

Comprehensive characterization of anthropogenic impacts on Mediterranean intermittent streams using cheminformatics and environmental risk assessment approaches

Olga Gómez Navarro

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Olga Gómez Navarro

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Comprehensive Characterization of Anthropogenic Impacts on Mediterranean Intermittent Streams using Cheminformatics and Environmental Risk Assessment Approaches

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Programa de doctorat

"Química Analítica i Medi Ambient"

Comprehensive characterization of anthropogenic impacts on Mediterranean intermittent streams using cheminformatics and environmental risk assessment approaches

Caracterització integral dels impactes antropogènics en rieres intermitents mediterrànies mitjançant enfocaments de quimioinformàtica i avaluació del risc ambiental

Olga Gómez Navarro

Memòria presentada en compendi d'articles per optar al títol de
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“And once the storm is over,
you won’t remember how you made it through,
how you managed to survive.
You won’t even be sure, whether the storm is really over.
But one thing is certain.
When you come out of the storm,
you won’t be the same person who walked in.
That’s what this storm’s all about.”

Haruki Murakami

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Table of contents

Abstract.....V

Resum.....VII

Abbreviations and Acronyms.....VIII

Thesis structure.....XI

Chapter 1. General Introduction.....3

1.1. Growing relevance of intermittent streams and their associated groundwater.....3

1.2. Impact of wastewater effluents on intermittent streams and associated groundwater.....6

1.3. Contaminants of emerging concern in surface waters and associated groundwater.....8

1.3.1. Presence and state of the art of CECs in Mediterranean countries.....8

1.3.2. Main sources of CECs in water streams and associated groundwater.....1

1.3.3. Degradation and transformation pathways of CECs.....13

1.3.3.1. Environmental and analytical challenges of TPs 15

1.3.4. Regulation of CECS and their TPs.....17

1.3.5. Environmental risk assessment of CECs and their TPs.....19

1.4. Analysis of pollutants in water samples.....20

1.4.1. Sample collection 20

1.4.2. Sample preparation.....20

1.4.2.1. Sample pretreatment.....20

1.4.2.2. Sample extraction.....21

1.4.3. Sample analysis.....22

1.5. Identification approaches for known and unknown compounds.....26

1.5.1. Cheminformatics approaches for identification of unknowns.....28

1.6. References.....30

Chapter 2. Objectives.....53

Chapter 3. Method Development for the Analysis of CECs in Surface Waters.....59

3.1. Introduction.....59

3.2. Methodology.....64

3.3. Results.....65

3.4. Discussion.....110

Chromatographic separation performance.....110

Acquisition methods evaluation.....112

Method performance comparison and outlook.....114

3.5. References.....116

Chapter 4. Characterizing Wastewater Impacts in the Mediterranean Region.....125

4.1. Introduction.....125

4.2. Methodology.....131

4.2.1. Sampling collection.....131

Water sampling.....132

Diatom and benthic invertebrate sampling 133

4.2.2. Environmental risk assessment.....133

4.3. Results 134

4.4. Discussion.....263

Occurrence of CECs in Mediterranean intermittent streams and associated groundwater.....263

Environmental risk assessment.....264

Integrated monitoring and assessment of multiple stressors.....266

4.5. References.....270

Chapter 5. Identification and Fate of Transformation Products in the Environment.....285

5.1. Introduction.....285

5.2. Methodology.....288

5.2.1. Lab-scale degradation experiments.....288

5.2.2. Data curation and prediction models for TPs.....289

5.2.3. Suspect and non-target screening through open-source cheminformatics approaches.....291

5.3. Results.....292

5.4. Discussion.....350

Fate of N-oxides in Mediterranean intermittent streams lab condition and real scenarios.....350

Environmental risk of transformation products.....352

Future perspectives and research needs for TPs.....353

Application of non-target screening.....354

5.5. References.....355

Chapter 6. Conclusions.....363

Abstract

Intermittent streams, characterized by variable and discontinuous flow regimes, are becoming increasingly common in the Mediterranean region due to climate change, population growth and land use changes, which further decrease water availability. As a result, the natural dilution capacity of streams is limited, making them highly vulnerable to both anthropogenic pressures and climate-driven hydrological variability. Among the key stressors, wastewater treatment plants (WWTPs) are one of the major sources of contaminants of emerging concern (CECs) and their transformation products (TPs). These are continuously being released into the environment and their detection in surface water can pose significant environmental risk due to their persistence, bioactivity, and incomplete removal during wastewater treatment. In this context, this thesis aimed to develop and apply robust analytical techniques for the detection, quantification and characterization of CECs and TPs, as well as to assess their potential environmental risks in Mediterranean freshwater systems, including both surface and groundwater, with a particular focus on under-studied regions as the Middle East North African (MENA) region.

An analytical method based on solid phase extraction combined with liquid chromatography coupled to high resolution mass spectrometry was developed and validated for the detection and quantification of 116 CECs. The method demonstrated high sensitivity and broad applicability in both surface and groundwater samples. It was applied for the analysis of samples collected across multiple locations in Southern European countries (Spain, Italy, France) and MENA countries (Algeria, Tunisia), enabling a comparative assessment of water quality between northern and southern Mediterranean regions. Special attention was given to MENA countries, where comprehensive monitoring efforts have been previously lacking.

Through an integrated approach combining chemical, ecological, and hydrological indicators, the study highlighted the cumulative impact of wastewater discharges on aquatic ecosystems and emphasized the need for region-specific monitoring frameworks. The importance of considering hydrological variability, particularly in low flow conditions, where natural dilution capacities of streams are limited was also emphasized, especially as water scarcity increases. The correlations between various indicators were assessed where CECs concentrations and benthic indices (ecological) revealed a moderate positive relationship.

Environmental risk assessments were conducted by calculating risk quotients to evaluate the potential ecological impact associated to the detected concentrations. This approach enabled the identification of several priority pollutants, including diclofenac, losartan and azithromycin, which were consistently found in surface water at ecologically relevant concentrations, posing potential environmental risks across the five Mediterranean countries studied. In Tunisian groundwater, compounds including azithromycin, ibuprofen and caffeine were also detected

at concentration levels indicating potential ecological risks. Notably the overlap in potential ecological risk, driven by the presence of common compounds as azithromycin in both Tunisian groundwater and surface water systems, underscores the ongoing pressures on the studied basin where agriculture, industry and tourism activities predominate. The findings reinforce the urgent need for increased monitoring frameworks, both regionally and internationally, to better manage and mitigate the impact of CECs on the environment.

Additionally, to investigate the fate and transformation pathways of CECs and their TPs in intermittent streams, open-source cheminformatics approaches were applied. A combination lab-scale experiments, data curation and prediction models were used to assess the environmental behaviour of TPs. N-oxide transformation products were found to remain persistent along the stream and were observed to be derived from WWTP point sources rather than in-stream formation. Unfortunately, lab-scale experiments did not successfully predict the high occurrence of N-oxide TPs observed in field studies. Suspect and non-target screening approaches, supported by open-source tools, were performed as complementary and valuable tools, to expand the target analysis and provide a deeper insight of the presence of TPs and other unknowns. These approaches enabled the identification of compounds not initially included in the analytical method, offering a broader understanding of the contaminant profiles in intermittent streams.

Overall, the findings presented in this thesis underscore the necessity of interdisciplinary strategies and adaptive water management to protect vulnerable freshwater, both intermittent streams and groundwater resources across the Mediterranean Basin.

Keywords: High Resolution Mass Spectrometry, Liquid Chromatography, Mediterranean Basin, Intermittent Streams, MENA Countries, Contaminants of Emerging Concern, Transformation Products, Environmental indicators

Resum

Les rieres intermitents, caracteritzades pel seu cabal variable i discontinu, són cada cop més freqüents a la conca mediterrània a causa del canvi climàtic, el creixement poblacional i els canvis d'ús del sòl. Aquestes alteracions redueixen la capacitat de dilució natural dels cursos d'aigua intermitents, fent-los especialment vulnerables tant a les pressions antropogèniques com a la variabilitat hidrològica.

Les estacions depuradores d'aigües residuals (EDARs) són una de les fonts principals de contaminants emergents (CECs) i dels seus productes de transformació (TPs). Aquests s'alliberen contínuament al medi ambient degut a l'eliminació incompleta durant els processos de tractament, fet que pot suposar un risc ambiental a causa de la seva persistència i bioactivitat.

Aquesta tesi té com a objectiu desenvolupar i aplicar tècniques analítiques per detectar, quantificar i caracteritzar els CECs i els seus TPs en cursos d'aigua intermitents de la regió del mediterrani, especialment en regions poc estudiades com els països MENA (Orient Mitjà i Nord d'Àfrica). Es va desenvolupar i validar un mètode d'alta sensibilitat i àmplia aplicabilitat mitjançant l'extracció en fase sòlida i cromatografia líquida acoblada a espectrometria de masses d'alta resolució per a 116 CECs.

Diferents països del Mediterrani van ser estudiats incloent Espanya, Itàlia, França, Algèria i Tunísia. Els resultats van mostrar l'impacte de les descàrregues d'aigües residuals sobre els ecosistemes aquàtics i la necessitat de marcs de seguiment específics per cada regió, destacant la importància de tenir en compte la variabilitat hidrològica, sobretot en condicions de baix cabal, quan la capacitat de dilució natural es veu limitada. Diversos compostos com el diclofenac, el losartan i l'azitromicina es van detectar en concentracions ecològicament rellevants en aigües superficials, indicant un risc ambiental a diversos països mediterranis. A Tunísia, també es van identificar riscos ecològics a les aigües subterrànies, fet que evidencia la necessitat de millores en la gestió dels recursos hídrics.

Per estudiar el destí i la transformació dels CECs i els seus TPs en rieres intermitents, es van utilitzar eines quimioinformàtiques de codi obert, experiments a escala laboratori i models de predicció de TPs. L'ús combinat d'anàlisi de cribratge de sospitosos i no dirigit va permetre aprofundir en la identificació i el comportament ambiental de TPs i altres compostos desconeguts en rieres intermitents.

Paraules claus: *Espectrometria de masses d'alta resolució, Cromatografia líquida, conca mediterrània, rieres intermitents, països MENA, contaminants de preocupació emergent, productes de transformació, indicadors ambientals*

Abbreviations and Acronyms

A

AGC	Automatic gain control
APCI	Atmospheric pressure chemical ionization
APPI	Atmospheric pressure photoionization

C

CAS	Chemical Abstracts Service
CCL	Contaminant Candidate List
CECs	Contaminants of emerging concern

D

DBPs	Disinfection byproducts
DDA	Data dependent acquisition
DIA	Data independent acquisition
DOM	Dissolved organic matter

E

ECHA	European Chemicals Agency
EI	Electron ionization
EPA	Environmental Protection Agency
ERA	Ecological risk assessment
ESI	Electrospray ionization

F

FWHM	Full width at half maximum

G

GC	Gas chromatography

H

HCD	Higher energy collision dissociation cell
HILIC	Hydrophilic interaction chromatography
HLB	Hydrophilic lipophilic balance
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry
HSDB	Hazardous Substance Data Bank

I

IBMWP	Index of Biological Monitoring Working Party
IC	Ion chromatography
IWRM	Integrated Water Resources Management

L

LC	Liquid chromatography
LLE	Liquid-liquid extraction
LOD	Limit of detection
Log D	Logarithm of the distribution coefficient between octanol and water

L

Log P	Logarithm of the partition coefficient between octanol and water
LOQ	Limit of quantification
LRMS	Low resolution mass spectrometry

M

MCX	Mixed cation exchange
MEC	Measured environmental concentration
MENA	Middle East North Africa
MoNA	MassBank of North America
MS	Mass spectrometry
m/z	mass to charge

N

NOM	Naturally organic matter
NTS	Non-target screening

O

ONAS	Office National de l'Assainissement/National Sanitation Utility (Tunisia)

P

p.e	Population equivalent
PFAs	Per- and Polyfluoroalkyl Substances
PNEC	Predicted no-effect concentrations
PRM	Parallel Reaction Monitoring

Q

Q	Quadrupole
QqQ	Triple Quadrupole
Q-TOF	Quadrupole Time-Of-Flight

R

REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RF	Radio frequency
RP	Reversed phase
RQ	Risk quotients

S

SAX	Strong anion exchange
SDGs	Sustainable Development Goals
SPE	Solid phase extraction

T

tDIA	Targeted data independent acquisition
TPs	Transformation products

U

UHPLC	Ultra high-performance liquid chromatography

W

WAX	Weak anion exchange
WCX	Weak cation exchange
WFD	Water Framework Directive
WWTP	Wastewater treatment plant

X

XlogP	Predicted partition coefficient

Thesis structure

This doctoral thesis is presented as a compendium of six scientific articles derived from the experimental results generated throughout this thesis and it is divided into six chapters.

Chapter 1 presents a general introduction related to intermittent streams and the associated groundwater, with a focus on the effects of low-flow regimes. It also outlines the impact of anthropogenic inputs and their environmental consequences, highlighting the need to develop improved analytical techniques to detect and monitor the presence of anthropogenic contaminants. The analytical techniques employed are explained alongside the various cheminformatics approaches adopted for the detection and quantification of contaminant of emerging concern (CECs) and their transformation products (TPs) in water samples.

Chapter 2 outlines the main aim of the thesis and defines the specific objectives.

Chapters 3, 4 and 5 present the results obtained in this thesis. Each chapter begins with a brief introduction, a description of materials and methods, the results with the corresponding scientific publications and a discussion on the most relevant aspects of the results.

Chapter 3 focuses on the development of an analytical methodology for the detection and quantification of targeted CECs in surface water and its subsequent application. The results are detailed in *Publication #1*.

Publication #1: **O. Gómez-Navarro**, F. Labad, D.P. Manjarrés-López, S. Pérez, N. Montemurro. (2023). HRMS-Targeted-DIA methodology for quantification of wastewater-borne pollutants in surface water, MethodsX, 10, 102093. https://doi.org/10.1016/J.MEX.2023.102093.

Chapter 4 comprises three publications on the occurrence and environmental risk of CECs in the Mediterranean basin. The impact of wastewater treatment plants in Spanish intermittent streams was studied and included as *Publication #2*. The analysis of groundwater samples from Tunisia, is presented in *Publication #3* aiming to provide a better understanding and reduce the knowledge gap in Southern Mediterranean countries. Finally, *Publication #4* provides a comprehensive study on several Mediterranean intermittent streams for which ecological, hydrological and chemical indicators were evaluated to enable a holistic characterization.

- *Publication #2*: **O. Gómez-Navarro**, L. Bertrans-Tubau, M. Abril, N. Montemurro, L. Proia, S. Pérez, Unveiling the impact of wastewater effluents on Mediterranean streams with seasonal flow variability. Submission to Environmental Science & Technology.

- *Publication #3*: F. Khezami[1], **O. Gómez-Navarro**[1], M.V. Barbieri, N. Khiari, A. Chkirbene, S. Chiron, S. Khadhar, S. Pérez. (2024). Occurrence of contaminants of emerging concern and pesticides and relative risk assessment in Tunisian groundwater. Science of The Total Environment, 906, 167319. https://doi.org/10.1016/j.scitotenv.2023.167319

[1] Co-first authorship, authors contributed equally to this work.

- *Publication #4*: **O. Gómez-Navarro**, A.M. De Girolamo, A.W. Lorenz, S. Khadhar, T.H. Debieche, F. Gentile, S. Chiron, S. Pérez. (2024). Characterization of anthropogenic impacts in Mediterranean intermittent rivers with chemical, ecological and hydrological indicators, Journal of Hazardous Materials, 480, 135951. https://doi.org/10.1016/j.jhazmat.2024.135951.

Chapter 5 presents the studies on the fate and environmental behaviour of CECs and related TPs. The chapter includes *Publication #5* which evaluates the environmental behaviour of TPs at both field and laboratory scales, and *Publication #6* which explores the use of open-source cheminformatics approaches for both suspect and non-target screening to identify TPs and other unknowns in intermittent river samples. *Publication #6* presents the results obtained during the pre-doctoral research stay carried out at the Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, under the supervision of Professor Dr. Emma L. Schymanski, within the Environmental Cheminformatics group.

- *Publication #5*: R. Manasfi, D. Tadić, **O. Gomez,** S. Perez, S. Chiron. (2022). Persistence of N-oxides transformation products of tertiary amine drugs at lab and field studies. Chemosphere, 309, 136661. https://doi.org/10.1016/j.chemosphere.2022.136661

- *Publication #6:* **O. Gómez-Navarro,** S. Perez, E.L. Schymanski, (2025). Integrating Suspect and Non-Target Screening with Open Cheminformatics Approaches to Identify Pharmaceutical Related-Transformation Products and previously unidentified Pesticides in a Mediterranean Stream. Submission to ACS ES&T Waters.

Finally, **Chapter 6**, summarizes the main conclusions drawn in this doctoral thesis.



Chapter 1

General Introduction

1.1 Growing relevance of intermittent streams and their associated groundwater

1.2 Impact of wastewater effluents on intermittent streams and associated groundwater

1.3 Contaminants of emerging concern in surface waters and associated groundwater

1.4 Analysis of pollutants in water samples

1.5 Identification approaches for known and unknown compounds

1.6 References

Chapter 1. General Introduction

1.1. Growing relevance of intermittent streams and their associated groundwater

Water is a fundamental resource for sustaining life and maintaining ecological balance [1], covering approximately 71% of the Earth's surface. However, freshwater only represents 2.5% of this water and a large proportion of it is unavailable, as it remains stored in glaciers, ice caps, the atmosphere and soil [2]. Groundwater represents around 30% of freshwater, whereas lakes and rivers represent less than 0.01% of all water on earth [3]. Despite the low percentage, freshwater bodies, particularly rivers, play a crucial role in the hydrological cycle. Rivers supply freshwater for human consumption, agriculture and energy generation, while they also connect terrestrial and marine ecosystems [4]. River water availability, however, is not constant throughout the year [5,6], particularly those in arid or seasonally variable climates, can experience notable variations in their flow.

Unlike perennial rivers, which maintain continuous flow throughout the year, intermittent streams and rivers, are defined as fluvial ecosystems that can temporally stop flowing or dry out completely during certain seasons, because of natural climate variations, regional hydrology and water scarcity [7–10]. Globally, these systems now represent over 50% of all river network [11]. These are commonly found in arid and semi-arid regions, often known as drylands [12] which are characterized by low and highly variable rainfall where evapotranspiration frequently exceeds precipitation [13]. This situation leads to significant water scarcity that impacts both natural ecosystems and anthropogenic systems, limiting the production of crops, livestock, wood and other essential environmental services. At the same time, climate change and increasing human water demands are accelerating the transition of perennial rivers to intermittent systems [14,15]. Due to all these factors, in recent years, low-flow, intermittency and drying conditions have been increasing in many parts of the world, including Europe [16]. This phenomenon is expected to continue and intensify in the coming years [17,18], leading to more frequent and severe hydrological drought conditions [11]. Recent studies predict that seasonal drying periods will start earlier and will persist longer [19].

The temporary nature of these water bodies affects their physico-chemical, ecological, and hydrological conditions, as the drastic changes and challenges during periods of flow or drought influence the biodiversity within the ecosystem, nutrient cycle, sediment transport and water quality [20–22]. Flow regimes are directly affected by seasonal variations in precipitations, with heavy rain triggering flooding and extended dry periods leading to flow

cessation [10].

As global water scarcity intensifies, especially in arid and semi-arid regions, the relationship between intermittent rivers and groundwater is becoming more significant. During rainy periods, intermittent rivers promote surface water infiltration into groundwater systems [23]. In drylands, this interaction becomes crucial, as groundwater becomes an important reserve when surface water sources are unavailable. Without intermittent rivers acting as seasonal aquifer recharge in drylands, groundwater levels would drop faster, exacerbating water scarcity and straining ecosystems [24]. This direct connection between surface water and groundwater also influences flow regimes, since geomorphic features such as channel morphology and sediment composition, play a key role in controlling hydrological processes including infiltration, recharge and runoff [12]. For example, narrow, steep channels enhance rapid runoff, while sediment characteristics such as porosity and impermeability play crucial role in water movement. While coarse, porous sediments like gravel promote infiltration, enhancing groundwater recharge and reducing surface water retention [25,26].

Despite the environmental relevance of intermittent rivers, they have not been as studied as perennial systems. In recent years, there has been a growing attention to understand their hydrology, geomorphology, biogeochemistry, ecology, management and conservation [7,27–29]. Nevertheless, a critical need remains for further research to assess how intermittency impacts ecological roles and the consequences of associated anthropogenic activities [30].

The Mediterranean basin is a prime example of a region with abundant intermittent rivers due to its semi-arid climate, which as described previously implies pronounced seasonal variability and often extreme flooding and drying periods [31,32]. In Figure 1.1 the Mediterranean intermittency and climate type distribution is shown (extracted from [15]). In the South Mediterranean area, non-perennial streams are more likely to occur due to the predominance of hot and arid climates, whereas the North Mediterranean experiences slightly lower probability of such streams, associated with cooler climate zones. However, as observed in Figure 1.1, the probability of river intermittence in Northern Mediterranean area is higher than in the Northern European countries [15,33]. While this thesis focuses on the Mediterranean region, intermittent streams are also found in other semi-arid and temperate regions worldwide, including North America, Australia, and southern Africa [11,26]. Comparative studies highlight the global importance of understanding flow intermittency shaped by climate change and human activity [10,27]. Studying Mediterranean intermittent streams provides globally relevant insights into water quality and aquatic conservation by

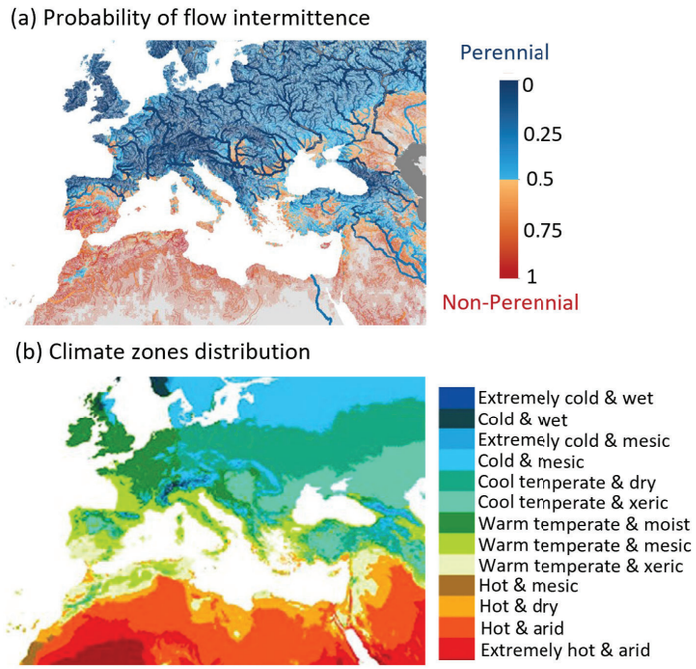


Figure 1.1. *Mediterranean and North European countries on the probability of flow intermittence (a) and climate zone distribution (b), extracted from Messager et al [15].*

examining flow variability and pollution dynamics in similar systems.

Nevertheless, the Mediterranean region is one of the most biologically diverse and ecologically complex areas in the world [32–34]. Its rich biodiversity results from its unique geography, colliding with 22 different countries across Europe, North Africa and the Middle East, its dynamic geological history and highly seasonal climate [31,33]. In addition, the pronounced seasonal variability and prolonged water scarcity during the dry season exert strong selective pressures on organisms, favouring those with specialized adaptations, thus contributing to the region's high species richness [32]. However, this area also faces challenges from both natural and human-induced factors [33,35]. Seasonal climate patterns strongly influence hydrological regimes, where, during summer, watercourses experience low flow, forming isolated pool or drying up completely. Anthropogenic pressures exacerbate the natural variability of the streams and associated groundwaters [10] with water extraction, specially for agricultural pollution, tourism and vacation uses, urban development and land-use changes all contributing to the degradation of these ecosystems [36]. These issues are particularly acute in South Mediterranean countries, notably in the Middle East North Africa (MENA) region, where extreme climate conditions, high evapotranspiration rates and chronic water scarcity further pressures freshwater ecosystems [37,38]. The MENA region is among the most water-stressed globally [39,40], with many

countries experiencing water availability per capita below international thresholds [41]. Additionally, the region's high dependence on agriculture, which consumes approximately 80% of total water withdrawals, places further strain on limited water resources [39,42]. The interplay of arid climates with intense human activities makes the MENA region particularly vulnerable to hydrological and ecological degradation. Also, these areas are highly impacted by wastewater effluents which often make up a significant portion of river or stream flow, particularly during the dry season [43].

1.2. Impact of wastewater effluents on intermittent streams and associated groundwater

Wastewater effluents from wastewater treatment plants (WWTPs) are a major contributor to anthropogenic stress on rivers, causing serious harm to hydrological integrity of rivers and surrounding groundwater resources, water quality and river ecosystems. Wastewater discharges, especially from large urban areas or industrial sites, can alter the natural flow patterns of rivers. For instance, during dry periods when flow is already low, wastewater released into rivers may cause an artificial increase in flow, which can affect the river's hydrological balance. At a European level, wastewater effluent discharges are subjected to strict regulations under the Urban Wastewater Treatment Directive, recently updated as Directive (EU) 2024/3019 [44], which sets stricter quality standards for effluent discharge into receiving water bodies. It is important to note that the updated Directive introduces stricter limits for nitrogen and total phosphorus depending on the WWTP size. Additionally, it introduces the implementation of removal of micropollutants for larger plants. Specific parameters related to water quality such as biochemical oxygen demand, chemical oxygen demand and nutrients concentrations are regulated to minimize their environmental impacts [45].

When wastewater is not treated properly, these pollutants end up in rivers, leading to harmful effects. High levels of nutrients, particularly nitrogen and phosphorus species, can lead to eutrophication, where excessive nutrient enrichment stimulates rapid algal blooms [46]. As algal blooms increase, these block and decrease in sunlight, affecting, negatively, the growth of aquatic vegetation which are crucial for aquatic life [47]. When algal blooms die and decompose, dissolved oxygen is consumed by microbial activity, leading to hypoxic or even anoxic conditions which can harm and even kill fish and other aquatic life [48]. In some situations, certain algae can even produce harmful algal toxins, posing additional risks to the aquatic ecosystem and human health [49]. Wastewater effluents can also introduce pathogenic bacteria, viruses and parasites from faecal contamination [50,51]. Microbial pollutants can cause health threats while also disrupting the balance of river ecosystems.

Hence, both nutrient and microbial levels have to be reduced to established limits before the discharge of the effluent to the receiving streams.

In Spain, the recent *Real Decreto* 1085/2024 [52], has replaced the previous *Real Decreto* 1620/2007 [53], introducing updated regulations for the treatment and reuse of wastewater effluents. This new directive aligns Spanish regulation with the EU Regulation 2020/741 [54], introducing stricter standards for water reuse and expanding the permitted applications for reclaimed water. The updated Spanish decree establishes maximum limits for different parameters depending on the intended use of treated wastewater, with reinforced quality control measures to ensure safety and environmental protection. It also introduces a classification system for reclaimed water quality, A+ the highest quality, hence stricter regulations and D the lowest.

In Table 1.1, a summary of the main regulated parameters and their respective maximum limits for treated wastewater effluents are shown. The values reflect current national legislation primarily according to the EU Directive 2024/3019 [44] and the *Real Decreto* 1085/2024 [52]. All the parameters are inputs which heavily influence river water chemistry and ecosystem functions, making strict monitoring and regulation essential. Regional authorities can set even stricter limits based on specific characteristics of the receiving environment.

Table 1.1. *Maximum concentration limits requirements for discharges from urban wastewater treatment plants (WWTPs), as established by Directive (EU) 2024/3019 and the maximum value for environmental discharge by the Spanish Real Decreto 1085/2024. Abbreviations: ND = Not defined.*

Parameter	Maximum limit	Regulation
pH	6.0-9.0	Directive (EU) 2024/3019
Biochemical oxygen demand (BOD_5)	≤ 25 mg/L	Directive (EU) 2024/3019
Chemical oxygen demand (COD)	≤ 125 mg/L	Directive (EU) 2024/3019
Total suspended solids (TSS)	≤ 35 mg/L	Directive (EU) 2024/3019
Total nitrogen	≤ 10-15 mg/L*	Directive (EU) 2024/3019
Total Phosphorus	≤ 1-2 mg/L *	Directive (EU) 2024/3019
Fecal coliforms	100 CFU/100 mL	*Real Decreto* 1085/2024
Escherichia coli	Not detectable in 100 mL	*Real Decreto* 1085/2024
Turbidity	5 NTU	*Real Decreto* 1085/2024
Intestinal nematodes	1 egg/10 L	*Real Decreto* 1085/2024

**Note: range depends on WWTP size.*

While intermittent rivers and associated groundwater are vital for sustaining ecosystems, their unique hydrological patterns also make them vulnerable to wastewater contamination. Low flow conditions can lead to increased concentration of pollutants [55], and in some cases where there is a constant inflow from WWTPs, temporary streams can transform into "artificial" perennial ones [56]. Under such circumstances, the effluent flow rate from WWTPs is usually higher than the natural river flow rate, and streams can become

wastewater dominated streams. During these low-flow or complete drying periods, organic pollutants, nutrients and organic waste increases [57] and can also accumulate in sediments or isolated pools, only to be mobilized during subsequent flow events. The intermittent nature of Mediterranean watercourses plus the increasing anthropogenic pressures, such as WWTPs and agricultural runoff, underscore the importance to understand the behaviour and impacts of contaminants in these dynamic systems. This includes not only investigating the surface water dynamics but also the associated groundwater. Wastewater can also end up affecting groundwater, as contaminants can infiltrate through soil and accumulate in the groundwater over time, posing long-term risks to drinking water supplies and irrigation water quality [58,59].

More broadly, freshwater systems play a very important role in maintaining ecological balance but face important threats from stressors such as eutrophication, emerging pollutants, climate change, habitat alteration and species invasions [60]. Water scarcity further exacerbates these challenges by disrupting the ecosystem health, hydrology and river chemistry [61]. Despite the continuous progress in the different fields, interactions and impacts of many stressors remain poorly understood [62]. This is why it is important to enhance scientific understanding and develop effective strategies to understand and manage better these vulnerable ecosystems.

To address these challenges, environmental indicators can serve as tools to assess freshwater health and monitor the effects of environmental stressors and to detect possible changes over time [63,64]. Indicators can range from physical parameters like temperature, electrical conductivity and water quality to biological markers such as species diversity and population trends [65,66], as well as hydrological indicators that monitor river flow dynamics [67,68]. Additionally, chemical indicators including contaminants of emerging concern (CECs) concentrations provide insights on the ecological state of the aquatic systems.

1.3. Contaminants of emerging concern in surface waters and associated groundwater

1.3.1. Presence and state of the art of CECs in Mediterranean countries

CECs refer to chemicals or substances identified by environmental monitoring efforts that may pose a potential adverse effect on the environment wildlife and human health [69] but have not yet been fully regulated. CECs encompass a broad range of both synthetic and natural chemicals, covering a wide variety of categories such as pharmaceuticals, personal care products, illicit drugs, pesticides and industrial compounds, as well as their

related transformation products (TPs). According to the U.S Environmental Protection Agency (EPA), these substances were previously not detected in water but are now found at significantly different levels than expected [70].

The presence of CECs in the aquatic environment has gained attention in the last decades, as their use, consumption and therefore detection is increasing. In recent years, CECs have been detected in various environmental matrices, from surface water [71] to groundwater [72–76], but also other matrices like sediments and soils [77,78], vegetables [79,80], fish [81,82] and even in human samples [83,84]. Among these, surface water is by far the most extensively studied matrix with a substantial number of publications concerning various CECs classes and wide range of concentrations.

The first analysis of CECs in the aquatic environment started in the late 70s, however, it was not since the 1990s-2000s that major improvements on chromatographic separations and detection techniques enabled their detection even at lower concentrations.

To visualize these increased trends, Figure 1.2 presents the *chemical stripes* for two commonly detected and reported CECs, estradiol and carbamazepine. This visualization tool (*chemical stripes*), developed as an R-package by Aurich et al., 2024 [85], uses publication data to map the evolution of a compound's scientific relevance over time [86,87]. Scientific literature is fundamental in understanding the global trends of chemical compounds throughout the years. PubChemLite [88] provides the metric Literature Count, which quantifies the number of references associated to a specific compound.

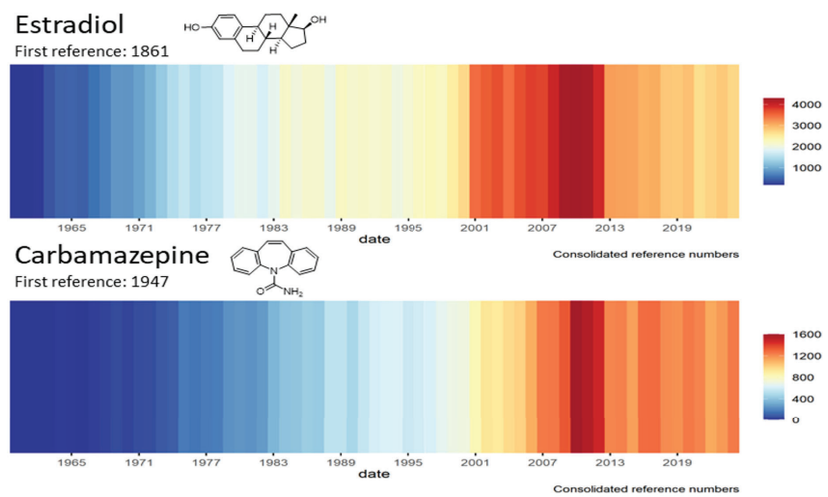


Figure 1.2. *Chemical stripes showing temporal trend based on literature counts for estradiol and carbamazepine [85].*

This count is calculated through several sources including peer-reviewed scientific papers, patents, book and regulatory reports. However, it only includes references from PubChem linked sources i.e., those research papers indexed in PubMed. Nevertheless, while it is not comprehensive of all literature, it offers a valuable overview of how the compounds scientific significance evolved over time (Figure 1.2).

Given the increasing detection of CECs in waterbodies, it is essential to examine how global scientific efforts have been distributed. While the presence of CECs has been studied European Union countries the most studied [89,90]. Southern Europe countries such as Spain, France and Italy have emerged as leading contributors, supported by EU directives and sustained research investments [90].

In contrast, the MENA region remains underrepresented in scientific literature. A critical review by Ouda et al., 2021 [91] highlighted the scarcity of studies on CECs in the MENA region. While pharmaceuticals and personal care products, have been detected in various water bodies, research is unevenly distributed also within the region, with countries like Iran and Tunisia receiving more attention. Another comprehensive review [92] analysed 14,400 papers and identified only 89 studies focusing on emerging pollutants in MENA countries between 1976 and 2020. The majority of these studies were part of international

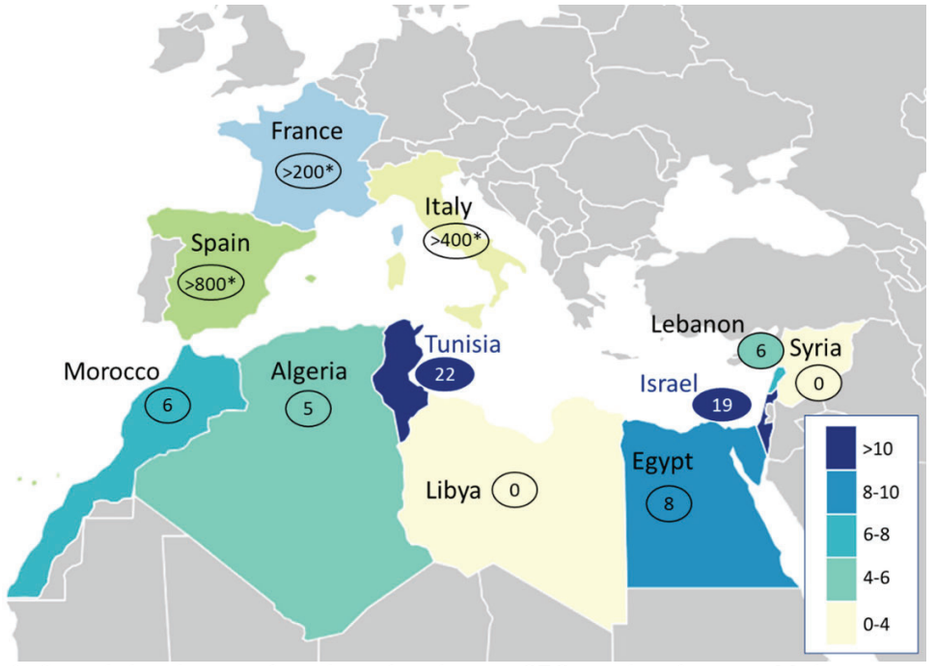


Figure 1.3. *Spatial distribution of number of studies on CECs, with a focus on Mediterranean countries, particularly those in the MENA region. Countries marked with an asterisk (*) include approximate values extracted from [90] on data collected between 2017 and 2021.*

projects funded by entities like the EU and the USA, indicating a reliance on external support for research in this area. This research gap is particularly concerning given the region's environmental vulnerabilities, growing urbanizations and reliance on limited water resources. To visualize these regional disparities, Figure 1.3 present the number of studies on CECs found in Mediterranean MENA countries alongside selected South European Mediterranean countries. For these latter, only approximate values are shown based on the bibliometric analysis by Yu et al., 2024 [90] from 2017 to 2021, due to the vast number of articles present in literature.

To provide further insights into the specific studies conducted in MENA Mediterranean countries, Table 1.2, presents a breakdown of the number of studies on CECs per country and type of environmental sample. Together with Figure 1.3, Table 1.2 underscores the uneven distribution of studies across the regions, particularly regarding surface water and groundwater matrices. This imbalance is especially concerning given that both water sources are critical for drinking water supply and are therefore highly relevant to public health and environmental safety. Thus, addressing these gaps is essential.

Table 1.2. *Summary of studies on CECs in the MENA region, including the number of studies per sample type being SW surface water, GW groundwater and others all other matrices studied (e.g., wastewater, sea water, sediments, soil, biota).*

Country	Number of studies	Type of samples studied			Reference
		SW	GW	Others	
Algeria	5	2	2	2	[93-97]
Egypt	8	5	-	3	[98-105]
Israel	19	4	5	12	[71,106-123]
Lebanon	9	4	4	2	[124-132]
Libya	0	-	-	-	[92]
Morocco	6	3	2	1	[71,133-137]
Syria	0	-	-	-	[92]
Tunisia	22	6	2	17	[71,138-157]

1.3.2. Main sources of CECs in water streams and associated groundwater

Understanding the main pollution sources of CECs is crucial to develop effective monitoring and mitigation strategies. For surface water, there are various CECs sources; some can be direct point sources, but indirect or diffuse, including multiple and sometimes unknown, sources also exist. Figure 1.4 illustrates the common pathways of CECs to the aquatic environment. Although WWTPs are the major source of CECs contamination there are many other possible sources. Agricultural activities can contribute significantly to the presence of herbicides and pesticides in water bodies, while livestock activities can contribute to pharmaceutical residues like antibiotics and hormones [158].

During periods of rainfall events, the detection of these contaminants may increase

because of high flow leading to agricultural runoff [159]. Hospitals also account for a significant load of CECs, as their concentration in hospital wastewater can be up to 100 times higher than that found in domestic wastewater [160]. Another important source are industrial discharges. Industrial activities contribute mainly to the presence of synthetic dyes, flame retardants and other industrial chemicals. These chemicals are often found at higher concentrations near industrial areas and can persist in the environment due to their hydrophobic nature [161–163].

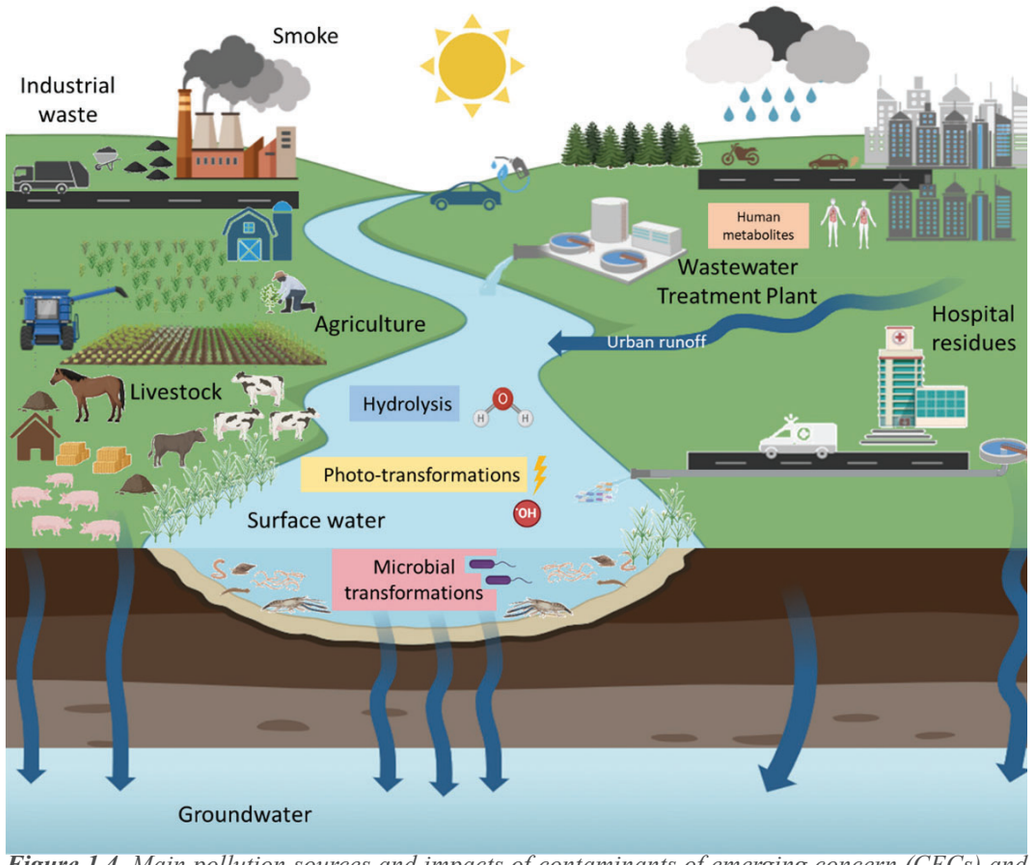


Figure 1.4. *Main pollution sources and impacts of contaminants of emerging concern (CECs) and TP reactions.*

Another important contribution to CECs contamination is sewage overflows. These should not be very common, however during high rainfall events and increased sewage inputs, sewage overflow events may occur leading to significant inputs of pharmaceuticals and domestic contaminants in water bodies near urban areas [164]. Many types of CECs end up in wastewater, in the case of pharmaceuticals, after ingestion our body metabolizes them, with more than 50% of the most commonly consumed are excreted as metabolites rather than as the parent compound [165]. Other CECs, after being used at homes are discarded

through the toilet or sinks. The same happens with industries and hospital residues, and all end up entering WWTPs. WWTPs tend to reduce pollution load before returning the treated wastewater back to the environment through a sequence of physicochemical and/or biological treatments capable of eliminating suspended solids, dissolved substances and pathogens, improving the water quality according to the established water regulations. Despite their great efficiency in the elimination of the organic matter load present in wastewater, conventional WWTPs are not designed to eliminate organic contaminants. Most CECs load often remain stable and only a partial elimination of CECs is generally achieved [166,167], the rest comes out in the effluent and ends up being released into the aquatic environment, distributed throughout the various ecosystems [168]. Consequently, CECs are detected in many water bodies such as rivers, seas, aquifers, as well as in biota.

While CECs sources in the aquatic environment are diverse, their behaviour and distribution across different compartments including sewage, surface water and groundwater vary considerably. Sewage typically contains the highest and most variable concentrations of CECs including both parent compounds and their TPs [160,165]. Once discharged into surface waters, CECs are subjected to dilution and possible transformation processes often leading to lower and altered contamination profiles both spatially and temporally [34,159,166,168]. In contrast, groundwater pollutants behave differently, where CECs tend to persist longer in aquifers due to limited dilution and slower movements, especially in regions where groundwater recharge is low [59,74,91].

1.3.3. Degradation and transformation pathways of CECs

Degradation and transformation of CECs can occur through both engineering and natural processes. Engineered treatment systems typically reduce CECs concentration before they enter the aquatic environment, while once they reach the aquatic system natural attenuation can occur. Natural attenuation can take place through various mechanisms that can either physically, chemically or biologically alter the concentration and composition of CECs (Figure 1.4). Physical processes such as dispersion and dilution do not chemically modify the compound but change their concentrations [169]. For instance, dilution processes are usual situations which occur when WWTP release their effluents to the receiving streams [56,170,171]. WWTP effluent flow rates are usually lower than the receiving river flow rates, hence CECs concentrations are diluted and thus their impact on the aquatic environment remains lower [171].

In addition to these physical mechanisms, degradation processes also play an important role in the reduction of CECs concentrations through structural modifications forming

TPs. Transformations that may occur in the aquatic system can be through either biotic or abiotic processes. Biotic transformations usually occur through human metabolism or microbial degradation processes. Nevertheless, microorganisms present in the environment can mineralize organic compounds as a source of energy or nutrients and break them down into simpler molecules. Alternatively, abiotic transformations generally occur through processes such as hydrolysis, reduction, chlorination, oxidation and photolysis.

Transformation products can be classified in several ways. Längin et al. [172], emphasized the importance of distinguishing between metabolites and TPs to avoid confusion when discussing their formation and behaviour, while Escher et al. [173,174] defined three main categories to classify TPs including metabolites, TPs and byproducts from advanced water processes, and TPs formed in natural environments. For the purposes of this work, both classification systems were integrated to provide a comprehensive understanding of TPs, and the following classification was applied to categorize them. Metabolites and TPs were differentiated, with this latter further divided based on the environment in which modifications can occur.

1) Metabolites:

Metabolites specifically refer to compounds formed within the biological systems of humans, animals or plants as a result of metabolism. Their formation occurs through biotic processes, where metabolism is divided into 2 phase reactions. Phase I metabolism involves the introduction of a new functional group in the molecule mainly through oxidation or hydrolysis reaction processes, while phase II metabolism involves conjugation reactions of endogenous substances, including processes such as glucuronidation or sulfation reactions [165].

2) Transformation products:

a. Engineered systems:

This category includes those TPs generated during specific treatments that trigger transformations, being WWTP and drinking water facilities the most common engineered systems. In these systems, advanced water treatments are applied where advanced oxidations processes highly predominate. Typical treatments include reactions such as chlorination, ozonation and advanced oxidation through e.g., UV/H_2O_2 [175]. All these treatments are primarily used to disinfect water and eliminate pathogens effectively. Despite their high efficiency in removing pathogens and micropollutants, the breakdown of parent compounds implies the formation of TPs and disinfection byproducts (DBPs) [176], some of which

may result in a higher toxicity [177]. The chemical disinfectants used are strong oxidants (e.g. chlorine, ozone) and react with naturally occurring organic matter (NOM) found in freshwater. Although NOM is the main precursor of DBPs, anthropogenic contaminants, even at low concentrations can also contribute to DBP formations, as they undergo changes during disinfection processes [178,179].

b. Natural systems:

In this category, sites where transformations can occur naturally are taken into account such as water bodies (including rivers, lakes, oceans) and land bodies (soil, sediments). Natural attenuation involves all processes, mainly biodegradation and photolysis, by which CECs are degraded and transformed into new molecules, without any anthropogenic intervention. The degradation of each compound may vary significantly depending on their physico-chemical properties as well as environmental conditions (temperature, pH, or oxygen levels) [180].

1.3.3.1. Environmental and analytical challenges of TPs

Analysing TPs is essential to better understand the compound fate and environmental risks, as TPs may differ largely from their parent compounds in terms of their properties, persistence and toxicity [181,182]. Despite the growing recognition concerning their importance, there is still a gap up to date on TPs information on both their occurrence and toxicity. While TPs are generally more polar and less toxic than their parent compounds, some TPs, can sometimes be more hazardous and resistant to degradation, posing environmental and public health risks [174,183]. Still very little is known and available data on TPs remains scarce, especially compared to the substantial number of publications concerning various CECs. To visualize these differences in research attention, and as previously described, *chemical stripes* were generated. In Figure 1.5, the literature stripes for the insecticide fipronil along with several of its known TPs are shown. The consolidated references published per year for each chemical as well as the year of the first reference are presented. The clear contrast in literature coverage between parent compounds and TPs are evident.

Nevertheless, efforts to track and study TPs have relatively improved with the development of chemical inventories such as the NORMAN List S68 [184] or the ZeroPM database [185], and open-source libraries like Drugbank [186,187] and PubChem. The latter includes transformation reaction data incorporated which significantly helps in TPs chemical identification [185].

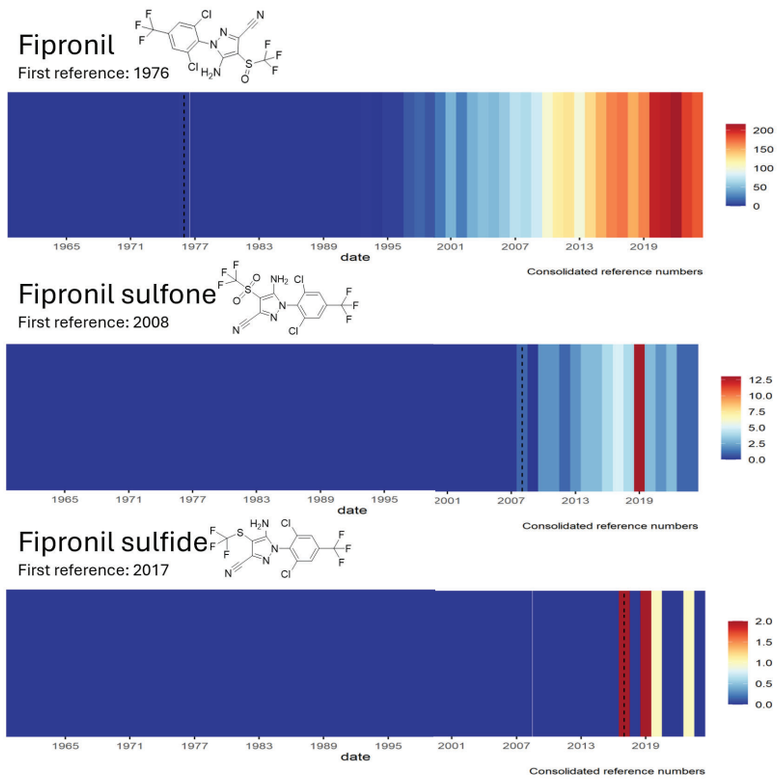


Figure 1.5. *Literature stripes visualizing trends over time for fipronil and its transformation products (TPs) in the scientific literature.*

The NORMAN List S68 (HSDBTPS) [184], is an open-source database compiled by the network of reference laboratories, research centres and related organizations. This list provides information on emerging pollutants and their TPs, incorporating data from the Hazardous Substances Data Bank (HSDB). Specifically, the S68 list contains metabolites and TPs extracted from relevant sections of the HSDB within PubChem, which is continuously being updated.

In addition to curated data, TP information can be expanded through other means such as transformation product prediction models (e.g., BioTransformer tool [188]) and lab-scale experiments. For example, photolysis lab-scale experiments can be conducted to simulate natural attenuation processes and to study transformation processes of contaminants [189–192]. At the same time, biodegradation studies can also be performed through sorption and biodegradation experiments of compounds in water-sediment systems [193–195].

Even though lab-scale processes help in elucidating and identifying TPs, challenges remain due to the low concentrations of TPs in the environment [196–198]. To address these, effective sample preparation steps are needed along with advanced analytical

instrumentation to overcome the limited availability of commercial reference standards and to improve spectral information for confirmation and quantification [199]. Additionally, methods that simulate TP formation have been developed [188,200]. These methods are faster, more cost-effective, and allow the simulation of TP structure for their retrospective detection in samples [201].

1.3.4. Regulation of CECS and their TPs

The Water Framework Directive (WFD) (2000/60/EC) [202] establishes the foundation for water protection across the European Union by specifying regulations for wastewater effluent quality. The recast Urban Wastewater Treatment Directive (Directive (EU) 2024/3019) [44] establishes further stringent requirements for several parameters (as previously mentioned in Table 1.1) to avoid deterioration of the receiving waters. To regulate hazardous substances, the Environmental Quality Standards Directive (2013/39/EU) [203] – an amendment to the WFD [202] – established limits for heavy metals, certain pesticides and industrial compounds, to prevent their accumulation in waterbodies. It also introduced the Watch Lists, marking the beginning of efforts to prioritize CECs.

Despite the progress in water quality regulations, many CECs remain unregulated, even though well-established parameters (Table 1.1) are routinely monitored. CECs regulation is still an ongoing concern, where further regulatory developments are expected in the future to address these pollutants more comprehensively. In the United States, the Drinking Water Contaminants Candidate List (CCL) serves as a framework for identifying unregulated pollutants, with EPA updating the list every five years. The most recent CCL5 [204], released in 2022, identified 66 chemical substances, some of which are recognized as CECs. In contrast, the European Union has implemented a broader regulatory approach that indirectly addresses CECs. The WFD has undergone several revisions, where the first Watch List was introduced in 2015 and to date the 5th Watch List has been published, expanding the scope of monitored contaminants [205]. The purpose of the Watch Lists is to identify and monitor potentially harmful substances before they are formally classified as priority substances, of which some are classified as CECs. Complementing the WFD, the REACH regulation sets requirements for registration, evaluation, authorization and restriction of chemicals [206], overseen by the European Chemicals Agency (ECHA). Figure 1.6 presents an overview of the main regulations and several regulatory frameworks that have been implemented over the past 50 years concerning water quality and hazardous pollutants, including CECs.

Among CECs, Per- and polyfluoroalkyl substances (PFAS) compounds are among the

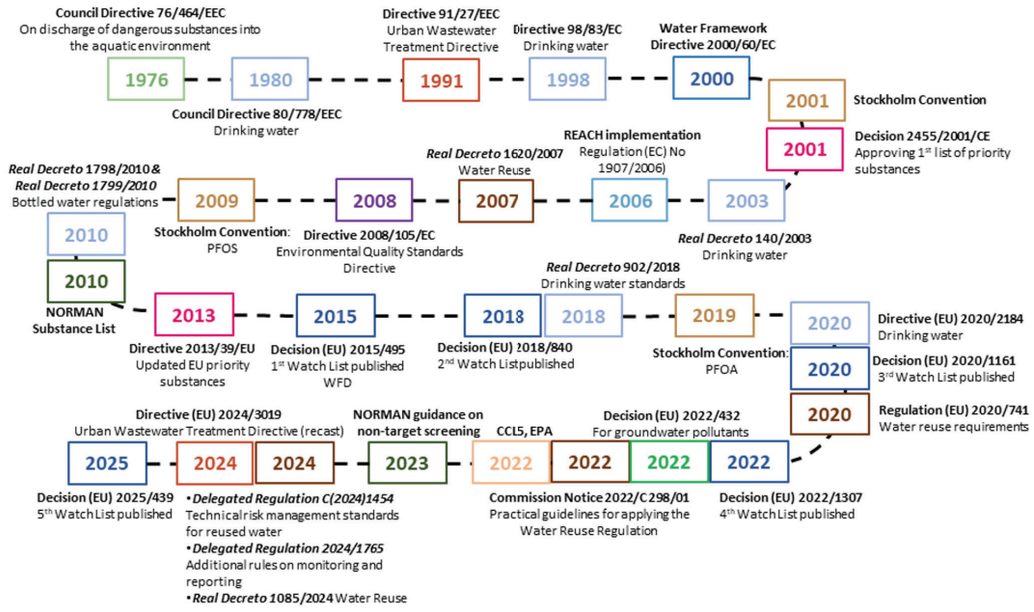


Figure 1.6. *Timeline of main Directives and regulations concerning CECs and water quality over the past years.*

most regulated and few compounds with specific regulations. In Europe, the Directive on Drinking Water Quality (EU 2020/2184) [207] established concentration limits for PFAS compounds, setting a maximum of 0.1 μg/L for individual substances and a combined limit of 0.5 μg/L for the total concentration of all PFAS detected in drinking water.

In the case of pharmaceuticals, these are regulated at EU level for human and veterinary use but remain largely unregulated in terms of their environmental impact. To address this gap, the European Watch Lists under the WFD have been a valuable tool for monitoring selected pharmaceuticals of emerging concern in surface water. Since the 1st Watch List in 2015, several pharmaceuticals including diclofenac and clarithromycin (1st Watch list examples [208]), venlafaxine and sulfamethoxazole (3rd Watch list examples [209]) and more recently fluoxetine and citalopram (5th Watch list examples [205]) have been included. Furthermore, with the recent updated Urban Wastewater Treatment Directive [44], large WWTPs are now required to implement a quaternary treatment to achieve 80% reduction in certain priority CECs.

Moreover, the NORMAN network (Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances) is collecting data on the occurrence and effects of CECs to inform future regulations, focusing specifically on those CECs that remain unregulated. In 2010 the NORMAN Substance List [210] was

created including over 100,000 substances. This list encompasses a large comprehensive database that serves as a tool for researchers, policymakers and environmental agencies to track and evaluate the presence of these substances in the environment.

Without established monitoring and thresholds, CECs pass through wastewater treatment processes as unchanged or transformed substances reaching the aquatic environment. The lack of regulations becomes alarming as new chemicals keep on being introduced and existing CECs degrade into TPs with unknown risks to ecosystem and human health [173,174,181].

1.3.5. Environmental risk assessment of CECs and their TPs

In recent years, various regulatory directives (WFD [202], REACH [206]) have been implemented to address the potential hazards posed by the presence of CECs and other anthropogenic factors to the environment. These risks can be systematically assessed using risk indices, which serve valuable tools to evaluate the environmental impacts of specific compounds [211].

One of the most widely used approaches for the characterization of the potential ecological risks of CECs in the aquatic environment is through ecotoxicological risk assessments (ERA) [212,213]. The EPA established the first guidelines for ERA back in the late 90s [214]. ERA evaluates adverse effects from exposure to stressors, such as CECs, and investigates the impact of their concentration on human health, invasive species and the environment. Through ERA, possible hazards are identified, the exposure is assessed, and the possible risks are characterised. Once the concentrations of CECs are evaluated, these are used to estimate the potential impacts on specific organisms and environmental compartments by evaluating its ecological risk. Potential risk posed by CECs are assessed through risk quotients (RQ). RQs can be calculated according to established guidelines [215] where the measured environmental concentration (MEC) is divided by its lowest predicted no-effect concentration (PNEC). PNEC values can be obtained from experimental ecotoxicity data or predicted models such as QSARs (Quantitative Structure-Activity Relationships). At a European level, there is an eco-toxicology database from the NORMAN network which includes PNEC values for over 90,000 substances and for different environmental matrices such as freshwater, marine water, sediments, fish and molluscs [216]. Up to date, this database does not include PNEC values for many TPs, however those prioritized, such as O-desmethylvenlafaxine are included.

1.4. Analysis of pollutants in water samples

In the following sections, the common procedures performed for nutrient analysis as well as for the analysis of CECs, including their TPs in water samples are detailed going from the initial stages involving sample collection to instrumental analysis.

1.4.1. Sample collection

Firstly, a sampling protocol needs to be properly designed to ensure reliable results, specifying the number of samples, the required volume, and protocols to cover field blanks, sample replicates and cleaning procedures to avoid cross-contamination. Sample collection should be performed in accordance with the standard ISO 5667-6:2014 [217], which provides detailed guidelines for water sampling for chemical and microbiological analysis. Depending on the parameters measured, polyethylene bottles (transparent or amber), Teflon or amber glass bottles are used. Amber materials are usually used to avoid possible photodegradation of light-sensitive compounds. In practice, water samples are often collected manually as grabbed samples, where the samples are collected at specific locations at a single point in time, making those samples representative of that specific time. However, automated samplers or passive samplers can also be employed. Automated samplers are designed to collect samples based on flow and time, providing more continuous data. Nonetheless, grab sampling is typically recommended for matrices where analyte concentration variation is minimal or where degradation rates of analytes are expected. Grab sampling is recommended by the WFD for water sample collection [202], and thus the technique applied in this thesis.

1.4.2. Sample preparation

Sample preparation is the critical and time-consuming step within the analytical workflow, as its main objective is to reduce the effects of endogenous/exogenous chemicals or complicated sample matrices. Its objectives are to stabilize the sample in a suitable solvent, concentrate the analytes at low concentration to improve sensitivity and shield analytical instruments from possible harm.

1.4.2.1. Sample pretreatment

Once the samples have been collected, samples are immediately refrigerated and transported to the laboratory. Upon arrival at the laboratory, samples are commonly filtered at 0.7, 0.45 µm or even smaller pore size to remove particulate matter that may interfere with the analysis. Samples are stored at 4ºC until analysis, avoiding possible degradation

of compounds if analysis is in the next 24 to 48 hours, if not, samples are frozen at -20ºC. When nutrient analysis is performed, a portion of the collected water samples is stored in acid washed bottles to prevent contamination [218,219].

Centrifugation, either in addition to or alternatively to filtration, is used as it helps to precipitate solids from the liquid phase and can also assist in the removal of oils and grease, particularly in more complex matrices [220,221]. However, a key drawback relies on the potential co-precipitation of target analytes with the suspended particles, which can lead to inaccuracies in the analysis. This issue can be controlled, to some extent, through the addition of isotopically labelled internal standards before filtration or centrifugation. This practice helps to prevent possible problems with analyte losses, compensates for any matrix effects and improves accuracy and reliability.

For nutrient analysis, especially for ammonium and phosphates, samples have to be acidified to pH < 2 to avoid possible microbial activity and stabilize ammonium and phosphate ions in solution [222]. Nutrient concentrations are commonly determined through standardized methods, outlined in guidelines such as Standard Methods for the Examination of Water and Wastewater [222] or EPA Methods for Chemical Analysis of Water and Wastes [223]. Common methods mainly involve colorimetric techniques, such as the Griess reaction for nitrate and nitrite [224], the indophenol blue method for ammonium [225,226], and the molybdenum blue method for phosphate [227]. Commercial test kits are now commonly used in laboratories due to their simplicity, fast and effective determination of concentration in water samples. Alternatively, ion chromatography (IC) can be used for the simultaneous analysis of multiple anions [228].

1.4.2.2. Sample extraction

For CECs analysis, sample extraction is important to isolate the analytes from the matrix and remove possible interferences to improve the selectivity of the analytical procedure. This step is critical to concentrate the analytes to detectable levels on analytical instruments. For liquid samples, the most common preconcentration method is still solid-phase extraction (SPE) [229,230]. SPE was developed in the 1970s, as a refinement of liquid-liquid extraction (LLE) and a less solvent intensive alternative. SPE consists of the use of a solid material for the enrichment of compounds of interest, providing the highest preconcentration factors and thorough sample clean-up [231]. The liquid sample is passed through the solid material which has specific physical or chemical properties where the analytes of interest are retained on the sorbent, washed to remove impurities and eluted with a compatible solvent. In Figure 1.7, the SPE principle is shown.

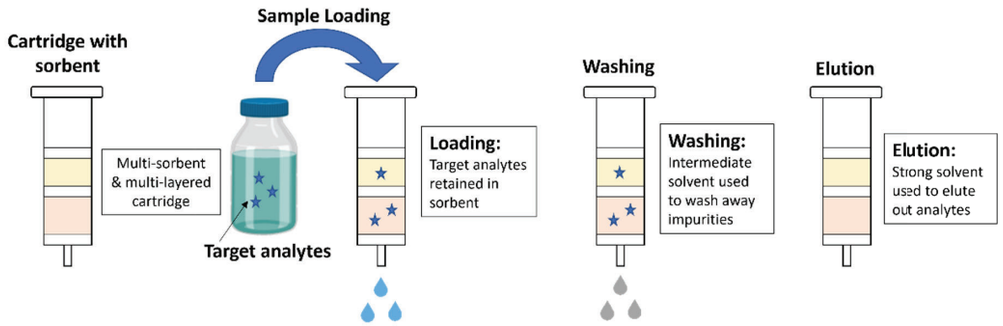


Figure 1.7. *Solid phase extraction (SPE) procedure.*

Many types of sorbents exist, with different binding chemistries, but hydrophilic-lipophilic balance (HLB) sorbent has been the most used for multi residual analyses [232]. For specific applications, ion exchange sorbents for either weak or strong cations/anions (e.g., WCX, SAX) are also commonly used. For polar compounds graphitized carbon black is sometimes employed as it has high retention capability. However, this sorbent, despite its many advantages, has a major drawback concerning desorption of the analytes of interest [232,233]. When addressing a wide scope of contaminants, which may vary in their polarity and physico-chemical characteristics, multi-sorbent and multi layered cartridges can be used as an alternative to single mode sorbent cartridges. When using mixed-mode cartridges, sorbents can be either blended, layered or even stacked. The use of mix-mode cartridges enhances selectivity by using a combination of sorbents, from HLB to ion exchange resins [190,234,235], but experience limitations for highly polar compounds. However, vacuum-assisted evaporation techniques for preconcentration of highly polar and moderately polar are becoming more and more popular, as it is a simple and straight forward method, with low risk of analyte losses [236,237].

1.4.3. Sample analysis

IC is a powerful analytical technique used to determine ionic species in various matrices [228,238,239]. It is highly employed for the analysis of inorganic nutrients, such as anions and cations. Recently IC has also been increasingly used for the analysis of polar organic compounds such as carbohydrates and sugar alcohols [240]. Further advances in IC through the coupling with MS allows the detection and quantification of even highly polar and mobile compounds with increased sensitivity and specificity [239,241].

Liquid (LC) and gas (GC) chromatography are widely used for the analysis of CECs. Chromatographic techniques play a key role in separating complex mixtures, ensuring individual components are isolated before being analysed by mass spectrometry (MS).

In LC, separation occurs based on the interaction and affinity of the analytes between the stationary phase and the mobile phase, while in GC a gas phase is used for the separation of volatile compounds or less polar compounds [242]. In LC, different types of stationary phases are applied depending on the aim of the analysis, including normal phase and reversed phase (RP), being the C18 reverse phase the most common, covering from low to medium polar compounds and the modified normal phase hydrophilic interaction chromatography (HILIC) columns are very promising for highly polar compounds [236].

In Figure 1.8, the common application of chromatographic techniques as well as the different ionization techniques are shown based on the polarity of the analytes. For LC, electrospray ionization (ESI) covers a wide range of polar compounds while electron ionization (EI) is commonly used for non-polar compounds analysed through GC. Atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) are other possible ionizations sources, however, in this thesis the only ionization technique applied was ESI.

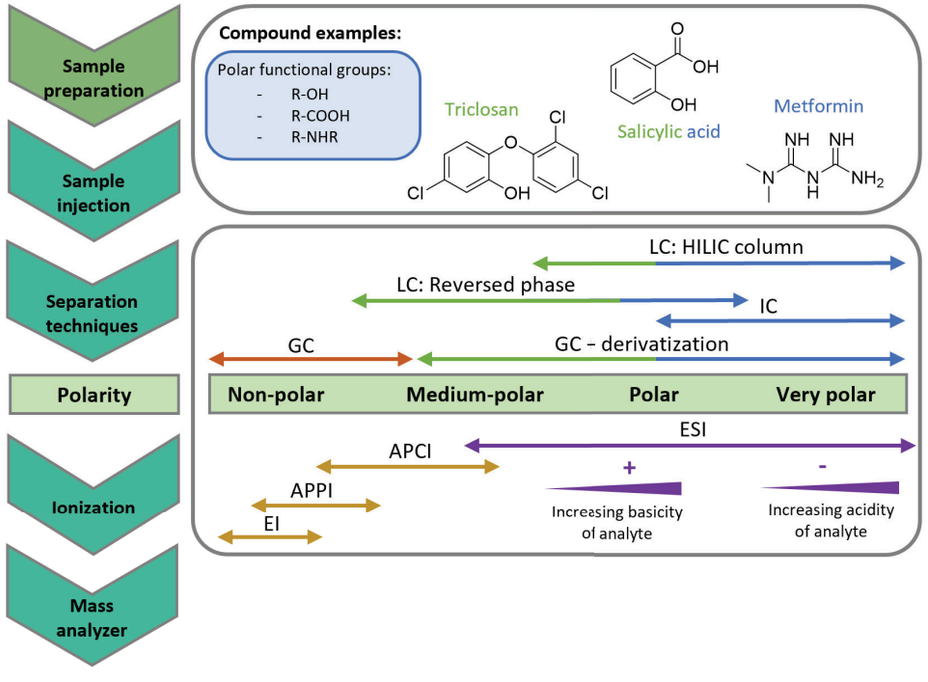


Figure 1.8. *Chromatographic separation and ionization sources for analytical methodologies based on the polarity of the compounds. Adapted from Hollender et al. [243] and Talavera-Andujar [244]. Abbreviations: Ion chromatography (IC), liquid chromatography (LC), gas chromatography (GC), hydrophilic interaction chromatography (HILIC), electron ionization (EI), atmospheric pressure photoionization (APPI), atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI).*

Mass spectrometry is a universal detection technique used for the identification and quantification of multiple small molecules and can be coupled to separation techniques, including both LC and GC. MS measures the mass to charge ratio (*m/z*) of ionized species, allowing the detection of individual components in the chromatographic effluent. Various mass analysers can be used for MS analysis, depending on the nature of the sample and the type of analysis being performed. Quadrupole (Q) mass analysers, including triple-quadrupole (QQQ) instruments are commonly used in targeted and routine studies due to their high sensitivity and selectivity, making them ideal for the quantification of targeted compounds. However, their main drawback relies on their low resolution, making them unsuitable for non-target applications. In contrast, Quadrupole Time-Of-Flight (Q-TOF) and Orbitrap instruments are frequently employed in suspect and non-target screening as they offer high resolution MS (HRMS). Mass resolution describes the ability to distinguish between ions differing in *m/z,* expressed as full width at half maximum (FWHM), which refers to the width of the peak at half its height, where narrower peaks indicate higher resolution. Q-TOF instruments combine the capabilities of tandem mass spectrometry (by isolating ion in the quadrupole) with the high mass resolution of a TOF analyser, achieving mass resolutions typically ranging from 40,000 to 60,000 FWHM at *m/z* 900-1000 [245]. For even higher mass resolutions applications, Orbitrap analysers, such as the Thermo Scientific Q-Exactive series, are preferred as they provide resolutions ranging from 70,000 to 280,000 FWHM at *m/z* 200, depending on the model and settings, and in some cases can achieve ultra-high resolutions of up to 1,000,000 FWHM [246,247].

Currently, LC coupled to a HRMS has become one of the leading technologies in the analysis of both target and non-target of biological and environmental samples [248].

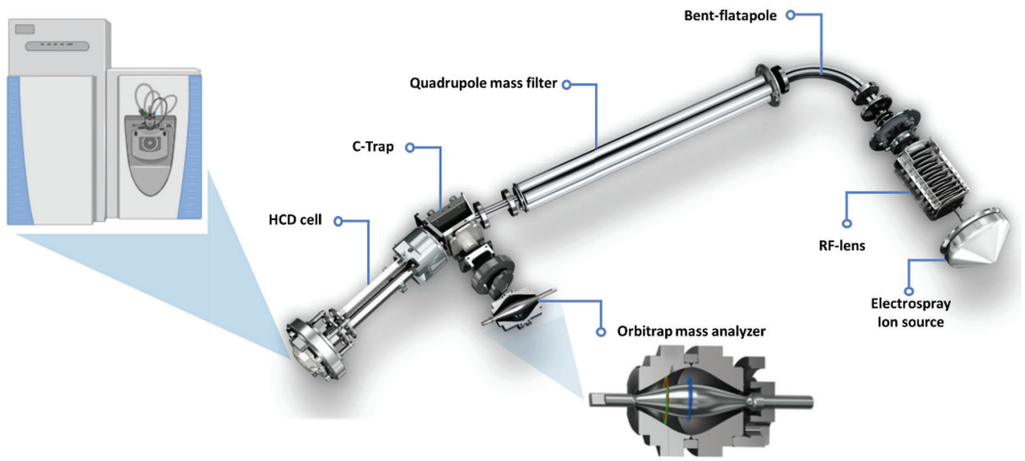


Figure 1.9. *Q-Exactive Orbitrap mass spectrometer main components [251].*

Although low resolution MS (LRMS) is approximately one order of magnitude more sensitive than HRMS, the continuous advances on HRMS instruments, particularly in Orbitrap analysers, have narrowed this gap enabling HRMS to be highly effective in routine screening and target analysis based on full-spectrum accurate mass acquisition [248]. Despite its lower sensitivity compared to LRMS, the versatility offered by HRMS, has made it a valuable alternative analytical tool for the study of CECs, including pharmaceuticals and their transformation products in surface water [249,250]. Thus, HRMS was applied in this PhD thesis where an Orbitrap mass analyser was used.

The basic principle of an Orbitrap instrument is illustrated in Figure 1.9. It consists of four key components: the quadrupole mass filter, a C-Trap for ion storage, a higher energy collision dissociation cell (HCD) and finally, the Orbitrap mass analyser for ion detection and mass analysis.

Ions are generated by electrospray ionization and enter the radio frequency (RF) lens through a heated capillary. The RF lens focuses and transmits the ions to the bent-flatapole, where they are redirected by 90 degrees, enabling natural species to be removed. Ions then enter the quadrupole mass filter, which isolates ions within a selected *m/z* range, using either wide (10-100 Da) or narrow (0.4 -2 Da) isolation windows. Unlike traditional tandem quadrupole mass spectrometers, the Orbitrap quadrupole is not used for scanning, instead its main function relies exclusively in the isolation of specific ions. Isolated ions are then accumulated in the C-trap, where the user can then decide whether to: 1) send the ions to the Orbitrap mass analyser for a full scan detection (MS1), or 2) direct ions to the HCD cell for ion fragmentation, then back through the C-Trap to the Orbitrap mass analyser for fragment detection (MS/MS). The C-Trap, controlled by an automatic gain control (AGC) and a charge detector, avoids ion overfilling and space charge effects, ensuring optimal ion management. The HCD cell, located immediately after the C-Trap, is responsible for fragmenting the ions which were previously filtered by the quadrupole. The Orbitrap mass analyser acts as both analyser and detector, where ions orbit a central electrode. These oscillations result in ion separation and produce a time-varying signal, known as transient, which is subsequently converted into an m/z spectrum, through the Fourier Transform analysis [251].

Data acquisition usually starts with a full-scan acquisition (obtaining MS1) across a specified *m/z* range, to determine the exact mass of precursor ions. This is followed by MS2 analysis, where selected ions are isolated in the quadrupole and fragmented in the HCD cell, enhancing structural elucidation. The two main acquisition strategies applied in HRMS are data-dependent acquisition (DDA) and data-independent acquisition (DIA),

each presenting their respective advantages depending on the research objective. HRMS acquisition methods used will be discussed in further detail in chapter 3.

Regardless of the acquisition method used, advanced data processing techniques for accurate compound identification are required.

1.5. Identification approaches for known and unknown compounds

Depending on the type and objectives of the analysis, different approaches can be employed such as target analysis for the quantification of specific compounds or suspect and non-target screening for tentative identification.

LRMS has traditionally been preferred for target analysis due to its robustness and historically higher sensitivity compared to early HRMS instruments, while HRMS has been primarily used for qualitative analysis. However, advancements in HRMS technology have significantly improved its sensitivity, making HRMS suitable also for target analysis with enhanced accuracy, even at low concentrations [221,252]. In this thesis HRMS was applied for targeted, suspect and non-target approaches.

In Figure 1.10, the different workflows that can be applied for the identification of CECs by HRMS are shown. Targeted analysis involves a selection of specific compounds of which reference standards are necessary for their confirmation. Inclusion lists with these compounds are then introduced and their presence is confirmed and quantified. Suspect screening involves the application of lists of possible compounds that are expected to be present, while for non-target screening the aim is to identify as many compounds as possible without prior information. For these latter, reference standards are not initially available and a large volume of data of high complexity is obtained.

The key challenge in non-targeted workflows relies on accurate interpretation and identification of unknowns. Therefore, access to comprehensive spectral libraries, molecular databases, and cheminformatic tools is essential for increasing confidence in compound annotation. These resources help assigning compounds to the correct classification level and improving overall identification reliability.

Compound annotation is the process of assigning chemical identities to detected features, which rely on various analytical techniques and data sources including spectral library matching and computational predictions. Schymanski et al., 2014 proposed a five-level system to classify compound annotations based on the available supporting evidence, and since then has been by far the most used classification rank in non-target approaches [254].

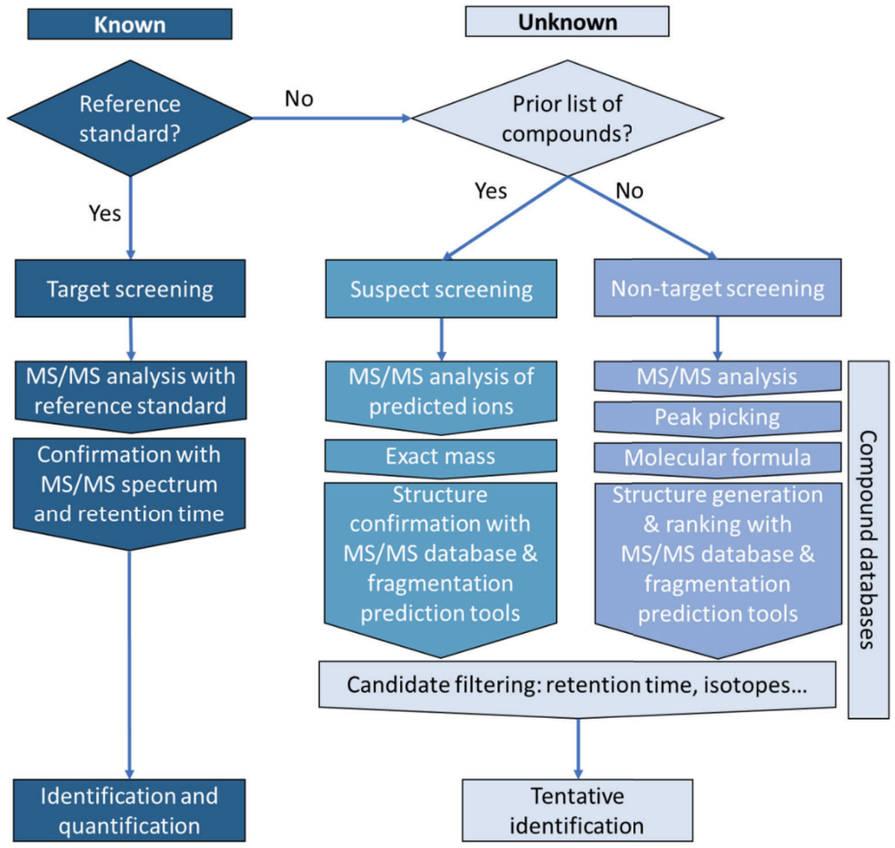


Figure 1.10. *Identification workflow for target, suspect and non-target analysis. Adapted from Hollender et al. [243] and Bletsou [253].*

The classification system (Figure 1.11) ranges from level 1, with compound confirmation with reference standards, to level 5, which includes unknown compounds with only an exact mass measurement. As observed in Figure 1.11, the higher the confidence level the stronger validation criteria are required such as spectral and retention time matching reference standards. While for lower confidence levels, there is a decrease in certainty where only molecular information (level 4), or m/z (level 5) is available. This confidence level system provides a structured approach, however there is not yet any universal standard system for compound annotation, making it challenging to compare results across studies.

As mentioned above, the major challenges rely on the interpretation and identification of unknown compounds, hence access to spectral libraries, comprehensive databases, and advanced cheminformatics tools are necessary.

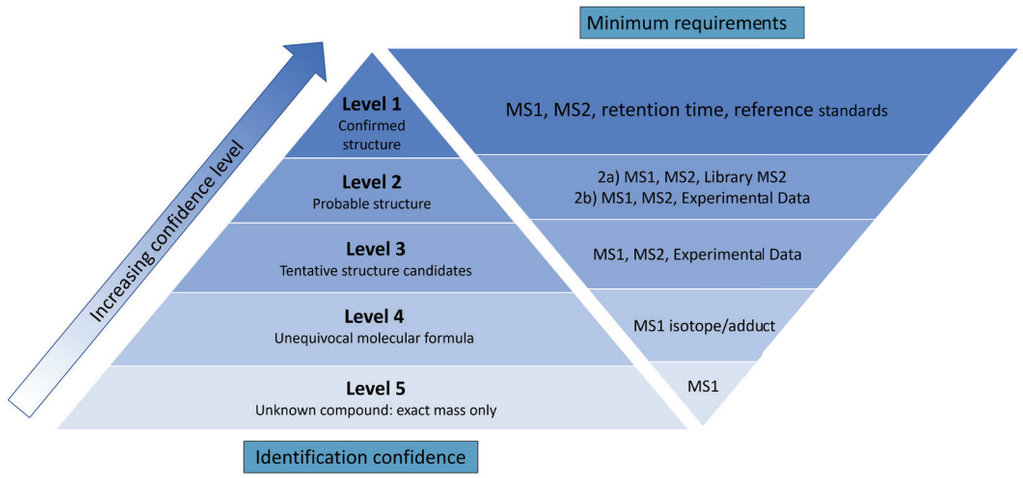


Figure 1.11. *Identification confidence levels proposed by Schymanski et al., 2014 [254]. Adapted from [254].*

1.5.1. Cheminformatics approaches for identification of unknowns

Cheminformatics is a multidisciplinary field that integrates chemistry, computer science, and information technology and plays a key role in the identification and characterization of unknown compounds in non-target screening workflows. Since reference standards are not available, accurate compound annotation relies on advanced cheminformatics tools for data interpretation, mass spectral analysis, chemical structural elucidation and database mining.

Among the most effective strategies for achieving a higher confidence level in compound annotation is mass spectral library matching. This approach involves comparing experimental product ion spectra against reference databases such as MassBank [255], MassBank of North America (MoNA) [256] and mzCloud [257]. If a compounds fragmentation spectrum closely matches an entry in a spectral database, its identity can be tentatively assigned at a level 2a or fully confirmed at a level 1 if reference standards are available, and retention time is matched (Figure 1.11). Despite this, spectral libraries remain limited, as only a small fraction of known chemicals are represented. When no high-confidence spectral match is found, alternative computation approaches such as in-silico fragmentation tools are used. These tools are used to predict possible molecular structures by simulating how molecules are fragmented in mass spectrometers, creating theoretical spectra that can be compared with experimental data. One commonly used tool is MetFrag [258] which predicts structures by generating possible molecular fragments from a proposed molecular formula. It retrieves potential structures from databases such as

PubChem [259] and ChemSpider [260], compares the predicted fragments to experimental MS/MS spectra, and ranks candidates based on spectral similarity.

Open data exchange helps the collaboration of scientific research and enables the scientific community to access, validate and enlarge shared information, methods and results. Closed data systems such as Chemical Abstracts Service (CAS) [261], which is the largest chemical database with over 200 million entries have largely predominated in this field but are not accessible for free [243]. However, open data initiatives like ChemSpider [260] and PubChem [259] (over 120 and 110 million chemicals respectively) are becoming more and more popular. Other open-source tools such as MassBank [255], PubChemLite [88,262], and R-based software tools, all help to contribute and benefit collective knowledge by making chemical data available to everyone [243].

To streamline and integrate mass spectral data processes, automated annotation software which combine multiple cheminformatic approaches are available. Depending on the analytical instrument used, each brand tends to have their own licensed software for non-target approaches; Compound discoverer (Thermofisher), MetaboScape (Bruker), MassHunter Profinder (Agilent) and Progenesis Q1 (Waters). Alternatively, open software programs exist such as MS-DIAL, MZmine [263] and the R package patRoon [264], which offer powerful alternatives for compound annotations without a license required. The patRoon package, developed by Rick Helmus et al., [264,265], was applied in this thesis. These tools help refine compound annotation by automating spectral matching, retention time prediction and in silico fragmentation analysis. They also provide the assignment to identification confidence levels by providing spectral confidence and structure predictions.

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Chapter 2

Objectives

Chapter 2. Objectives

The Mediterranean region is increasingly affected by water scarcity due to the combined effects of climate change, resulting in limited rainfall, and the growing freshwater demand driven by population growth and land use changes. This situation has led to an increasing number of intermittent rivers across this region. Despite their importance in biodiversity, nutrient cycling and water quality, intermittent streams remain underrepresented in both monitoring programs and scientific research, as studies have been mainly focused on perennial (permanent) rivers. At the same time, this knowledge gap is even more pronounced in Southern Mediterranean countries, such as the MENA region where low precipitation, and thus the probability of discontinuous flow is higher.

In this context, the aim of this thesis was to assess the impact of anthropogenic pressures on intermittent streams across different Mediterranean countries. This was accomplished through the following specific objectives:

- To develop and validate an analytical methodology based on SPE and high-resolution mass spectrometry for the determination of a wide range of contaminants of emerging concern in water samples.

- To investigate the effects of anthropogenic inputs, such as WWTP effluents on the chemical quality and to explore the associated environmental risk of the presence of CECS in intermittent Mediterranean streams and connected groundwater.

- To evaluate the environmental status of streams impacted by WWTPs combining chemical, hydrological and ecological indicators across five Mediterranean countries, aiming for a comprehensive characterization and thorough understanding of their current condition under anthropogenic pressures.

- To study the fate of CECs and their TPs present in intermittent streams through lab-scale experiments and cheminformatics approaches by suspect and non-target screening.

The increasing detection of CECs and their TPs in surface water presents significant challenges to both environmental sustainability and human health. These compounds, which are often not efficiently removed by conventional wastewater treatment processes, have been linked to adverse ecological impacts and potential risks to public health.

In alignment with the United Nations Sustainable Development Goals (SDGs), the research performed in this thesis addressed several key targets aimed at improving

water quality, promoting sustainable chemical management and safeguarding the aquatic environment (Figure 2.1). Specifically, this study contributed to:

- SDG 12: Responsible Consumption and Production – by applying sustainable chemical use and management for method development and analysis.

- SDG 6: Clean Water and Sanitation – through the study and monitoring of water pollution to support mitigation strategies.

- SDG 14: Life Below Water – by evaluating the impacts of CECs on aquatic life.

- SDG 3: Good Health and Well-Being – through the assessment of environmental risks with implications for human health.

- SDG 13: Climate Action – by considering how climate-induced changes in hydrology may influence contaminants behaviour.

By exploring the occurrence, behaviour and environmental risks of CECs and their TPs in Mediterranean surface water and associated groundwater, this thesis contributes actionable insights that support the achievement of these global sustainability goals.



Figure 2.1. *Sustainable Development Goals addressed in this thesis.*



Chapter 3

Method Development for the Analysis of CECs in Surface Waters

3.1 Introduction

3.2 Methodology

3.3 Results

3.4 Discussion

3.5 References

Chapter 3. Method Development for the Analysis of CECs in Surface Waters

3.1. Introduction

Chromatographic separation and detection methods are crucial for effective detection, identification and analysis of small organic molecules. Significant advances in mass spectrometry technologies have led to a wide variety of instruments available on the market, varying in mass resolution and mass acquisition speed. For example, Thermo Scientific has recently introduced the Orbitrap Astral mass spectrometer, which combines high acquisition speed of up to 200 Hz, enabling rapid data collection, and high resolution capabilities of up to 480,000 FWHM at *m/z* 200 [1].

However, when deciding which analytical technology to apply there are several considerations that must be taken into account. First of all, even though it might seem obvious, the availability of instrumentation in the laboratory is a key factor. In our case, we are fortunate to have access to a broad range of advanced instruments, including the Q-Exactive Orbitrap mass spectrometer, which was employed throughout this thesis. This instrument offers high-resolution capabilities of up to 140,000 FWHM at 200 *m/z*. This thesis focuses on LC-HRMS, chosen based on the physicochemical properties of our targeted compounds and the thesis objectives, as it allows both the identification of both known and unknown compounds. However, it is understood that other compounds may remain beyond our reach. In addition to chromatographic and mass spectrometry techniques, sample preparation and extraction are also essential steps in analytical method development. With this in mind, Figure 3.1 provides a schematic overview of the common steps involved in method development and validation.

The method development phase is essential to optimize the extraction process, chromatographic condition and mass spectrometry parameters. This process ensures that the method is capable of analysing the targeted compounds by selecting the appropriate extraction method, to achieve a sufficient enrichment factor, adequate solvents, chromatographic condition and mass spectrometry setting to maximize sensitivity and specificity of the targeted CECs. Once the method is developed, method validation is performed.

To ensure the reliability of our analytical method, the guidelines outlined in SANTE 11312/2021 [2] were followed. These guidelines provide a structured approach for method validation and quality control, covering key analytical aspects. Despite these guidelines

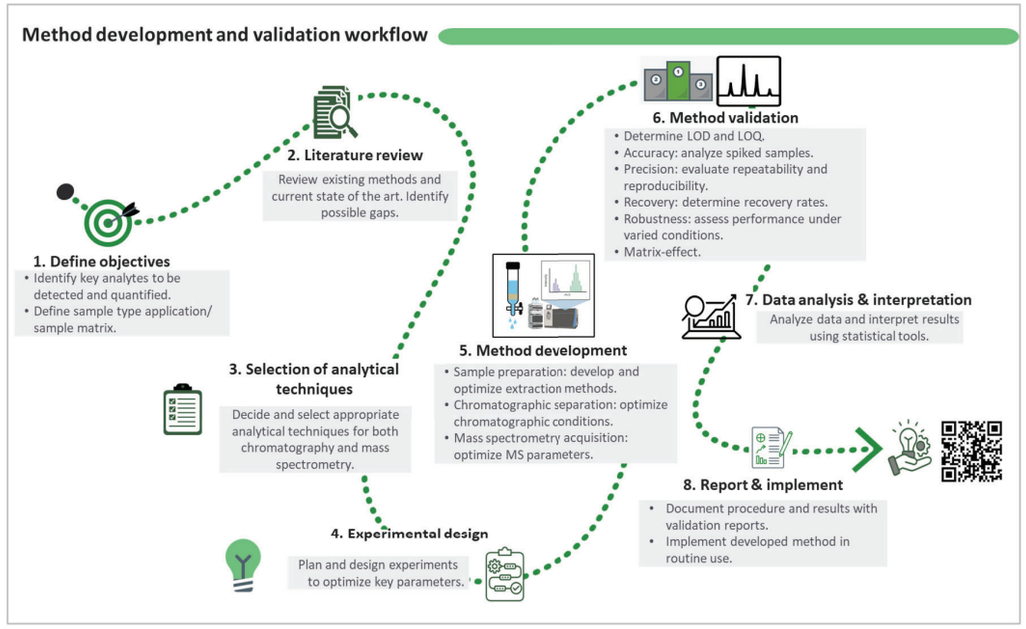


Figure 3.1. *Method development and validation workflow [2].*

were originally developed for pesticide residue analysis in food and feed, in the absence of standardized regulatory frameworks, they serve as a valuable resource, especially concerning the validation of analytical methods for CECs in surface water. Although a new version of the SANTE guidelines was released in 2023 [3], expanding on sample processing, confirmatory analysis, reporting protocols and uncertainty measurements, this thesis followed the 2021 version [2], aiming for a solid, robust and reliable analytical method. In Table 3.1 the key parameters that must be evaluated to ensure adequate method performance and reliability are described.

SPE is one of the most widely used techniques for the preconcentration of CECs in liquid samples [4,5]. This is due to the advantages it provides such as high enrichment factors as well as enhanced selectivity [6]. The latter largely depends on the type of sorbents used. Depending on the target analytes, different sorbents as well as the combination of these can be used. The choice of sorbent strictly depends on the physico-chemical characteristics of the target analytes [5,7]. Multi-layered SPE approaches, which combine different sorbents, are becoming an important alternative to single sorbent. Table 3.2 presents a comparison of several studies on the application of different sorbents, both individually (listed in separate rows) and in combination (within the same row), where good and promising recovery performance are observed.

Table 3.1. *Key parameters for method validation based on the SANTE guideline [2].*

Parameters to be evaluated	Description
Specificity	Assess the method's ability to selectively detect the target analytes in the presence of potential interferences. By means of procedure and experimental blanks i.e., quality controls and spiked sample in solvent and matrix-matched
Sensitivity	Determine the method's detection limit (LOD) and quantification limit (LOQ) for the analytes.
Linearity	Evaluate the method's linearity by constructing calibration curves over the desired concentration range.
Accuracy	Assess the method's accuracy by analysing spiked samples and comparing the measured concentrations to the known values.
Precision	Evaluate the method's repeatability (intra-day precision) and reproducibility (inter-day precision) by analysing multiple replicates of the same sample.
Recovery	Determine the method's recovery by comparing the measured concentration of spiked samples to the actual amount added
Robustness	Assess the method's robustness by evaluating its performance under slightly varied experimental conditions.
Matrix effects	Evaluate the impact of the sample matrix on the analytical method's performance and accuracy.

As it can be observed from Table 3.2, multi-layered and mixed-mode SPE cartridges show promising performances due to their enhanced selectivity and retention mechanisms [22]. Common sorbents applied in combination are Oasis HLB, Strata-X (polymeric reversed phase), Isolute ENV+ (non-polar polymeric) and ion exchange sorbents. The latter includes various types for example from MCX (for strong cation exchange) to WAX (weak anion exchange). Bond Elut PPL is also quite frequently used for hydrophilic compounds. Although Table 3.2 focuses on multi-layered applications, Oasis HLB remains one of the widely applied in both individual and combined sorbent strategies [4,5,7,23–25]. Its continued preference application as a single-layered cartridge is attributed to its simplicity, balanced extraction capabilities [26], and proven effectiveness in complex matrix [27].

Despite the sorbent selection for sample enrichment, the next steps including chromatographic separation and acquisition methods have the potential to increase the quality of the whole method performance [28]. LC-HRMS is currently the instrumentation of choice for identification of wide-scope CECs in water matrices [5] as previously explained in the introduction chapter.

The selection of an appropriate column, both in terms of chromatographic technique and separation mode is essential, especially for the analysis of a large number of compounds. Both high-performance liquid chromatography (HPLC) and ultra-high-performance liquid chromatography (UHPLC) are commonly used, however the latter offers the advantage

Table 3.2. Summary of studies where different sorbents (either in single or in combination) have been evaluated between 2010-2022 for water matrices including SW: surface water, SS: sea water, WW: wastewater, GW: groundwater, DW: drinking water, TW: tap water. Use of singles sorbents are per row, while multi sorbents are included in the same row. Abbreviations: ICM iodinated contrast media, PMOCs: persistent and mobile organic contaminants, NA: not available.

Reference	Sample type (volume)	Compounds analysed	Cartridges	Recovery performance
Nodler et al., 2010 [8]	SW, SS (500 mL)	46 pharmaceuticals	Oasis HLB	65% median for cationic
			Bond Elut Plexa	Low for ICM
			AbsElut Nexus	Low for ICM
			Bond Elut PPL	>82% for ICM
			Strata X	Very high (>120%) over-compensation effects
			Isolute ENV+	Similar to Strata X
Nurmi et al., 2011 [9]	WW (200 mL)	68 pesticides and 16 pharmaceuticals	Oasis MCX, Strata-X	2-183%
Huntscha et al., 2012 [10]	GW, SW, WW (50 mL)	88 polar micropollutants	Oasis HLB, Strata X-AW, Strata X-CW, Isolute ENV+	80 - 120%
Moschet el al., 2013 [11]	SW (1000 mL)	185 insecticides and fungicides	Oasis HLB, Strata X-AW, Strata X-CW, Isolute ENV+	91% of compounds within 75-125%
Ruff et al., 2015 [12]	SW (1000 mL)	132 CECs	Oasis HLB, Strata X-AW, Strata X-CW, Isolute ENV+	Average of 97%
Zonja et al., 2015 [13]	SW (800 mL)	6 ICM & TPs	Oasis HLB, Bond Elut PPL, Oasis MAX, Oasis MCX	71-128%
Singer et al., 2016 [14]	WW (1000 mL)	119 pharmaceuticals + 24 metabolites	Oasis HLB, Isolute ENV+, Strata XAW, Strata XCW	Median of 105%
Ibañez et al., 2017 [15]	WW (200 mL)	PhACs + metabolites	Strata X, Strata WAX, Strata WCX, IsoluteENV+	NA
Salas et al., 2017 [16]	SW, WW (500 mL)	13 PhACs	SCX, WCX, SAX, WAX	SCX/SAX: 45-88%
Osorio et al., 2018 [17]	SW (1000 mL)	117 CECs	Oasis HLB, Strata-X-AW, Strata-X-CW, Isolute ENV+	74% of the compound's acceptable performance
Köke et al., 2018 [18]	TW, WW, SW GW (100 mL)	Polar analytes	CHROMABOND, GCB, WCX, WAX	Mean recoveries (± standard deviation) 79 ± 6%
			HLB	HLB = 72 ± 3%
Montes et al., 2019 [19]	SW, DW	23 PMOCs	Oasis WCX	Oasis WCX and WAX resulted in improved extraction for more polar PMOCs.
			Oasis WAX	
			Oasis HLB	
Gago-Ferrero et al., 2020 [20]	WW (100mL)	195 analytes	Oasis HLB, Strata-X-CW, Strata-X-AW, Isolute ENV+	75% of compounds between 57-120%
Fagnani et al., 2022 [21]	SW (500mL)	Pharmaceuticals & TPs	HLB layer, Bond Elut PPL, WAX, WCX	86–132%

of sharper peaks, faster analysis times and higher resolution due to its use of smaller particle size compared to conventional HPLC [29]. Hence UHPLC columns are commonly preferred in HRMS [30,31]. Reversed phase C18 columns are widely used due to their broad applicability for non-polar to moderate polar compounds. However, for very polar substances, they present limited retentions, leading to early elution and poor separation. When polar compounds are targeted, alternative columns such as HILIC can be used as they offer improved retention for such highly polar analytes [32,33]. These, however, often require more complex optimization of mobile phases. Mixed-mode columns combine multiple retention mechanisms enabling broader retention of analytes of different polarities [34]. Thus, the choice of chromatographic column is fundamental, however, a compromise must be reached between the targeted compound retentions, method robustness and analytical performance.

Besides, the selection of the acquisition mode in LC-HRMS is another crucial step as it determines the method's detection sensitivity, selectivity and the ability to identify unknown compounds within complex mixtures. In Orbitrap HRMS systems, the main acquisition methods are DDA, DIA, and for targeted analysis Parallel Reaction Monitoring (PRM) [35,36]. The different acquisition methods vary depending on how ions are selected and measured in the MS/MS scan.

In DDA mode, the mass spectrometer first performs a full scan (MS1), detecting all ions in the sample. It then selects highly abundant precursor ions, within specific isolation windows (Da) for fragmentation. The selected precursor then undergoes fragmentation in the HCD producing MS/MS spectra with all the fragmented ions. Ions are fragmented based on their intensity making it "data-dependent". A major disadvantage of DDA is that low-intensity ions (below and established threshold) may not be fragmented, losing MS/MS data, especially in complex matrices [35–37].

PRM is a targeted HRMS technique in which specific ions (target analytes) are selected, isolated and fragmented, where the instrument monitors multiple specific fragment ions simultaneously. This means that all ions in the MS/MS scan are monitored and measured with accurate mass [38]. PRM enables higher specificity and sensitivity as monitoring multiple fragments helps distinguish the target analyte from other potential interferents in the sample and is also robust in the detection of low-abundance compounds [39,40].

In contrast, the DIA mode is a less selective acquisition mode. All ions are fragmented within the isolation window, regardless of the intensity of the ions (without the need of a threshold). This means that both high-intensity and low-intensity ions are fragmented generating MS/MS spectra, making it "data-independent". It uses sequential windows that cover a wide m/z range, fragmenting all ions within each window and acquiring all possible data on the fragmented ions [38]. A major advantage of DIA is that very low-intensity ions are fragmented, ensuring also a broader range of compounds to be detected. While DIA mode provides MS/MS spectra highly rich in information, complex data processing is required to link the fragment to the correct precursor ion. Recent advances in Orbitrap instruments have simplified this process as it is possible to relate the precursor ion (MS1) quickly and its fragments in the MS/MS spectrum, by setting sequential isolation of precursor ions over a defined mass range and adjusting specific *m/z* isolation window widths [38]. These refined isolation windows minimize the need for complex data handling, thereby, facilitating a more efficient targeted DIA (tDIA) approach.

In this context, the objective was to develop and validate a targeted HRMS method based on SPE for the analysis of over 100 CECs with a specific focus on waste-borne pollutants in surface water samples.

3.2. Methodology

The selection of the target CECs was based on their source (wastewater effluents), their environmental relevance, as some of them are considered priority substances [41] or included in the European Watch Lists [42–45], their high environmental occurrence and consumption/ usage, as well as their analytical amenability by LC-MS. In Table 3.3, the targeted compounds are listed, including relevant data such as molecular formula and weight, important compound identifiers and their predicted partition coefficient (XlogP). The selected compounds include pharmaceuticals, personal care products, some insecticides, industrial compounds and some TPs.

LC-HRMS using a Thermo Q-Exactive Orbitrap-MS was selected as the analytical technique, due to its proven high performance in environmental analysis [38] and availability in our facilities. Details on the method development in terms of chromatography and acquisition modes will be further discussed and evaluated in section 3.4. The method validation was performed at three relevant environmental concentrations (5, 50, 500 ng/L). Method validation details as well as the following steps including data analysis and report of method application, are presented and detailed in section 3.3 as part of Publication #1.

Briefly, the method was developed and validated using a pool of real surface water samples, collected from six streams in the Osona region of Catalonia, Spain. The extraction and preconcentration of the analytes were performed using a multi-layered and multi-sorbent cartridge (Figure 3.2), previously developed and optimized by our group [21].

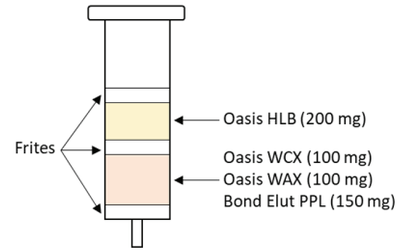


Figure 3.2. *Multi-sorbent solid phase extraction cartridge. Adapted from [21].*

For detection, a full scan (MS1) followed by a tDIA mode (MS/MS) were applied and for qualitative and quantitative data processing, Thermo Scientific Xcalibur and TraceFinder software were used, respectively. An in-house spectral library of MS/MS spectra for all the target analytes was generated from reference standards, including the unique fragmentation patterns of the compounds. These library spectra were then compared to those obtained from real samples. By matching the unknown spectra to the reference spectra in the library, the compounds were identified and quantified. For quantification, matrix-matched calibration curves were used.

3.3. Results

This chapter includes one article on the development and validation of the analytical method which will be subsequently applied to the samples analysed in the later chapters included in this thesis.

