicted data is generated using the US Environmental Protection Agency's EPISuite™) and chemical structures were made by ChemDraw 20.1.1.

Table 5. Fragrance allergens, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Fragrance allergen	CAS number	MW	Chemical structure
Pinene	80-56-8	136.2	
Limonene	5989-27-5	136.2	
Benzyl alcohol	100-51-6	108.1	
Linalool	78-70-6	154.2	
Methyl-2-octynoate	111-12-6	154.2	
Citronellol	106-22-9	156.3	
Citral	5392-40-5	152.2	
Geraniol	106-24-1	154.2	
Cinnamaldehyde	104-55-2	132.2	
Hydroxycitronellal	107-75-5	172.3	
Anise alcohol	105-13-5	138.2	
Cinnamyl alcohol	104-54-1	134.2	
Eugenol	97-53-0	164.2	
Methyleugenol	93-15-2	178.2	
Isoeugenol	97-54-1	164.2	

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Table 5. (cont.) Fragrance allergens, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

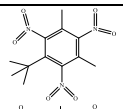
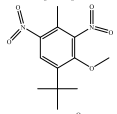
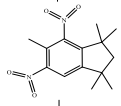
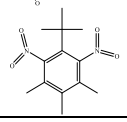
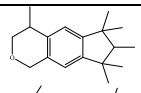
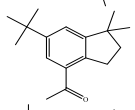
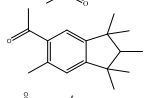
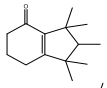
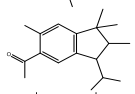
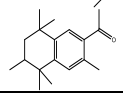
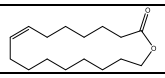
Fragrance allergen	CAS number	MW	Chemical structure
Coumarin	91-64-5	146.1	
α-isomethyl ionone	127-51-5	206.3	
Lilial®	80-54-6	204.3	
Amylcinnamal	122-40-7	202.3	
Lyral®	31906-04-4	210.3	
Amylcinnamyl alcohol	101-85-9	204.3	
Farnesol	4602-84-0	222.4	
Hexylcinnamal	101-86-0	216.3	
Benzyl benzoate	120-51-4	212.2	
Benzyl salicylate	118-58-1	228.2	
Benzyl cinnamate	103-41-3	238.3	

- Synthetic musks: natural musks, highly valued for their unique aroma, come from protected animal and plant species and are therefore not currently used. Therefore, synthetic musks seemed to be a good option, providing similar olfactory properties to natural musks, and lowering the cost of perfume. However, some of these synthetic musks are described as bioaccumulative and persistent xenobiotics, turned out to be associated with different types of dermatitis, carcinogenic effects and endocrine dysfunction. They are classified according to their chemical structure into nitrogenous (nitromusks), polycyclic (polymusks) and macrocyclic musks [46,47]. **Table 6** contains the synthetic musks studied, their CAS number, molecular weight (MW) and chemical structure. The data was

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obtained from ChemSpider (Predicted data is generated using the US Environmental Protection Agency's EPISuite™) and chemical structures were made by ChemDraw 20.1.1.

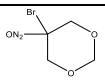
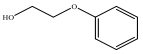
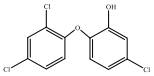
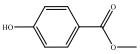
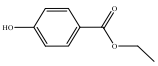
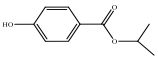
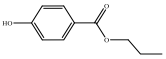
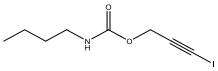
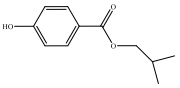
Table 6. Synthetic musks, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Synthetic musks	CAS number	MW	Chemical structure
Nitromusks			
Musk xylene	81-15-2	297.3	
Musk ambrette	83-66-9	268.3	
Musk moskene	116-66-5	278.3	
Musk tibetene	145-39-1	266.3	
Polimusks			
Galaxolide	1222-05-5	258.4	
Celestolide	33704-61-9	244.4	
Phantolide	15323-35-0	244.4	
Cashmeran	33704-61-9	206.3	
Traseolide	68140-48-7	258.4	
Tonalide	1506-02-1	258.4	
Macrocyclic			
Ambrettolide	7779-50-2	252.4	

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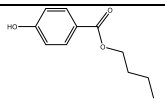
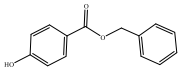
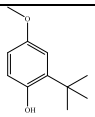
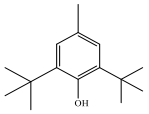
• Preservatives: these chemical compounds are added with the purpose of preventing deterioration due to microbial growth (antimicrobial function). In addition, other substances that are not strictly considered as preservatives, such as antioxidants and light absorbers, can also be added to protect cosmetics from undesirable oxidative processes and photochemical degradation and they are included in most methods to determine preservatives. Excessive exposure to preservatives can lead to contact allergy and skin dermatitis [48,49]. In **Table 7** the target preservatives and antioxidants, their CAS number, molecular weight (MW) and chemical structure are depicted. The data was obtained from ChemSpider (Predicted data is generated using the US Environmental Protection Agency's EPISuite™) and chemical structures were made by ChemDraw 20.1.1.

Table 7. Preservatives and antioxidants, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Preservatives	CAS number	MW	Chemical structure
Bronidox	30007-47-7	212.0	
Phenoxyethanol (PhEtOH)	122-99-6	138.2	
Triclosan (TCS)	3380-34-5	289.5	
Methyl paraben (MeP)	99-76-3	152.1	
Ethyl paraben (EtP)	120-47-8	166.2	
Isopropyl paraben (iPrP)	4191-73-5	180.2	
Propyl paraben (PrP)	94-13-3	180.2	
Iodopropynylbutyl carbamate (IPBC)	55406-53-6	281.1	
Isobutyl paraben (iBuP)	4247-02-3	194.2	

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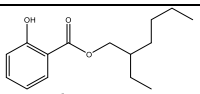
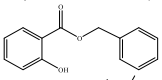
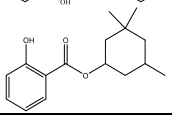
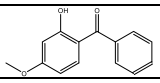
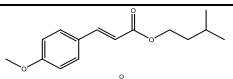
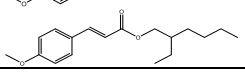
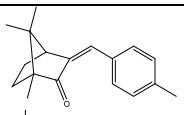
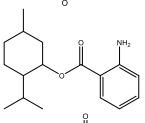
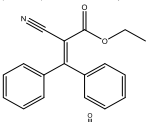
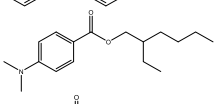
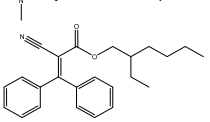
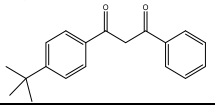
Table 7. (cont.) Preservatives and antioxidants, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Preservatives	CAS number	MW	Chemical structure
Butyl paraben (BuP)	94-26-8	194.2	
Benzyl paraben (BzP)	94-18-8	228.2	
Antioxidants			
Butylhydroxyanisole (BHA)	25013-16-5	180.2	
Butylhydroxytoluene (BHT)	128-37-0	220.3	

• UV filters: they are added to products with the aim of protecting the skin against certain UV radiation. Their function is based on absorbing, reflecting or scattering UV light. They can be inorganic (reflection or scattering) or organic (absorbance capacity in the UVA-UVB region). Among the organic ones they can be classified in different families depending on their chemical structure (benzophenones, salicylates, cinnamates, etc). Although UV filters are intended to protect consumers from harmful solar radiation, especially in sunscreens, some studies report possible adverse effects, such as endocrine disruption [50,51]. **Table 8** includes the target preservatives and antioxidants, their CAS number, molecular weight (MW) and chemical structure. The data was obtained from ChemSpider (Predicted data is generated using the US Environmental Protection Agency's EPISuite™) and chemical structures were made by ChemDraw 20.1.1.

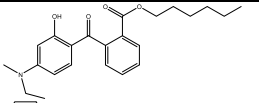
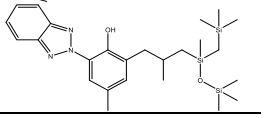
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Table 8. UV filters, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

UV filters	CAS number	MW	Chemical structure
Salicylates			
Ethylhexyl salicylate (EHS)	118-60-5	250.3	
Benzyl salicylate (BS)	118-58-1	228.2	
Homosalate (HMS)	118-56-9	262.4	
Benzophenone derivatives			
Benzophenone-3 (BP3)	131-57-7	228.2	
Cinnamates			
Isoamyl Methoxycinnamate (IAMC)	71617-10-2	248.3	
2-Ethylhexylmethoxycinnamate (2EHMC)	5466-77-3	290.4	
Others			
4-Methylbenzylidene Camphor (4MBC)	36861-47-9	254.4	
Menthyl anthranilate (MA)	134-20-3	275.4	
Etocrylene (ETO)	5232-99-5	277.3	
Ethylhexyl dimethyl p-aminobenzoic acid (EHPABA)	21245-02-3	277.4	
Octocrylene (OCR)	6197-30-4	361.5	
Butyl methoxy dibenzoyl methane (BMDM)	70356-09-1	310.4	

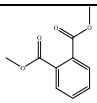
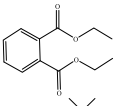
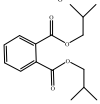
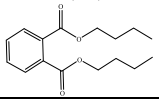
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Table 8. (cont.) UV filters, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

UV filters	CAS number	MW	Chemical structure
Diethylaminohydroxy benzoyl hexyl benzoate (DHHB)	302776-68-7	397.5	
Drometrizole Trisiloxane (DRT)	155633-54-8	501.8	

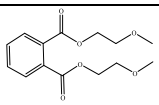
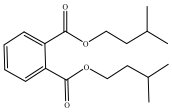
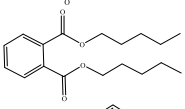
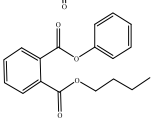
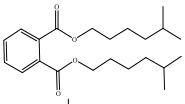
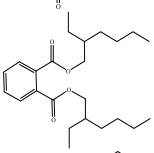
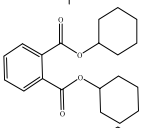
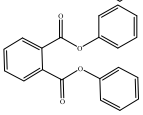
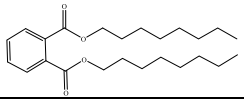
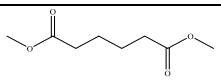
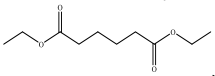
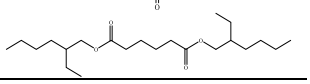
• Plasticizers: they are used as solvents, fragrance fixers and to promote skin penetration. However, they can also be transferred from the plastic container to the cosmetic product. They can be differentiated according to the acid from which they are formed: phthalates, esters of phthalic acid, and adipates, esters of adipic acid [52,53]. **Table 9** shows the target plasticizers (phthalates and adipates), their CAS number, molecular weight (MW) and chemical structure. The data was obtained from ChemSpider (Predicted data is generated using the US Environmental Protection Agency's EPISuite™) and chemical structures were made by ChemDraw 20.1.1.

Table 9. Plasticizers, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Plasticizers	CAS number	MW	Chemical structure
Phthalates			
Dimethyl phthalate (DMP)	131-11-3	194.2	
Diethyl phthalate (DEP)	84-66-2	222.2	
Diisobutyl phthalate (DIBP)	84-69-5	278.3	
Dibutyl phthalate (DBP)	84-74-2	278.3	

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Table 9. (cont.) Plasticizers, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Plasticizers	CAS number	MW	Chemical structure
Dimethoxyethyl phthalate (DMEP)	117-82-8	282.3	
Diisophentyl phthalate (DIPP)	605-50-5	306.4	
Dipentyl phthalate (DPP)	131-18-0	306.4	
Benzylbutyl phthalate (BBP)	85-68-7	312.4	
Diisoheptyl phthalate (DIHP)	41451-28-9	362.5	
Di-2(ethylhexyl) phthalate (DEHP)	117-81-7	390.6	
Dicyclohexyl phthalate (DCHP)	84-61-7	330.4	
Diphenyl phthalate (DPhP)	84-62-8	318.3	
Di-n-octyl phthalate (DNOP)	117-84-0	390.6	
Adipates			
Dimethyl adipate (DMA)	627-93-0	174.2	
Diethyl adipate (DEA)	141-28-6	202.2	
Di-2-(ethylhexyl) adipate (DEHA)	103-23-1	370.6	

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3.1.3 Legislation

Cosmetics marketed in Europe must comply with Regulation 1223/2009, that provides different list of substances that are continuously revised and updated. Annex II involve more than one thousand five hundred chemical compounds prohibited in cosmetic products. Annex III includes the lists of substances allowed for use as cosmetic ingredients, although most of them are restricted in terms of maximum permitted concentration depending on the use of the final product or the product type. In addition, other positive lists are included in Annexes IV, V and VI for colorants, preservatives and UV filters, respectively, in order to be allowed as ingredients with the corresponding functions in cosmetics.

The presence of perfuming and aromatic substances as fragrances (allergens and synthetic musks) shall be indicated by the terms "parfum" or "fragrance" according to Regulation 1223/2009. In addition, the mention of the 26 fragrance allergens on the product labelling is mandatory if their concentration exceeds 0.01% by weight (100 $\mu g\ g^{-1}$) for rinse-off products and 0.001% by weight (10 $\mu g\ g^{-1}$) for leave-on products.

However, some of the fragrances, both allergenic and synthetic musks, plasticizers, preservatives (Annex V) and UV filters (Annex VI) have restrictions on the maximum concentration allowed according to the type of product and/or the part of the body on which it is applied. In addition, it should be noted that some of them are listed in Annex II and therefore banned in cosmetic products. **Table 10** includes the current specific restrictions for the target PCPs. For those compounds that are not specified in the table there are no restrictions at this stage (May 2022), except all fragrance allergens included in this thesis (excluding pinene and mehytleugenol), that must comply with the product labelling mentioned above [39].

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Table 10. Specific restrictions of the studied PCPs in cosmetic products according to Regulation 1223/2009 [39].

PCPs	Restrictions
Fragrance allergens	
Methyleugenol	Fine fragrance (0.01%) Eau de toilette (0.004 %) Fragrance cream (0.002%) Other leave-on products and oral products (0.0002%) Rinse-off products (0.001%)
Isoeugenol	0.02% (except oral products)
Lilial®	Prohibited
Lyral®	Prohibited
Synthetic musks	
Musk xylene	All cosmetics except oral products: Fine fragrance (1,0 %) Eau de toilette (0,4 %) Other products (0,03%)
Musk ambrette	Prohibited
Musk moskene	Prohibited
Musk tibetene	Prohibited
Phantolide	Leave-on products (2%)
Tonalide	All cosmetics except oral products: Leave-on products (0.1%) except hydroalcoholic products (1%), fine fragrance (2.5%) and fragrance cream (0.5%). Rinse-off products (0.2%)
Preservatives	
Bronidox	Rinse-off products (0.1 %)
PhEtOH	1%
TCS	Toothpastes, hand soaps, body soaps/shower gels, deodorants (non-spray), face powders and blemish concealers, nail products for cleaning the fingernails and toenails before the application of artificial nail systems (0.3%) Mouthwashes (0.2%)
MeP, EtP	Single ester (0.4% as acid) Mixtures of esters (0.8% as acid)
iPrP	Prohibited
PrP, BuP	The sum of the individual concentrations (0.14% as acid) Mixtures of substances (0.8% as acid)* Prohibited in leave-on products designed for application on the nappy area of children under 3 years of age
IPBC	Rinse-off products (0.02%), prohibited in products for children under 3 years of age except bath products. Leave-on products (0.01%), prohibited in body lotion and body cream, and products for children under 3 years of age Deodorants/anti-persistent (0.0075%), prohibited in products for children under 3 years of age

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Table 10. (cont.) Specific restrictions of the studied PCPs in cosmetic products according to Regulation 1223/2009 [39].

PCPs	Restrictions
iBuP	Prohibited
BzP	Prohibited
UV filters	
EHS	5%
HMS	10%
BP	6% and it must be indicated in the label 'Contains benzophenone-3' if the concentration exceeds 0.5 % (not for product protection purposes).
IAMC	10%
2EHMC	10%
4MBC	4 %
OCR	10 % (as acid)
DHHB	10 %
DRT	15%
Plasticizers	
DIBP	Prohibited
DBP	Prohibited
DMEP	Prohibited
DIPP	Prohibited
DPP	Prohibited
BBP	Prohibited
DEHP	Prohibited
DCHP	Prohibited

*Mixture of substances mentioned in entry 12 and 12a of Regulation No 1223/2009, where the sum of the individual concentrations of butyland propylparaben and their salts does not exceed 0.14 %

There are products, so-called borderline products, that sometimes it is unclear whether they should be regulated as cosmetics or other legislation. This is the case of hydroalcoholic gels, included in the present thesis [54]. The World Health Organization (WHO) published a protocol to homogenise formulation and fabrication of this product, with the aim of assure their antimicrobial properties [55]. However, they can be considered biocides or cosmetic product. Biocide is defined as "*any substance or mixture, in the form in which it is supplied to the user, consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling*

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effect on, any harmful organism by any means other than mere physical or mechanical action" by Regulation No 528/2012 [56]. Nevertheless, if the main purpose of hydroalcoholic gels is cleaning or cleansing the skin, they are subject to the Cosmetic Products Regulation.

3.2 ANTIBIOTICS

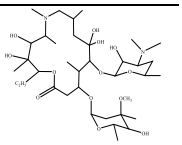
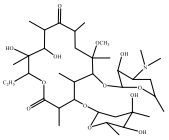
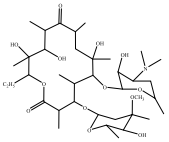
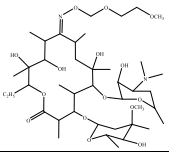
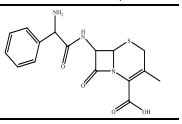
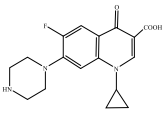
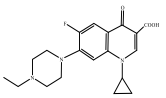
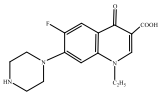
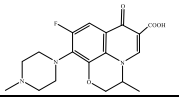
3.2.1 Introduction

Antibiotics are a class of pharmaceuticals which act on the metabolism of bacteria, fungi, and protozoa to inhibit microbial growth. They are used for the treatment of infectious diseases, although recently they have also been used for other therapies such as cancer treatment or organ transplantation. These chemical compounds can be classified according to their origin (natural, synthetic or semi-synthetic), chemical structure or mechanism of action. Despite being a very useful tool for curing diseases, both for human and veterinary, the misuse of these compounds has led to a rapid increase in antimicrobial resistance. They are mainly excreted as the active and metabolite forms via urine or faeces [57–59].

Table 11 depicts the families and antibiotics included in this report, their CAS number, molecular weight (MW) and chemical structure. The data was obtained from ChemSpider (Predicted data is generated using the US Environmental Protection Agency's EPISuite™) and chemical structures were made by ChemDraw 20.1.1.

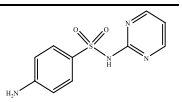
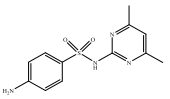
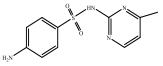
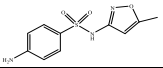
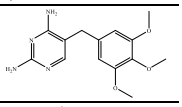
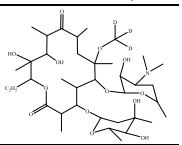
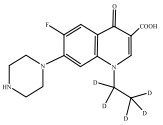
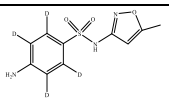
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Table 11. Families and antibiotics, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Family	Antibiotic	CAS number	MW	Chemical structure
Natural antibiotics				
Macrolides	Azithromycin (Azi)	83905-01-5	749.0	
	Clarithromycin (Clar)	81103-11-9	748.0	
	Erythromycin (Ery)	114-07-8	734.0	
	Roxithromycin (Rox)	80214-83-1	837.0	
Cephalosporins	Cephalexin (Ceph)	15686-71-2	347.4	
Synthetic antibiotics				
Fluoroquinolones	Ciprofloxacin (Cipro)	85721-33-1	331.3	
	Enrofloxacin (Enro)	93106-60-6	359.4	
	Norfloxacin (Nor)	70458-96-7	319.3	
	Ofloxacin (Oflo)	82419-36-1	361.4	

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Table 11. (cont.) Families and antibiotics, CAS number, molecular weight (MW, g mol^{-1}) and chemical structure.

Family	Antibiotic	CAS number	MW	Chemical structure
Sulfonamides	Sulfadiazine (SDZ)	68-35-9	250.3	
	Sulfadimidine (SDMD)	57-68-1	278.3	
	Sulfamerazine (SMZ)	127-79-7	264.3	
	Sulfamethoxazole (SMX)	723-46-6	253.3	
Diaminopyrimidines	Trimethoprim (TMP)	738-70-5	290.3	
Isotopically labelled compounds	Clarithromycin-D3 (Clar-D3)	959119-17-6	751.0	
	Norfloxacin-D5 (Nor-D5)	1015856-57-1	324.4	
	Sulfamethoxazole-D4 (SMX-D4)	1020719-86-1	257.3	

3.2.2 Occurrence in the environment

PPCPs are CECs as they can easily enter the environment and pose risks to ecosystems and human health. Antibiotics are one of the most interesting groups, since their residues as active substance or metabolites, lead to the emergence of antibiotic resistant bacteria and antibiotic resistance genes [60].

The main source of antibiotics into the environment is through wastewater treatment plants (WWTPs), as most of them are only

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partially degraded. The removal efficiency depends on the class of antibiotics, their physicochemical properties and the operating conditions of the WWTPs. In addition, antibiotics can reach the environmental compartments through other routes, such as liquid manure, aquaculture, the reuse of activated sludge as biofertilizers, etc [59].

The presence of antibiotics has been reported in wastewater, both influent and effluent, from different WWTPs such as urban, hospital or industrial. In addition, numerous studies have reported the presence of antibiotics in natural waters, including lakes, rivers, sea and groundwater, as well as in water for human consumption such as tap water or drinking water. On the other hand, they have also been found in soils, so that through leaching they can also easily reach the aquatic environment [61–65].

3.2.3 Photodegradation and advanced oxidation processes

Photodegradation is defined by the International Union of Pure and Applied Chemistry (IUPAC) as "*the photochemical transformation of a molecule into lower molecular weight fragments, usually in an oxidation process. This term is widely used in the destruction (oxidation) of pollutants by UV based processes*". As conventional WWTPs are not able to fully remove antibiotics, other processes are necessary to enable their complete elimination, being photodegradation an interesting alternative [66].

In the electromagnetic sector, UV radiation includes X-rays and visible light. In terms of wavelength range, UV radiation can be divided into three distinct regions: UVA (320-400 nm), which can be divided into UV-I or short UVA (340-400 nm) and UVA-II or long UVA (320-340 nm), UVB (290-320 nm) and UVC radiation (100-290 nm).

Photodegradation in water can be performed by direct or indirect photolysis. The first one is conducted when the substance absorbs light energy, causing it to decompose and, the second one occurs when light energy is absorbed by the photosensitisers (natural components of

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water) that prompt the photocatalytic reaction [67]. For UV light, low and medium pressure mercury vapor lamps are the most widely used. As regards the removal of antibiotics in this thesis, low-pressure mercury lamps emitting monochromatic UV light at 254 nm (UVC) were employed [68].

Advanced oxidation processes (AOPs) have been proposed in 1987 as a promising alternative for potable water purification [69]. However, nowadays it is well known that AOPs are very effective as tertiary treatment in WWTPs for the elimination of a wide variety of CECs.

AOPs are characterized by the generation of highly reactive oxidizing species (ROS), by using different oxidants (H_2O_2, $S_2O_8^{2-}$,O_3 etc) which produce radicals, as hydroxyl radical (HO•) and sulfate radical (SO_4^{-}•) among others. These formed radicals, under different chemical reactions, allow the degradation and mineralization of the organic compounds. In addition, catalysts can be used to accelerate reactions. Heterogeneous photocatalyst is based on direct or indirect absorption of radiant energy (UV or visible) by a solid photocatalyst (TiO_2, Ag_2MoO_4, etc) [70,71].

Several systems based on AOPs are proposed for the removal of different CECs. Recently, Vilar et al.in 2020 proposed the tube-in-tube membrane reactor as a promising alternative for water decontamination [72]. This reactor is a low footprint technology whose design enables the integration of different ROS generation mechanisms within a single unit operated in continuous-flow mode.

The degradation of antibiotics in aqueous media has been evaluated in several studies. However, during these processes, a transformation of the substances can occur, giving rise to by-products which may be more toxic and persistent than the parent substance, and therefore more harmful to the human health and environment [73,74].

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3.2.4 Legislation

There is still no legal framework concerning antibiotics in water matrices. Nevertheless, due to the enhanced interest in these compounds, some of them were included in the European Watch List of substances according to the Directive 2008/105/EC. The 1st Watch List included macrolide antibiotics (azithromycin, clarithromycin, and erythromycin) among other substances. In 2018 it was updated incorporating other antibiotics such as ciprofloxacin. The 3rd Watch List has been conceived in 2020, adding sulfamethoxazole and trimethoprim. Although no maximum permitted concentration is established, the analytical methodologies as well as the maximum acceptable limits of detection of the method are proposed [19,75].

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4. SAMPLE PREPARATION

4.1. INTRODUCTION

Sample preparation is a step usually employed to extract analytes from the sample matrix. This process specially depends on the physicochemical properties such as the polarity, solubility or volatility, among others, of the analytes, the matrix and its components, and the environmental conditions [76].

Conventional sample preparation procedures involve a high consumption of solvents and time, so that simple, faster, more cost-effective and environmentally friendly sample preparation methods are in continuous development [77].

Therefore, in this doctoral thesis different sample preparation approaches were employed. Solid-phase extraction (SPE) was used for the analysis of antibiotics and fungicides in waters. Fabric-phase sorptive extraction (FPSE) was also proposed for the fungicides determination in water and ultrasound-assisted extraction (UAE) was employed to extract these substances in vineyard matrices. UAE was also utilized after vortex extraction (VE) to extract phenolic compounds from honey. Solid-phase microextraction (SPME) was optimized for the analysis of personal care products (PCPs) in hydroalcoholic gels and a SPME miniaturized approach was developed for the determination of volatile and semi-volatile compounds in honey. Micro-matrix solid-phase dispersion (μMSPD) was employed for the analysis of PCPs in cosmetic products of different nature.

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4.2. SOLID-PHASE EXTRACTION (SPE)

4.2.1. Introduction

Solid Phase Extraction (SPE) is a sample preparation technique described by Braus in the 1950s [78], which allows the isolation of analytes of interest in liquid, fluid or gas matrices retaining them on a solid phase usually packed in a cartridge. In comparison with traditional extraction techniques, as liquid-liquid extraction (LLE), it provides simplicity, cost effectiveness, low solvent consumption and low processing time as well as the possibility to automate the whole process. SPE can be employed for clean-up, extraction, changing of solvents, preconcentration and fractionation of organic compounds [79,80].

4.2.2. Experimental procedure

In the SPE procedure, four steps are usually involved as illustrated in **Figure 4.1**:

1. Sorbent conditioning: it is usually necessary to pass a suitable solvent through the adsorbent to ensure efficient interaction with the analytes. Methanol (MeOH) is a typical solvent used for this step followed by the solvent in which the analyte is dissolved, or a solvent similar to the matrix sample [81].

2. Sample loading: the sample is passed through the sorbent and the analytes are retained on it.

3. Washing: allows carrying interfering substances using an adequate solvent, ensuring that the target compounds are not lost.

4. Elution: a solvent of suitable polarity is passed through the cartridge to achieve maximum recovery of analytes, obtaining a cleaned and concentrated extract.

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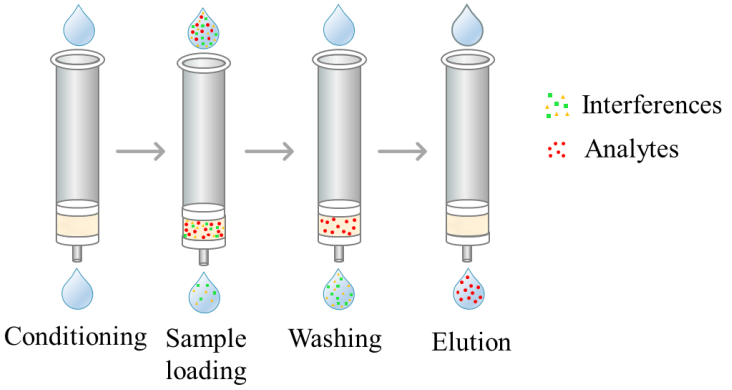


Figure 1. SPE procedure.

4.2.3. Parameters affecting SPE

There are some factors that can affect the SPE efficiency such as:

• Sorbent: it is one of the most critical parameters to obtain a successful extraction since it plays an important role in the efficiency and analyte selectivity. Typical sorbents can be classified into inorganic oxides (silica, alumina, florisil, etc.), low specificity sorbents (silica-based chemically bonded phases, porous polymers and carbon) and compound and group selective or high specificity sorbents (mixed-mode, ion exchange, macrocyclic, restricted access, affinity-based and molecularly imprinted polymers). Nevertheless, there is a wide variety of subcategories, with a current emphasis on biosorbents [82].

• Matrix: the sample can be liquid, viscous or solid. Liquid samples can pass through the SPE cartridge diluted or without any modification and viscous matrices (e.g. honey) often need to be diluted to facilitate extraction. Regarding solid samples (e.g. soil), prior extraction in a solvent mixture is necessary to further perform SPE, usually used in this case as a clean-up step.

• Sample volume: It is an important factor, especially for ultratrace water analysis. The maximum volume that can be

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concentrated without analytical lost (breakthrough volume) must be studied.

• Solvent: physico-chemical properties, such as polarity or density, may affect the extraction. The solvent must be suitable to break the sorbent-analyte interaction without eluting interferences.

• Flow rate: a too fast flow allows minimal preparation time but it might be inadequate for ensuring a suitable compound-sorbent interaction.

• Analytes: the properties of the compounds of interest (polarity, solubility…) determine the choice of the sorbent and solvent.

4.2.4. Applications

Solid-phase extraction is one of the most widely employed extraction techniques. In environmental matrices, such as wastewater or groundwater, sludge, etc, it is used for the extraction of a wide variety of compounds including personal and pharmaceutical care products, pesticides, among others [83–85]. In addition, it is also applied for other matrices such as biological (urine, plasma…), food (honey, milk..) or cosmetic samples to determine a wide variety of organic compounds [86–90].

SPE has been used for the analysis of fungicides employing cork by-products as sorbent in **Section IV**, Chapter 1.2. In addition, in Chapter 4.1 a method based on this technique is described to extract antibiotics from water, in this case using a conventional hydrophilic-lipophilic sorbent.

4.3. SOLID-PHASE MICROEXTRACTION (SPME)

4.3.1. Introduction

Solid-Phase Microextraction (SPME) was introduced in 1990 by Pawliszyn et al. [91] as a miniaturized alternative of SPE to apply not

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only in the laboratory, but also in the field. The SPME consists of using a fused silica fibre, chemically inert and very stable even at high temperatures, covered with an ab/adsorbent polymeric phase. To carry out extraction, the coated fused silica is placed in a holder resembling a syringe.

The main advantages of this extraction technique are its simplicity, since the device is easy to handle and to apply in the field, its ability to perform one-step sample preparation, and the high sensitivity as the extracted analytes are concentrated on the fibre and directly introduced in the chromatographic system (GC). In addition, the sample amount is usually low, and the use of organic solvents is negligible or even absent. Residue generation is also minimal, so that SPME can be considered an environmentally friendly sample preparation approach. Besides, it is easy to automate, which offers high time efficiency and increased simplicity [91,92].

4.3.2. Experimental procedure

The SPME procedure consists on [93]:

1. Extraction: the fibre is exposed to the sample placed in a sealed vial. In this way, there is a migration of the analytes from the sample to the fibre.

2. Desorption: the fibre with the retained and concentrated analytes can be directly introduced into the injector of a gas chromatograph to be thermally desorbed, or desorbed in a solvent for liquid chromatographic analysis.

The extraction can be carried out by two different extraction modes (see **Figure 4.2**):

• Headspace mode: the fibre is exposed to the headspace above the sample, whereby analytes first migrate from the sample to the headspace to be subsequently ab/adsorbed in/on the fibre. It is widely used for the extraction of volatile and semi-volatile substances.

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• Direct immersion mode: the fibre is directly introduced into the sample so that the compounds can migrate from the matrix to the fibre. This extraction mode is used for simple matrices and usually for slightly volatile compounds.

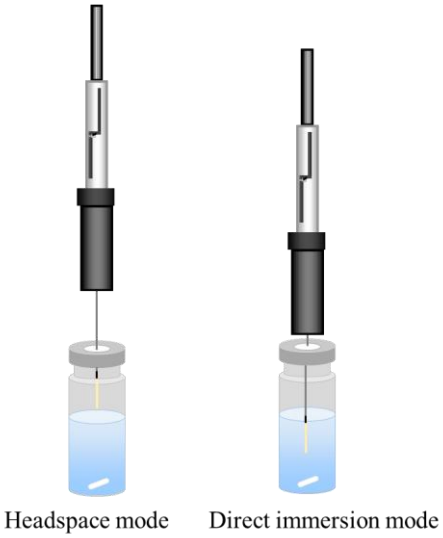


Figure 2. SPME extraction modes.

4.3.3. Parameters affecting SPME

Many parameters can affect SPME [93,94]:

• Fibre coating: there are many types of polymeric phases with different polarities and thickness. The fibre affinity for an analyte can be initially determined according to their polarity.

• Extraction time: the SPME is an equilibrium technique, implying that the highest analyte extraction is achieved in the time it takes to reach equilibrium. However, a shorter extraction time can be chosen if there is strict control of the process to ensure that good reproducibility is obtained.

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- Extraction temperature: the diffusion of the analytes to the fibre is usually favoured at higher temperatures. In addition, in headspace mode, the temperature increase eases the transfer of the analytes into the headspace. However, the fibre/sample distribution constant decreases with the temperature increase, as the absorption step is an exothermic process.

- Sample pH: the pH of the solution can affect the dissociation equilibrium of analytes with acidic or basic groups. Thus, to obtain high extraction efficiency, the pH must be two units below the pKa for acidic compounds and two units above for basic compounds.

- Salting-out effect: the ionic strength is increased when salts like NaCl or KCl are added in the aqueous solution, favouring a decrease of the analyte solubility in water, and promoting their migration to other phases of the system, such as the headspace and the fibre.

