TARGET ANALYSIS AND SUSPECT SCREENING OF WASTEWATER DERIVED CONTAMINANTS IN RECEIVING RIVERINE AND COASTAL AREAS AND ASSESSMENT OF ENVIRONMENTAL RISKS

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University of Girona

Doctoral Thesis

Target analysis and suspect screening of wastewater derived contaminants in receiving riverine and coastal areas and assessment of environmental risks

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Declare:

That the doctoral thesis entitled: **"Target analysis and suspect screening of wastewater derived contaminants in receiving riverine and coastal areas and assessment of environmental risks"** presented by **Mira Čelić** to obtain a doctoral degree from the University of Girona has been completed under our supervision and fulfills the requirements for the attainment of the international mention.

For all intents and purposes, we hereby sign this document.

Signature

Prof. Mira Petrović

Dr. Meritxell Gros

Girona, August 2020


The best
view comes
after the
hardest
climb!
-Anonymous-

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To my son Mark

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LIST OF PUBLICATIONS

The results of this Ph.D. thesis have been published in scientific journals included in the Journal Citation Report of the Institute of Scientific Information.

Chapter 1

Article №1:

Mira Čelić, Sara Insa, Biljana Škrbić, and Mira Petrović (2017) *Development of a sensitive and robust online dual column liquid chromatography-tandem mass spectrometry method for the analysis of natural and synthetic estrogens and their conjugates in river water and wastewater*. ***Analytical Bioanalytical Chemistry*** *409, 5427–5440.*

IF=3.637; Quartile 1 in category Environmental Sciences.

Article №2:

Mira Čelić, Biljana D. Škrbić, Sara Insa, Jelena Živančev, Meritxell Gros, and Mira Petrović (2020) *Occurrence and assessment of environmental risks of endocrine disrupting compounds in drinking, surface and wastewaters in Serbia.* ***Environmental Pollution*** *262, 114344.*

IF=6.792; Quartile 1 in category Environmental Sciences.

Chapter 2

Article №3:

Mira Čelić, Meritxell Gros, Marinella Farré, Damia Barceló, and Mira Petrović (2018) *Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain)*. ***Science of the Total Environment****, 652, 952-963.*

IF=6.551; Quartile 1 in category Environmental Sciences.

Chapter 3

Article №4:

Mira Čelić, Adrián Jaén-Gil, Susana Briceño-Guevara, Sara Rodriguez-Mozaz, Meritxell Gros, and Mira Petrović (2021) *Extended suspect screening to identify emerging organic contaminants in riverine and coastal ecosystems and assessment of environmental risks*. ***Journal of Hazardous Materials****, 404, 124102.*

IF=9.038; Quartile 1 in category Environmental Sciences.

LIST OF ABBREVATIONS

Abbreviation	Meaning
AA-EQS	Annual Average-Environmental Quality Standards
ACA	Catalan Water Agency
AFG	African Group
AOEOs	Alkylphenol Ethoxylates
APCI	Atmospheric-Pressure Chemical Ionization
APPI	Atmospheric-Pressure Photoionization
APs	Alkylphenols
ASG	Asian Group
BCF	Bioconcentration Factors
BPA	Bisphenol A
d.w.	Dry Weight
DDA	Data-Dependent Acquisition
DES	Diethylstilbestrol
E1	Estrone
E1-3G	Estone-3-glucuronide
E1-3S	Estrone-3-sulfate
17α-EE2	17α-Ethinylestradiol
17β-E2	17β-Estradiol
E2-17G	Estradiol-17-glucuronide
E3	Estriol
E3-16G	Estriol-16-glucuronide
E3-3S	Estriol-3-sulfate
EC	Emerging Contaminants
EC50	50% Effective Concentration
ECD	Electron Capture Detector
EDCs	Endocrine Disrupting Compounds
EEFi	Estradiol Equivalency Factor
EEG	Eastern Europe Group
EEQ	Estradiol Equivalents
EEQt	Total Estrogenic Activity
EQSs	Environmental Quality Standards
ESI	Electrospray Ionization
FID	Flame Ionization Detector
FLD	Fluorescence Detector
GC	Gas Chromatography
GRULAC	the group of Latin American and Caribbean States
HPLC	High-performance Liquid Chromatography
HQ	Hazard Quotient
HRMS	High Resolution Mass Spectrometry
ILISs	Isotopically Labelled Internal Standards
IRD	Isotopic Ratio Difference

Kd	Solid-Water Partitioning Coefficient
Kow	Octanol-Water Partition Coefficient
LC	Liquid Chromatography
LC50	50% Lethal Concentration
LC-HRMS	Liquid Chromatography – High Resolution Mass Spectrometry
LC-MS	Liquid Chromatography – Mass Spectrometry
LC-MS/MS	Liquid Chromatography – Tandem Mass Spectrometry
LOD	Limit of Detection
logD	Distribution Coefficient
LOQ	Limit of Quantification
LVI	Large-Volume Injection
MAC	Maximum Allowable Concentration
MDL	Maximum Acceptable Method Detection Limit
MEC	Measured Environmental Concentration
MRM	Multiple reaction monitoring
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NI	Negative Ionization
NOEC	Non-Observed Effect Concentrations
NP	Nonylphenol
NPEOs	Nonylphenoletoxylates
OP	Octylphenol
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
PCPs	Personal Care Products
PEC	Predicted Environmental Concentrations
PhACs	Pharmaceutically Active Compounds
PI	Positive Ionization
pKa	Acid Dissociation Constant
PLE	Pressurized Liquid Extraction
PNEC	Predicted No-Effect Concentration
PTOP	Para-*Tert*-Octylphenol
QqLIT	Quadrupole-Linear Ion Trap
QqQ	Triple Quadrupole
RE	Removal Efficiency
RQs	Risk Quotients
RT	Retention Time
SDME	Single Drop Microextraction
SME-PES	Sorptive Microextraction with Polyethersulfone
SPE	Solid-Phase Extraction
SPM	Suspended Particulate Matter
SRM	Selected Reaction Monitoring
TOF	Time-Of-Flight
UA-DLLME-SFO	Solidification of a Floating Organic Drop

UHPLC	Ultra-High-Performance Liquid Chromatography
UN	United Nations
USE	Ultrasonic Solvent Extraction
UV	Ultraviolet Detector
WEOG	Western Europe and Others Group
WHO	World Health Organization
WWTPs	Wastewater Treatment Plants

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Summary

The main objective of this thesis is to evaluate the impact of wastewater discharges on receiving riverine and coastal ecosystems by studying the occurrence and fate of emerging contaminants (ECs). Since there is a myriad of ECs present in the environment, this thesis mostly focused on two main groups of compounds: (i) endocrine disrupting compounds (EDCs), including natural and synthetic hormones, alkylphenols (APs), such as nonylphenol (NP) and octylphenol (OP), and the plasticizer bisphenol A (BPA), and (ii) pharmaceutically active compounds (PhACs). These compounds were selected because they are one of the ECs of major input into the environment, thus being considered as "pseudo-persistent" pollutants, and for their potential deleterious effects to non-target organisms. Besides these target ECs, this thesis also evaluated the occurrence of other groups of contaminants by using high resolution mass spectrometry (HRMS). Finally, an assessment of the environmental risks posed by the identified ECs has been done in order to select the compounds of major ecological concern and those that could be used as relevant markers of wastewater contamination in freshwater and marine ecosystems. To accomplish the thesis objectives, two different analytical approaches have been used: (i) quantitative target analytical methods to determine the two groups of selected ECs (13 target EDCs and 81 PhACs that belong to nineteen different therapeutic groups) using reference analytical standards, and (ii) suspect screening using HRMS and exact mass compound databases (with information for the detection of 360 ECs).

For the detection and quantification of EDCs in aqueous matrices, an online ultra-high-performance liquid chromatography – tandem mass spectrometry (UHPLC-MS/MS) method using a triple quadrupole (QqQ) tandem mass spectrometer was developed. The benefits of the proposed method, compared to previously published methodologies, include lower detection limits complying with EU requirements, minimum sample manipulation, reduced total analysis time, overall method accuracy and precision. The developed method was further applied in the monitoring of 13 selected EDCs in untreated urban and industrial wastewaters in Serbia to assess their impact on the Danube River basin and associated freshwaters used as sources for drinking water production. A comprehensive monitoring was performed, and samples were taken from major hot spots for environmental contamination in the area. Even though fecal pollution has been widely studied and is an ongoing problem in the Danube River basin, due to the absence of wastewater treatment plants (WWTPs), there is still a restricted number of surveys concerning EDCs in this area. Out of the 13 EDCs analyzed, estrone (E1) and its metabolite estrone-3-sulfate (E1-3S), were the most ubiquitous compounds in urban wastewater and corresponding surface water impacted sites, while BPA was the most frequently detected substance in industrial wastewaters and freshwater sites. For drinking water, BPA, NP and OP were the most abundant compounds, while estrogens were not found in any of the samples.

For PhACs, a multiresidue method based on UHPLC coupled to a hybrid quadrupole-linear ion trap (QqLIT) tandem mass spectrometer was applied for evaluating the impact of urban wastewater discharges on the vulnerable area of the Ebro Delta (Catalonia, Spain). The occurrence of PhACs was followed along the wastewater-recipient water chain until they reach estuaries and the Mediterranean Sea. In general, a decrease in PhACs concentration was observed from inland sampling points towards the sea, resulting from a dilution in the recipient marine water bodies. In total, 28 out of the 57 compounds detected in effluent wastewater were positively identified in estuary and seawaters, revealing that WWTP discharges are an important source of contamination in coastal environments. PhACs with the highest frequency of detection belonged to the groups of analgesics/anti-inflammatories (acetaminophen, salicylic acid), antihypertensives (valsartan), psychiatric drugs (carbamazepine) and antibiotics (clarithromycin, trimethoprim). Nevertheless, a reduced number of PhACs was detected in sediment samples, indicating that sorption is a minor natural attenuation pathway for these compounds.

To have a broader overview of the impact of wastewater discharges on the Ebro Delta area, and to track for potential contamination sources, a suspect screening method was developed for the fast and reliable identification of 360 multiple class ECs. The automated suspect screening methodology was based on liquid chromatography coupled to a HRMS, using an LTQ Orbitrap Velos mass spectrometer. Out of the 360 ECs screened for, 48 compounds were tentatively identified, and 37 compounds were fully confirmed using isotopically labelled standards. Among the confirmed suspects, the compounds detected with the highest degree of confidence were: pharmaceuticals (acetaminophen, tramadol, carbamazepine, citalopram, venlafaxine, lidocaine, lamotrigine, valsartan, metformin, and hydrochlorothiazide), pesticides, such as herbicides (metolachlor, terbuthylazine, and terbutryn), and fungicides (azoxystrobin, metalaxyl, prochloraz, propiconazole, and tebuconazole), stimulants (caffeine, and nicotine), drug of abuse (cocaine), and insect repellents (diethyltoluamide (DEET)).

Finally, in order to highlight the ECs of major ecological concern in each study area, a risk assessment evaluation was performed. For EDCs, ecological risks were characterized using risk quotients (RQ) and estrogenicity, while for PhACs a prioritization strategy based on the compound's concentration and frequency of detection in seawater, removal efficiencies (RE) in WWTP, bioaccumulation potential, toxicity to marine organisms and persistency was used. RQs showed that estradiol (E2) and E1, followed by BPA, were the EDCs showing the highest risks in wastewater, while in surface water, only E1 showed potential risks in two sampling sites. For estrogenicity, total estrogenic activity (EEQt) exceeded the 1 ng L^{-1} estradiol level threshold in 3 surface water samples, which are mostly influenced by industrial wastewater discharges, whereas in drinking water, EEQt was below the 1 ng L^{-1} threshold in all samples. For PhACs, the prioritization

strategy used highlighted the antibiotics (trimethoprim and sulfamethoxazole), psychiatric drugs (carbamazepine and citalopram), diuretic (hydrochlorothiazide), antihypertensives (irbersartan and valsartan), β-blocking agent (sotalol) and analgesics/anti-inflmmatories (diclofenac, salicylic acid, and acetaminophen) as the substances of major concern and those that could be used as relevant markers of wastewater contamination in coastal environments. The RQ-based method was also applied in the suspect screening study, and RQ>1 were estimated for 10 compounds, such as the pharmaceuticals telmisartan, venlafaxine, and carbamazepine, the herbicides terbutryn and terbuthylazine, as well as its degradation product desethylterbuthylazine, the insecticides azoxystrobin, tebuconazole, and prochloraz and the insecticide tebufenozide.

Resumen

La finalidad principal de esta tesis es evaluar el impacto de las aguas residuales en los ecosistemas de agua dulce y costeros, mediante el estudio de la presencia y destino de contaminantes emergentes (CEs). Dado que hay una gran cantidad de CEs presentes en el medio ambiente, esta tesis se ha centrado principalmente en dos grupos de contaminantes: (i) compuestos disruptores endocrinos (EDC), incluyendo las hormonas naturales y sintéticas, alquilfenoles (AP), como el nonilfenol (NP) y el octilfenol (OP), así como el plastificante bisfenol A (BPA); (ii) fármacos (PhACs). Esos compuestos se seleccionaron porque pertenecen a los CEs con mayor entrada en el medio ambiente, motivo por el cual se consideran contaminantes "pseudo persistentes", así como por sus posibles efectos nocivos para los organismos acuáticos. Además de estos CEs, esta tesis también evalúa la presencia de otros grupos de contaminantes, mediante el uso de espectrometría de masas de alta resolución (HRMS). Finalmente, se ha realizado una estimación de los riesgos planteados por los CEs identificados, con el objetivo de seleccionar los compuestos de mayor relevancia ecológica, los cuales se podrían usar como marcadores de contaminación de aguas residuales en los ecosistemas de agua dulce y marinos. Para lograr los objetivos de la tesis, se han utilizado dos enfoques analíticos diferentes: (i) métodos analíticos *target* para determinar los dos grupos de CEs seleccionados (13 EDCs y 81 PhACs que pertenecen a diecinueve grupos terapéuticos diferentes) empleando estándares analíticos de referencia; (ii) *suspect screening* usando HRMS y bases de datos de masa exacta (con información para la detección de 360 CEs).

Para la detección y cuantificación de EDCs en matrices acuosas, se desarrolló un método basado en la cromatografía de alto rendimiento (UHPLC-MS/MS) acoplada a un espectrómetro de masas en tándem de triple cuadrupolo (QqQ). Los beneficios de este método, en comparación con las metodologías publicadas anteriormente, son la obtención de límites de detección más bajos que cumplen con los requisitos de la UE, con una manipulación mínima de la muestra, una reducción del tiempo total de análisis y una mayor precisión. El método desarrollado se aplicó al estudio de la presencia de 13 EDCs en aguas residuales urbanas e industriales no tratadas de Serbia, para evaluar su impacto en la cuenca del río Danubio y las aguas dulces asociadas que se usan como fuentes para la producción de agua potable. Se realizó un estudio integral, cogiendo muestras de los principales focos de contaminación, con el fin de investigar la calidad del agua de la zona. Aunque se hayan realizado varios estudios sobre la contaminación fecal en la Cuenca del río Danubio, todavía existe poca información sobre la presencia de EDCs en la zona, debido a la ausencia de plantas de tratamiento de aguas residuales. De los 13 EDCs analizados, la estrona (E1) y su metabolito E1-3S, fueron los compuestos más ubicuos en las aguas residuales urbanas y superficiales. El BPA fue la sustancia detectada con mayor frecuencia en las aguas residuales industriales y las aguas dulces asociadas. BPA, NP y OP fueron los compuestos más

abundantes en el agua potable, mientras que los estrógenos no se detectaron en ninguna de las muestras.

Para el análisis de PhACs, se aplicó un método multi-residuo basado en la cromatografía líquida de alto rendimiento (UHPLC) acoplada a un espectrómetro de masas en tándem híbrido, de cuadrupolo y trampa de iones lineal (QqLIT), con el objetivo de evaluar el impacto de las descargas de aguas residuales urbanas en el área vulnerable del Delta del Ebro (Cataluña, España). Se estudió la presencia de PhACs dentro de la cadena agua residual y las aguas receptoras de sus descargas hasta los estuarios y el Mar Mediterráneo. En general, se observó una disminución en la concentración de PhACs en los puntos más cercanos al mar, como resultado de la dilución de los contaminantes en ese medio. En total, 28 de los 57 compuestos detectados en los efluentes de agua residual fueron también detectados en los estuarios y aguas de mar, hecho que revela que las descargas de las depuradoras son una fuente importante de contaminación de los ecosistemas costeros. Los PhACs detectados con mayor frecuencia pertenecen a los grupos de analgésicos/ antiinflamatorios (acetaminofén, ácido salicílico), antihipertensivos (valsartán), medicamentos psiquiátricos (carbamazepina) y antibióticos (claritromicina, trimetoprima). Sin embargo, se detectó un número reducido de PhACs en los sedimentos de agua dulce y marinos, lo que indica que la sorción es una vía de atenuación natural minoritaria para esos compuestos.

Con el fin de tener una visión más amplia del impacto ambiental de las descargas de aguas residuales en el área del Delta del Ebro, y para rastrear posibles fuentes de contaminación, se desarrolló un método de *suspect screening* para la identificación rápida y fiable de 360 CEs. La metodología automatizada desarrollada se basa en la cromatografía de líquidos acoplada a la espectrometría de masas de alta resolución HRMS, utilizando un espectrómetro de masas LTQ Orbitrap Velos. De los 360 CEs examinados, 48 compuestos fueron identificados de forma tentativa, mientras que la presencia de 37 de ellos se pudo confirmar mediante el uso de estándares marcados isotópicamente. Entre los compuestos confirmados con mayor precisión se incluyen: productos farmacéuticos (acetaminofén, tramadol, carbamazepina, citalopram, venlafaxina, lidocaína, lamotrigina, valsartán, metformina e hidroclorotiazida), pesticidas, como herbicidas (metolacloro, terbutilazina, y terbutrina) y fungicidas (azoxistrobina, metalaxil, procloraz, propiconazol y tebuconazol), estimulantes (cafeína y nicotina), drogas de abuso (cocaína) y repelentes de insectos (dietiltoluamida (DEET)).

Finalmente, para identificar los CEs de mayor interés ecológico en cada área, se realizó un estudio de evaluación de riesgos ambientales. Para los EDCs los riesgos ecológicos se caracterizaron mediante el cálculo de cocientes de riesgo (RQ) y estrogenicidad, mientras que para los PhACs se utilizó una estrategia de priorización basada en la concentración de los compuestos y la frecuencia de

detección en aguas de mar, la eficacia de eliminación en las depuradoras, su potencial de bioacumulación en organismos acuáticos, su toxicidad en organismos marinos y su persistencia. Los RQs mostraron que el estradiol (E2), E1 y el BPA son los EDC de mayor riesgo en aguas residuales, mientras que, en las aguas superficiales, solo E1 presentó riesgos potenciales en dos zonas de estudio. Los valores de estrogenicidad mostraron que la actividad estrogénica total (EEQt) excedió el umbral del nivel de estradiol de 1 ng L^{-1} en solo 3 muestras de agua superficial, que están influenciadas principalmente por descargas de aguas residuales industriales, mientras que en el agua potable EEQt estuvo por debajo del umbral de 1 ng L^{-1} en todas las muestras. Para los PhACs, la estrategia de priorización utilizada destacó los antibióticos (trimetoprima y sulfametoxazol), medicamentos psiquiátricos (carbamazepina y citalopram), diuréticos (hidroclorotiazida), antihipertensivos (irbersartán y valsartán), agentes bloqueadores β (sotalol) y analgésicos/antiinflamatorios (diclofenaco, ácido salicílico y acetaminofeno) como las sustancias de mayor preocupación y que podrían usarse como marcadores relevantes de contaminación de aguas residuales en ambientes costeros. El método basado en RQ también se aplicó en el estudio de *suspect screening*, y se estimaron RQ>1 para 10 compuestos, que incluyen los fármacos telmisartán, venlafaxina y carbamazepina, los herbicidas terbutilazina, desetilterbutilazina y terbutrina, los insecticidas azoxistrobina, tebuconazol, y procloraz y el insecticida tebufenozida.

Resum

La finalitat principal d'aquesta tesi és l'avaluació de l'impacte de les aigües residuals en els ecosistemes d'aigua dolça i costaners, mitjançant l'estudi de la presència i destí de contaminants emergents (CEs). Atès que hi ha una gran quantitat de CEs presents en el medi ambient, aquesta tesi s'ha centrat principalment en dos grups de contaminants: (i) compostos disruptors endocrins (EDC), que inclouen les hormones naturals i sintètiques, alquilfenols (AP), com el nonilfenol (NP) i el octilfenol (OP), i el plastificant bisfenol A (BPA); ii) fàrmacs (PhACs). Aquests compostos es van seleccionar perquè pertanyen als CEs amb major entrada al medi ambient, de manera que es consideren com a contaminants "pseudo persistents", a banda dels seus possibles efectes adversos per als organismes aquàtics. Apart d'aquests CEs, en aquesta tesi també s'ha estudiat la presència d'altres grups de contaminants mitjançant l'ús d'espectrometria de masses d'alta resolució (HRMS). Finalment, s'ha realitzat una avaluació dels riscos plantejats per la presència dels CEs identificats, amb l'objectiu de seleccionar els compostos de major rellevància ecològica i que es podrien utilitzar com a marcadors de contaminació d'aigües residuals en els ecosistemes d'aigua dolça i marins. Per aconseguir aquests objectius, s'han utilitzat dos enfocaments analítics diferents: (i) l'ús de mètodes analítics *target* per determinar els dos grups de CEs seleccionats (13 EDCs i 81 PhACs que pertanyen a dinou grups terapèutics diferents), utilitzant estàndards analítics de referència; (ii) *suspect screening* mitjançant l'ús de HRMS i bases de dades de massa exactes (amb informació per a la detecció de 360 CEs).

Per a la detecció i quantificació de EDCs en matrius aquoses, es va desenvolupar un mètode basat en la cromatografia d'alt rendiment (UHPLC-MS/MS) acoblada a un espectròmetre de masses en tàndem de triple quadrupol (QqQ). Els beneficis d'aquest mètode, en comparació amb les metodologies publicades anteriorment, són l'obtenció de límits de detecció més baixos que compleixen amb els requisits de la UE, amb una manipulació mínima de la mostra i una reducció del temps total d'anàlisi, a banda d'una major precisió. El mètode desenvolupat es va aplicar a l'estudi de la presència de 13 EDCS en aigües residuals urbanes i industrials no tractades de Sèrbia, per avaluar el seu impacte en la conca del riu Danubi i les aigües dolces associades que es fan servir com a fonts per a la producció d'aigua potable. Es va realitzar un estudi integral, agafant mostres dels principals focus de contaminació, per tal d'investigar la qualitat de l'aigua de la zona. Tot i que s'hagin realitzat diversos estudis sobre la contaminació fecal de la conca del riu Danubi, encara hi ha poca informació sobre la presència de EDCS a la zona, a causa de l'absència de plantes de tractament d'aigües residuals. Dels 13 EDCs analitzats, l'estrona (E1) i el seu metabòlit E1-3S, van ser els compostos més detectats a les aigües residuals urbanes i superficials, mentre que el BPA va ser la substància trobada amb major freqüència en les aigües residuals industrials i les aigües dolces

associades. BPA, NP i OP van ser els compostos més abundants en l'aigua potable, mentre que els estrògens no es van detectar en cap de les mostres.

Per a l'anàlisi de PhACs, es va aplicar un mètode multi-residu basat en la cromatografia líquida d'alt rendiment (UHPLC) acoblada a un espectròmetre de masses en tàndem híbrid, de quadrupol i trampa d'ions lineal (QqLIT), amb l'objectiu d'avaluar l'impacte de les descàrregues d'aigües residuals urbanes a l'àrea vulnerable del Delta de l'Ebre (Catalunya, Espanya). Es va estudiar la presència de PhACs en la cadena aigua residual i les aigües receptores de les seves descàrregues fins als estuaris i el Mar Mediterrani. En general, es va observar una disminució en la concentració de PhACs en els punts més propers al mar, com a resultat de la dilució dels contaminants en aquestes aigües. En total, 28 dels 57 compostos que es van detectar a les aigües residuals tractades també es van trobar als estuaris i aigües de mar, fet que revela que les descàrregues de les depuradores són una font important de contaminació dels ecosistemes costaners. Els PhACs detectats amb més freqüència pertanyen als grups d'analgèsics/antiinflamatoris (acetaminofén, àcid salicílic), antihipertensius (valsartan), medicaments psiquiàtrics (carbamazepina) i antibiòtics (claritromicina, trimetoprim). Tanmateix, es va detectar un nombre reduït de PhACs en els sediments d'aigua dolça i marins, la qual cosa indica que la sorció és una via d'atenuació natural minoritària per a aquests compostos.

Per tal de tenir una visió més àmplia de l'impacte ambiental de les descàrregues d'aigües residuals en l'àrea del Delta de l'Ebre, i per rastrejar possibles fonts de contaminació, es va desenvolupar un mètode de *suspect screening* per a la identificació ràpida i fiable de 360 CEs. La metodologia automatitzada que es va desenvolupar es basa en la cromatografia de líquids acoblada a l'espectrometria de masses d'alta resolució (HRMS), utilitzant un espectròmetre de masses LTQ Orbitrap Velos. Dels 360 CEs examinats, 48 compostos van ser identificats de manera temptativa, mentre que la presència de 37 d'ells es va poder confirmar mitjançant l'ús d'estàndards marcats isotòpicament. Entre els compostos confirmats amb major precisió s'inclouen: productes farmacèutics (acetaminofén, tramadol, carbamazepina, citalopram, venlafaxina, lidocaïna, lamotrigina, valsartan, metformina i hidroclorotiazida), pesticides, com herbicides (metolaclor, terbutilazina, i terbutrina) i fungicides (azoxistrobina , metalaxil, procloraz, propiconazol i tebuconazol), estimulants (cafeïna i nicotina), drogues d'abús (cocaïna) i repel·lents d'insectes (dietiltoluamida (DEET)).

Finalment, per identificar els CEs de major interès ecològic en cada àrea d'estudi, es va realitzar un estudi d'avaluació de riscos ambientals. Per als EDCs, els riscos ecològics es van caracteritzar mitjançant el càlcul de quocients de risc (RQ) i estrogenicitat, mentre que per als PhACs es va utilitzar una estratègia de priorització basada en la concentració dels compostos i la freqüència de detecció en aigües de mar, l'eficàcia d'eliminació en les depuradores, el seu potencial de

bioacumulació en organismes aquàtics, la seva toxicitat en organismes marins i la seva persistència. Els RQs van mostrar que l'estradiol (E2), E1 y el BPA són els EDCs de major risc en aigües residuals, mentre que en les aigües superficials només E1 va presentar riscos potencials en dues zones d'estudi. Els valors d'estrogenicitat van mostrar que l'activitat estrogènica total (EEQt) va excedir el llindar del nivell d'estradiol d'1 ng L^{-1} en només 3 mostres d'aigua superficial, que estan influenciades per descàrregues d'aigües residuals industrials, mentre que en l'aigua potable EEQt va estar per sota del llindar de 1 ng L^{-1} en totes les mostres. Pels PhACs, l'estratègia de priorització utilitzada va destacar els antibiòtics (trimetoprima i sulfametoxazol), medicaments psiquiàtrics (carbamazepina i citalopram), diürètics (hidroclorotiazida), antihipertensius (irbersartán i valsartan), agents bloquejadors β (sotalol) i analgèsics/antiinflamatoris (diclofenac, àcid salicílic i acetaminofen) com les substàncies de major preocupació i que podrien utilitzar-se com a marcadors de contaminació d'aigües residuals en ambients costaners. El mètode basat en RQ també es va aplicar en l'estudi de *suspect screening*, i es van estimar RQ > 1 per a 10 compostos, que inclouen els fàrmacs telmisartan, venlafaxina i carbamazepina, els herbicides terbutilazina, desetilterbutilazina i terbutrina, els insecticides azoxistrobina, tebuconazol, i procloraz i l'insecticida tebufenozida.

1. General Introduction

Water is one of the most important components in an ecosystem and is essential for human sustenance (Yu et al., 2006). With the increasing population density, industrial development, and agricultural growth, the demands for fresh water have drastically increased. Water resources are continuously being degraded because of the human impact in agricultural, industrial, urban and touristic activities (Kourgialas et al., 2018). Additionally, water bodies are under constant pressure due to the large quantities of wastewater released back to them. This, together with an increase in the number of new chemicals produced and applied in daily activities, that are released to the environment, has resulted in a risk of contamination of the water bodies. Indeed, up to date, numerous emerging contaminants (ECs) have been widely detected in aquatic ecosystems (Boleda et al., 2013; Masiá et al., 2013; Shraim et al., 2017; Vazquez-Roig et al., 2012).

The pollution of water bodies by ECs is an ubiquitous phenomenon around the world. The occurrence of ECs presents a global water quality challenge with potentially serious threats to human health and ecosystems (Desbiolles et al., 2018; Ivanová et al., 2018; Pino-Otín et al., 2017; Varela et al., 2016). ECs are continuously released into aquatic environments through multiple pathways, and some of them proved to be toxic for non-target aquatic organisms even at low concentration levels. Thus, the analysis and monitoring of these compounds in the environment is a hot topic of high environmental concern. The progresses in analytical instrumentation led to a detection of many harmful compounds at the levels at which they have a biological effect in the environment and several new or previously ignored and/or unrecognized contaminants have become under scrutiny, allowing us to expand our knowledge about their potential deleterious effects to the environment.

1.1 Sources and pathways of pollution on aquatic ecosystems

The quality of aquatic ecosystems is continuously being threatened by both point and non-point sources of pollution (**Fig. 1**). Point sources of pollution constitute a single identifiable source which originates from separate locations and can be predicted and calculated (Lapworth et al., 2012). For instance, industrial, hospital, and urban WWTPs effluents are the major point sources of pollution to the aquatic environment. On the contrary, non-point sources are attributed to diffuse pollution that is hard to be identified with a discrete location (Lapworth et al., 2012). Diffuse pollution is the release of potential pollutants from a range of activities that, individually, may have no effect on the water environment, but at the scale of a catchment can have a significant effect. Some examples of non-point sources are the discharges of unregulated domestic effluents from septic tanks, agricultural, and urban run-off resulting from the application of sewage sludge, domestic or animal waste as fertilizers in agricultural fields, and the leaking from sewage and landfills (Bueno et al., 2012). Diffuse pollution sources have generally lower

environmental loading than point sources because they have higher potential for natural attenuation in the soil and subsurface environments (Murray et al., 2010).

Among point sources of pollution, wastewater discharges constitute the primary route of entry of ECs into the aquatic environment. Wastewater discharges mostly include effluents from domestic, industrial, run-off or medical sources (i.e. hospitals) (Tran et al., 2018). ECs mostly enter the wastewater system through the excretion of unmetabolized consumed products in urine and feces, the rinsing off dermally applied products, the use of surfactants, detergents, and personal care products (PCPs) for household, clothing or personal cleansing or from their production and use in industrial activities (Tijani et al., 2016). Un-used and out-of-date products, such as medicines, also enter the environment via flushing into bins, drains, toilets, or disposal with industrial and household waste (Daughton and Ternes, 1999). In high income countries, wastewater is treated in wastewater treatment plants (WWTPs) before being discharged to the receiving aquatic environment. WWTPs were designed to remove pathogens and nutrients from wastewater without considering ECs, and numerous studies already showed that these compounds are not completely removed by conventional treatments applied in WWTPs, finding their way out in the aquatic environment through the treated effluents (Behera et al., 2011; Bolong et al., 2009; Kasprzyk-Hordern et al., 2009). In low- and middle-income countries, the connectivity to wastewater infrastructure is scarce, and in some cases, even inexistent. Thus, ECs enter the environment in a more diffusive manner (Kookana et al., 2014) and higher environmental risks are to be expected. Besides WWTP effluent discharges, ECs can also enter the aquatic environment through combined sewer overflows during wet weather conditions (Managaki et al., 2007). Numerous ECs have been quantified at ng L^{-1} and µg L^{-1} concentration levels in wastewaters and in various receiving water bodies, such as freshwater, ground and drinking water sources (Chen et al., 2013; Ferrer and Thurman, 2012; Garrido et al., 2016; Griffith et al., 2014).

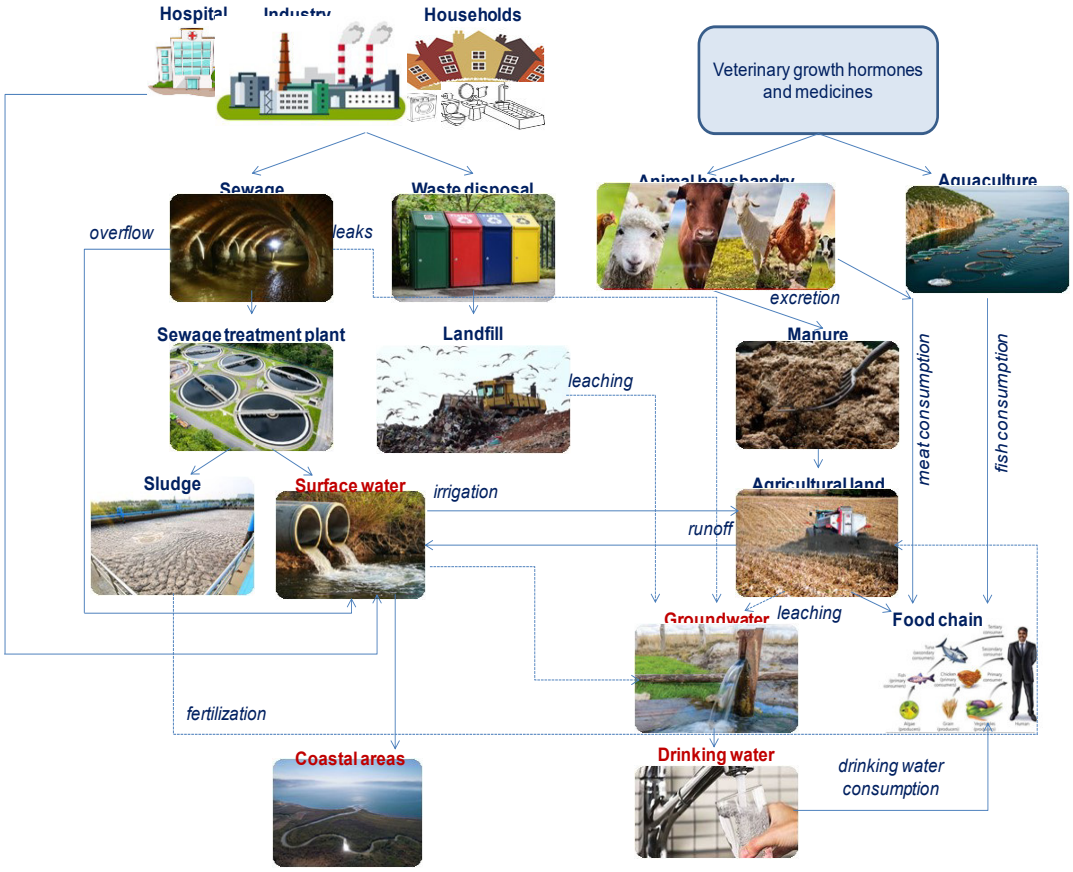


Fig. 1 *Sources and pathways of aquatic contamination in receiving riverine and coastal areas*

Besides freshwater bodies, marine ecosystems are also affected by ECs contamination through wastewater discharges (Klosterhaus et al., 2013). Indeed, ECs are released into seawater through the direct discharge of urban wastewater effluents, from submarine or marine WWTPs outfalls from boats, ships, and cruise liners (Fenet et al., 2014), or from runoff via rivers and streams that receive WWTPs effluents (Benotti and Brownawell, 2007; Farré et al., 2008; Lara-Martín et al., 2014). Other sources of ECs in the marine environment are aquaculture and pond-based farms in coastal areas (Le and Munekage, 2004; Rico and Van den Brink, 2014; Zou et al., 2011), and horticulture along rivers close to the sea (Jia et al., 2011; Kümmerer, 2009). Recreational activities, waste disposal, leachate from landfills, and seafills located in marine areas may also contribute to the loads of ECs entering coastal waterways (Bachelot et al., 2012; Langford and Thomas, 2008; Rodríguez-Navas et al., 2013). So far, data about ECs in seawater is scarce. Indeed, a limited number of studies that report on the occurrence of ECs in marine settings are available in the scientific literature (Arditsoglou and Voutsa, 2012; Benedé et al., 2014; Robles-Molina et al., 2014). However, coastal areas are subject to high environmental pressures due to the increase in human activities and they are of great concern because they constitute the ultimate receptacle of rivers and wastewater discharges and represent sensitive aquatic ecosystem. In many cases, these settings are vulnerable areas, with high ecological richness and diversity, where the input of ECs could cause potential undesired ecological effects.

1.2 Emerging contaminants (ECs)

ECs can be defined as pollutants that are currently not included in routine monitoring programmes at the European level and which may be candidates for future regulation, depending on research on their (eco)toxicity, potential health effects and public perception and on monitoring data regarding their occurrence in the various environmental compartiments (NORMAN Network, 2016). ECs are a group of natural and synthetic chemicals of wide use in everyday life and industrial applications (Jurado et al., 2012; Lapworth et al., 2012). ECs and their transformation products (TPs) have been widely detected in aquatic ecosystems at ppb or ppt concentration ranges throughout the globe (Birch et al., 2015; González-Alonso et al., 2017; Jia et al., 2011; Kasprzyk-Hordern et al., 2008). There are varying definitions for ECs, as well as discussion on the types of substances that should be included under this category. The term ECs does not necessarily include "new substances" only, (i.e. newly introduced chemicals and their degradation products and/or metabolites or by-products), but it also refers to compounds with recent detection in the environment and to compounds with previously unrecognized adverse effects on ecosystems. The latest advances and significant improvement in sample preparation and instrumental techniques allowed the detection of several contaminants in the last few decades that have been widely used for several years (Carmona and Picó, 2018), and these compounds are also considered as ECs.

The list of ECs is growing every day due to introduction of new commercial chemicals into the market, the disposal of chemicals of current widespread use and further identification of new molecules (Rodriguez-Narvaez et al., 2017). The NORMAN Network, which is based on an EU funded project (Dulio et al., 2018), created one of the world's largest classified databases of ECs occurring in the environment, for their monitoring and identification (NORMAN Network, 2016). Currently, the NORMAN list of ECs includes more than 1036 chemicals, and their TPs, which are categorized into 30 different classes, based on their origin and type. According to this classification list, some of the most prominent classes/categories of ECs are (NORMAN Network, 2016; Richardson and Ternes, 2014; Rodriguez-Narvaez et al., 2017):

- pharmaceutically active compounds (PhACs),
- personal care products (PCPs),
- endocrine disrupting compounds (EDCs),
- pesticides,
- disinfection by-products,
- industrial chemicals,
- artificial sweeteners and food additives,
- nanomaterials,
- sunscreens and UV filters,
- flame retardants,

- benzotriazoles and benzothiazoles,
- microplastics,
- per- and polyfluoroalkyl substances
- siloxanes, etc.

Due to the large diversity of ECs, this thesis mostly focused on two groups of contaminants of major concern:

i. EDCs, including natural and synthetic hormones, the alkylphenols (APs) nonylphenol (NP) and octylphenol (OP) and the plasticizer bisphenol A (BPA),
ii. PhACs

The selection of these compound groups was based on their high usage and continuous input into the aquatic environment and for the potential undesired ecotoxicological effects that they can cause to aquatic organisms and human health (Caliman and Gavrilescu, 2009; Liu and Wong, 2013).

1.2.1 Endocrine disrupting compounds

EDCs are widely spread contaminants of major public health concern, since they can interact with the endocrine system and produce adverse effects to wildlife and human health even at trace concentrations (Ascenzi et al., 2006). In the last few years, many countries and organizations have taken measures to promote the testing and assessment of EDCs in the environment. The United Nations (UN) Environment program and the World Health Organization (WHO) published *the State of the Science of Endocrine Disrupting Chemicals-2012*, to raise concern about these compounds to the public (WHO/UNEP, 2013). Among all EDCs, steroid estrogens (natural and synthetic hormones), APs and the plasticizer BPA are of particular interest due to their high estrogenic potency, since they could cause endocrine disruption, some of them even at low doses of exposure (below ng L^{-1}) (Brausch and Rand, 2011). Thus, these three groups of EDCs were selected as target compounds of interest in this thesis.

Natural and synthetic steroid estrogens. 17β-estradiol (17β-E2), is the most potent natural estrogenic hormone (Shore et al., 2004), which in the living organisms is transformed into estrone (E1) and further transforms into estriol (E3) (Liu et al., 2012), whereas synthetic estrogens include 17α-ethinylestradiol (17α-EE2) and diethylstilbestrol (DES). The synthetic estrogenic hormone 17α-EE2 is used as a main component in the contraceptive pills and menopause-related medicines (Zaccaroni et al., 2016). Estrogens are predominantly excreted into the environment through urine and feces of humans, livestock, and aquaculture in their biological inactive forms, as sulfate and glucuronide conjugate metabolites, and their existence in wastewater was reported by several researchers (D'Ascenzo et al., 2003; Gentili et al., 2002; Komori et al., 2004; Reddy et al., 2005). Indeed, in

some cases, higher concentrations of the metabolites have been reported, compared to the parent compounds (Kobayashi et al., 2006; Pedrouzo et al., 2009). Conjugated metabolites, which include estrone-3-sulfate (E1-3S), estriol-3-sulfate (E3-3S), estrone-3-glucuronide (E1-3G), estradiol-17-glucuronide (E2-17G) and estriol-16-glucuronide (E3-16G), are more water soluble and mobile than the parent compounds, and they can pose an environmental risk because they are hydrolyzed back to the biologically active free estrogen form in the environment or during wastewater treatment (Liu et al., 2009). In the last several years, natural and synthetic estrogens have been widely identified in different aquatic compartments (Amin et al., 2018; Galaon et al., 2016; Goeury et al., 2019; Le Coadou et al., 2017).

Steroid estrogens are of special interest because they may interfere with the normal growth and reproduction of human and wildlife species (Newbold et al., 2008). Estrogens can cause adverse effects to aquatic living organisms and, in some cases, to human health due to their high potency to act biologically even at ng L^{-1} concentration levels (Gerbersdorf et al., 2015). Compared with other EDCs, their estrogenic potency is 10.000 to 100.000 times higher than exogenous EDCs (i.e. pesticides, plasticizers, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs)) (Falconer et al., 2006; Gomes et al., 2003; Hanselman et al., 2003). This makes them the major contributors of estrogenic activities in wastewater and receiving water bodies (De Mes et al., 2005). Among estrogens, the hormone 17β-E2 has the highest estrogenic activity, and stability, and it has been extensively disseminated in the water bodies. It could induce male fish producing female attributes even at extremely low levels resulting in fish extinction (Yang et al., 2008). Apart from the endocrine disruption, the WHO has listed estrogens as group 1 carcinogens (Bilal and Iqbal, 2019).

Alkylphenols (APs), such as NP and OP, are industrial organic chemicals that result from the biodegradation of the synthetic non-ionic surfactants alkylphenol ethoxylates (AOEOs), widely used for industrial, agricultural, and domestic applications (Ying et al., 2002). NP is widely used in industry as raw material, additive in epoxy resins to enhance properties of polymerization, drying, plasticity (Casajuana and Lacorte, 2003), or it can be formed by the degradation of polyethoxylated nonylphenols, which are common surfactants applied as cleaning agent in bottling processing (Talmage, 1994). The main use of OP (80% of total quantity) is in the production of para-*tert*-octylphenol (PTOP)-based resins, which are used as tackifiers in tire manufacture (Quednow and Püttmann, 2009). It is estimated that 60-65% of all APs that enter conventional WWTPs are discharged into the environment, and around 25% of these are NPs (Ahel et al., 1994). Thus, their presence in surface water and sediments has been primarily attributed to their incomplete removal in wastewater treatment processes (Gomes et al., 2003; Purdom et al., 1994). APs disrupt the endocrine system by interfering with the estrogen receptor (Jobling and Sumpter, 1993). As a result, long-term toxic effects

on aquatic organisms can occur at concentrations of 3.3 µg L^{-1} (ECB, 2002). APs basically consist of an alkyl group, which can vary in size and position, attached to a phenolic ring. These compounds partition preferentially to the organic fraction of sediments and tend to bioaccumulate in aquatic organisms due to their pronounced lipophilicity (Esteban et al., 2014). Therefore, APs could be predominantly found in sediments and biological samples (Staples et al., 1998). However, NP and OP were also detected in water samples collected in several monitoring campaigns in European Rivers (Brix et al., 2010b; Esteban et al., 2014; Kasprzyk-Hordern et al., 2008; Loos et al., 2010; Pelayo et al., 2011; Quednow and Püttmann, 2009). Generally, the highest concentrations of APs were found in industrial areas, more likely attributed to industrial wastewater discharges (Petrovic et al., 2004).

Bisphenol A (BPA) is a polar monomer of polycarbonate plastic, used in canned foods and beverages as well as an intermediate component in the synthesis of polycarbonates, epoxy resins, flame retardants, and many other products (Staples et al., 1998). Furthermore, BPA is often used in manufacturing process of plastic products, such as water bottles, food containers, or electronics. Due to its wide use, it is an ubiquitous compound in the environment, entering water bodies through various pathways, such as indirect emissions, leaching and volatilization from plastic products after their usage, disposal, and incineration (Al-Odaini et al., 2010; Céspedes et al., 2008; Nurulnadia et al., 2014; Rodriguez-Mozaz et al., 2004; Selvaraj et al., 2014; Wenzel et al., 2004). Its release into the environment mainly occurs via wastewater from industrial plants producing plastic products, landfill leachates, via hydrolysis of BPA from plastics, or natural degradation of polycarbonate plastics due to moderate water solubility and low vapor pressure. The potential risks of BPA include reproduction, development, neurochemical, and behavioral effects (vom Saal and Hughes, 2005). Endocrine disrupting effects were observed *in vivo* with a relative potency ranging from 1.0×10^{-5} to 8.1×10^{-5} (relative to 17β-E2) (Petrovic et al., 2004).

1.2.2 Pharmaceutically active compounds

PhACs are an important class of ECs. They are widely used in both human and veterinary medicine and are essential to modern healthcare. PhACs are designed to be biologically active and include different compound groups such as antibiotics, analgesics and anti-inflammatory drugs, psychiatric drugs, blood lipid regulators, diuretics, antihypertensives, β-blockers, X-ray contrast media, or cytostatic drugs, among others (Daughton and Ternes, 1999). Prescription rates of pharmaceuticals have almost tripled in the last years and large number of PhACs are being prescribed worldwide, for both human and veterinary use (Boxall et al., 2012; Caldwell et al., 2014; Dong et al., 2013; Hughes et al., 2013). Furthermore, their usage and consumption are increasing consistently due to the expanding

population and the inverting age structure (Jelic et al., 2011). On a global scale, 3500 different PhACs were reported to be used by Caldwell et al. (2014) (Caldwell et al., 2014) while Boxall et al. (2012) (Boxall et al., 2012) reported 4000 different compounds. In Europe, 5000 PhACs have been reported by Hughes et al. (2013) (Hughes et al., 2013), while 10.000 have been reported for the US market by Dong et al. (2013) (Dong et al., 2013).

However, compared to high income countries, low income countries consume less PhACs by value of the worlds medicines (WHO, 2011), but have higher rate of infectious diseases and generally higher rate of over-the-counter self-medication (Ayukekbong et al., 2017; Segura et al., 2015). The large disparity in PhACs consumption figures significantly affects their environmental occurrence patterns. Several studies showed that the exposure of aquatic organisms to PhACs leads to a wide range of deleterious effects, such as lower growth, oxidative stress reproductive fitness impairment (Ericson et al., 2010; Gonzalez-Rey and Bebianno, 2014; Schmidt et al., 2011), and behavioral effects (Brodin et al., 2013). Their potential ecotoxicological and human health risks have raised interest about studying their occurrence and distribution in the aquatic environment (Tahar et al., 2017). Even though the occurrence, sources, and fate of PhACs in freshwater ecosystem have been widely reported (Daneshvar et al., 2010; Kasprzyk-Hordern et al., 2008; Pereira et al., 2017; Wu et al., 2017), information about their occurrence and fate in coastal settings, influenced by WWTP discharges, is still sparse (Ali et al., 2017; Fisch et al., 2017; Nödler et al., 2014).

1.3 Analysis of emerging contaminants

Impressive improvements in detection limits for the analysis of ECs, mostly due to the development of hyphenated chromatography-mass spectrometry (MS) techniques, have pushed the detectable target concentrations from the microgram to the nanograms or even picograms per liter range (Barceló and Petrovic, 2007). Together with the progresses in analytical instrumentation, extraction techniques have also become simpler, faster, and inexpensive to use, facilitating the analysis of ECs from complex environmental matrices (Runnqvist et al., 2010). All these improvements allowed the detection of ECs at the levels at which they have a biological effect in the environment. Recent trends have moved from conventional target analysis, including pre-selected target ECs, towards the use of high-resolution mass spectrometry (HRMS)-based methodologies, which allow the scrutiny of non-pre-selected, new, previously ignored and/or unrecognized contaminants.

Fig. 2 shows the differences between the so-called target methods and HRMS-based methodologies, by using the well-known iceberg principle. The iceberg shows the quantity of data that can be obtained when using different analytical approaches. For instance, when using target analysis, that focuses just on known

targeted and pre-selected ECs, limited information about the occurrence of ECs in the environment is obtained, as represented by the "tip of the iceberg" as "knowns". In target analytical methods, only a small amount of information is available or visible (in analytical terms a limited number of ECs is screened for), whereas there is still a lot of information that is unavailable or hidden in our samples. Thus, the current tendency is to use methodologies based on HRMS that can provide more information, other than just on a limited number of target ECs, and can provide data on the "hidden" information in a sample (the submerged part of the iceberg).

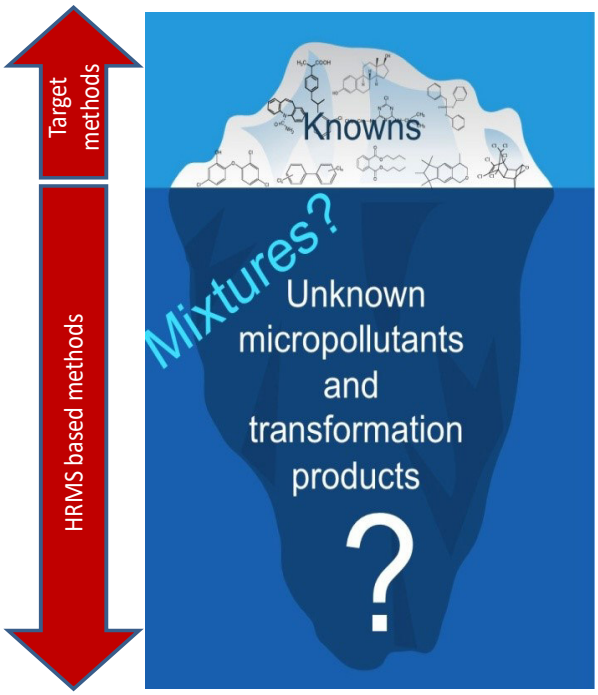


Fig. 2 *Iceberg effect in ECs analysis*

Although the new tendency in modern analytical approaches is to use HRMS based methods, the target methods by using liquid chromatography (LC) or gas chromatography (GC) coupled to tandem MS continue to be the methods of choice for the analysis of pre-selected target ECs due to their robustness, sensitivity, selectivity, repeatability, and reliability. The selection of LC and GC depends on the compound's physicochemical properties. Thus, the less volatile and polar compounds are analyzed with LC, whereas GC is used for volatile and semi-volatile compounds, and in some cases, it is necessary to perform an additional derivatization step to modify their structure and meet the conditions of GC-MS. Instruments that are most widely used in target analysis are triple quadrupole (QqQ) and hybrid quadrupole-linear ion trap (QqLIT) tandem mass spectrometers, operating at unit resolution. These instruments usually offer high sensitivity and selectivity, which are necessary characteristics for the identification and quantification of ECs at low concentration levels. With these instruments, the identification and quantification of target ECs is done by using the selected

reaction monitoring (SRM) mode, by recording precursor to product ion transitions. For each compound, two SRM transitions must be monitored to ensure a reliable identification (multiple reaction monitoring (MRM)), since the monitoring of only one transition might result in false positive identifications (Pozo et al., 2006). The most intense SRM transition is used for quantification and the other one for the confirmation of the chemical identity. Although these target methodologies have been successfully applied for studying the occurrence of a wide range of ECs in environmental samples, the information provided is just confined to a limited number of pre-selected chemicals. Indeed, the major bottleneck of conventional target analytical methods is the number of ECs that can be screened for, which in most of the available methods does not exceed more than 100 target chemicals (Ferrer and Thurman, 2012; Nödler et al., 2014). Additionally, for some analytes, only one and/or non-specific SRM transitions, such as the neutral loss of H_2O or CO_2, might occur, providing low confidence in compound identification.

These limitations could be overcome by using HRMS-based techniques, operating in full-scan screening mode, and that offer the possibility to detect hundreds (>100 compounds) of polar ECs. Thus, current trends have shifted towards the use of HRMS techniques either by operating in target, or suspect/non-target screening mode. **Fig. 3** shows the different approaches used in HRMS-based methods for the identification of known and unknown ECs. Two predominant workflows for the analysis of ECs could be distinguished: (i) target analysis of known ECs by using commercial standards, and (ii) suspect and non-target screening of non-pre-selected and/or unknowns, which do not require the use of commercial standards.

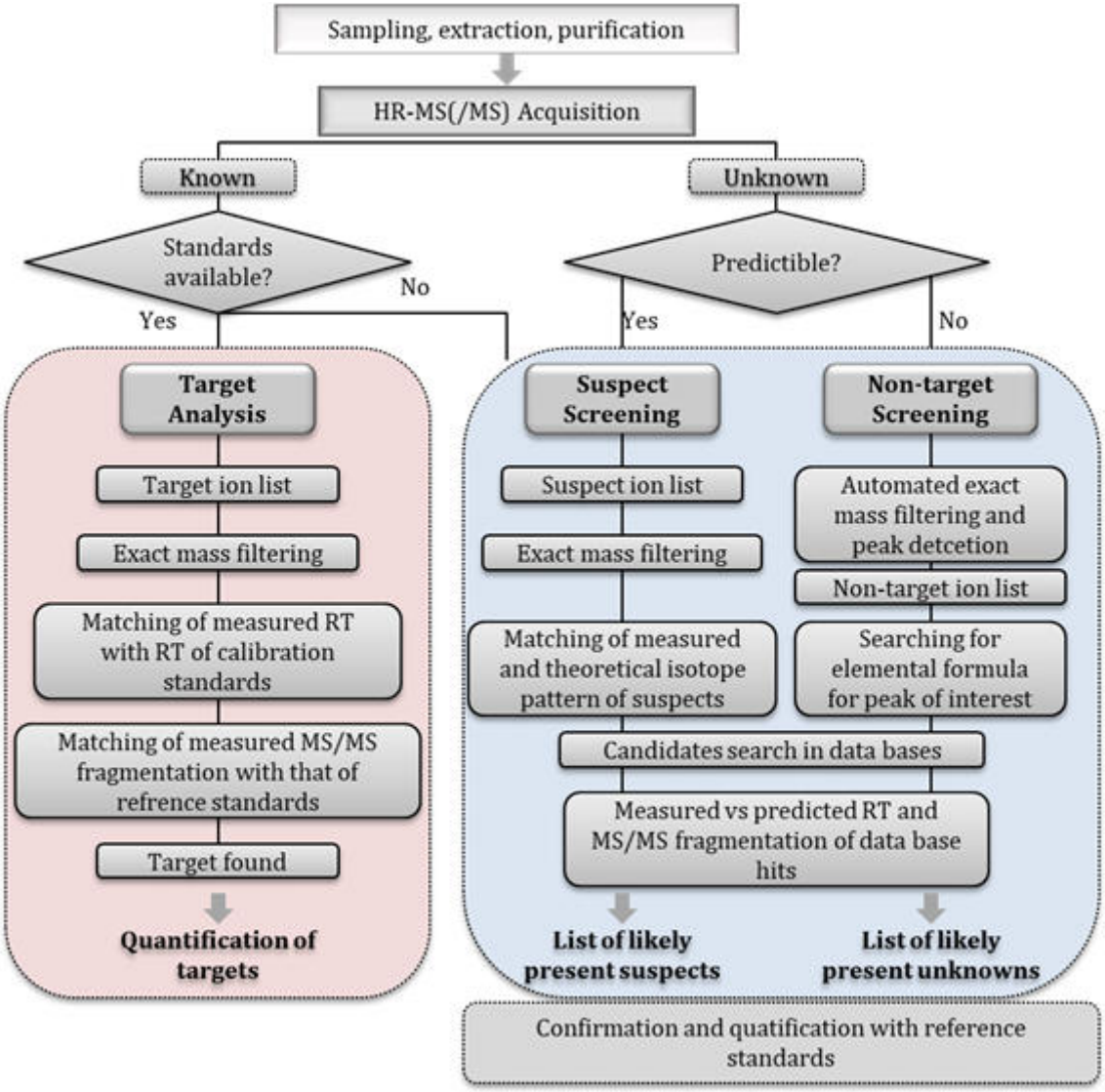


Fig. 3 *Workflows for (i) target analysis of known ECs, (ii) suspect and non-target screening of unknowns in environmental samples by using LC-HRMS adapted from Krauss et al. (2010) (Krauss et al., 2010)*

So far, liquid chromatography high resolution mass spectrometry (LC-HRMS)-based methods, using Orbitrap™ or quadrupole-time-of-flight (QTOF) mass spectrometers, have been successfully applied in environmental multi-residue analysis of ECs by using the target analysis mode (Al-Qaim et al., 2014; Farré et al., 2008; Ferrer and Thurman, 2012; Petrovic et al., 2006; Vergeynst et al., 2014). However, most of the current applications of LC-HRMS based methods focus on suspect or non-target screening workflows for the identification of unknown and un-selected ECs, their metabolites and TPs (Althakafy et al., 2017; Comtois-Marotte et al., 2017; Hernández et al., 2011; Ibáñez et al., 2017). Most HRMS instruments allow the recording of full-scan data together with product ion spectra within a single analytical run, achieving valuable structural information useful for compound identification. Suspect screening allows the detection of known compounds, suspected of being present in the samples without the use of reference

standards (Hernández et al., 2012, 2005; Krauss et al., 2010). In suspect screening, the occurrence of suspect compounds is suggested *a priori* and exact mass compound databases or suspect lists, which include information for several ECs, are being used. For the identification of detected compounds, the intrinsic exact mass and isotopic pattern of suspects are known *a priori.* Apart from that, retention time (RT) can be predicted, and measured fragment ions can be matched with predicted spectra. In non-target screening no *a priori* information is presumed, and molecular formulae and chemical structures must be suggested from the measured accurate mass, isotopic pattern, and fragment ions (Leendert et al., 2015). In both suspect and non-target screening, ECs are tentatively identified in the samples. For unequivocal identification and confirmation, and to achieve information about the concentration levels at which these compounds are present in the samples, reference standards are required (**Fig. 3**).

1.3.1 Target analysis of endocrine disrupting compounds

In recent years, the number of analytical methodologies available for the determination of hormones and other EDCs has grown considerably. **Table 1** shows several examples of different analytical methodologies developed and used for the analysis of EDCs. Selected methods include their analysis in influent and effluent wastewaters, surface, ground and drinking waters (**Table 1**). The type of analytes screened for, the matrix tested, the extraction method, the limit of detection (LOD) or limit of quantification (LOQ) achieved, and the instrumental techniques applied are compiled in a comparative way.

Estrogens have been analyzed by GC for a long time, being the flame ionization detector (FID), electron capture detector (ECD) or MS the most common detectors (Benedé et al., 2014; Cunha et al., 2015; Kotnik et al., 2014; Moeder et al., 2010; Regueiro et al., 2008; Zhang and Lee, 2012). Nowadays, the technique of choice for the analysis of polar EDCs in complex matrices has shifted towards LC coupled to mass or tandem mass spectrometry (MS or MS/MS), as they are less time-consuming than GC methods. The use of LC does not require the additional derivatization step or hydrolysis needed in GC, and it allows the ion fragmentation that is needed for an accurate and precise identification of the analytes. Despite a potential improvement in sensitivity and selectivity in GC-MS detection, the derivatization step increases the complexity of the process, along with the chance of error, increases sample preparation time and the analytical costs. Thus, LC coupled to MS or MS/MS has mostly replaced GC-MS for EDCs detection in environmental samples as summarized in **Table 1**. Among LC methods, current trends moved toward the use of ultra-high-performance-liquid chromatography (UHPLC) instead of conventional LC.

MS is the most commonly used instrumental technique for the determination and detection of EDCs, whereas fluorescence (FLD) and ultraviolet (UV) detectors have

been successfully applied in some particular cases, such as the determination of BPA, 4-NP, 4-OP, E3, E1, 17β-E2, and 17α-EE2 in seawater (Lisboa et al., 2013; López-Darias et al., 2010). In MS methods, electrospray ionization (ESI) is the most widely used ionization technique in the detection of EDCs in environmental samples (Al-Odaini et al., 2010; Chen et al., 2007; Ciofi et al., 2013; Gorga et al., 2013; Guo et al., 2013; Huerta-Fontela et al., 2010; Martín et al., 2015; Miège et al., 2009; Pedrouzo et al., 2009; Petrie et al., 2016; Ros et al., 2015; Vulliet et al., 2008). However, atmospheric-pressure chemical ionization (APCI) (Comtois-Marotte et al., 2017; Fayad et al., 2013) and atmospheric-pressure photoionization (APPI) (Viglino and Aboulfadl, 2008) are also widely employed. ESI is more susceptible to matrix effects (i.e. signal suppression or enhancement) than APCI or APPI, making necessary the use of quantification strategies to correct for these effects (García-Córcoles et al., 2019). QqQ tandem mass spectrometers are the most widely used MS instruments for the determination of EDCs. Some works also used hybrid QqLIT instruments, coupled to conventional LC, for the simultaneous multi-residue analysis of hormones and PhACs (Huerta-Fontela et al., 2008).

Sample treatment involves filtration in the case of aqueous samples, adjusting the pH if necessary, and extraction by solid-phase extraction (SPE). SPE is the most widely employed technique for the extraction of trace ECs from water sources and can be used in either off-line or on-line mode, coupled to the chromatographic and MS analysis. The preferred sorbents in SPE for the determination of EDCs are polymeric sorbent Oasis® HLB (Huerta-Fontela et al., 2010; Miège et al., 2009; Pedrouzo et al., 2009; Petrie et al., 2016) and mixed-mode cation-exchange sorbent Oasis® MCX (Al-Odaini et al., 2010), followed by other less employed sorbents such as octadecyl silica (C18) (Chen et al., 2007; Lisboa et al., 2013; Vulliet et al., 2008), and another polymeric sorbent Phenomenex® Strata X (Comotis-Marotte et al, 2017). Other extraction techniques have also been used, but are less common than SPE, such as ultrasound-assisted dispersive liquid-liquid microextraction, based on the solidification of a floating organic drop (UA-DLLME-SFO) (Martin et al, 2015), sorptive microextraction with polyethersulfone (SME-PES) (Ros et al, 2015), and single drop microextraction (SDME) (López-Darias et al., 2010).

Several methodologies developed for the analysis of EDCs are based on off-line SPE (Al-Odaini et al., 2010; Chen et al., 2007; Comtois-Marotte et al., 2017; Huerta-Fontela et al., 2010; Lisboa et al., 2013; Miège et al., 2009; Pedrouzo et al., 2009; Petrie et al., 2016; Vulliet et al., 2008), which is quite time-consuming, cumbersome and requires the use of large sample volumes (250-1000 mL) to achieve low LOD and LOQ. Another drawback of off-line SPE procedures is that they often require several steps before obtaining an extract with a suitable concentration for instrumental analysis, considering that only a small portion of this extract is injected onto the chromatographic column. The most recent methodologies for the analysis of EDCs in aqueous samples are focused on the use of on-line SPE methods coupled to instrumental techniques, mostly liquid

chromatography tandem mass spectrometry (LC-MS/MS) (Ciofi et al., 2013; Fayad et al., 2013; Gorga et al., 2013; Guo et al., 2013; Viglino and Aboulfadl, 2008; Wang et al., 2008). On-line SPE approaches offer several advantages compared to off-line techniques, such as a remarkable decrease in total analysis time, a reduction in the sample volumes and manipulation, which only requires sample filtration and pH adjustment in some cases, and increase in sample throughput, since the extraction of target analytes and their determination is done automatically, being able to achieve lower LOD (Guo et al., 2013). Thus, the on-line SPE methods are labor-saving and cost-effective in comparison with off-line SPE. Although currently available on-line methods can generally yield LODs in the sub-ng L^{-1} range, this is still particularly challenging for the analysis of hormones, because they are detected at low concentration levels in the environment and also because LODs required by European guidelines are very low. Currently, according to the new requirements in the field of water policy set by the European Union (EU) Water Framework Directive (WFD) and Watch List (WL), the maximum acceptable method detection limits (MDLs) for the determination of 17α-EE2 and 17β-E2/E1 are 0.035 ng L^{-1} and 0.4 ng L^{-1}, respectively (Decision 2015/495/EU, 2015; Decision 2018/840/EU, 2018). The achievement of such low MDLs in real environmental samples (i.e. surface water and specially wastewater) is extremely challenging and a demanding task that is difficult to fulfil with both on-line and off-line SPE methods coupled to tandem MS instruments (Guedes-Alonso et al., 2014). Even though few authors reported LODs for E1 and 17β-E2 in the range of the required 0.4 ng L^{-1} (Gorga et al., 2013; Kuster et al., 2008) none of the methods described in the literature achieved the detection limits set for 17α-EE2, which is 0.035 ng L^{-1}, making the on-line approach particularly suitable and necessary for such demanding task.

Table 1. *Overview of some of the most representative LC based methods for quantitative determination of EDCs in aqueous environmental samples*

Analyte	Sample type	Sample treatment	Sensitivity	Detection	Refernce
Hormones, antibiotics, PhACs	River water/Wastewater	Filter; SPE (HLB)	LOD 0.07-511 ng L^{-1}	UHPLC-QqQ-ESI-MS/MS	(Petrie et al., 2016)
Hormones, plasticizers, caffeine, and other EDCs	River water	UA-DLLME-SFO	LOD 10-1126 ng L^{-1}	HPLC-QqQ-ESI-MS/MS	(Martín et al., 2015)
BPA, APs, and hormones	Estuarine water/Wastewater	Filter; sorptive microextraction (PES)-LD	LOD 37-121 ng L^{-1}	HPLC-QqQ-ESI-MS/MS	(Ros et al., 2015)
BPA, 4-n-NP, 4-OP,4-t-OP, E3, E1, 17β-E2, and 17α-EE2	Seawater	Filter; SPE (C18)	LOD 4–56 μg L^{-1}	HPLC-FLD	(Lisboa et al., 2013)
BPA, 4-OP, 4-n-NP, and 4-t-OP others	Seawater	SDME; DLLME	LOD 4–39 ng mL^{-1} LOD 0.2-1.6 ng mL^{-1}	HPLC-UV	(López-Darias et al., 2010)
Hormones, PhACs	Wastewater	Filter; SPE (HLB)	LOQ 0.02-50 ng L^{-1}	UHPLC-QqLIT-ESI-MS/MS	(Huerta-Fontela et al., 2010)
Synthetic hormones, PhACs	River, dam water/Wastewater	Adjust pH (2); filter; SPE (MCX)	LOD 0.2-281 ng L^{-1}	HPLC-QqQ-ESI-MS/MS	(Al-Odaini et al., 2010)
	River water	Adjust pH (2)/ pH (7); filter; SPE (HLB)	LOD 2–30 ng L^{-1}	HPLC-QqQ-ESI-MS/MS	(Pedrouzo et al., 2009)
	River and housing water/Wastewater	Filter; SPE (HLB)	LOD <1 ng L^{-1}	HPLC-QqQ-ESI-MS/MS	(Miège et al., 2009)
17β-E2, α-E2, E3, E1, and 17α-EE2 (26 steriods)	Surface and groundwater	Filter; adjust pH (3); SPE (C18)	LOD 0.01–0.2 ng L^{-1}	HPLC- QqLIT-ESI-MS/MS	(Vulliet et al., 2008)
	River water/Wastewater effluent	Filter; high-flow SPE (C18)	LOD 0.78–7.65 ng L^{-1}	HPLC-QqQ-ESI-MS/MS	(Chen et al., 2007)
Estrogens and androgens	River water/Wastewater	Filter; On-line SPE	LOD 0.1–2.5 ng L^{-1}	HPLC-QqQ-ESI-MS/MS	(Guo et al., 2013)
Natural and synthetic estrogens and their conjugates, BPA, APs	River water/Wastewater	Filter; On-line SPE	LOD 0.004–62 ng L^{-1}	HPLC-QqQ-ESI-MS/MS	(Gorga et al., 2013)
Estrogenic and progestagenic steroid hormones	Wastewater	Filter; On-line SPE	LOD 8–60 ng L^{-1}	HPLC-QqQ-APCI-MS/MS	(Fayad et al., 2013)
Estrogenic steroids	River water/ Wastewter	Filter; On-line SPE	LOD 0.15–0.95 ng L^{-1}	HPLC-QqQ-ESI-MS/MS	(Ciofi et al., 2013)
Estrogens	River water	Filter; On-line SPE	LOD 0.98-78.1 ng L^{-1}	HPLC-UV	(Wang et al., 2008)
Natural hormones and related synthetic compounds	Lake water/Wastewater	Filter; adjust pH (2.4); On-line SPE	LOD 2–50 ng L^{-1}	HPLC-QqQ-APPI-MS/MS	(Viglino and Aboulfadl, 2008)

1.3.2 Target analysis of pharmaceutically active compounds

The need to monitor pharmaceutical residues in the environment resulted in the development of numerous analytical methods that allow their determination in several environmental matrices. **Table 2** shows several examples of some of the most recent methods, which are usually multi-residue methods that allows the simultaneous analysis of PhACs that belong to different therapeutic groups. Indeed, multi-residue analytical methods have become the preferred tools for tracing numerous PhACs in the environment. The most frequent pharmaceuticals included in such multi-residue methods are analgesic and anti-inflammatory drugs, antibiotics, lipid regulators, psychiatric drugs, tranquillizers, antidiabetics, anihypertensives, diuretics, antihelmintics, X-ray contrast agents, calcium channel blockers, and β-blockers. These drugs have high consumption rates worldwide and are the most ubiquitous in both surface and wastewaters.

According to **Table 2**, in these methods the sample preparation step is based on off-line SPE. The commercial extraction cartridges Oasis® HLB are the most widely used for the analysis of PhACs in aqueous matrices (Althakafy et al., 2017; Alygizakis et al., 2016; Boleda et al., 2013; Gros et al., 2012; Hernández et al., 2011; Sun et al., 2014). This cartridge is used for the simultaneous analysis of acidic, basic, and neutral compounds. Most of the off-line SPE methods use sample volumes ranging from 100-1000 mL, depending on the sample, and they are concentrated to an extract with smaller volume (i.e. 1 mL). Recent trends are also focused on the use of on-line SPE, coupled to liquid chromatography and tandem mass spectrometry, due to the advantages highlighted before, such as automation and less sample volumes required (García-Galán et al., 2016). However, off-line SPE is still the dominant method. For the analysis of PhACs in solid matrices, such as sediment, soils, or sewage sludge, the most widely used extraction methods are pressurized liquid extraction (PLE) (Jelić et al., 2009), ultrasonic solvent extraction (USE) (Comtois-Marotte et al., 2017), and QuEChERS *(Quick, Easy, Cheap, Effective, Rugged, and Safe)* (Chiaia-Hernandez et al., 2013). PLE and USE extracts are further purified by SPE. This step is also used to concentrate the PhACs in the samples and improve the methods LODs.

LC is the technique *per excellence* for the analysis of PhACs, allowing the detection of most compounds. As PhACs are polar compounds, GC is used in much fewer occasions (Azzouz and Ballesteros, 2012; Zhang et al., 2016). With respect to LC, the predominant trend has moved towards the use of UHPLC, which allows shorter separation time than conventional LC, reducing considerably analytical runs (Gago-Ferrero et al., 2015; Ibáñez et al., 2017). LC or UHPLC are mostly coupled to MS instruments, which offer high sensitivity, selectivity, and reliability, providing low LODs to detect PhACs in complex environmental samples. For PhACs, QqQ instruments are the most widely used. However, remarkable number of

applications rely on the use of hybrid QqLIT tandem mass spectrometers (**Table 2**).

Most of the methods are based on the use of ESI in either negative (NI) or positive (PI) ionization modes. For quantification purposes, SRM is used, in both QqQ and QqLIT instruments, achieving high sensitivity and selectivity. For instrumental analysis, recent trends are shifting towards the use of HRMS instead of QqQ or QqLIT instruments. The development of modern QTOF and Orbitrap high resolution mass spectrometers allowed the achievement of appropriate LODs and the identification of target compounds with high confidence due to exact mass measurements (Hernández et al., 2014). In the last few years, several multi-residue LC-HRMS methods were developed for the analysis of PhACs (**Table 2**) (Althakafy et al., 2017; Chiaia-Hernandez et al., 2013; Comtois-Marotte et al., 2017; Gago-Ferrero et al., 2015; Hernández et al., 2011; Ibáñez et al., 2017). One of the main advantages offered by HRMS instruments is that a larger number of target PhACs can be measured, compared to QqQ or QqLIT instruments. It is also noteworthy to mention that, in the most recent methodologies, not only the parent PhACs are targeted, but also their metabolites and TPs.

Table 2. *Selected multi-residue methodologies for the determination of PhACs in environmental matrices*

Sample type	N of compounds		Detection	Sensitivity	Ref
Surface and drinking waters	53 PhACs	SPE (HLB)	UHPLC-QqQ-ESI-MS/MS in MRM	LOD 0.5–10 ng L^{-1}	(Boleda et al., 2013)
Surface water and sediment	20 antibiotics	Water: SPE (HLB) Sediment: buffer solution	UHPLC-QqQ-ESI-MS/MS in MRM	LOQ (water) 0.03–1.68 ng L^{-1} LOQ (sediment) 0.01–0.56 ng g^{-1}	(Chen et al., 2014)
Seawater	158 PhACs and illicit drugs	SPE (HLB)	UHPLC-QqQ-ESI-MS/MS in MRM	LOD 0.01–28 ng L^{-1}	(Alygizakis et al., 2016)
Wastewater	50 PhACs and PCP	SPE (HLB)	LC-QqQ-ESI-MS/MS in MRM	LOD 0.01–5.0 ng L^{-1}	(Sun et al., 2014)
Sediment and sewage sludge	43 PhACs	PLE + SPE	HPLC-QqQ-EPI-MS/MS in MRM	LOQ 0.02–10.7 ng g^{-1}	(Jelić et al., 2009)
Wastewater, river, reservoir, sea, and drinking water	81 PhACs	SPE (HLB)	UPLC-QqLIT-ESI-MS/MS	LOD (drinking water) 0.01–7.2 ng L^{-1} LOD (surface water) 0.1–15.2 ng L^{-1} LOD (wastewater) 0.2–19 ng L^{-1}	(Gros et al., 2012)
Surface and wastewater	12 PhACs/20TPs	On-line SPE	LC-QqQ-ESI-MS/MS in MRM	LOD (wastewater) 0.03-42.4 ng L^{-1} LOD (surface water) 0.01-73.2 ng L^{-1}	(García-Galán et al., 2016)
Water	13 PhACs and PCP	SPE (HLB)	UHPLC-Orbitrap-HESI-MS/MS in MRM	LOD 0.01–0.61 ng L^{-1}	(Althakafy et al., 2017)
Water and solid samples	31 ECs (11 PhACs)	Water: SPE (Strata X-C) Solid samples: USE	LC-Orbitrap-APCI-MS/MS	LOD (water) 0.5–104 ng L^{-1} LOD (solids) 0.3–3.7 ng g^{-1}	(Comtois-Marotte et al., 2017)
Sediment	180 OP (9 PhACs)	PLEC modified QuEChERS	LC-Orbitrap-ESI and APPI	LOD 0.01–4 ng g^{-1}	(Chiaia-Hernandez et al., 2013)
Wastewater	45 PhACs	SPE (Strata X, Strata WAX, Strata WCX and Isolute ENVC)	UHPLC-QTOF-ESI	No data	(Ibáñez et al., 2017)
Wastewater	173 ECs (8 PhACs)	SPE (in-house cartridge)	UHPLC-QTOF-ESI MS/MS in Auto MS acquisition	No data	(Gago-Ferrero et al., 2015)
Wastewater	45 PhACs and their metabolites	SPE (HLB)	UPLC-QTOF-ESI-MS/MS in LE and HE function	No data	(Hernández et al., 2011)
Wastewater and sewage sludge	13 PhACs	SPE (ENVI-18), of the supernatant	GC-Single quadropole in SIM mode	LOD (wastewater) 0.2–4.8 ng L^{-1} LOD (sludge) 0.7–5.9 ng g^{-1}	(Zhang et al., 2016)
Surface water	23 PhACs	SPE (Empore™, 3 MSDB-RPS)	HPLC-Single quadropole, LC-MS, ESI	LOD 2.7–101 ng L^{-1}	(Nannou et al., 2015)
Soil, sediment, and sewage sludge	22 PhACs	Microwave-assisted (MAE) + continuous SPE (HLB)	GC-Single quadropole in SIM mode	LOD 0.8–5.1 ng kg^{-1}	(Azzouz and Ballesteros, 2012)

1.3.3 Suspect screening

Suspect screening goes beyond the conventional target analysis, extending it to the determination of non-previously selected compounds. Suspect screening approaches have been suggested as novel workflows to prioritize environmental pollutants using information on occurrence frequency and concentration-relevant indices (e.g., peak area) provided by qualitative analysis (Hollender et al., 2019; Singer et al., 2016). LC-HRMS, using either Orbitrap™ or QTOF mass spectrometers, are able to screen for a large number of ECs at trace levels in environmental samples within a single methodology, thereby reducing efforts and analysis time (Aalizadeh et al., 2016; Avagyan et al., 2016; Chiaia-Hernandez et al., 2014; Gago-Ferrero et al., 2015; Richardson and Ternes, 2018; Schymanski et al., 2014b).

Several suspect screening methodologies for the tentative identification of ECs in environmental samples have been published in the scientific literature (Assress et al., 2019; Campos-Mañas et al., 2019; Choi et al., 2020; Gago-Ferrero et al., 2018; Gros et al., 2017; Hug et al., 2014; Martínez Bueno et al., 2012; Mascolo et al., 2019; Masiá et al., 2014; Moschet et al., 2013; Nurmi et al., 2012; Park et al., 2018; Vergeynst et al., 2014). **Table 3** includes examples of 13 recent studies where suspect screening was used in the analysis of wastewater influents and effluents, surface, and coastal waters. The methods have screened for sets of suspects varying from about 69 chemicals (Vergeynst et al., 2014) up to almost 58.000 chemicals (Mascolo et al., 2019). In most of the studies, suspect compounds were selected to be screened for because they are known to occur in the aquatic environment (e.g., several pharmaceuticals, personal-care products and pesticides, and other classes of chemicals, such as surfactants). In suspect screening approaches, exact mass compound databases or “suspect lists” are used to screen for the occurrence of ECs in the samples. The databases (”suspect lists”) contain information about the compounds exact mass and predicted isotopic pattern. Generally, compounds included in suspect lists are selected based on their usage and consumption and their applications in industry or other every-day life activities (Hug et al., 2014). Their known and predicted TPs are sometimes also included. To narrow down the number of suspects, and focus on the ECs of major interest, sometimes prioritization strategies are applied (Gago-Ferrero et al., 2018).

Based on the information provided in **Table 3**, off-line SPE is also the technique of choice for sample preparation. After sample filtration, SPE extraction employing Oasis HLB cartridges is the most widely used (Campos-Mañas et al., 2019; Choi et al., 2020; Mascolo et al., 2019; Masiá et al., 2014). Besides the HLB polymeric material, several methods rely on the use of a self-packed-multi-layer SPE cartridge containing different sorbents, such as Isolute ENV+, Strata-X-AW, and X-CW, combined as a mixture to achieve enough enrichment for a broad range of

compounds (Gago-Ferrero et al., 2018; Moschet et al., 2013; Park et al., 2018). Although most authors aimed at developing sample-enrichment techniques using different polymers to retain a broad range of substances, some compounds are still preconcentrated selectively, and the achievement of acceptable recoveries for all compounds is a challenging task in this type of applications (Busetti et al., 2012). The new LC–MS systems even offer respectable sub-femtomole sensitivity, making large-volume injection (LVI)-based screening methods possible with direct injection and without any pre-concentration step leading to the detection of low abundance contaminants (Martínez Bueno et al., 2012; Vergeynst et al., 2014).

In suspect screening samples are typically analyzed by a combination of a full-scan and data-dependent acquisitions (DDA), when using Orbitrap systems, or full-scans at low and high collision energies with QTOF instruments. Even though UHPLC separation has been used in most of the studies, in order to provide a shorter chromatographic runs, HPLC is still widely applied and it can be sometimes preferred when multiple MS modes are alternated (e.g. full-spectrum MS, MS/HRMS and all ion-fragmentation HRMS for confirmatory purposes) in order to provide sufficient data points across the chromatographic peak (Leendert et al., 2015). For chromatographic analysis, generic stationary phases in both HPLC and UHPLC are being used, to ensure the detection of most ECs. Regarding the ionization, ESI is also the most widely used ionization source, analyzing the samples in both PI and NI modes (**Table 3**).

In suspect screening methodologies, data treatment for ECs identification encompasses the following steps:

- exact mass filtering (mono-isotopic ions; mass-error tolerance <5ppm)
- peak-noise filtering (blank-subtraction, signal-to noise, signal intensity, and peak shape filters),
- isotopic pattern matching (visual inspection, or comparison of the measured and theoretical isotope exact mass and/or ratios),
- RT verification (predicting the RT based on octanol-water partition coefficients (K_{OW}) or using linear solvation-energy relationship)
- fragmentation pattern verification.

In suspect screening, compound identification is based on exact mass measurements. Thus, the experimental exact mass is compared with the exact mass derived from the known molecular formula included in the exact mass databases or suspect lists, and the difference between both masses cannot be higher than 5ppm. Mass filtering and chromatographic alignment is performed for all exact masses in the full scan MS spectra and compounds are identified and filtered based on their mass accuracy error <5 ppm and an isotopic ratio difference (IRD) bellow 10%. For compound identification, some algorithms have been developed that can predict the compounds RT based on their physicochemical

properties. For a positive EC identification, the theoretical RT should match the experimental RT. Finally, to confirm the occurrence of ECs in the samples, the fragments of a suspect EC and their exact masses are investigated, to see if they match with those of the suspected structure. The fragmentation (MS/MS) spectra used to confirm suspect compounds are normally verified using spectral libraries, such as MassBank (http://massbanl.eu/MassBank/), METLIN (http://METLIN.scripps.edu), Drug BANK (htpps://www.drugbank.ca), ChemSpider (http://chemspider.com), and *in silico* fragmentation platforms (CFM-ID) (http://cfmid.wishartlab.com/), among others.

However, in these workflows, ECs are just tentatively identified in the samples. For final and unequivocal confirmation of the compound identity, the injection of reference standards is required but they may be acquired in a final stage when solid well-found evidence exists on the presence of the compound in the sample. In this way, laboratories do not need to acquire all reference standards before analysis, with the subsequent problems of availability (e.g., TPs), costs, and expiry dates (Ibáñez et al., 2014). The degree of confidence in compound identification in HRMS based methodologies, including suspect screening, was previously defined by Schymanski and coworkers (Schymanski et al., 2014a). In this classification a five-level identification confidence scheme is proposed, which starts with level 5 as the lowest level of confidence and moves up to level 1 with the highest degree of confirmation.

According to this system, identification levels are defined as follows:

(i) level 1 is only achieved when the occurrence of the candidate compounds is confirmed with reference analytical standards (highest degree of confidence),
(ii) level 2 is achieved when it is possible to assign a probable structure with a high degree of certainty to the tentative candidate compounds, but no standards are used for final confirmation.
(iii) level 3 is achieved when tentative candidate compounds are assigned to the molecular formulas,
(iv) level 4 refers to the assignment of an unequivocal molecular formula to these exact masses, and
(v) level 5 corresponds to the identification of exact masses only (**Fig. 4**).

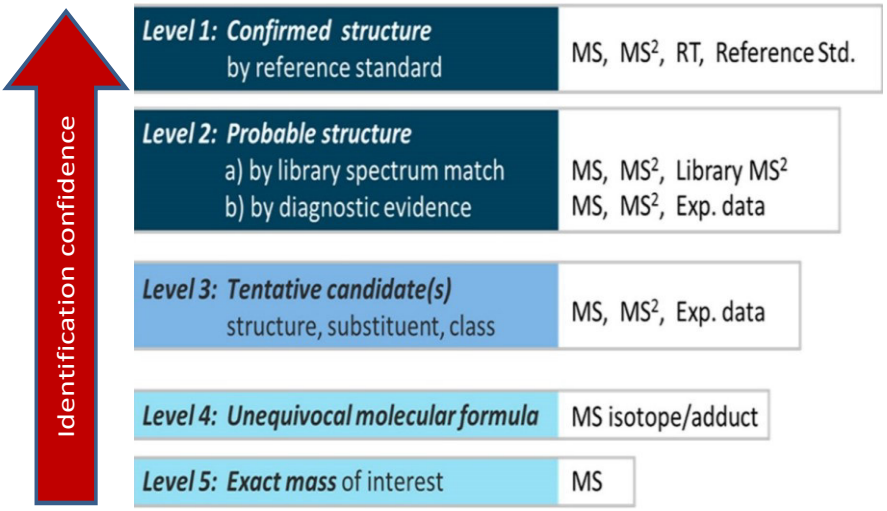


Fig. 4 *Proposed identification confidence levels in high resolution mass spectrometric analysis adapted from Schymanski et al. (2014a) (Schymanski et al., 2014a)*

One of the main drawbacks of suspect screening methodologies is the difficulty of identifying all suspects with the highest degree of confidence (level 1), since reference analytical standards are not always available for all compounds (Schymanski et al., 2014a). Indeed, most of ECs are identified under level 2 or 3. The lack of reference standards also hinders the quantification of ECs in environmental samples. To overcome this challenge, many authors use the corresponding or structurally related isotopically labelled internal standards (ILISs) for semi-quantitative analysis following compound identification (Kiefer et al., 2019), avoiding the high economic investments in purchasing reference standards for all ECs. Even though this strategy presents some limitations, since it is difficult to find suitable ILIS for all the substances tentatively identified, it allows estimating their concentrations in environmental samples (Choi et al., 2020; Park et al., 2018; Sjerps et al., 2016).