- Publication #1: **O. Gómez-Navarro,** F. Labad, D.P. Manjarrés-López, S. Pérez, N. Montemurro. (2023). HRMS-Targeted-DIA methodology for quantification of wastewater-borne pollutants in surface water, MethodsX, 10, 102093.https://doi.org/10.1016/J.MEX.2023.102093

Table 3.3. *Target compounds list (with TPs or metabolites marked in bold) including compound name, molecular formula and weight, CAS number, PubChem CID, InChIKey and XlogP. Abbreviations: Chemical abstracts service (CAS), Compound identifier (CID), International chemical identifier key (InChIKey) and Predicted partition coefficient (XlogP). XlogP extracted from [46]. Literature counts were extracted from [47,48].*

Compound name	Molecular formula	Molecular Weight	CAS number	PubChem CID	InChIKey	XLogP	Literature counts
1,7 Dimethylxanthine	C7H8N4O2	180.16	611-59-6	4687	QUNWUDVFRNGTCO-UHFFFAOYSA-N	-0.2	401
17-α Ethynilestradiol	C20H24O2	296.4	57-63-6	5994	BFPYWIDHMRZLRN-SLHNCBLASA-N	3.7	10486
1H-Benzotriazole	C6H5N3	119.12	95-14-7	7220	QRUDEWIWKLJBPS-UHFFFAOYSA-N	1.0	800
2.4-dihydroxybenzophenone (BP1)	C13H10O3	214.22	131-56-6	8572	ZXDDPOHVAMWLBH-UHFFFAOYSA-N	3.2	85
2.2'.4.4'-tetrahydroxybenzophenone (BP2)	C13H10O5	246.22	131-55-5	8571	WXNRYSGJLQFHBR-UHFFFAOYSA-N	2.4	64
4-Hydroxidiclofenac	**C14H11Cl2NO3**	**312.1**	**64118-84-9**	**116545**	**KGVXVPRLBMWZLG-UHFFFAOYSA-N**	**3.7**	**75**
4-Nitro Sulfamethoxazole	**C10H9N3O5S**	**283.26**	**29699-89-6**	**717516**	**XHJLDAHBWWMCRZ-UHFFFAOYSA-N**	**1.4**	**0***
5-Desamino-5 oxo 2,5 Lamotrigine	**C9H6Cl2N4O**	**257.08**	**252186-78-0**	**13556436**	**OVNGWOHWYFBGKF-UHFFFAOYSA-N**	**1.7**	**0***
5-methyl-1H-benzotriazole	C7H7N3	133.15	136-85-6	8705	LRUDIIUSNGCQKF-UHFFFAOYSA-N	1.4	27
5-nitro-Diclofenac	**C14H10Cl2N2O4**	**341.15**	**174316-61-1**	**9797358**	**MRJVSFHDJGEONX-UHFFFAOYSA-N**	**4.0**	**0***
6a-Methylprednisolone	C22H30O5	374.47	83-43-2	6741	VHRSUDSXCMQTMA-PJHHCJLFSA-N	1.9	24652
Acesulfame	C4H5NO4S	163.15	33665-90-6	36573	YGCFIWIQZPHFLU-UHFFFAOYSA-N	-0.3	357
Acetaminophen	C8H9NO2	151.16	103-90-2	1983	RZVAJINKPMORJF-UHFFFAOYSA-N	0.5	27656
Acridone	**C13H9NO**	**195.22**	**578-95-0**	**2015**	**FZEYVTFCMJSGMP-UHFFFAOYSA-N**	**3.0**	**263**
Adamantan-1-amine (Amantadine)	C10H17N	151.14	768-94-5	2130	DKNWSYNQZKUICI-UHFFFAOYSA-N	2.4	4358

Compound name	Molecular formula	Molecular Weight	CAS number	PubChem CID	InChIKey	XLogP	Literature counts
Alprazolam	C17H13ClN4	308.77	28981-97-7	2118	VREFGVBLTWBCJP-UHFFFAOYSA-N	2.1	2554
Atenolol	C14H22N2O3	266.34	29122-68-7	2249	METKIMKYRPQLGS-UHFFFAOYSA-N	0.2	7228
Azithromycin	C38H72N2O12	748.98	83905-01-5	447043	MQTOSJVFKKJCRP-BICOPXKESA-N	4.0	9497
Benzoylecgonine	**C16H19NO4**	**289.33**	**519-09-5**	**448223**	**GVGYEFKIHJTNQZ-RFQIPJPRSA-N**	**-0.3**	**1083**
B-estradiol	C18H24O2	272.38	50-28-2	5757	VOXZDWNPVJITMN-ZBRFXRBCSA-N	4.0	101020
Bezafibrate	C19H20ClNO4	361.82	41859-67-0	39042	IIBYAHWJQTYFKB-UHFFFAOYSA-N	3.8	1849
Bisphenol-A	C15H16O2	228.29	80-05-7	6623	IISBACLAFKSPIT-UHFFFAOYSA-N	3.3	9372
Bromazepam	C14H10BrN3O	316.15	1812-30-2	2441	VMIYHDSEFNYJSL-UHFFFAOYSA-N	1.7	503
Caffeine	C8H10N4O2	194.19	58-08-2	2519	RYYVLZVUVIJVGH-UHFFFAOYSA-N	-0.1	31617
Carazolol	C18H22N2O2	298.38	57775-29-8	71739	BQXQGZPYHWWCEB-UHFFFAOYSA-N	3.6	157
Carbamazepine	C15H12N2O	236.27	298-46-4	2554	FFGPTBGBLSHEPO-UHFFFAOYSA-N	2.5	15703
Carbamazepine-10,11-epoxide	**C15H12N2O2**	**252.27**	**36507-30-9**	**2555**	**ZRWWEEVEIOGMMT-UHFFFAOYSA-N**	**1.3**	**337**
Carisoprodol	C12H24N2O4	260.33	78-44-4	2576	OFZCIYFFPZCNJE-UHFFFAOYSA-N	1.9	503
Chloramphenicol	C11H12Cl2N2O5	323.13	56-75-7	5959	WIIZWVCIJKGZOK-RKDXNWHRSA-N	1.1	26247
Citalopram	C20H21FN2O	324.16	59729-33-8	2771	WSEQXVZVJXJVFP-UHFFFAOYSA-N	3.2	7544

Compound name	Molecular formula	Molecular Weight	CAS number	PubChem CID	InChIKey	XLogP	Literature counts
Clarithromycin	C38H69NO13	747.95	81103-11-9	84029	AGOYDEPGAOXOCK-KCBOHYOISA-N	3.2	9655
Climbazole	C15H17ClN2O2	292.76	38083-17-9	37907	OWEGWHBOCFMBLP-UHFFFAOYSA-N	3.7	65
Clofibric Acid	C10H11ClO3	214.64	882-09-7	2797	TXCGAZHTZHNUAI-UHFFFAOYSA-N	2.6	1639
Cocaethylene	**C18H23NO4**	**317.38**	**529-38-4**	**644006**	**NMPOSNRHZIWLLL-XUWVNRHRSA-N**	**2.7**	**391**
Cocaine	C17H21NO4	303.35	50-36-2	446220	ZPUCINDJVBIVPJ-LJISPDSOSA-N	2.3	37336
Codeine	C18H21NO3	299.36	76-57-3	5284371	OROGSEYTTFOCAN-DNJOTXNNSA-N	1.1	6047
Cotinine	C10H12N2O	176.22	486-56-6	854019	UIKROCXWUNQSPJ-VIFPVBQESA-N	-0.3	5978
Crotamiton	C13H17NO	203.28	483-63-6	688020	DNTGGZPQPQTDQF-UHFFFAOYSA-N	2.8	170
Cyclamate	C6H13NO3S	179.24	100-88-9	7533	HCAJEUSONLESMK-UHFFFAOYSA-N	0.6	692
Diazepam	C16H13ClN2O	284.74	439-14-5	3016	AAOVKJBEBIDNHE-UHFFFAOYSA-N	3.0	22145
Diclofenac	C14H11Cl2NO2	296.15	15307-86-5	3033	DCOPUUMXTXDBNB-UHFFFAOYSA-N	4.4	12297
Diltiazem	C22H26N2O4S	414.52	42399-41-7	39186	HSUGRBWQSSZJOP-RTWAWAEBSA-N	3.1	7642
Estriol	C18H24O3	288.38	50-27-1	5756	PROQIPRRNZUXQM-ZXXIGWHRSA-N	2.5	7027
Estrone	C18H22O2	270.37	53-16-7	5870	DNXHEGUUPJUMQT-CBZIJGRNSA-N	3.1	12185
Ezetimibe	C24H21F2NO3	409.43	163222-33-1	150311	OLNTVTPDXPETLC-XPWALMASSA-N	4.0	3338

Compound name	Molecular formula	Molecular Weight	CAS number	PubChem CID	InChIKey	XLogP	Literature counts
Fipronil	C12H4Cl2F6N4OS	437.15	120068-37-3	3352	ZOCSXAVNDGMNBV-UHFFFAOYSA-N	4.5	1397
Fipronil desulfinyl	**C12H4Cl2F6N4**	**389.08**	**205650-65-3**	**22673275**	**JWKXVHLIRTVXLD-UHFFFAOYSA-N**	**4.6**	**0***
Fipronil sulfide	**C12H4Cl2F6N4S**	**421.15**	**120067-83-6**	**9953940**	**FQXWEKADCSXYOC-UHFFFAOYSA-N**	**5.5**	**9**
Fipronil sulfone	**C12H4Cl2F6N4O2S**	**453.15**	**120068-36-2**	**3078139**	**LGHZJDKSVUTELU-UHFFFAOYSA-N**	**4.7**	**39**
Florfenicol	C12H14Cl2FNO4S	358.21	73231-34-2	114811	AYIRNRDRBQJXIF-NXEZZACHSA-N	0.8	975
Fluconazole	C13H12F2N6O	306.27	86386-73-4	3365	RFHAOTPXVQNOHP-UHFFFAOYSA-N	0.4	12727
Flumequine	C14H12FNO3	261.25	42835-25-6	3374	DPSPPJIUMHPXMA-UHFFFAOYSA-N	2.9	386
Fluticasone propionate	C25H31F3O5S	500.6	80474-14-2	444036	WMWTYOKRWGGJOA-CENSZEJFSA-N	4.0	3828
Furazolidone	C8H7N3O5	225.16	67-45-8	5323714	PLHJDBGFXBMTGZ-WEVVVXLNSA-N	-0.1	1397
Furosemide	C12H11ClN2O5S	330.74	54-31-9	3440	ZZUFCTLCJUWOSV-UHFFFAOYSA-N	2.0	14904
Gemfibrozil	C15H22O3	250.33	25812-30-0	3463	HEMJJKBWTPKOJG-UHFFFAOYSA-N	3.8	2171
Hydrochlorothiazide	C7H8ClN3O4S2	297.74	58-93-5	3639	JZUFKLXOESDKRF-UHFFFAOYSA-N	-0.1	7207
Hydroxychloroquine	C18H26ClN3O	335.87	118-42-3	3652	XXSMGPRMXLTPCZ-UHFFFAOYSA-N	3.6	7173
Hyoscine (scopolamine)	C17H21NO4	303.35	51-34-3	3000322	STECJAGHUSJQJN-USLFZFAMSA-N	0.9	9793
Ibuprofen	C13H18O2	206.28	15687-27-1	3672	HEFNNWSXXWATRW-UHFFFAOYSA-N	3.5	14112

Compound name	Molecular formula	Molecular Weight	CAS number	PubChem CID	InChIKey	XLogP	Literature counts
Indomethacin	C19H16ClNO4	357.79	53-86-1	3715	CGIGDMFJXJATDK-UHFFFAOYSA-N	4.3	30414
Irbesartan	C25H28N6O	428.53	138402-11-6	3749	YOSHYTLCDANDAN-UHFFFAOYSA-N	4.1	2074
Irgasan (Triclosan)	C12H7Cl3O2	289.54	3380-34-5	5564	XEFQLINVKFYRCS-UHFFFAOYSA-N	5.0	4260
Ketoprofen	C16H14O3	254.28	22071-15-4	3825	DKYWVDODHFEZIM-UHFFFAOYSA-N	3.1	4008
Lamotrigine	C9H7Cl2N5	256.09	84057-84-1	3878	PYZRQGJRPPTADH-UHFFFAOYSA-N	1.4	5285
Lamotrigine-N2-oxide	**C9H7Cl2N5O**	**272.09**	**136565-76-9**	**15089895**	**YGGNPWUXPLVPLK-UHFFFAOYSA-N**	**1.4**	**0***
Lidocaine	C14H22N2O	234.34	137-58-6	3676	NNJVILVZKWQKPM-UHFFFAOYSA-N	2.3	27885
Lincomycin	C18H34N2O6S	406.54	154-21-2	3000540	OJMMVQQUTAEWLP-KIDUDLJLSA-N	0.2	2326
Lorazepam	C15H10Cl2N2O2	321.2	846-49-1	3958	DIWRORZWFLOCLC-UHFFFAOYSA-N	2.4	4262
Losartan	C22H23ClN6O	422.9	114798-26-4	3961	PSIFNNKUMBGKDQ-UHFFFAOYSA-N	4.3	9482
Mefenamic Acid	C15H15NO2	241.29	61-68-7	4044	HYYBABOKPJLUIN-UHFFFAOYSA-N	5.1	1515
Meprobamate	C9H18N2O4	218.25	57-53-4	4064	NPPQSCRMBWNHMW-UHFFFAOYSA-N	0.7	2970
Methadone	C21H27NO	309.45	76-99-3	4095	USSIQXCVUWKGNF-UHFFFAOYSA-N	3.9	14142
Metoprolol	C15H25NO3	267.36	37350-58-6	4171	IUBSYMUCCVWXPE-UHFFFAOYSA-N	1.9	7389
Metronidazole	C6H9N3O3	171.15	443-48-1	4173	VAOCPAMSLUNLGC-UHFFFAOYSA-N	0.0	14762

Compound name	Molecular formula	Molecular Weight	CAS number	PubChem CID	InChIKey	XLogP	Literature counts
Midazolam	C18H13ClFN3	325.77	59467-70-8	4192	DDLIGBOFAVUZHB-UHFFFAOYSA-N	2.5	13881
Morphine	C17H19NO3	285.34	57-27-2	5288826	BQJCRHHNABKAKU-KBQPJGBKSA-N	0.8	48790
N-acetyl sulfamethoxazole	**C12H13N3O4S**	**295.32**	**21312-10-7**	**65280**	**GXPIUNZCALHVBA-UHFFFAOYSA-N**	**0.7**	**39**
Nalidixic acid	C12H12N2O3	232.24	389-08-2	4421	MHWLWQUZZRMNGJ-UHFFFAOYSA-N	1.4	4961
Naproxen	C14H14O3	230.26	22204-53-1	156391	CMWTZPSULFXXJA-VIFPVBQESA-N	3.3	6394
Neotame	C20H30N2O5	378.46	165450-17-9	9810996	HLIAVLHNDJUHFG-HOTGVXAUSA-N	-0.1	74
N-Methyl lamotrigine	**C10H9Cl2N5**	**270.12**	**1373243-86-7**	**90252513**	**WVJYMSFBMQWMAL-UHFFFAOYSA-N**	**2.1**	**0***
O-desmethylvenlafaxine	**C16H25NO2**	**263.19**	**93413-62-8**	**125017**	**KYYIDSXMWOZKMP-UHFFFAOYSA-N**	**2.6**	**444**
Oseltamivir	C16H28N2O4	312.4	196618-13-0	65028	VSZGPKBBMSAYNT-RRFJBIMHSA-N	1.1	4643
Oxazepam	C15H11ClN2O2	286.71	604-75-1	4616	ADIMAYPTOBDMTL-UHFFFAOYSA-N	2.2	1397
Paroxetine	C19H20FNO3	329.37	61869-08-7	43815	AHOUBRCZNHFOSL-YOEHRIQHSA-N	3.5	6199
Pentobarbital	C11H18N2O3	226.28	76-74-4	4737	WEXRUCMBJFQVBZ-UHFFFAOYSA-N	2.1	10755
Phenytoin	C15H12N2O2	252.27	57-41-0	1775	CXOFVDLJLONNDW-UHFFFAOYSA-N	2.5	16860
Primidone	C12H14N2O2	218.25	125-33-7	4909	DQMZLTXERSFNPB-UHFFFAOYSA-N	0.9	1627
Propranolol	C16H21NO2	259.34	525-66-6	4946	AQHHHDLHHXJYJD-UHFFFAOYSA-N	3.0	36639

Compound name	Molecular formula	Molecular Weight	CAS number	PubChem CID	InChIKey	XLogP	Literature counts
Propyphenazone	C14H18N2O	230.31	479-92-5	3778	PXWLVJLKJGVOKE-UHFFFAOYSA-N	1.7	183
Rosuvastatin	C22H28FN3O6S	481.54	287714-41-4	446157	BPRHUIZQVSMCRT-VEUZHWNKSA-N	1.6	3991
Saccharin	C7H5NO3S	183.18	81-07-2	5143	CVHZOJJKTDOEJC-UHFFFAOYSA-N	0.9	9895
Salicylic acid	C7H6O3	138.12	69-72-7	338	YGSDEFSMJLZEOE-UHFFFAOYSA-N	2.3	25762
Sertraline	C17H17Cl2N	306.23	79617-96-2	68617	VGKDLMBJGBXTGI-SJCJKPOMSA-N	4.8	5230
Sitagliptin	C16H15F6N5O	407.31	486460-32-6	4369359	MFFMDFFZMYYVKS-SECBINFHSA-N	0.7	2367
Sotalol	C12H20N2O3S	272.36	3930-20-9	5253	ZBMZVLHSJCTVON-UHFFFAOYSA-N	0.2	2793
Stevioside	C38H60O18	804.38	57817-89-7	442089	UEDUENGHJMELGK-HYDKPPNVSA-N	-1.2	578
Sucralose	C12H19Cl3O8	397.63	56038-13-2	71485	BAQAVOSOZGMPRM-QBMZZYIRSA-N	-1.5	601
Sulfadiazine	C10H10N4O2S	250.28	68-35-9	5215	SEEPANYCNGTZFQ-UHFFFAOYSA-N	-0.1	4938
Sulfadimethoxine	C12H14N4O4S	310.33	122-11-2	5323	ZZORFUFYDOWNEF-UHFFFAOYSA-N	1.6	973
Sulfamerazine	C11H12N4O2S	264.3	127-79-7	5325	QPPBRPIAZZHUNT-UHFFFAOYSA-N	0.1	639
Sulfamethazine	C12H14N4O2S	278.33	57-68-1	5327	ASWVTGNCAZCNNR-UHFFFAOYSA-N	0.3	1980
Sulfamethizole	C9H10N4O2S2	270.33	144-82-1	5328	VACCAVUAMIDAGB-UHFFFAOYSA-N	0.5	442
Sulfamethoxazole	C10H11N3O3S	253.28	723-46-6	5329	JLKIGFTWXXRPMT-UHFFFAOYSA-N	0.9	7616

Compound name	Molecular formula	Molecular Weight	CAS number	PubChem CID	InChIKey	XLogP	Literature counts
Sulfapyridine	C11H11N3O2S	249.29	144-83-2	5336	GECHUMIMRBOMGK-UHFFFAOYSA-N	0.0	678
Sulfathiazole	C9H9N3O2S2	255.32	72-14-0	5340	JNMRHUJNCSQMMB-UHFFFAOYSA-N	0.1	849
Sulisobenzone (BP4)	C14H12O6S	308.31	4065-45-6	19988	CXVGEDCSTKKODG-UHFFFAOYSA-N	2.2	70
Tramadol	C16H25NO2	263.38	27203-92-5	33741	TVYLLZQTGLZFBW-ZBFHGGJFSA-N	2.6	5139
Trimethoprim	C14H18N4O3	290.32	738-70-5	5578	IEDVJHCEMCRBQM-UHFFFAOYSA-N	0.9	8355
Valsartan	C24H29N5O3	435.52	137862-53-4	60846	ACWBQPMHZXGDFX-QFIPXVFZSA-N	4.4	4941
Valsartan acid	**C14H10N4O2**	**266.25**	**164265-78-5**	**19388302**	**USAWIVMZUYOXCF-UHFFFAOYSA-N**	**2.3**	**4**
Venlafaxine	C17H27NO2	277.4	93413-69-5	5656	PNVNVHUZROJLTJ-UHFFFAOYSA-N	2.9	4390
Verapamil	C27H38N2O4	454.6	52-53-9	2520	SGTNSNPWRIOYBX-UHFFFAOYSA-N	3.8	21095
Warfarin	C19H16O4	308.3	81-81-2	54678486	PJVWKTKQMONHTI-UHFFFAOYSA-N	2.7	27313
Zonisamide	C8H8N2O3S	212.23	68291-97-4	5734	UBQNRHZMVUUOMG-UHFFFAOYSA-N	0.2	1347

Publication #1

HRMS-Targeted-DIA methodology for quantification of wastewater-borne pollutants in surface water

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Method Article

HRMS-Targeted-DIA methodology for quantification of wastewater-borne pollutants in surface water

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ABSTRACT

A robust method was developed for the quantification of popular and highly occurrence contaminants of emerging concern from wastewater treatment plant effluents and is explained in detail. A homemade multi-layered and multi-sorbent solid phase extraction (SPE) cartridge was used to cover the wide range of polarities of the selected contaminants. A non-discriminant elution protocol was also applied. Liquid chromatography coupled to a high-resolution mass spectrometer (HRMS) Q-Exactive Orbitrap system was used for the separation and detection of the contaminants. A targeted data independent acquisition (DIA) mode with an inclusion list with the exact mass, retention time window and collision energy was tried for the first time obtaining good sensitivity, selectivity and high quality MS2 product ions.

- 116 compounds of a wide-scope of polarities and physic-chemical properties were validated using a surface water pool matrix.
- SPE followed by LC–HRMS with a targeted DIA was used for the method validation at three concentration levels 5, 50, 500 $\mu g\ L^{-1}$ in extract.
- Good recoveries were obtained between 70 and 120% for the majority of the selected contaminants. Matrix effect, precision, and linearity were also evaluated and results proved the suitability for the method application.

Specifications table

Subject area:	Environmental Science
More specific subject area:	*Environmental Analytical Chemistry – Organic Contaminants*
Name of your method:	*CECs targeted method for surface water*
Name and reference of original method:	*This analytical method is based on the method described by Enelton Fagnani, Nicola Montemurro and Sandra Pérez (2022). Multilayered solid phase extraction and ultra performance liquid chromatographic method for suspect screening of halogenated pharmaceuticals and photo-transformation products in freshwater - comparison between data-dependent and data-independent acquisition mass spectrometry*, Journal of Chromatography A https://doi.org/10.1016/j.chroma.2021.462760
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Method details

Background

Water is a basic need that is necessary in our day to day life and is impossible to do without, thus its constant use. After its use, different treatments are applied to ensure its return to the ecosystem of origin (back to rivers, sea or ocean) or for its future reuse. Wastewater is usually managed through wastewater treatment plants, (WWTPs), especially in developed countries, and it may contain several contaminants such as nutrients, metals, parasites and contaminants of emerging concern (CECs) [1]. However, it has been demonstrated that WWTPs are not sufficiently efficient in their removal and many wastewater-borne pollutants are continuously being released back into the environment. Through WWTPs, wastewater-borne pollutants are introduced into surface waters, where sometimes the wastewater effluent can even dominate those streams and rivers where low flow conditions are present. Wastewater-borne pollutants enclose various types of contaminants and are of growing concern. New pollutants are continuously detected and many compounds with different physicochemical properties and different polarities are found to be detected after the WWTP discharge [2,3]. However, discharge guidelines and standards are not always available, especially concerning most pharmaceuticals or personal care products even though their use is continuously increasing. Therefore, a targeted method was developed to be able to track and control the continuous release of these compounds by WWTPs as they should be monitored due to their potential undesirable effect on the environment.

In this work, an extraction method based on a multi-layered and multi-sorbent solid phase extraction was combined with a non-discriminant elution protocol to cover a wide range of different contaminants of emerging concern that end up in rivers mainly derived from WWTPs effluent. To study the method's effectiveness, the method was validated for a total of 116 compounds in surface water samples.

Chemicals and reagents

The analytes included for this targeted analysis were selected concerning their environmental occurrence as well as their presence in the list of priority substances in surface water from the EU Water Framework Directive [4] and previous group experience [5–7]. The list of selected compounds for a total of 116 compounds with a wide range of polarities (Log P ranging from -2.16 to 5.62) and different physicochemical properties are given in Table 1 including the name of the target compound, CAS number, molecular formula, monoisotopic mass, Log P and classification of compounds. Several classes of CECs are included in this list, mainly pharmaceuticals, UV filters, stimulants, sweeteners and some critical metabolites and transformation products (TPs). Extended details of the compounds are also included in Table SM 1. All analytical reference standards were of high purity and purchased from Sigma Aldrich (St. Louis, MO U.S). Isotopically labelled compounds used as surrogates for calibration purposes were purchased from Cerilliant (Sigma Aldrich, St. Lous, MO, U.S), Santa Cruz Biotechnology (Dallas, TX, US.), or Toronto Research Chemicals (Toronto, ON, Canada) and are listed in Table 5 in the supplementary material. For standard, samples, and calibration curve preparation, pure water, methanol (MeOH), acetonitrile (ACN) and ethyl acetate (EtOAc) of high-performance liquid chromatography (HPLC) grade were obtained from J.T. Backer (Deventer, The Netherlands). Formic acid (≥98.0%, ACS grade), ammonium acetate ($AcNH_4$), and concentrated ammonia solution (NH_4OH) (29.0%, ACS grade) were supplied by Sigma-Aldrich. Ammonium fluoride (NH_4F) was purchased from Fisher Chemical (Fisher Scientific SL, Madrid, Spain). The solid phase extraction (SPE) sorbents for the homemade SPE cartridges preparation were Oasis HLB, WAX, and WCX (60 µm particle size) purchased from Waters Corp., (Milford, MA, USA), while Bond Elut PPL (63–150 µm particle size) was purchased from Agilent Technologies Inc. (Santa Clara, CA, USA). Empty 6 mL polypropylene cartridges were purchased from Phenomenex Inc. (Torrance, CA, USA).

Sampling and sample pre-treatment

Surface water samples were collected from six different municipalities from Osona, a region in Catalonia, Spain. These municipalities were chosen as they have their own WWTP and a direct discharge into their respective municipality stream. For method development and validation, samples were taken from upstream points, approximately 100 m before the WWTP discharge point to avoid possible direct contamination from the discharge points and were collected in 1-L amber PET bottles, which were previously rinsed with sample water on site. The bottles were placed in a refrigerator at 4 °C under dark conditions and transported to the laboratory. Once in the laboratory, samples were immediately filtered with 0.7-µm glass microfiber filter GF/F from Whatman (UK). An aliquot was stored in 0.5 L flasks to be extracted directly or stored in the dark at -20 °C. The remaining portion was blended with all upstream points to generate a representative pool of contaminant-free surface water from the six different streams to be used for method validation and the corresponding matrix-matched curve. In Table 2 some basic water parameters are shown (pH and electrical conductivity (EC)) for each of the studied municipalities and the sample pool made.

Extraction procedure

Aliquots of 500 mL of surface water pool were extracted using a solid phase extraction. A homemade multi-sorbent SPE cartridge, previously developed by [8], was used for the extraction. This cartridge consisted of two layers, an upper layer containing 200 mg of Oasis HLB sorbent and a bottom layer containing a mixture of 150 mg of Bond Elut PPL, 100 mg of WAX and 100 mg WCX. Activation and conditioning of the cartridges were performed by passing 2 x 5 mL of MeOH followed by 2 x 5 mL of HPLC water.

Table 1
Target compounds name, CAS number, molecular formula, monoisotopic mass, Log P and compound classification.

Compound name	CAS number	Molecular Formula	Monoisotopic mass	Log P*	Classification
1,7 Dimethylxanthine	611–59–6	C7H8N4O2	180.0647	0.24	Stimulant
17-α Ethynilestradiol	57–63–6	C20H24O2	296.1776	3.9	Hormone
1H-Benzotriazole	95–14–7	C6H5N3	119.0483	1.3	Anticorrosive agent
2.4-Dihydroxybenzophenone (BP1)	131–56–6	C13H10O3	214.0630	3.48	UV filter
2.2′.4.4′-Tetrahydroxybenzophenone (BP2)	131–55–5	C13H10O5	246.0528	2.8	UV filter
4-Hydroxidiclofenac	64,118–84–9	C14H11Cl2NO3	311.0116	3.96	Anti-inflammatory TP / Metabolite
4-Nitro Sulfamethoxazole	29,699–89–6	C10H9N3O5S	283.0263	1.56	Antibiotic TP
5-Desamino-5 oxo 2,5 Lamotrigine	252,186–78–0	C9H6Cl2N4O	255.9919	2.24	Antiepileptic TP
5-methyl-1 H Benzotriazole	136–85–6	C7H7N3	133.0640	1.81	Anticorrosive agent
5-nitro-Diclofenac	66,505–80–4	C14H10Cl2N2O4	340.0018	4.26	Anti-inflammatory TP
6a-Methylprednisolone	83–43–2	C22H30O5	374.2093	1.56	Anti-inflammatory
Acesulfame	33,665–90–6	C4H5NO4S	162.9939	-0.55	Sweetener
Acetaminophen	103–90–2	C8H9NO2	151.0633	0.91	Analgesic
Acridone	578–95–0	C13H9NO	195.0684	4.2	Anticonvulsant TP
Adamantan-1-amine (Amantadine)	768–94–5	C10H17N	151.1361	1.47	Antiviral
Alprazolam	28,981–97–7	C17H13ClN4	308.0829	3.02	Anxiolytic
Atenolol	29,122–68–7	C14H22N2O3	266.1631	0.43	β-blocker agent
Azithromycin	83,905–01–5	C38H72N2O12	748.5085	2.44	Antibiotic
Benzoylecgonine	519–09–5	C16H19NO4	289.1314	-0.59	Metabolite
B-estradiol	50–28–2	C18H24O2	272.1776	4.01	Hormone
Bezafibrate	41,859–67–0	C19H20ClNO4	361.1081	3.99	Lipid regulator
Bisphenol-A	80–05–7	C15H16O2	228.1150	4.04	Plasticizer
Bromazepam	1812–30–2	C14H10BrN3O	315.0007	2.54	Anxiolytic
Caffeine	58–08–2	C8H10N4O2	194.0804	-0.55	Stimulant
Carazolol	57,775–29–8	C18H22N2O2	298.1681	2.71	β-blocker agent
Carbamazepine	298–46–4	C15H12N2O	236.095	2.77	Anticonvulsant
Carbamazepine-10,11-epoxide	36,507–30–9	C15H12N2O2	252.0899	1.97	Anticonvulsant TP/ Metabolite
Carisoprodol	78–44–4	C12H24N2O4	260.1736	1.92	Muscle relaxant
Chloramphenicol	56–75–7	C11H12Cl2N2O5	322.0123	0.88	Antibiotic
Citalopram	59,729–33–8	C20H21FN2O	324.1638	3.76	Antidepressant
Clarithromycin	81,103–11–9	C38H69NO13	747.4769	3.24	Antibiotic
Climbazole	38,083–17–9	C15H17ClN2O2	292.0979	4.34	Antibiotic
Clofibric Acid	882–09–7	C10H11ClO3	214.0397	2.9	Lipid regulators
Cocaethylene	529–38–4	C18H23NO4	317.1627	2.64	Metabolite
Cocaine	50–36–2	C17H21NO4	303.1471	2.28	Stimulant
Codeine	76–57–3	C18H21NO3	299.1521	1.34	Analgesic
Cotinine	486–56–6	C10H12N2O	176.095	0.21	Stimulant
Crotamiton	483–63–6	C13H17NO	203.131	3.09	anti-itching drug
Cyclamate	100–88–9	C6H13NO3S	179.0616	-1.61	Sweetener
Diazepam	439–14–5	C16H13ClN2O	284.0717	3.08	Anxiolytic
Diclofenac	15,307–86–5	C14H11Cl2NO2	295.0167	4.26	Anti-inflammatory
Diltiazem	42,399–41–7	C22H26N2O4S	414.1613	2.73	Antihypertensive
Estriol	50–27–1	C18H24O3	288.1725	2.67	Hormone
Estrone	53–16–7	C18H22O2	270.162	4.31	Hormone
Ezetimibe	163,222–33–1	C24H21F2NO3	409.149	4.56	Lipid regulator
Fipronil	120,068–37–3	C12H4Cl2F6N4OS	435.9387	4.49	Insecticide
Fipronil desulfinyl	205,650–65–3	C12H4Cl2F6N4	387.9717	4.53	Insecticide TP
Fipronil sulfide	120,067–83–6	C12H4Cl2F6N4S	419.9438	5.62	Insecticide TP
Fipronil sulfone	120,068–36–2	C12H4Cl2F6N4O2S	451.9336	4.6	Insecticide TP
Florfenicol	73,231–34–2	C12H14Cl2FNO4S	357.0005	0.67	Antibiotic
Fluconazole	86,386–73–4	C13H12F2N6O	306.1041	0.56	Antifugal
Flumequine	42,835–25–6	C14H12FNO3	261.0801	2.42	Antibiotic
Fluticasone propionate	80,474–14–2	C25H31F3O5S	500.1844	4.86	Anti-inflammatory
Furazolidone	67–45–8	C8H7N3O5	225.0386	0.87	Antibiotic
Furosemide	54–31–9	C12H11ClN2O5S	330.0077	1.75	Diuretic
Gemfibrozil	25,812–30–0	C15H22O3	250.1569	4.39	Lipid regulator
Hydrochlorothiazide	58–93–5	C7H8ClN3O4S2	296.9645	-0.58	Diuretic
Hydroxychloroquine	118–42–3	C18H26ClN3O	335.1764	1.26	Antimalarial
Hyoscine (scopolamine)	51–34–3	C17H21NO4	360.2175	-1.94	Anticholinergic
Ibuprofen	15,687–27–1	C13H18O2	206.1307	3.84	Anti-inflammatory
Indomethacin	53–86–1	C19H16ClNO4	357.0768	3.53	Anti-inflammatory

(*continued on next page*)

Table 1 (*continued*)

Compound name	CAS number	Molecular Formula	Monoisotopic mass	Log P*	Classification
Irbesartan	138,402–11–6	C25H28N6O	428.2325	4.47	Antihypertensive
Irgasan (Triclosan)	3380–34–5	C12H7Cl3O2	287.9512	4.98	Antimicrobial
Ketoprofen	22,071–15–4	C16H14O3	254.0943	3.61	Anti-inflammatory
Lamotrigine	84,057–84–1	C9H7Cl2N5	255.0079	1.93	Antiepileptic
Lamotrigine-N2-oxide	136,565–76–9	C9H7Cl2N5O	271.0028	1.76	Antiepileptic TP
Lidocaine	137–58–6	C14H22N2O	234.1732	2.84	Anesthetic
Lincomycin	154–21–2	C18H34N2O6S	406.2138	-0.32	Antibiotic
Lorazepam	846–49–1	C15H10Cl2N2O2	320.0119	4.06	Anxiolytic
Losartan	114,798–26–4	C22H23ClN6O	422.1622	4.06	Antihypertensive
Mefenamic Acid	61–68–7	C15H15NO2	241.1103	5.4	Anti-inflammatory
Meprobamate	57–53–4	C9H18N2O4	218.1267	0.93	Anxiolytic
Methadone	76–99–3	C21H27NO	309.2093	5.01	treatment of addiction
Metoprolol	37,350–58–6	C6H9N3O3	171.0644	1.76	β-blocker agent
Metronidazole	443–48–1	C15H25NO3	267.1834	-0.46	Antibacterial
Midazolam	59,467–70–8	C18H13ClFN3	325.0782	3.97	Anxyiolitic
Morphine	57–27–2	C17H19NO3	285.1365	0.9	opioid
N-acetyl sulfamethoxazole	21,312–10–7	C12H13N3O4S	295.0627	0.86	Antibiotic TP / Metabolite
Nalidixic acid	389–08–2	C12H12N2O3	232.0848	1.01	Antibiotic
Naproxen	22,204–53–1	C14H14O3	230.0943	2.99	Anti-inflammatory
Neotame	165,450–17–9	C20H30N2O5	378.2155	-0.08	Sweetener
N-Methyl lamotrigine	1,373,243–86–7	C10H9Cl2N5	269.0235	3.74	Antiepileptic TP/ Metabolite
O-desmethylvenlafaxine	93413dic-62–8	C16H25NO2	263.1885	2.29	Metabolite
Oseltamivir	196,618–13–0	C16H28N2O4	312.2049	1.16	Antiviral
Oxazepam	604–75–1	C15H11ClN2O2	286.0509	2.92	Anxiolytic
Paroxetine	61,869–08–7	C19H20FNO3	329.1427	3.15	Antidepressant
Pentobarbital	76–74–4	C11H18N2O3	226.1317	1.89	Anxiolytic
Phenytoin	57–41–0	C15H12N2O2	252.0899	2.15	Anticonvulsant
Primidone	125–33–7	C12H14N2O2	218.1055	1.12	Anticonvulsant
Propranolol	525–66–6	C16H21NO2	259.1572	2.58	β-blocker agent
Propyphenazone	479–92–5	C14H18N2O	230.1419	2.35	Analgesic
Rosuvastatin	287,714–41–4	C22H28FN3O6S	481.1683	1.92	Anti-inflammatory
Saccharin	81–07–2	C7H5NO3S	182.9990	0.45	Sweetener
Salicylic acid	69–72–7	C7H6O3	138.0317	1.98	Anti-inflammatory
Sertraline	79,617–96–2	C17H17Cl2N	305.0738	5.15	Antidepressant
Sitagliptin	486,460–32–6	C16H15F6N5O	407.1181	1.26	Antidiabetic
Sotalol	3930–20–9	C12H20N2O3S	272.1195	-0.4	β-blocker agent
Stevioside	57,817–89–7	C38H60O18	804.3780	-2.16	Sweetener
Sucralose	56,038–13–2	C12H19Cl3O8	396.0146	-0.47	Sweetener
Sulfadiazine	68–35–9	C10H10N4O2S	250.0524	0.39	Antibiotic
Sulfadimethoxine	122–11–2	C12H14N4O4S	310.0736	0.23	Antibiotic
Sulfamerazine	127–79–7	C11H12N4O2S	264.0681	0.52	Antibiotic
Sulfamethazine	57–68–1	C12H14N4O2S	278.0837	0.65	Antibiotic
Sulfamethizole	144–82–1	C9H10N4O2S2	270.0245	0.21	Antibiotic
Sulfamethoxazole	723–46–6	C10H11N3O3S	253.0521	0.79	Antibiotic
Sulfapyridine	144–83–2	C11H11N3O2S	249.0572	1.01	Antibiotic
Sulfathiazole	72–14–0	C9H9N3O2S2	255.0136	0.98	Antibiotic
Sulisobenzone (BP4)	4065–45–6	C14H12O6S	308.0355	3.52	UV filter
Tramadol	3715–90–0	C16H25NO2	263.1885	2.45	Analgesic
Trimethoprim	738–70–5	C14H18N4O3	290.1379	1.28	Antibiotic
Valsartan	137,862–53–4	C24H29N5O3	435.2271	4.59	Antihypertensive
Valsartan acid	164,265–78–5	C14H10N4O2	266.0804	2.56	Antihypertensive TP / Metabolite
Venlafaxine	93,413–69–5	C17H27NO2	277.2042	2.74	Antidepressant
Verapamil	52–53–9	C27H38N2O4	454.2832	5.04	Calcium channel blocking agent
Warfarin	81–81–2	C19H16O4	308.1049	2.74	Anticoagulant
Zonisamide	68,291–97–4	C8H8N2O3S	212.0256	0.11	Anticonvulsant

* Predicted values obtained from Chemaxon (www.chemaxon.com).

Before the sample loading, a mix of 80 isotopically labelled compounds was added to every sample. Then the samples were loaded on the previously activated cartridges at one drop every two seconds approximately for the maximum retention of the compounds. After the samples were loaded, cartridges were then dried passing a slight air current through the cartridges. Cartridges were then eluted with 2 x 3 mL 5% NH_3 in MeOH:EtOAc (50:50 v/v), followed by 2 x 3 mL of 2% formic acid in MeOH:EtOAc (50:50 v/v) and finally 3 mL of MeOH:EtOAc (50:50 v/v). The eluate (approximately 15 mL) was then evaporated to dryness under a gentle nitrogen stream and then reconstituted in 500 μL of H_2O:MeOH (95:5 v/v). A final concentration factor of 1000 was obtained.

Table 2
Sampled upstream points from each municipality and sample pool water parameters.

Municipalities studied (Osona, Spain)	pH	EC (μS cm^{-1})	Coordinates
Alpens	8.06	520	42°06′56.5″N 2°05′53.0″E
Folgueroles	6.81	617	41°56′39.0″N 2°18′34.0″E
Santa Eulàlia de Riuprimer	7.98	940	41°54′44.5″N 2°11′35.2″E
Taradell	7.66	572	41°52′52.0″N 2°16′33.9″E
Vidrà	7.6	501	42°07′13.9″N 2°18′50.0″E
Viladrau	7.72	170	41°50′51.6″N 2°22′21.1″E
Sample Pool	7.63	514	-

Table 3
Gradient profile, time, percentage of mobile phases and flow rate.

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow (min/mL)
0.0	5.0	95.0	0.2
0.3	5.0	95.0	0.2
10.0	30.0	70.0	0.2
13.3	65.0	35.0	0.2
15.5	100.0	0.0	0.2
17.3	100.0	0.0	0.2
17.7	5.0	95.0	0.2
19.0	5.0	95.0	0.2

Instrument analysis

The targeted contaminants of emerging concern were separated using ultra high-performance liquid chromatography in a Waters Acquity HSS T3 (C18) column (100 × 2.1 mm i.d., 1.8 µm particle size) thermostated at 40 °C using a Waters ACQUITY UHPLC system (Waters, Milford, MA). For the detection, a Q-Exactive Orbitrap mass spectrometer (Thermo-Fisher Scientific, Germany) equipped with heated electrospray ionization (HESI) was used. The tuning methods and parameters used for the MS acquisition were optimized in the following conditions; spray voltage was set to 3.5 kV (positive) and 2.5 kV (negative), S-Lens RF level, 60; capillary temperature, 350 °C; auxiliary gas temperature, 300 °C. Nitrogen was used as sheath gas flow rate 40; auxiliary gas flow rate 10 and sweep gas flow rate 2. For the positive electrospray ionization (ESI+), the mobile phases used were ACN and 5 mM $AcNH_4$ + 0.1% formic acid in water while for the negative electrospray ionization (ESI-) mode ACN and 2 mM NH_4F in water were used. The gradient profile was the same for both ionizations as shown in Table 3. The injection volume was set to 10 µL.

As for the mass spectrometer, a full-scan experiment followed by a targeted data-independent acquisition (targeted-DIA) mode were used with a specific predefined inclusion list which contained the exact mass of the selected precursor ion, optimized retention time windows and optimized collision energies in order to obtain high quality MS2 product ions (Table SM 2 and 3). For the retention time optimization, a mix of the analytes at 10 μg L^{-1} was initially evaluated with the specific selected column by a full-scan MS with a targeted data-dependent MS2 (dd-MS2) acquisition mode in order to obtain the compound retention times and study possible co-elutions. Then, the predefined inclusion list was compiled. The MS parameters for the selected method were optimized and described as follows: full MS (70,000 K resolution, AGC target 3x10^6, IT 150 ms, scan range 90–1000 m/z) followed by a DIA experiment to generate MS2 spectra (17,500 resolution, AGC 2x10^5, IT auto, Loop count 1, Top N 10, isolation window 1.5 m/z, and NCE 30 eV). As mentioned above, the inclusion list was included with the exact mass to generate as many micro-scanning events as precursor ions filtered. In this way, greater sensitivity and selectivity were achieved by this method. Further information on specific details is included in the supplementary material (Table SM 1) or elsewhere [4].

The guidance SANTE 11,312/2021 [9] was followed, and two ions were used for high-confidence peak identification criteria. For quantification, the presence of the precursor ion in the extracted chromatogram (signal-to-noise ratio >3) with a mass accuracy within 5 ppm and matched retention time within 0.1 min compared to the reference standard was applied. For further confirmation, the presence of at least one product ion in the MS2 spectrum with a mass error below 5 ppm was expected. Only the adduct ion obtained from the full-scan spectrum was used for the isotopically labelled compounds.

Method validation

Surface water pool samples were used for the method validation, and aliquots of 500 mL were spiked with the mix of the 116 selected targeted compounds. The method was validated, in triplicate, at three different concentration levels: 5, 50, and 500 μg L^{-1} in the extract, which would correspond to final sample concentrations of 5, 50, 500 ng L^{-1} respectively, after applying the method concentration factor of 1000. These concentrations were chosen in order to cover common environmental concentrations. The method was evaluated in terms of accuracy (recovery rates), matrix effect (ME), precision (% RSD), linearity (R^2), method detection limits (MDL), method quantification limits (MQL) according to [10], for all three validated concentrations.

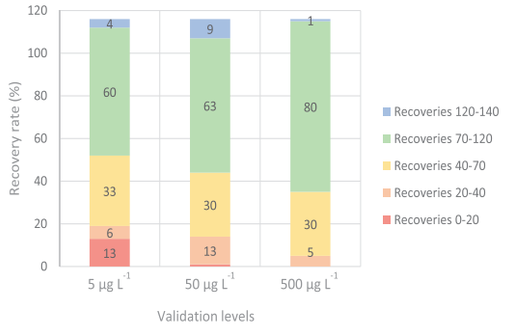


Fig. 1. Recovery rates for the three validated concentrations in surface water matrices.

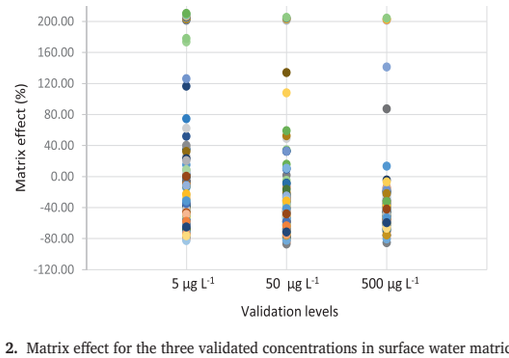


Fig. 2. Matrix effect for the three validated concentrations in surface water matrices.

In order to evaluate the accuracy of the method developed, recovery rates (RR) were calculated, for both intra and inter day precision. The recovery rates were calculated by comparing the peak area of the pool samples spiked before and after the method extraction. A blank sample without any spiked compounds was subtracted in each case (Eq. (1))

$$RR\ (\%) = \frac{Area_{pre-spiked} - Area_{blank}}{Area_{post-spiked} - Area_{blank}}\ x\ 100 \quad (1)$$

The recovery rates obtained for the present method are shown in Fig. 1. The recovery rates were generally good for most of the compounds ranging from 70 to 120% with the exception of some compounds such as cyclamate, irgasan, losartan, lidocaine or paroxetine which presented recovery values of 26, 20, 19, 38 and 19% respectively at the lowest level of validation. Nevertheless, for the high validation level (500 $\mu g\ L^{-1}$ in the extract) more than 80 compounds were recovered with recovery values higher than 70%. At the same time, those compounds with low recovery rates at the low validation level, are usually found at high concentrations in the environment [11,12] and with the use of isotopically labelled compounds these can be quantified and thus those compounds were included in the method anyway.

Matrix effect was also studied to relate either signal suppression or enhancement and was calculated for the selected compounds and for the three validation levels. Eq. (2) was applied where the area from a spiked matrix sample (post-spiked) was compared with the area given in a spiked solvent sample at the same given concentration. The area from a blank matrix (non-spiked) was subtracted.

$$ME(\%) = [[(Area_{matrix} - Area_{blank})/(Area_{solvent})] - 1] \times 100 \quad (2)$$

The matrix effects for the validated compounds for each validation level are shown in Fig. 2. Mainly ion suppression was observed in all three cases, and as the concentration increased, a slight increase or change from rather matrix enhancement to suppression was observed. There were some exceptional cases where an important enhancement was observed for hydroxychloroquine, azithromycin or carazolol.

Precision was evaluated by both intra and inter-day relative standard deviation (RSD), shown in Table 4, to study the repeatability and reproducibility. Good results were obtained for the studied method with intra-day RSD values from 0.43 to 20.63% and inter-day values from 0.06 to 19.31%, with the exception of crotamiton and fluticasone, which gave slightly higher RSDs. MLODs and MLOQs were estimated by the standard deviation of the intercept divided by the slope, multiplied by three and ten respectively. Linearity

Table 4
Relative standard deviation, method limits of detection and quantification, and linearity (R^2) for each compound.

Compound name	Intraday RSD (%)			Interday RSD (%)			MLOD (ng mL^{-1})	MLOQ (ng mL^{-1})	R^2
	5 ppb	50 ppb	500 ppb	5 ppb	50 ppb	500 ppb			
1,7 Dimethylxanthine	–	9.70	5.72	–	9.91	13.41	0.25	0.84	0.9975
17-α Ethynilestradiol	18.57	18.99	12.35	8.11	3.24	1.91	0.09	0.30	0.9997
1H-Benzotriazole	1.46	3.16	7.40	5.99	3.22	1.22	0.12	0.39	0.9990
2.4-Dihydroxybenzophenone (BP1)	7.73	16.45	4.83	1.06	1.44	1.44	0.52	1.75	0.9918
2.2′.4.4′-Tetrahydroxybenzophenone (BP2)	13.45	17.70	5.49	2.42	1.19	1.13	0.19	0.63	0.9994
4-Hydroxidiclofenac	20.63	2.45	15.06	10.27	1.52	2.02	0.97	3.24	0.9999
4-Nitro Sulfamethoxazole	4.41	9.54	5.03	3.93	1.41	0.68	0.20	0.68	0.9985
5-Desamino-5 oxo 2,5 Lamotrigine	2.54	6.99	7.35	3.45	1.63	1.19	0.23	0.77	0.9929
5-methyl-1 H Benzotriazole	1.96	4.84	7.52	8.51	1.02	0.29	0.26	0.88	0.9968
5-nitro-Diclofenac	9.33	17.91	11.24	3.11	1.85	1.32	0.25	0.82	0.9981
6a-Methylprednisolone	18.91	20.23	17.23	13.32	3.36	0.91	0.41	1.36	0.9949
Acesulfame	11.21	17.47	8.93	12.65	5.62	3.24	0.50	1.68	0.9935
Acetaminophen	2.67	0.93	5.21	6.21	18.55	1.80	0.12	0.41	0.9996
Acridone	5.80	4.38	7.88	0.06	1.92	0.54	0.16	0.54	0.9992
Adamantan-1-amine (Amantadine)	3.76	6.20	7.00	2.31	2.32	1.38	0.13	0.43	0.9994
Alprazolam	3.01	6.86	4.53	0.96	2.87	3.43	0.42	1.40	0.9976
Atenolol	9.77	9.49	12.04	3.97	14.84	6.46	0.10	0.34	0.9999
Azithromycin	–	2.76	1.51	–	2.45	1.26	0.34	1.14	0.9959
Benzoylecgonine	1.18	6.22	6.12	0.57	0.81	1.78	0.32	1.08	0.9990
B-estradiol	15.96	12.68	11.20	16.83	0.47	2.90	0.37	1.24	0.9977
Bezafibrate	0.63	7.09	5.88	3.86	1.79	0.77	0.14	0.48	0.9996
Bisphenol-A	–	30.90	12.72	–	1.51	0.46	0.07	0.25	0.9992
Bromazepam	7.01	4.55	7.14	6.32	3.88	9.65	0.31	1.02	0.9999
Caffeine	–	7.01	4.05	–	12.88	7.78	0.14	0.46	0.9952
Carazolol	5.65	6.13	7.31	8.27	11.33	8.31	0.06	0.21	0.9979
Carbamazepine	1.26	4.64	6.41	3.54	1.49	0.27	0.11	0.35	0.9987
Carbamazepine-10,11-epoxide	3.13	1.56	3.31	2.56	1.72	0.08	0.19	0.64	0.9982
Carisoprodol	0.66	10.32	4.84	3.62	2.36	1.02	0.20	0.68	0.9983
Chloramphenicol	4.88	7.01	5.70	1.90	1.49	0.28	0.86	2.85	0.9936
Citalopram	5.65	7.67	3.63	1.36	1.42	0.79	0.11	0.37	0.9993
Clarithromycin	4.42	3.91	4.38	9.25	1.98	0.92	0.15	0.50	0.9975
Climbazole	6.44	17.87	8.42	1.28	0.85	0.98	0.06	0.20	0.9997
Clofibric Acid	3.37	8.59	4.83	10.58	3.33	0.43	0.32	1.08	0.9997
Cocaethylene	1.63	2.98	3.42	1.07	0.19	0.28	0.11	0.37	0.9996
Cocaine	2.71	3.36	6.62	2.70	0.81	1.09	0.16	0.54	0.9994
Codeine	8.58	7.10	10.01	12.92	3.68	1.39	0.13	0.43	0.9997
Cotinine	19.56	11.20	10.17	12.18	2.75	1.32	0.15	0.51	0.9991
Crotamiton	30.55	31.50	27.56	4.12	1.51	1.66	0.21	0.69	0.9961
Cyclamate	13.85	14.23	9.67	15.32	13.00	2.98	0.53	1.78	0.9991
Diazepam	3.39	9.70	5.72	1.09	1.08	0.79	0.12	0.40	0.9990
Diclofenac	18.83	15.39	18.62	19.31	2.03	1.03	1.51	5.05	0.9994
Diltiazem	10.21	4.39	4.06	0.93	1.41	1.44	0.14	0.47	0.9974
Estriol	2.07	8.31	4.40	18.24	2.11	3.13	0.14	0.46	0.9978
Estrone	6.99	12.43	8.22	5.51	2.15	2.78	0.32	1.05	0.9997
Ezetimibe	11.37	13.90	11.22	4.11	0.74	4.11	0.40	1.34	0.9915
Fipronil	18.46	13.65	13.33	1.17	2.31	2.40	0.34	1.13	0.9912
Fipronil desulfinyl	12.92	14.83	14.70	2.22	2.16	2.76	0.33	1.11	0.9907
Fipronil sulfide	13.63	16.59	13.10	1.37	1.22	4.07	0.44	1.48	0.9995
Fipronil sulfone	15.39	18.69	12.65	1.88	0.84	6.79	0.26	0.87	0.9976
Florfenicol	4.98	8.73	5.51	1.99	0.80	0.86	0.67	1.22	0.9918
Fluconazole	3.80	8.28	7.69	0.17	1.28	0.82	0.19	0.63	0.9998
Flumequine	0.90	4.91	8.36	1.40	1.10	0.66	0.07	0.25	0.9999
Fluticasone propionate	27.83	16.35	24.48	35.25	29.63	27.57	1.27	4.22	0.9998
Furazolidone	9.02	10.73	7.77	4.93	0.73	1.42	0.20	0.66	0.9932
Furosemide	9.69	9.96	3.60	13.42	1.19	0.86	0.18	0.60	0.9993
Gemfibrozil	7.31	12.16	6.55	18.69	17.19	16.17	0.25	0.84	0.9991
Hydrochlorothiazide	18.46	13.66	15.83	11.15	2.52	3.33	0.04	0.15	0.9997
Hydroxychloroquine	13.72	10.43	4.87	17.81	15.51	6.85	1.84	6.15	0.9966

(continued on next page)

Table 4 (*continued*)

Compound name	Intraday RSD (%)			Interday RSD (%)			MLOD (ng mL^{-1})	MLOQ (ng mL^{-1})	R^2
	5 ppb	50 ppb	500 ppb	5 ppb	50 ppb	500 ppb			
Hyoscine (scopolamine)	3.45	6.21	7.68	2.81	1.82	0.54	0.11	0.37	0.9979
Ibuprofen	–	13.93	10.53	–	12.12	4.34	0.14	0.48	0.9988
Indomethacin	17.53	16.66	16.76	7.73	2.08	1.55	0.12	0.41	0.9997
Irbesartan	18.26	23.58	20.88	5.30	1.17	0.33	0.07	0.22	0.9996
Irgasan (Triclosan)	15.35	18.57	14.91	5.48	9.03	7.28	0.70	2.33	0.9978
Ketoprofen	8.86	7.50	6.98	10.92	0.70	1.27	0.36	1.18	0.9993
Lamotrigine	3.72	3.92	6.59	4.27	1.39	0.71	0.14	0.46	0.9976
Lamotrigine-N2-oxide	4.56	7.66	9.99	1.23	0.47	1.46	0.26	0.88	0.9915
Lidocaine	16.59	13.82	1.88	1.52	0.71	0.98	0.08	0.28	0.9998
Lincomycin	13.96	18.65	23.06	17.52	8.70	7.13	0.09	0.31	0.9963
Lorazepam	3.91	8.86	8.79	1.92	0.81	2.30	0.18	0.60	0.9987
Losartan	25.84	36.99	18.03	16.17	1.04	1.12	0.23	0.77	0.9965
Mefenamic Acid	15.79	17.58	12.63	5.70	2.13	1.19	0.46	1.53	0.9944
Meprobamate	6.70	5.04	0.52	2.09	0.78	0.89	0.75	2.50	0.9964
Methadone	3.36	12.36	3.68	1.16	0.57	0.38	0.02	0.07	0.9993
Metoprolol	1.96	6.95	5.65	1.14	0.06	0.23	0.06	0.20	0.9998
Metronidazole	5.75	4.12	8.38	4.27	1.73	3.04	0.02	0.08	0.9999
Midazolam	27.50	7.58	20.70	1.45	1.35	1.11	0.11	0.38	0.9936
Morphine	11.52	15.35	13.66	2.13	1.11	2.71	0.14	0.45	0.9936
N-acetyl sulfamethoxazole	2.69	8.43	7.86	5.55	1.35	0.53	0.16	0.52	0.9947
Nalidixic acid	0.47	4.65	9.51	2.10	1.13	0.11	0.12	0.41	0.9975
Naproxen	–	12.18	9.66	–	12.66	4.12	0.43	1.43	0.9973
Neotame	11.93	7.03	6.05	12.22	1.08	1.06	0.17	0.56	0.9962
N-Methyl lamotrigine	1.93	5.76	7.22	1.92	0.78	0.75	0.29	0.97	0.9932
O-desmethylvenlafaxine	5.72	4.45	2.49	1.27	0.45	1.25	0.19	0.63	0.9990
Oseltamivir	2.96	3.19	3.25	1.96	1.81	0.65	0.10	0.34	0.9997
Oxazepam	6.42	7.22	2.62	1.79	0.82	1.71	0.12	0.39	0.9998
Paroxetine	13.15	17.78	6.21	11.62	5.52	1.62	0.15	0.50	0.9994
Pentobarbital	4.38	4.57	1.31	1.53	1.14	1.34	0.16	0.55	0.9994
Phenytoin	3.53	8.81	6.36	1.40	1.17	1.67	0.27	0.90	0.9987
Primidone	–	9.60	11.60	–	7.33	1.60	1.89	6.31	0.9909
Propranolol	2.25	2.99	4.93	6.70	3.33	0.71	0.21	0.70	0.9984
Propyphenazone	1.38	11.66	1.34	2.27	1.47	0.48	0.18	0.60	0.9977
Rosuvastatin	2.43	9.24	6.10	3.99	0.28	0.47	0.20	0.66	0.9992
Saccharin	4.97	7.93	8.37	16.14	19.78	3.30	0.45	1.51	0.9993
Salicylic acid	6.81	17.00	5.82	12.27	16.50	7.62	0.75	2.50	0.9994
Sertraline	4.13	14.69	14.24	3.82	1.45	1.60	0.40	1.32	0.9951
Sitagliptin	16.37	6.38	2.58	9.34	0.69	0.37	0.21	0.70	0.9999
Sotalol	7.12	8.27	6.40	5.25	2.25	1.81	0.07	0.22	0.9990
Stevioside	–	8.31	8.31	–	5.68	1.25	0.35	1.18	0.9971
Sucralose	–	5.41	5.16	–	3.05	5.11	0.26	0.86	0.9974
Sulfadiazine	14.01	11.56	14.04	1.09	1.19	1.37	0.14	0.48	0.9999
Sulfadimethoxine	19.41	19.57	14.91	0.70	1.92	11.43	0.12	0.41	0.9995
Sulfamerazine	17.97	17.31	15.30	1.32	1.67	1.88	0.10	0.35	0.9992
Sulfamethazine	12.10	18.02	15.38	1.44	0.64	0.98	0.32	1.06	0.9972
Sulfamethizole	10.84	15.73	18.34	1.92	0.01	0.83	0.05	0.17	0.9999
Sulfamethoxazole	10.00	7.26	6.79	2.08	0.25	1.01	0.24	0.80	0.9914
Sulfapyridine	15.42	12.96	8.59	0.39	0.88	1.12	0.07	0.39	0.9991
Sulfathiazole	17.29	14.00	8.52	1.63	1.89	1.47	0.12	0.39	0.9988
Sulisobenzone (BP4)	8.27	10.55	5.02	3.02	0.35	1.06	0.10	0.34	0.9997
Tramadol	9.32	6.91	2.72	2.54	1.34	0.78	0.19	0.64	0.9907
Trimethoprim	1.67	3.36	6.72	0.89	0.76	1.07	0.14	0.47	0.9959
Valsartan	7.07	13.14	6.50	7.34	3.70	0.43	0.19	0.63	0.9998
Valsartan acid	22.81	5.10	2.81	18.09	1.71	0.88	0.61	2.05	0.9971
Venlafaxine	4.07	2.73	3.47	3.78	0.64	1.22	0.01	0.05	0.9999
Verapamil	9.33	16.19	10.66	1.66	0.72	0.72	0.11	0.36	0.9992
Warfarin	10.69	19.95	10.57	7.41	0.59	1.36	0.22	0.74	0.9955
Zonisamide	–	9.14	6.48	–	9.37	1.47	1.58	5.22	0.9993

range, MLODs and MLOQs values are given in concentrations found in final extract. The linearity interval of the matrix-matched calibration curve covered a wide range of concentrations from 0.05 to 1000 $\mu g\ L^{-1}$ with R^2 >0.99 for the majority of the compounds. The method also proved to be highly sensitive with MLODs in the range of 0.01–1.89 $\mu g\ L^{-1}$ and MLOQs ranging from 0.05 to 6.31 $\mu g\ L^{-1}$.

Quality assurance and quality control were ensured by preparing procedural blanks using HPLC water and repeating the exposed method procedure. No peaks were observed for any of the target analytes. Quality controls were also prepared by spiking the same

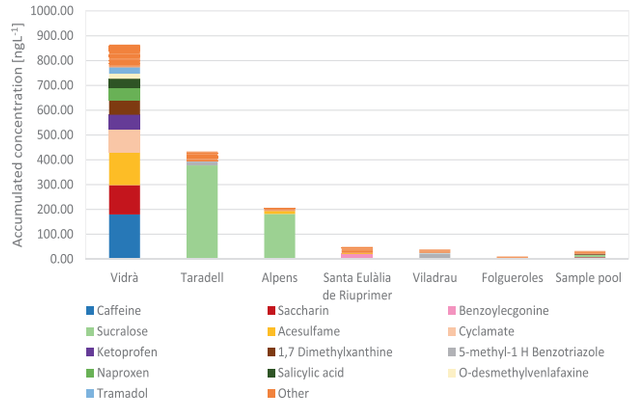


Fig. 3. Total concentration of wastewater borne contaminants in the different Catalan municipalities and the surface water sample pool.

solvent ratio as the final extracts with standards and isotopically labelled compounds to reach a concentration of 50 $\mu g\ L^{-1}$ in the vial, and these were injected and analysed along the sample sequence analysis to ensure reliable determination. To overcome matrix effects, isotopically labelled compounds were used in all samples as well as a matrix-matched calibration curve. For proper quantification, the matrix-matched calibration curve included 10 points with the 116 mix standards and 80 isotopically labelled compounds.

Method applicability

The WWTP discharge upstream samples of each municipality and the surface water pool used for the method validation and matrix-matched calibration curve (Table 2) were analysed as an example of method applicability and results are shown in Fig. 3. In general, very low levels of contamination were found. The most contaminated upstream was the Vidrà municipality with a total accumulated concentration of 864.2 ng L^{-1}, followed by the Taradell municipality with 433.7 ng L^{-1}. In Fig. 3, those contaminants present with at least 20 ng L^{-1} in one sample are shown. As expected, many of the selected contaminants were not detected (BP4, diclofenac, oxazepam, valsartan) as their presence is mainly expected in surface rivers after the wastewater treatment plant discharge. However, a total of 48 compounds of the 116 targeted compounds were detected in at least one of the samples. The highest concentrations found were mainly concerning those compounds used frequently in our day to day life such as sucralose (sweetener) with the highest concentration of 376.9 ng L^{-1} found in Taradell followed by caffeine (stimulant), acesulfame or saccharin (sweeteners) with maximum concentrations of 179.4, 130.3 and 119.0 ng L^{-1} respectively.

Overall, the method developed proved a very good performance for the quantification of a wide range of wastewater-borne pollutants with different polarities. Thus, it has a high applicability for monitoring and controlling the impact of wastewater-borne pollutants in surface water and to further explore possible other contamination sources other than the continuous WWTP discharges.

CRediT author statement

Olga Gómez-Navarro: Methodology, Validation, Formal analysis, writing - Original Draft; Writing - Original Draft; **Francesc Labad:** sampling, laboratory work; **Diana P. Manjarrés:** sampling, laboratory work; **Sandra Pérez:** Conceptualization, Supervision, Project administration, Writing - Review & Editing, Funding acquisition; **Nicola Montemurro*:** Supervision, Methodology, Validation, Writing - Review & Editing

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data Availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2023.102093.

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Publication #1

Supplementary Materials

HRMS-Targeted-DIA methodology for quantification of wastewater-borne pollutants in surface water

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Supplementary Material

Table SM 1. Target compound name, Cas number, Molecular formula, Isomeric SMILES and InChIKey

Compound name	Cas number	Molecular formula	Isomeric SMILES	InChIKey
1,7 Dimethylxanthine	611-59-6	C7H8N4O2	N1C=NC2=C1C(=O)N(C(=O)N2)C	QUNWUDVFRNGTCO-UHFFFAOYSA-N
17-α Ethynilestradiol	57-63-6	C20H24O2	C[C@]12CC[C@H]3[C@H]([C@@H]1CC[C@]2(C#C)O)CCC4=C3C=CC(=C4)O	BFPYWIDHMRZLRN-SLHNCBLASA-N
1H-Benzotriazole	95-14-7	C6H5N3	C1=CC2=NNN=C2C=C1	QRUDEWIWKLJBPS-UHFFFAOYSA-N
2.4-Dihydroxybenzophenone (BP1)	131-56-6	C13H10O3	C1=CC=C(C=C1)C(=O)C2=C(C=C(C=C2)O)O	ZXDDPOHVAMWLBH-UHFFFAOYSA-N
2.2'.4.4'-Tetrahydroxybenzophenone (BP2)	131-55-5	C13H10O5	C1=CC(=C(C=C1O)O)C(=O)C2=C(C=C(C=C2)O)O	WXNRYSGJLQFHBR-UHFFFAOYSA-N
4-Hydroxidiclofenac	64118-84-9	C14H11Cl2NO3	C1=CC=C(C(=C1)CC(=O)O)NC2=C(C=C(C=C2Cl)O)Cl	KGVXVPRLBMWZLG-UHFFFAOYSA-N
4-Nitro Sulfamethoxazole	29699-89-6	C10H9N3O5S	CC1=CC(=NO1)NS(=O)(=O)C2=CC=C(C=C2)[N+](=O)[O-]	XHJLDAHBWWMCRZ-UHFFFAOYSA-N
5-Desamino-5 oxo 2,5 Lamotrigine	252186-78-0	C9H6Cl2N4O	C1=CC(=C(C(=C1)Cl)Cl)C2=NN=C(NC2=O)N	OVNGWOHWYFBGKF-UHFFFAOYSA-N
5-methyl-1 H Benzotriazole	136-85-6	C7H7N3	CC1=CC2=NNN=C2C=C1	LRUDIIUSNGCQKF-UHFFFAOYSA-N
5-nitro-Diclofenac	174316-61-1	C14H10Cl2N2O4	ClC1=CC=CC(Cl)=C1NC2=CC=C([N+]([O-])=O)C=C2CC(O)=O	MRJVSFHDJGEONX-UHFFFAOYSA-N
6a-Methylprednisolone	83-43-2	C22H30O5	C[C@H]1C[C@H]2[C@@H]3CC[C@@]([C@]3(C[C@@H]([C@@H]2[C@@]4(C1=CC(=O)C=C4)C)O)C)(C(=O)CO)O	VHRSUDSXCMQTMA-PJHHCJLFSA-N
Acesulfame	33665-90-6	C4H5NO4S	CC1=CC(=O)NS(=O)(=O)O1	YGCFIWIQZPHFLU-UHFFFAOYSA-N
Acetaminophen	103-90-2	C8H9NO2	CC(=O)NC1=CC=C(C=C1)O	RZVAJINKPMORJF-UHFFFAOYSA-N
Acridone	578-95-0	C13H9NO	C1=CC=C2C(=C1)C(=O)C3=CC=CC=C3N2	FZEYVTFCMJSGMP-UHFFFAOYSA-N
Adamantan-1-amine (Amantadine)	768-94-5	C10H17N	C1C2CC3CC1CC(C2)(C3)N	DKNWSYNQZKUICI-UHFFFAOYSA-N
Alprazolam	28981-97-7	C17H13ClN4	CC1=NN=C2N1C3=C(C=C(C=C3)Cl)C(=NC2)C4=CC=CC=C4	VREFGVBLTWBCJP-UHFFFAOYSA-N
Atenolol	29122-68-7	C14H22N2O3	CC(C)NCC(COC1=CC=C(C=C1)CC(=O)N)O	METKIMKYRPQLGS-UHFFFAOYSA-N
Azithromycin	83905-01-5	C38H72N2O12	CC[C@@H]1[C@@]([C@@H]([C@H](N(C[C@@H](C[C@@]([C@@H]([C@H]([C@@H]([C@H](C(=O)O1)C)O[C@H]2C[C@@]([C@H]([C@@H](O2)C)O)(C)OC)C)O[C@H]3[C@@H]([C@H](C[C@H](O3)C)N(C)C)O)(C)O)C)C)O)(C)O	MQTOSJVFKKJCRP-BICOPXKESA-N
Benzoylecgonine	519-09-5	C16H19NO4	CN1[C@H]2CC[C@@H]1[C@H]([C@H](C2)OC(=O)C3=CC=CC=C3)C(=O)O	GVGYEFKIHJTNQZ-RFQIPJPRSA-N
B-estradiol	50-28-2	C18H24O2	C[C@]12CC[C@H]3[C@H]([C@@H]1CC[C@@H]2O)CCC4=C3C=CC(=C4)O	VOXZDWNPVJITMN-ZBRFXRBCSA-N
Bezafibrate	41859-67-0	C19H20ClNO4	CC(C)(C(=O)O)OC1=CC=C(C=C1)CCNC(=O)C2=CC=C(C=C2)Cl	IIBYAHWJQTYFKB-UHFFFAOYSA-N
Bisphenol-A	80-05-7	C15H16O2	CC(C)(C1=CC=C(C=C1)O)C2=CC=C(C=C2)O	IISBACLAFKSPIT-UHFFFAOYSA-N
Bromazepam	1812-30-2	C14H10BrN3O	C1C(=O)NC2=C(C=C(C=C2)Br)C(=N1)C3=CC=CC=N3	VMIYHDSEFNYJSL-UHFFFAOYSA-N

Compound name	Cas number	Molecular formula	Isomeric SMILES	InChIKey
Caffeine	58-08-2	C8H10N4O2	CN1C=NC2=C1C(=O)N(C(=O)N2C)C	RYYVLZVUVIJVGH-UHFFFAOYSA-N
Carazolol	57775-29-8	C18H22N2O2	CC(C)NCC(COC1=CC=CC2=C1C3=CC=CC=C3N2)O	BQXQGZPYHWWCEB-UHFFFAOYSA-N
Carbamazepine	298-46-4	C15H12N2O	C1=CC=C2C(=C1)C=CC3=CC=CC=C3N2C(=O)N	FFGPTBGBLSHEPO-UHFFFAOYSA-N
Carbamazepine-10,11-epoxide	36507-30-9	C15H12N2O2	C1=CC=C2C(=C1)C3C(O3)C4=CC=CC=C4N2C(=O)N	ZRWWEEVEIOGMMT-UHFFFAOYSA-N
Carisoprodol	78-44-4	C12H24N2O4	CCCC(C)(COC(=O)N)COC(=O)NC(C)C	OFZCIYFFPZCNJE-UHFFFAOYSA-N
Chloramphenicol	56-75-7	C11H12Cl2N2O5	C1=CC(=CC=C1[C@H]([C@@H](CO)NC(=O)C(Cl)Cl)O)[N+](=O)[O-]	WIIZWVCIJKGZOK-RKDXNWHRSA-N
Citalopram	59729-33-8	C20H21FN2O	CN(C)CCCC1(C2=C(CO1)C=C(C=C2)C#N)C3=CC=C(C=C3)F	WSEQXVZVJXJVFP-UHFFFAOYSA-N
Clarithromycin	81103-11-9	C38H69NO13	CC[C@@H]1[C@@](C[C@@H](C[C@H](C(=O)[C@@H](C[C@@]([C@@H]([C@H]([C@@H]([C@H](C(=O)O1)C)O[C@H]2C[C@@]([C@H]([C@@H](O2)C)O)(C)OC)C)O[C@H]3[C@@H]([C@H](C[C@H](O3)C)N(C)C)O)(C)OC)C)C)O)(C)O	AGOYDEPGAOXOCK-KCBOHYOISA-N
Climbazole	38083-17-9	C15H17ClN2O2	CC(C)(C)C(=O)C(N1C=CN=C1)OC2=CC=C(C=C2)Cl	OWEGWHBOCFMBLP-UHFFFAOYSA-N
Clofibric Acid	882-09-7	C10H11ClO3	CC(C)(C(=O)O)OC1=CC=C(C=C1)Cl	TXCGAZHTZHNUAI-UHFFFAOYSA-N
Cocaethylene	529-38-4	C18H23NO4	CCOC(=O)[C@@H]1[C@H]2CC[C@H](N2C)C[C@@H]1OC(=O)C3=CC=CC=C3	NMPOSNRHZIWLLL-XUWVNRHRSA-N
Cocaine	50-36-2	C17H21NO4	CN1[C@H]2CC[C@@H]1[C@H]([C@H](C2)OC(=O)C3=CC=CC=C3)C(=O)OC	ZPUCINDJVBIVPJ-LJISPDSOSA-N
Codeine	76-57-3	C18H21NO3	CN1CC[C@]23[C@@H]4[C@H]1CC5=C2C(=C(C=C5)OC)O[C@H]3[C@H](C=C4)O	OROGSEYTTFOCAN-DNJOTXNNSA-N
Cotinine	486-56-6	C10H12N2O	CN1[C@@H](CCC1=O)C2=CN=CC=C2	UIKROCXWUNQSPJ-VIFPVBQESA-N
Crotamiton	483-63-6	C13H17NO	CCN(C1=CC=CC=C1C)C(=O)/C=C/C	DNTGGZPQPQTDQF-XBXARRHUSA-N
Cyclamate	100-88-9	C6H13NO3S	C1CCC(CC1)NS(=O)(=O)O	HCAJEUSONLESMK-UHFFFAOYSA-N
Diazepam	439-14-5	C16H13ClN2O	CN1C(=O)CN=C(C2=C1C=CC(=C2)Cl)C3=CC=CC=C3	AAOVKJBEBIDNHE-UHFFFAOYSA-N
Diclofenac	15307-86-5	C14H11Cl2NO2	C1=CC=C(C(=C1)CC(=O)O)NC2=C(C=CC=C2Cl)Cl	DCOPUUMXTXDBNB-UHFFFAOYSA-N
Diltiazem	42399-41-7	C22H26N2O4S	CC(=O)O[C@@H]1[C@@H](SC2=CC=CC=C2N(C1=O)CCN(C)C)C3=CC=C(C=C3)OC	HSUGRBWQSSZJOP-RTWAWAEBSA-N
Estriol	50-27-1	C18H24O3	C[C@]12CC[C@H]3[C@H]([C@@H]1C[C@H]([C@@H]2O)O)CCC4=C3C=CC(=C4)O	PROQIPRRNZUXQM-ZXXIGWHRSA-N
Estrone	53-16-7	C18H22O2	C[C@]12CC[C@H]3[C@H]([C@@H]1CCC2=O)CCC4=C3C=CC(=C4)O	DNXHEGUUPJUMQT-CBZIJGRNSA-N
Ezetimibe	163222-33-1	C24H21F2NO3	C1=CC(=CC=C1[C@@H]2[C@H](C(=O)N2C3=CC=C(C=C3)F)CC[C@@H](C4=CC=C(C=C4)F)O)O	OLNTVTPDXPETLC-XPWALMASSA-N
Fipronil	120068-37-3	C12H4Cl2F6N4OS	C1=C(C=C(C(=C1Cl)N2C(=C(C(=N2)C#N)S(=O)C(F)(F)F)N)Cl)C(F)(F)F	ZOCSXAVNDGMNBV-UHFFFAOYSA-N
Fipronil desulfinyl	205650-65-3	C12H4Cl2F6N4	C1=C(C=C(C(=C1Cl)N2C(=C(C(=N2)C#N)C(F)(F)F)N)Cl)C(F)(F)F	JWKXVHLIRTVXLD-UHFFFAOYSA-N
Fipronil sulfide	120067-83-6	C12H4Cl2F6N4S	C1=C(C=C(C(=C1Cl)N2C(=C(C(=N2)C#N)SC(F)(F)F)N)Cl)C(F)(F)F	FQXWEKADCSXYOC-UHFFFAOYSA-N
Fipronil sulfone	120068-36-2	C12H4Cl2F6N4O2S	C1=C(C=C(C(=C1Cl)N2C(=C(C(=N2)C#N)S(=O)(=O)C(F)(F)F)N)Cl)C(F)(F)F	LGHZJDKSVUTELU-UHFFFAOYSA-N
Florfenicol	73231-34-2	C12H14Cl2FNO4S	CS(=O)(=O)C1=CC=C(C=C1)[C@H]([C@@H](CF)NC(=O)C(Cl)Cl)O	AYIRNRDRBQJXIF-NXEZZACHSA-N

Compound name	Cas number	Molecular formula	Isomeric SMILES	InChIKey
Fluconazole	86386-73-4	C13H12F2N6O	C1=CC(=C(C=C1F)F)C(CN2C=NC=N2)(CN3C=NC=N3)O	RFHAOTPXVQNOHP-UHFFFAOYSA-N
Flumequine	42835-25-6	C14H12FNO3	CC1CCC2=C3N1C=C(C(=O)C3=CC(=C2)F)C(=O)O	DPSPPJIUMHPXMA-UHFFFAOYSA-N
Fluticasone propionate	80474-14-2	C25H31F3O5S	CCC(=O)O[C@@]1([C@@H](C[C@@H]2[C@@]1(C[C@@H]([C@]3([C@H]2C[C@@H](C4=CC(=O)C=C[C@@]43C)F)F)O)C)C)C(=O)SCF	WMWTYOKRWGGJOA-CENSZEJFSA-N
Furazolidone	67-45-8	C8H7N3O5	C1COC(=O)N1/N=C/C2=CC=C(O2)[N+](=O)[O-]	PLHJDBGFXBMTGZ-WEVVVXLNSA-N
Furosemide	54-31-9	C12H11ClN2O5S	C1=COC(=C1)CNC2=CC(=C(C=C2C(=O)O)S(=O)(=O)N)Cl	ZZUFCTLCJUWOSV-UHFFFAOYSA-N
Gemfibrozil	25812-30-0	C15H22O3	CC1=CC(=C(C=C1)C)OCCCC(C)(C)C(=O)O	HEMJJKBWTPKOJG-UHFFFAOYSA-N
Hydrochlorothiazide	58-93-5	C7H8ClN3O4S2	C1NC2=CC(=C(C=C2S(=O)(=O)N1)S(=O)(=O)N)Cl	JZUFKLXOESDKRF-UHFFFAOYSA-N
Hydroxychloroquine	118-42-3	C18H26ClN3O	CCN(CCCC(C)NC1=C2C=CC(=CC2=NC=C1)Cl)CCO	XXSMGPRMXLTPCZ-UHFFFAOYSA-N
Hyoscine (scopolamine)	51-34-3	C17H21NO4	CN1[C@@H]2CC(C[C@H]1[C@H]3[C@@H]2O3)OC(=O)[C@H](CO)C4=CC=CC=C4	STECJAGHUSJQJN-USLFZFAMSA-N
Ibuprofen	15687-27-1	C13H18O2	CC(C)CC1=CC=C(C=C1)C(C)C(=O)O	HEFNNWSXXWATRW-UHFFFAOYSA-N
Indomethacin	53-86-1	C19H16ClNO4	CC1=C(C2=C(N1C(=O)C3=CC=C(C=C3)Cl)C=CC(=C2)OC)CC(=O)O	CGIGDMFJXJATDK-UHFFFAOYSA-N
Irbesartan	138402-11-6	C25H28N6O	CCCCC1=NC2(CCCC2)C(=O)N1CC3=CC=C(C=C3)C4=CC=CC=C4C5=NNN=N5	YOSHYTLCDANDAN-UHFFFAOYSA-N
Irgasan (Triclosan)	3380-34-5	C12H7Cl3O2	C1=CC(=C(C=C1Cl)O)OC2=C(C=C(C=C2)Cl)Cl	XEFQLINVKFYRCS-UHFFFAOYSA-N
Ketoprofen	22071-15-4	C16H14O3	CC(C1=CC(=CC=C1)C(=O)C2=CC=CC=C2)C(=O)O	DKYWVDODHFEZIM-UHFFFAOYSA-N
Lamotrigine	84057-84-1	C9H7Cl2N5	C1=CC(=C(C(=C1)Cl)Cl)C2=C(N=C(N=N2)N)N	PYZRQGJRPPTADH-UHFFFAOYSA-N
Lamotrigine-N2-oxide	136565-76-9	C9H7Cl2N5O	C1=CC(=C(C(=C1)Cl)Cl)C2=NN(C(=N)N=C2N)O	YGGNPWUXPLVPLK-UHFFFAOYSA-N
Lidocaine	137-58-6	C14H22N2O	CCN(CC)CC(=O)NC1=C(C=CC=C1C)C	NNJVILVZKWQKPM-UHFFFAOYSA-N
Lincomycin	154-21-2	C18H34N2O6S	CCC[C@@H]1C[C@H](N(C1)C)C(=O)N[C@@H]([C@@H]2[C@@H]([C@@H]([C@H]([C@H](O2)SC)O)O)O)[C@@H](C)O	OJMMVQQUTAEWLP-KIDUDLJLSA-N
Lorazepam	846-49-1	C15H10Cl2N2O2	C1=CC=C(C(=C1)C2=NC(C(=O)NC3=C2C=C(C=C3)Cl)O)Cl	DIWRORZWFLOCLC-UHFFFAOYSA-N
Losartan	114798-26-4	C22H23ClN6O	CCCCC1=NC(=C(N1CC2=CC=C(C=C2)C3=CC=CC=C3C4=NNN=N4)CO)Cl	PSIFNNKUMBGKDQ-UHFFFAOYSA-N
Mefenamic Acid	61-68-7	C15H15NO2	CC1=C(C(=CC=C1)NC2=CC=CC=C2C(=O)O)C	HYYBABOKPJLUIN-UHFFFAOYSA-N
Meprobamate	57-53-4	C9H18N2O4	CCCC(C)(COC(=O)N)COC(=O)N	NPPQSCRMBWNHMW-UHFFFAOYSA-N
Methadone	76-99-3	C21H27NO	CCC(=O)C(CC(C)N(C)C)(C1=CC=CC=C1)C2=CC=CC=C2	USSIQXCVUWKGNF-UHFFFAOYSA-N
Metoprolol	37350-58-6	C6H9N3O3	CC(C)NCC(COC1=CC=C(C=C1)CCOC)O	IUBSYMUCCVWXPE-UHFFFAOYSA-N
Metronidazole	443-48-1	C15H25NO3	CC1=NC=C(N1CCO)[N+](=O)[O-]	VAOCPAMSLUNLGC-UHFFFAOYSA-N
Midazolam	59467-70-8	C18H13ClFN3	CC1=NC=C2N1C3=C(C=C(C=C3)Cl)C(=NC2)C4=CC=CC=C4F	DDLIGBOFAVUZHB-UHFFFAOYSA-N
Morphine	57-27-2	C17H19NO3	CN1CC[C@]23[C@@H]4[C@H]1CC5=C2C(=C(C=C5)O)O[C@H]3[C@H](C=C4)O	BQJCRHHNABKAKU-KBQPJGBKSA-N
N-acetyl sulfamethoxazole	21312-10-7	C12H13N3O4S	CC1=CC(=NO1)NS(=O)(=O)C2=CC=C(C=C2)NC(=O)C	GXPIUNZCALHVBA-UHFFFAOYSA-N
Nalidixic acid	389-08-2	C12H12N2O3	CCN1C=C(C(=O)C2=C1N=C(C=C2)C)C(=O)O	MHWLWQUZZRMNGJ-UHFFFAOYSA-N

Compound name	Cas number	Molecular formula	Isomeric SMILES	InChIKey
Naproxen	22204-53-1	C14H14O3	C[C@@H](C1=CC2=C(C=C1)C=C(C=C2)OC)C(=O)O	CMWTZPSULFXXJA-VIFPVBQESA-N
Neotame	165450-17-9	C20H30N2O5	CC(C)(C)CCN[C@@H](CC(=O)O)C(=O)N[C@@H](CC1=CC=CC=C1)C(=O)OC	HLIAVLHNDJUHFG-HOTGVXAUSA-N
N-Methyl lamotrigine	1373243-86-7	C10H9Cl2N5	CNC1=C(N=NC(=N1)N)C2=C(C(=CC=C2)Cl)Cl	WVJYMSFBMQWMAL-UHFFFAOYSA-N
O-desmethylvenlafaxine	93413-62-8	C16H25NO2	CN(C)CC(C1=CC=C(C=C1)O)C2(CCCCC2)O	KYYIDSXMWOZKMP-UHFFFAOYSA-N
Oseltamivir	196618-13-0	C16H28N2O4	CCC(CC)O[C@@H]1C=C(C[C@@H]([C@H]1NC(=O)C)N)C(=O)OCC	VSZGPKBBMSAYNT-RRFJBIMHSA-N
Oxazepam	604-75-1	C15H11ClN2O2	C1=CC=C(C=C1)C2=NC(C(=O)NC3=C2C=C(C=C3)Cl)O	ADIMAYPTOBDMTL-UHFFFAOYSA-N
Paroxetine	61869-08-7	C19H20FNO3	C1CNC[C@H]([C@@H]1C2=CC=C(C=C2)F)COC3=CC4=C(C=C3)OCO4	AHOUBRCZNHFOSL-YOEHRIQHSA-N
Pentobarbital	76-74-4	C11H18N2O3	CCCC(C)C1(C(=O)NC(=O)NC1=O)CC	WEXRUCMBJFQVBZ-UHFFFAOYSA-N
Phenytoin	57-41-0	C15H12N2O2	C1=CC=C(C=C1)C2(C(=O)NC(=O)N2)C3=CC=CC=C3	CXOFVDLJLONNDW-UHFFFAOYSA-N
Primidone	125-33-7	C12H14N2O2	CCC1(C(=O)NCNC1=O)C2=CC=CC=C2	DQMZLTXERSFNPB-UHFFFAOYSA-N
Propranolol	525-66-6	C16H21NO2	CC(C)NCC(COC1=CC=CC2=CC=CC=C21)O	AQHHHDLHHXJYJD-UHFFFAOYSA-N
Propyphenazone	479-92-5	C14H18N2O	CC1=C(C(=O)N(N1C)C2=CC=CC=C2)C(C)C	PXWLVJLKJGVOKE-UHFFFAOYSA-N
Rosuvastatin	287714-41-4	C22H28FN3O6S	CC(C)C1=NC(=NC(=C1/C=C/[C@H](C[C@H](CC(=O)O)O)O)C2=CC=C(C=C2)F)N(C)S(=O)(=O)C	BPRHUIZQVSMCRT-VEUZHWNKSA-N
Saccharin	81-07-2	C7H5NO3S	C1=CC=C2C(=C1)C(=O)NS2(=O)=O	CVHZOJJKTDOEJC-UHFFFAOYSA-N
Salicylic acid	69-72-7	C7H6O3	C1=CC=C(C(=C1)C(=O)O)O	YGSDEFSMJLZEOE-UHFFFAOYSA-N
Sertraline	79617-96-2	C17H17Cl2N	CN[C@H]1CC[C@H](C2=CC=CC=C12)C3=CC(=C(C=C3)Cl)Cl	VGKDLMBJGBXTGI-SJCJKPOMSA-N
Sitagliptin	486460-32-6	C16H15F6N5O	C1CN2C(=NN=C2C(F)(F)F)CN1C(=O)C[C@@H](CC3=CC(=C(C=C3F)F)F)N	MFFMDFFZMYYVKS-SECBINFHSA-N
Sotalol	3930-20-9	C12H20N2O3S	CC(C)NCC(C1=CC=C(C=C1)NS(=O)(=O)C)O	ZBMZVLHSJCTVON-UHFFFAOYSA-N
Stevioside	57817-89-7	C38H60O18	C[C@@]12CCC[C@@]([C@H]1CC[C@]34[C@H]2CC[C@](C3)(C(=C)C4)O[C@H]5[C@@H]([C@H]([C@@H]([C@H](O5)CO)O)O)O[C@H]6[C@@H]([C@H]([C@@H]([C@H](O6)CO)O)O)O)(C)C(=O)O[C@H]7[C@@H]([C@H]([C@@H]([C@H](O7)CO)O)O)O	UEDUENGHJMELGK-HYDKPPNVSA-N
Sucralose	56038-13-2	C12H19Cl3O8	([C@@H]1[C@@H]([C@@H]([C@H]([C@H](O1)O[C@]2([C@H]([C@@H]([C@H](O2)CCl)O)O)CCl)O)O)Cl)O	BAQAVOSOZGMPRM-QBMZZYIRSA-N
Sulfadiazine	68-35-9	C10H10N4O2S	C1=CN=C(N=C1)NS(=O)(=O)C2=CC=C(C=C2)N	SEEPANYCNGTZFQ-UHFFFAOYSA-N
Sulfadimethoxine	122-11-2	C12H14N4O4S	COC1=NC(=NC(=C1)NS(=O)(=O)C2=CC=C(C=C2)N)OC	ZZORFUFYDOWNEF-UHFFFAOYSA-N
Sulfamerazine	127-79-7	C11H12N4O2S	CC1=NC(=NC=C1)NS(=O)(=O)C2=CC=C(C=C2)N	QPPBRPIAZZHUNT-UHFFFAOYSA-N
Sulfamethazine	57-68-1	C12H14N4O2S	CC1=CC(=NC(=N1)NS(=O)(=O)C2=CC=C(C=C2)N)C	ASWVTGNCAZCNNR-UHFFFAOYSA-N
Sulfamethizole	144-82-1	C9H10N4O2S2	CC1=NN=C(S1)NS(=O)(=O)C2=CC=C(C=C2)N	VACCAVUAMIDAGB-UHFFFAOYSA-N
Sulfamethoxazole	723-46-6	C10H11N3O3S	CC1=CC(=NO1)NS(=O)(=O)C2=CC=C(C=C2)N	JLKIGFTWXXRPMT-UHFFFAOYSA-N
Sulfapyridine	144-83-2	C11H11N3O2S	C1=CC=NC(=C1)NS(=O)(=O)C2=CC=C(C=C2)N	GECHUMIMRBOMGK-UHFFFAOYSA-N

Compound name	Cas number	Molecular formula	Isomeric SMILES	InChIKey
Sulfathiazole	72-14-0	C9H9N3O2S2	C1=CC(=CC=C1N)S(=O)(=O)NC2=NC=CS2	JNMRHUJNCSQMMB-UHFFFAOYSA-N
Sulisobenzone (BP4)	4065-45-6	C14H12O6S	COC1=C(C=C(C(=C1)O)C(=O)C2=CC=CC=C2)S(=O)(=O)O	CXVGEDCSTKKODG-UHFFFAOYSA-N
Tramadol	3715-90-0	C16H25NO2	C1CCC2=C(C1)C=CC=C2NC3=NCCN3.Cl	RZOXEODOFNEZRS-UHFFFAOYSA-N
Trimethoprim	738-70-5	C14H18N4O3	COC1=CC(=CC(=C1OC)OC)CC2=CN=C(N=C2N)N	IEDVJHCEMCRBQM-UHFFFAOYSA-N
Valsartan	137862-53-4	C24H29N5O3	CCCCC(=O)N(CC1=CC=C(C=C1)C2=CC=CC=C2C3=NNN=N3)[C@@H](C(C)C)C(=O)O	ACWBQPMHZXGDFX-QFIPXVFZSA-N
Valsartan acid	164265-78-5	C14H10N4O2	C1=CC=C(C(=C1)C2=CC=C(C=C2)C(=O)O)C3=NNN=N3	USAWIVMZUYOXCF-UHFFFAOYSA-N
Venlafaxine	93413-69-5	C17H27NO2	CN(C)CC(C1=CC=C(C=C1)OC)C2(CCCCC2)O	PNVNVHUZROJLTJ-UHFFFAOYSA-N
Verapamil	52-53-9	C27H38N2O4	CC(C)C(CCCN(C)CCC1=CC(=C(C=C1)OC)OC)(C#N)C2=CC(=C(C=C2)OC)OC	SGTNSNPWRIOYBX-UHFFFAOYSA-N
Warfarin	81-81-2	C19H16O4	CC(=O)CC(C1=CC=CC=C1)C2=C(C3=CC=CC=C3OC2=O)O	PJVWKTKQMONHTI-UHFFFAOYSA-N
Zonisamide	68291-97-4	C8H8N2O3S	C1=CC=C2C(=C1)C(=NO2)CS(=O)(=O)N	UBQNRHZMVUUOMG-UHFFFAOYSA-N

Table SM 2 MS detailed parameters

Full MS parameters	
Settings	
Chrom. Peak width (FWHM)	12 s
Microscans	1
Resolution	70k
AGC target	3e6
Maximum IT	150 ms
Number of scan ranges	1
Scan range	90 to 1000 m/z
Spectrum data type	Profile
MS/MS parameters	
Settings	DIA
Microscans	1
Resolution	17.5k
AGC target	2e5
Maximum IT	Auto
Loop count	1
MSX count	1
MSX isochronous ITs	On
Top N	-
Isolation window	1.5 m/z
Isolation offset	0.0 m/z
Scan range	-
Spectrum data type	Profile
(N) CE / stepped (N) CE	NCE: 30
Minimum AGC target	-
Intensity threshold	-
Apex trigger	-
Exclude isotopes	-
Dynamic exclusion	-

Table SM 3 Targeted-DIA Inclusion list for positive ESI

Polarity	Start (min)	End (min)	(N)CE	(N)CE type	Compound Name	Mass (m/z)
Positive	4.5	5.5	28	CE	1,7 Dimethylxanthine	181.072
Positive	6.5	7.5	20	CE	1H Benzotriazole	120.0556
Positive	13.5	14.5	18	CE	4-Hydroxidiclofenac	312.0189
Positive	9.7	10.7	25	CE	5-Desamino-5 oxo 2 Lamotrigine	256.9992
Positive	9.25	10	25	CE	5-methyl-1 H Benzotriazole	134.0713
Positive	12.6	13.6	22	CE	6a-Methylprednisolone	375.2166
Positive	4	4.75	20	CE	Acetaminophen	152.0706
Positive	12	13	30	NCE	Acridone	196.0757
Positive	7	7.8	15	CE	Adamantan-1-amine (Amantadine)	152.1434
Positive	13	14.1	37	CE	Alprazolam	309.0902
Positive	4	5	25	CE	Atenolol	267.1703
Positive	10.2	11.2	44	CE	Azithromycin	749.5158
Positive	7	8.2	26	CE	Benzoylecgonine	290.1387
Positive	13.8	15	14	CE	Bezafibrate	362.1154
Positive	12	13.1	31	CE	Bromazepam	316.008
Positive	5.5	6.5	27	CE	Caffeine	195.0877
Positive	10.8	11.8	40	NCE	Carazolol	299.1754
Positive	12.8	13.8	23	CE	Carbamazepine	237.1022
Positive	13.2	14.2	20	CE	Carisoprodol	261.1809
Positive	11.3	12.4	20	CE	Carbamazepine-10,11 epoxide	253.0972
Positive	12.2	13.3	26	CE	Citalopram	325.1711
Positive	13.1	14.1	40	CE	Clarithromycin	748.4842
Positive	14	15	30	CE	Climbazole	293.1051
Positive	9.8	10.8	30	CE	Cocaine	304.1543
Positive	11.2	12.3	25	CE	Cocaethylene	318.1700
Positive	5	6.1	42	CE	Codeine	300.1594
Positive	2.5	3.4	28	CE	Cotinine	177.1022
Positive	14.2	15.2	24	CE	Crotamiton	204.1383
Positive	14.5	15.5	35	CE	Diazepam	285.0789
Positive	15	16	23	CE	Diclofenac	296.024
Positive	12.5	13.5	24	CE	Diltiazem	415.1686
Positive	8.7	9.7	20	CE	Fluconazole	307.1113
Positive	13	14	30	CE	Flumequine	262.0874
Positive	15.3	16.3	20	CE	Fluticasone propionate	501.1917
Positive	8.4	9.5	18	CE	Furazolidone	226.0459
Positive	-	-	-	-	Hydroxychloroquine	336.1837
Positive	10.2	11.4	30	CE	Hyoscine (scopolamine)	360.2169
Positive	13.8	14.8	25	CE	Irbesartan	429.2397
Positive	13.8	14.8	25	CE	Ketoprofen	255.1016
Positive	8	9.3	40	CE	Lamotrigine	256.0151

Polarity	Start (min)	End (min)	(N)CE	(N)CE type	Compound Name	Mass (m/z)
Positive	7.5	8.5	18	CE	Lidocaine	235.1805
Positive	5.5	6.7	24	CE	Lincomycin	407.221
Positive	8	9.1	19	CE	Lamotrigine-N2-oxide	272.0100
Positive	13.3	14.3	26	CE	Lorazepam	321.103
Positive	13.2	14.2	20	CE	Losartan	423.1695
Positive	15.5	16.5	21	CE	Mefenamic acid	242.1176
Positive	10.2	11.5	13	CE	Meprobamate	219.1339
Positive	13	14.2	18	CE	Methadone	310.2165
Positive	4.3	5.3	17	CE	Metronidazole	172.0717
Positive	8.7	9.7	21	CE	Metoprolol	268.1907
Positive	12.2	13.3	32	CE	Midazolam	326.0855
Positive	3	4	40	CE	Morphine	286.1438
Positive	10.5	11.6	30	CE	N-acetyl Sulfamethoxazole	296.07
Positive	12.8	13.8	32	CE	Nalidixic acid	233.0921
Positive	12.5	13.5	30	CE	Neotame	379.2228
Positive	9.6	10.7	40	CE	N-Methyl lamotrigine	270.0308
Positive	7.7	9	21	CE	O-desmethylvenlafaxine	264.1958
Positive	11.1	12.1	18	CE	Oseltamivir	313.2122
Positive	13	14	25	CE	Oxazepam	287.0582
Positive	12.6	13.6	33	CE	Paroxetine	330.15
Positive	8	9	19	CE	Primidone	219.1128
Positive	11.7	12.75	23	CE	Propranolol	260.1645
Positive	13.2	14.3	30	CE	Propyphenazone	231.1492
Positive	13.5	14.5	43	CE	Rosuvastatin	482.1756
Positive	13	14.25	20	CE	Sertraline	306.0811
Positive	10	11	24	CE	Sitagliptin	408.1254
Positive	4	5	24	CE	Sotalol	273.1267
Positive	5.2	6.2	20	CE	Sulfadiazine	251.0597
Positive	6.5	7.6	21	CE	Sulfamerazine	265.0754
Positive	7.6	9	22	CE	Sulfamethazine	279.091
Positive	7.5	8.6	16	CE	Sulfamethizole	271.0318
Positive	10.3	11.3	20	CE	Sulfamethoxazole	254.0594
Positive	11.8	12.9	35	NCE	Sulfadimethoxine	311.0809
Positive	6.2	7.4	22	CE	Sulfapyridine	250.0645
Positive	5.8	7	15	CE	Sulfathiazole	256.0209
Positive	8.9	10	15	CE	Tramadol	264.1958
Positive	6.5	8	29	CE	Trimethoprim	291.1452
Positive	14	15	17	CE	Valsartan	436.2343
Positive	11	12.5	20	CE	Valsartan acid	267.0877
Positive	11	12	27	CE	Venlafaxine	278.2115
Positive	13	14	27	CE	Verapamil	455.2904

Polarity	Start (min)	End (min)	(N)CE	(N)CE type	Compound Name	Mass (m/z)
Positive	14.5	15.5	18	CE	Warfarin	309.1121
Positive	9	10	12	CE	Zonisamide	213.0328

Table SM 4. Targeted-DIA Inclusion list for negative ESI

Polarity	Start (min)	End (min)	(N)CE	(N)CE type	Comment	Mass (m/z)
Negative	14.3	15.3	55	NCE	17-a-Ethynilestradiol	295.1703
Negative	8.6	11.6	35	NCE	4-Nitro sulfamethoxazole	282.0190
Negative	2.4	3.4	20	CE	Acesulfame	161.9866
Negative	14	15	54	CE	B-estradiol	271.1703
Negative	13.7	14.7	31	CE	Bisphenol-A	227.1077
Negative	14	15	24	CE	BP1 (2.4-Dihydroxybenzophenone)	213.0557
Negative	12	13	19	CE	BP2 (2.2'.4.4'-Tetrahydroxybenzophenone)	245.0455
Negative	9.1	10.1	34	CE	BP4 (Sulisobenzone)	307.0281
Negative	11.3	12.3	13	CE	Chloramphenicol	321.0050
Negative	8.4	11.4	10	CE	Clofibric Acid	213.0324
Negative	4.1	5.1	34	CE	Cyclamate	178.0543
Negative	11.4	12.4	55	NCE	Estriol	287.1652
Negative	14.4	15.4	80	NCE	Estrone	269.1547
Negative	14.3	15.3	30	NCE	Ezetimibe	408.1416
Negative	15.5	16.5	20	CE	Fipronil	434.9314
Negative	15.7	16.7	10	CE	Fipronil desulfinyl	386.9644
Negative	15.9	16.9	25	CE	Fipronil sulfide	418.9365
Negative	15.8	16.8	25	CE	Fipronil sulfone	450.9263
Negative	10.3	11.3	14	CE	Florfenicol	355.9931
Negative	9.2	11.2			Furosemide	329.0004
Negative	15.2	16.2	14	CE	Gemfibrozil	249.1496
Negative	5.9	6.9	29	CE	Hydrochlorothiazide	295.9572
Negative	12.9	15.9			Indomethacin	356.0695
Negative	16	17	20	CE	Irgasan (Triclosan)	286.9439
Negative	10.5	14.5	13	CE	Naproxen	229.0870
Negative	12.5	14.5			NO2-Diclofenac	338.9939
Negative	12.9	13.9	60	NCE	Pentobarbital	225.1244
Negative	12.8	13.8	25	CE	Phenytoin	251.0826
Negative	3.3	4.3	36	CE	Saccharin	181.9917
Negative	4	5	16	CE	Salicylic acid	137.0244
Negative	12.1	13.1	20	CE	Stevioside	803.3706
Negative	7.1	8.1	22	CE	Sucralose	395.0072
Negative	5.9	6.9	20	CE	Sulfathiazole	254.0063

Table SM 5. Targeted compound mass spectrometer details, ionization, retention time, precursor and product ion mass and isotopically labelled standard used.