- Agitation: stirring the sample can facilitate the diffusion of analytes to the fibre, accelerating the extraction time.

- Addition of solvent: the addition of organic solvents or water in the case of solid samples can favour the diffusion of analytes from the matrix to the fibre, whereas solvents in aqueous solutions usually decrease the extraction efficiency.

- Sample and headspace volume: if the system consists of three phases (sample, fibre and headspace), the amount of analytes extracted follows the equation:

$$n = \frac{K_{fs} V_f V_s}{K_{fs} V_f + K_{hs} V_h + V_s} C_0$$

Where, n are the moles of analytes extracted; Kfs is the distribution constant between the fibre and sample and Khs between the headspace and sample; Vf, Vs, and Vh are the volumes of fibre, sample and headspace, respectively; C_0 is the initial analyte concentration in the sample.

Therefore, if the volume of the sample increases, the amount of analyte extracted will also increase until a certain volume from which

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the amount of analyte extracted will no longer increase. In this case, the volume of sample does not influence the amount of analyte extracted following the next equation:

$$n = K_{fs} V_f C_0$$

Regarding the headspace volume in eq. 1, when it increases, the amount of analyte extracted decreases.

4.3.4. Applications

SPME has been proposed for multiple applications, being one of the preferred extraction techniques to determine volatile and semivolatile organic compounds in food samples, as is the case of honey [95,96]. In addition, it has been applied for the determination of a wide variety of organic analytes, including personal care products, in environmental, biological, and cosmetic samples [97–100].

A miniaturized method based on SPME was developed in **Section IV**, Chapter 2.1, for the analysis of honey samples. Furthermore, in Chapter 3.2, SPME was applied for the determination of personal care products in hydroalcoholic gels.

4.4. FABRIC PHASE SORPTIVE EXTRACTION (FPSE)

4.4.1. Introduction

Fabric Phase Sorptive Extraction (FPSE) has been introduced as a novel sample preparation approach by Kabir and Furton in 2014 [101]. It can be considered as combination of the extraction mode of SPME (equilibrium extraction) and SPE (exhaustive extraction) into a single technology platform. FPSE allows direct extraction of the analytes present in the sample, since no other pre- or post-extraction procedures (filtration, centrifugation, solvent evaporation, etc.) are necessary, thus avoiding errors and additional costs.

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It consists of a flexible fabric (cellulose, polyester, or fiberglass) as the substrate, which is chemically coated with an ultra-thin coating with sol-gel organic-inorganic hybrid sorbent as the extraction medium [101,102].

4.4.2. Experimental procedure

It exists a protocol involving 3 steps to carry out the FPSE, described in **Figure 4.3** [103]:

1. Cleaning the membrane: the coated fabric is introduced in 2 mL of acetonitrile:methanol (ACN:MeOH, 50:50, v/v) solution during 5 minutes and then in 2 mL of ultrapure water to remove the residual organic solvents. This step can be repeated twice.

2. Extraction: the clean FPSE membrane is immersed in the sample contained in a vial in which the sorption of the target analytes by the sorbent will take place. After the extraction, the FPSE device is removed from the vial and left to dry.

3. Elution: the dried FPSE device is inserted in a vial to accomplish solvent desorption. It can be stirred, vortexed or centrifuged to favour the process.

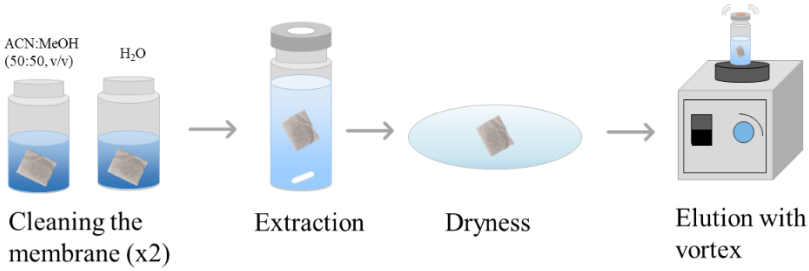


Figure 3. FPSE schematic procedure.

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4.4.3. Parameters affecting FPSE

The efficiency of the FPSE procedure can be increased/decreased depending on some parameters [104]:

- Fabric substrates: the different nature of the substrates is very important and determines the selectivity and the polarity of the FPSE media. The substrates can be hydrophilic (cotton cellulose), hydrophobic (polyester), or both (cotton-polyester).

- Sol-gel sorbent: the organic/inorganic polymer plays the most significant role in the sorbent selectivity. The polymer polarity (nonpolar, medium polar and polar) is determined according to the analytes.

- Extraction time: extraction efficiency is influenced by the time required to reach equilibrium.

- Agitation: stirring or sonication can promote the diffusion of analytes from the sample to the extraction phase, reducing the time needed to reach extraction equilibrium.

- Effect of salt content: the addition of salt to the sample matrix is an important parameter to evaluate, as it generally decreases the solubility of organic analytes providing higher extraction efficiency. However, it can increase the viscosity of the solution, reducing the diffusion rate of the analytes.

- Elution solvent and volume: the solvent should be selected according to the polarity of the analytes. In terms of volume, lower volumes can provide higher enrichment factors, although it is important to ensure that the device is completely immersed.

- Elution time: the desorption time is expected to be fast, because the solvent can easily penetrate the ultra-thin coating of the sol-gel sorbent as well as the pores of the textile substrate. Nevertheless, it highly depends on the affinity of the analytes for the solvent.

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• Sample volume: the amount of extracted analyte increases with the sample volume up to a certain volume which, according to equation 2 (Section 4.3.3), the amount of extracted analyte does not increase.

4.4.4. Applications

The variety of the available sorbents allows the application of the FPSE for different type of samples. It has been broadly used for the extraction of organic compounds in environmental, food and biological samples [103,105–108].

In **Section IV**, Chapter 1.2, a methodology based on FPSE has been developed for the determination of fungicides in environmental waters.

4.5. MATRIX SOLID-PHASE DISPERSION (MSPD)

4.5.1. Introduction

Matrix solid-phase dispersion (MSPD) was introduced by Barker and co-workers in 1989 [109]. It combines aspects of several analytical techniques, allowing the disruption and dispersion of the sample on a solid support, generating a unique chromatographic material. This sample procedure can be applied for the preparation, extraction and fractioning of solid, semi-solid or highly viscous samples.

MSPD allows extraction and cleaning to be performed at the same time, which reduces the chances of sample contamination during the procedure. In addition, it does not require the use of expensive and specific instrumentation, and the soft extraction conditions (ambient temperature and atmospheric pressure) allow using low volumes of organic solvents [110].

Besides, in order to further reduce the amounts of sample, dispersing agent and solvent, the MSPD miniaturization has been proposed, and called micro-MSPD (μMSPD) [111].

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4.5.2. Experimental procedure

The classic procedure implies the steps described below (see µMSPD procedure in **Figure 4.4**) [112]:

The solid, semi-solid or viscous sample is added in a mortar or a vial where it is mixed with a dispersing agent in order to achieve a complete disruption and dispersion of the sample. In addition, sometimes, a desiccant such as anhydrous sodium sulphate (Na_2SO_4) is also needed to obtain a finely divided material, dry enough for subsequent extraction.

1. Once the sample disruption and dispersion stage are completed, the resulting mixture is packed into an empty column, which is usually an empty syringe body, with a frit at the bottom. However, with the aim of miniaturize the procedure using the lowest amounts of sample and materials, a Pasteur pipette (approximately 150 mm) can be used with glass wool in the bottom in the case of µMSPD. In addition, in order to obtain a further degree of fractionation and an in-situ clean-up step, a small amount of Florisil or other sorbent agent can be added between the glass wool and the sample. Once the sample is packed, a second frit or glass wool is placed on top of the mixture and then compressed to avoid air bubbles.

2. The elution can be carried out according to two ways:

a) The analytes of interest are retained on the column and interfering compounds are eluted during the washing step. Then, the target compounds are eluted with another solvent.

b) The matrix interferences are retained on the column and the target analytes are eluted with a solvent.

3. The extract can be directly analysed or, if necessary, an additional cleaning step can be carried out.

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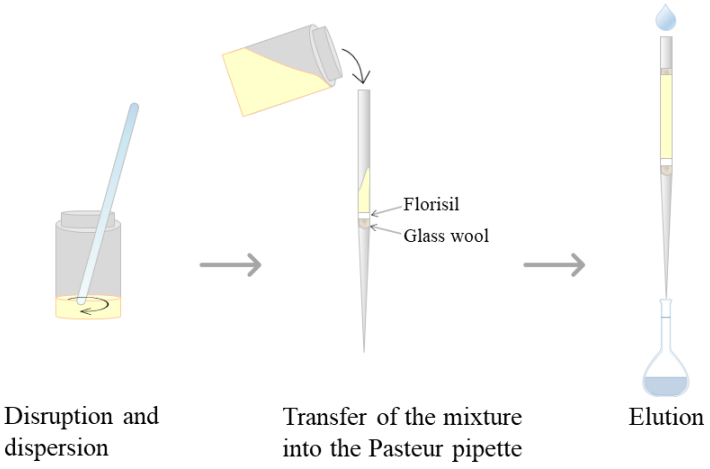


Figure 4. µMSPD schematic procedure.

4.5.3. Parameters affecting MSPD

Several factors can affect the MSPD extraction efficiency [110]:

- Dispersing agent: classical MSPD applications employ silica-based sorbents as dispersing agent. These materials have the additional advantage of containing free silanol groups, acting at the same time as dispersing and desiccant agents since they are able to form hydrogen bonds with water molecules. Usually, reversed-phase bonded silicas are employed (C8 and C18) but other modified reversed materials are also used for the extraction of more polar compounds, such as silica bonded to polar groups (e.g. amines) or non-bonded inorganic oxides (silica itself, alumina, Florisil).

- Matrix modification: sometimes, it is necessary to modify some matrix parameters acting on the pH adjustment, or with the addition of salts, chelating agents or de-chelating agents, antioxidants, etc...

This can be done during the sample blending and/or further on modifying the eluting solvent composition, with the aim of altering the ionization state of the sample components to assure that certain interactions occur between the solid support bonded-phase and/or the eluting solvent.

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• Solvent: the nature of the elution solvent affects the selectivity towards the analytes. The aim is desorbing the compounds of interest, while the matrix components are retained on the column.

4.5.4. Applications

MSPD has been used for a wide variety of compounds including PCPs and pesticides and many matrices (food, environmental, cosmetics) [113–116]. In addition, the µMSPD was also applied for the analysis of cosmetics and food samples [117–121]. In **Section IV**, Chapter 3.1 a method based on µMSPD has been developed to determine PCPs in cosmetic formulations. In addition, the MSPD viability was tested for the extraction of fungicides in leaves in Chapter 1.3.

4.6. ULTRASOUND ASSISTED EXTRACTION (UAE)

4.6.1. Introduction

Ultrasound Assisted Extraction (UAE) is a simple, fast and low-cost technique based on ultrasounds, sound waves with frequencies higher than the upper audible limit of human hearing. It consists of applying ultrasound energy to extract liquid or solid samples. This energy provokes the formation and implosion of bubbles according to a phenomenon called cavitation. The continuous agitation of the organic solvent facilitates the mass transfer processes between the two phases as the solvent penetrates further into the different matrices [122].

4.6.2. Experimental procedure

UAE can be performed employing an ultrasonic bath or an ultrasonic probe. In the first case, which is the most available and cheapest source of ultrasound irradiation, the sample (liquid or solid) is mixed with a solvent and immersed into a ultrasonic bath applying a

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specific and constant ultrasonic frequency (usually between 20 and 100 kHz). In case of using an ultrasonic probe, it can be inserted into the sample and more vigorous extractions can be performed [123].

4.6.3. Parameters affecting UAE

Several parameters can affect the extraction efficiency [124,125]:

• Solvent: the type, amount and the viscosity of solvent, as well as the ratio of solvent and solute, contribute to the transmission of ultrasonic energy. Furthermore, the solvent must be selected according to the analytes of interest.

• Temperature: cavitation is enhanced decreasing the temperature, since when the temperature is increased, the viscosity and surface tension are decreased, and induces a rise of vapor pressure. However, for extraction processes at lower acoustic intensity, higher temperatures can increase the number of cavitation bubble. For example, a larger contact area between the solid and the solvent is formed, increasing the diffusivity of the solvent and thus improving the desorption and solubility of the compounds of interest. Therefore, the temperature of the solvent should be controlled within a suitable range, since it is important to take into account the possible degradation of the analytes with the temperature.

• Extraction time: the time can affect the extraction since long extraction time enhances extraction yields but may induce undesirable changes for the extracted analytes.

3.6.4. Applications

UAE has been applied as a simple and fast extraction technique for the analysis of food, cosmetics or environmental samples [126–129].

In this thesis, an UAE procedure was optimized in Section IV, Chapter 1.3, for the determination of fungicides in leaves, grapes and

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soils. In Chapter 2.2, UAE was also employed after vortex extraction to extract phenolic compounds from honey.

4.7. VORTEX EXTRACTION (VE)

4.7.1. Introduction

Vortex extraction (VE) is a very simple and fast technique. In general, the effect of a vortex stirrer is to rotate the fluids and create a vortice, which size and shape depend on the velocity. It can be applied as a single step or followed by ultrasound application or centrifugation [130].

4.7.2. Experimental procedure

VE can be applied for the extraction of solid samples, adding a solvent and collecting the supernatant [131].

However, the extraction of certain substances in liquid samples can be performed by vortex-assisted liquid-liquid microextraction (VALLME). In this case, the dispersion of the extractant phase into the aqueous phase takes place and the phase separation of the resulting emulsion is carried out by centrifugation [132].

4.7.3. Parameters affecting VE

The organic solvent, the sample and solvent amount, and the pH and salt addition are factors that can clearly affect the extraction efficiency, as for most of the sample preparation procedures mentioned in the present thesis. This technique is also affected by the rotational speed of the vortex agitator and by the extraction time. For example, in VALLME, for immiscible liquids, high velocities result in the break-up of one of the two phases into fine droplets. The shorter diffusion distance and the higher specific surface area mean that the fine droplets formed can lead to fast extractions [130].

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4.7.4. Applications

VE has been utilized for the extraction of environmental, food and cosmetic samples [131,133]. VALLME was widely employed for several organic chemical compounds in different matrices, such as food, including honey, and environmental samples [134–136]. Vortex and ultrasound-assisted surfactant-enhanced emulsification microextraction was also used to determine pesticides residues in honey [137].

The work described in **Section IV**, Chapter 2.2 is based on the determination of phenolic compounds in honey by VE followed by UAE. Furthermore, this technique demonstrated its suitability for the extraction of fungicides in vine leaves as it is described in Chapter 1.3.

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5. ANALYSIS

5.1. INTRODUCTION

For the analysis of the studied analytes, chromatographic techniques combined with mass spectrometry have been employed. Gas chromatography (GC) has been used coupled to low resolution mass spectrometry. Mass spectrometry with a single quadrupole (MS) was utilized for the analysis of volatile and semivolatile compounds in honey. GC was also coupled with tandem mass spectrometry (MS/MS) for the analysis of fungicides in agricultural and environmental matrices and PCPs in cosmetic formulations.

Besides, polyphenolic compounds in honey samples were analysed by liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS). Furthermore, two approaches of high-resolution mass spectrometry (HRMS), employing single or triple quadrupole time-of-flight, were selected for the determination antibiotics and non-target analysis.

In addition, the total phenolic content (TPC) and the antioxidant activity (AA) were determined in Galician honey samples with absorbance measured with ultraviolet-visible (UV-Vis) spectrophotometry.

5.2. GAS CHROMATOGRAPHY (GC)

5.2.1. Introduction

Gas chromatography (GC) is a separation technique based on the distribution of the analytes between a gaseous mobile phase, called carrier gas, and the stationary phase, which is usually a non-volatile liquid immobilized on the surface of a solid (chromatographic column). GC is usually chosen for the analysis of volatile or semi-volatile compounds that are thermally stable.

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5.2.2. Mass spectrometry

Mass spectrometry is an analytical technique that provides both qualitative information (elemental composition of samples or chemical structure of substances) and quantitative (composition of samples or isotope ratios) information from the ions issued from the molecules studied. The combination of gas chromatography and mass spectrometry (GC-MS) provide qualitative and quantitative information, since mass spectrometry offers selectivity, specificity and sensitivity. The GC-MS coupling is probably one of the preferred hybrid techniques [138].

The GC-MS consists of a gas chromatograph directly connected to a mass spectrometer. The sample is introduced from the chromatographic system to the mass spectrometer where the analytes are ionized. A mass spectrometer is composed of three main parts: the ion source, the mass analyser and the detector. Hybridization between GC and MS or tandem mass spectrometry (MS/MS) is easy, since both analytical methodologies operate in gas phase. The sample is introduced directly into the mass spectrometer from the gas chromatograph via a directly heated interface that starts at the outlet of the column located inside the gas chromatograph and extending to the ion source of the mass spectrometer. Next, the sample is ionized.

The electronic impact (EI) is one of the most employed ionization sources (hard source). In this case, the molecules are bombarded with electrons at a specific energy causing the emission of an electron and forming positive molecular ions (M+), as well as fragment ions. Then, the ions are sent to the analyser to consequently go to the detector according to their mass-to-charge ratio (m/z), producing the mass spectrum of the substance [138].

There are different types of analysers such as quadrupoles, time of flight, ion trap, among others. In the present thesis, quadrupoles were employed coupled to gas chromatography. They consisted of four cylindrical and parallel bars that serve as electrodes, electrically connected to each other in opposite pairs on which a variable

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radiofrequency potential is applied. These analysers act as mass filters, because they only allow ions with a certain m/z ratio to pass through to the detector. Ions that do not have the m/z ratios recognised by the quadrupole reach the bars (cylinders) and become neutral molecules. This kind of mass analyser instruments are the most compact, cheapest and most powerful [138,139]. There are different ion monitoring modes:

- Full scan (FS): all masses are registered without filtration.
- Selected ion monitoring (SIM): only ions with a specific mas-to-charge (m/z) are filtered.

Moreover, GC can be coupled to a tandem mass spectrometer (MS/MS) instead of a simple quadrupole (MS). The most common tandem mass spectrometer is the triple quadrupole mass spectrometer (QqQ) which consists of the combination of 2 quadrupoles and a collision cell to achieve the filtration of ions with a specific m/z ratio and their subsequent fragmentation. This instrument can work in different ion monitoring modes:

- FS as single quadrupoles.
- Selective reaction monitoring (SRM): the first (Q1) and third (Q3) quadrupoles filter specific m/z of the ions, thus working in SIM mode. First, Q1 filter some ions and, after fragmentation by the action of the collision gas (an inert gas such as argon) in the second quadrupole (q2) or collision cell, selected product ions are analysed in Q3. Employing this mode, interferences are minimized or even avoided, providing a sensitive and selective tool that allows chromatograms with very low noise. **Figure 5.1** describes the configuration of a triple quadrupole mass spectrometer working in SRM mode.

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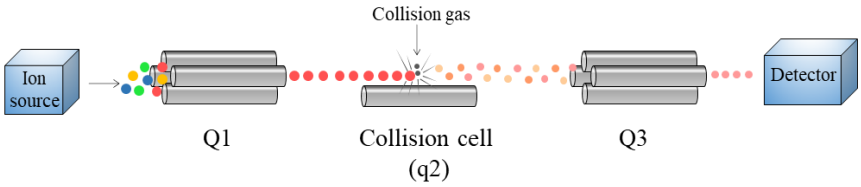


Figure 5. Scheme of a triple quadrupole mass spectrometer working in SRM mode.

GC coupled to MS in FS mode was applied for the analysis of volatile and semi-volatile compounds in honey (**Section IV**, Chapter 2.1). In addition, GC-MS/MS working in SRM mode was used for the determination of fungicides (**Section IV**, Chapter 1) and personal care products in cosmetic formulations (**Section IV**, Chapter 3).

5.3. LIQUID CHROMATOGRAPHY (LC)

5.3.1. Introduction

Liquid chromatography (LC) is based on the distribution of the analytes between a liquid mobile phase and a solid stationary phase (chromatographic column). The most commonly used liquid chromatography is partition chromatography (which was used in this doctoral thesis), but there are other types such as adsorption, ion exchange or molecular exclusion chromatography. In this case, the stationary phase consists of a packed column filled with silica porous particles coated with an organic film (octadecyl in this case) linked by chemical bonds. LC allows the separation of non-volatile, polar and/or thermally unstable compounds [138].

Ultra-high performance liquid chromatography (UHPLC) enables using of small particle-packed columns with small diameter, which withstand higher system pressures during chromatographic analysis. The advantages provided by UHPLC are and analysis time, a high peak resolution and excellent peak shape, as well as an increase in sensitivity [140].

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Both LC and UHPLC can be coupled to mass spectrometry.

5.3.2. Mass spectrometry

In this thesis, LC was used coupled to tandem mass spectrometry, using low-resolution triple quadrupole mass spectrometry, and high-resolution mass spectrometry (HRMS), employing a single or triple quadrupole time-of-flight analysers. As liquid samples cannot be introduced directly into the mass spectrometer, the ionization source must vaporize them transforming the molecules into gas-phase ions. Then, after leaving the ion source, the ions are filtered according to their m/z ratios in the analyser and passed to the detector [139].

There are different ion sources for generating gas-phase ions from the liquid phase and connecting LC with MS. The most commonly sources of atmospheric pressure ionization are atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI), both soft ionization sources. The latter, ESI, has been used in the present thesis, as well as HESI (heated electrospray ionization). In this source, first, a small flow of the sample solution is conducted through a capillary in which it is nebulized, forming charged droplets when a high voltage is applied. The droplets then shrink by evaporation of the solvent and disintegration of the droplets, and finally there is the emission or production of ions from the highly charged droplets (coulombic explosion) [141].

In the present work, LC-MS/MS has been employed to analyze phenolic compounds in honey samples in SRM acquisition mode (**Section IV**, Chapter 2.2). In addition, UHPLC-HRMS has been employed for the analysis of antibiotics and non-target compounds in water (**Section IV**, Chapter 4).

5.3.3. High-resolution mass spectrometry (HRMS)

High-resolution mass spectrometry (HRMS) provides MS/MS spectra and accurate monoisotopic mass measurements, which are

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highly applicable not only for the confirmation of targeted compounds but also for the identification of unknown compounds. In addition to the retention time and ion fragment ratio, HRMS also offers the exact mass, isotope profile and the possibility of spectral comparison against spectra libraries [142].

During this thesis, two approaches of HRMS based on single (QToF) and triple (QqTof) quadrupole time-of flight were used.

- The QToF operated in broadband collision-induced dissociation (bbCID), which is a Data Independent Acquisition (DIA) mode. It works in rapid and continuous cycles at low and high collision energy, simultaneously providing MS and MS/MS spectra and providing information on precursor ions and fragmentation patterns in a single analysis [143].

- The QqTof (TripleToF®) worked by a DIA approach based on SWATH^{TM} (Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra). MS/MS spectra are acquired for all precursor ions over a specified wide mass range, dividing the entire mass range into smaller m/z windows. This allows the analysis of each window to be repeated several times during the elution of a chromatographic peak; thus, for each detectable analyte in the sample passing through Q1, a complete MS and MS/MS spectrum is acquired. This tool also allows retroactive data mining without re-analysis, which is very useful for the identification of non-targeted unknown compounds [144]. **Figure 5.2** includes the scheme of a QqToF spectrometer.

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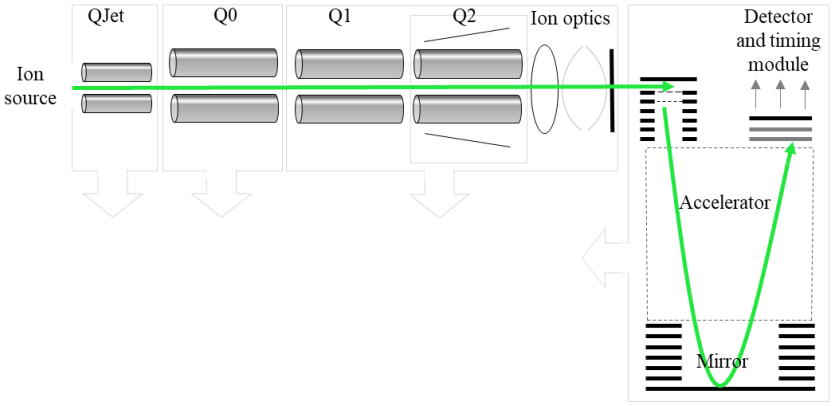


Figure 6. QqToF spectrometer scheme.

HRMS was utilized for the analysis of target antibiotics (QToF) and non-target approaches (TripleToF®) (**Section IV**, Chapter 4).

5.4. UV-Vis Spectroscopy

Spectroscopy is a science that studies the interaction of radiation with matter. UV-Vis molecular absorption spectroscopy is based on the measurement of transmittance (T) or absorbance (A). To perform this, a beam of light at a certain wavelength (λ, nm) is passed through a solution contained in a transparent bucket or cell which, depending on the spectral range of the measurement, is made of plastic, glass or quartz, and the amount of light absorbed by the solution is recorded/measured [139].

5.4.1 Total Phenolic Content and Antioxidant Activity

The chemical composition of honey can be studied in terms of total phenolic content (TPC) without having to selected phenolic compounds as targets. It can be determined according to the Folin–Ciocalteu (FC)

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colorimetric method described by Singleton and Rosssi [145], carrying out the measurement at 760 nm in a UV-Vis spectrophotometer.

Antioxidant activity (AA) is strongly correlated with the total phenolic content. It varies greatly depending on the botanical origin and its colour, while on a minor average it is considered to be affected by processing, handling and storage [146]. There is no official method to determine AA in honey, but several approaches can be applied as ORAC (oxygen radical absorbance capacity), FRAP assay (ferric reducing antioxidant power), DPPH scavenging (1,1-diphenyl-2-picrylhydrazyl), and TEAC (Trolox equivalent antioxidant activity) [147]. In this thesis, the AA was assessed employing the DPPH protocol by a modified method of Brand–Williams et al. [148] (absorbance measured at 515 nm).

Therefore, an UV-Vis spectrophotometer in the visible spectrum was employed to determine TPC and AA indexes in honey samples (**Section IV**, Chapter 2.2).

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III. METHODOLOGY

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The methodologies used throughout this thesis were based on sample preparation procedures that, as far as possible, should be miniaturized, simple, fast, low-cost, environmentally friendly and easy to implement in any laboratory.

Some of the procedures were based on solid-phase extraction technique that was used for the analysis of antibiotics in wastewater and natural water. This extraction technique was also applied for the determination of fungicides in water, in this case using by-products as an alternative and sustainable sorbent. Solid-phase microextraction (SPME) was optimized for the analysis of sixty personal care products (PCPs) in hydroalcoholic gels and, miniaturized SPME was applied for the determination of volatile and semi-volatile compounds in honey. In addition, micro-matrix solid-phase dispersion (μMSPD) was employed for the analysis of seventy-eight PCPs in cosmetic products of different nature and fabric phase sorptive extraction (FPSE), which can be considered as a combination of SPME and SPE, was used to determine fungicides in environmental waters.

On the other hand, as regards the analysis of fungicides in vine leaves as well as in grapes and soils, ultrasound assisted extraction (UAE) was utilized. This technique was also employed after vortex extraction (VE) to extract phenolic compounds from honey.

Chemometric tools were used for the optimization of the extraction efficiency of the sample preparation protocols mentioned above as well as for the result interpretation. These tools included design of experiments (DOE), analysis of variance (ANOVA), multiple-range least significant difference (LSD) tests and principal component analysis (PCA).

Instrumental determination was carried out employing chromatography coupled to mass spectrometry. Gas chromatography (GC) followed by mass spectrometry (MS) and tandem mass spectrometry (MS/MS) was used. Liquid chromatography (LC) coupled to MS/MS was also employed, and ultra-high performance LC (UHPLC) followed by high resolution mass spectrometry (HRMS), employing a single or triple quadrupole time-of-flight, was applied for target and non-target

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analysis operating in Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra (SWATH) acquisition mode.

In addition, the total phenolic content (TPC) and antioxidant activity (AA) of honey were measured by means of an ultraviolet-visible (UV-Vis) spectrophotometer.

As regards photodegradation experiments, a static laboratory-scale photoreactor using UVC radiation and advanced oxidation processes (AOPs), employing a tube-in-tube membrane reactor operating in continuous mode, were also tested for the removal of antibiotics in water.

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IV. RESULTS AND DISCUSSION

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CHAPTER 1. DETERMINATION OF FUNGICIDES IN AGRICULTURAL AND ENVIRONMENTAL SAMPLES

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Fungicides are a type of pesticides that are commonly used in viticulture and provide many benefits in terms of crop quality to prevent fungal infections. However, there are concerns about the presence of their residues on crops that may pose a risk to human health. Moreover, their continuous application induces their persistent entry into different compartments of the environment, and residues can easily reach aquatic ecosystems through leaching from crop soils. Therefore, it is necessary to evaluate the presence of these organic compounds in crops (leaves and grapes) as well as in the environmental compartments (soils and water).

In this chapter, three different works are included, concerning the identification and quantification of seventeen fungicides in natural waters, vine leaves, grapes and soils.

The first two studies deal with the simultaneous determination of fungicides in environmental waters. In the first one, a method based on FPSE followed by GC-MS/MS was developed. In the second one, cork by-products are proposed as green and sustainable sorbent of SPE for the determination of fungicides by gas chromatography coupled to tandem mass spectrometry.

The third work included an optimization and validation of an UAE methodology to extract fungicides from vine leaves. In addition, the presence of these organic compounds was evaluated in other types of leaves (tea), grapes and in the surrounding environment of vineyard areas, such as soil and water.

The three published articles are shown below.

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1.1.

Fabric phase sorptive extraction for the determination of 17 multiclass fungicides in environmental water by gas chromatography-tandem mass spectrometry

Maria Celeiro, Lua Vazquez, Piyaluk Nurerk, Abuzar Kabir, Kenneth G. Furton, Thierry Dagnac, Maria Llompart

Journal of Separation Science, 43 (2020), 1817–1829

ISSN: 1615-9306, Willey

DOI: 10.1002/jssc.201901232



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1.2.

Turning cork by-products into smart and green materials for solid-phase extraction - gas chromatography tandem mass spectrometry analysis of fungicides in water

Maria Celeiro, Lua Vazquez, Meruyert Sergazina, Santiago Docampo, Thierry Dagnac, Vitor J.P. Vilar, Maria Llompart

Journal of Chromatography A, 1628 (2020), 461437

ISSN: 0021-9673, Elsevier

DOI: 10.1016/j.chroma.2020.461437



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1.3.

Occurrence of fungicides in vineyard and the surrounding environment

Meruyert Sergazina, Lua Vazquez, Maria Llompart, Thierry Dagnac

Molecules, 26(20) (2021), 6152

ISSN: 1420-3049, MDPI

DOI: 10.3390/molecules26206152



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CHAPTER 2. CHARACTERIZATION OF HONEY: VOLATILE, SEMI-VOLATILE AND PHENOLIC COMPOUNDS

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Honey is a natural product mainly composed by sugars, water and other components like minerals, pollen grains and proteins among others. The identification of honey botanical origin is a valuable tool to assure honey quality, since its composition is strongly dependent on the plant species from which the nectar or honeydew was collected, and on other factors such as environmental climate and conditions.

In accordance with the geographical origin of the production, the European Union (EU) classifies Galician (NW Spain) honey under the Protected Geographical Identification (PGI) label, the only one with this registration in Spain. Hence, it is important to assess the chemical composition of honey, and especially Galician honeys, according to the aromatic and phenolic profiles.

This chapter contains two research articles. In the first study, a miniaturized methodology based on SPME followed by GC-MS was developed to determine volatile and semi-volatile compounds in honey samples from Galicia and foreign countries.

The second work involved a characterization of the content of phenolic compounds by LC-MS/MS in Galician honeys of different botanical origin, and aimed at searching possible chemical markers. In addition, the TPC and AA have been evaluated using an UV-Vis spectrophotometer.

Both studies have been published and are presented next.

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2.1.

Optimization of a miniaturized solid-phase microextraction method followed by gas chromatography mass spectrometry for the determination of twenty four volatile and semivolatile compounds in honey from Galicia (NW Spain) and foreign countries

Lua Vazquez, Maria Celeiro, Meruyert Sergazina, Thierry Dagnac, Maria Llompart

Sustainable Chemistry and Pharmacy, 21 (2021), 100451

ISSN: 2352-5541, Elsevier

DOI: 10.1016/j.scp.2021.100451



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2.2.

Evaluating the presence and contents of phytochemicals in honey samples: phenolic compounds as indicators to identify their botanical origin

Lua Vazquez, Daniel Armada, Maria Celeiro, Thierry Dagnac, Maria Llompart

Foods, 10(11) (2021), 2616

ISSN: 2304-8158, MDPI

DOI: 10.3390/foods10112616



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CHAPTER 3. DETERMINATION OF PERSONAL CARE PRODUCTS (PCPS) IN COSMETIC FORMULATIONS

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PCPs are a group of organic compounds that are added as ingredients to formulate cosmetic products of different nature. Since cosmetics are widely used all around the world, in order to ensure a high level of protection of human health, the EU established a Regulation (EC) Nº 1223/2009 that cosmetics must strictly comply with. Thus, this compliance must be verifiable, so greater analytical control of these compounds is necessary. In addition, PCPs are also considered emerging pollutants in the environment. Among PCPs, the most frequently found substances in cosmetic formulations are fragrance allergens, synthetic musks, preservatives, plasticizers and UV filters.

In this chapter, two works were carried out for the simultaneous analysis of PCPs in different cosmetic formulations by GC-MS/MS.

In the first study, the extraction of UV filters by µMSPD was optimized, and the method was extended to other families, including 78 compounds, in cosmetic samples of different nature.

Regarding the second work, a method based on SPME to determine fragrance allergens, synthetic musks, preservatives and plasticizers in hydroalcoholic gels was developed. This product has become one of the basic tools to prevent and mitigate transmission of COVID-19, being one of the most frequently diary used products worldwide.

Next, the two developed methods to analyse PCPs in cosmetic samples are presented.

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3.1.

Miniaturized matrix solid-phase dispersion for the analysis of ultraviolet filters and other cosmetic ingredients in personal care products

Maria Celeiro, Lua Vazquez, J. Pablo Lamas, Marlene Vila, Carmen Garcia-Jares, Maria Llompart

Separations, 6(2) (2019), 30

ISSN: 2297-8739, MDPI

DOI: 10.3390/separations6020030



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3.2.