Another drawback is the difficulty in treating HRMS data. A suite of commercial and open-access software has been developed for data treatment, and for peak deconvolution, isotope ratio calculation, RT alignment, statistical analysis, and spectral library search (Leendert et al., 2015). For instance, some of the most widely used software in the literature are Mass Hunter (Liu et al., 2019), MZmine (Hug et al., 2014), ChromaLynx (within MssLynx) (Ibáñez et al., 2008; Wood et al., 2017), MasterView™ software (Campos-Mañas et al., 2019), the Exact Finder ™ (Gros et al., 2017), Compound Discoverer (Park et al., 2018), Compass Data Analysis (Assress et al., 2019), among others. However, there is still the need for establishing systematic and more user-friendly data processing workflows that allow the identification of ECs using one single software platform. Even though several studies have already focused on developing automatized approaches for ECs identification in suspect screening, these methodologies still suffer from the large effort of manual data evaluation (Gago-Ferrero et al., 2018; Hug et al., 2014; Kiefer et al., 2019; Liu et al., 2019; Tian et al., 2019).

Table 3. *Examples of recent suspect screening LC-HRMS methods to identify ECs in environmental samples*

Matrix	Sample treatment	Analytical technique	Suspect screening list	Exact mass filter	Data analysis software	Tentatively identified compounds	Identity confirmed by reference standard	Ref.
Effluent, shoreline, and offshore	Filter, SPE (HLB)	HPLC-HRMS-ESI-Orbitrap MS	1862 organic pollutants	≤5 ppm	Compound Discoverer 2.0	30	17 (PPCPs, pesticides, PFASs, and their metabolites)	(Choi et al., 2020)
Wastewater	GμFF filter, pH 2, SPE (HLB-H disk),	UHPLC-ESI-QTOF MS	>300 emerging pollutants	≤5 ppm	Compass Data Analysis 4.3	12	1 hormone, 1 PhACs, 1 pesticide	(Assress et al., 2019)
Wastewater	GμFF filter, pH 8, SPE (HLB)	HPLC-ESI-QTOF MS	805 pesticides	≤5 ppm	MasterView™ 1.1 AnalysitTM TF 1.5 (Sciex)	105	19 pesticides	(Campos-Mañas et al., 2019)
WWTP effluent	pH 6.5, SPE with HLB, Isolute ENV+, Strata-X-AW, and X-CW	UHPLC-ESI-QTOF MS	160 emerging micropollutants	≤5 ppm	*in silico* fragmention softwer MetFrag and CFM-ID	36	23 (artificial sweetener, food preservatives, UV filters, corrosion inhibitors, anionic surfactants)	(Gago-Ferrero et al., 2018)
Wastewater	SPE with Oasis HLB and Isolute ENV+	UHPLC-ESI-Orbitrap MS	~1300 micropollutants	≤5 ppm	Exact Finder™ 2.5 UNIFI™	79	15 PFASs, 13 pesticides, 3 PFRs, 44 PPCPs	(Gros et al., 2017)
WWTP effluent	GF/F filter, 2x SPE with Chromabond HR-X sobent, pH 3, PTFE filter	HPLC-ESI-Orbitrap MS	1835 chemicals	≤7 ppm	MZmine v2.9	13	1 UV filter, 4 chemical sythesis intermediates, 1 PhACs	(Hug et al., 2014)
WWTP effluent	Filter, SPE (MCX, and Strata-X)	UHPLC-ESI-TOF MS	147 PhACs and 54 metabolites	5 mDa	ChromaLynx XS	25	4 PhACs	(Nurmi et al., 2012)
Estuary, lagoons, and sediment	Extraction in an ultrasonic bath, SPE (HLB)	UHPLC-ESI-QTOF MS	~58000 compounds	≤5 ppm	AB Sciex softwer (SciexOS 1.2, PeakView 2.2, MasterView 1.1, LibraryView 1.1.0)	23	4 PhACs, 1 corrosion inhibitor, 1 fungicide, 1 insect repellent, 1 flame retardant	(Mascolo et al., 2019)
Surface water	GF/F filter, SPE (HLB, Isolute ENV+, Strata-X-AW, and X-CW), cellulose acetate filter	UHPLC-ESI-Orbitrap MS	189 PhACs and PCPs	≤5 ppm	Compound Discoverer 2.0	58	38 PPCPs	(Park et al., 2018)
Surface water	Filters GF/F, and nylon membrane filter, SPE (HLB)	UHPLC–ESI-QTOF MS	1212 PhACs, 546 pesticides, 378 polyphenols, and 233 mycotoxins	≤5 ppm	Peak View 1.0, Formula Finder, and MultiQuant 2.0	42	n.a.	(Masiá et al., 2014)
Surface water	Filter GμFF, 0.1/0.02 % (v/v) formic acid, LVI	UHPLC-ESI-QTOF MS	69 PhACs	5-10 ppm	Masslynx 4.1	37	30 PhAc	(Vergeynst et al., 2014)
Surface water	pH 6.5−6.7, filter GF/F, SPE (HLB, Strata-X-AW, and X-CW, Isolute ENV+)	HPLC-ESI-Orbitrap MS	134 pesticides TPs	≤5 ppm	MassFrontier 6.0, MetFrag , and MassBank	n.a.	40 TPs	(Moschet et al., 2013)
Surface water	Direct sample injection, LVI, without prior sample treatment	HPLC-ESI-QTOF MS	1200 PPCPs	≤5 ppm	Analyst® TF 1.5 software (PeakView™ and MultiQuant™)	5	n.a.	(Martínez Bueno et al., 2012)

1.4 Occurrence and fate of emerging contaminants in freshwater and coastal ecosystems

Considering that conventional WWTPs are not able to fully remove most ECs (Aerni et al., 2004; Murray et al., 2010; Zhou et al., 2009), these compounds are continuously released into the aquatic environment. Indeed, as indicated in section 1.1., wastewater effluents are one of the major sources of pollution of aquatic ecosystems (Tran et al., 2018). Since the input of ECs to the aquatic environment is continuous, they are considered as "pseudo persistent" compounds. The fate, behavior and persistence of ECs in the aquatic environment depend on their degree of natural attenuation, which depend on their physicochemical properties and other environmental characteristics (Lapworth et al., 2012; Pal et al., 2010).

Once released into the surface and marine environment, ECs occurrence and fate in water systems are subjected to several natural attenuation processes (**Fig. 5**):

- transport (by dilution, dispersion, or diffusion),
- degradation (by photolysis, biodegradation, and hydrolysis),
- sorption to suspended particulate matter (SPM) and sediments,
- bioaccumulation in the tissues of aquatic organisms.

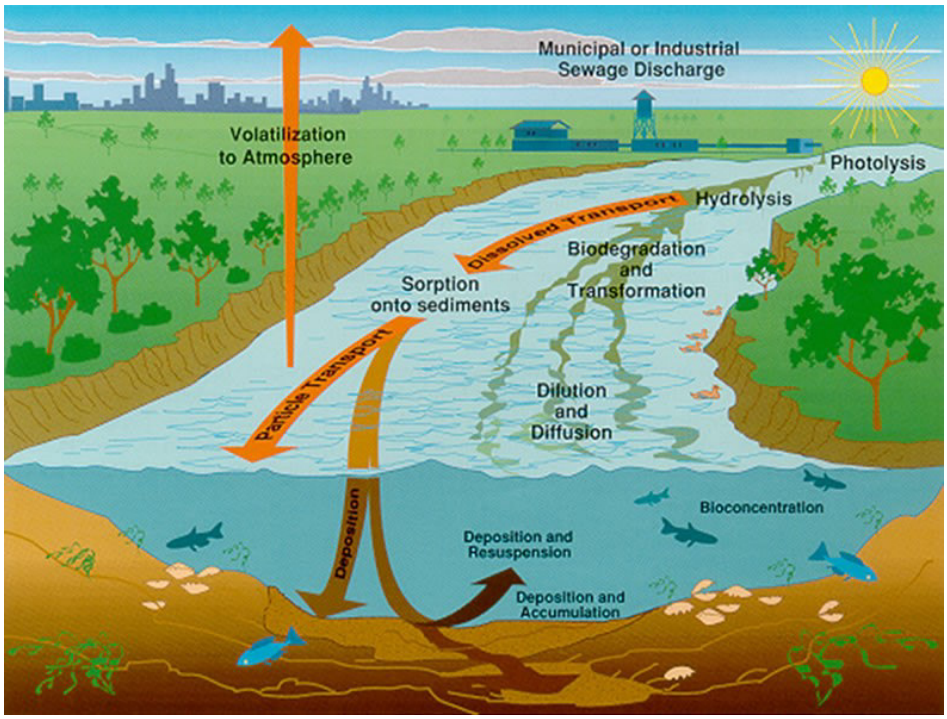


Fig. 5 *Fate of ECs in the aquatic environment; Source: Barber et al., 1995 (Barber et al., 1995)*

One of the most important factors that influence ECs concentrations in the aquatic environment is dilution, which is mostly related with the flow of the water bodies (i.e. rivers and streams). Thus, when ECs are released into aquatic ecosystems with low flow, there is a higher risk because the dilution is low. However, with increased flow, the dilution is high, and the risks consequently decrease. When degradation occurs, ECs could be completely mineralized, or they could be

transformed into TPs that may be subject to subsequent remineralization and thus have the potential to be permanently removed from the system. However, degradation does not necessarily remove ECs but rather transform them into new compounds, often with preserved biological activity, that might possess different chemical properties, hence decreasing or increasing the effects of the pollution in the environment (Halling-Sorensen, 2000). These TPs may also be more mobile and persistent than their corresponding parent compound (Agüera et al., 2013; Ekpeghere et al., 2018; Wilkinson et al., 2017; Yu et al., 2019). Furthermore, TPs can have even greater toxicity than parent compounds and they should not be neglected as target analytes in chemical and risk assessment analysis. Besides dilution and degradation, ECs can also sorb to suspended solids or sediments or deposit to particles, being stored in these environmental compartments. Several studies showed that sediments act as a sink of ECs, which may be released back to the aquatic environment by resuspension (Bayen et al., 2013; Carafa et al., 2007; Pignotti et al., 2017; Vryzas et al., 2009). Once released into aquatic systems, ECs can also accumulate in fat tissues of fish, mussels, or other organisms and seafood (Álvarez-Muñoz et al., 2015; Dodder et al., 2014; Martínez Bueno et al., 2014; Roch et al., 2019) and thus, they could be introduced into the human food chain (Done and Halden, 2015; Zenker et al., 2014).

Most studies focused their attention on the occurrence of ECs in freshwater ecosystems, while fewer studies have been conducted in the marine environment. In coastal settings, ECs levels are expected to be low due to dilution and diffusion and because of the complex hydrodynamics of the marine environment in coastal zones. The differences between the physicochemical properties of fresh and seawater, which include salinity, pH, and organic matter, may also have an impact in the environmental fate of ECs (Weigel et al., 2002). Vidal-Dorsch et al. (2012) observed significant dilution of WWTP effluent discharges in seawater at submarine outfalls and highlighted the difficulty of determining the environmental relevance of the low-level ECs observed in seawater (Vidal-Dorsch et al., 2012). Moreover, the environmental fate of ionizable compounds may be altered by the increased pH of seawater (Gaw et al., 2014). On the other hand, photodegradation may be a less important removal mechanism in coastal waters compared with more shallow freshwater environments due to light attenuation (Gaw et al., 2014). Indirect photodegradation mechanisms may differ to those occurring in freshwater due to differences in water composition (Choong et al., 2006; Ge et al., 2010). Reduced removal rates in surface seawaters can occur also during colder months due to lower temperatures, resulting in lower rates of biological activity enhancing the persistence of ECs in marine ecosystems (Choong et al., 2006). Even though direct effects of ECs are expected to be minor in coastal environments compared to freshwater ecosystems, some studies already reported the presence of ECs in marine settings (Birch et al., 2015; Brumovský et al., 2017; Koelmans et al., 2014; Lara-Martín et al., 2014; Nödler et al., 2014; Pignotti et al., 2017), and in marine

organisms (Klosterhaus et al., 2013; Nakata et al., 2012). Hence, it is essential to evaluate the contamination levels of coastal areas while identifying vulnerable marine sites to enhance the assessment of marine organisms' exposure.

1.4.1 Endocrine disrupting and pharmaceutically active compounds in riverine and coastal environments

Since EDCs and PhACs are not completely removed in the conventional treatments applied in WWTPs, they are continuously discharged into aquatic ecosystems. Recently published reviews focusing on the removal of PhACs and EDCs in WWTPs operating with conventional treatment techniques revealed that analgesics and anti-inflammatory drugs (i.e. acetaminophen, ibuprofen, naproxen, and salicylic acid) are, in general, the compounds that exhibited the highest removal efficiencies (RE)>70% (Couto et al., 2019; Luo et al., 2014). The exception is diclofenac, which experienced inefficient and variable removal rates (average 36%). Antibiotics generally show low REs of approximately 30% for compounds such as erythromycin, clarithromycin, roxithromycin, to moderate 65% removal for substances like sulfamethoxazole, trimethoprim, azithromycin, and ofloxacin. Concerning lipid regulators (i.e. bezafibrate, atorvastatin, and fluvastatin) and β-blockers (i.e. atenolol, metoprolol, and propanolol), these compounds are also moderately eliminated (38%–73%) in WWTPs. The anticonvulsant carbamazepine seemed to be the most persistent compound, showing poor removal rates (RE<40%), Among all the reviewed studies, the highest removal rate for carbamazepine was observed by K. Choi et al. (2008), with values reaching 62%. For EDCs, steroid hormones (17β-E2, E3, and 17α-EE2) showed high RE, which ranged from 72 to 100%. The two surfactants, NP and OP, showed REs of 78% and 84%, respectively (Luo et al., 2014). However, contradictory results have been reported for NP, with values ranging from 22% (Stasinakis et al., 2008) to 99% (Janex-Habibi et al., 2009). For BPA, reported REs were also high (82%) during wastewater treatment. According to the removal of PhACs and EDCs in WWTPs, a simple classification scheme is presented in **Table 4.**

Table 4. *Classification of PhACs and EDCs based on their removal efficiencies (RE) in WWTPs, adapted from Luo et al (2014) (Luo et al., 2014)*

Degree of removal	Compounds
Highly removed (>70%)	Acetaminophen, bisphenol A, estradiol, estriol, estrone, ethinylestradiol, gemfibrozil, ibuprofen, naproxen, nonylphenol, octylphenol, salicylic acid, fluoxetine, simvastatin
Moderately removed (40–70%)	Atenolol, bezafibrate, ketoprofen, nonylphenol, sulfamethoxazole, trimethoprim, azithromycin, ofloxacin, atorvastatin, fluvastatin
Poorly removed (<40%)	Carbamazepine, diclofenac, erythromycin, clarithromycin, roxithromycin, metoprolol, amlodipine, diazepam

Due to their incomplete removal in WWTPs and continuous input into the environment, these compounds have been frequently detected in freshwaters

worldwide (Amin et al., 2018; Bayen et al., 2013; K'oreje et al., 2012; López-Serna et al., 2012; Nazifa et al., 2020). Studies dealing with the occurrence of PhACs and EDCs in seawater are limited and most of them were done in temperate coastal waters (Bayen et al., 2013; Benotti and Brownawell, 2007; Jia et al., 2011; Magnér et al., 2010; Nakada and Kiri, 2008; Weigel et al., 2004; Wille et al., 2010). Given that a substantial fraction of the world's population lives in large, coastal cities, there is a current need to better understand the fate and risks derived from the exposure to PhACs and EDCs in coastal marine ecosystems.

So far, PhACs and EDCs releases could be traced to every continent of the planet where there are human activities. Even in the pristine Arctic and Antarctica environments and in northern Scandinavia, PhACs residues have been detected along with other trace organic contaminants (González-Alonso et al., 2017; Kallenborn et al., 2008). EDCs are globally ubiquitous as well, and their occurrence has been confirmed by numerous studies performed in various environmental compartments in different parts of the world (Belfroid et al., 1999; Campbell et al., 2006; De Mes et al., 2005; Kuster et al., 2008; Swartz et al., 2006; Zha et al., 2008; Zhang et al., 2009).

According to the database "Pharmaceuticals in the Environment" (www.uba.de/db-pharm, 2019) done by the German Environment Agency (Umweltbundesamt – UBA), among all published studies (until 2019) related with the occurrence of PhACs and hormones in the environment, there are 75 countries worldwide in which at least one PhACs or hormone was reported in the literature at concentrations exceeding the LOD of the analytical methods employed. **Fig. 6** shows the distribution of the published measured environmental concentrations (MECs), with countries with a high number of MECs marked in green and the countries with a low number of MECs highlighted in brown. The 75 countries in which PhACs and hormones have been detected in the environment include countries from all five UN regional groups, such as Western Europe and Others Group (North America, Australia, and New Zealand) (WEOG), the group of Latin American and Caribbean States (GRULAC), Eastern Europe Group (EEG), Asian Group (ASG), and African Group (AFG). Despite the global coverage, pronounced regional patterns in the number of positively detected MECs prevail. Most of the data are available for industrialized and developed regions such as Western Europe, North America, Asia, and Australia, while little information is available for developing countries with an insufficient number of wastewater treatment facilities, such as African continent and Eastern Europe countries. Furthermore, the majority of MECs available are from Germany, United Sates, Spain, and China (**Fig. 6**).

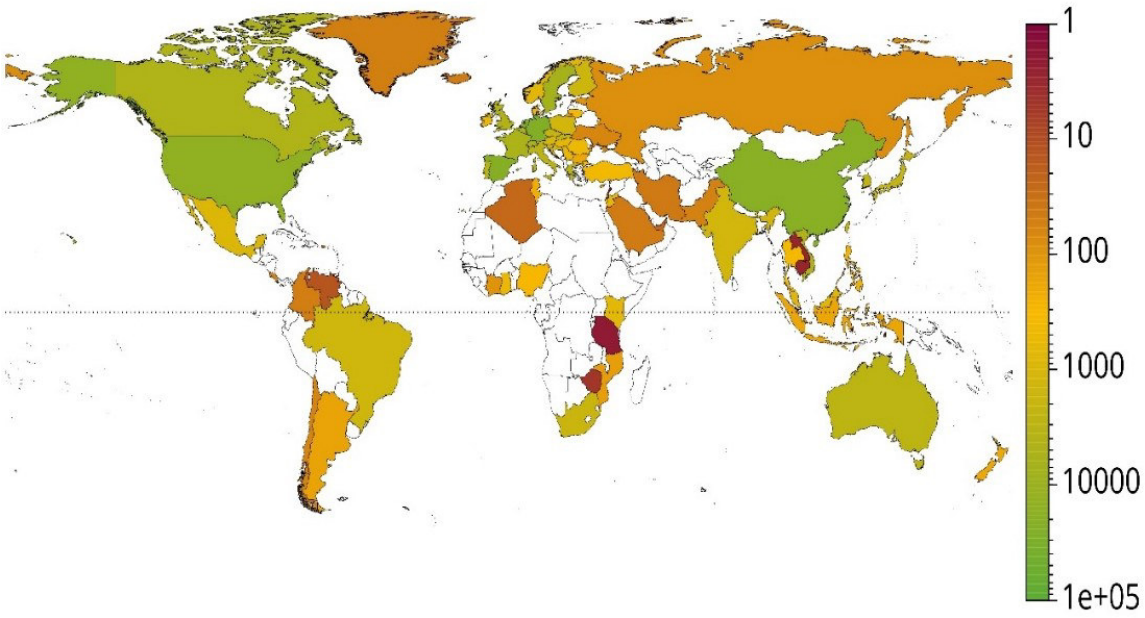


Fig. 6 *Map of worldwide number of measured environmental concentrations (MECs); Source: the database done by the German Environment Agency (www.uba.de/db-pharm, 2019)*

Globally, 771 different PhACs and hormones or their TPs were found above their respective LOD worldwide, and these compounds were mostly found in wastewater samples. In total, 528 out of the 771 measured compounds were positively detected in surface, ground or drinking water, and only 184 compounds were found in the sediments (www.uba.de/db-pharm, 2019). The substances that are analyzed the most belong to the therapeutic groups of antibiotics, analgesics, lipid-lowering drugs, and estrogens. Nevertheless, some regional differences between the low-income, middle-income, and high-income countries were notable, revealing different distribution patterns in the compounds detected. The key differences such as demographics, prescription practice, sewer connectivity, design of WWTPs, reuse of waste and wastewater affect the environmental occurrence of these compounds (Kookana et al., 2014). **Fig. 7** shows the patterns of PhACs and estrogens detected in different geographical regions, showing the groups of compounds of major detection in each area. Thus, antibiotics are the most widely detected in the Asian Group while estrogens are mostly found in Africa, analgesics in the Eastern Europe Group, and a range of different PhACs in Western Europe and Other Groups (**Fig. 7**).

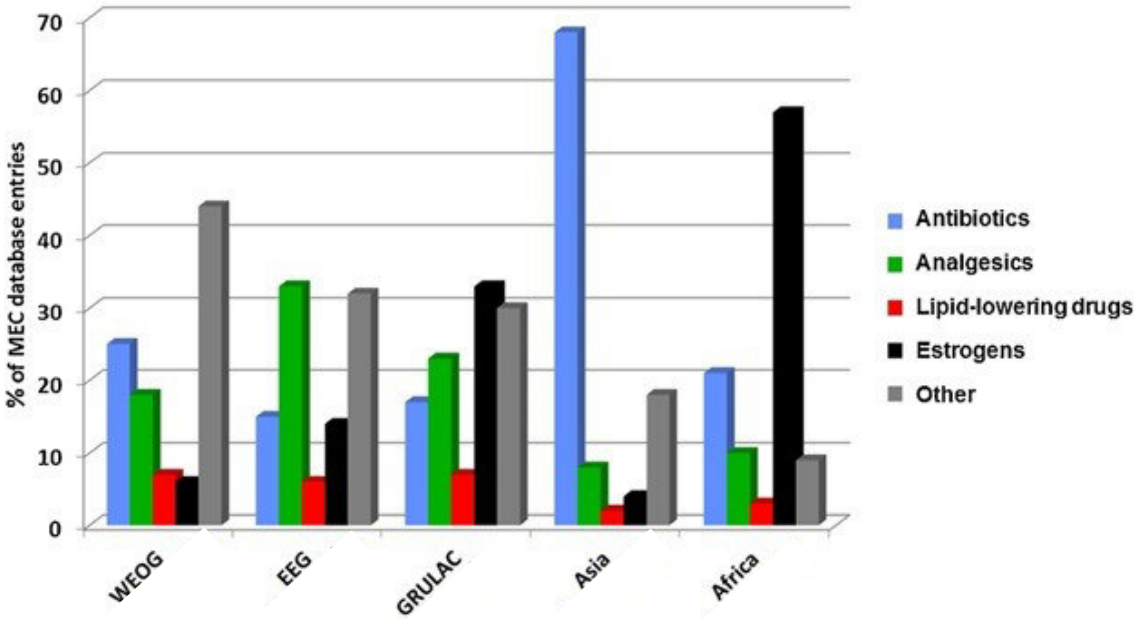


Fig. 7 *Regional patterns of different PhACs therapeutic groups detected in the environment in each UN region (Western Europe and Others Group (WEOG), Eastern Europe Group (EEG), the group of Latin American and Caribbean States (GRULAC), Asian Group (Asia), and African Group (Africa)); adapted from aus de Beek et al. (2016)* (aus de Beek et al., 2016)

Available data showed that, from all entries in the database, only 19 substances (13 PhACs and 4 estrogenic hormones) were detected in all five UN-regions (www.uba.de/db-pharm, 2019). **Fig. 8** shows the most prominent PhACs that were detected in all surface water compartments. These compounds include the analgèsics, diclofenac, ibuprofen, and naproxen, followed by the antiepileptic carbamazepine and the antibiotic sulfamethoxazole. Other compounds that have been globally detected are the antibiotics trimethoprim, sulfamethazine and ciprofloxacin, the estrogens E1, 17β-E2, 17α-EE2, and E3, the analgesics and anti-inflammatories paracetamol, ketoprofen, acetylsalicylic acid, and indomethacin and the lipid regulator clofibric acid (**Fig. 8**).

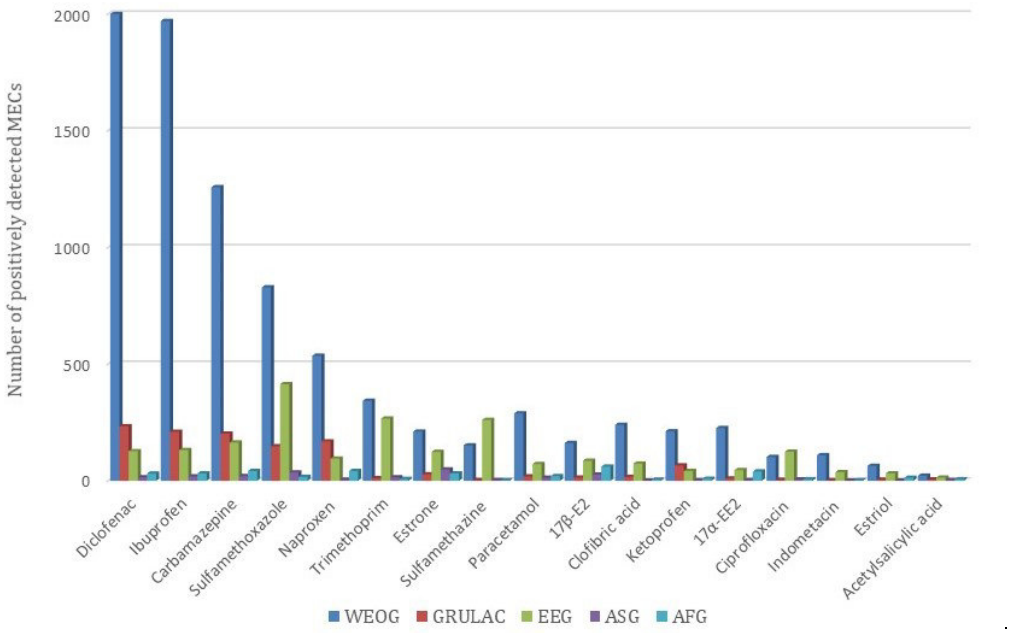


Fig. 8 *Number of MECs in surface waters (rivers, streams, lakes, seawater, and oceans) for substances occurring in all 5 UN-regions (Western Europe and Others Group (WEOG), the group of Latin American and Caribbean States (GRULAC), Eastern Europe Group (EEG), Asian Group (ASG), and African Group (AFG)); adapted from aus de Beek et al. (2016)* (aus de Beek et al., 2016)

Table 5 lists the concentration ranges of the 17 most detected PhACs and hormones in different types of water. PhACs concentrations in influent wastewater varied from 0.052 to 88.4 µg L^{-1}, depending upon the country, region, area, consumption patterns, and origin of the wastewater (i.e urban or industrial wastewater). For effluents, typical concentrations range was from 0.049 to 8.9 µg L^{-1}, depending on the compound characteristics and on the efficiency of the applied wastewater treatment processes. The concentration levels of estrogenic hormones (17β-E2, E1, E3, and 17α-EE2) in influent and effluent wastewaters, are usually quite lower, generally not exceeding 200 ng L^{-1}. E2 metabolites, E3 and E1, were found in some studies at higher concentrations in influent wastewater, exceeding 1 µg L^{-1} for E3 (Sim et al., 2011), and 273 ng L^{-1} in the effluent (Yarahmadi et al., 2018). The occurrence of these compounds can also originate from the transformation of estrogenic conjugates (metabolites) to the parent compound (Yu et al., 2019).

PhACs concentration ranges in rivers and streams are impressively wide, from low ng L^{-1} to low-medium µg L^{-1} range. Even though concentrations are often an order of magnitude lower than those found in WWTP effluents, some compounds such as antibiotics and analgesics (i.e. ciprofloxacin and ibuprofen) were found at maximum concentrations of 2500 µg L^{-1} and 303 µg L^{-1}, respectively in surface waters (Fick et al., 2009). With respect to estrogenic hormones, their concentration levels in surface water are much lower than those found for PhACs, usually in the low ng L^{-1} range and not exceeding 50 ng L^{-1}. Nevertheless, one study found unexpectedly high concentrations for E1 and E3 (560 and 170 ng L^{-1},

respectively) (Pelayo et al., 2011). In terms of frequency of detection, E1 is the most ubiquitous compound in natural waters.

The concentrations of PhACs in drinking water are even lower than those found in surface water, usually by about a factor of 10 (Aga, 2008). Estrogens are rarely detected in drinking water system, and when found, they are usually present at trace-level concentrations. For instance, in some monitoring campaigns, E1 and 17β-E2 were detected at concentrations of ~3 ng L^{-1} (Goeury et al., 2019; Kuch and Ballschmiter, 2001; Morteani et al., 2006), while E1 was detected at 0.4 ng L^{-1} (Loos et al., 2007), and E3 at 1 ng L^{-1} (Goeury et al., 2019).

The range of PhACs concentrations in seawater and oceans is quite low, due to higher dilution effect, with concentrations of positively detected compounds falling in the low 0.3-31 ng L^{-1} range (**Table 5**). However, some PhACs were found to be present in their maximum concentrations at several hundreds of ng L^{-1} or even µg L^{-1}, such as the analgesics paracetamol and ketoprofen, with concentration of 230 µg L^{-1} and 6.5 µg L^{-1}, respectively (Togola and Budzinski, 2008), which is subjected to intense tourist and urban pressure. Thus, their detection in these ecosystems confirms that coastal settings have become a vulnerable system to PhACs pollution, mostly because they are the ultimate receptacles of pollutants. In terms of estrogens, they are often monitored among other PhACs (Bayen et al., 2013; Patel et al., 2019), and their concentrations are usually below LOQ or in the low ng L^{-1} range. If their detection is achieved, concentration rarely exceed 10 ng L^{-1} range. In the coastal waters of Singapore, E1 concentration was 2.1 ng L^{-1} (Bayen et al., 2013), whereas in the Scheldt estuary E1 was observed as most frequently detected estrogen in water at concentrations up to 10 ng L^{-1} (Noppe et al., 2007). However, as previously mentioned, there is still a lack of knowledge about the occurrence of these contaminants in marine waters. Thus, future studies should be devoted to expanding the current knowledge about their occurrence and fate in the marine environment.

The occurrence of PhACs in sediments has not been widely studied (Antonić and Heath, 2007; Fairbairn et al., 2015; Ferreira et al., 2011), especially for marine sediments where data is very limited (Bayen et al., 2016; Huber et al., 2016; Klosterhaus et al., 2013; Moreno-González et al., 2015). Generally, these compounds are detected with low frequency in sediment and at lower concentrations compared to water resources. In addition, the concentrations found in marine sediments are generally lower than those observed in river sediments, and concentrations are in the low ng g^{-1} range (Zeng et al., 2008). PhACs are mainly hydrophilic compounds that tend to be present in the dissolved fraction. Furthermore, concentrations in marine settings are lower, thus explaining the lower concentrations in marine sediments. However, some PhACs can sorb onto solid fractions, and as a result, they can be transferred to sediments (Moreno-González et al., 2015). The studies available reported that PhACs are present in

sediments at concentrations ranging from 0.05 to 53.8 ng g^{-1} dry weight (d.w.). In some cases, concentrations up to 257.4 ng g^{-1} were found (Viganó et al., 2006). The most common PhACs found in sediments are antibiotics (trimethoprim, sulfamethoxazole, and sulfamethazine), analgesics and anti-inflammatories (ibuprofen and diclofenac), and antiepileptics (carbamazepine) (Li, 2014). On the other hand, steroid hormones are non-polar hydrophobic compounds that can easily adsorb onto river sediments (Yarahmadi et al., 2018). Steroid hormones have log Kow mostly between 3 and 6 and thus, river sediments likely act as an environmental sink for these compounds. Generally, concentrations ranged from 0.8-257.4 ng g^{-1} d.w. According to previous studies about the fate of estrogens in riverbeds, between 13% and 92% of estrogens ended up in the river sediments during the first hours of their discharge into the river (Jurgens et al., 1999; Lai et al., 2000). From the various steroid hormones investigated, E1 and 17α-EE2 were the most frequently detected compounds in sediments (López de Alda et al., 2002).

Table 5. *Concentration ranges of the most frequently detected PhACs and hormones in different environmental compartments**

Pharmaceuticals	Environmental Matrix	WWTP influent (untreated) (µg L^{-1})	WWTP effluent (treated) (µg L^{-1})	Surface water - river/stream (ng L^{-1})	Drinking water (ng L^{-1})	Surface water - sea or ocean (ng L^{-1})	Sediment - river/stream (ng g^{-1})
Diclofenac	Concentration range	1.8-181	1.02-24.3	185-18740	2.4-140	4-1500	5.9-14
	Total number of samples	3527	5011	5714	166	12	45
Ibuprofen	Concentration range	12-373.1	1.36-95	240.5-303000	23.8-1350	13-1500	0.1-0.4
	Total number of samples	3436	4153	5435	73	9	49
Carbamazepine	Concentration range	0.637-2.6	0.49-67.7	409.6-11561	17.5-601	23.6-119	0.052-2.9
	Total number of samples	1363	2682	7811	291	90	37
Sulfamethoxazole	Concentration range	2.3-309	0.24-24.8	119.7-29000	2.0-80	16.4-212.2	2.6-484700
	Total number of samples	1022	2355	2725	79	180	22
Naproxen	Concentration range	3.4-552	0.51-39.3	119.3-12300	38.2-55	1.36-2050	10.5-60
	Total number of samples	1087	2626	1915	29	9	13
Trimethoprim	Concentration range	0.76-162	0.87-95.1	71.5-13600	3.59-15	15.1-330	0.8-2600
	Total number of samples	1110	1153	1528	45	177	14
Sulfamethazine	Concentration range	3.6-189	0.158-25.4	64.4-9600	4.1-89.6	6.4-61	4.5-13.7
	Total number of samples	365	1268	956	2	41	21
Paracetamol	Concentration range	35.9-1200	3.49-280	331.5-37000	21.1-211	23-230000	0.2-0.74
	Total number of samples	765	852	658	25	2	5
Clofibric acid	Concentration range	0.568-61	0.049-3.3	68.4-7910	3.4-270	1.8-7.8	1.8-3.01
	Total number of samples	500	639	1840	213	1	13
Ketoprofen	Concentration range	2.95-233.6	0.35-6.5	90.4-9808	24.7-51	1.5-6050	53.8-320
	Total number of samples	458	1131	1664	52	5	13
Ciprofloxacin	Concentration range	1.8-160	185.8-31000	20509-2500000	13.3-13.3	31-66	1.9-74090
	Total number of samples	586	1218	514	10	31	25
Indometacin	Concentration range	0.052-1.6	0.240-5	28.4-2323	1.6-6	0.5-3	0.2-0.49
	Total number of samples	161	240	722	58	1	1
Acetylsalicylic acid	Concentration range	88.4-1731	8.9-193	1773-20960	2.3-13	-	n.d.
	Total number of samples	190	150	214	23	-	-
Hormones							
Estrone	Concentration range	0.2-129	0.074-32.9	39.8-5000	3.9-2.7	0.3-11	1.4-42.2
	Total number of samples	3019	3178	1865	106	35	84
17β-Estradiol	Concentration range	0.04-68.9	0.02-25.2	249.2-13450	590.1-2600	0.2-1.8	0.8-149
	Total number of samples	2979	2977	980	101	28	87
17α-Ethinylestradiol	Concentration range	0.03-140	0.028-5.1	143.2-5900	357.0-1900	4.7-17.9	189-35.6
	Total number of samples	2823	2957	1072	113	28	92
Estriol	Concentration range	0.22-110.7	0.227-83.4	10.8-480	0.7-0.72	n.d.	257.4-758.2
	Total number of samples	359	547	622	31	28	8

*Source: https://www.umweltbundesamt.de/en/database-pharmaceuticals-in-the-environment

n.d.- not detected;

For estrogenic hormones, their conjugated metabolites (i.e. sulfates and glucuronides), such as E1-3S, E3-3S, E2-17G, E1-3G, and E3-16G, have been also detected in environmental samples. However, these compounds have received less scientific attention, due to their low estrogenic potency (Tomšíková et al., 2012). A significant proportion of hormones is excreted by humans as conjugate metabolites, such as glucuronide or sulfate metabolites. Thus, their presence has been mainly investigated in wastewater samples (D'Ascenzo et al., 2003; Gentili et al., 2002; Komori et al., 2004; Reddy et al., 2005), and their concentrations were usually around 100 ng L^{-1}, except for the sulfate conjugate E1-3S, which was detected in influents wastewater at high ng L^{-1} levels (Kobayashi et al., 2006; Komori et al., 2004; Pedrouzo et al., 2009). These metabolites are converted to the active free estrogen form in WWTPs by E. coli bacteria and they are also relatively quickly de-conjugated to the parent compound in the environment (Andersen et al., 2003; Baronti et al., 2000). Even though these metabolites have been mostly detected in wastewaters, there are some studies reporting their occurrence in surface water at trace-levels, often close to or above the concentration at which they can exert estrogenic effects (>1–10 ng l^{-1}) (Brix et al., 2010b; Esteban et al., 2014; Komori et al., 2004). Sulfate conjugates are less prone to de-conjugation than glucuronides (Johnson and Williams 2004, Ma 2018) and those compounds are more frequently detected in aqueous environments. Estrogen glucuronides are often found below of their LOD (Gentili et al., 2002; Isobe et al., 2003; Komori et al., 2004), mostly related with their relatively quick de-conjugation in the environment.

Regarding other EDCs, such as the surfactants NP and OP, and the plasticizer BPA, their occurrence has been widely investigated in both aqueous and solid phases (Céspedes et al., 2008; Li et al., 2010; Pamplona-Silva et al., 2018; Zhang et al., 2009). In general, concentrations of NP, OP, and BPA are one order of magnitude higher than those reported for the rest of EDCs, and are usually the main contributors to the sum of all monitored EDCs in environmental samples (Brix et al., 2010b; Esteban et al., 2014; Pelayo et al., 2011). Their concentration in wastewater are generally in the upper ng L^{-1} range (Williams et al., 2019; Wu et al., 2017), and in some occasions, they even exceed 1 μg L^{-1} levels (Ballesteros-Gómez et al., 2007). Concerning natural waters, NP, OP, and BPA concentrations differ quite a lot in the literature, ranging from low ng L^{-1} (Leusch et al., 2018; Sodré et al., 2010) to even high μg L^{-1} (Brix et al., 2010b; Céspedes et al., 2006; Pelayo et al., 2011). The presence of these compounds is mostly related with industrial wastewater from manufacturing processes that discharge their effluents to freshwater ecosystems while their presence in coastal settings has not been so widely reported (Meador et al., 2016). Nevertheless, when found, concentrations are remarkable. NP was detected in Long Island Sound Estuary water at concentrations of 300 ng L^{-1} (Lara-Martín et al., 2014) and BPA was found to be one of the more ubiquitous compounds in Singapore's marine environment

reaching concentration >500 ng L^{-1} (Bayen et al., 2013), attributed to higher anthropogenic activities in coastal settings.

Table 6. *Concentration ranges of conjugated estrogens (E1-3S, E3-3S, E1-3G, E2-17G, and E3-16G), surfactants (NP and OP), and plasticizer (BPA) in the aquatic environment (see Table S1 in the Supplementary Material in Article 1 in the Annex)*

Compounds	wastewater			
	influent (ng L^{-1})	effluent (ng L^{-1})	surface water (ng L^{-1})	drinking water (ng L^{-1})
Conjugated estrogens				
E1-3S	12-170[a]	0.3[b]-42[i]	0.3-10[m]	<LOD
E3-3S	<LOD	<LOD	1.2-8.9[m]	<LOD
E1-3G	0.4[b]-88[a]	0.3[c]-140[a]	<LOD	<LOD
E2-17G	3[c]	0.7[c]	<LOD	<LOD
E3-16G	19[d]	n.d.	<LOD	<LOD
Surfactants				
4-NP	120[e]-4400[f]	<LOD	20[n]-17500[o]	2[q]-1073[r]
4-OP	<10-540[e]	<LOD-22[j]	6[g]-3980[o]	0.15[q]-24[s]
Plasticizer				
BPA	22[g]-1600[h]	4[k]-360[h]	3.9[p]-1510[o]	0.3[q]-317[t]

[a](Komori et al., 2004); [b](Reddy et al., 2005); [c](Ben et al., 2017); [d](D'Ascenzo et al., 2003); [e](Miloradov et al., 2014); [f](Terzić et al., 2008); [g](Leusch et al., 2018); [h](Ballesteros-Gómez et al., 2007); [i](Kobayashi et al., 2006); [j](Rubirola et al., 2017); [k](Wu et al., 2017); [l](Isobe et al., 2003); [m](Esteban et al., 2014); [n](Brix et al., 2010a); [o](Céspedes et al., 2006); [p](Goeury et al., 2019); [q](Kuch and Ballschmiter, 2001); [r](Casajuana and Lacorte, 2003); [s](Bono-Blay et al., 2012); [t](Li et al., 2010);

1.5 Risk assessment

Despite the low concentrations detected, the widespread occurrence of PhACs and EDCs in the environment has led to concerns over their potential impacts on aquatic organisms as well as to human health (Ankley et al., 2007; Brodin et al., 2017; Mennigen et al., 2011). Some of the ecotoxicological effects of PhACs have been already documented, showing that the exposure of aquatic organisms to certain PhACs leads to lower growth, oxidative stress reproductive fitness impairment, and behavioral effects (Oaks et al., 2004; Scholz and Klüver, 2009; Weatherly and Gosse, 2017). For EDCs, several studies already proved that these compounds could exert physiological effects on aquatic organisms at low ng L^{-1} levels (Céspedes et al., 2004; Christiansen et al., 2002; Desbrow et al., 1998; Hansen et al., 1998; Solé et al., 2000). These effects include feminization in males, decreased fecundity, developmental abnormalities, and intersex phenomena, among other alarming effects (Daughton and Ternes, 1999; Fent et al., 2006; Lai et al., 2002; Peng et al., 2008).

The most common approach to assess the potential risks posed by PhACs and EDCs in the environment is based on the estimation of risk or hazard quotients. In the case of EDCs other approaches are also used, which mostly include methods for the determination of their estrogenic activity in the aquatic environment (Damkjaer et al., 2018; Manickum and John, 2014; Wu et al., 2017; Yao et al., 2018; Yien Fang et al., 2019). Indeed, risk assessment strategies are also used to prioritize the compounds of major ecological concern. Sometimes, they are even used in

combination with other criteria, such as the compounds persistency in the environment or their potential to bioaccumulate in aquatic organism, etc., to highlight the substances of major concern (Gros et al., 2017; Kiefer et al., 2019; Park et al., 2018). However, risk-based assessment strategies proved to be a suitable tool for the identification of PhACs and EDCs that should be included in future monitoring programs and legislations.

Ecotoxicological risks of PhACs and EDCs are, in general, estimated for every single compound, and few studies considered the effects of compound mixtures (Cleuvers, 2003). Considering that PhACs and EDCs occur in the environment as mixtures, and they exist together with other pollutants, it is necessary to evaluate the ecotoxicological effects of these cocktails of contaminants. Some studies pointed out that the effects of mixtures most probably follow the concept of concentration addition. Thus, the overall toxicity can be estimated as the sum of each compound's individual toxicity (Fent et al., 2006).

1.5.1 Risk quotient and hazard quotient methods

Within risk assessment-based studies, the estimation of risk quotients (RQs) or hazard quotients (HQ) is the most common approach. RQs are a useful tool to identify the PhACs and EDCs that pose the highest environmental risks (Cooper et al., 2008; Escher et al., 2011; Ginebreda et al., 2010; Rivera-Jaimes et al., 2018; Verlicchi et al., 2012). RQ and HQ are calculated according to EU guidelines 93/67/EEC (93/67/EEC, 2003) as the ratio between MEC or predicted environmental concentrations (PEC) and their toxicity, usually expressed as predicted no-effect concentrations (PNEC, μg L^{-1}). PNECs are calculated using chronic toxicity data, through the non-observed effect concentrations (NOEC, μg L^{-1}), corrected with an assessment factor (AF) of 10 or 100. When NOEC values are not available, acute toxicity data from standard ecotoxicological tests (which are the 50% effective concentration (EC_{50}) or 50% lethal concentration (LC_{50}), respectively) can be used, after the correction with an AF of 1000. Toxicity data is generally determined for three different trophic levels, which include algae (*Selenastrum capricornutum)*, macroinvertebrates (*Daphnia magna),* and fish (*Pimephales promelas)*. Under this framework, the criteria for the risk rating is based on RQ < 0.1 "low risk", 0.1 ≤ RQ < 1 "medium risk", and RQ > 1 "high risk" (Cao et al., 2010; Hernando et al., 2006; Manickum and John, 2014; Wang and Zhu, 2017; Wu et al., 2017).

RQs and HQs are calculated using the following equation (1):

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

Information about the toxicity of ECs can be found in several available Ecotoxicological databases (Zillien et al., 2019). Ecotoxicity data employed in many risk-based prioritization studies are often derived from modelled data, using QSAR models, such as ECOSAR (Dong et al., 2013). One of the most extended and novel databases is the NORMAN Ecotoxicology Database, which is an open-access database that contains information for more than 39.000 substances (NORMAN Ecotoxicology Database, 2019). The values included in the NORMAN database (normally PNECs) were either predicted by models, such as QSAR, or were obtained experimentally and they include information about the toxicity of ECs towards different standard test organisms. For the calculation of RQ or HQ, the lowest available PNEC values are normally used, considering thus worst-case scenarios.

Most of the available ecotoxicity data is for trophic levels or endpoints that are representative for freshwater ecosystems, whereas toxicity data for marine organisms is not available. Indeed, the risks posed by ECs, such as PhACs and EDCs, to the marine environment have been poorly documented in the scientific literature (Minguez et al., 2014). To perform proper risk assessment studies for the marine environment, ecotoxicological data specific to marine species is needed (Marchand and Tissier, 2005; Minguez et al., 2016). In order to have an estimation of the ecotoxicological risks posed by ECs in marine ecosystems, PNECs can be estimated from those determined for freshwater organisms, but applying an additional assessment factor of 10 to the lowest acute toxicity values (Minguez et al., 2014).

According to a recently published review paper about risk-based studies of PhACs and hormones, a total of 73 compounds were identified as of highest priority (Burns et al., 2018). Therapeutic classes such as antibiotics (16), hormones (10), analgesics (9), antidepressants (6), lipid-lowering agents (5), and β-blockers (3) were flagged as the PhACs classes of highest priority (Burns et al., 2018). Of the entire list of identified priority compounds, the top 20 PhACs and hormones are listed in **Table 7**. It is worth mentioning that among this top 20 listed compounds, eight substances, namely 17α-EE2, 17β-E2, E1, ciprofloxacin, clarithromycin, erythromycin, amoxicillin, and azithromycin, are included in the WL of priority compounds (De Carvalho et al., 2015; Decision 2018/840/EU, 2018) (see Section 1.6).

Table 7. *Top 20 priority PhACs and hormones based on the literature*

N	Compound	Therapeutic class (N of compounds)[2]
1	Diclofenac	Analgesic (8)
2	Paracetamol	Analgesic (8)
3	Ciprofloxacin[1]	Antibiotic (16)
4	Ethinylestradiol[1]	Hormone (10)
5	Carbamazepine	Anticonvulsant (1)
6	Ibuprofen	Analgesic (8)
7	Clarithromycin[1]	Antibiotic (16)
8	Estradiol[1]	Hormone (10)
9	Sulfamethoxazole	Antibiotic (16)
10	Erythromycin[1]	Antibiotic (16)
11	Amoxicillin[1]	Antibiotic (16)
12	Propranolol	Beta-blocker (3)
13	Trimethoprim	Antibiotic (16)
14	Fluoxetine	Antidepressant (6)
15	Azithromycin[1]	Antibiotic (16)
16	Sertraline	Antidepressant (6)
17	Gemfibrozil	Lipid-lowering agent (5)
18	Mefenamic acid	Analgesic (8)
19	Estrone[1]	Hormone (10)
20	Amitriptyline	Antidepressant (6)

[1]- Compounds listed in the Watch List (Decision 2018/840/EU, 2018)

[2]- Total number of compounds from the same therapeutic group identified as priority

1.5.2 Estrogenicity

For EDCs, besides RQs, their potential to exert toxic effects is also evaluated by the estimation of their estrogenic activity (Solé et al., 2000; Wang et al., 2012; Yao et al., 2018; Zhang et al., 2011). Estrogenic factors are relative to the activity of the natural estrogen 17β-E2 and are expressed as estradiol equivalents (EEQ) by using the following equation (2):

$$\text{EEQ (ng E2 L}^{-1}) = C_i \times EEF_i \quad (2)$$

Where C_i (ng L^{-1}) is the MEC of each compound and estradiol equivalency factor (EEF_i) is the relative estrogenicity factor of the studied compounds, calculated as the ratio between the mean EC_{50} value of each compound relative to the EC_{50} of 17β-E2. Available EEF_i values in the literature for some target EDCs are summarized in **Table 8.**

Table 8. *Literature values of estradiol equivalency factor (EEFi) obtained for selected EDCs compounds in different bioequivalence experiments*

Compound	EEF_i ($\mu g\ L^{-1}$)[a]	EEF_i ($\mu g\ L^{-1}$)[b]	EEF_i ($\mu g\ L^{-1}$)[c]	EEF_i ($\mu g\ L^{-1}$)[d]	EEF_i ($\mu g\ L^{-1}$)[e]	EEF_i ($\mu g\ L^{-1}$)[f]
17β-E2	1	1	1	1	1	1
E1	0.06	$2.5x10^{-1}$	0.01886	-	-	0.01
E3	-	$5.9x10^{-3}$	0.3448	-	-	0.083
17α-EE2	-	1.25	0.1709	1.19	-	1.25
DES	-	-	0.04597	-	-	1.25
4-t-OP	$3x10^{-6}$	$4.5x10^{-6}$	$2.13x10^{-4}$	$7.8x10^{-6}$	$1.4x10^{-6}$	$8.33x10^{-5}$
4-NP	$1x10^{-6}$	$1.8x10^{-5}$	$5.05x10^{-4}$	$2.5x10^{-5}$	$2.3x10^{-5}$	$1.25x10^{-5}$
BPA	LE	$1.2x10^{-4}$	$2.43x10^{-5}$	$1.1x10^{-4}$	-	$2.5x10^{-5}$

[a](Song et al., 2006) [b](Beck et al., 2005) [c](Céspedes et al., 2004) [d](Rutishauser et al., 2004) [e](Van den Belt et al., 2003) [f](Gutendorf and Westendorf, 2001)

If one compound has several available EEFi values, some authors considered the average values of all the EEF_i reported (Brix et al., 2010b), whereas others just used the highest EEF_i, to consider worst-case scenarios (Brand et al., 2013; Zhao et al., 2009). The threshold for estrogenic activity is set at 1 ng E2 L^{-1}, since this is the level for estradiol to cause estrogenic effects in aquatic organisms, as it has been previously reported (Hansen et al., 1998; Purdom et al., 1994).

However, these assessments only concerned the toxicity caused by individual EDCs and underestimate the total risk of these compounds to aquatic life. Besides individual estrogenic effects, total estrogenic activities (EEQ_t) were also estimated since EDCs are present as mixtures in the environment (Cao et al., 2010; Manickum and John, 2014; Yien Fang et al., 2019). EEQ_t are calculated by summing individual EEQ_i, according to equation (3):

$$EEQ_t = C_1 \times EEF_1 + C_2 \times EEF_2 + \ldots \quad (3)$$

Even though the levels of EDCs in the aquatic environment are quite low, the calculated estrogenic potential could surpass the expected effect concentration of 1 ng E2 L^{-1} due to their estrogenic potency, as it has been previously reported (Brix et al., 2010b; Hansen et al., 1998; Purdom et al., 1994). Among EDCs, the hormone 17β-E2 is the most potent and stable (**Table 8**) and some previous reports indicated that "high risk" in investigated rivers are mostly posed due to presence of 17β-E2, followed by 17α-EE2 (Folmar et al., 2002; Manickum and John, 2014; Martín et al., 2012). Estrogen E1 is less potent, has much shorter half-life and therefore, a lower risk factor is generally associated with this hormone (Cao et al., 2010). However, potencies vary depending on the type of bioassay used and the method of determination (Hamid and Eskicioglu, 2012). Other EDCs, such as NP, OP, and BPA can also affect the reproduction of organisms that are exposed to them because they mimic the estrogenic hormones that are responsible for development and sexual behavior. For instance, NP mimics the natural hormone 17β-EE2 and competes with the endogenous hormones by binding to the ER (estrogen receptor) (Soares et al., 2008). However, compared to natural and

synthetic estrogens, the estrogenic potency of NP, OP, and BPA is 10.000 to 100.000 times lower (**Table 8**). This makes them lower contributors to estrogenic activities in the aquatic environment (De Mes et al., 2005). Considering that NP, OP, and BPA are present at higher concentrations than estrogenic hormones, their occurrence in waters may represent a potential estrogenic risk for the aquatic environment as well.

1.6 EU Legislation

Although ECs frequently occur in various environmental media in the world, they are not yet addressed comprehensively under existing regulations (Hecker and Hollert, 2011). In the EU, water pollution is regulated under the WFD (Directive 2000/60/EC, 2000), which established a framework for community action in the field of water policy. According to WFD, good ecological status of water bodies must be achieved and requires that the concentrations of specific pollutants do not exceed the established environmental quality standards (EQSs) set at member state level, designed to protect the environment and human health. EQSs are established for 45 chemical pollutants, known as priority substances of EU-wide concern, as set in Directive 2008/105/EC (Directive 2008/105/EC, 2008). This directive was lately amended by the Directive 2013/39/EU (Directive 2013/39/EU, 2013). The Directive 2013/39/EU includes a revised (second) list of priority substances (53), selected according to the levels found in surface water, production use and hazardousness. The first WL of compounds to be monitored Europe-wide, to gather data on their occurrence and select the most relevant to be included in the list of priority substances, was established in March 2015 (Decision 2015/495/EU, 2015). The WL comprised seventeen ECs, including neonicotinoid pesticides (5), macrolide antibiotics (3), hormones (3), herbicides (2), anti-inflammatory drug (1), pesticide (1), antioxidant (1), and UV-filter (1).

Although PhACs have been widely monitored in the environment, most of them are not included in environmental regulations yet. Initially, only four PhACs were included in the first WL (Decision 2015/495/EU, 2015) with the final goal to be added in the list of priority pollutants of the WFD (Directive 2000/60/EC, 2000). These PhACs include the analgesic and anti-inflammatory drug diclofenac and the macrolide antibiotics erythromycin, clarithromycin, and azithromycin. In a recent update of the compounds included in the WL (Decision 2018/840/EU, 2018), diclofenac was excluded, while two antibiotics, amoxicillin, and ciprofloxacin were incorporated, bringing it to the total number of five PhACs regulated compounds in the list. Among EDCs, the first WL initially included three hormones: E1, 17β-E2, and 17α-EE2 (Decision 2015/495/EU, 2015), which have been included in the second WL as well (Decision 2018/840/EU, 2018). Currently, the updated WL includes fifteen substances, among which eight are PhACs and EDCs. The WL includes three estrogen hormones (E1, 17β-E2, and 17α-EE2) and five antibiotics

(erythromycin, clarithromycin, azithromycin, amoxicillin, and ciprofloxacin), listed in the **Table 9.** In accordance with Article 8b of Directive 2008/105/EC (Directive 2008/105/EC, 2008), the European Commission identified possible methods of analysis and the maximum acceptable MDLs (ng L^{-1}) for the proposed substances.

Table 9. *The substances included in updated Watch List (WL) for Union-wide monitoring (Decision 2018/840/EU, 2018)*

Group of substances	Name of the substance	CAS number	EU number	Indicative analytical methods	MDL (ng L^{-1})
Hormones	17 α-ethinylestradiol*	57-63-6	200-342-2	Large-volume SPE-LC-MS/MS	0.035
	17 β-estradiol*	50-28-2	200-023-8	SPE-LC-MS/MS	0.4
	Estrone*	53-16-7	-	SPE-LC-MS/MS	
Macrolide antibiotics	Erythromycin*	114-07-8	204-040-1	SPE-LC-MS/MS	
	Clarithromycin*	81103-11-9	-	SPE-LC-MS/MS	19
	Azithromycin*	83905-01-5	617-500-5	SPE-LC-MS/MS	
Pesticide	Methiocarb	2032-65-7	217-991-2	SPE-LC-MS/MS or GC-MS	2
Neonicotinoids	Imidacloprid	105827-78-9/ 138261-41-3	428-040-8	SPE-LC-MS/MS	8.3
	Thiacloprid	111988-49-9	-	SPE-LC-MS/MS	
	Thiamethoxam	153719-23-4	428-650-4	SPE-LC-MS/MS	
	Clothianidin	210880-92-5	433-460-1	SPE-LC-MS/MS	
	Acetamiprid	135410-20-7	160430-64-8	SPE-LC-MS/MS	
Insecticide	Metaflumizone	139968-49-3	604-167-6	LLE-LC-MS/MS or SPE-LC-MS/MS	65
Penicilin antibiotic	Amoxicillin*	26787-78-0	248-003-8	SPE-LC-MS/MS	78
Fluoroquinolone antibiotic	Ciprofloxacin*	85721-33-1	617-751-0	SPE-LC-MS/MS	89

*EDCs and PhACs included in WL (three estrogen hormones and five antibiotics)

Other EDCs are not included in the WL but they are covered by different specific regulations depending on their uses (Cécile, 2019). The WFD (Directive 2000/60/EC, 2000) was the first Community measure to have brought some of EDCs under regulatory control. So far, about 435 different compounds were classified by the EU Commission as priority substances and 94 of them as EDCs with evidenced endocrine effects (Okkerman and Van der Putter, 2002). Some of them are well-known chemicals, such as APs (NP and OP), and the plasticizer (BPA), that belong to the category 1 of the Endocrine Disruptor Priority List for wildlife and human health (Directive 2008/105/EC, 2008). The implementation of the European Directive 2003/53/EC has prohibited the use of NP and related nonylphenoletoxylates (NPEOs) in industrial and commercial cleaning agents since January 2005 (Directive 2003/53/EC, 2003). In contrast to NP, OP has not been banned yet. However, concentrations of NP and OP could not exceed the annual average (AA-EQS) of 0.3 and 0.1 μg L^{-1}, respectively as specified in the Directive 2008/105/EC (Directive 2008/105/EC, 2008) (see **Table 10**). The maximum allowable concentration (MAC) of NP in inland surface water was set at 2 μg L^{-1} in

the same Directive (Directive 2008/105/EC, 2008). Recently, NP and OP were listed among the 45 priority substances in the European WFD (Directive 2013/39/EU, 2013), being NP classified as a priority hazardous substance. On the other hand, the use of BPA was restricted in certain food-contact materials (Directive 2018/213/EC, 2018) and this compound is gradually replaced with analog bisphenols (Gramec Skledar and Mašič, 2016). However, recent studies showed that these analogs might have similar or even higher toxicity (Hu et al., 2019).

Table 10. *Environmental quality standards (EQSs) set in Directive 2008/105/EC (Directive 2008/105/EC, 2008)*

Name of substance	CAS number	AA-EQS (μg L^{-1}) Inland surface waters	AA-EQS (μg L^{-1}) Other surface waters	MAC-EQS (μg L^{-1}) Inland surface waters	MAC-EQS (μg L^{-1}) Other surface waters
Nonylphenol	104-40-5	0.3	0.3	2.0	2.0
Octylphenol	104-66-9	0.1	0.01	not applicable	not applicable

Regarding marine and coastal waters, monitoring programs for continuous evaluation of their environmental status should be established according to the Marine Strategy Framework Directive (Directive 2008/56/EC, 2008). This Directive establishes a framework where Member States shall take the necessary measures to achieve or maintain good environmental status in the marine environment. For that purpose, strategies should be developed and implemented to protect, preserve, and reduce impacts on marine biodiversity, marine ecosystems, human health, or legitimate uses of the sea. Although the PhACs among other hazardous substances are set out in the Annex II from the same Directive, as a relevant indicator of pressure or impact of human activities in marine region, to date there is no specific regulation for these compounds.

2. Objectives

The overall objective of the thesis was to perform an integrated study to evaluate the impact of wastewater discharges and other anthropogenic pressures into receiving riverine and coastal ecosystems. Thus, special focus is given on identifying relevant wastewater derived contaminants and other relevant ECs through target and suspect screening approaches, to finally include them in risk assessment schemes.

For this purpose, in this thesis two different analytical approaches were used: (i) quantitative target analysis focusing on two groups of ECs (81 PhACs, belonging to nineteen different therapeutic groups and 13 EDCs, including hormones, NP, OP and BPA) and (ii) suspect-screening using exact mass compound databases with information for 360 ECs. Finally, an assessment of the environmental risks posed by the occurrence of the identified contaminants in both riverine and coastal ecosystems was performed. Furthermore, the risk assessment strategy allowed us to highlight the compounds of major ecological concern and those that could be used as relevant markers of contamination.

In this thesis, we focused on two different study areas: (1) the Danube River basin within the Northern Serbian territory (Vojvodina, Serbia), as riverine ecosystem, and (2) the vulnerable area of the Ebro Delta region, as a coastal setting. First area is of special interest because it receives the discharge of untreated urban and industrial wastewater and is subject to a high environmental pressure. Second area is in the mouth of the Ebro River (Catalonia, North-East of Spain). It is the third largest delta in the Mediterranean Sea and is one of the most important wetlands in the Mediterranean region. This is a site of special interest because its contamination might seriously endanger its ecological richness and biodiversity as well as compromise water quality.

The thesis starts with general introduction and results are presented in three major sections (Chapter 1, Chapter 2 and Chapter 3) (**Fig. 9**):

I. **Chapter 1-** Method development and occurrence of endocrine disrupting compounds is divided in two sections. The first part describes the development of an on-line SPE analytical method for the analysis of selected EDCs (the natural and synthetic estrogens) and their conjugates in surface and wastewater and is presented as a scientific publication (Article Nº1). In the second part, the developed analytical methodology was applied to study the occurrence and distribution of selected EDCs in drinking, surface and wastewater in the Danube River basin, in the region of Vojvodina, the North of Serbia (study area 1). In this study, the assessment of potential environmental and human health risks was also evaluated. The results are also presented as a scientific manuscript (Article Nº2).
II. **Chapter 2-** Occurrence of pharmaceutical residues in coastal areas shows the results of a comprehensive study about the occurrence, fate, and behavior of PhACs, as markers of wastewater contamination, in the

vulnerable coastal area of the Ebro Delta (North-East Spain) (study area 2). In addition, a prioritization strategy was performed as a part of a risk assessment approach to identify the most persistent and ecologically relevant PhACs. These results are presented as the third scientific publication (Article Nº3).

III. **Chapter 3-** Suspect screening: method development and identification of emerging contaminants in a coastal area describes the results related to the development of a novel suspect screening methodology, which was afterwards used to identify ECs and track for contamination sources in a coastal setting. In this case, the study area was also the Ebro Delta region (study area 2). In this study, a comprehensive risk assessment was also performed, and results are compiled in a scientific publication that has been published in the Journal of Hazardous Materials (Article Nº4).

Finally, the results of all the studies conducted during the thesis are discussed in the General Discussion, with a final section of future perspectives and the conclusions.

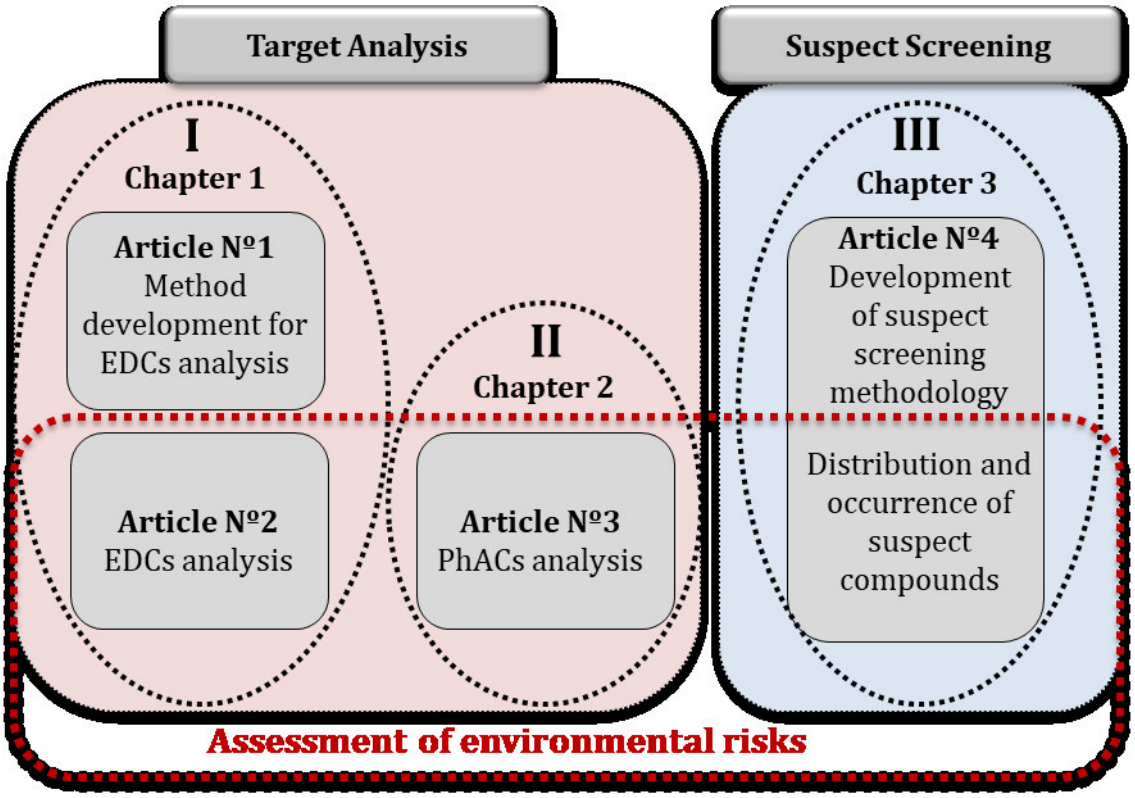
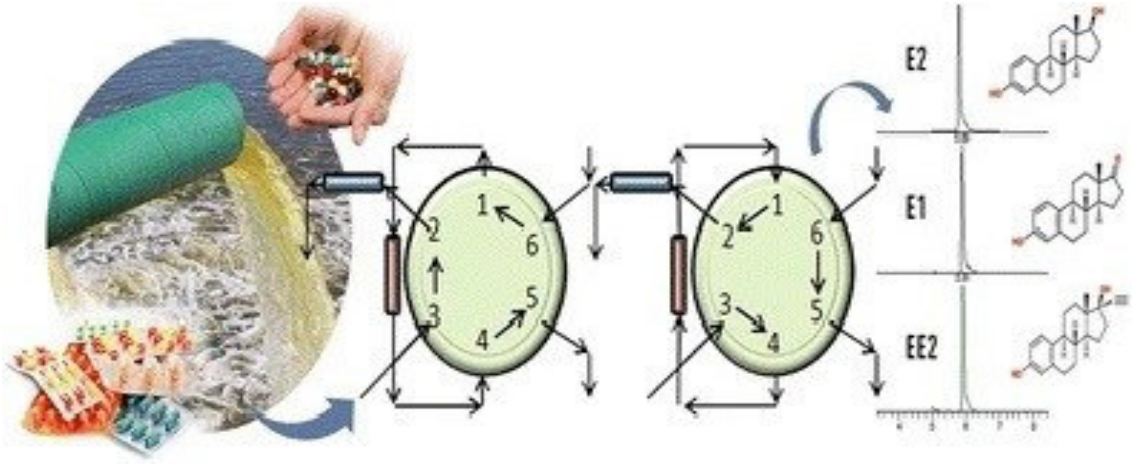


Fig. 9 *The schematic diagram of the structure of the thesis with specific objectives associated with the published articles*

3. Results

Chapter 1

Method development and occurrence of endocrine disrupting compounds



Article Nº1:

Mira Čelić, Sara Insa, Biljana Škrbić, and Mira Petrović

Development of a sensitive and robust online dual column liquid chromatography-tandem mass spectrometry method for the analysis of natural and synthetic estrogens and their conjugates in river water and wastewater

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RESEARCH PAPER

Development of a sensitive and robust online dual column liquid chromatography-tandem mass spectrometry method for the analysis of natural and synthetic estrogens and their conjugates in river water and wastewater

Mira Čelić [1,2] · Sara Insa [1] · Biljana Škrbić [2] · Mira Petrović [1,3]

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Abstract An online ultra-high-performance-liquid chromatography-triple quadrupole tandem mass spectrometry (UHPLC-MS/MS) method for detection and quantification of natural and synthetic estrogens and their conjugates in aqueous matrices was developed. Target compounds include the natural estrogen estradiol (E2) and its main metabolites estrone (E1) and estriol (E3), the synthetic estrogens ethinylestradiol (EE2) and diethylstilbestrol (DES) and their conjugates estrone 3-sulfate (E1-3S), estriol 3-sulfate (E3-3S), estradiol 17-glucuronide (E2-17G), estrone 3-glucuronide (E1-3G), and estriol 16-glucuronide (E3-16G). After pH adjustment, sample filtration and addition of internal standards (IS), water samples (5 mL) were preconcentrated on a Hypersil GOLD aQ column after which chromatographic separation was achieved on a Kinetex C18 column using methanol and water as a mobile phase. The experimental parameters, such as sample loading flow rate, elution time, the percentage of organic solvent in the aqueous-organic eluent mixture, pH, and volume of analyzed samples, were optimized in detail. The benefits of the method compared to previously published methods include minimum sample manipulation, lower detection limits, reduced total analysis time, and overall increased method accuracy and precision. Method detection limits (MDLs) are in subnanogram per liter, complying with the requirements of the EC Decision 2015/495 (Watch list) for hormones listed therein. Applicability of the developed method was confirmed by analysis of river and raw wastewater samples taken directly from urban sewerage systems before being discharged into the river.

Keywords Estrogens · Conjugated estrogens · Online solid phase extraction · Ultra-high-performance-liquid chromatography mass spectrometry (UHPLC-MS/MS) · River water · Wastewater

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Introduction

Endocrine-disrupting compounds (EDCs) have become a public health concern in modern times because of their detrimental effects on the human endocrine system [1, 2]. Among the various substances with reported endocrine-disrupting properties, natural and synthetic estrogens are of particular interest due to their high estrogenic potency [3–5] and the effects that they could cause to aquatic organisms even at below nanogram per liter concentrations [6, 7]. The three major naturally occurring estrogens in women are 17β-estradiol (E2) and its main metabolites estrone (E1) and estriol (E3). They are also used as contraceptives or medication for menopausal women, i.e., E2 and the synthetic estrogen 17β-ethinylestradiol (EE2) are active ingredients in a number of drugs used in physiological hormonal replacement therapies, treatment of prostate and breast cancer, and hair lotions for

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contrasting alopecia [8]. Both natural and synthetic steroids, in either conjugated (as glucuronides and sulfates, principally) or unconjugated form, are excreted in the urine of mammalians and enter the aquatic environment via wastewater treatment plant (WWTP) effluents or untreated discharges.

Estrogens have been detected by a variety of analytical procedures in influents and effluents of WWTPs and in surface water, groundwater, and drinking water [9–16]. The complexity of the environmental matrices and the very low environmental concentrations require the use of highly sensitive and selective methods to monitor these EDC compounds in water. Although a number of approaches for the detection of estrogens have already been published, their determination is still a difficult analytical task. New requirements in the field of water policy by the European Community Water Framework Directive have become of scientific interest and a new challenge in the analytical world [17]. According to the EC Decision 2015/495, estrogen compounds such as E1, E2, and EE2 are included in a novel Watch list of emerging pollutants, among other substances for monitoring in the European Union [18]. The maximum acceptable method detection limits (MDLs) required for EE2 and E1/E2 are 0.035 and 0.4 ng/L, respectively. However, achieving such low MDL in real environmental samples (i.e., surface water) is an extremely difficult achievement that requires either large volume off-line solid-phase extraction (SPE) or specifically optimized online approach combined with high sensitivity detection using the last generation mass spectrometry (MS/MS) instruments [19]. While several authors reported MDLs for E1 and E2 in the range of the required 0.4 ng/L [20, 21], none of the methods described in the literature achieve detection of EE2 at the required 0.035 ng/L making the online approach particularly suitable and necessary for such a demanding task.

Nowadays, the technique of choice for the analysis of this group of compounds of different polarities in complex matrices is liquid chromatography-tandem mass spectrometry (LC-MS/MS), as less demanding method than gas chromatography (GC-MS), since it does not require an additional step such as derivatization or hydrolysis, which may cause losing information about hormone conjugates (e.g., sulfate and glucuronide) [22]. According to published papers, estrogen glucuronides and sulfates have received less attention, because of their low estrogenic potency, and only a few papers took into account their environmental distribution and behavior [23–28]. However, their monitoring is especially relevant for WWTP samples, where deconjugation may occur resulting in an increase of free form [5, 23, 29, 30]. Many reported methods are based on off-line SPE [31–34], which makes them time-consuming and requires a large volume of samples (250–1000 mL) in order to achieve low MDLs. Other drawbacks of the off-line SPE procedures are that they often need many steps before reaching an extract concentration suitable for instrumental analysis, of which only a small portion is actually injected onto the chromatographic column. In recent years, labor-saving and cost-effective online SPE has become more popular as a sample preparation technique for water analysis due to the small amounts of solvents necessary and low sample volume required (mL range), as well as a remarkable decrease in the total analysis time and improvement of precision, accuracy, and sensitivity [21, 35–39]. Furthermore, the additional sample contamination and analyte losses, which may occur during the off-line SPE sample pretreatment, are avoided. Although currently available techniques could generally yield MDLs in the subnanogram per liter range, further improvements are demanded and the applicability of the proposed methodologies must be tested on real water samples. MDLs and method quantification limits (MQLs) were estimated with standard solutions and rarely in real matrices, so they were probably often underestimated [40]. It should be noted that in some cases, the MQLs reported [41, 42] were calculated by statistical extrapolations, and estrogen concentrations are quantified up to four orders of magnitude lower than the investigated linearity range [37].

In this context, the purpose of this work was to develop and validate a completely automated, reliable, and cost-effective method based on online SPE and ultra-high-performance-liquid chromatography mass spectrometry (UHPLC-MS/MS) for the determination of the most active and environmentally relevant estrogens at below the nanogram per liter levels in water thus complying with the required maximum acceptable MDLs set up in EC Decision 2015/495 [18]. Target compounds included the natural estrogen E2, its main metabolites E1 and E3, the synthetic estrogens EE2 and diethylstilbestrol (DES), and the conjugates estrone 3-sulfate (E1-3S), estriol 3-sulfate (E3-3S), estradiol 17-glucuronide (E2-17G), estrone 3-glucuronide (E1-3G), and estriol 16-glucuronide (E3-16G). The method was validated in real water matrices, such as raw wastewater and river water receiving municipal and industrial discharges.

Material and methods

Chemicals and materials

All pure standards of the target estrogens E2, E1, E3, EE2, DES, E1-3S, E3-3S, E2-17G, E1-3G, and E3-16G were purchased from Sigma-Aldrich (Germany). Isotopically labeled compounds, used as internal standards (IS), were E1-d_4, E2-d_2, EE2-d_4, and E1-3S-d_4 obtained from CDN Isotopes Pointe-Claire, Quebec, Canada. The chemical structures and physicochemical properties of target-free and conjugated estrogens are shown in the Electronic Supplementary Material (ESM) in Table S1. Both individual stock standard and isotopically labeled internal standard solutions were prepared on a

weight basis in methanol at a concentration of 1000 mg/L. After preparation, standards were stored at −20 °C. Working standard solutions, containing all estrogens, were prepared in water and were renewed before each analytical run by mixing appropriate amounts of the intermediate solutions. The standard mixtures were used as spiking solutions for the preparation of the standard curve and in the recovery study. Separate mixtures of isotopically labeled internal standards were obtained in methanol and further dilutions in water for online analysis. Samples for off-line analysis were prepared in methanol:water (10:90) corresponding to the initial conditions of the chromatographic run. Methanol and water were of HPLC grade purity from Fisher Scientific (Whitby, ON, Canada). Ammonium and sodium hydroxide were obtained from Scharlab SL (Barcelona, Spain). Glass microfiber filters GF/F (0.7 μm) were purchased from Whatman (Fairfield, CT, USA) and nylon membrane syringe filters (0.45 μm), used for sample filtration before HPLC analysis, were provided from Sigma-Aldrich (Germany).

Sample collection and preparation

The method was optimized using three types of water matrices, namely river water (Onyar, located in Girona, Catalonia, Spain), WWTP effluent, and influent water (WWTP Quart, Catalonia, Spain). The applicability of the developed method was tested on river (S1–S15) and raw wastewater samples (WW1–WW15) collected at 15 sampling sites in the north of Serbia (see Fig. S1 in the ESM). River water samples were taken from Danube Basin and its major tributaries Tisa and Sava, downstream of the discharges of raw wastewater. Sites affected by the discharges of urban and agricultural wastes were selected along the rivers as sampling points. Raw wastewater samples were taken directly from urban sewerage systems before discharge into the receiving rivers without any treatment. Information regarding the sampling sites is provided in the ESM Table S2. All samples were collected as grab samples in prewashed amber glass bottles and transported back to the laboratory under cooled conditions (4 °C) and immediately frozen at −20 °C until analysis. Before analysis, water samples were defrosted and filtered through 0.7-μm glass microfiber filter. Water samples were pH adjusted to 11 with sodium hydroxide and were filtered through 0.45-μm nylon membrane syringe filters. Finally, samples were placed in amber glass SPE vials (10 mL), and prior to HPLC analysis, a mix of IS (E1-d_4, E2-d_2, EE2-d_4, and E1-3S-d_4) was added to achieve a final concentration of 50 ng/L.

Analytical method

Chromatographic analysis was performed using an automated online preconcentration system EQuan™ coupled to a TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific) equipped with an electrospray (ESI) source. The base of the EQuan™ system is a high-end HPLC system with a high-performance injector that handles sample volumes from 10 μL up to 5 mL fully automated. The system consists of a PAL autosampler (CTC Analysis) and two quaternary pumps: a loading pump (Accela™ 600 pump) and an elution pump (Accela™ 1250 pump) both from Thermo Fisher Scientific, and a three-valve switching device unit with a six-port valve. A divert valve was programmed to control loading and elution of the two LC columns. The first column was used for preconcentration of the sample (20 × 2.1 mm, 12-μm particle size Hypersil GOLD aQ, Thermo Fisher Scientific) and the C18 reversed-phase column for chromatographic separation (50 × 2.1 mm, 1.7-μm particle size Kinetex, C18, Phenomenex) (see Fig. S2 in the ESM which shows the different valve positions of the online system during analysis).

UHPLC-MS/MS analysis conditions

After testing different volumes (1 to 5 mL), the injection volume was set to 5 mL for all types of matrices. The flow rate through the loading column was 1.75 mL/min during the charge step. In the transfer step, the flow rate was set at 0.5 mL/min, while during the analysis time, the flow rate through the eluting column was 0.4 mL/min. After the charging step, the six-port switching valve was activated and the analytes were transferred from the preconcentration column to the analytical column. When the transfer was finished, the switching valve was activated and the analytes were separated. Simultaneously, the preconcentration column was rinsed and conditioned. Chromatographic separation was performed under gradient elution conditions using methanol and water. The initial conditions were 50% methanol and they were held during 3.5 min. Then, the gradient was linearly increased to 70% methanol during 1 min. Afterwards, the gradient was linearly increased to 100% methanol in 3 min and kept isocratic for 1 min. Finally, at the end of the run, the initial conditions were reached. The duration of the whole analytical procedure was 10.5 min and all the steps were performed automatically. The overall chromatography conditions are shown in Table 1.

Off-line mode was only used in the optimization procedure to optimize chromatographic separation and to assess the relative recovery of the online extraction. Relative recovery was calculated by comparing the peak areas obtained in the online analyses of spiked HPLC samples with those obtained from the injection of standard mixture in off-line mode. The optimized conditions for the off-line analysis were also obtained using methanol and water as a mobile phase. The elution gradient was as follows: 10% methanol held isocratically during the first 2.5 min, linear increase to 100% methanol in 2.5 min, isocratic elution at 100% methanol during the next

Table 1 The optimized LC-LC conditions

Valve position	Start time (min)	Pump 1: load pump					Pump 2: elute pump				
		Injection volume: river, wastewater: 5 mL									
		Preconcentration column: 20 × 2.1 mm, 12 μm Hypersil GOLD aQ Accela 600 pump					Analytical column: 50 × 2.1 mm, 1.7 μm Kinetex, C18 Accela 1250 pump				
		Solvent A: methanol					Solvent A: methanol				
		Solvent C: water					Solvent D: water				
		Flow (mL/min)	Gradient	A%	C%	Description	Flow (mL/min)	Gradient	A%	D%	Description
Load	0:00	1.75	Step	2	98	Sample loading into the EQuan™ column	0.4	Step	50	50	Analytical column conditioning
Load	3:25	0.5	Ramp	2	98	Sample loading into the EQuan™ column	0.4	Step	50	50	Analytical column conditioning
Inject	3:50	0.5	Ramp	50	50	Analyte transfer to the analytical column	0.4	Ramp	50	50	Analyte transfer to the analytical column
Load	4:50	1	Ramp	100	0	EQuan™ column cleaning	0.4	Ramp	70	30	LC separation
Load	7:50	1	Ramp	100	0	EQuan™ column cleaning	0.4	Ramp	100	0	LC separation
Load	8:50	0.5	Ramp	100	50	EQuan™ column cleaning	0.4	Ramp	100	0	Analytical column cleaning
Load	9:50	1	Ramp	2	98	EQuan™ column conditioning	0.4	Ramp	50	50	Analytical column conditioning
Load	10:50	0.5	Step	2	98	EQuan™ column conditioning	0.4	Ramp	50	50	Analytical column conditioning

1.5 min, and return to the initial conditions at 6 min (see Table S3 in the ESM). The total elution time of the method was 6 min.

Mass spectrometry

The detection was carried out on a TSQ Vantage triple quadrupole (QqQ) mass spectrometer (Thermo Fisher Scientific), equipped with an ESI turbo spray ionization source. The optimized parameters which showed higher response for the majority of the compounds were as follows: spray voltage 2500 V, sheath gas pressure 40 (N_2), auxiliary gas pressure 20 (N_2), ion sweep gas pressure 0.5 (N_2), and with capillary and vaporizer temperature set at 300/350 °C, respectively. Natural and synthetic estrogens and conjugates were separated under negative ionization (NI) conditions as $[M-H]^-$. The cycle time was adjusted to 0.250 s, giving a minimum of 12 points per peak, and the first and third quadrupole (Q1 and Q3) were operated at unit resolution (0.7 Da FWHM) and with the second quadrupole (Q2) collision gas pressure set at 1.2 mTorr.

Quantitative analysis was performed using selected reaction monitoring (SRM) mode, for each compound, and the two most abundant transitions were monitored: the most intense one used for quantification and the other for confirmation of the chemical identity. In order to maximize sensitivity and peak reproducibility, data acquisition was performed under time-scheduled conditions. The optimized MS/MS parameters and time-scheduled conditions for SRM analysis of individual target compounds are shown in Table 2. The entire system was controlled via Xcalibur 2.2 software and data were processed using TraceFinder 3.1 (Thermo Fisher Scientific).

Method validation

The method was validated by using HPLC water and three environmental matrices, spiked with a standard mixture of target analytes and IS, which were added prior to analysis. Each of the experimental conditions evaluated was run in triplicate.

The relative recovery (RE) for the online method was evaluated by spiking HPLC grade water, confirming the efficiency of the analyte transfer to the online system. In order to allow the comparison, the same mass of mix standard of analytes was injected in the off-line and online system (0.5 ng injected on-column) [43]. The peak area ratio of the selected estrogen of a direct injection (20 μL) was compared with those of the online volume injection (5 mL). RE was calculated by using following equation:

Table 2 The optimized MS/MS parameters for SRM analysis of individual target compounds in NI ionization mode

Abbreviation	Name	Internal standards[a] (IS)	Start-stop time (min)[b]	Precursor ion (*m*/*z*)	S-lens (Hz)	SRM1[c] (*m*/*z*)	Collision energy (eV)	SRM2[d] (*m*/*z*)	Collision energy (eV)
Natural and synthetic estrogens and conjugates									
E2	Estradiol	Estradiol-d_2	5.00–7.00	271	105	183	40	145	41
E1	Estrone	Estrone-d_4	5.00–7.00	269	121	145	41	143	57
E3	Estriol	Estrone-d_4	3.50–5.50	287	117	171	38	145	43
EE2	Ethinylestradiol	Ethinylestradiol-d_4	5.00–7.00	295	129	145	43	143	55
DES	Diethylstilbestrol	Estrone-d_4	5.00–7.00	267	92	251	26	237	29
E1-3S	Estrone 3-sulfate	Estrone-d_4 sulfate	2.75–4.75	349	111	269	33	145	55
E3-3S	Estriol 3-sulfate	Estrone-d_4 sulfate	2.75–4.75	367	110	287	35	171	53
E2-17G	Estradiol 17-glucuronide	Estrone-d_4 sulfate	2.75–4.75	447	103	271	30	325	20
E1-3G	Estrone 3-glucuronide	Estrone-d_4 sulfate	2.75–4.75	445	100	269	40	113	22
E3-16G	Estriol 16-glucuronide	Estrone-d_4 sulfate	2.75–4.75	463	126	287	32	113	29
Internal standards									
E2-d2	Estradiol-d_2		5.00–7.00	273	106	185	43	147	44
E1-d4	Estrone-d_4		5.00–7.00	273	101	147	42	145	40
EE2-d4	Ethinylestradiol-d_4		5.00–7.00	299	160	161	38	147	41
E1-S-d4	Estrone-d_4 sulfate		2.75–4.75	349	110	269	34	143	50

SRM selected reaction monitoring

[a] Internal standard applied for the identification and quantification

[b] Time-scheduled conditions

[c] SRM1: for quantification

[d] SRM2: for confirmation

$$RE(\%) = \left[\frac{(A_{\text{online}}/AIS_{\text{online}})}{(A_{\text{off-line}}/AIS_{\text{off-line}})}\right] *100\%$$

Where $A_{\text{online}}/AIS_{\text{online}}$ is the peak area ratio of analyte to internal standard (IS) measured online, while $A_{\text{off-line}}/AIS_{\text{off-line}}$ is the peak area ratio of analyte to IS measured off-line. Relative recoveries are indicated in Fig. S3 in the ESM. In general, most of the target compounds were recovered at more than 80% efficiency. Calculated standard deviation of all compounds was low, indicating a good precision of the online extraction.