Compound Name	Ionization	Retention time (min)	Precursor (m/z)	Product ion (m/z)	Isotopically labelled standard
1,7 Dimethylxanthine	+	4.9	181.0720	124.0506	Caffeine 13C3
17-α Ethynilestradiol	-	14.7	295.1704	267.1393	17b-Estradiol D5
1H-Benzotriazole	+	7.4	120.0556	65.0389	Benzotriazole D4
2.4-Dihydroxybenzophenone (BP1)	-	14.54	213.0557	169.0663	17b-Estradiol D5
2.2'.4.4'-Tetrahydroxybenzophenone (BP2)	-	12.5	245.0455	109.0298	Ibersartan D6
4-Hydroxidiclofenac	+	14.2	312.0189	231.0448	Diclofenac 13C6
4-Nitro sulfamethoxazole	-	10	282.0190	138.0201	Sulfamethoxazole 13C6
5-Desamino-5 oxo 2,5 Lamotrigine	+	10.5	256.9991	229.0041	Lamotrigine 13C3
5-methyl-1 H Benzotriazole	+	10	134.0713	79.0543	Benzotriazole D4
5-nitro-Diclofenac	-	13.1	338.9945	295.0031	Ibersartan D6
6a-Methylprednisolone	+	13.3	375.2166	357.2064	Carbamazepine D10
Acesulfame	-	2.8	161.9867	82.0300	Sulfamethoxazole 13C6
Acetaminophen	+	4.7	152.0706	110.0600	Acetaminophen D4
Acridone	+	12.6	196.0757	167.0724	Carbamazepine D10
Adamantan-1-amine (Amantadine)	+	7.6	152.1434	135.1169	Benzotriazole D4
Alprazolam	+	13.8	309.0901	281.0714	Alpralozam D5
Atenolol	+	4.5	267.1703	190.0862	Sotalol D6
Azithromycin	+	10.9	749.5158	591.4216	Carbamazepine D10
Benzoylecgonine	+	7.8	290.1387	168.1019	Benzotriazole D4
B-estradiol	-	14.3	271.1704	145.0664	17b-Estradiol D5
Bezafibrate	+	14.4	362.1154	316.1101	Bezafibrate D4
Bisphenol-A	-	14.2	227.1078	211.0769	BisphenolA D8
Sulisobenzone (BP4)	-	9.5	307.0282	211.0405	Furosemide D5

Compound Name	Ionization	Retention time (min)	Precursor (m/z)	Product ion (m/z)	Isotopically labelled standard
Bromazepam	+	12.7	316.0080	209.0948	Bromazepam D4
Caffeine	+	6.4	195.0877	138.0663	Caffeine 13C3
Carazolol	+	11.5	299.1754	116.1070	Propranolol D7
Carbamazepine	+	13.3	237.1022	194.0964	Carbamazepine D10
Carbamazepine-10,11-epoxide	+	12	253.0972	180.0810	Carbamazepine D10
Carisoprodol	+	13.9	261.1809	62.0240	Carisoprodol D7
Chloramphenicol	-	11.7	321.0051	152.0357	Ibersartan D6
Citalopram	+	12.9	325.1711	109.0448	Midazolam 13C6
Clarithromycin	+	13.8	748.4841	158.1178	Carbamazepine D10
Climbazole	+	14.7	293.1051	69.0700	Climbazole D4
Clofibric Acid	-	9.2	213.0324	126.9960	Bezafibrate D4
Cocaine	+	10.5	304.1543	182.1178	Cocaine D5
Cocaethylene	+	12	318.1700	196.1335	Venlafaxine D6
Codeine	+	5.8	300.1594	165.0699	Codeine D3
Cotinine	+	3	177.1022	80.0495	Cotinine D3
Crotamiton	+	14.9	204.1383	69.0337	Climbazole D4
Cyclamate	-	4.5	178.0543	79.9576	Hydrochlorothiazide 13CD2
Diazepam	+	15	285.0789	193.0886	Climbazole D4
Diclofenac	+	15.4	296.0240	215.0497	Diclofenac 13C6
Diltiazem	+	13	415.1686	178.0321	Carbamazepine D10
Estriol	-	11.8	287.1653	171.0820	Bezafibrate D4
Estrone	-	14.9	269.1547	145.0664	Ibuprofen D3
Ezetimibe	-	14.93	408.1417	271.1144	Fipronil 13C3
Fipronil	-	16	434.9314	249.9588	Fipronil 13C3
Fipronil Desulfinyl	-	16.1	386.9644	350.9882	Fipronil 13C3
Fipronil sulfide	-	16.3	418.9365	313.9654	Fipronil 13C3

Compound Name	Ionization	Retention time (min)	Precursor (m/z)	Product ion (m/z)	Isotopically labelled standard
Fipronil sulfone	-	16.3	450.9263	183.0179	Fipronil 13C3
Florfenicol	-	10.8	355.9932	119.0506	Furosemide D5
Fluconazole	+	9	307.1113	220.0681	Fluconazole 13C3
Flumequine	+	13.6	262.0874	244.0768	Carbamazepine D10
Fluticasone propionate	+	15.9	501.1917	313.1596	Fluconazole 13C3
Furazolidone	+	9.2	226.0459	95.0366	Fluconazole 13C3
Furosemide	-	9.8	329.0004	204.9848	Furosemide D5
Gemfibrozil	-	15.9	249.1496	121.0663	Gemfibrozil D6
Hydrochlorothiazide	-	6.3	295.9572	268.9467	Hydrochlorothiazide 13CD2
Hydroxychloroquine	+	6	336.1837	247.0997	Caffeine 13C3
Hyoscine (scopolamine)	+	11.1	360.2169	138.0914	Cocaine D5
Ibuprofen	-	14.74	205.1234	159.1186	Ibuprofen D3
Indomethacin	-	13.8	356.0695	297.0567	Indomethacin D3/4
Irbesartan	+	14.5	429.2397	207.0918	Ibersartan D6
Irgasan (Triclosan)	-	16.5	286.9439	118.9248	Irgasan (Triclosan) 13C6
Ketoprofen	+	14.5	255.1016	105.0336	Valsartan D3
Lamotrigine	+	9	256.0151	172.9670	Lamotrigine 13C3
Lidocaine	+	8.2	235.1805	86.0965	Benzotriazole D4
Lincomycin	+	6.3	407.2210	126.1276	Caffeine 13C3
Lamotrigine-N2-oxide	+	8.8	272.0100	242.0122	Lamotrigine 13C3
Lorazepam	+	13.82	321.0192	275.0138	Lorazepam D4
Losartan	+	13.9	423.1695	207.0918	Sulfamethoxazole 13C6
Mefenamic Acid	+	14.5	242.1176	224.1070	Diclofenac 13C6
Meprobamate	+	11.1	219.1339	158.1176	Carisoprodol D7
Methadone	+	13.8	310.2165	265.1588	Carbamazepine D10
Metoprolol	+	9.4	268.1907	116.1070	Metoprolol D7

Compound Name	Ionization	Retention time (min)	Precursor (m/z)	Product ion (m/z)	Isotopically labelled standard
Metronidazole	+	5.1	172.0717	128.0454	Metronidazole D4
Midazolam	+	12.8	326.0855	291.1167	Midazolam 13C6
Morphine	+	3.6	286.1438	201.0910	Morphine D3
N-acetyl sulfamethoxazole	+	11.4	296.0700	134.0602	Sulfamethoxazole 13C6
Nalidixic acid	+	13.45	233.0921	205.0607	Carbamazepine D10
Naproxen	-	12.4	229.0870	158.0379	Ibersartan D6
Neotame	+	13.1	379.2227	172.1332	Carbamazepine D10
N-Methyl lamotrigine	+	10.4	270.0308	200.9979	Lamotrigine 13C3
O-desmethylvenlafaxine	+	8.4	264.1958	246.1853	Venlafaxine D6
Oseltamivir	+	11.8	313.2122	166.0863	Oseltamivir D3
Oxazepam	+	13.6	287.0582	241.0528	Lorazepam D4
Paroxetine	+	13.2	330.1500	192.1187	Paroxetine D4
Pentobarbital	-	13.62	225.1245	183.1395	Valsartan D3
Phenytoin	-	13.3	251.0826	102.0353	Ibersartan D6
Primidone	+	8.7	219.1128	162.0916	Sulfamethazine D4
Propranolol	+	12.4	260.1645	116.1070	Propranolol D7
Propyphenazone	+	13.8	231.1492	189.1024	Cotinine D3
Rosuvastatin	+	14	482.1756	258.1405	Bezafibrate D4
Saccharin	-	3.8	181.9917	61.9705	Hydrochlorothiazide 13CD2
Salicylic acid	-	4.45	137.0244	93.0349	Hydrochlorothiazide 13CD2
Sertraline	+	13.9	306.0811	158.9763	Sertraline D3
Sitagliptin	+	10.6	408.1254	174.0529	Sitagliptin D4
Sotalol	+	4.6	273.1268	213.0694	Sotalol D6
Stevioside	-	12.6	803.3707	641.3193	17b-Estradiol D5
Sucralose	-	7.5	395.0073	59.0137	Sucralose D6
Sulfadiazine	+	5.9	251.0597	156.0114	Sulfadiazine D4

Compound Name	Ionization	Retention time (min)	Precursor (m/z)	Product ion (m/z)	Isotopically labelled standard
Sulfamerazine	+	7.3	265.0754	156.0114	Sulfamethazine D4
Sulfamethazine	+	8.4	279.0910	204.0440	Sulfamethazine D4
Sulfamethizole	+	8.4	271.0318	156.0114	Sulfamethazine D4
Sulfamethoxazole	+	10.6	254.0594	156.0114	Sulfamethoxazole 13C6
Sulfadimethoxine	+	12.5	311.0809	156.0769	Sulfamethoxazole 13C6
Sulfapyridine	+	6.9	250.0645	156.0114	Sulfadiazine D4
Sulfathiazole	+	6.4	256.0209	156.0114	Sulfamethazine D4
Sulisobenzone (BP4)	-	9.5	307.0282	211.0405	Furosemide D5
Tramadol	+	9.5	264.1958	58.0654	Tramadol 13C3D3
Trimethoprim	+	7.3	291.1452	230.1162	Trimethoprim D9
Valsartan	+	14.6	436.2343	207.0918	Valsartan D3
Valsartan acid	+	12.1	267.0877	206.0596	Valsartan acid D4
Venlafaxine	+	11.6	278.2115	58.0654	Venlafaxine D6
Verapamil	+	13.6	455.2904	165.0910	Carbamazepine D10
Warfarin	+	15	309.1121	163.0389	Climbazole D4
Zonisamide	+	9.6	213.0328	150.0550	Zonisamide 13C6

Table SM 6. Retention time, type of ionization and isotopically labelled compound associated of targeted compounds

			Intraday 5 ppb		Intraday 50 ppb		Intraday 500 ppb		Interday 5 ppb		Intreday 50 ppb		Interday 500 ppb	
Compound name	ESI	RT (min)	RR (%)	ME (%)	RR (%)	ME (%)	RR (%)	ME (%)	RR (%)	ME (%)	RR (%)	ME (%)	RR (%)	ME (%)
1,7 Dimethylxanthine	+	4.9	-	-	140.70	-52.21	113.69	-69.17	-	-	126.79	-38.34	101.66	-60.90
17-α Ethynilestradiol	-	14.7	54.97	-38.05	51.39	-43.17	57.68	-45.65	52.98	-39.46	49.57	-46.33	56.43	-47.64
1H-Benzotriazole	+	7.4	59.91	7.91	70.41	-50.80	81.90	-56.02	58.68	26.73	73.13	-46.78	82.33	-53.50
2.4-Dihydroxybenzophenone (BP1)	-	14.3	87.36	-14.56	92.00	-31.51	94.08	-27.10	88.15	-20.39	90.82	-33.42	92.57	-29.46
2.2'.4.4'-Tetrahydroxybenzophenone (BP2)	-	12.5	92.53	-65.31	95.89	-79.92	88.76	-62.60	94.86	-68.12	95.19	-80.55	87.65	-63.59
4-Hydroxidiclofenac	+	14.2	46.37	-42.90	62.62	-48.00	68.10	-51.00	51.87	-52.36	61.99	-46.60	66.58	-48.40
4-Nitro Sulfamethoxazole	-	10	108.04	15.00	112.70	10.63	113.98	13.14	103.71	8.30	111.70	3.15	113.13	8.64
5-Desamino-5 oxo 2,5 Lamotrigine	+	10.5	92.24	-51.86	100.81	-57.44	103.33	-46.88	95.06	-48.19	101.61	-56.02	101.93	-45.50
5-methyl-1 H Benzotriazole	+	10	105.34	116.26	90.94	-45.35	94.76	-58.27	101.99	69.59	90.10	-58.82	94.66	-50.93
5-nitro-Diclofenac	-	13.1	85.44	-59.03	91.41	-55.52	89.66	-44.00	87.92	-61.95	89.70	-58.09	88.61	-46.58
6a-Methylprednisolone	+	13.3	99.77	-57.93	118.62	-82.62	98.33	-71.73	104.15	-54.85	116.13	-81.24	97.32	-70.71
Acesulfame	-	2.8	61.33	>200	49.99	1.40	43.30	-14.30	48.90	>200	46.98	3.58	41.74	-14.43
Acetaminophen	+	4.7	66.10	-59.90	66.18	-68.50	66.47	-60.20	69.20	-47.12	75.85	-65.62	66.92	-56.78
Acridone	+	12.6	107.85	-78.59	89.56	-76.70	90.17	-76.13	107.93	-79.09	90.76	-77.57	89.90	-76.87
Adamantan-1-amine (Amantadine)	+	7.6	95.35	-43.52	97.45	-53.79	101.89	-59.28	94.22	-37.72	99.38	-52.24	100.30	-57.67
Alprazolam	+	13.8	99.34	-40.80	103.29	-53.60	103.38	-38.70	100.19	-39.31	103.99	-53.27	105.14	-39.10
Atenolol	+	4.5	73.67	-29.22	72.61	-42.16	66.78	-14.65	79.07	-18.02	81.19	-53.55	91.14	-48.30
Azithromycin	+	10.9	-	-	25.09	>200	108.99	>200	-	-	25.81	>200	110.02	>200
Benzoylecgonine	+	7.8	109.15	-52.62	110.46	-68.13	112.97	-59.51	109.50	-50.02	109.92	-67.70	113.62	-59.37
B-estradiol	-	14.5	100.04	-6.59	65.63	-30.77	70.19	-33.25	92.63	-3.00	65.88	-34.82	67.92	-36.73
Bezafibrate	+	14.4	101.58	-36.10	108.10	-51.30	107.32	-63.30	99.64	-30.15	109.03	-48.94	106.89	-61.82
Bisphenol-A	-	9.5	-	-	77.15	-38.22	72.13	-38.80	-	-	77.51	-41.95	71.88	-41.40
Bromazepam	+	12.7	96.67	74.21	76.26	32.69	80.16	-30.26	100.02	71.10	75.42	30.15	85.20	-36.29
Caffeine	+	6.4	-	-	141.56	-26.26	113.70	-55.01	-	-	145.82	-16.79	113.71	-51.88
Carazolol	+	11.5	80.67	>200	69.91	>200	73.37	141.15	85.09	>200	74.68	>200	69.89	130.83
Carbamazepine	+	13.3	103.70	-39.07	107.86	-66.72	107.13	-53.06	104.14	-37.54	106.54	-66.32	107.17	-53.19

Compound name	ESI	RT (min)	Intraday 5 ppb RR (%)	Intraday 5 ppb ME (%)	Intraday 50 ppb RR (%)	Intraday 50 ppb ME (%)	Intraday 500 ppb RR (%)	Intraday 500 ppb ME (%)	Interday 5 ppb RR (%)	Interday 5 ppb ME (%)	Intreday 50 ppb RR (%)	Intreday 50 ppb ME (%)	Interday 500 ppb RR (%)	Interday 500 ppb ME (%)
Carbamazepine-10,11-epoxide	+	12.0	109.46	-46.38	91.89	-42.90	96.70	-51.57	106.35	-50.50	90.12	-45.64	96.60	-53.14
Carisoprodol	+	13.9	96.32	-48.40	111.22	-58.30	111.48	-49.80	100.52	-45.16	108.29	-55.66	110.90	-48.27
Chloramphenicol	-	11.7	101.89	-35.20	111.08	-36.09	106.75	-21.72	103.13	-39.50	109.29	-38.47	106.55	-25.12
Citalopram	+	12.9	53.13	173.26	56.05	-5.11	65.42	-45.76	53.41	178.83	59.28	6.12	59.52	-27.54
Clarithromycin	+	13.8	67.52	-6.30	65.45	-64.00	68.26	-50.20	66.03	-3.27	65.58	-62.45	67.56	-48.60
Climbazole	+	14.7	81.38	-62.80	71.46	-71.20	86.93	-67.40	82.57	-60.94	71.66	-70.27	86.85	-66.64
Clofibric Acid	-	9.2	116.95	-6.05	113.31	-30.49	109.00	-24.07	114.01	-3.36	111.98	-27.61	109.07	-20.14
Cocaine	+	10.5	74.86	>200	79.75	133.92	86.30	-19.80	76.10	>200	79.65	140.97	87.02	-19.53
Cocaethylene	+	12.0	88.49	35.05	78.81	-8.97	84.09	-21.15	89.48	28.31	78.70	-11.82	83.82	-23.03
Codeine	+	5.8	44.17	-46.51	47.11	-49.86	59.79	-46.76	42.88	-38.02	48.61	-48.50	60.36	-46.86
Cotinine	+	3.0	151.35	5.10	125.48	-49.56	126.62	-46.30	169.62	10.01	128.96	-47.21	128.57	-46.04
Crotamiton	+	14.9	44.19	-81.00	26.31	-85.00	30.73	-79.20	44.14	-79.35	26.75	-84.23	30.52	-78.34
Cyclamate	-	4.5	25.51	38.68	21.81	-34.47	20.22	-28.47	41.63	11.56	23.78	-36.64	20.71	-30.32
Diazepam	+	15	99.60	-72.00	101.95	-68.70	99.75	-57.20	98.95	-70.33	101.23	-67.66	100.35	-56.59
Diclofenac	+	15.4	55.91	-67.70	78.75	-62.10	81.77	-66.60	64.18	-62.47	77.98	-60.80	80.87	-64.94
Diltiazem	+	13	59.94	177.86	60.34	34.12	69.03	-47.48	59.30	184.66	59.63	38.31	70.07	-47.51
Estriol	-	11.8	84.73	51.77	89.07	-22.58	88.41	-19.08	106.98	46.45	86.95	-29.07	85.33	-26.63
Estrone	-	14.9	76.67	1.04	72.66	-27.75	73.70	-35.54	81.51	-5.51	70.90	-31.30	71.54	-37.89
Ezetimibe	-	14.9	47.91	39.84	25.71	-12.74	37.09	-68.73	45.86	32.25	25.50	-16.04	35.42	-68.88
Fipronil	-	16.0	63.04	-10.24	38.74	-20.60	58.79	-17.46	63.72	-16.05	39.18	-24.55	57.26	-18.77
Fipronil desulfinyl	-	16.1	65.37	-56.68	36.68	-57.87	57.59	-46.47	63.87	-58.43	36.59	-59.50	55.93	-46.64
Fipronil sulfide	-	16.3	51.92	-71.27	31.79	-73.08	51.44	-58.70	52.62	-72.68	31.93	-74.50	49.52	-58.55
Fipronil sulfone	-	16.3	52.32	-73.57	30.16	-76.95	49.50	-67.73	51.33	-74.54	30.44	-78.02	46.18	-66.22
Florfenicol	-	10.8	100.74	-36.23	110.28	-25.02	105.47	-16.20	100.57	-41.17	109.33	-28.44	105.22	-19.62
Fluconazole	+	9.0	103.22	-41.88	108.17	-52.37	110.89	-59.33	103.34	-32.70	108.11	-48.39	110.52	-56.78
Flumequine	+	13.6	90.24	-51.76	93.72	-66.79	97.10	-64.97	90.04	-49.80	92.57	-66.23	97.78	-64.39
Fluticasone propionate	+	15.9	53.36	-82.70	28.82	-82.80	45.07	-85.00	71.50	-81.18	54.57	-81.65	82.49	-83.94
Furazolidone	+	9.2	110.73	-69.68	121.04	-74.90	110.72	-69.07	106.75	-63.10	120.65	-71.50	111.97	-66.78

Compound name	ESI	RT (min)	Intraday 5 ppb RR (%)	Intraday 5 ppb ME (%)	Intraday 50 ppb RR (%)	Intraday 50 ppb ME (%)	Intraday 500 ppb RR (%)	Intraday 500 ppb ME (%)	Interday 5 ppb RR (%)	Interday 5 ppb ME (%)	Intreday 50 ppb RR (%)	Intreday 50 ppb ME (%)	Interday 500 ppb RR (%)	Interday 500 ppb ME (%)
Furosemide	-	9.8	55.42	-40.62	72.45	-51.76	80.81	-44.91	61.95	-44.60	73.40	-53.09	80.02	-45.47
Gemfibrozil	-	15.9	46.38	-41.53	45.61	-68.37	54.93	>200	46.86	-43.41	44.74	-68.75	53.43	>200
Hydrochlorothiazide	-	6.3	180.58	25.48	111.91	-50.50	104.07	-43.99	174.47	17.76	108.57	-51.48	103.70	-46.14
Hydroxychloroquine	+	6.0	29.63	>200	50.72	>200	60.44	>200	32.37	>200	52.03	>200	64.07	>200
Hyoscine (scopolamine)	+	11.1	102.06	-36.50	106.09	-54.40	102.65	-58.90	101.09	-34.05	104.95	-52.63	103.09	-58.26
Ibuprofen	-	14.7	-	-	180.72	15.58	97.26	-37.88	-	-	174.13	14.10	93.99	-36.42
Indomethacin	-	13.8	66.22	-61.76	61.06	-61.76	61.62	-59.95	63.83	-62.81	59.64	-61.35	60.65	-58.81
Irbesartan	+	14.5	34.61	-67.30	43.34	-78.60	58.65	-75.80	36.82	-64.86	43.39	-77.00	58.45	-74.46
Irgasan (Triclosan)	-	16.5	20.57	-67.41	20.42	-87.24	45.78	-85.59	20.79	-67.61	22.69	-87.65	42.90	-84.35
Ketoprofen	+	14.5	97.63	32.10	108.59	-41.80	104.57	-59.40	94.24	36.79	107.88	-38.33	105.43	-58.59
Lamotrigine	+	9.0	96.48	-72.74	96.04	-78.41	101.57	-69.20	93.50	-69.56	95.47	-77.29	101.23	-68.11
Lamotrigine-N2-oxide	+	8.2	86.42	-67.58	92.71	-71.31	88.20	-61.96	85.99	-65.41	92.40	-70.09	88.65	-61.07
Lidocaine	+	6.3	38.16	-48.86	35.97	-58.53	55.89	-52.60	38.60	-48.18	36.00	-58.13	55.94	-52.68
Lincomycin	+	8.8	48.83	-0.99	57.66	-14.43	46.71	-55.12	40.68	27.26	56.69	-44.31	50.84	-37.74
Lorazepam	+	13.8	98.41	-24.30	105.08	-37.20	102.31	-40.40	98.23	-19.87	104.11	-34.74	100.07	-37.38
Losartan	+	13.9	18.72	-48.00	37.05	-64.60	47.37	-61.40	19.89	-45.17	37.44	-63.20	47.27	-59.96
Mefenamic Acid	+	14.5	56.03	-72.50	55.98	-82.50	48.67	-80.70	59.36	-72.22	54.64	-81.65	49.15	-80.13
Meprobamate	+	11.1	106.69	-50.28	116.66	-45.64	111.14	-43.33	107.73	-42.30	116.63	-40.20	112.28	-40.22
Methadone	+	13.8	56.37	>200	57.57	9.50	63.74	-18.80	56.80	>200	57.24	11.75	63.91	-18.27
Metoprolol	+	9.4	43.21	-10.85	63.17	-45.87	70.60	-50.87	42.80	-6.45	63.14	-44.47	70.65	-50.33
Metronidazole	+	5.1	78.47	-58.27	86.42	-61.34	85.76	-63.14	82.22	-49.53	88.06	-58.26	82.87	-59.33
Midazolam	+	12.8	64.96	-75.34	66.60	-75.94	57.79	-75.53	65.34	-75.66	65.86	-76.49	57.35	-75.84
Morphine	+	3.6	47.94	-56.95	50.15	-67.75	61.41	-57.21	47.40	-46.98	50.65	-63.49	62.84	-54.37
N-acetyl sulfamethoxazole	+	11.4	101.71	-40.72	107.44	-45.85	107.08	-54.85	100.91	-33.87	109.14	-41.76	107.04	-52.81
Nalidixic acid	+	13.4	82.38	-63.80	86.72	-71.12	94.03	-61.00	84.32	-63.09	85.76	-70.71	94.15	-60.41
Naproxen	-	12.4	-	-	136.99	-49.79	110.00	-53.66	-	-	122.86	-42.84	105.90	-50.27
Neotame	+	13.1	66.60	61.86	93.22	48.71	96.19	-38.91	73.75	53.10	92.53	53.77	97.35	-37.90
N-Methyl lamotrigine	+	10.4	99.92	-25.72	105.99	-51.18	104.46	-49.95	101.61	-22.13	105.23	-49.51	103.64	-48.75

Compound name	ESI	RT (min)	Intraday 5 ppb RR (%)	Intraday 5 ppb ME (%)	Intraday 50 ppb RR (%)	Intraday 50 ppb ME (%)	Intraday 500 ppb RR (%)	Intraday 500 ppb ME (%)	Interday 5 ppb RR (%)	Interday 5 ppb ME (%)	Intreday 50 ppb RR (%)	Intreday 50 ppb ME (%)	Interday 500 ppb RR (%)	Interday 500 ppb ME (%)
O-desmethylvenlafaxine	+	8.4	67.49	-31.01	70.39	-61.64	80.06	-51.57	68.47	-30.78	70.76	-61.11	80.51	-50.82
Oseltamivir	+	11.8	73.34	22.60	64.47	32.48	72.76	-4.77	74.07	11.52	63.59	30.43	72.23	-5.74
Oxazepam	+	13.6	100.00	-34.80	111.61	-45.70	104.35	-46.00	99.96	-30.88	112.67	-43.45	104.07	-42.71
Paroxetine	+	13.2	18.69	>200	27.32	>200	35.35	87.05	17.14	>200	26.32	>200	35.70	94.46
Pentobarbital	-	13.6	102.66	-40.48	114.66	-41.87	112.10	-31.57	103.21	-41.59	116.16	-42.24	110.41	-29.81
Phenytoin	-	13.3	99.88	-37.83	105.81	-44.44	105.66	-35.14	101.50	-38.82	106.37	-44.90	103.78	-34.60
Primidone	+	8.7	-	-	85.86	-24.39	98.84	-54.74	-	-	93.11	-28.13	97.06	-52.82
Propranolol	+	12.4	80.05	126.03	68.30	32.22	74.71	-17.11	86.04	121.76	70.65	29.05	74.25	-19.03
Propyphenazone	+	13.8	100.49	-73.80	103.57	-75.00	106.26	-54.20	100.55	-73.13	101.95	-74.93	106.33	-54.92
Rosuvastatin	+	14	99.86	-42.20	105.06	-44.20	100.87	-52.60	99.39	-41.28	105.03	-43.06	101.35	-50.86
Saccharin	-	3.8	150.00	>200	53.94	107.70	102.01	-7.23	140.00	>200	47.81	95.21	104.36	-10.75
Salicylic acid	-	4.4	149.69	>200	128.79	10.50	107.37	-50.30	130.60	>200	115.69	8.06	114.25	-58.75
Sertraline	+	13.9	36.31	>200	26.83	>200	38.11	>200	37.89	>200	27.20	>200	37.53	>200
Sitagliptin	+	10.6	37.87	2.14	47.05	-32.99	49.72	-51.45	44.25	2.04	47.12	-30.75	49.57	-50.30
Sotalol	+	4.6	73.77	-46.79	85.24	-58.60	93.11	-54.88	76.30	-38.11	86.64	-53.57	93.84	-51.64
Stevioside	-	12.6	-	-	104.65	-42.86	98.36	-31.61	-	-	102.42	-45.97	96.97	-36.62
Sucralose	-	7.5	-	-	134.63	52.36	111.71	-22.16	-	-	134.79	40.23	107.28	-26.58
Sulfadiazine	+	5.9	81.74	-1.09	65.36	-9.04	77.76	-43.27	81.34	6.19	66.05	-6.22	78.93	-42.77
Sulfamerazine	+	7.3	85.68	-12.61	71.91	-16.68	67.93	-40.56	86.32	-8.23	72.76	-23.73	78.21	-40.07
Sulfamethazine	+	8.4	85.23	-12.08	70.15	-25.41	81.24	-36.02	86.36	-9.01	69.66	-22.69	82.21	-35.61
Sulfamethizole	+	8.4	72.53	-49.11	60.93	-44.96	73.27	-56.62	73.72	-46.21	60.70	-42.77	73.92	-55.85
Sulfamethoxazole	+	10.6	52.37	5.46	74.60	-42.71	85.18	-58.51	51.90	16.00	74.59	-38.66	85.45	-56.94
Sulfamethoxine (Sulfadimethoxine)	+	12.5	116.84	-76.45	82.97	-62.06	91.51	-67.05	118.20	-77.64	83.15	-63.99	90.55	-68.23
Sulfapyridine	+	6.9	81.02	-32.56	66.88	-30.38	78.70	-48.21	81.26	-26.03	67.48	-26.26	79.43	-46.70
Sulfathiazole	+	6.4	74.68	8.50	63.45	-36.12	79.34	-54.12	74.71	12.23	64.02	-34.48	79.80	-53.73
Sulisobenzone (BP4)	-	14.2	87.22	-47.63	99.23	-47.80	89.37	-39.44	84.46	-49.54	99.41	-49.08	89.41	-40.50
Tramadol	+	9.5	42.23	-34.82	51.65	-58.32	65.89	-57.07	43.16	-32.06	51.06	-57.40	65.34	-56.30
Trimethoprim	+	7.3	90.66	-58.02	96.22	-63.78	97.03	-56.07	90.73	-54.66	96.75	-61.89	97.93	-54.96

Compound name	ESI	RT (min)	Intraday 5 ppb RR (%)	Intraday 5 ppb ME (%)	Intraday 50 ppb RR (%)	Intraday 50 ppb ME (%)	Intraday 500 ppb RR (%)	Intraday 500 ppb ME (%)	Interday 5 ppb RR (%)	Interday 5 ppb ME (%)	Intreday 50 ppb RR (%)	Intreday 50 ppb ME (%)	Interday 500 ppb RR (%)	Interday 500 ppb ME (%)
Valsartan	+	14.6	64.86	20.30	88.14	-47.60	88.09	-48.20	62.49	32.20	84.95	-42.80	87.74	-46.08
Valsartan acid	+	12.1	53.91	-22.57	80.68	-31.83	89.62	-37.72	78.62	-30.40	80.79	-29.52	89.88	-35.82
Venlafaxine	+	11.6	75.43	-31.70	65.98	-41.59	73.77	-53.44	72.84	-35.33	66.11	-44.94	72.87	-54.76
Verapamil	+	13.6	50.75	>200	46.94	58.70	51.58	-32.50	49.97	>200	46.59	63.19	51.92	31.37
Warfarin	+	15.0	87.27	-65.10	92.00	-71.90	83.07	-59.70	89.94	-65.05	91.54	-69.82	83.47	-58.27
Zonisamide	+	9.6	-	-	122.28	-48.29	114.81	-42.25	-	-	116.01	-47.80	113.84	-43.22

3.4. Discussion

The resulting analytical method demonstrated an outstanding performance for the quantification of a large variety of CECs, which enabled the analysis of samples included in the publications in Chapter 4. As mentioned previously, to develop an analytical method, there are many different variables to be taken into account. In this section, the development of the method will be further discussed and the experiments performed to establish the validated method will be described in more detail to justify the resulting method. More specifically, the chromatographic separation performance and the evaluation of several acquisition methods will be discussed. As for the method optimization of the analyte extraction, the choice of sorbents and mobile phases was based on prior group experience [13,17,21], and since reliable results were achieved, no further tests were necessary.

Chromatographic separation performance

The selection of the chromatographic column is fundamental to achieve optimal separation and the best possible selectivity. Currently, LC separation for HRMS methodologies is mainly achieved using reverse-phase mode, in agreement with our method developed [5,49]. However, to cover wide-scope contaminants depending on the column used different compounds are retained. To effectively cover as many compounds as possible the separation efficiency of different stationary phases was evaluated by comparing four types of columns including two reversed-phase C18 column (HSS T3 and BEH C18), a mixed mode column (Atlantis Premier BEH C18 AX) and a hydrophilic interaction polar stationary phase (BEH Amide), all purchased from Waters Corporation. All columns had a length of 100 mm and an internal diameter of 2.1 mm. In Table 3.4 the individual column specifications and properties are presented while in Figure 3.3 the number of compounds retained based on the compound's partition coefficient (log P) for each of the studied columns are shown to further discuss their performance.

Selectivity is a fundamental aspect when assessing chromatographic separation, as it reflects the ability of a method or material to separate and distinguish between different analytes [50]. Retention is influenced by the interaction of the analytes and the stationary phase, being the polarity of the compound a significant characteristic to consider. Log P is commonly used to predict the degree of polarity of a compound. Log P takes into account the ratio of the concentration of a compound in a lipophilic (octanol) phase over a hydrophilic (water) phase [51]. Compounds with negative log P values are generally hydrophilic, while those with positive and higher log P values are more hydrophobic. Another quantitative parameter to assess polarity is the pH-dependent distribution coefficient (log D). In fact,

log D provides a more accurate prediction of retention behaviour for ionizable compounds, as it accounts for the compound's ionization state at a given pH [52]. However, in this study log P was used to provide a general overview of the analytes' hydrophobicity or hydrophilicity.

Table 3.4. *Chromatographic column main characteristics comparison. Data resumed in this table was extracted from [53].*

Characteristics \ Columns	HSS T3	BEH C18	BEH C18 AX Atlantis Premier	BEH Amide
Ligand density	1.6 µmol/m^2	3.1 µmol/m^2	1.6 µmol/m^2	7.5 µmol/m^2
Particle size	1.8 µm	1.7 µm	1.7 µm	1.7 µm
Carbon load	11%	18%	17%	12%
End capped	Yes	Yes	Yes	No
pH range	2-8	1-12	2-10	2-11
Temperature	45ºC	60-80ºC	60ºC	90ºC
Surface area	230 m^2/g	185 m^2/g	270 m^2/g	185 m^2/g
Stationary phase	Reversed phase	Reversed phase	Reversed phase & Anion exchange	Normal, hydrophilic
Bonding	Intermediate T3 C18 bonding	Trifunctional C18	Mixed mode C18/ anion-exchange bonding	Trifunctional amide
Substrate	High Strength Silica (HSS)	Bridged Ethylene Hybrid (BEH)	BEH	BEH
Performance benefits	- 100% aqueous stability - For polar compound retention	- Extreme pH stability - Large range of compounds applicability	- Excellent retention of polar acidic analytes - Excellent pH stability - 100% aqueous stability	- Rugged HILIC stationary phase - Excellent to separate wide range of very polar compounds
Structure				

Overall, 87% of the analytes were retained in at least one of the columns, being HSS T3 the column that enabled to retain the maximum number of compounds achieving a total of 116. In agreement with literature Acquity UPLC BEH Amide column effectively retained the highly polar compounds such as aspartame (log P = -2.22), 5-fluorouacil (log P = -0.66) and sulfaguanidine (log P = -0.52) [18,32,54]. These compounds are typically challenging

to retain with low polarity columns. Despite HSS T3 was less effective in retaining the most polar compounds it was still capable of retaining other polar compounds such as lincomycin (log P = -0.32) and hydrochlorothiazide (log P = -0.58) proving good application for large variety of compounds. BEH C18 AX Atlantis also demonstrated interesting potential for highly polar compounds, however fewer compounds were retained.

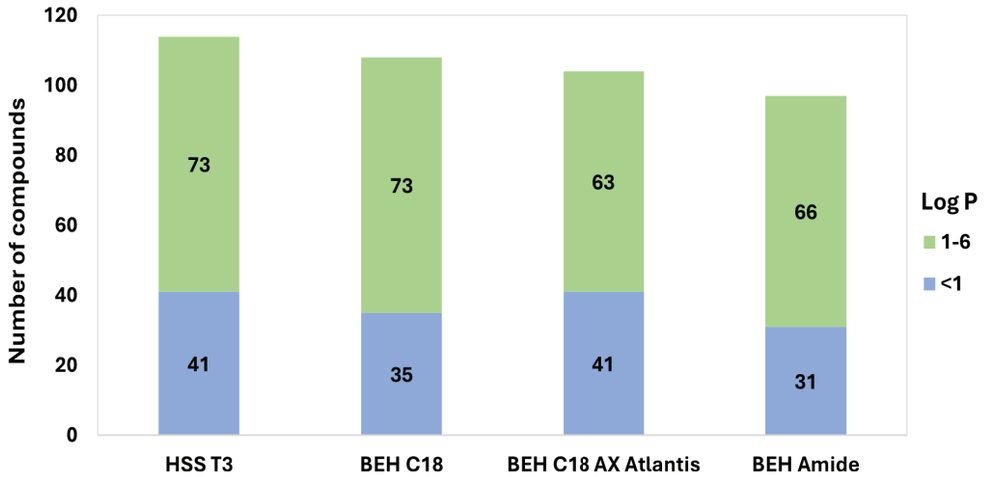


Figure 3.3. *Chromatographic column performance based on the log P of the targeted compounds.*

The superior performance of HSS T3 aligns with literature findings [55], where its polar end-capping enhances retention of small polar molecules compared to conventional C18 columns (BEH C18). Unlike standard C18, which rely on trimethylsilyl (-Si$(CH_3)_3$) groups to cap the residual silanols (improving peak shape for basic compounds but compromising polar interactions [56,57]), HSS T3 structure preserves these interactions, improving the retention of polar analytes [57]. Additionally, its larger surface area (230 m^2/g) further supports the increased polar interactions, exposing more silanol groups [56], contributing to its broad applicability, even in complex matrices [58,59].

To sum up, the HSS T3 provided the best overall retention and separation efficiency. However, for a better outreach of a larger number of polar compounds, the combination of both HSS T3 and HILIC column would be beneficial for further improving method coverage, as demonstrated by previous studies [32,60]. Future work could focus on optimizing this combined approach to achieve a wider scope of CECs in surface water.

Acquisition methods evaluation

DIA acquisition method was selected to validate the method as it provided superior performance in terms of sensitivity, selectivity, resolution and accuracy. While the results

section primarily focuses on the optimized DIA method, a comparative evaluation for the main HRMS targeted acquisition modes DDA, PRM and DIA was performed. These assessments, summarized in Figure 3.4, highlight better performance of DIA in terms of MS/MS fragmentation. The acquisition method performance and sensitivity were assessed by determining the number of product ions detected for each targeted compound at two environmentally relevant surface water concentrations: 10 ng/L (low) and 100 ng/L (high). To ensure a fair comparison across acquisitions methods, the same inclusion list was used and MS1 parameters were kept constant. Detection criteria were defined by the presence of the precursor ion (MS1), followed by at least one fragmentation ion (MS/MS), both ions within 5 ppm mass accuracy and within a retention time window of ± 0.1 min.

The tDIA method resulted in a greater number of compounds detected for which at least one product ion was observed in the MS/MS spectra for both concentration levels, compared to the DDA and PRM modes. The tDIA demonstrated its superior sensitivity and robust fragmentation, aligning with other studies that have demonstrated the application of DIA using variable isolation windows for the analysis of more complex matrices such as pesticides in fruits, vegetables and spices [61–63] and even for the analysis of veterinary residues in fish [64,65].

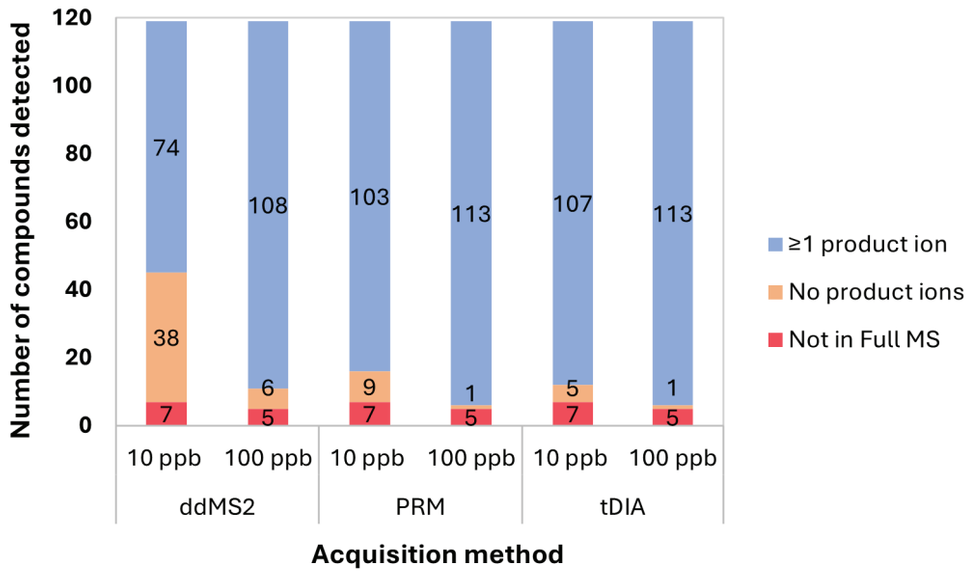


Figure 3.4. *Data acquisition methods comparison. Concentrations shown are vial concentrations in ppb, after a 1000-fold preconcentration of surface water samples.*

Moreover, although DIA is sometimes associated to a reduced number of points per chromatographic peak [38], our method optimization demonstrated that this potential

drawback can effectively be mitigated through the optimization of isolation windows and instrument settings. In fact, we successfully achieved more points per peak using the tDIA, than with DDA and PRM, further supporting its suitability for both screening and quantification.

Above all, the incorporation of optimized compound specific parameters in the inclusion list (e.g., exact mass, retention time window (±0.5min), and optimized collision energy) enabled easy and accurate MS1-MS2 ion matching, even for the fragmented ions at low signal intensities. This facilitated high-confidence identification of all targeted compounds, enhancing the reliability of the method in real sample applications.

Method performance comparison and outlook

The performance of the method, validated and published in Publication #1, confirmed its suitability for sensitive and reliable quantification of CECs in water samples. While full comparison across all validation parameters is limited by methodological differences between studies, LOQs and matrix effect offer a consistent basis for evaluation. The achieved LOQs (0.05-6.31 ng/L) were, in general, lower than those reported in the literature for HRMS methods [12,66], highlighting the method's enhanced sensitivity. An exception was noted for certain artificial sweeteners; acesulfame, cyclamate and saccharin, with LOQs of 1.36, 1.78 and 1.51 ng/L, respectively, which were slightly higher than the LOQ of 1 ng/L reported in [12]. Also, in comparison to LRMS methods, our LOQs were also lower for some compounds [67,68] and hence within the range necessary for effective environmental monitoring and quantification, even at low concentrations.

As for the matrix effect, even though the SANTE guideline [2] does not specify any specific value for this parameter, it recommends that there should be a minimal signal suppression or enhancement. In our study, the matrix effects lead mainly to ion suppression. The median matrix effects were -36%, -46% and -50% for the three validated levels respectively, while the average matrix effects were -3%, -29% and -37%, respectively. These trends suggest increased ion competition and possible ion source saturation at higher concentrations [69]. However, the use of matrix-matched calibration curves helped compensating potential matrix induced variations, either signal suppression or enhancement. Consequently, excessive matrix effects were corrected, ensuring accurate quantification. These matrix effects are consistent with values reported in other studies using HRMS for environmental analysis, where suppression in the range of -20% to -50% is commonly observed [70,71].

To sum up, a SWOT analysis outlining the *Strengths, Weaknesses, Opportunities and Threats*, is presented in Figure 3.5 to evaluate the final methodology and assess its practical implementation potential.

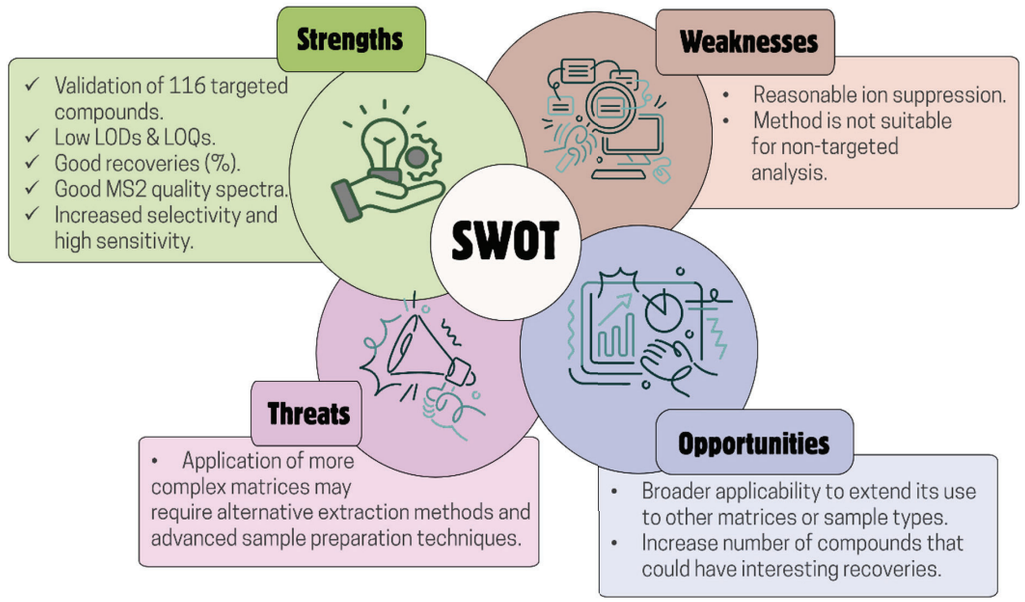


Figure 3.5. *SWOT analysis of the development methodology. (Strengths, Weaknesses, Opportunities and Threats).*

Following the SWOT analysis, the method's main advantage in enhanced sensitivity remains evident, and despite some limitations, it highlights areas for future improvement and application. As outlined in Publication #1, the method has been successfully implemented in surface water samples and shows strong potential for its broader use in other environmental monitoring matrices. In fact, it has already been implemented and validated for other water matrices such as groundwater [72], and the tDIA acquisition method has proven its effective high sensitivity in other complex matrices, such as biotic samples [73,74].

3.5. References

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Chapter 4

Characterizing Wastewater Impacts in the Mediterranean Region

4.1 Introduction

4.2 Methodology

4.3 Results

4.4 Discussion

4.5 References

Chapter 4. Characterizing Wastewater Impacts in the Mediterranean Region

4.1. Introduction

Freshwater ecosystems in the Mediterranean region are increasingly threatened by the combination of natural climate variability and intensifying anthropogenic pressures [1]. The region's characteristic climate, marked by hot and dry summer with sporadic intense rainfall in spring and autumn, leads to high hydrological variability, with many low-order streams exhibiting intermittent flow regimes [2,3]. These intermittent streams are often overlooked in traditional water quality assessments, as research has been focused on common permanent streams. Intermittent streams are increasing as water scarcity also intensifies [2,4,5].

Most river basins are subjected to several anthropogenic inputs, including WWTPs discharges, both industrial and municipal, urban and storm water runoff, affecting water quality [6]. Even though WWTPs are widely implemented to manage and treat wastewater, especially in developed countries, WWTP effluents may still contain several wastewater-borne pollutants including pharmaceuticals and other CECs, as conventional WWTP do not fully eliminate these [7]. As a result, CECs are continuously introduced in surface water behaving as pseudo-persistent contaminants, due to constant input and slow degradation rates [8]. Their contamination profiles often exhibit relatively stable concentrations over time [9], presenting a considerable challenge to the quality and safety of surface water resources globally.

Table 4.1 summarizes the occurrence of the targeted CECs in both surface water and groundwater matrices from reported studies. For surface water, studies specifically addressing intermittent streams are presented separately. While some studies have addressed the impacts of WWTP effluents on these streams, the focus has mainly been on regulated water quality parameters [10,11]. To the best of our knowledge, studies specifically on the occurrence of CECs are even more scarce, with some exceptions, for example concerning the presence of organotin compounds [12], but very few are focused on pharmaceuticals or the specific targeted compounds of this study. Additionally, challenges arise in identifying relevant studies, as the intermittent nature of some rivers is not explicitly described, or at that time were not considered as such, but some studies report hydrological patterns with distinct wet and dry seasons and flow variations [6,13–15].

Table 4.1. Concentration ranges of commonly detected CECs in surface water and groundwater.Adapted from Wilkinson et al., [16]. Abbreviations P: perennial rivers, INT: intermittent rivers, n.d.: not detected (this were only noted for contaminants in intermittent river studies) and NA: not available indicating compounds that were either not detected or not studied in the bibliographic search.

Compound name	SW (ng/L)	References P	References INT	GW (ng/L)	References
1,7 Dimethylxanthine	46.5-1,605	[17–19]	NA	1.66-104	[17–19]
17-α Ethynilestradiol	<0.98-57	[16,18,20,21]	NA	2.6-18.7	[22]
1H-Benzotriazole	3.4-56,000	[19,23]	NA	10-36,188	[19,23,24]
2.4-dihydroxybenzophenone (BP1)	1.9-39.9	[19,25,26]	NA	0.9-64.1	[19,25,27]
2.2'.4.4'-tetrahydroxybenzophenone (BP2)	0.28-7.66	[26]	NA	0.97-7.2	[19]
4-Hydroxidiclofenac	0.08	[19]	NA	13.2-154	[19,28,29]
4-Nitro Sulfamethoxazole	NA	NA	NA	0.02	[19]
5-Desamino-5 oxo 2,5 Lamotrigine	66-133.6	[19]	NA	37.7-132.2	[19]
5-methyl-1H-benzotriazole	809-2,201	[19]	NA	423-1,424	[19,24]
5-nitro-Diclofenac	0.16	[19]	NA	0.09	[19]
6a-Methylprednisolone	0.07	[19]	NA	7.05-43.1	[19]
Acesulfame	19-120,000	[19,30,31]	NA	<0.5-4,243	[19,31–33]
Acetaminophen	20.3-10,000	[7,14,16,19,21, 34–38]	[39]	0.7-1,890	[19,28,33,37,40–43]
Acridone	5.5-16.6	[19]	NA	0.5-30.3	[19,44]
Adamantan-1-amine (Amantadine)	5.3-189.4	[6,19,30]	NA	18.7-195.4	[19]
Alprazolam	n.d-13.2	[19,45]	[46]	0.6-11.4	[19,47,48]
Atenolol	<1-487	[6,14,16,18,19,37,49]	[39]	0.14-41.5	[19,33,37,40,47,49]
Azithromycin	3.6-2,356	[6,14,19,35]	NA	4.9-1,620	[28,35,47,50,51]
Benzoylecgonine	0.15	[19]	NA	0.35-20	[19,44,52]
B-estradiol	0.1-200	[7,16,20]	NA	0.02	[19]
Bezafibrate	n.d-15,000	[6,14,16,18,19]	[39,46]	1.8-31.4	[19,47,50,53]
Bisphenol-A	140-12,000	[7,16,19–21,36]	NA	268-12,000	[19,24,54,55]
Bromazepam	6-320	[45,56]	NA	NA	NA
Caffeine	3-40,000	[6,16–19,37,57]	NA	1.7-7,900	[17,19,32,33,37,40,42,48,52, 57]
Carazolol	n.d-1	[14]	[46]	NA	NA
Carbamazepine	<0.01-146,000	[14,16–19,37,57,58]	[39,46]	0.1-1,094	[17,19,24,28,32,33,35,37,43, 47,48,52,53,57–62]
Carbamazepine-10,11-epoxide	2.4-35.3	[19]	[46]	1.1-26.8	[19]
Carisoprodol	57-87	[63]	NA	12.4-58	[40,48]

Compound name	SW (ng/L)	References P	References INT	GW (ng/L)	References
Chloramphenicol	0.3-2,648	[6,19]	NA	5.8-28.1	[64,65]
Citalopram	n.d-219	[18,19,38]	[46]	7.4	[40]
Clarithromycin	5.2-2,400	[6,14,18,19,38]	[39,46]	0.7-20.5	[19,28,47]
Climbazole	2.3-27.7	[19,23]	NA	6.7-24.5	[19]
Clofibric Acid	0.02-250	[6,14,19,34,38]	NA	3.4-4,200	[19,28,44,47,53]
Cocaethylene	0.17	[19]	NA	0.06	[19]
Cocaine	0.24	[19]	NA	0.22-0.24	[19,52]
Codeine	3.8-1,000	[7,14,16,19,36]	[39]	1.7-214	[28,42,47]
Cotinine	1-181.3	[6,16,17,19,57]	NA	3-130	[17,19,57]
Crotamiton	0.6-0.8	[19]	NA	0.7-650	[19,33,54]
Cyclamate	66-112,800	[19,31]	NA	<0.1-14,829	[19,33,53,54]
Diazepam	n.d-69	[14,19,38,45,56]	[46]	0.7-100	[19,28,47,50]
Diclofenac	<0.5-19,000	[6,14,16–21,29,37,38,66]	[39,46]	0.3-7,000	[17,19,28,29,37,47,50]
Diltiazem	10.4-510.8	[19]	NA	0.02-0.5	[19,47]
Estriol	0.05-480	[6,18,19]	NA	0.13-3.1	[19,32]
Estrone	14.8-89	[18,19]	NA	0.06-20	[19,22,32,57]
Ezetimibe	NA	NA	NA	NA	NA
Fipronil	11.5-16.2	[19]	NA	0.31-0.63	[19]
Fipronil desulfinyl	0.62-12.2	[19]	NA	0.55-1.77	[19]
Fipronil sulfide	0.07	[19]	NA	0.07-28.22	[19]
Fipronil sulfone	0.05	[19]	NA	0.05-5.66	[19]
Florfenicol	31.5-99.9	[19]	NA	NA	[19]
Fluconazole	6.6-103.1	[6,17,19]	NA	6.48-124	[17,19,40,48]
Flumequine	0.07-344	[14,19,37]	NA	0.2-75	[28,33,37,47,51,52,64]
Fluticasone propionate	0.03-4.1	[67,68]	NA	NA	NA
Furazolidone	NA	NA	NA	17.6	NA
Furosemide	n.d-508.1	[6,14,19]	[46]	9.84-930	[19,28]
Gemfibrozil	4.6-7,800	[7,14,16,18,19,36]	[39,46]	<5-815	[19,28,32,53,60,69]
Hydrochlorothiazide	25-2,001	[19,37]	[46]	2-1,438	[19,37]
Hydroxychloroquine	6.02-92.7	[6,19,38]	NA	0.45-665	[19,28]
Hyoscine (scopolamine)	0.2	[19]	NA	0.06	[19]
Ibuprofen	1-2,796	[14,16,20,37,38,57]	NA	<2-65	[37,57,61,69,70]
Indomethacin	n.d-460.3	[6,14,19,37]	[46]	0.16-15	[19,37,53]

Compound name	SW (ng/L)	References P	References INT	GW (ng/L)	References
Irbesartan	6.4-160.1	[6,19,38]	NA	33-104.9	[19]
Irgasan (Triclosan)	140-2,300	[7,16,36]	NA	1.67-210	[33,55]
Ketoprofen	4.0-1,481	[6,14,19,29,34,37]	[46]	5.9-2,900	[19,22,24,27–29,37,47]
Lamotrigine	35.8-534.7	[17,19,38]	NA	15-649	[17,19,47]
Lamotrigine-N2-oxide	3.1-8.3	[19]	NA	2.4-15.9	[19]
Lidocaine	7.0-1,235.3	[6,19]	NA	0.03-76.9	[19,40,48]
Lincomycin	2-730	[19,37,38]	NA	0.3-1400	[19,33,37,47,55,64,65]
Lorazepam	n.d-643.1	[6,14,19]	[46]	16.8-60.4	[19,28]
Losartan	n.d-12.2	[6]	[46]	NA	NA
Mefenamic Acid	0.1-170	6,14,19,29,34,37]	NA	0.17-64.3	[19,27–29,37]
Meprobamate	1.4-594	[49,71,72]	NA	4.7-1,130	[19,48,49,52,73]
Methadone	2.27-12.14	[19]	NA	5.47-18.28	[19]
Metoprolol	<0.5-441	[6,14,16,17,19,20,37,38]	[39]	3.5-360	[17,19,28,37,47,50]
Metronidazole	0.1-292	[14,19,37]	[46]	21-64	[37]
Midazolam	0.76-1.61	[19]	NA	0.12-0.43	[19]
Morphine	100.8-230.3	[19]	NA	29.3-143.6	[19]
N-acetyl sulfamethoxazole	20.4-80.0	[19]	NA	0.22-5.5	[19,44]
Nalidixic acid	0.64-0.96	[19]	NA	1.14-20.5	[19,64]
Naproxen	0.22-633.4	[6,14,16,19,34,38,57,74]	[39,46]	0.03-333.7	[19,28,52,57,75]
Neotame	27	[31]	NA	NA	NA
N-Methyl lamotrigine	1.8-3.5	[19]	NA	1.65-3.66	[19]
O-desmethylvenlafaxine	621.3-1013	[19]	NA	2.74-660	[19]
Oseltamivir	2-58	[76]	NA	NA	NA
Oxazepam	0.9-61	[19,45]	NA	8.79-32.28	[19]
Paroxetine	n.d-104.3	[6,14,38]	[39,46]	30.2	[28]
Pentobarbital	2.4-45	[14,19]	NA	17.7-805.1	[19,28,54]
Phenytoin	5.9-74.3	[19,71]	NA	4.8-115	[19,48]
Primidone	0.1-179.4	[6,17,19,38]	NA	57.8-490	[19,50,73]
Propranolol	n.d-68.5	[6,14,19,38]	[46]	0.1-9.4	[19,28,33]
Propyphenazone	n.d-2,000	[14,18,19,29]	[39,46]	3.2-121	[19,28,29]
Rosuvastatin	0.15	[19]	NA	0.73-4.32	[19]
Saccharin	14-16,800	[17,19,23,31,66]	NA	0.11-13,301	[17,19,23,32,33,53]
Salicylic acid	29-309	[29,38,77]	NA	1-464	[29,77]

Compound name	SW (ng/L)	References P	References INT	GW (ng/L)	References
Sertraline	n.d-49	[6,19,38]	[46]	0.2-2.1	[19]
Sitagliptin	412-761	[19]	NA	0.03-0.83	[19]
Sotalol	0.7-164.7	[6,14,17,19]	[39,46]	8-33	[17,19,28,32,47]
Stevioside	NA	NA	NA	NA	NA
Sucralose	20-43,600	[19,31]	NA	4.5-50	[19,31,32,53]
Sulfadiazine	n.d-19,000	[14,18,19,37]	[39]	5.8-208	[19,28,37]
Sulfadimethoxine	141-15,000	[37,38]	NA	0.45-65.5	[33,37,52,64]
Sulfamerazine	1.5	[38]	NA	0.15-65.5	[19,33]
Sulfamethazine	1.4-114	[6,19,37]	[39]	5.6-160	[19,28,37,78]
Sulfamethizole	109-130	[37,38]	NA	0.35-28.7	[19,37,64]
Sulfamethoxazole	1-13,800	[14,16–19,37,66,79]	[39,46]	0.2-965	[16,17,19,28,33,37,42,43,47, 48,51,52,57,65,80,81]
Sulfapyridine	0.1-12,000	[6,17–19]	NA	4.32-56.4	[17,19]
Sulfathiazole	0.03-419	[6,19,37,38]	NA	2.5-46.2	[19,37,82]
Sulisobenzone (BP4)	<1-5,002	[16,19,25,26,83]	NA	0.2-36.6	[19,25]
Tramadol	52-7,700	[6,17–19]	NA	0.9-186	[17,19,48]
Trimethoprim	<1-2,560	[19,34,37,57,79]	[39,46]	0.2-730	[19,27,32,33,35,37,57]
Valsartan	6.2-7,500	[6,17–19]	[46]	5.85-2,762	[17,19,52]
Valsartan acid	1.84-2,167	[19]	NA	1.84-888	[19]
Venlafaxine	1.1-580	[16,18–20,66]	NA	1.05-84.3	[19,47]
Verapamil	n.d-1.42	[6]	[46]	0.03-0.61	[19]
Warfarin	0.58-1.42	[19]	[46]	0.3-5.13	[19,32]
Zonisamide	n.d-0.56	[19]	[46]	NA	NA

Intermittent streams are particularly vulnerable during dry periods of low flow, when dilution is minimal and WWTP effluents can become a dominant input source [84,85]. This vulnerability is especially critical in the MENA region, which faces some of the highest levels of water stress globally [86,87].

Surface waters are connected to shallow groundwater systems, enabling the infiltration of CECs from different diffuse or point sources [88]. In many Mediterranean and semi-arid areas, such as Tunisia, groundwater contamination is becoming of increasing concern due to the rise in water demand and the high dependency on groundwater as irrigation and drinking alternatives [89]. Groundwater is a vital water source, particularly in rural and semi-arid regions, where it often serves as the primary water supply [90]. In Tunisia, groundwater accounts for more than 50% of the country's total available water resources, and approximately 83% of the rural population relies on wells and boreholes for domestic use [91,92]. The vulnerability of shallow aquifers in densely populated or intensively farmed areas heightens the risk of contamination and underscores the need for comprehensive chemical assessments.

Many MENA countries, including Tunisia, have developed national water laws and policies that reflect principles of the Integrated Water Resources Management (IWRM), aiming to promote sustainable and coordinated use of water resources [93]. While these frameworks align and share similar principles to the EU WFD [94], these are often less comprehensive and less integrated. As a result, water quality monitoring and regulations are still developing. In Tunisia, significant progress has been made through the implementation of several legal instruments, including the amended Water Code [95] and the efforts of the National Sanitation Utility (ONAS) [96], which particularly focused on improving coastal regions where population density is higher. Nevertheless, various industries continue to discharge inappropriately their treated wastewaters into receiving streams, representing a serious threat to the freshwater ecosystem and shallow groundwater quality [97,98].

This chapter focuses on Mediterranean intermittent streams, largely affected by CECs contamination, and consequently their associated groundwater. North Mediterranean streams including sites from South European countries such as Spain, France and Italy, and South Mediterranean streams from the MENA countries, Tunisia and Algeria were studied. In the latter countries, WWTPs are sometimes over-exploited, and many industries directly release their wastewater to the river basins [99]. In addition to this, the lack of regulations regarding CECs concentration in surface water and the limited monitoring further underscore the need to investigate their presence and impact. While research on the occurrence of CECs in surface water and its associated groundwater has been continuously

increasing in European countries, United States and China, this has not been the case for the MENA countries, where studies remain lower than that reported in developed countries as previously reported [66,100,101]. This highlights the important knowledge gaps in low- and middle-income regions where regulations and water treatment infrastructures are limited.

Given the combined anthropogenic and climatic pressures in Mediterranean streams, there is an important need to assess the environmental status of their freshwater through integrated and site adapted approaches [102]. Achieving good ecological status is outlined in the WFD [94]. An effective way to address this challenge is through the application of environmental indicators, which are measurable variables, that allow the evaluation of ecosystem health, pollutions levels and influence of stressors over time. Indicators reflect the effects of stressors helping to understand and manage environmental health. To achieve this, chemical indicators, such as CECs monitoring, hydrological indicators and biological indicators serve as powerful tools. Among the latter, diatoms, are highly sensitive to environmental conditions, making them excellent bioindicators as they provide insights on relevant factors such as salinity, nutrient levels, organics pollution and hydrological disturbances [103]. On the other hand, benthic invertebrates, including for example insects, crustaceans or molluscs, also play a crucial role in stream ecosystems. Changes in their diversity, abundance and community structure can indicate specific environmental stressors [3,104].

Hydrological indicators are also fundamental when evaluating water quality and offer valuable tools for predicting pollutant concentrations and fate as well as assessing environmental risks [105,106]. However, the lack of hydrological data, particularly for intermittent rivers limits effective monitoring and modelling efforts [107].

In this context the objectives of this chapter were to study the effects of anthropogenic sources across the Mediterranean basin through the assessment and understanding of the effect of variable seasonal flow, the contamination of associated groundwater as well as performing a holistic approach covering chemical, ecological and hydrological indicators.

4.2. Methodology

4.2.1. Sampling collection

In this chapter water and biota samples from five Mediterranean countries, including surface water and groundwater, were collected.

Water sampling

In Figure 4.1 the countries studied, sampling region, sample water type and number of samples (N) are shown. Different intermittent streams from each country were sampled where the occurrence of CECs before and after the introduction of treated wastewater was analysed, thus several points along each study site were sampled. Associated groundwater samples from Tunisia were also sampled (Figure 4.1). Sampling campaigns for both matrices were performed during 2020 and 2023. A total of 291 samples were analysed, including 22 Mediterranean streams and 13 associated groundwater samples from Tunisia. This resulted in 3 different publications.

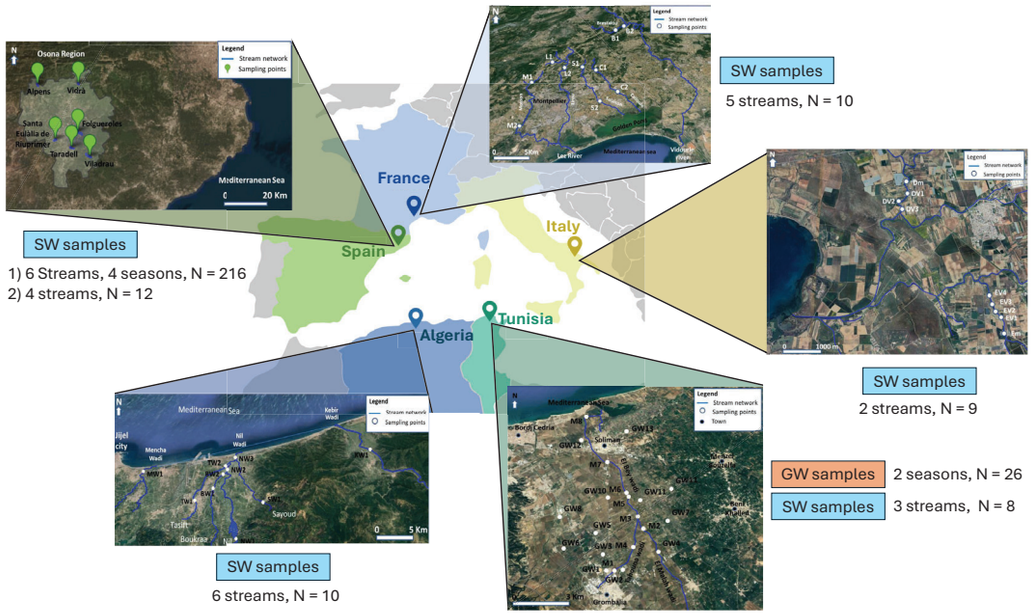


Figure 4.1. *Map of the five Mediterranean countries sampling included in this chapter, indicating location of sampling sites, number of streams, seasons, and number of samples (N).*

For the analysis of CECs, the method described in Publication #1 was applied in all studies [108]. For groundwater samples from Tunisia, the applicability of the method in groundwater was proved and validated in [19].

Physico-chemical parameters including temperature, pH, electrical conductivity and dissolved oxygen were measured in-situ with a portable water quality multiparameter probe (YSI professional plus, YSI Incorporated, USA). Water samples for the analysis of inorganic ions were filtered in-situ using 0.2 µm nylon filters (Merck, Germany). Phosphates were measured using colorimetric methods, while nitrates, nitrites and other inorganic ions were determined using ion chromatography. The analysis and reporting of inorganic ion concentrations were carried out in collaboration with the BETA Technological

Center analytical service (University of Vic, Spain).

Diatom and benthic invertebrate sampling

The interplay between diatoms and benthic invertebrates offers a comprehensive view of the ecological dynamics in intermittent rivers, particularly when trying to evaluate the streams response to anthropogenic pressures or Mediterranean dry summer periods. Sampling sites were selected from five countries, as shown in Figure 4.1, representing both impacted and unimpacted conditions. Sites were chosen to capture a degradation gradient, where both diatoms and benthic invertebrates were sampled for the study outlined in Publication #4. The methodology for sample collection is detailed in Publication #4. In Figure 4.2, examples of diatom and benthic invertebrate sampling collection conducted during field campaigns in Spain and France are shown, where I personally participated. These were conducted in 2021 during the COVID-19 pandemic, while for the remaining countries, field campaigns took place in 2022 (Italy) and 2023 (Tunisia), after travel restrictions had been lifted. Diatom and benthic invertebrate samples unfortunately were not possible in Algeria due to travelling restrictions.



Figure 4.2. *Diatom (left) and benthic invertebrate (right) sampling campaigns in Osona, Spain, and Montpellier, France, respectively during 2021.*

4.2.2. Environmental risk assessment

In all publications included in this chapter the following methodology was applied for the evaluation of the ERA, following the European guidelines. Individual RQ were calculated for each CEC through Eq. 4.1. PNEC values were obtained from the Norman

network database [109], where the lowest PNEC values in freshwater were used.

$$Risk\ quotients\ (RQ) = \frac{Measured\ environmental\ concentration\ (MEC)}{PNEC} \qquad \text{Eq. 4.1}$$

Based on the calculated RQ values, a range of risks from low to high are identified, where RQ ≥ 1 indicates a high risk, 0.1 ≤ RQ < 1 a medium risk, 0.01 ≤ RQ < 0.1 a low risk and finally RQ < 0.01 indicates an insignificant risk for aquatic ecosystems. In this chapter, RQs were calculated for the worst-case scenario, maximum concentrations detected were used for those compounds above PNEC in at least one sample.

4.3. Results

This chapter includes the following publications:

- Publication #2: **O. Gómez-Navarro,** L. Bertrans-Tubau, M.Abril, N. Montemurro, L. Proia, S. Pérez, Unveiling the impact of wastewater effluents on Mediterranean streams with seasonal flow variability. Submission to Environmental Science & Technology

- Publication #3: F. Khezami[1], **O. Gómez-Navarro**[1], M.V. Barbieri, N. Khiari, A. Chkirbene, S. Chiron, S. Khadhar, S. Pérez. (2024). Occurrence of contaminants of emerging concern and pesticides and relative risk assessment in Tunisian groundwater, Science of The Total Environment, 906, 167319. https://doi.org/10.1016/j.scitotenv.2023.167319

[1] Co-first authorship, authors contributed equally to this work.

- Publication #4: **O. Gómez-Navarro**, A.M. De Girolamo, A.W. Lorenz, S. Khadhar, T.H. Debieche, F. Gentile, S. Chiron, S. Pérez. (2024) Characterization of anthropogenic impacts in Mediterranean intermittent rivers with chemical, ecological and hydrological indicators, Journal of Hazardous Materials, 480, 135951. https://doi.org/10.1016/j.jhazmat.2024.135951

Publication #2

Unveiling the impact of wastewater effluents on Mediterranean streams with seasonal flow variability

O. Gómez-Navarro, L. Bertrans-Tubau, M. Abril, N. Montemurro, L. Proia, S. Pérez

Article

Unveiling the impact of wastewater effluents on Mediterranean streams with seasonal flow variability

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ABSTRACT

The Mediterranean region is characterized by its distinct climate, with hot dry summers, rainy autumn and spring and mild winters, which makes the Mediterranean ecosystem very rich and diverse, but also very sensitive to environmental changes. Climate change is increasingly impacting this region, leading to more frequent and severe droughts, altered precipitation pattens and rising temperatures as well as transforming perennial streams (those with year-round flow) into intermittent ones (those with seasonal flow). In dry periods or arid regions, wastewater discharges can be the only consistent source of flow in intermittent rivers. The aim of this study was to evaluate six streams in Northeast Spain representative of Mediterranean basins with variable flow regimes through the analysis of the presence and fate of contaminants of emerging concern (CECs) released by wastewater treatment plant (WWTP) discharge effluents. The WWTPs spatial and seasonal variability, flow contribution and the environmental risk associated to the detected CECs are discussed. A comprehensive analysis revealed the presence of 89 compounds including pharmaceuticals, personal care products and sweeteners, highlighting the impact of WWTP as primary sources of CECs. The results evidenced a high environmental risk associated with the concentration of eleven compounds. A comprehensive approach was performed to classify the streams based on the effluent contributions for flow rate, CECs and nutrients, observing situations of effluent-driven contributions. The results revealed the high sensitivity of these streams to hydrological stress whereas seasonal variations were less influential, due to COVID-19 restrictions. The study on the contribution of the WWTPs on the river system emphasized the importance of analysing WWTP discharges in relation to river flow, particularly in watercourses with high seasonal flow fluctuation to avoid scenarios of WWTP effluent-dominated streams. This study highlights the need to adopt regulatory measures based on the dilution capacity of the receiving streams to better protect the aquatic system, especially considering the expected increase in extreme climate conditions.

1. Introduction

Worldwide, freshwater ecosystems face numerous challenges from both natural and human-induced factors shifting some perennial streams to an intermittent flow state [1]. The Mediterranean Basin, which is characterized by hot, dry summers, intense rain events in autumn and spring and mild wet winters [2,3], has faced significant droughts in the past decade, and droughts are predicted to become more frequent and severe in the future [4]. These climatic patterns greatly influence the streams hydrology, often leading to high seasonal flow variability, and even flow intermittency. During summer, Mediterranean streams may experience largely reduced flow, form isolated pools, or completely dry up, depending on local geology, microclimate, and topography [5–7]. Low-order streams, which are the origin of the larger river network, often exhibit characteristics of intermittent rivers due to their dependency on rainfall and limited groundwater recharge. Despite their modest size, they are key contributors to the overall watershed function, supporting a variety of terrestrial and aquatic species and influencing nutrient cycling, water quality, sediment transport, and all processes related with ecosystem services provision [8,9]. However, beyond natural flow variability, these streams are subjected to significant anthropogenic

pressures including water extraction, agricultural pollution, urban development, and land-use changes, all of which exacerbate their natural variability and contribute to the degradation of these ecosystems. Among these pressures, wastewater treatment plants (WWTPs) represent a major source of alteration, as their effluents, although treated, still contain nutrients and organic pollutants such as contaminants of emerging concern (CECs) that can disrupt receiving streams [10]. This impact is particularly pronounced in Mediterranean regions during extended reduced flow periods when low flow streams, in contrast to perennial rivers, are especially vulnerable to such disturbances because of negligible WWTP effluent dilution at discharge points[11]. In case of extremely low or absent flow, WWTP effluents has been shown to account for between 3 to 100% of the receiving water flow, generating pronounced changes in water quality, ecological and physical status [10,12–15]. These low flow conditions lead to increased concentrations of nutrients and CECs [16], while in some cases, constant inflow from WWTPs may ensure perennial conditions at the cost of becoming effluent-dominated streams.

Research on Mediterranean flow-variable regime streams has mainly focused on nutrient pollution, including phosphates, nitrates and ammonium, originating from diffuse (e.g. agricultural runoff) and point sources (e.g. WWTP effluents) [17,18]. These inorganic pollutants can pose significant risks to water quality and the overall status of aquatic ecosystems [17–20]. In contrast, while the occurrence and risks of CECs in surface waters have been largely investigated [21–23], only a limited number of studies have reported their presence in low-flow streams, and research on the associated risks remains scarce [24,25]. Seasonal hydrology and climate patterns of the Mediterranean region influence chemical exposure, bioavailability and the responses and dynamics of biological communities [26,27]. While some studies explore the effects of contaminants on macroinvertebrate communities and fish [28–31], as well as seasonal and spatial distribution patterns [32,33], further research is needed to understand the combined impacts of nutrient and CECs contamination in flow-variable regime streams. Evaluating the effects of multiple stressors associated with human-induced contamination in low-flow streams is particularly challenging, due to the complexity of stressors, dynamic flow conditions and diverse ecological responses.

In this context, this study aimed to examine the influence and impacts of WWTP effluents on six low-flow streams from the Osona county in Catalonia, affected by common Mediterranean climate with direct WWTP effluent discharge. The main objectives being to: (1) Assess the effect of nutrients and CECs in six Mediterranean streams by analysing 3 strategic sampling points: (i) upstream the WWTPs; (ii) the WWTP effluent; (iii) downstream the WWTPs. (2) To study the spatial-temporal patterns of the different WWTP effluents on the selected streams, (3) To relate the fate of CECs to key environmental and chemical variables that influence their transport, (4) To evaluate and classify WWTP contributions to the receiving streams, (5) To perform an environmental risk assessment of the detected CECs.

2. Materials and methods

2.1. Chemicals and reagents

In this study, 100 CECs were selected for target analysis based on their high consumption/usage and documented environmental relevance, as reported by the European Commission and the European Chemical Agency, alongside previous studies on their occurrence [34–36]. The target compounds encompass a wide range of chemical with difference physicochemical characteristics, which are classified in the following categories; analgesics / anti-inflammatories (15%), anti-hypertensives (10%), antimicrobials (22%), hormones (4%), illicit drugs (3%), industrial compounds (2%), lipid regulators (4%), psychiatric drugs (18%), stimulants (3%), sweeteners (6%), UV filters (3%) and *others* (10%). Further information on specific details of the target compounds can be found in Table SM1 in the supplementary material

2.2. Description of the study area and samples collection

This study was conducted in six municipalities located in the Osona region, in Catalonia, Spain (Fig. 1). All six municipalities have their own WWTP which discharge its effluent into the corresponding receiving stream. The receiving streams are all low-order streams, meaning they originate from the upper zone and are small headwater tributaries, typically the initial contributors to larger rivers [37]. Detailed information is presented in Table 1, including the municipalities population, main land use, the name of the stream where the WWTP effluent discharges and information on the WWTP present at each site (information was extracted from the Statistical Institute of Catalonia (IDESCAT) [38] and the Catalan Water Agency (ACA) [39]) Two WWTPs include full biologic, nitrogen and phosphorus treatment (Taradell (TAL) and Santa Eulàlia de Riuprimer and (SEP)), while the remaining only include biologic treatment. The WWTP from TAL has the highest population equivalent design, while the one in Vidrà (VID) has the lowest, matching also the actual municipality population trend.

2.3. Sample collection

Surface water samples were collected using a 1 L amber PET bottles. Samples were filtered at 0.7 µm

Table 1. *Study area information extracted from IDESCAT [38] and ACA [39]*

Municipality	Land use	Population 2021	WWTP[2]	Discharge point	Treatment	Design Q[5] (L/s)	Design PE[6] (h/e)
Alpens	Forest	348	ALP	Riera d'Alpens	B[3]	2.3	833
Folgueroles	A+L[1]	1049	FOL	Torrent Folgueroles	B	14.5	2500
Taradell	A+L	6270	TAL	Riera de Taradell	BNP[4]	23.2	11667
Santa Eulàlia de Riuprimer	A+L	2195	SEP	Riu Meder	BNP	5.8	2000
Vidrà	Forest	182	VID	Riu Ges	B	2.2	752
Viladrau	Forest	1071	VLD	Riera Major	B	15.2	3499

[1]A+L: agriculture and livestock; [2] WWTP acronym name, [3]B: biologic; 4BNP: biologic, nitrogen and phosphorus; [5]Q: flow rate, [6]PE: population equivalent.

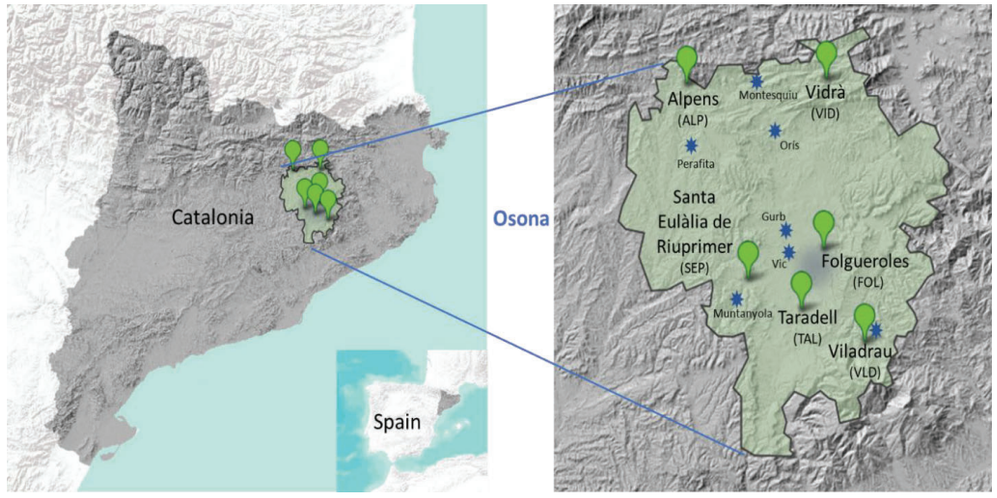


Fig. 1. *Sampling sites map with the six studied municipalities in green and the weather stations marked with a blue star.*

using glass fibre filters (GF/F from Whatman, UK) and stored in the freezer at -20ºC until extraction. For each study area (Fig.1), samples were collected: upstream of the WWTP location, at the WWTP discharge point and downstream point. Seasonal campaigns were carried out in the same way, in July 2020 (summer), November 2020 (autumn), March 2021 (winter), and May 2021 (spring) to cover seasonal variability. Meteorological data was also collected from 7 weather stations located in Osona [40] (Fig.1). Further information on precipitation is provided in Fig. SM1.

2.4. Water analysis: physico-chemical and nutrient analysis

Temperature, pH, electrical conductivity (EC) and dissolved oxygen were measured in-situ with a portable water quality multiparameter instrument (YSI professional plus, YSI Incorporated, USA). For the analysis of dissolved organic carbon (DOC) and inorganic ions, water samples were collected and filtered in situ using 0.20 µm pore-size nylon filters (Merck, Darmstadt, Germany). DOC was measuresd using a Multi 2100 N/C TOC Analyser (Analytik Jena AG; Jena, Germany), while ammonium ($N\text{-}NH_4^+$, mg $N\text{-}NH_4^+$ L^{-1}) and soluble reactive phosphorus (SRP) ($P\text{-}PO_4^{3-}$, mg $P\text{-}PO_4^{3-}$ L^{-1}) were determined using colorimetric methods with a bioNova nanophotometer (IMPLEN; München, Germany). Nitrate (NO_3, mg $N\text{-}NO_3^-$ L^{-1}), nitrite (NO_2^-, mg $N\text{-}NO_2^-$ L^{-1}), chloride (Cl^-, mg Cl^- L^{-1}) and sulphate (SO_4^{2-}, mg SO_4^{2-} L^{-1}) were analysed using ionic chromatography with a Dionex Integrion HPI System (Thermo Fisher Scientific; Sunnyvale, CA, USA) and an anionic column (Dionex IonPac AS18-Fast-4mm).

To calculate stream flow above the WWTP, the water travel time was measured using a conservative tracer, NaCl in this case. [33,41]. Briefly, the method involves the addition of fixed amount of NaCl to the stream to achieve an increase in EC of 20-50 mS/cm. The EC was then recorded at 5 second intervals downstream until a sudden increase was observed, indicating the arrival of the tracer. The flow rate was then calculated by integrating the EC curve to estimate the travel time, and hence, the stream flow rate. Flow rate measurements were taken after collecting water samples.

2.5. Sample extraction and analysis of CECs

Samples were processed using a previously published method for CECs analysis [42]. Briefly, 500 mL of sample were extracted by solid-phase extraction with custom made multi-layered and multi-sorbent cartridges. Prior to the extraction, samples were spiked with a mixture of internal standards, to ensure method performance. After loading, analytes were eluted with a series of solvent mixtures, evaporated and reconstituted in 500 µL, achieving an enrichment factor of 1000. Analysis was conducted using reversed-phase liquid chromatography coupled to a high-resolution mass spectrometer on a Q-Exactive Orbitrap (Thermo-Fisher Scientific, Germany). Chromatographic separation was performed using a Waters Acquity UPLC HSS T3 (Waters, Milford, MA), using both positive and negative ionization modes and an injection volume of 10 µL. The analytical method validation was also reported previously in [42], with limits of detection (LOD) and quantification (LOQ) ranging from 0.01 to 1.9 ng L^{-1} and from 0.05 to 6.3 ng L^{-1} respectively.

2.6. Data analysis

To evaluate the WWTP impact on the streams, a relative contribution of the WWTP effluent in terms of flow rate was calculated using Eq. 1. A CECs dilution factor was also calculated using Eq. 2 to further assess the effect of the WWTP discharge point contribution, based on the total concentration of CECs. Additionally, a mass load approach was also employed, considering the WWTP discharge point and downstream points of the study area system by applying Eq. 3. The equations used are shown below where Q is the flow rate in L/s for the upstream (US), discharge point (DP) and downstream (DS) sampling

points and ∑C represents the sum of the concentration of all the detected contaminants in ng/L.

$$\text{WWTP contribution to flow rate at DS (\%)} = \frac{Q_{DP}}{Q_{DS}} \, x \, 100 \qquad \text{Eq. 1}$$

$$\text{CECs dilution (\%)} = \left(1 - \frac{\sum C_{DS}}{\sum C_{DP}}\right) x \, 100 \qquad \text{Eq. 2}$$

$$\text{Load contribution} = \frac{\sum C_{DP} \, x \, Q_{DP}}{\sum C_{DS} \, x \, Q_{DS}} \qquad \text{Eq. 3}$$

2.7. Statistical analysis

Statistical analysis was performed using the open-source software R studio (version 4.4.0, 2024.04.2 + 764) with the packages “stats”, “ggplot2”, “ggpubr”, “dplyr” “tidyr”, “readxl”, “FactoMineR”, “corrplot” were employed. Compounds below LOQ in all samples were excluded and for those cases where compounds were not detected or detected at level below LOD or LOQ, LOD/2 or LOQ/2 were used for statistical purposes, (n = 78).

The distribution of the data was tested using the Shapiro Wilks test to which a non-normal distribution was obtained for our data. Hence nonparametric tests were employed. Specifically, Kruskal-Wallis test was used to evaluate differences among the sample types (i.e., US, DP, DS) and across seasons when more than two groups were involved. For pairwise comparisons between groups, Dunn’s post-hoc test was used following the Kruskal-Wallis test to determine which specific groups differed significantly. Bonferroni correction was applied to adjust p-values for multiple comparisons. Statistical significance was determined using a threshold of $p < 0.05$.

A principal components analysis (PCA) was also performed for the WWTP effluents to evaluate possible differences between WWTPs. The data used was normalized by log transformation to handle skewed data and scaling the data to ensure each variable contribute equally to the analysis regardless the original values. Additionally, a Spearman rank correlation analysis was performed to evaluate the relationships and patterns among multiple variables in the DS points.

2.8. Environmental risk assessment

Ecological risk assessment (ERA) was performed based on the established criteria according to the European Union technical guidance documents on risk assessment [43]. In this sense, risk quotients (RQs) were calculated for each compound by calculating the relationship between the measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC). PNEC values were extracted from the NORMAN Ecotoxicology Database [44] where the lowest PNEC value for freshwater was considered. The corresponding environmental risk associated to the compounds was specified based on the RQ values obtained according to [45] where:

- RQ ≥ 1 indicates a high risk for aquatic ecosystems.
- 0.1 ≤ RQ < 1 indicates a medium risk for aquatic ecosystems.
- 0.01 ≤ RQ < 0.1 indicates a low risk for aquatic ecosystems.
- RQ < 0.01 indicates an insignificant risk for aquatic ecosystems.

3. Results and discussion

3.1. Occurrence of contaminants of emerging concern

Of the 100 target compounds analysed, 89 were detected in at least one sample, of which 11 compounds were only detected at LOQ levels. Sixteen compounds were present at a frequency of detection (FoD) above 90% being acetaminophen benzotriazole, bisphenol-A, tramadol, venlafaxine the five compounds most frequently occurring. The highest concentrations detected, however, corresponded to acesulfame, caffeine, diclofenac, hydrochlorothiazide, and sucralose, with concentrations similar to those detected in other studies [46,47]. These findings are in agreement with previous findings as these are easily detectable compounds, due to their high consumption and persistence, as many are not fully removed during WWTP processes [48,49]. The artificial sweeteners, acesulfame and sucralose along with hydrochlorothiazide, a common diuretic, are particularly resistant to degradation, which allows

them to persist in treated wastewater and natural aquatic environments [50].

In Fig. 2 the total concentration per CECs class for each season, WWTP and sample type (US, DP and DS points) is shown. The dominant categories were artificial sweeteners, *others*, analgesics/anti-inflammatories and psychiatric drugs. In fact, the artificial sweeteners class prevailed in most of the cases, accounting up to a 75% of the total concentrations (Fig 2). Such high concentrations corresponded to the artificial sweeteners acesulfame, saccharin and sucralose. Specifically, sucralose, was the compound with highest concentration in 58% of the studied samples, reinforcing its known environmental occurrence with its high FoD agreeing with previous studies [47,51,52]. Our results show the clear impact of the WWTP effluent in all seasons and streams, consistent with previous research [24,53,54].

In most cases, a significant increase in CECs concentration was observed at the downstream sites compared to the upstream sites, confirming the role of the WWTP discharge as primary contributor of CECs contamination to the receiving water bodies. In general, the seasons in which the highest total concentrations were observed were winter and spring, especially in the SEP WWTP, which was the one with the highest total CECs concentration. This trend can be partly explained by changes in human activity

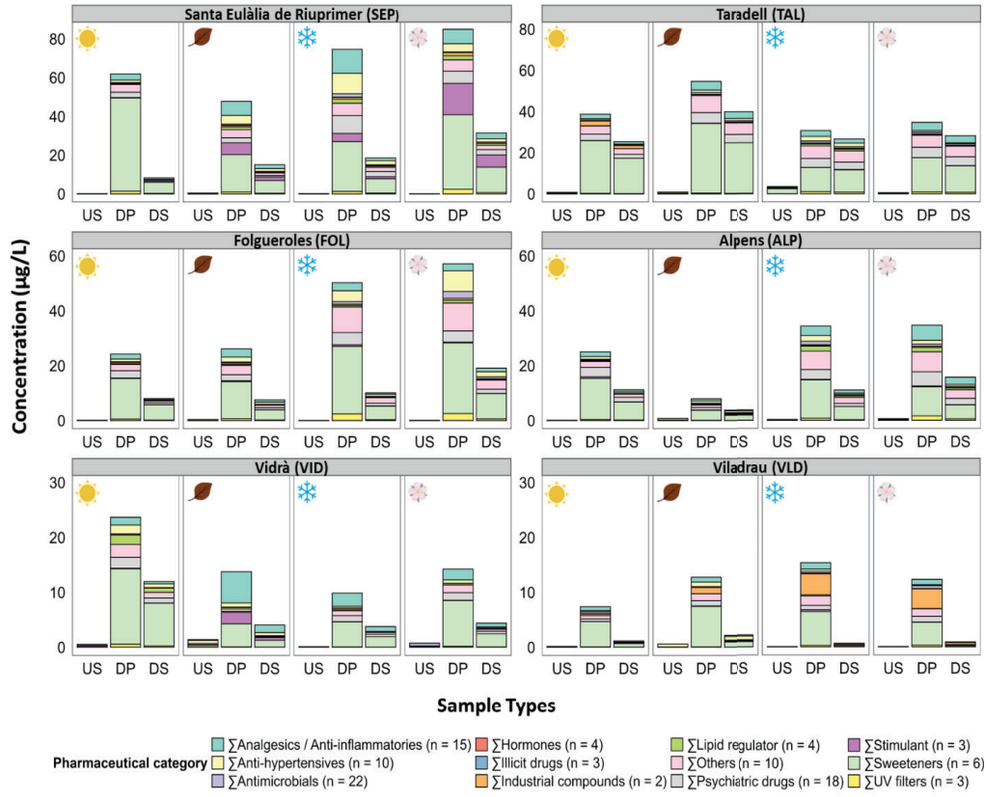


Fig. 2 *Total concentration of CECs in Mediterranean streams from the Osona region per season, WWTP and per CECs class. Number of compounds detected is shown above each bar. Note: The y-axis scales differ across rows to enhance a better visualization of the results. Being US: upstream point, DP: discharge point and DS: downstream point.*

Table 2. *Relative contribution of the WWTP effluent in terms of flow rate.*

WWTP	Contribution of WWTP effluent to river downstream (%)				CECs dilution (%)			
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
SEP	2	10	10	18	87	69	75	63
TAL	36	40	81	62	35	27	13	19
FOL	26	8	4	7	67	71	80	67
ALP	32	6	4	9	55	52	68	55
VID	87	5	11	41	50	71	62	69
VLD	3	2	1	2	84	83	96	93

patterns during the study period. During 2020, COVID-19 restrictions and lockdowns in Spain were still in place, while in 2021, restrictions started to decrease. These shifts in local population dynamics likely influenced wastewater compositions, particularly in small municipalities (population < 6500), such as our study sites. Small municipalities are more sensitive to population changes as they often have fewer industrial or commercial inputs. Thus, wastewater composition is more directly affected by residential patterns. The affected streams are low-order streams, typically located in the upper reaches of river system, with usually consistent flow, although they are highly influenced by local conditions such as rainfall, and severe drought periods. The dilution capacity of each receiving stream played a crucial role in the understanding the concentrations at the downstream points. To better understand these inputs and therefore the differences between up and downstream points, the WWTP discharge point contribution to the receiving stream in terms of flow rate (Eq.1) and the consequent dilution factor of the detected CECs to the receiving streams (Eq. 2) are shown in Table 2. Specific flow rates per season can be found in Table SM 3.

WWTP flow contributions to the river downstream were generally low, with seasonal averages below 40%, classifying the streams as effluent-impacted (<50%) except for TAL, which averaged 55% and was categorized as effluent-dominated based on Hamdhani et al. 2020 [10]. While summer exhibited higher contributions compared to other seasons, no significant differences were observed.

As shown in Fig. 2 and Table 2, TAL experienced highest total concentrations of CECs at downstream sites compared to other sites. This is due to TAL's low dilution effect of CECs compared to the other WWTPs. Its highest dilution was 35%, and the minimum 13%, both substantially lower than the minimum dilution observed in other WWTPs, which ranged from 50% to 83%. This disparity was most pronounced in winter when WWTP contributions to TAL reached 81%, resulting in the lowest dilution rate. Such values make the receiving TAL stream entirely WWTP effluent-dominated, reinforcing the evidence that even though WWTPs can efficiently reduce CECs concentrations up to a 60% [53], their concentration on the river downstream remains significant, especially in low-flow streams. In line with these observations, concentrations of CECs at downstream locations tend to be lower than the discharge point due to processes such as dilution, as previously explained, as well as sorption, biodegradation and photodegradation, which could further reduce CECs concentrations downstream [55,56]. SEP, however, showed the opposite effect. Despite detecting the highest concentrations in the discharge point, SEP had a greater dilution effect due to its low effluent flow contribution to the receiving streams (Table SM3). This underscores, the importance of sufficient river flow in mitigating the discharge impacts.

The observed upstream points, with a maximum total concentration of 3.5 µg L^{-1} in the case of TAL during winter (Fig. 2), could be attributed to the surrounding land uses. The TAL catchment area is predominantly

characterized by urban, agricultural and livestock activities, which likely contribute to the observed diffuse inputs. These inputs occurred when the stream had a very low flow rate (Table SM3), limiting the stream's dilution capacity. This reduction in dilution not only affects the WWTP effluent, as mentioned before, but also the influence of other potential contamination sources.

3.2. Variability in CECs concentrations across WWTPs and streams

Spatial differences between WWTPs and their receiving streams were evaluated, where a Kruskal-Wallis test revealed significant differences in CECs concentrations among the six WWTPs, considering data from the three types of sampling sites (upstream, discharge point and downstream). These results reflect variability in pollutant distribution across WWTPs and their respective receiving streams. To further identify specific differences between WWTPs, Dunn's post hoc test was applied, confirming significant pairwise differences in CECs levels among WWTPs. Notably, greater pairwise differences between WWTPs were observed in the downstream than in the other two points (Fig. SM1). These findings suggest that while the WWTPs are key contributors to the presence of CECs, downstream attenuation processes such as dilution, sorption, or maybe even degradation influence pollutant distribution. However, the flow variability of each stream also affects specially dilution, leading to varying impacts depending on the receiving river conditions (Table 2).

To further explore potential differences among WWTPs and their differentiation in CECs class compositions to the receiving streams, a principal components analysis (PCA) was performed on normalized data (Fig. 3) for the discharge points. The PCA reduced dimensionality and revealed patterns in CECs variability. PC1 and PC2 together explained a 67% of the total variance, enabling a clear differentiation among WWTPs. Distinct clustering

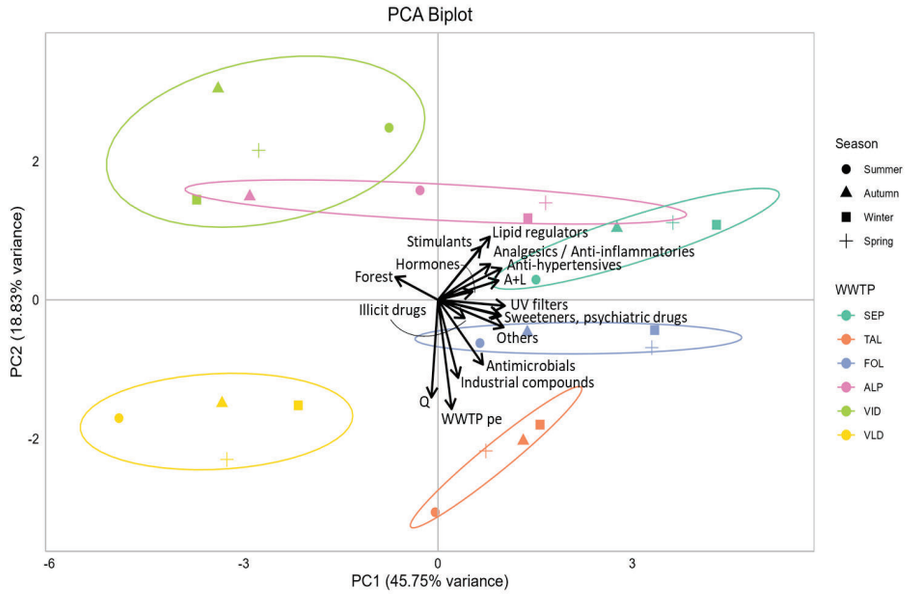


Fig. 3 *PCA biplot of the six studied WWTP. Discharge point concentrations per CECs class, flow rate, WWTP population equivalent and main land use, including forest and A+L (agriculture and livestock), were the features included.*

was observed for each WWTP, with VID and TAL clustering on opposite sides, reflecting differences in population size and total CECs concentration. Additionally, TAL was primarily driven by antimicrobials and industrial compounds, whereas VID showed an inversely relationship with these. At the same time, the negative PC2 space suggested contributions from specific industrials and agricultural activities. In contrast, the positive PC1 and PC2 space showed a strong association with lipid regulators, stimulants, anti-hypertensives and analgesics /anti-inflammatories, indicating that human pharmaceutical use was a key source. VLD notably clustered with no direct vectors pointing towards it, as it was the WWTP with lowest total CECs concentrations. Other WWTPs, exhibited a slight overlapping distribution, indicating that they share similar wastewater compositions or influences.

These findings highlight the potential for PCA to differentiate wastewater profiles and identify dominant sources of contamination. Although the PCA did not reveal seasonal clustering, as points grouped WWTP by rather than by season, this suggests that variations in WWTP composition were more strongly influenced by WWTP-specific characteristics and input sources rather than temporal effects in our study. Despite this general observation, significant seasonal differences ($p<0.05$) were observed in 20 compounds after performing a Kruskal-Wallis test for the detected compounds in the downstream points (Fig. 4). However, when pairwise comparisons were performed between seasons, no significant differences were observed indicating an overlap in the median concentrations and the absence of distinct seasonal variations in our study (Fig. 4).

To better understand the factors influencing the observed patterns, statistical differences were studied in relation to flow rate. For the upstream and WWTP discharge flow rate, no significant differences were observed when evaluated by season. In the Mediterranean region, climate is characterized by marked seasonality, with hot dry summers and mild winters, with most precipitation occurring in autumn and spring [57]. Hence lower river flow rates are typically observed during summer, while they tend to increase in autumn and spring. However, this seasonal pattern was not evident in our study. This can be explained by the monthly precipitation recorded during 2020 and 2021 (Fig. SM2). In January 2021, the rainfall was extremely low, even lower than the summer period during 2020, contributing to the reduced flow rates and consequently, less dilution of pollutants. This aligns with the Mediterranean winter drought trend attributed to anthropogenic climate change [58,59]. As a result, the pairwise concentration comparisons between seasons showed no significant differences as the river flow remained consistent with average CECs dilutions of 64, 65, 66 and 62% for each studied season summer, autumn, winter and spring respectively (Table 2).

In Fig. 4 the 20 compounds where significant differences between seasons were obtained are shown. Significant seasonal compounds are ordered in descending median concentration. Salicylic acid was the compound with highest median concentrations, with its lowest median concentrations observed in autumn. Salicylic acid is a naturally occuring compound involved in plant growth and stress responses [60], but it is also extensively used in skincare products, pharmaceuticals (notably as a metaboliote of acetyl salicylic acid), and industrial applications. Its diverse range of uses make its trends very difficult to explain as its environmental concentrations may reflect from both plant based sources and anthropogenic contributions. Nevertheless, given the proximity of WWTPs it is highly plausible that its presence is linked to the use of pharmaceutical, particularly acetyl salicylic acid. The observed decrease in median concentrations might reflect changes in pharmaceutical consumption patterns, such as the recommended reduced use of acetyl salicylic acid during COVID-19 pandemic [61]. The seasonal variations in concentrations may have

been influenced by the uncertainties concerning the use of acetyl salicylic acid during this period [62].