Development of a solid phase microextraction gas chromatography tandem mass spectrometry methodology for the analysis of sixty personal care products in hydroalcoholic gels – hand sanitizers – in the context of COVID-19 pandemic

Lua Vazquez, Maria Celeiro, Ana Castiñeira-Landeira, Thierry Dagnac, Maria Llompart

Analytica Chimica Acta, 1203 (2022), 339650

ISSN: 0003-2670, Elsevier

DOI: 10.1016/j.aca.2022.339650



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CHAPTER 4. DETERMINATION OF ANTIBIOTICS IN WATER AND DEGRADATION STUDIES

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The presence of pharmaceuticals in water is growing due to the increased use of these substances. Within these compounds considered as emerging pollutants, one of the most interesting groups are antibiotics, which produce microbial resistance because of their irresponsible and irrational use. As they are only partially removed in wastewater treatment plants, these substances are continuously entering the environment implying a risk not only to the aquatic ecosystem but also to the human health. Therefore, alternative procedures such as UVC photolysis or advanced oxidation processes (AOPs) are needed, appearing to be an option for the efficient removal of antibiotics and other emerging pollutants. However, during degradation by-products may be formed, so that their identification is also of great importance.

This chapter includes two works. In the first study, a SPE-UHPLC-HRMS methodology was optimized and validated to analyse and quantify 14 multiclass antibiotics in waste and natural waters. A non-target analysis was also performed for the identification of other antibiotics and other emerging contaminants, as well as a preliminary photodegradation study. In addition, some by-products formed during photodegradation were subsequently identified using a non-target approach.

The second work is based on advanced oxidation processes employing a tube-in-tube membrane reactor for the simultaneous removal of the 14 target antibiotics in demineralized water and urban wastewater. Furthermore, transformation products were determined under the most optimal treatments.

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4.1.

Complementarity of two approaches based on the use of Quadrupole Time-of-Flight High-Resolution Mass Spectrometry for the determination of multi-class antibiotics in wastewater and natural waters. Photodegradation studies and non-targeted screenings

Lua Vazquez, Maria Llompart, Thierry Dagnac

Environmental Science and Pollution Research

Under revision for publication

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Complementarity of two approaches based on the use of Quadrupole Time-of-Flight High-Resolution Mass Spectrometry for the determination of multi-class antibiotics in wastewater and natural waters. Photodegradation studies and non-targeted screenings

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ABSTRACT

The increasing use of human and veterinary antibiotics with their ineffective removal in wastewater treatment plants constitutes a major environmental problem. The development of analytical methodologies to monitor different antibiotics families in water and the implementation of alternatives for their efficient elimination is a great challenge. A method based on solid phase extraction followed by ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry has been developed to analyse multiclass antibiotics, including macrolides, cephalosporins, fluoroquinolones, sulfonamides and diaminopyrimidines, in waters. Several parameters affecting the extraction were optimized leading to a method that was validated in real samples of different origins. Urban and hospital wastewater samples, as well as natural waters, were analysed, confirming the entrance of antibiotics into the environment, constituting a risk to the aquatic environment. Non-target analysis based on data independent workflow was also performed, enabling the identification of 94 pollutants. Preliminary photodegradation experiments were assessed being, to our knowledge, the first study including the simultaneous photodegradation of such a high number of multi-class antibiotics. After UVC irradiation, many target compounds were totally removed, showing photodegradation offered a powerful

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tool for water decontamination. The identification of by-products using a non-target approach was carried out, as well.

Keywords: antibiotics; solid phase extraction; UHPLC-QToF-MS; high-resolution mass spectrometry; non-target analysis; photodegradation by-products.

1. Introduction

The presence and persistence of pharmaceuticals in water is growing, due to the increasing use of these substances by the population and in veterinary medicine (Čizmić et al. 2017; Kosma et al. 2020). Within these compounds, one of the most interesting groups are the antibiotics, which produce microbial resistance because of their irresponsible and irrational use, being mainly excreted as the active parent chemical. As in wastewater treatment plants (WWTPs) they are only partially removed, these substances are continuously entering into the environment (Carballa et al. 2004; Christou et al. 2017; Kosma et al. 2020; Berges et al. 2021) implying a significant risk to the aquatic ecosystem and human health. Alternative procedures such as photodegradation appear to be an option for the efficient removal of antibiotics and other emerging contaminants. However, during photodegradation by-products may be formed, so their identification is also of great importance (Sanchez-Prado et al. 2006; Wang and Lin 2012; Batchu et al. 2014; Jia et al. 2021; Klementová et al. 2022). Although antibiotics are present as a mixture in the environment, the reported photodegradation studies were performed for only few antibiotics and at high concentrations (mg L-1) in comparison with the usual levels existing in real samples. Moreover, most studies only implied the evaluation of single analytes and substances of the same family.

The European Union commission has established a Watch List under the Water Framework to monitor unregulated substances in the European waters, which can cause a significant risk to or via the aquatic environment (Loos et al. 2018). The 1st Watch List that included macrolide antibiotics (azithromycin, clarithromycin, and erythromycin)

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among other substances (Decision 2015/495/EU 2015) was updated adding new antibiotics such as ciprofloxacin and amoxicillin (Decision 2018/840/EU 2018). Knowing that the duration of a continuous monitoring period of the watch list for any individual substance should not exceed four years, a 3rd Watch List has been established (Decision 2020/1161/EU 2020) and all substances involved in the 1st Watch List were removed. In this way, macrolide antibiotics were not included in this last Watch List, while other substances such as sulfamethoxazole and trimethoprim antibiotics were incorporated, and amoxicillin and ciprofloxacin were maintained.

As regards sample preparation, the European Union suggests using Solid Phase Extraction–Ultra High Performance Liquid Chromatography tandem Mass Spectrometry (SPE-UHPLC-MS/MS) as analytical method for the monitoring of these substances in waters, although a specific method is not proposed (Decision 2015/495/EU 2015; Decision 2018/840/EU 2018; Decision 2020/1161/EU 2020). As reported in literature, SPE is the most employed technique regarding the sample preparation procedure (Tuc Dinh et al. 2011; Gros et al. 2013; Čizmić et al. 2017; Mokh et al. 2017; Yuan et al. 2019; Martínez-Orgániz et al. 2021; Zheng et al. 2022) and less studies apply other extraction techniques such as solid phase microextraction (SPME) (Mitani and Kataoka 2006; Balakrishnan et al. 2006; McClure and Wong 2007), dispersive solid phase extraction (d-SPE) (Herrera-Herrera et al. 2013a; Wang et al. 2019), liquid–liquid extraction (LLE) (Ashfaq et al. 2016) and dispersive liquid–liquid microextraction (DLLME) (Herrera-Herrera et al. 2013b). Regarding the analytical determination of antibiotics in wastewater, LC-MS/MS has been the most employed technique (Tong et al. 2009; Tuc Dinh et al. 2011; Rossmann et al. 2014; Čizmić et al. 2017; Mokh et al. 2017; Yuan et al. 2019; Martínez-Orgániz et al. 2021). Other detectors were also used, such as Diode Arrary Detectors (DAD), Orbitrap Mass Spectrometry (Orbitrap MS), Time of Flight Mass Spectrometry (ToF-MS) and Quadrupole Time of Flight Mass Spectrometry (QToF-MS) (Turiel et al. 2005; Pedrouzo et al. 2008; Diaz et al. 2013; Tylová et al. 2013; Kosma et al. 2020).

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Among the SPE methods, different parameters have been studied to obtain the highest extraction efficiency and selectivity for different antibiotic families in water. The type of cartridge and the sample pH are some of the most evaluated factors (Tong et al. 2009; Gros et al. 2013; Rossmann et al. 2014; Čizmić et al. 2017). Nevertheless, most studies did not evaluate the elution volume, an important parameter to minimize the use of organic solvents and to achieve maximum extract enrichment, and the amount of sample. In addition, very few studies evaluate the accuracy in water samples of different characteristics, which could seriously compromise the validity of the results.

One of the aims of this work consisted of the optimization of the SPE-UHPLC-QToF-MS methodology, to analyze and quantify antibiotic residues of different families in water, some of them included in the 3rd Watch List (ciprofloxacin, sulfamethoxazole and trimethoprim), as well as macrolides (azithromycin, clarithromycin, erythromycin and roxithromycin), which were removed from the 1st Watch List. Other antibiotics such as cephalosporines (cephalexin), fluoroquinolones (enrofloxacin, norfloxacin and ofloxacin) and sulfonamides (sulfadiazine, sulfadimidine and sulfamerazine) were targeted, as well. After being optimized, the method was extensively validated and applied to different water matrixes such as urban wastewater (effluent and influent), hospital wastewater, river and well water, collected in Galicia (NW Spain) and Porto (N Portugal). As second approach, a SWATH non-target analysis was carried out by means of UHPLC-QToF-HRMS for the identification of other antibiotics and other emerging pollutants. Preliminary photodegradation experiments and a tentative identification of the main photodegradation by-products were also conducted being, to our knowledge, the first study including the simultaneous photodegradation of such a high number (14) of multi-class antibiotics.

2. Materials and methods

2.1. Chemicals, materials and samples

Formic acid, methanol and ultrapure water MS grade were supplied by Scharlab (Barcelona, Spain) and ammonium formate was from

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Sigma Aldrich Chemie GmbH (Steinheim, Germany). Ciprofloxacin, enrofloxacin, norfloxacin, ofloxacin, sulfadiazine, sulfadimidine, sulfamerazine, sulfamethoxazole and trimethoprim were supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany), clarithromycin, erythromycin and roxithromycin by Glentham Life Sciences Ltd. (Wiltshire, UK) and azithromycin and cephalexin by Apollo Scientific Ltd (Cheshire, UK). Clarithromycin-D3, norfloxacin-D5 and sulfamethoxazole-D4 were purchased from LGC GmbH (Luckenwalde, Germany). Individual stock solutions of each compound (concentration about 1000 mg L-1) were prepared in methanol except for ciprofloxacin and ofloxacin, prepared in a water solution of HCl 0.4 % and NaOH 1 M, respectively. Stock solutions were stored in glass vials and protected from light at -20 ºC. All solvents and reagents were of analytical grade. Target compounds, their acronyms, the CAS numbers, their LC retention times, and the quantification and identification ions to ensure an unequivocal identification of the studied compounds are summarized in Table 1.

Oasis HLB (60 mg, 3 mL), Oasis HLB (200 mg, 6 mL) and Oasis HLB Prime (150 mg, 3 mL) were from Waters (Milford, MA, USA). Strata-X (60 mg, 3 mL), Strata-X (200 mg, 6 mL) and Strata-X Pro (200 mg, 3 mL) were from Phenomenex (Torrance, CA, USA).

Different real water samples, including five effluents (EF) and an influent (INF) from urban WWTPs, two effluents from hospital wastewater (HW), a river water (RW) located near a hospital as well as a well water (WW) from an agricultural-livestock area, were collected in Galicia (NW, Spain) and Porto (Portugal). Individual samples of 1 L were collected in amber glass bottles, and they were transported and stored at 4 °C in the dark. The samples were vacuum-filtered through 0.45 μm glass microfiber filters GF/A (70 mm diameter, Whatman, Kent, UK) before the extraction.

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Table 1. Studied compounds. Acronym, CAS number, retention time, quantification and identification ions.

Family	Antibiotic	Acronym	CAS number	Retention time (min)	Quantification ions	Identification ions
Macrolides	Azithromycin	Azi	83905-01-5	4.95	749.5158	591.4215, 158.1176, 116.107, 83.0491
	Clarithromycin	Clar	81103-11-9	8.14	748.4842	158.1176, 558.3637, 590.384, 83.0491
	Clarithromycin-D3	Clar-D3	959119-17-6	8.14	751.5030	590.3884, 158.1534
	Erythromycin	Ery	114-07-8	6.99	734.4685	116.107, 158.1176, 522.3425, 540.3531, 558.3637, 576.3742, 83.0491, 735.4719
	Roxithromycin	Rox	80214-83-1	8.33	837.5318	838. 5318, 158.1176, 679.4376, 116.107, 522.3273
Cephalosporins	Cephalexin	Ceph	15686-71-2	4.07	348.1013	106.0651, 158.027, 349.1041
Fluoroquinolones	Ciprofloxacin	Cipro	85721-33-1	4.24	332.1405	231.0564, 288.1507, 314.1299, 333.1435
	Enrofloxacin	Enro	93106-60-6	4.44	360.1718	245.1085, 316.182, 342.1612, 361.1749
	Norfloxacin	Nor	70458-96-7	4.19	320.1405	233.1085, 276.1507, 300.1343, 302.1299, 321.1435
	Norfloxacin-D5	Nor-D5	1015856-57-1	4.19	325.1719	307.1613, 326.1749
	Ofloxacin	Oflo	82419-36-1	4.16	362.1511	261.1034, 318.1612, 344.1405, 363.1541
Sulfonamides	Sulfadiazine	SDZ	68-35-9	3.83	251.0597	108.0444, 156.0114, 176.0124, 185.0822, 92.0495, 96.0556, 252.0622
	Sulfadimidine	SDMD	57-68-1	4.37	279.0910	108.0444, 124.0869, 156.0114, 186.0332, 204.0437, 92.0495, 92.0495
	Sulfamerazine	SMZ	127-79-7	4.12	265.0754	108.0444, 110.0713, 156.0114, 190.0281, 199.0978, 287.0573, 92.0495, 266.0779
	Sulfamethoxazole	SMX	723-46-6	4.49	254.0594	108.0444, 156.0114, 160.0869, 188.0818, 92.0495, 93.0573, 99.0553, 255.0620
	Sulfamethoxazole-D4	SMX-D4	1020719-86-1	4.49	258.0845	112.0695, 160.0365, 68.0574, 96.0746, 99.0553, 259.0871
Diaminopyrimidines	Trimethoprim	TMP	738-70-5	4.02	291.1452	123.0679, 230.1162, 245.1033, 261.0982, 275.1139, 292.148

2.2. Sample preparation and photodegradation experiments

The optimized procedure was as follow: the Oasis HLB (60 mg, 3 mL) cartridges were conditioned with 2 mL of methanol followed by 2 mL of ultrapure water and, then, 50 mL of sample acidified at pH 3 with HCl 2 % (v/v) were loaded through the sorbent at a flow rate of 3 mL min^{-1}, using vacuum with the SPE manifold (Visiprep™, Supelco, USA). Before drying the adsorbent under vacuum, cartridges were rinsed with 2 mL of ultrapure water. Once the cartridge was dried, the concentrated compounds were eluted with 1 mL of methanol. In the case of Oasis HLB (200 mg, 6 mL) cartridges, 200 mL of water and 5 mL of methanol as elution solvent were employed.

The study of the pH, the type of cartridges and breakthrough volume were carried out employing ultrapure water spiked at 2 μg L^{-1} with the target antibiotics. Regarding the elution volume assessment, the ultrapure water was fortified at 0.2 μg L^{-1} and the elution was achieved with two consecutive 1 mL fractions of methanol for Oasis HLB (60 mg, 3 mL) and 2.5 mL for Oasis HLB (200 mg, 6 mL).

Matrix effect was also evaluated and isotopically labelled surrogates (clarithromycin-D3, norfloxacin-D5 and sulfamethoxazole-D4) were added to the samples (0.25 μg L^{-1}).

In order to carry out the photodegradation experiments, 3 mL of ultrapure water containing the target compounds at 100 μg L^{-1} were placed in two quartz cuvettes that were located inside a lab-scale photoreactor equipped with two UVC lamp ($\lambda = 254$ nm, 8 W and 11 W), at a distance of 9 cm from the lamps. Solutions were irradiated under UVC light for different times and, afterwards, they were transferred to a glass vial and directly analyzed by UHPLC-QToF-MS. Dark tests (without UVC radiation) were also performed to ensure that no antibiotic degradation occurred in the absence of light.

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2.3. Instrumentation

2.3.1. Target analysis and quantification

Target compounds were determined by ultra-high performance liquid chromatography (Elute UHPLC 1300) coupled to quadrupole time-of-flight mass spectrometry (QToF-MS) (Compact-II Instrument, Bruker Daltonics, Bremen, Germany). The chromatographic separation was achieved with an Intensity Solo HPLC column C18-2 (100 mm × 2.1 mm, 1.8 μm; Bruker Daltonics, Bremen, Germany) and a precolumn, kept at 40 °C. The gradient elution is described in Table S1.

The mass spectrometer was operated in the electrospray ionization (ESI) in positive mode. Ion source conditions were as follow: capillary 4500 V, nebulizer 29 psi, drying gas 8 L min^{-1}, gas temperature 200 ºC. The QToF mass spectrometer operated in broadband collision-induced dissociation (bbCID) acquisition mode that provided MS and MS/MS spectra at the same time. Spectra were recorded over the range m/z 30−1000 with a scan rate of 2 HZ. To obtain the MS spectra the bbCID mode works with a Low Collision Energy of 6 eV, and to acquire the MS/MS spectra a High Collision Energy of 30 eV was applied. The system was operated by Compass HyStar software and O-TOF Control version 5.2. Quantitation and processing software was TASQ Version 2.1 (Build 201.2.4019).

2.3.2. Non-target analysis - Photoproduct identification

Both the non-target analysis and the tentative identification of photoproducts were carried out using a UHPLC system (Shimadzu Nexera X2) consisting of two high-pressure pumps (LC-30AD) and a SIL-30AC autosampler. QToF-High Resolution Mass Spectrometry (QToF-HRMS) non-target detection was performed by a SCIEX (Ontario, Canada) TripleTOF®5600+ equipped with a DuoSpray™ ion source and an Electrospray Ionization (ESI) probe working in positive mode. The chromatographic separation was achieved with a Kinetex Biphenyl (50 × 2.1 mm, 2.6 μm) column from Phenomenex (Torrance, CA, USA), kept at 40 ºC with a CTO-30 A column oven. The mobile phase consisted of water (A) and methanol (B), both buffered with 3 mM ammonium formate and both including formic acid (0.15%). The

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flow rate was kept to 0.20 mL min^{-1}, employing an 8-minute gradient elution profile (10% B to 100% B) reaching a total runtime of 15 min, and 10 μL of sample were injected in the analytical column.

As regards the HRMS conditions, the source temperature was set at 550ºC, the ion source gas at 42 (au, arbitrary units), the curtain gas at 32 (au), and the ion spray voltage floating at 5500 V. The HRMS workflow consisted of a TOF Full Scan, using 220 ms as accumulation time and 80 V as declustering potential in the ESI. Simultaneously, a data independent approach based on SWATH (Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra) was performed. For that, a wide mass range (80–1200 Da) was divided in 30 mass windows with an accumulation time of 30 ms for each one. The resulting total scan time per cycle was 1.16 s. The declustering potential was set to 80 V and the collision energy was 40 V with an energy spread of 20 V. The system was operated by Analyst®1.7.1 control software for data acquisition and by SCIEX OS 1.5.0.23389 for data treatment and library search.

3. Results and Discussion

3.1. Evaluation of SPE procedure

Influence of pH

One of the most critical parameters affecting extraction efficiency of antibiotics in water is the sample pH. Although it has been included in other studies, in most cases the conclusions are not clear, especially for multi-class antibiotics. For this reason, the influence of the pH was evaluated (pH values 2, 3 and 5.5). Experimental conditions are described in Section 2.2., and the obtained results are depicted in Fig. 1a. As it can be seen, the pH value was not significant for sulfonamide antibiotics, trimethoprim and some fluoroquinolones (enrofloxacin and ofloxacin), which achieved high recovery values for all pH tested. On the other hand, although azithromycin, clarithromycin and ciprofloxacin achieved higher recovery values at pH 2, compounds such as erythromycin, which according literature is converted into its main

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degradation product (erythromycn-H2O) at pH lower than 7 (Tuc Dinh et al. 2011), and roxithromycin, only reached values about 3 and 19 %, respectively. By contrast, under pH 3, the recoveries of these two compounds were much higher, although for others, especially azithromycin, the efficiency decreased. When sample pH was 5.5, recoveries were lower for most of compounds. Thus, pH 3 was selected to perform further experiments since it allowed recovery values within acceptable ranges from 52 % to 105 % for the simultaneous extraction of all target compounds. These results are in concordance with other studies in which acidic conditions with pH between 2.5 and 4, were chosen as the most efficient (Gros et al. 2013; Rossmann et al. 2014; Čizmić et al. 2017).

Type of cartridge

With the aim of attaining the highest extraction efficiency, four commercial polymeric SPE cartridges were tested: Oasis HLB (200 mg, 6 mL), Oasis HLB Prime (150 mg, 3 mL), Strata-X (200 mg, 6 mL) and Strata-X Pro (200 mg, 3 mL) (see experimental conditions in Section 2.2). As can be seen in Fig. 1b (four first bars for each compound), no significant differences were observed among the tested cartridges and recoveries ranged between 48-108 %.

On the other hand, as one of the recurrent objectives of sample preparation is to make the analytical methods as green and sustainable as possible, the use of lower amounts of sorbent, elution solvent and sample was tested employing Oasis HLB and Strata-X (60 mg, 3 mL). Fig. 1b (two last bars for each compound) shows the recoveries obtained for both cartridges compared with those obtained with the previous four ones. The results were similar for all compounds. Therefore, these last two cartridges were selected as the best choice since they enable a scale down method with less consumption of sorbent (60 mg instead of 200 mg) and organic solvent (1 mL instead of 5 mL), and a higher enrichment factor.

The efficiency of commercially packed cartridges and manual packed cartridges prepared in the lab was compared using the same materials. Manual packed cartridges were prepared with 60 mg of

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sorbent, in a 2 mL polypropylene cartridge with a cellulose filter at the top and bottom. For all target antibiotics, recovery values were lower, with fluoroquinolone antibiotic percentages dropping up to 20 times (Fig. S1). Thus, the commercial packed cartridges were selected to perform the SPE of antibiotics.

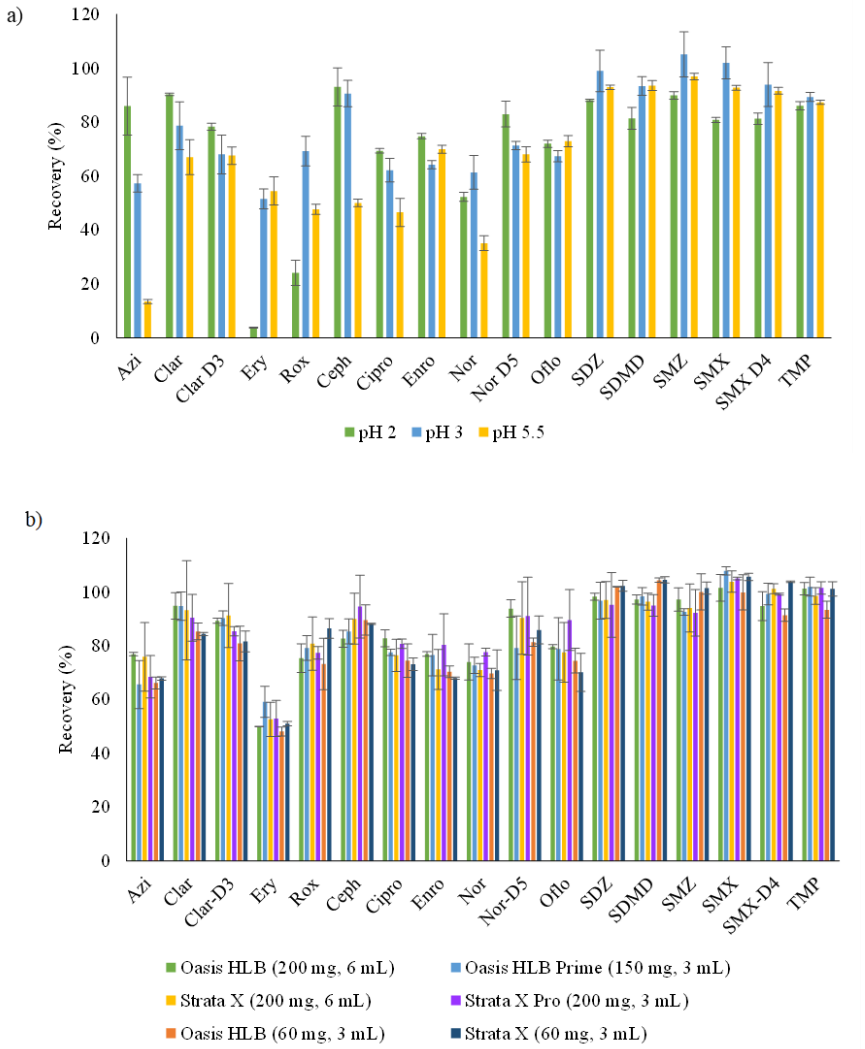


Fig. 1. Recovery of the SPE procedure evaluating: a) the influence of sample pH (2, 3 and 5.5) and b) different types of cartridges.

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Elution volume

In order to achieve the optimum enrichment factor of the target compounds and, taking into account that in most studies the elution is carried out with a higher volume of solvent (Gros et al. 2013; Čizmić et al. 2017), the elution volume influence was assessed collecting two consecutive and separate fractions of the SPE eluate (see Section 2.2). Recovery values for Oasis HLB (60 mg, 3 mL) depicted in Fig. S2a demonstrate that 1 mL of methanol was enough to elute the target compounds. In this case, recoveries in the second fraction were lower than 2 %. On the other hand, as can be seen in Fig. S2b, only three compounds were detected in the second fraction for Oasis HLB (200 mg, 6 mL). As clarithromycin recovery in the second 2.5 mL fraction was not negligible (7 %), 5 mL of solvent was selected as elution volume.

Hence, the use of 1 mL of methanol for Oasis HLB (60 mg, 3 mL) allowed a high enrichment factor and a lower consumption of organic solvent.

Breakthrough volume

Another important SPE parameter is the breakthrough volume that determines the maximum sample volume that can be loaded on the sorbent without analyte loss. Under the optimum conditions using Oasis HLB (60 mg, 3 mL) (see Section 2.2), different water sample volumes were evaluated: 10, 25, 50, 100 and 200 mL. As shown in Fig. S3, no breakthrough was observed for most of the target antibiotics. However, recovery values for erythromycin and cephalexin clearly decreased when the sample volume was greater than 50 mL, and recoveries of roxithromycin, enrofloxacin and trimethoprim started to decrease, as well. Therefore, 50 mL was selected as the optimum sample volume to perform the SPE procedure followed by the elution with 1 mL of MeOH, which implies an enrichment factor of 50 in case of quantitative recoveries.

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3.2. Method performance parameters

Calibration study was performed employing standard solutions prepared in methanol, containing the target compounds at different levels, covering a concentration range from 0.05 to 2000 µg L-1 (see specific ranges for each compound in Table 2) with fourteen levels and three replicates per level. The method showed a direct proportional relationship between the concentrations of each analyte and the chromatographic responses with coefficients of determination (R^2) higher than 0.9922 in all cases. The instrumental precision was evaluated within a day (n = 4) and among days (n = 6) at different concentration levels. RSD values for 50 µg L^{-1} are shown in Table 2. As can be seen, RSD values were for most compounds lower than 8 and 10 % for intra- and inter-day precision, respectively.

Recovery studies were carried out in ultrapure water at two fortification levels: 0.2 and 2 µg L^{-1} including all the target antibiotics. In all cases, fortifications were performed by triplicate employing two different SPE cartridges, Oasis HLB (60 mg, 3 mL) and Oasis HLB (200 mg, 6 mL), and were calculated by comparing the obtained chromatographic responses (area counts) with those obtained with standards prepared in methanol. As can be seen in Table 2, recoveries ranged between 60 and 104 % with RSD values lower than 10% for all target compounds, excluding erythromycin, giving recoveries about 40-50 %.

The LODs and LOQs of the method were calculated as the compound concentration giving a signal-to-noise ratio of three (S/N = 3) and ten (S/N = 10), respectively, since the target antibiotics were not detected in the whole method blanks. Oasis HLB (60 mg, 3 mL) and ultrapure water spiked at the lowest concentration level were employed to calculate the limits LODs and LOQs shown in Table 2, obtaining satisfactory results.

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Table 2. Method performance. Linearity, precision, mean recoveries in ultrapure water, LODs and LOQs.

Compound	Linearity		Recoveries in ultrapure water (%)				Mean recoveries in real samples (%)	Precision		LODs (ng L^{-1})	LOQs (ng L^{-1})
	Range (µg L^{-1})	R^2	0.2 µg L^{-1}		2 µg L^{-1}			RSD (%)			
			60 mg	200 mg	60 mg	200 mg		Intra-day (n=4)	Inter-day (n=6)		
Azi	0.1-1000	0.9961	64.3 ± 6.2	60.4 ± 2.3	66.1 ± 2.2	76.85 ± 0.67	104 ± 15	7.8	15	0.76	2.5
Clar	0.5-2000	0.9986	78.7 ± 4.5	77.3 ± 1.4	85.3 ± 3.1	94.8 ± 4.9	102.5 ± 5.6	2.5	15	2.8	9.3
*Clar-D3**			*73.5 ± 3.3*	*75.5 ± 4.3*	*75.1 ± 1.7*	*89.26 ± 0.93*		*1.1*	*11*	*1.2*	*4.1*
Ery	0.2-2000	0.9959	41.8 ± 4.6	39.2 ± 2.9	48.1 ± 1.7	49.92 ± 0.10	85.8 ± 7.0	2.1	7.6	3.0	10
Rox	0.2-2000	0.9993	61.5 ± 2.9	60.59 ± 0.28	73.2 ± 9.6	75.3 ± 5.3	100.9 ± 3.6	1.3	11	1.3	4.3
Ceph	5-2000	0.9962	86.43 ± 0.51	93.58 ± 0.92	89.5 ± 5.6	82.6 ± 3.2	78.2 ± 5.4	10	7.6	28	92
Cipro	0.2-2000	0.9948	78.4 ± 7.0	81.2 ± 4.8	78.8 ± 6.2	82.8 ± 3.2	89 ± 17	13	9.6	1.2	3.8
Enro	0.2-2000	0.9922	78.8 ± 6.0	76.1 ± 5.2	70.3 ± 2.2	76.87 ± 0.86	109 ± 16	7.3	7.5	1.2	3.7
Nor	1-2000	0.9926	73.0 ± 1.4	76.6 ± 4.6	69.7 ± 1.7	73.9 ± 6.7	77 ± 15	8.7	7.4	7.0	23
*Nor-D5**			*74.1 ± 7.0*	*82.7 ± 3.9*	*81.3 ± 1.5*	*93.8 ± 3.3*		*4.9*	*9.5*	*13*	*42*
Oflo	0.2-2000	0.9925	79.3 ± 4.4	73.81 ± 0.30	74.4 ± 4.6	79.7 ± 0.7	102 ± 11	6.1	12	1.3	4.4
SDZ	0.05-1000	0.9966	98.3 ± 5.4	96.1 ± 4.3	101.9 ± 1.1	98.2 ± 1.2	101.0 ± 9.0	2.9	8.0	0.37	1.2
SDMD	0.05-500	0.993	90.5 ± 1.8	93.96 ± 0.13	104.4 ± 1.0	97.2 ±1.7	94 ± 12	2.2	4.4	0.33	1.1
SMZ	0.1-1000	0.9955	89.8 ± 2.8	97.4 ± 4.3	100.0 ± 6.7	97.2 ± 4.3	96.8 ± 9.8	1.2	8.7	0.70	2.3
SMX	0.05-1000	0.9923	85.31 ± 0.93	96.0 ± 1.0	99.8 ± 6.6	101.5 ± 5.0	96.0 ± 5.8	3.0	11	0.40	1.3
*SMX-D4**			*95.9 ± 4.3*	*93.6 ± 1.6*	*91.3 ± 2.3*	*94.7 ± 5.3*		*3.4*	*9.0*	*1.7*	*5.6*
TMP	0.05-500	0.9927	91.4 ± 1.7	98.2 ± 3.4	93.4 ± 3.1	101.2 ± 2.3	93 ± 19	2.7	9.9	0.31	1.0

*Isotopically labelled surrogates were added at 0.25 µg L^{-1}.

Antibiotic recoveries in waters of different origin.

In order to assess the influence of the water matrix on the extraction process, recovery studies were performed in four real water matrixes of different origin at two concentration levels: 1 μg L^{-1} in well (WW) and river (RW); and 5 μg L^{-1} in effluents from urban (EF5) and hospital (HW2) wastewater treatment plants. In those cases, in which the compounds were initially detected in the samples, their responses were subtracted. Recovery values for ultrapure and the other water sample matrices are summarized in Fig. 2a. As it is observed, recovery values ranged between 40 and 90 % for most compounds, although especially for the most complex matrixes (urban and hospital wastewater), recoveries decrease for sulfonamides and trimethoprim, as well as for fluoroquinolone antibiotics. Thus, these differences observed between the recoveries obtained in different real water samples clearly show that the type of water matrix influences the extraction yield.

In an attempt to find out whether the low values were due to a low extraction efficiency or to matrix effects into the MS ionization source, some additional experiments were performed. SPE was applied to ultrapure water and the obtained methanol extracts were spiked at two different levels and compared with standard solutions. According to the results (Fig. S4a), no matrix effect occurred into the ionization source since responses were comparable between the fortified extracts and the standard solutions. In addition, recovery values between the spiked extracts and the obtained extracts after SPE using fortified ultrapure water were compared (Fig. S4b). Recovery values for extracts spiked after SPE were 40 % higher for erythromycin, demonstrating that the poor retention capacity of the sorbent was responsible of the low recoveries.

Consequently, isotopically labelled surrogate antibiotics have to be used to mitigate matrix effects. Three isotopically labelled compounds were selected according to their similarity with the compounds under study. In this way, clarithromycin-D3 was used to correct matrix effects for all macrolide antibiotics, norfloxacin-D5 for fluoroquinolones and sulfamethoxazole-D4 for sulfonamides and trimethoprim. Recovery values for cephalexin were not corrected since they were higher than 73

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% in all matrixes. As shown in Fig. 2b, the recoveries calculated using the isotopically labelled surrogates were quantitative for most compounds in the different water matrixes, achieving values above or close to 80 % in most cases even in complex matrices such as hospital and urban wastewater. In Table 2, the mean recovery values in real samples are included (individual values for each real sample can be seen in Fig. 2b).