Throughout the recovery evaluation, wastewater influent and effluent were spiked in triplicate at 50 ng/L and surface water at 5 ng/L (considering as much cleaner matrix). Concentrations obtained after online SPE procedure, calculated by internal standard calibration, were compared by the theoretical initial spiking levels. All the values shown in the "Results and discussion" section represent the mean of the triplicate measurements. Along the analysis, quality control standards (QCs) were injected in order to ensure a good performance of the analysis. QCs were daily prepared in two levels 5 and 50 ng/L with a mixture of target analytes and IS and were measured after every 10 injections during the analysis. Intraday and interday precision were determined from six repeated injections of a 5-ng/L standard mixture during the same day (repeatability) and in four successive days (reproducibility).

For quantification of the analytes, a calibration curve was constructed on the basis of seven points in the concentration range of 0.5–100 ng/L, whereas the corresponding IS was added at 50 ng/L. The IS were used to correct matrix effects and recovery of extraction. In the absence of appropriate isotopically labeled analogues, for some of the compounds, quantification was performed with the closely eluting IS (see Table 2). According to the Commission Decision 2002/657/CE, for positive identification, the following criteria were adopted: (1) LC chromatographic retention time agreement within 2%; and (2) relative abundance of the two transitions, selected as precursor ion and product ion, fall within a range ±20% [44]. Identification of the target analytes was accomplished by comparing the retention time and UHPLC-MS/MS signals of the target compounds in the samples with those of standards analyzed under the same conditions. Complete elution of analytes and absence of carryover were checked injecting blank samples after each batch of samples. Throughout the whole determination procedure, contamination of blanks (water and methanol) was never observed.

Results and discussion

Optimization of online procedure and LC-LC operational parameters

Optimization of the online procedure was done by a series of tests to achieve the optimum extraction performance. Throughout the study, the following key parameters have been tested as essential in the development of an online procedure: sample loading flow rate, elution time, the percentage of organic solvent in the aqueous-organic eluent mixture, pH, and volume of analyzed samples.

A comparative study employing three types of online columns with different chemical modifications (i.e., Hypersil GOLD™ aQ, Hypersil GOLD™ PEP, Hypersil GOLD™ PFP) was previously reported by Gorga et al. [21] for the analysis of endocrine disruptors including some natural and synthetic estrogens. In our approach, the Hypersil GOLD™ aQ provided good extraction recoveries for most of the analytes, including the E3 as the least hydrophobic one.

Different loading flow rates from the injection loop to the SPE column can cause great differences in the preconcentration efficiency, making its optimization a necessary step. After testing loading flow rates in the range from 1000 to 3000 μL/min, results showed that the preconcentration efficiency was increasing when flow rate was faster than 1000 μL/min because the free hormones were strongly retained in the cartridge, especially the less polar ones (i.e., E2, E1, EE2, and DES). However, an additional increase of flow rate produced losses in the chromatographic area response for E2, EE2, and DES (see Fig. S4 in the ESM). A similar behavior was observed for all conjugated estrogens. For the compound that elutes first in the chromatographic analysis (i.e., E3), a slight initial increase was observed by increasing flow rate to 1750 μL/min, but considerable analyte losses of this compound were observed at higher flow rates. Since conjugated forms of estrogens are less demanding compounds generally yielding the lowest MDL values, the negative impact of peak area response was considered acceptable. Therefore, a flow rate of 1750 μL/min was selected as a compromise in order to decrease sample loading time while allowing better interactions between the analytes in the water samples and the surface of the sorbent, giving the highest analyte peak response for the analyzed compounds.

The elution time is determined as the time required for target compounds to be completely eluted from the SPE column to the analytical column. A short elution time may lead to incomplete elution, while a long elution time may be time-consuming and increase the possibility of eluting the sample matrix [38]. In our study, chromatographic separation was performed under gradient elution conditions using methanol and water and the effect of elution time was checked for values of 5.25–6.75 min. As shown in Fig. S5 in the ESM, the chromatographic peak areas of estrogens proportionally increased with the elution time, with the effect being relevant especially for the less polar compounds which are strongly retained in the SPE column. Nevertheless, too slow elution from SPE preconcentration column results in peak broadening, which may cause a decrease in sensitivity [45]. Therefore, after running the experiments applying different elution times, the optimal elution time of 5.5 min was selected accordingly, whereupon all analyzed analytes could be eluted from the SPE column.

The mobile phase in the elution step was optimized to ensure the absolute desorption of the all analyzed estrogens and to obtain baseline separation of the analytes. The influence of methanol percentage ranging from 10 to 75% on chromatographic response has been investigated showing the best chromatographic response at methanol percentage of 50%. The additional increase in the methanol percentage up to 75% showed lower values in the mean chromatographic area for EE2, E2, E3, DES, and E1. In contrast, a different trend of chromatographic response was only observed for E3-3S. Similar results by using a higher ratio of methanol have been reported by Wang et al. [36] and Ciofi et al. [37] indicating that some of the estrogens such as E2, E1, and DES could not be baseline separated.

Influence of sample pH on the adsorption efficiency

Sample pH is reported as an important parameter that may influence greatly the extraction efficiency of the SPE procedure [36, 46, 47]. A majority of target analytes, mostly free estrogen compounds, have relatively high p*K*a vales (9–10) (see ESM Table S1) [48], so basic conditions should favor obtaining better results in the extraction process. Studies of Guo et al. [38] and Iparraguirre et al. [49] have shown that the addition of ammonia and basic conditions in mobile phases in ESI result in an improved ionization efficiency and consequently lower limits of detection of E1, E2, and EE2. Our preliminary results showed that using ammonia as modifier in the mobile phase yielded poor peak shape and overall not satisfactory results. However, increasing pH of the sample resulted in a remarkable improvement in terms of higher chromatographic response and signal to noise (S/N) (see Fig. S6 in the ESM). We compared both sodium and ammonium hydroxide to adjust sample pH, in the range between pH 8 and 11, and the first one provided better recoveries and response for the vast majority of estrogens analyzed. The results of the comparative study of different pH values of the samples are summarized in Fig. 1. As shown, an increase in pH to 11 resulted in the best ion response (peak area) for compounds requiring extremely low MDLs, i.e., E1, E2, and EE2.

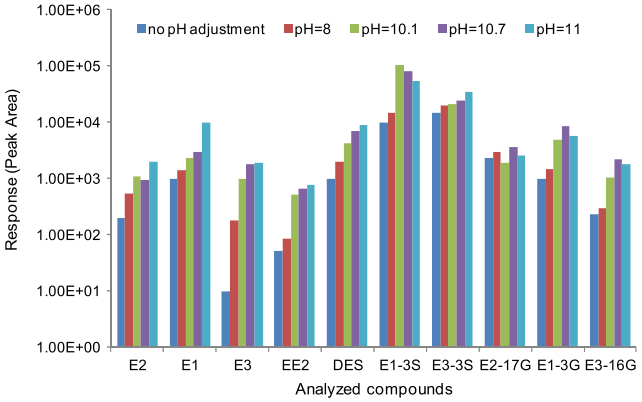

Fig. 1 The response comparison of 5 ng/L in HPLC water before and after pH adjustment

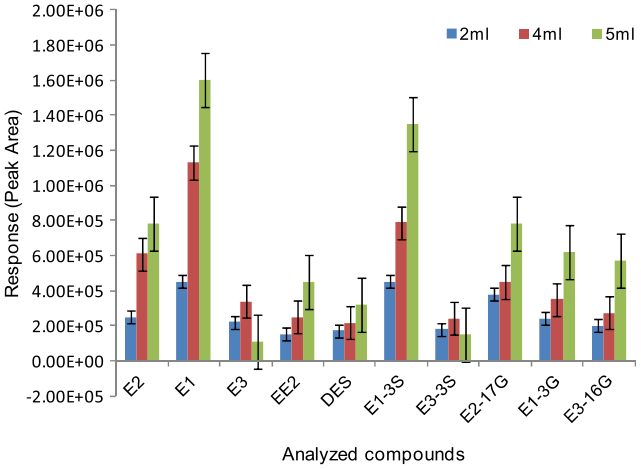

Fig. 2 Injection of different sample volumes (2, 4, and 5 mL) using HPLC water spiked at 5 ng/L ($n = 3$)

However, there was a concern that such a high pH may potentially affect the performance of the column, either in terms of extraction efficiency or in terms of chromatographic response. Also, there was an issue of premature aging of the column that may cause the pressure buildup and ultimate clogging when the online SPE column is coupled with an analytical column [50]. Thus, the column lifetime was considered as an important parameter and special attention was paid to evaluate the potential negative effect of using such high pH. However, no negative effect occurred, and it is worth mentioning that throughout all the optimization study, the same SPE column was used, and no apparent increase of column pressure, no remarkable changes on peak shapes, and decrease of preconcentration efficiency were observed, even after thousands of injections. Thus, the pH of the samples was adjusted to pH 11 for further analysis.

Sample volume

In order to evaluate the influence of the loading volume on the method detection limits, three different volumes (2, 4, and 5 mL) were tested. Both peak areas and the S/N response of analytes were monitored as a function of injection volumes. Results shown in Fig. 2 indicate that the peak areas for eight of ten analytes increased nearly proportionally with the introduction of higher sample volumes (5 mL), meaning that higher volumes are beneficial and needed to obtain low MDLs. Nevertheless, it was noted that the introduction of a larger volume of the sample caused an increase of unwanted interfering compounds, due to the presence of co-extracted substances in the environmental water that may differently affect the signal variability of each analyte. A strong impact of the interferences was observed for E3 and DES, whose peaks were accompanied by another peak caused by the presence of interferences, thus making the quantification of these compounds difficult (see Fig. S7 in the ESM). However, in this case, careful selection of retention time windows improved S/N and facilitated the quantification. Data acquisition performed under scheduled SRM showed that interferences were nearly eliminated and applied conditions show higher S/N ratios for the analyzed estrogens. Still, with increasing sample volumes, notable losses were observed for E3 and E3-3S, as previously observed by Naldi et al. [51]. Hence, a trade-off between higher sensitivity for all analytes and somehow higher MDLs for E3 and E3-3S was made.

Method performance evaluation

The matrix effect is a common problem in UHPLC-MS/MS, especially when using an ESI, which can have a significant impact on analyte signal and can influence the reproducibility, linearity, and accuracy of the method. It should be specifically evaluated, since it is strongly dependent on the sample type and is unpredictable [52].

Herein, the effects of sample matrix interferences on the online extraction were evaluated together with the matrix effects on efficiency of ionization and estimated as process efficiency (PE). As reported by other authors, process efficiency represents the combination between the matrix effect and the recovery of the sample [53–55]. PE was calculated by comparing the mean peak areas for target compounds in standard solution, with the mean peak area of the matrix spiked at the same concentration of the standard. The following equation was applied in the three evaluated matrices (i.e., river water, effluent, and influent):

$$\mathrm{PE}\ (\%) = \left(\frac{A_{\text{spiked matrix}} - A_{\text{unspiked matrix}}}{A_{\text{standard}}}\right) * 100$$

Where $A_{\text{spiked matrix}}$ is the average peak area of the analyte measured in the spiked sample matrix ($n = 3$), $A_{\text{unspiked matrix}}$ is the average peak area of the unspiked sample matrix ($n = 3$), and A_{standard} represents the average peak area of the standard solution spiked in HPLC grade water at the same

concentration of the standard (n = 3). Target compounds for PE evaluation were spiked at 5 ng/L (Fig. 3).

The values lower or greater than 100% indicate signal suppression or enhancement in the samples, respectively. As shown in Fig. 3, the results for river water evidenced high signal suppression for conjugated estrogens, while the signal enhancement was observed for the majority of the free estrogens. In more complex matrices, such as WWTP effluent and especially in WWTP influent, all compounds showed consequential ion suppression. As reported previously [56], signal suppression is strongly dependent on the amount of dissolved organic carbon (DOC), such as surfactants, volatile compounds, and co-eluting analytes present in the matrix [37]. Therefore, it is of crucial importance to account for the matrix effect in the analyte quantification. Herein, we used deuterated standards to overcome the matrix effect. Figure 3 shows that matrix components in river water, effluent, and influent wastewater samples had no considerable effect on signal responses of the target compounds after internal standard corrections since these labeled compounds have similar suppression/enhancement as the target compounds. This proves that using appropriate deuterated internal standards helps to overcome the matrix effect and to reliably determine estrogens at environmentally relevant concentrations.

The method performance was evaluated through estimation of the linearity, sensitivity (by calculating instrumental detection limits (IDL), MDL, and MQL), intraday (repeatability), and interday precision (reproducibility). Results for analytical method validation parameters obtained in HPLC water are summarized in Table 3, whereas Table 4 shows the parameters determined for each matrix (surface, effluent, and influent).

The chromatographic response demonstrated good linearity in the range from 0.5 to 100 ng/L, with excellent determination coefficients (r^2) of linear regressions greater than 0.9990 for all analyzed compounds. Excellent recoveries were obtained after spiking HPLC water in triplicate at 5 ng/L, with values over 87% and precision calculated as relative standard deviation (RSD, %) with not higher than 5%. IDL were calculated as S/N = 3 for the lowest point of the calibration curve constructed in HPLC grade water. Obtained IDL values ranged from 0.01 to 0.36 pg injected. These IDL values are in a similar range to those previously reported by Gorga et al. [21]. Repeatability and reproducibility were also acceptable showing %RSD values below 7.8 and 18.1%, respectively. The majority of obtained values fall below 7%, therefore evidencing the good precision of the method (Table 3).

MDLs and MQLs were experimentally estimated as the minimum detectable amount of analyte with a S/N of 3 and 10, respectively, in different matrices tested (Table 4). As mentioned before, the main analytical challenge in the analysis of hormones in environmental samples is the need to detect below nanogram per liter levels. Consequently, the excellent robustness and the capability to detect such low concentrations are essential in the analysis of estrogen compounds in real water samples. The MDLs and MQLs were matrix dependent with values ranging from 0.02 to 0.07 ng/L and 0.07 to 0.22 ng/L in river water, from 0.02 to 0.12 ng/L and 0.08 to 0.97 ng/L in WWTP effluent, from 0.03 to 0.3 ng/L and 0.10 to 1 ng/L in WWTP influent, respectively. It should be highlighted that the obtained MDL values obtained for river water comply with the required maximum acceptable method detection limit for surface waters (0.035 ng/L for EE2 and 0.4 ng/L for E1 and E2) set in the Watch list of the European Commission [18]. In river water, WWTP influent and effluent satisfactory recoveries with values over 73% and below 120% were obtained for the majority of compounds. The overall method precision, calculated as RSD (%), was also satisfactory and ranged from 1 to 15% for all compounds and matrices tested, showing higher RSD values for most compounds in wastewater (Table 4). The online SRM chromatogram obtained for all the compounds spiked at 5 ng/L in a real river water sample is shown in the ESM in Fig. S8.

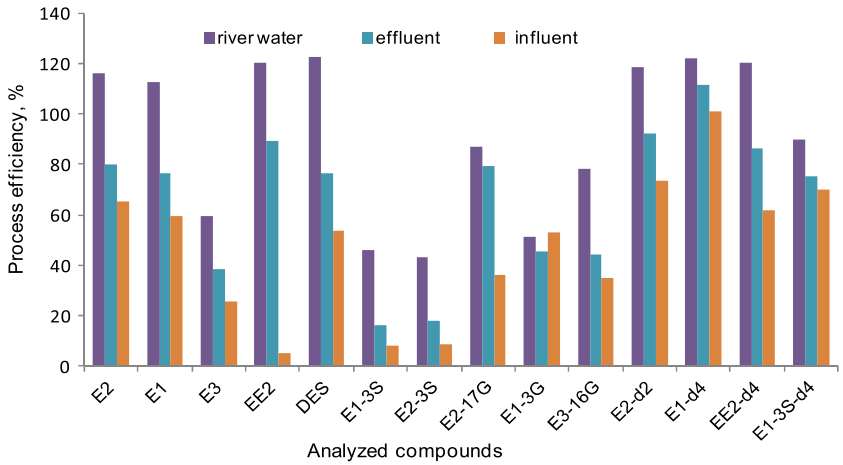

Fig. 3 Process efficiency observed in river water, WWTP effluent, and WWTP influent spiked at 5 ng/L (n = 3)

Table 3 Analytical method validation parameters: linearity, recovery, and precision obtained for target compounds in HPLC water; instrumental detection limits (IDLs); repeatability (intraday); and reproducibility (interday)

Compound	Linearity	Recovery, % (5 ng/L, $n = 3$)	(±RSD %)	IDL (pg)	Intraday (5 ng/L) (%RSD, $n = 6$)	Interday (5 ng/L) (%RSD, $n = 4$)
E2	0.9998	100.9	(±1.2)	0.240	1.4	2.1
E1	0.9999	102.3	(±2.0)	0.069	3.0	6.3
E3	0.9992	98.1	(±1.5)	0.285	2.0	10.4
EE2	0.9997	97.6	(±4.2)	0.141	4.9	7.2
DES	0.9990	95.9	(±2.5)	0.360	2.8	18.1
E1-3S	0.9998	107.5	(±4.4)	0.011	7.8	5.3
E3-3S	0.9995	94.0	(±2.9)	0.009	4.5	14.2
E2-17G	0.9996	97.6	(±2.1)	0.135	4.2	10.7
E1-3G	0.9999	98.2	(±3.2)	0.068	7.5	15.3
E3-16G	0.9994	87.6	(±4.0)	0.145	5.6	14.0

Comparison with previously published online SPE-UHPLC methods

The online SPE-UHPLC-MS/MS proposed herein for estrogen analysis in surface and wastewater was compared to the ones previously reported in the literature (Table 5). To the best of our knowledge, the lowest MDLs currently reported for estrogens are obtained by Gorga et al. [21], who investigated estrogens together with other related EDCs. Using a sample volume of 5 mL, the authors achieved MDLs for E1, E2, and EE2 of 0.05, 0.037, and 0.14 ng/L, respectively, for surface water. In the case of wastewater, injection volume was set at 2 mL and MDLs for compounds E1, E2, and EE2 of 0.14, 0.59, and 3.8 ng/L and 0.14, 5.4, and 4.2 ng/L were obtained for effluent and influent samples, respectively. Other authors also reported MDLs lower than the nanogram per liter level. For example, Guo et al. [38] developed a simple and suitable procedure for the simultaneous analysis of estrogen and androgen compounds in water samples by using 50 mL of sample reaching MDLs in the range of 0.5–2, 0.5–1, and 0.5–2 ng/L, respectively, for surface water, WWTP effluent, and influent. All other online methods reported much higher MDLs [37, 43, 51, 57].

By adjusting the pH of the sample to 11 and by optimizing all operational parameters, we have succeeded to obtain a robust method yielding noticeable lower MDLs complying therefore with the requirements of the EC Decision 2015/495 [18]. Figure 4 shows one of the online chromatograms obtained at 5 ng/L for E2, E1, and EE2 in surface water sample. As seen, the S/N depends on the compound and matrix complexity, but still high enough to meet the required criteria. As mentioned before, two SRM transitions were monitored for each compound. The first transition was used for quantification purposes, whereas the second one was used to confirm the identity of the target compounds, including the isotopically labeled internal standards (see Fig. S9 in the ESM). Furthermore, our method provides quantitative measurements

Table 4 Parameters indicating the performance of the analytical method: recovery and precision obtained for target compounds, method detection limits (MDL), and method quantification limits (MQL) in all matrices studied

	% Recovery (%RSD) ($n = 3$)			MDL (ng/L)			MQL (ng/L)		
	River water (5 ng/L)	Effluent (50 ng/L)	Influent (50 ng/L)	River water	Effluent	Influent	River water	Effluent	Influent
E2	96.6 (±1.0)	106.1 (±6.8)	103.2 (±8.4)	0.041	0.076	0.155	0.136	0.253	0.517
E1	99.7 (±2.2)	98.2 (±6.1)	94.5 (±12.3)	0.030	0.040	0.057	0.100	0.133	0.189
E3	84.3 (±3.1)	80.0 (±5.1)	76.2 (±9.5)	0.063	0.074	0.100	0.210	0.246	0.333
EE2	100.2 (±6.2)	112.3 (±7.5)	112.0 (±8.5)	0.035	0.078	0.260	0.115	0.967	0.867
DES	84.0 (±5.1)	78.5 (±7.5)	75.1 (±15.0)	0.067	0.120	0.300	0.223	0.400	1.000
E1-3S	93.1 (±3.5)	91.3 (±8.8)	83.5 (±13.5)	0.021	0.023	0.035	0.069	0.078	0.115
E3-3S	85.2 (±4.0)	75.5 (±11.1)	73.2 (±14.5)	0.022	0.034	0.031	0.075	0.113	0.102
E2-17G	95.7 (±7.4)	83.4 (±12.5)	75.8 (±14.1)	0.028	0.058	0.068	0.095	0.194	0.228
E1-3G	91.3 (±3.7)	85.2 (±7.3)	79.4 (±10.4)	0.037	0.064	0.099	0.123	0.214	0.331
E3-16G	80.2 (±3.0)	79.5 (±4.5)	73.0 (±12.5)	0.031	0.061	0.057	0.103	0.203	0.189

Table 5 Main characteristics of the analytical method previously published in the literature (detection, volume, IDL, and MDL values) compared with the method proposed herein, using online SPE coupled to UHPLC-MS/MS

Reference	Detection	Volume (mL)	IDL (pg)			MDL (ng/L)								
			Milli-Q water			River water			Effluent			Influent		
			E2	E1	EE2	E2	E1	EE2	E2	E1	EE2	E2	E1	EE2
Present study	ESI(−)/MS	5	0.240	0.069	0.141	0.041	0.030	0.035	0.076	0.040	0.078	0.155	0.057	0.260
Gorga et al. [21]	ESI(−)/MS	5 and 2	0.17	0.23	0.06	0.037	0.050	0.14	0.59	0.14	3.8	5.4	0.14	4.2
Ciofi et al. [37]	ESI(−)/MS	2.5	0.775	0.375	1.3	nr	nr	nr	nr	nr	nr	nr	nr	nr
Guo et al. [38]	ESI(−)/MS	50	25	5	25	1	0.5	0.5	1	0.5	1	0.5	0.5	0.5
Fayad et al. [43]	APCI(+)/MS	10	50	90	130	nr	nr	nr	21	16	18	24	23	21
Naldi et al. [51]	HESI(−)/MS	5 and 1	30.5	65	36	9.5	9.7	25	14	26	62	nr	nr	nr
Vega-Morales et al. [57]	ESI(−)/MS	5	6	6.5	4.5	nr	nr	nr	nr	nr	nr	nr	nr	nr

nr not reported values by the authors

of both free estrogens and conjugates in a single run for each sample.

Method application on real samples

As a part of the validation procedure, the method developed was applied to the analysis of the target analytes in real environmental water samples. The established method was successfully applied to assess the concentration of the estrogens in river water and raw wastewater collected in northern Serbia. The results are summarized in Table 6. Of the ten compounds investigated, eight estrogens were detected in raw wastewater, while six of them were detected in river water. Despite the very low MDL achieved with the proposed methodology, none of synthetic estrogens, such as EE2 and DES, were found in the analyzed samples.

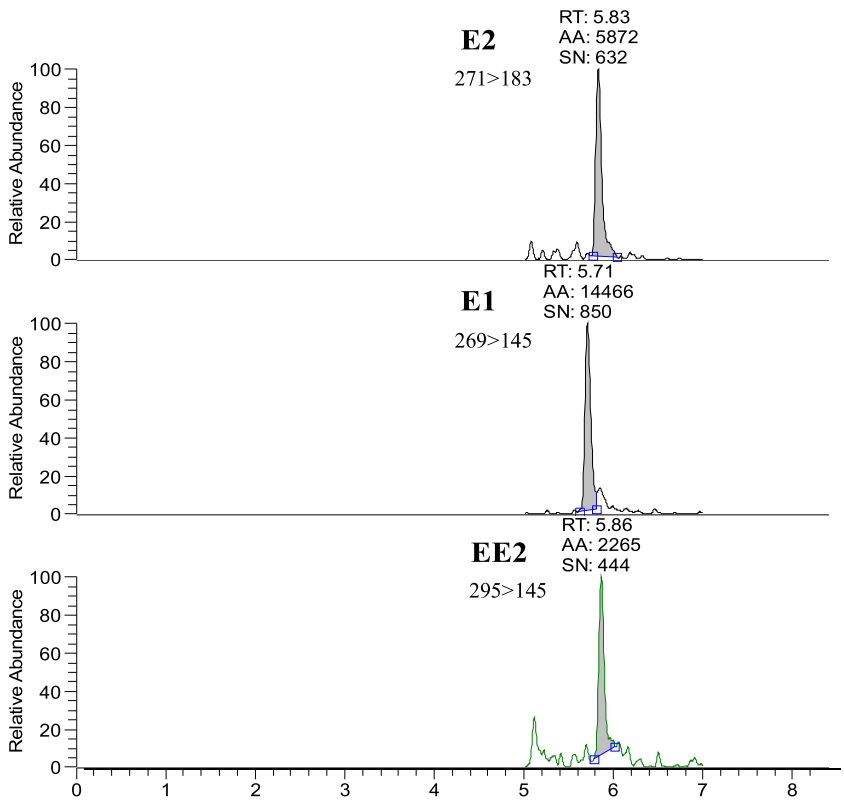

Fig. 4 SRM chromatograms of E2, E1, and EE2 obtained under NI conditions of spiked surface water samples at 5 ng/L

Table 6 Concentrations of estrogens, expressed in nanogram per liter, detected in river water and raw wastewater samples. EE2 and DES were not detected in any sample

Compound		River water (ng/L)	Raw wastewater (ng/L)
E2	Min-max (median)	<MDL	6.05–8.72 (8.48)
	Frequency of detection (%)	–	20
E1	Min-max (median)	0.162–2.29 (0.572)	1.55–3.59 (2.54)
	Frequency of detection (%)	26.7	26.7
E3	Min-max (median)	2.19–4.24 (2.62)	3.71–12.6 (8.38)
	Frequency of detection (%)	40.0	33.3
EE2	Min-max (median)	<MDL	<MDL
	Frequency of detection (%)	–	–
DES	Min-max (median)	<MDL	<MDL
	Frequency of detection (%)	–	–
E1-3S	Min-max (median)	0.072–6.24 (0.112)	2.53–6.81 (4.93)
	Frequency of detection (%)	46.7	80.0
E3-3S	Min-max (median)	0.892–7.79 (1.52)	24.1–44.1 (34.3)
	Frequency of detection (%)	80.0	60.0
E2-17G	Min-max (median)	9.34–17.9 (12.6)	22.1–69.1 (30.2)
	Frequency of detection (%)	53.3	20
E1-3G	Min-max (median)	1.64–3.97 (2.89)	4.16–6.57 (4.43)
	Frequency of detection (%)	46.7	20
E3-16G	Min-max (median)	<MDL	6.66–49.2 (34.1)
	Frequency of detection (%)	–	60

<*MDL* below detection limit

Levels detected in raw wastewater were similar in all analyzed samples and were in the high nanogram per liter range. Although the dilution could be significant in river water, the levels observed in wastewater discharged directly into the river without treatment are of potential concern and may result in river concentrations sufficiently high to induce estrogenic

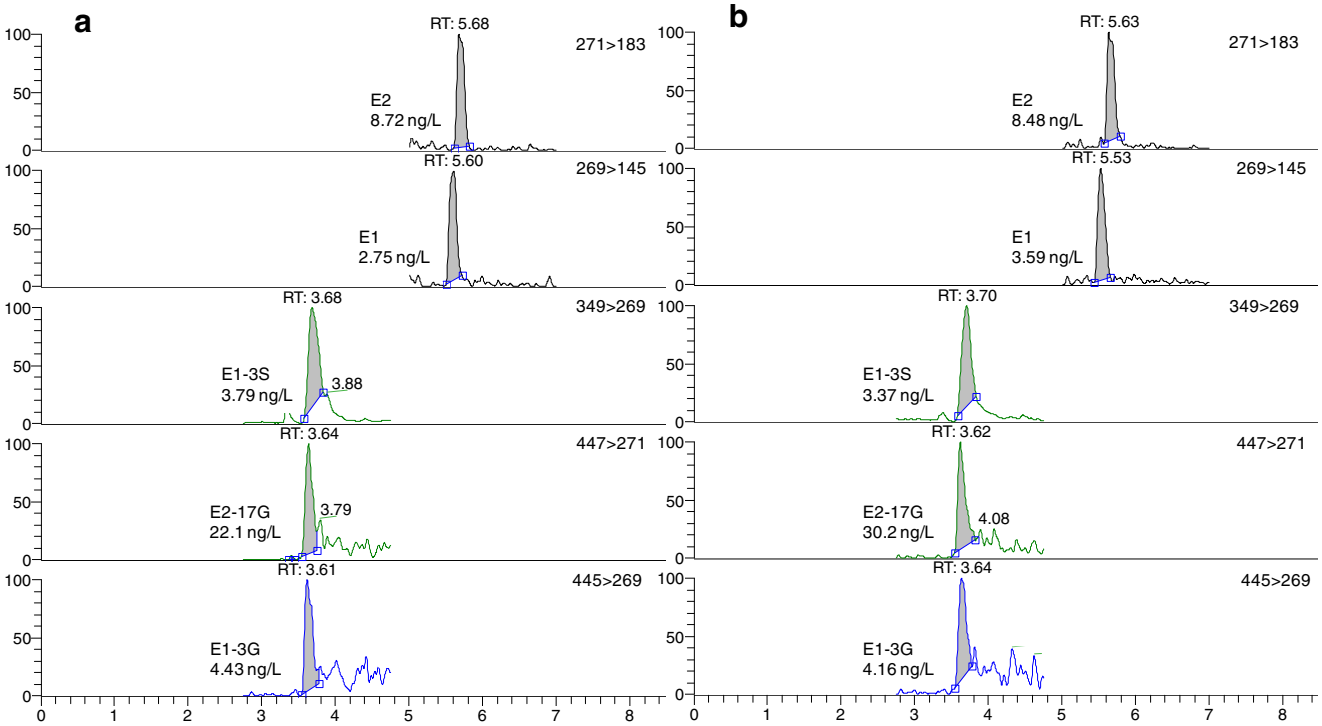

Fig. 5 SRM chromatograms of detected compounds in two analyzed wastewater samples: **a** WW5 and **b** WW15

activity in aquatic species. The mean concentrations of estrogens detected in river water and wastewater samples are summarized and given in the ESM on Tables 4Sa and 4Sb, respectively.

Figure 5 shows the SRM chromatograms obtained in the online analysis of the target analytes under the optimized conditions in two of the analyzed real samples of wastewater. Generally, the levels found in our study are in a similar range as those recently reported by other authors [21, 37, 38, 43]. However, it should be emphasized that this sampling campaign was not designed as an environmental monitoring of the analyzed compounds but only to test the applicability of the developed methodology. Thus, spatial and temporal variability was not taken into account.

Conclusions

A fully automatic online method was developed and validated for the analysis of natural and synthetic estrogens and their conjugates in both river and wastewater samples. By using the tandem mass spectrometry detection instrument, UHPLC-MS/MS has substantially improved the performance of chromatographic methods by reducing MDLs and aiding analyte identification. The integrated system improved analytical performance (analyte reliability and repeatability of the method), increased sample throughput, and reduced operating costs and contamination risks. The methodology also ensures cost efficiency, saves time of analysis, and minimizes errors from manual manipulation. This proposed method represents an improvement in terms of lower detection limits and higher selectivity, compared to the previously published online methods. An effective compromise was achieved by pH adjustment of the samples. The negative ion intensity was remarkably improved when pH was set at basic conditions into the samples, and a remarkable increase in the average recoveries was observed for most of the analyzed compounds. The proposed methodology is suitable to comply with the requirements for monitoring of hormones at the European level reaching low MDL set up in the EC Decision 2015/495. The applicability of the method was demonstrated on real samples by confirming widespread occurrence of free and conjugated estrogens in environmental waters.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Allner B, von der Gönna S, Griebeler EM, Nikutowski N, Weltin A, Stahlschmidt-Allner P. Reproductive functions of wild fish as bioindicators of reproductive toxicants in the aquatic environment. Environ Sci Pollut Res Int. 2010;17(2):505–18.
2. Jobling S, Beresford N, Nolan M, Rodgers-Gray T, Brighty GC, Sumpter JP, Tyler CR. Altered sexual maturation and gamete production in wild roach (*Rutilus rutilus*) living in rivers that receive treated sewage effluents. Biol Reprod. 2002;66(2):272–81.
3. Sumpter JP, Jobling S. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environ Health Perspect. 1995;103(7):173–8.
4. Thorpe KL, Cummings RI, Hutchinson TH, Scholze M, Brighty G, Sumpter JP, Tyler CR. Relative potencies and combination effects of steroidal estrogens in fish. Environ Sci Technol. 2003;37(6):1142–9.
5. Ternes TA, Stumpf M, Mueller J, Haberer K, Wilken RD, Servos M. Behavior and occurrence of estrogens in municipal sewage treatment plants—I. Investigations in Germany, Canada and Brazil. Sci Total Environ. 1999;225(1–2):81–90.
6. Hansen PD, Dizer H, Hock B, Marx A, Sherry J, McMaster M, Blaise C. Vitellogenin a biomarker for endocrine disruptors. TrAC Trends Anal Chem. 1998;17(7):448–51.
7. Larsson DGJ, Adolfsson-Erici M, Parkkonen J, Pettersson M, Berg AH, Olsson PE, Förlin L. Ethynilestradiol an undesired fish conctraceptive? Aquat Toxicol. 1999;45(2–3):91–7.
8. Kim J-H, Lee SY, Lee H-J, Yoon N-Y, Lee W-S. The efficacy and safety of 17α-estradiol (Ell-Cranell® alpha 0.025%) solution on female pattern hair loss: single center, open-label, non-comparative, phase IV study. Ann Dermatol. 2012;24(3):295–305.
9. Kuch HM, Ballschmiter K. Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC–(NCI)–MS in the picogram per liter range. Environ Sci Technol. 2001;35(15):3201–6.
10. Kim SD, Cho J, Kim IS, Vanderford BJ, Snyder SA. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. Water Res. 2007;41(5):1013–21.
11. Matějíček D, Kubáň V. Enhancing sensitivity of liquid chromatographic/ion-trap tandem mass spectrometric determination of estrogens by on-line pre-column derivatization. J Chromatogr A. 2008;1192(2):248–53.
12. Benotti MJ, Trenholm RA, Vanderford BJ, Holady JC, Stanford BD, Snyder SA. Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. Environ Sci Technol. 2009;43(3):597–603.
13. Pedrouzo M, Borrull F, Pocurull E, Marcé RM. Estrogens and their conjugates: determination in water samples by solid-phase extraction and liquid chromatography–tandem mass spectrometry. Talanta. 2009;78(4–5):1327–31.
14. López-Roldán R, de Alda ML, Gros M, Petrovic M, Martín-Alonso J, Barceló D. Advanced monitoring of pharmaceuticals and estrogens in the Llobregat River basin (Spain) by liquid chromatography–triple quadrupole-tandem mass spectrometry in combination with ultra performance liquid chromatography–time of flight-mass spectrometry. Chemosphere. 2010;80(11):1337–44.

15. Sodré FF, Pescara IC, Montagner CC, Jardim WF. Assessing selected estrogens and xenoestrogens in Brazilian surface waters by liquid chromatography–tandem mass spectrometry. Microchem J. 2010;96(1):92–8.
16. Chang H, Wan Y, Wu S, Fan Z, Hu J. Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters: comparison to estrogens. Water Res. 2011;45(2):732–40.
17. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. Off J Eur Commun. L327/1 (2000) 1–73.
18. Commission Implementing Decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council (notified under document C (2015) 1756).
19. Guedes-Alonso R, Montesdeoca-Esponda S, Sosa-Ferrera Z, Santana-Rodríguez JJ. Liquid chromatography methodologies for the determination of steroid hormones in aquatic environmental systems. Trends Environ Anal Chem. 2014;3-4:14–27.
20. Kuster M, López de Alda MJ, Hernando MD, Petrovic M, Martín-Alonso J, Barceló D. Analysis and occurrence of pharmaceuticals, estrogens, progestogens and polar pesticides in sewage treatment plant effluents, river water and drinking water in the Llobregat river basin (Barcelona, Spain). J Hydrol. 2008;358:112.
21. Gorga M, Petrovic M, Barceló D. Multi-residue analytical method for the determination of endocrine disruptors and related compounds in river and waste water using dual column liquid chromatography switching system coupled to mass spectrometry. J Chromatogr A. 2013;1295:57–66.
22. Vega-Morales T, Sosa-Ferrera Z, Santana-Rodríguez JJ. Determination of various estradiol mimicking-compounds in sewage sludge by the combination of microwave-assisted extraction and LC–MS/MS. Talanta. 2011;85(4):1825–34.
23. Belfroid AC, Van der Horst A, Vethaak AD, Schäfer AJ, Rijs GBJ, Wegener J, Cofino WP. Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. Sci Total Environ. 1999;225(1–2):101–8.
24. D'ascenzo G, Di Corcia A, Gentili A, Mancini R, Mastropasqua R, Nazzari M, Samperi R. Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. Sci Total Environ. 2003;302(1–3):199–209.
25. Gentili A, Perret D, Marchese S, Mastropasqua R, Curini R, Di Corcia A. Analysis of free estrogens and their conjugates in sewage and river waters by solid-phase extraction then liquid chromatography-electrospray-tandem mass spectrometry. Chromatographia. 2002;56(1):25–32.
26. Isobe T, Shiraishi H, Yasuda M, Shinoda A, Suzuki H, Morita M. Determination of estrogens and their conjugates in water using solid-phase extraction followed by liquid chromatography-tandem mass spectrometry. J Chromatogr A. 2003;984:195–202.
27. Labadie P, Budzinski H. Determination of steroidal hormone profiles along the Jalle d'Eysines River (near Bordeaux, France). Environ Sci Technol. 2005;39(14):5113–20.
28. Mouatassim-Souali A, Tamisier-Karolak SL, Perdiz D, Cargouet M, Levi Y. Validation of a quantitative assay using GC/MS for trace determination of free and conjugated estrogens in environmental water samples. J Sep Sci. 2003;26(1–2):105–11.
29. Johnson AC, Belfroid A, Di Corcia A. Estimating steroid oestrogen inputs into activated sludge treatment works and observations on their removal from the effluent. Sci Total Environ. 2000;256(2–3):163–73.
30. Baronti C, Curini R, D'Ascenzo G, Di Corcia A, Gentili A, Samperi R. Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. Environ Sci Technol. 2000;34(24):5059–66.
31. Al-Odaini NM, Zakaria MP, Yaziz MI, Surif S. Multi-residue analytical method for human pharmaceuticals and synthetic hormones in river water and sewage effluents by solid-phase extraction and liquid chromatography–tandem mass spectrometry. J Chromatogr A. 2010;1217:6791–806.
32. Huerta-Fontela M, Galceran MT, Ventura F. Fast liquid chromatography–quadrupole-linear ion trap mass spectrometry for the analysis of pharmaceuticals and hormones in water resources. J Chromatogr A. 2010;1217:4212–22.
33. Liu S, Ying G-G, Zhao J-L, Chen F, Yang B, Zhou L-J, Lai H-J. Trace analysis of 28 steroids in surface water, wastewater and sludge samples by rapid resolution liquid chromatography–electrospray ionization tandem mass spectrometry. J Chromatogr A. 2011;1218:1367–78.
34. Tomšíková H, Aufartová J, Solich P, Nováková L, Sosa-Ferrera Z, Santana-Rodríguez JJ, Nováková L. High-sensitivity analysis of female-steroid hormones in environmental samples. Trends Anal Chem. 2012;34:35–58.
35. Viglino L, Aboulfadl K, Prévost M, Sauvé S. Analysis of natural and synthetic estrogenic endocrine disruptors in environmental waters using online preconcentration coupled with LC-APPI-MS/MS. Talanta. 2008;76:1088–96.
36. Wang S, Huang W, Fang G, He J, Zhang Y. On-line coupling of solid-phase extraction to high-performance liquid chromatography for determination of estrogens in environment. Anal Chim Acta. 2008;606(2):194–201.
37. Ciofi L, Fibbi D, Chiuminatto U, Coppini E, Checchini L, Del Bubba M. Fully-automated on-line solid phase extraction coupled to high-performance liquid chromatography–tandem mass spectrometric analysis at sub-ng/L levels of selected estrogens in surface water and wastewater. J Chromatogr A. 2013;1283:53–61.
38. Guo F, Liu Q, Qu G-b, Song S-j, Sun J-t, Shi J-b, Jiang G-b. Simultaneous determination of five estrogens and four androgens in water samples by online solid-phase extraction coupled with high-performance liquid chromatography-tandem mass spectrometry. J Chromatogr A. 2013;1281:9–18.
39. Valsecchi S, Polesello S, Mazzoni M, Rusconi M, Petrovic M. On-line sample extraction and purification for the LC–MS determination of emerging contaminants in environmental samples. Trends Environ Anal Chem. 2015;8:27–37.
40. Miège C, Bados P, Brosse C, Coquery M. Method validation for the analysis of estrogens (including conjugated compounds) in aqueous matrices. TrAC Trends Anal Chem. 2009;28(2):237–44.
41. Qin F, Zhao YY, Sawyer MB, Li XF. Column-switching reversed phase-hydrophilic interaction liquid chromatography/tandem mass spectrometry method for determination of free estrogens and their conjugates in river water. Anal Chim Acta. 2008;627(1):91–8.
42. Vuillet E, Wiest L, Baudot R, Grenier-Loustalot M-F. Multi-residue analysis of steroids at sub-ng/L levels in surface and ground-waters using liquid chromatography coupled to tandem mass spectrometry. J Chromatogr A. 2008;1210(1):84–91.
43. Fayad PB, Prévost M, Sauvé S. On-line solid-phase extraction coupled to liquid chromatography tandem mass spectrometry optimized for the analysis of steroid hormones in urban wastewaters. Talanta. 2013;115:349–60.
44. European Commission. Council Regulation (EEC)-OJEC L 224-Annex 11990.
45. Soriano JM, Jiménez B, Redondo MJ, Moltó JC. Comparison of different sorbents for on-line liquid-solid extraction followed by high-performance liquid chromatographic determination of nitrogen-containing pesticides. J Chromatogr A. 1998;822(1):67–73.
46. Quintana JB, Carpinteiro J, Rodríguez I, Lorenzo RA, Carro AM, Cela R. Determination of natural and synthetic estrogens in water by gas chromatography with mass spectrometric detection. J Chromatogr A. 2004;1024(1–2):177–85.

47. Rodriguez-Mozaz S, Lopez de Alda MJ, Barceló D. Picogram per liter level determination of estrogens in natural waters and waterworks by a fully automated on-line solid-phase extraction-liquid chromatography-electrospray tandem mass spectrometry method. Anal Chem. 2004;76(23):6998–7006.
48. Lewis KM, Archer RD. pK values of estrone, 17β-estradiol and 2-methoxyestrone. Steroids. 1979;34(5):485–99.
49. Iparraguirre A, Navarro P, Rodil R, Prieto A, Olivares M, Etxebarria N, Zuloaga O. Matrix effect during the membrane-assisted solvent extraction coupled to liquid chromatography tandem mass spectrometry for the determination of a variety of endocrine disrupting compounds in wastewater. J Chromatogr A. 2014;1356:163–70.
50. Souverain S, Rudaz S, Veuthey JL. Restricted access materials and large particle supports for on-line sample preparation: an attractive approach for biological fluids analysis. J Chromatogr B. 2004;801(2):141–56.
51. Naldi AC, Fayad PB, Prévost M, Sauvé S. Analysis of steroid hormones and their conjugated forms in water and urine by on-line solid-phase extraction coupled to liquid chromatography tandem mass spectrometry. Chem Cent J. 2016;10:30.
52. Antignac JP, de Wasch K, Monteau F, De Brabander H, Andre F, Le Bizec B. The ion suppression phenomenon in liquid chromatography–mass spectrometry and its consequences in the field of residue analysis. Anal Chim Acta. 2005;529(1–2):129–36.
53. Buhrman DL, Price PI, Rudewiczcor PJ. Quantitation of SR 27417 in human plasma using electrospray liquid chromatography-tandem mass spectrometry: a study of ion suppression. J Am Soc Mass Spectrom. 1996;7(11):1099–105.
54. Trufelli H, Palma P, Famiglini G, Cappiello A. An overview of matrix effects in liquid chromatography–mass spectrometry. Mass Spectrom Rev. 2011;30(3):491–509.
55. Farré MJ, Insa S, Mamo J, Barceló D. Determination of 15 N-nitrosodimethylamine precursors in different water matrices by automated on-line solid-phase extraction ultra-high-performance-liquid chromatography tandem mass spectrometry. J Chromatogr A. 2016;1458:99–111.
56. EPA Method 539 Document No. 815-B-10-001 (2010) Determination of hormones in drinking water by solid phase extraction (SPE) and liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS).
57. Vega-Morales T, Sosa-Ferrera Z, Santana-Rodríguez JJ. Development and optimisation of an on-line solid phase extraction coupled to ultra-high-performance liquid chromatography-tandem mass spectrometry methodology for the simultaneous determination of endocrine disrupting compounds in wastewater samples. J Chromatogr A. 2012;1230:66–76.

Article №2:

Mira Čelić, Biljana Škrbić, Sara Insa, Jelena Živančev, Meritxell Gros, and Mira Petrović

Occurrence and assessment of environmental risks of endocrine disrupting compounds in drinking, surface, and wastewaters in Serbia

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Occurrence and assessment of environmental risks of endocrine disrupting compounds in drinking, surface and wastewaters in Serbia☆

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ABSTRACT

The present study is the first comprehensive monitoring of 13 selected endocrine disrupting compounds (EDCs) in untreated urban and industrial wastewater in Serbia to assess their impact on the Danube River basin and associated freshwaters used as sources for drinking water production in the area. Results showed that natural and synthetic estrogens were present in surface and wastewater at concentrations ranging from 0.1 to 64.8 ng L^{-1}. Nevertheless, they were not detected in drinking water. For alkylphenols concentrations ranged from 1.1 to 78.3 ng L^{-1} in wastewater and from 0.1 to 37.2 ng L^{-1} in surface water, while in drinking water concentrations varied from 0.4 to 7.9 ng L^{-1}. Bisphenol A (BPA) was the most abundant compound in all water types, with frequencies of detection ranging from 57% in drinking water, to 70% in surface and 84% in wastewater. Potential environmental risks were characterized by calculating the risk quotients (RQs) and the estrogenic activity of EDCs in waste, surface and drinking water samples, as an indicator of their potential detrimental effects. RQ values of estrone (E1) and estradiol (E2) were the highest, exceeding the threshold value of 1 in 60% of wastewater samples, while in surface water E1 displayed potential risks in only two samples. Total estrogenic activity (EEQ_t) surpassed the threshold of 1 ng E2 L^{-1} in about 67% of wastewater samples, and in 3 surface water samples. In drinking water, EEQ_t was below 1 ng L^{-1} in all samples.

1. Introduction

Endocrine disrupting compounds (EDCs) are contaminants of major public health concern, since they can interact with the endocrine system and produce adverse effects on human health and wildlife even at low concentrations (Ascenzi et al., 2006). Indeed, several studies already proved that they can exert physiological effects on aquatic organisms at low ng L^{-1} levels (Desbrow et al., 1998; Hansen et al., 1998; Solé et al., 2000; Christiansen et al., 2002; Céspedes et al., 2004). So far, about 435 different compounds were classified by the EU Commission as candidate EDCs and 94 of them as EDCs with evidenced endocrine effects (Okkerman and van der Putte, 2002). Among all EDCs, natural (estradiol (E2), estrone (E1), estriol (E3)) and synthetic hormones (ethinylestradiol (EE2), diethylstilbestrol (DES)), alkylphenols (nonylphenol (NP) and octylphenol (OP)), which are used in many domestic, industrial and agricultural applications, and are formed by degradation of non-ionic surfactants (Ying et al., 2002) and bisphenol A (BPA), used as plasticizer, are the most ubiquitous compounds in the environment (Wenzel et al., 2004; Al-Odaini et al., 2010; Selvaraj et al., 2014; Céspedes et al., 2008; Nurulnadia et al., 2014; Rodriguez-Mozaz et al., 2004). Natural estrogens are predominantly excreted from human and animal bodies in their conjugated forms, as sulfate and glucuronide metabolites, and their existence in wastewater was reported by several researchers (Gentili et al., 2002; D'Ascenzo et al., 2003; Komori et al., 2004; Reddy et al., 2005) and in some cases, at higher

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concentrations than the parent compounds (Kobayashi et al., 2006; Pedrouzo et al., 2009). Conjugated metabolites, which include estrone-3-sulfate (E1-3S), estriol-3-sulfate (E3-3S), estone-3-glucuronide (E1-3G), estradiol-17-glucuronide (E2-17G) and estriol-16-glucuronide (E3-16G), are more water soluble and mobile than the parent compounds, posing an environmental risk once they are hydrolyzed to the biologically active free estrogen form (Liu et al., 2009). Currently, some EDCs are already included in European legislations. Steroid estrogens, such as the synthetic hormone EE2, the natural hormone E2 and its metabolite E1, were included in the first "Watch List" (2015/495/EU) of emerging pollutants to be monitored Europe-wide, and eventually will be included in the list of priority pollutants of the Water Framework Directive (2000/60/EC) if proven to pose a risk. Alkylphenols, such as NP and OP, and the plasticizer BPA, belong to the category 1 of the Endocrine Disrupter Priority List (2008/105/EC) for wildlife and human health. Furthermore, NP and OP are listed among the 33 priority substances in the European Water Framework Directive (2013/39/EU), being NP classified as a priority hazardous substance. In addition, BPA has recently been included in the list of substances under the observation for possible inclusion as priority contaminant in the European Water Framework Directive (2000/60/EC).

Among the heterogeneous group of EDCs, natural and synthetic steroid hormones are considered to have the highest endocrine disrupting potency (Ternes et al., 1999). Compared with other EDCs, their estrogenic potency is 10.000–100.000 times higher than exogenous EDCs (i.e. pesticides, plasticizers, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs)) (Gomes et al., 2003; Hanselman et al., 2003; Falconer, 2006). This makes them the major contributors of estrogenic activities in wastewater and receiving water bodies (de Mes et al., 2005). EDCs are emitted to surface waters by point environmental sources, including treated or untreated wastewater from municipal and industrial origin, pulp mill or livestock activities and by diffuse sources such as urban and agriculture runoff (Heeb et al., 2012; Eggen et al., 2014). Several studies have reported that these substances are partially removed during wastewater treatment processes (Kolpin et al., 2002; Cui et al., 2006; Roslev et al., 2007; Xue et al., 2010; Chang et al., 2011; Vymazal et al., 2015), being finally discharged into surface water, where they have been found at concentrations ranging from sub ng L^{-1} to tens of ng L^{-1}. Due to their continuous input into the environment through point and diffuse sources, and the concern about their potential threat to human and wildlife reproduction, the identification and characterization of EDCs in the environment is of great scientific interest (Valcárcel et al., 2018). The discharge of untreated wastewater in areas with poor wastewater sanitation can pose even higher environmental and public health risks.

According to the Statistical Office of Serbia, approximately 98% of all wastewater (industrial, as well as urban wastewater) is being discharged without any treatment into receiving waters, which may have detrimental impacts on the water quality (The Statistical Office of the Republic of Serbiahttp://www.stat.gov.rs/en-US). Furthermore, some of the urban and rural settlements are not connected to the public sewerage network. Therefore, septic tanks (many of them are improperly designed) discharge wastewaters from households directly to receiving freshwater ecosystems (Kaštelan-Macan et al., 2007). Indeed, fecal pollution has been an ongoing problem in the Danube River basin due to the discharges of untreated wastewater (Kirschner et al., 2009; Milic et al., 2014), indicating that aquatic organisms and the human population are under constant environmental pressure. On the other hand, the Danube River, together with groundwater reservoirs, is the major source for drinking water production in Serbia. In fact, about 65% of the drinking water supply comes from groundwater and many aquifers are in the direct proximity of wastewater discharges. Thus, the contamination by EDCs seriously compromises the safety of drinking water supplies. Until now, some studies have been conducted to monitor the water quality throughout the whole Danube basin (Micić and Hofmann, 2009; Loos et al., 2010; Liska, 2015; Loos et al., 2017). Nevertheless, a restricted number of them investigated the occurrence of EDCs in the Danube River basin and its tributaries within Serbia (Terzic et al., 2008; Antonijević et al., 2014; Miloradov et al., 2014; Milanović et al., 2016). Most of the surveys performed up to date in this area included a very limited number of sampling sites and target EDCs while none of them evaluated the quality of drinking water sources intended for direct human consumption. Furthermore, there is still a lack of knowledge about the potential environmental risks associated with the occurrence of EDCs in the area. This information is much needed since most of the population heavily relies on low-quality water for drinking water purposes and other activities such as cooking, hygiene, irrigation and recreation as its contamination may have severe negative impacts for human health.

In this study we performed a comprehensive survey on the occurrence of selected EDCs, covering industrial chemicals (NP, OP, BPA), natural (E2, E1, E3), synthetic estrogens (EE2, DES) and their conjugates (E1-3S, E3-3S, E1-3G, E2-17G, E3-16G), in 30 sites, including 90 different samples, along the Danube River and its tributaries in Serbia. Potential environmental risks posed by the occurrence of these compounds were evaluated through the calculation of risk quotients (RQs) and estrogenic activity in waste, surface and drinking water. This study reflects the necessity for a thorough evaluation of water resources in Serbia and the establishment of treatment networks for the effective management of wastewater and control of drinking water resources.

2. Materials and methods

2.1. Chemicals

All pure standards of the target compounds E2, E1, E3, EE2, DES, E1-3S, E3-3S, E2-17G, E1-3G, E3-16G, NP, OP and BPA were purchased from Sigma-Aldrich (Germany). Isotopically labelled compounds, used as internal standards (IS), E1-d_4, E2-d_2, EE2-d_4, E1-3S-d_4, E2-17G-$^{13}C_4$, NP-d_4, OP-d_4, and BPA-d_4 were obtained from CDN Isotopes Pointe-Claire (Quebec, Canada). More information about standards, chemicals and reagents used can be found in the supplementary material (SM) (Section 2.1 Chemicals).

2.2. Description of the study area and sampling sites

The Danube River basin and its tributaries, Sava and Tisa rivers, located in the Northern Serbian territory, are inhabited with more than 3.5 million people. The territory has 24.848 km^2 and is mostly used for agriculture. The capital city of this area has more than 1.500.00 inhabitants, and it also includes 5 large cities, with >100.000 inhabitants, while 83% of the settlements have a population lower than 50.000 inhabitants. The irrigation canal system Danube-Tisa-Danube (DTD) has been one of the major hot-spots for environmental contamination in Serbia for decades, as it has been used for discharge of untreated urban and industrial wastewater. On the other hand, Veliki Bačka Canal, which is part of the DTD hydro-system and the connecting point between the Danube and Tisa rivers, is the most polluted one in the studied region, as the biggest proportion of industrial facilities, mostly involved in food processing (e.g. sugar factories, oil industries, fruit and vegetable processing and slaughterhouses) discharge their wastewater into the canal (Pantelić et al., 2012; Stojanović et al., 2014). Furthermore, the main towns and cities located in the Danube basin as well as Sava and Tisa rivers, use water from alluvial aquifers as sources for

drinking water production. Over 65.4% of the public water supply comes from underground and spring water, 26.2% of the water comes directly from watercourses, while 8.4% of community members take water from lakes and accumulations (http://www.stat.gov.rs/en-US), located in these heavily polluted sites.

In this study, 90 samples along 30 different sites were collected including: (i) untreated municipal and industrial wastewater, which were sampled directly from sewerage systems in selected settlements and towns along the study area (sites 1–30, Fig. 1); (ii) surface water samples that are heavily impacted by direct urban and industrial wastewater discharges, including sites along the Danube River (sites 1–8, Fig. 1) and its major tributaries, Tisa (sites 9–14, Fig. 1) and Sava river (points 15–19, Fig. 1); (iii) five hot-spots throughout the Veliki Bačka Canal (sites 20–25, Fig. 1); (iv) three spots along the irrigation canal system DTD (points 26–28, Fig. 1); (v) two samples from lakes Palić and Zobnatica, (points 29 and 30, Fig. 1). These sites were selected due to their vicinity to the main groundwater aquifers used for drinking water abstraction in the area and that suffer from intensive anthropogenic pollution; and (vi) drinking water samples from public fountains in selected residential areas (points 1–30, Fig. 1) used for direct human consumption. This water originates from different sources, is chlorinated upon collection, stored in reservoirs and distributed through public water system. The complete list of the analyzed samples and a description of the sampling sites is given in Table S1 in SM. Water samples were collected as grab samples in pre-cleaned 1L amber glass bottles. Upon reception at the laboratory, samples were filtered through glass fiber filters (GFF, 0.7 μm) in order to remove suspended particle matter from the aqueous phase and stored at −20 °C until analysis.

2.3. EDCs analysis

Prior to analysis, samples pH was further adjusted to 11 with sodium hydroxide (NaOH) and they were filtered through hydrophilic polyvinylidene fluoride (PVDF, 0.45 μm) membrane syringe filters. Further, samples were placed in amber glass 10 mL injection vial for on-line SPE analysis. Subsequently, isotopically labelled IS were added to each sample for a final concentration of 50 ng L^{-1} prior to HPLC analysis. For EDCs detection and quantification, an automated on-line solid phase extraction (SPE) pre-concentration system EQuan™ coupled to a triple quadrupole tandem mass spectrometer (QqQ) TSQ Vantage (Thermo Fisher Scientific, USA), equipped with an electrospray (ESI) ionization source, was used. The procedures for sample preparation and analysis are described in detail elsewhere (Čelić et al., 2017). The optimized MS/MS parameters for SRM analysis of selected EDCs are displayed in Table S2 while quality control parameters, such as extraction recoveries, method and quantification limits can be found in Table S3 in SM.

2.4. Risk assessment and estrogenic potential

Up to now several approaches have been used to characterize the risks posed by the occurrence of EDCs to aquatic organisms (i.e. fish) (Hernando et al., 2006; Cao et al., 2010; Manickum and John, 2014; Wu et al., 2017; Wang and Zhu, 2017; Yao et al., 2018; Damkjaer et al., 2018; Yien Fang et al., 2019; Esteban et al., 2014b). In the present study, the calculation of risk quotients (RQs) and estrogenic activity were used as two strategies to assess the potential risks of estrogenic compounds. Risk quotients (RQs) in waste and surface water were calculated by using the following equation (1):

$$RQ = MEC/PNEC, \quad (1)$$

where MEC (ng L^{-1}) is the measured environmental concentration (assumed to be the exposure concentration) of detected compounds, PNEC (ng L^{-1}) is the predicted no-effect concentration of selected EDCs obtained from the NORMAN database. For the

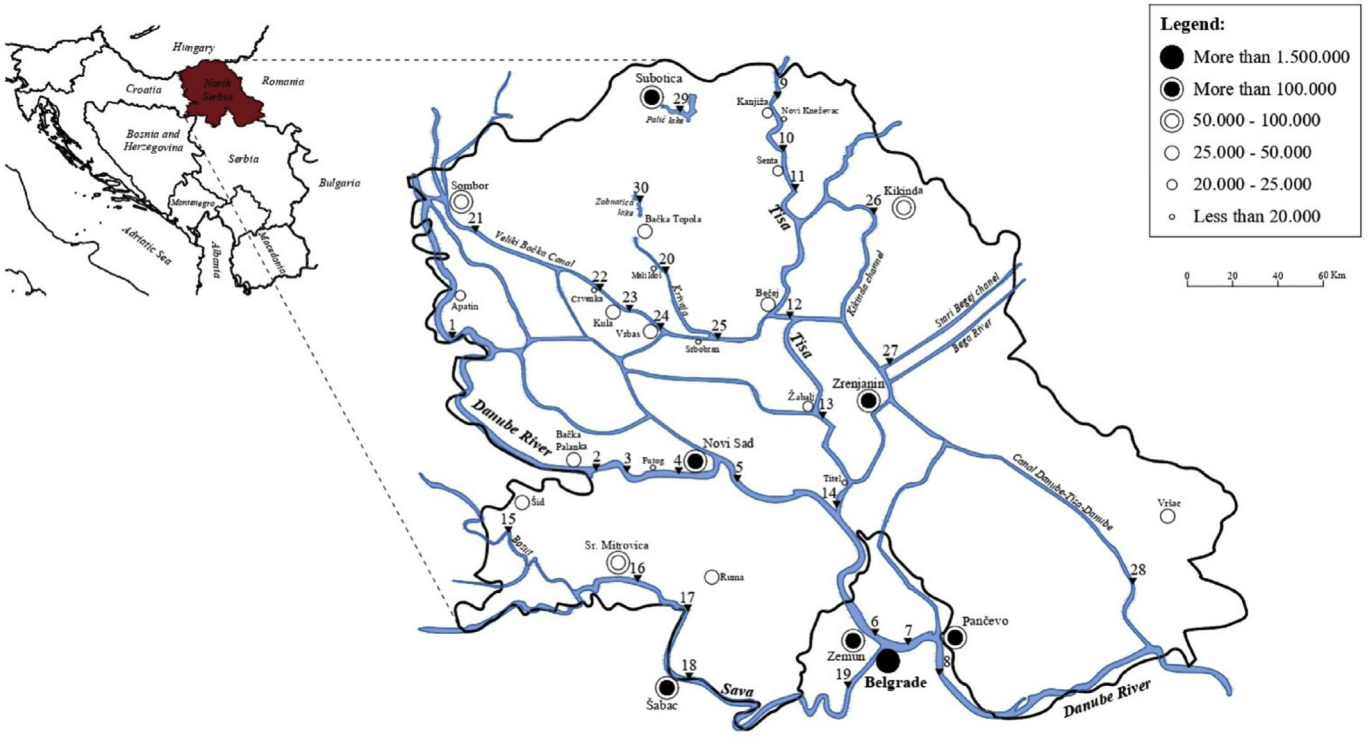

Fig. 1. Map showing the cities where wastewater and drinking water were collected. The triangles with the number indicate the sites of corresponding surface water collection.

calculation of RQs, the lowest available PNEC values were used (NORMAN-Network, http://www.norman-network.com/). The PNEC values for each compound are given in Table S4 in SM. The criteria for the risk rating was based on RQ $\geq$ 1: "high risk"; 1 > RQ $\geq$ 0.1: "medium risk"; and RQ < 0.1: "low risk".

In the second approach, the potential risk of estrogenic compounds to aquatic organisms and humans was evaluated via the calculated estrogenic activity for each individual compound in waste, surface and drinking water. Estrogenic factors are relative to the activity of the natural estrogen E2 and are expressed as estradiol equivalents (EEQ) by using the following formula (2):

$$EEQ\ (ng\ E2\ L^{-1}) = Ci*EEFi, \quad (2)$$

where C_i (ng L^{-1}) is the measured environmental concentration of each compound and EEF_i is the relative estrogenicity factor of the studied compounds, calculated as the ratio between the mean effective concentrations (EC_{50}) of each compound relative to the EC_{50} of E2. EEF_i values used in our study were taken from the literature and can be found in Table S5 in SM. Thus, estrogenic activity was only evaluated for the compounds whose EEF_i values were available (Gutendorf and Westendorf, 2001; Van den Belt et al., 2003; Rutishauser et al., 2004; Céspedes et al., 2004; Beck et al., 2005; Song et al., 2006). If the compounds have several EEF_i values, the highest determined EEF_i was used in this study in order to consider worst-case scenarios. The threshold for estrogenic activity was set at 1 ng E2 L^{-1}, since this is the level for estradiol to cause estrogenic effects in aquatic organisms, as it has been previously reported (Purdom et al., 1994; Hansen et al., 1998). Besides individual estrogenic effects, total estrogenic activities (EEQ_t) were also estimated, since these compounds are present as mixtures in the environment. EEQ_t were calculated by summing individual EEQ_i and they were calculated for each sampling site, according to equation (3):

$$EEQ_t = C_1\ x\ EEF_1\ +\ C_2\ x\ EEF_2 + \ldots \quad (3)$$

3. Results and discussion

3.1. Occurrence of EDCs in urban and industrial wastewater

Minimum, maximum and mean concentrations of the EDCs found in waste, surface and drinking water are summarized in Table 1, together with their total frequency of detection in each type of sample. Fig. 2A shows the compounds with the major contribution to the total concentration in both municipal and industrial wastewaters. Differences in the chemical profile are observed between these two types of wastewaters. In municipal wastewaters, natural and synthetic estrogens and their sulfate metabolites are the major contributors to the total concentration while in industrial wastewaters, alkylphenolic compounds and BPA are the most prominent compounds in terms of concentration (Fig. 2A).

In municipal wastewater samples E1 was the most widely detected compound (47% frequency of detection), with concentrations ranging from 0.3 to 14.9 ng L^{-1}, followed by E2 and E3, detected up to 17% of the samples with maximum concentrations of 9.8 ng L^{-1}. The synthetic estrogen EE2, the main active compound in contraceptive pills, and DES, used for hormone therapy, were not detected in any of the samples. Even though a significant proportion of natural and synthetic hormones occur in the aquatic environment as conjugated metabolites, such as glucuronide or sulfate conjugates, the estrogen glucuronides analyzed (E2-17G, E1-3G and E3-16G) were found below the detection limits in all wastewater samples analyzed. Nevertheless, sulfate conjugates E1-3S and E3-3S were found in both types of wastewater at similar concentrations (up to 17.7 ng L^{-1} and 30.1 ng L^{-1}, respectively). This can be attributed to the fact that glucuronide metabolites are more prone than sulfate conjugates to be degraded by bacteria, thus being less frequently detected in the environment (Baronti et al., 2000; Andersen et al., 2003; Komori et al., 2004; Ben et al., 2017). Even though estrogens were more ubiquitous in municipal wastewaters, concentrations detected in some industrial sites were higher than those observed in municipal sewage. This is for example the case for WW7 (wastewater collected in Serbia's capital city), where E1 and E3 maximum concentrations were 64.8 ng L^{-1} and 34.2 ng L^{-1}, respectively. This could be attributed to the fact that industrial wastewater is less diluted than urban wastewater.

Regarding industrial wastewater, alkylphenolic compounds (OP and NP) and BPA were the main contributors to the sum of all detected EDCs (Fig. 2A). BPA was the most ubiquitous compound, with concentrations ranging from 6.4 to 98.1 ng L^{-1} and with only one sample showing higher concentration (338 ng L^{-1} in WW8). This point is in the vicinity of an industry that is the major plastic producer in Serbia, which might explain the high BPA levels detected. Total alkylphenol concentrations ranged from 16.1 ng L^{-1}

Table 1
Summary of detected EDCs (ng L^{-1}) for each studied type of water, with total frequency of detection (Freq.), minimum (Min), maximum (Max) detected concentration and mean (Mean) value.

Sample type		Compound	E2	E1	E3	E1-3S	E3-3S	OP	NP	BPA
Wastewater	Municipal	Total freq. of detection	10%	47%	17%	57%	30%	23%	27%	47%
		Concetration range (ng L^{-1})	4.0–9.8	0.3–14.9	2.7–7.8	0.4–17.3	2.1–28.8	1.1–3.2	1.2–17.6	0.5–40.9
		Mean value (ng L^{-1})	7.2	5.9	4.9	4.4	6.6	1.9	4.9	6.8
	Industrial	Total freq. of detection	20%	37%	17%	27%	17%	37%	37%	37%
		Concetration range (ng L^{-1})	4.0–10.4	1.2–64.8	3.8–34.2	3.3–17.7	2.1–30.1	3.6–52.4	3.2–78.3	6.4–338.2
		Mean value (ng L^{-1})	7.9	15.8	15.8	8.4	12.9	15.4	35.9	78.8
Surface water	Municipal impact	Total freq. of detection	–	27%	17%	47%	33%	20%	27%	33%
		Concetration range (ng L^{-1})	<0.034	0.2–9.8	2.1–4.8	0.1–2.1	0.2–3.8	0.2-1.5	0.1–1.5	0.6–31.2
		Mean value (ng L^{-1})	<0.034	2.3	3.0	1.1	1.1	0.9	0.7	5.8
	Industrial impact	Total freq. of detection	–	33%	10%	20%	10%	33%	37%	37%
		Concetration range (ng L^{-1})	<0.034	0.1–2.3	0.4-1.5	0.3–7.2	0.8-4.1	0.7–37.2	0.2–36.6	1.8–105.7
		Mean value (ng L^{-1})	<0.034	1.1	0.9	1.9	2.2	5.5	10.3	26.2
Drinking water	Rural area	Total freq. of detection	–	–	–	–	–	13%	13%	20%
		Concetration range (ng L^{-1})	<0.037	<0.022	<0.132	<0.020	<0.024	1.1–2.8	1.7-3.9	2.6–6.2
		Mean value (ng L^{-1})	<0.037	<0.022	<0.132	<0.020	<0.024	1.8	2.6	4.0
	Urban area	Total freq. of detection	–	–	–	–	–	20%	27%	37%
		Concetration range (ng L^{-1})	<0.037	<0.022	<0.132	<0.020	<0.024	0.4-3.7	1.2–7.9	2.5–35.6
		Mean value (ng L^{-1})	<0.037	<0.022	<0.132	<0.020	<0.024	1.7	3.1	9.1

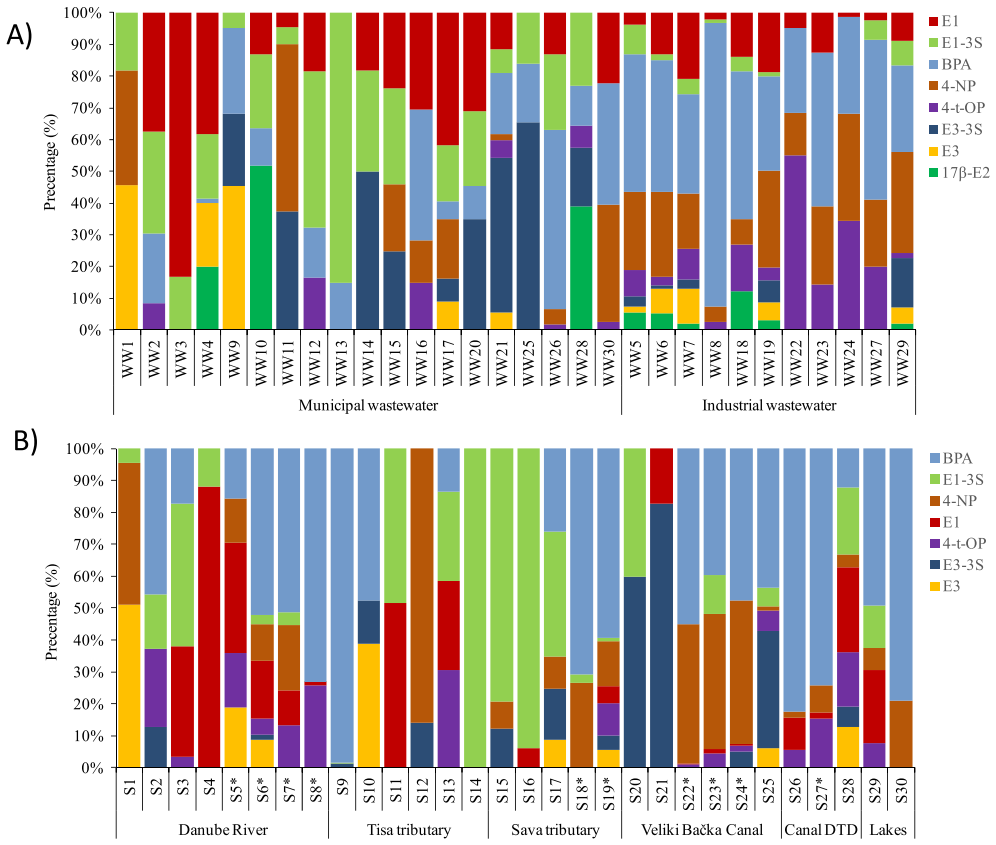

Fig. 2. Composition profile (%) of EDCs detected in A) wastewater and B) surface water samples (*with asterisks are marked sampling sites impacted by urban wastewater discharges).

to 104 ng L^{-1}. The highest concentration was found in wastewater coming from a food processing-based industrial area that discharges its wastewaters into the Veliki Bačka Canal. The maximum individual OP concentration was 52.4 ng L^{-1} (WW24), while NP was present in slightly higher concentrations, up to 78.3 ng L^{-1} (WW19). The high concentrations detected in this area might be explained by their vicinity to oil refinery, petrochemical, fertilizer, machinery and aircraft industries, which might be potential sources of alkylphenolic compounds.

The concentrations of estrogens, BPA and alkylphenolic compounds detected in wastewater samples are comparable with those found in previous studies at international level (Table S6a, SM) (D'Ascenzo et al., 2003; Isobe et al., 2003; Kobayashi et al., 2006; Ballesteros-Gómez et al., 2007; Avberšek et al., 2013; Ben et al., 2017; Wu et al., 2017; Rubirola et al., 2017; Zhang and Fent, 2018; Yarahmadi et al., 2018; Goeury et al., 2019; Williams et al., 2019) and they are also in good agreement with other studies performed within the Western Balkan region (Terzic et al., 2008; Miloradov et al., 2014), where alkylphenolic compounds and BPA were ubiquitous chemicals. Unfortunately, to the best of our knowledge, there is no available data on the occurrence of estrogens in wastewater in Serbia for comparison.

3.2. Distribution in wastewater impacted surface waters

The similarity in chemical profile between waste and surface waters indicated that wastewater discharges are the major contributors to the contamination by EDCs in receiving surface waters (Fig. 2B). Total EDCs concentrations were higher in industrial wastewater impacted sites (up to 145 ng L^{-1}, S8), compared to the sites with major urban wastewater influence (up to 31.7 ng L^{-1}, S9).

Estrogenic compounds were the main contributors to the total EDCs concentration in surface water impacted by urban wastewater discharges (Fig. 2B). E1 and E3 were detected in 27% and 17% of the samples investigated, at maximum concentrations of 9.8 ng L^{-1} and 4.8 ng L^{-1}, respectively. The highest total concentrations of estrogens and their conjugates were found at site S6 (16.9 ng L^{-1}), which is in the close vicinity of the main pharmaceutical industry in the region, suggesting that these compounds also originate from industrial discharges. As it was observed in wastewater samples, the most potent estrogenic compounds (E2, EE2 and DES) were not found in any of the surface water samples and only two sulfate conjugates were detected. E1-3S and E3-3S were found in 67% and 43%, of the samples at similar concentrations (from <LOD to 7.2 ng L^{-1}). Even though estrogens and their conjugates are detected at low ng L^{-1} levels, these concentrations are often close to or above the concentration at which they can exert estrogenic effects (>1–10 ng L^{-1}). For alkylphenols, OP and NP were found in 53% and 64% of the total number of samples, respectively. Their average concentrations (3.7 ng L^{-1} and 6.3 ng L^{-1}) were generally lower than those found in wastewater samples, reflecting high dilution factors and dispersion processes in the rivers. The highest total alkylphenols concentrations were 38.1 ng L^{-1} and 37.4 ng L^{-1}, which correspond to sampling sites S24 (Veliki Bačka Canal) and S8 (the Danube River, downstream the city of Pančevo), respectively. The first one, S24, is a highly polluted site due to the uncontrolled discharge of untreated wastewater and leakage from landfill sites, while S8 is a heavily industrialized area, with petrochemical and organochlorine industries, an oil refinery, a chemical fertilizer factory, machinery, and aircraft industries. Indeed, the presence of

these compounds in sediment samples has been also observed by other authors (Micić and Hofmann, 2009). It is worth pointing out that in any of the sampling sites the concentrations of NP exceeded the maximum allowable concentration (MAC) for inland surface water, set to be 2 μg L^{-1}, in accordance of the Directive 2008/105/EC (2008/105/EC). Furthermore, concentrations of NP and OP did not exceed the annual average level (AA) of 0.3 and 0.1 μg L^{-1}, respectively specified in the same Directive. Regarding BPA, it was detected in 70% of the samples and the concentrations ranged from non-detected to 106 ng L^{-1}, being the highest concentration found in site S8.

Fig. 3 compares the levels of estrogens, alkylphenols and BPA detected in our study with those previously found in the Danube River. For estrogens, E1 and E3 were also ubiquitous compounds and found at similar concentrations (Antonijević et al., 2014; Galaon et al., 2016; König et al., 2017), while some other authors detected somewhat higher levels for E3 up to 33 ng L^{-1} (Hashmi et al., 2018). EE2 was detected at very low concentrations, ranging from 0.05 ng L^{-1} to 0.16 ng L^{-1} (Hashmi et al., 2018; Antonijević et al., 2014) and up to 1.16 ng L^{-1} (Andrási et al., 2013). For BPA and NP, concentrations in our study differ than those from previous surveys, where concentrations were much higher (even six-fold) (Loos et al., 2010; Milanović et al., 2016; König et al., 2017). Nevertheless, lower OP concentrations were recorded in past monitoring campaigns, with levels not exceeding 10 ng L^{-1} (Miloradov et al., 2014; Loos et al., 2010). Comparing our results with other international studies, concentrations of estrogens are in good agreement with those previously reported (Isobe et al., 2003; Komori et al., 2004; Brix et al., 2010; Esteban et al., 2014b; Wang and Zhu, 2017), yet some authors reported higher values for estrogens (Sodré et al., 2010; Pelayo et al., 2011; Avberšek et al., 2013; Manickum and John, 2014; Rubirola et al., 2017; Yao et al., 2018). For

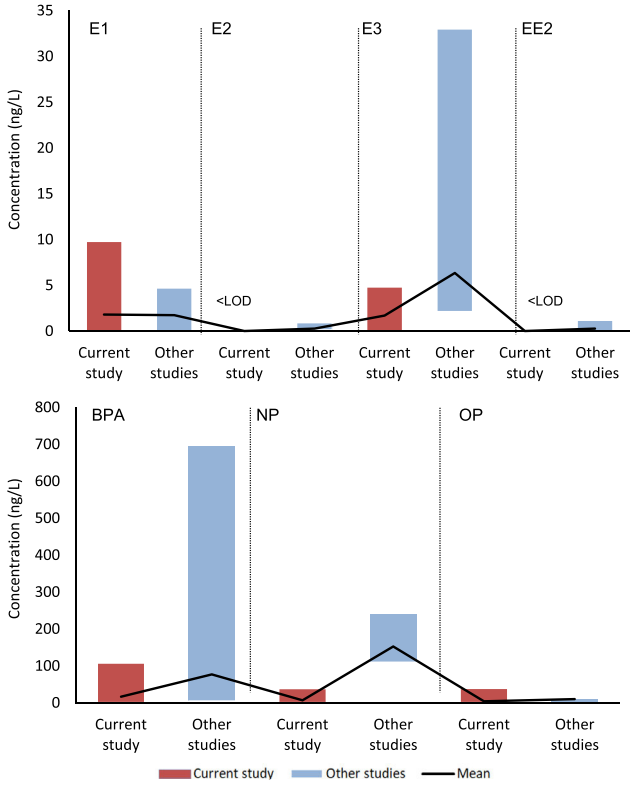

Fig. 3. The concentration levels of estrogens, alkylphenols and BPA detected in our study compared with those previously found in the Danube River.

BPA and alkylphenols, concentrations found in our study are somewhat lower than those previously reported at international level (Céspedes et al., 2006; Ballesteros-Gómez et al., 2007; Kasprzyk-Hordern et al., 2008; Quednow and Püttmann, 2009; Brix et al., 2010; Pelayo et al., 2011; Esteban et al., 2014b) (Table S6b, SM).

3.3. EDCs in drinking water

Drinking water samples were collected from 30 public fountains situated in markets or public places in selected urban settlements and towns. To the best of our knowledge, this is the first study that evaluates the occurrence of EDCs in drinking water in Serbia. Only one recent study by Miloradov and co-workers followed the fate of some EDCs in a drinking water treatment plant (Miloradov et al., 2014).

The plasticizer BPA was the most ubiquitous compound in drinking water, followed by NP and OP, while natural and synthetic estrogens and their conjugates were not found in any of the samples. BPA was found in 57% of the samples at an average concentration of 7.3 ng L^{-1}. NP and OP showed frequencies of detection of 40% and 33%, respectively and their individual concentrations ranged from 0.4 to 7.9 ng L^{-1}. The highest total EDCs concentration (39.8 ng L^{-1}) was observed at point DW8, which corresponds to a fountain located in the city of Pančevo, a heavily industrialized area. In this site, BPA was the major contributor to the total EDC concentration (detected at 35.6 ng L^{-1} and with a 90% contribution to the total concentration). The DWTP of this region supplies a population of more than 100.000, and the water is extracted from reservoirs for human potable supply located in the immediate vicinity of the Danube River. Site DW24 exhibited the second highest accumulated concentration (16 ng L^{-1}). In this case it was the alkylphenol NP the substance with major contribution to total EDCs concentrations, found at 7.9 ng L^{-1}.

Our results are in good agreement with previous studies where no estrogens were detected in drinking water (Esteban et al., 2014a; Naldi et al., 2016; Machado et al., 2016; Le Coadou et al., 2017; Leusch et al., 2018; Valcárcel et al., 2018) or when they were found, they were present at remarkably low concentrations (<LOD to 17 ng L^{-1}) (Kuch and Ballschmiter, 2001; Morteani et al., 2006; Loos et al., 2007; Benotti et al., 2009; Zacs et al., 2016; Goeury et al., 2019). For BPA, our results are consistent with those previously reported in other studies (Casajuana and Lacorte, 2003; Colin et al., 2014; Esteban et al., 2014a; Machado et al., 2016; Valcárcel et al., 2018), while in some other reports (Li et al., 2010; Bono-Blay et al., 2012) higher maximum concentrations levels (up to 317 ng L^{-1}) were found. Finally, for OP and NP, the concentration ranges reported in this work are in the same order of magnitude than those previously reported in other European countries (Kuch;Ballschmiter, 2001; Loos et al., 2007; Esteban et al., 2014a; Leusch et al., 2018). Only a restricted number of studies showed higher NP levels than those reported herein, such as the one performed by Valcárcel and co-workers (Valcárcel et al., 2018) in drinking water of the capital city of Spain, where NP reached 126 ng L^{-1} in some samples, or in the one carried out by Colin and colleagues in France (Colin et al., 2014), with NP levels up to 505 ng L^{-1} (Table S6c, SM).

3.4. Risk assessment

Estimated RQs in waste and surface waters are shown in Fig. 4. Based on the calculated individual RQ values, 18 out of 30 wastewater samples presented high risk (RQ > 1), with E1 and E2 being the compounds posing the highest risk (with maximum RQ values of 18 and 26, respectively), while for BPA, RQs exceeded the

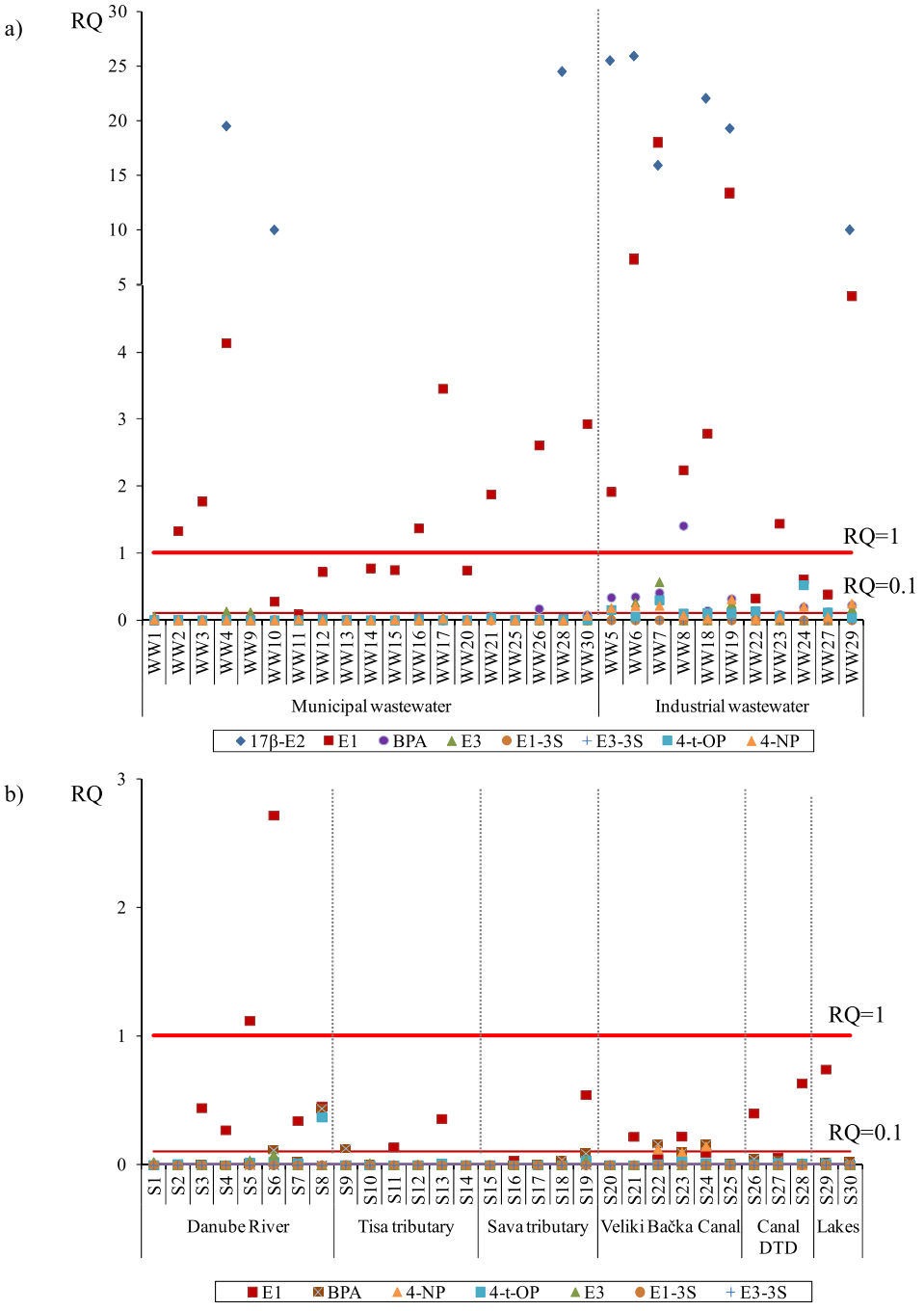

Fig. 4. Risk quotients (RQs) calculated for detected compound in each sampling site; RQ ≥ 1 High risk; 1 > RQ ≥ 0.1 Medium risk; RQ < 0.1 Low risk; RQ = MEC/PNEC (values from NORMAN database).

threshold of 1 in only one sample (RQ value 1.4). The potential environmental risks of detected EDCs in wastewaters were ordered from higher to lower RQs as E2>E1>BPA > E3>OP > NP > E1-3S > E3-3S, with values between 26 and 2×10^{-5}. For surface water, RQs achieved were one order of magnitude lower than those estimated for wastewater. RQs ranged from 5×10^{-6} to 2.7, and only one estrogen, E1, exceeded the threshold of 1 in 2 samples, revealing high risks for the aquatic organisms living in these freshwater ecosystems. In surface water, RQ were ordered from higher to lower values as E1>BPA > OP > NP > E3>E3-3S > E1-3S.