Sertraline an antidepressant, exhibited higher median concentrations in winter and spring. This season variation may be linked to the increased prevalence of mood disorders in population such as seasonal affective disorder which is more common during colder months, potentially leading to higher prescription of antidepressants like sertraline [63].

Paroxetine, another antidepressant, also exhibited significant differences, with median concentration peaking in autumn and sporadic high concentrations also observed in cooler seasons. In general the concentrations of paroxetine were lower than sertraline, which is consistent with the literature as sertraline is usually more prescribed than paroxetine [64,65]. Benzophenone-2 trends were particularly interesting, with significantly higher concentrations detected in the spring season 2021. Benzophenone-2 is a UV filter, hence a higher use or application is expected during seasons with higher UV exposure, such as summer and spring. These findings align with results from other studies where similar seasonal patterns were reported [66]. Notably, no detectable concentrations were observed during the summer 2020 season, coinciding with travelling restriction and lockdown measures in Spain due to the COVID-19 pandemic.

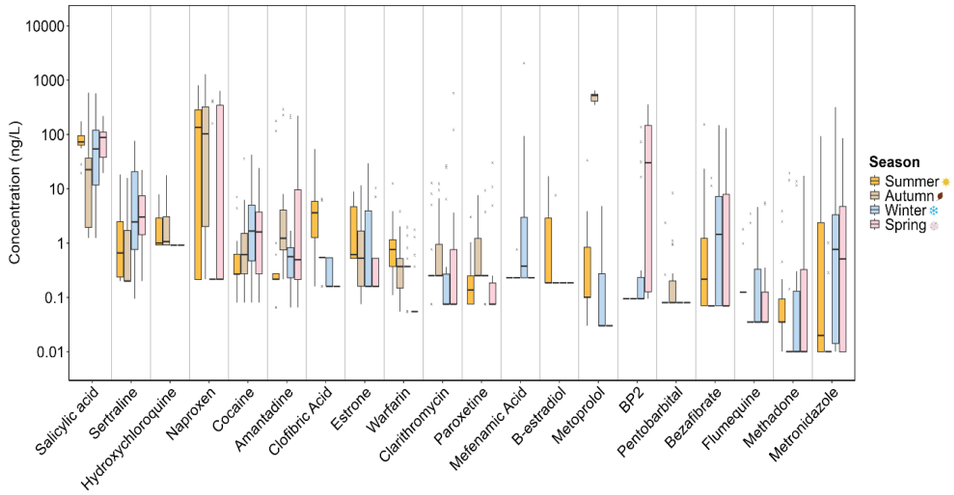


Fig. 4 Compounds with significant seasonal concentration variations. Boxplots represent the distribution of compounds concentration on a log10 scale for each season. Compounds are ordered by descending median concentration. Vertical lines separate individual compounds for clarity.

These limitations, likely led to a reduced use of UV filter products. In contrast, the peak of Benzophenone-2 concentrations observed in the following spring suggests a return to a more common usage pattern, also reflecting the gradual resumption to normal activities.

Another interesting significant seasonal variation was the case of hydroxychloroquine, an antimalarial drug which is also used for various autoimmune diseases. In 2019, hydroxychloroquine was proposed and recommended as a possible antiviral agent to treat COVID-19 [67]. In our study, higher concentrations were detected in the summer and autumn sampling seasons, while much lower concentrations were detected in winter and spring. This trend could be explained by the fluctuations in the number of COVID-19 cases, as in the autumn season there was a significant peak in cases [68], likely leading to an

increase in use of hydroxychloroquine, while the winter and spring seasons experienced relatively lower cases, reducing the demand of hydroxychloroquine treatments. However, by June 2020, early clinical studies began to report that HCQ showed no significant benefits in treating COVID-19 [69] and both the U.S. Food and Drug Administration (FDA) and the World Health Organization (WHO) started to advise against its use. By December 2020, the stance against the use of hydroxychloroquine solidified and both reaffirmed against its widespread use, thus further explaining our seasonal differences.

3.3. Fate and transport of CECs: analysis of environmental factors, land use and water quality parameters

To evaluate the fate and transport of pollutants in the aquatic environment, the relationship between different variables was assessed for the downstream sites using a Spearman's rank correlation (Fig. 5). Spearman correlations coefficient (r) and corresponding p-values are included in the supplementary material (Table SM4). A significant negative correlation was observed between the flow rate and the electrical conductivity as well as total CECs concentration. This can be explained because during low-flow periods e.g., water scarcity conditions, the reduced dilution capacity of the receiving streams results in higher concentrations of dissolved substances, including CECs, such as the case of TAL (Table 2), allowing these concentrations to dominate in the streams and exert stronger effects on organisms [70–72]. These relationships underscore the importance of flow in regulating water quality, specially under low-flow conditions. Other notable relationships were the positive correlation between

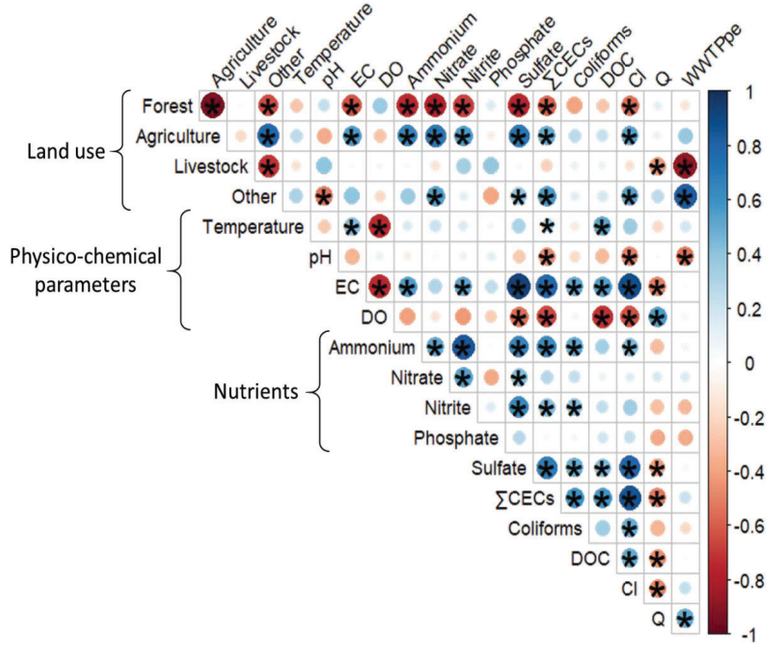


Fig. 5. Spearman correlation matrix showing the relationships between land use, physico-chemical parameters (EC: electrical conductivity, DO: dissolved oxygen), nutrients, total CECs concentration (∑CECs) and other wastewater treatment variables (DOC: dissolved organic carbon, Cl:chlorine, Q: effluent flow rate, WWTPpe: design population equivalent). Significant results (p- value<0.05) are shown with an asterisk. Correlation coefficients are represented by colour intensity: blue for a strong positive correlation and red for a strong negative correlation, white for no significant correlation.

temperature and CECs concentrations, as well as the negative correlation between dissolved oxygen and CECs concentration. These are consistent with other research studies [73], as they describe typical processes in rivers during dry seasons. When a river experiences a decrease in flow it usually implies an increase in temperature while oxygen levels decrease. The positive correlation between temperature and CECs can also be explained by the fact that the WWTP effluent has a higher temperature compared to that of the receiving river water, due to the WWTP treatment processes. At the same time, the WWTP effluent not only raises the overall river temperature but also contributes to a significant increase in CEC concentrations, as explained above, hence it is an expected relationship. Additionally, the input of nutrients from the effluent may lead to oxygen depletion in the river due to eutrophication, further reinforcing the observed negative correlation between dissolved oxygen and CECs.

Possible correlations with dominant land use in the catchment were also studied. In our region of study, the main land use surrounding three of the studied streams was forest (ALP, VID, and VLD), while in the three other study sites (FOL, SEP and TAL) agriculture and livestock land uses predominated. As expected, in Fig. 6 we can observe the positive correlation between most of the nutrients studied in the case where agriculture land use predominated, and a negative correlation for those sites with main forest land use. The same correlations were also observed for the CECs, implying that there was a lower contamination in the sites where forest land use predominated.

3.4. Holistic approach to classify effluent contributions

To evaluate the overall impact of WWTP effluents on the six Mediterranean streams, Eq. 3 was applied to calculate the WWTP contribution. Each stream was then classified based on the classification described by Hamdhani et al. 2020 [10] with adaptations made to address concentrations of CECs and nutrients. The WWTP stream contribution was categorized into three groups: effluent-impacted (<50%), effluent-dominated (>50%) and effluent-driven (>75%).

In Fig. 6, the effluent-to-natural stream classification is illustrated with further details provided in Table SM7. While the studied streams generally do not reach effluent-driven situations in terms of flow and phosphates concentrations, effluent-driven situations were more frequently observed for CECs and nitrates. In the case of CECs, particularly in summer, streams suffered higher impacts. TAL remained effluent-dominated throughout all 4 seasons, whereas VID presented effluent-driven scenarios in summer and spring, raising concerns about their environmental impact during these periods.

Regarding phosphates, VID presented an alarming scenario during two seasons, where the WWTP's lack of phosphate treatment contributed significantly to the stream's phosphate levels. Even though phosphate concentrations from the effluent remain well below the regulatory threshold of 1 mg/L [74] (ranging from 0.07 - 0.28 mg/L), the increased contribution from WWTPs in these areas signals potential long-term risks, particularly under low dilution conditions. Phosphates are a primary driver of eutrophication, high concentrations could lead to oxygen depletion, algal blooms and in the long-term habitat degradations if concentrations rise [75]. On the other hand, nitrate contributions were higher in autumn, being the most worrisome season, however WWTP nitrate concentrations were always lower than the established threshold of 10 mg/L[74]. Even though concentrations are within regulatory limits, Fig. 6 highlights the significant impact of WWTPs. These observations align with the revised European Urban Wastewater Treatment Directive [76], which incorporated more stringent nitrogen and phosphorus limits for smaller WWTPs and, for the first time addresses the reduction of CECs through quaternary treatments [76].

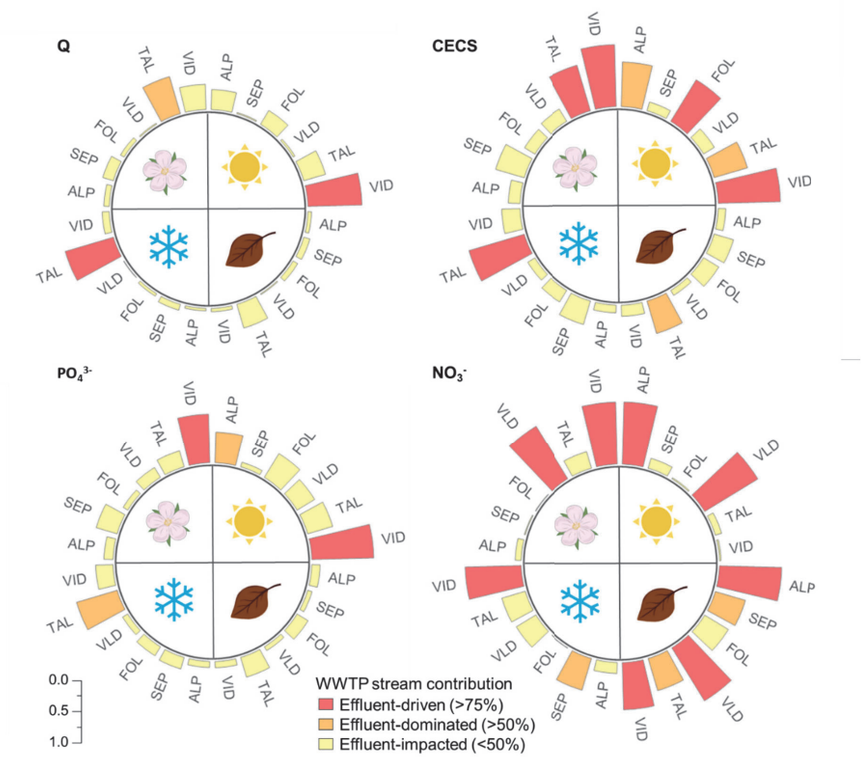


Fig. 6. Effluent-to-natural-stream classification per season and WWTP for Q (flow rate) and CECs, phosphate and nitrate concentrations.

3.5. Environmental risk assessment of CECs from WWTP effluents contribution in streams

An ERA was performed for all upstream and downstream points (Fig. 7), clearly demonstrating the influence of the WWTP discharge point on environmental risk (RQ values in Table SM4&5). Before the WWTP discharge point, there was generally no high risk associated to the upstream samples, except for 2 cases, where risk was detected for 3 compounds: fipronil (VID, autumn) and estrone and losartan (TAL, winter). In the case of TAL winter, possible diffuse sources, were likely responsible for the risk observed. Agriculture and livestock dominate land use in TAL, explaining the presence of estrone. This hormone, excreted by animals and found in manure and studies have confirmed estrone's presence in livestock wastewater and agricultural runoff, particularly in areas with intensive farming [77,78]. The presence of losartan on the other hand, is harder to understand as it is usually associated to human consumption, but it could also enter agricultural systems through the application of treated wastewater or biosolids as fertilizer [79].

On the other hand, in the downstream samples, concentrations of compounds at a high risk to the environment were detected in most WWTP for most of the seasons. A total of 11 compounds resulted in a high risk to the environment (Fig. 7) including azithromycin, caffeine, clarithromycin, diclofenac, estrone, fipronil, gemfibrozil, lorazepam, losartan, O-desmethylvenlafaxine, and sertraline. These

compounds are known for their persistence, potential bioaccumulation and specific toxic effects on aquatic organisms at different trophic levels. For instance, diclofenac and estrone are recognized endocrine disruptors, with known potential to interfere with hormonal systems in aquatic fauna [80,81]. Losartan, an anti-hypertensive, was one of the compounds which resulted with the highest risk in our study. This anti-hypertensive has been considered for monitoring by environmental agencies in several EU countries [82,83] and previous studies reported its toxic effect to different organisms [84,85]. While caffeine, even though it is a highly consumed chemical, recent studies have shown its capacity to induce disturbances in metabolic pathway in *Danio rerio* and demonstrated toxic effects on freshwater fish [86]. This

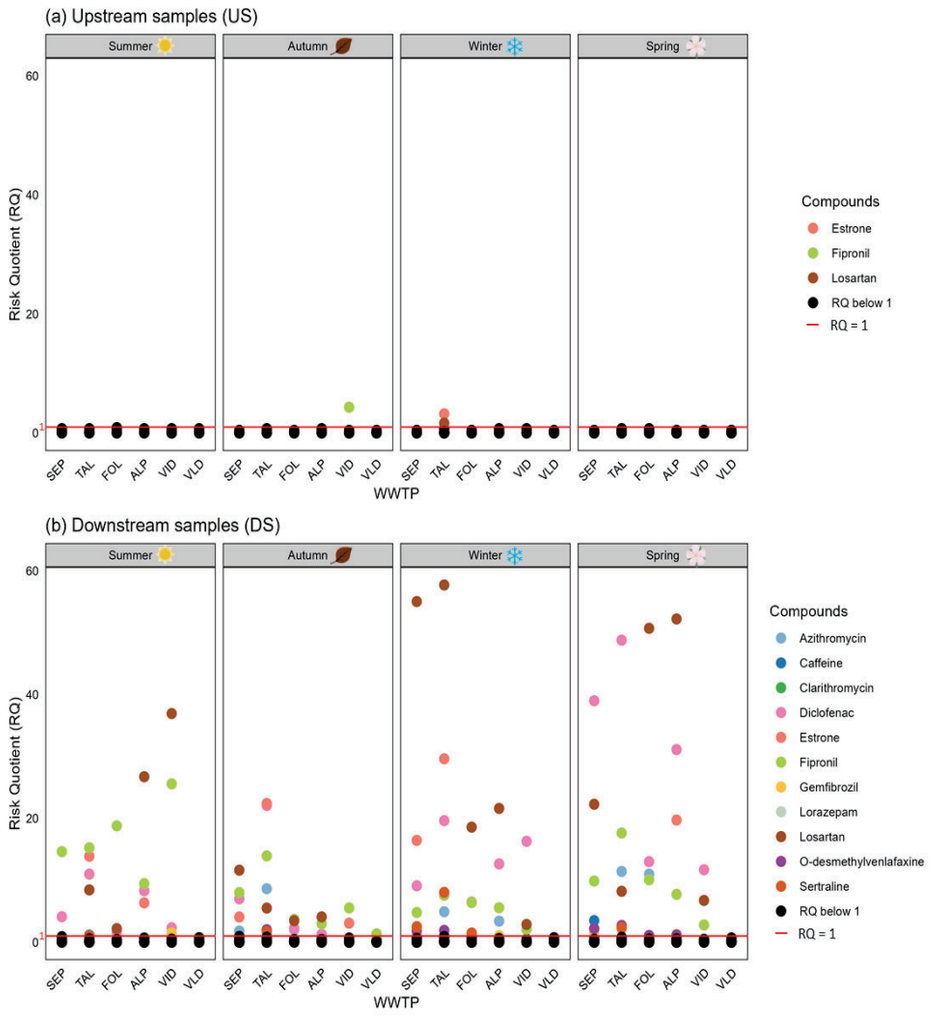


***Fig.** 7 Environmental risk assessment comparison between upstream and downstream points of the six wastewater treatme plants, where those compounds with RQ > 1 are plotted. RQ=1 is plotted as a red line.*

is due to the fact that, despite its low bioaccumulation potential, aquatic organisms and fish, in particular, are continuously exposed to this compound in the environment [87]. Both azithromycin and clarithromycin, two antibiotics, represent very high acute toxicity to aquatic organisms [88,89], have previously been monitored within the framework of EU water legislation and are known for their potential enhancement of antimicrobial resistance in aquatic environments. Even at very low concentrations their presence can promote the selection of antibiotic-resistant bacteria, increasing the persistence of antibiotic-resistant genes [90,91].

Interestingly, sweeteners were very prevalent in our study however none resulted in a high risk to the environment, despite their high concentration. This is in accordance with other studies [51,92] which indicate that sweeteners exhibit lower toxicity, reflected in their higher PNEC values (>18 µg/L) in comparison to other chemical compounds (0.36 ng/L) (see tables SM4&5). Their impact on the environment and biota is primarily as a source of labile carbon rather than as chemical stressors.

4. Conclusions

This study assessed the occurrence, seasonal variability, and environmental risks of CECs in Mediterranean low-order streams with marked flow variability. Among the 89 compounds detected, sweeteners and anti-hypertensives were particularly prevalent. The findings clearly demonstrated the significant impact of WWTPs, as a major source of CECs contamination, posing environmental risks.

Additionally, this study revealed the importance of river flow in regulating water quality, with WWTP impact varying based on flow conditions. Although dilution can mitigate some effects, the contaminant load remains high, emphasizing the need for more stringent regulations and monitoring to address the persistent impact of pollutants on aquatic ecosystems. Future discussions should integrate these results to strengthen policy recommendations and environmental management strategies.

Despite seasonal variations were not observed, WWTP-specific characteristics and input sources were the primary drivers of variability in CECs compositions. However, long-term studies that include high-flow conditions are needed to better understand seasonal variations and the impact of low-flow periods, which may be intensified by climate change.

ERA identified eleven compounds of concern, underscoring the need to improve wastewater treatments, particularly in small facilities discharging into low-flow streams. These streams are especially vulnerable due to their limited dilution capacity and high flow variability, intensifying the impacts of contaminants. While dilution plays a crucial role in reducing contaminants concentrations, it is not sufficient on its own to mitigate environmental risks. The implementation of regulatory measures to mitigate the release of worrisome concentrations of a wide range of bioactive compounds is crucial and should consider the dilution factor of the receiving stream to protect the aquatic ecosystem from anthropogenic impacts.

As intermittent or ephemeral watercourses are expected to become more frequent in the Mediterranean region due to climate change, it is crucial to enhance the understanding of their response to contaminants, to improve the overall management at catchment-scale and to provide evidence supporting the decision-making processes concerning the policies and regulation adaptations of river ecosystems in these areas.

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CRediT authorship contribution statement

Olga Gómez-Navarro: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Lluís Bertrans-Tubau:** Methodology, Investigation, Writing – review & editing. **Meritxel Abril:** Writing – review & editing, Validation, Conceptualization. **Nicola Montemurro:** Methodology, Writing – review & editing, **Lorenzo Proia:** Writing – review & editing, Validation, Conceptualization. **Sandra Perez:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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Publication #2

Supplementary Materials

Unveiling the impact of wastewater effluents on Mediterranean streams with seasonal flow variability

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Supplementary Material

Unveiling the impact of wastewater effluents on Mediterranean streams with seasonal flow variability

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Table of contents

Table SM 1. Target compound name, molecular formula, molecular weight, CAS number, PubChem CID, InChIKey, XlogP and classified category. S2
Table SM 2. Location of sampling sites. Seasonal average of hydrological, physical and chemical variables measured at the different sampling sites for each wastewater treatment plant. Abbreviation: electrical conductivity (EC), Temperature (T), dissolved oxygen (DO), dissolved organic carbon (DOC), chlorine (Cl). S6
Table SM 3. Seasonal flow rates. S7
Table SM 4. Spearman correlation p-values. S9
Table SM 5. Risk quotients for those compounds that resulted in a high risk in the upstream samples. S10
Table SM 6. Risk quotients for those compounds that resulted in a high risk in the downstream samples. S10
Table SM 7. Stream classification based on Hamdhani et. al 2020 [2]. S14

Figures

Fig. SM 1 Osona region monthly precipitation for 2020 and 2021 obtained from Servei Meteorològic de Catalunya, Estacions Meteorològiques [2]. S8

References S15

Table SM 1. Target compound name, molecular formula, molecular weight, CAS number, PubChem CID, InChIKey, XlogP and classified category.

Compound name	Molecular formula	Molecular Weight	CAS number	PubChem CID	InChIKey	XLogP	Category
1,7 Dimethylxanthine	C7H8N4O2	180.16	611-59-6	4687	QUNWUDVFRNGTCO-UHFFFAOYSA-N	-0.2	Stimulant
17-α Ethynilestradiol	C20H24O2	296.4	57-63-6	5994	BFPYWIDHMRZLRN-SLHNCBLASA-N	3.7	Hormones
1H-Benzotriazole	C6H5N3	119.12	95-14-7	7220	QRUDEWIWKLJBPS-UHFFFAOYSA-N	1.0	Industrial compounds
2.4-dihydroxybenzophenone (BP1)	C13H10O3	214.22	131-56-6	8572	ZXDDPOHVAMWLBH-UHFFFAOYSA-N	3.2	UV filters
2.2'.4.4'-tetrahydroxybenzophenone (BP2)	C13H10O5	246.22	131-55-5	8571	WXNRYSGJLQFHBR-UHFFFAOYSA-N	2.4	UV filters
6a-Methylprednisolone	C22H30O5	374.47	83-43-2	6741	VHRSUDSXCMQTMA-PJHHCJLFSA-N	1.9	Analgesics / Anti-inflammatories
Acesulfame	C4H5NO4S	163.15	33665-90-6	36573	YGCFIWIQZPHFLU-UHFFFAOYSA-N	-0.3	Sweeteners
Acetaminophen	C8H9NO2	151.16	103-90-2	1983	RZVAJINKPMORJF-UHFFFAOYSA-N	0.5	Analgesics / Anti-inflammatories
Acridone	C13H9NO	195.22	578-95-0	2015	FZEYVTFCMJSGMP-UHFFFAOYSA-N	3.0	Others
Adamantan-1-amine (Amantadine)	C10H17N	151.14	768-94-5	2130	DKNWSYNQZKUICI-UHFFFAOYSA-N	2.4	Others
Alprazolam	C17H13ClN4	308.77	28981-97-7	2118	VREFGVBLTWBCJP-UHFFFAOYSA-N	2.1	Psychiatric drugs
Atenolol	C14H22N2O3	266.34	29122-68-7	2249	METKIMKYRPQLGS-UHFFFAOYSA-N	0.2	Anti-hypertensives
Azithromycin	C38H72N2O12	748.98	83905-01-5	447043	MQTOSJVFKKJCRP-BICOPXKESA-N	4.0	Antimicrobials
Benzoylecgonine	C16H19NO4	289.33	519-09-5	448223	GVGYEFKIHJTNQZ-RFQIPJPRSA-N	-0.3	Illicit drugs
B-estradiol	C18H24O2	272.38	50-28-2	5757	VOXZDWNPVJITMN-ZBRFXRBCSA-N	4.0	Hormones
Bezafibrate	C19H20ClNO4	361.82	41859-67-0	39042	IIBYAHWJQTYFKB-UHFFFAOYSA-N	3.8	Lipid regulator
Bisphenol-A	C15H16O2	228.29	80-05-7	6623	IISBACLAFKSPIT-UHFFFAOYSA-N	3.3	Industrial compounds
Bromazepam	C14H10BrN3O	316.15	1812-30-2	2441	VMIYHDSEFNYJSL-UHFFFAOYSA-N	1.7	Psychiatric drugs
Caffeine	C8H10N4O2	194.19	58-08-2	2519	RYYVLZVUVIJVGH-UHFFFAOYSA-N	-0.1	Stimulant
Carazolol	C18H22N2O2	298.38	57775-29-8	71739	BQXQGZPYHWWCEB-UHFFFAOYSA-N	3.6	Anti-hypertensives
Carbamazepine	C15H12N2O	236.27	298-46-4	2554	FFGPTBGBLSHEPO-UHFFFAOYSA-N	2.5	Psychiatric drugs
Carisoprodol	C12H24N2O4	260.33	78-44-4	2576	OFZCIYFFPZCNJE-UHFFFAOYSA-N	1.9	Others
Chloramphenicol	C11H12Cl2N2O5	323.13	56-75-7	5959	WIIZWVCIJKGZOK-RKDXNWHRSA-N	1.1	Antimicrobials
Citalopram	C20H21FN2O	324.16	59729-33-8	2771	WSEQXVZVJXJVFP-UHFFFAOYSA-N	3.2	Psychiatric drugs
Clarithromycin	C38H69NO13	747.95	81103-11-9	84029	AGOYDEPGAOXOCK-KCBOHYOISA-N	3.2	Antimicrobials
Climbazole	C15H17ClN2O2	292.76	38083-17-9	37907	OWEGWHBOCFMBLP-UHFFFAOYSA-N	3.7	Antimicrobials
Clofibric Acid	C10H11ClO3	214.64	882-09-7	2797	TXCGAZHTZHNUAI-UHFFFAOYSA-N	2.6	Lipid regulator

Compound name	Molecular formula	Molecular Weight	CAS number	PubChem CID	InChIKey	XLogP	Category
Cocaethylene	C18H23NO4	317.38	529-38-4	644006	NMPOSNRHZIWLLL-XUWVNRHRSA-N	2.7	Illicit drugs
Cocaine	C17H21NO4	303.35	50-36-2	446220	ZPUCINDJVBIVPJ-LJISPDSOSA-N	2.3	Illicit drugs
Codeine	C18H21NO3	299.36	76-57-3	5284371	OROGSEYTTFOCAN-DNJOTXNNSA-N	1.1	Analgesics / Anti-inflammatories
Cotinine	C10H12N2O	176.22	486-56-6	854019	UIKROCXWUNQSPJ-VIFPVBQESA-N	-0.3	Stimulant
Crotamiton	C13H17NO	203.28	483-63-6	688020	DNTGGZPQPQTDQF-XBXARRHUSA-N	2.8	Others
Cyclamate	C6H13NO3S	179.24	100-88-9	7533	HCAJEUSONLESMK-UHFFFAOYSA-N	0.6	Sweeteners
Diazepam	C16H13ClN2O	284.74	439-14-5	3016	AAOVKJBEBIDNHE-UHFFFAOYSA-N	3.0	Psychiatric drugs
Diclofenac	C14H11Cl2NO2	296.15	15307-86-5	3033	DCOPUUMXTXDBNB-UHFFFAOYSA-N	4.4	Analgesics / Anti-inflammatories
Diltiazem	C22H26N2O4S	414.52	42399-41-7	39186	HSUGRBWQSSZJOP-RTWAWAEBSA-N	3.1	Anti-hypertensives
Estriol	C18H24O3	288.38	50-27-1	5756	PROQIPRRNZUXQM-ZXXIGWHRSA-N	2.5	Hormones
Estrone	C18H22O2	270.37	53-16-7	5870	DNXHEGUUPJUMQT-CBZIJGRNSA-N	3.1	Hormones
Fipronil	C12H4Cl2F6N4OS	437.15	120068-37-3	3352	ZOCSXAVNDGMNBV-UHFFFAOYSA-N	4.5	Others
Florfenicol	C12H14Cl2FNO4S	358.21	73231-34-2	114811	AYIRNRDRBQJXIF-NXEZZACHSA-N	0.8	Antimicrobials
Fluconazole	C13H12F2N6O	306.27	86386-73-4	3365	RFHAOTPXVQNOHP-UHFFFAOYSA-N	0.4	Antimicrobials
Flumequine	C14H12FNO3	261.25	42835-25-6	3374	DPSPPJIUMHPXMA-UHFFFAOYSA-N	2.9	Antimicrobials
Fluticasone	C25H31F3O5S	500.6	80474-14-2	444036	WMWTYOKRWGGJOA-CENSZEJFSA-N	4.0	Hormones
Furazolidone	C8H7N3O5	225.16	67-45-8	5323714	PLHJDBGFXBMTGZ-WEVVVXLNSA-N	-0.1	Antimicrobials
Furosemide	C12H11ClN2O5S	330.74	54-31-9	3440	ZZUFCTLCJUWOSV-UHFFFAOYSA-N	2.0	Others
Gemfibrozil	C15H22O3	250.33	25812-30-0	3463	HEMJJKBWTPKOJG-UHFFFAOYSA-N	3.8	Lipid regulator
Hydrochlorothiazide	C7H8ClN3O4S2	297.74	58-93-5	3639	JZUFKLXOESDKRF-UHFFFAOYSA-N	-0.1	Others
Hydroxychloroquine	C18H26ClN3O	335.87	118-42-3	3652	XXSMGPRMXLTPCZ-UHFFFAOYSA-N	3.6	Antimicrobials
Hyoscine (scopolamine)	C17H21NO4	303.35	51-34-3	3000322	STECJAGHUSJQJN-USLFZFAMSA-N	0.9	Others
Indomethacin	C19H16ClNO4	357.79	53-86-1	3715	CGIGDMFJXJATDK-UHFFFAOYSA-N	4.3	Analgesics / Anti-inflammatories
Irbesartan	C25H28N6O	428.53	138402-11-6	3749	YOSHYTLCDANDAN-UHFFFAOYSA-N	4.1	Anti-hypertensives
Ketoprofen	C16H14O3	254.28	22071-15-4	3825	DKYWVDODHFEZIM-UHFFFAOYSA-N	3.1	Analgesics / Anti-inflammatories
Lamotrigine	C9H7Cl2N5	256.09	84057-84-1	3878	PYZRQGJRPPTADH-UHFFFAOYSA-N	1.4	Psychiatric drugs
Lidocaine	C14H22N2O	234.34	137-58-6	3676	NNJVILVZKWQKPM-UHFFFAOYSA-N	2.3	Others
Lincomycin	C18H34N2O6S	406.54	154-21-2	3000540	OJMMVQQUTAEWLP-KIDUDLJLSA-N	0.2	Antimicrobials
Lorazepam	C15H10Cl2N2O2	321.2	846-49-1	3958	DIWRORZWFLOCLC-UHFFFAOYSA-N	2.4	Psychiatric drugs
Losartan	C22H23ClN6O	422.9	114798-26-4	3961	PSIFNNKUMBGKDQ-UHFFFAOYSA-N	4.3	Anti-hypertensives
Mefenamic Acid	C15H15NO2	241.29	61-68-7	4044	HYYBABOKPJLUIN-UHFFFAOYSA-N	5.1	Analgesics / Anti-inflammatories

Compound name	Molecular formula	Molecular Weight	CAS number	PubChem CID	InChIKey	XLogP	Category
Meprobamate	C9H18N2O4	218.25	57-53-4	4064	NPPQSCRMBWNHMW-UHFFFAOYSA-N	0.7	Psychiatric drugs
Methadone	C21H27NO	309.45	76-99-3	4095	USSIQXCVUWKGNF-UHFFFAOYSA-N	3.9	Analgesics / Anti-inflammatories
Metoprolol	C15H25NO3	267.36	37350-58-6	4171	IUBSYMUCCVWXPE-UHFFFAOYSA-N	1.9	Anti-hypertensives
Metronidazole	C6H9N3O3	171.15	443-48-1	4173	VAOCPAMSLUNLGC-UHFFFAOYSA-N	0.0	Antimicrobials
Midazolam	C18H13ClFN3	325.77	59467-70-8	4192	DDLIGBOFAVUZHB-UHFFFAOYSA-N	2.5	Psychiatric drugs
Morphine	C17H19NO3	285.34	57-27-2	5288826	BQJCRHHNABKAKU-KBQPJGBKSA-N	0.8	Analgesics / Anti-inflammatories
Nalidixic acid	C12H12N2O3	232.24	389-08-2	4421	MHWLWQUZZRMNGJ-UHFFFAOYSA-N	1.4	Antimicrobials
Naproxen	C14H14O3	230.26	22204-53-1	156391	CMWTZPSULFXXJA-VIFPVBQESA-N	3.3	Analgesics / Anti-inflammatories
Neotame	C20H30N2O5	378.46	165450-17-9	9810996	HLIAVLHNDJUHFG-HOTGVXAUSA-N	-0.1	Sweeteners
O-desmethylvenlafaxine	C16H25NO2	263.19	93413-62-8	125017	KYYIDSXMWOZKMP-UHFFFAOYSA-N	2.6	Psychiatric drugs
Oseltamivir	C16H28N2O4	312.4	196618-13-0	65028	VSZGPKBBMSAYNT-RRFJBIMHSA-N	1.1	Others
Oxazepam	C15H11ClN2O2	286.71	604-75-1	4616	ADIMAYPTOBDMTL-UHFFFAOYSA-N	2.2	Psychiatric drugs
Paroxetine	C19H20FNO3	329.37	61869-08-7	43815	AHOUBRCZNHFOSL-YOEHRIQHSA-N	3.5	Psychiatric drugs
Pentobarbital	C11H18N2O3	226.28	76-74-4	4737	WEXRUCMBJFQVBZ-UHFFFAOYSA-N	2.1	Others
Phenytoin	C15H12N2O2	252.27	57-41-0	1775	CXOFVDLJLONNDW-UHFFFAOYSA-N	2.5	Psychiatric drugs
Primidone	C12H14N2O2	218.25	125-33-7	4909	DQMZLTXERSFNPB-UHFFFAOYSA-N	0.9	Psychiatric drugs
Propranolol	C16H21NO2	259.34	525-66-6	4946	AQHHHDLHHXJYJD-UHFFFAOYSA-N	3.0	Anti-hypertensives
Propyphenazone	C14H18N2O	230.31	479-92-5	3778	PXWLVJLKJGVOKE-UHFFFAOYSA-N	1.7	Analgesics / Anti-inflammatories
Rosuvastatin	C22H28FN3O6S	481.54	287714-41-4	446157	BPRHUIZQVSMCRT-VEUZHWNKSA-N	1.6	Lipid regulator
Saccharin	C7H5NO3S	183.18	81-07-2	5143	CVHZOJJKTDOEJC-UHFFFAOYSA-N	0.9	Sweeteners
Salicylic acid	C7H6O3	138.12	69-72-7	338	YGSDEFSMJLZEOE-UHFFFAOYSA-N	2.3	Analgesics / Anti-inflammatories
Sertraline	C17H17Cl2N	306.23	79617-96-2	68617	VGKDLMBJGBXTGI-SJCJKPOMSA-N	4.8	Psychiatric drugs
Sitagliptin	C16H15F6N5O	407.31	486460-32-6	4369359	MFFMDFFZMYYVKS-SECBINFHSA-N	0.7	Others
Sotalol	C12H20N2O3S	272.36	3930-20-9	5253	ZBMZVLHSJCTVON-UHFFFAOYSA-N	0.2	Anti-hypertensives
Stevioside	C38H60O18	804.38	57817-89-7	442089	UEDUENGHJMELGK-HYDKPPNVSA-N	-1.2	Sweeteners
Sucralose	C12H19Cl3O8	397.63	56038-13-2	71485	BAQAVOSOZGMPRM-QBMZZYIRSA-N	-1.5	Sweeteners
Sulfadiazine	C10H10N4O2S	250.28	68-35-9	5215	SEEPANYCNGTZFQ-UHFFFAOYSA-N	-0.1	Antimicrobials
Sulfadimethoxine	C12H14N4O4S	310.33	122-11-2	5323	ZZORFUFYDOWNEF-UHFFFAOYSA-N	1.6	Antimicrobials
Sulfamerazine	C11H12N4O2S	264.3	127-79-7	5325	QPPBRPIAZZHUNT-UHFFFAOYSA-N	0.1	Antimicrobials
Sulfamethazine	C12H14N4O2S	278.33	57-68-1	5327	ASWVTGNCAZCNNR-UHFFFAOYSA-N	0.3	Antimicrobials
Sulfamethizole	C9H10N4O2S2	270.33	144-82-1	5328	VACCAVUAMIDAGB-UHFFFAOYSA-N	0.5	Antimicrobials

Compound name	Molecular formula	Molecular Weight	CAS number	PubChem CID	InChIKey	XLogP	Category
Sulfamethoxazole	C10H11N3O3S	253.28	723-46-6	5329	JLKIGFTWXXRPMT-UHFFFAOYSA-N	0.9	Antimicrobials
Sulfapyridine	C11H11N3O2S	249.29	144-83-2	5336	GECHUMIMRBOMGK-UHFFFAOYSA-N	0.0	Antimicrobials
Sulfathiazole	C9H9N3O2S2	255.32	72-14-0	5340	JNMRHUJNCSQMMB-UHFFFAOYSA-N	0.1	Antimicrobials
Sulisobenzone (BP4)	C14H12O6S	308.31	4065-45-6	19988	CXVGEDCSTKKODG-UHFFFAOYSA-N	2.2	UV filters
Tramadol	C16H25NO2	263.38	27203-92-5	33741	TVYLLZQTGLZFBW-ZBFHGGJFSA-N	2.6	Analgesics / Anti-inflammatories
Trimethoprim	C14H18N4O3	290.32	738-70-5	5578	IEDVJHCEMCRBQM-UHFFFAOYSA-N	0.9	Antimicrobials
Valsartan	C24H29N5O3	435.52	137862-53-4	60846	ACWBQPMHZXGDFX-QFIPXVFZSA-N	4.4	Anti-hypertensives
Venlafaxine	C17H27NO2	277.4	93413-69-5	5656	PNVNVHUZROJLTJ-UHFFFAOYSA-N	2.9	Psychiatric drugs
Verapamil	C27H38N2O4	454.6	52-53-9	2520	SGTNSNPWRIOYBX-UHFFFAOYSA-N	3.8	Anti-hypertensives
Warfarin	C19H16O4	308.3	81-81-2	54678486	PJVWKTKQMONHTI-UHFFFAOYSA-N	2.7	Others
Zonisamide	C8H8N2O3S	212.23	68291-97-4	5734	UBQNRHZMVUUOMG-UHFFFAOYSA-N	0.2	Psychiatric drugs

Table SM 2. Location of sampling sites. Seasonal average of hydrological, physical and chemical variables measured at the different sampling sites for each wastewater treatment plant. Abbreviation: electrical conductivity (EC), Temperature (T), dissolved oxygen (DO), dissolved organic carbon (DOC), chlorine (Cl).

WWTP	Sample Type	Location		Flow rate	EC	T	pH	DO	Ammonium	Phosphate	Nitrate	Nitrite	Sulphate	DOC	Cl	Coliforms
		Lat. N	Long. E	(L/s)	(µS/cm)	(ºC)		(mg/L)	(µg/L)	(µg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(CFU/100mL)
ALPE	US			13.12	657	10.65	7.86	10.75	75.03	53.61	0.88	0.01	89.11	14.46	36.74	1747
	DP	42º6'57"	2º5'53"	0.95	1316	14.98	7.78	6.14	661.45	1322.14	2.33	0.07	59.03	10.65	224.24	78950
	DS			14.07	925	12.19	7.86	7.80	254.70	710.02	1.44	0.07	74.45	9.20	128.21	29727
FOL	US			71.41	669	14.55	7.63	10.24	140.78	23.20	14.38	0.05	59.60	14.85	34.16	1029
	DP	41º56'39"	2º18'34"	6.19	1050	15.80	7.69	8.44	6089.45	319.32	1.87	0.20	67.09	11.42	137.57	63000
	DS			77.60	786	14.81	7.85	9.04	1884.44	106.96	11.98	0.26	64.04	6.81	72.07	12578
SEP	US			26.31	1062	11.43	8.14	9.64	67.46	50.79	6.07	0.05	252.57	6.92	106.25	726
	DP	41º54'44"	2º11'35"	1.93	1392	14.83	7.68	5.46	17311.47	706.36	1.52	1.13	118.49	8.31	241.10	259050
	DS			28.23	1172	13.18	7.89	8.38	5769.62	333.11	4.52	0.41	209.90	6.49	145.98	53254
TAL	US			18.49	602	11.68	7.70	9.21	98.33	67.87	11.82	0.04	80.05	7.39	40.49	2966
	DP	41º52'52"	2º16'34"	16.76	1044	15.23	7.68	8.24	376.71	80.72	1.13	0.04	63.57	8.30	221.48	33442
	DS			35.24	964	14.48	7.70	8.49	325.41	93.94	3.10	0.04	64.16	8.69	181.54	24717
VID	US			30.22	592	11.63	7.72	9.13	86.80	43.71	2.11	0.02	47.95	4.76	20.00	2452
	DP	42º7'14"	2º18'50"	2.64	621	13.48	7.80	8.26	867.86	228.14	2.42	0.08	43.95	7.67	39.08	187675
	DS			32.86	569	12.71	8.15	8.55	480.18	130.29	2.50	0.15	44.44	5.42	23.87	20017
VLD	US			531.65	160	11.20	7.60	10.88	25.21	20.79	0.65	0.07	10.29	2.91	15.58	180
	DP	41º50'51"	2º22'44"	7.08	1851	17.85	8.03	6.81	918.09	1821.68	4.55	0.09	33.72	14.55	116.53	28350
	DS			538.73	275	11.82	7.89	10.33	69.52	199.45	1.04	0.03	11.32	3.94	19.82	2790

Table SM 3. Seasonal flow rates.

WWTP	Season	US flow (L/s)	WWTP flow (L/s)	DS flow (L/s)
VID	Summer	0.11	0.74	0.85
VID	Autumn	100.63	5.75	106.38
VID	Winter	17.45	2.25	19.70
VID	Spring	2.68	1.83	4.51
TAL	Summer	33.78	19.34	53.12
TAL	Autumn	28.47	18.77	47.24
TAL	Winter	3.75	15.83	19.58
TAL	Spring	7.95	13.08	21.03
VLD	Summer	250.09	8.11	258.20
VLD	Autumn	480.68	7.60	488.28
VLD	Winter	985.91	5.59	991.50
VLD	Spring	409.94	7.01	416.95
FOL	Summer	20.40	7.09	27.50
FOL	Autumn	87.91	7.95	95.86
FOL	Winter	109.66	5.01	114.67
FOL	Spring	67.65	4.71	72.36
SEP	Summer	53.21	1.04	54.25
SEP	Autumn	22.25	2.53	24.78
SEP	Winter	21.49	2.34	23.82
SEP	Spring	8.28	1.79	10.08
ALPE	Summer	1.22	0.58	1.80
ALPE	Autumn	28.21	1.72	29.93
ALPE	Winter	12.97	0.54	13.51
ALPE	Spring	10.10	0.95	11.05

In Fig. SM1 you can see the monthly precipitation for 2020 and 2021 collected from the different weather stations.

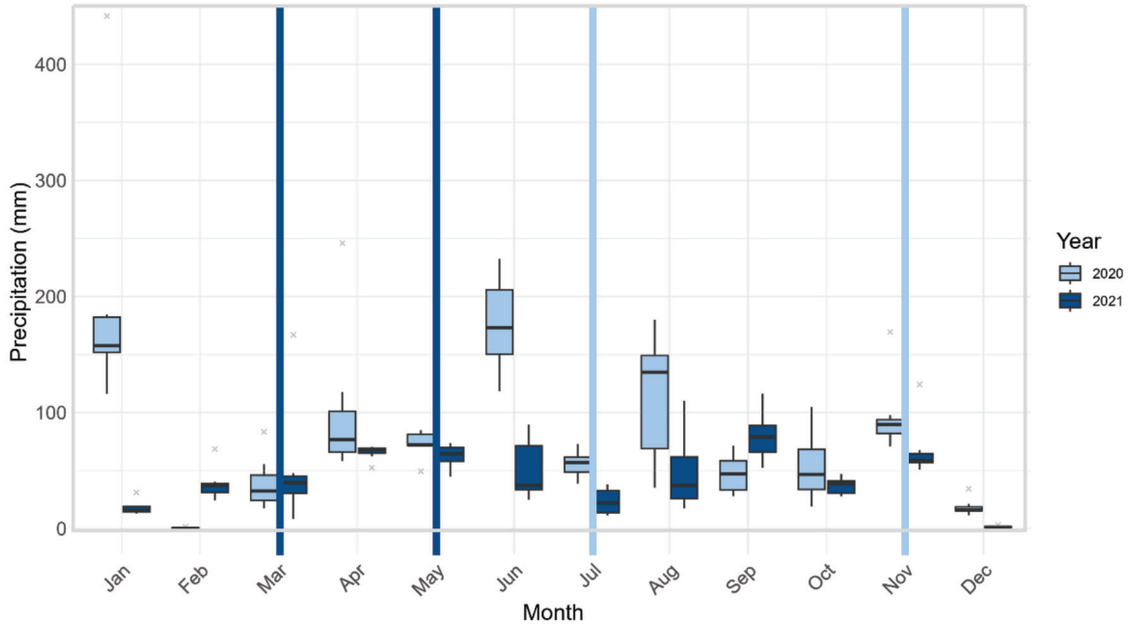


Fig. SM 1 Osona region monthly precipitation for 2020 and 2021 obtained from Servei Meteorològic de Catalunya, Estacions Meteorològiques [2]. Marked with the sampling months marked in blue, depending on the year.

Table SM 4. Spearman correlation p-values.

Forest	Agriculture	Livestock	Other	T	pH	EC	DO	NH4	NO3	NO2	PO4	SO4	CECs	Coliforms	DOC	Cl	Q	WWTPpe	
	6E-12	9E-01	5E-04	2E-01	3E-01	9E-04	1E-01	3E-05	5E-06	2E-04	5E-01	2E-05	2E-03	5E-02	2E-01	5E-03	6E-01	5E-01	**Forest**
6E-12		3E-01	1E-05	2E-01	8E-02	4E-03	3E-01	5E-04	8E-05	4E-03	6E-01	1E-04	1E-02	2E-01	3E-01	7E-03	7E-01	7E-02	**Agriculture**
9E-01	3E-01		9E-05	5E-01	6E-02	9E-01	8E-01	9E-01	5E-01	1E-01	6E-02	8E-01	3E-01	6E-01	7E-01	4E-01	3E-02	9E-09	**Livestock**
5E-04	1E-05	9E-05		1E-01	7E-03	6E-02	4E-01	8E-02	5E-03	6E-01	7E-02	5E-02	4E-03	5E-01	4E-01	7E-03	2E-01	6E-07	**Other**
2E-01	2E-01	5E-01	1E-01		2E-01	3E-02	6E-04	4E-01	3E-01	5E-01	8E-01	1E-01	4E-02	6E-01	9E-03	9E-02	4E-01	4E-01	**T**
3E-01	8E-02	6E-02	7E-03	2E-01		1E-01	7E-01	6E-01	9E-01	7E-01	5E-01	2E-01	2E-02	4E-01	1E-01	1E-02	9E-01	7E-03	**pH**
9E-04	4E-03	9E-01	6E-02	3E-02	1E-01		5E-04	6E-03	2E-01	1E-02	2E-01	2E-06	1E-05	1E-02	7E-03	3E-06	1E-02	1E+00	**EC**
1E-01	3E-01	8E-01	4E-01	6E-04	7E-01	5E-04		9E-02	6E-01	8E-02	3E-01	2E-02	4E-03	8E-01	1E-03	6E-03	2E-02	8E-01	**DO**
3E-05	5E-04	9E-01	8E-02	4E-01	6E-01	6E-03	9E-02		1E-02	2E-06	1E+00	7E-04	1E-03	1E-02	1E-01	3E-02	1E-01	7E-01	**Ammonium**
5E-06	8E-05	5E-01	5E-03	3E-01	9E-01	2E-01	6E-01	1E-02		7E-03	7E-02	3E-02	2E-01	3E-01	7E-01	5E-01	4E-01	5E-01	**Nitrate**
2E-04	4E-03	1E-01	6E-01	5E-01	7E-01	1E-02	8E-02	2E-06	7E-03		5E-01	1E-03	4E-02	4E-02	3E-01	1E-01	2E-01	1E-01	**Nitrite**
5E-01	6E-01	6E-02	7E-02	8E-01	5E-01	2E-01	3E-01	1E+00	7E-02	5E-01		2E-01	9E-01	8E-01	3E-01	3E-01	7E-02	8E-02	**Phosphate**
2E-05	1E-04	8E-01	5E-02	1E-01	2E-01	2E-06	2E-02	7E-04	3E-02	1E-03	2E-01		3E-04	2E-02	3E-02	6E-06	4E-02	8E-01	**Sulfate**
2E-03	1E-02	3E-01	4E-03	9E-02	2E-02	1E-05	4E-03	1E-03	2E-01	4E-02	9E-01	3E-04		3E-03	3E-03	2E-06	1E-02	3E-01	**Totalconcentration**
5E-02	2E-01	6E-01	5E-01	6E-01	4E-01	1E-02	8E-01	1E-02	3E-01	4E-02	8E-01	2E-02	3E-03		1E-01	2E-02	1E-01	4E-01	**Coliforms**
2E-01	3E-01	7E-01	4E-01	9E-03	1E-01	7E-03	1E-03	1E-01	7E-01	3E-01	3E-01	3E-02	3E-03	1E-01		1E-02	3E-02	1E+00	**DOC**
5E-03	7E-03	4E-01	7E-03	9E-02	1E-02	3E-06	6E-03	3E-02	5E-01	1E-01	3E-01	6E-06	2E-06	2E-02	1E-02		2E-02	3E-01	**Cl**
6E-01	7E-01	3E-02	2E-01	4E-01	9E-01	1E-02	2E-02	1E-01	4E-01	2E-01	7E-02	4E-02	1E-02	1E-01	3E-02	2E-02		1E-02	**Q**
5E-01	7E-02	9E-09	6E-07	4E-01	7E-03	1E+00	8E-01	7E-01	5E-01	1E-01	8E-02	8E-01	3E-01	4E-01	1E+00	3E-01	1E-02		**WWTPpe**

Table SM 5. Risk quotients for those compounds that resulted in a high risk in the upstream samples.

Classification	Compound	Season	WWTP	Concentration (ng/L)	PNEC (ng/L)	RQ	Risk
Others	Fipronil	Autumn	VID	3.3	0.77	4.33	High risk
Hormones	Estrone	Winter	TAL	1.2	0.36	3.23	High risk
Anti-hypertensives	Losartan	Winter	TAL	20.1	12	1.67	High risk

Table SM 6. Risk quotients for those compounds that resulted in a high risk in the downstream samples.

Classification	Compound	Season	WWTP	Concentration (ng/L)	PNEC (ng/L)	RQ	Risk
Anti-hypertensives	Losartan	Winter	TAL	692.6	12	57.72	High risk
Anti-hypertensives	Losartan	Winter	SEP	660.5	12	55.04	High risk
Anti-hypertensives	Losartan	Spring	ALPE	626.8	12	52.23	High risk
Anti-hypertensives	Losartan	Spring	FOL	608.7	12	50.72	High risk
Analgesics / Anti-inflammatories	Diclofenac	Spring	DTAL	2440.3	50	48.81	High risk
Analgesics / Anti-inflammatories	Diclofenac	Spring	SEP	1950.8	50	39.02	High risk
Anti-hypertensives	Losartan	Summer	VID	443.3	12	36.95	High risk
Analgesics / Anti-inflammatories	Diclofenac	Spring	ALPE	1556.3	50	31.13	High risk
Hormones	Estrone	Winter	TAL	10.7	0.36	29.63	High risk
Anti-hypertensives	Losartan	Summer	ALPE	320.9	12	26.74	High risk
Others	Fipronil	Summer	VID	19.7	0.77	25.58	High risk
Hormones	Estrone	Autumn	TAL	8.1	0.36	22.42	High risk
Anti-hypertensives	Losartan	Spring	SEP	267.5	12	22.29	High risk
Analgesics / Anti-inflammatories	Diclofenac	Autumn	TAL	1104.7	50	22.09	High risk
Anti-hypertensives	Losartan	Winter	ALPE	259.4	12	21.62	High risk
Hormones	Estrone	Spring	ALPE	7.1	0.36	19.74	High risk
Analgesics / Anti-inflammatories	Diclofenac	Winter	TAL	982.1	50	19.64	High risk
Others	Fipronil	Summer	FOL	14.5	0.77	18.79	High risk
Anti-hypertensives	Losartan	Winter	FOL	223.1	12	18.59	High risk
Others	Fipronil	Spring	TAL	13.6	0.77	17.65	High risk

Hormones	Estrone	Winter	SEP	5.9	0.36	16.45	High risk
Analgesics / Anti-inflammatories	Diclofenac	Winter	VID	815.1	50	16.30	High risk
Others	Fipronil	Summer	TAL	11.8	0.77	15.27	High risk
Others	Fipronil	Summer	SEP	11.3	0.77	14.63	High risk
Others	Fipronil	Autumn	TAL	10.7	0.77	13.95	High risk
Hormones	Estrone	Summer	TAL	5.0	0.36	13.89	High risk
Analgesics / Anti-inflammatories	Diclofenac	Spring	FOL	650.8	50	13.02	High risk
Analgesics / Anti-inflammatories	Diclofenac	Winter	ALPE	632.8	50	12.66	High risk
Analgesics / Anti-inflammatories	Diclofenac	Spring	VID	585.7	50	11.71	High risk
Anti-hypertensives	Losartan	Autumn	SEP	139.7	12	11.64	High risk
Antimicrobials	Azithromycin	Spring	TAL	217.2	19	11.43	High risk
Analgesics / Anti-inflammatories	Diclofenac	Summer	TAL	549.9	50	11.00	High risk
Antimicrobials	Azithromycin	Spring	FOL	208.9	19	11.00	High risk
Others	Fipronil	Spring	FOL	7.8	0.77	10.09	High risk
Others	Fipronil	Spring	SEP	7.6	0.77	9.89	High risk
Others	Fipronil	Summer	ALPE	7.3	0.77	9.47	High risk
Analgesics / Anti-inflammatories	Diclofenac	Winter	SEP	456.2	50	9.12	High risk
Antimicrobials	Azithromycin	Autumn	TAL	164.3	19	8.64	High risk
Anti-hypertensives	Losartan	Summer	TAL	101.5	12	8.46	High risk
Analgesics / Anti-inflammatories	Diclofenac	Summer	ALPE	416.0	50	8.32	High risk
Anti-hypertensives	Losartan	Spring	TAL	98.8	12	8.23	High risk
Psychiatric drugs	Sertraline	Winter	TAL	75.7	9.4	8.05	High risk
Others	Fipronil	Autumn	SEP	6.2	0.77	8.03	High risk
Others	Fipronil	Spring	ALPE	6.0	0.77	7.73	High risk
Others	Fipronil	Winter	TAL	5.8	0.77	7.59	High risk
Analgesics / Anti-inflammatories	Diclofenac	Autumn	SEP	350.7	50	7.01	High risk
Anti-hypertensives	Losartan	Spring	DVID	81.1	12	6.76	High risk
Analgesics / Anti-inflammatories	Diclofenac	Winter	FOL	324.9	50	6.50	High risk
Others	Fipronil	Winter	FOL	4.9	0.77	6.41	High risk
Hormones	Estrone	Summer	ALPE	2.3	0.36	6.37	High risk
Others	Fipronil	Winter	ALPE	4.3	0.77	5.57	High risk
Others	Fipronil	Autumn	VID	4.3	0.77	5.54	High risk

Anti-hypertensives	Losartan	Autumn	TAL	66.2	12	5.52	High risk
Antimicrobials	Azithromycin	Winter	TAL	93.6	19	4.92	High risk
Others	Fipronil	Winter	SEP	3.7	0.77	4.80	High risk
Analgesics / Anti-inflammatories	Diclofenac	Summer	SEP	205.8	50	4.12	High risk
Anti-hypertensives	Losartan	Autumn	ALPE	49.2	12	4.10	High risk
Hormones	Estrone	Autumn	SEP	1.5	0.36	4.08	High risk
Others	Fipronil	Autumn	FOL	2.8	0.77	3.68	High risk
Stimulant	Caffeine	Spring	SEP	4192.7	1200	3.49	High risk
Anti-hypertensives	Losartan	Autumn	FOL	41.8	12	3.48	High risk
Antimicrobials	Azithromycin	Winter	ALPE	64.6	19	3.40	High risk
Hormones	Estrone	Autumn	VID	1.1	0.36	3.08	High risk
Others	Fipronil	Autumn	ALPE	2.3	0.77	2.99	High risk
Anti-hypertensives	Losartan	Winter	VID	34.5	12	2.87	High risk
Others	Fipronil	Spring	VID	2.1	0.77	2.78	High risk
Psychiatric drugs	O-desmethylvenlafaxine	Spring	TAL	2384.9	880	2.71	High risk
Psychiatric drugs	Sertraline	Winter	SEP	23.6	9.4	2.51	High risk
Psychiatric drugs	Sertraline	Spring	TAL	22.2	9.4	2.36	High risk
Analgesics / Anti-inflammatories	Diclofenac	Summer	VID	117.1	50	2.34	High risk
Antimicrobials	Azithromycin	Winter	SEP	43.3	19	2.28	High risk
Analgesics / Anti-inflammatories	Diclofenac	Autumn	FOL	113.1	50	2.26	High risk
Psychiatric drugs	O-desmethylvenlafaxine	Spring	SEP	1946.1	880	2.21	High risk
Anti-hypertensives	Losartan	Summer	FOL	26.4	12	2.20	High risk
Others	Fipronil	Winter	VID	1.6	0.77	2.09	High risk
Antimicrobials	Azithromycin	Autumn	FOL	38.7	19	2.04	High risk
Psychiatric drugs	O-desmethylvenlafaxine	Autumn	TAL	1778.4	880	2.02	High risk
Psychiatric drugs	Lorazepam	Spring	TAL	190.8	96	1.99	High risk
Psychiatric drugs	O-desmethylvenlafaxine	Winter	TAL	1682.0	880	1.91	High risk
Psychiatric drugs	Lorazepam	Autumn	TAL	182.8	96	1.90	High risk
Psychiatric drugs	O-desmethylvenlafaxine	Winter	SEP	1616.3	880	1.84	High risk
Antimicrobials	Azithromycin	Autumn	SEP	34.3	19	1.81	High risk
Psychiatric drugs	Sertraline	Autumn	TAL	16.0	9.4	1.70	High risk
Analgesics / Anti-inflammatories	Diclofenac	Summer	FOL	84.8	50	1.70	High risk

Lipid regulator	Gemfibrozil	Spring	SEP	817.4	500	1.63	High risk
Antimicrobials	Azithromycin	Spring	SEP	31.0	19	1.63	High risk
Antimicrobials	Azithromycin	Summer	FOL	30.3	19	1.59	High risk
Lipid regulator	Gemfibrozil	Summer	VID	748.5	500	1.50	High risk
Psychiatric drugs	Sertraline	Winter	FOL	14.0	9.4	1.49	High risk
Lipid regulator	Gemfibrozil	Winter	TAL	738.9	500	1.48	High risk
Others	Fipronil	Autumn	VLD	1.0	0.77	1.35	High risk
Analgesics / Anti-inflammatories	Diclofenac	Autumn	ALPE	64.0	50	1.28	High risk
Lipid regulator	Gemfibrozil	Spring	ALPE	626.7	500	1.25	High risk
Antimicrobials	Azithromycin	Summer	TAL	23.2	19	1.22	High risk
Lipid regulator	Gemfibrozil	Winter	SEP	602.5	500	1.21	High risk
Antimicrobials	Azithromycin	Spring	ALPE	22.7	19	1.20	High risk
Psychiatric drugs	O-desmethylvenlafaxine	Spring	ALPE	1031.8	880	1.17	High risk
Psychiatric drugs	O-desmethylvenlafaxine	Spring	FOL	961.9	880	1.09	High risk
Lipid regulator	Gemfibrozil	Winter	ALPE	532.3	500	1.06	High risk
Psychiatric drugs	Lorazepam	Summer	TAL	100.8	96	1.05	High risk
Psychiatric drugs	O-desmethylvenlafaxine	Summer	TAL	918.8	880	1.04	High risk
Psychiatric drugs	Sertraline	Summer	TAL	9.8	9.4	1.04	High risk
Antimicrobials	Clarithromycin	Spring	FOL	122.0	120	1.02	High risk

Table SM 7. Stream classification based on Hamdhani et. al 2020 [2].

WWTP	Season	Q DP/DS	Classification	CECs	Classification	PO4	Classification	NO3	Classification
VID	Summer	0.87	Effluent-dominated	1.73	Effluent-dependent	1.06	Effluent-dependent	0.03	Effluent-impacted
VID	Autumn	0.05	Effluent-impacted	0.18	Effluent-impacted	0.10	Effluent-impacted	0.76	Effluent-dominated
VID	Winter	0.11	Effluent-impacted	0.30	Effluent-impacted	0.28	Effluent-impacted	0.90	Effluent-dominated
VID	Spring	0.41	Effluent-impacted	1.31	Effluent-dependent	0.79	Effluent-dominated	1.72	Effluent-dependent
TAL	Summer	0.36	Effluent-impacted	0.56	Effluent-dominated	0.42	Effluent-impacted	0.10	Effluent-impacted
TAL	Autumn	0.40	Effluent-impacted	0.54	Effluent-dominated	0.33	Effluent-impacted	0.54	Effluent-dominated
TAL	Winter	0.81	Effluent-dominated	0.93	Effluent-dominated	0.68	Effluent-dominated	0.39	Effluent-impacted
TAL	Spring	0.62	Effluent-dominated	0.76	Effluent-dominated	0.27	Effluent-impacted	0.28	Effluent-impacted
VLD	Summer	0.03	Effluent-impacted	0.20	Effluent-impacted	0.33	Effluent-impacted	6.26	Effluent-dependent
VLD	Autumn	0.02	Effluent-impacted	0.09	Effluent-impacted	0.07	Effluent-impacted	4.34	Effluent-dependent
VLD	Winter	0.01	Effluent-impacted	0.13	Effluent-impacted	0.10	Effluent-impacted	0.37	Effluent-impacted
VLD	Spring	0.02	Effluent-impacted	0.23	Effluent-impacted	0.18	Effluent-impacted	2.50	Effluent-dependent
FOL	Summer	0.26	Effluent-impacted	0.78	Effluent-dominated	0.45	Effluent-impacted	0.02	Effluent-impacted
FOL	Autumn	0.08	Effluent-impacted	0.29	Effluent-impacted	0.21	Effluent-impacted	0.44	Effluent-impacted
FOL	Winter	0.04	Effluent-impacted	0.22	Effluent-impacted	0.17	Effluent-impacted	0.01	Effluent-impacted
FOL	Spring	0.07	Effluent-impacted	0.19	Effluent-impacted	0.09	Effluent-impacted	0.01	Effluent-impacted
SEP	Summer	0.02	Effluent-impacted	0.14	Effluent-impacted	0.08	Effluent-impacted	0.16	Effluent-impacted
SEP	Autumn	0.10	Effluent-impacted	0.33	Effluent-impacted	0.07	Effluent-impacted	0.50	Effluent-dominated
SEP	Winter	0.10	Effluent-impacted	0.39	Effluent-impacted	0.19	Effluent-impacted	0.53	Effluent-dominated
SEP	Spring	0.18	Effluent-impacted	0.48	Effluent-impacted	0.35	Effluent-impacted	0.01	Effluent-impacted
ALPE	Summer	0.32	Effluent-impacted	0.72	Effluent-dominated	0.51	Effluent-dominated	1.47	Effluent-dependent
ALPE	Autumn	0.06	Effluent-impacted	0.12	Effluent-impacted	0.14	Effluent-impacted	1.80	Effluent-dependent
ALPE	Winter	0.04	Effluent-impacted	0.12	Effluent-impacted	0.10	Effluent-impacted	0.18	Effluent-impacted
ALPE	Spring	0.09	Effluent-impacted	0.19	Effluent-impacted	0.15	Effluent-impacted	0.10	Effluent-impacted

References

[1] Servei Meteorològic de Catalunya, Estacions Meteorològiques, (2021). https://www.meteo.cat/wpweb/climatologia/dades-i-productes-climatics/anuari-de-dades/estacions-meteorologiques/ (accessed October 28, 2024).

[2] H. Hamdhani, D.E. Eppehimer, M.T. Bogan, Release of treated effluent into streams: A global review of ecological impacts with a consideration of its potential use for environmental flows, Freshw Biol 65 (2020) 1657–1670. https://doi.org/10.1111/FWB.13519.

Publication #3

Occurrence of contaminants of emerging concern and pesticides and relative risk assessment in Tunisian groundwater

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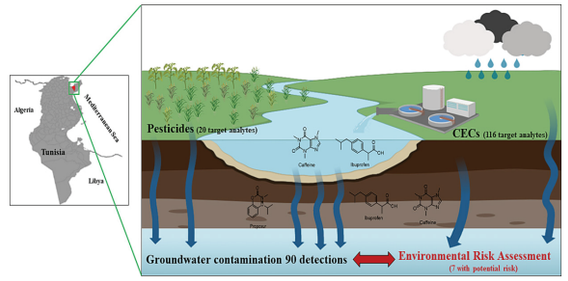
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HIGHLIGHTS

- CECs concentrations in Tunisian groundwater resemble those in industrialized countries.
- Toxic carbamates insecticides were detected in Tunisian groundwater samples.
- Caffeine, ibuprofen and proxopur posed the highest risk to the environment.
- Our results filled the gap on CECs and pesticides presence in Tunisian groundwater.

GRAPHICAL ABSTRACT



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ABSTRACT

Groundwater is an important source for drinking water supply, agricultural irrigation and industrial uses in the Middle East and North Africa region. Due to the growing need for groundwater use, groundwater quality studies on the presence of contaminants of emerging concern (CECs) and pesticides have gained attention. The Wadi El Bey is one of the most polluted areas in Tunisia. However, very limited data on CECs infiltration into aquifers has been described, in comparison to industrialized countries where groundwater contamination has been comprehensively addressed. To gain early insight into potential contamination, groundwater wells in northeast Tunisia, an area with high population density and intensive agricultural activity were sampled during two seasons and were analyzed with two high resolution mass spectrometry approaches: target and suspect screening. The latter was used for screening banned pesticides. A selection of 116 CECs of which 19 are transformation products (TPs) and 20 pesticides previously prioritized by suspect screening were screened in the groundwater samples. The results showed the presence of 69 CECs and 1 TP and 20 pesticides at concentrations per well, ranging between 43 and 7384 ng L^{-1} and 7.3 and 80 ng L^{-1}, respectively. CECs concentrations in Tunisian groundwater do not differ from those in industrialized countries. WWTPs were considered the main source of

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pollution, where the main classes detected were analgesics, antihypertensives and artificial sweeteners and especially caffeine, salicylic acid and ibuprofen were found to be ubiquitous. Regarding pesticides, triazines herbicides and carbamates insecticides pose the highest concern due to their ubiquitous presence, high leachability potential for most of them and high toxicity. The environmental risk assessment (ERA) highlighted the high risk that caffeine, ibuprofen, and propoxur may pose to the environment, and consequently, to non-target organisms. This study provides occurrence and ERA analysis of CECs and pesticides in Tunisian groundwater.

1. Introduction

Groundwater is a vital source of freshwater and plays a crucial role concerning global water demand in terms of drinking, agricultural and industrial purposes. In recent years, due to the growing need for groundwater use, groundwater quality recognition and studies on the presence of contaminants such as pesticides and their transformation products (TPs) (Kiefer et al., 2019; Bexfield et al., 2021) and contaminants of emerging concern (CECs) have gained attention. The presence of CECs and pesticides in groundwater can be attributed to various sources including point emissions and diffuse sources. Contamination by wastewater effluents is recognized as one of the main sources and usually present high concentrations of micropollutants (Ternes and Hirsch, 2000; Chaves et al., 2022). However, agricultural runoff, industrial discharges, septic systems and crop irrigation with reclaimed wastewater (Renau-Pruñonosa et al., 2020) also constitute important contributors to groundwater contamination. The dominant factors controlling the distribution of pesticides and their TPs in groundwater resources are well established with herbicides and their TPs being the most frequently detected compounds in aquifers (Kim et al., 2022). In contrast, CECs contamination from surface water bodies is much more difficult to predict (Labad et al., 2022). While global research concerning the presence of CECs and pesticides has increased, studies in the Middle East North Africa (MENA) region, including Tunisia, remain scarce (Haddaoui and Mateo-Sagaste, 2021). Up to date, there are only 13 studies carried out on the presence of CECs in the MENA region, including Algeria, Jordan, Lebanon, Palestine, Saudi Arabia and Tunisia (Mheidli et al., 2022; Kermia et al., 2016). In Tunisia, there is limited knowledge regarding the presence of CECs and pesticides, and the few studies published have mainly focused on WWTPs (Tahrani et al., 2018; Harrabi et al., 2018; Khazri et al., 2019). The knowledge gap becomes larger regarding the presence of CECs and pesticides in groundwater as well as the potential effects of wastewater recharge. The watershed of El Bey Wadi which covers a total area of approximately 664 km^2, includes several urban agglomerations such as Soliman, Bou Argoub, Grombalia, and Menzel Bouzelfa. This area presents vulnerability of its aquifers to contamination. This vulnerability is mainly attributed to the changes in the piezometric levels as a consequence of excessive extraction of groundwater, salinization, seawater intrusion in coastal phreatic aquifers and shallow groundwater contamination like nitrate contamination due to anthropogenic activities including the excessive use of chemical fertilizers and the return of irrigation water (Moussa and Zouri, 2011). In semi-arid countries, water shortage triggers the exploration of alternative water resources, such as groundwater for irrigation. Tunisia has not taken specific initiatives at a national level to study CECs and pesticides. Previous studies have demonstrated the presence of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyl (PCBs) pollutants as well as heavy metal pollution (Khadhar et al., 2018; Samia et al., 2018; Hassen et al., 2023). The challenging analysis of CECs and pesticides at trace levels mostly relies on international scientific collaborations. This has resulted in few data (Fries et al., 2016) and scientific research on CECs is still lagging in Tunisia (Haddaoui and Mateo-Sagaste, 2021). Consequently, evidence on many anthropogenic organic substances in groundwater is missing to adequately inform National policy on this topic.

In this context, this work investigated the occurrence of 116 CECs and 20 pesticides to help understand the occurrence and potential impacts of CECs in Tunisian groundwater. The watershed of El Bey Wadi and the associated shallow aquifer in Grombalia were selected for this study as they are surrounded by high anthropogenic activities, including agricultural activities and agglomerations. Two sampling campaigns in the Grombalia aquifer can provide valuable data to improve the understanding of the occurrence and distribution of CECs in groundwater samples from Tunisia. Analytical techniques such as liquid chromatography coupled to mass spectrometry (LC-MS) were employed to take advantage of the unique features of high resolution mass spectrometry (HRMS) to identify and quantify the diverse range of CECs and pesticides. Additionally, assessing the potential risks associated with these chemicals through environmental risk assessments can guide regulatory decisions and inform water management strategies. Hence, the specific objectives of this study were (i) to study the presence of CECs in groundwater from the Grombalia Plain using targeted analysis by means of LC-HRMS, (ii) to assess the presence of pesticides with an alternative analytical approach, applying a suspect screening protocol including quantification of the prioritized compounds with target analysis (iii) to assess the environmental risk associated with the occurrence of CECs and pesticide in groundwater. This study is the first to provide occurrence and environmental risk assessment analysis of CECs and pesticides in the northeast Tunisia, and will help update the current contamination profile for this ecosystem. The determination of CECs and pesticide occurrence was therefore an additional contribution to reaching a full picture of the contamination by organic and inorganic contaminants in this Tunisian area.

2. Materials and methods

2.1. Study area and sample collection

The study was carried out in the Grombalia Plain, situated in northeast Tunisia, in the Cap Bon peninsula bordered by the Mediterranean Sea to the north and the Djebel Ressa mountains to the south, covering a surface area of approximately 720 km^2. The Grombalia Plain is an arid and semi-arid region typical of the Mediterranean Basin and it is known for its fertile soil and Mediterranean climate, which make it ideal for agriculture. The Grombalia region has an average annual temperature of 17 °C (± 6) and an average annual precipitation of 486 mm, based on the rainfall records of the Grombalia rain gauge station (Lachaal et al., 2016), and a potential evapotranspiration with an average of 920 mm $year^{-1}$ (Charfi et al., 2013). The rain period extends from October to March representing >60 % of the annual precipitation (Lachaal et al., 2016). The humidity and temperature were similar between May and October. But, the data recorded in Grombalia obtained from the rain gauge station shows that monthly precipitation in October 2020 was 21 mm and as for May 2021, the rainfall recorded was 8 mm. As for annual temperature and rain (2020–2021) 20 °C and 411 mm were recorded. The watershed of Wadi El Bey (475 km^2) is located in the northeast and drains the Grombalia, Beni Khaled and Soliman plains. In Fig. 1, the study area as well as the different sampling points can be observed.

Two sampling campaigns were carried out, the first one in October 2020 and the second one in May 2021 period. Thirteen groundwater samples were collected in each campaign, wells GW1-GW13, distributed across the plain at both left and right of the Wadi El Bey (Fig. 1). Groundwater samples were purged from each well by pumping three

well volumes to remove the stagnant water and collected after several field parameters (such as temperature, pH and electrical conductivity) remained stable. The static water level of each well was measured using a piezometer and is reported in Table S1. All samples were collected in 1 L amber glass bottles and were rinsed previously with distilled water and then onsite with sample water and stored at 4 °C in a field refrigerator during their transport to the laboratory facilities.

2.2. Chemicals and reagents

One hundred and sixteen high purity analyte standards (> 98 %) were purchased from Sigma Aldrich (St. Louis, MO U.S.) for CECs monitoring. The number of analyzed CECs and TPs is summarised in Table 1 per compound class. Further details on the list of the 116 analytes are reported in Table S2. Analytical standards of twenty target pesticides, i.e. anabasine, atrazine, bendiocarb, bentranil, carbaryl, carbofuran, DEET, desethylatrazine, desethylterbuthylazine, dimethomorph, fenfuram, imidacloprid, isoproturon, methfuroxam, mexacarbate, propamocarb, propoxur, pyroquilon, spiroxamine, and terbuthylazine were purchased from Toronto Research Chemicals (Toronto, Canada). Isotopically labeled compounds of both CECs and pesticides were used as surrogates for calibration purposes; these were purchased from Cerilliant (Sigma Aldrich, St. Lous, MO, U.S.), Santa Cruz Biotechnology (Dallas, TX, US.), or Toronto Research Chemicals (Toronto, ON, Canada). Most standards as well as isotopically labeled standards were prepared in methanol at a concentration of 1000 mg L^{-1} and were stored at −20 °C until preparation of the working standard mixtures containing all 116 CECs (2 mg L^{-1}). High-performance liquid chromatography (HPLC) grade pure water, methanol, acetonitrile and ethyl acetate were supplied from J.T. Backer (Deventer, The Netherlands). Ammonium acetate, ammonia solution (29 %) and formic acid were purchased by Sigma-Aldrich. Ammonium fluoride was obtained from Fisher Chemical (Fisher Scientific SL, Madrid, Spain). For the solid phase extraction (SPE) cartridges preparation, different sorbents were purchased including; Oasis HLB, WAX, and WCX from Waters Corp., (Milford, MA, USA), and Bond Elut from Agilent Technologies Inc. (Santa Clara, CA, USA).

Table 1
Compound classification list.

Compound class	Number of compounds	Of which transformation products
Analgesics/anti-inflammatories	15	2
Anti-hypertensives	13	1
Antimicrobials	25	2
Artificial Sweeteners	6	–
Hormones	4	–
Illicit drugs	5	2
Industrial compounds	3	–
Insecticides	4	3
Lipid regulators	5	1
Others	6	–
Psychiatric drugs	24	6
Stimulants	3	2
UV filters	3	–

2.3. Target analysis: CECs

Water samples were stored at −20 °C until extraction. A target analysis was performed to study the presence of 116 CECs (Table S2). The analysis was performed following the method described in Gómez-Navarro et al. (2023). Briefly, the analysis was carried out with an off-line SPE, followed by ultra high-performance liquid chromatography coupled to a high resolution Q-Exactive mass spectrometer (UHPLC-qExactive-MS/MS). Before SPE, 500 mL of water samples were filtered at 0.7 μm glass fiber filters (Whatman, U.K.). Once filtered, the samples were spiked with a mix of isotopically labeled standards and were pre-concentrated using multi-layered and multi-sorbent SPE cartridges previously developed by Fagnani et al. (2022). These cartridges were constituted by two different layers, a top layer with 200 mg of Oasis HLB sorbent followed by a second layer containing a mixture of three

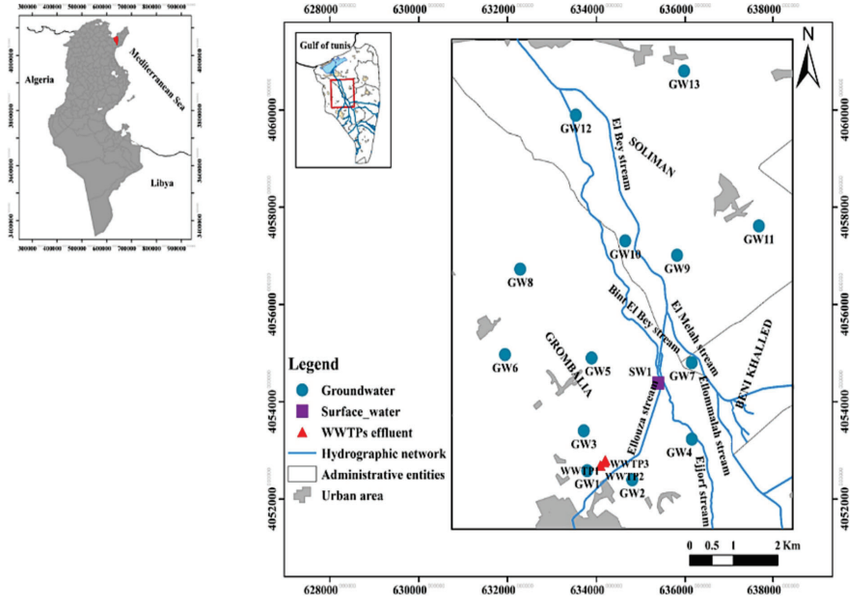


Fig. 1. Sampling sites and WWTP locations in the Grombalia plain.

different sorbents including WAX, WCX and Bond Elut PPL. A variety of sorbents were used to maximize the retention of the targeted CECs list which include contaminants covering a wide polarity range with log Ps ranging from −2.16 to 5.62. Once the samples were loaded, the cartridges were dried and eluted in three steps with a mixture of methanol: ethyl acetate (50:50 *v*/v), a first step with 2 × 3 mL with 5 % ammonia, followed by 2 × 3 mL with 2 % formic acid and finally 3 mL of the neutral mixture. The final extracts (15 mL) were then evaporated to dryness under a gentle nitrogen stream and reconstituted with 0.5 mL of methanol: water (5:95 v/v).

Analysis of the extracts was performed by LC-MS using a Waters ACQUITY UHPLC system (Waters, Milford, MA) and chromatographic separation was obtained using a Waters ACQUITY UHPLC HSS T3 (C18) column. A Q-Exactive Orbitrap mass spectrometer (Thermo-Fischer Scientific, Germany) equipped with heated electrospray ionization (HESI) was used for the detection. Further details concerning the method applied and its validation (linearity, detection and quantification limits (LOD, LOQ), etc.) are described in Gómez-Navarro et al. (2023). Thirty-three of the 116 CECs were analyzed in negative ionization mode while the 83 others were in positive ionization. For the compounds analyzed in positive ionization mode, the mobile phase consisted of acetonitrile as the organic phase and the aqueous phase consisted of water with 5 mMol of ammonium acetate and 0.1 % formic acid. While in negative ionization mode, the mobile phase consisted of acetonitrile and 2 mMol ammonium fluoride in water. The LC separation gradient consisted of 5 % acetonitrile for 0.3 min then ramped to 30 % from 0.3 to 10.0 min, followed by 65 % from 10.0 to 13.3 min, by 100 % from 13.3 to 15.5 and held at 100 % acetonitrile from 15.5 to 17.3 min. The column was re-equilibrated at 5 % acetonitrile for 1.3 min before the next injection. The data was then processed with the following software XcaliburTM version 4.1.31.9 and TraceFinder 4.1 for the quantification and confirmation of the targeted compounds. A matrix-matched calibration curve of ten concentration levels in the range of 0.05 to 1000 ng L^{-1} was used to compensate for matrix effects in the target analysis quantification. Isotopically labeled standards were included in all levels at a fixed concentration of 50 ng L^{-1}.

Quality assurance and quality control were achieved by analysing blank samples and quality controls (mix of standards at a known concentration, usually 50 ng L^{-1}) at the beginning and randomly along the sequence to control the instrument signal stability.

2.4. Suspect screening approach: pesticide analysis

For the extraction of the pesticides' analytes, 500 mL of sample were filtered using GF/F filters to remove possible suspended matter. Then, isotopically labeled standards were spiked (50 μL) and samples were extracted by SPE using 500 mg sorbent of Oasis HLB cartridges and finally eluted at a flow rate of 1 mL min^{-1} using 8 mL of acetonitrile.

First, the extracts were submitted to a suspect screening for the detection of pesticides, based on different parameters such as mass accuracy, both isotopic and fragmentation patterns, and retention time prediction. As a second step, prioritized compounds were quantified following a target methodology.

For the suspect screening, the analysis was performed following the method described in Tadić et al. (2022). Briefly, HPLC Accelera 600 pump coupled to a Q-Exactive Orbitrap mass spectrometer (Thermo Fischer Scientific, Les Ullis, France) with a HESI source was used for detection and the column for the chromatographic separation was a Waters XBridge BEH C18 (2.1 × 150 mm and 2.5 μm particle size). The injection volume was set at 10 μL and the flow rate applied was 0.3 mL min^{-1}. The separation gradient consisted of water and acetonitrile as mobile phases, both at 0.1 % of formic acid, starting with 10 % of water from 0 to 1 min, then increasing to a 90 % from 10 to 23 min, and finally 10 % of water from 24 to 29 min. Full scan data were acquired at a resolution of 70,000 and a scan range of 100–1000 *m/z*. Concerning the MS2, both data-dependent acquisition (DDA) and data-independent acquisitions (DIA) modes were employed at a resolution of 17,500 and a scan range of 50–1000 m/z. As for the identification parameters, the following were established for confirmation; the parent compound and at least 2 fragments detected within a mass error < 5 ppm, peak intensity higher than 10^4, as well as an isotopic match of at least 80 %. Once the initial screening was performed, potential candidates were chosen for subsequent DDA-MS2 analysis. The acquired spectra were compared against online available databases such as MassBank, MoNA, or mz cloud for final confirmation, reaching identification level 2a or 2b based on the obtained MS2 spectra (Schymanski et al., 2014).

For the targeted quantification of the prioritized pesticides, the method used was also developed by Tadić et al. (2022) where an Alliance HPLC system (Waters Series 2695) and a reverse-phase column, a Phenomenex Kinetex Polar C18 (100 mm × 4.6 mm I·D × 2.6 μm particle size) were used. In this case, the mobile phase consisted of Milli-Q and acetonitrile, both with 0.05 % formic acid, and the flow rate was set at 0.4 mL min^{-1}. The separation gradient consisted of acetonitrile increasing from 60 to 100 % for 10 min followed by 2 min stabilization and then back to initial conditions. As for the acquisition mode employed, a multiple reaction monitoring mode was used and all analytical data treatment was performed with MassLynx software from Waters where Quantlynx interface was used for the quantification of the potential targeted compounds. Further details concerning both the suspect screening and the target analysis as well as accuracy, precision, linearity, LOQs and LODs of the analytical methods are described in Tadić et al. (2022).

2.5. Environmental risk assessment

The environmental risk was assessed through risk quotients (RQs), defined as the ratio of exposure concentration and the threshold of adverse effects, in this case, the predicted no effect concentration (PNEC). The RQs were calculated using two different methods, the maximum concentration (Cmax) found (Eq. (1)) and the mean concentration (Cmean) found (Eq. (2)) all in ng L^{-1}, to predict both the possible worst-case scenario and a more conservative risk scenario respectively.

$$RQ = \frac{Cmax}{PNEC} \quad (1)$$

$$RQ = \frac{Cmean}{PNEC} \quad (2)$$

In all cases, the lowest PNEC values in freshwater were considered as quality targets for the calculation of risk assessment as suggested by experts for prioritization purposes. The lowest PNECs in freshwater are preferably based on experimental eco-toxicity data, but in case of no or insufficient empirical endpoints, QSAR predictions were used to estimate a provisional P-PNEC value to allow for a first screening. All the lowest PNECs were obtained from the NORMAN Ecotoxicology Database (https://www.norman-network.com/nds/ecotox/). A resulting RQ <1 would relate that the chemical exposure is less than the benchmark considered (PNEC), explaining that the exposure to that certain chemical is unlikely to result in adverse effects to the environment.

In order to assess the extent of water pollution in the different investigated sites of the Grombalia Plain, an additive model was used for the calculation of the total risk in any specific site (RQtot). The calculation is made by adding the individual RQ values (RQi) of all the compounds found in a specific sample, following the Eq. (3):

$$RQtot = \sum_{i=1}^{n} RQi \quad (3)$$

This model assumes an additive action of the contaminants, without considering the possible synergistic or antagonistic effects (Barbieri et al., 2021), as well as the specific behavior of each compound. Anyway, it can be used as a first approach to have a picture of the overall site-specific contamination due to the presence of different class of contaminants like the case of pesticides and pharmaceuticals in this

specific case study.

3. Results and discussion

3.1. Occurrence and spatial-temporal distribution in groundwater

3.1.1. Targeted CECs

To the best of our knowledge, there is a lack of data concerning the occurrence of CECs in groundwater from Tunisia. The list of CECs including commonly prescribed drugs, artificial sweeteners, insecticides, and UV filters was selected mainly based on their prevalence and abundance in the aquatic environment, as well as their widespread human use and consumption. Such a list of CECs was monitored for the first time in groundwater samples from the Grombalia Plain in Tunisia. Sixty-nine compounds, i.e. 59 % of the targeted compounds studied were detected in at least one sample from one campaign (above LOD), covering a broad range of different concentrations. Forty-seven compounds were not detected in any of the samples. Interestingly, commonly found CECs in groundwater, such as the insecticide fipronil and its TPs were not found in any of the samples. None of the illicit drugs analyzed were detected above LOQs in any of the samples, except for methadone which was detected in sample GW6 in the first campaign at a concentration of 54 ng L^{-1}. Concerning the psychiatric drugs, 15 out of 24 analyzed compounds were not detected in any of the samples. In contrast, several therapeutic classes, including antimicrobials, analgesics and anti-inflammatories, were detected in surface wells within the study area. However, it was challenging to compare these findings as no previous studies had been conducted on their occurrence in Tunisia or the southern Mediterranean region. For instance, 6a methyl prednisolone, an anti-inflammatory drug, was identified in surface wells for the

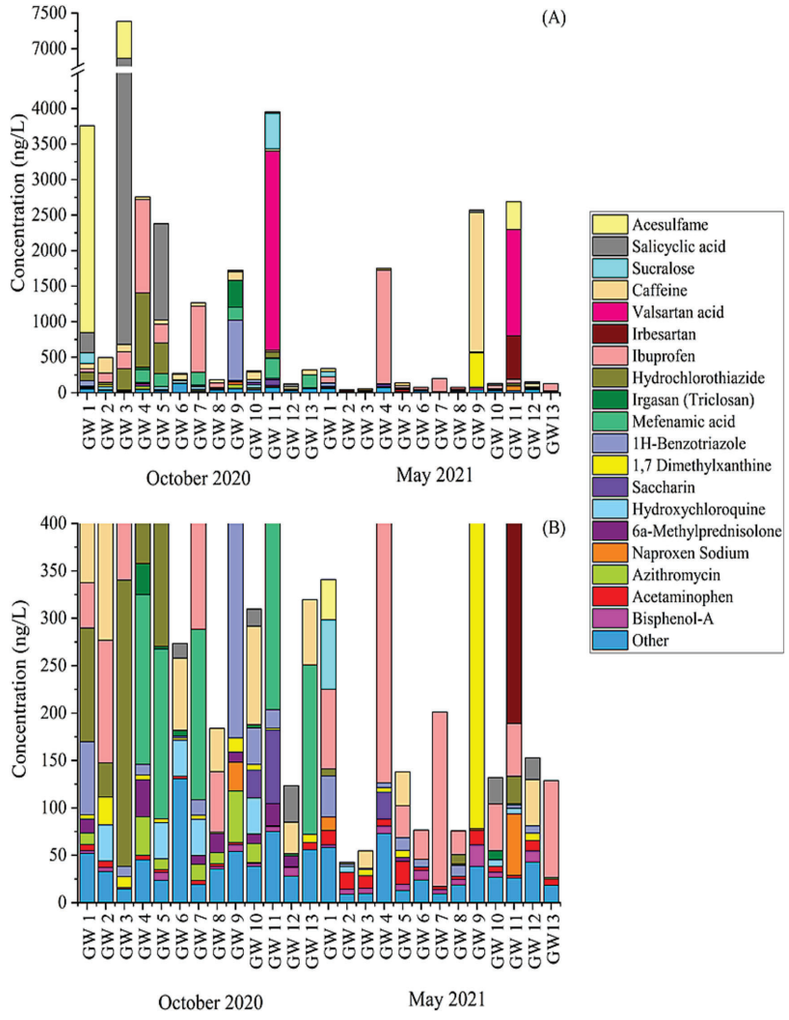


Fig. 2. (A) Total concentration of CECs in the Grombalia plain groundwater samples from both campaigns. (B) Zoom of (A) to show the other predominant CECs at lower concentration (<400 ng L^{-1}).

first time in Tunisia, with an average concentration of 16 ng L^{-1}. Furthermore, sweeteners, such as sucralose, for which there had been no recorded occurrences in Tunisian groundwater, were found at a concentration of 497.2 ng L^{-1}.

In Fig. 2, the total concentration found per compound is shown, for every sampled well and for both studied campaigns. Compound ubiquity was also assessed by calculating the frequency of detection (FoD) i.e. the number of samples above the LOD over the total number of samples. FoD as well as the concentration found in every sample are listed in Table S2 in the supplementary material. Salicylic acid, acesulfame, ibuprofen and caffeine were the contaminants found at the highest FoD and concentration. In terms of spatial variation, a gradient of concentrations was anticipated between the wells close to WWTP discharges and those closer to the Mediterranean Sea. As acesulfame and caffeine (CAF), often used as wastewater tracers, (Nödler et al., 2013) were detected, WWTP discharges were assumed to be the main source of contamination. Overall, the highest groundwater concentrations (> 2 μg L^{-1}) and the largest number of detected compounds were found in zones close to the Wadi El Bey (GW1, GW3, GW4, and GW5) and most likely recharged by the Wadi that receives large amounts of effluents from WWTPs. In contrast, the sampling points located far away from the Wadi (e.g., GW6, GW8, and GW13) or downstream the main WWTP discharges (e.g., GW12) yielded not only lower concentrations but also a much smaller number of detected compounds. One exception was GW11, where a local origin of contaminants was suggested. This gradient of concentrations from the highest urbanized area possibly meant dilution together with removal processes of compounds under oxidizing (surface water) and reducing (aquifer) conditions. The well GW11 reported high total concentrations in both campaigns, where valsartan acid was the compound detected at the highest concentration in both campaigns (Fig. 2). Valsartan acid is a transformation product of various sartan-class pharmaceuticals i.e., different hypertensives (Posselt et al., 2020). Consequently, valsartan acid was detected at important concentrations, and for the first time in groundwater samples from Tunisia. Interestingly, it was only detected in GW11. This well is located very near an urban area, thus we supposed that there was a larger consumption of sartan-class pharmaceuticals, resulting in the presence of valsartan acid which was detected at 2804 and 1492 ng L^{-1} in the first and second campaign respectively. In the first campaign, from the sartan-compounds included in our study, none were detected, while in the second campaign, irbesartan was detected at 614 ng L^{-1}. The consumption of other sartan groups was assumed, justifying the valsartan acid concentrations reported in GW11 well.

Surprisingly, cationic compounds were also detected such as the β-blocker metoprolol, the alprazolam benzodiazepine and the tramadol analgesic with FoD of 100 % in at least one of the campaigns and concentrations ranging from 0.25 to 5.51, 4.85 to 6.21, and 2.90 to 24 ng L^{-1}, respectively. These are rather expected to be retained in soil rather than in water. Consequently, the direct contamination of the aquifer by wastewater without passing through the soil layer could not be ruled out. Interestingly, tramadol was found in all samples in both campaigns similarly to previous groundwater studies (Labad et al., 2022), and other studies referring to other aquatic compartments where its presence has been reported in the environment (Golovko et al., 2020; Sadutto et al., 2021). The 100 % FoD of tramadol was most likely related to its high consumption and increased use during the Covid period in Tunisia. The occurrence of sulfamethoxazole (SMX) and crotamiton was more reasonable. SMX is a polar and negatively charged compound (at pH 7) which can easily leach into groundwater and has often been the only antibiotic found in several groundwater monitoring studies (Labad et al., 2022; López-Serna et al., 2013; Szekeres et al., 2018). SMX has been recently suggested to be included in the list of priority contaminants of the EU Groundwater Directive, while crotamiton has been proposed as a tracer of groundwater contamination by wastewater in urbanized areas due to its high environmental persistence similar to carbamazepine (CBZ) (Kuroda et al., 2012). SMX was detected in all samples but only during the first campaign in October 2020 with concentrations ranging from 0.40 to 9.32 ng L^{-1}. SMX is a commonly used livestock antibiotic, and has been previously reported in groundwater from Tunisia (Fries et al., 2016), both studied regions commonly use bovine manure to improve soil fertility and hence its presence was therefore not surprising. The difference between seasons could be explained due to the manure period application or by the fact that antibiotic consumption in humans usually increases during the winter season.

CAF and acetaminophen are readily biodegradable compounds but their high usage and the direct wastewater input into the aquifer might account for their occurrence in most of the sampling points (Fig. 2). Acetaminophen is one of the most popular and commonly used analgesic and antipyretic drug around the world (Al-Mashaqbeh et al., 2019), thus is high detection in all samples was not surprising. Caffeine is a commonly consumed stimulant and is usually used as a wastewater contamination tracer. CBZ and CAF would be the most commonly used WWTP contamination indicators. CBZ is usually more stable then CAF, and persists longer as its stability enables CBZ to migrate from the pollution source and enter the aquifer (Dvory et al., 2018). In this study, CBZ was not detected in any sample however CAF was detected in all samples with concentrations ranging from 33 to 219 ng L^{-1} in the first campaign and 0.23 (LOQ) to 1961 ng L^{-1} in the second campaign. CAF resulted an appropriate contamination indicator due to the proximity of pollution sources both in time and location (Dvory et al., 2018). CAF has been commonly found in groundwater samples even though with slight variations in concentration (Kruć et al., 2019; Labad et al., 2022; Loos et al., 2010). Its metabolite, 1,7 dimethylxanthine, was also detected in all samples and it was detected for the first time in Tunisian groundwater. Its concentration ranged from 0.42 to 29 ng L^{-1} and 0.42–485 ng L^{-1} in both campaigns respectively. Maximum concentrations of the caffeine metabolite certainly corresponded to the wells with maximum caffeine concentration as these are positively correlated.

Azithromycin (log $P = 2.4$) is a broad spectrum macrolide, with high activity against atypical respiratory pathogens, prescribed during the Covid pandemic as a potential Covid-19 treatment option in Tunisia. It has also been recently included in the EU Watch list of CECs due to its high environmental persistency and due to its contribution to the environmental spreading of resistant bacteria strains. Azithromycin was one of the antimicrobials found at the highest concentrations with concentrations from 11.3 to 54.2 ng L^{-1} and detected at a FoD of 52 %, but only detected during the first campaign, relating the detection with Tunisian Covid waves. In previous studies, azithromycin has been detected at similar concentrations and found to reach deep alluvial sediments and was already detected in groundwater samples below the streambed (Senta et al., 2021; Silori et al., 2022).

Temporal distribution was also studied and a generalized decrease was observed from the first campaign to the second campaign. The concentration of CECs sorted by the compound class described in Table 1 and per season can be observed in Fig. 3. The most concentrated CECs class were analgesics/anti-inflammatories, followed by anti-hypertensives. In the case of the anti-hypertensive class, in October 2020 an important dispersion is observed between samples, covering a wide range of different concentrations. In terms of total concentration per class of CECs, there is a relevant decrease, especially concerning analgesics/anti-inflamatories and antihypertensives. However, this decrease in concentrations between campaigns was not statistically significant, Anova tests were performed to study possible significant differences between campaigns, but the obtained p-values were > 0.05. Nevertheless, this decrease could be attributed mainly to the Tunisian Covid waves, as during the first campaign in October 2020, Tunisia was suffering from the second pandemic wave and hence a higher consumption of these was expected.

In the case of industrial compounds, including benzotriazole, its metabolite methyl benzotriazole and bisphenol A (BPA), again no statistical differences between seasons were observed. BPA was detected at FoD of 69 and 85 % and a mean concentration of 5 and 8 ng L^{-1} for the

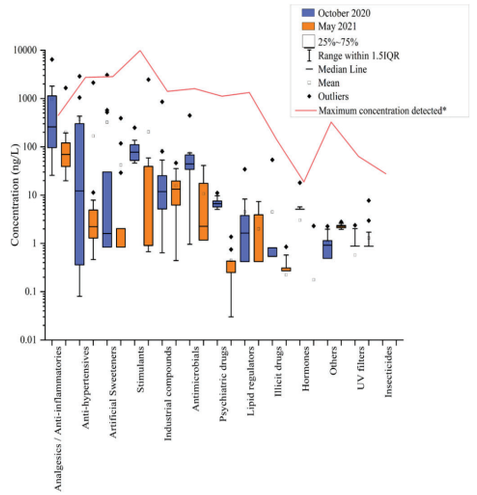


Fig. 3. Box-plot of the concentration per CECs classes for October 2020 (blue) and May 2021 (orange). The red line indicates the maximum concentration detected in several studies *(García-Gil et al., 2018; Hass et al., 2012; Labad et al., 2022; López-Serna et al., 2013; Szekeres et al., 2018; Wolf et al., 2012).

first and second campaign, respectively. The concentrations detected are quite low in comparison to other studies performed in Brazil, Austria or USA between others, where the average concentration range from 20 to 50 ng L^{-1} (Dueñas-Moreno et al., 2022). In accordance to Bono-Blay et al. (2012), BPA tends to be detected at lower concentrations in groundwater than surface water because of the strong attenuation processes, mainly adsorption and biodegradation that occur in the soil column. Benzotriazole was detected at high FoD in both campaigns 100 % and 92 % being the concentration of May 2021 lower. In October 2020, well GW9 presented a maximum concentration of 847 ng L^{-1}, similar maximum concentration was detected by Loos et al. (2010). This punctual high concentration peak leads to the fact that sporadic high concentrations were detected in some groundwater samples, providing evidence that there were periods of high contamination. However, these latter did not remain stable during the year and thus prove that groundwater was capable of recovering back to lower contamination levels.

The red line included in Fig. 3 shows the maximum concentrations of CECs detected in groundwater samples from different countries. Interestingly, concentrations found in the Grombalia Plain groundwater samples do not differ from those encountered in industrialized countries (Table SM 4). In most cases, the maximum concentrations would match outlier samples, except for analgesics and stimulants, where Tunisian sample concentrations were slightly higher. In the case of psychiatric drugs, in the Tunisian wells studied, a maximum concentration of 6 ng L^{-1} was detected, much lower than concentrations found in other groundwater studies (Hass et al., 2012). This could be explained by a possible lower consumption of psychiatric drugs in Tunisia, as only 7 of the 24 psychiatric drugs studied were detected above quantification limits, interestingly, one of the most ubiquitous, carbamazepine, was barely detected. However, to further confirm this assumption, surface water and WWTP effluents should be analyzed.

3.1.2. Pesticides

By applying the analytical approach described in the methodology section, a total of 20 compounds were detected in groundwater samples. Nine compounds were confirmed with analytical standards following the methodologies described above, including terbuthylazine, desethylterbuthylazine, desethylatrazine, isoproturon, imidacloprid, dimethomorph, propoxur, mexacarbate and DEET among which 5 pesticides (i.e., mexacarbate, propoxur, desethylatrazine, terbuthylazine and DEET) could be frequently quantified (see Table 2). Among the 10 remaining sporadically detected compounds, 8 were identified at a level of confidence 2a including anabasine, propamocarb, carbofuran, methfuroxam, bendiocarb, carbaryl, fenfuram, spiroxamine and 2 at a level of confidence 2b including pyroquilon and bentranil.

The most frequently quantified pesticides belong to the family of triazine herbicide and carbamate insecticide. Triazines have been highly used on several agricultural crops. The occurrence of triazine herbicides in groundwater has been found very commonly (Baran et al., 2021) with most likely no potential human-health concerns at the observed levels of detection (Bexfield et al., 2021). The frequent detection of triazines can also be expressed as a function of their groundwater ubiquity score (GUS) index, used to predict the behavior of pollutants in groundwater. Triazines are the compounds with the highest GUS index and detection frequency, indicating their high leachability. Atrazine and its degradation product desethylatrazine are by far the most abundant herbicides detected in shallow groundwater (Shomar et al., 2006; Tappe et al., 2002). Atrazine was not detected in the sampling sites, while its metabolite desethylatrazine was detected in 13 samples in both sampling campaign (Fig. 4). Some researchers have shown that pesticide metabolites are often detected in groundwater at higher concentrations compared to parent compounds (Kolpin et al., 2004; Lapworth and Gooddy, 2005) and that their presence can be more toxic than their parents (Sinclair and Boxall, 2003; Stuart et al., 2012).