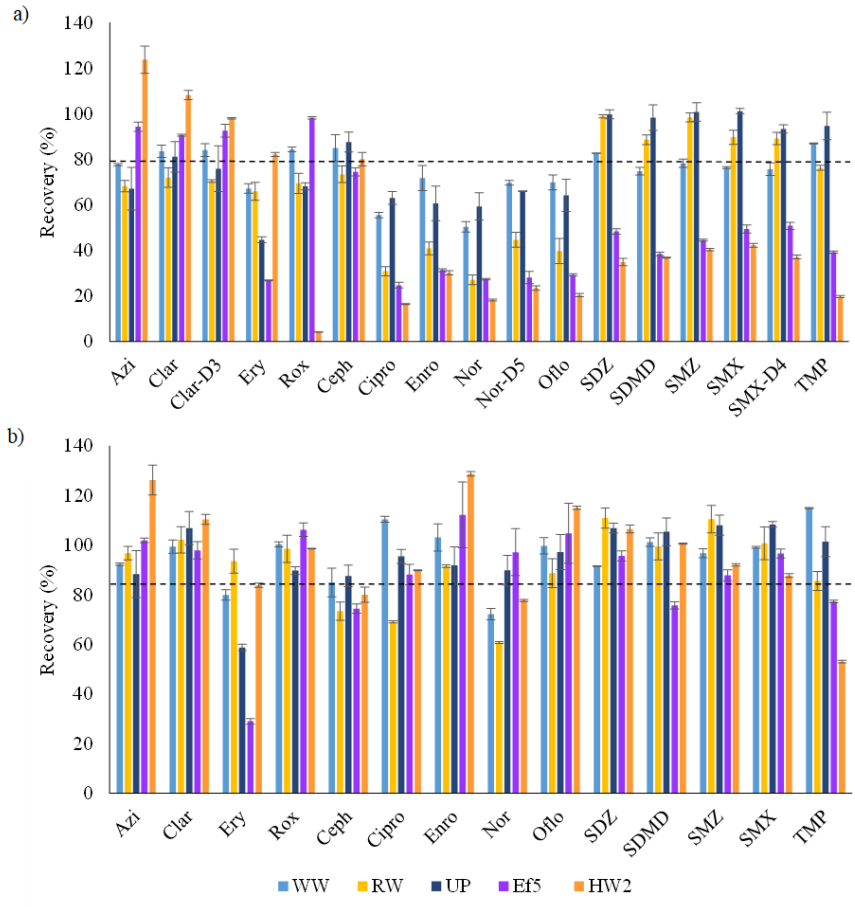


Fig. 2. Antibiotic recoveries obtained for real water matrixes fortified at 1 µg L $^{-1}$ for well (WW), river (RW) and ultrapure (UP) water and at 5 µg L $^{-1}$ for effluents from urban (EF5) and hospital (HW2) wastewater treatment plants: a) without considering isotopically labelled surrogates and b) considering isotopically labelled surrogates.

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3.3. Occurrence of antibiotics in water samples of different origin.

The presence of the studied macrolides, cephalosporins, fluoroquinolones, sulfonamides and diaminopyrimidines in real samples of different origin including urban wastewaters (Influent, INF and effluents, EF) hospital wastewater (HW), river (RW) and well water (WW) was investigated. Results are summarized in Table 3 and the detection frequency of the compounds in the urban and hospital wastewater samples is depicted in Fig. 3.

All target compounds were found in the samples except cephalexin and sulfamerazine. Azithromycin and trimethoprim were present in all the analyzed samples, followed by ciprofloxacin and ofloxacin detected in 9 out of the 10 samples, and enrofloxacin, norfloxacin and sulfamethoxazole in 8 samples. Clarithromycin, sulfadiazine and erythromycin were only present in wastewaters, at 75, 63 and 50 % detection frequencies, respectively (see Fig. 3). On the other hand, roxithromycin and sulfadimidine were only detected in one sample (EF5 and EF4, respectively). Regarding the number of compounds per sample, in two urban effluents (EF1, EF4) and in the influent (INF) up to 10 compounds of the 14 targets were detected. It is important to note that EF4 and INF were collected at a WWTP serving around 400,000 habitants, which can influence the presence of a high number of the target compounds. The other samples contained between 2 and 9 target compounds. Although the hospital effluents (HW1 and HW2) were not the samples with the highest number of detected compounds, they contained the highest concentrations in terms of sum of the target antibiotics (8.2 and 5.1 $\mu g\ L^{-1}$, respectively). Additionally, both hospital effluents also achieved the highest levels for some individual compounds, such as azithromycin and ofloxacin with concentrations up to 3.5 $\mu g\ L^{-1}$. These high concentrations for ofloxacin in hospital wastewater were in line with those reported by Gros et al. (2013) and the concentrations of the individual compounds in the effluent samples, which ranged between 0.0055 and 1.3 $\mu g\ L^{-1}$, were also similar with those obtained in other studies (Tylová et al. 2013; Gros et al. 2013; Rossmann et al. 2014). On the other hand, as it could be expected, in

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general fewer antibiotics were detected in river and well water and at lower concentrations (0.027 and 0.61 μg L^{-1} for the sum, respectively) than in wastewaters. The presence of 6 antibiotics in the well water sample may be due to its location in an agricultural-livestock area. Among the detected antibiotics, trimethoprim is classified as prudent use drugs by the Antimicrobial Advice Ad Hoc Expert Group (AMEG), and other four (ciprofloxacin, enrofloxacin, norfloxacin and ofloxacin) as antibiotics for use with limitation in cattle (European Medicines Agency 2019).

Therefore, these results clearly confirm the entrance of antibiotics into the environment, implying a significant risk to the aquatic environment and human health.

Table 3. Concentrations (μg L^{-1}) of the target antibiotics in different water samples. EF: urban effluent; INF: urban influent; HW: hospital effluent; WW: well water; RW: river water.

Comp.	EF1	EF2	EF3	EF4	EF5	INF1	HW1	HW2	WW	RW
Azi	1.3	0.15	0.49	0.67	0.81	0.056	3.4	1.9	0.0033	0.0077
Clar	0.14		0.24	0.11	0.19	0.10		0.034		
Ery	0.11				0.064	0.072		0.063		
Rox					0.043					
Cipro	0.15	0.10	0.22	0.11	0.14	0.08	0.66	0.18	0.23	
Enro	0.15	0.12	0.12	0.14	0.17	0.24			0.10	
Nor	0.22	0.15	0.27	0.28		0.83		0.21	0.072	
Oflo	0.88	0.074	0.19	0.62	0.18	0.43	3.5	2.5	0.19	
SDZ	0.010		0.010	0.010		0.095	0.35			
SDMD				0.0055						
SMX	0.12	0.029	0.023	0.091	0.15	0.087	0.10	0.073		
TMP	0.077	0.018	0.025	0.048	0.074	0.042	0.20	0.13	0.0016	0.019
Σantib.	3.2	0.64	1.6	2.1	1.8	2.0	8.2	5.1	0.60	0.027

Screening studies by a non-target SWATH approach

Unknown compounds were identified employing a data retrospective approach and spectral libraries containing more than 2.000 high-resolution mass spectra. SWATH workflow uses an

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identification protocol based on the exact masses of the unknown eluted compounds taking into account additional criteria for the confirmation such as the isotope profile and library score calculated with high-resolution MS/MS spectra. The combination of the three above-mentioned criteria provided a final combined score. All results showing a combined score below 50 were excluded from the final list of the tentatively identified compounds. The non-targeted compounds detected in the real samples and the related identification parameters are included in Table S2. The detection frequency of the non-target substances in the wastewater samples (urban and hospital) are summarized in Fig. 3.

Up to 94 compounds including antibiotics, anticonvulsants, analgesic, anti-inflammatory, β-blockers, stimulants and metabolites or pesticides among others were found in the analyzed samples. A high number of these compounds were found in the analyzed samples (Table S2), except in the well and river waters (6 and 10 compounds, respectively). As can be seen in Fig. 3, the most frequently detected compounds were the stimulant caffeine, the lipid-lowering agent fenofibric acid, the antiarrhythmic flecainide and the antipsychotic sulpiride, detected in the 100% of the wastewater samples, followed by the angiotensin receptor blockers irbersartan, telmisartan and valsartan, the insect repellent DEET and the non-steroidal anti-inflammatory fenbufen identified in 88 % of them.

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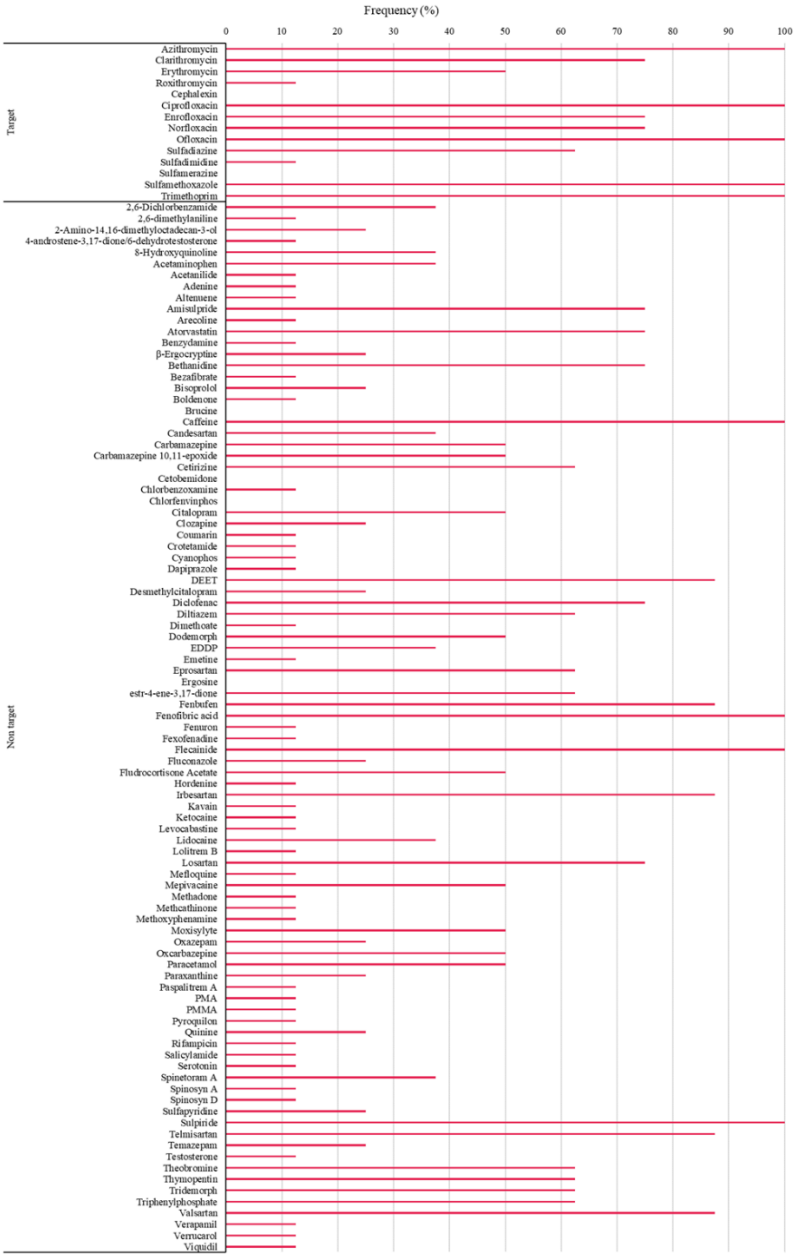


Fig. 3. Detection frequency of target and non-target compounds in urban and hospital wastewaters.

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3.4. Photodegradation experiments.

As it has been demonstrated, antibiotics can easily reach the aquatic environment, therefore the use and improvement of water decontamination procedures is of great interest to protect the environment. Thus, a preliminary study dealing with the simultaneous photodegradation of the 14 target multi-class antibiotics was carried out. The water solutions (100 µg L^{-1}) were irradiated under UVC light in a lab-scale photoreactor during different times: 5, 10, 20, 30, 40 and 60 min. The detailed experimental conditions are summarized in Section 2.2.

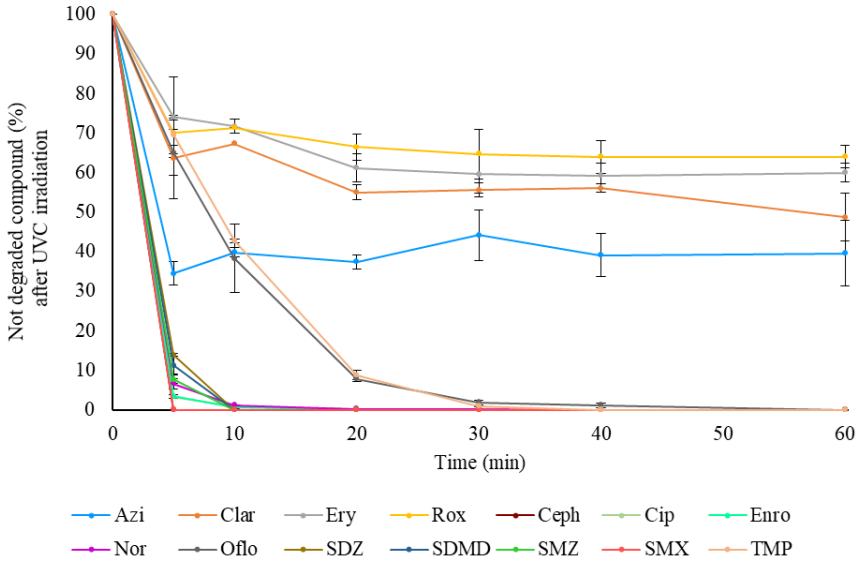


Fig. 4. Degradation kinetics observed for the 14 studied antibiotics under UVC irradiation. Initial concentration: 100 µg L^{-1}.

The degradation kinetics for the antibiotics are depicted in Fig. 4. As can be seen, cephalexin and sulfamethoxazole achieved complete degradation before the first 5 min. The other sulfonamide antibiotics and enrofloxacin were completely degraded after 5 min, and ciprofloxacin and norfloxacin after 10 min. Trimethoprim and ofloxacin reached a complete degradation, after 30 and 40 min, respectively. On the other hand, the macrolide degradation was much

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slower, reaching maximum degradation of 60 % within the first 5-20 minutes and remaining at the same percentage for all the time (60 min). Results were in concordance with those reported by Batchu et al. (2014) for UVC photolysis, also demonstrating the slow degradation of macrolide antibiotics (Batchu et al. 2014). Other photodegradation studies required longer times to remove the compounds (Trovó et al. 2009; Sirtori et al. 2010; Guo et al. 2013; Haddad and Kümmerer 2014).

Therefore, photodegradation seems to be a plausible option for the removal of most of the target antibiotics in water, and further studies including advanced oxidation processes could lead to efficient and novel procedures (Anjali and Shanthakumar 2019). Nevertheless, it is necessary to assess the possible generation of photoproducts as well as their stability.

Tentative identification of photoproducts by QToF-(SWATH)-HRMS

In addition to UHPLC for separation purposes, the triple TOF mass spectrometer was employed, implementing a data independent workflow (SWATH) to identify and characterize the highest number of photodegradation by-products of the targeted antibiotics. This non-targeted retrospective search of the maximum number of unexpected ions present in the samples enables performing fragmentation of all precursor ions entering the mass spectrometer in 20-30 m/z isolation windows.

A comprehensive bibliographic research dealing with degradation studies conducted with the targeted antibiotics in different aqueous media, and taking into account different types of photodegradation processes (solar radiation simulation or photocatalytic degradation) was carried out (Trovó et al. 2009; Sirtori et al. 2010; Sturini et al. 2012; Wang and Lin 2012; Guo et al. 2013; Haddad and Kümmerer 2014; Song et al. 2017; Wang et al. 2018; Zhang et al. 2019; Jia et al. 2021). It is important to underline that all these investigations only considered a few antibiotics and most of them dealt with only individual compounds or individual families. Therefore, it appears very necessary to develop multi-antibiotic determination methods since these

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substances are present simultaneously in natural waters. Hence, based on this quite exhaustive literature searching regarding the degradation of the 14 targeted precursor antibiotics, about 182 likely by-products could be identified.

As first approach, the by-products identification was achieved after importing a list (actually, chemical formula - m/z precursor ions were imported) of the 182 suspected targeted by-products and metabolites. This list was imported to SCIEX-OS software –Analytics. Twenty out of the 182 proposed by-products could be identified based on their exact mass and on the main fragments in their high-resolution MS/MS spectra. The molecular formula, precursor ion, mass error, structure and the bibliographic references of the identified photoproducts are summarized in Table 4.

Most of the identified by-products were detected at the maximum intensity within the first 5 and 10 min, except the transformation products from macrolides.

Among the 20 detected by-products, 7 of them were transformation products of fluoroquinolones, all of them reaching the maximum intensity at the experimental times of 5 and 10 minutes. Ciprofloxacin was the fluoroquinolone from which the highest number of degradation products could be identified (4), which arose from the breakdown of the piperazine ring, hydroxylation, and substitution of fluorine and defluorination. The photoproduct obtained from ofloxacin has the same molecular formula as one stemming from ciprofloxacin, hence the same exact mass ($C_{17}H_{18}O_4N_3F$, m/z 341.135).

On the other hand, up to 12 transformation products of the four targeted macrolides could also been detected and identified. The chemical formulas were imported from a recent study conducted by Jia et al. (2021), in which the transformation products of these four compounds were comprehensively evaluated. The identified by-products appeared with the highest intensity after 20 and 40 minutes of irradiation, while one of them (Clar-3) reached the highest intensity at the maximum study time (60 min). Most of the degradation reactions involved demethylation, hydroxylation or combination of both.

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Roxithromycin can also degrade into erythromycin as first stage, the latter giving rise to a final by-product with m/z 720.4528 as exact mass ($C_{36}H_{65}NO_{13}$). The main fragment observed in the MS/MS spectrum at m/z 562.3533 resulted from the loss of cladinose moiety. In addition, one by-product for each macrolide antibiotic could be identified with this chemical formula and exact mass (see Table 4). Therefore, it was not possible to differentiate them by HRMS without comparing their responses with those of their related standards.

The last characterized transformation product was identified as a by-product of sulfamerazine. It is formed by cleavage of the S-N bond and its maximum intensity was detected after 20 minutes of irradiation.

The highest responses were reached for two degradation products of clarithromycin and one of erythromycin, with m/z 748.4842 ($C_{38}H_{69}NO_{13}$), m/z 764.4791 ($C_{38}H_{69}NO_{14}$) and m/z 750.4634 ($C_{37}H_{67}NO_{14}$), as exact masses, respectively. The MS/MS spectra of these three degradations products also showed a main fragment resulting from the loss of cladinose moiety. As examples, the chromatographic peaks, isotopic profiles and MS/MS spectra of 4 of the identified by-products are shown in Fig. S5.

As second approach, a full non-targeted search was conducted using the same SWATH acquired data, but without entering any candidate precursor ion of likely by-products. The not irradiated "t=0" water sample was taken as reference sample. In this case, additional criteria besides exact mass of precursor ion was employed, such as mass accuracy of fragments, isotope score and formula finder score. High-resolution MS/MS spectra were also available in some cases.

About 30 precursor ions were identified in the different water samples in which photodegradation products were studied (irradiation times 5, 10 or 20 minutes). Among these 30 precursors, 7 were already present in the list of the 20 by-products previously identified (Table 4), thereby confirming the presence of these 7 degradation products. These precursor ions were m/z 245.093 (Cipro-2), 316.133 (Cipro-4), 348.135 (Cipro/Oflo-1), 732.451 (Ery-2), 750.463 (Ery-3), 764.478 (Clar-4) and 765.510 (Azi-2).

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Table 4. Data for the photodegradation products: parent compounds, photoproducts, molecular formulae, precursor ions, structures, mass errors, exposure times with maximum response and bibliographic references.

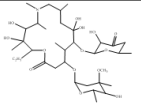
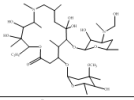
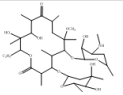
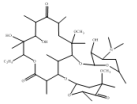
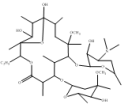
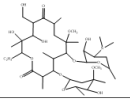
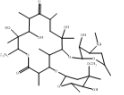
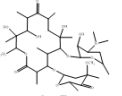
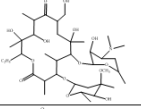
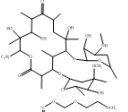
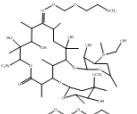
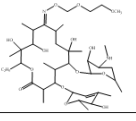
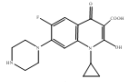
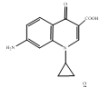
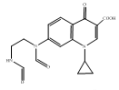
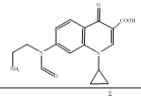
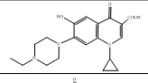
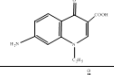
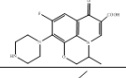
Parent compounds	Photoproducts	Molecular Formulae	Precursor ions (m/z)	Structures	Mass errors (ppm)	Exp times with maximum response (min)	Ref.
Azi	Azi/Clar/Ery/Rox-1	$C_{36}H_{65}NO_{13}$	720.453		-2.9	20	Jia et al. 2021
	Azi-2	$C_{38}H_{72}N_2O_{13}$	765.511		-2.1	30	
Clar	Azi/Clar/Ery/Rox-1	$C_{36}H_{65}NO_{13}$	720.453		-2.9	20	
	Clar-2	$C_{38}H_{67}NO_{13}$	746.469		-0.2	30	
	Clar-3	$C_{38}H_{69}NO_{13}$	748.484		-1.8	60	
	Clar-4	$C_{38}H_{69}NO_{14}$	764.479		-1.2	40	

Table 4. (cont.) Data for the photodegradation products: parent compounds, photoproducts, molecular formulae, precursor ions, structures, mass errors, exposure times with maximum response and bibliographic references.

Parent compounds	Photoproducts	Molecular Formulae	Precursor ions (m/z)	Structures	Mass errors (ppm)	Exp times with maximum response (min)	Ref.
Ery	Azi/Clar/Ery/Rox-1	$C_{36}H_{65}NO_{13}$	720.453		-2.9	20	Jia et al. 2021
	Ery-2	$C_{37}H_{65}NO_{13}$	732.453		-1.4	30	
	Ery-3	$C_{37}H_{67}NO_{14}$	750.463		-1.3	40	
Rox	Azi/Clar/Ery/Rox-1	$C_{36}H_{65}NO_{13}$	720.453		-2.9	20	
	Rox-3	$C_{41}H_{76}N_2O_{16}$	853.527		-1.3	30/40	
	Rox-4	$C_{39}H_{70}N_2O_{14}$	791.492		-4.2	40	

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Table 4. (cont.) Data for the photodegradation products: parent compounds, photoproducts, molecular formulae, precursor ions, structures, mass errors, exposure times with maximum response and bibliographic references.

Parent compounds	Photoproducts	Molecular Formulae	Precursor ions (m/z)	Structures	Mass errors (ppm)	Exp times with maximum response (min)	Ref.
Cipro	Cipro/Oflo-1	$C_{17}H_{18}O_4N_3F$	348.135		0.4	10	Haddad and Kümmerer 2014; Trovó et al. 2009; Wang et al. 2018
	Cipro-2	$C_{13}H_{12}N_2O_3$	245.092		-0.5	5	Guo et al. 2013; Wang et al. 2018
	Cipro-3	$C_{17}H_{17}N_3O_5$	344.124		-0.2	5	Haddad and Kümmerer 2014
	Cipro-4	$C_{16}H_{17}N_3O_4$	316.129		-0.2	5	Haddad and Kümmerer 2014
Enro	Enro-1	$C_{19}H_{23}O_4N_3$	358.176		2.5	5	Sturini et al. 2012
Nor	Nor-1	$C_{12}H_{12}O_3N_2$	233.092		-0.3	5	Zhang et al. 2019
Oflo	Cipro/Oflo-1	$C_{17}H_{18}O_4N_3F$	348.135		0.4	10	
SMZ	SMZ-1	$C_5H_7N_3$	110.071		2.0	20	Song et al. 2017

For the remaining 23 precursors, although the exact mass was established, it was not possible to identify the chemical structure or the compound from which they were formed. With this full non-targeted approach, it would be necessary to carry out studies for each individual compound. Nevertheless, the results showed that this tool could offer an alternative to identify transformation products.

4. Conclusions

A solid phase extraction methodology followed by ultra-high performance resolution liquid chromatography- quadrupole time-of-flight mass spectrometry (UHPLC-QToF-MS) was optimized for the simultaneous analysis of macrolides, cephalosporins, fluoroquinolones, sulfonamides and diaminopyrimidines in water samples. The optimum conditions implied the use of Oasis HLB (60 mg, 3 mL) as solid phase extraction, 50 mL of sample acidified to pH 3, and 1 mL of methanol as elution solvent. The methodology was successfully validated, and matrix effects were assessed demonstrating than the use of isotopically labelled surrogates was necessary. Finally, the validated method was applied to different water matrixes including urban (effluent and influent) and hospital wastewater, well water and river water, putting in evidence the presence of all target antibiotics in these real samples. In addition, a non-target approach based on a SWATH workflow was performed, demonstrating the presence of up to 94 compounds with a wide variety of substances including pesticides, drugs, and pharmaceuticals in all samples. The UVC photodegradation kinetics showed the total removal of many target compounds after less than 30 minutes of irradiation and twenty photodegradation by-products could be identified by means of the same SWATH workflow. Therefore, photodegradation appears as a good alternative to simultaneously remove the target antibiotics in ultrapure water, although studies in other water matrixes are also needed. However, for some compounds, especially macrolide antibiotics, which remained partially no degraded, advanced oxidation processes (AOPs), are deemed necessary to effectively remove these compounds and prevent them from reaching aquatic compartments.

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Supplementary material

Table S1. Gradient elution program for the liquid chromatography separation method.

Retention (min)	Flow (mL min^{-1})	% A	% B
0.0	0.200	96.0	4.0
0.1	0.200	96.0	4.0
1.0	0.200	81.7	18.3
2.5	0.223	50.0	50.0
14.0	0.400	0.1	99.9
16.0	0.480	0.1	99.9
16.1	0.480	96.0	4.0
19.0	0.480	96.0	4.0
19.1	0.200	96.0	4.0
20.0	0.200	96.0	4.0

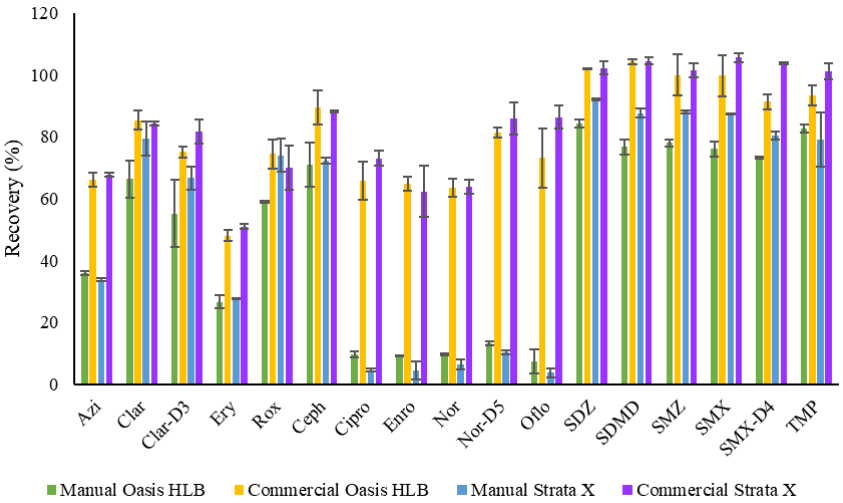


Fig. S1. Antibiotic recovery comparison between the manual and commercial cartridges for Oasis HLB and Strata X.

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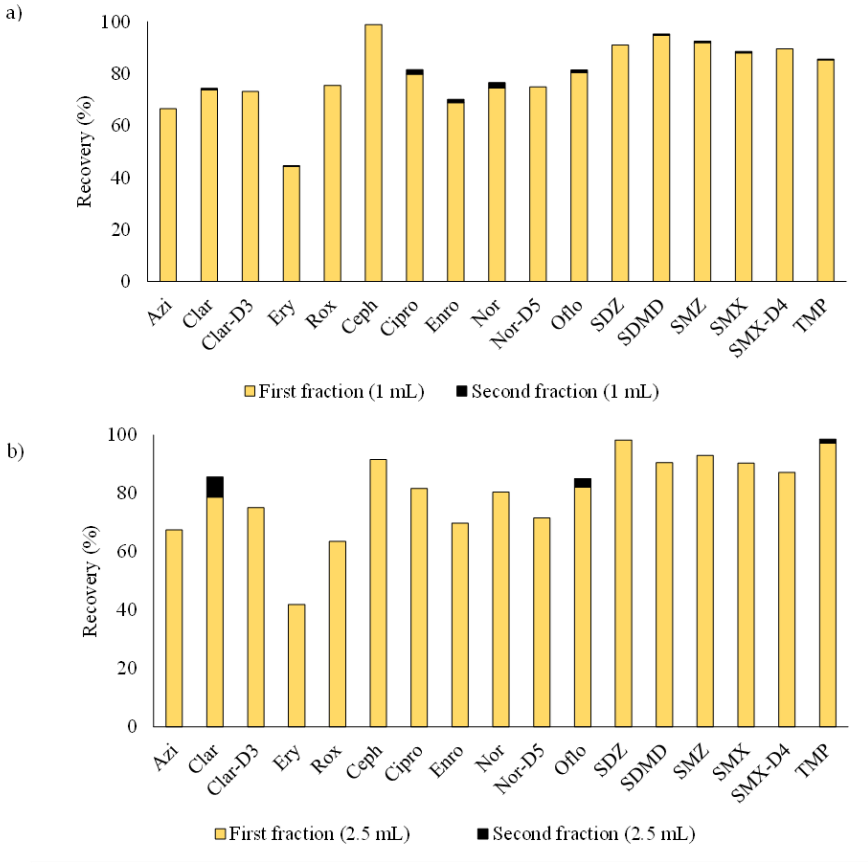


Fig. S2. Study of the elution volume on SPE efficiency for the target compounds: a) Oasis HLB (60 mg, 3 mL); and b) Oasis HLB (200 mg, 6 mL), respectively.

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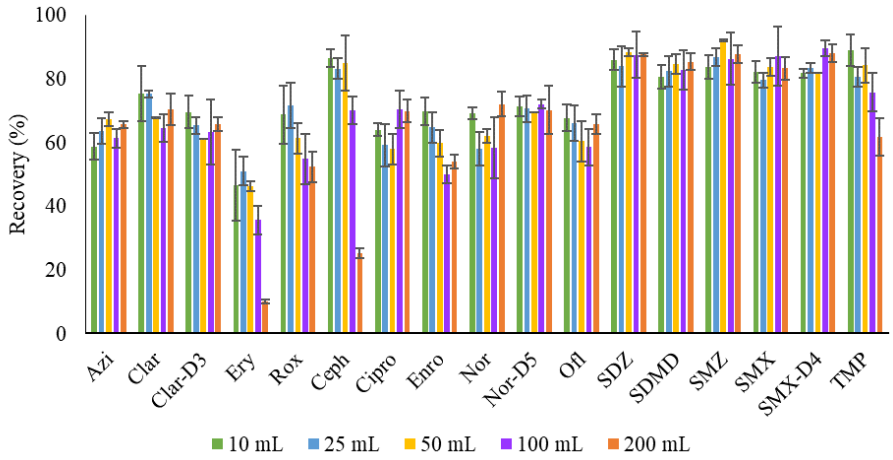


Fig. S3. Study of the sample breakthrough volume and its influence on SPE antibiotic recoveries.

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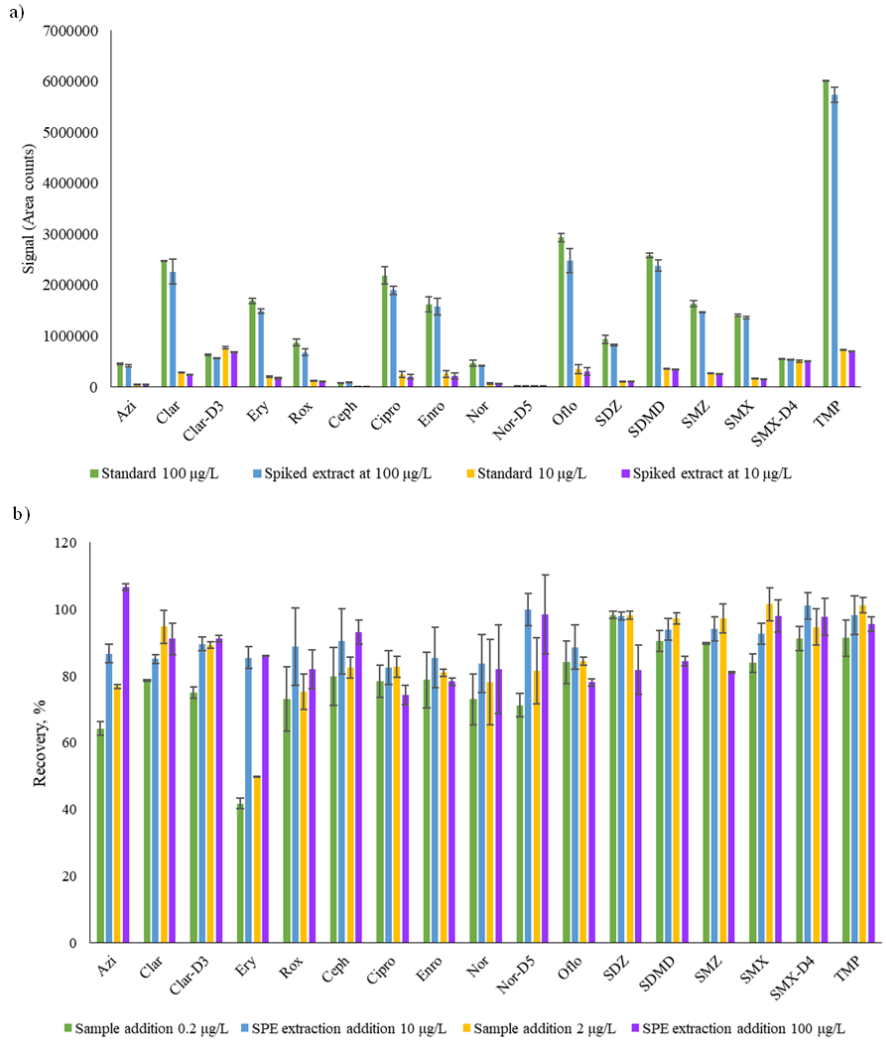


Figure S4. Evaluation of matrix effect into the ionization source: a) comparison of standard solutions and spiked extracts after SPE; b) recovery values between the spiked extracts and the obtained extracts after SPE, using fortified ultrapure water.