Fig. 5 shows the total estrogenic potential (EEQ_t) of waste, surface and drinking water, based on the maximum estradiol equivalency factors taken from the literature (see Table S5, SM). EEQ_t reached 34.4 ng E2 L^{-1}, 4.09 ng E2 L^{-1}, and 5.5 pg E2 L^{-1} for waste, surface, and drinking water, respectively. EEQ_t exceeded the estradiol level threshold (1 ng E2 L^{-1}) in 20 wastewater samples and in 3 surface waters (sites S5, S6 and S19). These sites are mostly influenced by industrial wastewater discharges (Fig. 5b), while estrogenicity in drinking water samples was below the threshold value of 1 ng E2 L^{-1} (Fig. 5c). The total estrogenic activity reported in wastewater was mostly attributed to the estrogens E2, E1 and E3, because of their higher EEFi values, in comparison with the EEFi values for alkylphenolic compounds and BPA (see Table S5, SM). Indeed, E1 was the major contributor to the calculated EEQt in

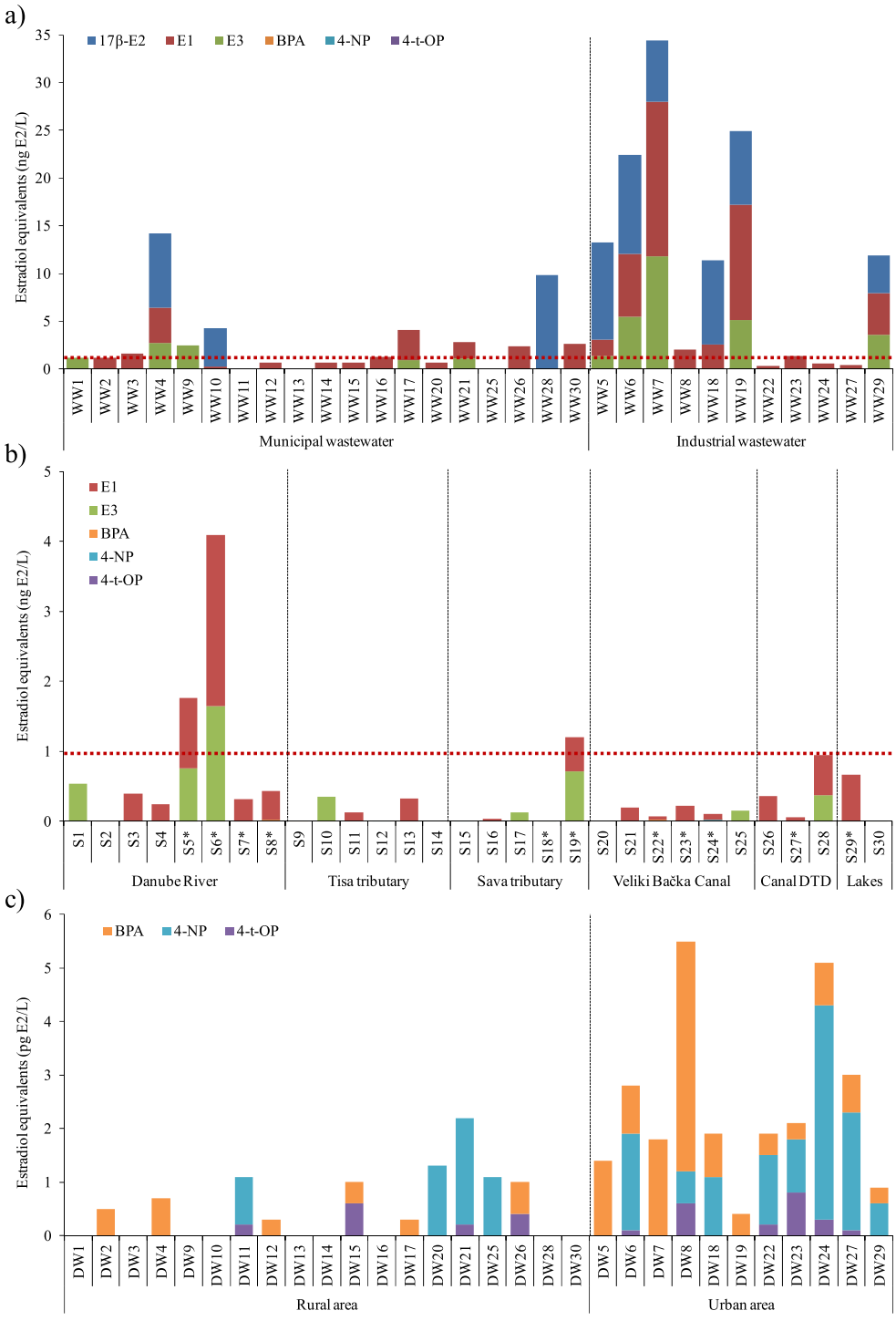

Fig. 5. Accumulated levels of β-estradiol equivalents (EEQ) calculated for each sampling site for a) wastewater; b) surface water, and c) drinking water.

surface water (from 0.03 to 2.5 ng E2 L^{-1}) despite the fact that it shows half of E2's potency. These results match well with previous studies, where E1 was found to be the most important EDCs among natural estrogens in terms of estrogenic potential (Manickum and John, 2014), while BPA showed minor contributions to total estrogenic activity (Brix et al., 2010).

Concerning drinking water samples, EEQ_t levels reached pg L^{-1} levels (Fig. 5c), and these values never exceeded the threshold level of 1 ng E2 L^{-1}. These results indicate that the levels at which these compounds are present in drinking water will not pose an appreciable risk to the human endocrine system, which coincides with previous investigations (Caldwell et al., 2010; Esteban et al., 2014a).

4. Conclusions

To date this is the first study that reports on the occurrence, fate and risk assessment of estrogenic compounds in waste, surface and drinking water in Serbia. The absence of wastewater treatment

results in the untreated sewage being discharged directly into the surrounding surface water bodies, thus representing a serious pollution source in the study area. The similarity in the chemical profile of EDCs in both waste and surface water indicates that industrial and urban wastewaters are the major contributors to the pollution by EDCs in the Danube River Basin. Out of the 13 EDCs analyzed, estrogen E1 and its metabolite E1-3S were the most ubiquitous compounds in urban wastewater and the corresponding surface water impacted sites, while BPA was the most frequently detected substance in industrial wastewaters and freshwater sites. The total concentration of EDCs was higher in industrial impacted sites in comparison with urban areas, indicating that industrial discharges are important contributors to the pollution of EDCs in the study area.

The calculated RQs for each the compounds detected in wastewater samples showed high risk for the estrogens E2 and E1, while BPA presented high risks in only one sampling site. In this area large volumes of this compound are used in industrial processes. Regarding RQs for surface water, values were mainly below 1, except for E1 in two sampling sites of major urban wastewater impact. These results suggest that the environmental risks associated to the occurrence of EDCs are low and below those thought to be of concern. For estrogenic activity, EEQt exceeded 1 ng L^{-1} estradiol level threshold (1 ng E2 L^{-1}) in 3 surface waters samples, which are mostly influenced by industrial wastewater discharges. These results indicate that aquatic organisms living in these sites might be subjected to some alterations in their endocrine system when chronically exposed to the detected EDCs levels. For drinking water, EEQts were one order of magnitude lower than the 1 ng E2 L^{-1} threshold, suggesting that the levels of EDCs present in drinking water might not induce negative effects on human's endocrine system. Although estimated RQs and EEQts indicate that negligible environmental and human health effects are to be expected at short term, certain actions should be taken to prevent EDCs contamination in the area, especially in source waters used for drinking water consumption.

CRediT authorship contribution statement

Mira Čelić: Investigation, Methodology, Formal analysis, Writing - original draft. **Biljana D. Škrbić:** Conceptualization, Supervision, Visualization. **Sara Insa:** Project administration, Data curation. **Jelena Živančev:** Data curation, Resources. **Meritxell Gros:** Writing - review & editing, Software. **Mira Petrović:** Validation, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114344.

References

2000/60/EC, 2000. Directive 2000/60/EC of the European parliament and of the council of 23 october 2000 establishing a Framework for community action in the field of water policy off. J. Eur. Union L327/1, 1–73.

2008/105/EC. Direcrtive 2008/105/EC on Environmental Quality Standards in the Field of Water Policy, Amending and Subsequently Repealing Council Directives.

2013/39/EU, 2013. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Off. J. Eur. Union L226/1, 1–17.

2015/495/EU, 2015. Decision 2015/495 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. Off. J. Eur. Union L78/40, 40–42.

Al-Odaini, N.A., Zakaria, M.P., Yaziz, M.I., Surif, S., 2010. Multi-residue analytical method for human pharmaceuticals and synthetic hormones in river water and sewage effluents by solid-phase extraction and liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1217 (44), 6791–6806.

Andersen, H., Siegrist, H., Halling-Sørensen, B., Ternes, T.A., 2003. Fate of estrogens in a municipal sewage treatment plant. Environ. Sci. Technol. 37 (18), 4021–4026.

Andrási, N., Molnár, B., Dobos, B., Vasanits-Zsigrai, A., Záray, G., Molnár-Perl, I., 2013. Determination of steroids in the dissolved and in the suspended phases of wastewater and Danube River samples by gas chromatography, tandem mass spectrometry. Talanta 115, 367–373.

Antonijević, M., Arsović, M., Čáslavský, J., Cvetković, V., Dabić, P., Franko, M., et al., 2014. Actual contamination of the danube and Sava rivers at belgrade (2013). J. Serb. Chem. Soc. 79 (9), 1169–1184.

Ascenzi, P., Bocedi, A., Marino, M., 2006. Structure–function relationship of estrogen receptor α and β: impact on human health. Mol. Aspect. Med. 27 (4), 299–402.

Avberšek, M., Žegura, B., Filipič, M., Uranjek-Ževart, N., Heath, E., 2013. Determination of estrogenic potential in waste water without sample extraction. J. Hazard Mater. 260, 527–533.

Ballesteros-Gómez, A., Ruiz, F.-J., Rubio, S., Pérez-Bendito, D., 2007. Determination of bisphenols A and F and their diglycidyl ethers in wastewater and river water by coacervative extraction and liquid chromatography–fluorimetry. Anal. Chim. Acta 603 (1), 51–59.

Baronti, C., Curini, R., D'Ascenzo, G., Di Corcia, A., Gentili, A., Samperi, R., 2000. Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. Environ. Sci. Technol. 34 (24), 5059–5066.

Beck, I.-C., Bruhn, R., Gandrass, J., Ruck, W., 2005. Liquid chromatography–tandem mass spectrometry analysis of estrogenic compounds in coastal surface water of the Baltic Sea. J. Chromatogr. A 1090 (1–2), 98–106.

Ben, W., Zhu, B., Yuan, X., Zhang, Y., Yang, M., Qiang, Z., 2017. Transformation and fate of natural estrogens and their conjugates in wastewater treatment plants: influence of operational parameters and removal pathways. Water Res. 124, 244–250.

Benotti, M.J., Trenholm, R.A., Vanderford, B.J., Holady, J.C., Stanford, B.D., Snyder, S.A., 2009. Pharmaceuticals and endocrine disrupting compounds in U.S. Drinking water. Environ. Sci. Technol. 43 (3), 597–603.

Bono-Blay, F., Guart, A., de la Fuente, B., Pedemonte, M., Pastor, M.C., Borrell, A., et al., 2012. Survey of phthalates, alkylphenols, bisphenol A and herbicides in Spanish source waters intended for bottling. Environ. Sci. Pollut. Control Ser. 19 (8), 3339–3349.

Brix, R., Postigo, C., González, S., Villagrasa, M., Navarro, A., Kuster, M., et al., 2010. Analysis and occurrence of alkylphenolic compounds and estrogens in a European river basin and an evaluation of their importance as priority pollutants. Anal. Bioanal. Chem. 396 (3), 1301–1309.

Caldwell, D.J., Mastrocco, F., Nowak, E., Johnston, J., Yekel, H., Pfeiffer, D., et al., 2010. An assessment of potential exposure and risk from estrogens in drinking water. Environ. Health Perspect. 118 (3), 338–344.

Cao, Q., Yu, Q., Connell, D.W., 2010. Fate simulation and risk assessment of endocrine disrupting chemicals in a reservoir receiving recycled wastewater. Sci. Total Environ. 408 (24), 6243–6250.

Casajuana, N., Lacorte, S., 2003. Presence and release of phthalic esters and other endocrine disrupting compounds in drinking water. Chromatographia 57 (9), 649–655.

Čelić, M., Insa, S., Škrbić, B., Petrović, M., 2017. Development of a sensitive and robust online dual column liquid chromatography-tandem mass spectrometry method for the analysis of natural and synthetic estrogens and their conjugates in river water and wastewater. Anal. Bioanal. Chem. 409 (23), 5427–5440.

Céspedes, R., Petrovic, M., Raldúa, D., Saura, Ú., Piña, B., Lacorte, S., et al., 2004. Integrated procedure for determination of endocrine-disrupting activity in surface waters and sediments by use of the biological technique recombinant yeast assay and chemical analysis by LC–ESI-MS. Anal. Bioanal. Chem. 378 (3), 697–708.

Céspedes, R., Lacorte, S., Ginebreda, A., Barceló, D., 2006. Chemical monitoring and occurrence of alkylphenols, alkylphenol ethoxylates, alcohol ethoxylates, phthalates and benzothiazoles in sewage treatment plants and receiving waters along the Ter River basin (Catalonia, N. E. Spain). Anal. Bioanal. Chem. 385 (6), 992–1000.

Céspedes, R., Lacorte, S., Ginebreda, A., Barceló, D., 2008. Occurrence and fate of alkylphenols and alkylphenol ethoxylates in sewage treatment plants and impact on receiving waters along the Ter River (Catalonia, NE Spain). Environ. Pollut. 153 (2), 384–392.

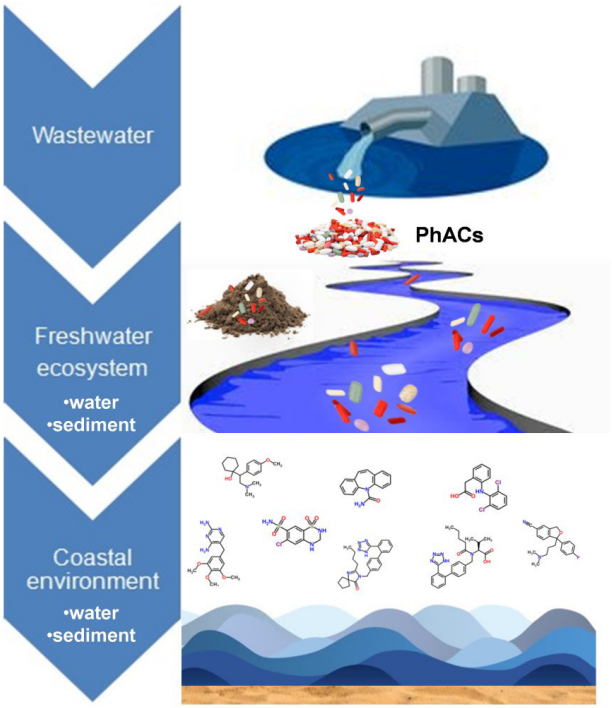
Chang, H., Wan, Y., Wu, S., Fan, Z., Hu, J., 2011. Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters:

comparison to estrogens. Water Res. 45 (2), 732–740.
Christiansen, L., Winther-Nielsen, M., Helweg, C., 2002. Feminization of Fish: the Effect of Estrogenic Compounds and Their Fate in Sewage Treatment Plants and Nature. Danish Envir Prot Agency.
Colin, A., Bach, C., Rosin, C., Munoz, J.-F., Dauchy, X., 2014. Is drinking water a major route of human exposure to alkylphenol and bisphenol contaminants in France? Arch. Environ. Contam. Toxicol. 66, 86–99.
Cui, C.W., Ji, S.L., Ren, H.Y., 2006. Determination of steroid estrogens in wastewater treatment plant of A controceptives producing factory. Environ. Monit. Assess. 121 (1), 409–419.
D'Ascenzo, G., Di Corcia, A., Gentili, A., Mancini, R., Mastropasqua, R., Nazzari, M., et al., 2003. Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. Sci. Total Environ. 302 (1), 199–209.
Damkjaer, K., Weisser, J.J., Msigala, S.C., Mdegela, R., Styrishave, B., 2018. Occurrence, removal and risk assessment of steroid hormones in two wastewater stabilization pond systems in Morogoro, Tanzania. Chemosphere 212, 1142–1154.
de Mes, T., Zeeman, G., Lettinga, G., 2005. Occurrence and fate of estrone, 17β-estradiol and 17α-ethynylestradiol in STPs for domestic wastewater. Rev. Environ. Sci. Biotechnol. 4 (4), 275.
Desbrow, C., Routledge, E.J., Brighty, G.C., Sumpter, J.P., Waldock, M., 1998. Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and in vitro biological screening. Environ. Sci. Technol. 32 (11), 1549–1558.
Eggen, R.I.L., Hollender, J., Joss, A., Schärer, M., Stamm, C., 2014. Reducing the discharge of micropollutants in the aquatic environment: the benefits of upgrading wastewater treatment plants. Environ. Sci. Technol. 48 (14), 7683–7689.
Esteban, S., Gorga, M., González-Alonso, S., Petrovic, M., Barceló, D., Valcárcel, Y., 2014a. Monitoring endocrine disrupting compounds and estrogenic activity in tap water from Central Spain. Environ. Sci. Pollut. Control Ser. 21 (15), 9297–9310.
Esteban, S., Gorga, M., Petrovic, M., González-Alonso, S., Barceló, D., Valcárcel, Y., 2014b. Analysis and occurrence of endocrine-disrupting compounds and estrogenic activity in the surface waters of Central Spain. Sci. Total Environ. 466–467, 939–951.
Falconer, I.R., 2006. Are endocrine disrupting compounds a health risk in drinking water? Int. J. Environ. Res. Publ. Health 3 (2), 180.
Galaon, T., Petre, J., Iancu, V.I., Cruceru, L., Vasile, G.G., Pascu, L.F., et al., 2016. Detection of estrogen hormones in Danube River and tributaries using liquid chromatography-mass spectrometry. Rev. Chem. 67 (8), 1474–1478.
Gentili, A., Perret, D., Marchese, S., Mastropasqua, R., Curini, R., Di Corcia, A., 2002. Analysis of free estrogens and their conjugates in sewage and river waters by solid-phase extraction then liquid chromatography-electrospray-tandem mass spectrometry. Chromatographia 56 (1), 25–32.
Goeury, K., Vo Duy, S., Munoz, G., Prévost, M., Sauvé, S., 2019. Analysis of Environmental Protection Agency priority endocrine disruptor hormones and bisphenol A in tap, surface and wastewater by online concentration liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1591, 87–98.
Gomes, R.L., Scrimshaw, M.D., Lester, J.N., 2003. Determination of endocrine disrupters in sewage treatment and receiving waters. Trac. Trends Anal. Chem. 22 (10), 697–707.
Gutendorf, B., Westendorf, J., 2001. Comparison of an array of in vitro assays for the assessment of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and xenoestrogens. Toxicology 166 (1), 79–89.
Hanselman, T.A., Graetz, D.A., Wilkie, A.C., 2003. Manure-borne estrogens as potential environmental Contaminants: A review. Environ. Sci. Technol. 37 (24), 5471–5478.
Hansen, P.D., Dizer, H., Hock, B., Marx, A., Sherry, J., McMaster, M., et al., 1998. Vitellogenin – a biomarker for endocrine disruptors. Trac. Trends Anal. Chem. 17 (7), 448–451.
Hashmi, M.A.K., Escher, B.I., Krauss, M., Teodorovic, I., Brack, W., 2018. Effect-directed analysis (EDA) of Danube River water sample receiving untreated municipal wastewater from Novi Sad, Serbia. Sci. Total Environ. 624, 1072–1081.
Heeb, F., Singer, H., Pernet-Coudrier, B., Qi, W., Liu, H., Longrée, P., et al., 2012. Organic micropollutants in rivers downstream of the megacity Beijing: sources and mass fluxes in a large-scale wastewater irrigation system. Environ. Sci. Technol. 46 (16), 8680–8688.
Hernando, M.D., Mezcua, M., Fernández-Alba, A.R., Barceló, D., 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta 69 (2 SPEC. ISS.), 334–342.
Isobe, T., Shiraishi, H., Yasuda, M., Shinoda, A., Suzuki, H., Morita, M., 2003. Determination of estrogens and their conjugates in water using solid-phase extraction followed by liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 984 (2), 195–202.
Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res. 42 (13), 3498–3518.
Kaštelan-Macan, M., Ahel, M., Horvat, A.J.M., Jabučar, D., Jovančić, P., 2007. Water resources and waste water management in Bosnia and Herzegovina, Croatia and the State Union of Serbia and Montenegro. Water Pol. 9 (3), 319–343.
Kirschner, A.K.T., Kavka, G.G., Velimirov, B., Mach, R.L., Sommer, R., Farnleitner, A.H., 2009. Microbiological water quality along the Danube River: integrating data from two whole-river surveys and a transnational monitoring network. Water Res. 43 (15), 3673–3684.
Kobayashi, Y., Okuda, T., Yamashita, N., Tanaka, H., Tanaka, S., Fuji, S., 2006. The behavior of free/conjugated estrogens during advanced wastewater treatment. Environ. Sanit. Eng. Res. 20, 55–58.
Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., et al., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. Environ. Sci. Technol. 36 (6), 1202–1211.
Komori, K., Tanaka, H., Okayasu, Y., Yasojima, M., Sato, C., 2004. Analysis and occurrence of estrogen in wastewater in Japan. Water Sci. Technol. 50 (5), 93–100.
König, M., Escher, B.I., Neale, P.A., Krauss, M., Hilscherová, K., Novák, J., et al., 2017. Impact of untreated wastewater on a major European river evaluated with a combination of in vitro bioassays and chemical analysis. Environ. Pollut. 220, 1220–1230.
Kuch, H.M., Ballschmiter, K., 2001. Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC–(NCI)–MS in the picogram per liter range. Environ. Sci. Technol. 35 (15), 3201–3206.
Le Coadou, L., Le Ménach, K., Labadie, P., Dévier, M.-H., Pardon, P., Augagneur, S., et al., 2017. Quality survey of natural mineral water and spring water sold in France: monitoring of hormones, pharmaceuticals, pesticides, perfluoroalkyl substances, phthalates, and alkylphenols at the ultra-trace level. Sci. Total Environ. 603–604, 651–662.
Leusch, F.D.L., Neale, P.A., Arnal, C., Aneck-Hahn, N.H., Balaguer, P., Bruchet, A., et al., 2018. Analysis of endocrine activity in drinking water, surface water and treated wastewater from six countries. Water Res. 139, 10–18.
Li, X., Ying, G.-G., Su, H.-C., Yang, X.-B., Wang, L., 2010. Simultaneous determination and assessment of 4-nonylphenol, bisphenol A and triclosan in tap water, bottled water and baby bottles. Environ. Int. 36 (6), 557–562.
Liska, I., 2015. Managing an international River basin towards water quality protection: The Danube Case. Handb. Environ. Chem. 39, 1–20.
Liu, Z.-h., Kanjo, Y., Mizutani, S., 2009. Urinary excretion rates of natural estrogens and androgens from humans, and their occurrence and fate in the environment: a review. Sci. Total Environ. 407 (18), 4975–4985.
Loos, R., Wollgast, J., Huber, T., Hanke, G., 2007. Polar herbicides, pharmaceutical products, perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and nonylphenol and its carboxylates and ethoxylates in surface and tap waters around Lake Maggiore in Northern Italy. Anal. Bioanal. Chem. 387 (4), 1469–1478.
Loos, R., Locoro, G., Contini, S., 2010. Occurrence of polar organic contaminants in the dissolved water phase of the Danube River and its major tributaries using SPE-LC-MS2 analysis. Water Res. 44 (7), 2325–2335.
Loos, R., Tavazzi, S., Mariani, G., Suurkuusk, G., Paracchini, B., Umlauf, G., 2017. Analysis of emerging organic contaminants in water, fish and suspended particulate matter (SPM) in the Joint Danube Survey using solid-phase extraction followed by UHPLC-MS-MS and GC–MS analysis. Sci. Total Environ. 607–608, 1201–1212.
Machado, K.C., Grassi, M.T., Vidal, C., Pescara, I.C., Jardim, W.F., Fernandes, A.N., et al., 2016. A preliminary nationwide survey of the presence of emerging contaminants in drinking and source waters in Brazil. Sci. Total Environ. 572, 138–146.
Manickum, T., John, W., 2014. Occurrence, fate and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa). Sci. Total Environ. 468–469, 584–597.
Mićić, V., Hofmann, T., 2009. Occurrence and behaviour of selected hydrophobic alkylphenolic compounds in the Danube River. Environ. Pollut. 157 (10), 2759–2768.
Milanović, M., Sudi, J., Grujić-Letić, N., Radonić, J., Turk-Sekulić, M., Vojinović-Miloradov, M., et al., 2016. Seasonal variations of bisphenol A in the Danube River by the municipality of novi sad, Serbia. J. Serb. Chem. Soc. 81 (3), 333–345.
Milic, N., Spanik, I., Radonic, J., Sekulic, M.T., Grujic, N., Vyviurska, O., et al., 2014. Screening analyses of wastewater and danube surface water in Novi Sad Locality, Serbia. Fresenius Environ. Bull. 23 (2), 372–377.
Miloradov, M., Mihajlovic, I., Vyviurska, O., Cacho, F., Radonic, J., Milic, N., et al., 2014. Impact of wastewater discharges to Danube surface water pollution by emerging and priority pollutants in the vicinity of Novi sad, Serbia. Fresenius Environ. Bull. 23, 2137–2145.
Morteani, G., Möller, P., Fuganti, A., Paces, T., 2006. Input and fate of anthropogenic estrogens and gadolinium in surface water and sewage plants in the hydrological basin of Prague (Czech Republic). Environ. Geochem. Health 28 (3), 257–264.
Naldi, A.C., Fayad, P.B., Prévost, M., Sauvé, S., 2016. Analysis of steroid hormones and their conjugated forms in water and urine by on-line solid-phase extraction coupled to liquid chromatography tandem mass spectrometry. Chem. Cent. J. 10, 30.
NORMAN-Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances (Accessed 10 September 2019).http://www.norman-network.com/.
Nurulnadia, M.Y., Koyama, J., Uno, S., Kito, A., Kokushi, E., Bacolod, E.T., et al., 2014. Accumulation of endocrine disrupting chemicals (EDCs) in the polychaete paraprionospio sp. from the yodo river mouth, osaka bay, Japan. Environ. Monit. Assess. 186 (3), 1453–1463.
Okkerman, P.C., van der Putte, I., 2002. Endocrine Disrupters: Study on Gathering Information on 435 Substances with Insufficient Data. RPS BKH Consultants B.V, Delft, p. 279.
Pantelić, M., Đurđev, B., Stankov, U., Dragićević, V., Dolinaj, D., 2012. Water quality as an indicator of local residents' attitudes towards tourism development: a case study of settlements along Veliki Bački Kanal, Vojvodina, Serbia. Knowl. Manag.

Aquat. Ecosyst. (404), 9.
Pedrouzo, M., Borrull, F., Pocurull, E., Marcé, R.M., 2009. Estrogens and their conjugates: determination in water samples by solid-phase extraction and liquid chromatography–tandem mass spectrometry. Talanta 78 (4–5), 1327–1331.
Pelayo, S., López-Roldán, R., González, S., Casado, M., Raldúa, D., Cortina, J.L., et al., 2011. A zebrafish scale assay to monitor dioxin-like activity in surface water samples. Anal. Bioanal. Chem. 401 (6), 1861.
Purdom, C.E., Hardiman, P.A., Bye, V.V.J., Eno, N.C., Tyler, C.R., Sumpter, J.P., 1994. Estrogenic effects of effluents from sewage treatment works. Chem. Ecol. 8 (4), 275–285.
Quednow, K., Püttmann, W., 2009. Temporal concentration changes of DEET, TCEP, terbutryn, and nonylphenols in freshwater streams of Hesse, Germany: possible influence of mandatory regulations and voluntary environmental agreements. Environ. Sci. Pollut. Control Ser. 16 (6), 630–640.
Reddy, S., Iden, C.R., Brownawell, B.J., 2005. Analysis of steroid conjugates in sewage influent and effluent by liquid Chromatography–Tandem mass spectrometry. Anal. Chem. 77 (21), 7032–7038.
Rodriguez-Mozaz, S., Marco, M.P., Lopez De Alda, M.J., Barceló, D., 2004. Biosensors for environmental monitoring of endocrine disruptors: a review article. Anal. Bioanal. Chem. 378 (3), 588–598.
Roslev, P., Vorkamp, K., Aarup, J., Frederiksen, K., Nielsen, P.H., 2007. Degradation of phthalate esters in an activated sludge wastewater treatment plant. Water Res. 41 (5), 969–976.
Rubirola, A., Boleda, M.R., Galceran, M.T., 2017. Multiresidue analysis of 24 Water Framework Directive priority substances by on-line solid phase extraction-liquid chromatography tandem mass spectrometry in environmental waters. J. Chromatogr. A 1493, 64–75.
Rutishauser, B.V., Pesonen, M., Escher, B.I., Ackermann, G.E., Aerni, H.-R., Suter, M.J.-F., et al., 2004. Comparative analysis of estrogenic activity in sewage treatment plant effluents involving three in vitro assays and chemical analysis of steroids. Environ. Toxicol. Chem. 23 (4), 857–864.
Selvaraj, K.K., Shanmugam, G., Sampath, S., Joakim Larsson, D.G., Ramaswamy, B.R., 2014. GC-MS determination of bisphenol A and alkylphenol ethoxylates in river water from India and their ecotoxicological risk assessment. Ecotoxicol. Environ. Saf. 99, 13–20.
Sodré, F.F., Pescara, I.C., Montagner, C.C., Jardim, W.F., 2010. Assessing selected estrogens and xenoestrogens in Brazilian surface waters by liquid chromatography–tandem mass spectrometry. Microchem. J. 96 (1), 92–98.
Solé, M., López de Alda, M.J., Castillo, M., Porte, C., Ladegaard-Pedersen, K., Barceló, D., 2000. Estrogenicity determination in sewage treatment plants and surface waters from the catalonian area (NE Spain). Environ. Sci. Technol. 34 (24), 5076–5083.
Song, M., Xu, Y., Jiang, Q., Lam, P.K.S., O'Toole, D.K., Giesy, J.P., et al., 2006. Measurement of estrogenic activity in sediments from Haihe and Dagu river, China. Environ. Int. 32 (5), 676–681.
Stojanović, V., Pantelić, M., Pavić, D., Nad, I., 2014. Remediation of Veliki bački kanal and sustainable use of resources in its surroundings. Geographica Pannonica 18 (4), 117–123.
Ternes, T.A., Stumpf, M., Mueller, J., Haberer, K., Wilken, R.D., Servos, M., 1999. Behavior and occurrence of estrogens in municipal sewage treatment plants — I. Investigations in Germany, Canada and Brazil. Sci. Total Environ. 225 (1), 81–90.
Terzic, S., Senta, I., Ahel, M., Gros, M., Petrovic, M., Barcelo, D., et al., 2008. Occurrence and fate of emerging wastewater contaminants in Western Balkan Region. Sci. Total Environ. 399 (1–3), 66–77.
The Statistical Office of the Republic of Serbia (Accessed 10 July 2019). http://www.stat.gov.rs/en-US.
Valcárcel, Y., Valdehíta, A., Becerra, E., López de Alda, M., Gil, A., Gorga, M., et al., 2018. Determining the presence of chemicals with suspected endocrine activity in drinking water from the Madrid region (Spain) and assessment of their estrogenic, androgenic and thyroidal activities. Chemosphere 201, 388–398.
Van den Belt, K., Verheyen, R., Witters, H., 2003. Comparison of vitellogenin responses in zebrafish and rainbow trout following exposure to environmental estrogens. Ecotoxicol. Environ. Saf. 56 (2), 271–281.
Vymazal, J., Březinová, T., Koželuh, M., 2015. Occurrence and removal of estrogens, progesterone and testosterone in three constructed wetlands treating municipal sewage in the Czech Republic. Sci. Total Environ. 536, 625–631.
Wang, J., Zhu, Y., 2017. Occurrence and risk assessment of estrogenic compounds in the East Lake, China. Environ. Toxicol. Pharmacol. 52, 69–76.
Wenzel, A., Böhmer, W., Müller, J., Rüdel, H., Schröter-Kermani, C., 2004. Retrosperctive monitoring of alkylphenols and alkylphenol monothoxylates in aquatic biota from 1985 to 2001: results from the German environmental specimen bank. Environ. Sci. Technol. 38 (6), 1654–1661.
Williams, M., Kookana, R.S., Mehta, A., Yadav, S.K., Tailor, B.L., Maheshwari, B., 2019. Emerging contaminants in a river receiving untreated wastewater from an Indian urban centre. Sci. Total Environ. 647, 1256–1265.
Wu, Q., Lam, J.C.W., Kwok, K.Y., Tsui, M.M.P., Lam, P.K.S., 2017. Occurrence and fate of endogenous steroid hormones, alkylphenol ethoxylates, bisphenol A and phthalates in municipal sewage treatment systems. J. Environ. Sci. 61, 49–58.
Xue, W., Wu, C., Xiao, K., Huang, X., Zhou, H., Tsuno, H., et al., 2010. Elimination and fate of selected micro-organic pollutants in a full-scale anaerobic/anoxic/aerobic process combined with membrane bioreactor for municipal wastewater reclamation. Water Res. 44 (20), 5999–6010.
Yao, B., Li, R., Yan, S., Chan, S.-A., Song, W., 2018. Occurrence and estrogenic activity of steroid hormones in Chinese streams: a nationwide study based on a combination of chemical and biological tools. Environ. Int. 118, 1–8.
Yarahmadi, H., Duy, S.V., Hachad, M., Dorner, S., Sauvé, S., Prévost, M., 2018. Seasonal variations of steroid hormones released by wastewater treatment plants to river water and sediments: Distribution between particulate and dissolved phases. Sci. Total Environ. 635, 144–155.
Yien Fang, T., Praveena, S.M., Aris, A.Z., Syed Ismail, S.N., Rasdi, I., 2019. Quantification of selected steroid hormones (17β-Estradiol and 17α-Ethynylestradiol) in wastewater treatment plants in Klang Valley (Malaysia). Chemosphere 215, 153–162.
Ying, G.-G., Williams, B., Kookana, R., 2002. Environmental fate of alkylphenols and alkylphenol ethoxylates—a review. Environ. Int. 28 (3), 215–226.
Zacs, D., Perkons, I., Bartkevics, V., 2016. Determination of steroidal oestrogens in tap water samples using solid-phase extraction on a molecularly imprinted polymer sorbent and quantification with gas chromatography-mass spectrometry (GC-MS). Environ. Monit. Assess. 188 (7), 433.
Zhang, K., Fent, K., 2018. Determination of two progestin metabolites (17α-hydroxypregnanolone and pregnanediol) and different classes of steroids (androgens, estrogens, corticosteroids, progestins) in rivers and wastewaters by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Sci. Total Environ. 610–611, 1164–1172.

Chapter 2

Occurrence of pharmaceutical residues in coastal areas



Article №3:

Mira Čelić, Meritxell Gros, Marinella Farré, Damia Barceló, and Mira Petrović

Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain),
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Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain)

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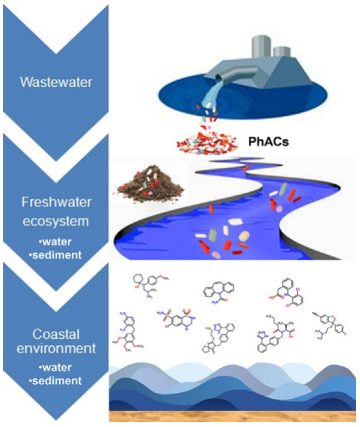
HIGHLIGHTS

- Distribution of PhACs followed along the wastewater-recipient water-sediment chain.
- WWTP discharges are an important source of PhACs contamination in the Ebro Delta.
- PhACs are subject to dilution once they reached freshwater and marine ecosystems.
- Sorption to sediments is a minor natural attenuation pathway.
- Ecologically relevant PhACs highlighted as markers of wastewater contamination.

GRAPHICAL ABSTRACT



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ABSTRACT

This study evaluated the occurrence and distribution of 81 pharmaceutically active compounds (PhACs) in the vulnerable area of the Ebro Delta region (Catalonia, Spain), to assess the environmental impact of wastewater treatment plants discharge to coastal environments. The occurrence of PhACs was followed along the wastewater-recipient water-sediment chain until they reach estuaries and the Mediterranean Sea. Water and sediment samples were collected in an integrated way at different sampling points covering three different seasons in reaches of the Ebro River located upstream and downstream from wastewater treatment plants (WWTPs), surrounding channels, estuaries, and the associated receiving seawater. 28 out of the 57 compounds detected in effluent wastewater were positively identified in estuary and seawaters, revealing that WWTP discharges are an important source of contamination in coastal environments and that PhACs are suitable markers of urban contamination in these areas. The substances with the highest frequency of detection belonged to the groups of analgesics/anti-inflammatories (acetaminophen, salicylic acid), antihypertensives (valsartan), psychiatric drugs (carbamazepine), and antibiotics (clarithromycin, trimethoprim). In general, a decrease in concentration was observed from inland sampling points towards the Mediterranean Sea, resulting from a dilution in the recipient marine water bodies. A reduced number of PhACs, at concentrations ranging from 0.1 to 12.5 ng g^{-1} dry weight (d.w.) was detected in sediment samples, indicating that sorption is a minor natural attenuation pathway for these compounds. Finally, a prioritization strategy, based on the compounds concentration and frequency of detection in seawater, removal efficiency in WWTP, bioaccumulation potential, toxicity to marine

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organisms and persistency, was used to highlight the PhACs of major ecological concern and that could be used as relevant indicators of wastewater contamination in coastal environments.

1. Introduction

Pharmaceutically active compounds (PhACs), which are widely used in both human and veterinary medicine, represent an important group of emerging organic contaminants. PhACs can enter the aquatic environment through different pathways, such as wastewater treatment plant (WWTP) discharges, hospital effluents, direct disposal of unused or expired drugs, manufacturing, landfill leachates, livestock activities, aquaculture, and soil fertilization with sewage sludge and/or livestock waste (Gros et al., 2013; Gaw et al., 2014; Chen et al., 2015). However, from all these sources, WWTP effluent discharges are considered as the main route of entry of these pollutants in environment (Ali et al., 2017). Several studies have reported that these substances are partially removed during wastewater treatment (Collado et al., 2014; Acuña et al., 2015) and thus, they are discharged into surface and coastal environments (Yoon et al., 2010; Birch et al., 2015). Even though the occurrence and behavior of PhACs in freshwater ecosystems has been widely reported (Kasprzyk-Hordern et al., 2008; Lin and Tsai, 2009; Daneshvar et al., 2010; Bu et al., 2013; Pereira et al., 2017; Wu et al., 2017; Lin et al., 2018; Bai et al., 2018), information about their occurrence and fate in vulnerable coastal settings, influenced by WWTP discharges, is still sparse (Nödler et al., 2014; Ali et al., 2017; Fisch et al., 2017). Indeed, a restricted number of studies focused their attention on natural protected coastal environments, and/or investigated the natural attenuation processes (i.e. sorption to sediments) to which PhACs are subjected since they enter the aquatic environment through WWTP effluents until they reach the open sea. The occurrence of PhACs in marine ecosystems can pose several negative impacts for both human health and the environment. Even though direct effects of single PhACs are expected to be minor, compared to freshwater ecosystems due to higher dilution factors, it is still necessary to investigate potential long-term effects and risks for marine organisms. Some studies have already reported that PhACs can bioaccumulate in fat tissues of fish, mussels, and other seafood (Martínez Bueno et al., 2014; Dodder et al., 2014; Álvarez-Muñoz et al., 2015) and thus, be introduced into the human food chain (Zenker et al., 2014; Done and Halden, 2015).

Although PhACs have been widely monitored, they are not included in environmental regulations yet. Only four PhACs were included in the first "Watch List" of emerging pollutants to be monitored Europe-wide (European Commission, 2015) with the final goal to be added in the list of priority pollutants of the Water Framework Directive (European Commission, 2000). These PhACs included the analgesic and anti-inflammatory drug diclofenac, and the macrolide antibiotics erythromycin, azithromycin, and clarithromycin. In a recent update of the compounds included in the "Watch List", diclofenac was excluded, while two antibiotics, amoxicillin and ciprofloxacin, were incorporated (European Commission, 2018), bringing it to the total number of five PhACs in the list.

In this context, the main objectives of this study were to increase the existing knowledge about the occurrence, distribution, and fate of a large number of multiple-class PhACs in coastal areas influenced by WWTP discharges and to propose a list of ecologically relevant PhACs as markers of wastewater contamination. This study focused its attention on the vulnerable area of the Ebro River Delta (Catalonia, north-east of Spain), which is the third largest delta in the Mediterranean Sea and one of the most important wetlands in the Mediterranean region. This is a site of special interest because its contamination might seriously endanger its ecological richness and biodiversity as well as compromise water quality. Historically, several monitoring campaigns were performed within the Ebro River Basin (Gros et al., 2010; Postigo et al., 2010; da Silva et al., 2011; López-Serna et al., 2012; Mastroianni et al., 2016; Pignotti et al., 2017). These studies focused their attention on the occurrence of wastewater derived contaminants (i.e. PhACs, perfluoroalkyl substances, drugs of abuse) in water, sediment and biota samples in the Ebro River and its tributaries, while only one of them investigated the Ebro Delta and its associated marine environment (Pignotti et al., 2017). However, this study only focused on perfluoroalkyl substances and did not include other relevant wastewater-derived organic contaminants such as PhACs. Thus, in this study, a comprehensive monitoring was performed, collecting water and sediment samples in different wastewater impacted sites along the Ebro Delta, to assess the persistence and attenuation of PhACs from the contamination source to receiving water bodies, channels, estuaries, and finally, the Mediterranean Sea. To this end, different types of samples were collected, covering three seasons: (i) influent and effluent wastewater samples, from the two main WWTPs in the area (Amposta and Sant Carles de la Rápita); (ii) water and sediments of the water bodies receiving WWTP effluent discharges; and (iii) water and sediments from channels, estuaries, and the sea. Among the channels monitored in this study, some of them collect and transport the sewage impacted freshwater used for the irrigation of rice fields, while others collect the water used in the fields and transport it to estuaries and the Mediterranean Sea.

2. Materials and methods

2.1. Chemicals

All pharmaceutical standards (Table S1, in Supporting Information (SI)) were of high purity grade (>90%). Compounds were purchased from Sigma–Aldrich (Steinheim, Germany), the US Pharmacopeia (USP) and the European Pharmacopeia (EP). Isotopically labeled compounds, used as internal standards (ISs), were atenolol-d_7, ronidazole-d_3, cimetidine-d_3, ofloxacin-d_3, sulfamethoxazole-d_4, phenazone-d_3, venlafaxine-d_6, citalopram-d_4, verapamil-d_6, carbamazepine-d_{10}, amlodipine-d_4, erythromycin-N,N$^{13}C_2$, fluoxetine-d_5, diazepam-d_5, warfarin-d_5, glibenclamide-d_3, atenolol-d_7, ronidazole-d_3, cimetidine-d_3, acetaminophen-d_4, valsartan-d_8, furosemide-d_5, meloxicam-d_3, bezafibrate-d_6, ibuprofen-d_3, indomethacine-d_4, dexamethasone-d_4, and gemfibrozil-d_6. These substances were purchased from Sigma-Aldrich and from Toronto Research Chemicals (Ontario, Canada). IS, used as surrogate standards, were sulfadoxine-d_3, sulfadimethoxine-d_6, and ketoprofen-d_3.

Individual stock standard, ISs, and surrogate solutions were prepared on a weight basis in methanol (at a concentration of 1000 mg L^{-1}), except ofloxacin and ciprofloxacin, which were dissolved in methanol adding 100 μL of NaOH 1 M, and cefalexin which was solved in high performance liquid chromatography (HPLC) grade water. After preparation, standards were stored at −20 °C. Antibiotic stock solutions (macrolides, tetracycline, sulfonamides, cephalosporins, and nitroimidazoles) were prepared every three months, while fluoroquinolone and tetracycline antibiotics were prepared monthly due to their limited stability. Stock solutions for the rest of substances were renewed every six months. A mixture containing all PhACs was prepared by appropriate dilution of individual stock solutions in methanol–water (10:90 v/v for water and 25:75, v/v for sediment samples, respectively). Working standard solutions were renewed before each analytical run. A separate mixture of IS, used for internal standard calibration, and surrogates were also prepared in methanol

and further diluted in methanol–water (10:90 v/v for water and 25:75, v/v for sediments). The cartridges used for solid phase extraction (SPE) were Oasis hydrophilic-lipophilic-balanced (HLB) sorbent (60 mg, 3 mL) and Oasis HLB (200 mg, 6 mL), both from Waters Corporation (Milford, MA, USA). Glass fiber filters (GFF) and polyvinylidene fluoride (PVDF) filters were purchased from Whatman (U.K.) and Merck Millipore (Darmstadt, Germany). HPLC grade methanol, acetonitrile, water (LiChrosolv), and formic acid 98% were supplied by Merck (Darmstadt, Germany). Ammonium hydroxyde, hydrochloric acid, and ethylenediaminetetraacetic acid disodium salt solution (Na_2EDTA) at 0.1 M were from Panreac. Nitrogen for drying was from Abelló Linde S.A. (Spain), and it was of 99.9% purity. A Milli-Q-Advantage system from Millipore Ibérica S.A. (Spain) was used to obtain HPLC-grade water.

2.2. Description of the study area

The Ebro Delta is located in the mouth of the Ebro River, in the province of Tarragona, Catalonia (NE Spain). It is the third largest wetland area in the western Mediterranean region, with a surface area of 320 km^2. It was declared a natural park (1984) and it was included in the Ramsar Convention list (1993) of wetlands of international importance, as defined for the conservation and sustainable utilization of wetlands. The area is composed of natural lagoons, bays, irrigation and drainage channels, salt pans and marshes that provide extensive habitats. The use of the Ebro Delta and the upper part of the Ebro basin (around 21.000 ha) is dedicated mainly to agricultural activities, such as rice and horticulture, as well as aquaculture. About 20% of the continental aquaculture production in Spain takes place in the Ebro Delta that is also one of the main shellfish producers. Currently, there are 13 aquaculture facilities in the area, mainly located near the Alfacs Bay. Furthermore, the Ebro Delta is also a touristic area, known for seafood, fisheries, and leisure activities in two semi-enclosed embayments, Alfacs and Fangar Bay (Fig. 1). Hydrodynamics in the Ebro Delta are influenced by the Mediterranean Sea and by freshwater inflows coming from the drainage channels that collect used water from the rice fields. This region experiences a huge variability of the precipitation regime, with high precipitation during late fall and winter (200–300 mm), and intensive summer drought (<50 mm). The climate is typically Mediterranean, with mean annual temperature between 12 and 22 °C (Sainz-Elipe et al., 2010). In the Ebro Delta there are eight WWTPs, located in several towns, such as Sant Carles de la Ràpita, Amposta, l'Ampolla, Deltebre, l'Aldea, Sant Jaume d'Enveja, Camarles and els Muntells. However, Amposta and Sant Carles de la Ràpita WWTPs have the highest population equivalents (PE), and thus, they can be considered as the main sources of wastewater contamination, in the surrounding freshwater and marine environment. Amposta (WWTP1) has primary and secondary wastewater treatment with activated sludge, with a total capacity of 27.500 PE. Sant Carles de la Ràpita (WWTP2) uses primary and secondary treatment, also based on conventional activated sludge, followed by tertiary treatment (sand filter), with a total capacity of 28.921 PE. Besides urban and agricultural activities, there are several chemical industries and a nuclear power plant in the area, which are also a non-negligible source of contamination.

2.3. Sampling sites and sample collection

A total of 156 samples, including 84 water and 72 sediments, were collected (Table S2, SI). In order to evaluate potential seasonal variations, sampling campaigns were carried out in different seasons, covering autumn (October–November 2015), winter (February–April 2016), and spring (May–June 2016) period. For freshwater (river, channels, estuaries, and sea), water and surface sediment samples were collected. For water the physicochemical parameters measured were: temperature, pH, oxygen, conductivity, salinity and flow, while for sediments the total organic carbon (TOC) was recorded. Physicochemical parameters of water samples were measured in-situ by using hand-held probes (WTW, Weilheim, Germany) and for sediments, TOC

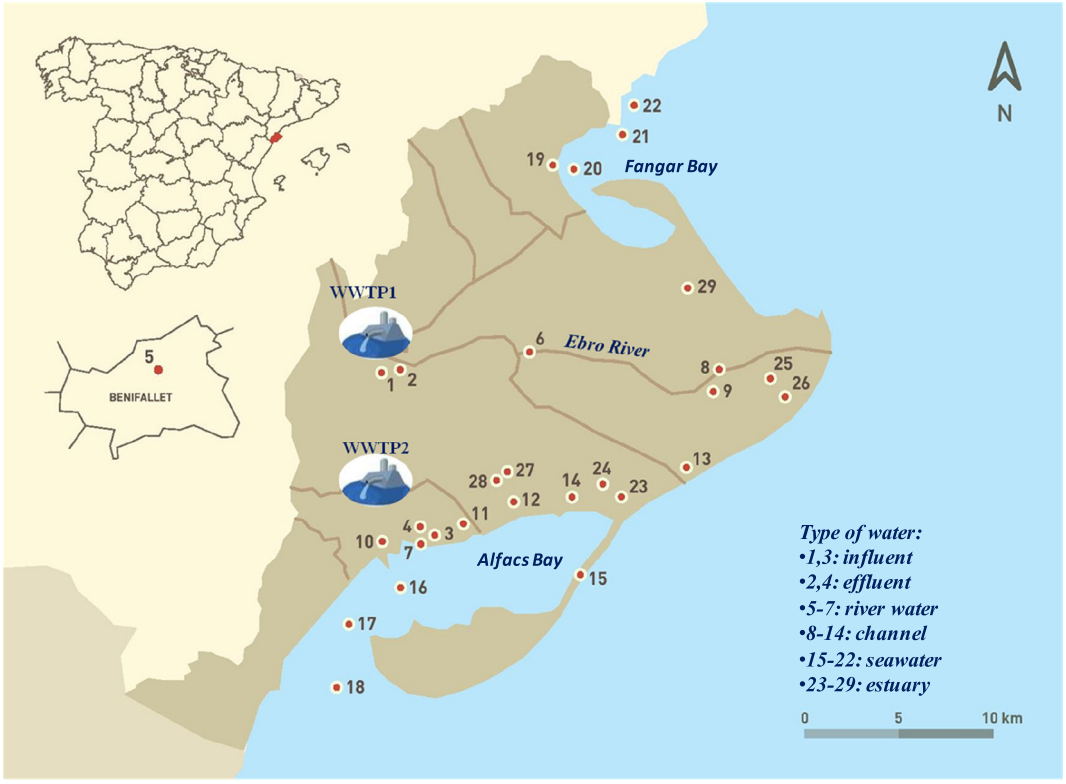

Fig. 1. Map indicating the sampling locations in the Ebro Delta.

was analyzed using a TOC analyzer (TOC-V CSH, Shimadzu), following the method Norma UNE-EN 1484. These parameters are reported in Table S3 in SI. Fig. 1 schematically shows the sampling area and the sampling sites. Samples taken include: (a) influent and effluent wastewaters from WWTP1-Amposta (sampling sites 1–2) and WWTP2-Sant Carles de la Ràpita (sampling sites 3–4); (b) water and sediment of the upstream (5) and downstream sites of WWTP1 (6) and WWTP2 (7). Effluents from WWTP1 are discharged into the Ebro River, whereas effluents from WWTP2 go to the emissary in Sant Carles de la Ràpita, being afterwards discharged directly to the sea (Alfacs Bay); (c) water and sediment from two irrigation channels (channels A and B; sampling sites 8–9) that are directly connected with the Ebro River, and are used to irrigate the rice and agricultural fields in the area and five drainage channels (C, D1–D4; sites 10–11–12–13–14) that collect used water from the rice fields and discharge their waters into the sea; (d) eight seawater and sediments, taken from the shore of Alfacs (sites 15–16) and Fangar (19–20) bays and offshore at the open sea adjacent to these bays (sites 17–18 and 21–22); and finally (e) sites 23–29 correspond to estuary water and sediment samples collected from different estuaries across the area (Illa de Buda, L'Encanyissada, La Tancada and Canal Vell), which collect the water coming from the surrounding drainage channels. Influent and effluent wastewater samples were 24 h composite samples, whereas the other types of water and sediment (0–20 cm) were grab samples. Water samples were collected in 1 L amber polyethylene bottles, previously rinsed with ultrapure water, whereas sediment samples were collected with a dredge and placed in an aluminum tray. Samples were transported in a refrigerated isothermal container and stored at −20 °C until analysis. All sediment samples were freeze-dried (LioAlfa 6, Telstar) at −80 °C and with 0.044 bar vacuum. The lyophilized samples were grounded and homogenized using a mortar and pestle and then sieved through a 125 μm sieve. Freeze-dried and sieved samples (˂125 μm fraction) were stored in pre-cleaned glass jars at −20 °C until laboratory processing.

2.4. Sample preparation

Water sample analysis was carried out following the method described by Gros et al. (Gros et al., 2012). Briefly, water samples were filtered through 1 μm GFF, followed by 0.45 μm PVDF, while seawater was filtered only through the 0.45 μm PVDF filters. A suitable volume of a 0.1 M Na_2EDTA solution was added to the different types of water to achieve a final concentration of 0.1% (g solute/g solution). 25 mL of wastewater influents, 50 mL of effluents, 100 mL of river, estuary and channel water, and 500 mL of seawater were measured. Water samples were spiked with an appropriate volume of a standard mixture containing surrogate standards, in order to have a concentration of 200 ng L^{-1} in influent, 100 ng L^{-1} in effluent wastewaters, 50 ng L^{-1} in river, channel and estuary water, and 10 ng L^{-1} for seawater. Samples were extracted by SPE using Oasis HLB (60 mg, 3 mL) cartridges, except for seawater where Oasis HLB (200 mg, 6 mL) were used. Cartridges were previously conditioned with methanol (5 mL) and HPLC water (5 mL). After sample loading, cartridges were rinsed with 6 mL of HPLC grade water, at a flow rate of 2 mL min^{-1}, and were dried for 5 min, to remove excess of water. Finally, analytes were eluted with 8 mL of pure methanol at a flow rate of 1 mL min^{-1}. Extracts were evaporated to dryness under a gentle stream of purified nitrogen (>99.9%) using the nitrogen evaporator Reacti-Therm III Heating Module (TS-18824) (Thermo Fisher Scientific, Driesch, Germany) and then reconstituted to 1 mL with a final proportion of methanol/water (10:90, v/v). Finally, 10 μL of a 1 ng L^{-1} standard mixture containing all isotopically labeled standards were added in the extract as IS.

Extraction and clean-up of sediment samples were carried out using the method described by Jelić et al. (Jelić et al., 2009). However, in our study the number of target analytes was increased to 38 compounds in comparison with the original method, where only 43 PhACs were determined. Aliquots of freeze-dried and sieved sediment (1 g) were mixed with Hydromatrix in 11 mL stainless steel extraction cells and extracted by pressurized liquid extraction (PLE) using a Dionex ASE 350 system (Dionex; Sunnyvale, CA). Sample extraction was performed using a methanol-water mixture (1:2, v/v) as extraction solvent, at 1500 psi and 100 °C in 3 static cycles, each one lasting 5 min. Finally, the extraction cell was flushed with 100% cell volume of fresh solvent. The extract obtained in PLE (22 mL) was diluted with 500 mL of HPLC water, in order to reduce the content of methanol (<5 vol%) and was extracted by SPE using Oasis HLB (200 mg, 6 mL) cartridges, following the same procedure as for water samples. Elution was performed with pure methanol (8 mL). The eluates were evaporated under nitrogen stream and reconstituted in 1 mL methanol-water mixture (25:75, v/v). Prior to analysis, the samples were passed through 0.45 μm PVDF filters and fortified with a standard mixture of ISs to have a concentration of 20 ng mL^{-1} in the extracts. All samples were processed in triplicate.

2.5. Instrumental analysis

PhACs detection and quantification in the samples was done by ultra-performance liquid chromatography (UPLC), using a Waters Acquity Binary Solvent Manager system (Waters Corporation, MA, USA) coupled to the 5500 QTRAP (Applied Biosystems, Foster City, CA, USA), a quadrupole linear ion trap tandem mass spectrometer ($QqLIT^-$), with a Turbo Ion Spray source, following the method developed by Gros and coworkers (Gros et al., 2012). Chromatographic separation of the PhACs analyzed under positive electrospray ionization (PI) was achieved using an Acquity HSS T3 column (50 mm × 2.1 mm i.d., 1.8 μm particle size), while an Acquity BEH C_{18} column (50 mm × 2.1 mm i.d., 1.7 μm particle size) was used for the compounds analyzed under negative electrospray ionization (NI). Both columns were purchased from Waters Corporation. For the analysis in PI mode, methanol and 10 mM formic acid/ammonium formate (pH 3.2) were used as a mobile phase at the flow rate of 0.5 mL min^{-1} while, for the analysis in the NI mode, acetonitrile and 5 mM ammonium acetate/ammonia (pH = 8) were used at the flow rate of 0.6 mL min^{-1}. The sample volume injected was 5 μL for both modes. For quantitative and detection purposes, two Selected Reaction Monitoring (SRM) transitions were monitored for each compound and a summary of the optimum SRM transitions and conditions is available in Table S4 in SI. All data was acquired and processed using Analyst 1.6.3 software.

2.6. Quality assurance and quality control

Quality parameters of the analytical methods, for the different types of water and sediments analyzed, included: extraction recoveries (% Rec) and method precision (%RSD), method detection (MDLs) and quantification limits (MQLs). Results are summarized in Tables S5 and S6 in SI, respectively. Eight-point calibration curves (0.1–100 μg L^{-1}) were generated using linear regression analysis. Calibration curves were injected at the beginning and the end of each sequence, and one calibration standard was measured repeatedly throughout the sequence, after every 20–25 injections, to check for signal stability. R-squared values were consistently >0.99 for all PhACs. Method and instrumental blanks were performed to account for any background levels of the analytes investigated. For water and sediment samples, recoveries were determined in triplicate by spiking a known concentration of target analytes and comparing the obtained concentrations after the whole analytical process with the initial spiking levels, calculated by internal standard calibration. Blanks (non-spiked samples) were also analyzed and the levels found, were subtracted from those obtained from spiked samples. Spiking concentrations used for water samples were: 400 ng L^{-1} for influent and effluent wastewaters, 50 ng L^{-1} for river, channels and estuary and 10 ng L^{-1} for seawater samples. For sediments, a standard mixture containing all target PhACs was added at a concentration of 25 ng g^{-1} dry weight. Spiked sediment samples were left 24 h for equilibration, before extraction. Spiked water and sediment samples were

used to determine MDL and MQL. MDL and MQL were established as the minimum detectable amount of analyte with a signal-to-noise ratio of 3 and 10, respectively. Quantification of the samples was based on peak areas and was performed by the internal standard calibration approach. For each compound its corresponding isotopically labeled analogue was used, except for those substances whose corresponding labeled compound was not available. In this case, the most similar labeled substance, in terms of chemical structure and chromatographic retention time, was used as IS (Table S1, SI). The identification and confirmation criterion for the analysis of the target compounds was based on the Commission Decision 2002/657/CE (European Comission, 2002), with the following criteria: (1) LC chromatographic retention time agreement within 2% between samples and standards; and (2) the ratio between the quantification and identification SRM transitions, which should fall within a range ± 20% in standards and samples.

2.7. Statistical analysis

Statistical analyses were performed with SPSS software, version 18.0 (SPSS Inc., Chicago, IL, USA). In all statistical analyses, undetected compounds and compounds below MQL were given the corresponding MDL/2 and MQL/2 value. Two nonparametric methods, the Mann-Whitney U and the Kruskal-Wallis test, were performed to assess the statistical differences in the total PhACs concentrations of all compounds and therapeutic groups between each sampling season. The existence and strength of relationships between variables was established by Pearson bivariate correlation coefficient. The significance level for all applied analysis was at level $p < 0.05$. All tests were performed at 95% confidence level.

3. Results and discussion

3.1. Occurrence of PhACs and removal in WWTP

The individual concentrations of the PhACs detected in influent and effluent wastewater samples, collected during three sampling seasons, at two WWTPs, are summarized in Table S7a in SI. Overall, considering all sampling campaigns, 63 out of the 81 monitored compounds were detected in influent, whereas nearly 57 out of 81 were found in effluent. The concentrations detected in both treatment facilities were similar and within the same order of magnitude. Concentration levels of detected PhACs in influent samples ranged from 5.1 ng L^{-1} to 45.3 μg L^{-1}, while in treated wastewater concentrations of PhACs from ng L^{-1} to 3.8 μg L^{-1} were found (Table S7b, SI). Indeed, Mann Whitney *U* test highlighted that there were no significant differences between the total concentrations of the different therapeutic groups in the two treatment facilities ($p > 0.05$). In raw wastewaters, the analgesics and anti-inflammatories were generally the most ubiquitous therapeutic group, followed by antihypertensives, lipid regulators, β-blockers, diuretics and antihelmintics. The highest maximum concentrations for individual compounds were attributed to ibuprofen (45.3 μg L^{-1}), acetaminophen (43.0 μg L^{-1}), and naproxen (26.3 μg L^{-1}) for analgesics/anti-inflammatories, gemfibrozil (11.8 μg L^{-1}) for lipid regulators, valsartan (18.9 μg L^{-1}) for antihypertensives, atenolol (6.1 μg L^{-1}) for β-blocking agents, levamisole (4.8 μg L^{-1}) for antihelmintics, hydrochlorothiazide (3.4 μg L^{-1}) and furosemide (3.4 μg L^{-1}) for diuretics (see Table S7a, SI). Antibiotics were ubiquitous compounds as well. However, their concentration levels ranged from <MDL to 2.8 μg L^{-1}. The most ubiquitous compounds within this group were azithromycin, ofloxacin, ciprofloxacin, sulfamethoxazole, and trimethoprim.

Concerning treated wastewaters, the levels of PhACs were generally lower than in the corresponding influent samples. In fact, there were statistically significant differences in total PhACs concentrations for the majority of therapeutic groups between influent and effluent wastewater (Mann Whitney *U* test, $p < 0.05$). Even though individual concentrations of detected compounds fell within the low μg L^{-1} range, a great number of compounds were still detected, indicating that PhACs are not completely removed during wastewater treatment, as it has been widely reported (Gracia-Lor et al., 2012; Al Aukidy et al., 2012; Collado et al., 2014; Chen et al., 2015; Aymerich et al., 2016; Rivera-Jaimes et al., 2018). The compounds detected at the highest maximum concentrations in effluents were valsartan (3.8 μg L^{-1}), followed by furosemide (1.6 μg L^{-1}), hydrochlorothiazide (1.5 μg L^{-1}), levamisol (1.4 μg L^{-1}), venlafaxine (1.2 μg L^{-1}), and azithromycin (1.1 μg L^{-1}) (see Table S7a, SI). In general, there were no statistically significant differences regarding the removal rates of the compounds and therapeutic groups in the two WWTP facilities (Kruskal-Wallis test; $p > 0.05$) within sampling campaigns. Thus, average removal efficiencies (RE), considering the reduction rates in each sampling campaign, are presented in Fig. S1 and Table S8 in SI. Target PhACs could be classified in different groups, according to their RE in WWTP: (a) compounds with high RE (>80%), including analgesics and anti-inflammatories (ibuprofen, acetaminophen, naproxen, salicylic acid), lipid regulators (gemfibrozil and atrovastatin), the antihypertensive valsartan, and the antidiabetic glibenclamide; (b) substances with moderate RE (35–80%), where most PhACs are fitted, including compounds such as antibiotics (trimethoprim, sulfamethoxazole, ofloxacin, erythromycin), the antihypertensive losartan, the lipid regulator bezafibrate, the diuretics furosemide and hydrochlorothiazide, the anthelmintic thiabendazole, the β-blocker metoprolol, the histamine H1 receptor antagonist desloratadine, and the analgesic diclofenac, among others; (c) compounds with poor reduction rates (˂35%), such as psychiatric drugs carbamazepine and venlafaxine, the antibiotics clarithromycin and azithromycin; and (d) compounds that showed no removal, such as ketoprofen, verapamil, iopromide, phenazone, and levamisol. The compounds included in groups (b), (c), and (d) were the most ubiquitous PhACs in wastewater effluents. Even though analgesics and anti-inflammatories showed high RE, they were present in the effluent samples at remarkable concentrations (up to 1.5 μg L^{-1} for ketoprofen). Concentration levels detected in influent and effluent wastewaters, as well as removal rates reported are in good agreement with those previously reported in the scientific literature (Gros et al., 2010; Behera et al., 2011; Fang et al., 2012; Collado et al., 2014; Lara-Martín et al., 2014). Nevertheless, for some compounds, the concentrations detected in this study were somewhat higher than those found in previous studies (Sim et al., 2010; Al Aukidy et al., 2012; Chen et al., 2015; Rivera-Jaimes et al., 2018). This could be attributed to the differences in seasonal drug consumption in each country (Rodríguez-Navas et al., 2013). In general, Kruskal-Wallis ($p > 0.05$) test indicated that there were no significant seasonal differences regarding PhACs concentrations in both influent and effluent wastewaters, except for some therapeutic groups, such as the antiplatelet agent (clopidogrel), the X-ray contrast agent (iopromide) and a drug to treat asthma (salbutamol).

3.2. PhACs in wastewater impacted freshwater bodies, the Ebro River, surrounding channels, estuaries and the Mediterranean Sea

In order to evaluate the input of PhACs into the Mediterranean Sea through WWTP effluents, water and sediment samples were collected from the Ebro River and emissary that receive wastewater discharges, as well as in irrigation and drainage channels, estuaries, and finally the Mediterranean Sea along three different seasons. First, the distribution of PhACs in water samples is discussed and after that, the sorption to sediments as a potential natural attenuation pathway is evaluated.

3.2.1. Occurrence in water samples

The individual concentrations of PhACs detected in water samples are summarized in Table S9 in SI. Fig. 2 shows cumulative levels (ng L^{-1}) of different therapeutic groups of PhACs detected in all sampling sites during three sampling seasons. For the wastewater impacted sites, samples were taken upstream (site number 5, CSW) and

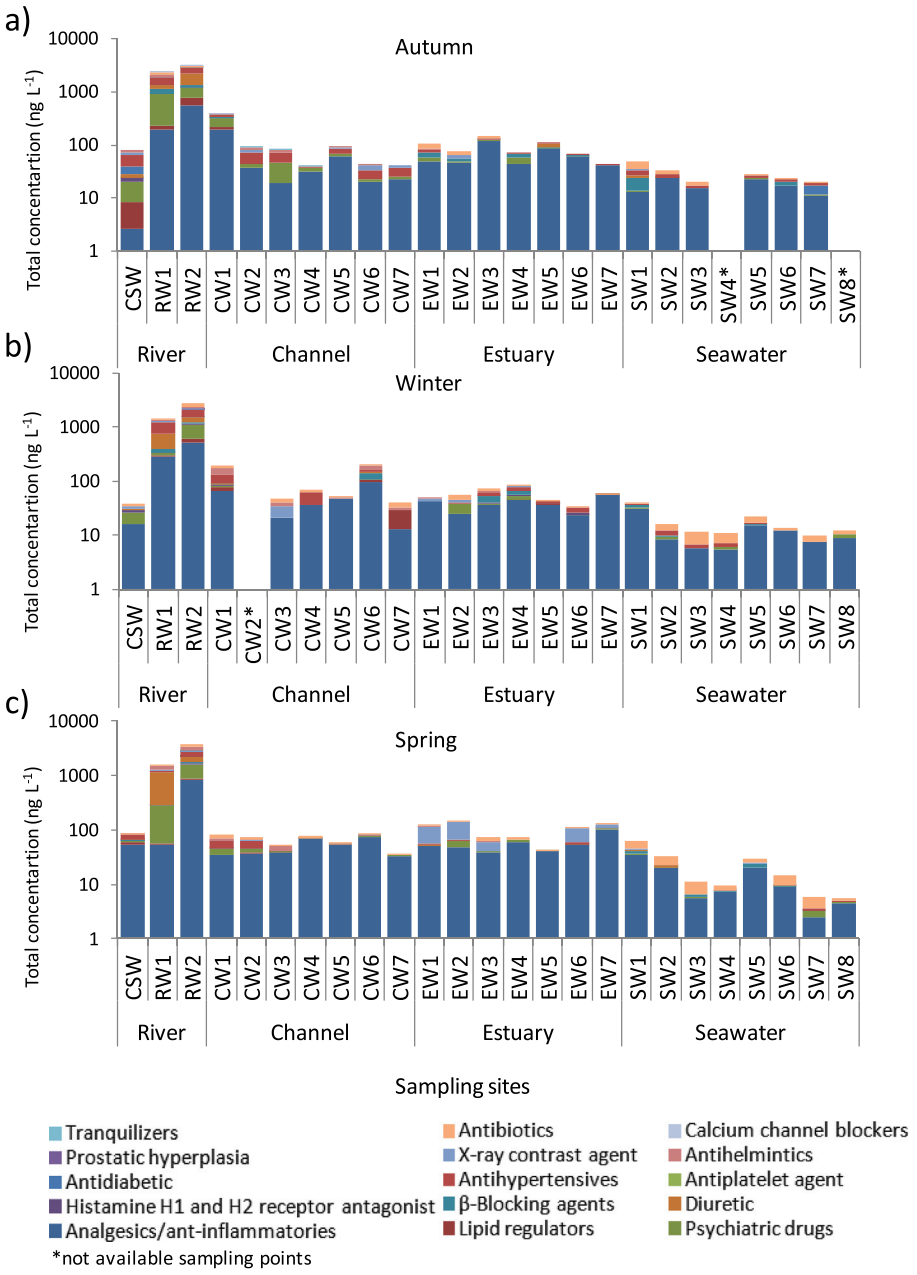

Fig. 2. Cumulative levels (ng L^{-1}) of different therapeutic groups of PhACs detected in freshwater and marine sampling sites during the autumn (a), the winter (b) and the spring season (c).

downstream from WWTP1 (site number 6, RW1) in the Ebro River, and downstream from WWTP2 in the emissary Sant Carles de la Rápita (site number 7, RW2), which discharges the effluents into the Mediterranean Sea (Fig. 1). The samples taken at the control site (CSW) correspond to the Ebro River, located near Xerta town. Even though this area was selected as a reference site, PhACs were detected at non-negligible concentration levels (<MQL up to 53.6 ng L^{-1} range), with a total maximum concentration of 87.4 ng L^{-1}. The occurrence of PhACs in this location could be attributed to both anthropogenic and agricultural inputs from the neighboring towns (Pignotti et al., 2017). Results indicated that a higher number of PhACs were detected in wastewater impacted sites (RW1 and RW2), compared with the upstream location (CSW). Mann-Whitney *U* test confirmed that there were significant differences between total PhACs concentrations (sum of all compounds) between upstream and downstream locations ($p < 0.05$). In these sites, 63 different PhACs out of the 81 monitored were detected, considering all sampling campaigns. 19 of these compounds were detected in at least two of the three sampling campaigns, whereas 11 were positively identified in only one season and the compounds found coincide with those present in effluent wastewaters. Thus, the most ubiquitous therapeutic groups, with individual compounds found at the highest concentrations (>100 ng L^{-1}) were: anti-hypertensives (valsartan, ibersartan, losartan), diuretics (hydrochlorothiazde, furosemide), analgesics and anti-inflammatories (diclofenac, phenazone, ketoprofen, acetaminophen), anthelmintics (levamisol, albendazole), phychiatric drugs (venlafaxine, lorazepam, carbamazepine), antibiotics (ofloxacin, azithromycin, ciprofloxacin), the lipid regulator (gemfibrozil), and the β-blocker (atenolol) (Table S10, SI). PhACs concentrations in RW1 were found with maximum concentration up to 289.7 ng L^{-1}, and this level is one order of magnitude lower than the maximum concentrations detected in the discharged wastewater effluent from WWTP1, indicating that PhACs are subject to a remarkable dilution factor when they are discharged into the Ebro River. Indeed, for the winter season, a dilution factor of 196 was estimated for site RW1 (2.28 m^3 s^{-1} of

WWTP1 effluent was mixed with 447 $m^3 s^{-1}$ of river flow), while a factor of 60 was calculated for autumn and spring (approximately 2.70 $m^3 s^{-1}$ of effluent being mixed with 161 $m^3 s^{-1}$ of river water). In fact, Kruskal-Wallis tests ($p < 0.05$) confirmed that there were statistically significant seasonal differences in total PhACs concentrations for site RW1 between different sampling seasons. In RW2, concentration levels were higher than those found in RW1, ranging from 1.2 to 571.3 ng L^{-1} and PhACs levels were within the same range as those detected in WWTP2 effluents. Indeed, Mann-Whitney *U* test confirmed that there were significant differences in the total PhACs concentrations (sum of all compounds) between the two wastewater-influenced sites ($p < 0.05$). However, Kruskal-Wallis tests ($p > 0.05$) indicated that there were no statistically significant seasonal differences in total PhACs concentrations in sampling site RW2; this could be explained by the fact that emissary RW2 is mostly composed by constant wastewater effluent released from WWTP2 Sant Carles de la Ràpita and is subject to minor changes in the flow, whereas PhACs present in RW1 are subject to a dilution factor that is more dependent on the Ebro River flow.

Regarding the channels, 46 different PhACs out of the 81 monitored were detected taking into account all sampling campaigns, where 13 of them were identified in at least two of three sampling events. The compounds most frequently detected in channel waters were salicylic acid (85% of the samples), carbamazepine (70%), thiabendazole (65%), and valsartan (55%), while the presence of antibiotics, such as ofloxacin, clarithromycin, sulfamethoxazole, and trimethoprim, was rather low (29–67%), being mostly found in irrigation channels A and B (sites 8–9) at concentrations ranging from 0.3 to 13.9 ng L^{-1} (Table S10, SI). Indeed, sites downstream WWTPs showed greater antibiotic concentrations than the ones recorded in the channels. Individual PhACs concentrations ranged from <MQL to 48.8 ng L^{-1} and a similar chemical profile than in wastewater impacted sites was observed. Specifically, the highest concentrations were detected for naproxen (48.8 ng L^{-1}), ibuprofen (45.3 ng L^{-1}), ketoprofen (39.2 ng L^{-1}), salicylic acid (38.8 ng L^{-1}), and acetaminophen (35.2 ng L^{-1}); all these substances belong to the group of analgesics and anti-inflammatories. Total PhAC concentrations in channels were higher in autumn and winter seasons (356.8 and 204.4 ng L^{-1}, respectively) in comparison to those found during the spring period (83.6 ng L^{-1}). Kruskal-Wallis test confirmed a significant difference between spring and autumn-winter interval in the channels ($p < 0.05$), mostly attributed to the low flow in the channels during autumn and winter seasons compared with the spring period. In fact, the flow in the irrigation channels A and B (sites 8–9) strongly fluctuates along seasons, as it depends on the flow in the Ebro River, while the flow in drainage channels C, D1-D4 (sites 10–14) depends on discharges from the rice fields during the year.

For estuarine water, seven samples were taken from four estuaries (La Tancada, L'Encanyissada, Illa de Buda and Chanal Vell), which mostly collect the waters that are coming from the irrigation and drainage channels. For seawater, —eight samples were collected from two bays (Fangar and Alfacs). Indeed, the point at Alfacs Bay (site 16) receives the discharge of RW2, which in turn, receives the effluents from WWTP2 (Table S2, SI). Fig. 3 illustrates the composition profiles (%) of the PhACs detected in estuarine and seawater samples during the three sampling campaigns. For estuarine water, 28 out of the 81 monitored PhACs were detected, taking in to account all sampling seasons,

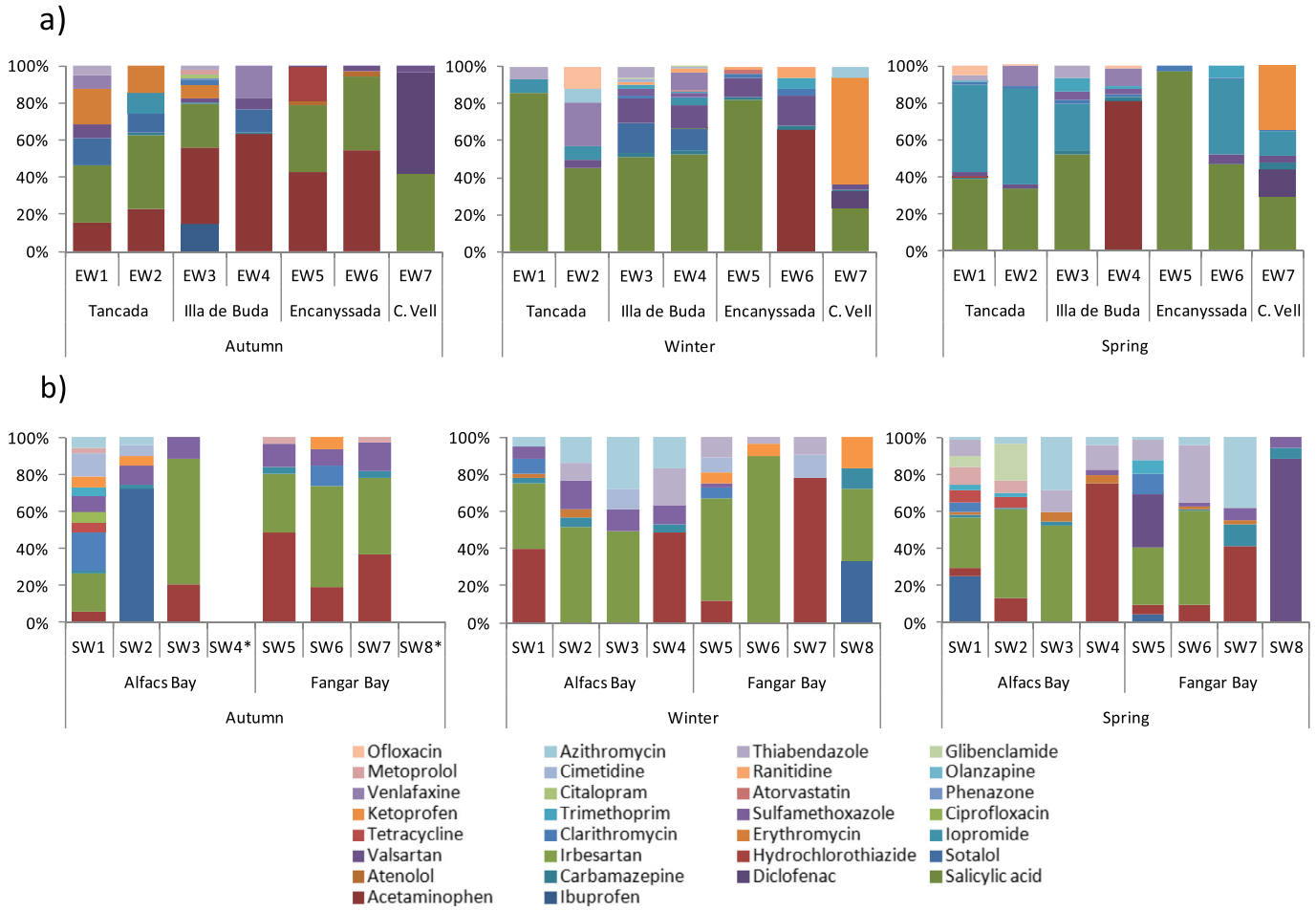

Fig. 3. Composition profile (%) of PhACs detected in (a) estuary and (b) seawater during three sampling campaigns.

and all these compounds were also found in wastewater effluent samples. The most frequently detected PhACs in estuaries (measured in >50% of the analyzed samples) were salicylic acid (86%), carbamazepine (81%), valsartan (76%), and clarithromycin (57%) (Table S11, SI). In a general extent, concentrations detected were between <MQL and 72 ng L^{-1}, with the X-ray agent (iopromide) being the compound detected at the highest concentrations. Regarding total PhACs concentrations in estuarine water, they were higher in autumn and spring season, compared to winter. This could be attributed to a better mixing between the water masses of estuaries and the sea and higher dilution during winter period, as suggested by the salinity values obtained (Table S3, SI). In autumn and spring, salinity values were twofold lower than in winter, indicating a lower mixture of these two water bodies (Fig. S2, SI). This trend has been also observed by other authors (Lara-Martín et al., 2014; Zhang et al., 2013).

For seawater, 17 out of the 81 PhACs investigated could be quantified (n = 24). Salicylic acid (73%), acetaminophen (68%), valsartan (68%), carbamazepine (64%), and trimethoprim (64%) were the compounds most frequently detected (>50%) along the three sampling events (Table S11, SI). Individual PhAC concentrations ranged from 0.1 to 23.9 ng L^{-1}. The highest individual concentrations were determined for ibuprofen at sampling site SW2 (23.9 ng L^{-1}), and for acetaminophen and salicylic acid both with a concentration of 16.5 ng L^{-1} at site SW1 (Alfacs Bay) (see Table S9, SI). Besides trimethoprim, other antibiotics such as sulfamethoxazole (45% frequency of detection), tetracycline and erythromycin were quite ubiquitous compounds in seawater samples as well (27% frequency of detection). These antibiotics were also present in effluent samples and wastewater-impacted sites. However, the antibiotic tetracycline was only found in seawater, in locations that are placed near aquaculture facilities, such as Alfacs Bay. This indicates that the occurrence of some antibiotics may not only be attributed to wastewater and river water discharges, but also to aquaculture facilities, where large amounts of antibiotics are being used. Nonetheless, the levels of tetracycline were rather low, at concentration levels ranging from 0.4 to 5.8 ng L^{-1}.

Comparing total PhACs concentrations in estuaries and seawater, they were lower in the latter (61.5 ng L^{-1}) in comparison with estuaries (146.9 ng L^{-1}). Among seawater, total PhACs concentrations were higher in point receiving WWTP2 discharges (up to 62 ng L^{-1}, sites 15–16) in comparison with offshore samples (up to 20 ng L^{-1}, sites 17–18). This could be attributed to the lower anthropogenic pressure and to a higher dilution in offshore waters. Pearson's correlation analysis was done to evaluate the correlations between total PhACs concentrations and the number of compounds versus salinity, for estuarine and seawater and a negative correlation was observed between these variables at each sampling site (Table S12, SI). A similar trend has also been observed by other authors (Zhang et al., 2013; Lara-Martín et al., 2014; Moreno-González et al., 2014). An increase in salinity was used to indicate a higher dilution of PhACs in offshore areas, in comparison with confined estuaries, which would explain the lower levels detected in the open sea sites in comparison with those located near the shoreline (Biel-Maeso et al., 2018). Kruskal-Wallis tests showed significant differences between seasons ($p < 0.05$), with lower concentrations detected in the winter period.

The concentration levels detected in this study for estuaries and seawater are in good agreement with those previously found in the scientific literature (Birch et al., 2015; Alygizakis et al., 2016; Biel-Maeso et al., 2018). Similar levels of ibuprofen, acetaminophen, and diclofenac were detected in previous studies (Gros et al., 2012; Nödler et al., 2014; Biel-Maeso et al., 2018), as it occurs for the psychiatric drug carbamazepine (Birch et al., 2015; Alygizakis et al., 2016; Biel-Maeso et al., 2018), a well-known indicator for wastewater contamination, the diuretic hydrochlorothiazide, the anti-hypertensives valsartan and irbesartan (Gros et al., 2012; Moreno-González et al., 2015; Alygizakis et al., 2016) and antibiotics (Gros et al., 2013; Alygizakis et al., 2016; González-Alonso et al., 2017). Nevertheless, other studies detected higher concentrations for some PhACs, such as analgesic and anti-inflammatory drugs acetaminophen, diclofenac, and ibuprofen (Lolić et al., 2015; Afonso-Olivares et al., 2013, González-Alonso et al., 2017), the beta-blocking agent atenolol (Ali et al., 2017; González-Alonso et al., 2017), while for the X-ray agent iopromide higher levels were observed in estuarine water than those reported in other coastal settings (Birch et al., 2015).