In contrast, the detection and quantification of several carbamate insecticides cause much more concern due to their high acute and chronic toxicity. The occurrence of propoxur and mexacarbate, two phenyl-, *N*-methyl-carbamates was not fully surprising because they are water soluble (1800 mg L^{-1} and 100 mg L^{-1}, respectively) and mobile in soil (Koc of 30 and 300, respectively). They have therefore the potential for leaching to groundwater. The main source of these insecticides was agricultural activities due to their low concentrations in the WWTP effluent discharge (10.2 and 0.2 ng L^{-1}, respectively). This is in contrast to desethylatrazine, DEET and imidacloprid for which concentrations peaked in WWTP effluents (1635, 85, 7 and 37.4 ng L^{-1}, respectively). DEET contamination is widespread in the aquatic environment as indicated by Turner et al. (2019) and Loos et al. (2010). Consequently, this compound was detected in all wells and in both sampling campaigns. There was no significant variation observed between the two sampling periods. DEET concentrations ranged from 7.3 to 9.6 ng L^{-1} in 2020 with a median of 7.8 ng L^{-1}, and from 7.7 to 8.8 ng L^{-1} in 2021 with a median of 8.2 ng L^{-1}. This is in contrast to the lower EU median concentration of 1 ng L^{-1}, and the higher values of 49.5 ng L^{-1} reported in Singaporean groundwater (Sorensen et al., 2015).

Additional three carbamate insecticides namely bendiocarb, carbofuran, and carbaryl, as well as one carbamate fungicide (propamocarb) were identified in this study even though they could not be quantified due to the lack of analytical standards. This result shows the vulnerability of the investigated shallow aquifer to insecticide spraying in agriculture. The USEPA classified carbamates as hazardous and included

Table 2
Concentration of the most frequently detected pesticides in groundwater samples.

Compound	Frequency	Range (ng L^{-1})	Mean[a] (ng L^{-1})
DEET	26/26	7.3–9.6	8.1
Mexacarbate	19/26	0.2–2.5	0.80
Propoxur	14/26	9.8–11.4	10.2
Desethylatrazine	13/26	0.2–47.3	16.4
Terbuthylazine	13/26	0.3–54.6	15.8

[a] Mean calculated only for detected compounds.

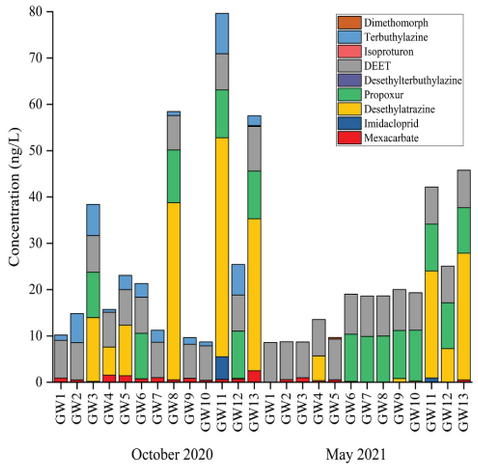


Fig. 4. Concentrations of prioritized pesticides in groundwater samples of the Grombalia aquifer.

them in their priority list (Boujelbane et al., 2022) because they are probable carcinogens for humans. Several studies revealed carbamates as responsible for altering the normal functioning of neurological, reproductive and immune systems (Morais et al., 2012). They are also highly toxic to birds, fish and benthic macroinvertebrates (Guarda et al., 2020). Mexacarbate is classified by the World Health Organization among class I "highly hazardous pesticides". Furthermore, even though carbamates pesticides are unauthorized by the EU, they are still approved in Tunisia for their use. Imidacloprid, a neonicotinoid insecticide was also found to be present in two GW samples at the maximum concentration of 4.9 ng L^{-1} (Fig. 4). This compound is highly soluble (610 mg L^{-1}), moderately mobile and persistent in soil (DT50 of 191 days), also being highly toxic to birds and honeybees and moderately toxic to mammals and fish (Lewis et al., 2016). Since investigated groundwater is not intended to human consumption and since the occurrence levels are very low (few ng L^{-1}), risks are likely very limited. However, taking in mind that this is the first study to provide insight into the occurrence of pesticides in the shallow aquifer in Grombalia, the first monitoring results support the need to collect more data, especially data on agricultural practices.

In terms of spatial distribution, the contamination pattern tends to increase alongside the SE-NW Grombalia regional groundwater flow towards the Gulf of Tunis (Moussa and Zouri, 2011), with higher concentrations in GW samples located closer to the Gulf. The occurrence of pesticides in groundwater after their application during seasonal agricultural practices strongly depends on their transport through the vadose zone, with the corresponding delay in time due to the usually slow transport of the compounds to the aquifer (Köck-Schulmeyer et al., 2013). Pesticides may accumulate along the river course and consequently, have increased concentrations at the northern sampling points after leaching into groundwater. Their distribution is also related to the wide agricultural practices in the Grombalia region, which economy is mainly based on citrus, vegetable crops (e.g., tomatoes, artichokes), vines and grain as main agricultural activities. In particular, 77.3 % of the irrigated area is planted with citrus (Lachaal et al., 2016). In fact, in the Nabeul district where the Grombalia region belongs, the production of citrus was about 255,000 tons in 2014, counting for 78 % of the total national production of citrus in Tunisia (Lachaal et al., 2018).

Contrariwise, the pesticides' temporal distribution is not significant. An overall higher amount of pesticides was detected in the first sampling campaign of October 2020 at specific locations in comparison with May 2021. However, no huge differences can be pointed out considering the low and generally similar detected levels over time. Many factors can contribute to the contamination profile of a specific area, with variability over time most likely related to the aquifer recharge, rainfall events, soil characteristics, irrigation practices, which facilitate the transport of pesticides into the groundwater, and water availability (Jurado et al., 2012). In the case of Wadi El Bey, the intermittent flow regime makes the area susceptible to dilution factor which should also be considered to outline contaminants' temporal distribution and pollutants leaching into groundwater. Often, the use of treated wastewater for agricultural irrigation may contribute to the increase in the concentration of pesticides in groundwater (Renau-Pruñonosa et al., 2020).

3.2. Environmental risk assessment

Environmental risk assessment (ERA) was performed for those substances that are ubiquitous in the groundwater samples analyzed and for those compounds which are included in the watch list and are worth considering. Fig. 5 shows the substances evaluated where in this study the approach proposed by Hernando et al. (2006), was applied. RQ above 1 is considered as high environmental risk (red), RQ between 0.1 and 1 medium (yellow) and below 0.1 as low risk (green). Further details about ERA are reported in the supplementary material (Table S5).

The obtained data shows that CAF and ibuprofen pose a high risk to the environment, as well as propoxur at a medium to a high level. In previous studies, caffeine has also been identified as a potential substance causing high risk to algae (Sadutto et al., 2021). CAF is a worldwide highly consumed stimulant, ingested by the population through various means including, soft drinks, coffee, tea and even medicines. These sources contribute to the introduction of CAF into the environment and is essential to consider its impact (Dafouz et al., 2018; Quadra et al., 2020). In Tunisia, the mean coffee consumption per day ranges between 1891 and 5810 mg per person (Quadra et al., 2020). The data obtained indicates that CAF poses a significant environmental risk. This concern was underscored in Quadra's 2020 study, which highlighted that developing countries like Tunisia, as well as Brazil, Turkey, Ethiopia, Vietnam, and Indonesia, could become contamination hotspots over time due to their inadequate sanitation and wastewater treatment technologies, coupled with higher per capita CAF consumption. At the same time, when comparing both campaigns, for the majority of the cases there is no difference in the environmental risk between campaigns. However, the most remarkable result would be azithromycin where the concentrations found during the first campaign result in a high risk to the environment, both in the conservative and worst-case scenario but the presence of this antibiotic remains undetectable during the second campaign. As mentioned previously, this change is mainly associated with the Covid waves, where during the first campaign in October 2020, Tunisia was in general suffering from the second pandemic wave, while in May 2021 the Covid situation was much calmer.

This situation is also well visible if we look at the results obtained after the calculation of the total risk (RQtot) derived by the sum of the individual risk of each compound in a specific sample (Table S5). In Fig. 5, the RQtot are represented with a star. RQtot were above 1 in all samples in both sampling campaigns except in two cases, GW2 and GW3 in 2021. As it can be observed in Fig. 5, this high environmental risk is mainly due to the presence of ibuprofen and CAF, azithromycin and propoxur, which in most cases directly overlap with the individual compound RQ due to the high risk presented individually. However, in the samples where their concentrations were quite low, the results obtained by the addition of the individual risk of all the compounds increase the total risk. This outcome confirms that even small concentrations can be harmful if we consider the whole pool of contaminants, which may be present in a water sample, pointing out the importance of studying the effects derived from the co-occurrence of

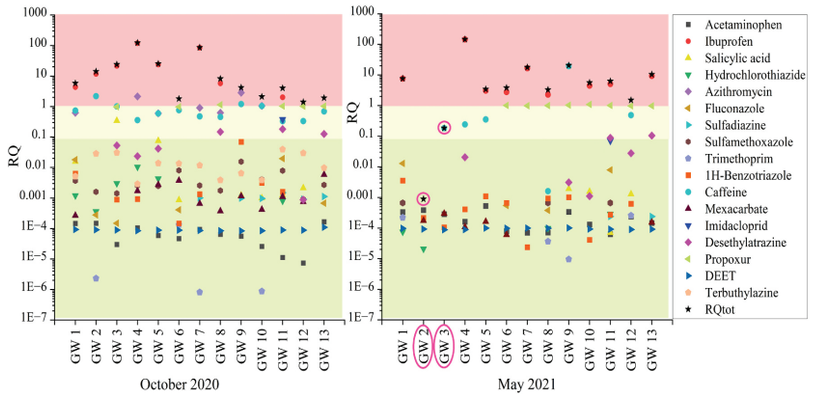


Fig. 5. Environmental risk associated to main ubiquitous substances found in groundwater samples from the Grombalia Plain.

different classes of compounds in order to determine the real risk they may pose to the environment.

Concurrently, the RQs obtained, both at an individual basis and as a whole, indicated that groundwater quality has reached a critical threshold concerning the presence of caffeine, ibuprofen and propoxur. This highlights the need for future studies and monitoring to ensure future viability of groundwater use.

4. Conclusions

This work provided information about the occurrence of CECs and pesticides in the Grombalia shallow aquifer, northeast of Tunisia, and constitutes one of the first studies reporting on the detection of a large number of CECs in a MENA country. Sixty-nine CECs and 20 pesticides were detected and quantified in groundwater samples. Their relative occurrence was very heterogeneous across the 13 sampling wells with large differences in total contaminant burden and individual contaminant concentration in the range of sub ng L^{-1} to a few μg L^{-1}. This study also demonstrates that there are no market differences between the concentrations of CECs found in groundwater samples from Tunisia, North Africa, compared to those found in the industrialized countries. Several wastewater contamination markers were confirmed such as CAF and its metabolite and some artificial sweeteners. This is in agreement with the observation of most of the detected CECs found in groundwater are frequently reported in WWTPs effluents indicating that human wastes are the main source of pollution. Furthermore, TPs/metabolites such as valsartan acid and 1,7 dimethylxanthine were detected for the first time in Tunisian groundwater, reaching concentrations of 2804 ng L^{-1} and 485 ng L^{-1}, respectively. Seasonal variations could be correlated to Tunisia's Covid waves, particularly during the first campaign in October 2020, when the country faced its second pandemic wave, resulting in increased consumption of analgesics and anti-inflammatories. Notably, azithromycin, prescribed as a Covid-19 treatment option in Tunisia, was detected at its highest concentrations during the first campaign, ranging from 11.3 to 54.2 ng L^{-1}, aligning with the timing of Tunisian Covid waves. Regarding pesticides, a suspect HRMS screening approach was very helpful for confirming the occurrence of a few toxic carbamate insecticides in investigated groundwater.

The environmental risk assessment indicated low risk for most of the contaminants studied, however CAF, ibuprofen and propoxur were the substances that may pose a high risk to the environment, affecting also the RQtot of the mixture of contaminants calculated per sample.

The results can help in filling the data gap on CECs and pesticides in groundwater contamination in the Tunisian territory and the MENA region and might provide important information for water resource management regarding the fate and behavior of persistent compounds (CECs and pesticides) in similar natural systems. However, more investigation on the mechanism of their input into groundwater from the contamination sources is crucial to better understand the fate of CECs and to establish possible mitigations and new regulations to limit their occurrence. The obtained data shows the highlighted the need for improving wastewater management and for implementing appropiate barriers to avoid leaching of CECs and pesticides into groundwater.

CRediT authorship contribution statement

Farah Khezami: Conceptualization, Methodology, Data Curation, Writing Original Draft.

Olga Gómez-Navarro: Conceptualization, Methodology, Data Curation, Writing Original Draft.

Maria Vittoria Barbieri: Conceptualization, Methodology, Writing Original Draft.

Nouha Khiari: Methodology, Visualization.

Anis Chkirbene: Sampling and Methodology.

Serge Chiron: Conceptualization, Methodology, Data Curation; Writing Original Draft, Visualization, Supervision, Funding acquisition.

Samia Khadhar: Methodology, Visualization, Funding acquisition.

Sandra Pérez: Conceptualization, Methodology Data Curation; Writing Original Draft, Visualization, Funding acquisition; Supervision.

All authors have contributed to manuscript reviewing and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.167319.

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Publication #3

Supplementary Materials

Occurrence of contaminants of emerging concern and pesticides and relative risk assessment in Tunisian groundwater

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Supplementary material

Occurrence of contaminants of emerging concern and pesticides and relative risk assessment in Tunisian groundwater.

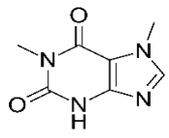
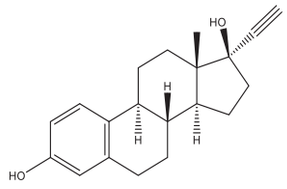
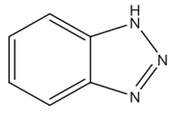
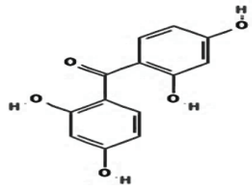
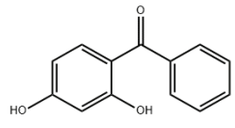
Farah Khezami[1], Olga Gómez-Navarro[1], Maria Vittoria Barbieri, Nouha Khiari, Anis Chkirbene, Serge Chiron, Samia Khadhar, Sandra Pérez*

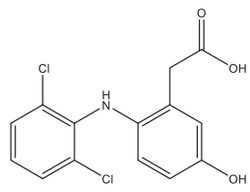
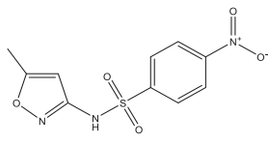
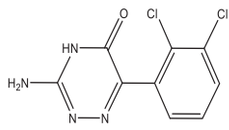
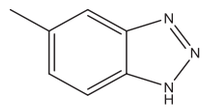
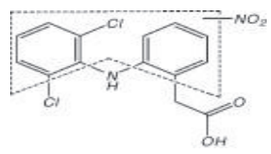
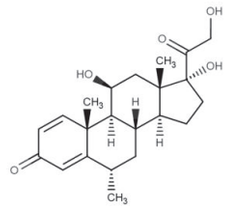
[1] *Co-first authorship, authors contributed equally to this work.*

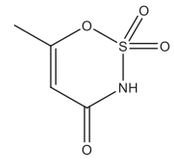
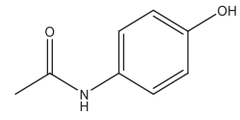
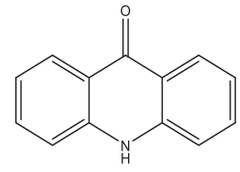
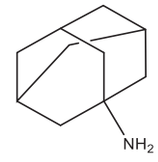
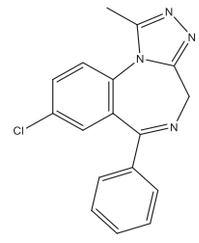
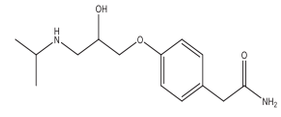
Table S1. Groundwater samples ID, aquifer levels, and temperature

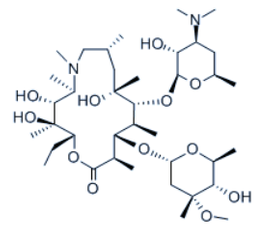
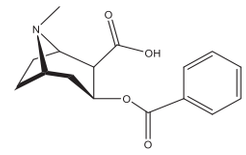
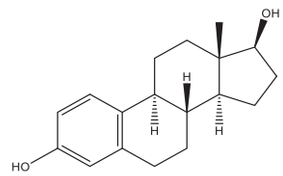
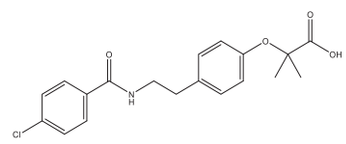
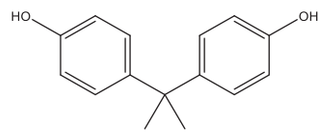
Groundwater sample ID	Static water level (m)	Temperature 2020 (ºC)	Temperature 2021 (ºC)
GW1	16.5	22.2	27.4
GW2	5.6	20.9	20.5
GW3	10.0	21.1	26.7
GW4	4.5	20.8	18.9
GW5	5.4	19.9	22.6
GW6	10.5	21.2	22.8
GW7	-	21.2	21.7
GW8	8.3	20.4	21.5
GW9	3.0	20.7	19.2
GW10	4.4	21.0	20.2
GW11	3.8	22.2	20.2
GW12	2.8	20.4	20.5
GW13	5.0	21.1	20.2

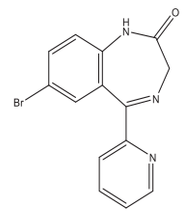
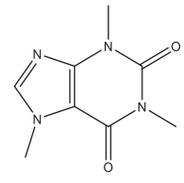
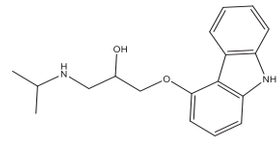
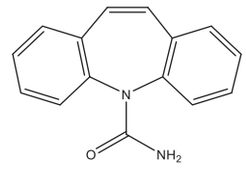
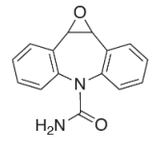
Table S2. List of CECs analysed

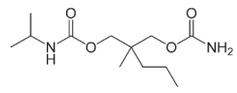
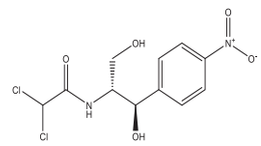
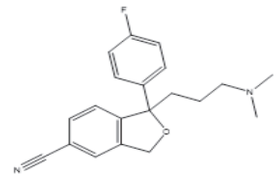
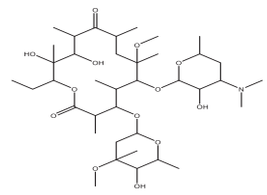
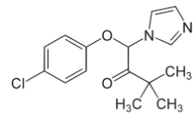
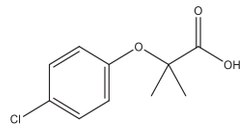
Compound name	CAS number	Molecular Formula	Monoisotopic mass	Classification	Structure
1,7 Dimethylxanthine	611-59-6	C7H8N4O2	180.0647	Stimulant	
17-α Ethynilestradiol	57-63-6	C20H24O2	296.1776	Hormones	
1H-Benzotriazole	95-14-7	C6H5N3	119.0483	Industrial compounds	
4.4'-Tetrahydroxybenzophenone (I	131-55-5	C13H10O5	246.0528	UV filter	
2.4-Dihydroxybenzophenone (BP1	131-56-6	C13H10O3	214.0630	UV filter	

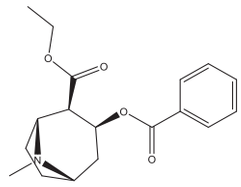
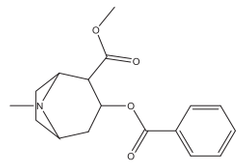
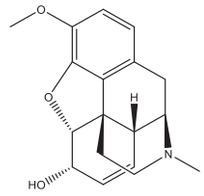
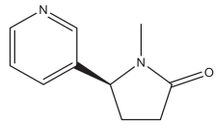
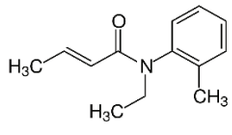
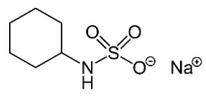
4-Hydroxidiclofenac	69002-84-2	C14H11Cl2NO3	311.0116	algesics / Anti-inflammatories	
4-Nitro Sulfamethoxazole	29699-89-6	C10H9N3O5S	283.0263	Antimicrobials	
5-Desamino-5 oxo 2,5 Lamotrigin	252186-78-0	C9H6Cl2N4O	255.9919	Psychiatric drugs	
5-methyl-1 H Benzotriazole	136-85-6	C7H7N3	133.0640	Industrial compounds	
5-nitro-Diclofenac	174316-61-1	C14H10Cl2N2O4	340.0018	algesics / Anti-inflammatories	
6a-Methylprednisolone	83-43-2	C22H30O5	374.2093	algesics / Anti-inflammatories	

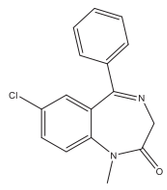
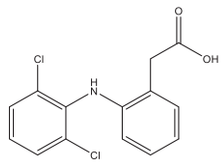
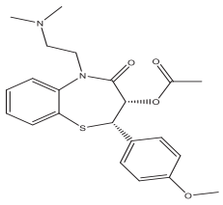
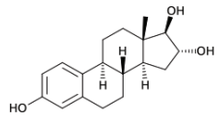
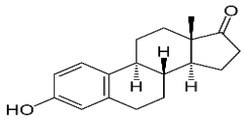
Acesulfame	33665-90-6	C4H5NO4S	162.9939	Artificial Sweeteners	
Acetaminophen	103-90-2	C8H9NO2	151.0633	algesics / Anti-inflammatories	
Acridone	578-95-0	C13H9NO	195.0684	Psychiatric drugs	
Adamantan-1-amine (Amantadine)	768-94-5	C10H17N	151.1361	Psychiatric drugs	
Alprazolam	28981-97-7	C17H13ClN4	308.0829	Psychiatric drugs	
Atenolol	29122-68-7	C14H22N2O3	266.1631	Anti-hypertensives	

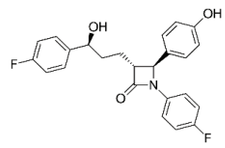
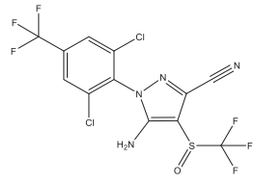
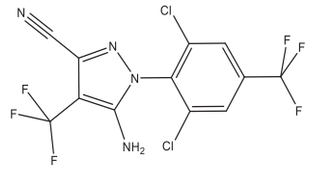
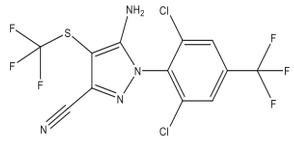
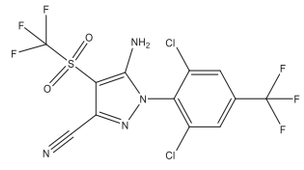
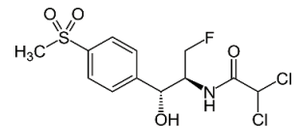
Azithromycin	83905-01-5	C38H72N2O12	748.5085	Antimicrobials	
Benzoylecgonine	519-09-5	C16H19NO4	289.1314	Illicit drugs	
B-estradiol	50-28-2	C18H24O2	272.1776	Hormones	
Bezafibrate	41859-67-0	C19H20ClNO4	361.1081	Lipid regulator	
Bisphenol-A	80-05-7	C15H16O2	228.1150	Industrial compounds	

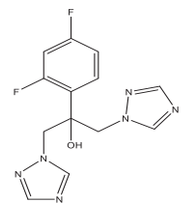
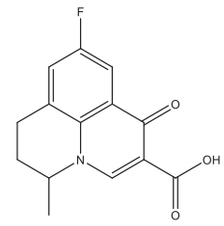
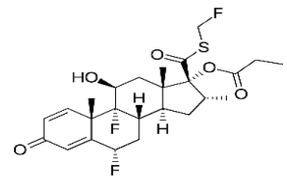
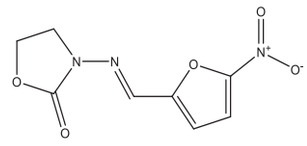
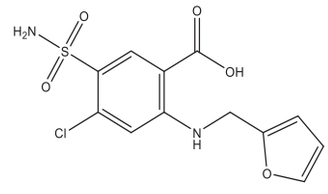
Bromazepam	1812-30-2	C14H10BrN3O	315.0007	Psychiatric drugs	
Caffeine	58-08-2	C8H10N4O2	194.0804	Stimulant	
Carazolol	57775-29-8	C18H22N2O2	298.1681	Anti-hypertensives	
Carbamazepine	298-46-4	C15H12N2O	236.0950	Psychiatric drugs	
Carbamazepine-10,11-epoxide	36507-30-9	C15H12N2O2	252.0899	Psychiatric drugs	

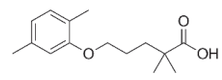
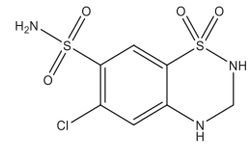
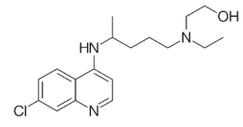
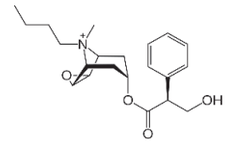
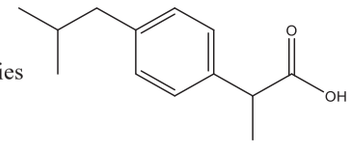
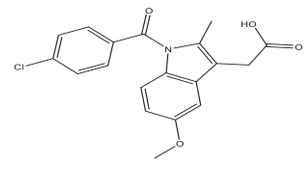
Carisoprodol	78-44-4	C12H24N2O4	260.1736	Others (Muscle relaxant)	
Chloramphenicol	56-75-7	C11H12Cl2N2O5	322.0123	Antimicrobials	
Citalopram	59729-33-8	C20H21FN2O	324.1638	Psychiatric drugs	
Clarithromycin	81103-11-9	C38H69NO13	747.4769	Antimicrobials	
Climbazole	38083-17-9	C15H17ClN2O2	292.0979	Antimicrobials	
Clofibric Acid	882-09-7	C10H11ClO3	214.0397	Lipid regulator	

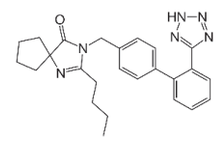
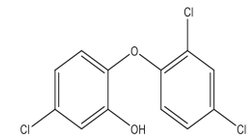
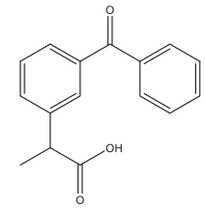
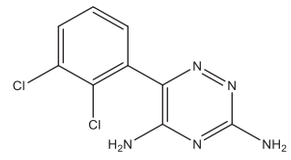
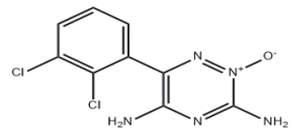
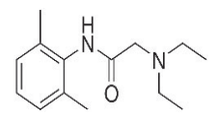
Cocaethylene	529-38-4	C18H23NO4	317.1627	Illicit drugs	
Cocaine	50-36-2	C17H21NO4	303.1471	Illicit drugs	
Codeine	76-57-3	C18H21NO3	299.1521	algesics / Anti-inflammatories	
Cotinine	486-56-6	C10H12N2O	176.0950	Stimulant	
Crotamiton	483-63-6	C13H17NO	203.1310	Others (Anti-itching)	
Cyclamate	100-88-9	C6H13NO3S	179.0616	Artificial Sweeteners	

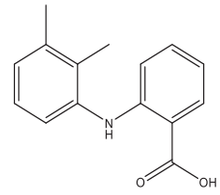
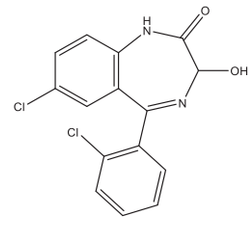
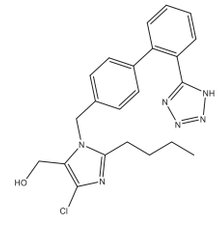
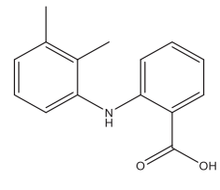
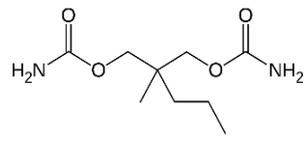
Diazepam	439-14-5	C16H13ClN2O	284.0717	Psychiatric drugs	
Diclofenac	15307-86-5	C14H11Cl2NO2	295.0167	algesics / Anti-inflammatories	
Diltiazem	42399-41-7	C22H26N2O4S	414.1613	Anti-hypertensives	
Estriol	50-27-1	C18H24O3	288.1725	Hormones	
Estrone	53-16-7	C18H22O2	270.1620	Hormones	

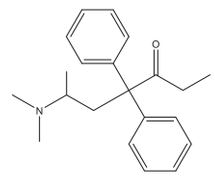
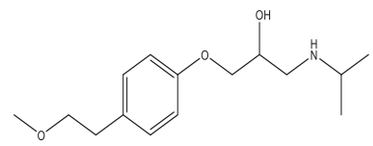
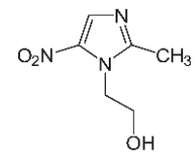
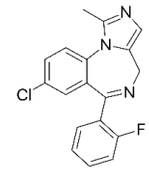
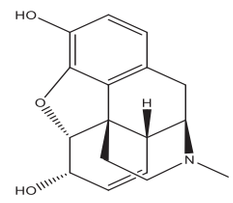
Ezetimibe	163222-33-1	C24H21F2NO3	409.1490	Lipid regulator	
Fipronil	120068-37-3	C12H4Cl2F6N4OS	435.9387	Insecticide	
Fipronil desulfinyl	205650-65-3	C12H4Cl2F6N4	387.9717	Insecticide	
Fipronil sulfide	120067-83-6	C12H4Cl2F6N4S	419.9438	Insecticide	
Fipronil sulfone	120068-36-2	C12H4Cl2F6N4O2S	451.9336	Insecticide	
Florfenicol	73231-34-2	C12H14Cl2FNO4S	357.0005	Antimicrobials	

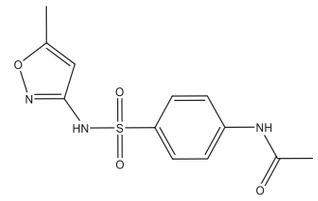
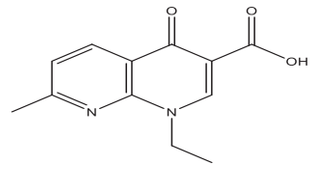
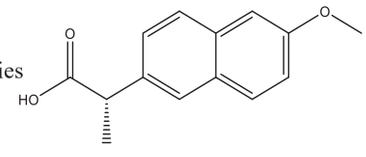
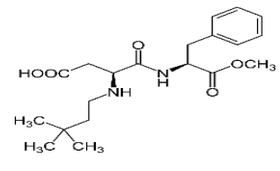
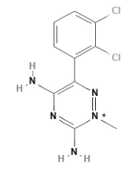
Fluconazole	86386-73-4	C13H12F2N6O	306.1041	Antimicrobials	
Flumequine	42835-25-6	C14H12FNO3	261.0801	Antimicrobials	
Fluticasone propionate	80474-14-2	C25H31F3O5S	500.1844	ılgesics / Anti-inflammatories	
Furazolidone	67-45-8	C8H7N3O5	225.0386	Antimicrobials	
Furosemide	54-31-9	C12H11ClN2O5S	330.0077	Anti-hypertensives	

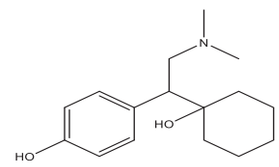
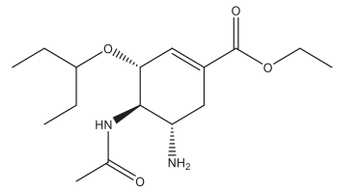
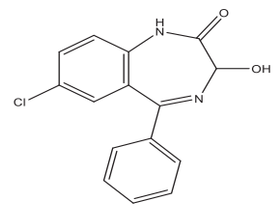
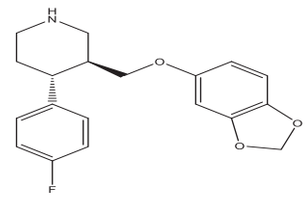
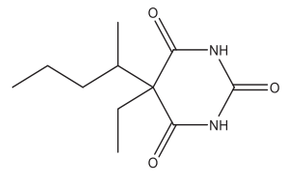
Gemfibrozil	25812-30-0	C15H22O3	250.1569	Lipid regulator	
Hydrochlorothiazide	58-93-5	C7H8ClN3O4S2	296.9645	Anti-hypertensives	
Hydroxychloroquine	118-42-3	C18H26ClN3O	335.1764	Antimicrobials	
Hyoscine (scopolamine)	149-64-4	C17H21NO4	360.2175	Others (Anticholinergic)	
Ibuprofen	15687-27-1	C13H18O2	206.1307	algesics / Anti-inflammatories	
Indomethacin	53-86-1	C19H16ClNO4	357.0768	algesics / Anti-inflammatories	

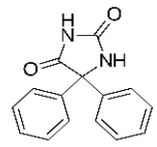
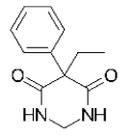
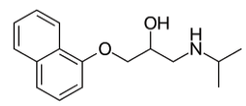
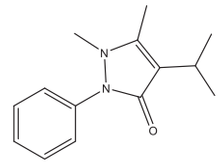
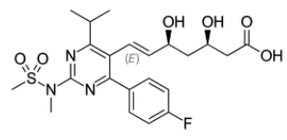
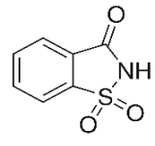
Irbesartan	138402-11-6	C25H28N6O	428.2325	Anti-hypertensives	
Irgasan (Triclosan)	3380-34-5	C12H7Cl3O2	287.9512	Antimicrobials	
Ketoprofen	22071-15-4	C16H14O3	254.0943	algesics / Anti-inflammatories	
Lamotrigine	84057-84-1	C9H7Cl2N5	255.0079	Psychiatric drugs	
Lamotrigine-N2-oxide	136565-76-9	C9H7Cl2N5O	271.0028	Psychiatric drugs	
Lidocaine	137-58-6	C14H22N2O	234.1732	Others (Anesthetic)	

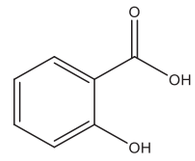
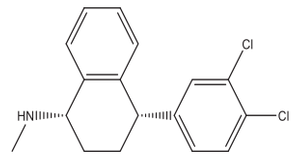
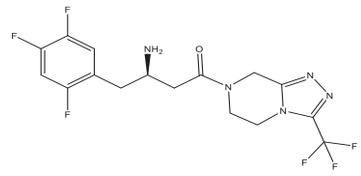
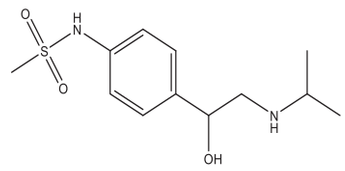
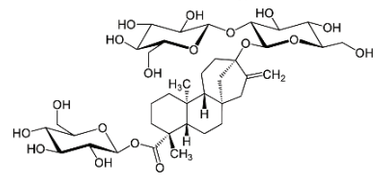
Lincomycin	859-18-7	C18H34N2O6S	406.2138	Antimicrobials	
Lorazepam	846-49-1	C15H10Cl2N2O2	320.0119	Psychiatric drugs	
Losartan	114798-26-4	C22H23ClN6O	422.1622	Anti-hypertensives	
Mefenamic Acid	61-68-7	C15H15NO2	241.1103	algesics / Anti-inflammatories	
Meprobamate	57-53-4	C9H18N2O4	218.1267	Psychiatric drugs	

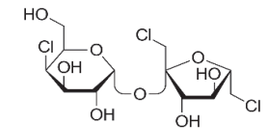
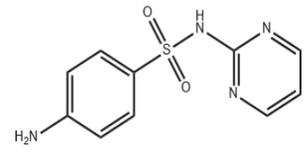
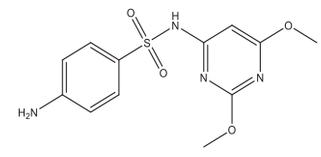
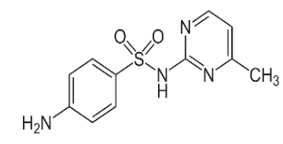
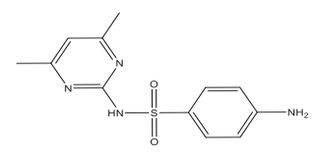
Methadone	76-99-3	C21H27NO	309.2093	Illicit drugs	
Metoprolol	37350-58-6	C6H9N3O3	171.0644	Anti-hypertensives	
Metronidazole	443-48-1	C15H25NO3	267.1834	Antimicrobials	
Midazolam	59467-70-8	C18H13ClFN3	325.0782	Psychiatric drugs	
Morphine	57-27-2	C17H19NO3	285.1365	Illicit drugs	

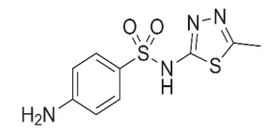
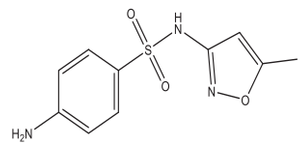
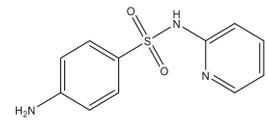
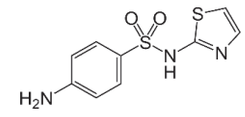
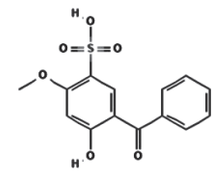
N-acetyl sulfamethoxazole	21312-10-7	C12H13N3O4S	295.0627	Antimicrobials	
Nalidixic acid	389-08-2	C12H12N2O3	232.0848	Antimicrobials	
Naproxen	22204-53-1	C14H14O3	230.0943	algesics / Anti-inflammatories	
Neotame	165450-17-9	C20H30N2O5	378.2155	Artificial Sweeteners	
N-Methyl lamotrigine	1373243-86-7	C10H9Cl2N5	269.0235	Psychiatric drugs	

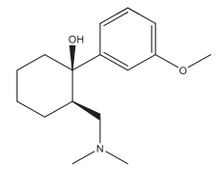
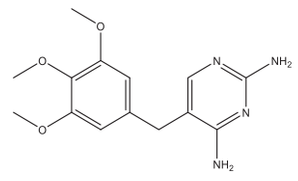
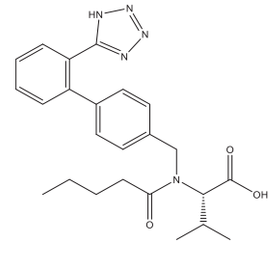
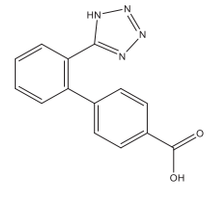
O-desmethylvenlafaxine	93413-62-8	C16H25NO2	263.1885	Psychiatric drugs	
Oseltamivir	196618-13-0	C16H28N2O4	312.2049	Antimicrobials	
Oxazepam	604-75-1	C15H11ClN2O2	286.0509	Psychiatric drugs	
Paroxetine	61869-08-7	C19H20FNO3	329.1427	Psychiatric drugs	
Pentobarbital	76-74-4	C11H18N2O3	226.1317	Psychiatric drugs	

Phenytoin	57-41-0	C15H12N2O2	252.0899	Psychiatric drugs	
Primidone	125-33-7	C12H14N2O2	218.1055	Psychiatric drugs	
Propranolol	525-66-6	C16H21NO2	259.1572	Anti-hypertensives	
Propyphenazone	479-92-5	C14H18N2O	230.1419	ılgesics / Anti-inflammatories	
Rosuvastatin	287714-41-4	C22H28FN3O6S	481.1683	Lipid regulator	
Saccharin	81-07-2	C7H5NO3S	182.9990	Artificial Sweeteners	

Salicylic acid	69-72-7	C7H6O3	138.0317	ılgesics / Anti-inflammatories	
Sertraline	79617-96-2	C17H17Cl2N	305.0738	Psychiatric drugs	
Sitagliptin	486460-32-6	C16H15F6N5O	407.1181	Others (Antidiabetic)	
Sotalol	3930-20-9	C12H20N2O3S	272.1195	Anti-hypertensives	
Stevioside	57817-89-7	C38H60O18	804.3780	Artificial Sweeteners	

Sucralose	56038-13-2	C12H19Cl3O8	396.0146	Artificial Sweeteners	
Sulfadiazine	68-35-9	C10H10N4O2S	250.0524	Antimicrobials	
Sulfadimethoxine	122-11-2	C12H14N4O4S	310.0736	Antimicrobials	
Sulfamerazine	127-79-7	C11H12N4O2S	264.0681	Antimicrobials	
Sulfamethazine	57-68-1	C12H14N4O2S	278.0837	Antimicrobials	

Sulfamethizole	144-82-1	C9H10N4O2S2	270.0245	Antimicrobials	
Sulfamethoxazole	723-46-6	C10H11N3O3S	253.0521	Antimicrobials	
Sulfapyridine	144-83-2	C11H11N3O2S	249.0572	Antimicrobials	
Sulfathiazole	72-14-0	C9H9N3O2S2	255.0136	Antimicrobials	
Sulisobenzone (BP4)	4065-45-6	C14H12O6S	308.0355	UV filter	

Tramadol	3715-90-0	C16H25NO2	263.1885	algesics / Anti-inflammatories	
Trimethoprim	738-70-5	C14H18N4O3	290.1379	Antimicrobials	
Valsartan	137862-53-4	C24H29N5O3	435.2271	Anti-hypertensives	
Valsartan acid	164265-78-5	C14H10N4O2	266.0804	Anti-hypertensives	

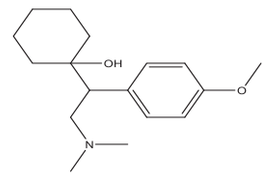
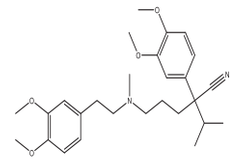
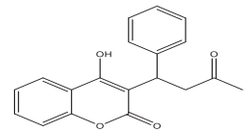
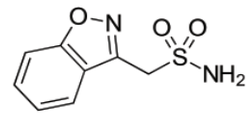
Venlafaxine	93413-69-5	C17H27NO2	277.2042	Psychiatric drugs	
Verapamil	52-53-9	C27H38N2O4	454.2832	Anti-hypertensives	
Warfarin	81-81-2	C19H16O4	308.1049	Others (Anticoagulant)	
Zonisamide	68291-97-4	C8H8N2O3S	212.0256	Psychiatric drugs	

Table S3. CECs concentration and frequency of detection per campaign

n.d.	not detected
BLOD	Below Limit of Detection
BLOQ	Below Limit of Quantification

		Concentration ng L^{-1}												
		October 2020												
Classification	Compound name	GW 1	GW 2	GW 3	GW 4	GW 5	GW 6	GW 7	GW 8	GW 9	GW 10	GW 11	GW 12	GW 13
Analgesics / Anti-inflammatories	4-Hydroxidiclofenac	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	5-nitro-Diclofenac	n.d.	n.d.	n.d.	n.d.	n.d.	BLOD	n.d.	n.d.	n.d.	n.d.	BLOQ	n.d.	n.d.
Analgesics / Anti-inflammatories	6a-Methylprednisolone	**14.83**	n.d.	n.d.	**38.87**	n.d.	n.d.	**9.27**	**20.09**	**10.67**	**10.09**	**23.64**	**11.63**	n.d.
Analgesics / Anti-inflammatories	Acetaminophen	**6.79**	**6.84**	**1.39**	**4.86**	**2.73**	**2.14**	**4.32**	**2.98**	**2.60**	**1.20**	**0.52**	**0.34**	**7.68**
Analgesics / Anti-inflammatories	Codeine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Diclofenac	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Fluticasone propionate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Ibuprofen	**47.83**	**129.38**	**237.55**	**1313.12**	**262.36**	BLOQ	**930.29**	**63.88**	BLOQ	BLOQ	**22.13**	BLOQ	n.d.
Analgesics / Anti-inflammatories	Indomethacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Ketoprofen	n.d.	n.d.	n.d.	n.d.	n.d.	BLOQ	n.d.	n.d.	BLOQ	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Mefenamic Acid	n.d.	n.d.	n.d.	**179.02**	**179.25**	n.d.	**179.81**	n.d.	**182.48**	n.d.	**278.92**	n.d.	**178.74**
Analgesics / Anti-inflammatories	naproxen	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**30.36**	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Propyphenazone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Salicylic acid	**284.98**	BLOQ	**6186.14**	n.d.	**1356.13**	**15.49**	n.d.	BLOQ	**22.13**	**18.03**	**22.67**	**38.63**	n.d.
Analgesics / Anti-inflammatories	Tramadol	**7.90**	**8.23**	**7.30**	**8.87**	**7.64**	**7.20**	**7.43**	**8.28**	**8.88**	**8.21**	**7.86**	**7.28**	**7.33**
Anti-hypertensives	Atenolol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**0.48**	n.d.	n.d.
Anti-hypertensives	Carazolol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anti-hypertensives	Diltiazem	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anti-hypertensives	Furosemide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**0.68**	**1.88**	n.d.	n.d.	n.d.
Anti-hypertensives	Hydrochlorothiazide	**120.00**	**36.06**	**301.94**	**1048.26**	**431.23**	BLOQ	n.d.	BLOQ	BLOQ	BLOQ	**80.82**	BLOQ	n.d.
Anti-hypertensives	Irbesartan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anti-hypertensives	Losartan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anti-hypertensives	Metoprolol	BLOQ	n.d.	n.d.	**1.71**	n.d.	**0.28**	BLOQ	n.d.	**5.15**	**0.79**	n.d.	n.d.	BLOQ
Anti-hypertensives	Propranolol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anti-hypertensives	Sotalol	**4.42**	**4.37**	n.d.	n.d.	n.d.	n.d.	n.d.	**4.33**	**6.30**	n.d.	n.d.	n.d.	n.d.
Anti-hypertensives	Valsartan	n.d.	**1.02**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anti-hypertensives	Valsartan acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	BLOD	n.d.	**2804.68**	n.d.	n.d.
Anti-hypertensives	Verapamil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	4-Nitro Sulfamethoxazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Azithromycin	**11.82**	n.d.	n.d.	**40.53**	**11.34**	n.d.	**17.19**	**11.85**	**54.21**	**19.99**	n.d.	n.d.	n.d.
Antimicrobials	Chloramphenicol	n.d.	BLOQ	BLOD	BLOD	BLOD	BLOD	n.d.	n.d.	BLOD	BLOD	BLOQ	n.d.	n.d.
Antimicrobials	Clarithromycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Climbazole	**3.14**	**3.00**	n.d.	n.d.	n.d.	**2.90**	**2.87**	n.d.	**3.62**	**2.86**	n.d.	n.d.	**2.98**
Antimicrobials	Florfenicol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Fluconazole	**18.78**	BLOQ	BLOD	n.d.	n.d.	BLOQ	n.d.	n.d.	n.d.	n.d.	**20.40**	n.d.	**0.70**
Antimicrobials	Flumequine	n.d.	BLOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**0.25**	n.d.	n.d.	n.d.	**2.14**
Antimicrobials	Furazolidone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Hydroxychloroquine	n.d.	**37.99**	n.d.	n.d.	**38.16**	**38.00**	**38.24**	n.d.	n.d.	**38.09**	n.d.	n.d.	n.d.
Antimicrobials	Irgasan (Triclosan)	n.d.	n.d.	n.d.	**32.77**	**2.73**	**5.90**	n.d.	n.d.	**377.99**	**3.44**	**10.72**	**2.28**	n.d.
Antimicrobials	Lincomycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	BLOQ	n.d.	n.d.
Antimicrobials	Metronidazole	**0.12**	**0.24**	**0.11**	n.d.	n.d.	n.d.	**0.11**	**0.13**	**0.18**	**0.17**	n.d.	n.d.	**0.15**

Antimicrobials	N-acetyl sulfamethoxazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**0.57**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	nalidixic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Oseltamivir	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Sulfadiazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**1.01**	n.d.	**0.99**	**0.97**	**1.50**	**0.92**	**1.12**
Antimicrobials	Sulfadimethoxine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Sulfamerazine	BLOD	n.d.	n.d.	n.d.	n.d.	n.d.	BLOD	n.d.	BLOD	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Sulfamethazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**25.47**
Antimicrobials	Sulfamethizole	**0.33**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**8.61**	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Sulfamethoxazole	**2.21**	**0.96**	**0.85**	**1.53**	**1.49**	**4.86**	**1.55**	**1.05**	**9.32**	**2.46**	**4.75**	**0.55**	**1.61**
Antimicrobials	Sulfapyridine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**0.45**	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Sulfathiazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Trimethoprim	n.d.	BLOQ	n.d.	n.d.	n.d.	**15.94**	BLOQ	n.d.	n.d.	BLOQ	n.d.	n.d.	n.d.
Artificial Sweeteners	Acesulfame	**2912.69**	BLOQ	**519.96**	BLOD	**0.78**	BLOQ	BLOQ	BLOQ	n.d.	BLOQ	n.d.	BLOQ	n.d.
Artificial Sweeteners	Cyclamate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Artificial Sweeteners	Neotame	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Artificial Sweeteners	Saccharin	BLOQ	BLOQ	n.d.	n.d.	n.d.	BLOQ	n.d.	BLOQ	BLOQ	**29.18**	**77.38**	n.d.	n.d.
Artificial Sweeteners	Stevioside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Artificial Sweeteners	Sucralose	**151.21**	BLOQ	BLOD	n.d.	BLOD	BLOQ	n.d.	n.d.	BLOQ	n.d.	**497.16**	n.d.	n.d.
Hormones	17-α Ethynilestradiol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hormones	B-estradiol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hormones	Estriol	BLOD	n.d.	n.d.	**10.43**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hormones	Estrone	n.d.	n.d.	n.d.	**7.65**	**5.09**	**5.03**	n.d.	**5.71**	**5.70**	n.d.	n.d.	n.d.	n.d.
Illicit drugs	Benzoylecgonine	BLOQ	BLOQ	n.d.	n.d.	BLOQ	n.d.	n.d.	BLOQ	BLOQ	n.d.	BLOQ	n.d.	n.d.
Illicit drugs	Cocaethylene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Illicit drugs	Cocaine	n.d.	BLOQ	n.d.	n.d.	BLOQ	BLOQ	n.d.	BLOQ	n.d.	BLOQ	n.d.	n.d.	BLOQ
Illicit drugs	Methadone	n.d.	n.d.	n.d.	n.d.	n.d.	**53.57**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Illicit drugs	Morphine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Industrial compounds	1H-Benzotriazole	**77.10**	BLOQ	**10.87**	**11.36**	BLOQ	**1.80**	**16.30**	BLOQ	**847.50**	**38.46**	**19.55**	BLOQ	BLOQ
Industrial compounds	5-methyl-1 H Benzotriazole	BLOQ	BLOQ	n.d.	BLOQ	**4.06**	BLOQ	BLOQ	BLOQ	BLOQ	**12.01**	BLOQ	BLOQ	BLOQ
Industrial compounds	Bisphenol-A	**2.73**	**4.54**	n.d.	n.d.	**8.58**	**0.59**	n.d.	**2.19**	**6.89**	**2.76**	**5.24**	**9.27**	n.d.

Insecticide	Fipronil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Insecticide	Fipronil desulfinyl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Insecticide	Fipronil sulfide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Insecticide	Fipronil sulfone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lipid regulator	Bezafibrate	**1.54**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**1.71**	n.d.	n.d.	**1.89**
Lipid regulator	Clofibric Acid	**1.35**	**1.60**	n.d.	n.d.	n.d.	**3.62**	n.d.	**1.22**	n.d.	n.d.	n.d.	n.d.	**5.99**
Lipid regulator	Ezetimibe	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lipid regulator	Gemfibrozil	**0.96**	**2.19**	n.d.	BLOQ	BLOQ	**30.71**	BLOQ	BLOQ	**2.39**	BLOQ	BLOQ	BLOQ	BLOQ
Lipid regulator	Rosuvastatin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Others	Carisoprodol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Others	Crotamiton	**2.12**	**0.70**	BLOQ	**0.79**	**0.78**	BLOQ	BLOQ	BLOQ	**0.71**	**0.79**	**1.05**	**0.77**	**1.55**
Others	Hyoscine (scopolamine)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Others	Lidocaine	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	**0.43**	BLOQ	**0.92**	BLOQ	BLOQ
Others	Sitagliptin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Others	Warfarin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	5-Desamino-5 oxo 2,5 L	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Acridone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Adamantan-1-amine (Amantadine)	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	**0.70**	BLOQ	BLOQ	**1.18**	BLOQ	n.d.	n.d.	BLOD
Psychiatric drugs	Alprazolam	**5.93**	**5.69**	**6.21**	**4.85**	**4.49**	**5.76**	**5.46**	**6.43**	**5.84**	**5.54**	**5.65**	**5.71**	**4.90**
Psychiatric drugs	Bromazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Carbamazepine	**2.47**	BLOQ	BLOQ	BLOQ	n.d.	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOD	BLOQ
Psychiatric drugs	Carbamazepine-10,11-epoxide	**0.74**	BLOD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Citalopram	n.d.	**3.17**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Diazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Lamotrigine	n.d.	**1.36**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**1.82**	n.d.	n.d.
Psychiatric drugs	Lamotrigine-N2-oxide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Lorazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Meprobamate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Midazolam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	N-Methyl lamotrigine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Psychiatric drugs	O-desmethylvenlafaxine	BLOQ	BLOQ	n.d.	BLOQ	BLOQ	BLOQ	BLOQ	n.d.	BLOQ	n.d.	BLOQ	n.d.	n.d.
Psychiatric drugs	Oxazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Paroxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Pentobarbital	n.d.	n.d.	n.d.	n.d.	n.d.	BLOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Phenytoin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Primidone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Sertraline	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Venlafaxine	n.d.	**0.17**	n.d.	n.d.	BLOQ	n.d.	BLOD	n.d.	BLOQ	BLOQ	BLOD	n.d.	BLOD
Psychiatric drugs	Zonisamide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Stimulant	1,7 Dimethylxanthine	**4.42**	**29.40**	**11.60**	**5.10**	**3.97**	**2.74**	**4.29**	**1.53**	**15.10**	**6.25**	**2.11**	BLOQ	**8.58**
Stimulant	Caffeine	**73.56**	**219.65**	**100.55**	**35.88**	**59.54**	**76.26**	**47.72**	**45.54**	**121.07**	**103.84**	**33.43**	**33.26**	**68.94**
Stimulant	Cotinine	BLOQ	BLOD	BLOQ	**9.38**	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	**28.35**	**12.86**	BLOQ
UV filter	2.2'.4.4'-Tetrahydroxybenzophenone (BP2)	BLOD	n.d.	n.d.	n.d.	n.d.	BLOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
UV filter	2.4-Dihydroxybenzophenone (BP1)	BLOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	BLOQ	n.d.
UV filter	Sulisobenzone (BP4)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**2.03**	**0.98**	**2.38**	n.d.	n.d.

		May 2021												
Classification	Compound name	GW 1	GW 2	GW 3	GW 4	GW 5	GW 6	GW 7	GW 8	GW 9	GW 10	GW 11	GW 12	GW13
Analgesics / Anti-inflammatories	4-Hydroxidiclofenac	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	5-nitro-Diclofenac	n.d.	n.d.	n.d.	n.d.	n.d.	BLOD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	6a-Methylprednisolone	n.d.	n.d.	n.d.	n.d.	**4.16**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Acetaminophen	**15.31**	**17.71**	**13.17**	**7.39**	**24.28**	**3.66**	**3.25**	**3.28**	**15.54**	**6.04**	**2.90**	**10.93**	**6.71**
Analgesics / Anti-inflammatories	Codeine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Diclofenac	n.d.	n.d.	n.d.	n.d.	n.d.	**5.94**	n.d.	**4.43**	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Fluticasone propionate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Ibuprofen	**84.12**	BLOQ	BLOQ	**1599.34**	**33.96**	**30.74**	**184.07**	**25.00**	BLOQ	**49.21**	**55.76**	BLOQ	**102.39**
Analgesics / Anti-inflammatories	Indomethacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Ketoprofen	n.d.	n.d.	n.d.	**48.71**	n.d.	n.d.	n.d.	n.d.	n.d.	**6.69**	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Mefenamic Acid	BLOQ	BLOQ	n.d.	BLOQ	BLOQ	n.d.	n.d.	n.d.	BLOQ	BLOQ	**1.40**	n.d.	n.d.
Analgesics / Anti-inflammatories	naproxen	**14.26**	n.d.	n.d.	n.d.	n.d.	BLOQ	n.d.	n.d.	**1.88**	n.d.	**64.69**	n.d.	n.d.
Analgesics / Anti-inflammatories	Propyphenazone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Analgesics / Anti-inflammatories	Salicylic acid	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	**34.50**	**27.98**	**1.27**	**23.19**	BLOQ
Analgesics / Anti-inflammatories	Tramadol	**4.99**	**4.89**	**5.19**	**5.04**	**4.93**	**4.87**	**4.99**	**4.84**	**4.90**	**4.94**	**5.09**	**4.82**	**4.97**
Anti-hypertensives	Atenolol	n.d.	n.d.	BLOD	BLOQ	n.d.	n.d.	n.d.	n.d.	BLOQ	n.d.	n.d.	n.d.	n.d.
Anti-hypertensives	Carazolol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anti-hypertensives	Diltiazem	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**2.01**	n.d.	n.d.	n.d.
Anti-hypertensives	Furosemide	BLOD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anti-hypertensives	Hydrochlorothiazide	**7.50**	**2.09**	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	**9.87**	BLOQ	BLOQ	**28.86**	BLOQ	BLOQ
Anti-hypertensives	Irbesartan	n.d.	BLOQ	n.d.	n.d.	n.d.	n.d.	n.d.	BLOQ	n.d.	n.d.	**614.04**	n.d.	n.d.
Anti-hypertensives	Losartan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anti-hypertensives	Metoprolol	**0.38**	**0.25**	**0.34**	**1.98**	**0.57**	**0.88**	**0.38**	**0.51**	**0.83**	**0.45**	**0.63**	**0.43**	**1.21**
Anti-hypertensives	Propranolol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.90	n.d.
Anti-hypertensives	Sotalol	n.d.	n.d.	**0.97**	n.d.	n.d.	**0.99**	n.d.	**0.80**	n.d.	**2.36**	n.d.	**0.93**	n.d.
Anti-hypertensives	Valsartan	n.d.	BLOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	BLOD	n.d.	n.d.	n.d.

Anti-hypertensives	Valsartan acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**1492.37**	n.d.	n.d.
Anti-hypertensives	Verapamil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	4-Nitro Sulfamethoxazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Azithromycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Chloramphenicol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Clarithromycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Climbazole	**0.95**	**1.92**	**0.91**	n.d.	**0.93**	**0.96**	n.d.	**1.01**	**1.11**	**1.14**	**0.98**	**1.05**	**1.42**
Antimicrobials	Florfenicol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Fluconazole	**13.47**	n.d.	n.d.	n.d.	n.d.	**0.57**	n.d.	**0.39**	n.d.	n.d.	**8.22**	n.d.	n.d.
Antimicrobials	Flumequine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**7.05**	n.d.	n.d.	n.d.
Antimicrobials	Furazolidone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Hydroxychloroquine	n.d.	**6.04**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**6.80**	**6.05**	n.d.	n.d.
Antimicrobials	Irgasan (Triclosan)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**1.60**	n.d.	**9.24**	n.d.	n.d.	n.d.
Antimicrobials	Lincomycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**1.40**	n.d.	n.d.
Antimicrobials	Metronidazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	N-acetyl sulfamethoxazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	nalidixic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Oseltamivir	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Sulfadiazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	BLOQ	n.d.	BLOQ
Antimicrobials	Sulfadimethoxine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Sulfamerazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Sulfamethazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	BLOQ
Antimicrobials	Sulfamethizole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Sulfamethoxazole	BLOQ	BLOD	BLOD	BLOD	BLOD	BLOD	n.d.	BLOQ	BLOD	BLOD	BLOQ	BLOD	BLOD
Antimicrobials	Sulfapyridine	n.d.	n.d.	n.d.	n.d.	n.d.	BLOD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Sulfathiazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antimicrobials	Trimethoprim	**26.30**	n.d.	n.d.	BLOQ	BLOQ	BLOQ	n.d.	**4.40**	**1.15**	BLOD	BLOQ	**30.75**	n.d.
Artificial Sweeteners	Acesulfame	**42.57**	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	n.d.	BLOQ	BLOQ	BLOQ	**391.45**	BLOD	BLOQ
Artificial Sweeteners	Cyclamate	**2.07**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Artificial Sweeteners	Neotame	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Artificial Sweeteners	Saccharin	n.d.	n.d.	n.d.	**28.34**	n.d.	BLOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Artificial Sweeteners	Stevioside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Artificial Sweeteners	Sucralose	**73.07**	n.d.	BLOQ	n.d.	n.d.	BLOQ	n.d.	BLOQ	n.d.	n.d.	n.d.	n.d.	n.d.
Hormones	17-α Ethynilestradiol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hormones	B-estradiol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hormones	Estriol	n.d.	n.d.	n.d.	**0.16**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hormones	Estrone	n.d.	n.d.	n.d.	**2.07**	**0.27**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	BLOD	n.d.
Illicit drugs	Benzoylecgonine	n.d.	BLOD	n.d.	BLOQ	n.d.	BLOD	n.d.	BLOD	n.d.	BLOQ	n.d.	n.d.	n.d.
Illicit drugs	Cocaethylene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Illicit drugs	Cocaine	n.d.	n.d.	BLOQ	n.d.	BLOQ	n.d.	BLOQ	n.d.	BLOQ	BLOQ	n.d.	BLOQ	n.d.
Illicit drugs	Methadone	n.d.	n.d.	n.d.	BLOQ	BLOQ	n.d.	BLOQ	n.d.	n.d.	BLOQ	n.d.	BLOQ	n.d.
Illicit drugs	Morphine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Industrial compounds	1H-Benzotriazole	**43.14**	**2.62**	**1.30**	**5.03**	**13.35**	**8.12**	**0.29**	**11.33**	**12.39**	**0.50**	**3.34**	**7.59**	n.d.
Industrial compounds	5-methyl-1 H Benzotriazole	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Industrial compounds	Bisphenol-A	**2.76**	**5.26**	**5.85**	**7.81**	**6.54**	**10.08**	**4.28**	**5.88**	**22.76**	**5.26**	n.d.	**11.54**	n.d.
Insecticide	Fipronil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Insecticide	Fipronil desulfinyl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Insecticide	Fipronil sulfide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Insecticide	Fipronil sulfone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lipid regulator	Bezafibrate	n.d.	BLOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lipid regulator	Clofibric Acid	**7.35**	n.d.	n.d.	**2.82**	n.d.	n.d.	n.d.	n.d.	**3.01**	n.d.	**6.79**	n.d.	**4.08**
Lipid regulator	Ezetimibe	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lipid regulator	Gemfibrozil	n.d.	n.d.	n.d.	n.d.	n.d.	BLOQ	n.d.	n.d.	**0.89**	BLOQ	n.d.	n.d.	n.d.
Lipid regulator	Rosuvastatin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Others	Carisoprodol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Others	Crotamiton	**2.65**	**1.99**	**2.07**	**2.16**	**2.10**	**1.97**	**2.22**	**2.32**	**2.47**	**2.25**	**1.97**	**1.81**	**2.12**
Others	Hyoscine (scopolamine)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**0.11**	n.d.	n.d.	n.d.
Others	Lidocaine	BLOQ	n.d.	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	n.d.	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Others	Sitagliptin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Others	Warfarin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	5-Desamino-5 oxo 2,5 L	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Psychiatric drugs	Acridone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Adamantan-1-amine (Amantadine)	BLOQ	BLOQ	BLOD	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOD	BLOQ	BLOQ	BLOQ	n.d.
Psychiatric drugs	Alprazolam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Bromazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Carbamazepine	BLOQ	BLOQ	n.d.	BLOQ	BLOQ	BLOQ	n.d.	BLOQ	n.d.	n.d.	BLOQ	BLOQ	BLOQ
Psychiatric drugs	Carbamazepine-10,11-epoxide	BLOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Citalopram	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Diazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Lamotrigine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**0.94**	n.d.	n.d.
Psychiatric drugs	Lamotrigine-N2-oxide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Lorazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Meprobamate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Midazolam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	N-Methyl lamotrigine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	O-desmethylvenlafaxine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	BLOQ	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Oxazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Paroxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Pentobarbital	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Phenytoin	BLOD	n.d.	n.d.	n.d.	n.d.	BLOD	n.d.	n.d.	BLOD	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Primidone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Sertraline	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Venlafaxine	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Psychiatric drugs	Zonisamide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Stimulant	1,7 Dimethylxanthine	BLOQ	BLOQ	**6.55**	**4.85**	**7.48**	BLOQ	BLOQ	BLOQ	**485.16**	BLOQ	BLOQ	**8.07**	**1.36**
Stimulant	Caffeine	BLOQ	BLOQ	**18.37**	**24.46**	**35.52**	BLOQ	BLOQ	BLOQ	**1960.58**	BLOQ	BLOQ	**48.70**	BLOQ
Stimulant	Cotinine	BLOQ	BLOQ	BLOQ	**10.04**	**0.85**	BLOQ	BLOQ	BLOQ	**23.70**	BLOQ	BLOQ	**2.25**	**4.64**
UV filter	2.2'.4.4'-Tetrahydroxybenzophenone (BP2)	n.d.	n.d.	n.d.	n.d.	**0.35**	n.d.	**0.33**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
UV filter	2.4-Dihydroxybenzophenone (BP1)	n.d.	n.d.	BLOQ	BLOQ	**2.65**	**7.74**	**1.40**	n.d.	BLOQ	BLOQ	n.d.	BLOQ	n.d.
UV filter	Sulisobenzone (BP4)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

		Frequency of dection		Total concentration (ng/L)		Average concentration (ng/L)	
Classification	Compound name	October 2020	May 2021	October 2020	May 2021	October 2020	May 2021
Analgesics / Anti-inflammatories	4-Hydroxidiclofenac	0%	0%	0.00	0.00	-	-
Analgesics / Anti-inflammatories	5-nitro-Diclofenac	8%	0%	0.00	0.00	-	-
Analgesics / Anti-inflammatories	6a-Methylprednisolone	62%	8%	139.09	4.16	17.39	4.16
Analgesics / Anti-	Acetaminophen	100%	100%	44.39	130.18	3.41	10.01
Analgesics / Anti-inflammatories	Codeine	0%	0%	0.00	0.00	-	-
Analgesics / Anti-inflammatories	Diclofenac	0%	15%	0.00	10.38	-	5.19
Analgesics / Anti-inflammatories	Fluticasone propionate	0%	0%	0.00	0.00	-	-
Analgesics / Anti-inflammatories	Ibuprofen	92%	100%	3006.54	2164.60	375.82	240.51
Analgesics / Anti-inflammatories	Indomethacin	0%	0%	0.00	0.00	-	-
Analgesics / Anti-inflammatories	Ketoprofen	15%	15%	0.00	55.40	-	27.70
Analgesics / Anti-inflammatories	Mefenamic Acid	46%	54%	1178.23	1.40	196.37	1.40
Analgesics / Anti-inflammatories	naproxen	8%	31%	30.36	80.84	30.36	26.95
Analgesics / Anti-inflammatories	Propyphenazone	0%	0%	0.00	0.00	-	-

Analgesics / Anti-inflammatories	Salicylic acid	77%	100%	7944.20	86.94	993.02	21.74
Analgesics / Anti-inflammatories	Tramadol	100%	100%	102.41	64.46	7.88	4.96
Anti-hypertensives	Atenolol	8%	15%	0.48	0.00	0.48	-
Anti-hypertensives	Carazolol	0%	0%	0.00	0.00	-	-
Anti-hypertensives	Diltiazem	0%	8%	0.00	2.01	-	2.01
Anti-hypertensives	Furosemide	15%	0%	2.56	0.00	1.28	-
Anti-hypertensives	Hydrochlorothiazide	85%	100%	2018.32	48.32	336.39	12.08
Anti-hypertensives	Irbesartan	0%	23%	0.00	614.04	-	614.04
Anti-hypertensives	Losartan	0%	0%	0.00	0.00	-	-
Anti-hypertensives	Metoprolol	54%	100%	7.92	8.84	1.98	0.68
Anti-hypertensives	Propranolol	0%	8%	0.00	0.90	-	0.90
Anti-hypertensives	Sotalol	31%	38%	19.42	6.05	4.86	1.21
Anti-hypertensives	Valsartan	8%	8%	1.02	0.00	1.02	-
Anti-hypertensives	Valsartan acid	8%	8%	2804.68	1492.37	2804.68	1492.37
Anti-hypertensives	Verapamil	0%	0%	0.00	0.00	-	-
Antimicrobials	4-Nitro Sulfamethoxazole	0%	0%	0.00	0.00	-	-
Antimicrobials	Azithromycin	54%	0%	166.93	0.00	23.85	#DIV/0!
Antimicrobials	Chloramphenicol	15%	0%	0.00	0.00	-	-
Antimicrobials	Clarithromycin	0%	0%	0.00	0.00	-	-
Antimicrobials	Climbazole	54%	85%	21.37	12.38	3.05	1.13
Antimicrobials	Florfenicol	0%	0%	0.00	0.00	-	-
Antimicrobials	Fluconazole	38%	31%	39.88	22.65	13.29	5.66
Antimicrobials	Flumequine	23%	8%	2.39	7.05	1.20	7.05
Antimicrobials	Furazolidone	0%	0%	0.00	0.00	-	-
Antimicrobials	Hydroxychloroquine	38%	23%	190.49	18.89	38.10	6.30
Antimicrobials	Irgasan (Triclosan)	54%	15%	435.83	10.84	62.26	5.42
Antimicrobials	Lincomycin	8%	8%	0.00	1.40	-	1.40
Antimicrobials	Metronidazole	62%	0%	1.21	0.00	0.15	-

Antimicrobials	N-acetyl sulfamethoxazole	8%	0%	0.57	0.00	0.57	-
Antimicrobials	nalidixic acid	0%	0%	0.00	0.00	-	-
Antimicrobials	Oseltamivir	0%	0%	0.00	0.00	-	-
Antimicrobials	Sulfadiazine	46%	15%	6.51	0.00	1.09	-
Antimicrobials	Sulfadimethoxine	0%	0%	0.00	0.00	-	-
Antimicrobials	Sulfamerazine	0%	0%	0.00	0.00	-	-
Antimicrobials	Sulfamethazine	8%	8%	25.47	0.00	25.47	-
Antimicrobials	Sulfamethizole	15%	0%	8.94	0.00	4.47	-
Antimicrobials	Sulfamethoxazole	100%	23%	33.19	0.00	2.55	-
Antimicrobials	Sulfapyridine	8%	0%	0.45	0.00	0.45	-
Antimicrobials	Sulfathiazole	0%	0%	0.00	0.00	-	-
Antimicrobials	Trimethoprim	31%	62%	15.94	62.60	15.94	15.65
Artificial Sweeteners	Acesulfame	69%	85%	3433.43	434.02	1144.48	217.01
Artificial Sweeteners	Cyclamate	0%	8%	0.00	2.07	-	2.07
Artificial Sweeteners	Neotame	0%	0%	0.00	0.00	-	-
Artificial Sweeteners	Saccharin	54%	15%	106.56	28.34	53.28	28.34
Artificial Sweeteners	Stevioside	0%	0%	0.00	0.00	-	-
Artificial Sweeteners	Sucralose	38%	31%	648.37	73.07	324.18	73.07
Hormones	17-α Ethynilestradiol	0%	0%	0.00	0.00	-	-
Hormones	B-estradiol	0%	0%	0.00	0.00	-	-
Hormones	Estriol	8%	8%	10.43	0.16	10.43	0.16
Hormones	Estrone	38%	15%	29.18	2.34	5.84	1.17
Illicit drugs	Benzoylecgonine	46%	15%	0.00	0.00	-	-
Illicit drugs	Cocaethylene	0%	0%	0.00	0.00	-	-
Illicit drugs	Cocaine	46%	46%	0.00	0.00	-	-
Illicit drugs	Methadone	8%	38%	53.57	0.00	53.57	-
Illicit drugs	Morphine	0%	0%	0.00	0.00	-	-
Industrial compounds	1H-Benzotriazole	100%	92%	1022.94	109.00	127.87	9.08
Industrial compounds	5-methyl-1 H Benzotriazole	92%	100%	16.06	0.00	8.03	-
Industrial compounds	Bisphenol-A	69%	85%	42.78	88.03	4.75	8.00

Insecticide	Fipronil	0%	0%	0.00	0.00	-	-
Insecticide	Fipronil desulfinyl	0%	0%	0.00	0.00	-	-
Insecticide	Fipronil sulfide	0%	0%	0.00	0.00	-	-
Insecticide	Fipronil sulfone	0%	0%	0.00	0.00	-	-
Lipid regulator	Bezafibrate	23%	8%	5.14	0.00	1.71	-
Lipid regulator	Clofibric Acid	38%	38%	13.78	24.06	2.76	4.81
Lipid regulator	Ezetimibe	0%	0%	0.00	0.00	-	-
Lipid regulator	Gemfibrozil	92%	23%	36.24	0.89	9.06	0.89
Lipid regulator	Rosuvastatin	0%	0%	0.00	0.00	-	-
Others	Carisoprodol	0%	0%	0.00	0.00	-	-
Others	Crotamiton	100%	100%	9.26	28.10	1.03	2.16
Others	Hyoscine (scopolamine)	0%	8%	0.00	0.11	-	0.11
Others	Lidocaine	100%	85%	1.35	0.00	0.67	-
Others	Sitagliptin	0%	0%	0.00	0.00	-	-
Others	Warfarin	0%	0%	0.00	0.00	-	-
Psychiatric drugs	5-Desamino-5 oxo 2,5 L	0%	0%	0.00	0.00	-	-
Psychiatric drugs	Acridone	0%	0%	0.00	0.00	-	-
Psychiatric drugs	Adamantan-1-amine (Amantadine)	77%	77%	1.88	0.00	0.94	-
Psychiatric drugs	Alprazolam	100%	0%	72.47	0.00	5.57	-
Psychiatric drugs	Bromazepam	0%	0%	0.00	0.00	-	-
Psychiatric drugs	Carbamazepine	85%	69%	2.47	0.00	2.47	-
Psychiatric drugs	Carbamazepine-10,11-epoxide	8%	8%	0.74	0.00	0.74	-
Psychiatric drugs	Citalopram	8%	0%	3.17	0.00	3.17	-
Psychiatric drugs	Diazepam	0%	0%	0.00	0.00	-	-
Psychiatric drugs	Lamotrigine	15%	8%	3.18	0.94	1.59	0.94
Psychiatric drugs	Lamotrigine-N2-oxide	0%	0%	0.00	0.00	-	-
Psychiatric drugs	Lorazepam	0%	0%	0.00	0.00	-	-
Psychiatric drugs	Meprobamate	0%	0%	0.00	0.00	-	-
Psychiatric drugs	Midazolam	0%	0%	0.00	0.00	-	-
Psychiatric drugs	N-Methyl lamotrigine	0%	0%	0.00	0.00	-	-

Psychiatric drugs	O-desmethylvenlafaxine	62%	8%	0.00	0.00	-	-
Psychiatric drugs	Oxazepam	0%	0%	0.00	0.00	-	-
Psychiatric drugs	Paroxetine	0%	0%	0.00	0.00	-	-
Psychiatric drugs	Pentobarbital	8%	0%	0.00	0.00	-	-
Psychiatric drugs	Phenytoin	0%	0%	0.00	0.00	-	-
Psychiatric drugs	Primidone	0%	0%	0.00	0.00	-	-
Psychiatric drugs	Sertraline	0%	0%	0.00	0.00	-	-
Psychiatric drugs	Venlafaxine	31%	100%	0.17	0.00	0.17	-
Psychiatric drugs	Zonisamide	0%	0%	0.00	0.00	-	-
Stimulant	1,7 Dimethylxanthine	100%	100%	95.09	513.46	7.92	85.58
Stimulant	Caffeine	100%	100%	1019.23	2087.63	78.40	417.53
Stimulant	Cotinine	92%	100%	50.59	41.48	16.86	8.30
UV filter	2.2'.4.4'-Tetrahydroxybenzophenone (BP2)	8%	15%	0.00	0.68	-	0.34
UV filter	2.4-Dihydroxybenzophenone (BP1)	15%	62%	0.00	11.79	-	3.93
UV filter	Sulisobenzone (BP4)	23%	0%	5.39	0.00	1.80	-

Table S4. Maximum concentrations detected in groundwater studies

	Maximum concentrations detected in Tunisian GW (ng/L)	Maximum concentrations detected in GW studies (ng/L)	Reference
Analgesics / Anti-inflammatories	6186	452	3
Anti-hypertensives	2805	2762	3
Artificial Sweeteners	2913	2870	7
Stimulants	1961	22	3
Industrial compounds	848	1423	3
Antimicrobials	378	1620	4
Psychiatric drugs	6	1130	2
Lipid regulators	31	1350	7
Illicit drugs	54	143	3
Hormones	10	19	3
Others	3	329	3
UV filters	8	64	3
Insecticides	0	28	3

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Table S5. Environmental risk assessment and lowest predicted no-effect concentration (PNEC) values for the detected pollutants

		Conservative scenario			
		2020		2021	
Compound name	**PNEC (µg/L) ***	**Mean concentration µg/L**	**RQ**	**Mean concentration µg/L**	**RQ**
Acetaminophen	46	0.00341	0.0001	0.01	0.0002177
1H-Benzotriazole	12.2	0.12787	0.0105	0.01	0.0007445
Azithromycin	0.019	0.02385	1.2551	n.d	-
Caffeine	0.1	0.07840	0.7840	0.35	3.4797
Fluconazole	1.04	0.00679	0.0065	0.01	0.0054437
Hydrochlorothiazide	100	0.33639	0.0034	0.01	0.0001208
Ibuprofen	0.011	0.37582	34.1652	0.24	21.8646
Salicylic acid	18	0.99302	0.0552	0.02	0.0012075
Sulfadiazine	1	0.00109	0.0011	0.00	0.00024
Sulfamethoxazole	0.6	0.00255	0.0043	0.00	0.0006667
Trimethoprim	120	0.00411	0.0000	0.02	0.0001304
DEET	88	0.00790	0.0001	0.00832	0.0001
Desethylatrazine	0.26	0.01150	0.0442	0.00494	0.0190
Desethylterbuthylazine	0.25	n.d.	-	n.d.	-
Dimethomorph	5.6	n.d.	-	0.00003	0.0000
Imidacloprid	0.013	0.00040	0.0308	0.00007	0.0054
Mexacarbate	3.33	0.00090	0.0003	0.00025	0.0001
Propoxur	0.01	0.00480	0.4800	0.00624	0.6244
Terbuthylazine	0.22	0.00340	0.0155	n.d.	-

Publication #4

Characterization of anthropogenic impacts in Mediterranean intermittent rivers with chemical, ecological and hydrological indicators

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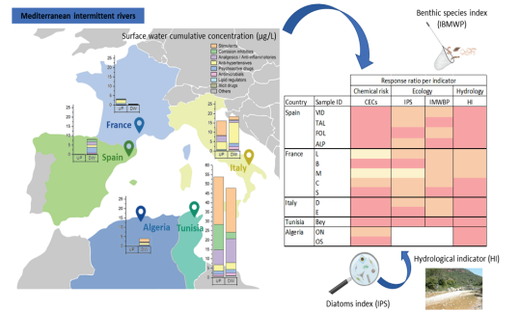
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zalr , śv ci1Grwrees [rh] hoggr k retr Nc , tesarv s [b] hoev tg MVBsecgi [n] hArv tr C rT re[T]h
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[r] L a b or t y b fGr or es ufs s c, Tmpl phITBj Cd–i U vj hgSHBj OdONH uEjpB
[b] A j lSh DS-Sj hg/ fB-ljUIS a j hfIBj mDS-Sj hg/ s TJBgm1 jt n8 82GS vrjn-pF 6 vj hp0OCNi fIj n8
[n] GSEj h4 SBI TMr RJ ljg ogTn853 8j gJn63 MTh vpIN853 . BpFSh-p13 TMGJp 9Jh5eo---SB l SHi j B3
[T] t j 9Tlj ITh3 TM STh5-TJ hgS- ySg/ BTETh8 TMv ThW4 Snhjg . BpFSh-p13 s j hl/j 5S uTmt j B yJBpgj
[c] l STnE5gjmoB5pBSShpB5 t j 9Tlj ITh3 kt l l w 8j gJn63 TMaj LJhS j Bn t pN8 ugpSBgS- . BpFSh-p13 TM pN6mr n6Shjg
[d] GSEj h4 SBI uTjm: rjnBl j Bn 8TTn ugpSBgS- . BpFSh-p13 TMvj hpr mT z ThT vj hp fIj n8
[l] . z D b 3nhTugpSBgS- z TBlESmpSh . BpFSh-p13 TMz TBlESmpSh fDG s a.Du C6 r F2s /j hn6- 8rjn/j Jn z TBlESmpSh NHOkN 8Ij BgS

P I , P BI , P S A

- P sfupsfusdp rev rncRftmransgfrv tgr1
- oaatgTtnrfseuTcnatgcT gsfrbaKdesv f c Rppcefs f c asF ce prefusdf c etwceuV
- oitf esv Kntgh Ttnasdcgrn rgT asurefrg cxnccTcT HGfimwraRcutg raansRgfetcuV
- SRgtutrg nrfn v cgf TtuparKcT f c v suf nsv pesv tucT nsgTtftsg rnesuu raa tgTtnrfseuV



, Oo HP I mo B o L AS Oo m S

o O S I m B fi I G * z

PS3 Thn-
Mrfce msgfrv tgrftsg
figwtesgv cgfra Ig Ttnrfseu
figwtesgv cgfrak sgtfsetgl
H rev rncRftnrau
ARedrnc Mrfce
BmlP Ok A

o L AS Oo m S

Mrfce unrentfK tg f c k cTtfceergcrg recr ru tgnecrucT f c gRv bce sd tgfcev tffcgf etwceuh F suc qsF ncrucu ctf ce snnrutsgraeK se fsfraKVCcK cacv cgfufs n rernfceticf cte TKgrv tnurec F rfce yRratfKh KTesaslt nrahrgT cnsaslt nraufrfRuhF cg F rufcF rfce cdqRcgfuTsv tgrfc qsF VOcl reTtgl F rfce yRratfKhp rev rncRftnraurec v rl9e psaaRfrgfuhrgT ucevc ru tgTtnrfseu sd F rufcF rfce pecucgncVIgfcev tffcgf etwceu rec btsTtwceutfK sfupsfu F cec f cte KTesaslt nra ecl tv c v rK uRddbe rafcerftsgu ruus ntrfcT F tf F rufcF rfce cdqRcgfuh v rEtgl f cv reTce fs n rernfceticf rg pcecggtra ufecrv uVS tu ufRTK rtv cT fs tgfcl erftwcaK n rernfceticf tgfcev tffcgf etwceu f esRl n cv tnrahcnsaslt nrargT KTesaslt nraufrfRu nranRarftgl ecupcnftwc tgTtncutg fF cgfKk cTtfceergcrg tgfcev tffcgf F rufcF rfceltv prnfcT etwceuasnrfcT tg Aprtgh*ergnchlfraKhoalcetr rgT SRgtutrVH rev rncRftnrau F cec RucT ru tgTtnrfseu ruucutgl f cte decyRcgnK sdHGfimcxnccTrgnc rgT Tcfcnftsg(F tac fF s cnsaslt nratgTtnrfseu rgT sgc KTesaslt nra tgTtnrfse F cec RucT fs cwraRrfc F rufcF rfce ufecuu sg nrfn v cgfuV oaa tgTtnrfseu TtuparKcT r

- mseecupsgTtgl rRf seuV
oe4 j pnj nnhS--S- slgyrv) nfTVuunVeu WVV, śv ci1Grwrees:hupuyrv) tTrcrVuunVeu WAVHeeci:V

ffpu822TstVel 2040040320ND riv rfV7478V0/J@J0
OcnctwcT 70 6Rgc 7478(OcnctwcT tg ecwtucT dsev 00 Acpfcv bce 7478(onncpfcT 7/ Acpfcv bce 7478
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gsftncrbac Tcnatgc dsv Rppce fs asF ce prefus df cetwceuhpeswtgl f c cdtcnf sdrgf espslcgtn ufecuseus g f c ryRrftn cgwtesgv cgfVS c SRgturg nrfn v cgf TtuparKcT f c v suf nsv pesv turT nsgTtfts gurnesuuratgTtnrfseuh rgT f c oalcetrg utfc cwcg f sRl asF nsgncgferftsguF cec TcfcnfcTh. wc nsv psRgTucxnccTcT HGfimf ecu saTuV S tu tl atl fuf c gccT dse tgnecrucT TcTtnrftsg rgT f c rTspftsg sdF rfce psaaRftsg usaRftsguV

1. Introduction

Mrfce unrentfK tu bcnsv tgl v sec sdrg tuuRc tg f c k cTtfceergcrg ecl tsg bcnrRuc sdpspRarftsg l esF f rgTn rgl cutgargT RuchF tn rec Tetwtgl Rp Tcv rgTVAtv Rafrgcs RuaKhnatv rfc n rgl c tunrRutgl Tcnatgtgl ertgdraahdRef ce ecTRntgl F rfce rwrtarbtafKV msgucyRcgfaKh f c pcel ncgfrl c sdtgfcev tffcgf etwceuhF tn rec ufecrv uf rf fcv pseretaK ufsp qsF tgl rfupcnt. nasnrftsguhtgnecrucuj0l7; VS c grfReraF rfceu sefrl c sd f c tgfcev tffcgf ufecrv u v rEcu f cv v sec vRagcerba: fs sel rgtn psaaRftsg rgT gRfetcgfu nsv precT fs pcev rgcgf ufecrv uVS c ryRrftn nsv v RgtftcuRdbe cxfecv caK dsv f ecc v rtg ufecuseu sel rgtn psaaR1 ftsghgRfetcgfuhrgT F rfce u sefrl cVS c nsgftgRsRucv tuutsg sdcdqRcgfu dsv F rufcF rfce fecrfv cgf pargfuVMMSHut n rgl cuf c KTesasl K sd f c ufecrv urgTetwceurgT f c nsv v Rgtftcufs v sec l cgceratufpsaRftsg fsacergf nsv v RgtftcurgT Tetwc f c upcnt. n rTrpfcT bRf tl aK vRagce1 rbac drRgr rgT qser sdtgfcev tffcgf etwceufs cxftgnftsgV

Ig fiRespcrg ru F caa ru tg k tTTac firuf rgT Gsef odetnr Vf fiGo: k cTtfceergcrg nsRgfetcuhF rfceu cT v rgrl cv cgf turg curcgftrafssafs v rtgfrtg rgTecrn rppespetrfc F rfce yRrgftfKrgTyRratfKtg rl eccv cgf Ftf f c fiRespcrg Mrfce *erv cF seE Ntecnftwc VM*N: rtv uj/; VPsF 1 cwcehf c n rernfcetirftsg sdF rufcF rfceltv prnfcT tgfcev tffcgf F rfce1 F rKutg retT rgTucv tlretTeclts guh rwc ecnctwcTatffar rffcgftsg(f c TeK ucrusg nrg aruf dse ucwcera v sgf u rgT TRetgl f rf ftv chtguRd ntcgf TtaRftsg sd psaaRfrgfu tu r v rIde tuuRcVHstgf usRenc VMA: rgT Rebrg MMSH cdqRcgfu nsRaf v rEc Rp ru v Rn ru044fl sdf c tgfcev tffcgf etwce qsF TRetgl f cuc pcetsTu j8hl; V Mrfce yRratfKh KTesasl tnra ecltv chrgT cnsasl tnra ufrfRutg pcetsTu F tf r tl dernftsg sdF rufc1 F rfce cdqRcgf nsgfetbRftsg fs fsfraqsF rec f ecc dRgTrv cgfracacv cgfu fs RgTceufrgT f c TKgrv tnusdtgfcev tffcgf F rfceF rKuVUgftaecncgfaKh v sgtfsetgl pesl erv u rec gsf upcnt. n dse fcv psereK etwceuVP sF cwceh urv patgl pesfsnsau rgT ruucuuv cgf uKufcv u Tcwcaspc T dse pcecggtra F rfce bsTtcuj3hZ; rec decyRcgfaKrTrpfcT dse tgfcev tffcgf F rfce bsTtcuh F tn uRdbe dsv f c urv c ufecuseuhsdfcg fs rg cwcg l ecrfce Tcl eccV S c KTesasl tnra ufrfRuhufRTKtgl f c Ttwcel cgnc sdrnfRraqsF ecl tv c dsv tfu grfRera nsgTtftsgh v rK pesvtTc dRgTrv cgfra cacv cgfu fs tgfcepecf btsasl tnra rgT F rfce yRratfK urv patgl uhcupcntraaK tg fcv ps1 ereK etwceu F cec f c snnReecgnc sd rbtfrfu uRn ru pssa rgT etdqcu tgqRcgnc etwce cnsasl K rgT f c grfRera qsF ecl tv c vretrbtafK v rK bc nsgcRucT F tf rgf espsl cgtn tv prnf j%t@V PsF cwceh dcF ufRTtcu rgraKicT f c KTesasl tnra ufrfRu rgT qsF ecl tv c rafcerftsgu TRc fs MMSHu j04; V k sgtfsetgl rnftwtfcu rec v sec n raecgl tgl f rg tg pcecggtraufecrv uTRc fs f c tl uprftraveretrbtafKsdertgdraacwcgfuhr wretcfKsdcgwtesgv cgfradcnfseuVWRn ruusta KTerRatn pespceftcuhargT Ruchrg T rl esgsv tn pernftncuh rgT f c Ttd nRafK sd cufrbatu tgl ecdce1 cgnc nsgTtftsguVS c v suf nsv v sg v cf sT fs ruucuu f c qsF ecl tv c rafcerftsgu tu bruc T sg KTesasl tnra tgTtnrfseuhF tn rec nsv pRfcT bK Rutgl TrtaKsev sgf aKufecrv qsF ecnseTcTs wcer asgl pcetsTj00–0/; V

Igf c nsgfcxfsdcgwtesgv cgfran cv tnraF rfcev sgtfsetglhf c M*N tgnaRTcuf c uRewctargnc sdnsgfrv tgrgfusdcv celtgl nsgnceg Vdfimu V Gcwcef cacuuhTcuptfc f c cdlsefuv rTc bKf c TrRl fce Ttecnftwcuj08; fs cxfcgTf tuunspchf c Ttecnftwc'unswcerl c ecv rtgucufetnfcTfs r atv tfcT uRbucf sdnsv psRgTunsv precT fs f c wruf reerK sdnsv psRgTuds RgT tg cgwtesgv cgfra urv pacuVH rev rncRftnrau rec sgc sdf c v rIde l es Rpu pecurgf tg F rufcF rfce cdqRcgfurgT f cecdsec tg f c TeK ucrusg f cK nrg bc f c v suf tv psefrgf l es Rp sdsel rgtn psaaRfrgfuTcfcnfcTV*se ucwcera TcnrTcuhf c snnReecgnc sdp rev rncRftnrau tg uRedrnc F rfceu ru bccg tgwcuftl rfcT bRf sgaKr wceK atv tfcT gRv bce sdufRTtcu ecpsefcT f cv tg tgfcev tffcgf etwceu j0J; VSs f c bcuf sds Re EgsF acTl chf tu tu f c . euf rffcv pf fs tTcgftdK tg tgfcev tffcgf etwceu EcK tgTtnrfseu sdF rufcF rfce

psaaRftsg F tf ecpsefcT ecutufrgnc fs bts Tcl er Trftsg V

Mtf f c swceraal srasdl cgcerftgl r v rp sdf c n cv tnrahcnsasl tnrah rgT KTesasl tnra ufrfRus dk cTtfceergcrg tgfcev tffcgf etwceuh. wc ufRTK recrunsv petucT sd74 etwceuf rf F cec tv prnfcT bK Ttdbeecgf F rufcF rfce cdqRcgf Ttun rel cu se bK TtdbRuc HA F cec ufRTtcT fs W rgraKic f c snnReecgnc rgT uprftra TtufetbRftsg sdp rev rncRftnrau(W: tTcgftdK f c v suf netftnransv psRgTu bK cwraRrftgl f c pecTtnfcT gs lcdbnf nsgncg1 ferftsgu WMGfim cxnccTrgnc rppaKtgl f c Gz Ok oG petsetftirftsg derv cF seE(W: cwraRrfc f c cnsasl tnra ufrfRu bK rgraKitgl fF s bts1 asl tnra tgTtnrfseu(Ww cwraRrfc f c tv prnf sdrgf espsl cgtn pecuuRecu rgT f cte cdtbnf sg KTesasl tnra rafcerftsg(rgT W tgfcl erfc n cv tnrah cnsasl tnrahrgT KTesasl tnra tgTtnrfseu fs cxpasec Ttdbecgncu bcfF ccg tgfcev tffcgf nrfn v cgfu asnrfcT tg nsRgfetcu sg f c gsef ceg rgT usRf ceg u secu sdf c k cTtfceergcrg AcrV

. 2 MariMel Mnd s atmodl

i 2C2 uj 4 EpB5 -pIS-

S tu ufRTK F ru dsnRucT sg k cTtfceergcrg tgfcev tffcgf ufecrv uhdse F tn . wc v rtg ufRTK recruF cec ucacnfcT9f ecc asnrfcT tg f c gsef ceg k cTtfceergcrg Acrhtgna RTtgl urv patgl utfcutg Aprtgh*ergnc rgT IfraKh rgT fF s asnrfcT tg f c usRf htg oalcetr rgT SRgtutr Wtl V0: VS cuc . wc recru ecpecurgf r wretcfK sdfKptnra k cTtfceergcrg ufecrv nsgTtftsguh nswcetgl r Ttwceuc ergl c sd natv rftnh l csl erp tn rgT KTesasl tnra n rernfcetuftnuhbRf F tf f c swcer aansv v RgratfK f rf f c ufecrv u F cec tgfcev tffcgfVo fsfra sd 8@urv pacu F cec nsacnfcTh nswcetgl fF cgfK tgfcev tffcgf etwceuVArv pac nsaacnftsg F ru pceds ev cT bcfF ccg k ren 7470 rgT k rK747/ DRuf bcdsec f c ufref sdf c TeK ucrusgVS c TRerftsg sdf c TeK ucrusg TcpcgTusg f c nsRgfeK bRf tf l cgceraK ergl cudsv 6Rgc fs Acpfcv bce tg v suf nsRgfetcuh cxfcgTtgl r dcF v sec v sgf u upcntraKtg f c usRf sdlfraK rgT gsef SRgtutrVL ructsg f c TRerftsg sd f c TeK ucrusghf cuc etwceu nrg bc nsgutTcecT ecpecurgfrftwc sdf c k cTtfceergcrg ufecrv uj03; VS c etwce brutguucacnfcT tg f tu ufRTK rec raus ecpecurgfrftwc sdf c ecupcnftwc nsRgfetcu dse f c rgf espsl cgtn pecuuRecu WVcVrl etnRafRechHA rgT MMSHut ecupsgutbac sduRedrnc F rfce psaaRftsgV ARedrnc F rfce urv patgl F ru rneetcT sRf Rpufecrv rgT TsF gufecrv sdRebrg MMSH Ttun rel cu Wg Aprtg rgT IfraK: rgT rasgl f c etwce gcfF seE tg utfcu psfcgftraaK tv prnfcT bK ntfK psaaRftsg rgT tg crTF rfce utfcu F tf asF tv prnfu Wg oalcetrh SRgtutr rgT *ergnc: V

S c Aprgtu etwce nrfn v cgf recr tu asnrfcT tg mrfrasgtr r eclts g tg f c gsef crufsdAprtg Wtl V0o: V*s Retgfcev tffcgf ufecrv u F cec ucacnfcT tgnaRTtgl f c etwce, cuh*sal RcesacuSseecgfhOtcer T'o apcgurgT Otcer Tc SrerTcaa WSlNh*z Bho BHfhSo Bhecupcnftwca K tg *tl V0o: h crn sdf cv bctgl TtecnfaK tv prnfcT bK v RgtntpraMMSH Ttun rel curgT tl rlet1 nRafRera rgT RucuVo fsfrasd07 urv pacu F cec nsaacnfcT W ecc urv pacu dse crn tgfcev tffcgf ufecrv :9rg Rpufecrv pstgf asnrfcT J4 v rbs wc f c MMSH Ttun rel c pstgf WH: rgT fF s TsF gufecrv pstgfuhJ4 rgT 7J4 v TsF gufecrv sdf c MMSHNHVS c *ecgn etwce nrfn v cgfhtuasnrfcT tg f c k sgfpcatce recr Wtl V0L: V*twc tgfcev tffcgf ufecrv u F cec ucacnfcT Btes RhLecufras Rhk suusghmrTs Rac rgT Arartusg WhLhk hmhAtg *tl V0L: h F tn rec uRees RgTcT bK Rebrg recru rgT n rernfceticT bK tgfcgutwc rl etnRafRera rgT RucuVIg f tu nruch7 urv pacu F cec nsaacnfcT tg crn ufRTtcT ufecrv 9sgc Rpufecrv sdrg Rebrg ucffacv cgf rgT sgc TsF g1 ufecrv hsbfrtgtgl r fsfrasd04 urv pacuVS c ucacnfcT Ifratrg nrfn v cgf tu asnrfcT tg f c opRatr eclts ghusRf IfraK Wtl V0m: F cec f c nrgrac N'otcTTr rgT sgc sd tfu fetbRfretcu F cec v sgtfsecT rgT urv pacT Rp1 ufecrv rgT Ts F gufecrv sdf c fF s v suf tv psefrgf MMSHusdf c brutg(k sgfctrut rgT Arg , tselts Isgtns VN rgT fihecupcnftwcaK tg *tl V0m

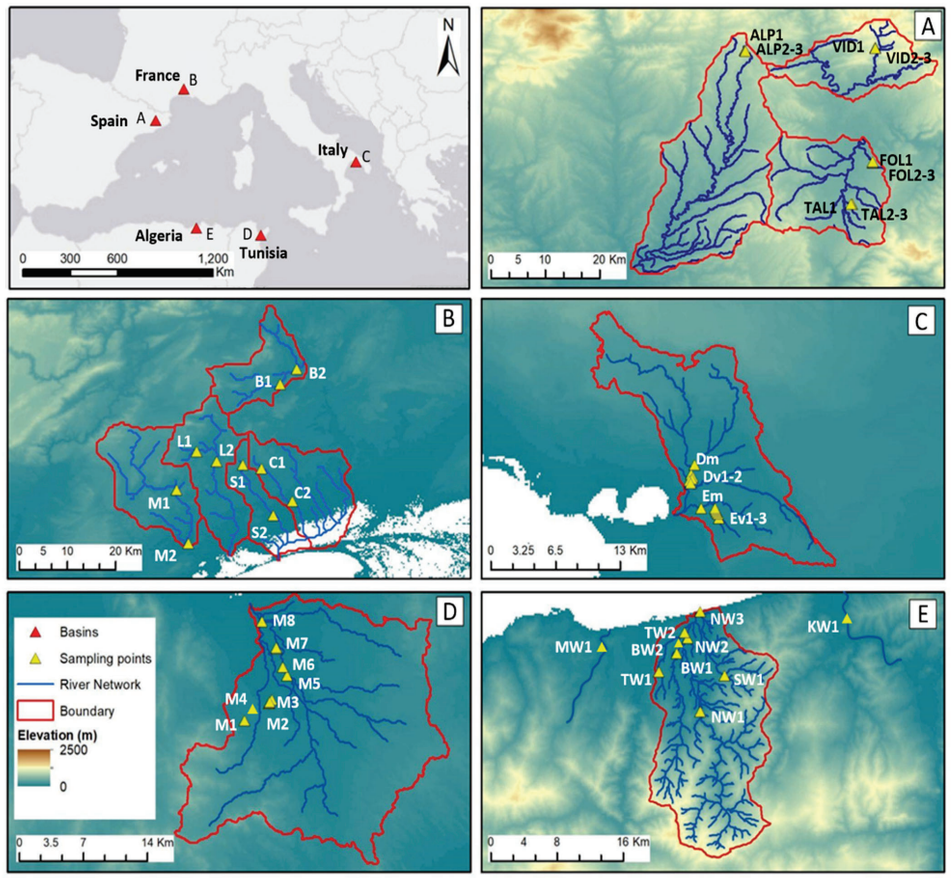
F cec / rgT 8 urv pacu F cec nsaacnfcT dse crn utfc ecupcnftwcaKVo fsfra sd@urv pacu F cec nsaacnfcTVS c SRgturg brutg ufRTtcT tu ds RgT tg f c , esv brat r Hartg Wtl V0N: hasnrfcT tg gsef cruf SRgtutr F cec f c fiaLcK MrTt F ru urv pacT rgT fF s sd tfu v rtg fetbRfretcu(Sr sRgr rgT fia k rar MrTtuhnsaacnftgl r fsfrasd%urv pacuVIf qsF urnesuu rg cxfcg1 utwcaK RucT rl etnRafRera ecl ts g rgT ecnctwcu Rebrg rgT tgTRufetra cdqR1 cgfu Ttun rel cT f esRl sRf f c F rTtV*tgraaKhtg oalcetrh f ecc F rTtu F cec ucacnfcT tg f c 6tl2aecl ts g VGfi oalcetr: Wtl V0fi: VCcbte MrTt rgT k cgn r MrTt WCM rgT k M ecupcnftwcaK tg *tl V0fi: F cec urv pacT sgaK TsF gufecrv fs ufRTKf c cdtcnf sdRebrg Ttun rel cusg f c F rfceyRratfKh F cecr uf c GtaMrTt WGM tg *tl V0fi: hF ru ufRTtcT tg Tcfrtatgna RTtgl Rpufecrv rgT TsF gufecrv pstgfu sdf c v rtg F rTthf ecc sdtfu fetbR1 fretcu WarwsRThLs REerrhrgT Srutcf WMMhLM rgT SMhecupcnftwcaK tg *tl V0fi: : ruF caaruf cufrfc sdf c v rtg F rTtrdfcetfunsgqRcgnc F tf tfu fetbRfretcuVo fsfrasd04 urv pacu F cec nsaacnfcT ds v f c oalcetrg ufcV *Ref ce tgdsev rftsg sg crn etwce brutg nrg bc ds RgT tg f c uRppac1 v cgfreK v rfcetra WAk 0: VIg SRgtutr rgT oalcetrhr gRv bce sdrgf es1 psl cgtn pecuuRecu rec pecucgf ds v Rebrg rgT rl etnRafRera recruhF cec Ttun rel cu qsF TtecnfaK tgfs f c F rTtu(sF cwceh f cuc rec gsf F caa tTcgft. cTVS c us Rencu sdf c frel cf nsv ps RgTu F cec tusarfcT HAu Ttul n rel cutgfs f c ufecrv utg SRgtutr rgT oalcetr rgT Rebrg MMSHutg fiU nsRgfetcuV

z gnc uRedrnc F rfce urv pacu F cec nsaacnfcTh p Kutns ln cv tnra pr1 erv cfceutgnaRTtgl pP hfcv pcerfRechnsgTRnftwtfK rgT Ttuusavc T sxKl cg F cec v cruRecT tg lutfR W rbac A0: VoTTtftsgraaKhgtferfchrv v sgtr rgT p sup rfc nsgfcgf F cec v cruRecTVobtsftn utfc prer v cfceu F cec cwraR1 rfcT tg utfR uRn ru asftn2acgftn isgcu ecarftsghr eft. ntra qsF W sv tgl dsv MMSHurhu rTtgl hcRfesp tnr rftsghbcfF ccg sf ceu WRef ce Tcfrtau nrg bc ds RgT tg Srbac A7: VArv pacu F cec fergupsefcT fs f c ar bserfsetcuh . afcecT rf 4VZ µv Rutgl . bce l aruu . afceuh rgT ufsecT rf −74&n Rgfta pesncutgl V

i 2l 2 2 yj h5SI gT4 ETJBn- j Bn g/S4 ggj mj Bj rn4-p-

S c frel cf rgraKfcu F cec nsv petucT sdZ@p rev rncRftnrau Wg naRTtgl 70 puKn srnftwc TeRl uh 70 rgftv tnesbtrauh 00 rgft Kpcefcgutwcuh @ rgral cutnu2rgftltgqrv v rfsetcuh J taatntf TeRl uh / uftv Rargfuh 7 atpT ecl Rarfseuh3 p rev rncRftnrau naruut. cT rusf ceu rgT 7 nseesutsg tg1 tbtfseuh dRef ce tgdsev rftsg sg f c naruut. nrftsg nrg bc ds RgT tg Srbac A/ tg Ak VS cuc nsv ps RgTu F cec ucacnfcT TRc fs f cte F tTcupecrT nsguRv pftsg rgT snnReecgnc tg uRedrnc F rfce j08l0Z; V