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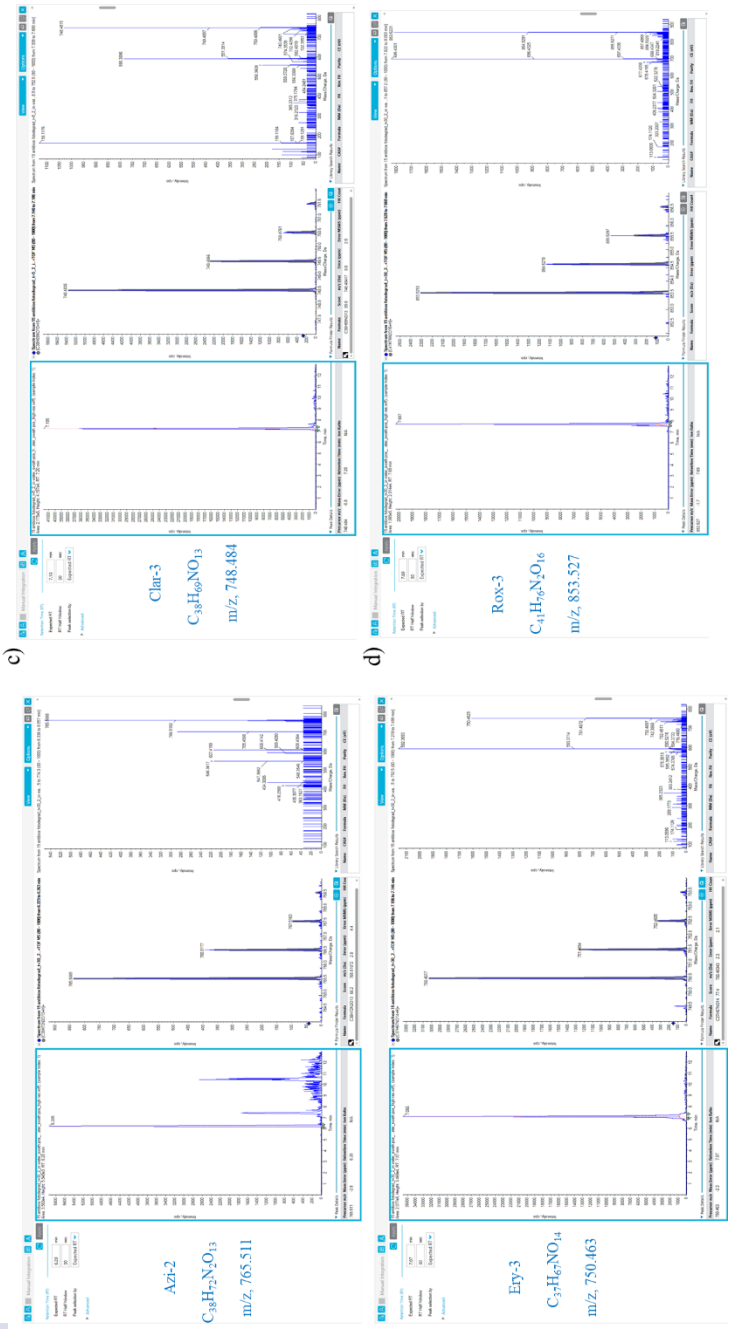


Fig. S5. Chromatographic peaks, isotopic profiles and MS/MS spectra of the photodegradation products: a) Azi-2; b) Clar-3; c) Ery-3 and d) Rox-2.

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Table S2. Non-target compounds identified in real water samples by QToF-HRMS.

Compound	RT (min)	Precursor Mass (m/z)	Sample	Peak Area	Signal Intensity	Mass error (ppm)	Library score	Combined score	Isotope Ratio Difference
2,6-Dichlorbenzamide	5.61	189.982	EF2	169500	24190	-2.2	97.6	83.0	3.8
			EF3	145000	23100	0.1	97.7	66.2	6.4
			EF4	70590	10840	-3.2	97.3	76.2	5.1
			INF1	70310	12330	-4.5	96.8	58.0	15.1
2,6-dimethylaniline	3.78	122.096	EF2	47440	1470	0.4	85.4	78.2	11.9
2-Amino-14,16-dimethyloctadecan-3-ol	8.58	314.342	EF3	174100	28830	0.5	93	88.3	6.1
			HW2	133300	26920	0.4	94.2	88.0	4.5
4-androstene-3,17-dione/6-dehydrotestosterone	8.59	287.201	HW2	31680	4804	-2.5	96.2	70.6	9.7
8-Hydroxyquinoline	3.93	146.06	EF2	57450	7753	-0.5	96.8	88.8	5.3
			EF3	73650	10700	-3.8	50.6	60.8	8.7
			HW2	661300	83450	-1.8	97.1	87.6	3
			RW	9606	1180	-7.2	93.4	51.7	10.7
			WW	16650	2590	-7.2	97.6	57.2	5.2
Acetaminophen	2.13	152.071	HW1	185500	19890	-3.4	91.3	59.1	4.1
			HW2	122000	11260	-1.4	86.5	61.8	5.1
			INF1	3034000	260100	-1.4	97	87.4	1.9
			RW	31530	3103	-3.6	69.4	71.8	1.7
Acetanilide	4.52	136.076	INF1	20520	2729	1.5	100	69.0	95.5
Adenine	0.91	136.062	INF1	69310	9077	-1	99.6	90.5	1.3
Altenuene	6.88	293.102	EF4	24520	4180	5.9	99.6	50.5	39.8
Amisulpride	5.14	370.18	EF1	862300	129200	-0.8	100	93.2	2.3
			EF4	652900	97400	-0.2	100	95.3	1.9
			EF5	1093000	164200	-2.7	100	80.4	4.3
			HW1	137700	21840	0.3	99.1	82.2	11.7
			HW2	404100	59660	0.4	100	86.4	7.3
			INF1	479900	69460	-0.7	100	92.1	3.1
Arecoline	4.32	156.102	EF3	39620	5317	0.3	98.7	72.5	1.1

Table S2. (cont.) Non-target compounds identified in real water samples by QToF-HRMS.

Compound	RT (min)	Precursor Mass (m/z)	Sample	Peak Area	Signal Intensity	Mass error (ppm)	Library score	Combined score	Isotope Ratio Difference
Atorvastatin	7.97	559.26	EF1	82050	13760	0.6	100	85.3	9.1
			EF2	32900	5762	-0.9	89.7	82.1	7.7
			EF3	20360	3361	0.4	83.8	67.1	37
			EF4	69900	11570	0.4	100	80.8	12.6
			HW2	32120	6669	-0.1	94.6	76.5	15.7
			INF1	72500	13280	-0.9	100	77.9	13
Benzydamine	7.1	310.191	HW2	1240000	198200	0.3	97.8	95.8	0.9
ß-Ergocryptine	7.14	576.318	EF5	1515000	216500	-1.2	96	80.0	9.7
			HW1	106500	17330	3.9	51.8	61.6	7.7
			RW	1466000	232200	3.4	74.1	66.6	7.7
			WW	376200	53060	3.6	60.9	66.0	4.6
Bethanidine	4.4	178.134	EF1	152200	23230	-2.9	85.2	74.2	7.1
			EF3	44110	5600	-1.2	86.8	69.1	17.9
			EF4	456000	67470	-1.9	86.1	84.1	2.9
			HW1	260800	35610	-1.5	85.8	83.9	4.6
			HW2	84110	13210	-3.6	84.5	76.8	1.2
			INF1	579000	83640	-2.5	85.9	82.4	1.8
Bezafibrate	7.27	362.115	HW1	80070	14220	-0.8	96	77.0	13.3
Bisoprolol	6.15	326.233	EF5	344000	57710	-5.8	99.1	56.8	2.8
			HW1	336000	45570	-0.7	99.4	59.1	11.1
Boldenone	8.59	287.201	HW2	31680	4804	-2.5	54.4	60.2	9.7
Brucine	8.00	395.197	WW	39370	5363	0.8	97.9	80.8	10.1
Caffeine	5.32	195.088	EF1	137900	18150	-0.5	83.3	80.5	8.4
			EF2	87480	13260	-3	93.9	78.8	4
			EF3	292300	38820	-0.4	96.2	95.3	0.9
			EF4	162100	24380	-1.8	79.4	80.0	2.4
			EF5	195400	33580	-1.6	97.3	61.0	7.4
			HW1	16490000	2086000	-0.9	98.4	93.5	0.4
			HW2	12880000	1824000	0.5	98.9	95.0	0.5
			INF1	5563000	699700	-1.3	98.3	89.1	1.6
			RW	94500	16690	-0.1	76.4	66.7	1.8

Table S2. (cont.) Non-target compounds identified in real water samples by QToF-HRMS.

Compound	RT (min)	Precursor Mass (m/z)	Sample	Peak Area	Signal Intensity	Mass error (ppm)	Library score	Combined score	Isotope Ratio Difference
Candesartan	7.58	441.167	EF3	99990	16920	-3.1	87.2	75.2	4.6
			EF4	44030	6331	-0.6	76.9	69.1	14.9
			HW2	84020	14370	-0.1	77.8	85.1	4.9
Carbamazepine	7.02	237.102	EF1	93180	13710	-0.8	95.7	71.5	17.8
			EF2	21080	3427	1.7	91.7	67.7	18
			EF4	65430	10520	-0.5	88.4	85.0	6.8
			HW2	25350	5385	-2	50	55.0	19.5
Carbamazepine 10,11-epoxide	5.69	253.097	EF1	184400	26330	-0.9	97.3	86.6	4.3
			EF4	163100	23230	-0.8	95.2	91.5	1.9
			EF5	499200	76060	-0.9	98	84.3	5.7
			INF1	74610	16000	-0.6	91.3	81.0	8.9
Cetirizine	7.43	389.163	EF1	103100	17720	-0.9	98.6	61.5	8.8
			EF3	81660	13210	0.7	98.6	69.5	2.6
			EF4	86290	12280	0.6	98.7	84.8	7.5
			HW1	334900	50060	-0.5	98.3	88.5	5.9
			HW2	173300	29470	-0.6	98.5	89.7	3.8
Cetobemidone	4.71	248.165	WW	58280	7664	-4.1	76.6	56.6	1.8
Chlorbenzoxamine	6.18	435.22	HW2	125400	15840	0.7	83	58.3	8.5
Chlorfenvinphos	8.24	358.977	RW	60750	9495	0.5	100	94.2	1.6
Citalopram	6.44	325.171	EF1	180600	30870	-3.4	100	59.2	34.6
			HW1	292200	49020	0.9	100	84.8	7.2
			HW2	320500	53910	3.9	98.3	54.8	22.9
			INF1	103700	14930	-3.2	100	59.7	27.1
Clozapine	6.3	327.137	EF3	29120	4554	2.4	91.4	60.7	34.1
			HW1	1792000	306800	0.3	99.4	90.7	5.9
Coumarin	6.41	147.044	HW2	86040	13060	0	93.5	71.1	52
Crotetamide	5.94	227.175	HW1	350500	50200	-3.1	99.6	82.8	1.5
Cyanophos	5.00	244.019	HW2	3088	639	0.5	73.8	66.2	119.4
Dapiprazole	6.15	326.234	HW1	323400	45070	-3.1	84.4	53.5	22.7
DEET	7.24	192.138	EF1	277800	43980	-2.1	82.1	83.8	0.4
			EF2	127600	18440	-1.4	82.5	61.6	4.4
			EF3	182400	30960	-2.8	82.4	61.2	1.8
			EF4	111200	16940	-0.3	67.1	82.0	2.3
			HW1	54580	11820	0.8	81.8	75.3	11.2
			HW2	37450	7402	0.3	67.1	72.9	11.3
			INF1	38000	7106	0.5	86.8	64.5	4.7
			WW	48020	8058	-2	83.8	53.5	10

Table S2. (cont.) Non-target compounds identified in real water samples by QToF-HRMS.

Compound	RT (min)	Precursor Mass (m/z)	Sample	Peak Area	Signal Intensity	Mass error (ppm)	Library score	Combined score	Isotope Ratio Difference
Desmethylcitalopram	6.44	311.155	EF4	63550	9766	-3.2	98.7	58.9	49.8
			HW1	83060	14850	-2.5	100	61.5	33.1
Diclofenac	7.95	296.024	EF1	42950	9974	0.9	100	92.5	1.9
			EF2	16910	3379	4.5	97.7	68.2	7.3
			EF3	106500	18120	0.5	100	89.7	4.6
			EF4	30590	6239	0.6	100	83.7	9.7
			EF5	274300	52150	-4.5	100	74.8	2.8
			INF1	12610	3726	5.2	97.9	64.6	10.6
Diltiazem	7.25	415.169	EF1	91180	13960	-0.5	92.6	60.5	9.2
			EF3	16100	3015	1.1	84.3	65.5	23.3
			EF4	79210	13660	1.8	95.2	64.6	26.9
			HW1	845300	138600	-0.7	100	94.2	1.1
			HW2	5700000	858400	0.1	100	96.9	1.3
Dimethoate	5.47	230.007	EF1	276800	38350	-3.2	99.4	83.0	1.8
Dodemorph	8.71	282.279	EF4	368500	68120	-1.8	75.5	82.6	2.2
			HW1	481900	90670	-0.4	77.8	89.0	2.2
			HW2	666500	123900	-1.5	73.8	83.2	2.2
			INF1	686900	147100	-0.2	80.4	91.1	0.3
EDDP	7.16	278.19	EF4	140000	21240	-1	95.7	78.9	10.5
			HW1	375400	55490	0.4	96.6	90.0	5.2
			INF1	112300	21560	-0.8	99.1	69.4	26.4
Emetine	9.11	481.306	EF1	13830	1602	-0.1	100	74.7	52.9
Eprosartan	6.65	425.153	EF1	1222000	162000	-2.3	100	84.9	2.1
			EF2	2262000	327200	-1.5	100	91.8	1.1
			EF4	483900	70760	0.6	78.5	88.5	0.8
			HW1	118100	18380	-0.3	98	90.4	5.1
			INF1	1117000	129700	-1	100	85.4	5.9
Ergosine	6.23	548.287	WW	17590	2027	0.8	84.4	66.1	34.9
estr-4-ene-3,17-dione	8.67	273.185	EF1	573700	110700	0.2	71.3	65.9	1.2
			EF2	506400	97140	-0.3	68.7	63.3	2.4
			EF3	419300	80690	-2.5	73.8	59.4	2.1
			EF4	327900	69860	-0.5	68.3	84.6	3.6
			INF1	240100	43170	-0.6	69.3	59.0	5.4
			RW	423700	78970	-0.8	67.8	53.8	8.9

Table S2. (cont.) Non-target compounds identified in real water samples by QToF-HRMS.

Compound	RT (min)	Precursor Mass (m/z)	Sample	Peak Area	Signal Intensity	Mass error (ppm)	Library score	Combined score	Isotope Ratio Difference
Fenbufen	7.46	255.102	EF1	175500	34410	-0.6	72.2	51.8	11.9
			EF2	38210	5583	0.9	98.8	64.0	6.8
			EF3	165400	28100	-1.7	86.7	62.0	4.4
			EF4	152800	24990	0.7	97.2	68.5	3.3
			HW1	1051000	165900	-0.7	67.8	83.5	3.4
			HW2	1227000	207200	0.3	86.4	93.5	0.6
			INF1	133100	21600	-0.6	85.4	75.5	12.7
Fenofibric acid	8.01	319.073	EF1	132900	25540	-1	99.2	87.3	6.1
			EF2	62050	11040	0.4	98.5	92.0	2.6
			EF3	120500	20150	0.5	98.6	93.8	1.5
			EF4	96080	16970	0.4	99.1	93.2	3.2
			EF5	123800	22860	-4.5	100	73.2	2.9
			HW1	341900	60230	-0.2	99.2	93.2	2.5
			HW2	45020	9081	-1.2	97.9	64.1	5.9
			INF1	88500	20780	0.1	99.1	89.8	6
Fenuron	5.01	165.102	EF1	7415000	901700	-3.4	57.4	70.0	1.5
Fexofenadine	7.21	502.295	EF3	20020	3227	-0.1	97.3	73.7	40.1
Flecainide	6.2	415.145	EF1	365300	62450	-1.7	99.2	85.6	3.6
			EF2	235200	34100	-3	99.4	73.8	7.6
			EF3	903200	116000	0.6	99.2	93.5	1.8
			EF4	373100	46720	-0.9	99.4	85.2	7.7
			EF5	778400	129400	-5.3	99.4	61.2	9.1
			HW1	194300	21130	1.3	96.9	78.0	11.1
			HW2	753200	108200	-0.9	99.5	90.1	4.2
			INF1	193800	30050	0.6	99.3	78.4	13.7
Fluconazole	5.52	307.111	EF1	34870	5151	2.4	95.3	66.9	13.3
			HW1	130500	18750	-0.2	99.8	72.6	1.5
Fludrocortisone Acetate	5.00	423.218	EF1	513000	59400	3.5	100	60.7	4.3
			EF5	203600	16730	-0.3	100	68.8	4.4
			HW1	5022000	345100	3	100	74.4	7.9
			HW2	2301000	217700	3.7	100	75.7	3.7
Hordenine	4.37	166.123	HW2	66380	9482	-2.6	97.4	64.6	2.5
Irbesartan	7.79	429.24	EF1	503300	84770	-0.6	96	86.6	6.8
			EF2	226300	38430	-0.3	97.2	89.3	6.4
			EF3	272200	43840	-0.7	96.8	83.1	8.3
			EF4	377000	64350	0.2	96.1	93.2	3.2
			EF5	2989000	490500	-7.3	96.9	62.1	2.5
			HW1	280500	48480	-0.3	96.4	85.2	9.4
			INF1	290200	58070	-0.1	96.9	92.8	3.9

Table S2. (cont.) Non-target compounds identified in real water samples by QToF-HRMS.

Compound	RT (min)	Precursor Mass (m/z)	Sample	Peak Area	Signal Intensity	Mass error (ppm)	Library score	Combined score	Isotope Ratio Difference
Kavain	7.44	231.102	HW1	987100	159400	0.2	72.5	88.8	2.1
Ketocaine	6.21	292.227	EF2	46920	7378	-1.9	82.3	77.7	6
Levocabastine	8.3	421.229	EF5	168400	29120	-1	95.3	73.9	15.8
			RW	41690	8261	-1.7	93	60.4	97.8
Lidocaine	4.71	235.18	EF3	761500	109700	-0.9	99.7	92.3	2.3
			HW1	153100	25400	-0.8	99.7	52.6	16.2
			HW2	486600	73880	-0.9	99.7	89.0	3.3
Lolitrem B	7.9	686.405	HW1	81600	10480	3.5	95.9	62.4	15.2
Losartan	7.42	423.169	EF1	293600	42820	-0.3	99.7	89.9	4.8
			EF3	242100	38280	-2.1	99.5	88.5	0.4
			EF4	243400	39800	-0.3	99.7	86.6	7.6
			HW1	177300	29910	-1.2	91.3	90.2	0.9
			HW2	82200	14370	0.7	97.3	83.5	8.4
			INF1	239800	36520	-0.8	99.6	92.9	1.7
Mefloquine	6.46	379.124	EF4	10800	2063	4.1	99.5	58.7	17.1
Mepivacaine	4.76	247.18	EF1	32190	3574	0.1	100	63.1	9.4
			HW1	60520	6677	-1.1	100	70.5	31.3
			HW2	76080	12850	-2.1	100	55.4	11.6
			INF1	17200	1986	-0.8	100	71.6	27.2
Methadone	7.43	310.217	HW1	2088000	328600	-0.6	94.6	89.4	3.3
Methcathinone	3.72	164.107	HW1	17420	2682	-0.2	74.5	67.9	50.3
Methoxyphenamine	5.06	180.138	INF1	13770	1505	-1.2	80.8	51.6	12.5
Moxisylyte	4.46	280.191	EF2	45950	7838	-0.9	59.2	83.4	2.2
			EF4	118200	18000	0.4	96.6	94.1	2.8
			HW1	299800	48770	-0.3	94.8	96.3	1
			INF1	104600	15270	-1.7	93.2	63.9	4.1
Oxazepam	7.32	287.058	EF1	53030	9163	-2.1	94.8	82.4	4.5
			EF5	123000	24610	-3.8	100	76.5	3.6
Oxcarbazepine	6.58	253.097	EF1	137000	18470	0.7	86.1	90.1	1.8
			EF3	30230	3770	3.3	89.9	50.5	11
			EF4	106100	14980	1.2	97.2	89.2	1.8
			EF5	746400	121500	-3.6	98.5	77.2	1.8
Paracetamol	2.14	152.071	EF4	7525	914	-2.4	57.7	50.1	6.7
			HW1	185500	19890	-3.4	99.7	61.3	4.1
			HW2	122000	11260	-1.4	99.6	65.1	5.1
			INF1	3034000	260100	-1.4	99.6	88.1	1.9
			RW	31490	3102	-3.6	89.9	76.9	1.7

Table S2. (cont.) Non-target compounds identified in real water samples by QToF-HRMS.

Compound	RT (min)	Precursor Mass (m/z)	Sample	Peak Area	Signal Intensity	Mass error (ppm)	Library score	Combined score	Isotope Ratio Difference
Paraxanthine	3.68	181.072	EF4	75490	8922	-0.6	83.9	67.7	22.6
			EF5	487200	67100	-5.4	69.6	62.0	3.8
Paspalitrem A	7.21	502.295	EF3	20020	3227	-0.1	83.9	70.3	40.1
PMA	4.37	166.123	HW2	66760	9502	-2.6	86.4	61.9	2.5
PMMA	4.23	180.138	EF5	20020	2788	-4.3	96.4	53.6	7.9
Pyroquilon	3.93	174.091	EF4	102700	13840	-1.7	70.6	79.7	3.7
Quinine	6.16	325.191	EF1	122500	16370	3.2	92.7	72.3	9.3
			EF2	61000	9421	0.9	71.9	81.0	3.7
Rifampicin	7.9	823.412	HW1	37500	6480	-2.7	75.1	72.5	6.3
Salicylamide	3.82	138.055	HW1	23430	3750	-1.2	57.3	54.0	6
Serotonin	3.79	177.102	EF3	6303	655	1.3	97.9	69.2	143.2
Spinetoram A	8.23	748.499	EF1	136900	9082	1.3	95.1	82.2	5.6
			EF3	108400	9076	0.3	98.7	79.0	12.7
			EF4	83750	5229	3.3	64.7	50.5	21
Spinosyn A	8.26	732.468	EF4	36600	5096	2.4	97	70.2	12.3
Spinosyn D	8.34	746.484	EF5	865100	102200	-0.3	68.3	72.1	12.8
Sulfapyridine	4.75	250.064	EF1	45150	6347	-0.9	96.7	90.1	2.3
			EF5	165800	27830	-4.1	97.6	62.8	12.5
Sulpiride	3.69	342.148	EF1	610100	92740	0.8	100	94.3	0.6
			EF2	74370	12180	-0.6	99.6	89.8	5.7
			EF3	630600	97530	0.2	100	94.1	1.8
			EF4	485400	78860	-0.4	100	93.6	1.7
			EF5	308100	46910	-0.6	100	88.9	4.3
			HW1	353300	55280	0.1	100	96.3	1.1
			HW2	442500	70220	-0.2	100	95.7	1.2
			INF1	393300	62520	-0.9	99.7	92.6	1.9
Telmisartan	8.72	515.244	EF1	764900	132000	0.1	100	97.0	1
			EF2	682300	122400	-0.1	100	96.4	1.2
			EF3	653800	112700	-0.9	100	92.2	2.5
			EF4	369500	57430	-1.1	100	90.1	3
			HW1	345700	61010	0.2	100	75.7	17.3
			HW2	52430	7962	-0.6	96	71.1	21.2
			INF1	271200	44530	-0.8	100	94.5	1.2
Temazepam	7.85	301.074	EF1	22220	4025	-0.9	100	86.1	7.6
			EF5	74280	13610	-7.5	98.8	59.3	3
Testosterone	8.06	289.216	INF1	30830	6295	3.5	74.3	53.8	27.8

Table S2. (cont.) Non-target compounds identified in real water samples by QToF-HRMS.

Compound	RT (min)	Precursor Mass (m/z)	Sample	Peak Area	Signal Intensity	Mass error (ppm)	Library score	Combined score	Isotope Ratio Difference
Theobromine	3.67	181.072	EF4	75490	8922	-0.6	56.9	60.9	22.6
			EF5	487200	67100	-5.4	96.6	68.7	3.8
			HW1	2140000	272200	-1.5	96	88.2	1.4
			HW2	1482000	179200	-2.2	96	87.1	0.7
			INF1	2420000	296000	-1.3	97.9	91.8	0.7
Thymopentin	6.78	680.373	EF2	369300	55910	-4.9	100	65.9	8
			EF4	15940	3212	-2.3	98.2	63.6	24.4
			HW1	1283000	198800	-5.6	100	68.0	4.1
			HW2	1822000	268100	-3.2	100	77.6	5.1
			INF1	384200	56220	-5.9	100	69.9	1.2
Tridemorph	8.94	298.31	EF1	4782	1481	-0.4	53.8	60.8	23.7
			EF2	6901	1783	0	53.8	61.6	21.4
			EF3	7128	1729	-3	53.8	52.5	16.4
			EF4	12910	2697	0.4	87.2	67.1	21.4
			HW2	8411	1902	-1.3	97.8	63.6	21.7
			RW	22510	5883	0	53.8	61.3	21.4
Triphenylphosphate	8.43	327.078	EF1	107500	21520	-0.8	61.1	81.4	3.5
			EF3	63700	10660	-0.4	67.9	87.8	1.5
			EF4	79980	15020	-0.6	88.3	89.7	3.3
			EF5	344000	70520	-6.8	89.3	60.6	1.9
			HW2	36040	7309	-1	55.6	74.8	6.1
			RW	416800	78600	-1.4	91.2	88.9	1.7
Valsartan	7.7	436.234	EF1	1663000	286700	2.1	99.6	88.9	0.5
			EF2	464300	77830	0.4	99	93.5	2.9
			EF3	608800	100800	-0.2	99.7	95.3	2
			EF4	1232000	220200	-0.2	99.3	97.1	0.1
			HW1	1462000	239300	-1.8	99.7	89.3	0.1
			HW2	720600	132700	-0.8	99.3	88.4	4.5
			INF1	1100000	178600	-0.3	99.6	94.4	2
Verapamil	7.01	455.29	HW1	471200	77020	-1	100	56.8	12.5
Verrucarol	6.19	267.159	EF1	51200	6102	-0.2	97.7	73.4	39.2
Viquidil	6.17	325.191	EF1	122500	16370	3.2	69.3	66.4	9.3

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4.2.

Tube-in-tube membrane photoreactor for antibiotic removal from urban wastewater

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Tube-in-tube membrane photoreactor for antibiotic removal from urban wastewater

ABSTRACT

In this work, a tube-in-tube membrane photoreactor, operating in continuous mode, was applied for the removal of fourteen antibiotics in demineralized water (DW) and urban wastewater (UWW) after secondary treatment. In this system, the oxidant (H_2O_2, $S_2O_8^{2-}$ or O_3) was permeated through the membrane pores and delivered to the catalyst nanoparticles (Ag_2MoO_4 or TiO_2), embedded in the shell side of the membrane, and to the annular reaction zone (ARZ). The ARZ is where the fortified water sample with the antibiotics of interest flows. The efficiency of photochemical and photocatalytic oxidation was evaluated as a function of water flow rate, oxidant dose and type, catalyst and aqueous matrix (DW vs UWW). Samples were collected at reactor outlet at different time intervals and directly analyzed by ultra-high performance liquid chromatography followed by quadrupole time-of-flight high resolution mass spectrometry (UHPC-QTOF-HRMS). The O_3 process proved to be the most efficient, resulting in >95% removal of 12 of the 14 antibiotics in UWW, followed by the Ag_2MoO_4/UVC/$S_2O_8^{2-}$ system (5 antibiotics removal >60% in UWW). A tentative identification of possible by-products formed during the degradation process has also been carried out by UHPC-QTOF-HRMS.

Keywords: Advanced oxidation processes, antibiotics, tube-in-tube membrane reactor, ozonation, photocatalysis, sulfate based AOPs.

1. Introduction

The presence of pharmaceuticals in water is growing, due to their increasing worldwide use by the population and veterinary practices. Within these contaminants of emerging concern (CECs), one of the most worrying groups are antibiotics since their chemical structures are resistant to conventional wastewater treatment processes. The Watch

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List under the Water Framework Directive comprises a wide range of compounds, including some antibiotics, which are under surveillance for potential risks and adverse effects in water [1,2].

Since biological treatments in wastewater treatment plants (WWTPs) are not capable to completely remove antibiotics, advanced oxidation processes (AOPs), characterized by the generation of highly reactive oxidizing species (ROS), appear as a good option for the tertiary treatment of urban wastewaters (UWW) enabling the removal of persistent organic pollutants [3]. For that, different systems employing UV radiation and a wide variety of photocatalysts and oxidants can be employed.

One of the most employed oxidant used in AOPs is hydrogen peroxide (H_2O_2), since it is a powerful oxidizer that can form hydroxyl radicals ($HO^{\bullet}$) through different activation mechanisms, such UV radiation, the use of photocatalysts, etc. In addition, AOPs based on sulfate radicals are gaining attention as a potential technology for the removal of CECs from waters. This process shows advantages such as high redox potential, long radical lifetime and wide pH range in the reactions, after being activated the peroxymonosulfate (HSO_5^-) or persulfate ($S_2O_8^{2-}$) by different sources (photolysis, heat, ultrasound, catalysts, etc.) giving rise to sulfate radicals ($SO_4^{-\bullet}$). Another strong oxidant widely applied for the water disinfection and degradation of organic pollutants is ozone (O_3). In this case, the degradation mechanism is based on direct oxidation and the generation of hydroxyl radicals ($HO^{\bullet}$) by ozone decomposition [4-7].

The tube-in-tube membrane photoreactor proposed by Vilar et al. (2020) [8], is a low footprint technology whose design enables the integration of different ROS generation mechanisms within a single unit operated in continuous-flow mode [9]. However, according to all reported studies using this type of reactor, its efficacy has only been studied for a maximum of one antibiotic, and it has never been applied for the simultaneous degradation of more than four compounds.

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[4,5,8,10,11]. In addition, the investigations related to antibiotics degradation in waters involve the study of few compounds, usually from the same family, and they have been performed at quite high concentrations, which are not in agreement with the levels usually found in real waters [12-14].

The aim of this project was the optimization of the operational conditions of a novel tube-in-tube membrane photoreactor, evaluating the performance of different oxidant-catalyst combinations for the simultaneous removal of fourteen antibiotics (including sulphonamides, fluoroquinolones, cephalosporins, macrolides and diaminopyrimidines) in demineralized water and urban wastewater after secondary treatment. The formation and presence of transformation products resulting from the treatment processes was studied by UHPLC-QToF-HRMS, as well. This work is the first time that a gaseous oxidant (O_3) is proposed for the removal of organic compounds by a tube-in-tube membrane reactor. In addition, to the best of our knowledge, the use of Ag_2MoO_4 as catalyst in this type of reactor has never been evaluated, as well as the simultaneous degradation of such a high number of analytes.

2. Materials and methods

2.1. Chemicals, reagents and samples

Ciprofloxacin, enrofloxacin, norfloxacin, ofloxacin, sulfadiazine, sulfadimidine, sulfamerazine, sulfamethoxazole and trimethoprim were supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany), clarithromycin, erythromycin and roxithromycin by Glentham Life Sciences Ltd. (Wiltshire, UK) and azithromycin and cephalexin by Apollo Scientific Ltd (Cheshire, UK). A mix stock solution (concentration about 5000 mg/L) was prepared in methanol except for ciprofloxacin and ofloxacin which individual solutions were prepared in an acidified water solution HCl 0.4 % (Merck, 37 %) and NaOH 1 M

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(Merck), respectively. Stock solutions were stored in glass vials and protected from light at -20 ºC. All solvents and reagents were of analytical grade. The 14 target antibiotics, their CAS numbers, retention times, quantification and identification ions used to ensure their unequivocal identification are summarized in Table 1, Chapter 4.1.

Sodium persulfate (Merck, 98%) and hydrogen peroxide (Labkem, 30% v/v) were used as oxidant, as well as ozone, which was generated from pure and dry oxygen using an ozone generator (BMT 802 N). Ethanol (Merck) and catalase from bovine liver (Sigma-Aldrich) were employed to stop the oxidation ability of $S_2O_8^{2-}$ and H_2O_2, respectively. Potassium iodide and sodium bicarbonate (Merck) was required to quantify $S_2O_8^{2-}$ and ammonium metavanadate (Merck) for H_2O_2.

As regards the treatments with the liquid oxidants (H_2O_2 or $S_2O_8^{2-}$), an ultrafiltration ceramic membrane (pore size 10 nm) coated with 29 mg TiO_2-P25 or 60 mg of Ag_2MoO_4 was used; while for the O_3 treatments, a microfiltration borosilicate membrane (pore size 5 µm) impregnated with 0.6 g of TiO_2-P25 was employed.

Formic acid, methanol, ultrapure water MS grade (Scharlab, Barcelona, Spain) and ammonium formate (Sigma-Aldrich) were used to prepared HPLC mobile phases.

Real wastewater (UWW) sample was collected downstream from the secondary settling tank of an urban wastewater treatment plant located in the north of Portugal and it was stored refrigerated at 4 ºC until use. Demineralized water (DW) (Panice® reverse osmosis system) was used for synthetic samples. Samples used as inlet streams of the tube-in-tube membrane reactor were spiked with 50 µg L^{-1} of each antibiotic.

2.2. Tube-in-tube membrane reactor

The technical characteristics and the operational parameters of the lab-scale tube-in-tube reactor were previously fully described by Vilar et al., (2020) [8]. To briefly summarize, the reactor is composed by an

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outer quartz tube (the "window"-radiation entrance to the reactor) and an inner tubular ultrafiltration ceramic membrane (for liquid oxidants) or a microfiltration borosilicate membrane (for O_3). Membrane acts as photocatalyst support and oxidant-catalyst/water contactor and it is sealed by two mobile polypropylene flanges and vertically fixed in a stainless steel structure. Besides, if necessary, to uniformly irradiate the annular reaction zone (ARZ) and membrane surface, four UVC lamps (Puritec HNS G5 from Osram, λmax = 254 nm; nominal power = 6 W; useful power = 1.7 W) were located externally to the quartz tube. In Figure 1 is included a scheme of the tube-in-tube membrane reactor.