3.2.2. Distribution of PhACs in sediment samples

The individual concentrations of PhACs detected in sediment samples are summarized in Table S13 in SI. Fig. 4 shows cumulative levels (ng g^{-1}) of different therapeutic groups of PhACs detected in all sampling sites during three sampling seasons. Target compounds were detected at low ng g^{-1} in sediment samples. In general, the concentrations found in marine sediments were lower than those observed in river and channel sediments. This could be attributed to the lower concentrations detected in water samples but also to the salinity and TOC content, as an additional factors that may control PhACs sorption onto natural sediment (Zeng et al., 2008; Wang et al., 2010). TOC was measured for each sampling site, in triplicate, and results are included in Table S3 in SI. Considering all sampling seasons, 25 out of the 81 monitored PhACs were detected in at least one sediment sample. Individual PhACs concentrations ranged from 0.1 to 12.5 ng g^{-1} dry weight (d.w.). Sediment samples collected nearby WWTP2 discharge outlet (site number 7, RS2) showed the highest total PhACs concentrations followed by channel A sediment (site number 8, CS1), which can be attributed to the proximity of WWTP and consequently high aqueous concentrations, and also the high TOC content in these two sediments; 4.8 and 5.9%, respectively (see Table S3 in SI). In fact, sediment taken from irrigation channel A (site 8) showed higher total PhACs concentration compared with other channels. Individual compounds detected at the highest concentrations were: ketoprofen (12.5 ng g^{-1} d.w.), followed by gemfibrozil (11.3 ng g^{-1} d.w.), hydrochlorothiazide (8.6 ng g^{-1} d.w.), and citalopram (7.8 ng g^{-1} d.w.). Other PhACs, such as thiabendazole and diclofenac were detected at maximum concentration of 7.5 ng g^{-1} and 6.8 ng g^{-1}, respectively (see Table S14). Surprisingly, concentrations in the site located downstream from WWTP1 (site 6, RS1) were rather low, with a total PhACs concentration of 5.3 ng g^{-1}, which could be explained by the sand texture and very low TOC content (in average 1.78%) of these sediments. Pearson's correlations showed significant positive correlation between total PhACs concentrations and TOC in sediments ($p < 0.05$), with higher PhACs levels in sites with higher TOC values (Table S15, SI). This is in good agreement with previous studies, where positive correlations between the PhACs detected in sediments and their organic content were found (Bayen et al., 2016; Wilkinson et al., 2018; Stewart et al., 2014). The low detection of PhACs in sediments could be also explained by their physicochemical properties. Most of the target compounds included in this study have hydrophilic characteristics with logKow values lower than 1. Thus, it is expected that target compounds with high Kow values will be sorbed onto the sediments. Even though this tendency is confirmed by Pearson's correlations, some compounds detected in sediments, such as the antibiotic azithromycin and the analgesic phenazone, with logKow values (<1) did not follow this trend. This point out that logKow may not be the only indicator to assess PhACs sorption onto sediments. Since PhACs are ionizable compounds, several authors have indicated that the use of the pH dependent logD and the compounds pKa are more suitable parameters to assess PhACs partitioning onto sediments (Schaffer et al., 2012; da Silva et al., 2011). Nevertheless, in this study, Pearson's correlations showed no correlations between PhACs concentrations in sediments and these two variables (logD and pKa) (Table S16, SI). No seasonal differences in total PhACs concentrations were observed ($p > 0.05$), which is in agreement with previous studies (Yang et al., 2010; Fairbairn et al., 2015; Moreno-González et al., 2015). The concentrations of PhACs detected in freshwater sediment were in good agreement with those

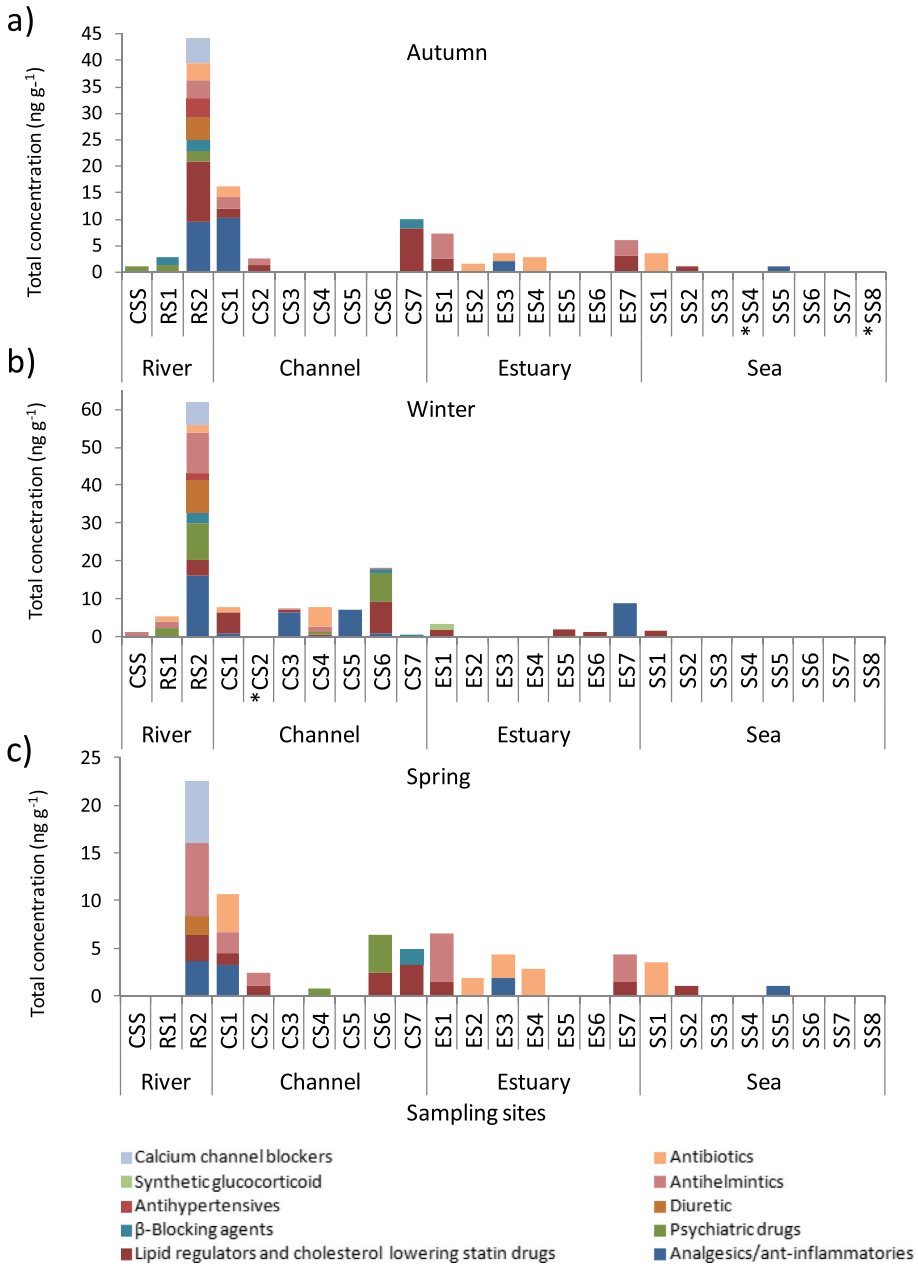

Fig. 4. Cumulative levels (ng g^{-1}) of different therapeutic groups of PhACs detected in the sediments during the autumn (a), the winter (b) and the spring season (c).

reported in previous studies (Yang et al., 2010; da Silva et al., 2011; Vazquez-Roig et al., 2012; Fairbairn et al., 2015; Carmona et al., 2017; Wilkinson et al., 2018; Koba et al., 2018) as well as for marine sediments (Yang et al., 2011; Stewart et al., 2014; Moreno-González et al., 2015; Huber et al., 2016; Bayen et al., 2016; Kim et al., 2017). Finally, for the PhACs detected in water and sediments, sorption coefficients (K_d, in L kg^{-1}) were calculated and values are reported in Table 1. K_d was calculated by dividing individual PhAC concentrations in the sediments (ng kg^{-1}) by the concentration in water sample (ng L^{-1}). For a specific compound, K_d values varied significantly between the different types of sediment, with values ranging from 1.3 to 3986.8 L kg^{-1} (log 0.1 and 3.6) in freshwater sediment and from 88.9 to 1416.7 L kg^{-1} (log 1.9 and 3.2) in estuarine and sea sediment. The compounds showing the highest K_d values, and thus, with the strongest sorption to sediments, were citalopram and thiabendazole (with K_d of 3986.8 and 2142.9 L kg^{-1}, respectively) followed by ibuprofen, erythromycin, and norverapamil (K_d values 916.7, 911.1 and 875.0 L kg^{-1}, respectively). These results indicate that sorption is a minor natural attenuation pathway for most PhACs in freshwater and marine ecosystems, except for a thiabendazole and citalopram, which showed a remarkable sorption potential (K_d higher than 1000 L kg^{-1}). The concentration levels and K_d values reported in this study match well with those found in the scientific literature (Yang et al., 2010; da Silva et al., 2011; Moreno-González et al., 2015; Fairbairn et al., 2015; Wilkinson et al., 2018; Koba et al., 2018).

3.3. Selection of ecologically relevant PhACs as markers of wastewater contamination

One of the main concerns related to the occurrence of PhACs in marine waters is their potential for bioaccumulation in biota and the negative effects that they may induce to marine organisms. Thus, it is necessary to assess any potential risk of exposure to aquatic species and ultimately, to seafood consumers. In order to highlight the most

Table 1
The partition coefficient K_d (L kg^{-1}) for those compounds present in water and sediment.

Compound/type of sediment	River	Channels	Estuary	Sea
	K_d (L kg^{-1})	K_d (L kg^{-1})	K_d (L kg^{-1})	K_d (L kg^{-1})
Thiabendazole	409.6–2142.9	104.8–956.5	88.9–1416.7	–
Citalopram	182.2–1264.4	3986.8	–	–
Norverapamil	875.0	–	–	–
Gemfibrozil	17.7–262.7	–	–	–
Carbamazepine	11.0–242.8	571.4	–	–
Propanolol	49.0–186.0	–	–	–
Clarithromycin	109.5	–	–	177.4
Bezafibrate	42.8–96.0	258.8	–	–
Ketoprofen	109.5	303.7–404.5	253.6	–
Hydrochlorothiazide	42.8–96.0	–	–	–
Diclofenac	57.9	–	–	–
Phenazone	6.0–28.3	–	–	–
Levamisol	11.5–24.3	3.9–11.5	–	–
Irbesartan	11.5–22.6	–	–	–
Ibuprofen	1.3–21.6	732.9	96.8	916.7
Furosemide	19.7	–	–	–
Azithromycin	10.4	–	–	–
Valsartan	5.8	–	–	–
Erythromycin	–	–	126.1–132.1	911.1
Sotalol	–	34.4	–	–

Table 3
Priority chemical markers based on the scoring system used (see Table 2).

Compound	Therapeutic group	Score
Venlafaxine	Psychiatric drug	17
Trimethoprim	Antibiotic	16
Hydrochlorothiazide	Diuretic	16
Carbamazepine	Psychiatric drug	16
Irbesartan	Antihypertensive	15
Diclofenac	Analgesics/anti-inflammatory	15
Citalopram	Psychiatric drug	15
Valsartan	Antihypertensive	14
Thiabendazole	Antihelmintic	14
Sulfamethoxazole	Antibiotic	14
Sotalol	β-Blocking agent	14
Salicylic acid	Analgesics/anti-inflammatory	14
Acetaminophen	Analgesics/anti-inflammatory	14
Ranitidine	Histamine H1 and H2 receptor antagonist	13
Olanzapine	Psychiatric drug	13
Ofloxacin	Antibiotic	13
Ketoprofen	Analgesics/anti-inflammatory	13
Ciprofloxacin	Antibiotic	13
Atenolol	β-Blocking agent	13
Metoprolol	β-Blocking agent	12
Iopromide	X-ray contrast agent	12
Glibenclamide	Antidiabetic	12
Cimetidine	Histamine H1 and H2 receptor antagonist	12
Tetracycline	Antibiotic	11
Phenazone	Analgesics/anti-inflammatory	11
Ibuprofen	Analgesics/anti-inflammatory	11
Clarithromycin	Antibiotic	11
Erythromycin	Antibiotic	10
Azithromycin	Antibiotic	8
Atorvastatin	Lipid regulator	6

ecologically relevant PhACs as suitable indicators for wastewater contamination, a prioritization strategy was applied. First, a list of the compounds that were detected in estuarine and seawater, as well as in wastewater effluent, was compiled and a scoring system was applied for the prioritization of the most ecologically relevant PhACs (see Table S17, SI). The scoring system used was based on the prioritization strategy published by Gros and coworkers (Gros et al., 2017) and included: (a) frequency of detection in estuary and seawater samples; (b) maximum concentrations in these samples; (c) ecotoxicity data, based on the hazard quotient (HQs) ratios; (d) bioconcentration factors (BCFs); (e) removal efficiencies in WWTPs; and (f) their estimated persistency in water (half-lives in days) (Table 2). HQs were calculated as the ratio between the highest measured environmental concentration (MEC) in estuarine and seawater and predicted no-effect concentration (PNEC). PNEC values were assessed for long-term exposure and they were estimated using Chronic Toxicity (ChV) data towards fish, mysids (seawater invertebrates), and green algae, divided by an assessment factor of 10. ChV values were obtained using the Ecological Structure Activity Relationships Predictive Model (ECOSAR V2.0). HQs obtained for each end-point are summarized in Table S18 in SI, and for our ranking, the highest value was considered. BCFs and half-lives were obtained from ChemSpider database. According to Table 2, each PhAC was given a score value (1–4) in each category (a–f) and the PhACs that showed higher total score values were suggested as the most environmentally relevant PhACs, and they can be considered as relevant chemical markers of wastewater contamination (Table 3). From our scoring list, the antidepressant venlafaxine was highlighted as the most relevant compound. Other PhACs, such as the antibiotics trimethoprim and sulfamethoxazole, psychiatric drugs carbamazepine and citalopram, the diuretic hydrochlorothiazide, antihypertensives ibersartan and valsartan, the β-blocking agent sotalol, and analgesics/anti-inflammatories diclofenac, salicylic acid, and acetaminophen were also placed in the list of the most highly ranked compounds.

Recent studies provided evidence that venlafaxine promote adverse effects at the physiological and behavioral levels in aquatic species (Best et al., 2014; Bisesi et al., 2014; Bidel et al., 2016; Maulvault et al., 2018). Furthermore, venlafaxine together with carbamazepine, sulfamethoxazole, azithromycin, and hydrochlorothiazide were determined at concentrations above MDL in bivalves from the Alfacs and Fangar bays, in the Ebro Delta region (Alvarez-Muñoz et al., 2015). On the other hand, numerous studies have pointed carbamazepine as the classical marker of wastewater contamination and this substance is one of the most frequently detected PhACs in marine species (Klosterhaus et al., 2013; Martínez Bueno et al., 2013; McEneff et al., 2014; Alvarez-Muñoz et al., 2015; Álvarez-Muñoz et al., 2015). Analgesics and anti-inflammatories, even though they are well removed during wastewater treatment, they still persist in the water environment and can also be considered as relevant markers of urban contamination. Several studies have already demonstrated that the exposure of marine organisms to

Table 2
Criteria and scoring system for prioritization of identifed PhACs in estuary and seawater.

Criteria[a]	Score			
	1	2	3	4
(a) Freq of detection (%)	<25%	25–50%	50–75%	>75%
(b) Max detected conc.	<1 ng L^{-1}	>1 ng L^{-1}	>5 ng L^{-1}	>10 ng L^{-1}
(c) HQ = MEC/PNEC	≤0.01	≥0.01	≥0.1	≥1
(d) BCF	<10	>10	>100	>1000
(e) RE (%) in WWTPs	100% in both WWTPs	>75% in both WWTPs	<75% in at least one WWTPs	<75% in both WWTPs
(f) $t_{1/2}$ in water (days)	–	≥15	≥37.5	≥60

[a] HQ = hazard quotient; MEC = measured environmental concentration; PNEC = predicted no effect concentration; BCF = bioconcentartion factor; RE = removal efficency; $t_{1/2}$ = half-life.

these group of compounds leads to a wide range of deleterious effects, such as lower growth, oxidative stress, reproductive fitness impairment, and endocrine disrupting effect among others (Ericson et al., 2010; Schmidt et al., 2011; Gonzalez-Rey and Bebianno, 2012; Gonzalez-Rey and Bebianno, 2014). It is worth highlighting that macrolide and fluoroquinolone antibiotics (i.e. clarithromycin, erythromycin, azithromycin and ciprofloxacin), which are included in the EU Watch List, were the compounds with the lowest score values within this prioritization strategy. However, this could be mainly attributed to the lack of data regarding their toxicity to marine organisms, used to predict HQs, BCFs, and half-life values (Table S18, SI).

4. Concluding remarks

This study revealed that WWTPs are an important source of PhACs contamination in coastal areas, as numerous compounds were detected in estuary and seawater. PhACs more frequently detected in the receiving water systems coincided, in a great extent, with those that are more ubiquitous in effluent wastewaters. Even though PhACs were subject to natural attenuation processes during their transport towards the open sea, they were still detected at remarkable concentrations in the offshore areas. PhACs attenuation was mainly attributed to dilution, while sorption to sediments showed to be a minor pathway, as highlighted by the low number of PhACs detected in these matrices. These results indicate that the continuous input of PhACs through waste and receiving water courses would favor their presence in seawater and this may negatively affect the organisms living in these ecosystems. Seasonality in PhACs concentration was observed in water but not in sediment. The prioritization strategy used here represents a robust tool to identify the most persistent and ecologically relevant PhACs as chemical markers of wastewater contamination in coastal environments. Nevertheless, this prioritization strategy showed that there is still a lack of data regarding PhACs toxicity to marine organisms. Thus, further research that assess the potential negative effects of PhACs to marine species and biota is necessary, in order to perform a proper risk assessment of these substances to marine settings.

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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.2018.10.290.

References

Acuña, V., von Schiller, D., García-Galán, M.J., Rodríguez-Mozaz, S., Corominas, L., Petrovic, M., et al., 2015. Occurrence and in-stream attenuation of wastewater-derived pharmaceuticals in Iberian rivers. Sci. Total Environ. 503-504, 133–141.

Afonso-Olivares, C., Torres-Padrón, M., Sosa-Ferrera, Z., Santana-Rodríguez, J., 2013. Assessment of the presence of pharmaceutical compounds in seawater samples from coastal area of Gran Canaria Island (Spain). Antibiot. 2 (2), 274.

Al Aukidy, M., Verlicchi, P., Jelic, A., Petrovic, M., Barcelò, D., 2012. Monitoring release of pharmaceutical compounds: occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy. Sci. Total Environ. 438, 15–25.

Ali, A.M., Rønning, H.T., Alarif, W., Kallenborn, R., Al-Lihaibi, S.S., 2017. Occurrence of pharmaceuticals and personal care products in effluent-dominated Saudi Arabian coastal waters of the Red Sea. Chemosphere 175 (Supplement C), 505–513.

Alvarez-Muñoz, D., Huerta, B., Fernandez-Tejedor, M., Rodríguez-Mozaz, S., Barceló, D., 2015. Multi-residue method for the analysis of pharmaceuticals and some of their metabolites in bivalves. Talanta 136, 174–182.

Álvarez-Muñoz, D., Rodríguez-Mozaz, S., Maulvault, A.L., Tediosi, A., Fernández-Tejedor, M., Van den Heuvel, F., et al., 2015. Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves, and fish from coastal areas in Europe. Environ. Res. 143, 56–64.

Alygizakis, N.A., Gago-Ferrero, P., Borova, V.L., Pavlidou, A., Hatzianestis, I., Thomaidis, N.S., 2016. Occurrence and spatial distribution of 158 pharmaceuticals, drugs of abuse and related metabolites in offshore seawater. Sci. Total Environ. 541, 1097–1105.

Aymerich, I., Acuña, V., Barceló, D., García, M.J., Petrovic, M., Poch, M., et al., 2016. Attenuation of pharmaceuticals and their transformation products in a wastewater treatment plant and its receiving river ecosystem. Water Res. 100, 126–136.

Bai, X., Lutz, A., Carroll, R., Keteles, K., Dahlin, K., Murphy, M., et al., 2018. Occurrence, distribution, and seasonality of emerging contaminants in urban watersheds. Chemosphere 200, 133–142.

Bayen, S., Estrada, E.S., Juhel, G., Kit, L.W., Kelly, B.C., 2016. Pharmaceutically active compounds and endocrine disrupting chemicals in water, sediments and mollusks in mangrove ecosystems from Singapore. Mar. Pollut. Bull. 109 (2), 716–722.

Behera, S.K., Kim, H.W., Oh, J.-E., Park, H.-S., 2011. Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea. Sci. Total Environ. 409 (20), 4351–4360.

Best, C., Melnyk-Lamont, N., Gesto, M., Vijayan, M.M., 2014. Environmental levels of the antidepressant venlafaxine impact the metabolic capacity of rainbow trout. Aquat. Toxicol. 155, 190–198.

Bidel, F., Di Poi, C., Budzinski, H., Pardon, P., Callewaert, W., Arini, A., et al., 2016. The antidepressant venlafaxine may act as a neurodevelopmental toxicant in cuttlefish (Sepia officinalis). NeuroToxicology 55, 142–153.

Biel-Maeso, M., Baena-Nogueras, R.M., Corada-Fernández, C., Lara-Martín, P.A., 2018. Occurrence, distribution and environmental risk of pharmaceutically active compounds (PhACs) in coastal and ocean waters from the Gulf of Cadiz (SW Spain). Sci. Total Environ. 612, 649–659.

Birch, G.F., Drage, D.S., Thompson, K., Eaglesham, G., Mueller, J.F., 2015. Emerging contaminants (pharmaceuticals, personal care products, a food additive and pesticides) in waters of Sydney estuary, Australia. Mar. Pollut. Bull. 97 (1), 56–66.

Bisesi, J.H., Bridges, W., Klaine, S.J., 2014. Reprint of: effects of the antidepressant venlafaxine on fish brain serotonin and predation behavior. Aquat. Toxicol. 151, 88–96.

Bu, Q., Wang, B., Huang, J., Deng, S., Yu, G., 2013. Pharmaceuticals and personal care products in the aquatic environment in China: a review. J. Hazard. Mater. 262, 189–211.

Carmona, E., Andreu, V., Picó, Y., 2017. Multi-residue determination of 47 organic compounds in water, soil, sediment and fish—Turia River as case study. J. Pharm. Biomed. Anal. 146, 117–125.

Chen, M., Cooper, V.I., Deng, J., Amatya, P.L., Ambrus, D., Dong, S., et al., 2015. Occurrence of pharmaceuticals in Calgary's wastewater and related surface water. Water Environ. Res. 87 (5), 414–424.

Collado, N., Rodriguez-Mozaz, S., Gros, M., Rubirola, A., Barceló, D., Comas, J., et al., 2014. Pharmaceuticals occurrence in a WWTP with significant industrial contribution and its input into the river system. Environ. Pollut. 185, 202–212.

da Silva, B.F., Jelic, A., Lopez-Serna, R., Mozeto, A.A., Petrovic, M., Barcelo, D., 2011. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. Chemosphere 85 (8), 1331–1339.

Daneshvar, A., Svanfelt, J., Kronberg, L., Prévost, M., Weyhenmeyer, G.A., 2010. Seasonal variations in the occurrence and fate of basic and neutral pharmaceuticals in a Swedish river–lake system. Chemosphere 80 (3), 301–309.

Dodder, N.G., Maruya, K.A., Lee Ferguson, P., Grace, R., Klosterhaus, S., La Guardia, M.J., et al., 2014. Occurrence of contaminants of emerging concern in mussels (*Mytilus* spp.) along the California coast and the influence of land use, storm water discharge, and treated wastewater effluent. Mar. Pollut. Bull. 81 (2), 340–346.

Done, H.Y., Halden, R.U., 2015. Reconnaissance of 47 antibiotics and associated microbial risks in seafood sold in the United States. J. Hazard. Mater. 282, 10–17.

Ericson, H., Thorsén, G., Kumblad, L., 2010. Physiological effects of diclofenac, ibuprofen and propranolol on Baltic Sea blue mussels. Aquat. Toxicol. 99 (2), 223–231.

European Comission, 2002. Commission decision (2002/657/EC) of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (notified under document number C (2002) 3044). Off. J. Eur. Union L 221/8.

European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. (OJ L 327, 22.12.2000). p. 1.

European Commission, 2015. Commission implementing decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. Off. J. Eur. Union L78/40 (2015), 20–30.

European Commission, 2018. Commission implementing decision (EU) 2018/840 of 5 June 2018 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council and repealing Commission Implementing Decision (EU) 2015/495 (notified under document C(2018) 3362). Off. J. Eur. Union L141/9.

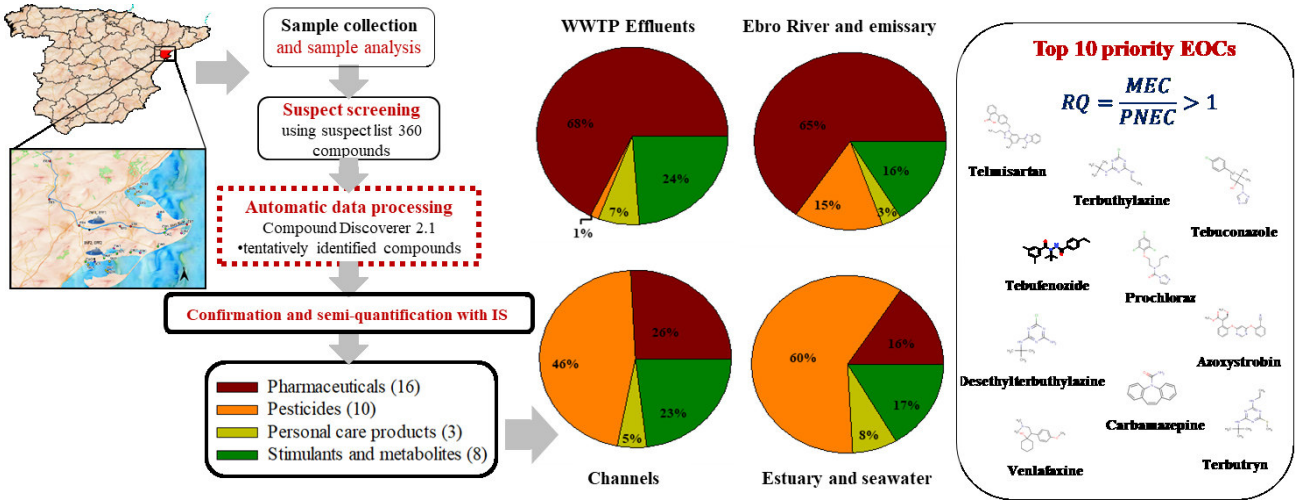
Fairbairn, D.J., Karpuzcu, M.E., Arnold, W.A., Barber, B.L., Kaufenberg, E.F., Koskinen, W.C., et al., 2015. Sediment–water distribution of contaminants of emerging concern in a mixed use watershed. Sci. Total Environ. 505, 896–904.

Fang, T.-H., Nan, F.-H., Chin, T.-S., Feng, H.-M., 2012. The occurrence and distribution of pharmaceutical compounds in the effluents of a major sewage treatment plant in Northern Taiwan and the receiving coastal waters. Mar. Pollut. Bull. 64 (7), 1435–1444.

Fisch, K., Waniek, J.J., Schulz-Bull, D.E., 2017. Occurrence of pharmaceuticals and UV-filters in riverine run-offs and waters of the German Baltic Sea. Mar. Pollut. Bull. 124 (1), 388–399.
Gaw, S., Thomas, K.V., Hutchinson, T.H., 2014. Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. Philos. Trans. R. Soc. B Biol. Sci. 369 (1656).
Gonzalez-Rey, M., Bebianno, M.J., 2012. Does non-steroidal anti-inflammatory (NSAID) ibuprofen induce antioxidant stress and endocrine disruption in mussel *Mytilus galloprovincialis*? Environ. Toxicol. Pharmacol. 33 (2), 361–371.
Gonzalez-Rey, M., Bebianno, M.J., 2014. Effects of non-steroidal anti-inflammatory drug (NSAID) diclofenac exposure in mussel *Mytilus galloprovincialis*. Aquat. Toxicol. 148, 221–230.
González-Alonso, S., Merino, L.M., Esteban, S., López de Alda, M., Barceló, D., Durán, J.J., et al., 2017. Occurrence of pharmaceutical, recreational and psychotropic drug residues in surface water on the northern Antarctic Peninsula region. Environ. Pollut. 229, 241–254.
Gracia-Lor, E., Sancho, J.V., Serrano, R., Hernández, F., 2012. Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia. Chemosphere 87 (5), 453–462.
Gros, M., Petrović, M., Ginebreda, A., Barceló, D., 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ. Int. 36 (1), 15–26.
Gros, M., Rodriguez-Mozaz, S., Barcelo, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. J. Chromatogr. A 1248, 104–121.
Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2013. Rapid analysis of multiclass antibiotic residues and some of their metabolites in hospital, urban wastewater and river water by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. J. Chromatogr. A 1292, 173–188.
Gros, M., Blum, K.M., Jernstedt, H., Renman, G., Rodríguez-Mozaz, S., Haglund, P., et al., 2017. Screening and prioritization of micropollutants in wastewaters from on-site sewage treatment facilities. J. Hazard. Mater. 328, 37–45.
Huber, S., Remberger, M., Kaj, L., Schlabach, M., Jörundsdóttir, H.Ó., Vester, J., et al., 2016. A first screening and risk assessment of pharmaceuticals and additives in personal care products in waste water, sludge, recipient water and sediment from Faroe Islands, Iceland and Greenland. Sci. Total Environ. 562, 13–25.
Jelić, A., Petrović, M., Barceló, D., 2009. Multi-residue method for trace level determination of pharmaceuticals in solid samples using pressurized liquid extraction followed by liquid chromatography/quadrupole-linear ion trap mass spectrometry. Talanta 80 (1), 363–371.
Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008. The effect of signal suppression and mobile phase composition on the simultaneous analysis of multiple classes of acidic/neutral pharmaceuticals and personal care products in surface water by solid-phase extraction and ultra performance liquid chromatography-negative electrospray tandem mass spectrometry. Talanta 74 (5), 1299–1312.
Kim, H.-Y., Lee, I.-S., Oh, J.-E., 2017. Human and veterinary pharmaceuticals in the marine environment including fish farms in Korea. Sci. Total Environ. 579, 940–949.
Klosterhaus, S.L., Grace, R., Hamilton, M.C., Yee, D., 2013. Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. Environ. Int. 54, 92–99.
Koba, O., Grabicova, K., Cerveny, D., Turek, J., Kolarova, J., Randak, T., et al., 2018. Transport of pharmaceuticals and their metabolites between water and sediments as a further potential exposure for aquatic organisms. J. Hazard. Mater. 342, 401–407.
Lara-Martín, P.A., González-Mazo, E., Petrovic, M., Barceló, D., Brownawell, B.J., 2014. Occurrence, distribution and partitioning of nonionic surfactants and pharmaceuticals in the urbanized Long Island Sound Estuary (NY). Mar. Pollut. Bull. 85 (2), 710–719.
Lin, A.Y.-C., Tsai, Y.-T., 2009. Occurrence of pharmaceuticals in Taiwan's surface waters: impact of waste streams from hospitals and pharmaceutical production facilities. Sci. Total Environ. 407 (12), 3793–3802.
Lin, H., Chen, L., Li, H., Luo, Z., Lu, J., Yang, Z., 2018. Pharmaceutically active compounds in the Xiangjiang River, China: distribution pattern, source apportionment, and risk assessment. Sci. Total Environ. 636, 975–984.
Lolić, A., Paíga, P., Santos, L.H.M.L.M., Ramos, S., Correia, M., Delerue-Matos, C., 2015. Assessment of non-steroidal anti-inflammatory and analgesic pharmaceuticals in seawaters of North of Portugal: occurrence and environmental risk. Sci. Total Environ. 508, 240–250.
López-Serna, R., Petrović, M., Barceló, D., 2012. Occurrence and distribution of multi-class pharmaceuticals and their active metabolites and transformation products in the Ebro River basin (NE Spain). Sci. Total Environ. 440, 280–289.
Martínez Bueno, M.J., Boillot, C., Fenet, H., Chiron, S., Casellas, C., Gómez, E., 2013. Fast and easy extraction combined with high resolution-mass spectrometry for residue analysis of two anticonvulsants and their transformation products in marine mussels. J. Chromatogr. A 1305, 27–34.
Martínez Bueno, M.J., Boillot, C., Munaron, D., Fenet, H., Casellas, C., Gómez, E., 2014. Occurrence of venlafaxine residues and its metabolites in marine mussels at trace levels: development of analytical method and a monitoring program. Anal. Bioanal. Chem. 406 (2), 601–610.
Mastroianni, N., Bleda, M.J., López de Alda, M., Barceló, D., 2016. Occurrence of drugs of abuse in surface water from four Spanish river basins: spatial and temporal variations and environmental risk assessment. J. Hazard. Mater. 316 (Supplement C), 134–142.
Maulvault, A.L., Santos, L.H.M.L.M., Paula, J.R., Camacho, C., Pissarra, V., Fogaça, F., et al., 2018. Differential behavioural responses to venlafaxine exposure route, warming and acidification in juvenile fish (*Argyrosomus regius*). Sci. Total Environ. 634, 1136–1147.
McEneff, G., Barron, L., Kelleher, B., Paull, B., Quinn, B., 2014. A year-long study of the spatial occurrence and relative distribution of pharmaceutical residues in sewage effluent, receiving marine waters and marine bivalves. Sci. Total Environ. 476-477, 317–326.
Moreno-González, R., Rodríguez-Mozaz, S., Gros, M., Pérez-Cánovas, E., Barceló, D., León, V.M., 2014. Input of pharmaceuticals through coastal surface watercourses into a Mediterranean lagoon (Mar Menor, SE Spain): Sources and seasonal variations. Sci. Total Environ. 490, 59–72.
Moreno-González, R., Rodriguez-Mozaz, S., Gros, M., Barceló, D., León, V.M., 2015. Seasonal distribution of pharmaceuticals in marine water and sediment from a Mediterranean coastal lagoon (SE Spain). Environ. Res. 138 (Supplement C), 326–344.
Nödler, K., Voutsa, D., Licha, T., 2014. Polar organic micropollutants in the coastal environment of different marine systems. Mar. Pollut. Bull. 85 (1), 50–59.
Pereira, A.M.P.T., Silva, L.J.G., Laranjeiro, C.S.M., Meisel, L.M., Lino, C.M., Pena, A., 2017. Human pharmaceuticals in Portuguese rivers: the impact of water scarcity in the environmental risk. Sci. Total Environ. 609, 1182–1191.
Pignotti, E., Casas, G., Llorca, M., Tellbüscher, A., Almeida, D., Dinelli, E., et al., 2017. Seasonal variations in the occurrence of perfluoroalkyl substances in water, sediment and fish samples from Ebro Delta (Catalonia, Spain). Sci. Total Environ. 607 (Supplement C), 933–943.
Postigo, C., López de Alda, M.J., Barceló, D., 2010. Drugs of abuse and their metabolites in the Ebro River basin: occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation. Environ. Int. 36 (1), 75–84.
Rivera-Jaimes, J.A., Postigo, C., Melgoza-Alemán, R.M., Aceña, J., Barceló, D., López de Alda, M., 2018. Study of pharmaceuticals in surface and wastewater from Cuernavaca, Morelos, Mexico: occurrence and environmental risk assessment. Sci. Total Environ. 613-614, 1263–1274.
Rodríguez-Navas, C., Björklund, E., Bak, S.A., Hansen, M., Krogh, K.A., Maya, F., et al., 2013. Pollution pathways of pharmaceutical residues in the aquatic environment on the island of Mallorca, Spain. Arch. Environ. Contam. Toxicol. 65 (1), 56–66.
Sainz-Elipe, S., Latorre, J.M., Escosa, R., Masià, M., Fuentes, M.V., Mas-Coma, S., et al., 2010. Malaria resurgence risk in southern Europe: climate assessment in an historically endemic area of rice fields at the Mediterranean shore of Spain. Malar. J. 9 (1), 221.
Schaffer, M., Boxberger, N., Börnick, H., Licha, T., Worch, E., 2012. Sorption influenced transport of ionizable pharmaceuticals onto a natural sandy aquifer sediment at different pH. Chemosphere 87 (5), 513–520.
Schmidt, W., O'Rourke, K., Hernan, R., Quinn, B., 2011. Effects of the pharmaceuticals gemfibrozil and diclofenac on the marine mussel (*Mytilus* spp.) and their comparison with standardized toxicity tests. Mar. Pollut. Bull. 62 (7), 1389–1395.
Sim, W.-J., Lee, J.-W., Oh, J.-E., 2010. Occurrence and fate of pharmaceuticals in wastewater treatment plants and rivers in Korea. Environ. Pollut. 158 (5), 1938–1947.
Stewart, M., Olsen, G., Hickey, C.W., Ferreira, B., Jelić, A., Petrović, M., et al., 2014. A survey of emerging contaminants in the estuarine receiving environment around Auckland, New Zealand. Sci. Total Environ. 468–469, 202–210.
Vazquez-Roig, P., Andreu, V., Blasco, C., Picó, Y., 2012. Risk assessment on the presence of pharmaceuticals in sediments, soils and waters of the Pego–Oliva Marshlands (Valencia, eastern Spain). Sci. Total Environ. 440, 24–32.
Wang, J., Hu, J., Zhang, S., 2010. Studies on the sorption of tetracycline onto clays and marine sediment from seawater. J. Colloid Interface Sci. 349 (2), 578–582.
Wilkinson, J.L., Hooda, P.S., Swinden, J., Barker, J., Barton, S., 2018. Spatial (bio)accumulation of pharmaceuticals, illicit drugs, plasticisers, perfluorinated compounds and metabolites in river sediment, aquatic plants and benthic organisms. Environ. Pollut. 234, 864–875.
Wu, M., Xiang, J., Chen, F., Fu, C., Xu, G., 2017. Occurrence and risk assessment of antidepressants in Huangpu River of Shanghai, China. Environ. Sci. Pollut. Res. 24 (25), 20291–20299.
Yang, J.-F., Ying, G.-G., Zhao, J.-L., Tao, R., Su, H.-C., Chen, F., 2010. Simultaneous determination of four classes of antibiotics in sediments of the Pearl Rivers using RRLC–MS/MS. Sci. Total Environ. 408 (16), 3424–3432.
Yang, Y., Fu, J., Peng, H., Hou, L., Liu, M., Zhou, J.L., 2011. Occurrence and phase distribution of selected pharmaceuticals in the Yangtze Estuary and its coastal zone. J. Hazard. Mater. 190 (1), 588–596.
Yoon, Y., Ryu, J., Oh, J., Choi, B.G., Snyder, S.A., 2010. Occurrence of endocrine disrupting compounds, pharmaceuticals, and personal care products in the Han River (Seoul, South Korea). Sci. Total Environ. 408 (3), 636–643.
Zeng, X., Mai, B., Sheng, G., Luo, X., Shao, W., An, T., et al., 2008. Distribution of polycyclic musks in surface sediments from the Pearl River Delta and Macao coastal region, South China. Environ. Toxicol. Chem. 27 (1), 18–23.
Zenker, A., Cicero, M.R., Prestinaci, F., Bottoni, P., Carere, M., 2014. Bioaccumulation and biomagnification potential of pharmaceuticals with a focus to the aquatic environment. J. Environ. Manag. 133, 378–387.
Zhang, R., Tang, J., Li, J., Zheng, Q., Liu, D., Chen, Y., et al., 2013. Antibiotics in the offshore waters of the Bohai Sea and the Yellow Sea in China: occurrence, distribution and ecological risks. Environ. Pollut. 174, 71–77.

Chapter 3

Suspect screening: method development and identification of emerging contaminants in a coastal area



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Mira Čelić, Adrián Jaén-Gil, Susana Briceño-Guevara, Sara Rodriguez-Mozaz, Meritxell Gros, and Mira Petrović

Extended suspect screening to identify emerging organic contaminants in riverine and coastal ecosystems and assessment of environmental risks

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Extended suspect screening to identify contaminants of emerging concern in riverine and coastal ecosystems and assessment of environmental risks

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ABSTRACT

A suspect screening methodology was developed for the fast and reliable identification of 360 contaminants of emerging concern (CECs) of anthropogenic origin in the vulnerable area of the Ebro Delta (Catalonia, Spain) and to track for potential contamination sources. The suspect screening methodology was combined with a risk assessment approach to prioritize the most ecologically relevant CECs. Out of the 360 suspects, 37 compounds were tentatively identified, 22 of which were fully confirmed using isotopically labelled standards. The detected suspect compounds included pesticides, pharmaceuticals, personal care products, stimulants and their metabolites. Pesticides were more ubiquitous in irrigation and drainage channels, while pharmaceuticals, stimulants, and personal care products were the most common in effluent wastewaters, in the receiving freshwater systems as well as in the marine environment. Ten compounds were found to be of high ecological concern, including the pharmaceuticals telmisartan, venlafaxine, and carbamazepine, the herbicides terbuthylazine, desethylterbuthylazine, and terbutryn, the fungicides azoxystrobin, tebuconazole and prochloraz and the insecticide tebufenozide. These compounds could be used as markers of anthropogenic contamination in riverine and coastal ecosystems.

1. Introduction

Effluents from wastewater treatment plants (WWTPs), agricultural activities, and industrial wastes are some of the major contamination sources of the aquatic environment (Mostofa et al., 2013; Fent et al., 2006). Wastewater effluents contain tens of thousands of contaminants of emerging concern (CECs) such as pharmaceuticals, pesticides, personal care products, industrial chemicals, their metabolites and transformation products (TPs). Since conventional WWTPs are not designed to remove them completely, these compounds are released into the receiving aquatic environments (Agüera et al., 2013).

Multi-residue analytical methodologies have been developed for the identification of several CECs, combining different detection and identification strategies. Target analysis is used for the identification of known CECs that are selected prior to sample analysis and for which analytical standards are commercially available. Although these methodologies have been widely used for studying the occurrence of a broad range of contaminants in environmental samples (Yoon et al., 2010; González-Alonso et al., 2017; Brumovský et al., 2017), information provided is just confined to a limited number of pre-selected chemicals, mostly not exceeding more than 100 compounds (María Baena-Nogueras et al., 2016; Borova et al., 2014; Ferrer and Thurman, 2012; Nödler et al., 2014). To overcome this limitation, high-resolution mass spectrometry (HRMS), using either Orbitrap or quadrupole-time-of-flight (QTOF) mass spectrometers, has been successfully applied in environmental analysis by using suspect or non-target screening approaches. Suspect screening is applied for the tentative identification of chemicals included in exact mass compound databases or suspect lists, while non-target screening allows the identification of unknown compounds without requiring any prior information (Aalizadeh et al., 2016). HRMS provides high reliability in compound identification, based on exact mass measurements of precursor and fragment ions and therefore, allows the detection of a larger number of CECs without requiring reference standards (a priori).

One of the most critical aspects of suspect screening methodologies is the need of establishing user-friendly data processing workflows. Even

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though several studies have already been focused on developing suspect screening approaches for CECs identification (Aalizadeh et al., 2016; Tian et al., 2019; Liu et al., 2019; Gago-Ferrero et al., 2015; Gros et al., 2017; Hug et al., 2014), there is still the need for setting-up systematic and more easy-to-use strategies for the rapid identification of relevant CECs (Brack et al., 2019). Another drawback of suspect screening methodologies is the difficulty of identifying suspect CECs with the highest degree of confidence, since reference analytical standards are not always available for the confirmation of all the compounds identified (Schymanski et al., 2014) and for their quantification. To overcome this challenge, the use of a selection of structurally related isotopically labelled internal standards (ILISs) is considered as the most suitable approach for semi-quantitative analysis and to help in the compounds identification (Kiefer et al., 2019), avoiding the high economic investments in purchasing reference standards for all CECs. Suspect compounds may have similar fragment ions than their corresponding analogous ILISs, thus enhancing the confidence in CECs identification. Even though it is difficult to find suitable ILISs for the quantification of all the substances tentatively identified, it allows the estimation of their concentrations in environmental samples (Sjerps et al., 2016; Park et al., 2018; Choi et al., 2020). On the other hand, the combination of suspect screening with risk assessment strategies may represent a valuable approach to further perform hazard-oriented studies and prioritize the most environmentally relevant CECs (Gros et al., 2017; Park et al., 2018; Campos-Mañas et al., 2019). Within risk assessment-based studies, the estimation of risk quotients (RQs) is one of the most widely used approaches and is a useful tool to identify CECs posing the highest environmental risks (Manickum and John, 2014; Lolić et al., 2015; Biel-Maeso et al., 2018; Yien Fang et al., 2019).

In this context, the objectives of the present study encompassed: (i) the development of a suspect screening approach for the fast identification of a large list of anthropogenic CECs in environmental samples; (ii) to study their occurrence and distribution along the Ebro Delta region and its surrounding freshwater and coastal environment to track for the most relevant contamination sources and (iii) highlight the contaminants of major ecological concern by performing an environmental risk assessment study. Compound identification was performed by using an in-house suspect list provided by the Catalan Water Agency (ACA) that contained information of 360 compounds of major use and consumption in Catalonia, as well as their generated TPs and metabolites. The list included pharmaceuticals (1 3 0), pesticides (125), antibiotics (42), psychoactive drugs (11), hormones (9), stimulants (8), personal care products (6), artificial sweeteners (4), phenols (3), antibacterial agents (2), plasticizers (2) and other compounds such as phthalates, UV filters, corrosion inhibitors, surfactants, and synthetic musks (18) (Table S1 in the Supplementary Material). The suspect screening methodology used the software Compound Discoverer 2.1 (Thermo Fisher Scientific) and the software-linked databases mzCloud, mzVault, and ChemSpider for compound identification. This methodology allows the identification and confirmation of CECs within one single platform and it aims at providing a reliable and user-friendly tool for compound identification, which is one of the major bottlenecks in HRMS-based strategies. Toxicity data used for the risk assessment evaluation was obtained from the NORMAN Ecotoxicology Database (NORMAN Ecotoxicology Database, 2019), which contains predicted-no-effect-concentrations (PNECs) for more than 39,000 substances. The combination of the suspect screening approach with a risk-assessment intends to be a suitable tool for the identification of the most relevant anthropogenic contaminants to be included in future monitoring programs and legislations.

2. Materials and methods

2.1. Chemicals

ILISs used in the study were obtained from Sigma-Aldrich (Steinheim, Germany) and Santa Cruz Biotechnology (Heidelberg, Germany) (Table S2 in the Supplementary Material). These compounds were the same substances, or structurally related, to the CECs that were tentatively identified in the samples. Individual stock solutions of ILISs were prepared on a weight basis in methanol (at a concentration of 1000 mg L^{-1}). After preparation, standards were stored at − 20 °C. A mixture containing 26 ILISs was prepared by appropriate dilution of individual stock solutions in methanol–water (50:50 v/v) before sample injection. HPLC grade water and solvents methanol (MeOH) and acetonitrile (ACN) were supplied by Fisher Scientific (Geel, Belgium). Ammonia (NH_3), formic acid, and ethyl acetate (purity ≥99.9% for trace analysis) were purchased from Panreac or Fluka (Buchs, Switzerland). Nitrogen of 99.9% purity for drying was from Abelló Linde S.A. (Spain). Empty solid phase extraction (SPE) polypropylene tubes (6 mL) and the SPE sorbent materials Sepra ZT (Strata-X), Sepra ZT-WCX (Strata-X-CW) and ZT-WAX (Strata-X-AW) were obtained from Phenomenex (Torrance, USA). The Isolute ENV+ SPE sorbent material and the frits (20 μm, 6 mL) were from Biotage (Ystrad Mynach, UK). Glass fiber filters (GFF, 0.7 μm) were purchased from Whatman (U.K.) and hydrophilic polyvinylidene fluoride (PVDF, 0.45 μm) membrane filters were provided from Sigma-Aldrich (Germany). Regenerated cellulose syringe filters (RC) of 15 mm diameter and 0.2 μm pore size were obtained from Phenomenex (Torrance, CA, USA).

2.2. Sampling

Samples were collected in 29 sampling points in reaches of the Ebro River Delta region, an area of high ecological value and richness (Fig. S1 in the Supplementary Material). The samples collected include: (a) influent (INF) and effluent (EFF) wastewaters from the two main WWTPs in the area, Amposta (INF1 and EFF1) and Sant Carles de la Ràpita (INF2 and EFF2), both covering > 75% of the wastewater by population in the Ebro River Delta region; (b) samples from the Ebro River located upstream (ER1-ER4) and downstream (ER5-ER7) WWTP1, and a sample downstream WWTP2 (SCR). Wastewater effluents from WWTP1 are discharged into the Ebro River itself (site ER5), whereas effluents from WWTP2 go to the emissary in Sant Carles de la Ràpita (site SCR); (c) irrigation (CW1-CW3) and drainage channels (CW4-CW7) used for the irrigation of rice fields in the area; (d) the associated receiving estuaries (EW1-EW4) and (e) eight seawater samples taken from two shores, Alfacs (SW1-SW2) and Fangar (SW5-SW6) bays, and offshore waters at the open sea adjacent to these bays (SW3, SW4,SW7 and SW8). Site SW1 is of special interest since it receives the discharge of SCR (the emissary of Sant Carles de la Ràpita). Detailed information about sampling sites and the physicochemical parameters measured in-situ for each water sample are summarized in Table S3 in the Supplementary Material. Influent and effluent wastewaters were 24 h composite samples, collected in pre-cleaned high-density polyethylene (HDPE) bottles, whereas the other types of water were grab samples taken in polyethylene bottles (PET). Samples were transported in a refrigerated isothermal container at 4ºC to the laboratory and were stored at − 20 °C until analysis.

2.3. Sample preparation

Samples were extracted in triplicate following the methodology described in detail elsewhere (Gago-Ferrero et al., 2015). Analytical blanks (ultrapure water) were performed and analyzed in the same way as water samples. Briefly, samples were filtered through 0.7 μm GFF followed by 0.45 μm PVDF membrane filters and pH was adjusted to 6.5 with formic acid. SPE was conducted using manually packed mixed-mode cartridges comprising of 200 mg of Strata-X and 350 mg of the mixture Strata-X-AW: Strata-X-CW: Isolute ENV+ (1:1:1.5). Different sample volumes were used depending on the samples, such as 500 mL for surface (river, emissary, and channels) and marine water (estuary and seawater), 200 mL for effluent and 100 mL for influent wastewater.

Different sample volumes were used for the different type of samples to reduce any potential matrix effects and to achieve the required pre-concentration factors to ensure the detection of suspects compounds. After sample enrichment, SPE cartridges were rinsed with 6 mL of HPLC water and were further air-dried for 5 min to remove the remaining water. The elution was conducted with 4 mL of methanol/ethyl acetate (50:50, v/v) containing 2% ammonia followed by 2 mL of methanol/ethyl acetate (50:50, v/v) with 1.7% formic acid. The extracts were combined and evaporated under a gentle nitrogen stream and reconstituted to 0.5 mL with a final proportion of methanol/HPLC-grade water (50:50, v/v). Finally, the extracts were filtered through a 0.2 μm RC syringe filters.

2.4. LC-HRMS analysis

Sample extracts and analytical blanks were further analyzed using liquid chromatography, with an Accela system coupled to a LTQ Orbitrap Velos HRMS instrument (Thermo Fisher Scientific, USA). The method used was adapted from the one described in Jaén-Gil and co-workers (Jaén-Gil et al., 2019). For chromatographic separation, 10 μL were injected in a ZORBAX Eclipse XDB-C18 (150 mm × 4.6 mm, 5 μm) column operating at room temperature using a constant flow rate of 0.5 mL min^{-1}. Samples were injected twice, in positive (+) and negative (-) ionization mode, respectively. Aqueous mobile phases used were 10 mM formic acid/ammonium formate (pH 3.0) and 5 mM ammonium acetate/ammonia (pH 8.0) for (+) and (-), respectively. ACN was used as the organic mobile phase in both cases. The chromatographic gradient used was: initial mobile phase composition (95% A) held for 1 min, followed by a decrease in composition of solvent A to 5% within 9 min, then to 0% in 3 min, and these conditions were held for 2 min. Finally, initial conditions were restored to 95% A in 1 min and were held for 1 more min. The total MS run time was 17 min

The suspect list, which had information about the monoisotopic masses for each of the 360 substances (see Table S1 in the Supplementary Material), was loaded into the LTQ-Orbitrap Velos prior to sample injection using Aria software under Xcalibur 2.1 (Thermo Fisher Scientific). Samples were injected in data-dependent acquisition (DDA) mode. Firstly, MS data was recorded in full scan mode, scanning from *m/z* 100–1000 range at a resolving power of 60,000 FWHM. Then, MS/MS fragmentation was performed for the three most intense ions included in the suspect list, at a scan range from *m/z* 50–500 and a resolving power of 30,000 FWHM. The heated electrospray ionization interface (HESI) was used for compound ionization, operating in (+) and (-) mode with the following parameters: spray voltage, 3.5 kV; source heater temperature, 300 °C; capillary temperature, 350 °C; sheath gas flow, 40 (arbitrary units); and auxiliary gas flow, 20 (arbitrary units). Fragmentation parameters used were collision-induced dissociation (CID) at normalized collision energy of 30 eV (activation Q of 0.250 and an activation time of 30 ms) in an isolation width of 2 Da.

2.5. Data processing and evaluation of suspects

For the identification of suspect compounds, a data processing workflow was developed using Compound Discoverer 2.1 software (Thermo Fisher Scientific Inc., Waltham, MA). The different steps followed in the optimized workflow are described in detail in Fig. 1, and include: a) mass range selection from *m/z* 100–1000 and a retention time from 0.5 to 17 min, b) chromatographic alignment with a mass tolerance of ±5 ppm and a retention time tolerance of 0.3 min, c) detection of unknown compounds at a mass tolerance of ±5 ppm, minimum peak intensity of 10^5, a signal to noise (S/N) ratio of 10, isotopic intensity tolerance of 30% and a minimum peak width of 0.5 min, and d) grouping compounds detected at a mass tolerance of ±5 ppm and a retention time tolerance of 0.3 min. Further information of the selected parameters is presented in the Supplementary Material, Table S4. Identification of tentative compounds after data filtering was performed

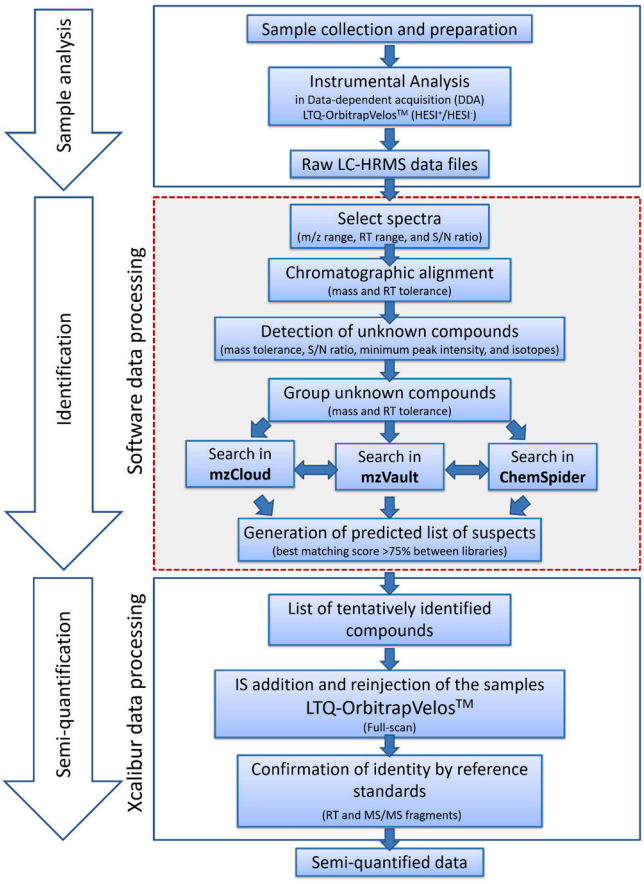

Fig. 1. Workflow for the identification of micropollutants in the suspect screening approach.

within the Compound Discoverer software, by comparison between experimental and theoretical accurate masses and MS/MS ion spectra using the software-linked libraries mzCloud, mzVault, and ChemSpider. Databases mzCloud and mzVault provide accurate MS/MS fragment data for many CECs and TPs (MS/MS spectral libraries), while ChemSpider contains information about elemental composition, molecular weight, or monoisotopic masses, and provides a number of citations associated to each compound (Little et al., 2012). Only those compounds that showed a high degree of similarity between the experimental and theoretical accurate masses and spectra, specifically by using the established threshold value (≥75%), were considered as tentatively identified in the samples. The occurrence of the compounds tentatively identified, and that showed a similar matching higher than or equal to 75%, was further verified by manual evaluation using the Xcalibur software (Thermo Scientific, USA). After data reduction and compound identification, final confirmation of the tentatively identified compounds was done by comparing their retention times and MS/MS fragments with those from ILISs. ILISs were added at a concentration of 500 μg L^{-1} in the sample extracts, which were reinjected and analyzed again. Samples spiked with ILISs prior to SPE were also processed to have information about any losses that could occur during the analytical process and to account for any potential matrix effects. The degree of confidence in compound identification was done following the classification defined by Schymanski et al., 2014 (Schymanski et al., 2014). In this classification a five-level identification confidence scheme is proposed, which starts with level 5 as the lowest level of confidence and moves up to level 1 with the highest degree of confirmation. According to this system, identification levels are defined as follows: (i) level 5 corresponds to the identification of exact masses only, (ii) level 4 refers to the assignment of an unequivocal molecular formula to these exact

masses, (iii) level 3 is achieved when tentative candidate compounds are assigned to the molecular formulas, (iv) level 2 is able to assign a probable structure to the tentative candidate compounds and (v) level 1 is only achieved when the occurrence of the candidate compounds is confirmed with reference analytical standards (highest degree of confidence). In our study, the compounds tentatively identified were those that comply with level 1 and 2 specifications.

2.6. Environmental risk assessment

The potential risks of the identified compounds were assessed using the RQ approach. RQs were calculated as the ratio between the compounds measured concentration (Ci, ng L^{-1}), determined by semi-quantification using the ILISs, and predicted no-effect concentrations (PNEC, μg L^{-1}). The NORMAN database (NORMAN Ecotoxicology Database, 2019) was used to obtain the PNEC values. In this database, PNECs are calculated based on ecotoxicological data reported for different trophic levels (algae, daphnids and fish), which were either predicted by QSAR or obtained experimentally. For the calculation of RQs, the lowest available PNEC values, for the most sensitive daphnids species assayed (worst-case scenario), were considered. Table S5 in Supplementary Material shows the PNECs used in this study. The risk ranking criterion applied was: RQ < 0.1 minimal risks, 0.1 ≤ RQ < 1 median risks, and RQ > 1 high risks towards aquatic organisms (Manickum and John, 2014; Cao et al., 2010; Wu et al., 2017; Wang and Zhu, 2017).

3. Results and discussion

3.1. Identification and semi-quantification of suspects

The screening of suspects by applying the methodology described in Section 2.5 was performed for each of the different sample types separately. Table 1 shows the results obtained and the number of positive findings after each data filtration step. Mass filtering and chromatographic alignment was performed for all exact masses detected in positive $[M+H]^+$ and negative $[M-H]^-$ ionization modes in the full scan MS spectra. In order to increase confidence in compound identification, peaks fulfilling the afore-mentioned pre-set criteria (i.e. intensity>10^5, S/N > 10, signal 10 times higher than the blank sample) were screened for throughout the chromatogram. In this first step, compounds were identified and filtered based on their mass accuracy (<5 ppm) and isotope ratio difference (IRD < 10%). After applying these criteria, the number of positive features was reduced by 38%, from 360 to 137. From these 137 selected features, their MS/MS fragmentation spectra were compared with the information provided in the software-linked databases mzCloud, mzVault, and ChemSpider, and measured fragments were compared with the predicted fragments, for each candidate substance based on their exact masses. As an example, the MS/MS spectra identification for one of the confirmed suspects is presented in Fig. 2. The figure at the top compares the experimental and theoretical MS/MS spectra predicted by mzCloud, while the figure below matches the experimental and theoretical MS/MS spectra predicted by mzVault. The percentages indicate the similarity between both spectra. Within each figure, the theoretical fragment ions, which are predicted by the databases, are included below, and the experimental fragments are indicated at the top. To further increase the confidence of identification, only the suspects whose theoretical and experimental MS/MS spectra had a match higher than or equal to 75% were considered as positively identified. Furthermore, only those compounds that had at least two characteristic MS/MS fragments, with a mass error <5 ppm in two MS/MS spectral libraries (mzCloud and mzVault), and detected in the web database ChemSpider, were considered as positively identified in the samples. At this stage, exclusion of suspects that did not fulfill the established criteria was 68 features, resulting in a 50% of data reduction. A manual evaluation of the remaining 69 features was performed to avoid any false positive findings, checking the isotopic patterns and peak shapes of the extracted-ion chromatograms (XIC). Furthermore, blanks were carefully evaluated and only those compounds whose peak area was higher than three times the peak area in the blanks were considered as present in the samples. After manual inspection, 21 peaks were excluded. After all these steps, 48 CECs were tentatively identified in the samples with confidence level 2 (Table S6 in the Supplementary

Table 1
Reduction of positive findings after each filtering step in the automated data processing workflow.

Suspect screening	Number of features	$HESI^+$/ $HESI^-$
List of suspects	360	
Software data processing		
Number of suspect detected	137	(72 +/65-)
Number of excluded compounds that did not fulfill the established criteria	68	(37^+/31^-)
Total number of suspects identified (searched in databases)	69	(35 +/34-)
Removed after manual investigation of chromatograms	21	(3^+/18^-)
Number of tentatively identified compounds	48	(34^+/14^-)
Tentative candidates rejected after ILIs addition	11	(3^+/8^-)
Fully confirmed suspects	37	(31^+/6^-)

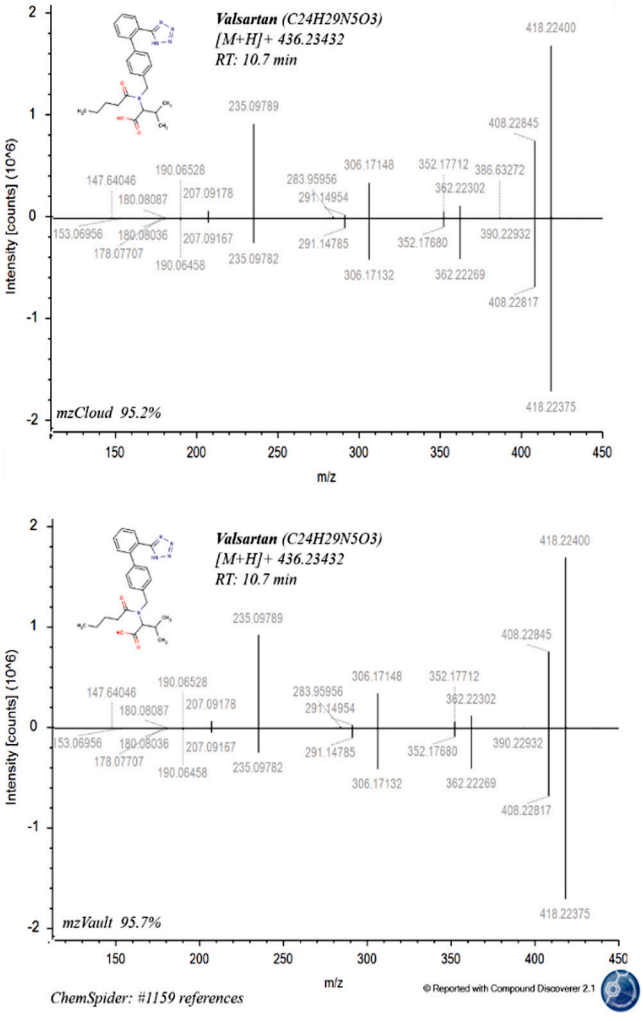

Fig. 2. Illustrative example for the identification of valsartan with the Software Compound Discoverer 2.1.

Table 2
List of confirmed (and semi-quantified) compounds (37) organized into different chemical groups (level 1 and level 2 refer to the degree of confidence in their identification).

Group	Use/Origin	Compound	Internal standard	RT (min)	Molecular formula	m/z (Expected)	m/z (Measured)	HESI mode	Identification level
Pharmaceuticals, metabolites, and TPs (16)	Analgesics/anti-inflammatories	Mesalamine	Acetaminophen-d_4	5.5	$C_7H_7NO_3$	153.04259	154.04987	(+)	*level-2*
		Acetaminophen	Acetaminophen-d_4	6.7	$C_8H_9NO_2$	151.06333	152.07061	(+)	*level-1*
		4-Acetamidoantipyrine	Antipyrine-d_3	7.0	$C_{13}H_{15}N_3O_2$	245.11643	246.12370	(+)	*level-2*
		4-Formylaminoantipyrine	Antipyrine-d_3	7.0	$C_{12}H_{13}N_3O_2$	231.10078	232.10805	(+)	*level-2*
	Opoid analgesic	Tramadol	Tramadol-^{13}C, d_3	7.3	$C_{16}H_{25}NO_2$	263.18853	264.19581	(+)	*level-1*
	Intravenous/topical analgesic	Lidocaine	Lidocaine-d_{10}	7.5	$C_{14}H_{22}N_2O$	234.17321	235.18049	(+)	*level-1*
	Psychoactive drugs	Carbamazepine	Carbamazepine-d_{10}	9.6	$C_{15}H_{12}N_2O$	236.09496	237.10224	(+)	*level-1*
		Citalopram	Citalopram-d_4	8.8	$C_{20}H_{21}FN_2O$	324.16379	325.17107	(+)	*level-1*
		Venlafaxine	Venlafaxine-d_6	8.2	$C_{17}H_{27}NO_2$	277.20418	278.21146	(+)	*level-1*
		Didesmethylvenlafaxine	Venlafaxine-d_6	7.0	$C_{15}H_{23}NO_2$	249.17288	250.18016	(+)	*level-2*
	β-Blocking agent	Atenolol acid	Atenolol-d_7	6.7	$C_{14}H_{21}NO_4$	267.14706	268.15433	(+)	*level-2*
	Anticonvulsant	Lamotrigine	Lamotrigine-$^{13}C_3$	7.7	$C_9H_7Cl_2N_5$	255.00785	256.01513	(+)	*level-1*
	Antihypertensives	Valsartan	Valsartan-d_8	10.7	$C_{24}H_{29}N_5O_3$	435.22704	434.21976	(-)	*level-1*
		Telmisartan	Valsartan-d_8	11.1	$C_{33}H_{30}N_4O_2$	514.23688	513.22959	(-)	*level-2*
	Antidiabetic	Metformin	Metformin-d_6	2.9	$C_4H_{11}N_5$	129.10145	130.10872	(+)	*level-1*
	Diuretic	Hydrochlorothiazide	Hydrochlorothiazide-d_2	7.7	$C_7H_8ClN_3O_4S_2$	296.96447	295.95720	(-)	*level-1*
Pesticides and metabolites (10)	Herbicide	Metolachlor	Metolachlor-d_{11}	13.1	$C_{15}H_{22}ClNO_2$	283.13391	284.14118	(+)	*level-1*
		Terbutryn	Terbutryn-d_3	12.6	$C_{10}H_{19}N_5S$	241.13612	242.14339	(+)	*level-1*
		Terbuthylazine	Terbuthylazine-d_5	12.0	$C_7H_{12}ClN_5$	201.07812	202.08540	(+)	*level-1*
		Desethylterbuthylazine	Terbuthylazine-d_5	10.0	$C_7H_{12}ClN_5$	201.07812	202.08540	(+)	*level-2*
	Fungicide	Azoxystrobin	Azoxystrobin-d_4	11.8	$C_{22}H_{17}N_3O_5$	403.11682	404.12410	(+)	*level-1*
		Metalaxyl	Metalaxyl-$^{13}C6$	10.8	$C_{15}H_{21}NO_4$	279.14706	280.15433	(+)	*level-1*
		Prochloraz	Prochloraz-d_4	13.1	$C_{15}H_{16}Cl_3N_3O_2$	375.03081	376.03809	(+)	*level-1*
		Propiconazole	Propiconazole-d_3	13.1	$C_{15}H_{17}Cl_2N_3O_2$	341.06978	342.07706	(+)	*level-1*
		Tebuconazole	Tebuconazole-d_9	12.4	$C_{16}H_{22}ClN_3O$	307.14514	308.15242	(+)	*level-1*
	Insecticide	Tebufenozide	Metalaxyl-$^{13}C_6$	12.9	$C_{22}H_{28}N_2O_2$	352.21508	351.20780	(-)	*level-2*
	Personal care products	Diethyltoluamide (DEET)	DEET-d_{10}	10.9	$C_{12}H_{17}NO$	191.13101	192.13829	(+)	*level-1*
		Panthenol	Triclosan-methyl-ether-d_3	6.1	$C_9H_{19}NO_4$	205.13141	206.13868	(-)	*level-2*
		Galaxolidone	Oxybenzone-d_5	14.8	$C_{18}H_{24}O_2$	272.17763	273.18491	(-)	*level-2*
Others (11)	Stimulants and metabolites	Caffeine	Caffeine-^{13}C	7.0	$C_8H_{10}N_4O_2$	194.08038	195.08765	(+)	*level-1*
		Xanthine	Caffeine-^{13}C	3.6	$C_5H_4N_4O_2$	152.03343	151.02615	(-)	*level-2*
		1-Methylxanthine	Caffeine-^{13}C	5.7	$C_6H_6N_4O_2$	166.04908	165.04180	(-)	*level-2*
		Theophylline	Caffeine-^{13}C	6.5	$C_7H_8N_4O_2$	180.06473	179.05745	(-)	*level-2*
		1,7- Dimethyluric acid	Caffeine-^{13}C	4.0	$C_7H_8N_4O_3$	196.05964	196.05950	(-)	*level-2*
		Nicotine	DL-Nicotine- d_3	4.4	$C_{10}H_{14}N_2$	162.11570	163.12298	(+)	*level-1*
		Cotinine	DL-Nicotine- d_3	5.1	$C_{10}H_{12}N_2O$	176.09496	177.10224	(+)	*level-2*
	Drug of abuse	Cocaine	Cocaine-d_3	8.1	$C_{17}H_{21}NO_4$	303.14706	304.15433	(+)	*level-1*

Material). From those 48, 34 features were determined using (+) and 14 hits using (-) mode.

For compound final confirmation, 26 ILISs were used (see Table S2 in Supplement Material). ILISs addition was performed to confirm the presence of the tentatively identified analogous substances with level 1 confidence and to semi-quantify the CECs in the samples (Section 3.3). For the substances (mesalamine, telmisartan, panthenol and tebufenozide), who's analogous ILISs were not available, the most similar standard in terms of chemical structure and chromatographic retention time was used for semi-quantification. For TPs, the ILISs corresponding to their parent compound was used for quantification. All these compounds were classified as level 2, since the exact same isotopically labelled analogous was not available for confirmation with level 1 confidence. CECs were confirmed by comparing spiked controls with the real samples using a mass and RT tolerance error of ±5 ppm, and ±2 min, respectively. In addition, eleven other substances that were tentatively identified using the database available were rejected, due to their absence from the suspect list, or because some of them are so far not known to be of environmental relevance. In total, out of the 37 identified suspects, 22 were finally confirmed with their analogous ILISs with level 1 and 15 were identified under level 2 (Table 2) and all the 37 compounds were semi-quantified using response factors. Concentrations achieved were afterwards corrected by the corresponding ILISs recovery, in order to correct for any potential analyte losses during SPE extraction and to overcome matrix effects. Table 2 shows the list of the confirmed suspects and the ILISs used for their semi-quantification. Compounds are classified in different groups according to their specific usage and are marked with level 1 or 2 category. It is important to highlight that the reliability in the identification of compounds labelled as level 2 is high, since their occurrence was confirmed by MS/MS spectra. In Table 2, 4 compounds, such as mesalamine, telmisartan, panthenol, and tebufenozide and 11 TPs namely 4-acetamidoantipyrine, 4-formylaminoantipyrine, didesmethylvenlafaxine, atenolol acid, desethylterbuthylazine, galaxolidone, xanthine, 1-methylxanthine, theophylline, 1,7-dimethyluric acid, and cotinine, are displayed as level 2. After the final step of ILISs matching (Table 2), 22 compounds were confirmed by a match of MS, MS/MS fragments (m/z, intensity), and RT with the reference standards and thus marked as level 1 and they were: (i) 10 pharmaceuticals, including acetaminophen, tramadol, carbamazepine, citalopram, venlafaxine, lidocaine, lamotrigine, valsartan, metformin, and hydrochlorothiazide, (ii) 8 pesticides, namely the herbicides metolachlor, terbuthylazine, and terbutryn, and the fungicides azoxystrobin, metalaxyl, prochloraz, propiconazole, and tebuconazole; and (iii) 4 compounds classified as others such as, the stimulants caffeine and nicotine, the drug of abuse cocaine and the insect repellents diethyltoluamide (DEET).

3.2. *Distribution patterns of CECs in wastewaters and receiving riverine and coastal areas*

Suspect screening revealed the presence of 26 CECs and 11 TPs from different chemical classes (Table 2) in all collected samples. The identified CECs were divided in the following categories: (i) pharmaceuticals, (ii) pesticides, (iii) personal care products, and (iv) stimulants and their metabolites (Fig. 3). Fig. 3 graphically shows the compound groups that contribute the most to the total concentration of CECs detected (composition profiles, expressed in %, of the total concentration of each group relative to the total concentration of CECs detected) in the different types of samples analyzed: (a) effluents, (b) Ebro River and emissary Sant Carles de la Ràpita, c) channels, and (d) estuary and seawater samples. Concerning the distribution patterns in effluent wastewaters, pharmaceuticals were the most remarkable compound group, in terms of concentrations. This finding is in good agreement with previous studies where pharmaceuticals were the dominant CECs group in urban wastewater effluents, mostly attributed to their wide use (Glauner et al., 2016; Assress et al., 2019). Comparing the CECs distribution patterns in effluent wastewaters (Fig. 3a) with the other types of samples, a similar profile is observed between wastewater effluents and sites that are directly affected by WWTPs discharges, such as Ebro River samples and emissary of Sant Carles de la Ràpita (which receive WWTP effluent discharges) (Fig. 3b). The presence of pharmaceuticals in the river and in the emissary (pharmaceuticals contribute to 65% to the total pollutant load) is higher than in irrigation and drainage channels (26%) and in estuary and seawater (16%) (Fig. 3). This change in composition profile could be explained by their dilution once they are discharged into freshwater and coastal environments. Nevertheless, some of the most prominent pharmaceuticals in terms of frequency of detection and concentration were present in marine samples, indicating that WWTP effluents are a non-negligible source of contamination in the coastal environment.

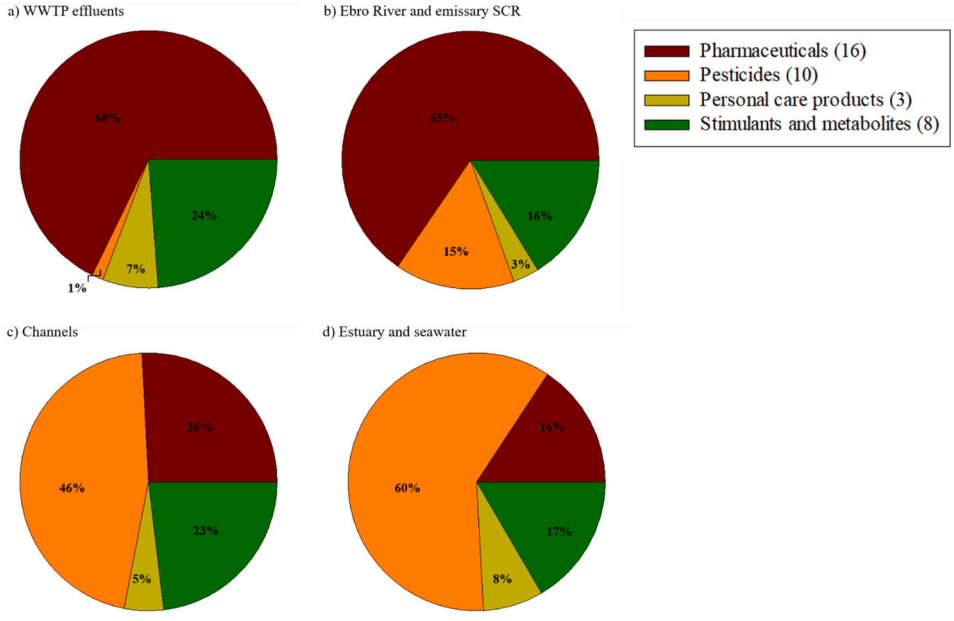

Fig. 3. Contribution of each compound group in the total concentration of CECs detected (composition profiles, expressed in %) across the different sample types.

Fig. 3c also shows that pesticides were the most abundant group of compounds in drainage and irrigation channels (46% of the total distribution), as expected, due to the spread agricultural activity in the region. Indeed, the most important economic activity in the Ebro Delta is agriculture (specifically rice production) (Kuzmanović et al., 2015). Regarding seawater (Fig. 3d), pesticides were also the most abundant group of compounds (60% of the total distribution), indicating that agriculture is an important source of pollution of the surrounding coastal environment and it is even a more prominent source of pollution than WWTPs. In contrast, effluents contain only 1% of pesticides, while samples from Ebro River and receiving estuary had only a 15%. Following the composition profiles of the groups labeled as personal care products and stimulants and their metabolites, they have a similar distribution pattern throughout all the samples, even in coastal settings. However, concentrations of individual compounds were much lower in seawater in comparison with other samples. This similarity in composition profile indicates that these chemical compounds originated from various anthropogenic activities (Kuzmanović et al., 2016) and not only from WWTP discharges.

3.3. Environmental relevance of suspects

Overall, pharmaceuticals was the group of compounds showing the highest total concentrations (low to high ng L^{-1} levels) in all samples, followed by pesticides, personal care products, stimulants and their derivates which showed similar range of concentrations (low to medium ng L^{-1} levels) (Fig. 4). The specific concentration levels for each compound, along with their frequency of detection as a result of semi-quantitative analysis, can be found in Table S7 in the Supplementary Material.

In total, eleven human-use pharmaceuticals and five TPs (Fig. 4a), belonging to different therapeutic groups, such as analgesics/anti-inflammatories, psychiatric drugs, β-blocking agents, cardiovascular drugs, anticonvulsants, antihypertensives, antidiabetics and diuretics were detected, at concentrations ranging from 6.3 to 9959 ng L^{-1} in the influents, and from 0.6 to 3915 ng L^{-1} in effluents (Table S7 in Supplementary Material). Concentrations of individual substances detected in WWTPs match well with previous studies (Borova et al., 2014; Glauner et al., 2016; Lindberg et al., 2014). In other samples, pharmaceuticals concentrations fell within the ng L^{-1} level along the catchment of the Ebro River, from approximately 0.3 to 266 ng L^{-1} in irrigation and drainage channels, and from 0.2 up to 98.4 ng L^{-1}, in coastal areas indicating that these substances are diluted once they reach receiving freshwater and coastal ecosystems (Čelić et al., 2019). For pharmaceuticals total concentrations, the site showing the highest levels was SCR, the emissary in Sant Carles de la Ràpita (up to 2187 ng L^{-1}). This site is mostly composed by constant wastewater effluent released from WWTP2 Sant Carles de la Rapita and is subject to minor changes in its flow (Pignotti et al., 2017).

Metformin (93%), telmisartan (56%), carbamazepine (52%), lamotrigine (44%), valsartan (41%) and venlafaxine (41%) were the compounds most frequently detected in the samples. Specifically, the highest individual concentrations were determined for the antihypertensives telmisartan (534.7 ng L^{-1}) and valsartan (457.8 ng L^{-1}), the antidiabetic metformin (192.3 ng L^{-1}), the psychiatric drugs carbamazepine (170.4 ng L^{-1}) and venlafaxine (144.1 ng L^{-1}), and the cardiovascular drug lidocaine (126.3 ng L^{-1}) in the emissary (site SCR). These levels are in the same range as those found in previous studies that focused on natural aquatic ecosystems impacted by WWTP discharges (Kasprzyk-Hordern et al., 2008; Daneshvar et al., 2010; Gros et al., 2012; Gago-Ferrero et al., 2017). Among the most abundant pharmaceuticals in this study metformin, telmisartan and lidocaine are compounds less frequently measured in routine monitoring programs in comparison with other drugs and their presence has been mostly overlooked in previous monitoring campaigns in Spain. However, their presence has been reported in other countries, at similar concentrations to those

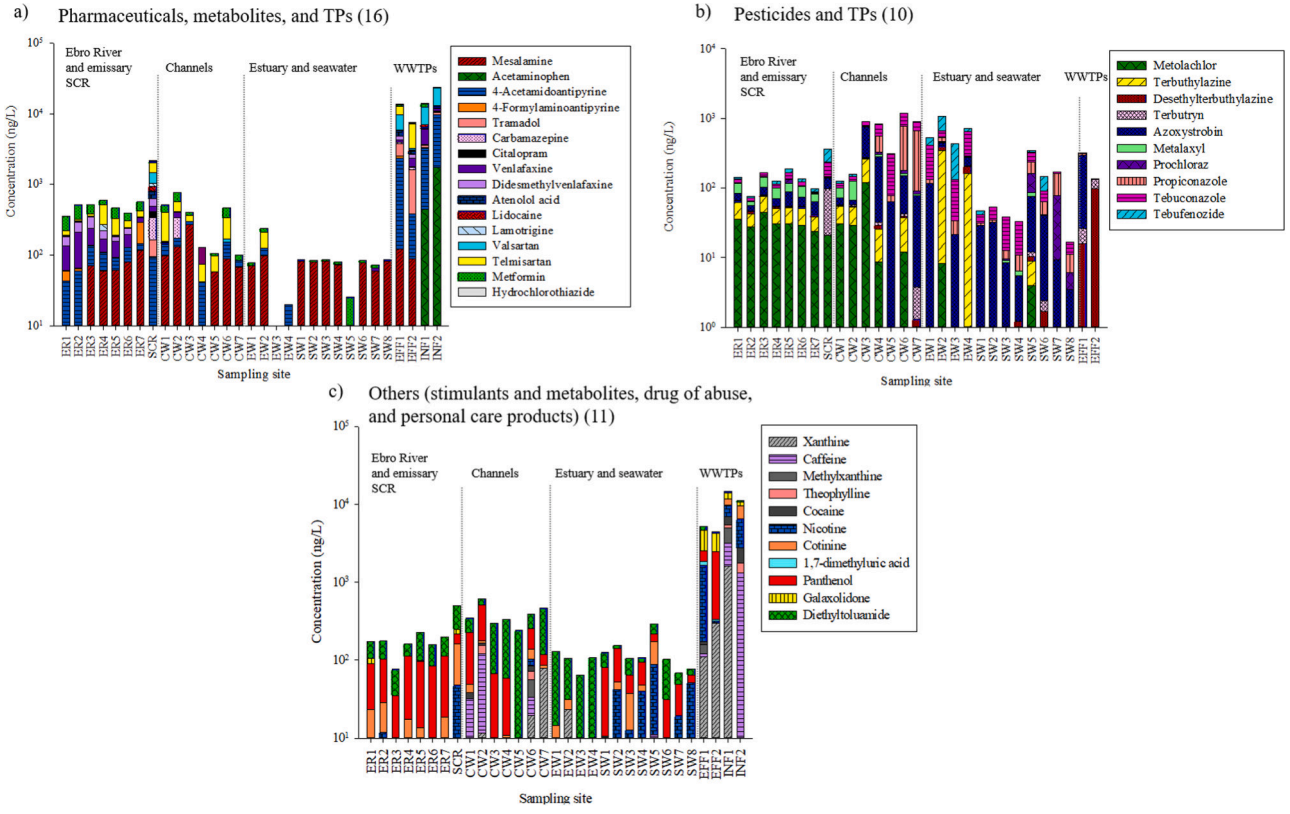

Fig. 4. Cumulative levels (ng L^{-1}) of suspects detected in different sampling sites in the study area.

observed in our study (Park et al., 2018; Choi et al., 2020; Bradley et al., 2016; Loos et al., 2013).

Besides reporting the occurrence of parent pharmaceuticals, with the suspect screening approach we were able to monitor the presence of some relevant metabolites, such as mesalamine (74%), an active metabolite of the anti-inflammatory sulphasalizine, two metabolites of the analgesic antipyrine (4-acetamidoantipyrine, 59% and 4-formylaminoantipyrine, 22%), the metabolites of the β-blocking agent atenolol (atenolol acid, 37%) and the antidepressant venlafaxine (didesmethylvenlafaxine, 26%), being mostly found in wastewater-impacted sites. Among all TPs, mesalamine (5-aminosalicylic acid), which is also available as a pharmaceutical product used to treat ulcerative colitis (Abdelrahman et al., 2020), was the most abundant TP and it was detected at levels up to 266 ng L^{-1}. To the authors' best knowledge, the occurrence of mesalamine was previously un-reported. Furthermore, this study provides data, for the first time, for the occurrence of 4-acetamidoantipyrine and 4-formylaminoantipyrine in surface and wastewater in Spain. However, these TPs have been identified in wastewaters or landfill leachate samples in other countries (Gago-Ferrero et al., 2020; Müller et al., 2011). Similar concentration levels than those reported herein were found in other suspect screening studies for atenolol acid and didesmethylvenlafaxine (Nödler et al., 2014; Park et al., 2018; Gago-Ferrero et al., 2020; Schlüsener et al., 2015; Arpin-Pont et al., 2016).

Focusing on marine water, only 5 out of the 16 pharmaceuticals were found, including metformin, mesalamine, carbamazepine, venlafaxine, and 4-formylaminoantipyrine. More specifically, metformin and mesalamine were the compounds present in almost all seawater samples. These substances were some of the most widely detected in effluents as well, indicating that WWTP discharges are an important source of these compounds in coastal water. To the best of our knowledge metformin is not so widely reported in marine waters (Ali et al., 2017), while mesalamine and 4-formylaminoantipyrine were previously unreported in other coastal settings.

For pesticides, 10 compounds belonging to different groups, such as herbicides (4), fungicides (5) and insecticides (1), were detected throughout the study area (Fig. 4b). Previous studies already reported that pesticides are one of the most abundant groups of pollutants in the Ebro Delta (Masiá et al., 2013). The most prominent pesticides detected in all freshwater samples were the fungicides azoxystrobin (100%), propiconazole (100%), and tebuconazole (100%) with particularly high concentrations in channels up to 599 ng L^{-1}. Other frequently detected pesticides, and found in lower concentration ranges, were metalaxyl (fungicide, 74%), metolachlor (herbicide, 70%), terbutryn (herbicide, 63%), tebufenozide (insecticide, 59%) and prochloraz (fungicide, 44%). All these compounds are widely used in agriculture. Thus, their high occurrence in the samples can be mostly related with the intensive agricultural activity in the area, since they were also widely detected in previous studies focusing on the monitoring of pesticides in Ebro Delta (Kuzmanović et al., 2015; Hildebrandt et al., 2008). The use of the suspect screening approach allowed the identification of desethylterbuthylazine in 85% of the samples, one of the most relevant degradation products of the herbicide terbuthylazine. Indeed, desethylterbuthylazine was more frequently detected than its parent compound. However, its concentrations were 7-fold lower, reaching concentration up to 48.4 ng L^{-1}. Similar concentrations of desethylterbuthylazine were obtained in other monitoring campaigns performed in the Ebro River basin (Hildebrandt et al., 2008; Moreno-González et al., 2013; Carafa et al., 2007).

Even though pesticides were present at low ng L^{-1} levels in marine waters, ranging from 0.3 to 88.4 ng L^{-1}, they were quite ubiquitous in the coastal environment. Some compounds, namely tebuconazole, propiconazole, prochloraz and azoxystrobin were found to be the most prominent pesticides in seawater, in terms of frequency of detection and concentration levels. However, pesticides concentrations determined in our study were lower compared to those reported in other coastal settings (Nödler et al., 2014; Carafa et al., 2007), but higher than those found in other littoral systems (Brumovský et al., 2017; Loos et al., 2013; Weigel et al., 2002), mostly related with seasonal application patterns.

Concerning the other group of compounds, several personal care products, stimulants and their derivates were also present in the samples (Fig. 4c). Total concentrations (sum of all compounds) from this group ranged from 64.3 to 616 ng L^{-1}. Individual substances that stood out in freshwater samples were DEET (present in 100% of the samples) and panthenol (found in 81% of the samples). These compounds were detected at highest maximum concentrations up to 343 ng L^{-1}. Regarding marine water samples, panthenol and DEET were ubiquitous CECs (found in all the samples) with mean concentrations of 42.7 and 36.9 ng L^{-1}, respectively. DEET is the main active ingredient in insect repellents for human use and it has also been applied in agriculture, e.g. to grazing cattle (Weigel et al., 2002; Riha et al., 1991). Its ubiquity in the area might be explained by a higher use of DEET in warm seasons to prevent mosquito bites. Even though DEET has been widely reported in other studies (Gago-Ferrero et al., 2017; Sui et al., 2010) its occurrence in the Ebro Delta region has not been reported yet. Panthenol has been rarely monitored in coastal settings, but it has been recently identified in few screening studies (Gago-Ferrero et al., 2015; Gago-Ferrero et al., 2020).