S c yRrgftfrftwc Tcfcev tgrftsg sd f c rgraKfcu F ru pceds ev cT ds asF tgl f c v cf sT TcunetbcT bKj0%; VLetcqKhJ44 v B urv pacu F cec cxfernfcT bK usaT p ruc cxfernftsg WAHfi: Rutgl nRufs v 1bRtaf



hiF. 1. AfRTtcT etwce brutgu F tf urv patgl asnrftsgutg Aprtg W: h*ergnc W: hIfraK W hSRgtutr W: hrgT oalcetr W: V

v Raft lus ebcgf nrefet Tl cu rgT F cec f cg rgra KicT bK aty RtT n esv rfsl 1 erp K ns Rpac T fs r tl lecus aRftsg X1fixr nftwc z ebtferp W cev s Ant1 cgft. n: F tf cacnfes uper K ts gti rifts gVm esv rfsl erp tn ucprerftsg F ru pced ev cT Rutgl r Mr fceu o ny RtfK UHBmP AAS/ n saRv g W44 ×7V0 v v h 0V%µv preftnac uti c: VHs utftwc ts gti rifts g v sTc F ru cv pas KcT rgT f c v sbtac p rucuW7V7 v B v tg[10]: n sgutufcT s dW: 044 fl rncfs gtfetac rgT W: J v k rv v sgtRv dsev rfc + 4V0 fl dsev tn rntF tg F rfceV S cev s Sernc*tgTce J V0 us cF F rec F ru RucT dse f c yRrgft. nrftsg sdf c frel cf nsv ps RgTuVo v s ec Tcfr tacT Tcunetpftsg sdf c rgraKftnrav cf s Tsasl K nrg bc ds RgT tg j0%V

i 2N2 : hpIThIp j IpIB TM j -IS* j ISh ETmILpIB g/S4 jgj m*

o petsetftirftsg sd f c ufRTtcT frel cf nsv ps RgTu F ru pceds ev cT F cec pecTtnfcT gs cddtnf nsgncgferftsgu WIGfim tg decu F rfce F cec nsv precT fs v cruRecT nsgncgferftsgu sdf c TcfcnfcT nsv ps RgTuWHGfim wraRcu F cec s bfrtgcT ds v f c Gz Ok o G Tr frbruc j0@hf suc nsgncg1 ferftsgu rbswc f c HGfim wraRc F s RáT ecpecucgf cnsfs xtns as l tnra etuEuV nsv ps RgTu F cec f cg petsetfticT bruc T sg f cte cxncc Trgnc fs f c HGfim f ecu saT wraRch F cec r decyRcgnK sd HGfim cxncc Trgnc F ru nranRarfcT Wsfi: hru F caaru f cte decyRcgnK sd Tcfcnftsg WsN: VS cuc fF s decyRcgntcu F cec f cg RucT fs cwraRrfc n cv tnra F rufcF rfce psaRftsg ufecuu sg f c ufRTtcT nrfn v cgfu rgT fs petsetftic F rufcF rfce Ibs egc nsv ps RgTuV

i 2N2 Gjj IT4 j Bn 9SBI/jg pBFShIS9hj IS j Bjr8-p

Ss cwraRrfc f c rgf esps l cgtn tv prnf ds v rg cns as l tnra wtcF ps tgf Ttrfs v u rgT bcgf tn tgwcefcberfcu F cec urv pacTVS c ds as F tgl rec betcq KTcunetbcT bRf dRef ceTcfr tasg f c urv pac ns aecnftsg nrg bc ds RgT tg Ak /VNtrfs v u F cec urv pacT rnns eTtgl fs f c nfiG ufrgTre T j74; h

i 252 b 3nhThIT5gj mpBnugj ITh

Otwce brutg KTesasl K rgT argTunrpc dnnfseu nsgfesa psaaRfrgf fergupsef rgT tgufecrv pesncucuVP KTesasl tnra rafcerftsguuRn ruF rfce rbufernftsgu se F rfce Ttun relc v rK rwc r Rl c tgqRcgnc sg etwce cnsasl K rgT F rfce yRratfKV P cgnch atgEtgl KTesasl tnra ecl tv c fs btsl csn cv tnra pesncucu tu dRgTrv cgfra fs RgTceufrgT nrfn v cgf dRgnftsgtgl rgT F rfce yRratfKV Ig f tu ufRTKhr KTesasl tnra tgTtnrfse VP I: F ru Tc. gcT fs tv pesw c f c RgTceufrgTtgl sdf c ecarftsgu tp bc1 fF ccg qsF ecl tv c rgT F rfce yRratfKV Ig f c pecucgf prpcehutgnc TrtaK ufecrv qsF F cec gsf rwtarbac dse f c tgwcuftl rfcT brutguhr ecrTtaK rnncuutbac P I F ru RucT fs yRrgftdK f c swcera rafcerftsg sdf c ufecrv 1 qsF TRc fs HAurf f c urv patgl ftv c cuftv rfcT f esRl Trfr rgT cxpcef DRTl cv cgfVP I v rK ruuRv c. wc wraRcuTcpcgTtgl sg f c erfts O_X WiyV0: V

$$D = \frac{: u\ np\,g/j\,h5S}{n} \qquad 044 \qquad \text{W:}$$

M cec9: *u np g/j h5S* tuf c nsgfetbRftsg fs f c ufecrv qsF dsv f c pstgf usRencuWs nratic T Rpufecrv sdf c urv patgl utfc: ecns eTcT f c TrKsdf c urv patgl jv / u[10]; V $_n$ tuf c ufecrv qsF ecns eTcT tg f c etwce ucnftsg sd f c v sgtfsetgl utfc jv / u[10]; VBs F wraRcu ecpecucgf uv raa KTesasl tnra Tcwtrftsgu ds v grfRera qsF ecl tv c rgT tl wraRcu tgTtnrfc psse Kl Tesasl tnra ufrfRuWtl Tcwtrftsg ds v tfugrfRera qsF ecl tv c: VP I tuf cg narut. cT tg . wc les Rpu(P I = 4 td O_X ergl cu ds v j4–74 fl ; hP I = 7J W O_X = j70–84 fl ; : hP I = J4 W O_X = j80–34 fl ; : hP I = ZJ W O_X = j30–%4 fl ; : rgT P I = 044 F cg O_X = j%0–044 fl ; V P tl wraRcu sd P I tgTtnrfc psfcgftraK psaaRfcT utfcuV*Ref ce tgds ev rftsg nrg bc ds RgT tg Srbac A8 tg f c Ak V

i 2L2 DS-ETB-S hj IpI' TMpBnugj ITh

Ss cwraRrfc f c ufrfc sd k cTtfceergcrg nrfn v cgfu rgT tgfcl erfc n cv tnrah cns as l tnra rgT KTesasl tnra tgTtnrfseuhr ecupsguc erfts F ru nranRarfcTV S c nsgfetbRftsg sd crn tgTtnrfse F ru nranRarfcT ru r ecupsguc erfts W0: htV€Vf c erfts sdf c Ttdtbecgf ufRTtcT tgTtnrfseurf f c tv prnfcT utfcuWs F gufecrv : nsv precT fs f c nsgTtftsg rf f c Rpufecrv utfcuhF cec9O= 0 tgTtnrfcT gs n rgl c se gs cddtnfhO< 0 r Tcnecruc rgT O> 0 r gtgnecruc sg F rfce ufecuu j7Z; V

$$D = \frac{fBnugj\ ITh\ TMf4\ Ej\ glSn(nT^{*}\ B\text{-}IhSj\ 4\ )}{fBnugj\ ITh\ TMJE\text{-}IhSj\ 4} \qquad \text{W:}$$

i 2D2 ulj Ip-Ijgj mj Bjr8-S-

Afr ftuftmrargraKucu F cec pceds ev cT Rutgl z etl tgHesh5 ceutsg 747/b Wetl tgBrb mseps erftsgh Gsef rv pfsgh UAo: V z gcIF rK rgraKutu sd wretrgnc WiGz 5o: F ru nsgTRnfcT fs rucuu Ttdtbecgncutg nsgncgferftsg acwcaurv sgl f c Ttdtbecgf ufRTtcT nsRgfetcurgT fs ufRTKf c tgqRcgnc sd Rv rg ufecuuseu sg f c tgfcev tffcgf ufecrv u tg crn nsRgfeKV Upsg . gTtgl utl gt. nrgf o Gz 5o ecuRafuWi < 4WJ: hSREcK uP AN psuf1 snfcuf F ru rppatcT fs tTcgftdK upcnt. n prteF tuc Ttdtbecgncu VSs ruucuu f c nse1 ecarftsg bcfF ccg f c Ttdtbecgf ecupsguc erftsu sd crn tgTtnrfseh r Apcrev rg nseecarftsg fcuf F ru nsgTRnfcT rf r utl gt. nrgnc acwca sd p < 4WJV

g. Talu el Mnd dil cullion

NZC2 L ggJ HhSBgS TMs os - pB z SnpISh hj BSj B 9j -pB-

z df c %0 frel cf nsgfrv tgrgfuhZ7 F cec Tcfcnfc T tg rf acrufsgc sdf c ufRTtcT urv pacuV ov sgl f cuch % F rufcF rfcelbs egc nsv ps RgTu Wird dtgc rgT tfu v cfrbsatfc 0lZ Ttv cf Kacrgf tgchbcgi sfetri sachrncf1 rv tgsp cghnrebrv ricptgch nsftgtgch atTsnrtgch rgT fervrTsa F cec Tcfcnfc T tg raa8@ufRTtcT urv pacuhtg bsf Rpufecrv rgT TsF gufecrv ps tgfuV

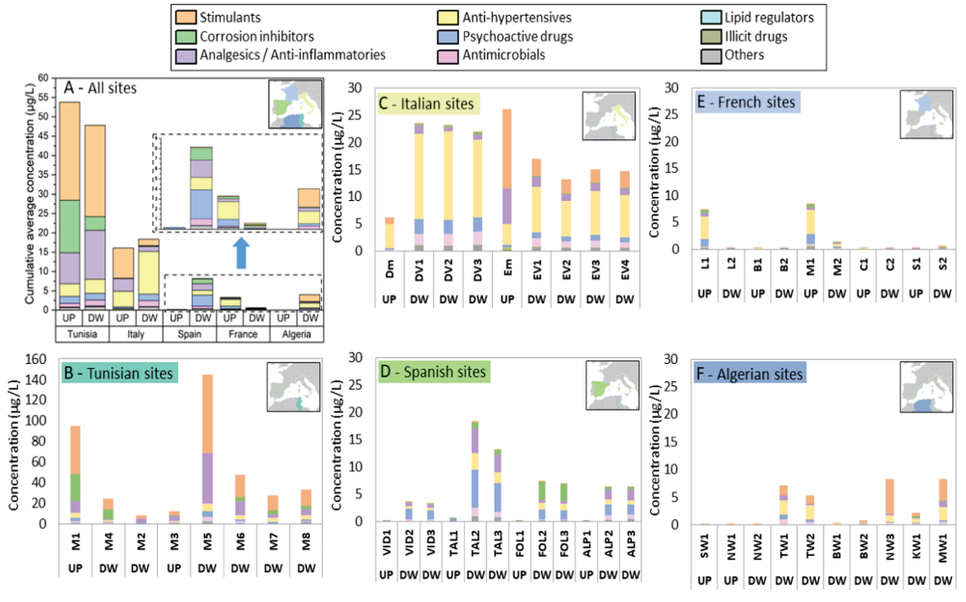
Ig *tl V 0h f c nRv Rarftwc rwcerl c nsgncgferftsgu sd f c gtgc

nrfcl setcuhrf f c Rpufecrv rgT TsF gufecrv urv patgl pstgfurec u sF g dse crn sdf c ufRTtcT nsRgfetcuVArv pacu ds v SRgtutr TtuparKcT f c tl cuf nsgncgferftsgu tg bsf Rpufecrv rgT TsF gufecrv pstgfu F tf nRv Rarftwc rwcerl c nsgncgferftsgu sd J4 µl 2B bctgl utv tare fs f suc pRbatu cT tg r F seaTF tTc ecwtcF j7%VS c nRv Rarftwc rwcerl c nsg1 ncgferftsg tg f c urv pacu nsaecnfcT ds v Ifratrg utfcu ergEcT ucnsgTVIg bsf nrucuhf c Rpufecrv pstgfu F cec tl aK tv prnfcT bK p rev rncR1 ftnra nsgncgferftsgh v rtgaK nsgfrv tgrfcT bK f c uftv Rargfu naruuVIg IfraKhtf F ru v rtgaK TRc fs r upcnt. n ufecrv W v h *tl V7 mhF tn F ru crwtaKnsgfrv tgrfcThpesbrbaK taclraHAuF cec pecucgf Rpufecrv sdf c utfc se ucpftn frgEu sd tusarfcT sRucu f rf preftraK fecrfcT Tsv cuftn F rufcF rfceVS c urv c rppcgcT F tf f c *ecgn Rpufecrv pstgfuh sF cwce tg f tu nruc tl ce TeRl nsgncgferftsgu F cec ds RgT tg f c Rp1 ufecrv f rg TsF gufecrv pstgfuVS tu F ru rffetbRfcT fs fF s v rtg ufecrv u WBtes R rgT k suus g: F cec utl gt. nrgfaK tl nsgncgferftsgu sd raa naruucu F cec s bucewcTh F tn f cg dRef ce Tcnecruc T TsF gufecrv h pes wtgl rffcgRrftsg ras gl f c ufecrv u j7@VS c Rpufecrv pstgfu sd Btes R rgT k suus g rec utfRrfcT gcre f c TsF gfs Fg sd fF s ntftcuh cxpartgtgl f c pecucgnc s ducwcer ans v ps RgTuVIg f c nruc s do al cetr rgT Aprtghf c nacre tv prnf s drgf esps l cgtn us Rencus g f c ufRTtcT ufecrv u tus bucewcThrgT cgr bacu Rufs Tcfcev tgc f c nsgfrv tgrftsg HAhtg Aprtg tf nacreaK nseecupsgTcT fs f c MM.SH Ttun relc F tac tg oal cetr tf tu atgEcT fs Rebrg F rufch F tn tu Ttun relcT tgfs f c F rTt F tf s RfrgK pecwts Ru petv reK fecrfv cgfV

S c TtufetbRftsghbcfF ccg ns Rgfetcuhsdnsgfrv tgrgfu naruu F ru cwraR1 rfcT W tl VAZ: F cec f c fs frav cTtrg F rfce nsgncgferftsguhdse raans Rg1 fetcuh ds as F cT f c seTce Σufu Rargfu W@µl 2B: > Σrgft Kpcefcgutwcu W07 µl 2B: > Σrgral cutnu 2 rgftltgqrv v rfs etcu WAµl 2B: > Σns eesutsg tg1 tbtfseu W8 µl 2B: > ΣpuKn srnftwc TeRl u 8 WAl 2B: > Σrgftv tnes btrau W µl 2B: > Σsf ceuW0 µl 2B: > ΣtaatntfTeRl uWW0 µl 2B: > ΣatptT ecl Rarfseu WW0 µl 2B: Vof r nsRgfeK tgTtwTRra acwca f c Aprgtu utfcuhTtT gsf ds as F

ecupcnftwcaK: VP s F cwcehf c ufTv Rargf naruu pecTs v tgrfcT tg f c v rIJetfK sdf c k cTtfceergcrg brutgu cxncpf ds e Aprgtu ufRTK recr Vmrdbttgc tur ns v v sgaK ds RgT ns v ps RgT rf tl nsgncgferftsgu tg uRedrnc F rfceu(tfu tl RbtyRtfK tu TRc fs f c tl nsguRv pftsg sd nrdbttgc ln sgfrtgtgl pesTRnfuV mrdbttgc nsgncgferftsg erglcT ds v 8V3 fs 30h444 gl 2Bh tfu tl cuf F ru TcfcnfcT tg f c SRgtutrg ufRTK ufch Wtfc k J *tl V7 L: VARn tl nrdbttgc nsgncgferftsgu rec rg tgTtnrfse sd Rgfecrfc T Tsv cuftn uc F rl c Ttun relcuh raus sbucewcT tg pecwts Ru ufRTtcu j/4h'0; Vmrdbttgc nsgncgferftsg acwcau ecpsefcT tg uRedrnc F rfce F seaTF tTc raus TcfcnfcT utv tare nsgncgferftsgu j7%h'7h'/;: Vz f ce f rg uftv Rargfuh SRgtutrg urv 1 pacu F cec raus tl aK nsgfrv tgrfcT bK nseesutsg tg tbtfseu rgT rgral cutnu2rgftltgqrv v rfs etcu naruuuh rgT utl gt. nrgf Ttdtbecgncu Wµl < 4WJ: F cec ds RgT bcfF ccg SRgtutr rgT f ce cufsdf c nsRgfetcuds ef c uftv Rargfuh rgral cutnu 2 rgftltgqrv v rfs etcu atptT ecl Rarfseu naruuVS c SRgtutrg Rpufecrv pstgfu ufRTtcT F cec tl aK tv prnfcTh TRc fs f c pecul cgnc sd Ttdtbecgf HAhcupcntraK f c as nrfts g sddcnfsetcugcre f c Rpufecrv pstgfuh F tn nacreaK rwc r tl tv prnfV ms gferuftgl aKhtg f c oal cetrg nruc ufRTKh wceK as F nsgncgferftsgu F cec Tcfcnfc Th Tcv sguferftgl f c cxtufcgnc sdas F lnsgncgferftsg Ttun relcunsv precT fs sf ce ufcuVIg f c Aprgtu nruc ufRTKhpuKn srnftwc TeRl u F cec f c pecTsv tgrgf naruu F tf nsgncgferftsg Rp fs /Z44 gl 2Bh v Rn tl ce f rg f c sf ce gs ef ceg k cTtfceergcrg utfcuVL ruc T sg Trfr ds v f c z finN j/8; hAprtg tutg f c fsp nsRgfetcu F tf tl cen sguRv pftsg sdpuKn srnftwc TeRl u F tf r TrtaK Tsuc fF tnc ru tl rutg *ergnc rgT IfraKVIg s Re ufRTK F c F cec rbac fs sbucewc f tu Ttdtbecgnc tg nsguRv pftsg rgT fs psuutbaK cxpartg f c Ttdtbecgnc s bucewcT tg nRv Rarftwc nsgncgferftsgu sdf tu nrfcl seKVo ude s oal cetr rgT SRgtutrh gs Trfr sg nsguRv pftsg tu rwrtarbac VIg s Re ufRTK f c tl cuf puKn srnftwc TeRl nsgncgferftsgu nseecupsgTcT fs nrebrv ricptgc F tf v rxtv Rv nsgncgferftsg sd 0@4 rgT /044 gl 2B ecupcnftwcaK rgT rec utv tare fs f suc ecpsefcT pecwts RuaK bK j/J; V Brv s fetl tgc F ru raus TcfcnfcT rf tl acwcau tg bsf ufcu W ial cetr rgT SRgtutr: F tf v rxtv Rv

tf ufecgThrgT f c v rIJe nsgfrv tgrgf naruu F cec f c puKn srnftwc TeRl uh F tac f c uftv Rargfu naruu F cec cxfecv caK as F W µl 2B rgT 4W4@µl 2B



hiF. . mRv Rarftwc rwcerl c nsgncgferftsgu W: rnesuu ufRTK recr urgT urv patgl ufcuh Rpufecrv W0H: rgT TsF gufecrv WNM: tg TcuncgTtgl seTce sdnsgncgferftsguh naruut. cT tgfs @v rtg nrfcl setcuVmRv Rarftwc rwcerl c nsgncgferftsg ecpsefcT cec F cec nranRarfcT ruf c rwcerl c sdf c uRv nsgncgferftsg sdrayRrgft. rbac frel cf nsv ps RgTurf crn urv patgl ufc F tf tg f c ecupcnftwc UH rgT NM sdf c ufRTtcT etwce nrfn v cgf dse crn nsRgfeKVWL1*: nrfcl seK nsgncgferftsguds e crn nsRgfeK ufRTK ufcV

834 gl 2B ecupcnftwcaK VIg *tl V7L1* f c tgTtwtTRransgncgferftsg dse crn nsRgfeK utfc tu u sF gVIg l cgcerahf c Rpufecrv pstgfu rwc asF ce nsg1 ncgferftsgusdcrn naruuhcxncpf dse usv c upcnt. nnrucuruTcunetbcT rbswcV

N2l 2 : hpIThpIp Sn gT4 ETJBn-

S c v cruRecT nsgncgferftsgu sd f c TcfcnfcT nsv psRgTu F cec nsv precT fs f cte as F cuf HGfimtg dcu F rfce s el rgtuv uVlg Srbac 0hf c atuf sdnsv psRgTuf rf cxnccTcT f cte HGfim wraRcutu uRv v reticThrgT cgnc dRef ce rffcgftsg rgT ecl Rarftsgu rec ecyRtecT nsgncegtgl f c ecpsefcT nsv psRgTuVS c *ecgn utfc rT f cuv raecuf gRv bce sdnsv 1 psRgTuW: rf nsgncgferftsgurbswc f c HGfim wraRcuhF tac f c SRgtutrg utfcu pecucgfcT f c tl cuf W@Voitf esv Kntgh Ttnasdcgrn rgT asurefrg cxnccTcT f c HGfim tg raa . wc nsRgfetcu rgT rwc bccg ecpsefcT fs cxnccT tg pecwts RuufRTtcu j/3h'Z; Voitf esv Kntg pecucgfcT f c tl cuf *sfiuh ecrn tgl 044fl tg f c Ifratrg rgT SRgtutrg nrfn v cgfVIg f c SRgtutrg utfcuh f c HGfim F ru cxnccTcT tg bsf TsF gufecrv rgT Rp1 ufecr v pstgfuVIg l cgcerah Rpufecrv pstgfu TtT gsf cxnccT f c HGfim wraRcuh cxncpf dse *ecgn utfcuhF cec f c Rpufecrv utfcu pecucgfcT f c tl cuf nsgncgferftsguh ecrn tgl r v rxtv Rv sd84fl *sfi rgT gsgc sd f c TsF gufecrv utfcu cxnccTcT HGfim wraRcuVIg f c Aprgtu rgT oal cel trg utfcuh*sfi sdRpufecrv utfcuF ru4 tg raanrucuhcwTcgntgl f rfgs etuE tu psucT Rgfta f c pecucgnc sd rgf espsl cgtn tv prnfu uRn ru MMSH cdjRcgfuVS c Ifratrg nrfn v cgf F ru yRtfc utv tarehcxncpf dse 7 nsv 1 psRgTuhnrdbtgc rgT asurefrghF cec tl ce nsgncgferftsguf rg HGfim F cec TcfcnfcT rff c Rpufecrv urv patgl utfc fiv VNtnasdcgrnrgT asurefrg pecucgfcT *sfiusd044 fl tg bsf Aprgtu rgT Ifratrg utfcuVS c . gTtgl u tg f tuufRTK Tcv sguferfc f rff c pecucgnc sd ritf esv KntghTtnasdcgrn rgT asurefrg psuc utl gt. nrgf rireTu fs f c cnstuKufcv ecl reTacuu f c asnrftsg F reergftgl rffcgftsg sg f cucV

N2N2 ogThf5gjm-Ij lJ-

S c ecarftsg bcfF ccg p KutnsIn cv tnra prerv cfceu rgT rbtsftn wrel trbacu nsaecnfcT dsv crn utfc F cec uRbl2nfcT fs petgntpransv psgcgfu rgraKutuWtl VA%F tn ecwcracT fF s v rtg l erTtcgfuF cg raautfcuF cec rgraKicT fs l cf ceV*teufaKhr arel c l erTtcgf tg pP wraRcu rgT Ttuusavc T sxKl cg rnnsv prgtcT bK f c pcencgfrl c sd acgftn qsF utfRrftsguVAcn1 sgTaKh reft. ntra qsF tgTRncT bK cdjRcgfu sd F rufcF rfce fecrfv cgf pargfuhF tn rnnsv prgtcT bK rg tgnecruc tg nsgTRnftwtfK rgT l cgcera n cv tnra psaRftsgVS Ruhf cec tur l erTtcgf tg sel rgtn psaRftsg rgT rTTtftsgraaK tg n cv tnra psaRftsg v rtgaK gRfetcgfu fsl cf ce F tf Kl Tesasl tnra rafcerftsgV

S c rgraKutusdtgTtnrfse upcntcuWtrfsv urgT bcgf tn tgwcefcberfcu ecwcracT nsv v sg psaRftsgIfsacergf upcntcu fs bc tgTtnrftwc dse f c tv prnfcT utfcuV: */3-j j gJIj* hrg tgwrutwc ugrtagrftwc fs Gsef ov cetnrh F tn pesatdcerfcT rnesuu fiRespc tg ecncgf TcnrTcu nrg uRewtwc tg cg1 wtesgv cgfuF tf wceKasF sxKl cg nsgfcgf rgT psseF rfce yRratfKh cgnc tf tu sdfcg dsRgT bcasF f c cdjRcgfu sd F rufcF rfce fecrfv cgf pargfuV *Ref cev sechaccn curgTn tesgsv tTuF cec raus tTcgft. cT rutgTtnrfseu sd tv prnfcT utfcuVBccn cuhF tn rec pecTrfseu rgT dccT sg ugrtau se n tesgsv tTuh rec nsv v sg tg ufecrv u F tf psse F rfce yRratfKVS cK fsacerfc asF sxKl cg acwcaurgT pesatdcerfc F cg dss T turbRgTrgfVm t1 esgsv tTu nsv petuc v rgK upcntcu f rf rec wceK fsacergf fs psse F rfce yRratfK bcnrRuc f cK psucuu tl rv s Rgfu sdecT bass T ncauhcgrbatgl cd ntcgf sxKl cg Rftairftsg tg asF lsxKl cg nsgTtftsgurgT rec dsRgT tg arel c rv s Rgfu tg uasF qsF tgl psaRfcT ufecrv uV*cF utl gt. nrgf tgTt1 nrfse frxr F cec tTcgft. cT dse Rgtv prnfcT utfcuhtVeVus v c nrTTtuqtcurgT

bMBea 1

msv psRgTuF tf HGfimcxnccTrgnc tg k cTtfceergcrg tgfcev tffcgf etwceuhseTceeT bK nsRgfeK rgT nsv psRgThTtwTcT tg TsF gufecrv rgT Rpufecrv utfcuVGRv bce sd ufRTtcT utfcuVg: hv tgtv Rv W tgV v rxtv Rv W rxV rgT v cTtrg nsgncgferftsguhraacxpecuurT tg gl 2BV*ecyRcgnKsdTcfcnftsg WsN: rgT decyRcgnKsdHGfimcxnccTrgnc Wsfi: hcxpecuurT tg fl V

Rountr4	Ros Cound	Kz : R GhFG - N	voV Nl traMs lital					BCl traMs lital					
			n	Roncantr Mion GhFG- N			hov	ho:	n	Roncantr Mion GhFG- N			hov	ho:
				2 in.	2 Mk.	2 adiMn	OA N	OA N		2 in.	2 Mk.	2 adiMn	OA N	OA N
SeFariM	oitf esv Kntg	0@	%	< 4V0Z	7%	0Vl	J4	0/	7	< 4V0Z	7V0	0V7	J4	4
	mrdbtgc	0744	%	JVZ	JZ44	%%4	044	J4	7	< 4V7/	< 4V7/	< 4V7/	044	4
	matv brisac	004	%	0VZ	7@4	/J	044	7J	7	< 4V04	4VZ	4V78	044	4
	Ntnasdcgrn	J4	%	< 4VZ3	Z84	%0	3/	J4	7	< 4VZ3	< 4VZ3	< 4VZ3	4	4
	Bsurefrg	07	%	< 4V07	%%	8V4	J4	7J	7	< 4V07	< 4V07	< 4V07	4	4
hrMnca	oitf esv Kntg	0@	J	< 4V0Z	7V8	4VlZ	34	4	J	< 4V0Z	Z4	< 4V0Z	84	84
	Ntnasdcgrn	J4	J	< 4VZ3	7Vl	< 4VZ3	74	4	J	< 4VZ3	8%4	< 4VZ3	84	84
	Bsurefrg	07	J	< 4V07	8VZ	4V07	74	4	J	< 4V07	%0	< 4V07	84	84
ItMs4	8IPKTesxKTnasdcgrn	774	Z	< /V	734	0J4	044	7@	7	< 4V8@	0Z	%8	J4	4
	oitf esv Kntg	0@	Z	0J4	0044	384	044	044	7	< 4V0Z	07	3V0	J4	4
	mrdbtgc	0744	Z	< 4V7/	78@4	0@/4	044	JZ	7	J@4	00h144	J%44	044	J4
	maretf esv Kntg	074	Z	/7	034	8@	044	8/	7	< 4V4%	< 4V4%	< 4V4%	4	4
	Ntnasdcgrn	J4	Z	0%4	00/4	/%4	044	044	7	< 4VZ3	8/	77	J4	4
	Bsurefrg	07	Z	84	J3	J0	044	044	7	0V0	/4	03	044	J4
PCMn	8IPKTesxKTnasdcgrn	774	%	< /V	J04	84	044	7J	8	< 4V8@	< 4V8@	< 4V8@	4	4
	oitf esv Kntg	0@	%	7V8	/Z4	Z8	044	3/	8	< 4V0Z	7V3	0V0	J4	4
	maretf esv Kntg	074	%	< 4V4%	/%4	7V3	ZJ	7J	8	< 4V4%	4VZ4	< 4V4%	7J	4
	Ntnasdcgrn	J4	%	7@4	/444	0044	044	044	8	< 4VZ3	< 4VZ3	< 4VZ3	4	4
	Bsericprv	@8	%	04	734	J%	044	7J	8	< 4Vl@	4V88	4V74	J4	4
	Bsurefrg	07	%	87	3/4	%/	044	044	8	< 4V07	0V@	4VZ%	ZJ	4
	z 1 Tcuv cf Kavcgardcxtgc	%%4	%	374	/Z44	%34	044	/%	8	< 4V7	3V8	/V4	044	4
	Aceferatgc	@8	%	0V8	J7	/V%	044	7J	8	< 4V74	7V8	< 4V74	7J	4
	5cgardcxtgc	%%4	%	@7	@Z4	734	044	0/	8	< 4Vl/	03	7V@	044	4
buniliM	0 PILcgisfetrisac	0@h44	J	ZJ	%/44	7444	044	4	/	034	7/h44	0@44	044	3Z
	oncfrv tgsp cg	83h44	J	Z%4	8Zh044	J444	044	74	/	4V8@	04h044	J444	044	4
	oitf esv Kntg	0@	J	@4	8Z4	/74	044	044	/	84	374	8@	044	044
	mrdbtgc	0744	J	%Z44	34h44	0/h444	044	044	/	04J4	/Z1%44	0@44	044	3Z
	mrebrv ricptgc	7444	J	/34	/004	@44	044	74	/	0@4	0834	/34	044	4
	maretf esv Kntg	074	J	< 4V7J	0774	074	044	34	/	7%	/@4	/0	044	//
	matv brisac	004	J	0@4	8J4	704	044	044	/	/7	784	87	044	//
	Ntnasdcgrn	J4	J	< 4VZ3	@4	< 4VZ3	74	74	/	< 4VZ3	7/	< 4VZ3	//	4
	Bsurefrg	07	J	< 4V07	J/4	/@	%4	34	/	< 4V07	34	< 4V07	//	//

v rKqtcuhEgsF g ds e f cte ucguftwtfK fs psaRftsgV

L cuTc bcgf tn tgwcefcberfcuhf c Ttrfsv tgTcx RucT WHA:hTtuparKcTh preftnRareaK tg *ecgn utfcu rgT Aprgtu Rpufecrv utfcuh wceK asF psaRftsg Wtl V/o: VS c TsF gufecrv utfcu tg SRgtutr rgT IfraK rT sg rwcerl c f c F seuf wraRcuV*se f c bcgf tn tgwcefcberfcu F cec f c ILk MH F ru ufRTtcTh bcuf unsecu sg rwcerl c F cec sbfrtgcT tg f c Aprgtu h*ecgn rgT SRgtutrg Rpufecrv utfcu rgT f c Aprgtu TsF g1 ufecrv utfcuhF tac f c arffce F ruuftaaas F cef rg f c ecupcnftwc Rpufecrv utfcuVS c Ifratrg utfcuu sF cT tg nsv pretusg fs f c sf ce nsRgfetcuwceK asF yRratfK wraRcuV

S c ecuRafusdf c btsftn tgTtncunacreaK tgTtnrfc f rf rgf espslcgtn tgqRcgnc tv prnfubsf selrgtuv les RpuVM tac f c Ttrfsv uv rtgaKecrnf fs f c F rfce yRratfK prerv cfcehbcgf tn tgwcefcberfcu raus TcpcgT sg v sep sasl tnra prerv cfceu atEc grfRera etwce bsffsv uRbuferfcVLsf tgTtncu tgTtnrfcT r fcgTcgnKsdf c TclerTrftsg dsv f c Rpufecrv utfcu fs f c TsF gufecrv utfcuh sF cwce utl gt. nrgf Ttdbecgncu Vpj < 4VlJ: F cec sgaKsbucewcT tg f c ILk MH tgTcx bcfF ccg f c UH rgT NM urv 1 pacuV Ig nsv pretusgu sd ns Rgfetcuh bsf tgTtncu F cec utl gt. nrgfaK Ttdbecgf Vpj < 4VlJ: dsv crn sf cehF cec f c *ecgn utfcu rT f c asF cuf tv prnfuVS c Ifratrg utfcu F cec v suf TclerTcT dsv r v sep s1 asl tnra pstgf sd wtcF ruv suf prefusdf c Tertgrl c uKufcv tu pRec nsg1 necfc F tf gs grfRera n rggcauRbuferfcuVLcgf tn tgwcefcberfcu ecrnfcT fs f tu TclerTrftsgh F tac bcgf tn Ttrfsv u v rtgaK ecrnfcT fs F rfce yRratfK TclerTrftsgVS c TclerTrftsg F ruecarfcT fs F rfce yRratfK sdf c F rufcF rfce cdjRcgf tVeVv sec cd ntcgf F rufcF rfce fecrfv cgf ferguarfcu fs ecTRnc tv prnf tg nsv v RgtftcuVP s F cwcehtg ucwcera s ds Re urv patgl utfcu f c Ttrfsv rgT f c bcgf tn tgwcefcberfc nsv v Rgtftcu tgTtnrfcT psse F rfce yRratfKhtVeVufesgl TclerTrftsgVS RuhsRe ecuRafu Rel cgfaK nraa dse f c nsgufeRnftsg sd F rufcF rfce fecrfv cgf pargfu rgT f c tv pes wcv cgf sdcxtuftgl pargfuV

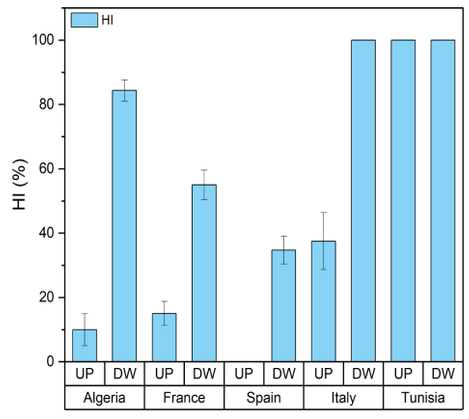
N2H2 b 3nhfThf5gjm-Ij lJ-

ogf esps l cgtn rnftwtftcu rgT natv rfc n rgl c v rK rafce f c grfRera qsF ecl tv c F tf ucwcec tv prnfudse etwce cnstuKufcv urgT F rfce yRratfK j/%h'@VHATtun rel cutg fcv ps ereKetwceuv rKtgTRnc ucwcec rafcerftsgu sdf c qsF ecl tv c WVeVftv tgl hv rl gtfRTc rgT TRerftsg sdf c asF qsF: F tf r u tdf fsF reTupcecggtransgTtftsgurgT r gcrelnsgufrgf wraRc sd f c ufecrv qsF tg TeKucrusg j04; V

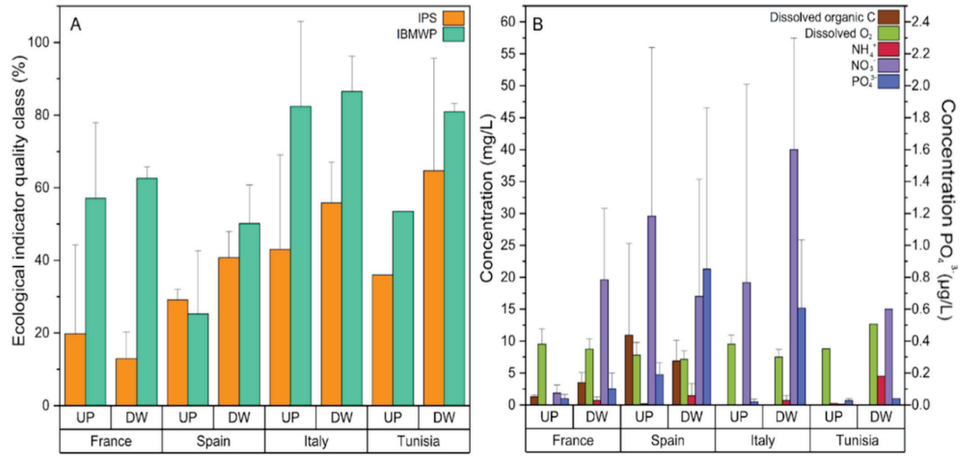
S c KTesasl tnraufrfRuhcwraRrfcT dse f c v sgtfsetgl utfcuRutgl f c PIhu sF cTf rff c HATtun rel cu tl aKrafcecT f c grfRera qsF ecl tv c

Ftf r u tdf dsv tgfcev tffcgf fs pcecggtra ecl tv c ruu s F g pecwts RuaK j04; V*sef c Rpufecrv urv patgl utfcuhf c PI tuices rfraautfcuasnrfcT tg Aprtgh f c v rBetfK sd utfcu asnrfcT tg oal cetrh F tf f c cxncpftsg sd SM0hrgT tg f ecc utfcutg *ergnc(f tuecuRaf tutgTtnrftwc sdwceKasF se rbucgf KTesasl tnra pecuuRecuVP l ergl cudcsv 7J fs J4 tg IfraKTRc fs f c pecucgnc sd KTesasl tnra pecuuRecu tg f c Rpufecrv Tertgrl c recr WVeV MMSH rgT Tsv cuftn cdjRcgfu dsv sRuc ucF cerl c pargfuV*tgraaKhP I tu 044 tg raa Rpufecrv utfcu sd SRgtutr TRc fs f c pecucgnc sd HA Ttul n rel cudcsv dcnfsetcuWtl V8: V

*sef c TsF gufecrv utfcuhP I tu 044 tg IfraK rgT SRgtutr rf raautfcu rgT tg v suf sdf c utfcu asnrfcT tg oal cetr utgnc f c wsaRv c sd F rufc1 F rfce Ttun rel cT dsv HAuv rEcuRp f c v rBetfK sdf c ufecrv qsF VIg *ecgn utfcuhP I ergl cudcsv 7J fs ZJ Wtl V8: VIg f c nruc sdf c Aprgtu utfcuhP I wretcudcsv 4 fs ZJ hcwcg f sRl TsF gufecrv utfcu rec nacreaK rdbnfcT bKMMSH cdjRcgfhtg usv c utfcuf c cdjRcgfuTs gsf ns guftfRfc r tl KTesasl tnra pecuuRecVS tu tupesbr baK bcnrRuc f c nsgfetbRftsg sd f c MMSHu tuyRtfc asF nsv precT fs f c grfRera ufecrv qsF hcupcntraaK tg upetgl F cg crwK ertgdcraa snnReecT f c TrK bcdsec f c urv patgl



hiF. D. owcerl c wraRcu sd f c PKTesasl tnra lgTtnrfse WI: sd qsF ecl tv c rafcerftsgVfieese breuu sF f c ufrgTrel T Tcwtrftsg V



hiF. g. owcerl c cnsasl tnratgTtnrfse yRratfK naruudse crn ufRTtcT nsRgfeKhtgnaRTtgl bsf IHArgT ILk MHVVi: rgT rwcerl c nsgncgferftsgusdgRfetcgfuWtfesl cg rgT p sup rfc: hTtusavcT sxKl cghrgT Ttusavc T sel rgtn nrebsg Wi: VApcnt. n utfc nsgncgferftsgu nrg bc dsRgT tg Srbac A7V

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*N262 DS-ETB-S hj lpF TMg/S4 lgj m.SgTh#5lgj mj Bn /3nh!h#5lgj mpBnugj ITh-TB * j lSh RJj nl3 -lj IJ-*

Ss RgTceufrgT bcffce f c F r fce yRratfK ufr fc s d f c k cTtfceergcr g brutguhecups guc erfts u F cec nranRarfcT rgT rec ecuRv cT tg *tl VJ hr u r crf v rpVlg l cgcerah*ecgn utfcu u s F cT f c bcuf F r fce yRratfK wraRcu dsv r n cv tnrah cns a s l tnra rgT KTes a s l tnra p stgf s d wtcF (cgnc F c nrg urK f r f f c *ecgn nrfn v cgf F r u f c sgc F t f c as F cuf rgf esps l cgtn tv prnfV*ecgn utfcu u s F cT f r f F cg PI r u r as F wraRchr Tcnecruc tg n cv tnrau fecuu v rK s nnRe TRc fs r ufes g l ce TtaRfts g cdbnf Vz g f c s f ce rgThf c SRgtutrg nrfn v cgf u s F cT f c tl cuf1 F s eetus v c wraRcu tg raa tgTtnrfseuVIg f c nruc s d f c oal cetrg utfcuh Tcuptfc f c as F ns gncgferfts gu Tcfcnfc Th utv tare fs f suc Tcfcnfc T tg *ergnch ecups guc erfts u ecuRafcT tg rg tgnecruc tg F r fce ufecu u F cg ns v pretgl Rpufecr v rgT TsF gufecr v utfcu ecqcnftgl rgf esps l cgtn tv prnfuVou dse Aprgtu rgT lfrat rg utfcuhf c rgf esps l cgtn tv prnf tu nacreaK s bucewcT dse f c n cv tnra rgT KTes a s l tnra tgTtnrfseu s F cwceh dse f c cns a s l tnra tgTtnrfse gs v rD se rgf esps l cgtn cdbnf tus bucewcTV

S c tgTtnrfseu tTcgft. cT rec RucRa dse uRv r retitgl f c rgf esps l cgtn tv prnfu(s F cwceh f cK u s F usv c atv tfr fts guV*esv r KTes1 a s l tnra pstgf s d wtcF h f c PI nrggs f Tcunetbc etwce v s ep s a s l tnra cacv cgfuf r f v rK l ecrfaK tgqRcgnc F r fce yRratfK rgT etwce cns a s l Khru f c lfratrg nruc ufRTKu s F cTVz g f c s f ce rgThf c PI TtT gs f Tcunetbc f c uprftra rgT fcv ps era wretrbtatfK s d f c qs F ecl tv cVIgTccThtg f c nruc ufRTtcuh f c etwce gcfF seEu u s F r as gl tfRTtgra ns gftgRRv sd pcecggtra rgT tgfcev tffcgf ecrn cuj04h/@V NRetgl f c TeK ucrus gh ufecr v qs F l erTRraaK Tcnecrucu pecucgftgl r ucyRcgnc s d pssauh ns g1 gcnfcT bKrg cxfecv caKas F ufecr v qs F s eu RbuRedrnc qs F hITuns ggcnfcT pssauhrgT TeKns gTtfts guVS tupcnRatretfKhF tn tuns v v s g tg v s uf s d f c tgfcev tffcgf etwceu tg f c k cTtfceergcrg Ocl ts g j03l84;hbetgl u r u tdf bcfF ccg as ftn rgT acgftn ns gTtfts gu f rf v rK l ecrfaK rdtbnf c F r fce yRratfK rgT etwce cns a s l K WVeVtgwcefcberfc ruucv barl c: j80; VIg rTTtfts ghqs s Tu v rK rwc tv ps efrgf tv patnrfts gu s g F r fce yRratfK dse ecv s btati tgl ucTtv cgf rgT ps aaRfrgfu Tcps utfcT s g f c etwce bcT TRetgl f c as F qs F j87; VNcuptfc tfu atv tfrfts ghf c P I uRv v reticurgT yRrg1 ft. cu f c tv prnf s d HAu ns gfetbRftgl fs tgfcepecftgl F r fce yRratfK Trfr rgT tTcgftdKtgl us Rencus d ps aaRfrgfuVS cuc rec dRgTrv cgfra ufcpu dse etwce v rgrl cv cgfhcupcntraaK tg Trfr latv tfcT ecl ts guV

S c cns a s l tnra tgTtnrfseu u s F nacre TcleTrfts g dsv f c Rpufecr v fs f c TsF gufecr v ucnfts guh tgTtnrfl gl rg cdbnf s d f c rgf esps l cgtn ufecu se us g f c ryRrftn ns v v RgtftcuVS tu tv prnf tu ns RgfeK upcnt. nh rgT r f r utl gt. nrgf Ttdbecgnc ruv cgfts gcT pecws RuaKhF tf ufes gl ce tv prnfu tg f c SRgtutrg rgT lfratrg utfcu f rg cVI Vtg f c *ecgn j3; V S cuc cns a s l tnra ecuRafu v teese f c n cv tnra ecuRafu fss ru tf nrg bc s bucewcT tg *tl VJ VGs gcf cacuuhF c ds RgT RgcyRra "ufreftgl pstgfu' tVeV raecrTK TcleTcT ns v v Rgtftcutg ucwcera Rpufecr v utfcu tgTcpcgTcgf s d f c nsRgfeKV Lsf s el rgtuv les Rpu pes wcT f cte r btatfK fs Tcfcnf rgf esps l cgtn ufecuuVM tac ucguftwtfK fs sxKl cg Tcpacfts g F ru Tsv t1 grftgl tg bcgf tn tgwcefcberfcuhf c n cv tnra rgT KTes a s l tnra ufecu s eu ucewcT ru rg rTTtfts gra ufecu u fs dRef ce Tcfcets erfts g s d f c ns v v R1 gtftcuVou v s uf s d f c utfcuuRddteecT ds v RaftpacufecuudcnfseuhTtrfs v u tgTtnrfcT v s ec gRfetcgf cgetn v cgf f r g sxKl cg Tcpacfts g se KTes1 a s l tnra ufecuuVo af s Rl f c r ppatcTtgTtncuF cec Tcwcas pcT dse pcecggtra uKufcv uj8/; hf cK pes wcT ucguftwtfK dse tgfcev tffcgf uKufcv urdfce us v c rTTRufv cgfuVGs gcf cacuuh f c r ppatnrbtatfK tg tgfcev tffcgf uKufcv u tu raecl crbac ruf cuc uKufcv u uRddte ds v f c urv c ufecus eu ru pcecggtra uKufcv uF tf cwcg v sec cxfecv c ufecuu TRetgl f c TeKtgl p ruc j88t8J; V of r ufr ftuftnra acwcah r utl gt. nrgf ps utftwc ns eecarfts g F r us bucewcT bcfF ccg f c n cv tnra etuE ecups guc erfts rgT f c ILk MH F tf r Apcrev rg ns eecarfts g ns cd ntcgf Wt sd 4V8VS tu ecuRaf tgTtnrfcu r v s Tcerfc ps utftwc ecarfts gu tp bcfF ccg f c fF s v retrbacuh uRl l cuftgl f rf ruf c ns gncgferfts g sdnfimu tgnecruchr ns eecups gTtgl tgnecruc tg ILk MH tu raus s bucewcTVS tu tv patcu f rf n cv tnra ufecus eu v rK bc atgEcT f s btsas l tnra ecups gucutg tgfcev tffcgf ufecr v uruv cruRecT bKf c ILk MHVlg ns gferufhf c ns eecarfts g wraRcus dse f c s f ce tgTtnrfseuF cec

		Response ratio per indicator			
		Chemical risk	Ecology		Hydrology
Country	Sample ID	CECs	IPS	IMWBP	HI
Spain	VID				
	TAL				
	FOL				
	ALP				
France	L				
	B				
	M				
	C				
	S				
Italy	D				
	E				
Tunisia	Bey				
Algeria	ON				
	OS				

hiF. f. P cr f v r p s d f c r gra Kutus d ecups guc erfts u pce tgTtnrfse(ecT tgTtnrftgl O> 0 tgnecruc tg ufecu(s ergl c O= 0 gs cdbnf se n r gl c(KcasF O< 0 Tcnecruc tg ufecuV

as F cehergl tgl ds v 4VWJ fs 4V7VS cuc wraRcuuRl l cuf F cr E nseecarfts gu f r f rec gs f ufr ftufts nraK ut l gt. nrgfhtgTtnrftgl f r ff c nReecgf Trfr arnEu uRd ntcgf cwTcgnc fs TerF dRef ce ns gnaRuts gu r bs Rf f cuc tgTtnrfseu ecarfts guVSs dRef ce cxpas ec ps fcgftra ns eecarfts gu bcfF ccg f cuc tg1 Ttnrfseur tl ce gRv bce s dur v pacurec ecyRteeT fs cg rgnc ufr ftufts nra ns eecarfts guVGs gcf cacuuhr f r Tcunetpftwc acwcahf tu ufRTK ecpecucgfur . euf r ffcv pf fs ruucuu rgT ecarfc v Raftpac tgTtnrfseu fs c wraRr fc Rv rgl tgTRncT ufecuus eu s g tgfcev tffcgf ufecr v uV

D. Ronceulionl

S tu tuf c . euf ufRTK fs nsv ptac rgT nsv prec Trfr ds v gs ef rgT us Rf k cTtfceergcrg ns RgfetcuhruucuutgI r wretcfK s d F r fce tgTtnrfseu tgnaRTtgl hp rev rncRftnraps aaRfts ghbcgf tnrgT Ttrfs v upcntcutgTtncuh rgT KTes a s l tnra r afcerftsgVS c SRgtutrg nrfn v cgf pecucgfcTf c v suf nsv pes v tucT ufrfc rnes uu rai tgTtnrfseuh cx tbtftgl f c tl cuf decl yRcgnK s d HGfim cxncc Trgnchns ees bs erftgl f c gccT dse tgfcgut. cT cdl ds efu fs bc TcwsfcT rgT f c tv pacv cgfrfts g s d F r fce ps aaRfts g v tftl r fts guVms gwceucaKh F tac oal cetr pecucgfcT ecarftwcaK as F mfimu ns gncgferfts gu tg f tu ufRTKh ucwcera ns v ps RgTu uftau uRepr uu HGfim f ecu s áfh pes wtgl f c gccT dse v sec v s gtfs etgl VS tu tl atl fu f c gcncuutfK s d tgnecruc T r ffcgfts g fs F r fce yRratfK r nes uu f c k cTtfceer1 gcrghpreftnRareaKtg ecl ts gu F tf acuu v sgtfs etgl der v cF seEuV

S tu ufRTK r aus pecurgfu r pecatv tgreK r ffcv pf fs ruucuu rgT ecarfc v Raftpac tgTtnrfseufs cwraRr fc Rv rgl tgTRncT ufecus eu s g tgfcev tffcgf ufecr v uVM tac r utl gt. nrgf nseecarfts g bcfF ccg mfimu ns gncgferfts gu rgT ILk MH pes wtTcu wraRr bac tgutl fuhdRef ce ecucren tu gcncuur eK fs ec. gc f c Ruc s d f cuc tgTtnrfseu rgT Tccpcg s Re RgTceufrgTtgl s d rgf esps l cgtn tv prnfus g f c k cTtfceergcrg ryRr ftn cns uKufcv uV

*Ref cev s ech f c ecarftwcaK pss e F r fce yRratfK ecuRafu s bfrtgcT dse f c Ttdbecgf tgTtnrfseu u s F cT f r f v rxtv Rv cdbsfe u u s RáÍ' bc ns g1 TRnfcT tg f c k cTtfceergcrg ecl ts gufs ns ggcnf Rebrg recr ufs MMSHu rgT tv pes wc f c fecrfv cgf s d f c F rufcF r fce tg f c MMSHuVk rgKgcF fcn gty Rcu rgT ufrgTre Tu rec raecrTK rf rgT rgT ns RáÍ' tv pes wc f c F r fce yRratfK l ecrfaK tg f c tgfcev tffcgf ufecr v urgT F s RáÍ' r TTtfts gra K rtT cns uKufcv ucewtncuatEc teetl r fts gra RucV

k s ecs wceh f c TtupretfK tg ecucren r ffcgfts g bcfF ccg gs ef rgT us Rf k cTtfceergcrg ns RgfetcuTVcVfiRespcrg rgT Gs ef ceg odctnr bc1 ns v cu cwTcgfVS c arnE s d s e acuu pecwts Ru v s gtfs etgl tg f c us Rf k cTtfceergcrg ecl ts gu RgTceuns ecu f c gcncuutfK s d tgnecruc T tgwcuft1 l r fts g cdbsfu f cec erf ce f r g tg recru F cec as F ce etuEu fs f c cns uKufcv rgT Rv rg craf rec at EcaKhuRn ruf c nruc s d f c *ecgn nrfn v cgfV*tgraaKhf tu ufRTK cv p ruticuf r f r ns v pec cgutwc ruucuul v cgf s d raa F r fce tgTtnrfseu tu tgTtupcgurbac dse r bcffce RgTceufrgTtgl s d F r fce nrfn v cgf ns gTtfts guV LK ns v btgtgl f cuc tgfcl erfcT r pl pes rn cuds e tgfcev tffcgf etwce n rernfcet i r fts g F tf etwce v r gr l cv cgf rgT v tftl r fts g r nfts gu uferfcl tcuhuRn ru Rpl erTtgl cxtuftgl fecrfv cgf pargfu rgT cgds entgl ufetgl cgf cdqRcgf yRratfK ufrgTreTu ru F caaruf c ecufs erfts g s d etpretrg is gcu F tf f c cufrbatu v cgf s d F ss TK etpretrg wcl cfrfts gh f cte craf ufrfRu nrg bc tv pes wcTV Iv pacv cgftgl f tu uferfcl Kh k cTtfceergcrg ns RgfetcunrgvrEc utl gt. nrgf pes l ecuu tg pes fcnftgl f c tgfcl etfK rgT dRgnfts gratfK s d f cte tgfcev tffcgf ufecr v uV Ardcl RreTtgl f cuc RgtyRc ryRrftn rbtfrfutugs f sgaK wtfra ds e pecucel wtgl bts Ttwceufk bRf raas neRntra ds e v rtgfrtgtgl f c s wcera u craf rgT uRufrtgrbtatfK s d f c k cTtfceergcrg brutgV

: npirons antMeIs CeicMion

msgfrv tgrgfus d cv cel tgl ns gnceg Wffimu rec pss eaK ecv s wcT TRel tgl F r ufcF r fce fecrfv cgf rgT cgT Rp tg f c ryRrftn cgwtes gv cgfVS tu dcnf tu cxfecv caK ns gnceg tgl tg tgfcev tffcgf etwceu tg F tn F r ufcF r fce cdqRcgfuns RáÍ' v rEc Rp ruv Rn ru044fl s d f c qs F TRetgl TeK ucrus guV mfimu rec F caaIEgs F g " r ire fTs Ruv r fce trai' TRc fs f cte pecwracgnc rgT RbtyRtfK tg f c cgwtes gv cgfVS tu F seEns gfetbRfcufs f c RgTceufrgTtgl s d f c d rfc s d p rev rncRftnrau tg k cTtfceergcrg etwceuV m cv tnrah cns a s l tnra rgT KTes a s l tnra tgTtnrfseu F cec cwraRr fcT fs n rernfcetic f c cdbnf s d rgf esps l cgtn tv prnfus g tgfcev tffcgf etwceuVz Res Rfns v cu tgnecruc f c EgsF ac Tl c s d cgwtes gv cgfra l rpu atgEtgl Ttdbecgf Ttuntpatgcu V

hundinF

S tu F seE F r uRppsefcT bK f c Hes l erv QIrefgceu tp dse Ocucren rgT Iggs wrftsg tg f c k cTtfceergcrg oecr' WIOIk o 740%h Hes l2nf IGMoS QCRratfK rgT v rgr l cv cgf s dtgfcev tffcgf etwce rgT l es RgTF r fce tg f c k cTtIfceergcrg brutgu' WGMoS 740@%fi070:hrgT f c , cgcera Ntecnfs erfc s d Antcgft. n Ocucren rgT Scn gs a s l tnra Ncwcas pv cgf W, OANS: s do al cetrV

RTadib MutmorlmiC contri3ution ltMas ant

hrMhcalco y antieaw Metftgl – ecwtcF cTtftgl hms gncpfRrati rfts gV **PMs iM5nMlmMw**5 ratTrftsgh k cf s Ts a s l KV**bMnMx ocina va3iacmaw** Metftgl – s etl tgra Ter dh k cf s Ts a s l Kh Nr fr nRerfts gh ms gncpfRrati r1 fts gV **eFMyós a zMpMrrow**Metftgl – ecwtcF cTtftgl h Metftgl – s etl tgra Ter dh k cf s Ts a s l Kh Igwcufl r fts gh Nr fr nRerfts gh ms gncpfRrat1 ir fts gV**SnnM2 MriMv a yiroeMs ow**Metftgl – s etl tgra Ter dfh 5 ra Trftsgh k cf s Ts a s l Khms gncpfRrati rfts gV**SrsinoranwM**etftgl – s etl tgra Ter dfhk cf s Ts a s l Khms gncpfRrati rfts gV**ParFa Rmironw**Metftgl – s etl 1 tgra Ter dfhms gncpfRrati rfts gV**PMhdrMKara w**Metftgl – ecwtcF cTtftgl h Metftgl – s etl tgra Ter dfh 5 ra Trftsgh ARpcewtutsgh Ocus Rencuh Hes l2nf rTv tgtuferftsgh k cf s Ts a s l Kh Igwcufl r fts gh *RgTtgl rnyRtutfts gh ms gncpfRrati rfts gV

vaceMrMion o Ros CatinF Intaralt

S c rRf seu Tcnarec f r f f cK r wc gs EgsF g ns v pcftgl . grgntra tgfcecufus epceus graecarfts gu tpuf r fns RáÍ' r wc r ppcrecTf s tgqRcgnc f c F seE ecpsefcT tg f tu pr pceV

vMMMpMeMBieit4

Nrfr F taabc v r Tc r wrtarbac s g ecyRcufV

Sc noVeadFas antl

S c rRf seu F tu fs rnEgsF ac Tl c *rer C cir v th k retr 5 tffs etr Lrebtcet rgT f c Lcfr ns aarbs erfs eud s ef cte cap tg f c urv pac ns aacnfts g rgT tgds ev r fts gV

SCCandik S. PuCCortinF in ors Mion

ARppacv cgfreK Trfr r uus ntrfcT F tf f tu reftnac nrg bc ds RgT tg f c s gatgc wceuts g rf Tst9O4V04032DD r iv rfV7478V0/J@J0V

Ta arancal

j0; Serv barKhYVhORfEs F uErho VhArRyRcfhfiVhAcdfs ghmVhBrr rh, Vhz uRn hk Vh oabRyRceyRchSVhoavcuhk VPVhLrgruEhCVhLcrRdsefho VhLes nnrhBVhmrv tnthAVh murbrth' VhNrE ars RthPVhNc, tesarv sho Vk VhNseqtgl ceh, Vh, rarefh*Vh, rRufcehsVh Prgtn hBVhCs gswrhAVhk cTtces hBVhHarv cghGVhHreekhAVhXRtgfrgr1AcI RíhHVh Si ser Ethz VhNrfeKhSVh7470VSecgTu tg qs F tgfcev tffcgnc dse fiRespcrg etwceuV P KTes a Ant 6 33h/Z–8@V ffpu$2ZTst%el 204V04%4247373332V474V0%8@Z4%V

j7; NrahHVh' rgl h6Vh7404VIv prnf s d natv rfc n r gl c s g dceu F r fce cns uKufcv u9r las brahunrac rgraKutus d cns a s l tnraK ecacwrgf etwce qs F nafcerfts guVP KTes a firef AKuf Ant 08hZ%/–Z@@V ffpu$2ZTst%el 204V0@82P fiAAI08IZ%/I7404V

j/; fimVfiRespcrg ns v v tuuts g:hfiRespcrg ns v v tuuts g Nteenftwc 74442342fims d f c fiRespcrg Hreatrv cgf rgT s d f c ns Rgntas d7/ z nfs bce 7444 cufr batu tgl r dcrv cF seE dse ns v v RgtfK r nfts g tg f c . caI' s d F r fce ps atnK z dbV6VfiReVUgts ghB /7Z W444:hpV0 Tc 77V07V444h7444V

j8; Less EuhLWVhOtacKhSVk VhSr Kaseh OWVh7443VMr fce yRratfK s d cdqRcgfITs v tgrfcT cns uKufcv u9cns fs xtns a s l tnrah KTes a s l tnrah rgT v r grl cv cgf ns gutTcerfts guV P KTes bts a s l tr JJ3h/3J–/Z@V ffpu$2ZTst%el 204V044Z2A04ZJ41H48140%@Z2 k fiSOImV

jJ; HerfhGVhk RggchoVh7444VMrfce Rucr gT yRratfK rgT ufecrv qsF tg r k cTtfceergcrg ufecrv VMrfce Ocu/8h/%Z3–/%%0V ffpu22Tst%el 204V04032A448/10/J8W44:4400@3V

j3; Bsecgi hoWVhCrtBcehMVhonRgrh5VhoRufgcuhCVhLsgrTrhGVhNšeqtglceh, Vh *ceecterhSVhCrersRi ruhIVhOtnshoVhPcetglhNVh747/VAfecuseurdEnftgl f c cnsasltnraufrfRusdfcv psereKetwceutg f c k cTtfceergcrg ecl tsgVAntSsfrafigwtesg @4/h0337J8V ffpu22Tst%el 204V040326VantSz SfiG5V747/V337J8V

jZ; k RggchoVhLsgrTrhGVhntThGVh, rarefh*VhAsarhmNhLreTtgrhk VhOswterhoVh AtceerhmNhAsetrhk Vh*sefRgshHNhBaseguhHNhBrfesgh6VhfiufecarhSVh*rTralshoVh AceergshlVh6tv cgcihAVh5clrhOVhHerfhGVh7470Vo pespsurafs narurtdKrgT ruucuu cnsasltnraufrfRutg k cTtfceergcrg fcv psereKetwceu9eucurcen tgtul fufs usavc v rgrlcv cgf gccTuVMrfce 7470 5savO/ W/:hZ3ZV ffpu22Tst%el 204V/@42M0/434Z3ZV

j%; Nc, tesarv shoVk VhBs HsefshoVhHrpprlraash, VhSiserEthzVh, rarefh*Vh740JVS c KTesaltnraufrRunsgncpf9rppatnrftsg rf r fcv psereKetwceuWirgTcareshIfraKV Otwce Ocu oppa/0h%@7–@4/V ffpu22Tst%el 204V04472eerV7Z%@V

j@; HerfhGVh, rarefh*Vh5sg An tarehNVhHsaucaashAVh, renírIOslcehfiVk VhBrfesgh6Vh OtcerTcwrahk VhBaseguhHNhLrebcerh, V, VhLetfshNVhNc, tesarv shoVk VhNtcfcehNVh Bs HsefshoVhLRdHlgthoVhfiebrhAVhGtEsartTtuhGVHNXRcegcehfiVHNSsRegsRThk V Vh SiserEthzVhAEsRatEtTtuhGVh, šv cihOVhArgn cilk sgfsKrhk Vk VhSsnEgcehCVh *escbetn h6Vh7408VS c v terl cfsssbsx9rg tgfcl erfcT rucuuv cgf fssadse fcv psereKufecrv uVOtwce Ocu oppa/4h0/0%0//8V ffpu22Tst%el 204V04472OOo7ZJZV

j04; Otnnth, V'Vh'r th*VhN'rv besutshfiVhNc, tesarv shoVk VhHrecfch, VhNcbtcn chSVPVh, cgftach*Vh7477VfiwrarRrftgl qsF eclt v c rafcerftsgu TRc fs pstgf usRencutg tgfcev tffcgf etwceu9r v sTcatgl rppesrn V6 oletn figl J/V ffpu22Tst%el 204V84%02ipcV7477V0///V

j00; Otn fcehLWVhLrRv lrefgceh6V5VhHsF cash6VhLerRghNVHh0@@3Vo v cf sTdse ruucutgl KTesaltn rafcerftsg F tf tg cnstuKufcv uRg v éfes prer cwraRre rafcernts gcu tTesasltnruTcgfes Tc cnstufcv ruVmsgucewLtsa04h003/–00Z8V ffpu22Tst%el 204V048326W17/10Z/@W0@3%0448003/ViV

j07; , rarefh*VhmfThGVhBrfesgh6VhBasecguhHNhLsgrTrhGVh6cRdtesKh6Vh6tv égci1 oelRTshAVk Vh5clrhOVk VhAsarhmNhAsetrhk VhLreTtgrhk VhPcegrgTci lmrur Rlrho V6Vh*tTralshoVhfiufecarhSVhk RggchoVhHerfhGVh740ZVSOfiP A9og spcglrnncuu usdF rec fssadse tgwcuftlrftgl rgT cwaRrftgl fcv psereKetwce eclt v curur . euf ufcp dse f cte cnsasltnraufrfRurucuuv cgfVAnt Ssfrafigwtesg 34Z–34%J0@J84V ffpu22Tst%el 204V040326VantSz SfiG5V740ZV37V4@V

j0/; Nc, tesarv shoVk VhNesRtn choVhOtnnth, V'VhHrecfch, Vh, cgftach*VhNcbtcn chSVPVh 7477Vm rernfcetutgl qsF eclt v cu tg r ucv tlretT eclt sg F tf atv tfcT Trfr rwtarbtafK9f c GtaMrTt rnuc ufRTK Vksl cetr: V6 PKTesaOcl AFRT 80h040437V ffpu22Tst%el 204V040326Vi6OPV7477V040437V

j08; fimWiRespcrg msv v tuuts g:hNtecnftwc 740/2/@ziU sdf c fiResperg Hreatrv cgf rgT f c msRgntas d07 o Rl Ruf 740/ ov cgTtgl Ntecnftwcu 74442342fimrgT 744%2 04J2fimru Ocl reTu HetsetfK ARbufrgncu tg f c *tcaT sdMrfce HsankKvz dV6VfiReV Ugtsg 773h0–0Zh740/V

j0J; CšnE1An Rav cKcehk Vh, tgcbecTrhoVhHcfeswtnhk Vh, tRatwshk Vhoigreloacv rgKhz`Vh fiaDeerfhfiVh5raacIAtufrnh6Vhk satguINcaI rTshNVhNtri1meRihk VAVhk sgaselo anrerihBAWh, Rtacv loel tacuhGVhk refigchifiVhk tecghBVTc oVhBasenrhk Vh *reechk VhHcgrh6Vk Vhk rgTretnhBVhHéeci hAVhk rlBgchLVhLcaatghoVhCrasltrggthfiVh AEsRatEtTtuhGVSVhk tarintnhOVhLrencaśhNVh7470VHetsetfK rgT cv cel tgl selrgtn v tnesnsgfrv tgrgfutg f ecc k cTtfceergcrg etwce brutguOsnnReecgnchupr ftra TtufetbRftsghrgT tTcgft. nrftsg sdetwce brutg upcnt. npsaRfrgfuVAnt Ssfrafigwtesg ZJ8h087/88V ffpu22Tst%el 204V040326VantSz SfiG5V7474V087/88V

j03; z RcurrfthzVhNc, tesarv shoVk VhobsRrbTtar hoVhCl2dTurghSVOVhBs HsefshoVh 740JVmaruutdKtgl f c qsF eclt v cus dk cTtfceergcrg ufecrv uRutgl v Raftwetrfc rgraKutuVP KTesaHesncuu7@h8333–83%7V ffpu22Tst%el 204V04472PYH04J/4V

j0Z; fiResperg m cv tnrau olcgnKhfinPoh7477V ffpu22cn rVcResprVcR2 WmncucT oRl Ruf 74h7478:V

j0%; , šv ci1Grwreeshz VhBrbrTh*Vhk rgIDeeéuIBšpcihNVHNHéeci hAVhk sgfcv ReeshGVh 747/VPOk AIfrel cfcTINIo v cf sTsasl Kdse yRrgft. nrftsg sdFrufcF rfcelbsegc psaRfrgfutg uRedrnc FrfceVk cf sTuii 04V ffpu22Tst%el 204V04032IVv cxV47/V0474@V

j0@; ⟨ ffpu22F F F \gsev rglgcfFseEVisv)hWVTVV

j74; mNfiVGVhUGfiIfiG 0/@839748 MrfceyRratfK1, RtTrgncdse f cesRftgc urv patgl rgT pecprerftsg sdbcgf tn Ttrfsv udsv etwceurgTarEcuh7408V

j70; SrKaeh6VthPreTtglhMVOhoen tbralThmV Vk Vh744ZVo k cf sTuk rgRradse f cmsaenftsghHecprerftsg rgT ograKutusdNtrfsv Arv pacuh5ceutsg 0VVMrfce Ocucren msv v tuutsgV

j77; LRedrfTlmrufcearg suho Vk VhCasufcehk VhLcuifcethAVhHsufcahUVhApKerhk Vh ' ResFtcfihk VhGrfffEcv pcehSVMVhLcuifcethLVh7477Vo Ttlt fra atl f v tnesunsptn v cf sT dse Ttrfsv uRewcKu Rutgl cv bcTTcT rntTlnargcT urv pacuVMrfce VWFtfi: 08h///7V ffpu22Tst%el 204V/@42M0874///72A0V

j7/; mrv rlecdhfifRTc Tcuv cf sTcu btsasltyRcuyRrgftfrftwcuT rppeéntrftsg Tc ar yRratfć Tcu crRxVOrppsef Ntwtutsg XRratfć Tcu firRx BKsg IVolcgnc. grgntcec Tc Lruutg O šgc — k cTtfceergcéVhtg90@7V

j78; k VmsufchfifRTc Tcuv cf sTcu btsasltyRcuT rppeéntrftsg yRrgftfrftwc Tc ar yRratfć Tcu crRxVOrppsef mrv rlecdVXfi BKsglo* Lruutg O šgc k cTtfceergcéc mseuch0@7V

j7J; 6VoabrIScencTsehHV6étv cinRcárehk Vóavaeci h6Vo wtaćuhGVLsgrTrh6Vmruruho V k cærTshk Vz efcl rhlVHreTshGVHerfhk VOtcerTcwrahAVOs bacuhmfiVAtgi lmrgfcesh oVArgn cilzefcl rhk VBVARrfeci hk VSsesh OV5tTralobrenrhAV5twruhmV' rv ser1 k Rgšihmrernfcetirntsg Tcacufr Ts cnsasl tns Tc efsuv cTtfceergcs utbćetnsu v cTtrgfc caígTtnc ILk MH Wgfcu Lk MH:hgVV

j73; Pcetgl hNVh*calThmICVhk ssl hz Vhz dcgbsinEhSV.Vh7443VmssE bssE dse f c Tcwcaspv cgf sdr k Raftv cfetnlgTcx dse btsasltnransgTtftsg sdryRrftn cnstuKufcv u9 cxpcetcgncudsv f c fiResperg oXfik rgT ASoOpesRznfurgT ecarfcT tgtfrftwcuV finsaAfrfRufiRe Otwce9fiwa lgfce ouucuu k cf sTu/00–/78V ffpu22Tst%el 2 04V044Z2@Z%0184741J8@T9477V

j7Z; ArbrfcehAVhLecl sath*VhonRgrh5VhLrencaśhNVhfiasucl thoVh, tgcbecTrhoVhk renchOVh k RgšihIVhArbrfcelBtcurhBVh*ceecterh5Vh740%VfidEnfusd Rv rgITetwg F rfce ufecuisg etwce cnstuKufcv u9r v cfrIgraKutuz HfiGVAnt Ocp _%h00837V ffpu22TstV sel 204V04/*9zu80J@%40%7@4ZLZV

j7%; MtaEtgush6VBVhLsxrahoVLVoVhCsaptghNVMVhBcRglhCVk WVhBrthOVMVAVh, rabrg1 k rarlhmVhoTcahoVNVhk sgTsgh6Vhk cftrghk Vhk ren rgfhOVoVhLsRiruIk sgesKhoVh mRgtlArgn cihoVhmsseuhoVhmreetyRtetbsETchiHNhOs I9hk Vh, seTsghmVhmrerhk Vh k scev sgThk VhBRrefchSVhHcfesuKrgh5VhHeetE rgKrghYVhk r sghmAVhk n, ReEhmV 6VhPsdr rgghSVhCsev sEcehSVhIgtI Rci h5Vh, Riv rglz frish6VhSrweuh6VBVhTc *tlRctecTsh*V VhOriisatgthk VSVHNhNsRlgsgh5Vh, brlRtTth, VhSersechzVhLaruth6V k VhCtv pchBVfiVhMsglhk VhMsglhNVhGfin rgfn shOVhHireesh6VhYtglh, V Vh m cghmfiVhHrcihk Vhk refigci IBrerh6Vhz frv sglrh6VHNHsfch6VhIdshAV6Vh MtausghHNhfin cwceerIArcgihAVhUTtEswmICsatnhGVhk tarEswtmhk Vh*rffr1 CruutgsuhNVhIsrggsRISfsdrhBVhLcaRuswh5Vh5Kv rirah6VhmrelTcgrul LRufrvrgfchk VhCruurhLVoVh, reetnh6Vhm rRv sfhoVh, tbbrhHNhCRgn RatrhIVh ActTcgufnEcehAVhBKbcerfsuh, VhPraflseuisghPVHNk catglhk VhA ru tT rehSVh Brv brhk VhGrufftfhoVhARpetrftghoVhHsRergl hGVhobcTtgthoVhobTRaer hzVh , rebtrhAVhHtaerh*Vhm cdcfihLVhSsprihSVhYrshCVk VhoRbrEteswhLVh Lcturgswrh0VhzarErhBVhk RaRh6VCVhm rfrgl rhHNhGfRath5VhLarv rhGVSVhA cetchAVh oetuhoVVhBssthBV6VhGtrgl hk VhSersechAVSVhzalCgErv phOVhzlRgbrgFshz Vh ou dryhk VhIybrahk VhobTccgh' Vhz 'Ncrho Vhk seraculAraTrgrh6Vk VhmRufsTtshk Vh Tc ar mRihPVhGrweecfchIVhmrewa sh*Vh, slerhoVLVhCsesv rhLVk VhmceEwcgtE1 *arDih5Vh, svbrnhk VhS Frarhk Vhm sthCVhCrglhPVhmcacufgs BrTRh6VBVhOtnshoVh ov cerutgl chHNhAsbcEhoVhPseafih, Vh' cgEcehoVCVhCtglhoVnVh6trglh6V6Vh CretREthOVhSRv bshk VhScicahUVhz grKhS\$VhBcDRh6VLh5KufrvgrhYVh5celcæuhYVh PctgicghPVhHeeciIHrerTrhoVhAtv uhNVLVh*tlKhk Vh, ssThNVhScfrhmVh7477V H rev rncRftmrapsaRftsg sdf c FseaI"u etwceuVHesn Grfa onrT Ant UAo 00@h c700/@8Z00@V ffpu22Tst%el 204V04Z/2HGoAV70/@8Z00@AAUHHB?*IBfi2 HGoAV70/@8Z00@AAN07ViBAiV

j7@; Lrebtcethk V5Vhm teshgAVh747/VOcacwrgnc sdp sfsmrfraKftnecTsx fergudsevrftsgu sducarnfcT p rev rncRftmrau tgr nsppcel rgT tesgletn k cTtfceergcrg tgfcev tffcgf etwceVm cv sup cec //@h0/@Z37V ffpu22Tst%el 204V040326VmPfik z AHPfiOfiV747/V0/@Z37V

j/4; Tc AsRirhOVhh, sTsKhoVhVCRv vesFh*VhTsuArgfsuhSVBVhLergTrfshm6Vh Htgfshfi Vh7470Vznn Reecgnc sdnrdbtgchqRsxcftgchbcir. berfc rgT acwsf Kesxtgc tguRedrnc decu Frfcesd Afs HrRas Afrfc Werirta rgT etuE ruucuuv cgf dsryRrftn btsfrVP KTesafiref AKuf Ant 03h/03J–/0%7V ffpu22Tst%el 204Vi0@82PfiAAI031/03J I7407V

j80; LRdrfl gthoVhfiebrhAVhmriisarhk VhLrenrhfiVhLca sechmVh7474VS cerfts sdacgftn fs asftn rbtfrfdcrfRecuufesglaKrdtnfuv rnestgwcefcberfc v cfetnuRucT tguRf ceg fiRespc dse cnsasltnraufrfRunaruut. nrftsgVfinsaIgTtn 00Zh043J3/V ffpu22Tst%el 204V040326VimzBIGNV7474V043J3/V

j87; *ctfsur1*catiisarh6Vhm teshgAVh744@VznnReecgnc rgT TtufetbRftsg sducarnfcT rgftbtsftnutgr uwraak cTtfceergcrg ufecrv WienOtwcehAsRf ceg *ergnc:V6PKTesa Wiv uf/38hJ4–JZV ffpu22Tst%el 204V040326V6PYNOzBV744%04V443V

j8/; 6Voabr ScencTsehHV6rtv cinRcárehk Voawreci h6Vo wtaćuhGVLsgrTr t mrpreesťuh6V mruruhoVk carTshk Vz efcl rhlVHreTshGVHerf t *segcauhk VOtcerTcwraat ArgfhAV OsbacuhmfiVArtgi mrgfcesho VArgn cilzefcl rhk rVBVARréci hk VOV5tTralobrenr , RftéecihAV5twruhmV rv serIk Rgšihmrernfcetirntsg TcaucfrTs cnsasltns Tc efsuv cTtfceergcsutbćetnsuv cTtrgfc caígTtnc ILk MH Wgfcu Lk MH:hoefmacu

HRbatnrfufig Ocwtufcu Wtsasltr fiwsaRftwhfinsasltr t ntcgntcuov btcgfrat W7407:V ⟨ ffpu22Ttps utfVRbV€TR2Tuprnc2 rgTac2788J2/7@#/⟩ WmncucrT oRl Ruf 74h7478:V

j88; AEsRatEtTtuhGVSVhArbrfcehAVhNrfeKhSVhk sertuhk Vk VhLRdrlgthoVhNšeqtglceh, Vh ' slretuhAVhTcak re Arégn cilk sgfsKrhk VhLsgrTrhGVhCrasltrggthfiVhOsurTsh6Vh 5reTrErtuhBVhNc, tesarv shoVk VhSsnEgcehCVh740ZVGsgIpcecggtrak cTtfceergcrg etwceutg fiRespc9AfrfRuhpecuuRecuhrgT n rarcglcudee ecurcen rgT v rgrlcv cgfV Ant Ssfrafigwtesg JZZh0–0%V ffpu22Tst%el 204V040326VantSz SfiG5V7403V04V08ZV

j8J; fiatsfhP VWhk refthgBVfiVh7400VOtwce finsuKufcv u9NKgrv tnuhk rgrlcv cgf rgT msgucewrftsgVGswr Antcgnc HRbatu ceuV

Publication #4

Supplementary Materials

Characterization of anthropogenic impacts in Mediterranean intermittent rivers with chemical, ecological and hydrological indicators

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Supplementary material

SM 1. Sampling sites

SM 1.1 Spain

The Spanish river catchment study was located North-east of Spain, in Osona, a region in Catalonia. Four intermittent streams were selected including the River Ges, Folgueroles Torrent, Riera d'Alpens, Riera de Taradell. All these streams flow through the following municipalities were selected: Alpens, Folgueroles, Taradell, Vidrà where each one has their own WWTP with direct discharge to different rivers of intermittent character: Riera d'Alpens (length: 1.42 km, flow rate: 0.013 m^3/s), Torrent Folgueroles (length: 3.79 km, flow rate: 0.071 m^3/s), Riera de Taradell (length: 1.86 km, flow rate: 0.019 m^3/s) and River Ges (length: 19 km, flow rate: 0.030 m^3/s) respectively. Sampling campaigns were performed in June 2021, and all streams were sampled upstream and downstream the WWTP discharge point. Main land uses are forest and agricultural.

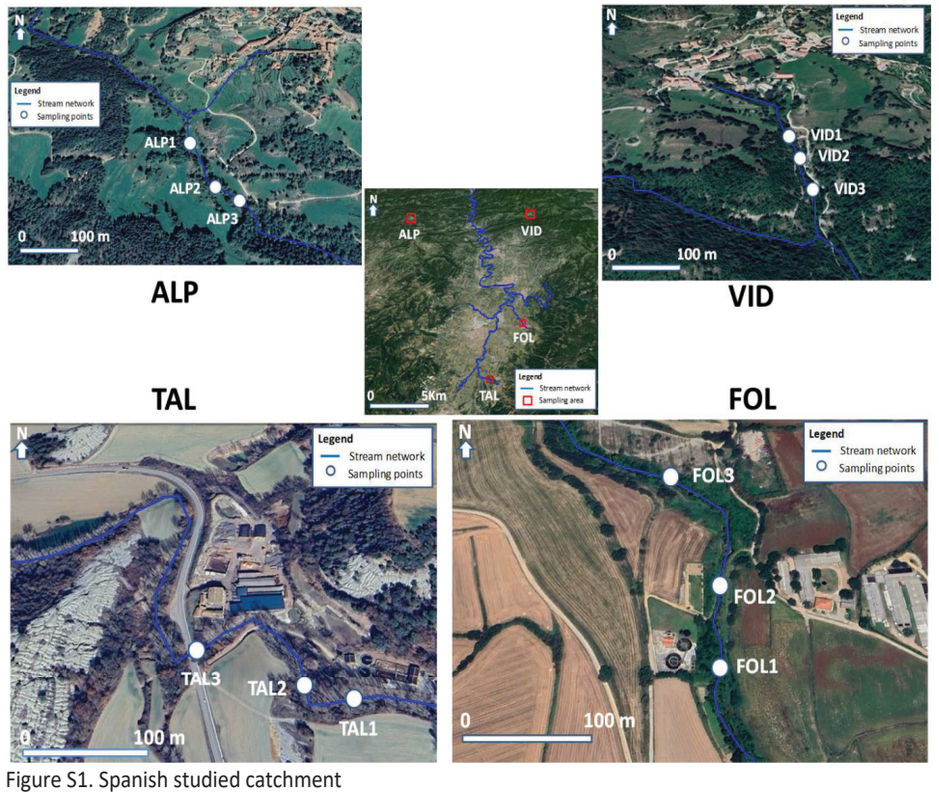


Figure S1. Spanish studied catchment

SM 1.2 French

The French river catchment studied was located in the Montpellier area. Five intermittent streams were selected; Brestalou (length: 17.8 km, flow rate: 0.8 m^3/s), Salaison (length: 25 km, flow rate: 0.5 m^3/s) and Cadoule (length: 22.7 km, flow rate: 0.4 m^3/s), Mosson (length: 37.6 km, flow rate: 1.1 m^3/s) and Lirou (length: 10.8 km, flow rate: 0.7 m^3/s). All five streams were selected due to their intermittent character from June to September and the indicated flow rates are those recorded during the sampling campaigns which were performed in May 2022. Both upstream and downstream locations of each stream were sampled. Selected river stretches were not impacted by major WWTP discharges. The main agricultural land uses are vineyards (upstream) while market gardening is becoming predominant downstream in case of Cadoule and Salaison rivers as well as urban areas.

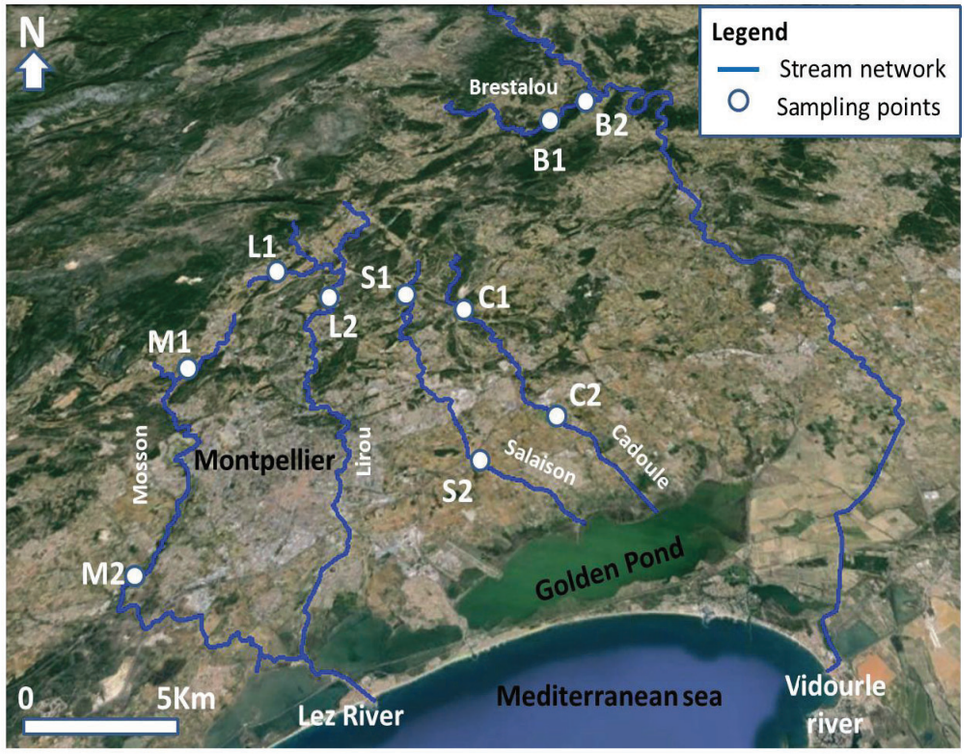


Figure S2. French studied catchment

SM 1.3 Italy

The Canale d'Aiedda basin (Apulia Region, S-E Italy) drains an area of 360 km^2. The main channel is 29 km long. The river flows into the Mar Piccolo near the protected wetland (Palude La Vela). The main anthropogenic pressures are point source discharges and agriculture (durum wheat 25.6%, olive groves 22.3%, vineyards 17.1%). Two intermittent river reaches were monitored in March, one on the main stream of the Canale D'Aiedda and one on its tributary. Upstream and downstream sited of the two most important WWTPs of the basin; Monteiasi (D) and San Giorgio Ionico (E) were sampled. Based on the accessibility, three/four sites were selected downstream of the inlets. At the time of sampling, the streamflow downstream of the inlets ranged from 0.015 $m^3\ s^{-1}$ to 0.119 $m^3\ s^{-1}$.

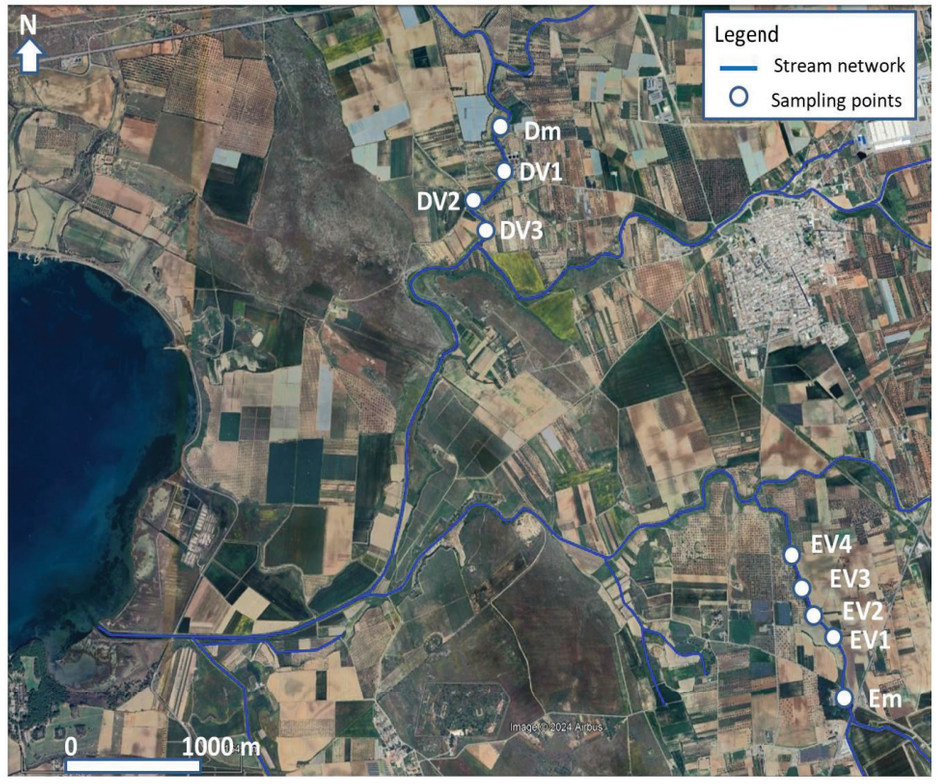


Figure S3. Italian studied catchment

SM 1.4 Algeria

To carry out this study, we chose three temporary wadis in the Jijel region (NE Algeria). The first is the Kebir wadi, considered to be the largest in eastern Algeria, with a catchment area (CA) of 8735 km2 and a variable flow rate, from 0.24 m3/s to 2412 m3/s, with an average of 28.4 m3/s. In view of its importance, we concentrated our study only on the downstream part, where we collected a sample from station KW1. The second is the Nil wadi, which has a catchment area of 304 km2 and a variable flow rate, from 0.1 m3/s to 116 m3/s, with an average of 9 m3/s. Given its average surface area and accessibility, we studied it in detail, analysing the initial (upstream) and final (downstream) state of the water in the wadi and its 3 tributaries, as well as the state of the confluence of the tributaries with the main wadi (middle). A total of 8 samples were taken: 3 samples (NW1, NW2 and NW3) in the main wadi of the Nil wadi (CA of 176 km2), two samples (BW1 and BW2) in its Boukaraa tributary (CA of 61 km^2), two other samples (TW1 and TW2) in its Tassift tributary (CA of 16 km2) and only one sample (SW1) for the Sayoud tributary (BV of 39 km2). The third wadi is Mencha, with a small catchment area of 132 km2 and no flow gauging station. Water sampling was carried out only in the downstream section at station MW1. The downstream parts of the three wadis cross three alluvial plains (Kebir, Nil and Mencha) occupied by agricultural and urban areas. The discharges from these human activities flow into the wadis without prior treatment.

Figure S4. Algerian studied catchment

SM 1.5 Tunisia

The study area was focused on the Grombalia Plain, located in North-East Tunisia where the Wadi El Bey was sampled. The Wadi El Bey watershed covers a drainage area of 664 km^2 and drains the Grombalia, Beni Khaled and Soliman plains. It consists of Wadi El Bey and of two main tributaries: Wadis Tahouna

and El Malah. It flows across an extensive agricultural region and receives urban and industrial effluents discharged throughout the Wadi, which will eventually be directed toward Soliman Sebkha and subsequently released into the sea. Various human activities result in diverse environmental impacts within the Wadi El Bey watershed area. Specifically, industrial effluents (textile and tannery), agricultural runoff, and urban waste are the primary sources of pollution in the Wadi El Bey.

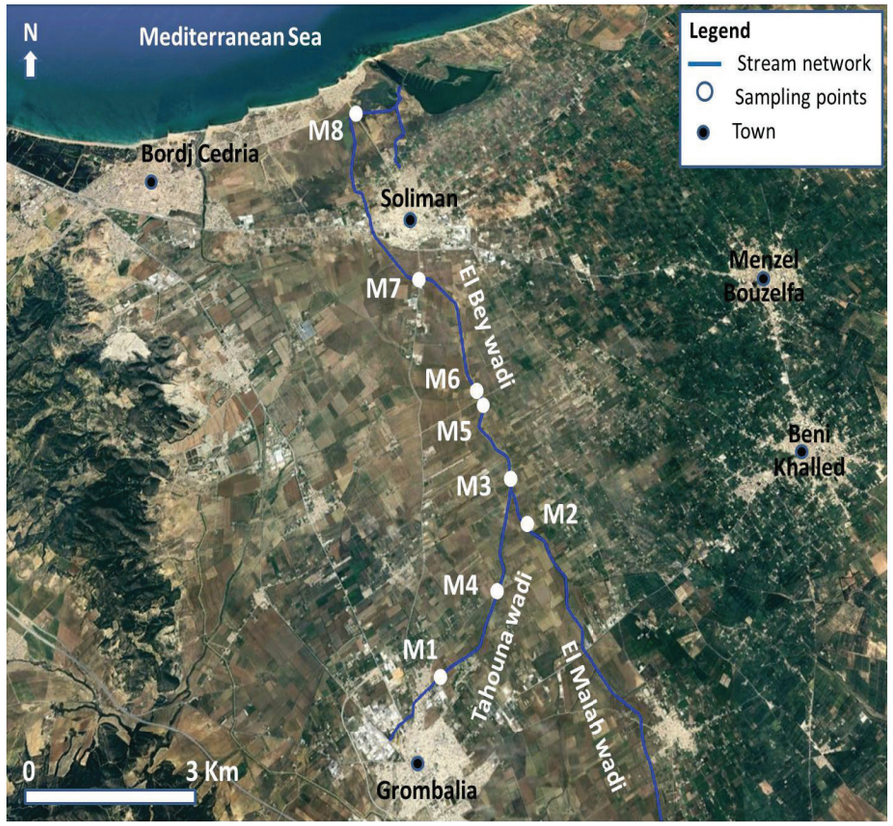


Figure S5. Tunisian studied catchment

SM 1.6 Physico-chemical parameters

Table S1. Physic-chemical parameters collected in each site

Country	Sample ID	Conductivity (µS/cm)	DOC (mg/L)	Dissolved O_2 (mg/L)	NH_4^+ (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	TN (mg/L)	PO_4^{3-} (µg/L)	TP (µg/L)
France	B1	608	1.46	8.06	0.00	1.56	NA	NA	0.02	NA
	B2	604	2.28	10.04	0.23	12.8	NA	NA	0.05	NA
	C1	800	0.98	8.88	0.00	0.92	NA	NA	0.08	NA

	C2	749	3.22	8.81	0.55	14.5	NA	NA	0.18	NA
	L1	860	1.24	9.71	0.00	3.65	NA	NA	0.03	NA
	L2	668	2.15	8.11	0.11	8.95	NA	NA	0.10	NA
	M1	929	1.72	7.57	0.00	2.64	NA	NA	0.05	NA
	M2	716	4.23	10.42	0.78	26	NA	NA	0.16	NA
	S1	772	0.95	13.47	0.00	0.50	NA	NA	0.02	NA
	S2	656	5.81	6.38	1.64	36	NA	NA	0.12	NA
Italy	Dm	1088	NA	10.00	0	55	0.09	NA	0.04	0.03
	DV1	829	NA	7.61	0.05	51	0.14	NA	0.88	1.07
	DV2	816	NA	9.32	0.07	53	0.15	NA	0.92	1.09
	DV3	823	NA	7.66	0.28	54	0.16	NA	0.95	1.22
	Em	383	NA	8.00	0.02	2.5	0.00	NA	0.01	0.02
	EV1	778	NA	6.29	1.51	24	0.21	NA	0.08	0.13
	EV2	805	NA	6.74	1.57	18	1.01	NA	0.21	0.29
Spain	ALP1	949	32.45	9.45	0.26	3.53	0.07	1.34	0.27	0.20
	ALP2	1629	11.07	5.31	1.07	2.20	0.24	2.31	2.51	1.76
	ALP3	1625	12.74	4.72	0.70	4.51	0.41	1.89	2.46	1.78
	FOL1	879	3.62	8.93	0.09	58	0.18	20.48	0.09	0.02
	FOL2	1174	5.96	8.93	5.01	40	0.78	18.33	0.23	0.12
	FOL3	1273	6.63	7.16	3.87	52	1.78	24.57	0.30	0.12
	TAL1	706	4.60	5.02	0.12	45	0.20	9.16	0.21	0.09
	TAL2	1501	4.78	7.54	0.25	8.53	0.10	3.93	0.18	0.10
	TAL3	1423	5.29	7.52	0.20	8.57	0.11	3.73	0.30	0.25
	VID1	898	3.09	7.90	0.16	11.05	0.12	3.43	0.19	0.06
	VID2	822	3.68	7.69	0.17	9.83	0.13	2.55	0.35	0.14
	VID3	816	5.07	8.01	0.13	10.7	0.12	3.72	0.47	0.18
Tunisia	T1	2040	NA	8.80	0.25	0.10	0.01	NA	0.02	NA
	T2	7080	NA	12.7	4.49	15	2.22	NA	0.04	NA

SM 1.7 Abiotic site parameters collected

Table S1. Abiotic parameter, abbreviations and units recorded and measured at the sampling sites.