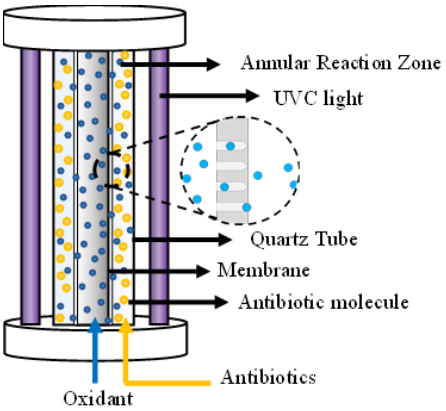


Figure 1. Tube-in-tube reactor scheme.

The membrane shell-side was coated with Ag_2MoO_4 or TiO_2-P25 by dip-coating. The procedure was adapted from Castellanos et al. (2020) [9].

2.3. Experimental procedure

The experiments using the tube-in-tube membrane photoreactor were carried out in continuous mode (hydraulic residence time (HRT) between 3.4-10.2 s), dosing the selected oxidant (H_2O_2, $S_2O_8^{2-}$ or O_3).

With the back pressure regulator (BPR) fully open, the ceramic tubular membrane was internally filled using a syringe pump (Nexus

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6000 from Chemyx Inc.) with the $S_2O_8^{2-}$ (67.75 g/L) or H_2O_2 stock solution (6.0 g/L). After that, the BPR was fully closed to gradually inject the oxidant using the syringe pump, until first drops appear in the membrane shell-side. Then, in order to remove the excess of oxidant on the membrane shell-side, 1 L of demineralised water was pumped through the annular reaction zone (ARZ). Afterwards, 5 L of fortified water with the target antibiotics ($[Antib]_{inlet}$= 50 µg/L of each; T=25ºC; pH~7) was pumped at a known flow rate ($Q_{inlet,Antib}$= 15, 30 and 40 L/h) through the ARZ while the syringe pump forced the oxidant solution through the membrane pores ($[S_2O_8^{2-}]_{ARZ}$= 0.3, 0.6 and 1.2 mM; $[H_2O_2]_{ARZ}$= 1.2 mM) and UVC lamps were turned on (the system was surrounded by an aluminium shell to protect direct eye contact from the radiation). Once the steady state was reached, samples were collected at reactor outlet at different time intervals. After sampling (1 mL), ethanol (100 µL) was added to quench $S_2O_8^{2-}$, and catalase (30 µL of 100 mg/L solution) was added in the case of H_2O_2 experiment.

Regarding to the ozonation process, the same procedure was applied ($Q_{inlet,Antib}$= 50 and 150 L/h), without the previous step of filling the membrane with the oxidant, since in this case, O_3 was directly pumped from a generator ($[O_3]_{ARZ}$= 2, 6, 12 and 18 mg/L). The collected samples were directly analyzed.

2.4. Analytical procedure

The residual H_2O_2 concentration was measured by the metavanadate method (λ = 450 nm) [15] and $S_2O_8^{2-}$ concentration according to the method proposed by Liang et al. (2008) [16] (λ = 352 nm) using a UV–visible spectrophotometer. Inlet and outlet gaseous ozone concentrations were monitored by means of a UV-based ozone analyzer (BMT 964), after passing through a sample gas dehumidifier (BMT DH3b).

The 14 antibiotics were determined by ultra-high performance liquid chromatography (Elute UHPLC 1300) coupled to quadrupole time-of-flight mass spectrometry (QToF) (Compact-II Instrument,

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Bruker Daltonics, Bremen, Germany). The chromatographic separation was achieved with an Intensity Solo HPLC column C18-2 (100 mm × 2.1 mm, 1.8 μm; Bruker Daltonics, Bremen, Germany) and a pre-column, kept at 40 °C. The mobile phase consisted of water/methanol (99:1) (A) and methanol (B), both containing 0.01 % (v/v) of formic acid and 5 mM ammonium formate. The gradient conditions were 4 % B increasing to 18.3 % in 1 min at a flow rate of 200 μL/min; 50 % B until min 2.5 at 223 μL/min; 99.9 % B until min 14 at 400 μL/min and then, the flow was increased to 480 μL/min for 2 min. To return to initial conditions in 0.1 min at 480 μL/min the gradient of B decreased to 4 % and held until min 19. Finally, the flow rate decreased to 200 μL/min in 0.1 min and held until min 20 (see chromatographic conditions (retention time, quantification and identification ions) in Table 1, Chapter 4.1).

The mass spectrometer was operated in the electrospray ionization (ESI) in positive mode. Ion source conditions were as follow: capillary 4500 V, nebulizer 29 psi, drying gas 8 L min^{-1}, gas temperature 200 ºC. The QToF mass spectrometer operated in broadband collision-induced dissociation (bbCID) acquisition mode that provided MS and MS/MS spectra at the same time. Spectra was recorded over the range m/z 30−1000 with a scan rate of 2 HZ. To obtain the MS spectra the bbCID mode works with a Low Collision Energy of 6 eV, and to acquire the MS/MS spectra a High Collision Energy of 30 eV was applied. The system was operated by Compass HyStar software and O-TOF Control version 5.2. Quantitation and processing software was TASQ Version 2.1 (Build 201.2.4019).

In an attempt to tentatively identify transformation products (TPs) generated during the photochemical/photocatalytic process, a UPLC system (Shimadzu Nexera X2) consisting of two high-pressure pumps (LC-30AD) and a SIL-30AC autosampler was employed. HRMS detection was performed employing a SCIEX (Ontario, Canada) TripleTOF®5600+ equipped with a DuoSprayTM ion source and an Electrospray Ionization (ESI) probe. A Kinetex Biphenyl (50 × 2.1 mm,

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2.6 µm) column from Phenomenex (Torrance, CA, USA), kept at 40 ºC with a CTO-30 A column oven, was utilized to achieved the chromatographic separation. The mobile phase consisted of water (A) and methanol (B), both buffered with 3 mM ammonium formate and both containing formic acid (0.15%). The flow rate was kept to 0.20 mL/min, employing an 8-minute gradient elution profile (10% B to 100% B) reaching a total runtime of 15 min, and 10 µL of sample were injected in the analytical column.

As regards the HRMS conditions, the source temperature was set at 550ºC, the ion source gas at 42 (au, arbitrary units), the curtain gas at 32 (au), and the ion spray voltage floating at 5500 V. The HRMS workflow consisted of a TOF Full Scan, using 220 ms as accumulation time and 80 V as declustering potential in the ESI. Simultaneously, a data independent approach based on SWATH (Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra) was performed. For that, a wide mass range (80–1200 Da) was divided in 30 mass windows with an accumulation time of 30 ms for each one. The resulting total scan time per cycle was 1.16 s. The declustering potential was set to 80 V and the collision energy was 40 V with an energy spread of 20 V. The system was operated by Analyst®1.7.1 control software for data acquisition and by SCIEX OS 1.5.0.23389 for data treatment and library search.

3. Results and discussion

As previously commented, the aim of this work is to optimize the operational conditions of the tube-in-tube membrane photoreactor for the simultaneous degradation of 14 antibiotics of different families by evaluating different oxidants and other parameters, such as catalyst, being the first time that Ag_2MoO_4 is proposed for this type of reactor.

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3.1 Liquid oxidants

3.1.1 Effect of the oxidant and catalyst

First, the degradation of the antibiotics was assessed by UVC radiation, as well as their combination with an oxidant and/or a catalyst. For that the flow rate and oxidant concentration were fixed (Q_{inlet}=30 L/h; $[S_2O_8^{2-}]_{ARZ}=[H_2O_2]_{ARZ}$ =1.2 mM) employing a solution of demineralized water (DW) fortified with the 14 antibiotics at 50 µg/L.

UVC radiation using the tube-in-tube reactor allowed low degradation of all antibiotics, being cephalexin the one which reached the highest removal efficiency (16 %). This may be due to the low hydraulic residence time (HRT). As it is depicted in Figure 2a, the obtained results using H_2O_2 showed a slight improvement comparing with the results using only photolysis with UVC. In this case, the removal rates reached 45 % for cephalexin, and around 30 % for enrofloxacin, norfloxacin, ciprofloxacin and sulfamethoxazole. On the other hand, a clear improvement in elimination efficiency was observed with $S_2O_8^{2-}$, as both cephalexin and the sulphonamides (except sulfamethoxazole) degraded around 50 %.

In addition to oxidants, heterogeneous photocatalysis can enhance the oxidation efficiency of the analytes of interest. In this context, the removal of the 14 antibiotics was studied using TiO_2-P25 or $AgMo_2O_4$ under UVC radiation. Removal percentages revealed slightly better results employing the UVC/$AgMo_2O_4$ approach (see Figure 2b). However, degradation with UVC/catalyst showed no improvement over UVC/oxidant, except for sulfamethoxazole which was 25 % removed with the UVC/Ag_2MoO_4 treatment. Thus, it is important to evaluate the UVC process with the catalyst/oxidant combination.

As can be seen in Figure 2c, the combination of $S_2O_8^{2-}$ with catalyst, leads to better results than H_2O_2 for almost all compounds. The degradation employing H_2O_2, regardless of whether the catalyst was

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TiO_2 or Ag_2MoO_4, was lower than 35 % for the 14 target antibiotics. Thus, H_2O_2 was discarded for the following studies.

So that, considering only the oxidant $S_2O_8^{2-}$, although the efficiency removal for TiO_2 is higher than using only oxidant or only catalyst, when combined with Ag_2MoO_4 the performance increases. Under these conditions, degradation between 87 and near 100 % (considering the analytical detection limit) was achieved for the fluoroquinolones, sulfonamides and cephalexin. In contrast, azithromycin was 45 % degraded; the rest macrolides achieved degradation rates of around 20 % and trimethoprim was barely removed (7 %).

Therefore, UVC/Ag_2MoO_4/$S_2O_8^{2-}$ was selected for further experiments, in order to select the most favourable conditions.

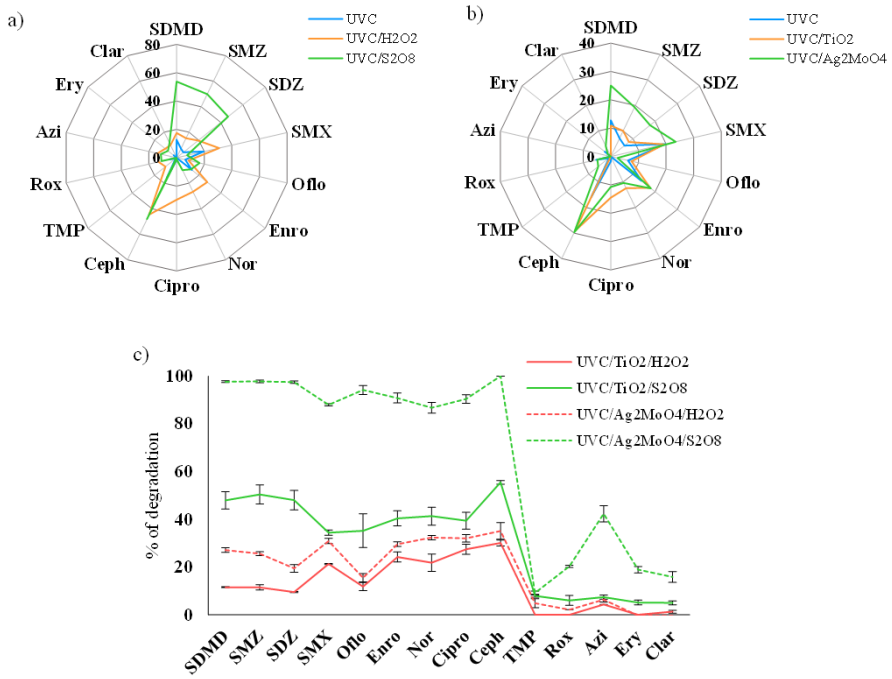


Figure 2. Removal percentages for the 14 antibiotics after treatment with: a) UVC and UVC/oxidant; b) UVC and UVC/catalyst; c) UVC/catalyst/oxidant.

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3.1.2. Effect of UVC, $S_2O_8^{2-}$ concentration and pH

In order to obtain the best removal efficiency, $S_2O_8^{2-}$ direct reaction with antibiotics, in the absence of UVC light and with UVC light, considering different doses in the ARZ (0.3, 0.6 and 1.2 mM) were also tested at a flow rate of 30 L/h.

Regarding the degradation that takes place in the absence of UVC, as it is shown in Figure 3a, it was inferior in practically all cases than in the presence of UVC. However, results for some of the target antibiotics reached slightly higher (sulfadiazine, azithromycin) or similar results (ofloxacin, sufamerazine), employing a dose of 1.2 mM without UVC than with 0.3 mM with UVC.

On the other hand, considering the dose of $S_2O_8^{2-}$ under UVC radiation, as expected, the higher the dose in the ARZ, the higher the degradation. Therefore, the maximum dose ($[S_2O_8^{2-}]_{ARZ}$=1.2 mM) appeared to be the best option under the UVC/Ag_2MoO_4/$S_2O_8^{2-}$ treatment for the antibiotics removal, although three macrolides and trimethoprim were hardly degraded (< 20%).

The pH of the sample may affect the formation of persulfate radicals and, consequently, the removal efficiency. In this context, the possible effect of the pH of the sample was studied (Q_{inlet}= 30L/h and $[S_2O_8^{2-}]_{ARZ}$=0.6 mM) in different ranges: pH=3, pH=8 and pH~7, which is the natural pH of the test sample.

As can be seen in Figure 3b, the pH does not influence the degradation, since differences were not higher than 10 %. Hence, the following experiments were carried out using the water sample without pH modification and the highest persulfate dose.

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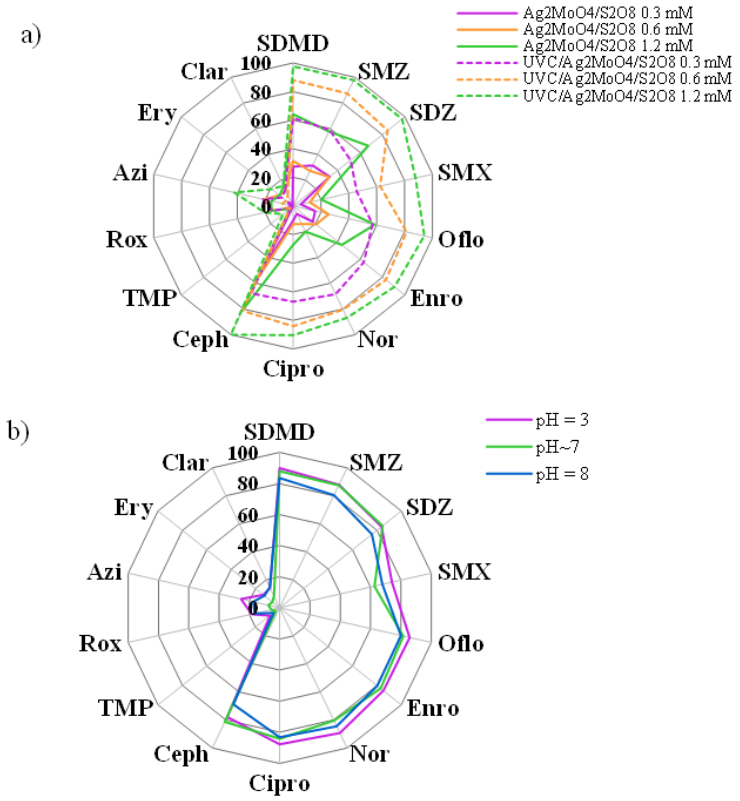


Figure 3. Removal percentages for the 14 antibiotics evaluating: a) UVC radiation and different doses of $S_2O_8^{2-}$ employing the $Ag_2MoO_4/S_2O_8^{2-}$ system; b) different sample pH for the UVC/$Ag_2MoO_4/S_2O_8^{2-}$ treatment.

3.1.3. Evaluation of the annular flow rate

Under the selected optimal conditions, the annular flow rate (Q_{inlet}) was assessed, using the same concentration of $S_2O_8^{2-}$ in the annular reaction zone ($[S_2O_8^{2-}]_{ARZ}$= 1.2 mM).

As it is shown in Figure 4, for the flow rate of 40 L/h the removal efficiency of all target antibiotics decreased, possibly due to the decrease on the residence time (4.6 s). For the other two flow rates tested, similar results were obtained for almost all compounds. At a flow rate of 15 L/h a slightly higher conversion of trimethoprim and

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macrolides was observed, and degradation remains on average close to 30%. However, since no significant differences were achieved and in order to obtain a higher productivity, a flow rate of 30 L/h was selected, offering a hydraulic residence time of 6.1 s.

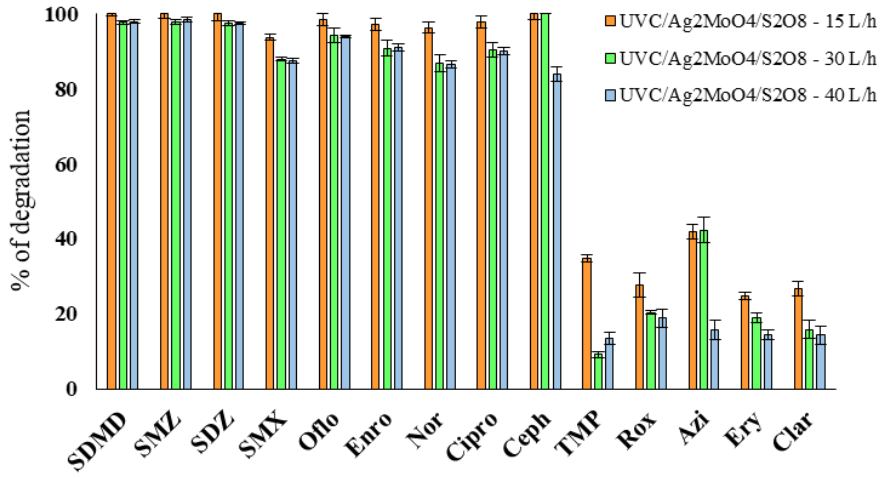


Figure 4. Flow rate assessment for the removal of the 14 antibiotics under the UVC/Ag_2MoO_4/$S_2O_8^{2-}$ treatment.

3.1.4. Effect of water matrix

Once the operational conditions of the tube-in-tube membrane reactor were optimized, to test this approach in real applications, the removal of the 14 antibiotics by the UVC/Ag_2MoO_4/$S_2O_8^{2-}$ photochemical system was evaluated for an urban wastewater (UWW) sample after a secondary treatment enriched with 50 mg/mL of the analytes of interest. Figure 5 shows the comparison with a solution of antibiotics in demineralized water and the real water under the same conditions of flow rate, oxidant and antibiotics concentration. A decrease in the conversion of antibiotics was observed for the real wastewater. It should be noted that this decrease can be mainly associated with the presence of light absorbing species and scavengers of ROS, such as natural organic matter and bicarbonates.

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In UWW the degradation of sulphonamides decreased by about 2.5 times (mean degradation rates of 37 % instead of 95 %); for cephalexin which in demineralized water (DW) was removed by near 100%, in the UWW matrix the degradation rate was 75%. The lower efficacy in real water was in concordance with the results obtained in other studies for other pharmaceuticals and hormones employing persulfate as oxidant [5,17].

However, fluoroquinolones were practically unaffected by the matrix, as the removal efficiency decreased from average values of 90 % in the DW to 80 % in UWW. Macrolides and trimethoprim were also unaffected by real water, as they were slightly degraded even in DW.

In order to give rise to higher degradation rates in wastewater with similar conversions to the synthetic solution, a much higher amount of oxidant would be needed to overcome matrix effects.

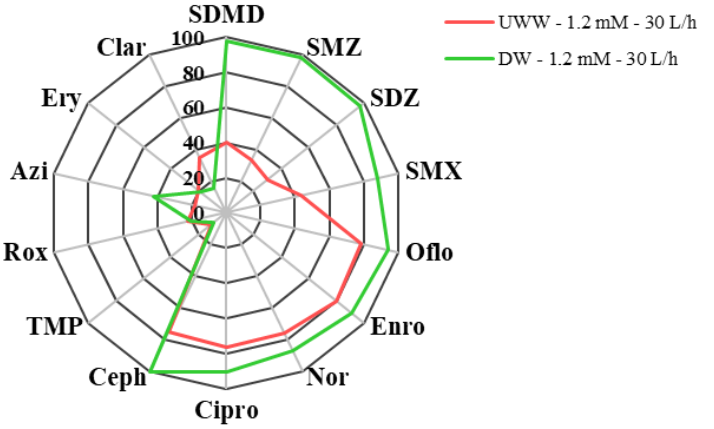


Figure 5. Removal efficiency of the 14 antibiotics by UVC/Ag_2MoO_4/$S_2O_8^{2-}$ treatment employing demineralized water (DW) or urban wastewater (UWW) under optimal conditions.

3.2. Gas oxidant

Although the use of $S_2O_8^{2-}$ oxidant allows high removal efficiency for some of the target antibiotics, the conversion of macrolides and

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trimethoprim is almost negligible. In this context, other processes which enhance the simultaneous removal of the 14 target antibiotics are needed. Hence, the use of O_3 as oxidant in the tube-in-tube membrane reactor was for the first time proposed. In this case, TiO_2-P25 was used as catalyst.

3.2.1. Effect of O_3 concentration

The employment of O_3 as oxidant was evaluated in DW working at a flow rate of 150 L/h (HRT=3.4 s), testing three different doses: 2, 6 and 12 mg/L. As can be seen in Figure 6, lower ozone concentrations in the annular reaction zone (2 mg/L) resulted in lower degradation rates (from 30 % for ciprofloxacin, norfloxacin and enrofloxacin; to 95 % for azithromycin) than for higher concentrations. By increasing the presence of O_3 up to 6 mg/L, degradation rates were very satisfactory for all compounds (>83 % in all cases). For the highest concentration (12 mg/L), the conversions were above 99 % for the 14 antibiotics. Thus, in demineralized water, the use of O_3 at a concentration of 6 mg/L in the ARZ was enough to obtain high removal efficiency of the antibiotics of interest.

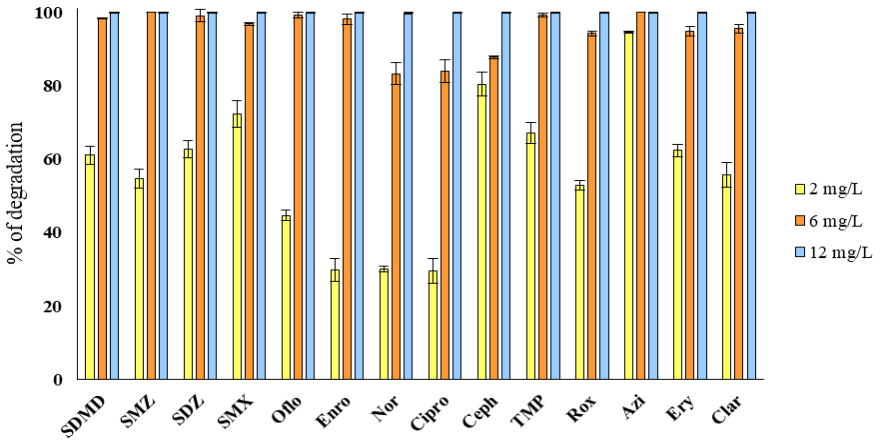


Figure 6. Assessment of the concentration of O_3 in the annular reaction zone for the removal of the 14 antibiotics.

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3.2.2 Water matrix

As in DW the use of O_3 as an oxidant appeared to be a very effective alternative for the removal of all target antibiotics, tests were carried out with water from a WWTP after secondary treatment. For this purpose, the UWW was fortified with the antibiotics of interest at the same concentration level (50 µg/L).

As shown in Figure 7, in UWW there was a clear decrease in the percentage of degradation at a flow rate of 150 L/h and $[O_3]_{ARZ}$=6 mg/L compared to DW. While in DW the degradation percentages were around 90 %, in UWW sulphonamides were 50 % degraded, cephalexin and trimethoprim 30 % and the remaining compounds (fluoroquinolones and macrolides) were hardly degraded (approx. 10 %).

However, increasing the residence time and O_3 dosage by 3 times, from Q_{inlet}=150 L/h and $[O_3]_{ARZ}$=6 mg/L to Q_{inlet}=50 L/h and $[O_3]_{ARZ}$=18 g/L (giving rise to a HRT of 10.2 s), allowed attaining levels of antibiotic removal similar to those obtained in demineralized water, with all antibiotics removed above 70%.

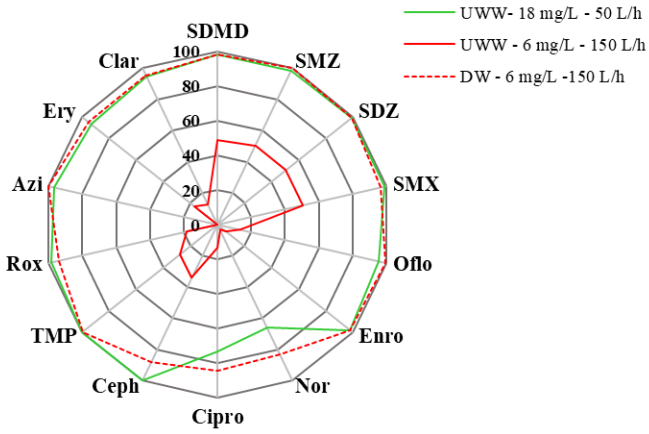


Figure 7. Removal efficiency of the 14 antibiotics under the ozonation process using demineralized water (DW) (Q_{inlet}=150L/h or 50 L/h; $[O_3]_{ARZ}$= 6 mg/L or 18 mg/L) or urban wastewater (UW) (Q_{inlet}=150L/h; $[O_3]_{ARZ}$= 6 mg/L).

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3.3. Identification of transformation products

For the identification of possible transformation products (TPs) formed during the degradation processes, spiked DW with 300 µg/L of each targeted antibiotic was used. As regards the analysis, a UHPLC-QTOF-HRMS system, which allows both the quantification of targeted compounds and the complete non-target search of unexpected ions present in the samples, was employed. For this purpose, a data independent workflow (SWATH) was implemented to identify the main by-products, consisting of the fragmentation of all precursor ions entering the mass spectrometer in a wide range of masses, divided in 20–30 m/z windows.

As first step, a previous bibliographic research dealing with different degradation processes of the targeted antibiotics was carried out [18,27]. The TPs identification was achieved after importing the list of the suspected targeted by-products and metabolites. By means of this approach, 8 by-products could be identified based on their exact mass, accepting only mass error below 8 ppm. The identified TPs after the two optimized oxidative treatments are included in Table 1.

As regards UVC/Ag_2MoO_4/$S_2O_8^{2-}$ treatment, only one degradation product could be identified. This transformation product ($C_{17}H_{18}O_4N_3F$, m/z 348.135) may have been formed from ofloxacin and/or ciprofloxacin.

The remaining 7 identified transformation products were obtained stemming of macrolide antibiotics during ozonation. One of the identified by-products showed the same chemical formula and exact mass ($C_{36}H_{65}NO_{13}$, m/z 720.453) and could come from the targeted four macrolides. Therefore, based on the HRMS criteria, it was not possible to differentiate it and establish from which macrolide this by-product could have originated.

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Table 1. Identified transformation products for O_3 and UVC/Ag_2MoO_4/$S_2O_8^{2-}$ treatments.

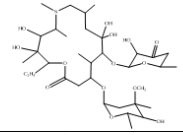
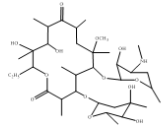
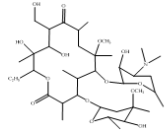
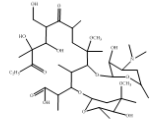
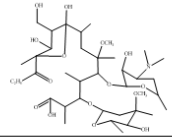
Parent compound	Transformation products	Molecular Formulae	Precursor ions (m/z)	Structures	Mass error (ppm)	Ref.
O_3						
Azi	Azi/Clar/Ery/Rox-1	$C_{36}H_{65}NO_{13}$	720.453		0.9	[25]
Clar	Azi/Clar/Ery/Rox-1	$C_{36}H_{65}NO_{13}$	720.453		0.9	
	Clar-2	$C_{38}H_{69}NO_{14}$	764.479		1.0	
	Clar-3	$C_{38}H_{69}NO_{15}$	780.474	a.  b. 	-0.1	

Table 1. (cont.) Identified transformation products for O_3 and UVC/Ag_2MoO_4/$S_2O_8^{2-}$ treatments.

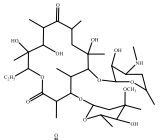
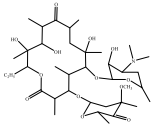
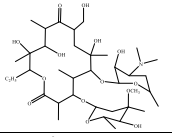
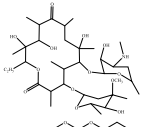
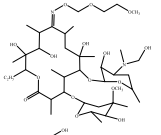
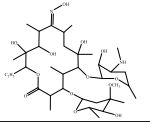
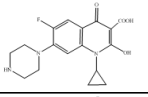
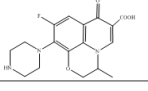
Parent compound	Transformation products	Molecular Formulae	Precursor ions (m/z)	Structures	Mass error (ppm)	Ref.
Ery	Azi/Clar/Ery/Rox-1	$C_{36}H_{65}NO_{13}$	720.453		0.9	[25]
	Ery-2	$C_{37}H_{65}NO_{13}$	732.453		1.9	
	Ery-3	$C_{37}H_{67}NO_{14}$	750.463		1.7	
Rox	Azi/Clar/Ery/Rox-1	$C_{36}H_{65}NO_{13}$	720.453		0.9	
	Rox-2	$C_{41}H_{76}N_2O_{16}$	853.527		2.0	
	Rox-3	$C_{36}H_{66}N_2O_{13}$	735.464		7.9	

Table 1. (cont.) Identified transformation products for O_3 and UVC/$Ag_2MoO_4/S_2O_8^{2-}$ treatments.

Parent compound	Transformation products	Molecular Formulae	Precursor ions (m/z)	Structures	Mass error (ppm)	Ref.
UVC/$Ag_2MoO_4/S_2O_8^{2-}$						
Cipro	Cipro/Oflo-1	$C_{17}H_{18}O_4N_3F$	348.135		-0.8	[18,21, 24]
Oflo	Cipro/Oflo-1	$C_{17}H_{18}O_4N_3F$	348.135		-0.8	[27]

In addition to this compound, other two by-products could be identified for each of the following macrolides: clarithromycin, erythromycin and roxithromycin. It is important to note that for Clar-3 ($C_{38}H_{69}NO_{15}$, m/z 780.474), two possible chemical structures (Clar-3a and Clar-3b) were proposed, which correspond to two isomeric structures that cannot be distinguished by HRMS.

It is worth noting that most of the obtained transformation products with the tube-in-tube membrane reactor working in continuous mode, except Clar-3 and Rox-3, were also formed after UVC radiation in the lab-scale photoreactor (see Chapter 4.1).

4. Conclusions

The operational conditions of a tube-in-tube membrane by applying different oxidant-catalyst combinations (either liquid or gaseous oxidants), under continuous mode and low residence time, have been optimized employing demineralized water. The photoreactor versatility was also demonstrated for the removal of a mixture of 14 antibiotics in an urban wastewater after secondary treatment. The best treatment performance was achieved with O_3 treatment (removal >70% for all antibiotics and >95% for 12 of the 14), followed by UVC/Ag_2MoO_4/$S_2O_8^{2-}$ (>60% removal for 5 of the 14 antibiotics). Finally, a tentative identification of degradation by-products has been also carried out, demonstrating the presence of seven macrolide transformation products during the ozonation process and one from ciprofloxacin/ofloxacin using the UVC/Ag_2MoO_4/$S_2O_8^{2-}$ system. Therefore, the tube-in-tube membrane reactor offers a suitable and high throughput system for the antibiotic removal, as it allows high degradation rates without forming too many degradation by-products.

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V. GENERAL DISCUSSION

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In this doctoral thesis, several analytical methodologies have been developed to isolate and determine a wide range of analytes in different matrices such as environmental, agronomic, food and cosmetic samples. Taking into account all the optimized methods, more than one hundred and fifty target substances were involved. Among the studied substances fungicides, volatile, semi-volatile and phenolic compounds present in honey samples, as well as pharmaceutical and personal care products (PPCPs), including antibiotics, fragrances (allergens and synthetic musks), UV filters, preservatives and plasticizers were included. In addition, non-target screening was carried out in some investigations.

One of the trends and most critical stage in analytical chemistry is the sample preparation step. In order to achieve sustainable features, the simplicity, minimization of the cost and time of preparation, the use of low volumes of reagents and solvents and the generation of the least amount of residues, are some of the most important factors to consider. In addition, it is important to achieve high sensitivity and selectivity of the analytes of interest, demonstrating that the developed methodology presents high performance and throughput and that it is easy to implement in any laboratory. The selection of the extraction technique has to be adequate for both the analytes and the matrix. Furthermore, the applied instrumentation is also crucial to select the sample preparation technique. During this thesis, different analytical techniques based on chromatography coupled to mass spectrometry were employed. Gas or liquid chromatography was selected according to the application and/or the analytes of interest and the detectors employed were based on low (simple or tandem mass spectrometry) and high resolution (simple and a triple quadrupole-time of flight detector). On the other hand, different chemometric tools were used to achieve the optimal conditions and the highest efficiency for the extraction of the target substances, as well as for the interpretation of the results obtained.

In this section, the most critical aspects of the experimental work and the most outstanding results obtained, highlighting the advantages and disadvantages of the developed methods are summarized. A brief

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discussion for each chapter, comparing the included publications in each one, is reported. In addition, a general discussion for all methodologies developed during the present thesis is also provided.

1.- Determination of fungicides in agricultural and environmental samples

Fungicides are a type of pesticides commonly used in viticulture to avoid fungi infection or even some bacteria in crops, such as grey rot (*Botrytis cinerea*), downy mildew (*Plasmopara viticola*) and powdery mildew (*Uncinula necator*). Galicia (NW Spain) is a wine-producing region in a large part of its territory, and due to the extreme climatic conditions, with hot summers and cold and rainy winters and autumns, the use of fungicides for the conservation of vineyards is necessary. Hence, the continuous application of these compounds induces their entry into different environmental compartments, since residues can easily reach soils and then, through leaching, they can reach aquatic ecosystems, posing a risk to environmental and human health. Therefore, the occurrence of these compounds in both crop and environmental compartments is of great interest.