Nicotine-related compounds (nicotine 48%; cotinine 85%) and caffeine-related compounds (caffeine, 30%; xanthine, 26%; theophylline, 7%; 1-methylxanthine, 4%), were also ubiquitous substances, revealing anthropogenic contamination sources in the area. Cotinine, which is the major urinary metabolite of nicotine, was found to be more abundant than its parent compound, and has been suggested as a potential marker for domestic wastewater contamination of surface waters (Metcalfe et al., 2003; Glassmeyer et al., 2005; Buerge et al., 2008). Caffeine is consumed in large amounts in many countries in the form of beverages, or as analeptic and in combination with analgesics to enhance their effect. Consequently, caffeine and its derivates were detected in many studies (Choi et al., 2020; Gago-Ferrero et al., 2017; He et al., 2018; Senta et al., 2015), and it has also been proposed as a marker of anthropogenic contamination (Esteban et al., 2014). The drug of abuse cocaine and the TP of the synthetic musk galaxolide (galaxolidone) were only sporadically (<15%) detected across the study area in low ng L^{-1} levels, being their use associated to urban settlements as well.

In seawater samples, nicotine (detected in 6 sites), and its metabolite cotinine (5 sites) were also ubiquitous substances, while caffeine (2 sites) and cocaine (only in one site) were almost exclusively detected in bays and harbor areas at low ng L^{-1} and their occurrence might be mostly attributed to human activities and tourism in the area. The concentration range of stimulants and their metabolites in seawater (4.7–86.1 ng L^{-1}) is similar to those found in other studies in marine environments (Nödler et al., 2014; Biel-Maeso et al., 2018; Kim et al., 2017; Alygizakis et al., 2016).

3.4. Risk assessment

The RQs calculated for the suspects were used to identify the most ecologically relevant compounds, which could be considered as suitable markers of anthropogenic contamination in freshwater and coastal ecosystems.

In total, RQ > 1 was estimated for 10 chemicals indicating that their concentrations found in the receiving water environment can pose a high risk for aquatic organisms. These compounds include the pharmaceuticals telmisartan, venlafaxine and carbamazepine, the herbicides terbuthylazine, desethylterbuthylazine and terbutryn, the fungicides azoxystrobin, tebuconazole and prochloraz and the insecticide tebufenozide (Fig. 5). This threshold for pharmaceuticals was exceeded at sampling locations downstream WWTP discharges such as SCR and ER5-ER7 (Fig. 5b), and RQ for pesticides was exceeded in sites located in channels, estuary and seawater mostly influenced by anthropogenic and agricultural activities (Fig. 5c and d). The compound with the highest

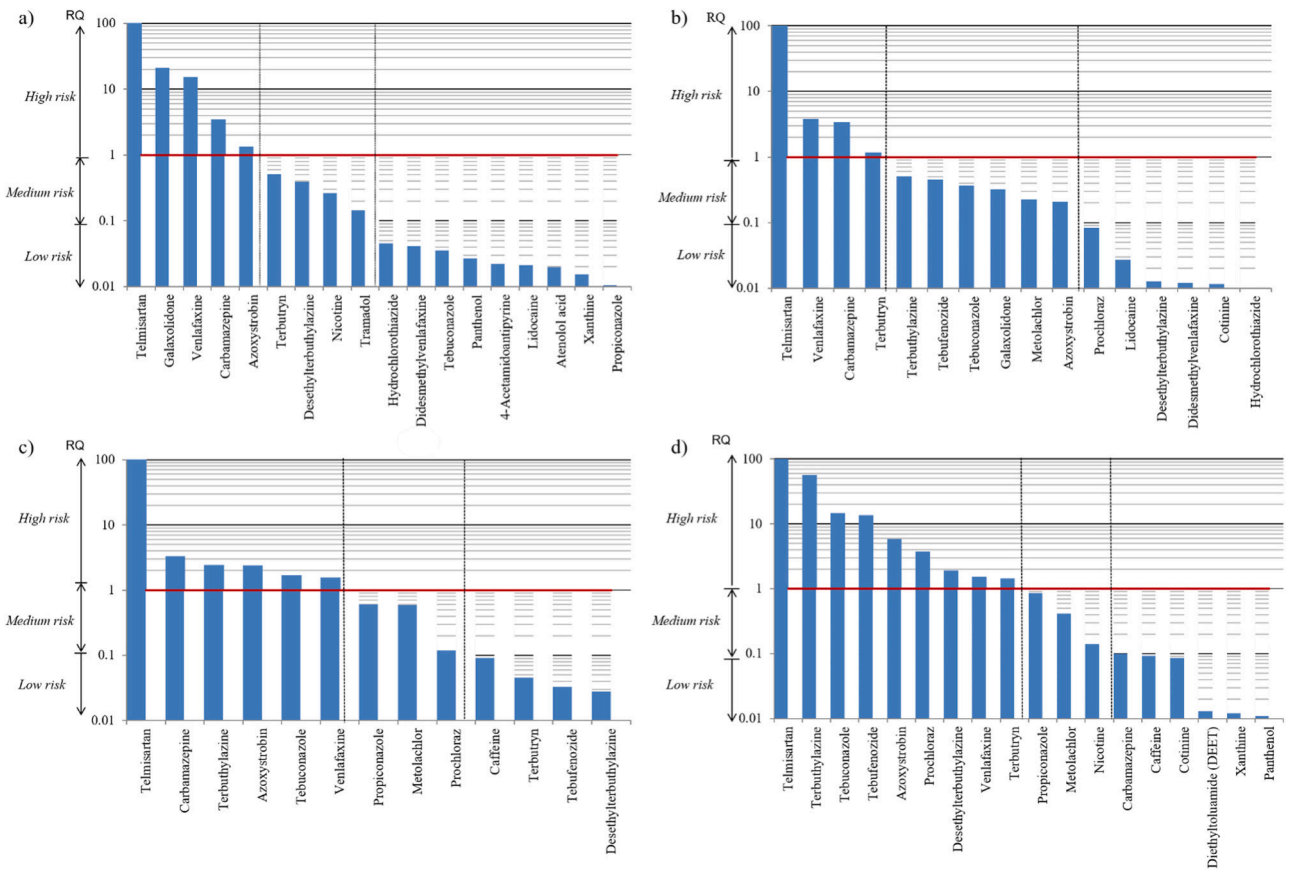

Fig. 5. Estimated risk quotient ratios for the CECs identified in a) WWTP effluents, b) Ebro River and Emissary, c) channels, and d) estuary and seawater.

RQ in all types of samples, ranging from 28 to 972, was the antihypertensive telmisartan (Fig. 5), attributed to its low PNEC values (0.5 ng L^{-1}), showing toxic potential at environmentally relevant concentrations (see Table S5). In seawater, besides telmisartan, terbuthylazine, tebuconazole and tebufenozide (RQ>10) were the most important compounds from an ecotoxicological point of view for marine organisms. Seven out of the ten compounds highlighted as relevant chemicals belong to the group of pesticides, explaining the importance of the intensive agricultural activities developed in the Ebro Delta area. Similar compounds were reported as of major concern in a risk assessment study carried out by Kuzmanovic et al. (2015) across Iberian rivers (including the Ebro river), where pesticides were also highlighted as priority pollutants among a wide range of organic contaminants (Kuzmanović et al., 2015). Our findings match well with other prioritization strategies based on risk assessment and exposure effects performed by other authors (von der Ohe et al., 2011; Besse and Garric, 2008; Donnachie et al., 2016). It is worth mentioning that one out of the seven ranked pesticides, terbutryn, is included in the list of priority substances of the European Water Framework Directive (EU WFD) (European Comission, 2013). It is classified as toxic to aquatic wildlife with long lasting effects (Mascolo et al., 2019). These results show that, even though pesticides are used in Europe following strict guidelines to reduce detrimental impacts in the environment (European Comission, 2009), they are still persistent and CECs of ecological concern in the environment. This highlights the need for establishing even more stringent guidelines and assessments for these substances.

4. Conclusions

This study demonstrates that suspect screening approaches based on HRMS are a powerful tool to identify the most relevant CECs, TPs and metabolites in environmental samples and allows the identification of previously unreported substances. Additionally, the data treatment tool for suspect screening analysis developed herein, based on exact mass, retention time and spectral matching using databases, showed its capability for the simultaneous unequivocal identification of 37 compounds, which are not usually included in target methods, attaining high degree of confidence in their identification using just one software package. The use of isotopic reference standards allowed us to confirm most of the identified compounds and evaluate the occurrence of suspects along the Ebro Delta. The combination of the suspect screening strategy with a risk-assessment based prioritization provided information about the compounds that can be considered of major risk for the aquatic environment and that should be classified as priority substances in future monitoring programs. Overall, suspect screening offers a new perspective in the context of regulatory frameworks and could improve current water quality monitoring programs and further exposure assessments.

CRediT authorship contribution statement

Mira Čelić: Conceptualization, Methodology, Software, Data curation, Writing-original draft, Writing - review & editing; **Adrián Jaén-Gil**: Methodology, Software, Data curation; **Susana Briceño-Guevara**: Methodology; **Sara Rodríguez-Mozaz**: Conceptualization, Writing-original draft, Writing - review & editing; **Meritxell Gros**: Supervision, Conceptualization, Writing original draft, Writing - review & editing; **Mira Petrović**: Supervision, Conceptualization, Writing original draft, Writing - review & 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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2020.124102.

References

Aalizadeh, R., Thomaidis, N.S., Bletsou, A.A., Gago-Ferrero, P., 2016. Quantitative structure-retention relationship models to support nontarget high-resolution mass spectrometric screening of emerging contaminants in environmental samples. J. Chem. Inf. Model. 56, 1384–1398. https://doi.org/10.1021/acs.jcim.5b00752.

Abdelrahman, M.M., Habib, N.M., Emam, A.A., Mahmoud, H.M., Abdelwhab, N.S., 2020. Chromatographic determination of sulfasalazine and its active metabolites: greenness assessment and application to spiked human plasma. Biomed. Chromatogr. 34, 0–2. https://doi.org/10.1002/bmc.4804.

Agüera, A., Bueno, M., Fernández-Alba, A., 2013. New trends in the analytical determination of emerging contaminants and their transformation products in environmental waters. Environ. Sci. Pollut. Res. Int. 20 https://doi.org/10.1007/s11356-013-1586-0.

Ali, A.M., Rønning, H.T., Al Arif, W.M., Kallenborn, R., Kallenborn, R., 2017. Occurrence of pharmaceuticals and personal care products in effluent-dominated Saudi Arabian coastal waters of the Red Sea. Chemosphere 175, 505–513. https://doi.org/10.1016/j.chemosphere.2017.02.095.

Alygizakis, N.A., Gago-Ferrero, P., Borova, V.L., Pavlidou, A., Hatzianestis, I., Thomaidis, N.S., 2016. Occurrence and spatial distribution of 158 pharmaceuticals, drugs of abuse and related metabolites in offshore seawater. Sci. Total Environ. 541, 1097–1105. https://doi.org/10.1016/j.scitotenv.2015.09.145.

Arpin-Pont, L., Martínez-Bueno, M.J., Gomez, E., Fenet, H., 2016. Occurrence of PPCPs in the marine environment: a review. Environ. Sci. Pollut. Res. 23, 4978–4991. https://doi.org/10.1007/s11356-014-3617-x.

Assress, H.A., Nyoni, H., Mamba, B.B., Msagati, T.A.M., 2019. Target quantification of azole antifungals and retrospective screening of other emerging pollutants in wastewater effluent using UHPLC –QTOF-MS. Environ. Pollut. 253, 655–666. https://doi.org/10.1016/j.envpol.2019.07.075.

Besse, J.P., Garric, J., 2008. Human pharmaceuticals in surface waters. Implementation of a prioritization methodology and application to the French situation. Toxicol. Lett. 176, 104–123. https://doi.org/10.1016/j.toxlet.2007.10.012.

Biel-Maeso, M., Baena-Nogueras, R.M., Corada-Fernández, C., Lara-Martín, P.A., 2018. Occurrence, distribution and environmental risk of pharmaceutically active compounds (PhACs) in coastal and ocean waters from the Gulf of Cadiz (SW Spain). Sci. Total Environ. 612, 649–659. https://doi.org/10.1016/j.scitotenv.2017.08.279.

Borova, V.L., Maragou, N.C., Gago-Ferrero, P., Pistos, C., Thomaidis, N.S., 2014. Highly sensitive determination of 68 psychoactive pharmaceuticals, illicit drugs, and related human metabolites in wastewater by liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 406, 4273–4285. https://doi.org/10.1007/s00216-014-7819-3.

Brack, W., Hollender, J., de Alda, M.L., Müller, C., Schulze, T., Schymanski, E., Slobodnik, J., Krauss, M., 2019. High-resolution mass spectrometry to complement monitoring and track emerging chemicals and pollution trends in European water resources. Environ. Sci. Eur. 31 https://doi.org/10.1186/s12302-019-0230-0.

Bradley, P.M., Journey, C.A., Button, D.T., Carlisle, D.M., Clark, J.M., Mahler, B.J., Nakagaki, N., Qi, S.L., Waite, I.R., VanMetre, P.C., 2016. Metformin and other pharmaceuticals widespread in wadeable streams of the southeastern United States. Environ. Sci. Technol. Lett. 3, 243–249. https://doi.org/10.1021/acs.estlett.6b00170.

Brumovský, M., Bečanová, J., Kohoutek, J., Borghini, M., Nizzetto, L., 2017. Contaminants of emerging concern in the open sea waters of the Western Mediterranean. Environ. Pollut. 229, 976–983. https://doi.org/10.1016/j.envpol.2017.07.082.

Buerge, I.J., Kahle, M., Buser, H.R., Müller, M.D., Poiger, T., 2008. Nicotine derivatives in wastewater and surface waters: application as chemical markers for domestic wastewater. Environ. Sci. Technol. 42, 6354–6360. https://doi.org/10.1021/es800455q.

Campos-Mañas, M.C., Plaza-Bolaños, P., Martínez-Piernas, A.B., Sánchez-Pérez, J.A., Agüera, A., 2019. Determination of pesticide levels in wastewater from an agro-food industry: target, suspect and transformation product analysis. Chemosphere 232, 152–163. https://doi.org/10.1016/j.chemosphere.2019.05.147.

Cao, Q., Yu, Q., Connell, D.W., 2010. Fate simulation and risk assessment of endocrine disrupting chemicals in a reservoir receiving recycled wastewater. Sci. Total Environ. 408, 6243–6250. https://doi.org/10.1016/j.scitotenv.2010.08.059.

Carafa, R., Wollgast, J., Canuti, E., Ligthart, J., Dueri, S., Hanke, G., Eisenreich, S.J., Viaroli, P., Zaldívar, J.M., 2007. Seasonal variations of selected herbicides and related metabolites in water, sediment, seaweed and clams in the Sacca di Goro coastal lagoon (Northern Adriatic). Chemosphere 69, 1625–1637. https://doi.org/10.1016/j.chemosphere.2007.05.060.

Čelić, M., Gros, M., Farré, M., Barceló, D., Petrović, M., 2019. Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain). Sci. Total Environ. 652 https://doi.org/10.1016/j.scitotenv.2018.10.290.

Choi, Y., Kim, K., Kim, D., Bang Moon, H., Jeon, J., 2020. Ny-Ålesund-oriented organic pollutants in sewage effluent and receiving seawater in the Arctic region of Kongsfjorden. Environ. Pollut. 258 https://doi.org/10.1016/j.envpol.2019.113792.

Daneshvar, A., Svanfelt, J., Kronberg, L., Prévost, M., Weyhenmeyer, G.A., 2010. Seasonal variations in the occurrence and fate of basic and neutral pharmaceuticals in a Swedish river-lake system. Chemosphere 80, 301–309. https://doi.org/10.1016/j.chemosphere.2010.03.060.

von der Ohe, P.C., von der Ohe, V., Slobodnik, J., De Deckere, E., Kühne, R., Ebert, R.U., Ginebreda, A., De Cooman, W., Schüürmann, G., Brack, W., 2011. A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive. Sci. Total Environ. 409, 2064–2077. https://doi.org/10.1016/j.scitotenv.2011.01.054.

Donnachie, R.L., Johnson, A.C., Sumpter, J.P., 2016. A rational approach to selecting and ranking some pharmaceuticals of concern for the aquatic environment and their relative importance compared with other chemicals. Environ. Toxicol. Chem. 35, 1021–1027. https://doi.org/10.1002/etc.3165.

Esteban, S., Gorga, M., González-Alonso, S., Petrovic, M., Barceló, D., Valcárcel, Y., 2014. Monitoring endocrine disrupting compounds and estrogenic activity in tap water from Central Spain. Environ. Sci. Pollut. Res. 21, 9297–9310. https://doi.org/10.1007/s11356-014-2847-2.

European Comission, 2009. Regulation (EC) No 1107/2009. Off. J. Eur. Union 309, 1–50.

European Comission, 2013. Directive 2013/39/EU. Off. J. Eur. Union 226, 1–17.

Fent, K., Weston, A.A., Caminada, D., 2006. Ecotoxicology of human pharmaceuticals. Aquat. Toxicol. 76, 122–159. https://doi.org/10.1016/j.aquatox.2005.09.009.

Ferrer, I., Thurman, E.M., 2012. Analysis of 100 pharmaceuticals and their degradates in water samples by liquid chromatography/quadrupole time-of-flight mass spectrometry. J. Chromatogr. A 1259, 148–157. https://doi.org/10.1016/j.chroma.2012.03.059.

Gago-Ferrero, P., Schymanski, E.L., Bletsou, A.A., Aalizadeh, R., Hollender, J., Thomaidis, N.S., 2015. Extended suspect and non-target strategies to characterize emerging polar organic contaminants in raw wastewater with LC-HRMS/MS. Environ. Sci. Technol. 49, 12333–12341. https://doi.org/10.1021/acs.est.5b03454.

Gago-Ferrero, P., Gros, M., Ahrens, L., Wiberg, K., 2017. Impact of on-site, small and large scale wastewater treatment facilities on levels and fate of pharmaceuticals, personal care products, artificial sweeteners, pesticides, and perfluoroalkyl substances in recipient waters. Sci. Total Environ. 601–602, 1289–1297. https://doi.org/10.1016/j.scitotenv.2017.05.258.

Gago-Ferrero, P., Bletsou, A.A., Damalas, D.E., Aalizadeh, R., Alygizakis, N.A., Singer, H. P., Hollender, J., Thomaidis, N.S., 2020. Wide-scope target screening of>2000 emerging contaminants in wastewater samples with UPLC-Q-ToF-HRMS/MS and smart evaluation of its performance through the validation of 195 selected representative analytes Pablo. J. Hazard. Mater. 387, 121712 https://doi.org/10.1016/j.jhazmat.2019.121712.

Glassmeyer, S.T., Furlong, E.T., Koplin, D.W., Cahill, J.D., Zaugg, S.D., Werner, S.L., Meyer, M.T., Kryak, D.D., 2005. Transport of chemical and microbial compounds from known wastewater discharges: potential use as indicators of human fecal contamination. Environ. Sci. Technol. 39, 5175, 5169.

Glauner, T., Wüst, B., Faye, T., Sales, T., Gmbh, S., Strasse, H., A.T.F.S.A. S, Ulis, L., 2016. A comprehensive workflow for target, suspect, and non-target screening by LC/MS demonstrated for the identification of CECs in effluents from waste water treatment plants.

González-Alonso, S., Merino, L.M., Esteban, S., López de Alda, M., Barceló, D., Durán, J. J., López-Martínez, J., Aceña, J., Pérez, S., Mastroianni, N., Silva, A., Catalá, M., Valcárcel, Y., 2017. Occurrence of pharmaceutical, recreational and psychotropic drug residues in surface water on the northern Antarctic Peninsula region. Environ. Pollut. 229, 241–254. https://doi.org/10.1016/j.envpol.2017.05.060.

Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem. J. Chromatogr. A 1248, 104–121. https://doi.org/10.1016/j.chroma.2012.05.084.

Gros, M., Blum, K.M., Jernstedt, H., Renman, G., Rodríguez-Mozaz, S., Haglund, P., Andersson, P.L., Wiberg, K., Ahrens, L., 2017. Screening and prioritization of micropollutants in wastewaters from on-site sewage treatment facilities. J. Hazard. Mater. 328, 37–45. https://doi.org/10.1016/j.jhazmat.2016.12.055.

He, K., Echigo, S., Asada, Y., Itoh, S., 2018. Determination of caffeine and its metabolites in wastewater treatment plants using solid-phase extraction and liquid

chromatography-tandem mass spectrometry. Anal. Sci. 34, 349–354. https://doi.org/10.2116/analsci.34.349.
Hildebrandt, A., Guillamón, M., Lacorte, S., Tauler, R., Barceló, D., 2008. Impact of pesticides used in agriculture and vineyards to surface and groundwater quality (North Spain). Water Res. 42, 3315–3326. https://doi.org/10.1016/j.watres.2008.04.009.
Hug, C., Ulrich, N., Schulze, T., Brack, W., Krauss, M., 2014. Identification of novel micropollutants in wastewater by a combination of suspect and nontarget screening. Environ. Pollut. 184, 25–32. https://doi.org/10.1016/j.envpol.2013.07.048.
Jaén-Gil, A., Castellet-Rovira, F., Llorca, M., Villagrasa, M., Sarrà, M., Rodríguez-Mozaz, S., Barceló, D., 2019. Fungal treatment of metoprolol and its recalcitrant metabolite metoprolol acid in hospital wastewater: biotransformation, sorption and ecotoxicological impact. Water Res. 152, 171–180. https://doi.org/10.1016/j.watres.2018.12.054.
Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res. 42, 3498–3518. https://doi.org/10.1016/j.watres.2008.04.026.
Kiefer, K., Müller, A., Singer, H., Hollender, J., 2019. New relevant pesticide transformation products in groundwater detected using target and suspect screening for agricultural and urban micropollutants with LC-HRMS. Water Res. 165, 114972 https://doi.org/10.1016/j.watres.2019.114972.
Kim, H.Y., Lee, I.S., Oh, J.E., 2017. Human and veterinary pharmaceuticals in the marine environment including fish farms in Korea. Sci. Total Environ. 579, 940–949. https://doi.org/10.1016/j.scitotenv.2016.10.039.
Kuzmanović, M., Ginebreda, A., Petrović, M., Barceló, D., 2015. Risk assessment based prioritization of 200 organic micropollutants in 4 Iberian rivers. Sci. Total Environ. 503–504, 289–299. https://doi.org/10.1016/j.scitotenv.2014.06.056.
Kuzmanović, M., López-Doval, J.C., De Castro-Català, N., Guasch, H., Petrović, M., Muñoz, I., Ginebreda, A., Barceló, D., 2016. Ecotoxicological risk assessment of chemical pollution in four Iberian river basins and its relationship with the aquatic macroinvertebrate community status. Sci. Total Environ. 540, 324–333. https://doi.org/10.1016/j.scitotenv.2015.06.112.
Lindberg, R.H., Östman, M., Olofsson, U., Grabic, R., Fick, J., 2014. Occurrence and behaviour of 105 active pharmaceutical ingredients in sewage waters of a municipal sewer collection system. Water Res. 58, 221–229. https://doi.org/10.1016/j.watres.2014.03.076.
Little, J.L., Williams, A.J., Pshenichnov, A., Tkachenko, V., 2012. Identification of "known unknowns" utilizing accurate mass data and chemspider. J. Am. Soc. Mass Spectrom. 23, 179–185. https://doi.org/10.1007/s13361-011-0265-y.
Liu, L., Aljathelah, N.M., Hassan, H., Leitão, A., Bayen, S., 2019. Development of a liquid chromatography-quadrupole-time-of-flight-mass spectrometry based method for the targeted and suspect screening of contaminants in the pearl oyster Pinctada imbricata radiata. Environ. Pollut. 253, 841–849. https://doi.org/10.1016/j.envpol.2019.07.047.
Lolić, A., Paíga, P., Santos, L.H.M.L.M., Ramos, S., Correia, M., Delerue-Matos, C., 2015. Assessment of non-steroidal anti-inflammatory and analgesic pharmaceuticals in seawaters of North of Portugal: occurrence and environmental risk. Sci. Total Environ. 508, 240–250. https://doi.org/10.1016/j.scitotenv.2014.11.097.
Loos, R., Carvalho, R., António, D.C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K., Haglund, P., Fick, J., Lindberg, R.H., Schwesig, D., Gawlik, B.M., 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Res. 47, 6475–6487. https://doi.org/10.1016/j.watres.2013.08.024.
Manickum, T., John, W., 2014. Occurrence, fate and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa). Sci. Total Environ. 468–469, 584–597. https://doi.org/10.1016/j.scitotenv.2013.08.041.
María Baena-Nogueras, R., Pintado-Herrera, M.G., González-Mazo, E., Lara-Martín, P.A., 2016. Determination of pharmaceuticals in coastal systems using Solid Phase Extraction (SPE) followed by ultra performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS). Curr. Anal. Chem. 12, 183–201. https://doi.org/10.2174/1573411012666151009193254.
Mascolo, G., Murgolo, S., Stefani, F., Viganò, L., 2019. Target and suspect contaminants of emerging concern in the Po River Delta lagoons. Estuar. Coast. Shelf Sci. 230 https://doi.org/10.1016/j.ecss.2019.106424.
Masiá, A., Ibáñez, M., Blasco, C., Sancho, J.V., Picó, Y., Hernández, F., 2013. Combined use of liquid chromatography triple quadrupole mass spectrometry and liquid chromatography quadrupole time-of-flight mass spectrometry in systematic screening of pesticides and other contaminants in water samples. Anal. Chim. Acta 761, 117–127. https://doi.org/10.1016/j.aca.2012.11.032.
Metcalfe, C.D., Miao, X.-S., Koenig, B.G., Struger, J., 2003. Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the lower Great Lakes, Canada. Environ. Toxicol. Chem. 22, 2881–2889.
Moreno-González, R., Campillo, J.A., León, V.M., 2013. Influence of an intensive agricultural drainage basin on the seasonal distribution of organic pollutants in seawater from a Mediterranean coastal lagoon (Mar Menor, SE Spain). Mar. Pollut. Bull. 77, 400–411. https://doi.org/10.1016/j.marpolbul.2013.09.040.
Mostofa, K.M.G., Liu, C.Q., Vione, D., Gao, K., Ogawa, H., 2013. Sources, factors, mechanisms and possible solutions to pollutants in marine ecosystems. Environ. Pollut. 182, 461–478. https://doi.org/10.1016/j.envpol.2013.08.005.
Müller, A., Schulz, W., Ruck, W.K.L., Weber, W.H., 2011. A new approach to data evaluation in the non-target screening of organic trace substances in water analysis. Chemosphere 85, 1211–1219. https://doi.org/10.1016/j.chemosphere.2011.07.009.
Nödler, K., Voutsa, D., Licha, T., 2014. Polar organic micropollutants in the coastal environment of different marine systems. Mar. Pollut. Bull. 85, 50–59. https://doi.org/10.1016/j.marpolbul.2014.06.024.
NORMAN Ecotoxicology Database, 2019. ⟨https://www.norman-network.com⟩ (accessed December 19, 2019).
Park, N., Choi, Y., Kim, D., Kim, K., Jeon, J., 2018. Prioritization of highly exposable pharmaceuticals via a suspect/non-target screening approach: a case study for Yeongsan River, Korea. Sci. Total Environ. 639, 570–579. https://doi.org/10.1016/j.scitotenv.2018.05.081.
Pignotti, E., Casas, G., Llorca, M., Tellbüscher, A., Almeida, D., Dinelli, E., Farré, M., Barceló, D., 2017. Seasonal variations in the occurrence of perfluoroalkyl substances in water, sediment and fish samples from Ebro Delta (Catalonia, Spain). Sci. Total Environ. 607– 608, 933–943. https://doi.org/10.1016/j.scitotenv.2017.07.025.
Riha, J., Minar, J., Vlcek, A., Bartonkova, D., 1991. The efficacy of the repellents diethyltoluamide and fenylpropandiol applied to grazing 1st-calvers. Vet. Med. 36, 65–69.
Schlüsener, M.P., Hardenbicker, P., Nilson, E., Schulz, M., Viergutz, C., Ternes, T.A., 2015. Occurrence of venlafaxine, other antidepressants and selected metabolites in the Rhine catchment in the face of climate change. Environ. Pollut. 196, 247–256. https://doi.org/10.1016/j.envpol.2014.09.019.
Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ. Sci. Technol. 48, 2097–2098. https://doi.org/10.1021/es5002105.
Senta, I., Gracia-Lor, E., Borsotti, A., Zuccato, E., Castiglioni, S., 2015. Wastewater analysis to monitor use of caffeine and nicotine and evaluation of their metabolites as biomarkers for population size assessment. Water Res. 74, 23–33. https://doi.org/10.1016/j.watres.2015.02.002.
Sjerps, R.M.A., Vughs, D., Van Leerdam, J.A., Thomas, L., Van Wezel, A.P., 2016. Data-driven prioritization of chemicals for various water types using suspect screening LC-HRMS. Water Res. 93, e34 https://doi.org/10.1016/j.watres.2016.02.034.
Sui, Q., Huang, J., Deng, S., Yu, G., Fan, Q., 2010. Occurrence and removal of pharmaceuticals, caffeine and DEET in wastewater treatment plants of Beijing, China. Water Res 44, 417–426. https://doi.org/10.1016/j.watres.2009.07.010.
Tian, L., Verreault, J., Houde, M., Bayen, S., 2019. Suspect screening of plastic-related chemicals in northern pike (Esox lucius) from the St. Lawrence River, Canada. Environ. Pollut. 255 https://doi.org/10.1016/j.envpol.2019.113223.
Wang, J., Zhu, Y., 2017. Occurrence and risk assessment of estrogenic compounds in the East Lake, China. Environ. Toxicol. Pharmacol. 52, 69–76. https://doi.org/10.1016/j.etap.2017.03.018.
Weigel, S., Kuhlmann, J., Hühnerfuss, H., 2002. Drugs and personal care products as ubiquitous pollutants: occurrence and distribution of clofibric acid, caffeine and DEET in the North Sea. Sci. Total Environ. 295, 131–141. https://doi.org/10.1016/S0048-9697(02)00064-5.
Wu, Q., Lam, J.C.W., Kwok, K.Y., Tsui, M.M.P., Lam, P.K.S., 2017. Occurrence and fate of endogenous steroid hormones, alkylphenol ethoxylates, bisphenol A and phthalates in municipal sewage treatment systems. J. Environ. Sci. 61, 49–58. https://doi.org/10.1016/j.jes.2017.02.021.
Yien Fang, T., Praveena, S.M., Aris, A.Z., Syed Ismail, S.N., Rasdi, I., 2019. Quantification of selected steroid hormones (17B-Estradiol and 17A-Ethynylestradiol) in wastewater treatment plants in Klang Valley (Malaysia). Chemosphere 215, 153–162. https://doi.org/10.1016/j.chemosphere.2018.10.032.
Yoon, Y., Ryu, J., Oh, J., Choi, B.G., Snyder, S.A., 2010. Occurrence of endocrine disrupting compounds, pharmaceuticals, and personal care products in the Han River (Seoul, South Korea). Sci. Total Environ. 408, 636–643. https://doi.org/10.1016/j.scitotenv.2009.10.049.

4. General Disscussion

4.1 Development of an analytical method for EDCs determination and new EU requirements

The new requirements in the field of water policy set by the WFD (Directive 2000/60/EC, 2000), which were recently complemented with Decision 2018/840 (Decision 2018/840/EU, 2018) and in the WL, have become of scientific interest and a new challenge in the analytical chemistry field. Chemical monitoring of estrogens, fulfilling the criteria set in the WL, is especially challenging because the analytical methods used by Member States for their monitoring have to meet the minimum requirements for MDLs, which are equal to the proposed EQSs of 0.4 ng L^{-1} for E1 and 17β-E2, and 0.035 ng L^{-1} for 17α-EE2. However, achieving such low MDLs in real environmental samples is an extremely difficult task that requires either the use of large sample volumes in off-line SPE or specifically optimized on-line SPE approaches coupled to the lastest generation of MS instruments (Guedes-Alonso et al., 2014).

The analysis of EDCs based on previously available methods is challenging because most of them fail to achieve such low detection and quantification limits, which exceed the established EQSs. Furthermore, many reported methods are based on off-line SPE (Al-Odaini et al., 2010; Huerta-Fontela et al., 2010; Liu et al., 2011; Tomšíková et al., 2012), which is time-consuming and require large sample volumes (250-1000 mL) in order to achieve low MDLs. Other drawbacks of these procedures are that they often need many steps before obtaining an extract concentration suitable for instrumental analysis, and only a small portion is injected onto the chromatographic column. Considering these limitations, in recent years, on-line SPE has become the preferred technique for sample preparation because it is labor-saving and more cost-effective than off-line SPE methodologies. With these methods, low sample volumes are required (mL range) and smaller amounts of solvents are necessary for the analysis. By applying on-line methods, the additional sample contamination and analyte losses, which may occur during off-line SPE sample pretreatment, are avoided. In addition, they do not require *a priori* sample extraction treatment, minimizing sample manipulation, they decrease total analysis time and increase overall method accuracy, precision, and sensitivity. Although currently available on-line methods can detect estrogenic substances at sub-ng or even pg-levels (Ciofi et al., 2013; Gorga et al., 2013; Guo et al., 2013; Viglino and Aboulfadl, 2008; Wang et al., 2008), most of them still fail in the achievement of such low MDLs for some compounds. Thus, further improvements are necessary to achieve the low MDLs established in the EU directives for all compounds and the applicability of the new methodologies must be validated on real water samples. This is specially challenging for the determination and quantification of 17α-EE2 in surface and wastewater samples due to the low MDLs required. Even though the analysis of the other estrogenic compounds is more feasible, since MDLs are higher and are achievable with most of the analytical methods already available, most routine analytical methods used

by the Member States cannot meet the requirements set for 17α-EE2 (Loos et al., 2018). In this context, one of the tasks of this thesis was the development and validation of an automated, reliable, robust, and cost-effective method for the specific determination of the most active and environmentally relevant estrogens in water, complying with the current WFD requirements set in the EU WL (Decision 2015/495/EU, 2015; Decision 2018/840/EU, 2018) (Chapter 1 in the Results section, Article Nº1). The method is based on on-line SPE coupled to UHPLC and QqQ-MS/MS.

The optimization of the on-line procedure was done by a series of tests to achieve the optimum extraction performance. Throughout the study, the experimental parameters, such as the sample loading flow rate, elution time, the mobile phase, sample pH, and volume were optimized in detail. A comparative study employing different conditions was performed and optimal conditions were selected based on monitored peak areas and S/N responses of the analytes. After all these tests, the Hypersil GOLD™ aQ (20 x 2.1 mm, 12 μm) column was selected as the optimal one for compounds preconcentration and Kinetex C18 (50 x 2.1 mm, 1.7 μm) for chromatographic separation. One of the key parameters tested in our study was the sample pH adjustment, which had a strong impact on the achievement of good extraction recoveries and appropriate MDLs. An increase in the sample pH resulted in a remarkable improvement in terms of higher chromatographic response and signal to noise ratio (S/N). As most target analytes, (especially free estrogen compounds), have relatively high acid dissociation constant (pKa) values (9-10), basic conditions favored their retention onto the cartridges and, thus, the higher extraction efficiencies were achieved. Indeed, the tests at different sample pHs (in the range between pH 8 and 11), showed that an increase to pH 11 resulted in the best ion responses (peak area) for estrogenic compounds, obtaining extremely low MDL, especially for E1, 17β-E2, and 17α-EE2. Sample pHs exceeding 11 were not tested, since such high pH may potentially affect the performance of the extraction column, leading to its premature aging, which may cause the pressure buildup and ultimate clogging (Souverain et al., 2004). Thus, the column lifetime was also considered when optimizing the extraction conditions and special attention was paid to evaluate the potential negative effects of using high sample pHs. In our method, with the pH selected (pH 11), even after hundreds of injections, no negative effects were observed.

Another challenge that hampers the achievement of the EU requirements in terms of MDLs are the matrix effects. In complex matrices, such as untreated wastewaters, these effects are remarkable and they directly affect the achievement of low MDLs. Indeed, wastewater is considered as the worst-case regarding matrix effects (Benijts et al., 2004), which can have a significant impact on analyte signal and can influence reproducibility, linearity, and accuracy of the method. To take into consideration the matrix effects of different waters, extraction recoveries, MDLs, and MQLs were calculated for each type of sample. In our approach, the

effect of sample matrix interferences on the on-line extraction was evaluated together with matrix effects on the ionization efficiency and were estimated through the process efficiency (PE). As reported by other authors, PE represents the combination between matrix effect and extraction recoveries (Farré et al., 2016; Trufelli et al., 2011). Furthermore, we used ILISs to overcome and correct for any potential matrix effects. Our findings proved that the use of appropriate ILIS can effectively overcome matrix effects and provide a reliable determination and quantification of estrogens in complex environmental samples.

Overall, the benefits of the method developed in this thesis, compared to other methodologies available in the literature, are the lower detection limits achieved, which comply with the EU requirements, the minimum sample manipulation, the reduced total analysis time, and the overall method accuracy and precision. Furthermore, the method developed in this thesis is specific for the analysis of estrogens, which require specific analytical conditions that are difficult to achieve when these compounds are analyzed together with other ECs. The methodology described in Gorga et al. (2013) achieved the lowest currently reported MDLs for E1, 17β-E2, and 17α-EE2 of 0.05, 0.037, and 0.14 ng L^{-1}, respectively, for surface water, by using a sample volume of 5 mL. In the case of wastewater, the injection volume was set at 2 mL and MDLs for compounds E1, 17β-E2, and 17α-EE2 were 0.14, 0.59, and 3.8 ng L^{-1} for efluent wastewater and 0.14, 5.4, and 4.2 ng L^{-1} for influent wastewater samples (Gorga et al., 2013). Some other authors also reported MDLs below the nanogram per liter level for some EDCs (Guo et al., 2013; Viglino and Aboulfadl, 2008; Wang et al., 2008). For example, Guo et al. (2013) developed a simple and suitable procedure for the simultaneous analysis of estrogen and androgen compounds in water samples by using 50 mL of sample reaching MDLs in the range of 0.5–2, 0.5–1, and 0.5–2 ng L^{-1}, respectively, for surface water, WWTP effluent, and influent (Guo et al., 2013). Nevertheless, all other online methods reported much higher MDLs (Ciofi et al., 2013; Fayad et al., 2013; Naldi et al., 2016; Vega-Morales et al., 2012). The applicability of the method developed in this thesis was further confirmed by the analysis of waste and surface water samples.

4.2 Occurrence and assessment of the environmental risks posed by EDCs in riverine ecosystems

The on-line SPE methodology originally developed for the analysis of estrogens (Section 3.1), was expanded and validated for the monitoring of other relevant EDCs, such as APs, NP, and OP, and the plasticizer BPA. The method was applied in a comprehensive survey that had as an objective to investigate the occurrence of EDCs in waste, surface, and drinking water in the Danube River basin in the North of Serbia (Chapter 1 in the Results section, Article Nº2). The study included 30 sampling sites and comprised a total of 90 samples, including untreated municipal

and industrial wastewater, surface waters that are heavily impacted by direct wastewater discharges and drinking water. The complete list and description of the sampling sites is given in Table S1 in the Supplementary Material of Article Nº2 in the Annex. Sampling sites along the Danube River basin and associated freshwaters used as sources for drinking water production were chosen as major hot spots for EDCs contamination. This area is of major interest because it has been classified as one of the most contaminated sites in Serbia for decades, as freshwaters receive continuous discharges of untreated urban and industrial wastewater. Indeed, approximately 98% of all wastewater is being discharged without any treatment into receiving waters, which may have detrimental impacts on the water quality in the area (The Statistical Office of the Republic of Serbia, 2020). Furthermore, most urban and rural settlements are not connected to public sewage network. In fact, fecal pollution has been an ongoing problem in the Danube River basin due to the discharges of untreated wastewater (Kirschner et al., 2009; Milic et al., 2014), indicating that aquatic organisms and human population in the area are under constant environmental pressure. Until now, there has been a restricted number of studies that investigated the occurrence of EDCs in the Danube River basin and its tributaries within Serbia (Antonijević et al., 2014; Milanovic et al., 2016; Miloradov et al., 2014; Terzić et al., 2008). Most of the studies performed focused on other areas in the Danube River basin while none of them evaluated the quality of drinking water sources intended for direct human consumption, and thus, the potential human health risks associated to the occurrence of these compounds.

The analysis of wastewaters revealed that the compounds with major contribution to the total concentration in municipal wastewaters are natural and synthetic estrogens and their sulfate metabolites, while in industrial wastewaters alkylphenolic compounds (NP and OP), and BPA are the most prominent compounds. Even though estrogens were more ubiquitous in municipal wastewater, the concentrations detected in some industrial wastewaters were higher than those observed in municipal sewage. This could be attributed to the fact that industrial wastewater is less diluted than urban wastewater. In municipal wastewater, E1 was the most widely detected compound (47% frequency of detection), with concentrations ranging from 0.3 to 14.9 ng L^{-1}, followed by 17β-E2 and E3 detected in 17% of the samples with maximum concentrations of 9.8 ng L^{-1}. Regarding industrial wastewater, BPA was the most ubiquitous compound, with concentrations ranging from 6.4 to 98.1 ng L^{-1}, and only in one sampling site concentrations reached up to 338 ng L^{-1}. This point is in the vicinity of an industry that is the major plastic producer in Serbia, which might explain the high BPA levels detected. For APs, NP and OP, maximum concentration of 78.3 and 52.4 ng L^{-1} were found in wastewater coming from a food processing-based industrial area. The synthetic estrogen 17α-EE2 and DES and the estrogen glucuronide metabolites (E2-17G, E1-3G, and E3-16G) were never detected. For the

metabolites, sulfate conjugates, E1-3S and E3-3S, were found in both types of wastewater at similar concentrations (up to 17.7 ng L^{-1} and 30.1 ng L^{-1}, respectively), while glucuronide metabolites were never detected. This could be explained by the fact that glucuronide metabolites are more prone to be degraded by bacteria than sulfate conjugates, thus being less frequently detected in the environment (Andersen et al., 2003; Ben et al., 2017; Komori et al., 2004).

Concerning the occurrence of EDCs in receiving freshwater ecosystems, the similarity in chemical profile between surface and wastewaters indicated that wastewater discharges are the major contributors to the contamination by EDCs in the area. Estrogenic compounds were the main contributors to the total EDCs concentration in surface water impacted by urban wastewater discharges, whereas NP, OP and BPA were the most prominent in industrialized area. The concentration levels detected for all EDCs were generally lower than those found in wastewater samples, reflecting that these compounds are subject to high dilution factors and dispersion processes once they enter freshwater ecosystems. Sulfate conjugates, E1-3S (67%) and E3-3S (43%) were the most frequently detected estrogenic compounds, followed by E1 (27%) and E3 (17%). Even though estrogens and their conjugates were detected at low ng L^{-1} levels, these concentrations are often close to or above the concentration at which they can exert estrogenic effects (>1-10 ng L^{-1}). Nevertheless, the most potent estrogenic compounds, such as 17β-E2, 17α-EE2, and DES, were not found above the MDL in any of the surface water samples. APs, NP (53%) and OP (64%), were mostly found in industrial sites, most probably due to the uncontrolled discharges of untreated wastewater from petrochemical and organochlorine industries, oil refinery, a chemical fertilizer factory, machinery, and aircraft industries. However, their maximum concentrations were below 40 ng L^{-1}, and in none of the sampling sites NP concentrations exceeded the MAC for inland surface water, set to 2 μg L^{-1} (Directive 2008/105/EC, 2008). Furthermore, it is also worth pointing out that in none of the sampling sites the concentrations of NP and OP exceeded the annual average (AA) level of 0.3 and 0.1 μg L^{-1}, specified in the same Directive. Regarding BPA, it was the most ubiquitous compound, detected in 70% of the samples, reaching maximum concentrations of 106 ng L^{-1}.

In drinking water samples, the plasticizer BPA (57%) was the most ubiquitous compound, followed by NP (40%) and OP (33%), while natural and synthetic estrogens and their conjugates were not found in any of the samples. Individual concentrations were in the low ng L^{-1} range, specifically from 0.4 to 7.9 ng L^{-1}. The highest total EDCs concentration observed was 39.8 ng L^{-1}, being BPA the major contributor (90%) to this total concentration. This high level was observed in the sampling site corresponding to a fountain located in a heavily industrialized area, whose water is extracted from reservoirs for human potable supply located in the immediate vicinity of the Danube River.

Besides studying the occurrence of EDCs, the environmental risks posed by these substances were also characterized by using two approaches: (i) RQs and (ii) estrogenicity, through the calculation of EEQt in waste, surface, and drinking water as an indicator of their potential detrimental effects. RQ values of E1 and 17β-E2 were the highest, exceeding the threshold value of 1 in 60% of wastewater samples, while in surface water only E1 displayed potential risks in just two sites, corresponding to areas of major urban wastewater impact. The potential environmental risks of detected EDCs in wastewaters were ordered from higher to lower RQs as 17β-E2>E1>BPA>E3>OP>NP>E-3S>E3-3S, with values between 26 and 2 x 10^{-5}. For surface water, RQs achieved were one order of magnitude lower than those estimated for wastewater, and they were ordered from higher to lower values as E1>BPA>OP>NP>E3>E3-3S>E1-3S, ranging from 2.7 to 5 x 10^{-6}. EEQt surpassed the threshold of 1 ng E2 L^{-1} in about 67% of wastewater samples, and in 3 surface water samples mostly influenced by industrial wastewater discharges. EEQt in wastewater was mainly attributed to the estrogens 17β-E2, E1 and E3, while E1 was found to be the most important EDCs among natural estrogens in terms of estrogenic potency in surface water. So far, there are no results available for comparison with previous risk assessment-based studies performed in this area. However, our findings confirm that the detected concentrations raise concerns about their potential environmental effects in receiving freshwater ecosystem. Indeed, our findings match well with previous studies, where E1 was found to be the most important EDCs among natural estrogens in terms of estrogenic potential (Manickum and John, 2014), while BPA showed minor contributions to total estrogenic activity (Brix et al., 2010b; Caldwell et al., 2010; Esteban et al., 2014). In drinking water, EEQt was below 1 ng L^{-1} in all samples, suggesting that the levels of EDCs present in drinking water might not induce negative effects to human's endocrine system. Although estimated RQs and EEQts indicate that negligible environmental and human health effects are to be expected at short term, some effects might occur after chronic exposures to these substances and certain actions should be taken to prevent EDCs contamination in the area, especially in source waters used for drinking water production.

4.3 Occurrence of PhACs in coastal areas

In order to evaluate the input of PhACs into a vulnerable marine setting in the Mediterranean Sea through WWTP effluents, water and sediment samples were collected in different locations in the Ebro Delta region in Catalonia, Spain (Chapter 2 in the Results section, Article Nº3). A total of 156 samples, including 84 water and 72 sediments were collected from 29 sampling sites comprising the two main WWTPs in the area, the main stretch of the Ebro River, several irrigation and drainage channels, estuaries, and the Mediterranean Sea. Samples were taken along three different seasons (autumn, winter, and spring). The complete list and

description of the sampling sites is given in Table S2 in the Supplementary Material of Article Nº3 in the Annex. The area was chosen for its biodiversity and natural significance as well as economic importance. The Ebro Delta is the third largest wetland area in the western Mediterranean region. It is composed of natural lagoons, bays, salt pans, marshes, irrigation, and drainage channels that provide extensive habitats. During the last years, this area has become a fragile ecosystem for anthropogenic contamination due to the agricultural and aquaculture production, and touristic activities.

Firstly, the distribution of 81 PhACs in wastewater samples was evaluated together with their impact in the surrounding freshwater bodies and coastal areas. Natural attenuation processes, such as dilution and sorption to sediments were also investigated. The concentration levels of the PhACs detected in influent wastewaters ranged from 5.1 ng L^{-1} to 45.3 μg L^{-1}, while in treated wastewater, concentrations were from 1 ng L^{-1} to 3.8 μg L^{-1}. The individual concentrations of the PhACs detected in wastewater samples, along the three sampling campaigns, are displayed in Table S7a in the Supplementary Material of Article Nº3 in the Annex. As it can be observed, in treated wastewater, a great number of compounds were still detected, indicating that PhACs are not completely removed during wastewater treatment, as it has been widely reported (Al Aukidy et al., 2012; Aymerich et al., 2016; Chen et al., 2015; Collado et al., 2014; Rivera-Jaimes et al., 2018). Overall, the concentrations of PhACs detected in wastewaters, in both treatment facilities and along the three sampling campaigns, were similar and within the same order of magnitude.

Wastewater impacted freshwater sites showed a similar chemical profile as effluent wastewaters. Indeed, the most frequently detected PhACs in effluent wastewaters were also the ones most frequently detected in freshwater and coastal areas. However, the concentrations of PhACs in freshwater bodies located downstream the WWTPs were one order of magnitude lower than those found in wastewater effluents, indicating that PhACs are subject to a remarkable dilution factor when they are discharged into the receiving aquatic environments. Specifically, in wastewater-impacted sites, the PhACs showing the highest maximum concentrations (>100 ng L^{-1}) were: the anti-hypertensives valsartan, irbersartan and losartan, the diuretics hydrochlorothiazide and furosemide, the analgesics and anti-inflammatories diclofenac, phenazone, ketoprofen, and acetaminophen, the anti-helmintics levamisol and albendazole, the psychiatric drugs venlafaxine, lorazepam, and carbamazepine, the antibiotics ofloxacin, azithromycin, and ciprofloxacin and the β-blocker atenolol. In Ebro River there were statistically significant seasonal differences in total PhACs concentrations between different sampling seasons, indicating that PhACs are subject to the dilution when they are discharged in riverine ecosystem (Kruskal-Wallis test, $p<0.05$). Indeed, for the winter season, estimated dilution factor was three times higher compared to dilution factor calculated for autumn and spring. On the

contrary, in emissary there were no statistically significant seasonal differences in total PhACs concentrations (Kruskal-Wallis test, $p>0.05$), explained by the fact that emissary is mostly composed by constant wastewater effluent released from WWTP and subjected to minor changes in the flow.

Regarding the irrigation, drainage channels and estuaries, the PhACs that were widely detected in effluent wastewaters were also some of the most ubiquitous compounds in these samples. In channels, the most frequently detected compounds were salicylic acid, carbamazepine, thiabendazole, and valsartan. The compounds detected at the highest concentrations (<50 ng L^{-1}) belonged to the group of analgesics and anti-inflammatories, including naproxen, ibuprofen, ketoprofen, salicylic acid, and acetaminophen. In estuaries, the most frequently detected compounds were salicylic acid, carbamazepine, valsartan, and clarithromycin, with the X-ray agent (iopromide) being the compound detected at the highest concentrations. Seasonal and temporal differences in channels and estuaries were observed when considering the total PhACs concentrations determined during all three sampling seasons (Kruskal-Wallis test, $p<0.05$). In the channels, total PhACs concentrations were higher in autumn and winter seasons in comparison to those found during spring, mostly attributed to the differences in the flow between the seasons. Regarding estuaries, higher total PhACs concentrations were observed in autumn and spring, compared to winter, mostly attributed to a better mixing between the water masses of the estuaries and sea and higher dilution factors in the winter period.

In seawater, salicylic acid, acetaminophen, valsartan, carbamazepine, and trimethoprim were the compounds most frequently detected (>50%) along the three sampling seasons. The highest individual concentrations (all in the low ng L^{-1} range) were mostly found for ibuprofen, acetaminophen and salicylic acid in the sites located near the shore-line, while in offshore waters lower total PhACs levels were observed, due to lower anthropogenic pressure and a higher dilution. Antibiotics such as trimethoprim, sulfamethoxazole, tetracycline, and erythromycin were widely detected in seawater, especially in locations that are placed near aquaculture facilities. These antibiotics, except tetracycline, were also present in effluent samples and wastewater impacted sites. This indicates that the occurrence of some antibiotics may not only be attributed to wastewater discharges, but also to aquaculture facilities, where large amounts of antibiotics are being used. Regarding seasonal differences in seawater, Kruskal-Wallis tests showed significant differences between sampling events ($p<0.05$), with lower concentration observed for winter period.

A reduced number of PhACs was detected in sediments, compared to water samples. Concentrations were in low ng g^{-1} d.w. range. The low detection of PhACs in sediments could be explained by their physicochemical properties. Most of the target compounds included in this study have hydrophilic characteristics, with

$logK_{OW}$ values lower than 1. Thus, it is expected that compounds with low K_{OW} values will be less sorbed onto the sediments. Nevertheless, the detection of some PhACs, such as phenazone in sediments, which has $logK_{OW}$ values (<1) pointed out that $logK_{OW}$ may not be the only indicator to assess PhACs sorption onto sediments. Since PhACs are ionizable compounds, several authors have indicated that the use of the pH dependent $logK_{OW}$, logD and the compounds pKa are more suitable parameters to assess PhACs partitioning onto sediments (da Silva et al., 2011; Schaffer et al., 2012).

Sediment samples collected near WWTPs discharge outlets and in the channels were those showing the highest total PhACs concentrations. This can be attributed by the proximity of WWTP and, consequently, to high aqueous concentrations, and also to their high total organic carbon (TOC) content. Individual compounds detected at the highest concentrations (<13 ng g^{-1} d.w.) were the analgesics and anti-inflammatories ketoprofen and diclofenac, the lipid regulator gemfibrozil, the diuretic hydrochlorothiazide, the psychiatric drug citalopram and the anti-helmintic thiabendazole. Our results showed significant positive correlations between total PhACs concentrations and TOC in sediments (Pearson's correlations; $p<0.05$), with higher PhACs levels in sites with higher TOC values. This is in a good agreement with previous studies, where positive correlations between the PhACs detected in sediments and their organic content were found (Bayen et al., 2016; Stewart et al., 2014; Wilkinson et al., 2018). In general, the concentrations found in marine sediments were lower than those observed in freshwater sediments. This could be attributed to the lower concentrations detected in water samples but also to the salinity and TOC content, as other additional factors that may control PhACs sorption onto natural sediment (Wang et al., 2010; Zeng et al., 2008).

For the PhACs detected in water and sediments, solid-water partitioning coefficients (K_d) were calculated (in L kg^{-1}) by dividing individual PhAC concentrations in the sediments (ng kg^{-1}) by the concentration detected in water sample (ng L^{-1}). For a specific compound, log K_d values varied significantly between the different types of sediment, with values ranging from 0.1 and 3.6 in freshwater sediment and from 1.9 to 3.2 in estuarine and sea sediment. The compounds showing the highest K_d values, and thus, with the strongest sorption to sediments, were citalopram and thiabendazole followed by ibuprofen, erythromycin, and norverapamil. These results indicate that sorption is a minor natural attenuation pathway for most PhACs in freshwater and marine ecosystems, except for thiabendazole and citalopram, which showed a remarkable sorption potential. The concentration levels and K_d values reported in this study match well with those found in the scientific literature (da Silva et al., 2011; Fairbairn et al., 2015; Koba et al., 2018; Moreno-González et al., 2015; Wilkinson et al., 2018; Yang et al., 2010).

In this study, a prioritization strategy was applied to highlight the PhACs of major ecological significance and those that could be considered as relevant markers of wastewater contamination in coastal areas. This strategy was a multiparameter approach that considered the compounds frequency of detection in estuary and seawater samples, maximum concentrations in these samples, ecotoxicity data, based on the HQ ratios, bioconcentration factors (BCFs), RE in WWTPs, and their estimated persistency in water (half-lives in days) (see **Table 2** in Article Nº3). With the prioritization approach, a score value was given to each PhACs, and the compounds having the highest scores were those considered of major ecological concern. According to the score values obtained, the most ecologically relevant PhACs were: the antidepressant venlafaxine, the antibiotics trimethoprim and sulfamethoxazole, psychiatric drugs carbamazepine and citalopram, the diuretic hydrochlorothiazide, antihypertensives irbersartan and valsartan, the β-blocking agent sotalol and analgesics/ant-inflammatories diclofenac, salycilic acid, and acetaminophen. It is worth highlighting that macrolide antibiotic (i.e. erythromycin, clarithromycin, and azithromycin) and fluoroquinolone antibiotic (ciprofloxacin), which are included in the EU WL, were the compounds with the lower score values within this prioritization strategy. However, this could be mainly attributed to the lack of data regarding their toxicity to marine organisms, used to predict parameters included in the prioritization, such as HQs, BCFs, and half-life values. Nevertheless, this strategy is a robust tool to highlight relevant chemicals and showed that there is still a lack of data regarding PhACs toxicity to marine organisms. Thus, further research that focus on assessing the potential negative effects of PhACs to marine species and biota is necessary, to perform a proper risk assessment of these substances to marine settings.

4.4 Suspect screening

Wastewater effluents contain tens of thousands of ECs such as PhACs, pesticides, PCPs, industrial chemicals, as well as their metabolites, and TPs. Target multi-residue analytical methodologies are suitable to study the occurrence of a broad range of selected groups of ECs. Nevertheless, these methods are useful to study just a limited number of pre-selected chemicals, and mostly do not allow the monitoring of more than 100 compounds (Baena-Nogueras et al., 2016; Borova et al., 2014; Ferrer and Thurman, 2012; Nödler et al., 2014). Thus, the new trends in the analytical chemistry field shifted towards the use of HRMS-based methodologies that allow the detection of a larger number of ECs (>100), without requiring reference standards (*a priori*). However, one of the most critical aspects of suspect screening methodologies is the need for establishing automated, faster, and user-friendly data processing workflows.

Thus, in this thesis, an automated suspect screening approach was developed for the fast and reliable identification of a large list of anthropogenic ECs in

environmental samples using one single software platform (Chapter 3 in the Results section, Article Nº4). With this methodology, ECs are identified with a high degree of confidence, even if reference analytical standards are not available, thanks to the use of the software-linked databases m/zVault, m/zCloud, and ChemSpider. In fact, this is one of the main advantages of the developed methodology, in comparison with other methods available in the scientific literature, that use several platforms for ECs identification and confirmation. The developed methodology was applied to track for contamination sources in the Ebro Delta region, a vulnerable marine setting with high biodiversity and ecological richness. Besides identifying ECs, a comprehensive risk assessment, using a toxicological database with information for more than 39.000 substances, was performed to identify compounds that can be used as relevant markers of anthropogenic contamination.

For sample preparation, an established sample preparation methodology was used (Gago-Ferrero et al., 2015). Samples were extracted by using manually packed mixed-mode SPE cartridges, that used a combination of different polymers, such as Strata-X-AW, Strata-X-CW, and Isolute ENV+ (1:1:1.5). The use of several polymeric materials is a common practice and advisable in any suspect screening methodology in order to ensure high enrichment and pre-concentration of several ECs that have different physicochemical characteristics. After extraction, samples and analytical blanks were further analyzed by LC coupled to a HRMS instrument (LTQ-Orbitrap Velos™, Thermo Fisher Scientific). Compound identification was performed using an in-house suspect list provided by the Catalan Water Agency (ACA) that contained information for 360 ECs of major use and consumption in Catalonia. The list had information on the monoisotopic masses of each of the 360 suspects and included: PhACs, metabolites and TPs (130), pesticides and TPs (125), antibiotics (42), psychoactive drugs (11), hormones (9), stimulants (8), PCPs (6), artificial sweeteners (4), phenols (3), antibacterial agents (2), plasticizers (2), and other compounds such as phthalates, UV filters, corrosion inhibitors, surfactants, and synthetic musks (18) (Table S1 in the Supplementary Material of Article Nº4 in the Annex). The suspect list was loaded into the LTQ-Orbitrap Velos™ prior to sample injection and samples were injected in DDA mode. For the identification of suspect compounds, an automated data processing workflow was developed using the software Compound Discoverer 2.1, using the software-linked databases m/zCloud, m/zVault, and ChemSpider for compound identification. The use of one single software platform is a major advancement, compared to other suspect screening methodologies, since it allows a faster and reliable identification of ECs in environmental samples. The developed methodology allowed the tentative identification of 48 compounds. ECs identification was done following the criteria set by Schymanski et al. (Schymanski et al., 2014a). The identification confidence proposed therein starts with level 5 as the lowest level of confidence based on a selection of an exact mass of interest, and moves up to level 1 as the

highest degree of confidence which is a confirmation of a structure with reference standards (Section 1.3.3).

Out of these 48 suspects, 37 could be fully confirmed using ILISs. ILISs were also used for the semi-quantification of the detected ECs. Detected compounds included: 16 PhACs, their metabolites, and TPs, 10 pesticides and their TPs, 8 stimulants and their metabolites, 3 PCPs, and drug of abuse. With the suspect screening approach used in this study we were able to monitor the occurrence of previously unreported ECs in the Ebro Delta and other compounds that are rarely monitored. Furthermore, it allowed the detection of relevant PhACs metabolites, which were even more prominent than their parent compound, such as mesalamine, 4-acetamidoantipyrine, 4-formylaminoantipyrine, atenolol acid, and didesmethylvenlafaxine, desethylterbuthylazine (the degradation product of the herbicide terbuthylazine), cotinine (the major urinary metabolite of nicotine) and caffeine-related metabolites, such as xanthine, theophylline, and 1-methylxanthine. Some of these metabolites, such as 4-acetamidoantipyrine and 4-formylaminoantipyrine were reported for the first time in surface and wastewater in Spain, while the occurrence of mesalamine had never been reported before.

Overall, PhACs were the group of compounds showing the highest total concentrations (low to high ng L^{-1} levels) in all samples, followed by pesticides, PCPs and stimulants and their derivates, which showed a similar concentration range (from low to medium ng L^{-1} levels) (**Fig. 4** in Article Nº4). Concentration levels for each of the detected ECs, and their frequency of detection can be found in Table S7 in the Supplementary Material of Article Nº4 in the Annex. Results of the monitoring study revealed that pesticides were more ubiquitous in irrigation and drainage channels, while PhACs, stimulants and PCPs were the most ubiquitous in effluent wastewaters and in their associated receiving freshwater systems (**Fig. 3** in Article Nº4). Pesticides showed particularly high concentrations (up to 599 ng L^{-1}) in the irrigation channels and this can be mostly related with the intensive agricultural activities developed in the area in the rice fields. For PhACs, the site showing the highest total concentrations was the emissary in Sant Carles de la Ràpita (up to 2187 ng L^{-1}). This site is mostly composed by constant wastewater effluent released from WWTP2 in Sant Carles de la Ràpita and is subject to minor changes in its flow throughout the year (Pignotti et al., 2017).

In marine water, PhACs and pesticides were the most ubiquitous compounds. For PhACs, 5 compounds were found, including metformin, mesalamine, carbamazepine, venlafaxine, and 4-formylaminoantipyrine, which were also widely detected in effluents wastewaters, indicating that WWTP discharges are an important source of these compounds in coastal waters. The occurrence of metformin is not so widely reported in marine waters (Ali et al., 2017), while to the author's best knowledge, this is the first study that reports on the occurrence of mesalamine and 4-formylaminoantipyrine in a coastal setting. For pesticides,

individual concentrations ranged from 0.3 to 88.4 ng L^{-1} and the most widely detected compounds were tebuconazole, propiconazole, prochloraz, and azoxystrobin. The levels found in this study were lower than those observed in other coastal areas (Carafa et al., 2007; Nödler et al., 2014), but higher than those found in other littoral systems (Brumovský et al., 2017; Loos et al., 2013; Weigel et al., 2002), mostly related with seasonal application patterns. Nicotine and its metabolite cotinine were also ubiquitous substances in marine waters, while caffeine and cocaine were almost exclusively detected in bays and harbor areas at low ng L^{-1} (4.7-86.1 ng L^{-1}).

Finally, with the risk assessment study, ten compounds were found to be of high ecological concern (RQ>1), including the PhACs telmisartan, venlafaxine, and carbamazepine, the herbicides terbutryn and terbuthylazine, as well as its degradation product desethylterbuthylazine, the fungicides azoxystrobin, tebuconazole, and prochloraz, and the insecticide tebufenozide. Seven out of the ten compounds highlighted as relevant chemicals belong to the group of pesticides, explaining the importance of the intensive agricultural activities, specifically in the production of rice, developed in the Ebro Delta area. Similar compounds were reported as of major concern in previous risk assessment studies across Iberian rivers (including the Ebro River), where pesticides were also highlighted as priority pollutants among a wide range of ECs (Kuzmanović et al., 2015). It is worth mentioning that one out of the seven ranked pesticides, terbutryn, is included in the list of priority substances of the EU WFD (Directive 2013/39/EU, 2013). It is classified as toxic to aquatic wildlife with long lasting effects (Mascolo et al., 2019). These results show that, even though pesticides are used in Europe following strict guidelines to reduce detrimental impacts in the environment (Regulation (EC) No 1107/2009., 2009), they are still persistent and ECs of ecological concern in the environment. This highlights the need for establishing even more stringent guidelines and assessments for these substances.

5. Conclusions

A summary of the main conclusions for each chapter of this thesis is presented below.

Chapter 1:

Development of a sensitive and robust online dual column liquid chromatography-tandem mass spectrometry method for the analysis of natural and synthetic estrogens and their conjugates in river water and wastewater

- A fully automatic online-SPE-UHPLC-MS/MS method was developed for the analysis of natural and synthetic estrogens and their conjugates in surface and wastewater.
- The on-line methodology improved analytical performances over other available methods, especially those based on off-line SPE (i.e. increased reliability and sensitivity, sample throughput, reduction of operating costs, minimization of potential errors and contamination risks, and lower MDLs).
- An effective compromise was achieved when sample pH was adjusted at basic conditions (pH 11), resulting in increased signal intensities and extraction recoveries for most of the target compounds. Sample pH adjustment had a significant effect on the achievement of lower MDLs.
- The developed method represents an improvement compared to other previously published on-line SPE methods, in terms of lower detection limits and higher selectivity, suitable to comply with the requirements set up for hormones in the EC Decision 2018/840. Indeed, this method was able to decrease the MDL for 17α-EE2, compared to other methods, and it is one of the few methodologies available in the literature that complies with the requirements set by the EC.
- The applicability of the developed method was validated with the analysis of waste and surface water samples.

Occurrence and assessment of environmental risks of EDCs in drinking, surface, and wastewaters in Serbia

- The on-line SPE method originally developed for estrogens, was expanded for the monitoring of other relevant EDCs and proved its suitability for the analysis of all target compounds in environmental samples.
- The comprehensive survey performed along the Danube River basin and associated freshwaters used for drinking water production in northern Serbia showed a widespread occurrence of EDCs in the study area.
- Out of the 13 EDCs analyzed, the estrogen E1, and its metabolite E1-3S, were the most ubiquitous compounds in urban wastewater and the receiving surface water sites, while BPA was the most frequently detected compound in industrial wastewaters and associated freshwater sites.

- The similarity in the chemical profile of EDCs in both waste and surface water indicates that the lack of a proper wastewater treatment network represents a major pollution source by EDCs in the study area.
- The evaluation of environmental risks by the calculation of RQs indicated that the hazards posed by the detected EDCs in surface water are low, except for E1 in two sampling sites influenced by urban wastewater discharges where RQ exceeded the threshold of 1.
- The estimation of risks through the calculation of EEQ_t exceeded the 1 ng L^{-1} estradiol level threshold (1 ng E2 L^{-1}) in 3 surface waters sites, which are mostly influenced by industrial wastewater discharges. For drinking water, EEQ_ts were one order of magnitude lower than the 1 ng E2 L^{-1} threshold, suggesting that the levels of EDCs present in drinking water might not induce negative effects on human's endocrine system.
- Although estimated RQs and EEQ_ts indicate that negligible environmental and human health effects are to be expected at short term, some effects might occur after chronic exposures. Thus, certain actions should be made to prevent EDCs contamination in the area, especially in source waters used for drinking water production.

Chapter 2:

Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain)

- A comprehensive spatiotemporal monitoring indicated that PhACs are ubiquitous compounds in the Ebro Delta region.
- PhACs more frequently detected in the receiving water systems coincided, in a great extent, with those that are more ubiquitous in effluent wastewaters, reveling that WWTPs are an important source of PhACs contamination in coastal areas.
- Even though PhACs were subjected to natural attenuation processes during their transport towards the open sea, they were still detected at remarkable concentrations in seawater, being even detected in offshore areas.
- PhACs attenuation was mainly attributed to dilution, while sorption to sediments showed to be a minor pathway for most substances, as highlighted by the low number of PhACs and concentration levels detected.
- Seasonal variations in total PhACs concentration were observed for water samples, but not for sediments.
- The multi-criteria prioritization strategy used proved to be a robust tool to identify the most persistent and ecologically relevant PhACs that can be used as chemical markers of wastewater contamination in coastal environments.
- Results indicate that the continuous input of PhACs through waste and receiving water courses into the coastal environment deserves further

attention, since knowledge about their fate and behavior in marine settings is still scarce. Further studies focused on investigating the potential negative effects of PhACs on marine organisms are necessary to accurately assess potential environmental risks.

Chapter 3:

Extended suspect screening to identify organic ECs in riverine and coastal ecosystems and assessment of environmental risks

- Suspect screening approaches based on HRMS are a powerful tool to identify the most relevant ECs, TPs, and metabolites in environmental samples and they allow the identification of previously unreported substances.
- The developed automated data treatment tool for suspect screening analysis (based on exact mass, RT, and spectral matching using databases) showed its capability for the simultaneous unequivocal identification of 48 compounds, which are often not included in target methods, attaining high degree of confidence in their identification using just one software package.
- The use of isotopically labelled standards allowed the confirmation and semi-quantification of most of the identified compounds in the samples to evaluate their occurrence and distribution along the Ebro River Delta.
- The combination of the suspect screening with a risk-assessment based prioritization provided information about the compounds that can be considered as major risk for the aquatic environment and that should be classified as priority substances in future monitoring programs.

6. Future Perspectives

Target analysis by using QqQ or QqLIT instruments has shown its suitability for the analysis of pre-selected ECs, allowing their confirmation and quantification even down to sub-ng L^{-1} levels. Even though conventional target analytical approaches are still widely used for the analysis of pre-selected chemicals, their main disadvantage is that the number of compounds monitored are only "the tip of the iceberg" of the numerous chemicals entering the environment, including those that are potentially toxic to organisms and humans.

The introduction of HRMS, with the development of modern QTOF and Orbitrap instruments, arose new opportunities in the environmental chemistry field. HRMS allows the determination of contaminants by enlarging target analysis with the possibility to perform the screening of a wider number of compounds. On the other hand, suspect screening, using suspect lists and non-target screening workflows, allows the detection of previously unknown contaminants, expanding the knowledge about their environmental occurrence and fate. Thanks to their unique ability to detect analytes based on accurate mass, full-spectrum HRMS can simultaneously gain qualitative and quantitative information of a virtually unlimited number of analytes. This means that thousands of substances can in principle be detected simultaneously with high sensitivity and accuracy, including substances that have never been identified before. Another key advantage of HRMS, compared to low resolution mass spectrometers, is that a "digital archive" of full scan HRMS analyses and product ion spectra can be used for retrospective analysis, if new concerns or new knowledge on specific substances are required (Hollender et al., 2019). HRMS can also be used on the identification of new metabolites or TPs of organic contaminants as well as on the studying their dissipation/degradation in the environment.

Due to the large range of possibilities offered by suspect and non-target screening workflows, HRMS will replace target analysis and will become the technique of choice in the environmental chemistry field in the next few years. The use of analytical approaches based on HRMS have expanded in the last few years at a rapid pace. The costs for HRMS instruments have dropped and the technology performance has simultaneously improved with regards to mass resolving power, sensitivity, acquisition speed, and simplification through software solutions. The instruments are becoming available in many regional and national environmental monitoring laboratories and the availability of analytical experts professionally trained for their use has also increased.

Regarding suspect screening, it is becoming increasingly popular and is especially attractive when appropriate suspect lists are available. Suspect screening approaches have a huge potential for the identification of previously unreported and relevant contaminants and for the prioritization of substances to include in monitoring programs. However, one of the most important drawbacks that still exist is the availability and accessibility to relevant suspect lists or exact mass

databases. For instance, it would be necessary that information on compounds usage and production (i.e. industrial chemicals, PhACs, etc.) is accessible for the scientific community (via open-access databases) in order to facilitate the identification of relevant and new contaminants. Several organizations and laboratories are working on the development of relevant suspect lists to enhance compound identification on the global level. One example is the initiative promoted by the NORMAN network consortium, who launched an integrated and interactive platform for archiving, processing, and data mining of a large amount of LC-HRMS data. This platform is called Digital Sample Freezing Platform (DSFP) and incorporates all the recent developments in the HRMS screening methods within the NORMAN Network. The aim of this platform is to create an extended list to be used for suspect screening that allowed the detection of tens of thousands of ECs and their TPs in environmental samples in a systematic and consistent way, with the goal of becoming a European and possibly global standard. The platform integrates tools for storing raw HRMS chromatograms of samples, each containing typically several thousands of compounds, in a uniform mzML format independent from vendor software. At present, only those organizations and researchers contributing with their data can use the platform. It is planned to open DSFP to the public after a thorough testing of all its functionalities with a reference set of 'big data' and when a critical mass of samples is achieved. However, any organization can become a member of the NORMAN network according to its Statutes and researchers willing to join the activities of the network are invited to contribute to further development of DSFP and participate in network activities (Alygizakis et al., 2019).

It is worth mentioning that the identification of contaminants through suspect screening, even when using suspect lists, is not trivial and is a challenging task. Fragmentation spectra can vary significantly, depending on the HRMS instrument used, thus making the comparison of MS/MS spectra obtained with different instruments difficult. Another drawback of suspect screening approaches, and a subject that still needs improvement, is the development of more automated and user-friendly methodologies for the identification of ECs in environmental samples, using one single software platform. Currently, some other suspect screening methodologies have been developed, but most of them rely on the use of different platforms for ECs identification.

Non-target screening is also gaining popularity in the environmental chemistry field. Its application is mostly based on the identification of unknown chemicals, metabolites, and TPs. To enable a non-target screening of environmental samples with several thousand peaks within a reasonable time frame, advanced software solutions are needed with capabilities for automated batch processing and fast database queries. Now, integration within handy workflows of software packages is mainly available in the field of metabolomics, which is only partly applicable to environmental samples. However, the non-targeted screening presents important

drawbacks when compounds are present at low concentrations, especially in more complex-matrix samples, due to the difficulties in the component's detection step. Identification of non-target contaminants is greatly facilitated when the compound detected is included in the home-made libraries, otherwise the elucidation of the compound becomes an analytical challenge where the possibilities of success are rare. Further efforts should be made to speed the development of more intelligent softwares for screening unknown compounds. So far, results of the content of a sample can be gained with the thorough manual inspection, but larger scale automated data treatment is still hindered by the lack of advanced features in the software. Most probably, the impact of LC-HRMS in environmental analysis will increase within the coming years and this technology will make the environment more transparent regarding the occurrence and fate of ECs (Gosetti et al., 2016).

These non-target approaches are also gaining increased attention in the omics field, to assess for any potential harmful effects of the exposure of non-target organisms to ECs. In particular, the inclusion in metabolomics studies of the new analytical platforms coupled to HRMS, facilitates separation, detection, characterization, and quantification of new metabolites and related metabolic pathways. In addition, it can be used to detect molecular-level changes in organisms exposed to target contaminants and real mixtures of contaminants at environmentally relevant concentrations in the ng L^{-1} to low mg L^{-1} range (Álvarez-Muñoz and Farré, 2020). However, the use of metabolomics as an early warning signal for environmental biomonitoring and ecological risk assessment continues being one of the main challenges currently faced by scientists (Bahamonde et al., 2016). Indeed, tracking the occurrence of new and non-monitored contaminants in the aquatic environment and studying their potential deleterious effects to non-target organisms is of paramount importance and a matter that deserves further scientific efforts.

Finally, suspect and non-target screening methodologies should be used together with risk-assessment based strategies to identify the most ecologically relevant chemicals and those that should be included in future monitoring programs and legislations. For example, HRMS techniques could be used in support to the EU Watch List mechanisms. For instance, suspect screening could be established as a preliminary step to screen for the occurrence of a large number of substances, together with their TPs and metabolites, followed by a risk-assessment based prioritization and the compounds highlighted could be further monitored and quantified with target analysis at a second stage. This practice would avoid long years of monitoring efforts for lists of compounds that might eventually be proven as irrelevant chemicals, compared to others that could be of major interest for a specific study.

References

93/67/EEC, 2003. Technical guidance document on risk assessment in support of European Commission Directive 93/67/EEC on risk assessment for new notified substances. Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances, and Directive 98/8/EC of.

Aalizadeh, R., Thomaidis, N.S., Bletsou, A.A., Gago-Ferrero, P., 2016. Quantitative Structure-Retention Relationship Models to Support Nontarget High-Resolution Mass Spectrometric Screening of Emerging Contaminants in Environmental Samples. J. Chem. Inf. Model. 56, 1384–1398. https://doi.org/10.1021/acs.jcim.5b00752

Aerni, H.R., Kobler, B., Rutishauser, B. V., Wettstein, F.E., Fischer, R., Giger, W., Hungerbühler, A., Marazuela, M.D., Peter, A., Schönenberger, R., Vögeli, A.C., Suter, M.J.F., Eggen, R.I.L., 2004. Combined biological and chemical assessment of estrogenic activities in wastewater treatment plant effluents. Anal. Bioanal. Chem. 378, 688–696. https://doi.org/10.1007/s00216-003-2276-4

Aga, D.S. (Ed. ., 2008. Fate of Pharmaceuticals in the Environment and in Water Treatment Systems. Boca Raton: CRC Press, https://doi.org/10.1201/9781420052336.

Agüera, A., Bueno, M., Fernández-Alba, A., 2013. New trends in the analytical determination of emerging contaminants and their transformation products in environmental waters. Environ. Sci. Pollut. Res. Int. 20. https://doi.org/10.1007/s11356-013-1586-0

Ahel, M., Giger, W., Koch, M., 1994. Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment-I. Occurrence and transformation in sewage treatment. Water Res. 28, 1131–1142. https://doi.org/10.1016/0043-1354(94)90200-3

Al-Odaini, N.A., Zakaria, M.P., Yaziz, M.I., Surif, S., 2010. Multi-residue analytical method for human pharmaceuticals and synthetic hormones in river water and sewage effluents by solid-phase extraction and liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1217, 6791–6806. https://doi.org/10.1016/j.chroma.2010.08.033

Al-Qaim, F.F., Abdullah, M.P., Othman, M.R., Latip, J., Zakaria, Z., 2014. Multi-residue analytical methodology-based liquid chromatography-time-of-flight-mass spectrometry for the analysis of pharmaceutical residues in surface water and effluents from sewage treatment plants and hospitals. J. Chromatogr. A 1345, 139–153. https://doi.org/10.1016/j.chroma.2014.04.025

Al Aukidy, M., Verlicchi, P., Jelic, A., Petrovic, M., Barcelò, D., 2012. Monitoring release of pharmaceutical compounds: Occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy. Sci. Total Environ. 438, 15–25. https://doi.org/10.1016/j.scitotenv.2012.08.061

Ali, A.M., Rønning, H.T., Al Arif, W.M., Kallenborn, R., Kallenborn, R., 2017. Occurrence of

pharmaceuticals and personal care products in effluent-dominated Saudi Arabian coastal waters of the Red Sea. Chemosphere 175, 505–513. https://doi.org/10.1016/j.chemosphere.2017.02.095

Althakafy, J.T., Kulsing, C., Grace, M.R., Marriott, P.J., 2017. Liquid chromatography – quadrupole Orbitrap mass spectrometry method for selected pharmaceuticals in water samples. J. Chromatogr. A 1515, 164–171. https://doi.org/10.1016/j.chroma.2017.08.003

Álvarez-Muñoz, D., Farré, M., 2020. Future trends in environmental metabolomics analysis. Environ. Metabolomics 339–341. https://doi.org/10.1016/b978-0-12-818196-6.00012-1

Álvarez-Muñoz, D., Rodríguez-Mozaz, S., Maulvault, A.L., Tediosi, A., Fernández-Tejedor, M., Van den Heuvel, F., Kotterman, M., Marques, A., Barceló, D., 2015. Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves, and fish from coastal areas in Europe. Environ. Res. 143, 56–64. https://doi.org/10.1016/j.envres.2015.09.018

Alygizakis, N.A., Gago-Ferrero, P., Borova, V.L., Pavlidou, A., Hatzianestis, I., Thomaidis, N.S., 2016. Occurrence and spatial distribution of 158 pharmaceuticals, drugs of abuse and related metabolites in offshore seawater. Sci. Total Environ. 541, 1097–1105. https://doi.org/10.1016/j.scitotenv.2015.09.145

Alygizakis, N.A., Oswald, P., Thomaidis, N.S., Schymanski, E.L., Aalizadeh, R., Schulze, T., Oswaldova, M., Slobodnik, J., 2019. NORMAN digital sample freezing platform: A European virtual platform to exchange liquid chromatography high resolution-mass spectrometry data and screen suspects in “digitally frozen” environmental samples. TrAC - Trends Anal. Chem. 115, 129–137. https://doi.org/10.1016/j.trac.2019.04.008

Amin, M.M., Bina, B., Ebrahimi, A., Yavari, Z., Mohammadi, F., Rahimi, S., 2018. The occurrence, fate, and distribution of natural and synthetic hormones in different types of wastewater treatment plants in Iran. Chinese J. Chem. Eng. 26, 1132–1139. https://doi.org/10.1016/j.cjche.2017.09.005

Andersen, H., Siegrist, H., Halling-Sørensen, B., Ternes, T.A., 2003. Fate of Estrogens in a Municipal Sewage Treatment Plant. Environ. Sci. Technol. 37, 4021–4026.

Ankley, G.T., Brooks, B.W., Huggett, D.B., Sumpter, J.P., 2007. Repeating history: Pharmaceuticals in the environment. Environ. Sci. Technol. 41, 8211–8217. https://doi.org/10.1021/es072658j

Antonić, J., Heath, E., 2007. Determination of NSAIDs in river sediment samples. Anal Bioanal Chem 387, 1337–1342. https://doi.org/10.1007/s00216-006-0947-7

Antonijević, M.D., Arsović, M., ŕáslavsky, J., Cvetković, V., Dabić, P., Franko, M., Ilić, G., Ivanović, M., Ivanović, N., Kosovac, M., Medić, D., Najdanović, S., Nikolić, M., Novaković, J., Radovanović, T., Ranić, D.,,ajatović, B.,,pijunović, G., Stankov, I., Tǫović, J., Treþe, P., Vasiljević, O., Schwarzbauer, J., 2014. Actual contamination of the Danube and Sava Rivers at Belgrade (2013). J. Serbian Chem. Soc. 79, 1169–1184. https://doi.org/10.2298/JSC131105014A

Arditsoglou, A., Voutsa, D., 2012. Occurrence and partitioning of endocrine-disrupting compounds in the marine environment of Thermaikos Gulf, Northern Aegean Sea, Greece. Mar. Pollut. Bull. 64, 2443–2452. https://doi.org/10.1016/j.marpolbul.2012.07.048

Ascenzi, P., Bocedi, A., Marino, M., 2006. Structure-function relationship of estrogen receptor α and β: Impact on human health. Mol. Aspects Med. 27, 299–402. https://doi.org/10.1016/j.mam.2006.07.001

Assress, H.A., Nyoni, H., Mamba, B.B., Msagati, T.A.M., 2019. Target quantification of azole antifungals and retrospective screening of other emerging pollutants in wastewater effluent using UHPLC –QTOF-MS. Environ. Pollut. 253, 655–666. https://doi.org/10.1016/j.envpol.2019.07.075

aus de Beek, T., Weber, F.-A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., Kuster, A., 2016. Pharmaceuticals in the environment-global occurrences and perspectives. Environ. Toxicol. Chem. 35, 823–835. https://doi.org/10.1002/etc.3339

Avagyan, R., Åberg, M., Westerholm, R., 2016. Chemosphere Suspect screening of OH-PAHs and non-target screening of other organic compounds in wood smoke particles using HR-Orbitrap-MS. Chemosphere 163, 313–321. https://doi.org/10.1016/j.chemosphere.2016.08.039

Aymerich, I., Acuña, V., Barceló, D., García, M.J., Petrovic, M., Poch, M., Rodriguez-Mozaz, S., Rodríguez-Roda, I., Sabater, S., von Schiller, D., Corominas, L., 2016. Attenuation of pharmaceuticals and their transformation products in a wastewater treatment plant and its receiving river ecosystem. Water Res. 100, 126–136. https://doi.org/https://doi.org/10.1016/j.watres.2016.04.022

Ayukekbong, J.A., Ntemgwa, M., Atabe, A.N., 2017. The threat of antimicrobial resistance in developing countries: Causes and control strategies. Antimicrob. Resist. Infect. Control 6, 1–8. https://doi.org/10.1186/s13756-017-0208-x

Azzouz, A., Ballesteros, E., 2012. Combined microwave-assisted extraction and continuous solid-phase extraction prior to gas chromatography-mass spectrometry determination of pharmaceuticals, personal care products and hormones in soils, sediments and sludge. Sci. Total Environ. 419, 208–215.

https://doi.org/10.1016/j.scitotenv.2011.12.058

Bachelot, M., Li, Z., Munaron, D., Le Gall, P., Casellas, C., Fenet, H., Gomez, E., 2012. Organic UV filter concentrations in marine mussels from French coastal regions. Sci. Total Environ. 420, 273–279. https://doi.org/10.1016/j.scitotenv.2011.12.051

Baena-Nogueras, R.M., Pintado-Herrera, M.G., González-Mazo, E., Lara-Martín, P.A., 2016. Determination of Pharmaceuticals in Coastal Systems Using Solid Phase Extraction (SPE) Followed by Ultra Performance Liquid Chromatography – tandem Mass Spectrometry (UPLC-MS/MS). Curr. Anal. Chem. 12, 183–201. https://doi.org/10.2174/1573411012666151009193254

Bahamonde, P.A., Feswick, A., Isaacs, M.A., Munkittrick, K.R., Martyniuk, C.J., 2016. Defining the role of omics in assessing ecosystem health: Perspectives from the Canadian environmental monitoring program. Environ. Toxicol. Chem. 35, 20–35. https://doi.org/10.1002/etc.3218

Ballesteros-Gómez, A., Ruiz, F.J., Rubio, S., Pérez-Bendito, D., 2007. Determination of bisphenols A and F and their diglycidyl ethers in wastewater and river water by coacervative extraction and liquid chromatography-fluorimetry. Anal. Chim. Acta 603, 51–59. https://doi.org/10.1016/j.aca.2007.09.048

Barceló, D., Petrovic, M., 2007. Emerging contaminants in wastewaters. TrAC - Trends Anal. Chem. 26, 1019. https://doi.org/10.1016/j.trac.2007.10.006

Baronti, C., Curini, R., D'Ascenzo, G.D., Corcia, A. Di, Gentili, A., Samperi, R., 2000. Monitoring Natural and Synthetic Estrogens at Activated Sludge Sewage Treatment Plants and in a Receiving River Water. Environ. Sci. Technol. 34, 5059–5066.