Parameter	Abbreviation	Unit
relation lotic/lentic zones (share of lentic zones)	lentic	%
straightening	straight	yes/no
cross section mean bankfull depth	incision	m
cross section average stream width	width	m
dams/weirs number	dams	n
stagnation	stag	yes/no
water abstraction	abstr	yes/no
artificial perennial flow	artflow	yes/no
source pollution	poll	yes/no
eutrophication	eutro	yes/no
sewage overflows	sewage	yes/no
bank fixation shoreline	bankfix	yes/no
bed fixation stone	bedfix	yes/no
shading at zenith (foliage cover)	shading	%
shoreline covered with woody riparian vegetation at sampling site	ripveg	%
riparian cover structure tree cover	trees	%
riparian cover structure shrub cover	shrubs	%
riparian cover structure helophyte cover	helo	%
land use in the floodplain 30-100m both sides native deciduous forest	forest	%
land use in the riparian corridor 0-30m both sides open grass-/bushland	bush	%
land use in the riparian corridor 0-30m both sides reeds	reeds	%
land use in the riparian corridor 0-30m both sides naturally unvegetated	unveg	%
land use in the riparian corridor 0-30m both sides macchie	macchie	%
land use in the floodplain 30-100m both sides intense farming	crops	%
land use in the floodplain 30-100m both sides pasture	pasture	%
land use in the riparian corridor 0-30m both sides urban sites (resid.)	urban	%
odours	odours	yes/no
foam	foam	yes/no
pH-value	pH	
conductivity	cond	µS/cm
litter	litter	yes/no
turbidity	turb	yes/no
water temperature	T	°C
dissolved oxygen content	O2dis	mg/l
oxygen saturation	O2sat	%
Mineral substrates megalithal (> 40cm)	mega	%
Mineral substrates macrolithal (>20cm to 40cm)	macro	%
Mineral substrates mesolithal (>6cm to 20cm)	meso	%
Mineral substrates microlithal (>2cm to 6cm)	micro	%
Mineral substrates akal (>0.2cm to 2cm)	akal	%
Mineral substrates psammal/psammopelal	psam	%
Mineral substrates argyllal (<6µm)	argyl	%
Mineral substrates technolithal 2 (concrete walls or stream bed)	techno	%
Organic substrates algae	algae	%
Organic substrates submerged macrophytes	subm	%
Organic substrates emergent macrophytes	emm	%
Organic substrates living parts of ter. plants	lptp	%
Organic substrates xylal (wood)	xylal	%
Organic substrates CPOM	CPOM	%
Organic substrates FPOM	FPOM	%
Organic substrates / sewage bacteria	bakt	%
Total dissolved solids	TDS	
Ammonia	NH4	ug/L
Nitrite	NO2	mg/L
Nitrate	NO3	mg/L
Phosphate	PO4	ug/L
Total phosphorous	TP	ug/L
Total nitrogen	TN	mg/L
Dissolved organic carbon	DOC	mg/L
Chloride	Cl	mg/L
Discharge	Q	L/s
% of contribution of WWTP to stream flow at DW	Qsew	%

Copper	Cu	µg/L

SM 2. Target compounds classification

Table S3. Target compounds classification

Classification		
Pharmaceutical (79)	Psychoactive drugs (21)	5-Desamino-5 oxo 2,5 lamotrigine, acridone, amantadine, alprazolam, bromazepam, carbamazepine, carbamazepine epoxide, citalopram, diazepam, lamotrigine, lamotrigine-N-oxide, lorazepam, meprobamate, N-methyl lamotrigine, O-desmethylvenlafaxine, oxazepam, paroxetine, primidone, sertraline, venlafaxine, zonisamide
	Antimicrobials (21)	Azithromycin, clarithromycin, climbazole, fluconazole, linconmycin, metronidazole, N-acetyl sulfamethoxazole, nalidixic acid, sulfadiazine, sulfadimethoxine, sulfamethazine, sulfamethoxazole, sulfapyridine, sulfathiazole, trimethoprim
	Antihypertensives (11)	Atenolol, carazolol, diltiazem, irbesartan, losartan, metoprolol, propranolol, sotalol, valsartan, valsartan acid, verapamil
	Analgesics / anti-inflammatories (9)	4-hydroxidiclofenac, acetaminophen, codeine, diclofenac, ketoprofen, propyphenazone, tramadol
	Illicit drugs (5)	Benzoylecgonine, cocaine, methadone, morphine
	Stimulants (3)	1,7 Dimethylxanthine, caffeine, cotinine
	Lipid regulators (2)	Bezafibrate, rosuvastatin
	Other (6)	Carisoprodol, crotamiton, hyoscine, lidocaine, sitagliptin, warfarin
Corrosion inhibitors (2)		1-H-benzotriazole,5-methyl-1 H Benzotriazole

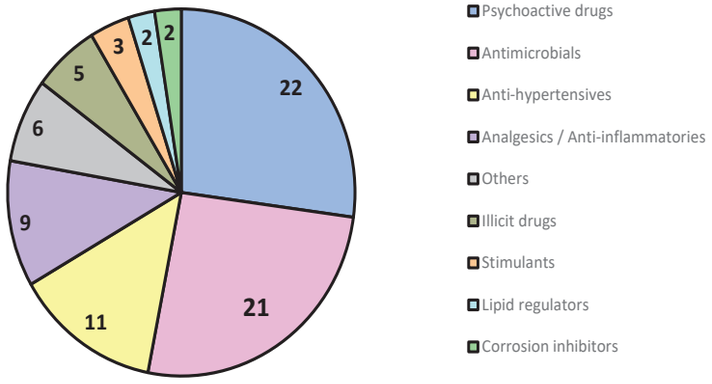


Figure S6. Compound categories distribution

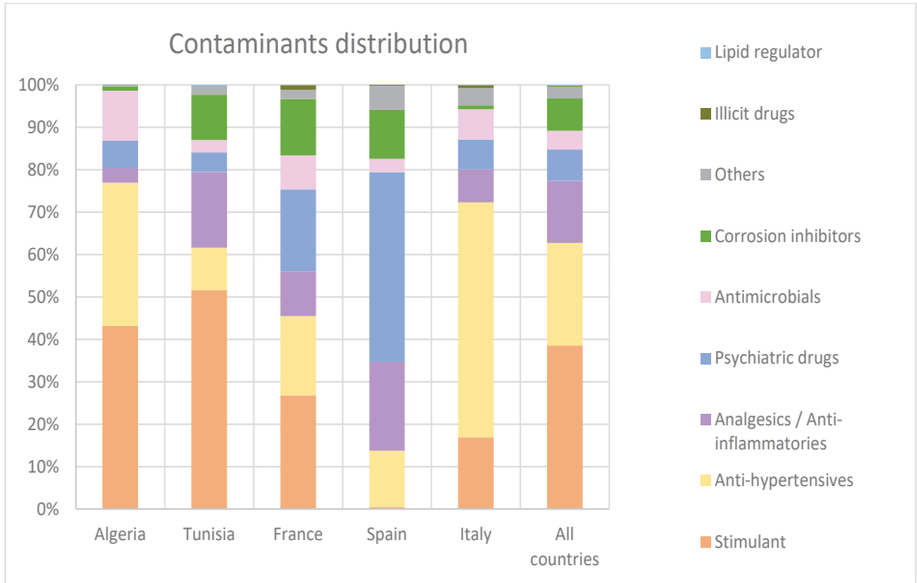


Figure S7. Detected target contaminants distribution based on the total median concentrations

SM 3. Ecological indicator sampling collection

SM 3.1. Diatom analysis

For the diaotm analysis, diatoms were sampled following the CEN standard, where five fist-sized cobbles were scrubbed using a fresh toothbrush at each sampling site. The resulting biofilm-scrapings were added to 45 mL of ethanol for preservation. In the lab, preparations followed the hot H_2O_2-HCl method. After three pre-washes with water, including homogenization and centrifugation the samples were cooked at 90°C with hydrogen peroxide (H_2O_2). Two to four drops of hydrochloric acid were added to the cold samples. After the reaction had subsided, the samples were repeatedly washed. The density of the samples was observed under the microscope to reach an acceptable diatom cell density. The samples were dripped onto coverslips in combination with ammonium chloride to prevent clumping of the silicate shells. After drying, the coverslips were mounted on slides. The slides were left to dry for 1-2 weeks. The finished diatom slides were imaged following the protocol described by. An area of 4 x 4 mm was imaged and stacked and stitched into one virtual slide where all diatoms were identified.

SM 3.2. Benthic invertebrate analysis

Benthic invertebrates were sampled using a square handnet (25 x 25 cm, 500 µm mesh size) in a representative 100 m reach of the sites. First, the substrate composition in the representative reach was evaluated in 10% steps and recorded. Then, 10 subsamples were taken appropriate to the substrate composition, i.e. one subsample for each 10% of substrate (e.g. 30% sand results in 3 samples in sand). The subsamples were transferred into white trays and inspected for benthic invertebrates. Each invertebrate specimen was directly identified at least on family level and recorded on the site protocol. For better identification, a magnification glass was used. The individuals were later on released back to the streams. The final taxa abundances were extrapolated to one square meter.

The ecological quality for the benthic invertebrates was calculated by applying the Spanish Index of Biological Monitoring Working Party (IBMWP). This index was also developed to characterize different types of pollution and has a low score of 0, but does not provide a fixed upper value. We calculated, according to, the 95th percentile of the range of all values, as the fixed upper value.

Finally, for a better comparison of the results of both organism groups, both indices were transformed to percentage related to the possible range of values. Here, low values represent good quality and high values represent poor quality.

Samples were collected from Spain, France, Italy and Tunisia. However, the ecological state of the Algerian sites was not studied due to restrictions to access the sampling sites.

SM 3.3. Abiotic site parameters principal components analysis (PCA)

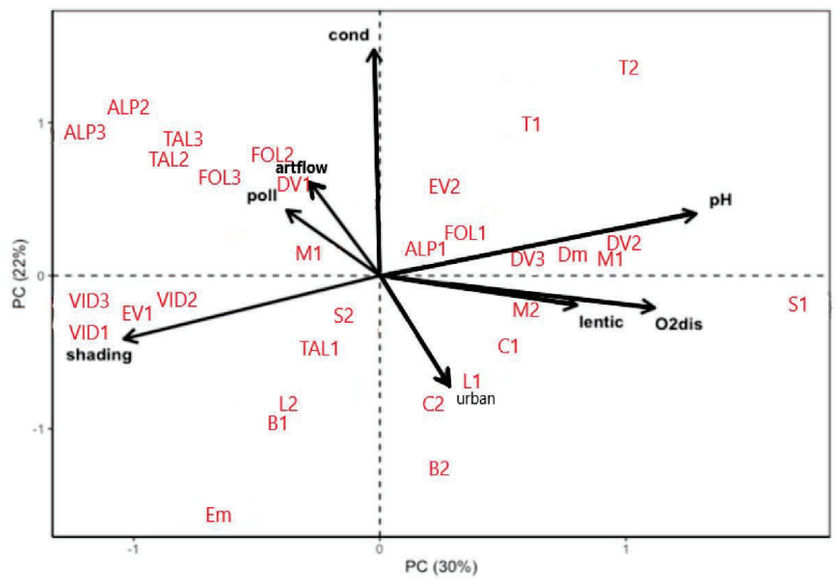


Figure S8. PCA-plot of the most important abiotic site parameter (black arrows) of all sampling sites (red abbreviations).

SM 4. Hydrological indicator of alteration

Table S4 shows the 5 proposed categories for the calculated hydrological indicator of alteration.

Table S2. Hydrological Indicator of alteration (HI).

HI	$R_Q = \frac{PS\ discharge}{Q_d}\ x\ 100$
100	80% - 100%
75	60% - 80%
50	40% - 60%
25	20% - 40%
0	20% - 0%

Where: *PS discharge* is the contribution to the streamflow from the point sources the day of sampling (localized upstream of the sampling site) [$m^3\ s^{-1}$]. Q_d is the streamflow recorded in the river section of sampling [$m^3\ s^{-1}$].

In table S4, the results of the calculated HI are presented for every studied country during the spring season.

Table S3. Hydrological indicator of alteration

Country	Stream / Location name	Sampling name	Position	Hydrological Indicator of alteration (HI)
Algeria	Sayoud	SW1	Upstream	0
	Nil	NW1	Upstream	0
		NW2	Medium	25
		NW3	Downstream	75
	Boukraa	BW1	Upstream	75
		BW2	Downstream	100
	Tassift	TW1	Upstream	100
		TW2	Downstream	100
	Mencha	MW1	Downstream	100
	Kebir	KW1	Downstream	100
France	Lirou	L1	Upstream	25
		L2	Downstream	25
	Brestalou	B1	Upstream	0
		B2	Downstream	25
	Mosson	M1	Upstream	50
		M2	Downstream	75
	Cadoule	C1	Upstream	0
		C2	Downstream	75
	Salaison	S1	Upstream	0
		S2	Downstream	75
Spain	Vidrà	VID1	Upstream	0
		VID2-3	Downstream	50
	Folgueroles	FOL1	Upstream	0
		FOL2-3	Downstream	0
	Alpens	ALP1	Upstream	0
		ALP2-3	Downstream	0
	Taradell	TAL1	Upstream	0
		TAL2-3	Downstream	75
Italy	San Giorgio Ionico	Em	Upstream	50
		EV1-2	Downstream	100
	Monteiasi	Dm	Upstream	25
		DV1-3	Downstream	100
Tunisia	Melha Wadi	M1	Upstream	100
		M2	Downstream	100
		M4	Downstream	100
	El Bey Wadi	M3	Upstream	100
		M5	Downstream	100
		M6	Downstream	100
		M7	Downstream	100
		M8	Downstream	100

12

4.4. Discussion

Occurrence of CECs in Mediterranean intermittent streams and associated groundwater

To explore the occurrence patterns and variability of the studied CECs across different countries, a violin plot is presented as Figure 4.3. This plot includes CECs concentration values from surface water collected at downstream points of WWTPs across all studies. For groundwater, as no additional datasets were presented, concentrations reported in a Spanish groundwater study by Labad et al., 2023 [19], which applied the same analytical method for the same targeted contaminants, were included for comparative purposes with the Tunisian catchment concentrations. Figure 4.3 enables the visualization of the distribution and overall trends of CECs concentrations across the studied countries. In the case of the

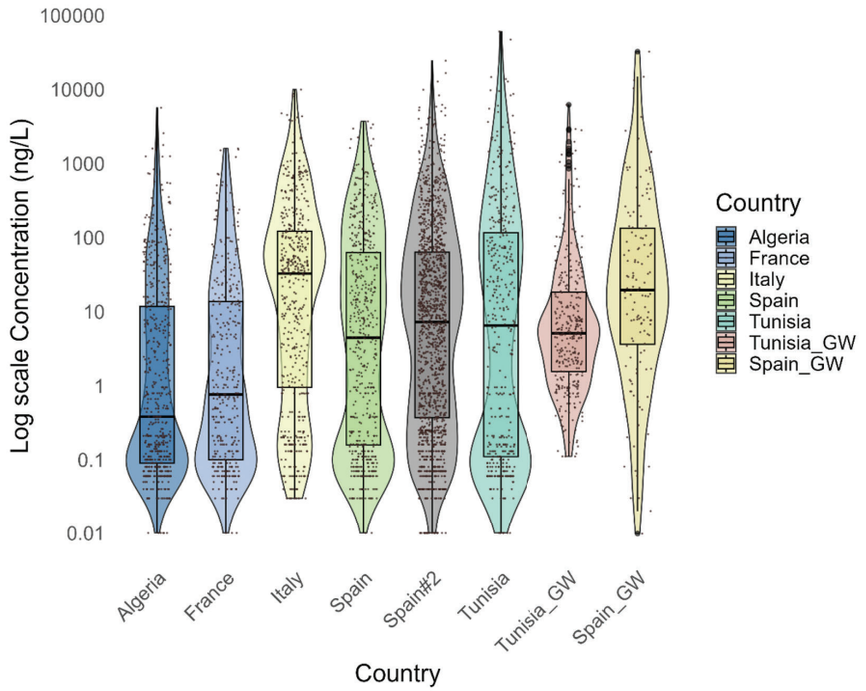


Figure 4.3. *Distribution of compound concentrations across studied countries. Violin plots show the distribution, with overlaid boxplots representing the interquartile range (IQR) and median. Points represent each compound concentration for every site. Note: Spain#2 includes the sites reported in publication #2.*

The Algerian and French catchments, as detailed in Publication #4, presented the lowest median concentrations, both below 1 ng/L. This is also reflected with the violin plot, where a wider section, hence distribution of lower concentrations, centered around 0.1 ng/L is observed. These patterns could be indicative of more efficient wastewater treatment

infrastructure, lower industrial inputs or higher stream flow rates and hence greater dilution capacity. Conversely, while the Spanish and Tunisian catchments also showed a high frequency of low concentration values, their violin plots displayed a more dispersed shape with additional widening at higher concentrations. This suggests a broader distribution of CECs levels, indicating more pronounced anthropogenic pressures.

Italian samples exhibited a broader distribution at higher concentrations, close to its median concentration, exceeding those observed in both Spanish and Tunisian sites. This elevated median concentration is attributed to the predominance of antihypertensives compound in the Italian catchment. However, broad distributions with extended upper tails were observed in all cases. Overall, the distributions revealed considerable heterogeneity in CECs contamination profiles among the different countries. The higher variance observed across countries could reflect environmental dynamics such as variability in hydrological flow regimes, seasonal fluctuations and differences in water management practices.

For groundwater, the Spanish samples presented a higher median concentration than those from Tunisia. This difference is primarily attributed to the high levels of artificial sweeteners detected in the Spanish samples, which, despite their elevated concentrations, are not considered to pose a significant risk to the environment nor human health risk as explained by Labad et al., 2023 [19]. Interestingly, the Spanish groundwater study exhibited higher median concentrations than most of the surface water catchments, revealing a high contamination profile in groundwater samples, likely driven by river water infiltration processes [19].

Environmental risk assessment

RQs were calculated for a worst-case scenario using the maximum concentrations detected, across all samples analysed. For a comparison and evaluation of the state of the Tunisian groundwater, maximum concentrations of CECs from Labad et al., 2023 [19] were used. In Figure 4.4, a heat map based on the RQs of those compounds that exceeded PNEC values in at least one sample are represented. A dendrogram is also illustrated to study possible clustering between sites.

High-risk profiles were observed in surface waters from Spain, Italy and Tunisia, consistent with previous findings [88,100], highlighting these regions as contamination hotspots. Interestingly, groundwater and surface water samples from France and Algeria, clustered together showing lower overall risk levels. Compounds such as diclofenac, losartan and azithromycin were repeatedly detected at ecologically relevant levels both in surface and groundwater samples, further supporting their inclusion in European and

regional monitoring programs [110–113].

While Figure 4.4 also highlights a high-risk profile for the Spanish and Italian catchments, Publication #4 indicates that, unlike Tunisia where even the upstream sites show significant risk, the upstream areas in Spain and Italy did not display comparable levels of impact. This pattern suggests chronic exposure to untreated or partially treated wastewater in Tunisia, aligning with the conditions described in Publication #3. Whereas for the sites in Spain and Italy, the WWTP input impact is much evident.

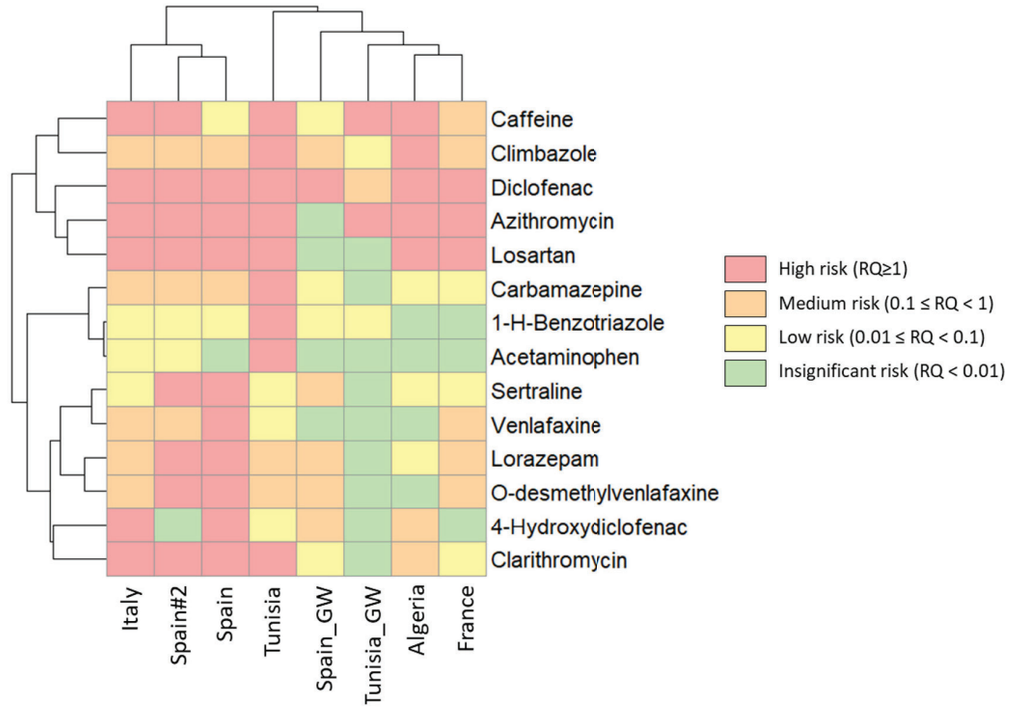


Figure 4.4. *Heat map of ecological risk assessment based on maximum detected concentrations of prioritized CECs exceeding risk thresholds (RQ>1) across the studied sites. All samples are integrated, both groundwater and surface water. Note: Spain#2 includes the sites reported in publication #2.*

High RQs, as a consequence of the presence of concentrations above PNEC values were also observed and reported in several studies from the MENA region [114]. This contamination not only degrades ecosystem quality but also raises serious concerns for public health, especially in rural areas where untreated groundwater is often used for drinking and irrigation.

Through Publications #3 and #4, when viewed together, a better understanding of the

Wadi El Bey basin from the Grombalia region is revealed. They highlight the presence of CECs from anthropogenic inputs in both groundwater and surface water, where the studied sites exhibited an increase in stress across all indicators in publication #4. Notably, several compounds, including caffeine and azithromycin, revealed RQs above 1 indicating a compromised state as sample concentrations reached a critical threshold. This overlap in potential ecological risk for the aquatic organisms between groundwater and surface water systems highlights the systemic issue on poor water resource management, especially in this basin where agriculture, industry and tourism activities predominate [114,115]. Despite legal framework, various industries discharge their inappropriately treated wastewater in this Wadi, which represents a serious threat on the shallow groundwater quality [97]. While Tunisia's water management laws show partial alignment with some principles of the EU WFD, important differences remain. For instance, the WFD's provides a higher emphasis on ecological status and river basin management approach, which is not strongly embedded in most MENA frameworks, but it is also facing implementation barriers in Europe itself, where many water bodies have yet to achieve a good ecological status [116]. At the same time effective monitoring systems are crucial for WFD implementation. In the case of Tunisia, especially on groundwater contamination very few research articles focused on CECs contamination are available [59,62]. To the best of our knowledge, Publication #3 was one of the first studies which included the study of more than 100 CECs in Tunisian groundwater samples. According to Mheidli et al., 2022 [114], the number of occurrence studies in the MENA region has been increasing in recent years and observed that Tunisia was the country with a notable growth adding six studies between 2017 and 2022. Despite this significant increase, the total number of studies reported in Tunisia based on [114] remains low, with only 11 reported by that time. The contributions of Publications #3 and #4 therefore represent valuable additions to the growing understanding and help narrow the regional knowledge gap of CECs on both surface and groundwater.

Integrated monitoring and assessment of multiple stressors

As intermittent waterways face increasing anthropogenic pressures, targeted studies that enhance and combine hydrological, ecological and chemical monitoring as well as pollutant modelling are crucial. Such interdisciplinary approaches not only enhance scientific robustness but are also essential to develop sustainable, climate resilient water management strategies, especially in vulnerable freshwater systems.

Publication #2 emphasized the importance of hydrological flow dynamics in shaping contaminant concentrations and ecological risks, particularly in intermittent streams where dilution capacity varies or can be minimum. In these situations, wastewater effluents may

dominate the streamflow, amplifying chemical exposure and risks [117]. Effluent-dominated flow regimes have been previously documented in Mediterranean catchments [118,119] and underscore the need for dynamic hydrological models to predict environmental risks under these changing flow conditions.

The recast of the Urban Wastewater Treatment Directive (2024) [120], which introduces stricter treatment requirements for smaller agglomerations based on population equivalent (p.e) thresholds. Although it does not explicitly focus on flow regimes, the introduction of tertiary treatment for smaller agglomerations reflects a growing recognition of the pressures that even small discharges can pose, especially in systems with limited flow or dilution capacity. Moreover, the new directive includes provisions to manage urban stormwater overflows in agglomerations over 100,000 p.e., aiming to reduce episodic pollution spikes that typically occur during heavy rainfall with high runoff and low flow periods. These regulatory developments signal an increased awareness of the interaction between flow regimes and pollution dynamic. Publication #4 also remarked on the lack of hydrological data [107], interrupting potential advances on hydrological modelling of CECs fate. As observed in recent work by De Girolamo et al., 2025 [121], the integration of hydrological and chemical modelling can provide accurate assessments of pollutant behavior and environmental risk, especially in intermittent rivers where dilution capacity is limited. The study on modelling azithromycin fate in Mediterranean rivers demonstrated how modelling approaches that account flow variability help improve the ability to predict ecological risks under different scenarios. These models would be very useful during dry periods when residence time of contaminants may increase.

In addition to chemical and hydrological assessments, ecological indicators help evaluate the aquatic ecosystems' environmental condition. As detailed in Publication #4, an effective tool is the Iberian Biological Monitoring Working Party (IBMWP) index [122], a widely applied biological assessment metric. IBMWP is based on the presence and tolerance of benthic macroinvertebrates to pollution, which serve as reliable bioindicators of water quality [103,104,123]. This index is relevant for Mediterranean river systems due to its sensitivity to organic pollution and its ability to reflect ecological responses to combined pressures, including hydromorphological changes commonly observed in semi-arid environments [124,125]. Publication #4 revealed a moderate positive correlation between CECs concentrations and IBMWP score. This result suggests that chemical contamination can be directly reflected in biological communities, reinforcing the utility of macroinvertebrate-based indices for pollution assessments consistent with findings in other studies [124]. Kuzmanovic et al., 2016 [126] obtained similar correlations, however, in this study the SPEAR index (species at risk index) was applied observing a decline in sensitive

macroinvertebrate taxa with the presence of organic compounds.

Publication #4 also revealed weak correlations between the other indices, underscoring the complexity of stressors' interactions in intermittent streams, which are highly sensitive to multiple and fluctuating environmental pressures [124]. Hydrological fluctuations in intermittent streams, characterized by shifts between lotic (flowing) and lentic (stagnant) conditions, highly affects biotic communities [127,128]. Thus, ecological indices are essential tools to better understand the effects of anthropogenic inputs. Although not typically used for chemical risk assessment, benthic macroinvertebrates serve as reliable indicators for assessing the ecological impact of these contaminants. Monitoring changes in macroinvertebrate along with understanding potential bioaccumulation patterns are essential for comprehensive environmental risk assessments of CECs.

While individual scientific fields are able to identify, detect and quantify stressors, translating these findings into actionable environmental indicators remains a challenge, especially due to the lack of standardized protocols to assess the combined effects of multiple stressors. This limits the ability to gain a more comprehensive view of the ecosystem dynamics, particularly in intermittent streams which are highly sensitive to both climatic variability and human activities. The findings from Publication #4, highlighted the necessity for strengthening collaborations across scientific disciplines were clearly identified.

The need for integrated policies combining chemical, hydrological and ecological strategies is evident. Despite recent regulatory updates representing progress, broader inclusions of flow variability and climate adaptative strategies are necessary. In regions like Tunisia and the broader Mediterranean region, where droughts and reduced river flows are increasingly common, avoiding high CEC concentrations due to low dilution is essential. The publications presented in this chapter provide important insights of the intensifying anthropogenic pressures on water systems and reinforce the need of more comprehensive water management.

The fact that WWTPs represent one of the main sources of organic contaminants into the environment [129], together with the growing concern about the potential adverse effects that these substances can have on the ecosystem and humans [130,131], has aroused the interest of public administration and scientists in recent decades to find a way to mitigate this type of pollution. The most direct approach involves the implementation of advanced tertiary treatments after conventional secondary processes, that are capable of eliminating CECs residues. In this sense, the application of a wide range of processes has

been investigated, including membrane filtration, ozonation, activated carbon adsorption and advanced oxidation processes [132,133]. While these technologies significantly improve effluent quality, their high operational and maintenance costs often limit their implantation, especially in low-and middle-income countries or regions like MENA, where infrastructures and economic resources are limited [134]. Nature-based solutions, such as constructed wetlands, are more sustainable alternatives with increasing attention as low cost and ecologically sustainable alternatives. These systems have not only proven promising removal of nutrients, pathogens [135] but also for certain CECs [136,137], making them particularly interesting for regions like MENA countries [138,139].

Despite the need to implement improved water treatment technologies to avoid CECs introduction into the environment, special attention must be given to a critical challenge, the formation of subsequent TPs. These may exhibit altered toxicity, persistence or mobility compared to the parent compound [140]. Even though TPs are rarely monitored and even less regulated, efforts should be made to better understand their fate. Hence to expand on the target analysis and study further possible TPs, through suspect and non-target approaches will be further explored in the following chapter.

4.5. References

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Chapter 5

Identification and Fate of Transformation Products in the Environment

5.1 Introduction

5.2 Methodology

5.3 Results

5.4 Discussion

5.5 References

Chapter 5. Identification and Fate of Transformation Products in the Environment

5.1. Introduction

CECs can undergo several types of transformations in the environment, involving chemical, biological and physical processes, all of which give rise to TPs. When these transformations occur in living organisms, especially through metabolic pathways, the resulting compounds are more specifically referred to as metabolites. In contrast the term of TPs usually encompasses products derived from engineered processes or environmental transformations. Apart from transformations in the environment, structural changes of synthetic organic compounds may already have taken place during the passage through the WWTP before being discharged as wastewater effluents. Within the broad group of CECs, comprehensive knowledge on metabolic pathways of pharmaceuticals in the human body allow to anticipate which metabolites may be of environmental relevance [1]. From a chemical perspective, common transformation reactions include hydrolysis of cleavable bonds, oxidations of carbon and heteroatoms, reduction reactions and conjugations with endogenous molecules such as glucuronic acid or sulfate, provided by the responsible organism [2]. Other typical processes involve hydroxylation, dealkylation, methylation and demethylation [2]. Both metabolites and TPs exhibit altered physico-chemical properties and biological activity compared to their parent compound. For simplicity, in this chapter all environmentally detected derivatives will be referred to as TP, unless otherwise specified.

In the past decades, research has been predominantly focused on parent compounds, with TPs receiving in comparison little attention [1,3]. Most current monitoring schemes have been focused on quantifying specific, previously selected compounds, based on prioritization strategies [4,5]. This approach, as demonstrated in the previous chapters, is very useful to understand and get an overview of the input of WWTPs, especially in low-flow river systems. However, its main limitation lies in its inability to detect unknown compounds outside the predefined spectrum of analytes. Here is when suspect and non-target screening (NTS) approaches appear as powerful tools for the detection and identification of a broader range of compounds and possibly even TPs [6].

Knowledge on TPs has been limited and largely neglected in management of pollutants for several reasons: (i) they are usually formed by oxidation reactions and therefore are more polar than the parent compound, reducing the risk of bioaccumulation, (ii) increased polarity of TPs commonly goes hand in hand with reduced affinity to molecular targets within environmental organisms attenuating potentially adverse effects with respect to

the precursor compounds (iii) TPs are usually detected in the environment at very low concentrations due to slow formation kinetics [7]. However, there has been increasing attention in the detection of TPs [1] as in some cases the previously mentioned arguments do not apply, as TPs can sometimes be equally or more environmentally relevant than the parent compound in terms of bioaccumulation potential, intrinsic ecotoxicological effects and abundance [7]. Thus, in some situations, TPs can result in greater mobility or persistence as well as higher toxicity [8,9]. At the same time, under certain conditions, some TPs, particularly those derived from pharmaceuticals, can act as prodrugs and revert back to their parent compounds [10], leading to a potential increase in the parent concentration in the environment [11]. For instance, N-oxides of certain pharmaceuticals, such as venlafaxine-N-oxide, are known to act as prodrugs [12]. These reversible transformations add further complexity to the understanding of TP fate and persistence in the aquatic environment.

Moreover, the study of TPs presents several analytical challenges including the absence of analytical reference materials for unequivocal identification, their detection at typically low concentration in environmental samples, limited spectral data, and scarce information on their transformation pathways [13]. Together, these factors make TP detection and structure elucidation a complex and cumbersome task. The workflow of this process requires the combination of lab-scale experiments for the study of transformation pathways of CECs, database searches and computational predictions, each contributing to different classification levels [7]. Nevertheless, understanding the extent of attenuation of TPs is crucial for assessing their long-term impact and potential risks on the aquatic ecosystem.

Among the various classes of CECs, tertiary amine compounds have gained attention due to their widespread presence in pharmaceuticals, such as venlafaxine (antidepressant) or tramadol (analgesics). These compounds are characterized by a nitrogen atom bonded to three carbon- alkyl groups. This chemical structure makes them susceptible to N-oxidation, in which an oxygen atom is transferred to the nitrogen atom. N-oxide derivatives are of particular interest due to their frequent occurrence and dual origin. Many N-oxides are formed as common metabolites of pharmaceuticals and natural compounds, often arising as being less toxic, more polar and more stable than the respective parent compounds [14,15]. Additionally, N-oxides are often generated as TPs in WWTPs, in activated sludge [16], and during advanced treatment processes such as ozonation, further raising concerns about their potential environmental impact [8,17]. Furthermore, the attenuation of these N-oxides in natural systems is a critical factor in understanding their environmental fate. The process by which these compounds degrade or persist in aquatic environments, influenced by factors such as sorption, photodegradation, and biodegradation, is a key area of investigation. Among the targeted compounds analysed in this thesis, 9 are tertiary amine-containing

compounds. Table 5.1 lists the targeted tertiary amine compounds along with their main N-oxide transformation. In addition, lamotrigine is included. Although it is not a tertiary amine its N-oxide was studied, as well as two other common tertiary amines with reported N-oxides transformations [18].

Table 5.1. *Tertiary amine-containing compounds and their possible N-oxide transformation.*

Compounds	Formula	Tertiary Amine?	Forms N-oxide?	Main Environmental transformation reaction
Citalopram	$C_{20}H_{21}FN_2O$	☑	☑	N-demethylation; N-oxidation
Clarithromycin	$C_{38}H_{69}NO_{13}$	☑	☑	N-demethylation and N-oxidation of desosamine; lactone hydrolysis
Lidocaine	$C_{14}H_{22}N_2O$	☑	☑	N-dealkylation; N-oxidation to lidocaine-N-oxide
Lincomycin	$C_{18}H_{34}N_2O_6S$	☑	☑	N-demethylation; N-oxidation; degradation of sugar or thioether groups
Paroxetine	$C_{19}H_{20}FNO_3$	☑	☑	N-demethylation; N-oxidation
Tramadol	$C_{16}H_{25}NO_2$	☑	☑	O-demethylation to O-desmethyltramadol; N-oxidation to tramadol-N-oxide
Venlafaxine	$C_{17}H_{27}NO_2$	☑	☑	O-demethylation to O-desmethylvenlafaxine; N-oxidation to venlafaxine-N-oxide.
Verapamil	$C_{27}H_{38}N_2O_4$	☑	☑	N-demethylation; N-oxidation.
O-desmethylvenlafaxine	$C_{16}H_{25}NO_2$	☑	☑	N-oxidation to O-desmethylvenlafaxine-N-oxide
Lamotrigine	$C_9H_7Cl_2N_5$	☒	☑	N-oxidation to lamotrigine-N2-oxide
Lamotrigine-N2-oxide	$C_9H_7Cl_2N_5O$	-	-	Minor back-transformation to parent compound
N-Methyl lamotrigine	$C_{10}H_9Cl_2N_5$	☒	☑	N-oxidation; minor hydroxylation possible
Amisulpride	$C_{17}H_{27}N_3O_4S$	☑	☑	N-oxidation; minor hydroxylation
Sulpiride	$C_{15}H_{23}N_3O_4S$	☑	☑	N-oxidation; minor hydroxylation possible

Recent publications have emphasized the main challenges of TPs analysis and detection highlighting key issues concerning the lack of reliable identification methods, the poor characterization of TP formation during water treatment, and the insufficient integration and harmonization of TPs in regulatory frameworks [7].

In this context, this chapter tries to expand the current understanding of TPs arising

from CECs in intermittent streams. A common technique to assess and identify TPs is through controlled lab-scale experiments, where degradation experiments are performed to simulate possible TP formation occurring in the environment [19,20]. However, other approaches, such as curation of existing data and the use of prediction models are also available and are becoming more and more efficient [7,21].

5.2. Methodology

5.2.1. Lab-scale degradation experiments

Different degradation processes can occur in the environment, influencing the fate of contaminants. Among the most relevant degradation processes that occur in surface waters are photodegradation, triggered by the exposure to sunlight, and biodegradation by microbial activity [22,23]. Figure 5.1 illustrates common lab-scale experimental setups for both photo- and bio- degradation studies.

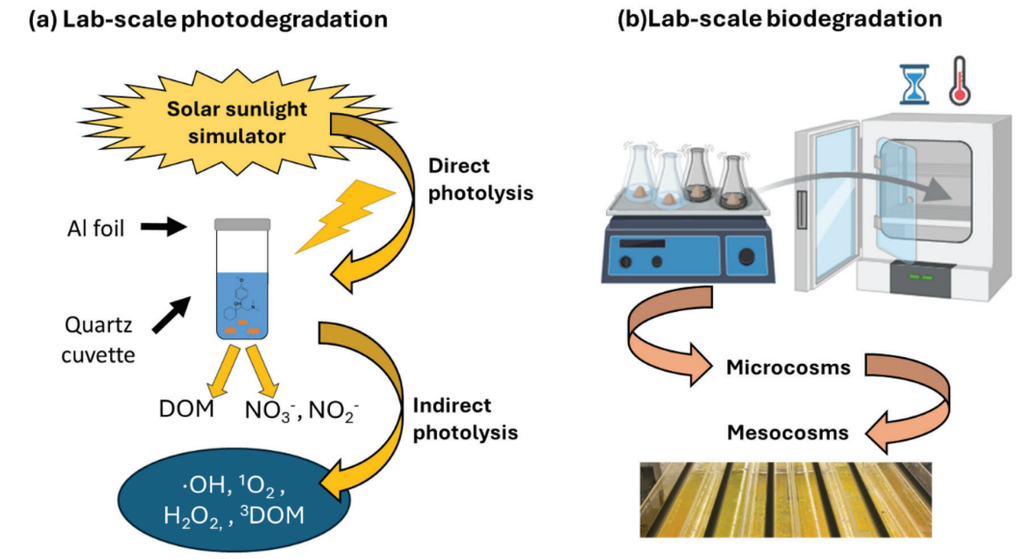


Figure 5.1. *Examples of (a) photo-degradation and (b) biodegradation lab-scale experiments for the study of TPs formations, simulating environmental conditions.*

Photolysis studies are conducted under either natural sunlight or artificial light sources, using solar simulators such as xenon arc lamps to irradiate the samples [22,24]. In case of photodegradation, two main pathways take place: direct photolysis, where the compounds itself absorb photons and undergo transformation, and indirect photolysis, which occurs through the interaction with photosensitive species commonly present in surface water such as dissolved organic matter (DOM), nitrates or nitrites. Upon activation by sunlight, these substances produce reactive intermediates (e.g., hydroxyl radicals, singlet oxygen)

that subsequently react with contaminants, thereby enabling accelerating degradation processes [23,25,26]. Both direct and indirect photolysis are driven by solar radiation primarly in wavelengths above 290 nm, in the UV-A (315-400 nm) and UV-B (280-315 nm) ranges [26]. However, the key difference lies in the light absorbing species. While direct photolysis experiments are relatively simple to set up, lab-based studies of indirect photolysis require the addition of photosensitive species, e.g. humic acids and/or nitrates to real or artificial fresh water to simulate indirect reactions [27–29]. Incorporating both direct and indirect photolysis experiments is essential to better simulate real case scenarios and hence obtain more meaningful results.

For biodegradation studies, water/sediment incubation experiments are commonly performed [30–32]. River conditions are simulated by collecting both water and sediments samples which are then incubated under controlled environmental conditions (Figure 5.1). In more advanced studies, microcosm systems allow higher levels of control and manipulation, while mesocosm studies provide even more realistic simulations of environmental conditions, by scaling up the system and including more ecological complexity [33,34].

Both approaches are valuable tools for assessing the environmental fate of contaminants and their TPs. Controlled lab-scale degradation studies can help identify TPs by simulating environmental conditions to which parent compounds are exposed in surface waters [22,23]. However, for both photo- and bio- degradation approaches, several factors influence degradation processes and the nature of the resulting TPs. Thus, experimental design is crucial in order to generate environmentally relevant TPs [35]. This includes controlling key parameters such as pH, temperature, oxygen levels and turbidity as well as external variables like solar irradiance. Although not all variables can be directly controlled, such as precipitation and flow variations, considering the influence of the previously mentioned parameters is important to avoid unrealistic scenarios which may lead to misinterpretation of environmental relevance or persistence. Adopting established protocols such as the OECD 308 guidelines on studying transformations in aquatic sediment systems [36], can help reduce variability and minimize the risk of misinterpretation.

5.2.2. Data curation and prediction models for TPs

Novel approaches for data curation and prediction of unknown pollutants are rapidly evolving, with open-source approaches becoming more available. The use of open-source software enables access to multiple researchers, which can modify and adapt these for their specific needs. Additionally, it promotes continuous improvements through global

community contributions. In this thesis an R-based tool developed by the Environmental Cheminformatic group at the University of Luxembourg was applied to search for possible TPs. *ShinyTPs* was developed by Palm et al., 2023 [37] and as part of the ZeroPM project, aiming to help curate TP information extracted from text-mined data from publicly available databases, particularly PubChem [38]. PubChem database incorporates content from the HSDB which contains detailed information on environmental fate, metabolism and biotransformation pathways. By this what *ShinyTPs* provides is the gathering and organization of TP information from public chemical databases. It collects, checks, organizes and verifies available data on possible transformation reactions on known TPs providing usable and reliable information for further studies. Thus, *ShinyTPs* aids in the identification and prioritization of possible TPs. This tool was applied to generate a suspect screening list of TPs derived from our targeted list of contaminants. In Figure 5.2, an example workflow of a curated reaction of estriol is shown, as well as the incorporation and update of the curated TPs in PubChem transformations.

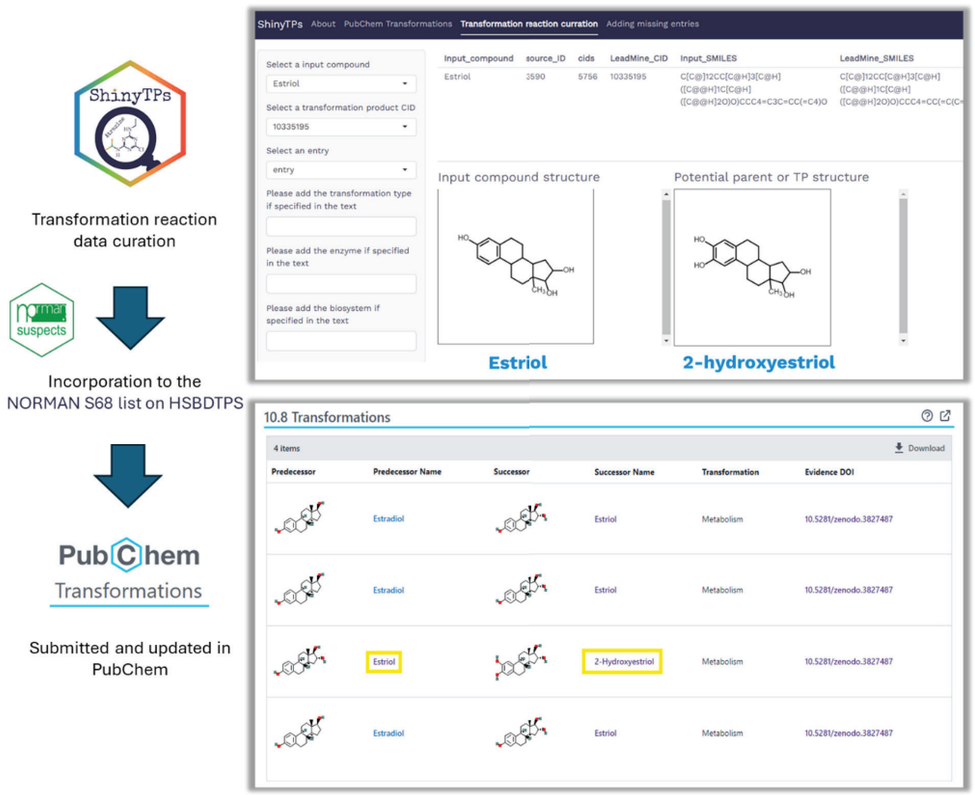


Figure 5.2. *Example of ShinyTPs curated reaction, incorporation to the Norman S68 list [39] and updated PubChem transformations. Abbreviations: HSDBTPS Hazardous Substance Data Bank transformation products.*

From our data curation of TPs, a suspect list of 270 TPs was generated (Publication #6). New curated TPs were added to the Norman S68 list [39] and the PubChem Transformation database [38] was updated as demonstrated in Figure 5.2.

The process of data curation, especially for TPs, is crucial to integrate and refine TP prediction tools such as Biotransformer [40,41]. As curated TPs are incorporated into databases such as PubChem, these serve as valuable reference sources for prediction algorithms to forecast potential metabolic pathways and environmental degradation products. To further expand the list of possible TPs, Biotransformer was used within *patRoon* [42]. Biotransformer is an advanced predictive modelling tool used to simulate how a given compound can degrade or transform in biological and natural environmental. It uses powerful models based on known chemical transformation pathways and machine learning algorithms, reaction networks and chemical structure analysis to predict possible TPs. In this case Biotransformer was used to simulate the transformations contaminants may undergo in environmental systems, hence abiotic and environmental microbial transformations were selected.

5.2.3. Suspect and non-target screening through open-source cheminformatics approaches

For suspect and non-target screening the open-source R-tool *patRoon* [42,43] was used for the identification of TPs and other unknowns. *PatRoon* provides a comprehensive workflow for HRMS data, incorporating tools for feature detection, annotation, prioritization and visualization, making it a very good and suitable tool within environmental cheminformatics.

To enhance annotation robustness, a set of complementary cheminformatics resources were integrated into the *patRoon* workflow. PubChemLite served as a compound database for environmental screening, supplying relevant chemical metadata. MetFrag was employed for in silico fragmentation modelling based on proposed molecular formulas, allowing alignment of theoretical spectra with experimental MS/MS data. MassBank contributed curated reference spectra, enabling direct empirical comparisons for compound verification. This layered approach of metadata retrieval, spectral prediction, and library matching reinforced confidence scoring and supported level assignments within the Schymanski framework [44].

All selected features were annotated using the level-based identification confidence scheme described by Schymanski et al., 2014 [44]. Compound annotation by library spectral match was performed using the individual MoNA score within *patRoon*. The individual

MoNA score is a spectral similarity score calculated based on comparing experimental MS/MS spectrum of a sample to reference spectra available in the MoNA database. The score ranges from 0 to 1, where 1 indicates a perfect match between experimental and reference spectra, leading to compound identification confidence of level 2 or 3 based on Schymanski et al., 2014 [44]. The criteria used to annotate the features was that described by Talavera Andújar et al., 2022 [45]. In Table 5.2 the associated rules applied for the established levels are presented.

Table 5.2. *Identification confidence level system used to annotate compounds based on Talavera Andújar et al., 2022 [45,43].*

Identification level	Description (*patRoon*)	Rules
1	Target match	Confirmation with reference standards
2a	Good MS/MS library match	Individual MoNA Score is at least 0.9 and no other candidates were matched with the MS library.
3a	Fair library match	The individualMoNAScore or libMatch is at least 0.4.
3b	Known MS/MS match	At least 3 (or all if the suspect list contains less) fragments from the suspect list must match.
3c	Good in-silico MS/MS match	The annotation MS/MS similarity (annSimComp column) is at least 0.7.
4a	Good formula MS/MS match	Suspect is top ranked formula candidate, annotation MS/MS similarity (annSimForm column) is at least 0.7 and isotopic match (isoScore) of at least 0.5. The latter two scores are at least 0.2 higher than next best ranked candidate.
4b	Good formula isotopic pattern match	Suspect is top ranked formula candidate and isotopic match (isoScore) of at least 0.9 and at least 0.2 higher than next best ranked candidate.
5	Unknown	All else.

5.3. Results

This chapter includes the following publications:

- Publication #5: R. Manasfi, D. Tadić, **O. Gomez**, S. Perez, S. Chiron. (2022). Persistence of N-oxides transformation products of tertiary amine drugs at lab and field studies. Chemosphere, 309, 136661. https://doi.org/10.1016/j.chemosphere.2022.136661

- Publication #6: **O. Gómez-Navarro,** S. Perez, E. L Schymanski (2025). Integrating Suspect and Non-Target Screening with Open Cheminformatics Approaches to Identify Pharmaceutical Related-Transformation Products and previously unidentified Pesticides in a Mediterranean Stream. Submission to ACS ES&T Waters.

Publication #5

Persistence of N-oxides transformation products of tertiary amine drugs at lab and field studies

R. Manasfi, D. Tadić, O. Gomez, S. Perez, S. Chiron

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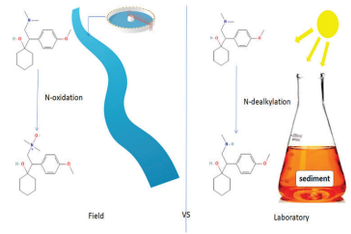
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1. Introduction

x drncE dncufcaj vc rigt p e dncgfrnucdp rf ducf Lcukfot p p t fe f dugf d gfrcp eidug IFv nf dwt fncp l cudncfucTet rfv gcufgut vTAnfot gent rKf HwAt vTAfE drncE dncufcaj vc nfod fl ut Legcf Fc chnrfrvoAfdrfrvrnde cgf rnucdp j t E rfgvue TfgutvTAnIfnAckfdwt fucwdrcfoAcp eodwfrvoAfdrfl Adui p docvneodwf ucregvcrf e nt f rvuedocf E dncuf d gf ot uucrl t ge Tf rAdwE f Tut v gE dncufE enAfep l donrft fd; vdneofrl coecrfdnfc Leut p c ndwkfucwi Ld nfot oc nudnet rfyeKKf< (fµT. *Dfrvo Afdrfhr Afy bt vgfcnfdWf6266(DftufFc nAeofp dout e Lcunc Fudncrfy Huent cfcnfdWf6266DKP irnucdp fdnnc vdi nt ftafl Adup docvneodwfe fueLcufAdrfFcc fudnAcufE cwdoR t E wg Tcgfc Lc f nAt vTAfE enAf df Tucdnf Ldued Fewrkfe fe nc rerkf Fcn E cc fot p l t v grfd gf dp t Tfc Leut p c ndwfot gent rfy Hoc dfcnfdWf62(: DK- t fgdnc Fdnnc vi dnet ft afl duc nfot p l t v grfAdrfucoceLcgfnAcfTucdncrnfat ovrKP fot nudrnlf rnvgecrft fdnnc vdnet ft af nud rat up dnet fl ut gvonrfy- OrDfe fueLcufducf wp encgfy flAefcnfdWf6262@x uencufcnfdWf62(8DFvnfoueneodwE Ac fot regi cue Tfl t nc nedwfep l donrft fFet geLcurerklfre ocfrt p cf- Orfod fucnde fFet i w Teodwfdone Lenecrfy flAefcnfdWf6262DK, de fadort urfdaecore TfnAcfcs nc nft af e irnucdp fdnnc vdnet fe nc rerkft afot p l t v gr. - Orfe owge Tfrt ul net If l At rt ifd gfFet nud rat up dnet rfdufcfrnewfdfp dncufta fgerovrret fy Jdc Tcuf cnfdWf62(U@VoAp enf cnfdWf626(DKP fnAerfot ncs nlf nist segcrft afguvTrf Fcdue Tfncuneduk fdp e cfducf- OrfE AeoAfwfRcwkfgercuLcfnt fFcfe Lcrne Tdncgf Fcodvrcfrt p cft afnAcp fAdLcfFcc fucl t uncgfnt fFcfudnAcufl curenc nf- Orf y, cucvfcnfdWf62() DKP fdggent Ifrt p cfnist segcrfE cucfrAt E fnt fFcfp t ucf l t nc nf dnf nAceuf Avp d f l Adup dot ww Teodwkf nduICnf nAd f nAceuf ot uuci rl t ge Tfl duc nfot p l t v grKfN cft afnAcfp t nnfedp t vrfcs dp l wft afnAcrcferf dp enuel nkw cimit segcIfdfguvTfvrcgfe fl rkoAednuofp cgcoe cfy bt gnfcnfdWf (U)(DK Zc vdadse cimit segcf d gf Nigcrp cnAkvkc vdadse cimit segcIf nudp dgt vimit segcfAdLcfFcc fdwt fl dnc nrgfdrfl ut guvTrfd gfp dTAnfFcfd f c Leut p c ndwfrt vuocft afnAceufot uucrl t ge Tfl duc nfot p l t v grKf

- Acft oovuuc ocft afrcwonrgfmit segcrft afncunedukfdp e crfe fudLE fgt i p crnofE dncE dncufduercrfaut p fAvp d fp cndFt wp rfy bdTt iqcucut fcnfdWf 62(/DKx Ac f t Ml dnet f erf vrcgf drf ncundukf nucdnp c nf at uf t ulTd eof p eout l t wwnd nrfucp t Ldwfl vul t rcrIfmit segcrfTc cudncrgfe fAeTAfdp t v nf drf- Orfy, cucvfcnfdWf62() DKmit segdnet fucdonet nfdwt fot rnenvncfdfp d7t uf p eout Fedwf Fet nud rat up dnet fl dnAE dkfat ufdwRkwfncunedukfdp e crKBt rci ; vc nkdfmit segcrfE cucfat v gfnt fFcfat up cgfe fdonLdncgfrwgTcfE Acucf at ufe rnd ocIfmit segeMtgfLc vdadse cfE drfat v gfnt fFcfucgvocgfFdoRfnt f Lc vdadse cf t uf avunAcuf nud rat up cgf p de vkf nAut vTAf migcdvRkwdnet f yb vgcf cnf dWf 62(3DK Vvul uere TwIf t f reT ehod nf ucp t Ldwf t af rvvlwergcimit segcIf dp ervvlwergcimit segcf d gf wdp t nuE e cimit segcf E drf t FrcuLcgfe fFc oAirodwf donLdncgfrwgTcfFet gcTudgdnet fcsl cuep c nnIf e geodne TfnAcf AeTAf Fet w Teodwf l curenrc ocf t af nAcrcf mit segeMtgf fot p i l t v grfy l t wp d fcnfdWf62(3DK, t ucf ucoc nkIf dp ervvlwergcimit segcIf rvvlwergcimit segcf d gf Lc vdadse cimit segcf E cucf dwt fgcncorncgf v gcuf rep vwdncgfAkl t uucAeofM cfot genet rIfE Acucfn Ackfnc gcgfnt fdloovp vwdncf t Lcufnp cfd gfE Acucf Lc vdadse cimit segcfot oc nudnet rfcs occgcgfnAt rcf t af migcrp cnAkwdncgf d gf Nigcrp cnAkwdncgf Lc vdadse cfy Ot rrcwf cnfdWf 6262DKP f ot nudrnlf df ot p l uc Ac reLcf wncudnvucf rcduoAf ucLcdwgf Lcukf wp encgfe at up dnet f ucTduge Tf dFet nof t uf Fet nof nud rat up dnet rf t af mit segcrft afncunedukfdp e crfe fucoceLe Tfrvuodocf E dncufycRTKfueLcuDK- Aerf E ewf Fcfn Acfp de fot nueFvnet ft afnAerfE t uRfFkfot p Fe e Tfwdf Ffd gfh cwgf rnvgecrf dnfueLcuf Fdrcf j t E fot genet rfd gfFkfvre Tfnudp dgt vimit segcIf Lc vdadse cimit segcIf Nigcrp cnAkvkc vdadse cimit segcIf dp ervvlwergcimif t segcf d gf rvvlwergcimit segcf drf l ut Fcf dwRkwfncunedukf dp e cf mit segcrfK Vl coe hoft F7cone Lcrfe owgcfyeDp t ent ue Tft afrcwonrgfmit segcrfdw Tfn Acf ueLcufrnucnoAfnt fgerovrrfnAceufl t nc nedwfl curenrc ocIfyeeDfe Lcrne Tdne TfnAcf at up dnet ft afmit segcrfd gfnAceufrndFewrkfe fE dncufv gcufl At rt vkrerfd gf e fE dncu. rcgep c nfrkrncp rfv gcufdcut Feofot genet rfd gfyeeDfgerovrre Tf nAcf ucwLd ocft afmit segdnet fd gfmigcdvRkwdnet fucdonet rfdnfvdFfd gf hcvgfrodwekf

2. Material and s etmodh

. IvF CQu2 seHho

H dvkncodvfrnd gdugrft afrndulTcncgfot p l t v grfe owge TfLc vdadse clf Lc vdadse cimit segcIfmigcrp cnAkvkc vdadse clfNigcrp cnAkvkc vdadse clf Nigcrp cnAkvkc vdadse cimit segcIf dnc t wtvlf oduFdp dMel e clf nudp dgt vlf migcrp cnAkwudp dgt vlf Nigcrp cnAkwudp dgt vlf nudp dgt vimit segcIf dp ei rvvlwergcIf dp ervvlwergcimit segcIf rvvlwergcf d gf rvvlwergcimit segcf E cucf l vuoAdrcgfaut p f- t ut nt fz crcduoAf BAcp eodwfy- t ut nt lfBd dgdDKqt uf csnudnet fl ut ocrrf d gf*Bf d dvkrerIf vwudil vucf E dncuf y%Ox DfE drf t Fi nde cgf vre Tf df, ewal t ucf rkrncp @S O*BiTudgcf docnt enuewf yHBmDf d gf p cnAd t v6y, cNS DE cucfl vuoAdrcgfaut p fBduwffiuFdfz cdTc nrfVHHWKfyZ dvf gcfz cvewlfqud ocDKqt up eofdoegfyqHDfl vuerrfl KfKHBVfucdTc nfd dvkncodvf TudgcfE drfl vuoAdrcgfaut p fVel'p diHvguoAfyVnf5 vc ne iqdwaLEcufkqud ocDKf

*JU8Sja enlHscuo9mBu o h uhHHSf c*K (Kf Tf t af gert gevp f Akgut Tc f l At rl Adncf gcAkgudncIf(8f Tf t afoenuof doegfp t t Akgudncf d gf2f8) 6f Tf fiG- HfE cucfgerrt wncgfe f(22fp *ft afS O*BfE dncuKf

. E F GHnyt o,sEho

qecwgfrvuLckrfE cucfot gvoncgfdw Tfdf(K)fRp frnucnoAft afnAcfBuecvw f zeLcufE enAfnAucfrdp l we Tfrencrfy V(IfV6fd gfV8IfccfqeTfV(fe fVvl l t ure Tf GdnfDf wodncgfe fnAcf t unAcu fl dunft af, t rl cwucufoenkf yqud ocDK- Acf rnvgecgfducdfE drfdfrp dwf, cgercucd cd fodnoAp c nfE AeoAfE drfp de ukf t oovl ecgfFkfLe ckdugIfE enAfdfl ut w Tcgfgukfrcdrt fd gfdfp cd fd vdwf dLcudTcfude adwft afW62fp pK- Acfrnucno Afucoce Lcgf E dncufaut p ft cfre Twf ot re vt vrf E drncE dncufl t e rirt vuocf aut p f d fcaj vc nfgeroAduTcf t af df l rko Aednuef Atrl enfdvyx x - OlfV6DK- Acf donLdncgfrwgTcfx x - Oft vnedwf E drfof regcucgfnAcfl ue oel dvfrt vuocft af l Adup docvneodwf E enAfp e ep dwf csl corrcgf t il t e nfrt vuocIfucl ucrc ne Tfdfrvendf wfrnvgkfrencfat ufe Lcri

nfTdne TfnAcfadncft afnAcrcfoAcp eodwfe frvuedocfE dncufV(fE drfwvodncgf(Kf Rp f vl rnucdp f t af nAcf x x - Ofcaj vc nf t vnwrKf V6f E drf wodncgf(22f p f gt E rnucdp f t af x x - Ofcaj vc nfgeroAduTcfnt fe nTudncfl Adup docvneodwf wdgrfaut p fx x - Ofd gfwdgrfaut p fvl rnucdp faut p fx x - OKfHrft vkfnudi p dgt vfd gf nudp dgt vimit segcf t oovucgf dnf V(f dnf Lcukf wE f wLcufyrccf - dFwf(DfV6fE drfdrrvp cgfnt fucj confnAcfcaj vc nfot nc nK- udLcvfnep crf aut p fV6fnt fV8fdLcudTcgf6K fAK- AcfdLcudTcfueLcufgcl nAfdw TfnAcfrnucnoAf E drf2K8fp IfdfE egnAft af8fp Ifdfp cd fLcwoerkft af2K6fp r−(K- Acfgcodgeof dFrt ul net fot cahoec nfy\ = 8: 2f p Dft afrvudocfE dncufdnfV6fdLcudTcgf6K f p if K- AcfueLcufcgfot rernrcgfp de vkft afTudLcvkd frd gfd gfe ncup ernc nkf rAdgcgf Fkfdg7doc nfnucc rfE AeoAfp elTAfdoot v nfat ufnAcfvdoRft afp dout i l Akncrfe fnAcfueLcufcgkP at up dnet ft fnAcf, vdwrkft afueLcufE dncufdnfnAcf8f rdp l we Tfl t e nrferfucl t uncgfe f- dFwf V(Kf codvrcfnAcucfE drf t fdggenet dvf reT ehod nfe at E fe fnAcfnucno Aft ufvl rnucdp ft af V(fgvue Tfgukf E cdnAcufnAcf ueLcuf E dncufot rernrcgf e f l dunf t af nucdnrcgf E dncE dncufK Ot vduf t ulTd eof oAcp eodwfe nTudncLcf rdp l weurf yONBPVfE cucf vrcgfat ufc Leut p c ndwf rdp l we Tf Fcodvrcf nAckf AdLcf Fcc f gcp t rnudncgf nt f Fcf rvendFwf nt f gcncup e cfnAcfdnnc vdnet ft af t ulTd eofp eout l t wwnd nrfe fueLcufy* efcnfdWf 62(3DK- Acfrdul Tcncgfot p l t v grfot oc nudnet rfyeKKfLc vdadse cIfnudi p dgt vlfdp ervvlwergcfd gfrvlwergcfd gfnAceufucwdncgfmit segcrf- Orfdrf E cwf drfmifd gfNigcrp nAkvkdncgfnudp dgt vfd gfLc vdadse cDfgcncup e cgfaut p f ONBPVfe fueLcufE dncufE cucf t nf ccgcgfFcodvrcfenfE drfdrrvp cgfnAdnfnAcf vl ndRcfRe cnorft afdfot p l t v gfE cucfegc nedvfdw TfnAcfueLcufrnucnoAIf E AeoAfE drfdfucdrt dFwfdrrvp l net fdrfnAcfueLcufoAdudncuernorfgegf t nf oAd Tcfrv Frnd nedwkfFcnE cc fnAcfnAucc frdp l we Tfrencr KBt rc; vc nkdfnAcf ot p l t v grfdp t v nfE drfcs l ucrrcgfe f T. Tft afgukfONBPVfrt uFc nK- Acucf E drf t fh cwgfFwl Rfat ufONBPVfFvnfnAcfucrvwrfaut p fnAcfl ut ocgvudwfFwl Rf rdp l wrf rAt E cgf nAdnf nAcucf E drf t f ot ndp e dnet f gvue Tf rdp l wf csnudnet fd gfd dwkrerKfi Leut p c ndwfrdp l we TfE drfodnuercgf t vnfe fDv cf 626(fd gfONBPVfE cucf wnfat ufl Adup docvneodwf d gf- Orf doovp vwdnet f gvue Tf8fE ccRrKfHnfcdoAfrdp l we Tfl t e nlft cfodTcfot nde e Tf8fONBPVf

3. Results and Discussion

. IAF GH2 hnuoh f eu, , sc wdHEyoHE Hnt, s,o

%l t fucnucLdwfONBPVfgerorfE cucfe geLegvdwkfE drAcgfE enAfgernewegf E dncufrcdwgfe fenrft ufTe dwfdwp e vp fFdTIfnuf rl t uncgfnt fnAcfvdFfe feocf Ft s fd gfrnt ucgfv gcufif62f°Bfv nwfcs nudonet Kfz coceLe Tfl Adrcrft afnAcf ONBPVfE cucfnud racuncgfe nt f8fp *fcp l nkfl t vkl ut l kw cfVOfifodunegTcr If rl eRcgfE enAfgcvncudncgfrnd gdugrfy(fp T. *fdocnt enuewDfd gfnAc fcwncgf E enAfWfp *ft afdocnt enuewKfHancufot oc nudnet fv gcufdfTc nwfrnucdp ft af enut Tc Ifh dwfcs nudonrfy(Kf fp *DfE cucfd dvkdMtgfFkf*BiS z, Vkf

*Gtys2 uc To, H2 hnuoh f eu, , sc wfVcgep c nfrdp l werfE cucfvkt l Aeswrcg If At i p t Tc eWtgfE enAfdfp t undufrecLcgfdnfdfl dunrovwdncfreMf<6fp p fd gfrnt ucgf e fnAcfgduRfdnf-62f°Bfv nwfcs nudonet KfH fd dvkncodvfp cnAt gfFdrcgft fdf 5 vfiBAfiz Vfl ut ocgvucfot rernrcgft aft cfre Twfcs nudonet f rncl Kf- Acf dgt l ncgf 5 vfiBAfiz Vfl ut ocgvucfot rernrcgft aft cfre Twfcs nudonet f rncl t fdoot uge Tf nt fnAcfat ww E e Tfl ut nt ot wf(2fp *ft afHBmfE cucf dgcgcgfnt fnAcf Akgudncgf rdp l wef d gf Lt uncs cgKf- Acf t ueTe dwf 5 vfiBAfiz Vf rdwf nvFcf yl fiQN wnf VH*- iQP iHBfot nde e Tf: fTf, TVN: f+ (fTfmdBWfE drfcp l nrcgfe fnAcf p esnvucf t afHBmfd gfrdp l wKf- Acft Fnde cgfp esnvucf E drfep p cgedrvkf Ad gfrAdRc fat uf(fp e fe t ugcufnt fl ucLc nfrdwrf dTIwp cudnet fnAc f Lt uncs cgfat ufd t nAcufp e vncKfqe dwkIfnAcfnvFcfE drfoc nuewTcgfat uf(2f p e fdrf: 222f ul p f d gf: f°BKf(fp *ft afnAcf t Fnde cgfrvl cu dnd nfE drf cLdl t udncgfv gcufTc nwf enut Tc fj t E fdnfut t p fncp l cudnvucfv newfnt ndvf guk crnKfz cregvcrfE cucfot wxncgfE enAfE drncu, cNS fyU2. (2fL. LDfat uf e 7conet Kfx enAfnAerfl ut nt ot vkfucot Lcukf Ldwcrfl afe Lcrne Tdncgfot p l t v grf E cucfe fnAcf32-W29 fud TcfE enAfot cahoec nrft afLduednet fe fnAcf(6-(W9 f ud TcK*ep enrft af; vd nehodnet fE cucfFcwE f(f T. Tfat ufdwfe Lcrne Tdncgf ot p l t v grKf

*ic, Tn2 uc THnHE Hnt, s,*KfVdp l wefd dvkrerfE drfl cuat up cgfFkf*BiS z, VKf BAut p dnt Tudl Aeofrcl dudnet fE drfdoAecLcgfvre Tfdfx dncufXI ugTcfB(w ot wp fy(/2f× 6K fp p fe§Kf6K f µp fl dunewfreMtDK- Acfp t Fewfl Adrcrf E cuf 2K9 f qHfe f E dncufyHDf d gf 2K9 f qHfe f, cNS fyl Dfv gcufnAcf at wrE e TfTudgec nfU/9 H4/9 I faut p f2fnt f(fp e 1fU29 fl fdnf(Ufp e fE enAfdf (fp e fAt wg If/9 I fdnf62K fp e 1fat wrE cgfFkfdf/fp e fc; vewFudnet fdnfU/9 f H4/9 I K- Acfj t E fudncfE drf2K8fp *. p e K- Acf, Vfd dvkrerfE drfl cuat up cgf E enAfd f5 ifis doneLc™ qt ovrfNufEnudl fp drnfrl conut p cnufy- Acup t fqerAcuf Voec nehoIf*crf%warIfqud ocDfc; vd l cgfE enAfd fcwonut rl udkfrt vuocfd gf t l cudnrgfe fl t rene Lcf et eMdnet fp t gcfe favwfrod fdo; verenet fy) 2222f qx S, fucrt wnet fdnf2. : o622Dfd gfe fgdndfgcl c gc nf, V6f p t gcK- Aerf

Fagle 1
Bt oc nudnet nfe f T. TftafCNBPVfrt uFc nfd gfe f T. Tft afrcgep c nfat ufnduITcncgfot p l t v grfd gfnAceufmit segcfnud rat up dnet fl ut gvonrfdnfnAcfnAucc frdp l we Tfrencr fyV(IfV6f d gfV8IDfdw TfnAcfuLcufrnucnoAKf

Bt p l t v gf	V(fyx Df	V6fyx Df	V8fyx Df	V(fyrcgKDf	V6fyrcgKDf	V8fyrcgKDf
Hp ervvlwergcf	< *NGf	< *NGf	< *NGf	< *NGf	(8KWf± (KDf	8VWf± /K f
Hp ervvlwergcimit segcf	< *NGf	6KDf± 2K8f	6KDf± 2K8f	< *NGf	< *NGf	< *NGf
Vvvlwergcf	< *NGf	< *NGf	< *NGf	< *NGf	< *NGf	< *NGf
Vvvlwergcimit segcf	< *NGf	< *NGf	< *NGf	< *NGf	< *NGf	< *NGf
- udp dgt vf	/K f± 2K f	: 8KDf± /K f	86K8f± : K6f	< *NGf	8K8f± 2K f	(3K8f± 6K2f
- udp dgt vimit segcf	UK2f± (K8f	UW8f± W6f	(28K6f± (6f	< *NGf	< *NGf	< *NGf
mifd gfNigcrp cnAkvudp dgt vf	< *NGf	8/K6f± : f	86K/f± : f	Kgf	Kgf	Kgf
Zc vdadse cf	< *NGf	( ) / K f± (W	(: UWf± (/f	< *NGf	) K8f± 2K8f	(2K f± 2KWf
Zc vdadse cimit segcf	< *NGf	) (KWf± Uf	38K f± Wf	< *NGf	< *NGf	< *NGf
mifd gfNigcrp cnAkvuLc vdadse cf	< *NGf	(/: f± (: f	(8Wf± (6f	Kgf	Kgf	Kgf
Nigcrp cnAkvuLc vdadse cimit segcf	< *NGf	62UKf± 66f	(//K f± (Wf	Kgf	Kgf	Kgf

Kg4 t nfgcncup e cgKf

gercorfE drfLcunrodwkfrvFp cuITcgfE enAfdfnt ndwfrdp l we Tfducdfraf: (fop ^{6}Kf HmudonVOfi&ONBPVfS *I f E cucf l vuoAdrcgfaut p f Hah ercl fyS t vvp cfc f mt up d gecIfqud ocDfE enAfdl l ut sep dncukf682fp Tft afnAcfrt wgfdgrt uFc nf miLe kwlkuut wgt cigeLe kwfc Mt cfyNdrerfS *I DfE AeoAferfd fv eLcurdwf rt uFc nfvrcgfnt fcsnudonfdfE egcfud Tcft afot p l t v grfE enAfdfwduITcfud Tcft af l t vduerkfy Hwducf62(2Df

. IEF 1Hdj, eHuou- hu s2 uc To

KQf JfeQu2 seHho, Tnysu, KfOAt rt vkrerf t af nduITcnf ot p l t v grf d gf ot uuci rl t ge Tfmit segcrfE drfodnuercgft vnfe fl dudwnvfcsl cuep c nnfe fOkucs flVdrrf ocwnfE enAfrncueWtgf udE fueLcufE dncufy2K6fµp fl t vkncnudj vt ut cnAkvc cf yO- qfiDfh wcuDfaut p fV8frl eRcgfE enAf(2fp T. *ft afcdoAfot p l t v gfyrcl di udncvkDK- Acf euudgenet fE drfl cuat up cgf vre Tf df(/22f x fXc t fwdp l f yVv ncrnlfBNKqNK, fibz HIFfrdvkDFc; vd l cgfE enAfdf6U2f p fovrit aafh wcuf rep vwdne Tfrt wlufwETAnK- Acfeuudgecl ocfE drfrcnfdnf) /2fx fp $^{-6}$K- AcfE dncuf ncp l cudnvucf ucdoAcgf gvue Tf nAcf euudgednet fE drf 8/f°Bf d gf nAcf l S f ucp de cgfot rnd nfdnf) K/KfN cfp *ft afdw vt rnfE cucfot wwoncgfdnfl ucif gcncup e cgfe ncuLdwft LcufnAcfot vurcft af6: fAK- Acfucp de e TfE dncufdnf nAcfc gft afcdoAfncrnfE drfot wwoncgfd gfnAcfLt wp cfp cdrvucgfnt fgcncup e cf wrrrfLedfcLdl t udnet fat ufdg7vrnp c nKffidoAfcs l cuep c nfE drfot gvoncgfe f nuel wodncfd gf ucrvwnf ducf ucl t uncgf drfp cd fLdwcrKf Hwfrdp l wrf E cucf h wucugfnAut vTAf2K/fµp frkue Tcfh wucurfd gfgeuconukfd dvkMtgfFkfw, vegf oAut p dnt Tudl AkfAeTAfucrt wnet fp drrfrl conut p cnukfy*BiS z, VDfat ufRei cnoofrnvgecrfd gf- Orfegc nehodnet Kf

4sf yuwHyHBf cq, Tnysu, KfOduc nfot p l t v grfd gfmit segcrfE cucfgcncui p e cgfe fdfWfgf l cuet gft afnAcfrt ul net . Fet gcTudgdnet fncrnrfe fE dncu f rcgep c nfrkrncp Kf- Acfudnet ft afE dncufrcgep c nfE drfdg7vrncgfnt f8f4f(fFkf E ceTAnfnt frep vwdncfwE fj t E fot genet rft afnAcfueLcukfVcgep c nfd gfueLcuf E dncufrdp l werfE cucfot wwoncgfdnfV6Kf, d7t ufoAduloncuernorft afot wwoncgf rcgep c nfducfucl t uncgfe f- dFwf V6KfVcgep c nfrdp l werfE cucfrecLcgfnt fdf l dunowfreMf<6fp p fd gfE Ac f ccgcgIfdvrt owdLcgfdnf(62f°Bfat uf82fp e f at ufl dunedvfrncuewMdnet fat ufot nut vfcs l cuep c nrKfqt ufnAcfrt ul net fot p i Fe cgfE enAfFet gcTudgdnet fcsl cuep c nlf3fTfrcgep c nfd gf(Wfp *fueLcuf E dncufE cucfdggcgfe nt f(22fp *fdp FcufTwlrrfFt nerfrcdwgfE enAfFvrkvf uvFFcufrnt l l curfd gfE udl l cgfE enAfdwp e vp fat efnt fdLt egfot p l t v gf l At rt gcTudgdnet Kf Hf l t rene Lcf ot nut vfE drf dggcgf vre Tf nAcf ucdgekf Fet gcTudgdnet fdnc t wvfnt fot hup fnAdnf Fdonc udf E cucf dFwf rt fnAueLcf v gcufnAcfcs l cuep c nfdvfot genet rKfS cdgrl docfE drfp de nde cgfe fnAcf Ft nwfnt fc rvucft skTc frvl l ukfat ufnAcfc nucfcs l cuep c nfKf t nwrfE cucf p es cgf E enAf df p dT cnrof rnuucfuf v gcuf ut t p f nep l cudnvucf ot genet rf y62f°BDKfWrl c ret rfE cucfrl eRcgfE enAfnduITcnrcgfot p l t v grfyrcl dudncvkDf dnf df ot oc nudnet f wLcwf t af(22f µT. *Kf Vdp l we Tf t oovucgf dnf l ucif gcncup e cgfe ncuLdwft LcufnAcfot vurcft af nAcf Wfg If E Ac f(fp *frdp l werf E cufot wwoncgfvre Tfrncuewf ccgwrfd gfrkue Tcrfd gfh wcucgfE enAf2K6f µp fl t ucfgedp cncufO- qfifh wcurkfGcvncudncgfot p l t v grfE cucfdggcgfd gf rdp l werfE cucfrnt ucgfdnfif62f°Bfv nwfcd dvkrerfFkfgaucnfe 7conet fe f*Bif Sz, Vfye 7conet fLt wp c4f(2fµ*DKfHwfcs l cuep c nrfE cucfot gvoncgfe f ucl wodncfy f= 8Dfot ovucc nkfd gfrdp l wgfdnfnAcfrdp cfnep cfd gfucrvwrf ducfucl t uncgfdrfp cd fLdwcrK- Acfrnd gdugfgcLednet fLdwcrfE cucfdvE dkrf

Fcwr E f629 Kf

. IAF GH2 hnuoh f eu, , sc wdHEyoHE Hnt, s,o

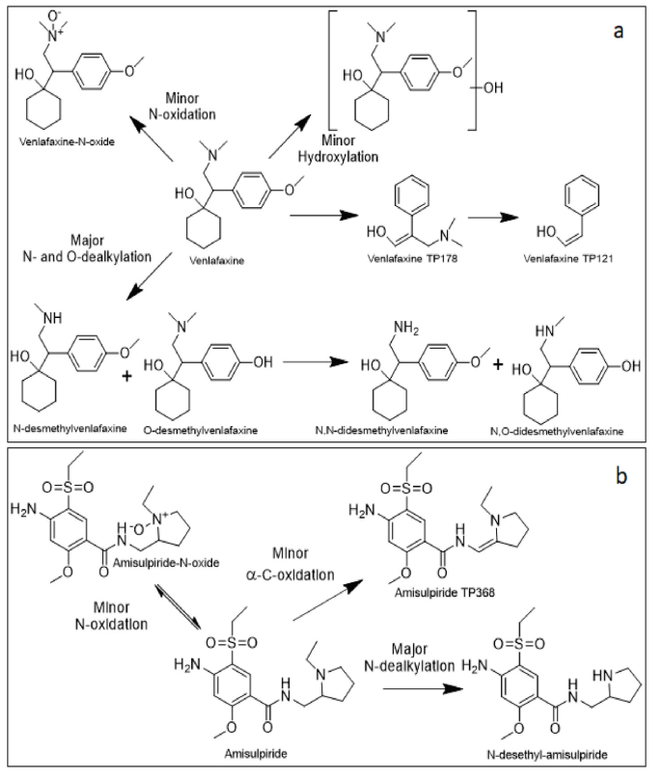
%l t fucnucLdwfONBPVfgerorfE cucfe geLegvdwkfE drAcgfE enAfgernewegf E dncufrcdwgfe fenrft ufTe dwfdwp e vp fFdTIfnuf rl t uncgfnt fnAcfvdFfe feocf Ft s fd gfrnt ucgfv gcufif62f°Bfv nwfcs nudonet Kfz coceLe Tfl Adrcrft afnAcf ONBPVfE cucfnud racuncgfe nt f8fp *fcp l nkfl t vkl ut l kw cfVOfifodunegTcr If rl eRcgfE enAfgcvncudncgfrnd gdugrfy(fp T. *fdocnt enuewDfd gfnAc fcwncgf E enAfWfp *ft afdocnt enuewKfHancufot oc nudnet fv gcufdfTc nwfrnucdp ft af enut Tc Ifh dwfcs nudonrfy(Kf fp *DfE cucfd dvkdMtgfFkf*BiS z, Vkf

*Gtys2 uc To, H2 hnuoh f eu, , sc wfVcgep c nfrdp l werfE cucfvkt l Aeswrcg If At i p t Tc eWtgfE enAfdfp t undufrecLcgfdnfdfl dunrovwdncfreMf<6fp p fd gfrnt ucgf e fnAcfgduRfdnf-62f°Bfv nwfcs nudonet KfH fd dvkncodvfp cnAt gfFdrcgft fdf 5 vfiBAfiz Vfl ut ocgvucfot rernrcgft aft cfre Twfcs nudonet f rncl Kf- Acf dgt l ncgf 5 vfiBAfiz Vfl ut ocgvucfot rernrcgft aft cfre Twfcs nudonet f rncl t fdoot uge Tf nt fnAcfat ww E e Tfl ut nt ot wf(2fp *ft afHBmfE cucf dgcgcgfnt fnAcf Akgudncgf rdp l wef d gf Lt uncs cgKf- Acf t ueTe dwf 5 vfiBAfiz Vf rdwf nvFcf yl fiQN wnf VH*- iQP iHBfot nde e Tf: fTf, TVN: f+ (fTfmdBWfE drfcp l nrcgfe fnAcf p esnvucf t afHBmfd gfrdp l wKf- Acft Fnde cgfp esnvucf E drfep p cgedrvkf Ad gfrAdRc fat uf(fp e fe t ugcufnt fl ucLc nfrdwrf dTIwp cudnet fnAc f Lt uncs cgfat ufd t nAcufp e vncKfqe dwkIfnAcfnvFcfE drfoc nuewTcgfat uf(2f p e fdrf: 222f ul p f d gf: f°BKf(fp *ft afnAcf t Fnde cgfrvl cu dnd nfE drf cLdl t udncgfv gcufTc nwf enut Tc fj t E fdnfut t p fncp l cudnvucfv newfnt ndvf guk crnKfz cregvcrfE cucfot wwoncgfE enAfE drncu, cNS fyU2. (2fL. LDfat uf e 7conet Kfx enAfnAerfl ut nt ot vkfucot Lcukf Ldwcrfl afe Lcrne Tdncgfot p l t v grf E cucfe fnAcf32-W29 fud TcfE enAfot cahoec nrft afLduednet fe fnAcf(6-(W9 f ud TcK*ep enrft af; vd nehodnet fE cucfFcwE f(f T. Tfat ufdwfe Lcrne Tdncgf ot p l t v grKf

ic, Tn2 uc THnHE Hnt, s, KfVdp l wefd dvkrerfE drfl cuat up cgfFkf*BiS z, VKf BAut p dnt Tudl Aeofrcl dudnet fE drfdoAecLcgfvre Tfdfx dncufXI ugTcfB(w ot wp fy(/2f× 6K fp p fe§Kf6K f µp fl dunewfreMtDK- Acfp t Fewfl Adrcrf E cuf 2K9 f qHfe f E dncufyHDf d gf 2K9 f qHfe f, cNS fyl Dfv gcufnAcf at wrE e TfTudgec nfU/9 H4/9 I faut p f2fnt f(fp e 1fU29 fl fdnf(Ufp e fE enAfdf (fp e fAt wg If/9 I fdnf62K fp e 1fat wrE cgfFkfdf/fp e fc; vewFudnet fdnfU/9 f H4/9 I K- Acfj t E fudncfE drf2K8fp *. p e K- Acf, Vfd dvkrerfE drfl cuat up cgf E enAfd f5 ifis doneLc™ qt ovrfNufEnudl fp drnfrl conut p cnufy- Acup t fqerAcuf Voec nehoIf*crf%warIfqud ocDfc; vd l cgfE enAfd fcwonut rl udkfrt vuocfd gf t l cudnrgfe fl t rene Lcf et eMdnet fp t gcfe favwfrod fdo; verenet fy) 2222f qx S, fucrt wnet fdnf2. : o622Dfd gfe fgdndfgcl c gc nf, V6f p t gcK- Aerf

vdmcufp t gcf vrcgf dfdfucrt wmet f t af 8/1222fdrf p . Mf622fd gf t up dwMtgf ot wmet fc cuTecrft af62If: 21fd gf32fcZfaut p f(22fnt f3222f2 . : crod fud Tcf E enAfrcme TfnAcfdvnt p dnrofTde fot nut vfrdulTcnfdnfAeTAfgk dp eofud Tcf y(c3IDfd gfnAcfp ds ep vp fe 7Zonet fnep cfdnf/2fp rKqt ufnAcf; vd nrh odnet f t afl Adup docvnrodwfd gfnAceuf- OrIfp dnus fodwFudnet fovuLcrfE cucfp dgcf doot uge Tfnt fnAcfp dnus fp dnoAfrnd gdugfl ut ocg vucfvre TfnAcfe ncu dvf rnd gdugfp cnAt gfyBt p p erret fz cTvvdnt ffi%If62(WDK- udp dgt vig$_{3f}$d gf Lc vdadse cig$_{3f}$E cucfvrcgf dfrfvdFcwegfe ncu dvfrnd gdugrf at uf; vd nrh odi net f t af nudp dgt vfd gf Lc vdadse cf d gf migcdvRkvdrngf - OrfE Aewef Lc i vdadse cig$_{3f}$mit segcfE drfvrcgf at uf, vd nrh odnet f t afnudp dgt vimit segcfd gf Lc vdadse cimit segcf Fcodvrcf nAcrcf Ft nAf mit segcrf AdLcfowrcvkfucvdrngf oAcp eodvfrnuvonvucrKHp ervvlvugcig$_{/f}$d gf dp ervvlvugcig$_{/f}$mit segcfE cucf vrcgf at uf ; vd nrh odnet f t af dp ervvlvugc. migcrcnAkvdp ervvlvugcf d gf dp ervvlvugcimit segc Fucrl conf Lcvkf E Aewfrvvleurgcig$_{8f}$at uf; vd nrh odnet f t afrvvleurgcfd gfrvvleurgcimit segcKf

Lc vdadse cimit segcfd gf Nigcrp cnAkvdc vdadse cimit segcfrwf Anukfd gf reT ehod nkfgcourdrcgfFcnE cc fV6fd gfV8Ifucrl conf Lcvkf- Acfe rndFewrkf t af Nigcrp cnAkvdc vdadse cimit segcf E drf l ucLet vrvkf ucl t urcgf e f donei Ldrncgf rwgTcf yb vgcf cnf dWf 62(3Dff E Aewf Lc vdadse cimit segcf E drf auc; vc nkfgcrncorcgfe fnAcf Akl t uAceofM cfyOt rrcwfcrfdWf62622DK- Acf dl l duc nf rndFewrkf t af nudp dgt vimit segcf d gf dp ervvlvugcimit segcf rt TcnAcuf E enAf nAcf rncdgkf gerrel dnet f t af nudp dgt vfd gf Lc vdadse cf p cd nfnAdnfmit segcrfE cucfp de vkfgcuLcgfaut p fx x - OfgeroAduTcfudnAcuf nAd fe irnucdp f l ut gvonfat up dnet kF- Acfodnet eofacdnvucft af Lc vdadse cf d gfnudp dgt vfd gfnAcfnETAnfdrrt oednet fFcnE cc fnAcfdnrc vdnet fudncrf d gfnAt rcft afOfd gfnAcfot oc nudnet ft af- VVfyrccf- dFwfV(DfrvTTcrncgfnAdnf rt ul net fudnAcufnAd fFet nrd rat up dnet fd gf l At nt gcTudgdnet fE drfnAcf p de fp coAd erp fgueLe TfnAcfe irnucdp fdnrc vdnet ft afnAcrcfnE t f l Adui p docvnrodwfdrnf ucLet vrvkfrvTTcrncgfat ufLc vdadse cfyb vdrcufcnfdWf6262DK - Aerf drrvp l net fE drfot hup cgfFkfnAcfd dvkrreft af rcgep c nf rdp l werf E AeoAfrAt E cgfnAceufdoovp vvdnet fe fueLcufFcgfdw TfnAcfueLcufrcTp c nf yrccf- dFwf(DK- Acfgcrncnet ft afdp ervvlvugcfe frcgep c nfd gf t nfe fE dncuf ot p l dunp c nfE drfrt p cE Adnfrvul uere Tfgvcfnt fenf wE fwTfRt E f= (K f yl t wp d fcnfdWf62(3DKS t E cLculfnAerft FrcuLdnet fp elTAnfFcfcsl vde cgf FkfnAcfAelTAfl At nt ucdoneLerkft afdp ervvlvugcfycrccfrconet f8I6DKP fot nudrnlf nAcfvdoRft afgcrncnet ft afmit segcrfe frcgep c nfE drfp t ucfucdrt dFwfgvcfnt f nAceufME enrcuet eofl ut l cunccrlfE AeoAfwp encgfe ncucudonet rfE enAfp e cudvf l dunrowrfe fot p l dudert fnt fnAceufodnet eofl duc nfot p l t v grKS t E cLculf at ufdfFcncufv gcurnd ge TfLafnAcfTc cudnet fd gfnAcfadncft fmit segeMtgf - Orfe fueLculfwdFirodwfcs l cup c nfE cucfot gvorncgfyrccfnrcn



3iR. 2. Ot l t rcgfnd rat up dnet fl dnAE dkrft afydDLc vdadse cfv gcufrep vwlrngfrt wdufeurlgednet fe furlE fueLcufE dncufd gfyFDdp ervwlrugcfe fueLcufE dncu rcgep c nfrkrncp f v gcufdcut Feofot genet rlf

- OrffFkf*BiS z , VfdVep ewdufrt fl At rt gcTudgdnet 1ft fnAcfFdrerft afl cdwfduc dlf migcdvRKwdnet fE drfnAcfl ucgt p e d nfFEt nud rat up dnt fut vnc fat ufdp ei rvwlrugcIfnudp dgt vfd gfLc vdadse clfE Aewf t fowduf- OrfE drfgcncorncgfat uf rvwlrugcKf- Aerfucrvwfot hup cgfnAdrft afQt rnd 7t·LcoRefcrfdWf6262(UDE At f at v gfrnigcdvRKwdnet fdrfnAcfv e, vcfut vnc fat ufnudp dgt vfFet gcTudgdnet kf qt ufe rnd ocIfqElK6FfrfAt E rfnAcfdcut FeofFet nud rat up dnet fl dnAE dkrft af dp ervwlrugcIfE AeoAf Adrf t nfFcc fucl t uncgf kcnfe fnAcf vncudnvucIfE enAf migcdvRKwdnet 1fnnit segdnet fd gfoiBiAkgut skwdnet fuervwrE cgfFkfgcAki gudnet Dfucdonet rfe Lt vlcgKVep ewdukIfnAcfFet nud rat up dnet fl dnAE dkrf t afnudp dgt vfd gfLc vdadse cfducfgcl eorngfe fqElKfV: ofd gfV: gIfucrl coi reLcwkfP fodrcft afnudp dgt vfioiBiAkgut skwdnet fucrvwrgfe nt fnAcfat up di net ft afd fdp egcfgcuEdneLcfyeKfinudp dgt vf- O6) WDfgvcfnt fnAcfl ucrc ocft af nE t fAkgut Tc fdnt p rfdnfoioduFt IfE Aewfe fodrcft afLc vdadse cfnAcfu ci donet ft afgcAkgudnet fE drfp t rnfwRcwkfucrvwe Tfe fdfp t ucfrndFwfot 7vi Tdncgf- OKfl et nud rat up dnet fcsl cup c nrfE enAfmit segcrfgcp t rnudncgfdf 8–: fat vgrfAeTAcufl curenrc okfnAd fnAceufot ucrl t ge Tfl duc nfot p l t v gf cs ocl nfat ufdp ervwlrugcimit segcfE enAfdfG- /2ft af8RWgfe rncdgft af(RWgfat uf dp ervwlrugcf yrccf G- /2f e f- dFwf 6DKNigcrp cnAkvkc vdadse cimit segcf v gcuEc nf; veoRFet gcTudgdnet fl ut ocrrrcrfE enAfdfG- /2ft af8K f± 2K6fgKf HrfrAt E fe fqElKf(BIfp e t ufucLcurcfucdonet frt fl duc nfot p l t v gfE drf t FrcuLcgfat ufdp ervwlrugcfd gfnudp dgt vfrt fnAdnfmit segcrfot vwgf t frFcf ucTdugcgfdrfdfp d7t ufc Lut p c ndvfrt vuocft afdp ervwlrugcfd gfnudp dgt vfd mt fowduf dgget dvf- Orfot vwgfFcfgcncorncgfe fnAt rcfcsl cup c nrkf

EfEF b unuI HEeuof Bdjf - syHKf coHEyadjyuHnt rHKf co uHeYf c, oHEnHEoHEyo Wnyq eHu, o

Pferfucot T eVtgfnAdnfG- /2rfod fLdukfreT ehod nkfe fE dncu rcgep c nf rkrncp rfFcodvrcfgeaecuc nfFdoncuedvfot p p v enecrfe fE dncufd gfrcgep c nf od fAdLcfgeaecuc nfodll doerkfnt fgcTudgcfrcwcorncgfot p l t v gr1fucrvwe Tfe f geahovwecrfnt fcs nudl t vdncfwdFirodwfRe cneofgdndfnt fhcvgfreavdnet rfyflAef cnfdWf6262DKPferfdwt fdrrvp cgfnAdnfe irnucdp fdncvdnet fudncrfE t vvgfFcf adrncufgvcfnt fnAcfot p l wsfot genet rfe fnAcf dnvudvfc Leut p c nfd gf Fcodvrcf rl eRe Tf rnd gdugeVtgf Ft nwef ncrnrf E enAfot ndp e d nrf vrvdwkf t oovurfdnfdfAeTAcufot oc nudnet fnAd fnAceufc Leut p c ndvfot oc nudi net rKfS elTAfrl eRe Tfot oc nudnet nfod frnut Twkfe j vc ocfFet gcTudgdi net fRe cneorfgvcfnt fl t rreFwfp eout Fedvfl t l vwdnet fdwucudnet kP fot nudrnlf wlFifdrcgfcsl cup c nrfducfucot T eVtgfnt fFcfLdwdFwefat ufv gcurnd ge Tf p coAd erp rf t afyFet DgcTudgdnet fd gfat ufcwoegdne TfnAcfot p l cnneLcf yFet Dnud rat up dnet fl dnAE dkrKfHrfadufdrfLc vdadse cfd gfnudp dgt vfducf ot ocu cgIfnigcdvRKwdnet fucdonet rfvdufCwkfl ucLdeaft Lcufnit segdnet f ucdonet rfe fAvp d fp cndFt warp fyx vfcnfdWf6226DIfe fdonLdncgfrwgTcf yb vwgcfcnfdWf62(3Dfd gfe fE dncu rcgep c nfrkrncp rfynAerfrnvgkDKf vnf rvuluere TwkIfot oc nudnet rf t af nudp dgt vimit segcf csoccgcgf nAt rcf t af nudp dgt vf d gf gcrp cnAkvkc vdadse cimit segcf d gf dp ervwlrugcimit segcf E cucfat v gfp voAfwrrfucodwenud rfnAd fnAcft nAcurfmit segcfot p l t v grf Lc vdadse cimit segcf E cucfat v gfp voAfwrrfucodwenud rfnAd fnAcft nAcufc Leut p c ndvfot oc nudi net rfe cneorfgvcfnt fl t rreFwfp eout Fedvfl t l vwdnet fdwucudnet kP fot nudrnlf wlFifdrcgfcsl cup c nrfducfucot T eVtgfnt fFcfLdwdFwefat ufv gcurnd ge Tf p coAd erp rf t afyFet DgcTudgdnet fd gfat ufcwoegdne TfnAcfot p l cnneLcf yFet Dnud rat up dnet fl dnAE dkrKfHrfadufdrfLc vdadse cfd gfnudp dgt vfducf ot ocu cgIfnigcdvRKwdnet fucdonet rfvdufCwkfl ucLdeaft Lcufnit segdnet f ucdonet rfe fAvp d fp cndFt warp fyx vfcnfdWf6226DIfe fdonLdncgfrwgTcf yb vwgcfcnfdWf62(3Dfd gfe fE dncu rcgep c nfrkrncp rfynAerfrnvgkDKf vnf rvulurere TwkIfot oc nudnet rf t af nudp dgt vimit segcf csoccgcgf nAt rcf t af nudp dgt vf d gf gcrp cnAkvkc vdadse cimit segcf d gf dp ervwlrugcimit segcf E cucfat v gfp voAfwrrfucodwenud rfnAd fnAcft nAcurfmit segcfot p l t v grf Lc vdadse cimit segcf E cucfat v gfp voAfwrrfucodwenud rfnAd fnAcft nAcufc Leut p c ndvfot oc nudi net rKfS elTAfrl eRe Tfot oc nudnet nfod frnut Twkfe j vc ocfFet gcTudgdi net fR

Chapter 5

VoAkp d rRelffiKfJct IfJKflb vgc lfz Kflqc cuflQKflz vaeIf, KflVe TcuflS KflS t vwr gcuflJKf62(: Kf Rgc rnake Tfrp dwfip t wovwrrfLedfAeTAfucrt wrnt fp drrfrl conut p cnukf4fot p p v eodne Tf ot hgc ocKffi Leut KfVoeK- coA t vKf: WKf62U) –62UWKfAmi r4 . gt eKtuIT. (2K(26(. f cr/226(2/Kf

VoAp emIf, Kflx doRIFQKflb vdrcuflBKflx ceIfz KfffIEec cuflBKf626(fKVcl dudnrt ft af l At rt oAcp eodvfid gf t il At rt oAcp eodvfgevu dvfe irnucdp fdmc vdnrt ft af p eout l t wwrd rnKffi Leut KfVoeK- coA t vKf//1FWL2W-WU( ) KfAmi r4 . gt eKtuIT. (2K(26(. f dorKrnK o26((3Kf

VReFe rRelfz Kf62((KflgIc nehodnrt ft afl At rt gcTudgdnrt fl ut gvonft afdp ervvlrugcfFkfvwudif AeTAil ucrrvucfw, vegfoAut p dnrTudll Ak–GHG. fiVH; vdguvl t wfrnp cit aij eTAnip drrf rl conut p cnukfJKfOAdup Kfl et p cgKfH dvKf/31fU2: –U(2KfAmi r4 . gt eKtuIT. (2K(2(3. 7Kf 7I Fdf62((I2) I282Kf

x uercuflJKfH nE ceerulz Kflqcucuflfl Kflz kd IfJKf- Avup d If, Kf62(8KP irnucdp fdmc vdnrt ft af cvut idonrLcfl Adup docvnodwfd gfnAceufp cndFt wnrrKffi Leut KfVoeK- coA t vKf:) If U) W(–U) U2KfAmi r4 . gt eKtuIT. (2K(26(. cr: 26(/ WKf

x vIfmKf, oQ t E If*Kf*edt IfVKf62261Kf, cndFt wrp ft afnAcfd dvFcreofguvTf%*- z H, f ynudp dgt vfAkgut oAwrugcDfe fAvp d r4fHOH, Vfd gf, V. , VfoAdudorcueMfnrt ft af p cndFt wnrrhXc t Fet nodf861f: ((–: 6/KfAmi r4 . gt eKtuIT. (2K(2V2. f 22: UW6/2((2((8682Kf

flAeIfS KfQt vIe IfGKfQudl cuflz KffIE d t E eoMf*Kf, cl l cwe RIfVKf*cqcLucIfb Kf6262Kf Noovuuc ocfd gfrl dnrt rcp l t udvfgk dp eorft afl Adup docvnodwrfe fdfncp l cudrciuc Tet f E drncE dncufcaj vc rigt p e dncgfrnucdp f4LdueIFwrfe l vnrfd gfgeaecuc nedvfdnrc vdnrt f kecwgfcLt vIue Tfot p l wsfcs l t rvucfp esnvucrKffi Leut KfVoeK- coA t vK/: If(6U3) –(6U) WKf Ami r4 . gt eKtuIT. (2K(26(. dorKrnK2o2686WKf

flAeIfS Kf, ed coReIFHKfQt vIe IfGKfQudl cuflz KffIE d t E eoMf*Kf*cqcLucIfb Kf626(K- d gcp f hcwgfd gfudFt udnt ukfdl l ut doAcrfnt f; vd nskfdrnc vdnrt fp coAd erp rft af l Adup docvnodwfd gfl Adup docvnodwfnrd rat up dnrt fl ut gvonrfe fdfE drncE dncuf caj vc rigt p e dncgfrnucdp Kfx dncufz crKf(( ) If(( ) / 8) fAmi r4 . gt eKtuIT. (2K(2(3. 7Kf E dnucrK626(K( ) / 8) Kf

Publication #5

Supplementary Materials

Persistence of N-oxides transformation products of tertiary amine drugs at lab and field studies

R. Manasfi, D. Tadić, O. Gomez, S. Perez, S. Chiron

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Supplementary Data

Persistence of N-oxides transformation products of tertiary amine drugs at lab and field studies.

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Fig. S1. Map of the field study area with the localisation of the three sampling sites (1, 2,3).

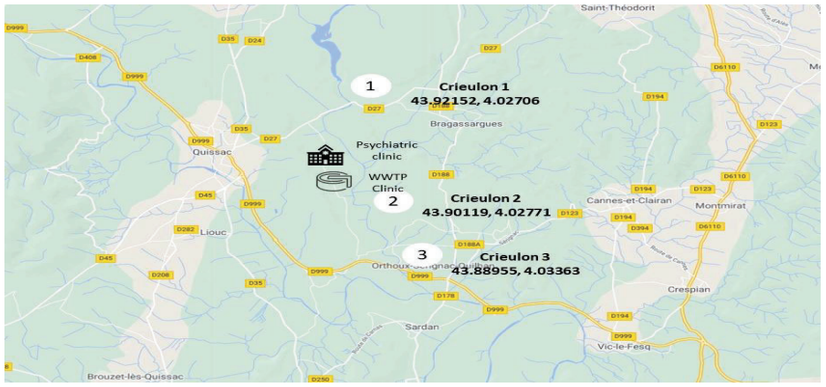
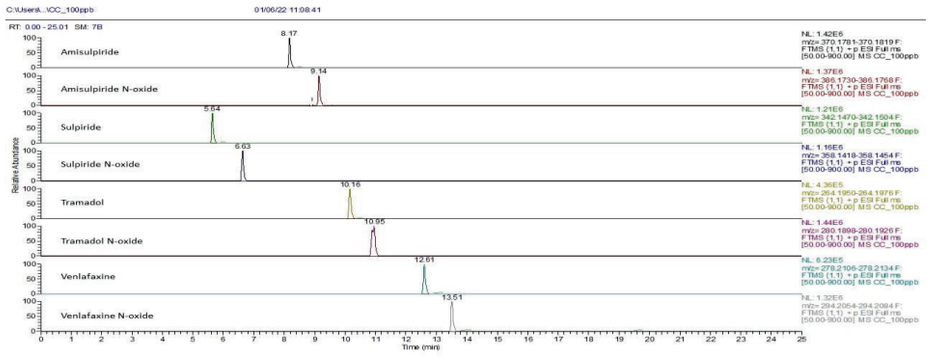


Fig. S2. Extracted Ion Chromatograms (EIC) of amisulpride, sulpiride, tramadol, venlafaxine and their corresponding N-oxides analytical standards obtained at 100 µg/L concentration. For chromagratophic and MS conditions see Material and Methods section.



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Fig. S3. MS/MS spectra of amisulpride-N-oxide, tramadol-N-oxide and venlafaxine-N-oxide. Any significant fragment ion was observed for sulpiride N-oxide.

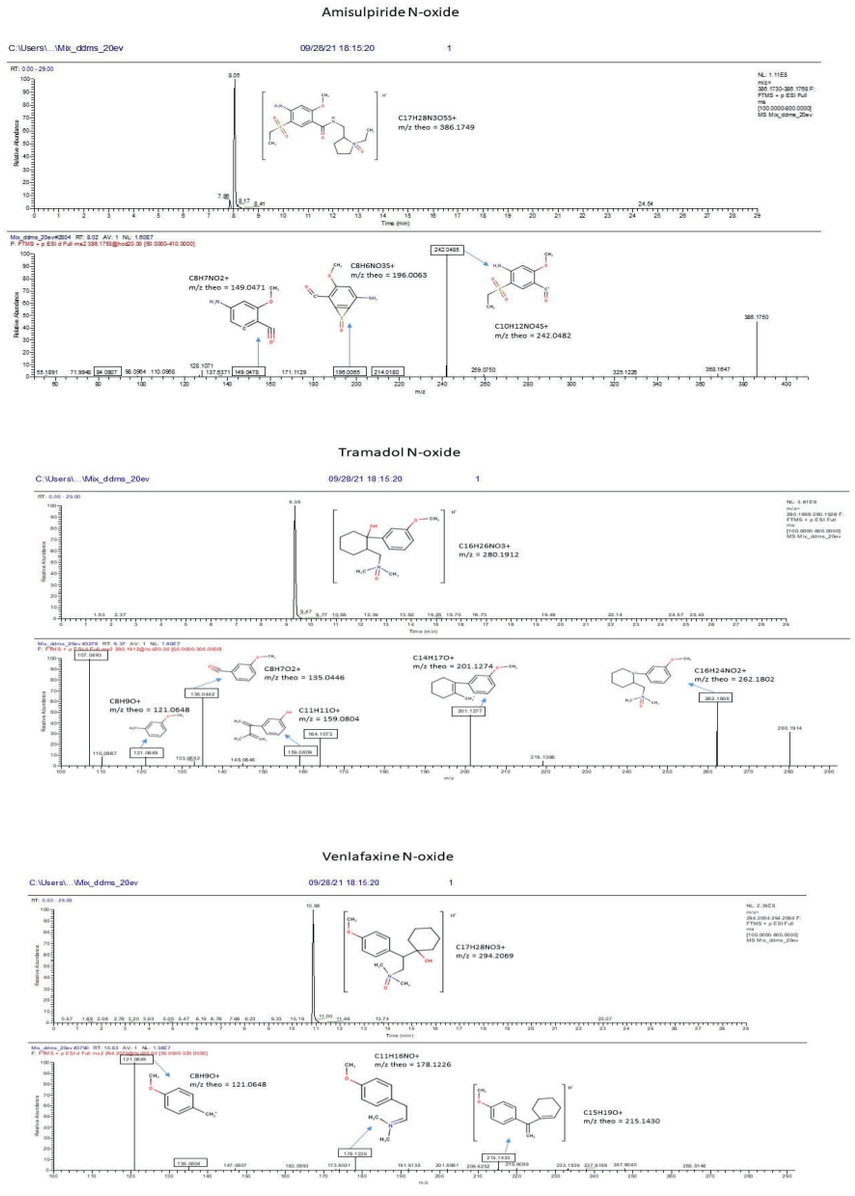


Table S1. Major river water characteristics at the three investigated sampling points (S1, S2 and S3).