In this context, the sample preparation techniques employed for the extraction of seventeen fungicides in water samples, collected in vineyard areas, were based on fabric phase sorptive extraction (FPSE) and solid-phase extraction (SPE) using cork by-products as sorbent, demonstrating that both were suitable. Ultrasound assisted extraction (UAE) was optimized and validated for the analysis of these substances in vine leaves and applied to grapes and soils from vineyard areas. In all cases, gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) was used because it offers a suitable option for the determination of nonpolar and low polar fungicides, such as those selected in this thesis. Nevertheless, liquid chromatography can be also a good option to determine these compounds, even though the extraction methods must sometimes be adapted to use this tool. The most used acquisition mode is the selective reaction monitoring (SRM) since it allows a sensitive and selective detection, with very low noisy

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chromatograms for the identification of the target fungicides, monitoring 2 or 3 transitions per compound.

Although the use of fungicides is indispensable for ensuring food safety, concerns have been raised about their presence in crops (leaves and grapes) and in the surrounding environment (water and soil) where they were spread. In this way, the UAE method was developed for the determination of 17 of these organic compounds in vine leaves. Different studies were carried out to obtain the best extraction efficiency, in which parameters and stages such as the extraction technique, the type of solvent, the dryness of the leaves, among others, were assessed. UAE has been compared with other extraction techniques such as matrix solid-phase dispersion (MSPD) and vortex extraction (VE), demonstrating that the latter was also suitable to determine fungicides in vine leaves. The type of solvent was evaluated using chemometric tools like the analysis of variance (ANOVA) or multiple-range least significant difference (LSD) tests. Once the most appropriate conditions were selected, the method was validated in terms of analytical quality parameters showing an adequate precision with relative standard deviations (RSD) below 9% and recovery values between 80 and 113% for all compounds at three concentration levels, except for two of them (fenhexamid and pyraclostrobin). The linearity was also evaluated, and results demonstrated that the quantification could be performed by external calibration for almost all studied fungicides, excluding fenhexamid and pyraclostrobin. For these compounds, matrix-matched calibration is highly recommended and estimated recovery values were 87–106% at the three concentration levels.

Furthermore, it is important to note that the developed method is also suitable and applicable to other leaves such as tea, as the studies conducted in this thesis showed. Other matrices such as grapes and soil were also analysed applying the UAE-GC-MS/MS methodology. Microwave assisted extraction (MAE) revealed similar results as UAE for the determination of the 17 target fungicides in soils. However, UAE was selected with the aim of using the lowest amounts of sample and solvent and minimizing the cost, time and residues.

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On the other hand, as it was mentioned above, fungicides can be present in waters, usually at trace levels, making essential the development of sensitive analytical methodologies in water samples of different origin. In order to determine this wide group of pesticides in this matrix, two approaches have been optimized.

SPE has been the most widely employed technique to extract fungicides from aqueous samples due to its extraction capacity, clean-up, high enrichment factor and simplicity. Undoubtedly, the most critical element to obtain a successful SPE technique is the sorbent selection, since it plays an important role in the efficiency and analyte selectivity. For this reason, a cork by-products has been proposed as biosorbent to determine these substances in water. Natural cork is a heterogeneous material with a chemical composition mainly formed by hydrophobic biopolymers and hydrophilic polysaccharides, which provide sites for the sorption of organic compounds. Besides, it can be easily thermally treated to enhance the possible interactions with less polar organic compounds. Therefore, the main advantages of this eco-natural material are that it is a renewable, biodegradable and low-cost alternative sorbent favouring the circular economy

FPSE is a novel sample preparation technique, which offers a number of advantages over conventional sample preparation techniques, since it innovatively combines the equilibrium extraction mode of the solid-phase microextraction (SPME) and the exhaustive extraction of the SPE, in a single technology platform.

Both extraction techniques employed for the analysis of fungicides in water were optimized with design of experiments (DOE). The SPE protocol employed a multifactorial design 2^3 with two central points (2^3 + 2). The factors evaluated were the type of cork (natural cork or thermally treated cork), extraction solvent and sample pH. For the FPSE procedure, a half fraction screening design 2^{5-1} plus two central points was conducted, giving rise to 18 experiments. In this case, the studied parameters were the sample volume, desorption solvent, desorption time, NaCl addition, and sample pH.

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The methodologies were validated in terms of linearity, limits of detection (LODs) and quantification (LOQs), precision (repeatability and reproducibility) and accuracy, showing satisfactory results in all cases. In addition, matrix effects were assessed by performing recovery studies in different water matrices. As regards the investigation based on SPE using cork by-products, ANOVA and multiple range LSD tests were carried out in order to evaluate whether the type of water matrices and the addition levels were comparable and/or statistically different.

The recovery values achieved in different matrices in both studies also demonstrated the suitability and high throughput of the related methods, except for one fungicide (metalaxyl) for which the results were not as good as expected. Although this compound was poorly recovered in some of the studied water matrices (i.e. tap water) using FPSE, the mean recovery values for this compound were around 30% in all matrices using SPE, including ultrapure water. Thus, it seems that the method based on FPSE-GC-MS/MS is more efficient for the determination of metalaxyl in environmental water samples. Nevertheless, both procedures appeared to be suitable alternatives for the determination of trace levels of fungicides in water samples, since LODs and LOQs were at the low ppt (ng L^{-1}). Consequently, they are promising techniques for the extraction of other organic compounds from the aquatic environment.

Therefore, the presence of fungicides at high concentrations in crops and in the surrounding environment, surpassing the maximum residue levels (MRLs) for some target compounds in vine leaves and grapes and the EU maximum permitted concentrations for pesticides in groundwater and water intended for human consumption, revealed that the application modes should be improved. This is of great importance because of the likely environmental consequences in vineyard areas, as well as for the consumer safety concerning grapes, wine and even vine leaves, which are used to cook numerous recipes in many cultures, both fresh and preserved.

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2.- Chapter 2. Characterization of honey: volatile, semi-volatile and phenolic compounds

Honey is a natural food product well known for its high nutritional value, due to the presence of sugars, proteins, amino acids, enzymes, organic acids, vitamins, minerals, phenolic and volatile compounds. The properties of honey are defined by the plant species from which the nectar or the honeydew was collected, and the geographical origin. The aromatic profile of honey is important since it forms its organoleptic characteristics, identity and quality. In addition, the phenolic compounds present in honey give it antimicrobial and antioxidant capacities. A high proportion of the produced honey in Galicia is recognised under the Protected Geographical Identification (PGI) denomination. Therefore, it is important to identify possible chemical markers since honey is among the top 10 products with the highest adulteration rate in the European Union. Furthermore, honey will be of a different botanical origin depending on the area in which it is produced. Thus, it is also of great interest to be able to differentiate it according to the floral origin of the nectar extracted by bees (multifloral or monofloral), which determines honey properties.

As it is well known, honey is a very complex matrix which requires a sample preparation step prior its analysis. The sample preparation techniques involved for the analysis of honey in this thesis were solid-phase microextraction (SPME) for the determination of volatile and semi-volatile compounds by gas chromatography followed by mass spectrometry with a single quadrupole (GC-MS), and a simple combination of VE followed by UAE to assess the presence of phenolic compounds by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The acquisition mode for the GC-MS analysis was full scan (FS), monitoring mass/charge (m/z) fragments between 35 and 400, since an initial screening analysis was first carried out with a multifloral type honey sample, and the most abundant compounds were selected as targets to conduct the optimization study. Regarding LC-MS/MS, SRM was chosen to identify and quantify the phenolic compounds. In addition, the adaptation of two protocols previously reported in the literature, were applied to evaluate the total

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phenolic content (TPC) and the antioxidant activity (AA) employing an UV-Vis spectrophotometer.

SPME is one the preferred extraction techniques for the analysis of volatile and semi-volatile compounds in honey. However, with the aim of minimize the cost, the amount of sample and waste generation, the SPME miniaturization (mini_SPME), enabling the use of a very small amount of honey (200 mg), seemed a good option, since the chromatographic responses were quite comparable when using 10 times more sample (2 g). A mixed level fractional factorial DOE ($3 \cdot 2^{3-1} + 2$ central points) was employed to evaluate four critical parameters that could affect the mini_SPME effectiveness for the determination of 24 selected compounds: temperature, fibre coating, salting out effect (addition of NaCl, w/v) and dilution in water (w/v). The method was validated achieving good linearity, LODs and LOQs and precision. In order to assess the method accuracy, recovery experiments were conducted in four different types of honey (multifloral, honeydew, chestnut and heather honey). Finally, the optimized method was applied for the identification of the 24 previous selected compounds and quantification of 9 of them in a broad range of honeys from Galicia and foreign countries (France, Greece, Italy and Kazakhstan). The botanical origin of the analysed honey samples was multifloral, honeydew, chestnut, heather, blackberry, eucalyptus, orange, thyme and lavender. In addition, a preliminary study was conducted to identify non-target species. Nevertheless, a statistical approach would be further necessary to check whether the compounds could be considered as markers for Galician honeys.

On the other hand, a methodology which combines VE with UAE, utilizing aqueous based solvents, was employed for the evaluation of phenolic compounds in honey. Since honey is a complex matrix, SPE is usually employed to remove matrix components such as polysaccharides. Nevertheless, in this investigation, after a previous optimization, external calibration was employed and accuracy was assessed fortifying a multifloral honey with the 41 target phenolic compounds, including aldehydes, acids, flavonoids (flavonols, flavones…) and coumarins, applying a quite simple and fast strategy.

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Twenty-five of the 41 target compounds were identified and quantified in the analysed samples. In addition, TPC and AA were also evaluated according to modified protocols previously reported using a UV-Vis spectrophotometer. A chemometric study has been carried out employing statistical tools such as ANOVA and principal component analysis (PCA) to obtain models that allowed classifying 91 different Galician honeys according to their botanical origin, including multifloral, honeydew, heather, chestnut, chestnut/honeydew, blackberry and eucalyptus honeys. These studies were based on TPC, AA, individual phenolic concentration and/or ∑phenolic compounds showing significant differences depending on the honey variety.

Hence, despite the complexity of the matrix, both proposed methods, the first one to identify and quantify volatile and semi-volatile compounds and the second one relying on phenolic compounds, proved to be suitable. The powerful of chemometric tools was demonstrated to differentiate the honey variety employing the TPC and AA indexes and the phenolic compound contents. However, results did not allow differentiating Galician honeys from other foreign honeys, although they allowed distinguishing between the different honey varieties. In addition, a more in-depth study in which a larger number of samples would be analysed would be necessary to find significant differences, especially for volatiles and semi-volatile compounds.

3.- Chapter 3. Determination of personal care products (PCPs) in cosmetic formulations

Cosmetics products are defined by the Regulation EC No 1223/2009 as *"any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours".* In this context, as it is well known, cosmetics such as make up, hair-care products, creams, among others, are widely used consumer products all

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over the world. Besides, nowadays, one of the most frequently daily used products are hydroalcoholic gels, since they became essential to prevent the spread of COVID-19 virus and they are massively employed for the general population and several times every day.

A large number of organic compounds, considered personal care products (PCPs), are present in cosmetic formulations, such as fragrance allergens, preservatives, plasticizers, synthetic musks, and ultraviolet (UV) filters, among others. Fragrances (allergens and synthetic musks), usually added to cosmetics with the intention of providing a pleasant scent, are the main responsible of allergic contact dermatitis. UV filters are intended to protect consumers against the harmful solar radiation and their presence is especially important in sunscreens, but they can also be found in a broad range of daily care products. Preservatives are added to cosmetic formulations to protect products and consumers against microbial growth, whereas plasticizers can be added to the product or transferred from the plastic containers to the product. The presence of most of these compounds in cosmetics is associated with contact allergies, skin dermatitis and even, in some cases, carcinogenic effects and endocrine dysfunction.

Cosmetic products marketed (whether produced in the EU or not) in the European Union must follow the applicable legislation. Therefore, they must comply with Regulation EC No 1223/2009, and this compliance must be verifiable from an analytical point of view. In this context, cosmetics analysis is a challenge task due to the wide variety and complexity of the samples, composed by a high number of substances of different nature.

Two analytical methodologies involving the use of µMSPD and SPME to determine a high number of PCPs in cosmetics of different nature were developed. In both cases, the PCPs were analysed by GC according to the chemical nature of the target analytes and MS/MS detection in SRM mode was chosen to improve analytical selectivity and sensitivity.

A methodology based on µMSPD was optimized for the analysis of 14 multiclass UV filters. This is a very novel approach as this technique

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has never been applied for the extraction of these substances in cosmetic formulations. Factors affecting the process such as the dispersing agent, and volume and type of elution solvent were studied by means of a DOE. In this case, a multifactor categorical $4 \cdot 2^2$ design involving 16 randomized experiments was selected. Once the method was optimized, the μMSPD-GC-MS/MS performance was assessed and extended to other PCPs, such as fragrance allergens, synthetic musks, preservatives and plasticizers, reaching 78 PCPs. The method showed good linearity and precision. Recovery studies were carried out fortifying a moisturizing hand cream, and the obtained results ranged from 70 to 110 % for all compounds. The fast and green developed method demonstrated high throughput and suitability, allowing the simultaneous analysis in a single extraction of 78 compounds with very different chemical nature, in a wide variety of cosmetic products (sunscreens, creams, make up, lipsticks…).

Regarding the analysis of hydroalcoholic gels, until the development of the present method, SPME had never been applied for the simultaneous extraction of multianalyte PCPs in cosmetic samples, including in this case 60 compounds, such as fragrance allergens, synthetic musks, preservatives and plasticizers. For the optimization of this protocol, a multifactorial categorical design ($3 \cdot 2^2$) was carried out evaluating the main factors affecting the sample preparation step and their interactions. The parameters included in the DOE were the fibre coating, the extraction mode and the temperature. Under the optimized conditions, the SPME-GC-MS/MS method was validated in terms of linearity, precision and accuracy, achieving good results and following as far as possible the application of the ISO 12787 international standard "Cosmetics–Analytical methods–Validation criteria for analytical results using chromatographic techniques". After that, the method was applied to real hydroalcoholic gel samples in order to assess their compliance with the applicable legislation (Regulation EC No 1223/2009), as well as with the World Health Organization recommendations to homogenise the hydroalcoholic gel formulation and fabrication, assuring their antimicrobial properties.

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Thus, both methodologies were simple, suitable, green, and fast allowing the determination of a high number of PCPs in cosmetic products of different nature. In both cases, matrix calibration was not required, which further simplifies the procedures facilitating their use in any routine laboratory. The presence of prohibited compounds in different cosmetic products, according to EC Regulation No. 1223/2009, was demonstrated, as well as that of fragrance allergens at concentrations above the label limit (10 μg g^{-1}) in hydroalcoholic gels. Furthermore, none of the formulations of the analysed samples of hand sanitizers complied with the WHO recommendations. Although, hydroalcoholic gels can be considered a borderline product, results showed that they the analysed sample were cosmetics and not biocidal products. Therefore, the obtained results revealed the need for a greater control over cosmetic products to ensure consumer safety without causing undesirable side effects.

4.- Chapter 4. Determination of antibiotics in water and degradation studies

Due to the increasing use of pharmaceuticals by the population and in veterinary medicine, their presence and persistence in water is growing. Among these compounds, one of the most interesting groups is the antibiotics, which can cause microbial resistance due to irresponsible and irrational uses. As they are only partially removed in wastewater treatment plants, these substances continuously enter the environment, posing a significant risk not only to the aquatic ecosystem, but also to human health. Thus, alternative procedures such as photodegradation and advanced oxidation processes (AOPs) seem to be good options for the efficient removal of antibiotics and other emerging pollutants.

SPE was employed for the isolation of 14 multi-class antibiotics in wastewaters and natural water, including macrolides, cephalosporins, fluoroquinolones, sulfonamides and diaminopyrimidines. The analysis was carried out by two approaches based on high-resolution mass spectrometry, employing a simple and a triple quadrupole-time of flight detectors, coupled to ultra-high performance liquid chromatography.

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The extraction method was optimized evaluating different parameters such as sample pH, amount of sample and solvent, and the SPE cartridge, which consisted in this case of a conventional hydrophilic-lipophilic sorbent, well known under the commercial name of Oasis HLB (Waters company). Under the optimal conditions, the method was validated achieving good linearity, precision and LODs and LOQs. The recovery values were satisfactory in ultrapure water, being above 80 % except for two macrolides, azithromycin and erythromycin. Therefore, experiments were conducted to evaluate whether the low values were due to a low extraction efficiency or to matrix effects into the MS ionization source. For this purpose, responses of fortified extracts before and after the SPE process were compared, showing that there was no significant ionization suppression into the source, but the poor retention capacity of the sorbent was responsible of the low recoveries. Additionally, recovery studies in four different real matrices (urban and hospital effluents, well water and river water) were conducted, observing that the type of water matrix influenced the extraction performance. The recovery percentages particularly decreased for the most complex matrices (urban and hospital wastewater), consequently the use of isotopically labelled surrogates deemed necessary. After that, the target antibiotics were quantified in wastewater and natural waters.

To analyse the target antibiotics, an ultra-high performance liquid chromatography coupled to a quadrupole time-of-flight (UHPLC-QToF) mass spectrometer was used. Another high-resolution mass spectrometry configuration was utilized for conducting non-target studies, employing a SCIEX TripleTOF (QqTof) instrument in SWATH^{TM} (sequential windowed acquisition of all theoretical mass spectra) mode. The SWATH workflow is based on the selection of a m/z range in which all analytes are included, then the range is divided into numerous small m/z windows (typically between 20 and 30) and MS/MS spectra are registered for all the detected peaks. In this way, since a TOF full scan and MS/MS spectra for all peaks are obtained simultaneously, it is not necessary to introduce mass transitions or expected masses in the acquisition method. One of the powerful features enabled by this scanning mode is the possibility to perform non-target retrospective searches using the same raw acquisition files.

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This approach allowed to identify up to 94 non-target chemical compounds, including another antibiotics, but also drugs, pesticides…, taking into account the exact masses of the unknown eluted compounds and other additional confirmation criteria such as the isotope profile and library score calculated with high-resolution MS and MS/MS spectra, providing a final combined score.

On the other hand, since the occurrence of the target antibiotics was demonstrated in real water samples, suggesting that they can easily reach the aquatic environment, the use and improvement of water decontamination procedures is of great interest to protect the environment and human health. As such, a preliminary study dealing with the simultaneous photodegradation of the 14 target multi-class antibiotics employing a lab-scale reactor with two UVC lamps has been carried out. Results showed that photodegradation seemed to be a useful option for the removal of most of the target antibiotics in ultrapure water. However, studies in other real water matrices are also needed as well as other investigations including advanced oxidation processes (AOPs), since they could lead to efficient and novel procedures.

In this context, a low footprint tube-in-tube membrane reactor based on AOPs working in continuous mode has been tested for the removal of target antibiotics in both demineralised water (DW) and urban wastewater (UWW). The operating conditions have been optimized by evaluating different parameters such as liquid (H_2O_2, $S_2O_8^{2-}$) and gaseous (O_3) oxidants, highlighting that it is the first time that a gaseous oxidant is proposed for this type of reactor. In addition, the catalyst (Ag_2MoO_4 or TiO_2), the use of UVC radiation, and sample flow rate were also assessed. It is also important to mention that Ag_2MoO_4 had never been proposed as a catalyst for the tube-in-tube membrane reactor. Promising removal efficiency was obtained not only for DW, but also for UWW. On the other hand, by-products may be formed during the degradation processes, so that their identification is also of great importance, as well as the investigation of their formation route. In this thesis, a tentative identification was conducted in HRMS by implementing a SWATH acquisition, being able to find and identify up to 20 photoproducts after applying UVC radiation and 8 after

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employing the tube-in-tube membrane reactor (1 for UVC/Ag_2MoO_4/$S_2O_8^{2-}$ treatment and 7 for the ozonation process). It is worth noting that most degradation studies and the subsequent identification of possible by-products are usually carried out for only few antibiotics and for compounds of the same family, although co-occurrence of antibiotics is very frequent in the environment. As an example, the tube-in-tube membrane reactor has never been applied for the simultaneous degradation of more than four pharmaceuticals.

Hence, to summarize briefly, seven different sample preparation techniques (FPSE, SPE, UAE, SPME, VE, MSPD and MAE) were tested along the present thesis. As common characteristics, all the optimized methods and extraction techniques follow as much as possible the principles of "green analytical chemistry", demonstrating their suitability for the analysis of different matrices. As an example, SPME was feasible both for the extraction of volatile and semi-volatile compounds in honey as well as for a wide variety of PCPs in cosmetic formulations, such as hydroalcoholic gels. In all cases, an attempt was made to minimize the amounts of sample and solvents employed, so that all procedures were as environmentally friendly, simple and miniaturized as possible. Besides, they were of easy implementation in any laboratory and some of them of easy automation, such as SPE or SPME.

On the other hand, six matrices of quite different nature were considered, including environmental, agronomic, food and cosmetic samples. Among the environmental ones, a wide variety of waters were analysed, such as rainwater, irrigation water, river, well water, urban and hospital wastewaters, and others utilized for recovery studies (tap water, spring water, fountain water, etc). In addition, the presence of fungicides was evaluated in soil samples and in food, leaves and grapes. These compounds were also determined in tea samples. Nevertheless, honey was the main food matrix characterized, being probably the most complex one to handle during this thesis, since its high viscosity makes difficult to work with. Finally, the cosmetic products that were included in this thesis involved creams (moisturizing facial cream, vitalizing

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cream…), leave-on hair serum, sunscreens, make-ups, lipstick and solar stick. In addition, hydroalcoholic gels were also analysed, due to their frequent use to prevent and mitigate SARS-CoV-2 virus dissemination during Covid-19 pandemic.

Therefore, it is important to highlight the complexity that comes from the optimization of so many sample preparation techniques for such a wide variety of matrices. In addition, the fact that these methods are multi-analyte, up to 78 target compounds in some of the investigations, makes the optimization and analysis more complex. It should be taken into account that more than 150 target compounds have been considered and that non-target compounds and photodegradation by-products have been identified, as well.

All the proposed analytical methodologies published in scientific papers and included in this thesis were validated. Linearity was assessed and external calibration was adequate for all the methods developed, although for complex matrices, compounds acting as internal standards and isotopically labelled surrogates were considered necessary (i.e. honey, wastewaters). The adequacy of the developed methods to detect trace and ultra-trace levels with sufficient confidence has been demonstrated, achieving low LODs and LOQs. In addition, these methods showed good precision, with satisfactory results for both inter-day (reproducibility) and intra-day (repeatability).

Recovery studies were also carried out in different matrices and at different concentration levels in most cases, demonstrating the accuracy of the developed methods. In addition, all the methodologies were applied to real samples for each class of compounds, demonstrating in all cases the occurrence of some of the target analytes. Besides, the quantification of the target substances has been carried out and the observed concentration levels were compared with those included in the legislation in force, where applicable.

On the other hand, the great potential of chemometric tools has been again demonstrated. In most of the investigations, DOE was used to simultaneously evaluate the influence of critical parameters affecting the extraction efficiency, taking into account not only the selected

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factors but also their interactions, reaching optimal conditions for all analytes. ANOVA was confirmed as a powerful statistical parameter, since it was applied for optimization, but also for the interpretation of the results. Multiple-range LSD tests were also applied with the aim of reaching the best extraction efficiency. Finally, PCA confirmed its suitability to describe differences between data set, for example to identify groups of honey from different botanical origins.

Different degradation processes for the simultaneous removal of antibiotics were assessed. For this purpose, photolysis was tested using a laboratory-scale static photoreactor and, advanced oxidation processes were applied employing a tube-in-tube membrane reactor operating in continuous mode. Degradation efficiencies showed that both systems appeared to be good options for antibiotics removal in water.

Analytical techniques based on chromatography coupled to mass spectrometry proved to be appropriate for the determination of the analytes of interest for which they were selected. Two types of chromatography, gas and liquid, were used for compound separations. Regarding the detection, instruments employed were based on two mass spectrometry approaches, using low-resolution and high-resolution instruments. For low resolution, mass spectrometry with a simple quadrupole was selected to determine volatiles and semi-volatiles in honey, obtaining good responses for the identification and quantification of the selected substances. Meanwhile, tandem mass spectrometry (triple quadrupole detector) coupled with gas chromatography was employed for the analysis of PCPs and fungicides and, coupled with liquid chromatography for phenolic compound analysis. This detector provides the unequivocal identification of the compounds as well as a high selectivity and sensitivity.

Concerning the high resolution, two approaches were used with single and triple quadrupole time-of-flight detectors. It proved to be a powerful tool, especially for the identification of unknown compounds such as by-products generated during degradation processes, often at very low concentrations.

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In addition, other instrumentation employed in the present thesis is an UV-Vis spectrophotometer, used for the assessment of the TPC and AA indexes in honey samples

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VI. CONCLUSIONS

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During the development of the present thesis, new analytical methodologies were optimized and applied to the determination of a wide variety of analytes in different matrices.

The sample preparation procedures were developed according to principles of green chemistry, trying to minimize the amount of sample, solvent and residues generated. In addition, all of them were simple, fast, low-cost and of easy implementation in any laboratory. The extraction techniques used for the analysis of fungicides were solid-phase extraction (SPE) and fabric phase sorptive extraction (FPSE) in water and ultrasound assisted extraction (UAE) in leaves, grapes and soil. Regarding the analysis of honey, solid-phase microextraction (SPME) for volatile and semi-volatile compounds and vortex extraction (VE) followed by UAE for phenolic compounds were employed. Micro-matrix solid-phase dispersion (μMSPD) and SPME were applied for analysing personal care products (PCPs) in cosmetic formulations and SPE for antibiotics in water.

The analysis was carried out employing gas (GC) or liquid chromatography (LC). GC was coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS), which provide excellent selectivity and sensitivity. LC was coupled to MS/MS or high-resolution mass spectrometry (HRMS), the latter being especially useful for the identification of non-target compounds such as by-products generated during degradation processes. Ultraviolet-visible (UV-Vis) spectrophotometry was also used to measure the total phenolic content (TPC) and the antioxidant activity (AA) of honey samples.

In addition, different reactors and technologies based on UVC photolysis and advanced oxidation processes (AOPs) were implemented in order to remove antibiotics from different water samples.

The extraction efficiency of the methods was optimized employing chemometric tools such as analysis of variance (ANOVA), multiple-range least significant difference (LSD) tests or design of experiments (DOE), enabling the influence assessment of the main factors affecting

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the extraction process and their interactions. In addition, for the interpretation of the results, ANOVA and principal component analysis (PCA) were also used.

The protocols were validated in terms of analytical quality parameters: linearity, intra-day and inter-day precision, accuracy and limits of detection and quantification. As the results were satisfactory in all cases, the methods were subsequently applied on real samples.

The general conclusions for each chapter are included below:

Chapter 1. Determination of fungicides in agricultural and environmental samples

- Two approaches based on FPSE and SPE followed by GC-MS/MS were optimized, by means of experimental design, and validated to determine fungicides in water samples.

- Cork by-products appeared to be a promising low-cost, renewable and biodegradable sorptive alternative phase to extract organic compounds from the water.

- The presence of fungicides at concentrations up to hundreds of μg L^{-1} in environmental waters demonstrated the entrance of fungicide residues through runoff rain and irrigation water, which can easily reach different environmental compartments.

- UAE is a suitable extraction technique for the analysis of fungicides in vine and tea leaves, grapes, and soils.

- Fungicide application modes should be improved since their residues can easily reach the surrounding environmental areas.

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Chapter 2. Characterization of honey: volatile, semi-volatile and phenolic compounds

- A methodology based on miniaturized SPME followed by GC-MS has been proposed for the simultaneous analysis of different volatile and semi-volatile compounds in honey.

- The extraction efficiency of twenty-four target compounds was assessed by DOE and they were investigated in twelve Galician honeys and eight foreign honeys. Nine of them were quantified, demonstrating the suitability of the proposed method.

- A simple, fast and environmentally friendly procedure based on VE-UAE-LC-MS/MS was optimized to analyse phenolic compounds in 91 honeys from Galicia.

- Statistical tools such as ANOVA and PCA revealed that phenolic compounds could be used as indicators to identify the honey floral origin since significant differences were obtained for TPC, AA and ∑phenolic compounds concentrations depending on the honey variety.

Chapter 3. Determination of personal care products (PCPs) in cosmetic formulations

- A new analytical methodology based on μMSPD-GC–MS/MS has been developed for the simultaneous analysis of multiclass organic UV filters and applied for other PCPs in cosmetic products, such as sunscreen, make up, and hair-care products, among others.

- A protocol based on SPME-GC-MS/MS to determine PCPs, including fragrance allergens, synthetic musks, preservatives and plasticizers in hydroalcoholic gels was optimized. This high throughput and environmentally friendly

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procedure was shown to be fast, simple and of easy implementation in any routine laboratory.

- Some forbidden compounds for their use as ingredients in cosmetics according to the Regulation EC No 1223/2009 were found in cosmetic products.

- Greater control over the formulations of the cosmetic products is necessary to ensure consumer safety without causing undesirable side effects.

Chapter 4. Determination of antibiotics in water and degradation studies

- A SPE method followed by two approaches based on UHPLC-HRMS was optimized and successfully validated for the simultaneous analysis of macrolides, cephalosporins, fluoroquinolones, sulfonamides and diaminopyrimidines in wastewater and natural waters.

- A non-target screening based on a SWATH^{TM} workflow was performed, demonstrating the presence of up to 94 compounds including pharmaceuticals, drugs and pesticides, among others.

- Photodegradation appears as a good alternative to simultaneously remove the target antibiotics in ultrapure water. Twenty by-products could be identified by HRMS after UVC photolysis.

- The operational conditions of a tube-in-tube membrane reactor, which is based on AOPs, have been optimized for the simultaneous degradation of the target antibiotics.

- The low footprint tube-in-tube membrane reactor offers high antibiotic removal efficiencies under different treatments in demineralized water and urban wastewater.

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ANNEX I: LIST OF PUBLICATIONS

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- Maria Celeiro, Lua Vazquez, Piyaluk Nurerk, Abuzar Kabir, Kenneth G. Furton, Thierry Dagnac, Maria Llompart. Fabric phase sorptive extraction for the determination of 17 multiclass fungicides in environmental water by gas chromatography-tandem mass spectrometry, *Journal of Separation Science*, 43 (2020), 1817–1829

  DOI: 10.1002/jssc.201901232
  Impact factor: **JCR**: 3.645. **CiteScore**: 5.5. **SJR**: 0.72 (2020)
  JCR-Q2: Analytical Chemistry. **CiteScore, SJR-Q2**: Analytical Chemistry; Filtration and Separation
  Contribution: Formal analysis, Validation, Investigation, Data curation.

- Maria Celeiro, Lua Vazquez, Meruyert Sergazina, Santiago Docampo, Thierry Dagnac, Vitor J.P. Vilar, Maria Llompart. Turning cork by-products into smart and green materials for solid-phase extraction - gas chromatography tandem mass spectrometry analysis of fungicides in water, *Journal of Chromatography A,* 1628 (2020), 461437

  DOI: 10.1016/j.chroma.2020.461437
  Impact factor: **JCR**: 4.759. **CiteScore**: 6.9. **SJR**: 1.011 (2020)
  JCR-Q1: Analytical Chemistry; Biochemical, Research methods. **CiteScore-Q1**: Organic Chemistry; Analytical Chemistry; Biochemistry. **SJR**: Analytical Chemistry; Medicine (miscellaneous); Organic Chemistry (2020)
  Contribution: Formal analysis, Validation, Investigation.

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- Meruyert Sergazina, Lua Vazquez, Maria Llompart, Thierry Dagnac. Occurrence of fungicides in vineyard and the surrounding environment, *Molecules*, 26(20) (2021), 6152

  DOI: 10.3390/molecules26206152
  Impact factor: **JCR**: 4.412. **CiteScore**: 4.7 (2020). **SJR**: 0.705 (2021)
  JCR-Q2: Chemistry, Multidisciplinary; Biochemistry & Molecular Biology. **CiteScore-Q2**: Chemistry (miscellaneous); Pharmaceutical Science; Physical and Theoretical Chemistry; Organic Chemistry; Analytical Chemistry; Drug Discovery (2020); **SJR-Q1**: Pharmaceutical Science (2021)
  Contribution: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing-original draft preparation.

- Lua Vazquez, Maria Celeiro, Meruyert Sergazina, Thierry Dagnac, Maria Llompart. Optimization of a miniaturized solid-phase microextraction method followed by gas chromatography mass spectrometry for the determination of twenty four volatile and semivolatile compounds in honey from Galicia (NW Spain) and foreign countries, *Sustainable Chemistry and Pharmacy*, 21 (2021), 100451

  DOI: 10.1016/j.scp.2021.100451
  Impact factor: **JCR**: 4.508. **CiteScore**: 4.5 (2020). **SJR**: 0.681 (2021)
  JCR-Q2: Environmental science; Chemistry, Multidisciplinary. **CiteScore-Q1**: Management, Monitoring, Policy and Law (2020). **SJR-Q2**: Environmental Chemistry; Management, Monitoring, Policy and Law; Pharmaceutical Science; Pollution (2021).
  Contribution: Formal analysis, Validation, Investigation, Data curation, Software, Writing - original draft, Writing - review & editing.

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- Lua Vazquez, Daniel Armada, Maria Celeiro, Thierry Dagnac, Maria Llompart. Evaluating the presence and contents of phytochemicals in honey samples: phenolic compounds as indicators to identify their botanical origin, *Foods*, 10(11) (2021), 2616

  DOI: 10.3390/foods10112616
  Impact factor: **JCR**: 4.350; **CiteScore**: 3.0 (2020); **SJR**: 0.726 (2021)
  JCR-Q2: Food Science & Technology; **CiteScore-Q1**: Health Professions (miscellaneous) (2020); **SJR-Q1**: Food Science & Technology, Health Professions (miscellaneous), Health (social science) and Plant Science (2021).
  Contribution: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing-original draft preparation.

- Maria Celeiro, Lua Vazquez, J. Pablo Lamas, Marlene Vila, Carmen Garcia-Jares, Maria Llompart. Miniaturized matrix solid-phase dispersion for the analysis of ultraviolet filters and other cosmetic ingredients in personal care products, *Separations*, 6(2) (2019), 30

  DOI: 10.3390/separations6020030
  Impact factor: **JCR**: 1.900. **CiteScore**: 2.4. **SJR**: 0.485 (2019)
  JCR, CiteScore, SJR-Q3: Analytical Chemistry (2019)
  Contribution: Validation, Formal analysis.