Bayen, S., Segovia, E., Juhel, G., Wei, L., Kelly, B.C., 2016. Pharmaceutically active compounds and endocrine disrupting chemicals in water , sediments and mollusks in mangrove ecosystems from Singapore. MPB 109, 716–722. https://doi.org/10.1016/j.marpolbul.2016.06.105

Bayen, S., Zhang, H., Desai, M.M., Ooi, S.K., Kelly, B.C., 2013. Occurrence and distribution of pharmaceutically active and endocrine disrupting compounds in Singapore's marine environment: Influence of hydrodynamics and physical-chemical properties. Environ. Pollut. 182, 1–8. https://doi.org/10.1016/j.envpol.2013.06.028

Beck, I.-C., Bruhn, R., Gandrass, J., Ruck, W., 2005. Liquid chromatography–tandem mass spectrometry analysis of estrogenic compounds in coastal surface water of the Baltic Sea. J. Chromatogr. A 1090, 98–106. https://doi.org/https://doi.org/10.1016/j.chroma.2005.07.013

Behera, S.K., Kim, H.W., Oh, J.E., Park, H.S., 2011. Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the

largest industrial city of Korea. Sci. Total Environ. 409, 4351–4360. https://doi.org/10.1016/j.scitotenv.2011.07.015

Belfroid, A.C., Horst, A. Van Der, Vethaak, A.D., Schafer, A.J., 1999. Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands.

Ben, W., Zhu, B., Yuan, X., Zhang, Y., Yang, M., Qiang, Z., 2017. Transformation and fate of natural estrogens and their conjugates in wastewater treatment plants: Influence of operational parameters and removal pathways. Water Res. 124, 244–250. https://doi.org/10.1016/j.watres.2017.07.065

Benedé, J.L., Chisvert, A., Salvador, A., Sánchez-Quiles, D., Tovar-Sánchez, A., 2014. Determination of UV filters in both soluble and particulate fractions of seawaters by dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry. Anal. Chim. Acta 812, 50–58. https://doi.org/10.1016/j.aca.2013.12.033

Benijts, T., Lambert, W., De Leenheer, A., 2004. Analysis of Multiple Endocrine Disruptors in Environmental Waters via Wide-Spectrum Solid-Phase Extraction and Dual-Polarity Ionization LC-Ion Trap-MS/MS. Anal. Chem. 76, 704–711. https://doi.org/10.1021/ac035062x

Benotti, M.J., Brownawell, B.J., 2007. Distributions of pharmaceuticals in an urban estuary during both dry- and wet-weather conditions. Environ. Sci. Technol. 41, 5795–5802. https://doi.org/10.1021/es0629965

Bilal, M., Iqbal, H.M.N., 2019. Persistence and impact of steroidal estrogens on the environment and their laccase-assisted removal. Sci. Total Environ. 690, 447–459. https://doi.org/10.1016/j.scitotenv.2019.07.025

Birch, G.F., Drage, D.S., Thompson, K., Eaglesham, G., Mueller, J.F., 2015. Emerging contaminants (pharmaceuticals, personal care products, a food additive and pesticides) in waters of Sydney estuary, Australia. Mar. Pollut. Bull. 97, 56–66. https://doi.org/10.1016/j.marpolbul.2015.06.038

Boleda, M.R., Galceran, M.T., Ventura, F., 2013. Validation and uncertainty estimation of a multiresidue method for pharmaceuticals in surface and treated waters by liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1286, 146–158. https://doi.org/10.1016/j.chroma.2013.02.077

Bolong, N., Ismail, A.F., Salim, M.R., Matsuura, T., 2009. A review of the effects of emerging contaminants in wastewater and options for their removal. Desalination 239, 229–246. https://doi.org/10.1016/j.desal.2008.03.020

Bono-Blay, F., Guart, A., de la Fuente, B., Pedemonte, M., Pastor, M.C., Borrell, A., Lacorte, S.,

2012. Survey of phthalates, alkylphenols, bisphenol A and herbicides in Spanish source waters intended for bottling. Environ. Sci. Pollut. Res. 19, 3339–3349. https://doi.org/10.1007/s11356-012-0851-y

Borova, V.L., Maragou, N.C., Gago-Ferrero, P., Pistos, C., Thomaidis, N.S., 2014. Highly sensitive determination of 68 psychoactive pharmaceuticals, illicit drugs, and related human metabolites in wastewater by liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 406, 4273–4285. https://doi.org/10.1007/s00216-014-7819-3

Boxall, A.B.A., Rudd, M.A., Brooks, B.W., Caldwell, D.J., Choi, K., Hickmann, S., Innes, E., Ostapyk, K., Staveley, J.P., Verslycke, T., Ankley, G.T., Beazley, K.F., Belanger, S.E., Berninger, J.P., Carriquiriborde, P., Coors, A., DeLeo, P.C., Dyer, S.D., Ericson, J.F., Gagné, F., Giesy, J.P., Gouin, T., Hallstrom, L., Karlsson, M. V., Joakim Larsson, D.G., Lazorchak, J.M., Mastrocco, F., McLaughlin, A., McMaster, M.E., Meyerhoff, R.D., Moore, R., Parrott, J.L., Snape, J.R., Murray-Smith, R., Servos, M.R., Sibley, P.K., Straub, J.O., Szabo, N.D., Topp, E., Tetreault, G.R., Trudeau, V.L., Van Der Kraak, G., 2012. Pharmaceuticals and personal care products in the environment: What are the big questions? Environ. Health Perspect. 120, 1221–1229. https://doi.org/10.1289/ehp.1104477

Brand, W., de Jongh, C.M., van der Linden, S.C., Mennes, W., Puijker, L.M., van Leeuwen, C.J., van Wezel, A.P., Schriks, M., Heringa, M.B., 2013. Trigger values for investigation of hormonal activity in drinking water and its sources using CALUX bioassays. Environ. Int. 55, 109–118. https://doi.org/10.1016/j.envint.2013.02.003

Brausch, J.M., Rand, G.M., 2011. A review of personal care products in the aquatic environment: Environmental concentrations and toxicity. Chemosphere 82, 1518–1532. https://doi.org/10.1016/j.chemosphere.2010.11.018

Brix, R., Postigo, C., González, S., Villagrasa, M., Navarro, A., Kuster, M., de Alda, M.J.L., Barceló, D., 2010a. Analysis and occurrence of alkylphenolic compounds and estrogens in a European river basin and an evaluation of their importance as priority pollutants. Anal. Bioanal. Chem. 396, 1301–1309. https://doi.org/10.1007/s00216-009-3358-8

Brix, R., Postigo, C., González, S., Villagrasa, M., Navarro, A., Kuster, M., De Alda, M.J.L., Barceló, D., 2010b. Analysis and occurrence of alkylphenolic compounds and estrogens in a European river basin and an evaluation of their importance as priority pollutants. Anal. Bioanal. Chem. 396, 1301–1309. https://doi.org/10.1007/s00216-009-3358-8

Brodin, T., Fick, J., Jonsson, M., Klaminder, J., 2013. Dilute concentrations of a psychiatric

drug alter behavior of fish from natural populations. Science (80-.). 339, 814–815. https://doi.org/10.1126/science.1226850

Brodin, T., Nordling, J., Lagesson, A., Klaminder, J., Hellström, G., Christensen, B., Fick, J., 2017. Environmental relevant levels of a benzodiazepine (oxazepam) alters important behavioral traits in a common planktivorous fish, (Rutilus rutilus). J. Toxicol. Environ. Heal. Part A 80, 963–970. https://doi.org/10.1080/15287394.2017.1352214

Brumovský, M., Bečanová, J., Kohoutek, J., Borghini, M., Nizzetto, L., 2017. Contaminants of emerging concern in the open sea waters of the Western Mediterranean. Environ. Pollut. 229, 976–983. https://doi.org/10.1016/j.envpol.2017.07.082

Bueno, M.J.M., Gomez, M.J., Herrera, S., Hernando, M.D., Agüera, A., Fernández-Alba, A.R., 2012. Occurrence and persistence of organic emerging contaminants and priority pollutants in five sewage treatment plants of Spain: Two years pilot survey monitoring. Environ. Pollut. 164, 267–273. https://doi.org/10.1016/j.envpol.2012.01.038

Burns, E.E., Carter, L.J., Snape, J., Thomas-Oates, J., Boxall, A.B.A., 2018. Application of prioritization approaches to optimize environmental monitoring and testing of pharmaceuticals. J. Toxicol. Environ. Heal. - Part B Crit. Rev. 21, 115–141. https://doi.org/10.1080/10937404.2018.1465873

Busetti, F., Backe, W.J., Bendixen, N., Maier, U., Place, B., Giger, W., Field, J.A., Field, J., 2012. Trace analysis of environmentalmatrices by large-volume injection and liquid chromatography-mass spectrometry. Anal Bioanal Chem 402, 175–186.

Caldwell, D.J., Mastrocco, F., Margiotta-Casaluci, L., Brooks, B.W., 2014. An integrated approach for prioritizing pharmaceuticals found in the environment for risk assessment, monitoring and advanced research. Chemosphere 115, 4–12. https://doi.org/10.1016/j.chemosphere.2014.01.021

Caldwell, D.J., Mastrocco, F., Nowak, E., Johnston, J., Yekel, H., Pfeiffer, D., Hoyt, M., DuPlessie, B.M., Anderson, P.D., 2010. An assessment of potential exposure and risk from estrogens in drinking water. Environ. Health Perspect. 118, 338–344. https://doi.org/10.1289/ehp.0900654

Caliman, F.A., Gavrilescu, M., 2009. Pharmaceuticals, personal care products and endocrine disrupting agents in the environment - A review. Clean - Soil, Air, Water 37, 277–303. https://doi.org/10.1002/clen.200900038

Campbell, C.G., Borglin, S.E., Green, F.B., Grayson, A., Wozei, E., Stringfellow, W.T., 2006. Biologically directed environmental monitoring , fate , and transport of estrogenic endocrine disrupting compounds in water : A review 65, 1265–1280.

https://doi.org/10.1016/j.chemosphere.2006.08.003

Campos-Mañas, M.C., Plaza-Bolaños, P., Martínez-Piernas, A.B., Sánchez-Pérez, J.A., Agüera, A., 2019. Determination of pesticide levels in wastewater from an agro-food industry: Target, suspect and transformation product analysis. Chemosphere 232, 152–163. https://doi.org/10.1016/j.chemosphere.2019.05.147

Cao, Q., Yu, Q., Connell, D.W., 2010. Fate simulation and risk assessment of endocrine disrupting chemicals in a reservoir receiving recycled wastewater. Sci. Total Environ. 408, 6243–6250. https://doi.org/10.1016/j.scitotenv.2010.08.059

Carafa, R., Wollgast, J., Canuti, E., Ligthart, J., Dueri, S., Hanke, G., Eisenreich, S.J., Viaroli, P., Zaldívar, J.M., 2007. Seasonal variations of selected herbicides and related metabolites in water, sediment, seaweed and clams in the Sacca di Goro coastal lagoon (Northern Adriatic). Chemosphere 69, 1625–1637. https://doi.org/10.1016/j.chemosphere.2007.05.060

Carmona, E., Picó, Y., 2018. The Use of Chromatographic Methods Coupled to Mass Spectrometry for the Study of Emerging Pollutants in the Environment. Crit. Rev. Anal. Chem. 48, 305–316. https://doi.org/10.1080/10408347.2018.1430555

Casajuana, N., Lacorte, S., 2003. Presence and release of phthalic esters and other endocrine disrupting compounds in drinking water. Chromatographia 57, 649–655. https://doi.org/10.1007/BF02491744

Cécile, M., 2019. How to regulate endocrine disrupting chemicals? Feedback and future development. Curr. Opin. Endocr. Metab. Res. 7, 21–25. https://doi.org/10.1016/j.coemr.2019.04.009

Céspedes, R., Lacorte, S., Ginebreda, A., Barceló, D., 2008. Occurrence and fate of alkylphenols and alkylphenol ethoxylates in sewage treatment plants and impact on receiving waters along the Ter River (Catalonia, NE Spain). Environ. Pollut. 153, 384–392. https://doi.org/10.1016/j.envpol.2007.08.026

Céspedes, R., Lacorte, S., Ginebreda, A., Barceló, D., 2006. Chemical monitoring and occurrence of alkylphenols, alkylphenol ethoxylates, alcohol ethoxylates, phthalates and benzothiazoles in sewage treatment plants and receiving waters along the ter River basin (Catalonia, N. E. Spain). Anal. Bioanal. Chem. 385, 992–1000. https://doi.org/10.1007/s00216-006-0448-8

Céspedes, R., Petrovic, M., Raldúa, D., Saura, Ú., Piña, B., Lacorte, S., Viana, P., Barceló, D., 2004. Integrated procedure for determination of endocrine-disrupting activity in surface waters and sediments by use of the biological technique recombinant yeast assay and chemical analysis by LC-ESI-MS. Anal. Bioanal. Chem. 378, 697–708. https://doi.org/10.1007/s00216-003-2303-5

Chen, C., Li, J., Chen, P., Ding, R., Zhang, P., Li, X., 2014. Occurrence of antibiotics and antibiotic resistances in soils from wastewater irrigation areas in Beijing and Tianjin, China. Environ. Pollut. 193, 94–101. https://doi.org/10.1016/j.envpol.2014.06.005

Chen, C.Y., Wen, T.Y., Wang, G.S., Cheng, H.W., Lin, Y.H., Lien, G.W., 2007. Determining estrogenic steroids in Taipei waters and removal in drinking water treatment using high-flow solid-phase extraction and liquid chromatography/tandem mass spectrometry. Sci. Total Environ. 378, 352–365. https://doi.org/10.1016/j.scitotenv.2007.02.038

Chen, M., Cooper, V.I., Deng, J., Amatya, P.L., Ambrus, D., Dong, S., Stalker, N., Nadeau-Bonilla, C., Patel, J., 2015. Occurrence of Pharmaceuticals in Calgary's Wastewater and Related Surface Water. Water Environ. Res. 87, 414–424. https://doi.org/10.2175/106143015X14212658614199

Chen, W., Xu, J., Lu, S., Jiao, W., Wu, L., Chang, A.C., 2013. Fates and transport of PPCPs in soil receiving reclaimed water irrigation. Chemosphere 93, 2621–2630. https://doi.org/10.1016/j.chemosphere.2013.09.088

Chiaia-Hernandez, A.C., Krauss, M., Hollender, J., 2013. Screening of lake sediments for emerging contaminants by liquid chromatography atmospheric pressure photoionization and electrospray ionization coupled to high resolution mass spectrometry. Environ. Sci. Technol. 47, 976–986. https://doi.org/10.1021/es303888v

Chiaia-Hernandez, A.C., Schymanski, E.L., Kumar, P., Singer, H.P., Hollender, J., 2014. Suspect and nontarget screening approaches to identify organic contaminant records in lake sediments. https://doi.org/10.1007/s00216-014-8166-0

Choi, Y., Kim, K., Kim, D., Moon, H. bang, Jeon, J., 2020. Ny-Ålesund-oriented organic pollutants in sewage effluent and receiving seawater in the Arctic region of Kongsfjorden. Environ. Pollut. 258. https://doi.org/10.1016/j.envpol.2019.113792

Choong, A.M.F., Teo, S.L.M., Leow, J.L., Koh, H.L., Ho, P.C.L., 2006. A preliminary ecotoxicity study of pharmaceuticals in the marine environment. J. Toxicol. Environ. Heal. - Part A Curr. Issues 69, 1959–1970. https://doi.org/10.1080/15287390600751371

Christiansen, L., Winther-Nielsen, M., Helweg, C., 2002. Feminization of Fish: the Effect of Estrogenic Compounds and Their Fate in Sewage Treatment Plants and Nature. Danish Environ. Protecion Agency.

Ciofi, L., Fibbi, D., Chiuminatto, U., Coppini, E., Checchini, L., Del Bubba, M., 2013. Fully-automated on-line solid phase extraction coupled to high-performance liquid chromatography-tandem mass spectrometric analysis at sub-ng/L levels of selected estrogens in surface water and wastewater. J. Chromatogr. A 1283, 53–61.

https://doi.org/10.1016/j.chroma.2013.01.084

Cleuvers, M., 2003. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicol. Lett. 142, 185–194. https://doi.org/https://doi.org/10.1016/S0378-4274(03)00068-7

Collado, N., Rodriguez-Mozaz, S., Gros, M., Rubirola, A., Barceló, D., Comas, J., Rodriguez-Roda, I., Buttiglieri, G., 2014. Pharmaceuticals occurrence in a WWTP with significant industrial contribution and its input into the river system. Environ. Pollut. 185, 202–212. https://doi.org/https://doi.org/10.1016/j.envpol.2013.10.040

Comtois-Marotte, S., Chappuis, T., Vo Duy, S., Gilbert, N., Lajeunesse, A., Taktek, S., Desrosiers, M., Veilleux, É., Sauvé, S., 2017. Analysis of emerging contaminants in water and solid samples using high resolution mass spectrometry with a Q Exactive orbital ion trap and estrogenic activity with YES-assay. Chemosphere 166, 400–411. https://doi.org/10.1016/j.chemosphere.2016.09.077

Cooper, E.R., Siewicki, T.C., Phillips, K., 2008. Preliminary risk assessment database and risk ranking of pharmaceuticals in the environment. Sci. Total Environ. 398, 26–33. https://doi.org/https://doi.org/10.1016/j.scitotenv.2008.02.061

Couto, C.F., Lange, L.C., Amaral, M.C.S., 2019. Occurrence, fate and removal of pharmaceutically active compounds (PhACs) in water and wastewater treatment plants—A review. J. Water Process Eng. 32, 100927. https://doi.org/10.1016/j.jwpe.2019.100927

Cunha, S.C., Pena, A., Fernandes, J.O., 2015. Dispersive liquid-liquid microextraction followed by microwave-assisted silylation and gas chromatography-mass spectrometry analysis for simultaneous trace quantification of bisphenol A and 13 ultraviolet filters in wastewaters. J. Chromatogr. A 1414, 10–21. https://doi.org/10.1016/j.chroma.2015.07.099

D'Ascenzo, G., Di Corcia, A., Gentili, A., Mancini, R., Mastropasqua, R., Nazzari, M., Samperi, R., 2003. Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. Sci. Total Environ. 302, 199–209. https://doi.org/10.1016/S0048-9697(02)00342-X

da Silva, B.F., Jelic, A., López-Serna, R., Mozeto, A.A., Petrovic, M., Barceló, D., 2011. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. Chemosphere 85, 1331–1339.

Damkjaer, K., Weisser, J.J., Msigala, S.C., Mdegela, R., Styrishave, B., 2018. Occurrence, removal and risk assessment of steroid hormones in two wastewater stabilization pond systems in Morogoro, Tanzania. Chemosphere 212, 1142–1154. https://doi.org/10.1016/j.chemosphere.2018.08.053

Daneshvar, A., Svanfelt, J., Kronberg, L., Prévost, M., Weyhenmeyer, G.A., 2010. Seasonal variations in the occurrence and fate of basic and neutral pharmaceuticals in a Swedish river-lake system. Chemosphere 80, 301–309. https://doi.org/10.1016/j.chemosphere.2010.03.060

Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and personal care products in the environment: Agents of subtle change? Environ. Health Perspect. 107, 907–938. https://doi.org/10.1289/ehp.99107s6907

De Carvalho, N., Ceriani, R.L., Ippolito, A., Lettieri., T., 2015. JRC technical report: Development of the first watch list under the environmental quality standards directive. EUR 27142. Publications Office of the European Union. Accessed June 3, 2020. https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technica.

De Mes, T., Zeeman, G., Lettinga, G., 2005. Occurrence and fate of estrone, 17β-estradiol and 17α- ethynylestradiol in STPs for domestic wastewater. Rev. Environ. Sci. Biotechnol. 4, 275–311. https://doi.org/10.1007/s11157-005-3216-x

Decision 2015/495/EU, 2015. Commission implementing decision (EU) 2015/495 of 20 March 2015 establishing a Watch List of Substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. Off. J. Eur. Union L78/40 (2015), 20–30.

Decision 2018/840/EU, 2018. Commission implementing decision (EU) 2018/840 of 5 June 2018 establishing a Watch List of Substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council and repealing Comm. Off. J. Eur. Union L141/9.

Desbiolles, F., Malleret, L., Tiliacos, C., Wong-Wah-Chung, P., Laffont-Schwob, I., 2018. Occurrence and ecotoxicological assessment of pharmaceuticals: Is there a risk for the Mediterranean aquatic environment? Sci. Total Environ. 639, 1334–1348. https://doi.org/10.1016/j.scitotenv.2018.04.351

Desbrow, C., Routledge, E.J., Brighty, G.C., Sumpter, J.P., Waldock, M., 1998. Identification of Estrogenic Chemicals in STW Effluent. 1. Chemical Fractionation and in Vitro Biological Screening. Environ. Sci. Technol. 32, 1549–1558. https://doi.org/10.1021/es9707973

Directive 2000/60/EC, 2000. Directive 2000/60/EC of the European Parliament and of the Council, of 23 October 2000, establishing a framework for community action in the field of water policy. Off J Eur Communities 2000; L32701–72. (22.12.2000).

Directive 2003/53/EC, 2003. Directive 2003/53/EC of the European Parliament and of the Council of 18 June 2003 amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and

preparations (nonylphenol, non. Off. J. Eur. Union L 178/24.

Directive 2008/105/EC, 2008. Directive 2008/105/EC of the European Parliament and of the Council, of 16 December 2008, on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/ EEC, 84/491/E. Off J Eur Communities 2008; L34884–97.

Directive 2008/56/EC, 2008. The marine strategy framework directive. Off. J. Eur. Union L164/19.

Directive 2013/39/EU, 2013. Directive 2013/39/EU of the European Parliament and of the Council, of 12 August 2013, amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Off J Eur Union 2013; L22601–17.

Dodder, N.G., Maruya, K.A., Lee Ferguson, P., Grace, R., Klosterhaus, S., La Guardia, M.J., Lauenstein, G.G., Ramirez, J., 2014. Occurrence of contaminants of emerging concern in mussels (Mytilus spp.) along the California coast and the influence of land use, storm water discharge, and treated wastewater effluent. Mar. Pollut. Bull. 81, 340–346. https://doi.org/10.1016/j.marpolbul.2013.06.041

Done, H.Y., Halden, R.U., 2015. Reconnaissance of 47 antibiotics and associated microbial risks in seafood sold in the United States. J. Hazard. Mater. 282, 10–17. https://doi.org/10.1016/j.jhazmat.2014.08.075

Dong, Z., Senn, D.B., Moran, R.E., Shine, J.P., 2013. Prioritizing environmental risk of prescription pharmaceuticals. Regul. Toxicol. Pharmacol. 65, 60–67. https://doi.org/10.1016/j.yrtph.2012.07.003

Dulio, V., van Bavel, B., Brorström-Lundén, E., Harmsen, J., Hollender, J., Schlabach, M., Slobodnik, J., Thomas, K., Koschorreck, J., 2018. Emerging pollutants in the EU: 10 years of NORMAN in support of environmental policies and regulations. Environ. Sci. Eur. 30. https://doi.org/10.1186/s12302-018-0135-3

ECB, 2002. 4-nonylphenol (branched) and nonylphenol. European Union Risk Assessment Report, Luxembourg [WWW Document]. -.

Ekpeghere, K.I., Sim, W.J., Lee, H.J., Oh, J.E., 2018. Occurrence and distribution of carbamazepine, nicotine, estrogenic compounds, and their transformation products in wastewater from various treatment plants and the aquatic environment. Sci. Total Environ. 640–641, 1015–1023. https://doi.org/10.1016/j.scitotenv.2018.05.218

Ericson, H., Thorsén, G., Kumblad, L., 2010. Physiological effects of diclofenac, ibuprofen and propranolol on Baltic Sea blue mussels. Aquat. Toxicol. 99, 223–231. https://doi.org/10.1016/j.aquatox.2010.04.017

Escher, B.I., Lawrence, M., Macova, M., Mueller, J.F., Poussade, Y., Robillot, C., Roux, A.,

Gernjak, W., 2011. Evaluation of Contaminant Removal of Reverse Osmosis and Advanced Oxidation in Full-Scale Operation by Combining Passive Sampling with Chemical Analysis and Bioanalytical Tools. Environ. Sci. Technol. 45, 5387–5394. https://doi.org/10.1021/es201153k

Esteban, Gorga, M., Petrovic, M., González-Alonso, S., Barcelo, D., Valcárcel, Y., 2014. Analysis and occurrence of endocrine-disrupting compounds and estrogenic activity in the surface waters of Central Spain. Sci. Total Environ. 466–467, 939–951. https://doi.org/10.1016/j.scitotenv.2013.07.101

Fairbairn, D.J., Karpuzcu, M.E., Arnold, W.A., Barber, B.L., Kaufenberg, E.F., Koskinen, W.C., Novak, P.J., Rice, P.J., Swackhamer, D.L., 2015. Science of the Total Environment Sediment – water distribution of contaminants of emerging concern in a mixed use watershed. Sci. Total Environ. 505, 896–904. https://doi.org/10.1016/j.scitotenv.2014.10.046

Falconer, I.R., Chapman, H.F., Moore, M.R., 2006. Endocrine-Disrupting Compounds: A Review of Their Challenge to Sustainable and SafeWater Supply andWater Reuse. Inc. Env. Toxicol 21, 181–191. https://doi.org/10.1002/tox.20172

Farré, M., Gros, M., Hernández, B., Petrovic, M., Hancock, P., Barceló, D., 2008. Analysis of biologically active compounds in water by ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. 22, 41–51. https://doi.org/10.1002/rcm.3324

Farré, M.J., Insa, S., Mamo, J., Barceló, D., 2016. Determination of 15 N-nitrosodimethylamine precursors in different water matrices by automated on-line solid-phase extraction ultra-high-performance-liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1458, 99–111. https://doi.org/https://doi.org/10.1016/j.chroma.2016.06.064

Fayad, P.B., Prévost, M., Sauvé, S., 2013. On-line solid-phase extraction coupled to liquid chromatography tandem mass spectrometry optimized for the analysis of steroid hormones in urban wastewaters. Talanta 115, 349–360. https://doi.org/10.1016/j.talanta.2013.05.038

Fenet, H., Arpin-Pont, L., Vanhoutte-Brunier, A., Munaron, D., Fiandrino, A., Martínez Bueno, M.J., Boillot, C., Casellas, C., Mathieu, O., Gomez, E., 2014. Reducing PEC uncertainty in coastal zones: A case study on carbamazepine, oxcarbazepine and their metabolites. Environ. Int. 68, 177–184. https://doi.org/10.1016/j.envint.2014.03.025

Fent, K., Weston, A.A., Caminada, D., 2006. Ecotoxicology of human pharmaceuticals. Aquat. Toxicol. 76, 122–159. https://doi.org/10.1016/j.aquatox.2005.09.009

Ferreira, B., Jelic, A., López-serna, R., Mozeto, A.A., Petrovic, M., Barceló, D., 2011. Chemosphere Occurrence and distribution of pharmaceuticals in surface water , suspended solids and sediments of the Ebro river basin , Spain. Chemosphere 85, 1331–1339. https://doi.org/10.1016/j.chemosphere.2011.07.051

Ferrer, I., Thurman, E.M., 2012. Analysis of 100 pharmaceuticals and their degradates in water samples by liquid chromatography/quadrupole time-of-flight mass spectrometry. J. Chromatogr. A 1259, 148–157. https://doi.org/10.1016/j.chroma.2012.03.059

Fick, J., Soderstrom, H., Lindberg, R.H., Phan, C., Tysklind, M., Larsson, D.G.J., 2009. Contamination of surface, ground, and drinking water from pharmaceuticals production. Environ. Toxicol. Chem. 28, 2522–2527.

Fisch, K., Waniek, J.J., Schulz-Bull, D.E., 2017. Occurrence of pharmaceuticals and UV-filters in riverine run-offs and waters of the German Baltic Sea. Mar. Pollut. Bull. 124, 388–399. https://doi.org/10.1016/j.marpolbul.2017.07.057

Folmar, L.C., Hemmer, M.J., Denslow, N.D., Kroll, K., Chen, J., Cheek, A., Richman, H., Meredith, H., Grau, E.G., 2002. A comparison of the estrogenic potencies of estradiol, ethynylestradiol, diethylstilbestrol, nonylphenol and methoxychlor in vivo and in vitro. Aquat. Toxicol. 60, 101–110. https://doi.org/https://doi.org/10.1016/S0166-445X(01)00276-4

Gago-Ferrero, P., Krettek, A., Fischer, S., Wiberg, K., Ahrens, L., 2018. Suspect screening and regulatory databases : A powerful combination to identify emerging micropollutants. https://doi.org/10.1021/acs.est.7b06598

Gago-Ferrero, P., Schymanski, E.L., Bletsou, A.A., Aalizadeh, R., Hollender, J., Thomaidis, N.S., 2015. Extended Suspect and Non-Target Strategies to Characterize Emerging Polar Organic Contaminants in Raw Wastewater with LC-HRMS/MS. Environ. Sci. Technol. 49, 12333–12341. https://doi.org/10.1021/acs.est.5b03454

Galaon, T., Petre, J., Iancu, V.I., Cruceru, L., Vasile, G.G., Pascu, L.F., Lehr, C.B., 2016. Detection of estrogen hormones in Danube River and tributaries using liquid chromatography-mass spectrometry. Rev. Chim. 67, 1474–1478.

García-Córcoles, M.T., Rodríguez-Gómez, R., de Alarcón-Gómez, B., Çipa, M., Martín-Pozo, L., Kauffmann, J.M., Zafra-Gómez, A., 2019. Chromatographic Methods for the Determination of Emerging Contaminants in Natural Water and Wastewater Samples: A Review. Crit. Rev. Anal. Chem. 49, 160–186. https://doi.org/10.1080/10408347.2018.1496010

García-Galán, M.J., Petrovic, M., Rodríguez-Mozaz, S., Barceló, D., 2016. Talanta Multiresidue trace analysis of pharmaceuticals , their human metabolites and

transformation products by fully automated mass spectrometry. Talanta 158, 330–341. https://doi.org/10.1016/j.talanta.2016.05.061

Garrido, E., Camacho-Muñoz, D., Martín, J., Santos, A., Santos, J.L., Aparicio, I., Alonso, E., 2016. Monitoring of emerging pollutants in Guadiamar River basin (South of Spain): analytical method, spatial distribution and environmental risk assessment. Environ. Sci. Pollut. Res. 23, 25127–25144. https://doi.org/10.1007/s11356-016-7759-x

Gaw, S., Thomas, K. V., Hutchinson, T.H., 2014. Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. Philos. Trans. R. Soc. B Biol. Sci. 369. https://doi.org/10.1098/rstb.2013.0572

Ge, L., Chen, J., Wei, X., Zhang, S., Qiao, X., Cai, X., Xie, Q., 2010. Aquatic photochemistry of fluoroquinolone antibiotics: kinetics, pathways, and multivariate effects of main water constituents. Environ. Sci. Technol. 44, 2400–2405. https://doi.org/10.1021/es902852v

Gentili, A., Perret, D., Marchese, S., Mastropasqua, R., Curini, R., Di Corcia, A., 2002. Analysis of free estrogens and their conjugates in sewage and river waters by solid-phase extraction then liquid chromatography-electrospray-tandem mass spectrometry. Chromatographia 56, 25–32. https://doi.org/10.1007/BF02490242

Gerbersdorf, S.U., Cimatoribus, C., Class, H., Engesser, K.H., Helbich, S., Hollert, H., Lange, C., Kranert, M., Metzger, J., Nowak, W., Seiler, T.B., Steger, K., Steinmetz, H., Wieprecht, S., 2015. Anthropogenic Trace Compounds (ATCs) in aquatic habitats - Research needs on sources, fate, detection and toxicity to ensure timely elimination strategies and risk management. Environ. Int. 79, 85–105. https://doi.org/10.1016/j.envint.2015.03.011

Ginebreda, A., Muñoz, I., de Alda, M.L., Brix, R., López-Doval, J., Barceló, D., 2010. Environmental risk assessment of pharmaceuticals in rivers: Relationships between hazard indexes and aquatic macroinvertebrate diversity indexes in the Llobregat River (NE Spain). Environ. Int. 36, 153–162. https://doi.org/https://doi.org/10.1016/j.envint.2009.10.003

Goeury, K., Vo Duy, S., Munoz, G., Prévost, M., Sauvé, S., 2019. Analysis of Environmental Protection Agency priority endocrine disruptor hormones and bisphenol A in tap, surface and wastewater by online concentration liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1591, 87–98. https://doi.org/10.1016/j.chroma.2019.01.016

Gomes, R.L., Scrimshaw, M.D., Lester, J.N., 2003. Determination of endocrine disrupters in sewage treatment and receiving waters. TrAC - Trends Anal. Chem. 22, 697–707. https://doi.org/10.1016/S0165-9936(03)01011-2

González-Alonso, S., Merino, L.M., Esteban, S., López de Alda, M., Barceló, D., Durán, J.J., López-Martínez, J., Aceña, J., Pérez, S., Mastroianni, N., Silva, A., Catalá, M., Valcárcel, Y., 2017. Occurrence of pharmaceutical, recreational and psychotropic drug residues in surface water on the northern Antarctic Peninsula region. Environ. Pollut. 229, 241–254. https://doi.org/10.1016/j.envpol.2017.05.060

Gonzalez-Rey, M., Bebianno, M.J., 2014. Effects of non-steroidal anti-inflammatory drug (NSAID) diclofenac exposure in mussel Mytilus galloprovincialis. Aquat. Toxicol. 148, 818–831. https://doi.org/10.1016/j.aquatox.2014.01.011

Gorga, M., Petrovic, M., Barceló, D., 2013. Multi-residue analytical method for the determination of endocrine disruptors and related compounds in river and waste water using dual column liquid chromatography switching system coupled to mass spectrometry. J. Chromatogr. A 1295, 57–66. https://doi.org/10.1016/j.chroma.2013.04.028

Gosetti, F., Mazzucco, E., Gennaro, M.C., Marengo, E., 2016. Contaminants in water: non-target UHPLC/MS analysis. Environ. Chem. Lett. 14, 51–65. https://doi.org/10.1007/s10311-015-0527-1

Gramec Skledar, D., Mašič, L., 2016. Bisphenol A and its analogs: Do their metabolites have endocrine activity? Environ. Toxicol. Pharmacol. 47, 182–199. https://doi.org/10.1016/j.etap.2016.09.014

Griffith, D.R., Kido Soule, M.C., Matsufuji, H., Eglinton, T.I., Kujawinski, E.B., Gschwend, P.M., 2014. Measuring free, conjugated, and halogenated estrogens in secondary treated wastewater effluent. Environ. Sci. Technol. 48, 2569–2578. https://doi.org/10.1021/es402809u

Gros, M., Blum, K.M., Jernstedt, H., Renman, G., Rodríguez-Mozaz, S., Haglund, P., Andersson, P.L., Wiberg, K., Ahrens, L., 2017. Screening and prioritization of micropollutants in wastewaters from on-site sewage treatment facilities. J. Hazard. Mater. 328, 37–45. https://doi.org/10.1016/j.jhazmat.2016.12.055

Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem. J. Chromatogr. A 1248, 104–121. https://doi.org/10.1016/j.chroma.2012.05.084

Guedes-Alonso, R., Montesdeoca-Esponda, S., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2014. Liquid chromatography methodologies for the determination of steroid hormones in aquatic environmental systems. Trends Environ. Anal. Chem. 3, 14–27. https://doi.org/10.1016/j.teac.2014.10.001

Guo, F., Liu, Q., Qu, G. bo, Song, S. jun, Sun, J. teng, Shi, J. bo, Jiang, G. bin, 2013. Simultaneous determination of five estrogens and four androgens in water samples by online solid-phase extraction coupled with high-performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1281, 9–18. https://doi.org/10.1016/j.chroma.2013.01.044

Gutendorf, B., Westendorf, J., 2001. Comparison of an array of in vitro assays for the assessment of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and xenoestrogens. Toxicology 166, 79–89. https://doi.org/https://doi.org/10.1016/S0300-483X(01)00437-1

Halling-Sorensen, B., 2000. Environmental risk assessment of antibiotics: comparison of mecillinam, trimethoprim and ciprofloxacin. J. Antimicrob. Chemother. 46, 53–58. https://doi.org/10.1093/jac/46.suppl_1.53

Hamid, H., Eskicioglu, C., 2012. Fate of estrogenic hormones in wastewater and sludge treatment: A review of properties and analytical detection techniques in sludge matrix. Water Res. 46, 5813–5833. https://doi.org/https://doi.org/10.1016/j.watres.2012.08.002

Hanselman, T.A., Graetz, D.A., Wilkie, A.C., 2003. Manure-Borne Estrogens as Potential Environmental Contaminants: A Review. Environ. Sci. Technol. 37, 5471–5478. https://doi.org/10.1021/es034410+

Hansen, P.-D., Dizer, H., Hock, B., Marx, A., Sherry, J., McMaster, M., Blaise, C., 1998. Vitellogenin – a biomarker for endocrine disruptors. TrAC Trends Anal. Chem. 17, 448–451. https://doi.org/https://doi.org/10.1016/S0165-9936(98)00020-X

Hecker, M., Hollert, H., 2011. Endocrine disruptor screening: Regulatory perspectives and needs. Environ. Sci. Eur. 23, 1–14. https://doi.org/10.1186/2190-4715-23-15

Hernández, F., Ibáñez, M., Bade, R., Bijlsma, L., Sancho, J. V., 2014. Investigation of pharmaceuticals and illicit drugs in waters by liquid chromatography-high-resolution mass spectrometry. TrAC - Trends Anal. Chem. 63, 140–157. https://doi.org/10.1016/j.trac.2014.08.003

Hernández, F., Ibáñez, M., Gracia-Lor, E., Sancho, J. V., 2011. Retrospective LC-QTOF-MS analysis searching for pharmaceutical metabolites in urban wastewater. J. Sep. Sci. 34, 3517–3526. https://doi.org/10.1002/jssc.201100540

Hernández, F., Pozo, Ó.J., Sancho, J. V., López, F.J., Marín, J.M., Ibáñez, M., 2005. Strategies for quantification and confirmation of multi-class polar pesticides and transformation products in water by LC-MS2 using triple quadrupole and hybrid quadrupole time-of-flight analyzers. TrAC - Trends Anal. Chem. 24, 596–612. https://doi.org/10.1016/j.trac.2005.04.007

Hernández, F., Sancho, J. V., Ibáñez, M., Abad, E., Portolés, T., Mattioli, L., 2012. Current use of high-resolution mass spectrometry in the environmental sciences. Anal. Bioanal. Chem. 403, 1251–1264. https://doi.org/10.1007/s00216-012-5844-7

Hernando, M.D., Mezcua, M., Fernández-Alba, A.R., Barceló, D., 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta 69, 334–342. https://doi.org/https://doi.org/10.1016/j.talanta.2005.09.037

Hollender, J., van Bavel, B., Dulio, V., Farmen, E., Furtmann, K., Koschorreck, J., Kunkel, U., Krauss, M., Munthe, J., Schlabach, M., Slobodnik, J., Stroomberg, G., Ternes, T., Thomaidis, N.S., Togola, A., Tornero, V., 2019. High resolution mass spectrometry-based non-target screening can support regulatory environmental monitoring and chemicals management. Environ. Sci. Eur. 31. https://doi.org/10.1186/s12302-019-0225-x

Hu, Y., Zhu, Q., Yan, X., Liao, C., Jiang, G., 2019. Occurrence, fate and risk assessment of BPA and its substituents in wastewater treatment plant: A review. Environ. Res. 178, 108732. https://doi.org/https://doi.org/10.1016/j.envres.2019.108732

Huber, S., Remberger, M., Kaj, L., Schlabach, M., Jörundsdóttir, H.Ó., Vester, J., Arnórsson, M., Mortensen, I., Schwartson, R., Dam, M., 2016. Science of the Total Environment A fi rst screening and risk assessment of pharmaceuticals and additives in personal care products in waste water , sludge , recipient water and sediment from Faroe Islands , Iceland and Greenland. Sci. Total Environ. 562, 13–25. https://doi.org/10.1016/j.scitotenv.2016.03.063

Huerta-Fontela, M., Galceran, M.T., Martin-Alonso, J., Ventura, F., 2008. Occurrence of psychoactive stimulatory drugs in wastewaters in north-eastern Spain. Sci. Total Environ. 397, 31–40. https://doi.org/10.1016/j.scitotenv.2008.02.057

Huerta-Fontela, M., Galceran, M.T., Ventura, F., 2010. Fast liquid chromatography-quadrupole-linear ion trap mass spectrometry for the analysis of pharmaceuticals and hormones in water resources. J. Chromatogr. A 1217, 4212–4222. https://doi.org/10.1016/j.chroma.2009.11.007

Hug, C., Ulrich, N., Schulze, T., Brack, W., Krauss, M., 2014. Identification of novel micropollutants in wastewater by a combination of suspect and nontarget screening. Environ. Pollut. 184, 25–32. https://doi.org/10.1016/j.envpol.2013.07.048

Hughes, S.R., Kay, P., Brown, L.E., 2013. Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ. Sci. Technol. 47, 661–677. https://doi.org/10.1021/es3030148

Ibáñez, M., Borova, V., Boix, C., Aalizadeh, R., Bade, R., Thomaidis, N.S., Hernández, F., 2017.

UHPLC-QTOF MS screening of pharmaceuticals and their metabolites in treated wastewater samples from Athens. J. Hazard. Mater. 323, 26–35. https://doi.org/10.1016/j.jhazmat.2016.03.078

Ibáñez, M., Sancho, J. V., McMillan, D., Rao, R., Hernández, F., 2008. Rapid non-target screening of organic pollutants in water by ultraperformance liquid chromatography coupled to time-of- light mass spectrometry 27, 481–489. https://doi.org/10.1016/j.trac.2008.03.007

Ibáñez, M., Sancho, J. V, Bijlsma, L., Nuijs, A.L.N. Van, Covaci, A., Hernández, F., 2014. Trends in Analytical Chemistry Comprehensive analytical strategies based on high-resolution time-of-flight mass spectrometry to identify new psychoactive substances 57, 107–117. https://doi.org/10.1016/j.trac.2014.02.009

Isobe, T., Shiraishi, H., Yasuda, M., Shinoda, A., Suzuki, H., Morita, M., 2003. Determination of estrogens and their conjugates in water using solid-phase extraction followed by liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 984, 195–202. https://doi.org/10.1016/S0021-9673(02)01851-4

Ivanová, L., Mackuľak, T., Grabic, R., Golovko, O., Koba, O., Staňová, A.V., Szabová, P., Grenčíková, A., Bodík, I., 2018. Pharmaceuticals and illicit drugs – A new threat to the application of sewage sludge in agriculture. Sci. Total Environ. 634, 606–615. https://doi.org/10.1016/j.scitotenv.2018.04.001

Janex-Habibi, M.-L., Huyard, A., Esperanza, M., Bruchet, A., 2009. Reduction of endocrine disruptor emissions in the environment : The benefit of wastewater treatment. Water Res. 43, 1565–1576. https://doi.org/10.1016/j.watres.2008.12.051

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sánchez, R., Ventura, F., Petrovic, M., Barcelo, D., 2011. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Res. 45, 1165–1176. https://doi.org/10.1016/j.watres.2010.11.010

Jelić, A., Petrović, M., Barceló, D., 2009. Multi-residue method for trace level determination of pharmaceuticals in solid samples using pressurized liquid extraction followed by liquid chromatography/quadrupole-linear ion trap mass spectrometry. Talanta 80, 363–371. https://doi.org/10.1016/j.talanta.2009.06.077

Jia, A., Hu, J., Wu, X., Peng, H., Wu, S., Dong, Z., 2011. Occurrence and source apportionment of sulfonamides and their metabolites in Liaodong Bay and the adjacent Liao River basin, North China. Environ. Toxicol. Chem. 30, 1252–1260. https://doi.org/10.1002/etc.508

Jobling, S., Sumpter, J.P., 1993. Detergent components in sewage effluent are weakly oestrogenic to fish: An in vitro study using rainbow trout (Oncorhynchus mykiss)

hepatocytes. Aquat. Toxicol. 27, 361–372. https://doi.org/10.1016/0166-445X(93)90064-8

Jurado, A., Vàzquez-Suñé, E., Carrera, J., López de Alda, M., Pujades, E., Barceló, D., 2012. Emerging organic contaminants in groundwater in Spain: A review of sources, recent occurrence and fate in a European context. Sci. Total Environ. 440, 82–94. https://doi.org/10.1016/j.scitotenv.2012.08.029

Jurgens, M.D., Williams, R.J., Johnson, A.C., 1999. Fate and Behaviour of Steroid Oestrogens in Rivers: A Scoping Study. Environment Agency, Bristol, UK, p. 94.

K'oreje, K.O., Demeestere, K., De Wispelaere, P., Vergeynst, L., Dewulf, J., Van Langenhove, H., 2012. From multi-residue screening to target analysis of pharmaceuticals in water: Development of a new approach based on magnetic sector mass spectrometry and application in the Nairobi River basin, Kenya. Sci. Total Environ. 437, 153–164. https://doi.org/10.1016/j.scitotenv.2012.07.052

Kallenborn, R., Fick, J., Lindberg, R., Moe, M., Nielsen, K.M., Tysklind, M., Vasskog, T., 2008. Pharmaceutical Residues in Northern European Environments : Consequences and Perspectives, in: Pharmaceuticals in the Environment. Sources, Fate, Effects and Risk, Third Ed. Springer, Berlin Heidelberg,. pp. 61–74.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res. 43, 363–380. https://doi.org/10.1016/j.watres.2008.10.047

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res. 42, 3498–3518. https://doi.org/10.1016/j.watres.2008.04.026

Kiefer, K., Müller, A., Singer, H., Hollender, J., 2019. New relevant pesticide transformation products in groundwater detected using target and suspect screening for agricultural and urban micropollutants with LC-HRMS. Water Res. 165, 114972. https://doi.org/10.1016/j.watres.2019.114972

Kirschner, A.K.T., Kavka, G.G., Velimirov, B., Mach, R.L., Sommer, R., Farnleitner, A.H., 2009. Microbiological water quality along the Danube River: Integrating data from two whole-river surveys and a transnational monitoring network. Water Res. 43, 3673–3684. https://doi.org/https://doi.org/10.1016/j.watres.2009.05.034

Klosterhaus, S.L., Grace, R., Hamilton, M.C., Yee, D., 2013. Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. Environ. Int. 54, 92–99.

https://doi.org/10.1016/j.envint.2013.01.009

Koba, O., Grabicova, K., Cerveny, D., Turek, J., Kolarova, J., Randak, T., Zlabek, V., Grabic, R., 2018. Transport of pharmaceuticals and their metabolites between water and sediments as a further potential exposure for aquatic organisms. J. Hazard. Mater. 342, 401–407. https://doi.org/https://doi.org/10.1016/j.jhazmat.2017.08.039

Kobayashi, Y., Okuda, T., Yamashita, N., Tanaka, H., Tanaka, S., Fuji, S., 2006. The behavior of free/conjugated estrogens during advanced wastewater treatment. Env. Sanit Eng Res 20, 55–58.

Koelmans, A.A., Besseling, E., Foekema, E.M., 2014. Leaching of plastic additives to marine organisms. Environ. Pollut. 187, 49–54. https://doi.org/10.1016/j.envpol.2013.12.013

Komori, K., Tanaka, H., Okayasu, Y., Yasojima, M., Sato, C., 2004. Analysis and occurence of estrogen in wastewater in Japan. Water Sci. Technol. 50, 93–100. https://doi.org/10.2166/wst.2004.0314

Kookana, R.S., Williams, M., Boxall, A.B.A., Larsson, D.G.J., Gaw, S., Choi, K., Yamamoto, H., Thatikonda, S., Zhu, Y.G., Carriquiriborde, P., 2014. Potential ecological footprints of active pharmaceutical ingredients: An examination of risk factors in low-, middle- and high-income countries. Philos. Trans. R. Soc. B Biol. Sci. 369. https://doi.org/10.1098/rstb.2013.0586

Kotnik, K., Kosjek, T., Krajnc, U., Heath, E., 2014. Trace analysis of benzophenone-derived compounds in surface waters and sediments using solid-phase extraction and microwave-Assisted extraction followed by gas chromatography-mass spectrometry. Anal. Bioanal. Chem. 406, 3179–3190. https://doi.org/10.1007/s00216-014-7749-0

Kourgialas, N.N., Karatzas, G.P., Dokou, Z., Kokorogiannis, A., 2018. Groundwater footprint methodology as policy tool for balancing water needs (agriculture & tourism) in water scarce islands - The case of Crete, Greece. Sci. Total Environ. 615, 381–389. https://doi.org/10.1016/j.scitotenv.2017.09.308

Krauss, M., Singer, H., Hollender, J., 2010. LC-high resolution MS in environmental analysis: From target screening to the identification of unknowns. Anal. Bioanal. Chem. 397, 943–951. https://doi.org/10.1007/s00216-010-3608-9

Kuch, H.M., Ballschmiter, K., 2001. Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range. Environ. Sci. Technol. 35, 3201–3206. https://doi.org/10.1021/es010034m

Kümmerer, K., 2009. Antibiotics in the aquatic environment - A review - Part I. Chemosphere 75, 417–434. https://doi.org/10.1016/j.chemosphere.2008.11.086

Kuster, M., López de Alda, M.J., Hernando, M.D., Petrovic, M., Martín-Alonso, J., Barceló, D., 2008. Analysis and occurrence of pharmaceuticals, estrogens, progestogens and polar pesticides in sewage treatment plant effluents, river water and drinking water in the Llobregat river basin (Barcelona, Spain). J. Hydrol. 358, 112–123. https://doi.org/10.1016/j.jhydrol.2008.05.030

Kuzmanović, M., Ginebreda, A., Petrović, M., Barceló, D., 2015. Risk assessment based prioritization of 200 organic micropollutants in 4 Iberian rivers. Sci. Total Environ. 503–504, 289–299. https://doi.org/10.1016/j.scitotenv.2014.06.056

Lai, K.M., Johnson, K.L., Scrimshaw, M.D., Lester, J.N., 2000. Binding of Waterborne Steroid Estrogens to Solid Phases in River and Estuarine Systems 34, 3890–3894.

Lai, K.M., Scrimshaw, M.D., Lester, J.N., 2002. The Effects of Natural and Synthetic Steroid Estrogens in Relation to their Environmental Occurrence. Crit. Rev. Toxicol. 32, 113–132. https://doi.org/10.1080/20024091064192

Langford, K.H., Thomas, K. V., 2008. Inputs of chemicals from recreational activities into the Norwegian coastal zone. J. Environ. Monit. 10, 894–898. https://doi.org/10.1039/b806198j

Lapworth, D.J., Baran, N., Stuart, M.E., Ward, R.S., 2012. Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. Environ. Pollut. 163, 287–303. https://doi.org/10.1016/j.envpol.2011.12.034

Lara-Martín, P.A., González-Mazo, E., Petrovic, M., Barceló, D., Brownawell, B.J., 2014. Occurrence, distribution and partitioning of nonionic surfactants and pharmaceuticals in the urbanized Long Island Sound Estuary (NY). Mar. Pollut. Bull. 85, 710–719. https://doi.org/10.1016/j.marpolbul.2014.01.022

Le, T.X., Munekage, Y., 2004. Residues of selected antibiotics in water and mud from shrimp ponds in mangrove areas in Viet Nam. Mar. Pollut. Bull. 49, 922–929. https://doi.org/10.1016/j.marpolbul.2004.06.016

Le Coadou, L., Le Ménach, K., Labadie, P., Dévier, M.H., Pardon, P., Augagneur, S., Budzinski, H., 2017. Quality survey of natural mineral water and spring water sold in France: Monitoring of hormones, pharmaceuticals, pesticides, perfluoroalkyl substances, phthalates, and alkylphenols at the ultra-trace level. Sci. Total Environ. 603–604, 651–662. https://doi.org/10.1016/j.scitotenv.2016.11.174

Leendert, V., Van Langenhove, H., Demeestere, K., 2015. Trends in liquid chromatography coupled to high-resolution mass spectrometry for multi-residue analysis of organic micropollutants in aquatic environments. TrAC - Trends Anal. Chem. 67, 192–208. https://doi.org/10.1016/j.trac.2015.01.010

Leusch, F.D.L., Neale, P.A., Arnal, C., Aneck-Hahn, N.H., Balaguer, P., Bruchet, A., Escher, B.I.,

Esperanza, M., Grimaldi, M., Leroy, G., Scheurer, M., Schlichting, R., Schriks, M., Hebert, A., 2018. Analysis of endocrine activity in drinking water, surface water and treated wastewater from six countries. Water Res. 139, 10–18. https://doi.org/10.1016/j.watres.2018.03.056

Li, W.C., 2014. Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil. Environ. Pollut. 187, 193–201. https://doi.org/10.1016/j.envpol.2014.01.015

Li, X., Ying, G.G., Su, H.C., Yang, X.B., Wang, L., 2010. Simultaneous determination and assessment of 4-nonylphenol, bisphenol A and triclosan in tap water, bottled water and baby bottles. Environ. Int. 36, 557–562. https://doi.org/10.1016/j.envint.2010.04.009

Lisboa, N.S., Fahning, C.S., Cotrim, G., Dos Anjos, J.P., De Andrade, J.B., Hatje, V., Da Rocha, G.O., 2013. A simple and sensitive UFLC-fluorescence method for endocrine disrupters determination in marine waters. Talanta 117, 168–175. https://doi.org/10.1016/j.talanta.2013.08.006

Liu, J.L., Wong, M.H., 2013. Pharmaceuticals and personal care products (PPCPs): A review on environmental contamination in China. Environ. Int. 59, 208–224. https://doi.org/10.1016/j.envint.2013.06.012

Liu, L., Aljathelah, N.M., Hassan, H., Leitão, A., Bayen, S., 2019. Development of a liquid chromatography-quadrupole-time-of-flight-mass spectrometry based method for the targeted and suspect screening of contaminants in the pearl oyster Pinctada imbricata radiata. Environ. Pollut. 253, 841–849. https://doi.org/10.1016/j.envpol.2019.07.047

Liu, S., Ying, G.G., Zhao, J.L., Chen, F., Yang, B., Zhou, L.J., Lai, H. jie, 2011. Trace analysis of 28 steroids in surface water, wastewater and sludge samples by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry. J. Chromatogr. A 1218, 1367–1378. https://doi.org/10.1016/j.chroma.2011.01.014

Liu, S., Ying, G.G., Zhao, J.L., Zhou, L.J., Yang, B., Chen, Z.F., Lai, H.J., 2012. Occurrence and fate of androgens, estrogens, glucocorticoids and progestagens in two different types of municipal wastewater treatment plants. J. Environ. Monit. 14, 482–491. https://doi.org/10.1039/c1em10783f

Liu, Z. hua, Kanjo, Y., Mizutani, S., 2009. Urinary excretion rates of natural estrogens and androgens from humans, and their occurrence and fate in the environment: A review. Sci. Total Environ. 407, 4975–4985. https://doi.org/10.1016/j.scitotenv.2009.06.001

Loos, R., Carvalho, R., António, D.C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K., Haglund, P., Fick, J.,

Lindberg, R.H., Schwesig, D., Gawlik, B.M., 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Res. 47, 6475–6487. https://doi.org/10.1016/j.watres.2013.08.024

Loos, R., Locoro, G., Comero, S., Contini, S., Schwesig, D., Werres, F., Balsaa, P., Gans, O., Weiss, S., Blaha, L., Bolchi, M., Gawlik, B.M., 2010. Pan-European survey on the occurrence of selected polar organic persistent pollutants in ground water. Water Res. 44, 4115–4126. https://doi.org/10.1016/j.watres.2010.05.032

Loos, R., Marinov, D., Sanseverino, I., Napierska, D., Lettieri, T., 2018. Review of the 1st Watch List under the Water Framework Directive and recommendations for the 2nd Watch List. Luxembourg. https://doi.org/10.2760/614367.

Loos, R., Wollgast, J., Huber, T., 2007. Polar herbicides , pharmaceutical products , perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and nonylphenol and its carboxylates and ethoxylates in surface and tap waters around Lake Maggiore in Northern Italy 1469–1478. https://doi.org/10.1007/s00216-006-1036-7

López-Darias, J., Germán-Hernández, M., Pino, V., Afonso, A.M., 2010. Dispersive liquid-liquid microextraction versus single-drop microextraction for the determination of several endocrine-disrupting phenols from seawaters. Talanta 80, 1611–1618. https://doi.org/10.1016/j.talanta.2009.09.057

López-Serna, R., Petrovic, M., Barceló, D., 2012. Direct analysis of pharmaceuticals , their metabolites and transformation products in environmental waters using on-line TurboFlow TM chromatography – liquid chromatography – tandem mass spectrometry 1252, 115–129. https://doi.org/10.1016/j.chroma.2012.06.078

López de Alda, M.J., Gil, A., Paz, E., Barceló, D., 2002. Occurrence and analysis of estrogens and progestogens in river sediments by liquid chromatography-electrospray-mass spectrometry. Analyst 127, 1299–1304. https://doi.org/10.1039/b207658f

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci. Total Environ. 473–474, 619–641. https://doi.org/10.1016/j.scitotenv.2013.12.065

Magnér, J., Filipovic, M., Alsberg, T., 2010. Application of a novel solid-phase-extraction sampler and ultra-performance liquid chromatography quadrupole-time-of-flight mass spectrometry for determination of pharmaceutical residues in surface sea water. Chemosphere 80, 1255–1260. https://doi.org/10.1016/j.chemosphere.2010.06.065

Managaki, S., Murata, A., Takada, H., Bui, C.T., Chiem, N.H., 2007. Distribution of macrolides, sulfonamides, and trimethoprim in tropical waters: Ubiquitous occurrence of

veterinary antibiotics in the Mekong Delta. Environ. Sci. Technol. 41, 8004–8010. https://doi.org/10.1021/es0709021

Manickum, T., John, W., 2014. Occurrence, fate and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa). Sci. Total Environ. 468–469, 584–597. https://doi.org/10.1016/j.scitotenv.2013.08.041

Marchand, M., Tissier, C., 2005. L'analyse du risqué chimique en milieu marin: l'approche méthodologique européenne. Éd. Ifremer, 126p (in French).

Martín, J., Camacho-Muñoz, D., Santos, J.L., Aparicio, I., Alonso, E., 2012. Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal. J. Hazard. Mater. 239–240, 40–47. https://doi.org/https://doi.org/10.1016/j.jhazmat.2012.04.068

Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2015. Determination of hormones, a plasticizer, preservatives, perfluoroalkylated compounds, and a flame retardant in water samples by ultrasound-assisted dispersive liquid-liquid microextraction based on the solidification of a floating organic drop. Talanta 143, 335–343. https://doi.org/10.1016/j.talanta.2015.04.089

Martínez Bueno, M.J., Boillot, C., Munaron, D., Fenet, H., Casellas, C., Gómez, E., 2014. Occurrence of venlafaxine residues and its metabolites in marine mussels at trace levels: Development of analytical method and a monitoring program. Anal. Bioanal. Chem. 406, 601–610. https://doi.org/10.1007/s00216-013-7477-x

Martínez Bueno, M.J., Ulaszewska, M.M., Gomez, M.J., Hernando, M.D., Fernández-Alba, A.R., 2012. Simultaneous measurement in mass and mass/mass mode for accurate qualitative and quantitative screening analysis of pharmaceuticals in river water. J. Chromatogr. A 1256, 80–88. https://doi.org/10.1016/j.chroma.2012.07.038

Mascolo, G., Murgolo, S., Stefani, F., Viganò, L., 2019. Target and suspect contaminants of emerging concern in the Po River Delta lagoons. Estuar. Coast. Shelf Sci. 230. https://doi.org/10.1016/j.ecss.2019.106424

Masiá, A., Campo, J., Blasco, C., Picó, Y., 2014. Ultra-high performance liquid chromatography – quadrupole time-of-flight mass spectrometry to identify contaminants in water : An insight on environmental forensics &. J. Chromatogr. A 1345, 86–97. https://doi.org/10.1016/j.chroma.2014.04.017

Masiá, A., Ibáñez, M., Blasco, C., Sancho, J. V., Picó, Y., Hernández, F., 2013. Combined use of liquid chromatography triple quadrupole mass spectrometry and liquid chromatography quadrupole time-of-flight mass spectrometry in systematic

screening of pesticides and other contaminants in water samples. Anal. Chim. Acta 761, 117–127. https://doi.org/10.1016/j.aca.2012.11.032

Meador, J.P., Yeh, A., Young, G., Gallagher, E.P., 2016. Contaminants of emerging concern in a large temperate estuary. Environ. Pollut. 213, 254–267. https://doi.org/10.1016/j.envpol.2016.01.088

Mennigen, J.A., Stroud, P., Zamora, J.M., Moon, T.W., Trudeau, V.L., 2011. Pharmaceuticals as Neuroendocrine Disruptors: Lessons Learned from Fish on Prozac. J. Toxicol. Environ. Heal. Part B 14, 387–412. https://doi.org/10.1080/10937404.2011.578559

Miège, C., Bados, P., Brosse, C., Coquery, M., 2009. Method validation for the analysis of estrogens (including conjugated compounds) in aqueous matrices. TrAC - Trends Anal. Chem. 28, 237–244. https://doi.org/10.1016/j.trac.2008.11.005

Milanovic, M., Sudji, J., Grujic-Letic, N., Radonic, J., Turk-Sekulic, M., Vojinovic-Miloradov, M., 2016. Seasonal variations of bisphenol A in the Danube River by the municipality of Novi Sad, Serbia. J. Serbian Chem. Soc. 2016 81, 333–345.

Milic, N., Spanik, I., Radonic, J., Sekulic, M.T., Grujic, N., Vyviurska, O., Milanovic, M., Sremacki, M., Miloradov, M. V, 2014. Screening analyses of wastewater and danube surface water in Novi Sad Locality, Serbia. Fresenius Environ. Bull. 23, 372–377.

Miloradov, M.V., Mihajlović, I., Vyviurska, O., Cacho, F., Radonić, J., Milić, N., Spanik, I., 2014. Impact of wastewater discharges to Danube surface water pollution by emerging and priority pollutants in the vicinity of Novi sad, Serbia. Fresenius Environ. Bull. 23, 2137–2145.

Minguez, L., Farcy, E., Ballandonne, C., Lepailleur, A., Serpentini, A., Lebel, J.-M., Bureau, R., Halm-Lemeille, M.-P., 2014. Acute toxicity of 8 antidepressants: What are their modes of action? Chemosphere 108, 314–319. https://doi.org/https://doi.org/10.1016/j.chemosphere.2014.01.057

Minguez, L., Pedelucq, J., Farcy, E., Ballandonne, C., Budzinski, H., Halm-Lemeille, M.P., 2016. Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. Environ. Sci. Pollut. Res. 23, 4992–5001. https://doi.org/10.1007/s11356-014-3662-5

Moeder, M., Schrader, S., Winkler, U., Rodil, R., 2010. At-line microextraction by packed sorbent-gas chromatography-mass spectrometry for the determination of UV filter and polycyclic musk compounds in water samples. J. Chromatogr. A 1217, 2925–2932. https://doi.org/10.1016/j.chroma.2010.02.057

Moreno-González, R., Rodriguez-Mozaz, S., Gros, M., Barceló, D., León, V.M., 2015. Seasonal distribution of pharmaceuticals in marine water and sediment from a mediterranean coastal lagoon (SE Spain). Environ. Res. 138, 326–344.

https://doi.org/10.1016/j.envres.2015.02.016

Morteani, G., Mo, P., Fuganti, A., Paces, T., 2006. Input and fate of anthropogenic estrogens and gadolinium in surface water and sewage plants in the hydrological basin of Prague (Czech Republic) 257–264. https://doi.org/10.1007/s10653-006-9040-6

Moschet, C., Piazzoli, A., Singer, H., Hollender, J., 2013. Alleviating the Reference Standard Dilemma Using a Systematic Exact Mass Suspect Screening Approach with Liquid Chromatography-High Resolution Mass Spectrometry. Anal. Chem. 85. https://doi.org/10.1021/ac4021598

Murray, K.E., Thomas, S.M., Bodour, A.A., 2010. Prioritizing research for trace pollutants and emerging contaminants in the freshwater environment. Environ. Pollut. 158, 3462–3471. https://doi.org/10.1016/j.envpol.2010.08.009

Nakada, N., Kiri, K., 2008. Evaluation of Pharmaceuticals and Personal Care Products as Water-soluble Molecular Markers of Sewage 6347–6353.

Nakata, H., Shinohara, R.I., Nakazawa, Y., Isobe, T., Sudaryanto, A., Subramanian, A., Tanabe, S., Zakaria, M.P., Zheng, G.J., Lam, P.K.S., Kim, E.Y., Min, B.Y., We, S.U., Viet, P.H., Tana, T.S., Prudente, M., Frank, D., Lauenstein, G., Kannan, K., 2012. Asia-Pacific mussel watch for emerging pollutants: Distribution of synthetic musks and benzotriazole UV stabilizers in Asian and US coastal waters. Mar. Pollut. Bull. 64, 2211–2218. https://doi.org/10.1016/j.marpolbul.2012.07.049

Naldi, A.C., Fayad, P.B., Prévost, M., Sauvé, S., 2016. Analysis of steroid hormones and their conjugated forms in water and urine by on-line solid-phase extraction coupled to liquid chromatography tandem mass spectrometry. Chem. Cent. J. 10, 1–17. https://doi.org/10.1186/s13065-016-0174-z

Nannou, C.I., Kosma, C.I., Albanis, T.A., 2015. Occurrence of pharmaceuticals in surface waters: analytical method development and environmental risk assessment. Int. J. Environ. Anal. Chem. 95, 1242–1262. https://doi.org/10.1080/03067319.2015.1085520

Nazifa, T.H., Kristanti, R.A., Ike, M., Kuroda, M., Hadibarata, T., 2020. Occurrence and distribution of estrogenic chemicals in river waters of Malaysia. Toxicol. Environ. Health Sci. 12, 65–74. https://doi.org/10.1007/s13530-020-00036-8

Newbold, R.R., Padilla-Banks, E., Jefferson, W.N., Heindel, J.J., 2008. Effects of endocrine disruptors on obesity. Int. J. Androl. 31, 201–208. https://doi.org/10.1111/j.1365-2605.2007.00858.x

Nödler, K., Voutsa, D., Licha, T., 2014. Polar organic micropollutants in the coastal environment of different marine systems. Mar. Pollut. Bull. 85, 50–59. https://doi.org/10.1016/j.marpolbul.2014.06.024

Noppe, H., Verslycke, T., Wulf, E. De, Verheyden, K., Monteyne, E., Caeter, P. Van, Janssen, C.R., De, H.F., 2007. Occurrence of estrogens in the Scheldt estuary : A 2-year survey 66, 1–8. https://doi.org/10.1016/j.ecoenv.2006.04.005

NORMAN Ecotoxicology Database, 2019. NORMAN Ecotoxicology Database; www.norman-network.com [WWW Document]. URL https://www.norman-network.com (accessed 12.19.19).

NORMAN Network, 2016. Glossary of Terms. WWW Document. http://www.norman-network.net/?q=node/9. (Accessed 18 April 2020).

Nurmi, J., Pellinen, J., Rantalainen, A.L., 2012. Critical evaluation of screening techniques for emerging environmental contaminants based on accurate mass measurements with time-of-flight mass spectrometry. J. Mass Spectrom. 47, 303–312. https://doi.org/10.1002/jms.2964

Nurulnadia, M.Y., Koyama, J., Uno, S., Kito, A., Kokushi, E., Bacolod, E.T., Ito, K., Chuman, Y., 2014. Accumulation of endocrine disrupting chemicals (EDCs) in the polychaete Paraprionospio sp. from the Yodo River mouth, Osaka Bay, Japan. Environ. Monit. Assess. 186, 1453–1463. https://doi.org/10.1007/s10661-013-3466-y

Oaks, J.L., Gilbert, M., Virani, M.Z., Watson, R.T., Meteyer, C.U., Rideout, B.A., Shivaprasad, H.L., Ahmed, S., Jamshed, M., Chaudhry, I., Arshad, M., Mahmood, S., Ali, A., Khan, A.A., 2004. Diclofenac residues as the. Nature 427, 630–633.

Okkerman, P. and, Van der Putter, I., 2002. Study on gathering information on 435 substances with insufficient data.

Pal, A., Gin, K.Y.H., Lin, A.Y.C., Reinhard, M., 2010. Impacts of emerging organic contaminants on freshwater resources: Review of recent occurrences, sources, fate and effects. Sci. Total Environ. 408, 6062–6069. https://doi.org/10.1016/j.scitotenv.2010.09.026

Pamplona-Silva, M.T., Elisa, D., Mazzeo, C., Bianchi, J., Marin-Morales, M.A., 2018. Estrogenic Compounds : Chemical Characteristics , Detection Methods , Biological and Environmental Effects.

Park, N., Choi, Y., Kim, D., Kim, K., Jeon, J., 2018. Prioritization of highly exposable pharmaceuticals via a suspect/non-target screening approach: A case study for Yeongsan River, Korea. Sci. Total Environ. 639, 570–579. https://doi.org/10.1016/j.scitotenv.2018.05.081

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman, C.U., Mohan, D., 2019. Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. Chem. Rev. 119, 3510–3673. https://doi.org/10.1021/acs.chemrev.8b00299

Pedrouzo, M., Borrull, F., Pocurull, E., Marcé, R.M., 2009. Estrogens and their conjugates: Determination in water samples by solid-phase extraction and liquid chromatography-tandem mass spectrometry. Talanta 78, 1327–1331. https://doi.org/10.1016/j.talanta.2009.02.005

Pelayo, S., López-Roldán, R., González, S., Casado, M., Raldúa, D., Cortina, J.L., Piña, B., 2011. A zebrafish scale assay to monitor dioxin-like activity in surface water samples. Anal. Bioanal. Chem. 401, 1861–1869. https://doi.org/10.1007/s00216-011-5288-5

Peng, X., Yu, Y., Tang, C., Tan, J., Huang, Q., Wang, Z., 2008. Occurrence of steroid estrogens, endocrine-disrupting phenols, and acid pharmaceutical residues in urban riverine water of the Pearl River Delta, South China. Sci. Total Environ. 397, 158–166. https://doi.org/https://doi.org/10.1016/j.scitotenv.2008.02.059

Pereira, A.M.P.T., Silva, L.J.G., Lino, C.M., Meisel, L.M., Pena, A., 2017. A critical evaluation of different parameters for estimating pharmaceutical exposure seeking an improved environmental risk assessment. Sci. Total Environ. 603–604, 226–236. https://doi.org/10.1016/j.scitotenv.2017.06.022

Petrie, B., Youdan, J., Barden, R., Kasprzyk-Hordern, B., 2016. Multi-residue analysis of 90 emerging contaminants in liquid and solid environmental matrices by ultra-high-performance liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1431, 64–78. https://doi.org/10.1016/j.chroma.2015.12.036

Petrovic, M., Eljarrat, E., Lopez De Alda, M.J., Barceló, D., 2004. Endocrine disrupting compounds and other emerging contaminants in the environment: A survey on new monitoring strategies and occurrence data. Anal. Bioanal. Chem. 378, 549–562. https://doi.org/10.1007/s00216-003-2184-7

Petrovic, M., Gros, M., Barcelo, D., 2006. Multi-residue analysis of pharmaceuticals in wastewater by ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry. J. Chromatogr. A 1124, 68–81. https://doi.org/10.1016/j.chroma.2006.05.024

Pignotti, E., Casas, G., Llorca, M., Tellbüscher, A., Almeida, D., Dinelli, E., Farré, M., Barceló, D., 2017. Seasonal variations in the occurrence of perfluoroalkyl substances in water, sediment and fish samples from Ebro Delta (Catalonia, Spain). Sci. Total Environ. 607–608, 933–943. https://doi.org/10.1016/j.scitotenv.2017.07.025

Pino-Otín, M.R., Muñiz, S., Val, J., Navarro, E., 2017. Effects of 18 pharmaceuticals on the physiological diversity of edaphic microorganisms. Sci. Total Environ. 595, 441–450. https://doi.org/10.1016/j.scitotenv.2017.04.002

Pozo, O.J., Guerrero, C., Sancho, J. V., Ibáñez, M., Pitarch, E., Hogendoorn, E., Hernández, F., 2006. Efficient approach for the reliable quantification and confirmation of

antibiotics in water using on-line solid-phase extraction liquid chromatography/tandem mass spectrometry. J. Chromatogr. A 1103, 83–93. https://doi.org/10.1016/j.chroma.2005.10.073

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler, C.R., Sumpter, J.P., 1994. Estrogenic Effects of Effluents from Sewage Treatment Works. Chem. Ecol. 8, 275–285. https://doi.org/10.1080/02757549408038554

Quednow, K., Püttmann, W., 2009. Temporal concentration changes of DEET, TCEP, terbutryn, and nonylphenols in freshwater streams of Hesse, Germany: Possible influence of mandatory regulations and voluntary environmental agreements. Environ. Sci. Pollut. Res. 16, 630–640. https://doi.org/10.1007/s11356-009-0169-6

Reddy, S., Iden, C.R., Brownawell, B.J., 2005. Analysis of steroid conjugates in sewage influent and effluent by liquid chromatography-tandem mass spectrometry. Anal. Chem. 77, 7032–7038. https://doi.org/10.1021/ac050699x

Regueiro, J., Llompart, M., Garcia-Jares, C., Garcia-Monteagudo, J.C., Cela, R., 2008. Ultrasound-assisted emulsification-microextraction of emergent contaminants and pesticides in environmental waters. J. Chromatogr. A 1190, 27–38. https://doi.org/10.1016/j.chroma.2008.02.091

Regulation (EC) No 1107/2009., 2009. European Comission. Off. J. Eur. Union 309, 1–50.

Richardson, S.D., Ternes, T.A., 2018. Water Analysis: Emerging Contaminants and Current Issues. https://doi.org/10.1021/acs.analchem.7b04577

Richardson, S.D., Ternes, T.A., 2014. Water analysis: Emerging contaminants and current issues. Anal. Chem. 86, 2813–2848. https://doi.org/10.1021/ac500508t

Rico, A., Van den Brink, P.J., 2014. Probabilistic risk assessment of veterinary medicines applied to four major aquaculture species produced in Asia. Sci. Total Environ. 468–469, 630–641. https://doi.org/10.1016/j.scitotenv.2013.08.063

Rivera-Jaimes, J.A., Postigo, C., Melgoza-Alemán, R.M., Aceña, J., Barceló, D., López de Alda, M., 2018. Study of pharmaceuticals in surface and wastewater from Cuernavaca, Morelos, Mexico: Occurrence and environmental risk assessment. Sci. Total Environ. 613–614, 1263–1274. https://doi.org/https://doi.org/10.1016/j.scitotenv.2017.09.134

Robles-Molina, J., Gilbert-López, B., García-Reyes, J.F., Molina-Díaz, A., 2014. Monitoring of selected priority and emerging contaminants in the Guadalquivir River and other related surface waters in the province of Jaén, South East Spain. Sci. Total Environ. 479–480, 247–257. https://doi.org/10.1016/j.scitotenv.2014.01.121

Roch, S., Walter, T., Ittner, L.D., Friedrich, C., Brinker, A., 2019. Science of the Total Environment A systematic study of the microplastic burden in freshwater fi shes of

south-western Germany - Are we searching at the right scale ? Sci. Total Environ. 689, 1001–1011. https://doi.org/10.1016/j.scitotenv.2019.06.404

Rodriguez-Mozaz, S., Marco, M.P., Lopez De Alda, M.J., Barceló, D., 2004. Biosensors for environmental monitoring of endocrine disruptors: A review article. Anal. Bioanal. Chem. 378, 588–598. https://doi.org/10.1007/s00216-003-2385-0

Rodriguez-Narvaez, O.M., Peralta-Hernandez, J.M., Goonetilleke, A., Bandala, E.R., 2017. Treatment technologies for emerging contaminants in water: A review. Chem. Eng. J. 323, 361–380. https://doi.org/10.1016/j.cej.2017.04.106

Rodríguez-Navas, C., Björklund, E., Bak, S.A., Hansen, M., Krogh, K.A., Maya, F., Forteza, R., Cerdà, V., 2013. Pollution pathways of pharmaceutical residues in the aquatic environment on the island of Mallorca, Spain. Arch. Environ. Contam. Toxicol. 65, 56–66. https://doi.org/10.1007/s00244-013-9880-x

Ros, O., Vallejo, A., Blanco-Zubiaguirre, L., Olivares, M., Delgado, A., Etxebarria, N., Prieto, A., 2015. Microextraction with polyethersulfone for bisphenol-A, alkylphenols and hormones determination in water samples by means of gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry analysis. Talanta 134, 247–255. https://doi.org/10.1016/j.talanta.2014.11.015

Rubirola, A., Boleda, M.R., Galceran, M.T., 2017. Multiresidue analysis of 24 Water Framework Directive priority substances by on-line solid phase extraction-liquid chromatography tandem mass spectrometry in environmental waters. J. Chromatogr. A 1493, 64–75. https://doi.org/10.1016/j.chroma.2017.02.075

Runnqvist, H., Bak, S.A., Hansen, M., Styrishave, B., Halling-Sørensen, B., Björklund, E., 2010. Determination of pharmaceuticals in environmental and biological matrices using pressurised liquid extraction-Are we developing sound extraction methods? J. Chromatogr. A 1217, 2447–2470. https://doi.org/10.1016/j.chroma.2010.02.046

Rutishauser, B. V, Pesonen, M., Escher, B.I., Ackermann, G.E., Aerni, H.-R., Suter, M.J.-F., Eggen, R.I.L., 2004. Comparative analysis of estrogenic activity in sewage treatment plant effluents involving three in vitro assays and chemical analysis of steroids. Environ. Toxicol. Chem. 23, 857–864. https://doi.org/10.1897/03-286

Schaffer, M., Boxberger, N., Börnick, H., Licha, T., Worch, E., 2012. Sorption influenced transport of ionizable pharmaceuticals onto a natural sandy aquifer sediment at different pH. Chemosphere 87, 513–520. https://doi.org/https://doi.org/10.1016/j.chemosphere.2011.12.053

Schmidt, W., O'Rourke, K., Hernan, R., Quinn, B., 2011. Effects of the pharmaceuticals gemfibrozil and diclofenac on the marine mussel (Mytilus spp.) and their comparison with standardized toxicity tests. Mar. Pollut. Bull. 62, 1389–1395.

https://doi.org/10.1016/j.marpolbul.2011.04.043

Scholz, S., Klüver, N., 2009. Effects of endocrine disrupters on sexual, gonadal development in fish. Sex. Dev. 3, 136–151. https://doi.org/10.1159/000223078

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014a. Identifying small molecules via high resolution mass spectrometry: Communicating confidence. Environ. Sci. Technol. 48, 2097–2098. https://doi.org/10.1021/es5002105

Schymanski, E.L., Singer, H.P., Longrée, P., Loos, M., Ruff, M., Stravs, M.A., Ripollés Vidal, C., Hollender, J., 2014b. Strategies to characterize polar organic contamination in wastewater: Exploring the capability of high resolution mass spectrometry. Environ. Sci. Technol. 48, 1811–1818. https://doi.org/10.1021/es4044374

Segura, P.A., Takada, H., Correa, J.A., El Saadi, K., Koike, T., Onwona-Agyeman, S., Ofosu-Anim, J., Sabi, E.B., Wasonga, O. V., Mghalu, J.M., dos Santos, A.M., Newman, B., Weerts, S., Yargeau, V., 2015. Global occurrence of anti-infectives in contaminated surface waters: Impact of income inequality between countries. Environ. Int. 80, 89–97. https://doi.org/10.1016/j.envint.2015.04.001

Selvaraj, K.K., Shanmugam, G., Sampath, S., Joakim Larsson, D.G., Ramaswamy, B.R., 2014. GC-MS determination of bisphenol A and alkylphenol ethoxylates in river water from India and their ecotoxicological risk assessment. Ecotoxicol. Environ. Saf. 99, 13–20. https://doi.org/10.1016/j.ecoenv.2013.09.006

Shore, L.S., Reichmann, O., Shemesh, M., Wenzel, A., Litaor, M.I., 2004. Washout of accumulated testosterone in a watershed. Sci. Total Environ. 332, 193–202. https://doi.org/10.1016/j.scitotenv.2004.04.009

Shraim, A., Diab, A., Alsuhaimi, A., Niazy, E., Metwally, M., Amad, M., Sioud, S., Dawoud, A., 2017. Analysis of some pharmaceuticals in municipal wastewater of Almadinah Almunawarah. Arab. J. Chem. 10, S719–S729. https://doi.org/10.1016/j.arabjc.2012.11.014

Sim, W., Lee, J.-W., Lee, E.-S., Shin, S.-K., Hwang, S.-R., Oh, J.-E., 2011. Occurrence and distribution of pharmaceuticals in wastewater from households , livestock farms , hospitals and pharmaceutical manufactures. Chemosphere 82, 179–186. https://doi.org/10.1016/j.chemosphere.2010.10.026

Singer, H.P., Wössner, A.E., McArdell, C.S., Fenner, K., 2016. Rapid Screening for Exposure to “non-Target” Pharmaceuticals from Wastewater Effluents by Combining HRMS-Based Suspect Screening and Exposure Modeling. Environ. Sci. Technol. 50, 6698–6707. https://doi.org/10.1021/acs.est.5b03332

Sjerps, R.M.A., Vughs, D., Leerdam, J.A. Van, Thomas, L., Wezel, A.P. Van, 2016. Data-driven

prioritization of chemicals for various water types using suspect screening LC-HRMS. Water Res. 93, e34–e34. https://doi.org/10.1016/j.watres.2016.02.034

Soares, A., Guieysse, B., Jefferson, B., Cartmell, E., Lester, J.N., 2008. Nonylphenol in the environment: A critical review on occurrence, fate, toxicity and treatment in wastewaters. Environ. Int. 34, 1033–1049. https://doi.org/https://doi.org/10.1016/j.envint.2008.01.004

Sodré, F.F., Pescara, I.C., Montagner, C.C., Jardim, W.F., 2010. Assessing selected estrogens and xenoestrogens in Brazilian surface waters by liquid chromatography–tandem mass spectrometry. Microchem. J. 96, 92–98. https://doi.org/https://doi.org/10.1016/j.microc.2010.02.012

Solé, M., De Alda, M.J.L., Castillo, M., Porte, C., Ladegaard-Pedersen, K., Barceló, D., 2000. Estrogenicity determination in sewage treatment plants and surface waters from the catalonian area (NE Spain). Environ. Sci. Technol. 34, 5076–5083. https://doi.org/10.1021/es991335n

Song, M., Xu, Y., Jiang, Q., Lam, P.K.S., O'Toole, D.K., Giesy, J.P., Jiang, G., 2006. Measurement of estrogenic activity in sediments from Haihe and Dagu River, China. Environ. Int. 32, 676–681. https://doi.org/https://doi.org/10.1016/j.envint.2006.03.002

Souverain, S., Rudaz, S., Veuthey, J.L., 2004. Restricted access materials and large particle supports for on-line sample preparation: An attractive approach for biological fluids analysis. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 801, 141–156. https://doi.org/10.1016/j.jchromb.2003.11.043

Staples, C.A., Dorn, P.B., Klecka, G.M., O'Block, S.T., Harris, L.R., 1998. A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere 36, 2149–2173. https://doi.org/10.1016/S0045-6535(97)10133-3

Stasinakis, A.S., Gatidou, G., Mamais, D., Thomaidis, N.S., Lekkas, T.D., 2008. Occurrence and fate of endocrine disrupters in Greek sewage treatment plants 42, 1796–1804. https://doi.org/10.1016/j.watres.2007.11.003

Stewart, M., Olsen, G., Hickey, C.W., Ferreira, B., Jelić, A., Petrović, M., Barcelo, D., 2014. A survey of emerging contaminants in the estuarine receiving environment around Auckland, New Zealand. Sci. Total Environ. 468–469, 202–210. https://doi.org/https://doi.org/10.1016/j.scitotenv.2013.08.039

Sun, Q., Lv, M., Hu, A., Yang, X., Yu, C.P., 2014. Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in a wastewater treatment plant in Xiamen, China. J. Hazard. Mater. 277, 69–75. https://doi.org/10.1016/j.jhazmat.2013.11.056

Swartz, C.H., Reddy, S., Benotti, M.J., Yin, H., Barber, L.B., Brownawell, B.J., Rudel, R.A., 2006.

Steroid Estrogens, Nonylphenol Ethoxylate Metabolites, and Other Wastewater Contaminants in Groundwater Affected by a Residential Septic System on Cape Cod, MA. Environ. Sci. Technol. 40, 4894–4902. https://doi.org/10.1021/es052595+

Tahar, A., Tiedeken, E.J., Clifford, E., Cummins, E., Rowan, N., 2017. Development of a semi-quantitative risk assessment model for evaluating environmental threat posed by the three first EU watch-list pharmaceuticals to urban wastewater treatment plants: An Irish case study. Sci. Total Environ. 603–604, 627–638. https://doi.org/10.1016/j.scitotenv.2017.05.227

Talmage, S., 1994. Environmental and human safety of major surfactants: alcohol ethoxylates and alkylphenol ethoxylates. A Rep. to Soap Deterg. Assoc. Boca Rat. Lewis Publ.