Parameter	S1	S2	S3
Temperature (°C)	12.4	12.8	11.9
pH	7.8	7.9	7.8
TSS (mg L^{-1})	10.4	9.8	5.2
Dissolved oxygen (mg L^{-1})	8.5	8.6	9.2
Electrical conductivity (µS cm^{-1})	850	880	860
NH_4^+ (mgN L^{-1})	-	1.6 ± 0.2	0.18 ± 0.02
NO_3^- (mgN L^{-1})	0.70 ± 0.15	6.34 ± 0.5	7.26 ± 0.6
DOC (mgC L^{-1})	2.5 ± 0.5	8.2 ± 1.2	6.6 ± 1.1
TDP (mgP L^{-1})	-	0.46 ± 0.18	0.22 ± 0.11

Table S2. Average major element composition (in weight percent oxide) of the riverbed sediment at the three investigated sampling points (S1, S2 and S3) and in river suspended particulate matter (SPM) collected at S3.

%	S1	S2	S3	SPM
Al_2O_3	14.30	15.14	15.45	14.84
Fe_2O_3	5.14	4.81	5.56	5.82
MnO	-	-	-	0.12
CaO	1.52	1.26	1.73	2.18
MgO	1.74	2.25	1.96	2.51
Na_2O	2.61	2.48	2.15	2.72
K_2O	1.96	1.86	1.67	2.83
TOC (%)	0.2	0.2	0.2	n.d.

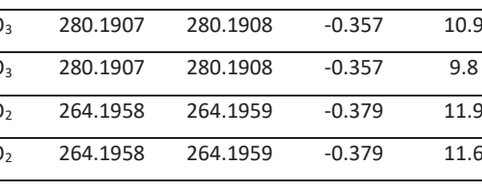
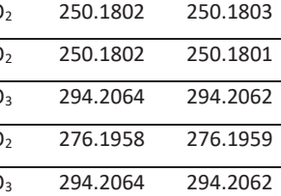
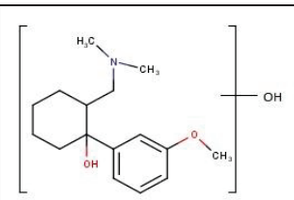
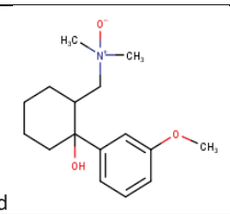
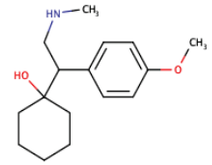
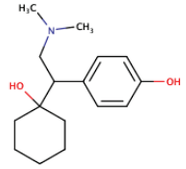
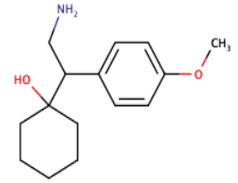
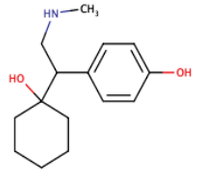
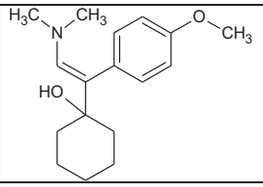
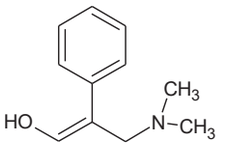
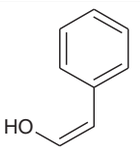
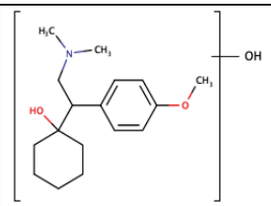
n.d. = not determined

Table S3. Detected transformation products in photodegradation/biodegradation experiments or field studies and their respective mass errors in ppm, retention time (RT) and confidence level of identification according to Schymanski rules.

Transformation product	Molecular formula	Theoretical m/z $[M+H]^+$	Detected m/z	Mass error (ppm)	RT (min)	Confidence level
O-desmethyltramadol	$C_{15}H_{23}NO_2$	250.1802	250.1803	-0.399	9.5	1
N-desmethyltramadol	$C_{15}H_{23}NO_2$	250.1802	250.1801	0.399	9.6	1

Tramadol TP278	$C_{16}H_{23}NO_3$	278.1751	278.1752	-0.359	10.0	2
Tramadol-N-oxide	$C_{16}H_{25}NO_3$	280.1907	280.1908	-0.357	10.9	1
Hydroxylated Tramadol	$C_{16}H_{25}NO_3$	280.1907	280.1908	-0.357	9.8	2
N-desmethylvenlafaxine	$C_{16}H_{25}NO_2$	264.1958	264.1959	-0.379	11.9	1
O-desmethylvenlafaxine	$C_{16}H_{25}NO_2$	264.1958	264.1959	-0.379	11.6	1
Didesmethylvenlafaxine1	$C_{15}H_{23}NO_2$	250.1802	250.1803	-0.400	9.3	2
Didesmethylvenlafaxine2	$C_{15}H_{23}NO_2$	250.1802	250.1801	0.400	9.4	2
Venlafaxine-N-oxide	$C_{17}H_{26}NO_3$	294.2064	294.2062	0.680	13.5	1
Venlafaxine TP 278	$C_{17}H_{25}NO_2$	276.1958	276.1959	-0.367	12.6	2
Hydroxylated Venlafaxine (TP294)	$C_{17}H_{27}NO_3$	294.2064	294.2062	0.680	11.3	2
Venlafaxine TP 178	$C_{11}H_{15}NO$	178.1226	178.1224	0.566	6.5	2
Venlafaxine TP 121	C_8H_8O	121.0646	121.0643	0.563	5.2	3
Amisulpiride TP368	$C_{17}H_{25}N_3O_4S$	368.1639	368.1637	0.543	8.9	3
N-desethylamisulpiride	$C_{15}H_{23}N_3O_4S$	342.1482	342.1483	-0.292	6.7	1
Amisulpride-N-oxide	$C_{17}H_{27}N_3O_5S$	386.1744	386.1748	-1.036	9.1	1

Table S4. Chemical structures of transformation products detected in photodegradation/biodegradation experiments or in field studies.

Transformation product	Molecular structure
N- and O-desmethyltramadol	 and
Tramadol TP278	
Tramadol N-oxide and hydroxylated Tramadol (TP280)	 and 
N- and O-desmethyl venlafaxine	 and 
Didesmethylvenlafaxine1 and 2	 and 
Venlafaxine TP 278	
Venlafaxine TP 178	
Venlafaxine TP121	
Hydroxylated Venlafaxine (TP294)	

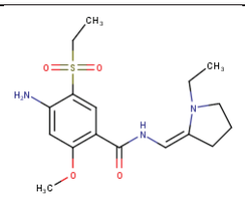
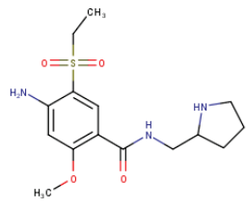
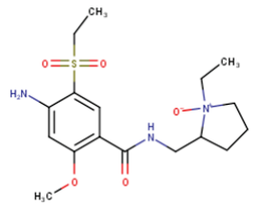
Amisulpiride (TP368)	
N-desethylamisulpiride	
Amisulpiride-N-oxide	

Fig. S4a. Proposed transformation pathways of amisulpride under photochemistry.

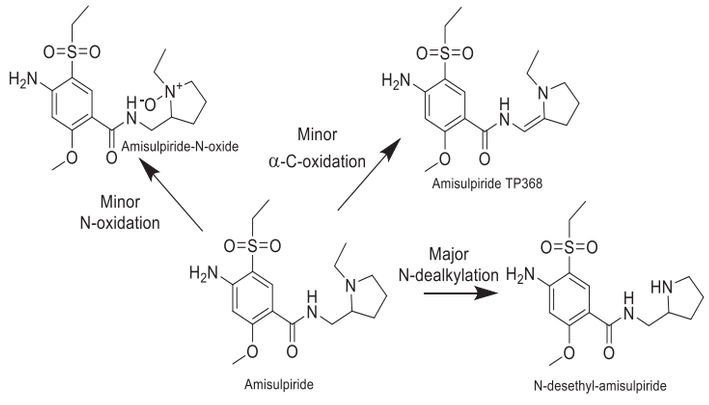


Fig. S4b. Proposed transformation pathways of tramadol under photochemistry.

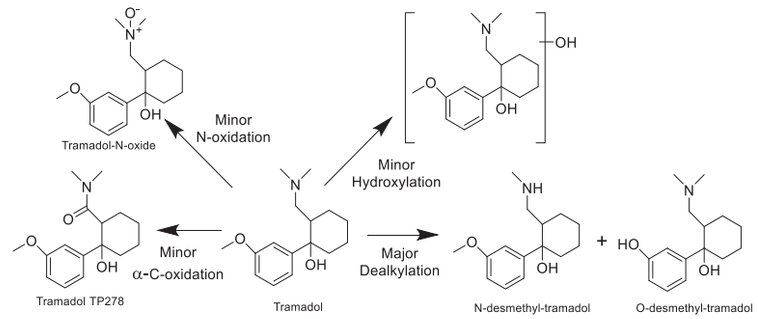


Fig. S4c. Proposed biotransformation pathways of tramadol under aerobic biodegradation in water/sediment system.

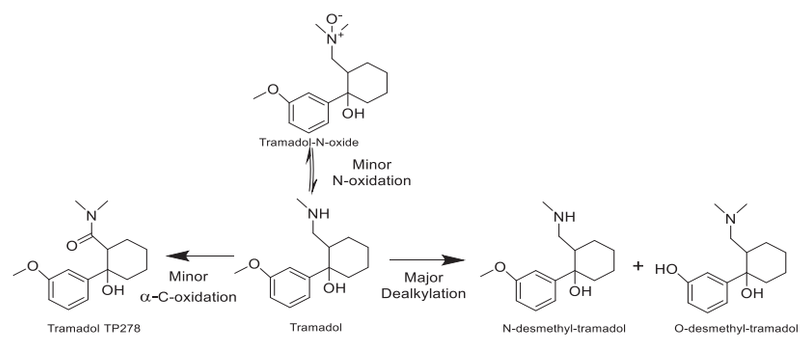
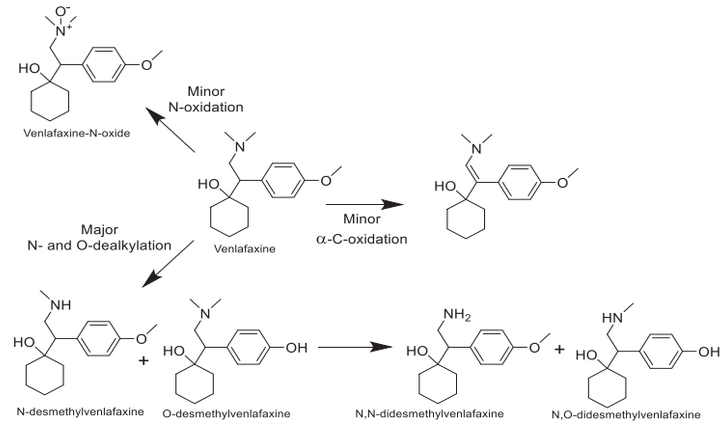


Fig. S4d. Proposed biotransformation pathways of venlafaxine in water/sediment system under aerobic conditions.



Publication #6

Integrating Suspect and Non-Target Screening with Open Cheminformatics Approaches to Identify Pharmaceutical Related-Transformation Products and previously unidentified Pesticides in a Mediterranean Stream

O. Gómez-Navarro, S. Pérez, E. L. Schymanski

Article

Integrating Suspect and Non-Target Screening with Open Cheminformatics Approaches to Identify Pharmaceutical Related-Transformation Products and previously unidentified Pesticides in a Mediterranean Stream

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Transformation products
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ABSTRACT

Transformation products (TPs) of contaminants of emerging concern are increasingly recognized for their potential environmental relevance, yet their occurrence, fate, and risks remain poorly understood, especially in hydrologically vulnerable regions such as the Mediterranean Basin. This study combined suspect and non-target screening (NTS) with open source cheminformatics approaches to explore pharmaceutical-related TPs and other unknowns in a low-flow, effluent-dominated stream in Catalonia, Spain. The selected Mediterranean stream is highly influenced by wastewater treatment plant discharges and is representative of intermittent flow regimes exacerbated by climate change. Liquid chromatography coupled to high resolution mass spectrometry was used for the analysis and the open source R package patRoon was used for feature annotation and compound identification. An extensive suspect screening workflow was applied incorporating >2000 TP candidates generated via ShinyTPs (to obtain reference TPs) and BioTransformer (predicted TPs). Thirty-four TP features were annotated at level 2a and 3a and eleven of these were confirmed at level 1 via reference standards and were subsequently quantified. Their occurrence and distribution across the stream as well as seasonal variability were analyzed, accompanied with an environmental risk assessment on TPs with available predicted no effect concentrations. This revealed that O-desmethylvenlafaxine posed a high ecological risk, while hydroxydiclofenac posed medium risk. Metabolites of cocaine were also annotated and together with other TPs showed low or negligible risk levels. Although the original focus was on pharmaceuticals, thirteen pesticides were tentatively identified through NTS, several of which are banned substances, highlighting the need for further investigation. This study demonstrates the power of integrating open cheminformatics tools with advanced analytical workflows to enhance TP detection and environmental interpretation; helping select features for confirmation efforts with reference standards and identifying other avenues for future studies.

1. Introduction

Contaminants of emerging concern (CECs) such as pharmaceuticals, personal care products, pesticides and industrial chemicals, are extensively used and have become ubiquitous in the aquatic environment [1,2]. However, conventional targeted analysis only detects predefined CECs, providing a limited understanding of their behavior and persistence in the environment. To address this, suspect and non-target screening approaches (collectively NTS) are increasingly employed, as these allow the detection of unknowns, enabling to better understand CECs fate through the identification of their transformation products (TPs)[3,4]. In some cases, certain TPs may exhibit higher persistence, increased mobility, and enhanced biological activity compared to their parent compounds, hence TPs are receiving growing recognition and understanding their fate in the environmental becomes essential [5,6].

TPs originate from the degradation of parent compounds, with wastewater treatment plants (WWTPs) being one of their major sources into the aquatic environment [3,7]. Although WWTPs play a crucial role in water quality improvement and partial contaminant removal [8,9], these are also hotspots for TP formation [10,11]. This occurs especially during the

Article

biological treatment, leading to biotic TPs, and during chemical processes driving to abiotic transformations [12,13]. In most studies on WWTP TP formation, the primary reactions include hydroxylation, oxidation and dealkylation [14]. Beyond WWTPs, natural attenuation processes, such as photolysis, microbial degradation, and sorption, can take place in surface water, sediments and soil, contributing to the diverse and complex environmental fate of TPs [13,15–17]. Additional sources of CECs also exist such as hospital residues, agricultural application, urban runoff and landfill leachates, adding up to the complexity of CECs fate and the elucidation of TPs resulting from natural attenuation.

Despite their environmental relevance, TPs remain an analytical challenge due to their structural diversity, their presence at low concentrations and their frequent lack of reference standards [18]. Advanced analytical techniques such as liquid chromatography high-resolution mass spectrometry (LC-HRMS) combined with cheminformatics tools are crucial to address these challenges and to elucidate TPs. Through suspect and non-target screening methodologies, identification of known and unknown TPs is possible. LC-HRMS provides high sensitivity and allows precise identification based on accurate mass measurement and fragmentation patterns [19] enabling to assign identification confidence levels [20]. Even though this implies complex data processing and interpretation, these can be addressed through the use of open source software tools such as ShinyTPs [21], for reference TP data curation, BioTransformer for TP predictions [22] and patRoon [23,24], which enables both suspect and NTS approaches.

The need for further identification and understanding of TPs is particularly interesting in regions characterized by semi-arid climates and low-flow hydrological regimes, such as the Mediterranean region. Intermittent streams are becoming more common in this region as climate change intensifies, leading to rising temperatures, altered precipitation patterns and more frequent and severe droughts [25,26]. These changes are significantly altering river flow regimes, causing perennial rivers to transition to low-flow or intermittent streams [26,27]. Under low flow situations, the dilution capacity of WWTP effluents can be limited, due to the low river flow, and streams can even become effluent dominated [28,29]. This makes them particularly vulnerable to potential accumulation and persistence of both CECs and their TPs in the aquatic environment [30]. Despite the growing evidence of TP formation both in WWTPs and under possible environmental conditions, their behavior, distribution, and fate in such hydrologically vulnerable systems remain poorly understood [31–33].

In this context the aim of this study was to elucidate and investigate the occurrence and environmental behavior of TPs in a Mediterranean low-flow stream that receives substantial input from WWTP effluents. A suspect and non-target screening workflow, based on open source cheminformatics tools, was applied to detect and tentatively identify TPs from CECs precursors and other unknowns across an effluent-impacted stream. This study seeks to improve the understanding of TPs by bridging the gap between advanced analytical workflows and environmental interpretation of TP behavior in vulnerable freshwater systems. By linking analytical chemistry with regional hydrological vulnerability, this work provides key insights into transformation dynamics with direct implications for monitoring strategies and regulatory frameworks across water-stressed regions. To deepen the understanding of TP dynamics, a low flow and effluent-dominated stream was selected as a case study, with a focus on exploring TPs fate and assessing their environmental relevance, as well as the identification of other possible compounds.

2. Materials and methods

2.1. Sample description

The study was focused on la Riera de Taradell (TAL), located in the Osona region in Catalonia, Spain. It is a low-order stream, tributary of the Gurri river and presents a common Mediterranean flow regime, characterized by seasonal variability with periods of

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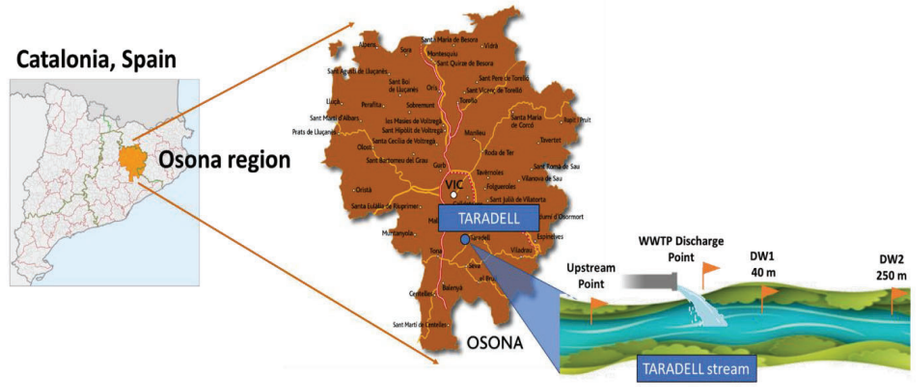


Figure 1. Sampling site and sampling sites of the Taradell stream in Osona, Catalonia, Spain

low flow or even dryness. Precipitation mainly occurs in spring and autumn, with little precipitation during summer and winter, although interannual precipitation can fluctuate greatly. The hydrological regime is marked by the seasonal pattern, highly influenced by high-volume runoff generated by short, intense storms [34]. The stream is surrounded by riparian vegetation and agricultural fields and impacted with the direct discharge of a WWTP plant. The natural low flow and constant WWTP input make this an effluent-dominated stream [35]. Samples were collected at an upstream point located 60 m above the WWTP, at the WWTP discharge point and at two downstream points: 40 m (DW1) and 250 m below the WWTP (DW2). These 4 sampling points were used to study the fate of contaminants and the possible generation or release of TPs. Two seasons were investigated, during summer 2020 and winter 2021, to study the effect of low-flow conditions in an effluent dominated stream and the effect on TPs. Seasonal flow rates are reported in Table S1. During the summer season, the downstream flow rate was 0.053 m^3/s, with WWTP contributing 36% of this flow. In contrast, during the winter season, the downstream flow rate was 0.016 m^3/s with a WWTP contribution of 81%.

2.2. Sample extraction and instrumental analysis

Samples were extracted as described in Gómez-Navarro *et al.* [36]. Briefly, samples were extracted through solid phase extraction (SPE) using a multi-layered and multi-sorbent cartridge to enhance the retention of compounds across a broad range of polarities at an enrichment factor of 1000. Samples were then analyzed by ultra-high performance liquid chromatography (UHPLC) coupled to a high-resolution mass spectrometer Q-Exactive Orbitrap (Thermo-Fisher Scientific, Germany). The chromatographic separation was achieved using the reversed phase Acquity HSS T3 C18 column (100 × 2.1 mm internal diameter, 1.8 µm particle size) (Waters, Milford, MA) at 40 °C using a Waters UHPLC system (Waters, Milford, MA). NTS mass spectrometry data was obtained in data-dependent acquisition mode, as described in Krier *et al.* [4] . The targeted method described previously [36] was applied for further confirmation and quantification of detected TPs.

2.3. Computational methods

2.3.1. TPs suspect list

A suspect list was generated for possible transformation products for 112 targeted compounds. First of all, ShinyTPs [21], an R package with Shiny interface [37] was used to find and curate existing

transformation reactions related to the targeted compounds in the Hazardous Substances Data Bank (HSDB) dataset. Over 400 reactions were curated, generating a suspect list including a total of 270 TPs. Although most of these TPs were already documented in open libraries, 40 newly curated reactions related to 11 pharmaceuticals were not already openly available and were thus added into the HSDBTPS dataset (S68 on the NORMAN Suspect List Exchange) [38]. Also, BioTransformer [22,39] a computational tool to predict potential TPs, was used to explore further the environmental fate of the target compounds. Environmental transformation predictions were performed using patRoon[24], which integrates BioTransformer with the environmental mode ('*env*') for two generations. A suspect list of parent compounds was prepared, and the *generateTPs* function was applied to simulate the environmental transformations, from both microbial metabolism and abiotic processes. A resulting suspect list of 2299 TPs was obtained. The two lists were combined obtaining a final suspect list of 2569 suspects (Table S2).

2.3.2. Suspect and non-target screening

The workflow applied for this study combined both a suspect and a non-target screening approach, as shown in Figure 2. The suspect screening included both the curated list of TPs from ShinyTPs alongside the predicted TPs from BioTransformer. The final suspect list (Table S2) was incorporated into the MS workflow in patRoon. Simultaneously, an NTS was also performed to tentatively identify additional compounds present. The NTS complemented this by enabling the detection of other unknown features beyond the suspect lists.

Data processing

All data analysis was performed in Rstudio (R version 4.4.1). Firstly, the HRMS raw files were converted into the open mzML format using *MScovert* from ProteoWizard [40]. Data processing (suspect and non-target screening) were performed using patRoon [23,24]. Features were extracted and grouped using the XCMS3 algorithm, applying *CentWave* with pre-defined parameters for peak width, signal-to-noise thresholds, and mass error tolerances. Detected features were grouped and aligned across analyses using the *PeakDensity* and Orbitrap alignment methods.

For the suspect screening the *screenSuspects* function was used to match detected features to suspect candidates based on retention time, mass to charge

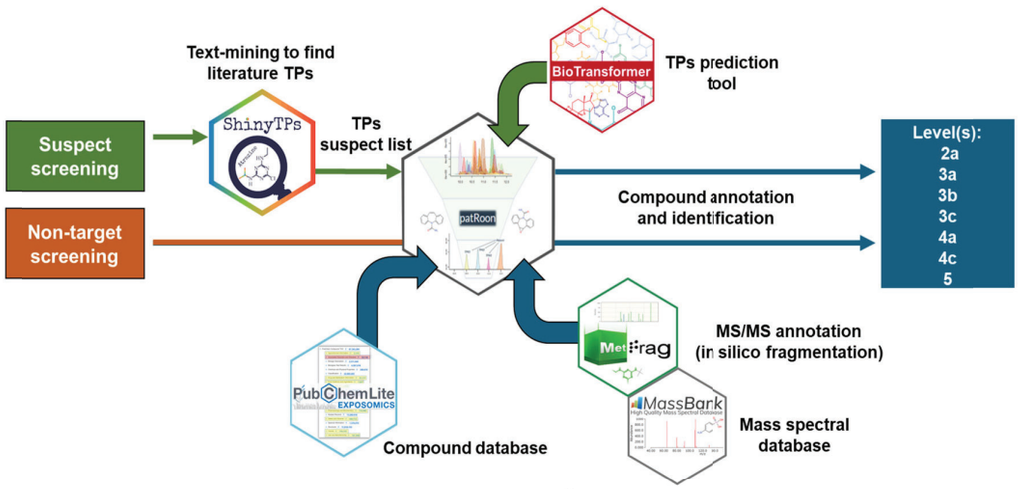


Figure 2. Suspect and non-target screening using open-source HRMS patRoon [22].

(*m/z*) windows, and adduct information. MS peak lists were generated with *generateMSPeakLists*, followed by intensity-based filtering of MS/MS spectra to prioritize high-quality peaks. The resulting features were filtered, annotated, and scored using established thresholds and spectral matching against reference libraries. Compound identification was performed with MetFrag [41] using the PubChemLite database [42–44]. To annotate features to the respective identification confidence levels [20], the *individualMoNAscore* value was used. This score indicates the quality of match between the samples and spectra in the MS libraries, where the higher the score the better match. The criteria used to annotate the features to the respective confidence level was that described for suspect and non-target screening in Talavera Andújar *et al.* [45]. Final results were summarized and exported using patRoon's built-in reporting functions for further analysis.

For data visualization, intensity values of the tentatively annotated features were log-transformed and Pareto-scaled to normalize variance and the processed data were subsequently analyzed using MetaboAnalyst [46,47].

2.3.3. Ecological risk assessment

An ecological risk assessment of the quantified TPs was performed where risk quotients (RQs) were calculated for those compounds where predicted no effect concentrations (PNEC) values were available in NORMAN Ecotoxicity Database [48]. Potential risks of the compounds were assessed by calculating individual RQs where Eq. 1 was applied. Lowest PNEC values were used for a more conservative scenario. PNEC values used are found in Table S5.

$$Risk\ quotients\ (RQ) = \frac{Measured\ environmental\ concentration\ (MEC)}{PNEC} \quad \text{Eq. 1}$$

3. Results and discussion

3.1. Suspect and NTS approaches

The combination of suspect screening and NTS enabled a comprehensive evaluation of TP identification, offering insights into their environmental behavior, potential persistence, and the identification of previously unreported or unknown transformation products.

A total of 49,880 feature groups were initially detected during the HRMS data processing with patRoon. Of the suspects (Table S2), 86% (2200/2569) were matched to at least one feature group during the initial suspect screening step, which is based on accurate mass, retention time and expected adducts. To enable structural annotation, MS/MS peak lists were generated for subsequent *in silico* fragmentation using MetFrag and compound annotations. After several filtering steps, the 8,514 features found with NTS and 9,694 suspect-feature matches were reduced to higher confidence features. The number of Level 1 (reference standard), 2a (good library match), 3a (tentative candidate *individualMoNAScore* > 0.4) and 3c (tentative candidate annotation MS/MS similarity > 0.7) were 11, 8, 15 and 139 for suspect screening and 25, 55, 29 and 25 for non-target screening workflows, respectively. Further details on the annotated features can be found in Tables S3 and S4. In some cases, multiple features were tentatively annotated as the same compound, but with differing retention times, potentially reflecting the presence of isomers, in source fragments, adducts, or co-eluting compounds. In the case of tramadol and O-desmethylvenlafaxine where different features were annotated, the use of reference standards and retention times enabled the identification of both cases to level 1 identification confidence. This highlights the inherent complexity and uncertainty in non-target identification workflows.

A larger number of features were annotated at a higher confidence level through NTS than through TPs suspect screening, despite the thorough number of suspect TPs included in the suspect list. This can be partially explained due to the typically low concentrations at which TPs are present in the environment, which may have prevented the triggering of MS/MS via DDA acquisition in this set-

up [49,50]. However, at the same time, NTS using PubChemlite enabled the identification of a broader range of chemicals. As previously observed in other studies[51,52], WWTP serve as input sources, not only of pharmaceuticals but also pesticides and industrial compounds. Therefore, NTS allowed for the annotation of additional compounds originating from WWTPs, of which 25 were confirmed to level 1 through reference standards. Nevertheless, 34 TPs were tentatively identified, of which 8 were annotated at level 2a and 11 at level 1. The challenge of TPs identification and fate, not only relies on NTS complexity but also on the overlapping nature of transformation pathways, as previously noted in literature [53,54]. In some cases, the transformations of certain compounds can lead to the formation of structurally related precursors, further complicating the interpretation of environmental pathways.

Many TPs identified in this study could also arise as human biological metabolites or TPs occurring in the WWTP. Transformation pathways can overlap (e.g. hydroxylation, demethylation) and it is not always possible to distinguish between environmental TPs and endogenous metabolites [53].

3.2. Curated transformation products behavior and seasonal differences

To explore spatial and seasonal differences in the distribution of tentatively identified pharmaceutical related TPs curated through ShinyTPs, a heat map was generated using Z-score normalized intensity data (Figure 3). From the 173 annotated TPs in Table S3, a representative subset of 16 TPs was selected for detailed visualization. These included N-oxides, demethylated metabolites, and acidic hydrolysis products, primarily derived from commonly used pharmaceuticals and illicit drugs.

The color scale represents Z-scores, where values range from -2 to 2, where positive values indicate above average intensities and negative values indicate lower than average intensities relative to the mean intensity of each TP across all samples. Hence, the color scale reflects the degree of deviation from the mean, highlighting possible patterns of TP distribution. Figure 3 allows a comparison of

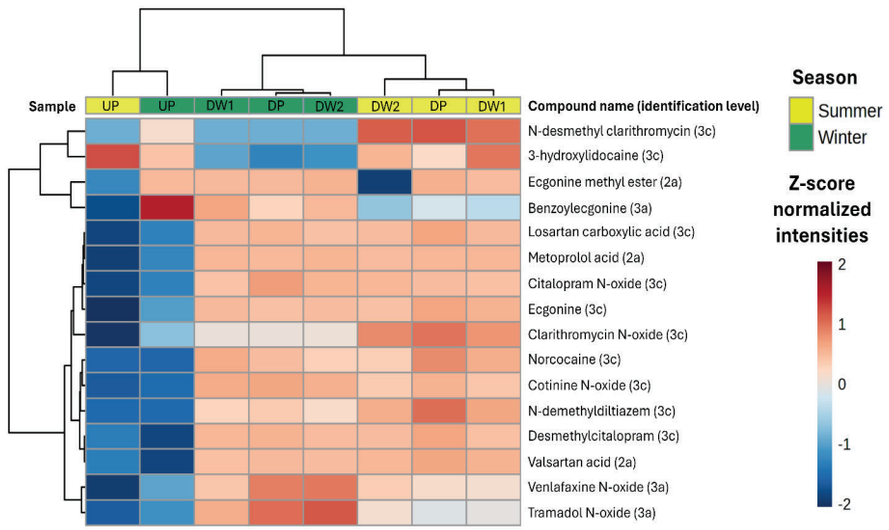


Figure 3. Heatmap on normalized intensities of the tentatively annotated TPs ShinyTPs curated extracted transformation products of the suspect screening dataset.

annotated compound-specific patterns, highlighting potential hotspots, possible degradation trends, and seasonal variability across the catchment.

The TPs included in Figure 3, are indicative of well-established degradation pathways including human metabolism, oxidation, and hydrolysis. Their detection is consistent with previously reported pathways and suggests a degree of environmental persistence. Many of these compounds are also of interest due to their potential biological activity [11,53–55], raising concern about their long-term presence and impact in aquatic environments.

Moreover, Figure 3 reveals differences between upstream and downstream samples for both seasons, indicating clear anthropogenic pressures through the WWTP effluent input. However, the annotated features included compounds, such as benzoylecgonine, norcocaine and ecgonine, all metabolites of cocaine, which were observed across multiple sites, including the upstream site during the winter season. Their presence suggests drug consumption in the region and implies that other sources beyond WWTP can be present, in line with other studies on wastewater-based epidemiology, which have demonstrated the use of these metabolites as environmental markers of illicit drug consumption patterns [56,57].

Among the annotated compounds, six N-oxide pharmaceutical derivatives were identified, of which lamotrigine N-oxide was confirmed with the highest identification confidence level 1, while the other at level 3. N-oxides can originate from WWTP during advanced oxidation processes [5,58] or in activated sludge [59] as well as a result of human metabolism [60,61]. N-oxides are generally more stable than their parent compounds, which can lead to increased persistence as reported in several studies [58,62]. However further confirmation and quantification is necessary to further evaluate the fate of TPs and ecological risk as performed in section 3.3 for lamotrigine N-oxide and other TPs. Overall, the patterns observed in Figure 3 underscores the presence and potential environmental relevance of several identified TPs, curated through ShinyTPs, and highlight their role in shaping seasonal and spatial trends within the studied stream.

3.3. Quantification and temporal trends of TPs along the stream

Eleven TPs were confirmed at a confidence level 1 with their respective reference standard and hence

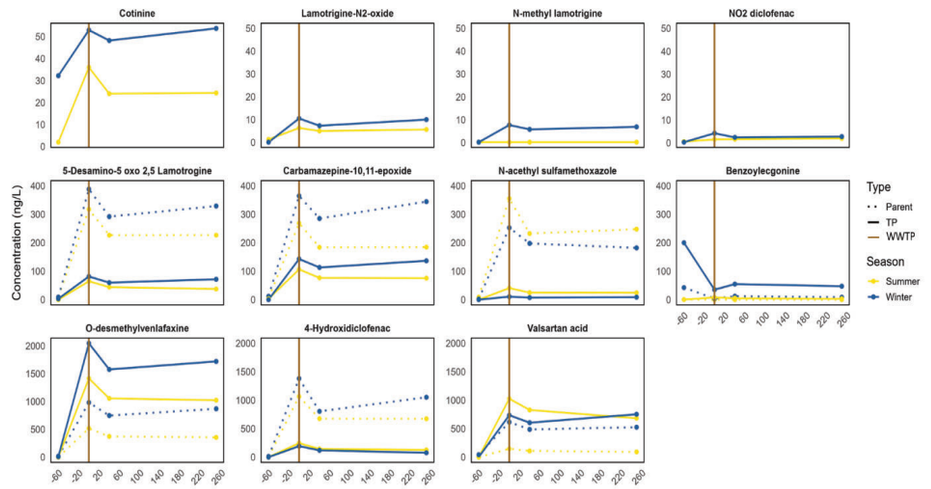


Figure 4. Transformation products (solid line) and parent compound (dotted line) concentration along the stream during summer and winter seasons.

quantification was possible (Table S6). In Figure 4 the fate of these TPs along the studied stream can be observed, for both seasons.

In general, TP concentrations were higher in winter than in summer, since the stream dilution capacity was lower (Table S1) and the WWTP effluent input was observed to be the main input of TPs, as previously reported [3,63]. Seasonal differences were also observed in the TP precursors and are explained by Covid-19 lockdown restrictions, leading to lower targeted CECs concentrations in the WWTP [35]. Interestingly, in the winter season, benzoylecgonine was detected already at the upstream point, and the WWTP input was barely observed. Benzoylecgonine is a primary metabolite of cocaine and its higher concentration before the WWTP effluent could be explained by possible diffuse contamination point either from surface runoff or anthropogenic activities. Ecgonine methyl ester, another metabolite of cocaine was annotated at level 2a, however this pattern was not observed with its intensities.

The general trend observed in Figure 4 after the WWTP input was a dilution of the effluent with the receiving stream flow. However, in some cases, especially in the winter seasons, a slight concentration increase of some TPs was observed. Among the detected TPs, three lamotrigine metabolites, 5-desamino-5-oxo-2,5-lamotrigine, N-methyl-lamotrigine, and lamotrigine-N2-oxide, were identified, quantified, and their environmental fate was explored. Their presence has been previously reported in surface water [10] with comparable median concentrations, generally at low ng L^{-1} concentrations. In particular, 5-desamino-5-oxo-2,5-lamotrigine was found at a median concentration of 42 ng L^{-1}, closely aligning with the 52 ng L^{-1} observed in our dataset. As reported by Zonja et al., 2016 [10], N-methyl lamotrigine was detected below its quantification limits during the summer campaign, whereas higher concentrations were found during the winter campaign, a seasonal trend also reflected by lamotrigine concentrations. Higher concentrations were observed for the TP 5-desamino-5-oxo-2,5-lamotrigine, commonly formed through anaerobic wastewater treatments, but it can also persist in the receiving streams with minor attenuation of the parent compound outside WWTPs. This raises attention on potential long-term exposure and ecotoxicological implications in the receiving water bodies.

For the TP NO_2-diclofenac, similar concentrations were reported by Osorio et al. (2016) [64], in both WWTP and surface waters. Its formation has been attributed to microbial transformation processes during nitrification/ denitrification processes in activated sludge, as reported by previous studies [64–66]. These studies also noted that it exhibits higher toxicity than the parent compound, however to date, the NORMAN ecotoxicology database [48] does not include information on this TPs hence further understanding of its ecotoxicological risk in freshwater was not possible.

Despite the insights gained on TP environmental fate, fully understanding their behavior remains challenging due to the multitude of interacting variables involved, ranging from hydrological dynamics and physicochemical properties to microbial activity and seasonal differences. Although in-stream attenuation of precursor compounds may occur, the subtle downstream increase in TP concentrations—particularly the consistent pattern across different compounds—suggests that incomplete mixing of WWTP effluent with the receiving stream could still be a contributing factor, as reported in other studies on mixing dynamics[67,68]. This trend was especially evident during the winter campaign. Such patterns, also reflected in corresponding parent compounds, supporting the hypothesis of incomplete mixing and raise the possibility of possible diffuse or delayed sources contributing to the load rather than in-stream transformation.

3.4. Ecological risk assessment of TPs along the stream

The potential ecological risks associated with the TP concentrations are summarized in Table 1, for those compounds where PNEC values were available (Table S5). As it can be observed, in general no high risk was associated with the detected TPs, except for O-desmethylvenlafaxine. O-desmethylvenlafaxine is a primary human metabolite of the antidepressant venlafaxine, an active pharmaceutical ingredient marketed independently and also a degradation TP of venlafaxine [69–71]. Its multiple roles complicate its classification as TP, especially due to its direct administration and subsequent widespread detection in the environment. It is frequently detected in both influent and effluent of WWTPs, with high environmental concentrations, reflecting its low removal rates [72]. It was also included in the European Watchlist (3rd and 4th) [73,74] under the Water Framework Directive, highlighting the required monitoring due to its potential risk.

All upstream sites (UP) had insignificant risk associated with them, except for the benzoylecgonine case, where, as mentioned previously, a higher concentration due to a diffuse point source was detected in the UP point. Hydroxy-diclofenac in-stream concentrations were associated with a medium risk. The dilution of WWTP effluent in the stream is low due to the reduced stream flow rate. Regulations should take into account the limited dilution capacity of streams to avoid potential high-risk scenarios resulting from insufficient dilution. Further efforts are also needed to expand the availability of PNEC values of other TPs (such as NO2 diclofenac and 2 lamotrigine TPs) to better understand the potential risks or not of TPs in the environment.

3.5. Tentatively identified pesticides from Non-Target Screening

Through the NTS, 13 pesticides were annotated, of which 10 were tentatively identified at a confidence level 2a and three at level 3a (Table SM7). Their intensities and distribution across samples and seasons were further studied. In Figure 5 a heatmap is used to illustrate their relative abundance, again based on Z-score normalized intensities across the studied sites. Figure 5 reveals potential notable pattens in the occurrence of pesticides. Tentatively identified pesticides were also clustered by season rather than sample type except for the upstream points, as shown by the dendrogram (Figure 5). The clustering highlights again how WWTP were the main source of input. Seasonal differences were observed between the two sampling campaigns specially for 4 pesticides carbendazim, thiamethoxam, acetamiprid and hexazinone, where their presence was not observed during the winter campaign. The seasonal nature of pesticides, as previously reported in [51], may explain their absence in winter, which is due to the seasonal application of pesticides application, in particular acetamiprid as its use is still approved.

Table 1. Ecological risk assessment for TPs. High risk (RQ≥1) is shown in red, medium risk (0.1≤RQ<1) in orange, low risk (0.01≤RQ<0.1) in yellow and insignificant risk (RQ<0.01) in green.

Sample	Season	4-hydroxy diclofenac	Benzoyl-ecgonine	Carbamazepine epoxide	Cotinine	Lamotrigine N2-oxide	N-acetyl sulfamethoxazole	O-desmethyl venlafaxine	Valsartan acid
UP	Summer	0.002	0.0002	0.001	0.0002	0.002	0.00003	0.001	0.000003
DP	Summer	1.12	0.004	0.04	0.004	0.01	0.02	1.61	0.003
DW1	Summer	0.65	0.002	0.03	0.002	0.01	0.01	1.20	0.003
DW2	Summer	0.58	0.001	0.03	0.002	0.01	0.01	1.16	0.002
UP	Winter	0.002	0.09	0.0001	0.003	0.0002	0.00003	0.01	0.000003
DP	Winter	0.88	0.02	0.06	0.01	0.02	0.004	2.32	0.002
DW1	Winter	0.54	0.03	0.04	0.005	0.01	0.003	1.79	0.002
DW2	Winter	0.35	0.02	0.05	0.01	0.02	0.004	1.95	0.002

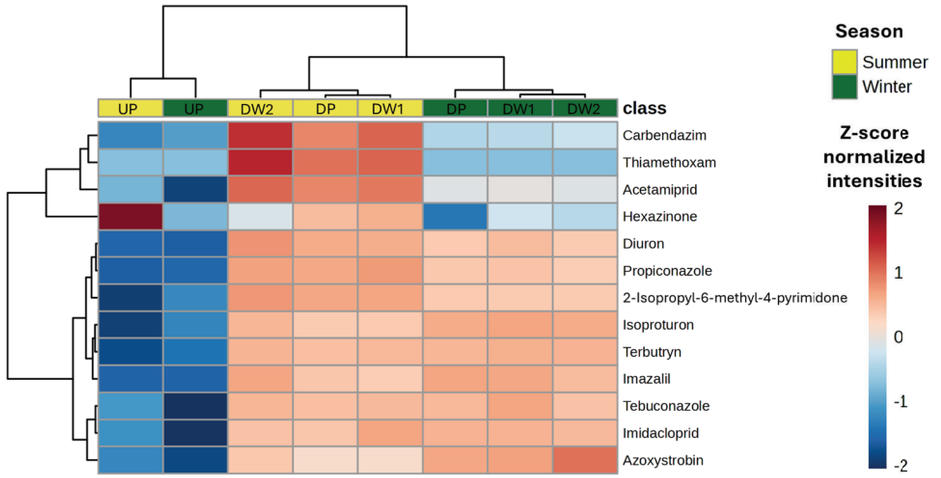


Figure 5. Heat map and dendrogram based on the normalized intensities of tentatively identified pesticides.

The dataset also suggests the potential presence of banned or restricted-use pesticides (8/13), which were not identified in upstream samples but appeared downstream and in WWTP effluents. This spatial difference points to WWTPs as key point sources for the presence of these substances likely due to inputs from domestic, industrial, or urban activities. These inputs can be attributed either to illegal use or from their remobilization from soils or sediments to which they could have adsorbed and accumulated during their application in the past due to their physico-chemical properties [75,76]. Compounds such as isoproturon, diuron and terbutryn, which are no longer authorized in the EU, may still be entering wastewater streams through residual use, improper disposal, or leaching from contaminated materials. Notably, these compounds are known to have moderate to high persistence in aquatic environments and can accumulate in sediments or groundwater, posing risks to aquatic organisms. Hexazinone was the only compound also observed upstream in summer, hinting at possible diffuse or agricultural sources beyond WWTP discharge.

Interestingly a pesticide TP, 2-isopropyl-6-methyl-4pyrimidone (IMP), a degradation product of diazinon, was also tentatively identified at level 2a. Precious studies have revealed that hydrolysis is the major process of degradation of diazinon in water, with IMP being its main TP [77,78].

These findings reinforce the need for integrated monitoring strategies that address not only current-use pesticides but also legacy substances that persist in the environment long after their formal withdrawal from use. Their continued presence raises concerns regarding chronic exposure risks to aquatic life and the potential for groundwater contamination. In addition, this analysis underscores the utility of non-target screening as a surveillance tool, capable of detecting unexpected or banned contaminants that may not be included in routine monitoring frameworks, however compound confirmation is necessary through reference standards, and quantification of these, for a better and more precise understanding of their environmental fate.

4. Conclusions

This study conducted an intensive TP suspect screening approach using the open source software patRoon, using LC-HRMS to investigate CECs fate in an intermittent effluent-dominated Spanish stream.

Over 2000 suspect TPs were compiled using the combination of ShinyTPs and the TPs generation linked to Biotransformer within patRoon. The suspect screening allowed to annotate 34 TP features at level 2a and 3a, eleven of which were confirmed to level 1. Eleven TPs were quantified, and their environmental fate was assessed, increasing the understanding of TP occurrence and distribution along the studied stream. Potential ecological risk assessment was performed for the quantified TPs, where only O-desmethylvenlafaxine was observed to have a potential high risk and hydroxydiclofenac concentrations were associated with a medium risk. The concentration of the other TPs was associated with a low or even insignificant risk. In addition, the NTS helped to complement previous studies by revealing additional highly occurrence compounds. Thirteen pesticides were tentatively identified at identification level 2-3, of which many of them their used is prohibited.

Overall, this study highlights and proves the potential and promising application of such open source cheminformatics tools for comprehensive environmental monitoring of contaminants, especially transformation products.

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Publication #6

Supplementary Materials

Integrating Suspect and Non-Target Screening with Open Cheminformatics Approaches to Identify Pharmaceutical Related-Transformation Products and previously unidentified Pesticides in a Mediterranean Stream

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Supplementary Material

Integrating Suspect and Non-Target Screening with Open Cheminformatics Appr Pharmaceutical Related-Transformation Products and previously unidentified Pe Mediterranean Stream

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Table S1	Case study stream flow rates
Table S2	Suspect list of TPs including curated TPs from ShinyTPs and Biotransfo
Table S3	Tentatively identified features from suspect list
Table S4	Tentatively identified features from NTS
Table S5	PNEC values
Table S6	Transformation products and precursor concentrations
Table S7	Tentatively identified pesticides NTS

TableS1 Case study stream flow rates

WWTP	Season	US flow (m3/s)	WWTP flow (m3/s)	DS flow (m3/s)
TAL	Summer	0.034	0.019	0.053
TAL	Autumn	0.028	0.019	0.047
TAL	Winter	0.004	0.016	0.020
TAL	Spring	0.008	0.013	0.021

TableS3 Tentatively identified features from suspect list

group	ret	mz	susp_name_orig	idlevel
M200_R111_9281	1.84	200.1282	Ecgonine methyl ester	2a
M207_R407_10041	6.78	207.1491	Lidocaine-TP2	2a
M254_R627_15745	10.45	254.0591	Sulfamethoxazole	2a
M267_R717_17482	11.95	267.0874	Valsartan acid	1
M267_R717_17483	11.95	267.0874	Losartan-TP41	2a
M268_R374_17656	6.24	268.1540	Metoprolol acid	2a
M285_R493_19770	8.21	285.1807	Oseltamivir Acid	2a
M287_R817_19937	13.61	287.0582	Lorazepam-TP2	2a
M291_R728_20432	12.14	291.2065	Verapamil-TP9	2a
M296_R669_20958	11.16	296.0698	N-acetyl sulfamethoxazole	1
M122_R296_3023	4.93	122.0964	Lidocaine-TP6	3a
M236_R357_13619	5.96	236.1644	2-Aminomethyl-1-(m-methoxyphenyl)cyclohexanol	3a
M236_R820_13620	13.66	236.1645	2-Aminomethyl-1-(m-methoxyphenyl)cyclohexanol	3a
M236_R551_13623	9.19	236.1642	2-Aminomethyl-1-(m-methoxyphenyl)cyclohexanol	3a
M250_R405_15308	6.74	250.1799	N-Desmethyltramadol	3a
M253_R574_15626	9.56	253.0968	Carbamazepine-10,11-epoxide	3a
M253_R671_15627	11.18	253.0969	Carbamazepine-10,11-epoxide	3a
M253_R478_15629	7.97	253.0965	Carbamazepine-10,11-epoxide	3a
M253_R719_15630	11.99	253.0970	Carbamazepine-10,11-epoxide	1
M264_R496_17142	8.27	264.1955	Desvenlafaxine	1
M264_R565_17143	9.41	264.1951	Desvenlafaxine	3a
M271_R414_18027	6.89	271.1071	(10s,11s)-10,11-Dihydroxy-10,11-dihydro-5h-dibenzo[b,f]azepine-5-carboxamide	3a
M271_R574_18030	9.56	271.1073	(10s,11s)-10,11-Dihydroxy-10,11-dihydro-5h-dibenzo[b,f]azepine-5-carboxamide	3a
M280_R597_19167	9.96	280.1904	Tramadol N-Oxide	3a
M286_R390_19860	6.50	286.1432	Morphine	3a
M290_R461_20288	7.68	290.1383	Benzoylecgonine	1
M294_R733_20782	12.22	294.2063	Venlafaxine n-oxide	3a
M300_R288_21452	4.79	300.1592	Codeine-TP14	3a
M312_R853_22749	14.21	312.0188	4'-Hydroxydiclofenac	1
M76_R83_1304	1.39	76.0758	Diltiazem-TP42	3c
M89_R84_1845	1.39	89.0710	Diazepam-TP21	3c
M90_R82_1877	1.36	90.0914	Hydroxychloroquine-TP5	3c
M95_R125_2007	2.08	95.0604	Sulfapyridine-TP3	3c
M99_R1059_2123	17.65	99.0553	Sulfamethoxazole-TP2	3c
M100_R214_2162	3.57	100.1120	Cyclohexylamine	3c
M102_R118_2250	1.96	102.1277	Neotame-TP11	3c
M102_R174_2251	2.90	102.1277	Neotame-TP11	3c
M116_R422_2785	7.03	116.1068	N-Cyclohexylhydroxylamine	3c
M118_R116_2864	1.93	118.0862	Valsartan-TP20	3c
M122_R369_3007	6.15	122.0599	Bezafibrate-TP9	3c
M122_R260_3009	4.33	122.0600	Bezafibrate-TP9	3c
M132_R116_3567	1.93	132.1020	Lidocaine-TP5	3c
M136_R418_3766	6.97	136.1122	2-Butenamide, N-ethyl-N-(2-methylphenyl)--TP7	3c
M136_R261_3768	4.34	136.1122	2-Butenamide, N-ethyl-N-(2-methylphenyl)--TP7	3c
M137_R357_3795	5.95	137.0597	Metoprolol-TP18	3c
M138_R143_3857	2.39	138.0915	4-Amino-3,5-dimethylphenol	3c
M140_R587_3966	9.78	140.1069	Benzoylecgonine-TP5	3c
M140_R281_3970	4.69	140.1070	Benzoylecgonine-TP5	3c
M141_R187_4011	3.12	141.0659	1,3-Dimethyluracil	3c
M141_R227_4012	3.78	141.0659	1,3-Dimethyluracil	3c
M152_R278_4789	4.63	152.0706	Atenolol-TP3	3c
M152_R218_4794	3.64	152.0707	Atenolol-TP3	3c
M153_R973_4858	16.21	153.0548	Atenolol-TP15	3c
M153_R1026_4875	17.10	153.0912	Metoprolol-TP3	3c
M153_R472_4876	7.87	153.0910	Metoprolol-TP3	3c
M155_R199_5026	3.31	155.1179	Losartan-TP38	3c
M163_R590_5665	9.83	163.0864	Demethylcotinine	3c
M166_R349_5900	5.82	166.0862	Neotame-TP13	3c
M166_R594_5905	9.90	166.0863	Neotame-TP13	3c
M168_R116_6070	1.94	168.0656	3-Hydroxyacetaminophen	3c
M177_R636_6906	10.61	177.1121	alpha-l-Cladinose	3c
M177_R526_6912	8.77	177.1122	alpha-l-Cladinose	3c
M179_R171_7058	2.85	179.1180	Glycinexylidide	3c
M179_R568_7059	9.47	179.1176	Glycinexylidide	3c
M179_R518_7061	8.63	179.1179	Glycinexylidide	3c
M180_R12_7113	0.20	180.0656	Cotinine-TP13	3c
M180_R823_7156	13.71	180.1385	Bezafibrate-TP12	3c
M182_R262_7326	4.36	182.1177	Verapamil-TP45	3c
M182_R616_7359	10.27	182.1904	Dicyclohexylamine	3c
M182_R960_7363	15.99	182.1906	Dicyclohexylamine	3c
M182_R699_7364	11.65	182.1905	Dicyclohexylamine	3c
M182_R544_7365	9.07	182.1905	Dicyclohexylamine	3c
M182_R815_7369	13.59	182.1906	Dicyclohexylamine	3c
M182_R871_7370	14.51	182.1906	Dicyclohexylamine	3c
M182_R904_7371	15.07	182.1906	Dicyclohexylamine	3c
M182_R753_7372	12.54	182.1906	Dicyclohexylamine	3c
M186_R376_7777	6.26	186.1125	Ecgonine	3c
M186_R506_7779	8.44	186.1125	Ecgonine	3c
M186_R263_7780	4.38	186.1126	Ecgonine	3c
M193_R449_8427	7.49	193.0972	cotinine N-oxide	3c
M193_R258_8429	4.30	193.0972	cotinine N-oxide	3c
M194_R438_8566	7.30	194.1539	1-Acetamidoadamantane	3c
M195_R694_8672	11.56	195.1493	Irbesartan-TP1	3c
M196_R528_8784	8.80	196.1332	Verapamil-TP7	3c
M198_R289_9038	4.82	198.1125	Methyl 3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate-TP2	3c
M200_R678_9282	11.30	200.1281	Ecgonine methyl ester	3c
M200_R281_9286	4.68	200.1281	Ecgonine methyl ester	3c
M207_R303_10015	5.06	207.1127	Phenylethylmalonamide	3c
M207_R854_10043	14.24	207.1493	Monoethylglycinexylidide	3c
M214_R403_10918	6.72	214.1436	Cocaethylene-TP5	3c

M214_R611_10919	10.18	214.1434	Cocaethylene-TP5	3c
M218_R281_11396	4.68	218.1386	N-(3,3-dimethylbutyl)-L-aspartic acid	3c
M225_R349_12228	5.81	225.1232	4-(3-Amino-2-hydroxypropoxy)phenylacetamide	3c
M225_R413_12230	6.89	225.1231	4-(3-Amino-2-hydroxypropoxy)phenylacetamide	3c
M225_R131_12233	2.18	225.1233	4-(3-Amino-2-hydroxypropoxy)phenylacetamide	3c
M233_R637_13231	10.61	233.0919	Phenobarbital	3c
M236_R820_13620	13.66	236.1645	Tramadol-TP8	3c
M236_R551_13623	9.19	236.1642	Tramadol-TP8	3c
M237_R799_13713	13.32	237.1023	Diazepam-TP8	3c
M237_R636_13714	10.60	237.1019	Diazepam-TP8	3c
M239_R512_13968	8.53	239.1175	Carbamazepine-TP3	3c
M243_R689_14439	11.48	243.1337	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(3-hydroxy-1-methylbutyl)-	3c
M243_R185_14440	3.09	243.1338	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(3-hydroxy-1-methylbutyl)-	3c
M250_R571_15309	9.51	250.1797	O-Demethyltramadol	3c
M250_R479_15310	7.99	250.1799	O-Demethyltramadol	3c
M251_R416_15447	6.93	251.1750	3-Hydroxylidocaine	3c
M253_R574_15626	9.56	253.0968	3-Hydroxycarbamazepine	3c
M253_R671_15627	11.18	253.0969	3-Hydroxycarbamazepine	3c
M253_R478_15629	7.97	253.0965	3-Hydroxycarbamazepine	3c
M253_R719_15630	11.99	253.0970	3-Hydroxycarbamazepine	3c
M254_R864_15825	14.40	254.1750	O-Demethylmetoprolol	3c
M255_R636_15918	10.60	255.1124	Licarbazepine	3c
M255_R731_15919	12.18	255.1125	Licarbazepine	3c
M255_R811_15922	13.51	255.1128	Licarbazepine	3c
M255_R540_15923	9.00	255.1123	Licarbazepine	3c
M257_R617_16144	10.28	256.9989	5-Desamino-5 oxo 2,5 Lamotrigine	1
M264_R496_17142	8.27	264.1955	N-Desmethylvenlafaxine	3c
M264_R565_17143	9.41	264.1951	N-Desmethylvenlafaxine	3c
M268_R219_17658	3.65	268.1541	Metoprolol acid	3c
M269_R752_17752	12.53	269.0919	5-(4-Hydroxyphenyl)-5-phenylimidazolidine-2,4-dione	3c
M271_R414_18027	6.89	271.1071	Diphenylhydantoic acid	3c
M271_R713_18028	11.88	271.1070	Diphenylhydantoic acid	3c
M271_R574_18030	9.56	271.1073	Diphenylhydantoic acid	3c
M271_R378_18034	6.31	271.1108	Sotalol-TP1	3c
M273_R1017_18327	16.96	273.1849	Estradiol	3c
M273_R944_18328	15.73	273.1847	Estradiol	3c
M276_R997_18673	16.61	276.1582	4-Hydroxypropranolol	3c
M280_R538_19168	8.97	280.1902	Tramadol N-Oxide	3c
M280_R706_19171	11.76	280.1904	Tramadol N-Oxide	3c
M282_R628_19349	10.46	282.0539	Carboxy-sulfamethoxazole	3c
M283_R168_19526	2.79	283.1651	Hydroxyatenolol	3c
M283_R438_19527	7.30	283.1650	Hydroxyatenolol	3c
M283_R353_19529	5.88	283.1650	Hydroxyatenolol	3c
M284_R969_19663	16.14	284.1857	alpha-Hydroxymetoprolol	3c
M285_R923_19752	15.38	285.1486	Estriol-TP5	3c
M285_R331_19768	5.51	285.1800	Oseltamivir Acid	3c
M286_R390_19860	6.50	286.1432	Norcodeine	3c
M286_R923_19861	15.38	286.1438	Morphine	3c
M287_R950_19991	15.83	287.1641	Estriol-TP1	3c
M288_R362_20087	6.03	288.1590	Codeine-TP20	3c
M289_R868_20198	14.46	289.1796	Estriol	3c
M289_R944_20203	15.74	289.1798	Estriol	3c
M290_R461_20288	7.68	290.1383	Norcocaine	3c
M290_R421_20289	7.01	290.1381	(3S,4R)-3-benzoyloxy-8-methyl-8-azabicyclo[3.2.1]octane-4-carboxylic acid	3c
M290_R194_20290	3.23	290.1385	(3S,4R)-3-benzoyloxy-8-methyl-8-azabicyclo[3.2.1]octane-4-carboxylic acid	3c
M290_R793_20291	13.21	290.1386	(3S,4R)-3-benzoyloxy-8-methyl-8-azabicyclo[3.2.1]octane-4-carboxylic acid	3c
M291_R830_20436	13.83	291.2091	3-(3,4-Dimethoxyphenyl)-2-methyl-6-methylaminohexane-3-carbonitrile	3c
M294_R526_20785	8.77	294.2060	Venlafaxine n-oxide	3c
M301_R861_21549	14.35	301.0739	Temazepam	3c
M302_R794_21705	13.23	302.1752	Codeine-TP7	3c
M307_R797_22226	13.28	307.1176	1-(hydroxymethyl)-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-ol	3c
M311_R769_22691	12.81	311.1555	Desmethylcitalopram	3c
M312_R853_22749	14.21	312.0188	3'-Hydroxydiclofenac	3c
M318_R717_23475	11.96	318.1698	Cocaethylene	3c
M318_R449_23476	7.49	318.1696	Cocaethylene	3c
M341_R786_25689	13.10	341.1661	Citalopram N-oxide	3c
M344_R699_26008	11.65	344.1653	Citalopram-TP1	3c
M359_R725_27277	12.09	359.1422	Diltiazem-TP22	3c
M359_R581_27278	9.69	359.1417	Diltiazem-TP22	3c
M373_R732_28409	12.20	373.1578	Deacetyldiltiazem	3c
M401_R686_30515	11.44	401.1525	N-Demethyldiltiazem	3c
M430_R600_32498	10.00	430.0925	(2S,3S,4S,5R)-3,4,5-trihydroxy-6-[4-[(5-methyl-1,2-oxazol-3-yl)sulfamoyl]anilino]oxane-2-carboxylic acid	3c
M436_R834_32925	13.90	436.1969	(2S,3S,4S,5R)-3,4,5-trihydroxy-6-[1-naphthalen-1-yloxy-3-(propan-2-ylamino)propan-2-yl]oxyoxane-2-carboxylic acid	3c
M437_R852_32976	14.20	437.1489	Losartan carboxylic acid	3c
M437_R766_32977	12.76	437.1488	Losartan carboxylic acid	3c
M439_R787_33108	13.11	439.1644	Losartan metabolite M2	3c
M439_R454_33109	7.57	439.1662	Losartan metabolite M2	3c
M734_R823_47601	13.72	734.4695	N-Desmethyl Clarithromycin	3c
M734_R776_47602	12.94	734.4693	N-Desmethyl Clarithromycin	3c
M177_R260_6903	4.34	177.1022	Cotinine	1
M272_R519_18118	8.66	272.0098	Lamotrigine-N2-oxide	1
M270_R614_17864	10.24	270.0304	N-methyl lamotrigine	1

TableS4 Tentatively identified features from NTS

group	ret	mz	compoundName2	idlevel	approach
M114_R1038_2657	17.29	114.0663	60-27-5	2a	NTS
M121_R177_2988	2.95	121.0760	benzamidine	2a	NTS
M152_R445_4833	7.42	152.1434	amantadine	1	NTS
M235_R485_13525	8.08	235.1803	lidocaine	1	NTS
M242_R961_14322	16.02	242.1175	mefenamic acid	1	NTS
M242_R930_14334	15.50	242.1433	Terbutryn	2a	NTS
M247_R487_14967	8.11	247.1801	mepivacaine	2a	NTS
M256_R773_16097	12.88	256.1694	diphenhydramine	2a	NTS
M326_R770_24221	12.83	326.0856	midazolam	1	NTS
M425_R664_32168	11.07	425.1868	clindamycin	2a	NTS
M177_R241_6923	4.01	177.1386	1-Benzylpiperazine	2a	NTS
M253_R755_15674	12.59	253.1627	HEXAZINONE	2a	NTS
M389_R811_29668	13.51	389.1626	cetirizine	2a	NTS
M248_R247_15012	4.12	248.0497	Emtricitabine	2a	NTS
M304_R317_21936	5.28	304.2013	Vildagliptin	2a	NTS
M455_R823_34134	13.72	455.2906	VERAPAMIL	1	NTS
M171_R297_6366	4.95	171.1127	Levetiracetam	2a	NTS
M327_R758_24364	12.64	327.1368	clozapine	2a	NTS
M252_R181_15508	3.01	252.1090	2'-deoxyadenosine	2a	NTS
M321_R827_23702	13.78	321.0192	lorazepam	1	NTS
M548_R872_39594	14.53	548.2430	Darunavir	2a	NTS
M192_R858_8361	14.29	192.1384	Diethyltoluamide	2a	NTS
M296_R929_20950	15.49	296.0240	diclofenac	1	NTS
M429_R872_32456	14.53	429.2399	irbesartan	1	NTS
M327_R913_24325	15.22	327.0081	13674-84-5	2a	NTS
M415_R787_31482	13.11	415.1452	flecainide	2a	NTS
M370_R804_28150	13.40	370.0833	lansoprazole	2a	NTS
M271_R524_18069	8.73	271.1802	doxylamine	2a	NTS
M308_R582_22314	9.70	308.0502	clopidogrel carboxylic acid	2a	NTS
M379_R789_28882	13.14	379.2228	Neotame	1	NTS
M256_R378_16014	6.30	256.0207	sulfathiazole	1	NTS
M182_R121_7309	2.02	182.0814	L-tyrosine	2a	NTS
M313_R699_22930	11.66	313.2119	oseltamivir	1	NTS
M220_R438_11626	7.30	220.1330	Ritalinic acid	2a	NTS
M423_R838_32028	13.97	423.1695	losartan	1	NTS
M246_R390_14800	6.49	246.1234	4-Acetamidoantipyrine	2a	NTS
M261_R832_16758	13.86	261.1809	carisoprodol	1	NTS
M342_R301_25770	5.01	342.1478	sulpiride	2a	NTS
M278_R690_18942	11.49	278.2113	venlafaxine	1	NTS
M212_R544_10640	9.07	212.1504	Terbutylazine-2-hydroxy	2a	NTS
M180_R696_7171	11.60	180.1747	memantine	2a	NTS
M180_R696_7171	11.60	180.1747	rimantadine	2a	NTS
M313_R877_22917	14.62	313.1910	praziquantel	2a	NTS
M163_R141_5685	2.35	163.1230	nicotine	2a	NTS
M240_R679_14087	11.31	240.1149	bupropion	2a	NTS
M256_R586_16023	9.77	256.0591	Imidacloprid	2a	NTS
M325_R776_24139	12.94	325.1711	citalopram	1	NTS
M436_R880_32932	14.67	436.2344	valsartan	1	NTS
M408_R630_30979	10.50	408.1250	Sitagliptin	1	NTS
M316_R763_23208	12.72	316.0081	bromazepam	1	NTS
M268_R171_17621	2.85	268.1038	adenosine	2a	NTS
M292_R476_20466	7.93	292.0262	Thiamethoxam	2a	NTS
M204_R892_9717	14.87	204.1384	Crotan	1	NTS
M183_R678_7439	11.31	183.0782	TRIETHYL PHOSPHATE	2a	NTS
M593_R903_42134	15.05	593.1845	GNF-Pf-2160	2a	NTS
M310_R836_22618	13.93	310.2165	methadone	1	NTS
M221_R500_11759	8.33	221.1647	prilocaine	2a	NTS
M307_R539_22225	8.98	307.1107	fluconazole	1	NTS
M279_R1013_19035	16.88	279.1591	dibutyl phthalate	2a	NTS
M219_R532_11497	8.87	219.1126	primidone	1	NTS
M226_R817_12384	13.62	226.1663	TERBUMETON	2a	NTS
M226_R817_12384	13.62	226.1663	SECBUMETON	2a	NTS
M204_R376_9697	6.26	204.1130	4-Aminoantipyrine	2a	NTS
M317_R528_23376	8.80	317.1642	timolol	2a	NTS
M404_R922_30713	15.36	404.1243	Azoxystrobin	2a	NTS
M370_R466_28174	7.77	370.1788	amisulpride	2a	NTS
M240_R249_14117	4.15	240.1592	Salbutamol	2a	NTS
M362_R869_27514	14.48	362.1153	bezafibrate	1	NTS
M232_R389_13108	6.49	232.1078	4-Formylaminoantipyrine	2a	NTS
M579_R547_41379	9.11	579.1710	Kaempferitrin	2a	NTS
M230_R857_12893	14.29	230.1751	Icaridin	2a	NTS
M285_R905_19722	15.08	285.0790	diazepam	1	NTS
M559_R916_40248	15.26	559.2610	atorvastatin	2a	NTS
M153_R344_4885	5.73	153.1022	2-Isopropyl-6-methyl-4-pyrimidone	2a	NTS
M207_R854_10043	14.24	207.1493	Isoproturon	2a	NTS
M223_R638_11935	10.63	223.0743	Acetamiprid	2a	NTS
M308_R940_22353	15.67	308.1521	Tebuconazole	2a	NTS
M342_R961_25751	16.02	342.0771	Propiconazole	2a	NTS
M233_R857_13203	14.29	233.0243	Diuron	3a	NTS
M297_R819_21071	13.65	297.0557	Imazalil	3a	NTS
M192_R387_8320	6.45	192.0767	Carbendazim	3a	NTS
M262_R816_16827	13.59	262.0874	Flumequine	2a	NTS
M195_R373_8629	6.22	195.0878	Caffeine	1	NTS
M146_R622_4369	10.36	146.0601	INDOLE-3-CARBOXALDEHYDE	3a	NTS
M193_R554_8402	9.23	193.0496	scopoletin	3a	NTS
M515_R891_37650	14.85	515.2448	Telmisartan	3a	NTS
M112_R142_2573	2.37	112.0869	histamine	3a	NTS
M327_R978_24337	16.31	327.0780	TRIPHENYL PHOSPHATE	3a	NTS
M340_R736_25595	12.26	340.1541	Canadine	3a	NTS
M187_R281_7870	4.68	187.0965	67950-95-2	3a	NTS
M441_R825_33238	13.75	441.1671	candesartan	3a	NTS
M184_R130_7574	2.16	184.1194	Hydroxysimazine	3a	NTS
M251_R543_15418	9.06	251.1386	Lacosamide	3a	NTS

M139_R89_3888	1.48	139.0503	urocanic acid	3a	NTS
M217_R854_11226	14.24	217.0496	Bergapten	3a	NTS
M152_R302_4815	5.04	152.1070	(-)-Norephedrine	3a	NTS
M300_R978_21517	16.30	300.2897	sphingosine	3a	NTS
M326_R701_24292	11.68	326.2322	BISOPROLOL	3a	NTS
M194_R425_8537	7.08	194.1175	MDMA	3a	NTS
M217_R854_11226	14.24	217.0496	methoxsalen	3a	NTS
M187_R472_7873	7.87	187.0966	67950-95-2	3a	NTS
M184_R289_7575	4.82	184.1195	66753-06-8	3a	NTS
M168_R356_6089	5.93	168.1019	phenylephrine	3a	NTS
M123_R512_3046	8.53	123.0552	nicotinamide	3a	NTS
M150_R495_4672	8.25	150.1277	METHAMPHETAMINE	3a	NTS
M168_R261_6086	4.35	168.1019	phenylephrine	3a	NTS
M149_R125_4590	2.09	149.1075	3-(pyrrolidin-2-yl)pyridine	3a	NTS
M150_R256_4671	4.27	150.1278	METHAMPHETAMINE	3a	NTS
M123_R554_3047	9.23	123.0552	nicotinamide	3a	NTS
M216_R469_11129	7.82	216.0879	kinetin	3c	NTS
M130_R104_3428	1.74	130.0500	L-Pyroglutamic acid	3c	NTS
M129_R343_3390	5.72	129.1022	Piperidine-4-carboxamide	3c	NTS
M204_R847_9712	14.11	204.1385	R-Deprenyl N-Oxide	3c	NTS
M227_R340_12508	5.67	227.1752	Crotetamide	3c	NTS
M168_R189_6088	3.14	168.1020	phenylephrine	3c	NTS
M176_R606_6793	10.10	176.0702	1-methoxyindole-3-carbaldehyde	3c	NTS
M216_R401_11090	6.69	215.9782	941-57-1	3c	NTS
M291_R430_20401	7.16	291.1445	trimethoprim	3c	NTS
M229_R961_12700	16.01	229.0860	oxybenzone	3c	NTS
M168_R460_6090	7.67	168.1019	phenylephrine	3c	NTS
M226_R558_12385	9.31	226.1659	TERBUMETON	3c	NTS
M226_R558_12385	9.31	226.1659	SECBUMETON	3c	NTS
M192_R387_8320	6.45	192.0767	Carbendazim	3c	NTS
M186_R822_7819	13.71	186.2219	TRIBUTYLAMINE	3c	NTS
M344_R953_26032	15.89	344.2217	Hydroxybutorphanol tartrate	3c	NTS
M195_R248_8630	4.13	195.0878	caffeine	3c	NTS
M214_R923_10908	15.38	214.1262	Octhilinone	3c	NTS
M188_R519_7952	8.65	188.0819	metamitron-desamino	3c	NTS
M163_R141_5685	2.35	163.1230	33089-74-6	3c	NTS
M247_R850_14933	14.17	247.1329	santonin	3c	NTS
M272_R757_18198	12.62	272.2006	dextromethorphan	3c	NTS
M208_R787_10141	13.12	208.1334	MDEA	3c	NTS
M502_R854_36853	14.23	502.2960	fexofenadine	3c	NTS
M208_R839_10142	13.99	208.1334	MDEA	3c	NTS

TableS5 Tentatively identified pesticides NTS

Compound	ID level	rt	m/z	Use in EU	other
Terbutryn	2a	15.50	242.1433	banned	
2-Isopropyl-6-methyl-4-pyrimidone	2a	5.73	153.1022	NA	TP of diazinon
Isoproturon	2a	14.24	207.1493	banned	
Imidacloprid	2a	9.77	256.0591	banned	
Thiamethoxam	2a	7.93	292.0262	banned	
Acetamiprid	2a	10.63	223.0743	approved	
Tebuconazole	2a	15.67	308.1521	approved	
Fluconazole	1	8.98	307.1107	NA	
Azoxystrobin	2a	15.36	404.1243	approved	
Propiconazole	2a	16.02	342.0771	banned	
Diuron	3a	14.29	233.0243	banned	
Imazalil	3a	13.65	297.0557	approved	
Carbendazim	3a	6.45	192.0767	banned	
Hexazinone	2a	12.59	253.1627	banned	
Flumequine	1	13.59	262.0874	NA	
Nicotine	3a	2.35	163.1230	NA	
Caffeine	1	6.22	195.0878	NA	

TableS6 Transformation products and precursor concentrations

Sample	Distance	Season	Type	Compound	Concentration (ng/L)
UP	-60	Summer	Parent	4-Hydroxidiclofenac	0.7
DP	0	Summer	Parent	4-Hydroxidiclofenac	1066.39
DW2	40	Summer	Parent	4-Hydroxidiclofenac	676.93
DW7	250	Summer	Parent	4-Hydroxidiclofenac	674.94
UP	-60	Winter	Parent	4-Hydroxidiclofenac	12.9
DP	0	Winter	Parent	4-Hydroxidiclofenac	1381.17
DW2	40	Winter	Parent	4-Hydroxidiclofenac	804.76
DW7	250	Winter	Parent	4-Hydroxidiclofenac	1052.04
UP	-60	Summer	TP	4-Hydroxidiclofenac	0.5
DP	0	Summer	TP	4-Hydroxidiclofenac	245.94
DW2	40	Summer	TP	4-Hydroxidiclofenac	143.87
DW7	250	Summer	TP	4-Hydroxidiclofenac	127.5
UP	-60	Winter	TP	4-Hydroxidiclofenac	0.5
DP	0	Winter	TP	4-Hydroxidiclofenac	193.5
DW2	40	Winter	TP	4-Hydroxidiclofenac	119.65
DW7	250	Winter	TP	4-Hydroxidiclofenac	76.48
UP	-60	Summer	Parent	5-Desamino-5 oxo 2,5 Lamotrogine	9.02
DP	0	Summer	Parent	5-Desamino-5 oxo 2,5 Lamotrogine	317.59
DW2	40	Summer	Parent	5-Desamino-5 oxo 2,5 Lamotrogine	226.68
DW7	250	Summer	Parent	5-Desamino-5 oxo 2,5 Lamotrogine	226.85
UP	-60	Winter	Parent	5-Desamino-5 oxo 2,5 Lamotrogine	9.04
DP	0	Winter	Parent	5-Desamino-5 oxo 2,5 Lamotrogine	388.4
DW2	40	Winter	Parent	5-Desamino-5 oxo 2,5 Lamotrogine	291.96
DW7	250	Winter	Parent	5-Desamino-5 oxo 2,5 Lamotrogine	329.03
UP	-60	Summer	TP	5-Desamino-5 oxo 2,5 Lamotrogine	0.15
DP	0	Summer	TP	5-Desamino-5 oxo 2,5 Lamotrogine	64.87
DW2	40	Summer	TP	5-Desamino-5 oxo 2,5 Lamotrogine	44.1
DW7	250	Summer	TP	5-Desamino-5 oxo 2,5 Lamotrogine	37.48
UP	-60	Winter	TP	5-Desamino-5 oxo 2,5 Lamotrogine	3.34
DP	0	Winter	TP	5-Desamino-5 oxo 2,5 Lamotrogine	81.6
DW2	40	Winter	TP	5-Desamino-5 oxo 2,5 Lamotrogine	59.94
DW7	250	Winter	TP	5-Desamino-5 oxo 2,5 Lamotrogine	71.79
UP	-60	Summer	Parent	Benzoylecgonine	0.5
DP	0	Summer	Parent	Benzoylecgonine	0.08
DW2	40	Summer	Parent	Benzoylecgonine	0.08
DW7	250	Summer	Parent	Benzoylecgonine	0.08
UP	-60	Winter	Parent	Benzoylecgonine	42.38
DP	0	Winter	Parent	Benzoylecgonine	4.21
DW2	40	Winter	Parent	Benzoylecgonine	12.49
DW7	250	Winter	Parent	Benzoylecgonine	8.65
UP	-60	Summer	TP	Benzoylecgonine	0.5
DP	0	Summer	TP	Benzoylecgonine	7.9
DW2	40	Summer	TP	Benzoylecgonine	5.06
DW7	250	Summer	TP	Benzoylecgonine	2.98
UP	-60	Winter	TP	Benzoylecgonine	200.11
DP	0	Winter	TP	Benzoylecgonine	34.99
DW2	40	Winter	TP	Benzoylecgonine	54.79
DW7	250	Winter	TP	Benzoylecgonine	47.32
UP	-60	Summer	Parent	Carbamazepine-10,11-epoxide	5.74
DP	0	Summer	Parent	Carbamazepine-10,11-epoxide	268.53
DW2	40	Summer	Parent	Carbamazepine-10,11-epoxide	184.03
DW7	250	Summer	Parent	Carbamazepine-10,11-epoxide	184.66
UP	-60	Winter	Parent	Carbamazepine-10,11-epoxide	12.29
DP	0	Winter	Parent	Carbamazepine-10,11-epoxide	364.57
DW2	40	Winter	Parent	Carbamazepine-10,11-epoxide	285.29
DW7	250	Winter	Parent	Carbamazepine-10,11-epoxide	344.61
UP	-60	Summer	TP	Carbamazepine-10,11-epoxide	3.12
DP	0	Summer	TP	Carbamazepine-10,11-epoxide	106.52
DW2	40	Summer	TP	Carbamazepine-10,11-epoxide	76.72
DW7	250	Summer	TP	Carbamazepine-10,11-epoxide	75.45
UP	-60	Winter	TP	Carbamazepine-10,11-epoxide	0.15
DP	0	Winter	TP	Carbamazepine-10,11-epoxide	143.04
DW2	40	Winter	TP	Carbamazepine-10,11-epoxide	113.24
DW7	250	Winter	TP	Carbamazepine-10,11-epoxide	136.67
UP	-60	Summer	TP	Cotinine	1.96
DP	0	Summer	TP	Cotinine	36.01
DW2	40	Summer	TP	Cotinine	24.02
DW7	250	Summer	TP	Cotinine	24.37
UP	-60	Winter	TP	Cotinine	32.2
DP	0	Winter	TP	Cotinine	52.97
DW2	40	Winter	TP	Cotinine	48.22
DW7	250	Winter	TP	Cotinine	53.74
UP	-60	Summer	TP	Lamotrigine-N2-oxide	1.34
DP	0	Summer	TP	Lamotrigine-N2-oxide	6.33
DW2	40	Summer	TP	Lamotrigine-N2-oxide	5.03
DW7	250	Summer	TP	Lamotrigine-N2-oxide	5.66
UP	-60	Winter	TP	Lamotrigine-N2-oxide	0.1
DP	0	Winter	TP	Lamotrigine-N2-oxide	10.51
DW2	40	Winter	TP	Lamotrigine-N2-oxide	7.31
DW7	250	Winter	TP	Lamotrigine-N2-oxide	10.01
UP	-60	Summer	Parent	N-acethyl sulfamethoxazole	8.92
DP	0	Summer	Parent	N-acethyl sulfamethoxazole	355.5
DW2	40	Summer	Parent	N-acethyl sulfamethoxazole	232.31
DW7	250	Summer	Parent	N-acethyl sulfamethoxazole	247.67
UP	-60	Winter	Parent	N-acethyl sulfamethoxazole	2.04
DP	0	Winter	Parent	N-acethyl sulfamethoxazole	252.82
DW2	40	Winter	Parent	N-acethyl sulfamethoxazole	197.69
DW7	250	Winter	Parent	N-acethyl sulfamethoxazole	182.03
UP	-60	Summer	TP	N-acethyl sulfamethoxazole	0.08
DP	0	Summer	TP	N-acethyl sulfamethoxazole	40.74
DW2	40	Summer	TP	N-acethyl sulfamethoxazole	24.79
DW7	250	Summer	TP	N-acethyl sulfamethoxazole	24.55
UP	-60	Winter	TP	N-acethyl sulfamethoxazole	0.08

DP	0	Winter	TP	N-acethyl sulfamethoxazole	10.64
DW2	40	Winter	TP	N-acethyl sulfamethoxazole	7.21
DW7	250	Winter	TP	N-acethyl sulfamethoxazole	8.68
UP	-60	Summer	TP	N-methyl lamotrigine	0.15
DP	0	Summer	TP	N-methyl lamotrigine	0.15
DW2	40	Summer	TP	N-methyl lamotrigine	0.15
DW7	250	Summer	TP	N-methyl lamotrigine	0.15
UP	-60	Winter	TP	N-methyl lamotrigine	0.15
DP	0	Winter	TP	N-methyl lamotrigine	7.67
DW2	40	Winter	TP	N-methyl lamotrigine	5.77
DW7	250	Winter	TP	N-methyl lamotrigine	6.87
UP	-60	Summer	TP	NO2 diclofenac	0.52
DP	0	Summer	TP	NO2 diclofenac	1.48
DW2	40	Summer	TP	NO2 diclofenac	1.55
DW7	250	Summer	TP	NO2 diclofenac	1.9
UP	-60	Winter	TP	NO2 diclofenac	0.25
DP	0	Winter	TP	NO2 diclofenac	4.17
DW2	40	Winter	TP	NO2 diclofenac	2.35
DW7	250	Winter	TP	NO2 diclofenac	2.7
UP	-60	Summer	Parent	O-desmethylvenlafaxine	1.93
DP	0	Summer	Parent	O-desmethylvenlafaxine	516.9
DW8	40	Summer	Parent	O-desmethylvenlafaxine	372.99
DW3	250	Summer	Parent	O-desmethylvenlafaxine	354.81
UP	-60	Winter	Parent	O-desmethylvenlafaxine	16.49
DP	0	Winter	Parent	O-desmethylvenlafaxine	981.03
DW8	40	Winter	Parent	O-desmethylvenlafaxine	747.91
DW3	250	Winter	Parent	O-desmethylvenlafaxine	869.08
UP	-60	Summer	TP	O-desmethylvenlafaxine	0.6
DP	0	Summer	TP	O-desmethylvenlafaxine	1413.93
DW2	40	Summer	TP	O-desmethylvenlafaxine	1054.52
DW7	250	Summer	TP	O-desmethylvenlafaxine	1023.14
UP	-60	Winter	TP	O-desmethylvenlafaxine	7.26
DP	0	Winter	TP	O-desmethylvenlafaxine	2045.38
DW2	40	Winter	TP	O-desmethylvenlafaxine	1575.36
DW7	250	Winter	TP	O-desmethylvenlafaxine	1719.91
UP	-60	Summer	TP	Valsartan acid	1
DP	0	Summer	TP	Valsartan acid	1026.27
DW2	40	Summer	TP	Valsartan acid	828.96
DW7	250	Summer	TP	Valsartan acid	682.32
UP	-60	Winter	TP	Valsartan acid	1
DP	0	Winter	TP	Valsartan acid	737.01
DW2	40	Winter	TP	Valsartan acid	604.6
DW7	250	Winter	TP	Valsartan acid	752.53
UP	-60	Summer	Parent	Valsartan acid	0.1
DP	0	Summer	Parent	Valsartan acid	147.21
DW2	40	Summer	Parent	Valsartan acid	108.71
DW7	250	Summer	Parent	Valsartan acid	92.06
UP	-60	Winter	Parent	Valsartan acid	42.37
DP	0	Winter	Parent	Valsartan acid	614.83
DW2	40	Winter	Parent	Valsartan acid	486.99
DW7	250	Winter	Parent	Valsartan acid	525.54

Table S7 PNEC values

Compound	CAS number	PNEC (µg/L)
4'-Hydroxydiclofenac	64118-84-9	0.22
5-Desamino-5 oxo 2,5 Lamotrigine	252186-78-0	NA
Benzoylecgonine	60305-56-8	2.18
Ecgonine methyl ester	136765-34-9	88.8
Carbamazepine-10,11-epoxide	36507-30-9	2.57
Cotinine	486-56-6	10
Lamotrigine-N2-oxide	136565-76-9	0.56
N-acethyl sulfamethoxazole	21312-10-7	2.38
N-methyl lamotrigine	1152091-68-3	NA
NO2 diclofenac	-	NA
O-desmethylvenlafaxine	93413-62-8	0.88
Valsartan acid	164265-78-5	320

*PNEC values obtained from https://www.norman-network.com/nds/ecotox/lowestPnecsIndex.php

5.4. Discussion

The studies compiled in this chapter contribute to a deeper understanding of the fate of N-oxides TPs from tertiary amine drugs while cheminformatics approaches for suspect and non-target screening helped to expand the understanding of the presence of CECs TPs in Mediterranean intermittent streams.

Fate of N-oxides in Mediterranean intermittent streams lab condition and real scenarios

The findings in Publication #5 demonstrated that N-oxide transformation products can exhibit high environmental persistence. However, environmental persistence of N-oxides was compound-specific. In the case of amisulpride-N-oxide, lab-scale experiments underestimated their environmental presence in river systems, as the obtained short degradation half-life of 3 h was not in line with its presence in the river samples. Interestingly, O-desmethylvenlafaxine N-oxide presented a shorter half-life compared to venlafaxine N-oxide (12 and 88 h respectively), suggesting that O-dealkylation may enhance degradation potential rather than prolong persistence. This contrasts with the general effect of N-oxidation, which is typically associated to higher environmental stability, due to reduced reactivity and enhanced polarity [14,46]. Conversely, the removal of a methyl group from oxygen (e.g., O-demethylation), may increase the compound's susceptibility to environmental breakdown, possibly enhanced by hydrophilicity or altered interactions with microbial enzymes [46,47]. In contrast, in a study on advanced oxidation processes [48], the opposite was observed for the non-N-oxides parent compounds. While 99% of venlafaxine degraded in 5 min, O-destheylvenlafaxine showed slower removal and degraded in 30 min. This suggest that under advanced oxidation conditions, O-desmethylvenlafaxine is more resistant to hydroxyl radical attack compared to venlafaxine. This highlights how different mechanisms can lead to contrasting environmental fates for the same compounds. Zucker et al., (2018) [49] observed that N-oxide venlafaxine persisted more than venlafaxine in biological treatment systems and that it is less reactive to ozone (compared to venlafaxine), however it was more reactive with hydroxyl radicals.

Publication #5 revealed that N-oxidation was a minor transformation pathway with the presence of N-oxides in river water being attributed primarily to their release with WWTP discharges rather than possible in-stream transformation. In contrast, Rúa-Gómez et al., 2013 [26] reported that indirect photodegradation played a significant role in the degradation of venlafaxine and tramadol and could be a feasible mechanism along the watercourse. Despite this, the half-lives of the selected compounds at lab-scale experiments remain high, indicating limited in-stream attenuation for both parent and N-oxides.

In Publication #6 five N-oxides were plausibly detected, including both tramadol and venlafaxine N-oxides which were tentatively annotated. Their persistence in river systems was assessed (Figure 5.3a) by plotting the peak intensities of these features against the distance of the sampling sites from the WWTP discharge point (DP) in order to assess their fate through changes in abundance. Parent concentrations were also plotted (Figure 5.3b) to elucidate if dilution processes or in-stream degradation governed the TP behaviour.

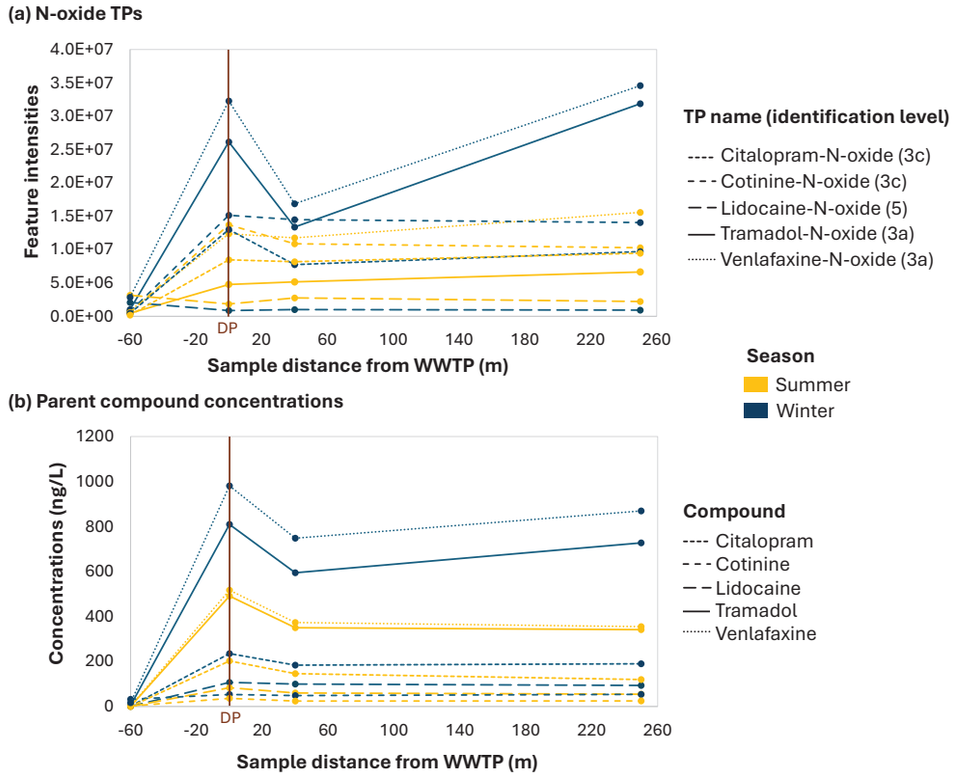


Figure 5.3. Behaviour of plausibly detected N-oxide transformation products (TPs) (a), including tentatively annotated compounds (Level 3) and one feature detected at Level 5 confidence, along with their corresponding parent compound concentrations (b) across the stream transect. Data extracted from publication #6. Abbreviations DP: WWTP discharge point.

Although lidocaine N-oxide was included in the N-oxide results, its identification confidence was only level 5. This feature shares the same molecular formula ($C_{14}H_{22}N_2O_2$), monoisotopic mass (250.1681 Da) and retention time with 3-hydroxylidocaine, which was assigned a higher confidence level (level 3) based on structural plausibility and available MS/MS data. Given the lack of definitive MS/MS data and reference standards, the identity of lidocaine N-oxide remains highly uncertain. Therefore, while this feature was included in the N-oxide dataset for comparison purposes, it may more plausibly correspond to the TP 3-hydroxylidocaine. Its confirmation would require the use of reference standards.

These findings highlight the need for environmental assessment to account for the persistence of TPs, whether they derive from in-stream attenuation or WWTP discharges. This is particularly relevant concerning N-oxides, which, despite their higher polarity, exhibit resistance to degradation and may accumulate in aquatic systems. Their higher dissipation half-lives in water/sediment suggests possible sorption to sediments under favourable conditions, underscoring the importance of understanding their formation, stability and degradation pathways. Such knowledge is crucial to improve water quality monitoring and regulatory frameworks.

Overall, the results obtained in this chapter highlight the complexity of TP fate in the environment. Predicting and understanding TP behaviour remains challenging, even with controlled lab-scale experiments, due to the vast number of dynamic and interacting environmental factors.

Environmental risk of transformation products

The persistence of N-oxides raises concerns about their potential ecological risks. However, evaluating these risks is difficult due to the lack of standards for quantification and available PNEC values. In Table 5.3, available PNEC values (Norman Ecotoxicology Database [50]) for parent compounds, their N-oxides, and other related TPs are presented.

In general, N-oxides present higher PNEC values than their corresponding parent compounds, indicating that higher concentrations would be required to pose the same level of ecological risk. This suggests that despite their observed persistence in the aquatic environment, N-oxide may pose comparatively lower toxicological risks. However, in some cases, N-oxides presented lower PNEC values than the parent compound namely for citalopram, lamotrigine, lidocaine and amisulpride (highlighted in red in Table 5.3), thus deserving further attention. In particular, the long-term presence of N-oxides calls for further action to assess potential chronic effects and accumulation over time.

Furthermore, to date, there are still compounds, particularly TPs, without available PNEC values neither in the Norman database nor from publicly available QSAR-derived estimates. To the best of our knowledge, this includes the TP O-desmethylvenlafaxine-N-oxide (Table 5.3), one of many TPs for which no toxicity data is available. Not only does the identification of TPs remain essential but also the study of their associated risk to the environment.

Table 5.3. *PNEC values for parent and TPs available on the Norman Ecotoxicology database [50]. NA: not available. Note: N-oxides with lower PNEC than parent compound are highlighted in red.*

Compound	CAS number	PNEC (µg/L)
Citalopram	59729-33-8	16
Citalopram-N-oxide	63284-72-0	7.91
Cotinine	486-56-6	10
Cotinine N-oxide	36508-80-2	42.6
Lidocaine	137-58-6	600
Lidocaine-N-oxide	2903-45-9	13.6
3-Hydroxylidocaine	392-17-6	13.1
Lamotrigine	84057-84-1	8
Lamotrigine-N-oxide	136565-76-9	0.56
Tramadol	27203-92-5	8.65
Tramadol N-oxide	147441-56-3	59.4
Venlafaxine	93413-69-5	0.88
Venlafaxine N-Oxide	1094598-37-4	34.2
O-Desmethylvenlafaxine	93413-62-8	0.88
O-desmethylvenlafaxine-N-oxide	1021933-95-8	NA
Clarithromycin	81103-11-9	0.12
Clarithromycin N-oxide	118074-07-0	1.67
Codeine	76-57-3	7.19
Codeine N-oxide	3688-65-1	32.4
Amisulpride	71675-85-9	140
Amisulpride N-oxide	71676-01-2	8.4
Sulpiride	15676-16-1	4.1
Sulpiride N-oxide	2214214-03-4	20.1

Future perspectives and research needs for TPs

Despite the advances in experimental and computational approaches, a major bottleneck persists in the harmonization of TP data across studies and platforms [7]. Addressing this requires not just technical tools, but community-wide consensus on reporting standards, greater accessibility to reference standards for TP verification, and shared, openly accessible data [44,51].

While experimental lab-scale approaches present promising results, these also present certain limitations as real conditions are hard to fully represent, due to the additional environmental and sometimes uncontrolled influences. Additionally, TPs are usually present at very low concentrations, hence sample treatment for effective enrichment is necessary, however a compromise needs to be reached as the preconcentration also enriches parent compounds present.

Another major limitation of suspect screening for TPs lies in the absence of TPs reference standards. This makes it difficult to confidently confirm compound identities,

as identification often relies on in silico fragmentation and spectral library matches. This increases the uncertainty and risk of false positives, particularly when MS/MS spectra are of low quality, due the low TPs concentration, or when multiple isomers are plausible.

To overcome these challenges, the integration of lab-scale experiments, data curation and prediction models as well as updates on databases remain essential for a better understanding of the fate and identification of TPs. As Zahn et al., 2024 [7] highlighted, there is a crucial need to implement data standards and a repository for TPs to enable and facilitate the understanding of TPs. Increased data-sharing and stronger collaborations between researchers and chemical producers are necessary to significantly enhance accuracy and ensure that regulatory TP frameworks remain relevant.

Application of non-target screening

The application of NTS approaches, enables to expand and better understand the occurrence of contaminants in aquatic environments by providing a more comprehensive characterization of chemical pollutants by detecting both known and unknown substances, including previously unmonitored TPs. Through Publication #6 the use of NTS allowed for tentatively identification of several pesticides. Specifically, ten pesticides were tentatively identified at a confidence level 2a, including a TP of diazinon, highlighting the potential of NTS to uncover both parent compounds and their degradation products. NTS serves as a potential tool to guide future targeted analysis by flagging compounds with high occurrence or persistence in vulnerable environments such as low flow streams. Moreover, integrating NTS results into monitoring strategies for the selection of priority substances for quantitative analysis and later a risk assessment evaluation is essential, to capture other contaminants that may be overlooked.

5.5. References

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Chapter 6

Conclusions

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The diverse nature and widespread use of CECs pose a significant challenge from both analytical detection and environmental monitoring perspectives. These challenges are further pronounced in Mediterranean intermittent streams, which are highly sensitive to climate and hydrological variability as well as anthropogenic pressures. As water scarcity intensifies, scenarios of droughts and reduced river flow are becoming more common. These situations lead to a reduced dilution of anthropogenic pressures such as WWTP effluents, and hence, a higher concentration of CECs in freshwater systems.

This thesis underscores the importance of integrating robust analytical techniques and cheminformatics approaches to better characterize and understand the occurrence, transformation and fate of CECs and their TPs in Mediterranean intermittent streams and associated groundwater. It provides novel insights into their dynamics and reinforces the need for integrated, multidisciplinary strategies to guide future monitoring efforts, improve environmental risk assessment, and support more adaptative water management practices.

The main conclusions drawn from this thesis are as follows:

- The developed and validated method, which combines SPE for preconcentration and extraction with detection and quantification by LC-HRMS, demonstrated high accuracy and efficiency with recoveries between 70 and 120% for 80 compounds. The optimized pre-defined inclusion list with specific compound windows with retention time and collisions energy enhanced the method's sensitivity enabling lower detection and quantification limits compared to other LC-HRMS reported methods. The method also demonstrated reliable performance for its application in both surface and groundwater samples.

- The application of the developed method demonstrated strong potential for its broad use in environmental monitoring strategies, enabling the assessment of the concentration of 116 CECs over more than 200 samples across Mediterranean intermittent streams, from Southern Europe to North African countries. This work contributed to the collection of valuable data on CECs concentrations and enhances our understanding of how anthropogenic activities are affecting vulnerable freshwater systems. In particular, the importance of river flow in regulating water quality was highlighted, showing that WWTP impacts are strongly influenced by flow conditions. During low flow periods, intermittent streams may become dominated by WWTP effluent, which can even serve as the primary water input, leading to reduced dilution and hence elevated CECs concentrations. The findings underscore the need for more stringent regulations and monitoring contemplating

hydrological conditions.

- This thesis expanded the monitoring efforts on the occurrence of CECs by including Mediterranean streams and groundwater samples from the MENA countries, helping to address the current global imbalance of CECs studies, which are currently heavily skewed towards high-income countries. While the Tunisian catchment presented the most compromised state, conversely the Algerian catchment presented relatively low CECs concentrations. Given the increasing anthropogenic pressures and water scarcity in the MENA countries, these results highlight the urgent need for more thorough treatments, further monitoring and stringent water quality regulations.

- The findings also highlight the need to integrate flow variability and climate adaptative measures into regulatory frameworks, as changes in hydrological regimes, especially in low-flow streams, can lead to effluent-dominated situations, reducing the streams' dilution capacity and increasing CECs concentrations. In some cases, contributions from WWTPs accounted for up to 81% of the total stream flow, emphasizing their dominant role in shaping water quality. Spatial and seasonal analysis revealed that WWTP-specific characteristics and input sources had a greater influence on contaminant profiles than seasonal variation, likely due to altered patterns during COVID-19 restrictions. Overall, the results remark the need to adapt and consider flow-dependent risk scenarios in both monitoring and management strategies, especially considering the expected rise in extreme climate event.

- Synergistic studies combining ecological, hydrological and chemical indicators, allowed a more comprehensive understanding of environmental stressors. The preliminary attempt presented enabled to observe a moderate positive correlation between the chemical and ecological indicators, where higher CECs concentrations implied a decline in biological quality, providing valuable insights. By incorporating chemical, hydrological, and ecological indicators, a holistic comprehension of the environmental status of WWTP impacted streams was achieved, however the lack of standardized protocols to evaluate the combined effects of multiple stressors stills remains a key challenge in understanding the dynamics of intermittent streams, which are highly sensitive to both climate variability and anthropogenic pressures and thus further studies are necessary. Integrated assessments may serve as valuable tools to develop effective mitigation measures for vulnerable systems such as Mediterranean intermittent streams.

- Risk assessment approaches are a valuable tool to estimate the risk associated with the CECs concentrations. Several CECs, especially diclofenac, losartan and

azithromycin, were repeatedly detected at ecologically relevant concentrations across the studied Mediterranean surface waters and groundwater samples. Despite diclofenac and azithromycin are already present in the Watchlists, this further supports and highlights the need for the inclusion of other potentially relevant contaminants in European and MENA monitoring programs and Watchlists. Further efforts should be made to establish environmental limits to those CECs that can pose a high risk. However, RQs are only qualitative measures, and further efforts are still needed to establish PNEC values for many unknown compounds, including TPs. Additionally, a comprehensive ecological risk assessment should incorporate environmental dynamics such as flow variability and contaminant persistence to achieve a more holistic understanding of the potential impacts of contaminants over time. At the same time, the integration of macroinvertebrate-based indices for pollution assessment can significantly enhance ecological risk assessments by providing critical biological insights into ecosystem health.

- Understanding the environmental fate of TPs remains a significant scientific challenge as a consequence of the complexity of transformation pathways together with the highly variable conditions and influences across ecosystems. While lab-scale experiment simulations help to predict possible scenarios and offer a valuable insight into environmental transformation pathways, these do not always mimic real environmental situations and strongly rely on compound specifications as observed for the studied N-oxides, where tramadol-N-oxide and amisulpride-N-oxide remained stable along the river stretch, while O-desmethylvenlafaxine-N-oxide and venlafaxine-N-oxide were less stable. Through the use of complementary approaches such as literature data curation and transformation prediction models, these enable to cover a wider scope of other possible transformation pathways previously reported in the literature. A suspect list of over 2000 TPs was generated allowing the subsequent tentatively annotated of 34 TPs (level 2a and 3a), of which 11 were latter quantified and their environmental fate was assessed. In this context, the use of suspect and non-target screening approaches play a crucial role in the identification of possible transformation products and serve as important complementary tools to targeted analysis. The integration of all three aspects is essential for a better understanding of possible environmental scenarios.

Overall, this thesis enabled the development and validation of a targeted high-resolution method for the quantification of a wide range of wastewater borne contaminants across several intermittent streams across Mediterranean countries. It narrowed the knowledge gap on the occurrence of CECs in two MENA countries, Algeria and Tunisia, including surface water and groundwater samples and provided a comprehensive characterization of Mediterranean streams through the integrated evaluation of chemical, ecological

and hydrological indicators. Furthermore, the study of the fate of TPs through lab-scale experiments and cheminformatics approaches underscored the potential of open source based approaches and the integration of experimental data, literature data curation and prediction models.