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- Lua Vazquez, Maria Celeiro, Ana Castiñeira-Landeira, Thierry Dagnac, Maria Llompart. Development of a solid phase microextraction gas chromatography tandem mass spectrometry methodology for the analysis of sixty personal care products in hydroalcoholic gels - hand sanitizers - in the context of COVID-19 pandemic, *Analytica Chimica Acta*, 1203 (2022), 339650

  DOI: 10.1016/j.aca.2022.339650
  Impact factor: **JCR**: 6.558. **CiteScore**: 9.3 (2020). **SJR**: 1.105 (2021)
  JCR-Q1: Analytical Chemistry. **CiteScore-Q1**: Spectroscopy; Analytical Chemistry; Biochemistry; Environmental Chemistry (2020). **SJR-Q1**: Analytical Chemistry; Spectroscopy (2021)
  Contribution: Formal analysis, Validation, Data curation, Investigation, Writing – original draft, Writing – review & editing.

- Lua Vazquez, Maria Llompart, Thierry Dagnac. Complementarity of two approaches based on the use of Quadrupole Time-of-Flight High-Resolution Mass Spectrometry for the determination of multi-class antibiotics in wastewater and natural waters. Photodegradation studies and non-targeted screenings, *Environmental Science and Pollution Research.*

  Under revision for publication
  Impact factor: **JCR**: 4.223. **CiteScore**: 5.5 (2020) **SJR**: 0.831 (2021)
  JCR-Q2: Environmental sciences. **CiteScore-Q1**: Pollution; Health, Toxicology and Mutagenesis (2020). **SJR-Q1**: Health, Toxicology and Mutagenesis (2021)
  Contribution: Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing.

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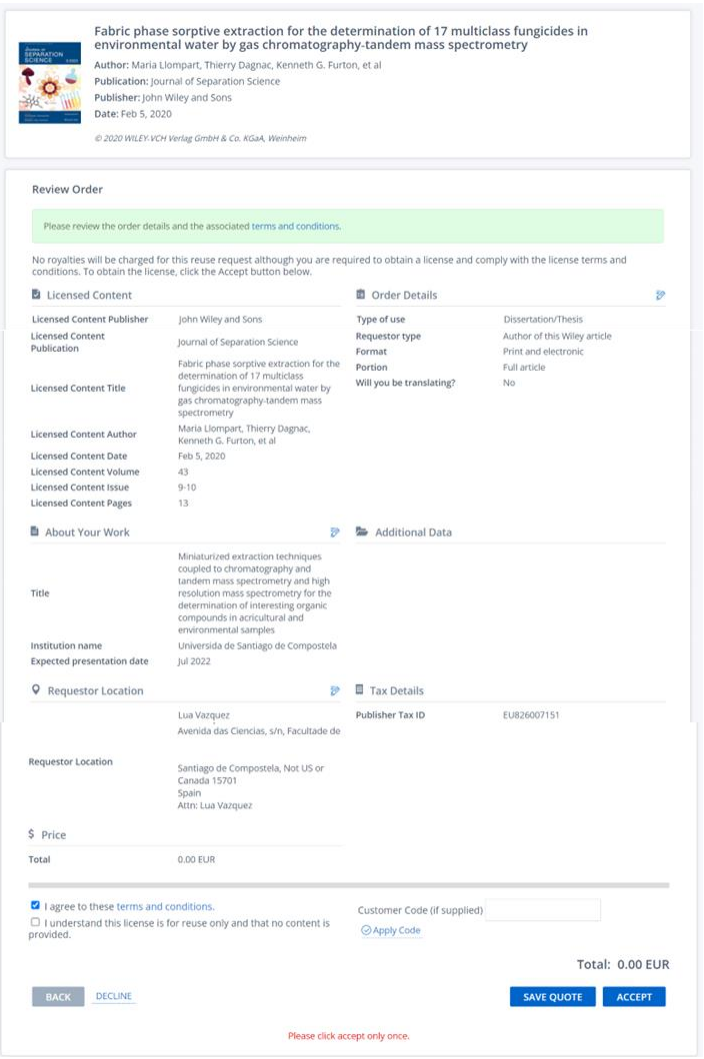
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ANNEX II: JOURNAL PERMISSIONS

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- M. Celeiro, L. Vazquez, P. Nurerk, A. Kabir, K. G. Furton, T. Dagnac, M. Llompart. Fabric phase sorptive extraction for the determination of 17 multiclass fungicides in environmental water by gas chromatography-tandem mass spectrometry, *J. Sep. Sci.*, 43 (2020), 1817–1829



Fabric phase sorptive extraction for the determination of 17 multiclass fungicides in environmental water by gas chromatography-tandem mass spectrometry

Author: Maria Llompart, Thierry Dagnac, Kenneth G. Furton, et al
Publication: Journal of Separation Science
Publisher: John Wiley and Sons
Date: Feb 5, 2020

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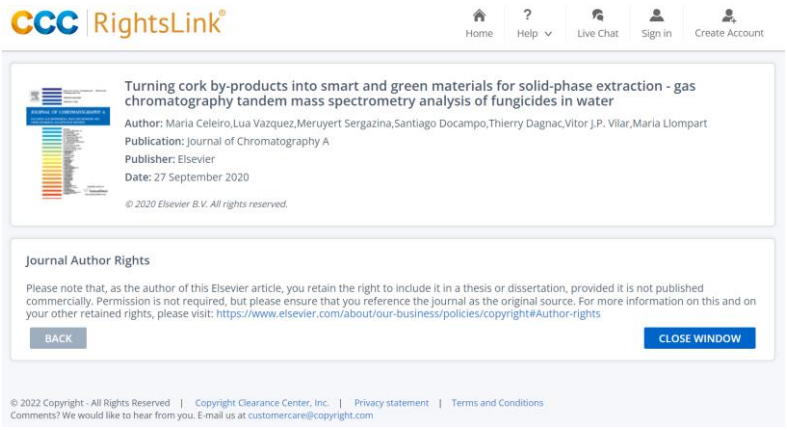
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- L. Vazquez, M. Celeiro, M. Sergazina, T. Dagnac, M. Llompart. Optimization of a miniaturized solid-phase microextraction method followed by gas chromatography mass spectrometry for the determination of twenty four volatile and semivolatile compounds in honey from Galicia (NW Spain) and foreign countries, *Sustain. Chem. Pharm.*, 21 (2021), 100451



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- M. Sergazina, L. Vazquez, M., T. Dagnac. Occurrence of fungicides in vineyard and the surrounding environment, *Molecules*, 26(20) (2021), 6152 (*Open Access*)
- L. Vazquez, D. Armada, M. Celeiro, T. Dagnac, M. Llompart. Evaluating the presence and contents of phytochemicals in honey samples: phenolic compounds as indicators to identify their botanical origin, *Foods*, 10(11) (2021), 2616 (*Open Access*)
- M. Celeiro, L. Vazquez, J. P. Lamas, M. Vila, C. Garcia-Jares, M. Llompart. Miniaturized matrix solid-phase dispersion for the analysis of ultraviolet filters and other cosmetic ingredients in personal care products, *Separations*, 6(2) (2019), 30 (*Open Access*).

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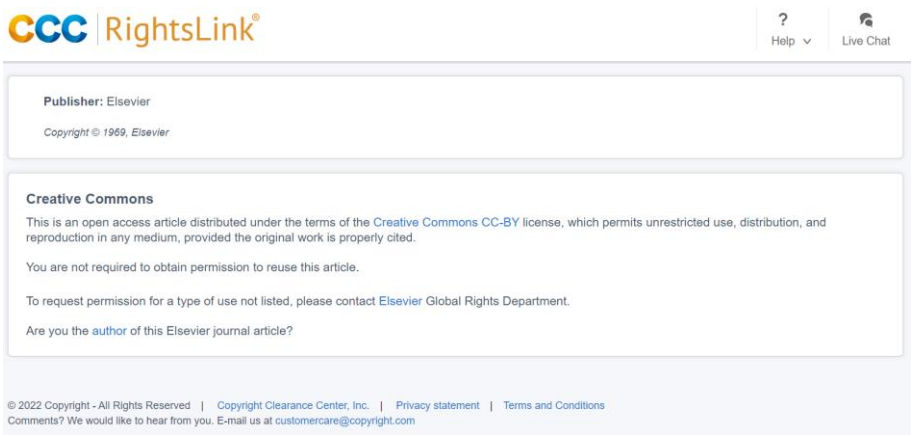
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- L. Vazquez, M. Celeiro, A. Castiñeira-Landeira, T. Dagnac, M. Llompart. Development of a solid phase microextraction gas chromatography tandem mass spectrometry methodology for the analysis of sixty personal care products in hydroalcoholic gels - hand sanitizers - in the context of COVID-19 pandemic, *Anal. Chim. Acta*, 1203 (2022), 339650 (*Open Access*).



- L. Vazquez, M. Llompart, T. Dagnac. Complementarity of two approaches based on the use of Quadrupole Time-of-Flight High-Resolution Mass Spectrometry for the determination of multi-class antibiotics in wastewater and natural waters. Photodegradation studies and non-targeted screenings, *Enviro. Sci. Pollut. R.*

Environmental Science and Pollution Research

Complementarity of two approaches based on the use of Quadrupole Time-of-Flight High-Resolution Mass Spectrometry for the determination of multi-class antibiotics in wastewater and natural waters. Photodegradation studies and non-targeted screenings

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Abstract:	The increasing use of human and veterinary antibiotics with their ineffective removal in wastewater treatment plants constitutes a major environmental problem. The development of analytical methodologies to monitor different antibiotics families in water and the implementation of alternatives for their efficient elimination is a great challenge. A method based on solid phase extraction followed by ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry has been developed to analyse multiclass antibiotics, including macrolides, cephalosporins, fluoroquinolones, sulfonamides and diaminopyrimidines, in waters. Several parameters affecting the extraction were optimized leading to a method that was validated in real samples of different origins. Urban and hospital wastewater samples, as well as natural waters, were analysed, confirming the entrance of antibiotics into the environment, constituting a risk to the aquatic environment. Non-target analysis based on data independent workflow was also performed, enabling the identification of 94 pollutants. Preliminary photodegradation experiments were assessed being, to our knowledge, the first study including the simultaneous photodegradation of such a high number of multi-class antibiotics. After UVC irradiation, many target compounds were totally removed, showing photodegradation offered a powerful tool for water decontamination. The identification of by-products using a non-target approach was carried out, as well.

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ANNEX IV: RESUMO

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A presente tese céntrase no desenvolvemento e a optimización de metodoloxías analíticas para determinación dunha gran variedade de compostos orgánicos de interese agroalimentario e medioambiental, así como fármacos e produtos de coidado persoal. Para este propósito, utilizáronse diversas técnicas de extracción seguidas dunha análise empregando distintos tipos de cromatografía acoprada á espectrometría de masas, tanto de baixa como de alta resolución.

Unha das tendencias e parámetros máis críticos na química analítica é a etapa de preparación da mostra. Co obxectivo de obter unhas características sostibles, a simplicidade, diminución dos costos e do tempo de preparación, o baixo uso de reactivos e disolventes, así como a redución de residuos xerados son algún dos factores máis importantes a ter en conta. Ademais, é primordial lograr unha alta selectividade e sensibilidade para os analitos de interese, demostrando que o método proposto presenta un alto rendemento e é fácil de implantar en calquera laboratorio. A selección da técnica de extracción ten que ser adecuada para os compostos, para a matriz e tamén hai que ter en conta a instrumentación utilizada.

Para desenvolver ditas metodoloxías analíticas leváronse a cabo distintas investigacións divididas en catro capítulos e descritas a continuación:

1.- Determinación de funxicidas en mostras agrícolas e ambientais

Os funxicidas son un tipo de pesticidas utilizados de forma usual en viticultura para evitar ou previr a infección por fungos ou incluso algunhas bacterias nos cultivos. Entre estas enfermidades atópanse a podremia gris (*Botrytis cinerea*), o mildio (*Plasmopara viticola*) e o oídio (*Uncinula necator*). Galicia é unha rexión cunha alta produción vitivinícola en gran parte do seu territorio. Como as condicións climáticas son extremas, é dicir, con veráns calorosos e invernos e outonos fríos e chuviosos, o uso de funxicidas é necesario para a conservación das vides. Deste xeito, a aplicación continuada destes compostos provoca a súa entrada en diferentes compartimentos ambientais, xa que os residuos poden chegar facilmente aos solos e

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logo, por medio da lixiviación, poden alcanzar os ecosistemas acuáticos, supoñendo un risco para a saúde humana e ambiental. Polo tanto, a determinación destes compostos presenta un gran interese tanto nos cultivos como nos compartimentos ambientais.

As técnicas de preparación da mostra empregadas para a extracción de dezasete funxicidas en mostras de auga baseáronse na extracción sortiva en fase téxtil (FPSE) e na extracción en fase sólida (SPE), usando subprodutos de cortiza como adsorbente. En canto á análise destes compostos en cultivos, optimizouse e validouse a extracción asistida por ultrasóns (UAE) en follas de vide, aínda que tamén se aplicou en uvas e en mostras ambientais, como solos recollidos en zona de viñedos. En todos os casos utilizouse cromatografía de gases seguida por espectrometría de masas en tándem (GC-MS/MS), xa que é unha opción adecuada para a determinación de funxicidas non polares ou pouco polares, como os seleccionados nesta tese. Non obstante, a cromatografía líquida tamén podería ser unha boa opción para analizar estes compostos.

Aínda que o uso de funxicidas é indispensable para a seguridade alimentaria, a súa presenza nos cultivos (follas e uvas) e no medio ambiente (solo e auga) causa certa preocupación. Deste xeito, desenvolveuse un método de UAE para determinar 17 funxicidas en follas de vide. Realizáronse diferentes estudos para obter a mellor eficiencia de extracción, como a avaliación da técnica de extracción, o tipo de disolvente ou a posibilidade de secar as follas, entre outros. UAE comparouse con outras técnicas como a dispersión de matriz en fase sólida (MSPD) ou a extracción por vórtice (VE), demostrando que esta última tamén é adecuada para determinar estes compostos nas follas. O tipo de disolvente avaliouse mediante ferramentas quimiométricas como a análise de varianza (ANOVA) ou probas de mínima diferencia significativa (LSD) de rango múltiple. Unha vez obtidas as condicións óptimas, o método validouse mostrando boa linearidade, precisión con desviacións estándar relativas (RSD) por debaixo do 9 % e valores de recuperación estudados a tres niveis de concentración oscilando entre o 80 e o 113 % para case todos os casos.

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Ademais, é importante sinalar que o método tamén é adecuado e aplicable para outro tipo de follas, como o té. Outras matrices, como uvas ou solos, tamén se analizaron mediante a metodoloxía baseada en UAE-GC-MS/MS. Por outro lado, a extracción asistida por microondas (MAE) revelou resultados similares aos obtidos por UAE para o estudo dos funxicidas de interese en solos. Non obstante, seleccionouse UAE co obxectivo de utilizar a menor cantidade de mostra e disolvente e minimizar os costos.

Por outra banda, como os funxicidas adoitan estar presentes en niveis traza nas augas, o desenvolvemento de métodos analíticos sensibles para a extracción de ditos compostos nesta matriz presentan gran interese. Deste xeito, leváronse a cabo a optimización de dous enfoques para determinar funxicidas en augas.

A SPE é a técnica máis empregada para extraer funxicidas en mostras acuosas debido á súa capacidade de extracción, o alto factor de enriquecemento e a súa simplicidade. Sen dúbida, o elemento máis crítico para obter unha técnica de SPE favorable é a selección do adsorbente, xa que xoga un papel moi importante para a eficacia e selectividade dos analitos de interese. Por esta razón, propúxose utilizar subprodutos de cortiza como adsorbente de SPE para a extracción dos 17 funxicidas obxectivo do estudo. A cortiza é un material heteroxéneo cunha composición química formada principalmente por biopolímeros hidrofóbicos e polisacáridos hidrófilos, que proporcionan sitios para a adsorción de compostos orgánicos. Ademais, pode ser tratado térmicamente para mellorar posibles interaccións con compostos orgánicos menos polares. Polo tanto, as principais vantaxes deste material eco-natural son que é un adsorbente alternativo renovable, biodegradable e de baixo costo que favorece a economía circular.

En canto á FPSE, é unha novidosa técnica de preparación da mostra que presenta unha serie de vantaxes sobre técnicas convencionais, xa que combina o modo de extracción en equilibrio da microextracción en fase sólida (SPME) coa extracción exhaustiva da SPE nunha única plataforma.

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Ambas técnicas de extracción empregadas para a análise de funxicidas en auga foron optimizadas mediante deseños de experimentos. O protocolo de SPE implica un deseño multifactorial, 2^3 con dous puntos centrais. Os factores avaliados foron o tipo de cortiza, o disolvente de extracción e o pH da mostra. Para o procedemento FPSE, realizouse un deseño de cribado de media fracción 2^{5-1} máis dous puntos centrais, que deron lugar a 18 experimentos. Neste caso, os parámetros de estudo foron o volume de mostra, o disolvente de desorción, o tempo de desorción, a adición de NaCl e o pH da auga.

En ambos casos, as metodoloxías foron validadas en termos de linearidade, precisión (repetibilidade e reproducibilidade), exactitude, límites de detección (LODs) e cuantificación (LOQs), mostrando resultados satisfactorios en todos os casos. Tamén se avaliaron os posibles efectos da matriz mediante estudos de recuperación realizados en diferentes matrices de auga. Aínda que un dos funxicidas diana (metalaxil) presentou problemas de recuperación nalgunhas das matrices de auga utilizando FPSE (por exemplo, a auga da billa), no caso de SPE os valores medios de recuperación de dito composto atopáronse ao redor do 30 % en todas as matrices, incluso en auga ultrapura. Deste xeito, o método baseado en FPSE-GC-MS/MS resultou ser máis eficiente para a extracción do metalaxil en auga, aínda que, ambos procedementos poden considerarse alternativas adecuadas para a determinación de niveis traza de funxicidas en mostras de auga. En consecuencia, son técnicas prometedoras para a extracción doutros compostos orgánicos que poden estar presentes no medio acuático.

Finalmente, os tres protocolos aplicáronse a mostras reais demostrando a presenza de funxicidas en altas concentracións nos cultivos e no medio ambiente. Algunha das substancias obxectivo superaron os límites máximos de residuos (MRLs) establecidos pola Comisión Europea en follas de vide e en uvas, e a concentración máxima permitida para pesticidas en augas subterráneas e en augas destinadas ao consumo humano. Estes resultados revelaron que os modos de aplicación dos funxicidas sobre os cultivos deben mellorarse.

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2.- Capítulo 2. Caracterización do mel: compostos volátiles, semivolátiles e fenólicos

O mel é un produto alimenticio natural coñecido polo seu alto valor nutricional, debido á presenza de azucre, proteínas, aminoácidos, enzimas, ácidos orgánicos, vitaminas, minerais, compostos fenólicos e volátiles. A orixe botánica e xeográfica do mel condiciona a súas propiedades. O seu perfil aromático configura as súas características organolépticas, a súa identidade e a súa calidade e, por outro lado, os compostos fenólicos presentes na mel demostraron a súa alta capacidade antimicrobiana e antioxidante. Unha alta proporción do mel producido en Galicia está recoñecido baixo a denominación de Identificación Xeográfica Protexida (IXP), polo que é importante identificar posibles marcadores químicos nestes meles. Ademais, dependendo da zona na que se produza o mel, terá unha orixe botánica diferente, polo que tamén é de gran interese poder diferencialo segundo a orixe floral do néctar extraído polas abellas.

O mel é unha matriz complexa que precisa dun paso de preparación da mostra previo á súa análise. As técnicas de preparación da mostra implicadas para a análise do mel nesta tese son a SPME para a determinación de compostos volátiles e semivolátiles por GC-MS, e unha combinación de VE seguida de UAE para avaliar a presenza de compostos fenólicos por cromatografía líquida seguida de espectrometría de masas en tándem (LC-MS/MS). Por outro lado, leváronse a cabo protocolos para o estudo do contido fenólico total (TPC) e a actividade antioxidante (AA) empregando un espectrofotómetro ultravioleta-visible (UV-Vis).

A SPME é unha das técnicas preferidas para a extracción de volátiles e semivolátiles no mel. Non obstante, co obxectivo de reducir o costo, a cantidade de mostra e os residuos xerados, a miniaturización da SPME (mini_SPME), que permite utilizar unha cantidade moi pequena de mel (200 mg), pareceu ser unha boa opción, xa que as respostas cromatográficas resultaron ser bastante similares que cando se usa 10 veces máis de cantidade de mel (2 g). Empregouse un deseño experimental factorial de nivel mixto ($3 \cdot 2^{3-1}$ + 2 puntos centrais) para avaliar catro parámetros críticos que poden afectar á eficacia da

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mini_SPME, para a determinación de 24 substancias previamente seleccionadas: temperatura, recubrimento da fibra, adición de NaCl e dilución. O método validouse satisfactoriamente, logrando boa linearidade, precisión, LODs e LOQs, e estudáronse recuperacións en catro tipos diferentes de mel. Finalmente, o método aplicouse para la identificación dos 24 compostos e a cuantificación de 9 deles nunha ampla gama de variedades galegas e estranxeiras (Francia, Grecia, Italia e Kazajstán). Non obstante, sería necesaria unha aproximación estatística adicional para comprobar se os compostos se poden considerar como marcadores dos meles galegos.

Por outra banda, para a investigación de compostos fenólicos no mel, utilizouse unha metodoloxía moi simple que combina VE con UAE, utilizado disolventes de base acuosa. Dado que o manexo do mel é bastante complexo, normalmente para o estudo de substancias fenólicas emprégase SPE para eliminar compoñentes da matriz que poden interferir na análise, como os azucres. Non obstante, nesta investigación, tras unha previa optimización, demostrouse que a calibración externa era adecuada para poder cuantificar 41 compostos fenólicos aplicando unha estratexia bastante máis rápida e sinxela. Nas mostras reais analizadas puidéronse identificar e cuantificar 25 dos 41 analitos obxectivo. Ademais, para a avaliación do TPC e da AA utilizáronse adaptacións de protocolos previamente propostos na bibliografía. Para a interpretación dos resultados, fíxose un estudo quimiométrico facendo uso de distintas ferramentas estatísticas, como ANOVA e análise de compoñentes principais (PCA) para obter modelos que permitiran clasificar 91 meles galegos diferentes, segundo a súa orixe botánica. Entre estes meles atopáronse meles de tipo multifloral, melada, breixo, castiñeiro, castiñeiro/melada, silva e eucalipto. Estes estudos baseáronse no TPC, AA e na concentración fenólica individual e/ou a concentración total destas substancias, mostrando diferenzas significativas de acordo coa variedade de mel.

3.- Capítulo 3. Determinación de produtos de coidado persoal (PCPs) en formulacións cosméticas

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Os cosméticos, como as cremas faciais e corporais, a maquillaxe, os produtos para o coidado do cabelo, entre outros, son produtos de amplo consumo en todo o mundo. Hoxe en día un dos produtos cosméticos máis utilizados son os xeles hidroalcólicos, xa que se empregan con moita frecuencia varias veces ao día ao volverse esenciais para a prevención do contaxio por SARS-CoV-2 e evitar a súa propagación, especialmente en forma de Covid-19.

Nas formulacións cosméticas están atópanse gran número de compostos orgánicos, como por exemplo as fragrancias alerxénicas, conservantes, plastificantes, almiscres sintéticos, filtros ultravioleta (UV), entre outros. As fragrancias (alérxenos e almiscres sintéticos), que adoitan engadirse aos cosméticos coa intención de proporcionar un aroma agradable, son as principais responsables da dermatite alérxica de contacto. Os filtros UV están destinados a protexer aos consumidores contra a radiación solar e a súa presenza é especialmente importante nos protectores solares, pero tamén poden encontrarse nunha ampla gama de produtos de coidado diario. Os conservantes engádense ás fórmulas cosméticas para protexer os produtos e aos consumidores contra o crecemento microbiano, mentres que os plastificantes ademais de ser engadidos, poden pasar dos envases de plástico ao propio produto. A presencia de moitas destas substancias en cosmética, asóciase a alerxias de contacto, dermatite cutánea e incluso, nalgún caso, a efectos cancerígenos e disfuncións endócrinas.

Os produtos cosméticos comercializados na Unión Europea deben seguir a lexislación vixente, polo tanto, deben cumprir co Regulamento CE nº 1223/2009, e dito cumprimento debe ser verificable analiticamente. Neste contexto, a análise de cosméticos é unha tarefa difícil debido á gran variedade e complexidade das mostras, compostas por un elevado número de substancias de diferente natureza.

Desenvolvéronse dúas metodoloxías analíticas utilizando µMSPD e SPME, para determinar un alto número de PCPs en diferentes cosméticos. En ambos casos, os PCPs analizáronse por GC debido á natureza química dos analitos obxectivo. En canto á detección, elixiuse a espectrometría de masas en tandem para mellorar a selectividade e sensibilidade analítica.

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Optimizouse un método baseado en µMSPD para as análises de 14 filtros UV, tratándose dunha aproximación moi novedosa xa que nunca se aplicara dita técnica para a extracción destas substancias en formulacións cosméticas. Os factores que afectan ao proceso, como o axente dispersante e a cantidade e o tipo de disolvente de elución, estudáronse mediante un deseño de experimentos. Neste caso seleccionouse un deseño multifactorial $4 \cdot 2^2$, que incluía 16 experimentos aleatorios. Unha vez optimizado o método, avaliouse o rendemento da metodoloxía µMSPD-GC-MS/MS e estendeuse a outros PCPs, como alérxenos, almiscres sintéticos, conservantes e plastificantes, dando un total de 78 PCPs. O método presentou unha boa linearidade e precisión e, a maiores, despois de realizar estudos de recuperación fortificando unha crema de mans hidratante, obtivéronse rendementos entre o 70 e o 110 % para todos os compostos. O protocolo establecido permitiu a análise simultánea nunha soa extracción dun total de 78 compostos con natureza química moi diferente nunha ampla variedade de produtos cosméticos (protectores solares, cremas, maquillaxe, barras de beizos...).

En canto á análise de xeles hidroalcólicos empregouse a SPME que, ata o método desenvolvido nesta tese nunca se aplicara para a extracción simultánea multianalitos de PCPs en mostras cosméticas. Neste caso, incluíronse un total de 60 substancias, abarcando fragrancias alerxénicas, almiscres sintéticos, conservantes e plastificantes. Para a optimización deste protocolo realizouse un deseño categórico multifactorial ($3 \cdot 2^2$) no que se avaliaron os principais factores e as súas interaccións. Os parámetros incluídos en dito deseño foron o recubrimento da fibra, o modo de extracción e a temperatura. En condicións optimizadas, o método SPME-GC-MS/MS foi validado en termos de linearidade, precisión e exactitude, obtendo bos resultados e seguindo na medida do posible a norma internacional ISO12787 "*Cosmetics-Analytical methods-Validation criteria for analytical results using chromatographic techniques*". Posteriormente, o método aplicouse a mostras reais de xeles hidroalcólicos para comprobar o cumprimento da lexislación aplicable (Regulamento CE nº 1223/2009), así como as recomendacións da Organización Mundial da Saúde (OMS)

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de homoxeneizar a formulación e fabricación dos xeles hidroalcólicos, asegurando as súas propiedades antimicrobianas.

Así, ambas metodoloxías resultaron ser sinxelas, adecuadas, verdes e rápidas para determinar un elevado número de PCPs en produtos cosméticos de distinta natureza. En ambos casos, non se necesitou calibración en matriz, o que simplifica aínda máis os procedementos e facilita o seu uso en calquera laboratorio de rutina. Demostrouse a presenza de compostos prohibidos segundo o Regulamento CE nº 1223/2009, en diferentes produtos cosméticos, así como de fragrancias alerxénicas en concentracións superiores a 10 μg g^{-1}, que é o límite establecido a partir do cal debe constar a súa presenza na etiqueta en xeles hidroalcólicos. Ademais, ningunha das formulacións das mostras analizadas de xeles hidroalcólicos cumpriu as recomendacións da OMS. Polo tanto, os resultados obtidos revelaron a necesidade dun maior control sobre os produtos cosméticos para garantir a seguridade do consumidor sen causar efectos secundarios indesexables.

4.- Capítulo 4. Determinación de antibióticos en auga e estudos de degradación

Debido ao crecente uso de produtos farmacéuticos tanto por parte da poboación como en actividade veterinaria, a súa presenza e persistencia na auga é cada vez maior. Entre estes compostos, un dos grupos que presenta máis interese son os antibióticos, que poden causar resistencia microbiana debido a un uso irresponsable e irracional. Como soamente son parcialmente eliminados nas plantas de tratamento de augas residuais, estas substancias entran continuamente no medio ambiente, o que supón un risco importante non só para o ecosistema acuático, se non tamén para a saúde humana. Deste xeito, procedementos alternativos como os procesos de fotodegradación e oxidación avanzada parecen ofrecer unha boa opción para a eliminación eficiente de antibióticos e doutros contaminantes emerxentes.

Empregouse a SPE para o illamento de 14 antibióticos en augas residuais e naturais, incluíndo macrólidos, cefalosporinas, fluoroquinolonas, sulfonamidas e diaminopirimidinas. A análise

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levouse a cabo mediante dous enfoques baseados na espectrometría de masas de alta resolución (HRMS), utilizando un detector de tempo de voo de cuadrupolo simple e un de triple cuadrupolo.

O método de extracción optimizouse empregando diferentes parámetros como o pH da mostra, a cantidade de mostra e disolvente, e o cartucho SPE, entre outros. Neste caso, o cartucho SPE foi un adsorbente hidrofílico-lipófilico convencional, coñecido co nome comercial de Oasis HLB (casa comercial Waters). Posteriormente, a metodoloxía validouse logrando boa linearidade, precisión, LODs e LOQs. Por outro lado, os valores de recuperación obtidos en auga ultrapura foron satisfactorios, estando por encima do 80 % excepto para dous compostos (os macrólidos azitromicina e eritromicina). Polo tanto, estudouse se estes baixos valores eran debidos a unha baixa eficiencia de extracción ou a efectos matriz xerados na fonte de ionización do espectrómetro de masas. Para isto, compararonse extractos enriquecidos antes e despois do proceso de SPE, mostrando que non existía supresión de ionización da fonte, e que a escasa capacidade de retención do adsorbente era a responsable das baixas recuperacións. Ademais, leváronse a cabo estudos de recuperación en catro matrices reais diferentes, observando que o tipo de matriz de auga si que influíu no rendemento da extracción. Para as matrices máis complexas (augas residuais urbanas e hospitalarias) as porcentaxes de recuperación diminuían considerablemente, polo que era necesario o uso de compostos marcados isotopicamente. Unha vez validado o método, cuantificáronse os antibióticos diana en augas residuais e naturais.

Para analizar os antibióticos diana, utilizouse un espectrómetro de masas de cromatografía líquida de ultra alta eficacia acoprado a un cuadrupolo-tempo de voo (UHPLC-QToF). Pola contra, empregouse outra configuración de espectrometría de masas de alta resolución para un estudio non obxectivo, usando un instrumento SCIEX TripleTOF (QqQTof) en modo SWATH^{TM} (adquisición secuencial por ventás de todos os espectros de masas teóricos). O fluxo de traballo SWATH basease na selección dun rango de m/z no que se inclúen todos os analitos, logo o rango divídese en numerosas ventás de m/z (normalmente entre 20 e 30) e rexístranse os espectros MS/MS para

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todos os picos detectados. Deste xeito, dado que se obtén de forma simultánea un barrido completo da mostra e os espectros MS/MS para todos os picos, non é necesario introducir nin masas precursoras nin transicións de masas. Unha das potentes características que permite este modo de escaneamento é a posibilidade de realizar buscas retrospectivas sen obxectivo utilizando o mesmo arquivo bruto de adquisición de datos.

Este enfoque permitiu identificar ata 94 compostos non diana, entre os cales se atoparon outros antibióticos, drogas, fármacos, pesticidas, etc..., tendo en conta as masas exactas dos compostos descoñecidos e outros criterios adicionais para a confirmación, como o perfil isotópico e a puntuación de librería calculada cos espectros MS/MS de alta resolución, proporcionando unha puntuación final combinada.

A presenza dos antibióticos en mostras reais indicou a facilidade coa que poden alcanzar o medio acuático. Polo tanto, o uso e a mellora dos procedementos de purificación da auga é de gran interese para protexer o medio ambiente. Para iso, realizouse un estudo preliminar no que se abordou a fotólise UVC simultánea dos 14 antibióticos diana empregando un reactor estático a escala de laboratorio. É importante destacar que, aínda que estas substancias están presentes como una mestura no medio ambiente, poucos estudos avalían a degradación simultánea dun número tan elevado de antibióticos multiclase. Os resultados obtidos mostraron que a fotodegradación parecía ser unha opción útil para a eliminación da maioría dos antibióticos diana en auga ultrapura. Non obstante, é necesario realizar estudos adicionais noutras matrices de augas reais.

Deste xeito, testáronse procesos de oxidación avanzada (AOPs) caracterizados pola xeración de especies oxidantes altamente reactivas. Para iso, optimizáronse as condición operacionais dun reactor de membrana tubo en tubo, traballando en modo continuo. Avaliáronse tanto oxidantes líquidos (H_2O_2, $S_2O_8^{2-}$) como gasosos (O_3), destacando que foi a primeira vez que neste tipo de reactor se utilizou un oxidante gasoso. Tamén se estudiaron distintos catalizadores (Ag_2MoO_4 e TiO_2), o uso de radiación UVC e o caudal de mostra. Estes tratamentos resultaron ser unha alternativa prometedora para a eliminación de

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compostos en augas residuais urbanas, especialmente o baseado na ozonización.

Por outro lado, durante a degradación poden formarse subprodutos, polo que a súa identificación tamén é de gran importancia. Nesta tese, levouse a cabo unha identificación destes subprodutos mediante a implantación dunha adquisición SWATH, podendo encontrar e identificar ata 20 produtos de transformación baixo a fotólise UVC.

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The studies conducted in this thesis focus on the development and optimization of analytical methodologies for the determination of organic compounds of agronomic, environmental, and cosmetic interest. These analytes include fungicides, volatile, semi-volatile and phenolic compounds present in honey, as well as pharmaceuticals and personal care products, including fragrances, UV filters, plasticizers, preservatives and antibiotics.
Miniaturized extraction techniques followed by chromatography coupled with mass spectrometry, tandem mass spectrometry and high-resolution mass spectrometry, were used and extensively applied to a wide variety of real samples.
In addition, innovative strategies based on photodegradation and advanced oxidation processes, have been evaluated for the removal of antibiotics from water.