Terzić, S., Senta, I., Ahel, M., Gros, M., Petrović, M., Barcelo, D., Müller, J., Knepper, T., Martí, I., Ventura, F., Jovančić, P., Jabučar, D., 2008. Occurrence and fate of emerging wastewater contaminants in Western Balkan Region. Sci. Total Environ. 399, 66–77. https://doi.org/https://doi.org/10.1016/j.scitotenv.2008.03.003

The Statistical Office of the Republic of Serbia, 2020. http://www. stat.gov.rs/en-US; Accessed 1 June 2020;

Tian, L., Verreault, J., Houde, M., Bayen, S., 2019. Suspect screening of plastic-related chemicals in northern pike (Esox lucius) from the St. Lawrence River, Canada. Environ. Pollut. 255. https://doi.org/10.1016/j.envpol.2019.113223

Tijani, J.O., Fatoba, O.O., Babajide, O.O., Petrik, L.F., 2016. Pharmaceuticals, endocrine disruptors, personal care products, nanomaterials and perfluorinated pollutants: a review. Environ. Chem. Lett. 14, 27–49. https://doi.org/10.1007/s10311-015-0537-z

Togola, A., Budzinski, H., 2008. Multi-residue analysis of pharmaceutical compounds in aqueous samples ☪ 1177, 150–158. https://doi.org/10.1016/j.chroma.2007.10.105

Tomšíková, H., Aufartová, J., Solich, P., Nováková, L., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2012. High-sensitivity analysis of female-steroid hormones in environmental samples. TrAC - Trends Anal. Chem. 34, 35–58. https://doi.org/10.1016/j.trac.2011.11.008

Tran, N.H., Reinhard, M., Gin, K.Y.H., 2018. Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. Water Res. 133, 182–207. https://doi.org/10.1016/j.watres.2017.12.029

Trufelli, H., Palma, P., Famiglini, G., Cappiello, A., 2011. An overview of matrix effects in liquid chromatography–mass spectrometry. Mass Spectrom. Rev. 30, 491–509. https://doi.org/10.1002/mas.20298

Van den Belt, K., Verheyen, R., Witters, H., 2003. Comparison of vitellogenin responses in

zebrafish and rainbow trout following exposure to environmental estrogens. Ecotoxicol. Environ. Saf. 56, 271–281. https://doi.org/https://doi.org/10.1016/S0147-6513(03)00004-6

Varela, A.R., Nunes, O.C., Manaia, C.M., 2016. Quinolone resistant Aeromonas spp. as carriers and potential tracers of acquired antibiotic resistance in hospital and municipal wastewater. Sci. Total Environ. 542, 665–671. https://doi.org/10.1016/j.scitotenv.2015.10.124

Vazquez-Roig, P., Andreu, V., Blasco, C., Picó, Y., 2012. Risk assessment on the presence of pharmaceuticals in sediments, soils and waters of the Pego-Oliva Marshlands (Valencia, eastern Spain). Sci. Total Environ. 440, 24–32. https://doi.org/10.1016/j.scitotenv.2012.08.036

Vega-Morales, T., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2012. Development and optimisation of an on-line solid phase extraction coupled to ultra-high-performance liquid chromatography-tandem mass spectrometry methodology for the simultaneous determination of endocrine disrupting compounds in wastewater samples. J. Chromatogr. A 1230, 66–76. https://doi.org/10.1016/j.chroma.2012.01.077

Vergeynst, L., Langenhove, H. Van, Joos, P., Demeestere, K., 2014. Suspect screening and target quantification of multi-class pharmaceuticals in surface water based on large-volume injection liquid chromatography and time-of-flight mass spectrometry i, 2533–2547. https://doi.org/10.1007/s00216-014-7672-4

Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. Sci. Total Environ. 429, 123–155. https://doi.org/https://doi.org/10.1016/j.scitotenv.2012.04.028

Vidal-Dorsch, D.E., Bay, S.M., Maruya, K., Snyder, S.A., Trenholm, R.A., Vanderford, B.J., 2012. Contaminants of emerging concern in municipal wastewater effluents and marine receiving water. Environ. Toxicol. Chem. 31, 2674–2682. https://doi.org/10.1002/etc.2004

Viganó, L., Mandich, A., Benfenati, E., Bertolotti, R., Bottero, S., Porazzi, E., Agradi, E., 2006. Investigating the Estrogenic Risk Along the River Po and Its Intermediate Section. Arch. Environ. Contam. Toxicol. 51, 641–651. https://doi.org/10.1007/s00244-005-0129-1

Viglino, L., Aboulfadl, K., 2008. Talanta Analysis of natural and synthetic estrogenic endocrine disruptors in environmental waters using online preconcentration coupled with LC-APPI-MS / MS 76, 1088–1096.

https://doi.org/10.1016/j.talanta.2008.05.008

vom Saal, F.S., Hughes, C., 2005. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. Environ. Health Perspect. 113, 926–933. https://doi.org/10.1289/ehp.7713

Vryzas, Z., Vassiliou, G., Alexoudis, C., Papadopoulou-Mourkidou, E., 2009. Spatial and temporal distribution of pesticide residues in surface waters in northeastern Greece. Water Res. 43, 1–10. https://doi.org/10.1016/j.watres.2008.09.021

Vulliet, E., Wiest, L., Baudot, R., 2008. Multi-residue analysis of steroids at sub-ng / L levels in surface and ground-waters using liquid chromatography coupled to tandem mass spectrometry 1210, 84–91. https://doi.org/10.1016/j.chroma.2008.09.034

Wang, J., Zhu, Y., 2017. Occurrence and risk assessment of estrogenic compounds in the East Lake, China. Environ. Toxicol. Pharmacol. 52, 69–76. https://doi.org/10.1016/j.etap.2017.03.018

Wang, L., Ying, G.-G., Zhao, J.-L., Yang, X.-B., Chen, F., Tao, R., Liu, S., Zhou, L.-J., 2010. Occurrence and risk assessment of acidic pharmaceuticals in the Yellow River, Hai River and Liao River of north China. Sci. Total Environ. 408, 3139–3147. https://doi.org/https://doi.org/10.1016/j.scitotenv.2010.04.047

Wang, L., Ying, G.G., Chen, F., Zhang, L.J., Zhao, J.L., Lai, H.J., Chen, Z.F., Tao, R., 2012. Monitoring of selected estrogenic compounds and estrogenic activity in surface water and sediment of the Yellow River in China using combined chemical and biological tools. Environ. Pollut. 165, 241–249. https://doi.org/10.1016/j.envpol.2011.10.005

Wang, S., Huang, W., Fang, G., He, J., Zhang, Y., 2008. On-line coupling of solid-phase extraction to high-performance liquid chromatography for determination of estrogens in environment. Anal. Chim. Acta 606, 194–201. https://doi.org/10.1016/j.aca.2007.11.030

Weatherly, L.M., Gosse, J.A., 2017. Triclosan exposure, transformation, and human health effects. J. Toxicol. Environ. Heal. Part B 20, 447–469. https://doi.org/10.1080/10937404.2017.1399306

Weigel, S., Berger, U., Jensen, E., Kallenborn, R., Thoresen, H., Heinrich, H., 2004. Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromsø / Norway with emphasis on ibuprofen and its metabolites 56, 583–592. https://doi.org/10.1016/j.chemosphere.2004.04.015

Weigel, S., Kuhlmann, J., Hühnerfuss, H., 2002. Drugs and personal care products as ubiquitous pollutants: Occurrence and distribution of clofibric acid, caffeine and DEET in the North Sea. Sci. Total Environ. 295, 131–141. https://doi.org/10.1016/S0048-9697(02)00064-5

Wenzel, A., Böhmer, W., Müller, J., Rüdel, H., Schröter-Kermani, C., 2004. Retrosperctive Monitoring of Alkylphenols and Alkylphenol Monothoxylates in Aquatic Biota from 1985 to 2001: Results from the German Environmental Specimen Bank. Environ. Sci. Technol. 38, 1654–1661. https://doi.org/10.1021/es035032b

WHO, 2011. The world medicines situation 2011. Medicines Prices, Availability and Affordability, The World Medicines Situation.

WHO/UNEP, 2013. WHO (WorldHealth Organization)/UNEP (United Nations Environment Programme) (2013) State of the Science of Endocrine Disrupting Chemicals–2012, Geneva. http://www.who.int/cehpublications/endocrine/en/index.html. Accessed 30 March 2020.

Wilkinson, J., Hooda, P.S., Barker, J., Barton, S., Swinden, J., 2017. Occurrence, fate and transformation of emerging contaminants in water: An overarching review of the field. Environ. Pollut. 231, 954–970. https://doi.org/10.1016/j.envpol.2017.08.032

Wilkinson, J.L., Hooda, P.S., Swinden, J., Barker, J., Barton, S., 2018. Spatial (bio)accumulation of pharmaceuticals, illicit drugs, plasticisers, perfluorinated compounds and metabolites in river sediment, aquatic plants and benthic organisms. Environ. Pollut. 234, 864–875. https://doi.org/https://doi.org/10.1016/j.envpol.2017.11.090

Wille, K., Noppe, H., Verheyden, K., Bussche, J. Vanden, Wulf, E. De, Caeter, P. Van, 2010. Validation and application of an LC-MS / MS method for the simultaneous quantification of 13 pharmaceuticals in seawater 1797–1808. https://doi.org/10.1007/s00216-010-3702-z

Williams, M., Kookana, R.S., Mehta, A., Yadav, S.K., Tailor, B.L., Maheshwari, B., 2019. Emerging contaminants in a river receiving untreated wastewater from an Indian urban centre. Sci. Total Environ. 647, 1256–1265. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.08.084

Wood, P.T., Christiaan, C. Du, Steenkamp, A., Duvenage, C., Rohwer, E.R., 2017. Database-driven screening of South African surface water and the targeted detection of pharmaceuticals using liquid chromatography - High resolution mass spectrometry *. Environ. Pollut. 230, 453–462. https://doi.org/10.1016/j.envpol.2017.06.043

Wu, Q., Lam, J.C.W., Kwok, K.Y., Tsui, M.M.P., Lam, P.K.S., 2017. Occurrence and fate of endogenous steroid hormones, alkylphenol ethoxylates, bisphenol A and phthalates in municipal sewage treatment systems. J. Environ. Sci. 61, 49–58. https://doi.org/10.1016/j.jes.2017.02.021

www.uba.de/db-pharm, 2019. Database “Pharmaceuticals in the Environment” [WWW Document]. Ger. Environ. Agency (Umweltbundesamt – UBA); Acessed 15 May 2020.

Yang, J.-F., Ying, G.-G., Zhao, J.-L., Tao, R., Su, H.-C., Chen, F., 2010. Simultaneous determination of four classes of antibiotics in sediments of the Pearl Rivers using RRLC–MS/MS. Sci. Total Environ. 408, 3424–3432. https://doi.org/https://doi.org/10.1016/j.scitotenv.2010.03.049

Yang, L.H., Ying, G.G., Su, H.C., Stauber, J.L., Adams, M.S., Binet, M.T., 2008. Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga Pseudokirchneriella subcapitata. Environ. Toxicol. Chem. 27, 1201–1208. https://doi.org/10.1897/07-471.1

Yao, B., Li, R., Yan, S., Chan, S.A., Song, W., 2018. Occurrence and estrogenic activity of steroid hormones in Chinese streams: A nationwide study based on a combination of chemical and biological tools. Environ. Int. 118, 1–8. https://doi.org/10.1016/j.envint.2018.05.026

Yarahmadi, H., Duy, S.V., Hachad, M., Dorner, S., Sauvé, S., Prévost, M., 2018. Seasonal variations of steroid hormones released by wastewater treatment plants to river water and sediments: Distribution between particulate and dissolved phases. Sci. Total Environ. 635, 144–155. https://doi.org/10.1016/j.scitotenv.2018.03.370

Yien Fang, T., Praveena, S.M., Aris, A.Z., Syed Ismail, S.N., Rasdi, I., 2019. Quantification of selected steroid hormones (17B-Estradiol and 17A-Ethynylestradiol) in wastewater treatment plants in Klang Valley (Malaysia). Chemosphere 215, 153–162. https://doi.org/10.1016/j.chemosphere.2018.10.032

Ying, G.G., Williams, B., Kookana, R., 2002. Environmental fate of alkylphenols and alkylphenol ethoxylates - A review. Environ. Int. 28, 215–226. https://doi.org/10.1016/S0160-4120(02)00017-X

Yu, J.T., Bouwer, E.J., Coelhan, M., 2006. Occurrence and biodegradability studies of selected pharmaceuticals and personal care products in sewage effluent. Agric. Water Manag. 86, 72–80. https://doi.org/10.1016/j.agwat.2006.06.015

Yu, W., Du, B., Yang, L., Zhang, Z., Yang, C., Yuan, S., Zhang, M., 2019. Occurrence, sorption, and transformation of free and conjugated natural steroid estrogens in the environment. Environ. Sci. Pollut. Res. 26, 9443–9468. https://doi.org/10.1007/s11356-019-04402-z

Zaccaroni, M., Seta, D. Della, Farabollini, F., Fusani, L., Dessì-Fulgheri, F., 2016. Developmental Exposure to Very Low Levels of Ethynilestradiol Affects Anxiety in a Novelty Place Preference Test of Juvenile Rats. Neurotox. Res. 30, 553–562. https://doi.org/10.1007/s12640-016-9645-1

Zeng, X., Mai, B., Sheng, G., Luo, X., Shao, W., An, T., Fu, J., 2008. Distribution of polycyclic musk in surface sediments from the Pearl River Delta and Macao coastal region,

South China. Environ. Toxicol. Chem. 27, 18–23.

Zenker, A., Cicero, M.R., Prestinaci, F., Bottoni, P., Carere, M., 2014. Bioaccumulation and biomagnification potential of pharmaceuticals with a focus to the aquatic environment. J. Environ. Manage. 133, 378–387. https://doi.org/10.1016/j.jenvman.2013.12.017

Zha, J., Sun, L., Zhou, Y., Spear, P.A., Ma, M., Wang, Z., 2008. Assessment of 17 α - ethinylestradiol effects and underlying mechanisms in a continuous , multigeneration exposure of the Chinese rare minnow (Gobiocypris rarus) ☆ 226, 298–308. https://doi.org/10.1016/j.taap.2007.10.006

Zhang, H., Lee, H.K., 2012. Simultaneous determination of ultraviolet filters in aqueous samples by plunger-in-needle solid-phase microextraction with graphene-based sol-gel coating as sorbent coupled with gas chromatography-mass spectrometry. Anal. Chim. Acta 742, 67–73. https://doi.org/10.1016/j.aca.2012.03.016

Zhang, M., Mao, Q., Feng, J., Yuan, S., Wang, Q., Huang, D., Zhang, J., 2016. Validation and application of an analytical method for the determination of selected acidic pharmaceuticals and estrogenic hormones in wastewater and sludge. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 51, 914–920. https://doi.org/10.1080/10934529.2016.1191304

Zhang, X., Li, Q., Li, G., Wang, Z., Yan, C., 2009. Levels of estrogenic compounds in Xiamen Bay sediment , China. Mar. Pollut. Bull. 58, 1210–1216. https://doi.org/10.1016/j.marpolbul.2009.03.011

Zhang, Z., Feng, Y., Gao, P., Wang, C., Ren, N., 2011. Occurrence and removal efficiencies of eight EDCs and estrogenicity in a STP. J. Environ. Monit. 13, 1366–1373. https://doi.org/10.1039/c0em00597e

Zhao, J.L., Ying, G.G., Wang, L., Yang, J.F., Yang, X.B., Yang, L.H., Li, X., 2009. Determination of phenolic endocrine disrupting chemicals and acidic pharmaceuticals in surface water of the Pearl Rivers in South China by gas chromatography-negative chemical ionization-mass spectrometry. Sci. Total Environ. 407, 962–974. https://doi.org/10.1016/j.scitotenv.2008.09.048

Zhou, J.L., Zhang, Z.L., Banks, E., Grover, D., Jiang, J.Q., 2009. Pharmaceutical residues in wastewater treatment works effluents and their impact on receiving river water. J. Hazard. Mater. 166, 655–661. https://doi.org/10.1016/j.jhazmat.2008.11.070

Zillien, C., van Loon, C., Gülpen, M., Tipatet, K., Hanssen, B., Beeltje, H., Roex, E., Oldenkamp, R., Posthuma, L., Ragas, A.M.J., 2019. Risk-management tool for environmental prioritization of pharmaceuticals based on emissions from hospitals. Sci. Total Environ. 694, 133733. https://doi.org/10.1016/j.scitotenv.2019.133733

Zou, S., Xu, W., Zhang, R., Tang, J., Chen, Y., Zhang, G., 2011. Occurrence and distribution of antibiotics in coastal water of the Bohai Bay, China: Impacts of river discharge and aquaculture activities. Environ. Pollut. 159, 2913–2920. https://doi.org/10.1016/j.envpol.2011.04.037

Annex

Supplementary material of Chapter 1

Article Nº1:

Mira Čelić, Sara Insa, Biljana Škrbić, and Mira Petrović

Development of a sensitive and robust online dual column liquid chromatography-tandem mass spectrometry method for the analysis of natural and synthetic estrogens and their conjugates in river water and wastewater

Analytical Bioanalytical Chemistry *409 (2017) 5427–5440.*

Supplementary Material has been reformed to match the style of the thesis.

Development of a sensitive and robust on-line dual column liquid chromatography – tandem mass spectrometry method for the analysis of natural and synthetic estrogens and their conjugates in river water and wastewater

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This file is 14 pages and contains 5 figures and 3 tables.

Fig. S1 Influence of the methanol percentage in the eluent mixture dispended by pump 1 during the "loading phase" obtained using Hypersil GOLD™aQ (20 x 2.1 mm, 12µm) EQuan column. Mean chromatographic areas (n=3) of target estrogens are shown as a function of the methanol percentage in the eluent mixture.

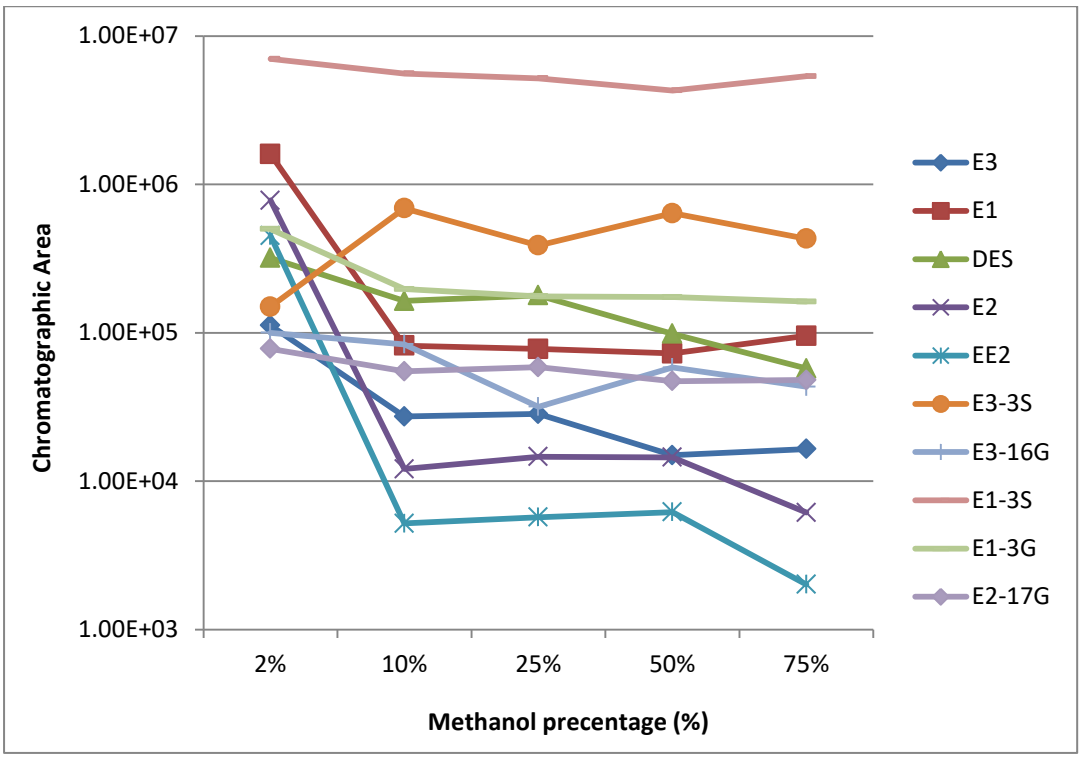


Fig. S2 Effect of loading speed (1000-3000 µl/min) for spiked free and conjugated estrogens (mean ± SD, n=3, 50 ng/L) peak areas from sample loop (2 mL) to on-line SPE column in analyte-free matrix.

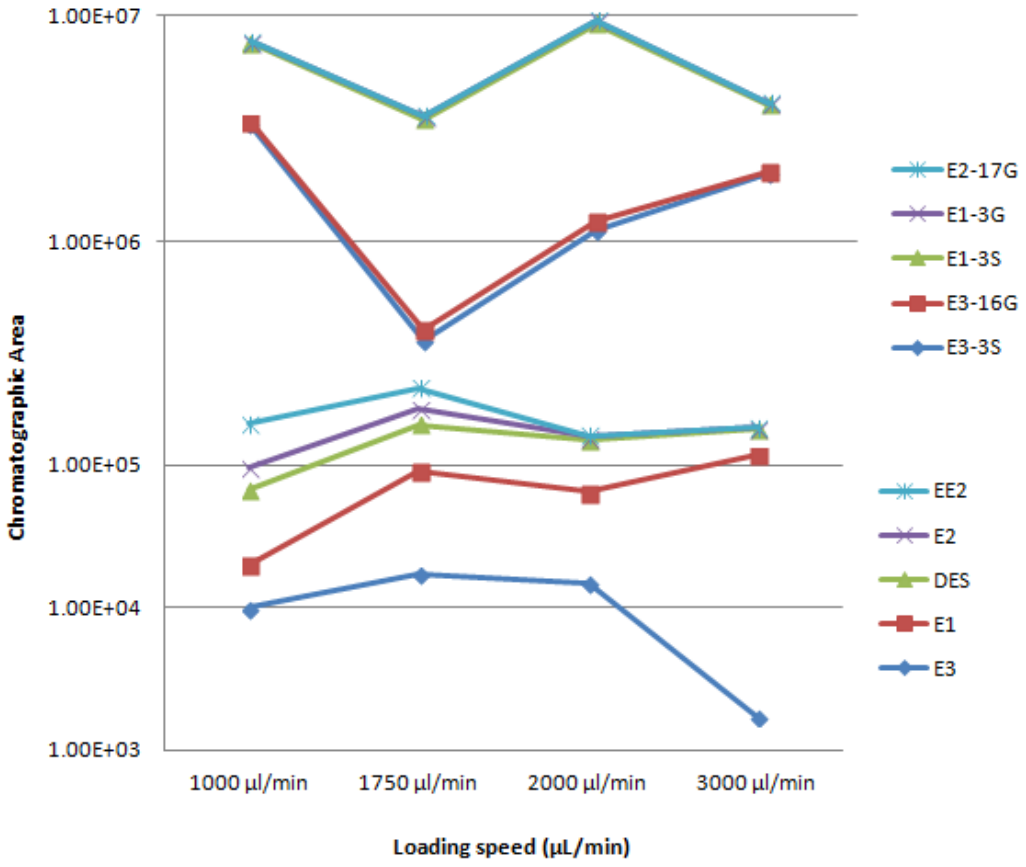


Fig. S3 The response changes of the analyzed compounds under the different elution times.

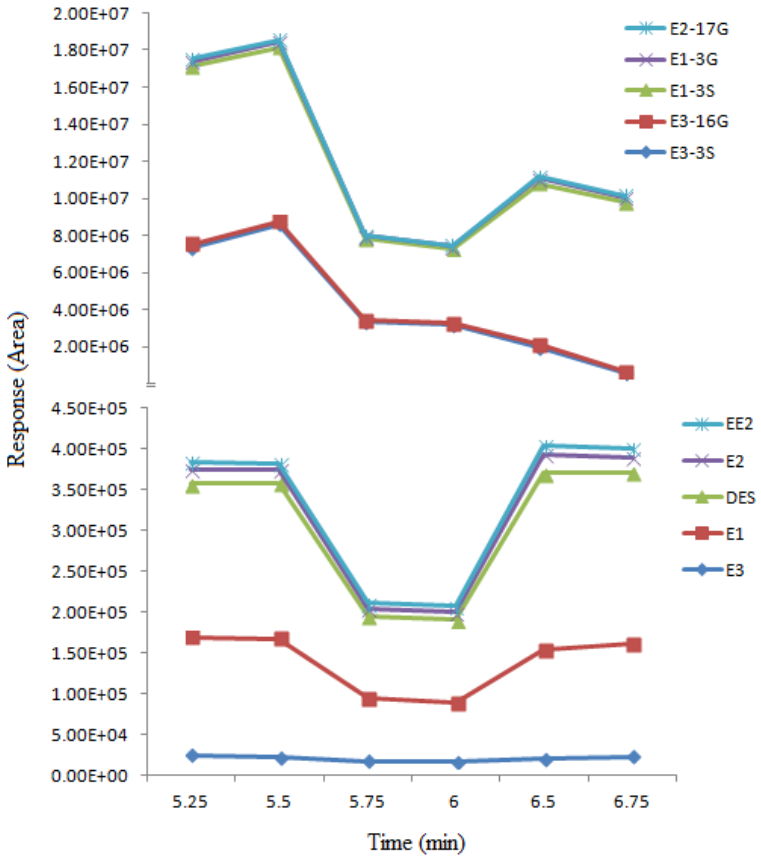


Fig. S4 Relative recovery values (mean ± SD) evaluated for the on-line SPE method. The mean peak areas (n=3, 100 ng L^{-1}) of the selected steroid hormones of a direct chromatographic injection (20 µL, off-line injection) were compared with those of the on-line high volume injection (5 mL) used for sample analysis.

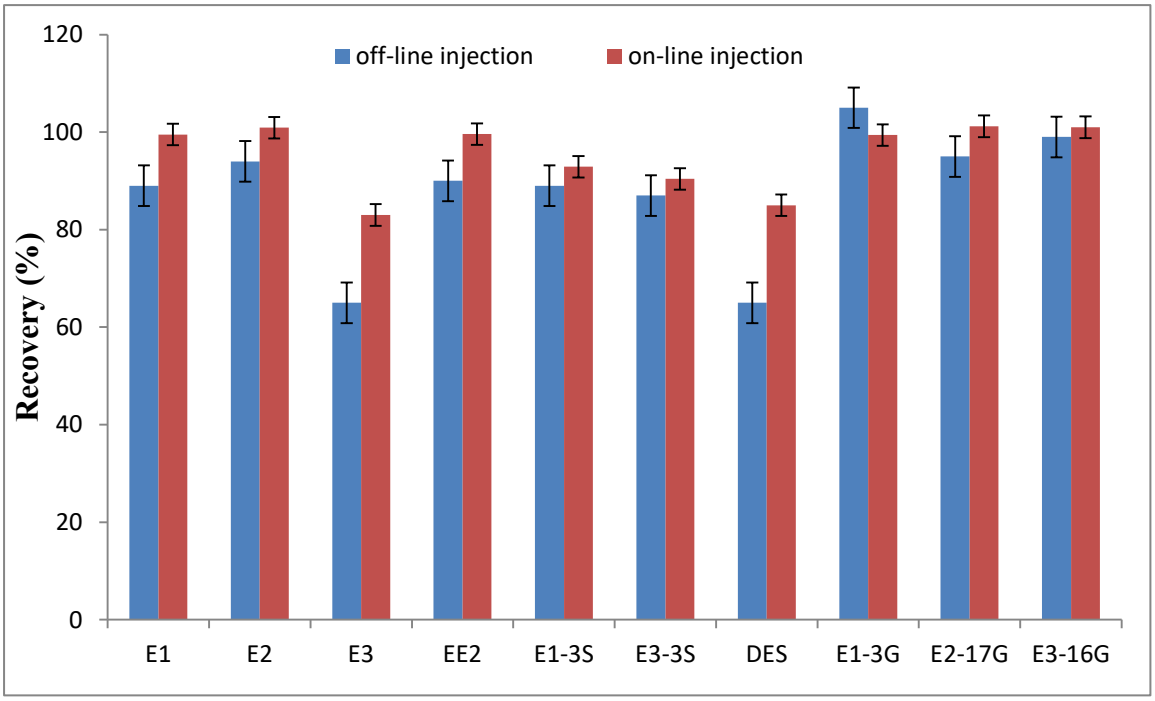


Fig. S5 SRM chromatograms (first transition) corresponding to the on-line SPE-LC-ESI-MS-MS analysis of 5ml of surface water spiked at 5ng/L with mix of target estrogens and conjugated forms.

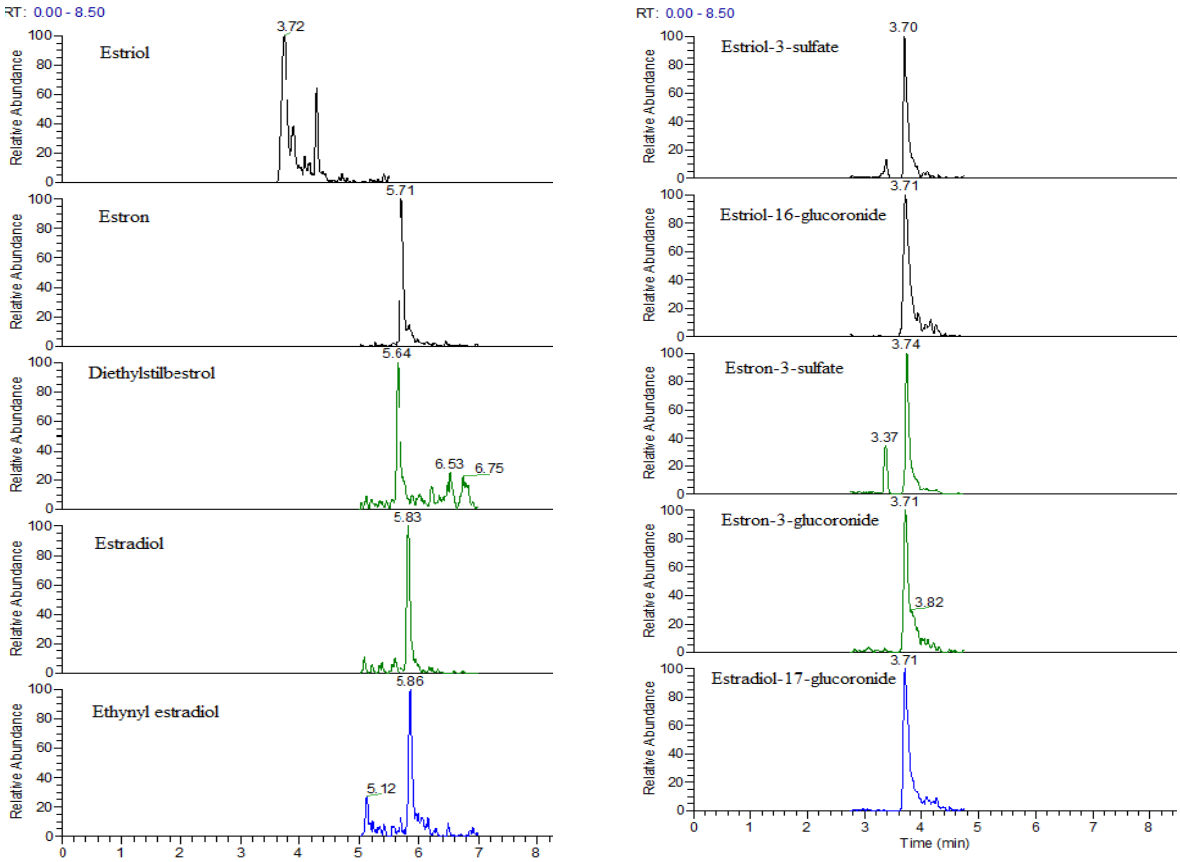
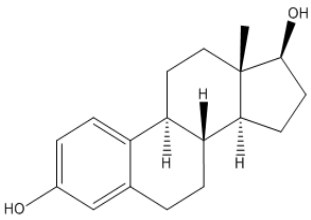
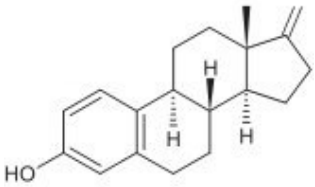
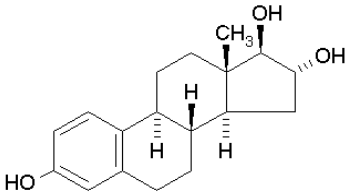
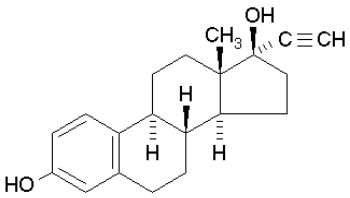
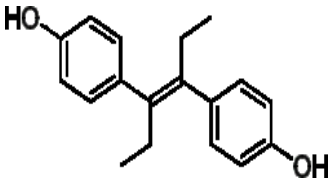
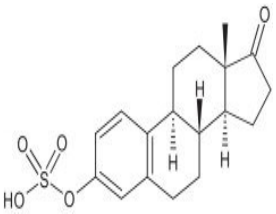
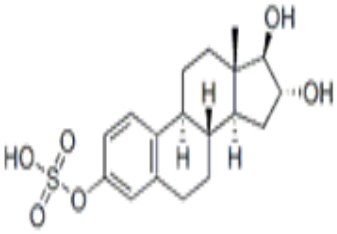
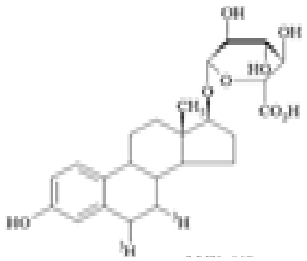
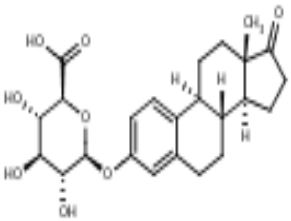
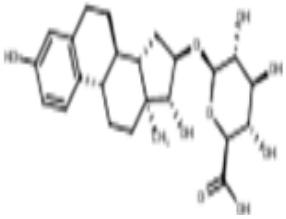


Table S1. Abbreviation, CAS number, chemical structures, K_{OW} (logP) and pKa values.

Abbreviation	CAS number	Chemical structure	K_{OW} (log P)[a]	pKa[b]
E2	50-28-2		4.146	10.27
E1	53-16-7		3.624	10.25
E3	50-27-1		2.527	10.25
EE2	57-63-6		4.106	10.24
DES	56-53-1		5.33	10.18

(continued next page)

Table S1. continued

Abbreviation	CAS number	Chemical structure	K_{OW} (log P)[a]	pKa[b]
E1-3S	438-67-5		2.81	-3.84
E3-3S	481-95-8		1.713	-3.82
E2-17G	1806-98-0		3.807	2.82
E1-3G	2479-90-5		1.144	2.8
E3-16G	1852-50-2		1.22	3.46

[a] The data was collected from the SRC PhysProp database. http://www.syrres.com [1]

[b] The pK_a values for compounds are experimental and taken from the literature [2]

Table S2. Description of sampling sites (code, river, location, description site and coordinates of sampling site).

	Code	River	Location	Description site	Coordinates of sampling site
Tisa basin	S1	Tisa	Kanjiza-N.Knezevac	Upper course of Tisa	46º02'42.3'' N 20º05'06.6''E
	S2	Tisa	Senta-Coka	Upstream of food industry from Senta town	45º56'05.4'' N 20º05'34.5''E
	S3	Tisa	Zabalj-Zrenjanin	Middle stretch of Tisa. Downstream of industry	45º23'51.8'' N 20º12'42.0''E
	S4	Tisa	Titel	Before the confluence with Danube River	45º11'51.7'' N 20º18'47.5''E
Danube basin	S5	Danube	Backa Palanka	Upper course of Danube River	45º14'0.21'' N 19º22'55.3''E
	S6	Danube	Celarevo	Upstream of Novi Sad city	45º14'17.7'' N 19º33'42.0''E
	S7	Danube	Novi Sad	Upstream of discharger	45º14'12.2'' N 19º50'57.7''E
	S8	Danube	Novi Sad	First major city in the mainstream	45º15'43.1'' N 19º51'26.9''E
	S9	Danube	Novi Sad	Industrial area, near refinery oil	45º15'53.4'' N 19º52'44.4''E
	S10	Danube	Sremski Karlovci	Downstream of Novi Sad city	45°12'23.8"N 19°56'25.4"E
	S11	Danube	Zemun	Before the confluence with Sava river and downstream from pharmaceutical industry	44°51'54.6"N 20°22'52.8"E
	S12	Danube	Beograd	In Belgrade city	44°49'41.0"N 20°29'29.6"E
Sava basin	S13	Sava	Beograd	On the confluence with Danube River	44°48'09.9"N 20°26'28.1"E
	S14	Sava	Sabac	Upstream the confluence with Danube River.	44°45'29.9"N 19°42'53.8"E

	S15	Sava	Sremska Mitrovica	Downstream of inlet of municipal wastewater	44°57'33.7"N 19°37'53.7"E

River basin/ Code		Town/city	Description site	Coordinates of sampling site
Tisa basin	WW1	Kanjiza	The town has a population of 9.871, the Potisje-Tondach roof tile factory. Other industries are FIM Kanjiža, Keramika Kanjiža, various paprika refining firms	46.0616°N 20.0470°E
	WW2	Senta	It is situated on the bank of the Tisatributary, the town has a population of 18.704	45.9260°N 20.0781°E
	WW3	Zrenjanin	The city's population is 76.511, the third largest city in Vojvodina	45.3816°N 20.3686°E
	WW4	Titel	The town of Titel has a population of 5.247	45.2164°N 20.2832°E
Danube basin	WW5	Backa Palanka	It is situated on the left bank of the Danube, 55.528	45.2497°N 19.3968°E
	WW6	Celarevo	its population numbers 5.423 people, beer industry	45.2821°N 19.5712°E
	WW7	Novi Sad	Is the second largest city of Serbia, the capital of the autonomous province of Vojvodina, on the banks of the Danube river, the city has a population of 250.439, the most fertile agricultural region in Serbia	45.2671°N 19.8335°E
	WW8	Novi Sad		45.2511°N 19.8549°E
	WW9	Novi Sad		45.2622°N 19.8560°E
	WW10	Sremski Karlovci	It is situated on the bank of the river Danube, 8 km from Novi Sad, it has a population of 8.750 inhabitants	45.1810° N 19.9443°E
	WW11	Zemun	Zemun has two large and still growing industrial zones, heavy agricultural machines and appliances pharmaceuticals, plastics, food, candies, and chocolate industry	44.8532° N, 20.3556°E
	WW12	Beograd	The capital and largest city of Serbia 1.34 million of inhabitants	44.7866°N 20.4489°E
a bas	WW13	Beograd	It is located at the confluence of the Sava and Danube rivers	44.8192°N 20.4438°E

	WW14	Sabac	It is situated along the Sava river, the city has a population of 53.919	44.7489° N 19.6908°E
	WW15	Sremska Mitrovica	It is situated on the left bank of the Sava river, 37.751 of inhabitants	44.9795° N 19.6210°E

Table S3. The LC-Conditions for the off-line analysis.

Pump 2:	Elute Pump			
	Injection volume: 20 µL (surface river water, effluent and influent water)			
	Analytical column:	50mm x 2.1 mm i.d., 1.7 µm C18 reversed-phase column, Kinetex C18 100A,		
	Solvent A:	Methanol		
	Solvent B:	Water		
Start time (min)	Flow (mL/min)	Gradient	A%	B%
0:00	0.4	Step	10	90
0:75	0.4	Step	10	90
2:50	0.4	Ramp	-	100
4:00	0.4	Ramp	-	100
5:00	0.4	Ramp	10	90
6:00	0.4	Ramp	10	90

NI, negative ionization

Table S4a. Concentrations levels of estrogens in river water samples (ng/L) taken from river basins of the Province of Vojvodina

Sample code	E2	E1	E3	EE2	DES	E1-3S	E3-3S	E2-17G	E1-3G	E3-16G
S1	nd	nd	64.8	nd	nd	0.93	4.46	17.9	4.16	35.1
S2	nd	nd	nd	nd	nd	nd	2.96	22.4	4.42	45.9
S3	nd	nd	75.2	nd	nd	0.22	2.78	23.3	4.21	52.1
S4	nd	nd	57.9	nd	nd	nd	2.18	21.7	nd	nd
S5	nd	nd	45.9	nd	nd	nd	1.82	15.9	3.20	nd
S6	nd	nd	43.8	nd	nd	0.27	2.26	19.0	5.09	nd
S7	nd	nd	58.3	nd	nd	9.98	nd	14.7	5.66	nd
S8	nd	2.29	nd	nd	nd	0.39	7.79	nd	nd	nd
S9	nd	nd	32.2	nd	nd	nd	nd	9.02	nd	nd
S10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S11	nd	4.51	nd	nd	nd	nd	3.43	nd	1.89	nd
S12	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S13	nd	nd	nd	nd	nd	nd	0.83	nd	nd	nd
S14	nd	nd	47.6	nd	nd	nd	nd	9.38	1.64	20.4
S15	nd	nd	69.4	nd	nd	nd	2.40	21.6	3.86	41.4

Table S4b. Concentrations levels of estrogens in raw wastewater samples (ng/L) from cities and towns from Province of Vojvodina

Sample code	E2	E1	E3	EE2	DES	E1-3S	E3-3S	E2-17G	E1-3G	E3-16G
WW1	nd	16.2	nd	nd	nd	nd	nd	nd	nd	nd
WW2	nd	7.66	nd	nd	nd	nd	nd	nd	nd	nd
WW3	6.05	2.32	nd	nd	nd	nd	nd	28.4	nd	83.8
WW4	nd	1.55	nd	nd	nd	nd	nd	36.5	6.43	nd
WW5	8.72	2.75	nd	nd	nd	nd	nd	25.7	4.16	103
WW6	nd	10.3	350	nd	nd	6.18	44.0	65.6	6.57	112
WW7	21.4	9.63	413	nd	nd	6.22	34.1	nd	nd	304
WW8	7.54	6.33	372	nd	nd	5.78	34.2	nd	nd	319
WW9	7.16	10.9	371	nd	nd	4.64	36.3	nd	nd	341
WW10	nd	12.1	393	nd	nd	4.12	36.4	nd	nd	366
WW11	nd	10.0	345	nd	nd	4.66	35.7	nd	nd	398
WW12	nd	nd	nd	nd	nd	6.81	24.1	nd	nd	nd
WW13	nd	nd	nd	nd	nd	34.3	24.5	nd	96.8	nd
WW14	15.2	102	nd	nd	nd	16.0	27.7	nd	28.3	492
WW15	8.48	3.59	nd	nd	nd	nd	3.22	30.2	4.43	106

Article Nº2:

Mira Čelić, Biljana Škrbić, Sara Insa, Jelena Živančev, Meritxell Gros, and Mira Petrović

Occurrence and assessment of environmental and human health risks of endocrine disrupting compounds in drinking, surface and wastewaters in Serbia
***Environmental Pollution** 262 (2020) 114344.*

Supplementary Material has been reformed to match the style of the thesis.

Occurrence and assessment of environmental risks of endocrine disrupting compounds in drinking, surface, and wastewaters in Serbia

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2. Materials and Methods

2.1 Chemicals

All pure standards of the target compounds E2, E1, E3, EE2, DES, E1-3S, E3-3S, E2-17G, E1-3G, E3-16G, NP, OP and BPA were purchased from Sigma-Aldrich (Germany). Isotopically labeled compounds, used as internal standards (IS), E1-d_4, E2-d_2, EE2-d_4, E1-3S-d_4, E2-17G-$^{13}C_4$, NP-d_4, OP-d_4, and BPA-d_4 were obtained from CDN Isotopes Pointe-Claire (Quebec, Canada). Physiochemical properties of endocrine disrupting compounds (EDCs) analysed are shown in Table S1.

Individual stock standard and isotopically labeled internal standard solutions were prepared on a weight basis in methanol (at a concentration of 1000 mg L^{-1}). After preparation, standards were stored at −20ºC. A mixture containing all target compounds was prepared by appropriate dilution of individual stock solutions in HPLC grade water. A separate mixture of IS were also prepared in methanol and further diluted in HPLC grade water. Glass fiber filters (GFF) were purchased from Whatman (U.K.) and hydrophilic polyvinylidene fluoride (PVDF) membrane syringe filters were provided from Sigma-Aldrich (Germany). HPLC grade purity methanol and water were supplied by Fisher Scientific (Whitby, ON, Canada). Sodium hydroxide (NaOH) was obtained from Scharlab SL (Barcelona, Spain).

2.2 EDCs analysis

The optimized MS/MS parameters for SRM analysis of selected EDCs are displayed in Table S2. The EQuan™ system was used for online pre-concentration of the samples and chromatographic separation of sample components. On-line SPE pre-concentration was achieved using an Acquity BEH C18 column (50 mm x 2.1 mm i.d., 1.7 μm, Kinetex, Phenomenex), while for the chromatographic separation of selected EDCs an Hypersil GOLD aQ column (20 x 2.1 mm i.d., 12 μm column, Thermo Fisher Scientific) was used. The injection volume was set at 5 mL for drinking and surface water, while 2 mL was used for wastewater. Chromatographic separation was performed under gradient elution conditions using methanol and water as mobile phases. The duration of the whole analytical procedure was 10.5 min and all the steps were performed automatically. The instrument was controlled via Xcalibur 2.2 software and data collection was processed using TraceFinder 3.1 software (ThermoScientific).

2.3 Quality assurance and quality control

In order to prevent possible contamination during sampling and analysis, especial care was taken to avoid sample contact with products known to contain alkylphenols and BPA. The absence of contamination was assessed by pouring LC-MS grade water into collection bottles and performing the overall analytical procedure. Further, to prevent cross-contamination, the sample loop and extraction column were flushed at a 2 mL min^{-1} flow with 98% of methanol after every extraction and conditioned with appropriate eluent before enrichment of the next sample.

Eight-point calibration curves (0.1-500 ng L^{-1}) were generated using linear regression analysis. Calibration curves were injected at the beginning and the end of each sequence. Procedural blanks (Mili-Q water, 5mL) were injected in every 2 vials (the sample and its spike) to prevent carryover. Calibration check standards (two concentration levels 5 ng L^{-1} and 50 ng L^{-1}) were measured repeatedly throughout the sequence, after the injection of every 10 samples and at the end of the analytical runs, to determine possible EDCs background levels and to check for signal stability, respectively. R-squared values were consistently greater than 0.99 for all selected EDCs. For the samples with concentrations above the linearity range, the samples were diluted and further injected.

Relative recoveries were estimated by spiking three types of water at a known concentration of target analytes, by triplicate and using two concentration levels and are listed in the Table S3, SM. Concentrations after the on-line analytical process were compared with the initial spiking levels, calculated by internal standard calibration. Blanks (non-spiked samples) were also analyzed and the levels found were subtracted from those obtained from spiked samples. Spiking concentrations used were: 50 ng L^{-1} and 200 ng L^{-1} for wastewater, 10 ng L^{-1} and 50 ng L^{-1} for river water, 5 ng L^{-1} and 20 ng L^{-1} for drinking water.

Spiked real water samples were used to determine limit of detection (LOD) and limit of quantification (LOQ) for each water type. LOD and LOQ were established as the minimum detectable amount of analyte that results in a reproducible measurement of peak areas consistent with calibration curve, with a signal to noise (S/N) ratio set as 3 and 10, respectively (see Table S3, SM). Identification of target analytes was accomplished by the retention time (within 2% between the standard and a sample) and

the MRM ratio (within 20% between standard and samples) (2002/657/EC). Quantification of target EDCs in the samples was based on peak areas and it was performed by the internal standard calibration approach. For each compound, its corresponding isotopically labeled analogue was used, except for those substances whose corresponding labeled compound was not available. In this case, the most similar labeled substance, in terms of chemical structure and chromatographic retention time, was used as IS.

Table S1. Sampling locations and information about sampling sites.

N[a]	Place of sampling	Number of habitantes[b]	Sample acronyms			[c]Wastewater samples	[d]Surface water samples	[e]Drinking water samples	Coordinates	
			wastewater	surface water	drinking water				X	Y
1	Apatin	20.000-25.000	WW1	S1	DW1	municipal	Danube River	public fountain	45.672998	18.986873
2	Bačka Palanka	25.000-50.000	WW2	S2	DW2	municipal	Danube River	public faucet, market place	45.249981	19.399669
3	Futog	<20.000	WW3	S3	DW3	municipal	Danube River	public faucet, market place	45.242880	19.715767
4	Novi Sad	>100.000	WW4	S4	DW4	municipal	Danube River	public faucet , beache zone	45.236094	19.847877
5	Novi Sad	>100.000	WW5	S5	DW5	municipal+industrial	Danube River	public fountain, city centar	45.202312	19.933597
6	Zemun	>100.000	WW6	S6	DW6	municipal+industrial	Danube River	public fountain	44.863627	20.367199
7	Beograd	>1.500.000	WW7	S7	DW7	municipal+industrial	Danube River	public fountain	44.802946	20.475952
8	Pančevo	>100.000	WW8	S8	DW8	municipal+industrial	Danube River	public fountain	44.872699	20.650325
9	Kanjiža	20.000-25.000	WW9	S9	DW9	municipal	Tisa tributary	public faucet, market place	46.061898	20.055457
10	Novi Kneževac	<20.000	WW10	S10	DW10	municipal	Tisa tributary	public faucet, market place	46.045313	20.092405
11	Senta	20.000-25.000	WW11	S11	DW11	municipal	Tisa tributary	public faucet, market place	45.919199	20.086324
12	Bečej	25.000-50.000	WW12	S12	DW12	municipal	Tisa tributary	public fountain	45.612330	20.038319
13	Zabalj	20.000-25.000	WW13	S13	DW13	municipal	Tisa tributary	public fountain	45.393433	20.123758
14	Titel	<20.000	WW14	S14	DW14	municipal	Tisa tributary	public fountain	45.216352	20.286101
15	Šid	25.000-50.000	WW15	S15	DW15	municipal	Borsut channel	public faucet, market place	45.126937	19.226621
16	Sr.Mitrovica	50.000-100.000	WW16	S16	DW16	municipal	Sava tributary	public faucet, market place	44.966271	19.604898
17	Ruma	25.000-50.000	WW17	S17	DW17	municipal	Sava tributary	public faucet, market place	45.013720	19.799578
18	Šabac	>100.000	WW18	S18	DW18	municipal+industrial	Sava tributary	public faucet, market place	44.750932	19.701439
19	Beograd	>1.500.000	WW19	S19	DW19	municipal+industrial	Sava tributary	public fountain	44.802946	20.475952
20	Mali Iđoš	<20.000	WW20	S20	DW20	municipal	Krivaja channel	public fountain	45.708862	19.664130
21	Sombor	50.000-100.000	WW21	S21	DW21	municipal	Veliki Bačka Canal	public fountain	46.773612	19.114040
22	Crvenka	<20.000	WW22	S22	DW22	municipal+industrial	Veliki Bačka Canal	public fountain	45.657536	19.458021
23	Kula	25.000-50.000	WW23	S23	DW23	municipal+industrial	Veliki Bačka Canal	public fountain	45.608887	19.532192
24	Vrbas	25.000-50.000	WW24	S24	DW24	municipal+industrial	Veliki Bačka Canal	public fountain	45.574509	19.649013
25	Srbobran	<20.000	WW25	S25	DW25	municipal	Veliki Bačka Canal	public fountain	45.546971	19.791672
26	Kikinda	50.000-100.000	WW26	S26	D26	municipal	Kikinda channel	public faucet, market place	45.832885	20.465570
27	Zrenjanin	>100.000	WW27	S27	DW27	municipal+industrial	Sari Begej channel	public fountain	45.381562	20.388485
28	Vršac	25.000-50.000	WW28	S28	DW28	municipal	Canal Danube-Tisa-Danube	public fountain	45.119761	21.290061

29	Subotica	>100.000	WW29	S29	DW29	municipal+industrial	Lake "Palić"	public fountain, city centar	46.104470	19.667348
30	Bačka Topola	25.000-50.000	WW30	S30	DW30	municipal	Lake "Zobnatica"	public fountain	45.809003	19.636836

[a]N-cardinal number of sample; [b]Total number of populations according to Republican Statistical Office www.stat.gov.rs; [c]Wastewater samples were collected from municipal wastewater collector; [d] Surface water samples were collected downstream of each wastewater discharge in order to assess quality of recipient water; [e]Samples of drinking water are representing public distribution supply systems from the area;

Table S2. The optimized MS/MS parameters for SRM analysis of selected EDCs in negative (NI) ionization mode.

Abbrevation	Corresponding Internal Standard[a]	CAS number	t_R (min)	Precursor ion (m/z)	S-Lens (Hz)	SRM1[b] (m/z)	Collision Energy (eV)	SRM2[c] (m/z)	Collision Energy (eV)
E2	Estradiol-d_2	50-28-2	5.66	271	105	145	40	183	41
E1	Estrone_d_4	53-16-7	5.54	269	121	145	41	143	57
E3	Estradiol_d_2	50-27-1	4.27	287	117	171	38	1145	43
EE2	Ethinylestradiol_d_4	57-63-6	5.69	295	129	145	43	143	55
DES	Estrone_d_4	56-53-1	5.56	267	92	251	26	237	29
E1-3S	Estrone-2,4,16,16-d4 3-sulfate	1240-04-6	3.66	349	111	269	33	145	55
E3-3S	Estrone-2,4,16,16-d4 3-sulfate	5150-64-1	3.61	367	110	287	35	171	53
E2-17G	Estradiol-17 glucuronide-^{13}C4	15087-02-2	3.65	447	103	271	30	325	20
E1-3G	Estradiol-17 glucuronide-^{13}C4	15087-01-1	3.64	445	100	269	40	113	22
E3-16G	Estradiol-17 glucuronide-^{13}C4	1852-50-2	3.62	463	126	287	32	113	29
OP	4-tert-Octylphenol_d_{17}	1806-26-4	7.19	205	87	133	19	134	20
NP	4-Nonylphenol_d_4	104-40-5	7.53	219	91	133	21	147	22
BPA	Bisphenol A_d_4	80-05-7	5.10	227	125	212	21	133	27

[a]Internal standard applied for the identification and quantification; [b]SRM1: selected reaction monitoring for identification; [c]SRM2: : selected reaction monitoring for quantification

Table S3. Validation parameters including: linearity (r^2), instrumental detection and quantification limits (IDL/IQL, pg L^{-1}), repeatability (intra-day, RSD %) and reproducibility (inter-day, RSD, %) precision, relative recoveries (±RS, %), limit of detection (LOD, ng L^{-1}) and limit of quantification (LOQ, ng L^{-1}) obtained in each type of water samples analyzed.

Abbr.	Instrumental parametres				[a]%Relative recovery (±RSD)			LOD/LOQ (ng L^{-1})		
	r^2	IDL/IQL (pg L^{-1})	Intra-day RSD, %	Inter-day, RSD, %	drinking water	surface water	wastewater	drinking water	surface water	wastewater
17β-E2	0.9999	0.09/.29	1.5	3.8	98.8±1.4	94.9±9.0	113.0±7.8	0.037/0.123	0.034/0.112	0.166/0.553
E1	0.9992	0.14/0.41	2.7	6.3	99.1±1.6	99.2±6.8	91.7±14.3	0.022/0.072	0.024/0.080	0.179/0.597
E3	0.9984	0.22/0.68	3.2	10.4	99.6±10.4	121.1±35.1	110.5±23.8	0.132/0.439	0.092/0.307	0.338/1.126
17α-EE2	0.9999	0.14/0.43	2.6	7.2	98.9±1.2	97.7±5.5	90.5±13.2	0.035/0.118	0.035/0.118	0.219/0.729
DES	0.9997	0.13/0.41	1.9	3.5	107.0±10.6	106.0±12.3	101.5±12.2	0.033/0.109	0.034/0.113	0.220/0.735
E1-3S	0.9997	0.12/0.35	5.5	7.6	84.4±17.8	78.5±25.0	80.6±19.7	0.020/0.066	0.007/0.023	0.014/0.045
E3-3S	0.9995	0.10/0.32	3.8	12.7	103.3±19.9	73.1±21.0	118.7±19.2	0.024/0.081	0.014/0.045	0.067/0.222
E2-17G	0.9999	0.17/0.52	2.9	9.5	101.2±15.3	78.2±28.6	78.5±15.5	0.006/0.020	0.025/0.082	0.260/0.866
E1-3G	0,9992	0.12/0.35	4.7	11.4	79.9±18.2	89.3±24.3	84.4±16.4	0.006/0.018	0.043/0.143	0.173/0.576
E3-16G	0.9986	0.42/1.26	3.3	12.1	108.0±12.6	80.6±16.7	121.3±12.7	0.035/0.116	0.055/0.184	0.369/1.229
4-t-OP	0.9982	0.16/0.50	5.7	7.5	99.0±4.0	103.7±5.4	100.8±5.4	0.037/0.123	0.020/0.066	0.183/0.611
4-NP	0.9997	0.12/0.36	2.6	10.8	97.9±7.9	95.5±16.4	98.0±4.6	0.037/0.123	0.020/0.066	0.172/0.573
BPA	0.9987	0.17/0.51	3.5	4.2	100.0±0.8	100.2±5.5	76.8±12.8	0.037/0.123	0.046/0.153	0.308/1.027

[a]Relative recovery obtained from two spiking levels in the matrix (5 and 50ng L^{-1}, n=6);

Table S4. Compounds with their lowest PNEC values (µg/L) obtained from NORMAN Database.

Compound name	NORMAN PNEC ID	CAS	PNEC type	Scientific name	Endpoint/ /Duration/ Effect	AF	Derivation method	Lowest PNEC freshwater (µg/L)	Data source name	Data source link	Source
17-beta-Estradiol	PNEC-ID-0040671	50-28-2	EQS chronic water (=AA-EQS)	HC5	n.r./n.r./n.r.	2	SSD[a]	0.0004	CIRCA web server of the EC	(1)	(1)
Estrone	PNEC-ID-0040728	53-16-7	EQS-proposal	Danio rerio	n.r./n.r./n.r.	10	Deterministic[b]	0.0036	n.r.	n.r.	n.r.
Estriol	PNEC-ID-0040670	50-27-1	PNEC experimental	n.r.	n.r./n.r./n.r.	n.r.	n.r.[c]	0.06	n.r.	n.r.	(2)
Ethinylestradiol	PNEC-ID-0040783	57-63-6	EQS chronic water (=AA-EQS)	n.r.	n.r./n.r./n.r.	2	n.r.[a]	3.50E-05	CIRCA web server of the EC	(1)	(1)
Diethylstilbestrol	PNEC-ID-0010599	6898-97-1	P-PNEC pred	Pimephales promelas	LC50/96h/ mortality	1000	Deterministic[d]	0.44	NORMAN SusDat: Suspect List Exchang	-	(3)
Estrone-sulfate	PNEC-ID-0040653	481-97-0	P-PNEC pred	Selenastrum capricornutum	LC50/96h/ mortality	1000	Deterministic[d]	20.5	NORMAN SusDat: Suspect List Exchang	(2)	(3)
Estriol-sulfate	PNEC-ID-0014821	3067-19-4	P-PNEC pred	Selenastrum capricornutum	IC50/72h/ growth rate	1000	Deterministic[d]	21.2	NORMAN SusDat: Suspect List Exchang	-	(3)
β-Estradiol-17β-glucuronide	PNEC-ID-0014955	1806-98-0	P-PNEC pred	Selenastrum capricornutum	IC50/72h/ growth rate	1000	Deterministic[d]	6.5	NORMAN SusDat: Suspect List Exchang	-	(3)
Estrone-3-glucuronide	PNEC-ID-0014079	2479-90-5	P-PNEC pred	Selenastrum capricornutum	IC50/72h/ growth rate	1000	Deterministic[d]	8.3	NORMAN SusDat: Suspect List Exchang	-	(3)
4-octylphenol	PNEC-ID-0040379	1806-26-4	EQS chronic water (=AA-EQS)	n.r.	n.r./n.r./n.r.	n.r.	n.r.[c]	0.1	CIRCA web server of the EC	(1)	(4)
4-nonylphenol	PNEC-ID-0040088	104-40-5	EQS chronic water (=AA-EQS)	n.r.	n.r./n.r./n.r.	n.r.	n.r.[c]	0.25	CIRCA web server of the EC	(1)	(4)
Bisphenol A	PNEC-ID-0040998	80-05-7	EQS chronic water (=AA-EQS)	Salmo trutta	n.r./n.r./n.r.	10	Deterministic[b]	0.24	Proposals for Acute and Chronic Quality Standards	(3)	(5)

Justification:
[a] Species sensitivity distribution (SSD) method; [b] Long-term results (e.g. EC10 or NOECs) from at least three species (normally fish, Daphnia and algae) representing three trophic levels; [c] n.r.; [d] One predicted short-term L(E)C50 from each of three trophic levels (i.e. base set)
Data source link: (1) https://circabc.europa.eu; (2) https://46.229.226.228/nds/s; (3) http://www.ecotoxcentre.ch/expert-service/quality-standards/proposals-for-acute-and-chronic-quality-standards

(1) DIRECTIVE (2011/876/EC)
(2) (Caldwell Daniel et al., 2010)

(3) (Aalizadeh et al., 2017)
(4) (2013/39/EU)
(5) (EQS Dossier OZ (2016))

Table S5. Literature values of estradiol equivalency factor (EEF) obtained for selected EDCs compounds in the different bioequivalence experiments.

Compound	EEF_i ($\mu g\ L^{-1}$)[a]	EEF_i ($\mu g\ L^{-1}$)[b]	EEF_i ($\mu g\ L^{-1}$)[c]	EEF_i ($\mu g\ L^{-1}$)[d]	EEF_i ($\mu g\ L^{-1}$)[e]	EEF_i ($\mu g\ L^{-1}$)[f]	Max ($\mu g\ L^{-1}$)	Max ($ng\ L^{-1}$)
E2	1	1	1	1	1	1	1	1000
E1	0.06	$2.5x10^{-1}$	0.01886	-	-	0.01	$2.5x10^{-1}$	250
E3	-	$5.9x10^{-3}$	0.3448	-	-	0.083	0.3448	344.8
EE2	-	1.25	0.1709	1.19	-	1.25	1.25	1250
DES	-	-	0.04597	-	-	1.25	1.25	1250
4-OP	$3x10^{-6}$	$4.5x10^{-6}$	$2.13x10^{-4}$	$7.8x10^{-6}$	$1.4x10^{-6}$	$8.33x10^{-5}$	$2.13x10^{-4}$	0.213
NP	$1x10^{-6}$	$1.8x10^{-5}$	$5.05x10^{-4}$	$2.5x10^{-5}$	$2.3x10^{-5}$	$1.25x10^{-5}$	$5.05x10^{-4}$	0.505
BPA	LE	$1.2x10^{-4}$	$2.43x10^{-5}$	$1.1x10^{-4}$	-	$2.5x10^{-5}$	$1.2x10^{-4}$	0.12

EEFi- the relative estrogenicity factor (E2 equivalent factor) defined as the mean effective concentrations (EC50) of each compound relative to the EC50 of 17β-estradiol;

EC50- The contaminant concentration that produces a 50% maximal response estrogenic;

LE-did not reach 50% of max E2 response in bioassay

[a] (Song et al., 2006)
[b] (Beck et al., 2005)
[c] (Céspedes et al., 2004)
[d] (Rutishauser et al., 2004)
[e] (Van den Belt et al., 2003)
[f] (Gutendorf et al., 2001)

Table S6. Occurrence and fate of selected EDCs detected in a) wastewater, b) surface water and c) drinking water other international studies.

Location	Category	Concentration of selected EDCs found in international studies													Reference
		a) Concentration range in wastewater (ng L^{-1})													
		4-NP	4-t-OP	BPA	17β-E2	E1	E3	17α-EE2	DES	E1-3S	E2-3S	E3-3S	E1-3G	E2-17G	E3-16G	
Northern Serbia	raw ww	**<LOD-78.3**	**<LOD-52.4**	**<LOD-338.2**	**<LOD-10.4**	**<LOD-64.8**	**<LOD-34.2**	<LOD	<LOD	**<LOD-17.7**	-	**<LOD-30.1**	<LOD	<LOD	<LOD	(Current study)
India	raw ww	-	-	**95-299**	**4-28**	**26-124**	-	-	-	-	-	-	-	-	-	(Williams et al., 2019)
Malasia	influent	-	-	-	**88.2-93.9**	-	-	**0.2-4.9**	-	-	-	-	-	-	-	(Yien Fang et al., 2019)
	efffluent	-	-	-	**35.1-85.2**	-	-	**0.02-1**	-	-	-	-	-	-	-	
Iran	influent	-	-	-	**11-35**	**50-140**	-	**0.3-2.6**	-	-	-	-	-	-	-	(Amin et al., 2018)
	efffluent	-	-	-	**0.1-3.8**	**2-57**	-	**0.02-0.36**	-	-	-	-	-	-	-	
Canada	influent	-	-	**<LOD -44**	**<LOD -15**	**<LOD-21**	**<LOD-92**	<LOD	-	-	-	-	-	-	-	(Goeury et al., 2019)
	efffluent	-	-	**60-234**	**31-122**	**14-44**	**<LOD-24**	<LOD	-	-	-	-	-	-	-	
Germany	wastewater	<LOD	<LOD	**540**	<LOD	-	-	<LOD	-	-	-	-	-	-	-	
Australia	wastewater	<LOD	<LOD	**130**	<LOD	-	-	<LOD	-	-	-	-	-	-	-	
France	wastewater	<LOD	<LOD	**22**	<LOD	-	-	<LOD	-	-	-	-	-	-	-	(Leusch et al., 2018)
South Africa	wastewater	<LOD	<LOD	**200**	<LOD	-	-	<LOD	-	-	-	-	-	-	-	
The Netherlands	wastewater	<LOD	<LOD	<LOD	<LOD	-	-	<LOD	-	-	-	-	-	-	-	
Spain	wastewater	<LOD	<LOD	<LOD	<LOD	-	-	<LOD	-	-	-	-	-	-	-	
Switzerland	influent	-	-	-	<LOD-**8.7**	**2.3-37.0**	<LOD	<LOD	-	-	-	-	-	-	-	(Zhang et al., 2018)
	efffluent	-	-	-	<LOD	**0.3-0.9**	<LOD **-0.8**	<LOD	-	-	-	-	-	-	-	
Canada	influent	-	-	-	**75.3-273.9**	**38.9-233.6**	**249.5-495.3**	**19.2-32.1**	-	-	-	-	-	-	-	
	effluent	-	-	-	<LOD-**147**	<LOD-**129.7**	<LOD-**264.4**	<LOD-**15.3**	-	-	-	-	-	-	-	(Yarahmadi et al., 2018)
East China	influent	-	-	-	**4.6-42.1**	**40.8-278.5**	**15.1-364.9**	-	-	**4.8**	**5.5**	-	**4.2**	**3**	-	(Ben et al., 2017)
	effluent	-	-	-	**1.4**	**7.7**	-	-	-	**0.8**	-	-	**0.3**	**0.7**	-	
Spain	effluent	<LOD	<LOD **-22**	-	<LOD	**<LOQ-40**	-	<LOD	-	-	-	-	-	-	-	(Rubirola et al., 2017)
China	influent	<LOD	-	**109-615**	**1.6-3.3**	**11-33**	**67.8**	<LOD	<LOD	-	-	-	-	-	-	(Wu et al., 2017)
	efffluent	<LOQ	-	**4-205**	-	**0.5-60.5**	-	-	-	-	-	-	-	-	-	
Canada	raw ww	-	-	-	<LOD	<LOD	<LOD	<LOD	-	<LOD	<LOD	-	<LOD	<LOD	-	
	effluent	-	-	-	<LOD	<LOD	<LOD	<LOD	-	<LOD	<LOD	-	<LOD	<LOD	-	(Naldi et al., 2016)
Iran	influent	-	-	-	**1.02-8.0**	**6.54-18.76**	**-**	**4.18-11.7**	-	-	-	-	-	-	-	(Mohagheghian et al., 2014)
	effluent	-	-	-	**0.5-2.20**	**1.04-4.99**		**0.5-2.58**	-	-	-	-	-	-	-	
South Africa	influent				**20-199**	**13-351**	**3-9**	**10-95**	-	-	-	-	-	-	-	(Manickum et al., 2014)
	effluent	-	-	-	**4-107**	**3-78**	<LOD	**1-8**	-	-	-	-	-	-	-	
Slovenia	influent	-	-	**-**	**1.3-10.1**	**6.2-82.7**	**37-119**	**<LOD**	-	-	-	-	-	-	-	(Avberšek et al., 2013)

	effluent	-	-	-	**<LOD-9.0**	**2.2-51.1**	**<LOD**	**<LOD**	-	-	-	-	-	-	-	
Korea	influent	-	-	-	<LOD	**13.-52**	**46-1130**	<LOD	-	-	-	-	-	-	-	(Sim et al., 2011)
	effluent	-	-	-	<LOD	**1-79**	**160-273**	<LOD	-	-	-	-	-	-	-	
Spain	influent	-	-	-	<LOD	<LOD	<LOD	**154**	-	**52-160**	**<LOQ-76**	-	<LOD	<LOQ	-	(Pedrouzo et al., 2009)
	effluent	-	-	-	<LOD	<LOD	<LOD	<LOD	-	**<LOQ-35**	**<LOQ**	-	<LOD	<LOD	-	
Spain	influent	-	-	**960-1600**	-	-	-	-	-	-	-	-	-	-	-	(Ballesteros-Gómez et al., 2007)
	effluent	-	-	**260-360**	-	-	-	-	-	-	-	-	-	-	-	
Japan	influent	-	-	-	**<LOD-57**	**259-326**	<LOD	-	-	**98-145**	**419-957**	-	**20-26**	-	-	(Kobayashi et al., 2006)
	effluent	-	-	-	**4.6-14**	**<LOD -17**	**<LOD-151**	-	-	**32-42**	**57-77**	-	**13-20**	-	-	
Japan	influent	-	-	-	-	-	-	-	-	**2.9**	**6.4**	-	-	-	-	(Nakada et al., 2006)
	effluent	-	-	-	-	-	-	-	-	**2.1**	**3.3**	-	-	-	-	
United States	influent	-	-	-	-	-	-	-	-	**34.1**	**3.2**	-	**0.4**	<LOD	-	(Reddy et al., 2005)
	effluent	-	-	-	-	-	-	-	-	**0.3**	<LOD	-	<LOD	<LOD	-	
Japan	influent	-	-	-	-	-	-	-	-	**1.2**	**66.7**	-	-	<LOD	-	(Sueoka et al., 2005)
	effluent	-	-	-	-	-	-	-	-	<LOD	**1.8**	-	-	<LOD	-	
Japan	influent	-	-	-	-	-	-	-	-	**12-170**	**26-410**	-	**<LOD-88**	-	-	(Komori et al., 2004)
	effluent	-	-	-	-	-	-	-	-	**7.5-34**	**27-94.0**	-	**34-140**	-	-	
Italy	raw ww	-	-	-	**9**	**58**	**62**	-	-	**25**	**9.0**	**-**	**10.0**	<LOQ	**39**	(D'Ascenzo et al., 2003)
	influent	-	-	-	**11**	**44**	**72**	-	-	**25**	**3.3**	**-**	**4.3**	<LOD.	**19**	
	effluent	-	-	-	**1.6**	**17**	**2.3**	-	-	**9**	<LOD	-.	**0.7**	<LOD	<LOD	
Japan	effluent	-	-	-	**0.3-2.5**	**2.5-34**	<LOD	-	-	**0.3-2.2**	**<LOD -1**	-	-	<LOD	-	(Isobe et al., 2003)
Italy	influent	-	-	-	-	-	-	-	-	**8**	-	-	**6**	-	-	(Gentili et al., 2002)
	effluent	-	-	-	-	-	-	-	-	**3**	-	-	**3**	-	-	

b) Concentration range in surface water (ng L^{-1})

Location	Category	4-NP	OP	BPA	17β-E2	E1	E3	17α-EE2	DES	E2-3S	E1-3S	E3-3S	E1-3G	E2-17G	E3-16G	Reference
Northern Serbia	river and channels	**<LOD-36.6**	**<LOD-37.2**	**<LOD-105.7**	<LOD	**<LOD -9.8**	**<LOD-4.8**	<LOD	<LOD	-	**<LOD-7.2**	**<LOD-4.1**	<LOD	<LOD	<LOD	Currrent study
Switzerland	river	-	-	-	<LOD	**0.2-0.9**	<LOD	<LOD	-	<LOD	-	-	-	-	-	(Zhang;Fent 2018)
Canada	river	-	-	-	**<LOD**	<LOD	<LOD	**<LOD**	-	<LOD	-	-	-	-	-	(Yarahmadi et al., 2018)
Canada	surface water	-	-	**3.9-17**	**<LOD -1.7**	**<LOQ-0.5**	**<LOD -92**	**<LOQ**	-	-	-	-	-	-	-	(Goeury et al., 2019)
Germany	surface water	<LOD	<LOD	**13**	<LOD	-	-	<LOD	-	-	-	-	-	-	-	(Leusch et al., 2018)
Australia	surface water	**240**	<LOD	**100**	<LOD	-	-	<LOD	-	-	-	-	-	-	-	
France	surface water	<LOD	**6**	**28**	<LOD	-	-	<LOD	-	-	-	-	-	-	-	
South Africa	surface water	<LOD	<LOD	**23**	<LOD	-	-	<LOD	-	-	-	-	-	-	-	
The Netherlands	surface water	<LOD	<LOD	<LOD	<LOD	-	-	<LOD	-	-	-	-	-	-	-	

Spain	surface water	<LOD	<LOD	**5**	<LOD	-	-	<LOD	-	-	-	-	-	-	-	
China	surface water	-	-	-	**10**	**26**	**31**	>LOQ	-	**14**	-	-	-	-	-	(Yao et al., 2018)
China	lakes	-	-	-	**<LOD-17.6**	**<LOD -9.6**	-	**<LOD-16.8**	-	-	-	-	-	-	-	(Wang et al., 2017)
Spain	surface water	**<LOD-138**	<LOD	-	**<LOD -30**	**<LOQ-34**	-	<LOD	-	-	-	-	-	-	-	(Rubirola et al., 2017)
Canada	river	-	-	-	<LOD	<LOD	<LOD	<LOD	-	<LOD	<LOD	-	<LOD	<LOD	-	(Naldi et al., 2016)
Spain	river	**96-1483**	**42-474**	**6.5-126**	<LOD	**4.5-17**	<LOD	<LOD	<LOD	-	**8.1-10**	**1.2-8.9**	<LOD	<LOD	<LOD	(Esteban et al., 2014b)
South Africa	upstream	-	-	-	**1-28**	**2-10**	<LOD	**<LOD-3**	-	-	-	-	-	-	-	(Manickum;John 2014)
	downstream	-	-	-	**2-66**	**1.0-32**	**<LOD-2**	**1-4**	-	-	-	-	-	-	-	
Slovenia	upstream	-	-	-	**<LOD-1.6**	**<LOD-2**	**<LOD**	**<LOD**	-	-	-	-	-	-	-	(Avberšek et al., 2013)
	downstream	-	-	-	**<LOD-3.1**	**<LOD-7.4**	**<LOD-79.8**	**<LOD**	-	-	-	-	-	-	-	
Spain	river	**440-6200**	**<LOD-150**	**40-130**	**<LOD-130**	**<LOD-560**	**<LOD-170**	-	-	-	-	-	-	-	-	(Pelayo et al., 2011)
Brazil	surface water	<LOD	<LOD	**25-83**	**<LOD-7.3**	**2.4-39**	**<LOD-2.3**		-	-	-	-	-	-	-	(Sodré et al., 2010)
Spain	river water	**20-530**	**60-880**	-	<LOD	**4.4-5.8**	<LOD	<LOD	<LOD	-	**0.4-1.4**	<LOD	<LOD	<LOD	<LOD	(Brix et al., 2010)
Spain	Ebro river	-	-	-	<LOD	<LOD	<LOD	<LOD	-	<LOD	<LOD	-	<LOD	<LOD	-	(Pedrouzo et al., 2009)
Germany	river water	**<LOD-770**	**-**	-	-	-	-	-	-	-	-	-	-	-	-	(Quednow et al., 2009)
United Kingdom	Taff, Ely River	-	**<LOD-536**	**<LOD-68**	-	-	-	-	-	-	-	-	-	-	-	(Kasprzyk-Hordern et al., 2008)
Spain	river water	**-**	**-**	**100-320**	-	-	-	-	-	-	-	-	-	-	-	(Ballesteros-Gómez et al., 2007)
Spain	Ter River	**<LOD-17500**	**<LOD-3980**	**60-1510**	-	-	-	-	-	-	-	-	-	-	-	(Céspedes et al., 2006)
Japan	surface	-	-	-	<LOD	<LOD	<LOD	<LOD	-	<LOD	**<LOD-6.4**	-	<LOD	<LOD	<LOD	(Komori et al., 2004)
Japan	river	-	-	-	**0.6-1**	**3.4-6.6**	<LOD	<LOD	-	**0.2-0.8**	**0.3-0.9**	-	-	<LOD	-	(Isobe et al., 2003)
	lake	-	-	-	<LOD	**0.2-0.8**	<LOD	<LOD	-	**0.2-0.4**	**0.3-0.8**	-	-	<LOD	-	
Italy	surface	-	-	-	<LOD	<LOD	<LOD	<LOD	-	**-**	**0.3-0.8**	-	<LOD	-	-	(Gentili et al., 2002)

c) Concentration range in drinking water (ng L^{-1})

Location	**Category**	**17β-E2**	**E1**	**E3**	**17α-EE2**	**4-NP**	**4-t-OP**	**BPA**	**Reference**
Northern Serbia	drinking water	<LOD	<LOD	<LOD	<LOD	**<LOD-7.9**	**<LOD-3.7**	**<LOD-35.6**	Current study
Canada	drinking water	**<LOQ-2.8**	<LOD	**<LOQ-1.2**	<LOD	**-**	**-**	<LOQ	(Goeury et al., 2019)
Spain	drinking water	<LOD	<LOD	<LOD	<LOD	**15.2-126.4**	<LOD	**51.2**	(Valcárcel et al., 2018)
Germany	drinking water	<LOD	**-**	**-**	<LOD	<LOD	<LOD	<LOD	(Leusch et al., 2018)
Australia	drinking water	<LOD	**-**	**-**	<LOD	<LOD	<LOD	<LOD	
France	drinking water	<LOD	**-**	**-**	<LOD	**34**	<LOD	<LOD	
South Africa	drinking water	<LOD	**-**	**-**	<LOD	<LOD	<LOD	<LOD	
The Netherlands	drinking water	<LOD	**-**	**-**	<LOD	<LOD	<LOD	<LOD	
Spain	drinking water	<LOD	**-**	**-**	<LOD	**36**	<LOD	<LOD	
France	bottled water	<LOD	<LOD	<LOD	<LOD	**30**	**2**	-	(Le Coadou et al., 2017)
Brazil	drinking water	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**11-19**	(Machado et al., 2016)

Latvia	drinking water	**0.09-0.15**	<LOD	<LOD	<LOQ	-	-	-	(Zacs et al., 2016)
Canada	drinking water	<LOD	<LOD	<LOD	<LOD	-	-	-	(Naldi et al., 2016)
France	tap water	-	-	-	-	**<LOD-505**	<LOD	**9-50**	(Colin et al., 2013)
Spain	Tap water	<LOD	<LOD	<LOD	<LOD	**2.5-20.5**	**2.6-9.7**	**3.7-50.3**	(Esteban et al., 2014a)
Serbia	raw drinking water	-	-	-	-	**110**	<LOD	-	(Miloradov et al., 2014)
Spain	springs/boreholes	-	-	-	-	<LOD-**58**	**2-24**	**31-203**	(Bono-Blay et al., 2012)
China	drinking water	-	-	-	-	**186-1073**	-	**2.3-317**	(Li et al., 2010)
	bottled water	-	-	-	-	**108-298**	-	**17.6-285**	
U.S.A.	source water	**0.5-17**	**0.2-0.9**	-	**<LOQ-1.4**	**80-130**	**<LOQ-25**	**5.4-14**	(Benotti et al., 2009)
	Category	<LOD	<LOD	<LOD	<LOD	<LOQ-100	<LOD	<LOD	
	distribution systems	<LOD	<LOD	<LOD	<LOD	<LOQ-110	<LOD	<LOD	
South China	source water	-	-	-	-	**28.0-8890**	-	**2.2-1030**	(Li et al., 2010)
Northern Italy	drinking water	-	-	-	**0.4**	**15**	-	-	(Loos et al., 2007)
Czech Republic	drinking water	<LOD-**2.6**	<LOD	<LOD	<LOD	-	-	-	(Morteani et al., 2006)
Catalonia, Spain	drinking water	-	-	-	-	<LOD-**24**	-	**6-25**	(Casajuana et al., 2003)
	bottled water	-	-	-	-	**30.0-1730**	-	**3-11**	
South Germany	drinking water	**0.2-2.1**	**0.2-0.6**	-	-	**2-15**	**0.15-5**	**0.3-2**	(Kuch et al., 2001)

Supplementary material of Chapter 2

Article Nº3:

Mira Čelić, Meritxell Gros, Marinella Farré, Damia Barceló, and Mira Petrović

Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain),
Science of The Total Environment *652 (2018) 952-963.*

Supplementary Material has been reformed to match the style of the thesis.

Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain)

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Table S1. Therapeutical groups and target compounds.

Therapeutic groups	Trivial name	IUPAC Name	CAS number	Internal standard
Analgesics/ant-inflammatories (14)	Ketoprofen	2-(3-Benzoylphenyl)propanoic acid	22071-15-4	Ibuprofen_d_3
	Naproxen	(2S)-2-(6-Methoxy-2-naphthyl)propanoic acid	22204-53-1	Ibuprofen_d_3
	Ibuprofen	2-(4-Isobutylphenyl)propanoic acid	15687-27-1	Ibuprofen_d_3
	Indomethacine	[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid	53-86-1	Indomethacine_d_4
	Acetaminophen	N-(4-Hydroxyphenyl)acetamide	103-90-2	Acetaminophen_d_4
	Salicylic acid	2-Hydroxybenzoic acid	69-72-7	Acetaminophen_d_4
	Diclofenac	{2-[(2,6-Dichlorophenyl)amino]phenyl}acetic acid	15307-79-6	Ibuprofen_d_3
	Phenazone	1,5-Dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one	60-80-0	Phenazone_d_3
	Propylphenazone	1,5-dimethyl-2-phenyl-4-propan-2-pylpyrazol-3-one	479-92-5	Phenazone_d_3
	Piroxicam	4-Hydroxy-2-methyl-N-(2-pyridinyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide	36322-90-4	Meloxicam_d_3
	Meloxicam	4-hydroxy-2-methyl-N-(5-methyl-1,3-thiazol-2-yl)-1,1-dioxo-1$l^{6},2-benzothiazine-3-carboxamide	59804-37-4	Meloxicam_d_3
	Tenoxicam	4-hydroxy-2-methyl-1,1-dioxo-N-pyridin-2-ylthieno[2,3-e]thiazine-3-carboxamide	59804-37-4	Meloxicam_d_3
	Oxycodone	(5α)-14-Hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-one	124-90-3	Carbamazepine-d_{10}
	Codeine	(5α,6α)-3-Methoxy-17-methyl-7,8-didehydro-4,5-epoxymorphinan-6-ol	76-57-3	Carbamazepine-d_{10}
Lipid regulators and cholesterol lowering statin drugs (5)	Bezafibrate	2-(4-{2-[(4-Chlorobenzoyl)amino]ethyl}phenoxy)-2-methylpropanoic acid	41859-67-0	Bezafibrate_d_6
	Gemfibrozil	5-(2,5-Dimethylphenoxy)-2,2-dimethylpentanoic acid	25812-30-0	Gemfibrozil_d_6
	Pravastatin	(3R,5R)-3,5-Dihydroxy-7-[(1S,2S,6S,8S,8aR)-6-hydroxy-2-methyl-8-{[(2S)-2-methylbutanoyl]oxy}-1,2,6,7,8,8a-hexahydro-1-naphthalenyl]heptanoic acid	81131-70-6	Gemfibrozil_d_6
	Fluvastatin	(3R,5S,6E)-7-[3-(4-Fluorophenyl)-1-isopropyl-1H-indol-2-yl]-3,5-dihydroxy-6-heptenoic acid	93957-54-1	Gemfibrozil_d_6
	Atorvastatin	(3R,5R)-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid	134523-03-8	Gemfibrozil_d_6
Psychiatric drugs (15)	Carbamazepine	5H-Dibenzo[b,f]azepine-5-carboxamide	298-46-4	Carbamazepine-d_{10}
	2-Hydroxycarbamazepine[a]	2-Hydroxy-5H-dibenzo[b,f]azepine-5-carboxamide	68011-66-5	Carbamazepine-d_{10}
	10,11-epoxycarbamazepine[a]	1A,10B-dihydro-6H-dibenzo[B,F]oxireno[D]azepine-6-carboxamide	36507-30-9	Carbamazepine-d_{10}
	Acridone[a]	9(10H)-Acridinone	578-95-0	Carbamazepine-d_{10}
	Setraline	(1S,4S)-4-(3,4-dichlorophenyl)-N-methyl-1,2,3,4-tetrahydronaphthalen-1-amine	79559-97-0	Fluoxetine_d_5
	Citalopram	1-[3-(Dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile	59729-32-7	Citalopram-d_4
	Venlafaxine	1-[2-(Dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol	99300-78-4	Venlafaxine_d_6
	Olanzapine	2-Methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzodiazepine	132539-06-1	Carbamazepine-d_{10}

	Trazadone	2-{3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}[1,2,4]triazolo[4,3-a]pyridin-3(2H)-one	25332-39-2	Fluoxetine_d_5
	Fluoxetine	N-Methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]-1-propanamine	56296-78-7	Fluoxetine_d_6
	Norfluoxetine[a]	3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine	83891-03-6	Fluoxetine_d_7
	Paroxetine	(3S,4R)-3-[(2H-1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine	110429-35-1	Fluoxetine_d_8
	Diazepam	7-Chloro-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one	439-14-5	Diazepam_d_5
	Lorazepam	7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2H-1,4-benzodiazepin-2-one	846-49-1	Diazepam_d_5
	Alprazolam	8-Chloro-1-methyl-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine	28981-97-7	Diazepam_d_5
Histamine H1 and H2 receptor antagonist (5)	Loratadine	Ethyl 4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidinecarboxylate	79794-75-5	Cimetidine_d_3
	Desloratadine[a]	8-Chloro-11-(4-piperidinylidene)-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridine	100643-71-8	Cimetidine_d_3
	Ranitidine	N-(2-[(5-[(dimethylamino)methyl]furan-2-yl)methylthio]ethyl)-N'-methyl-2-nitroethene-1,1-diamine	66357-59-3	Cimetidine_d_3
	Famotidine	3-[({2-[(diaminomethylidene)amino]-1,3-thiazol-4-yl}methyl)sulfanyl]-N-sulfamoylpropanimidamide	76824-35-6	Cimetidine_d_3
	Cimetidine	1-Cyano-2-methyl-3-(2-{[(4-methyl-1H-imidazol-5-yl)methyl]sulfanyl}ethyl)guanidine	51481-61-9	Cimetidine_d_3
β-Blocking agents (6)	Atenolol	2-{4-[2-Hydroxy-3-(isopropylamino)propoxy]phenyl}acetamide	29122-68-7	Atenolol_d_7
	Sotalol	N-{4-[1-Hydroxy-2-(isopropylamino)ethyl]phenyl}methanesulfonamide	959-24-0	Atenolol_d_7
	Propanolol	1-(Isopropylamino)-3-(1-naphthyloxy)-2-propanol	318-98-9	Atenolol_d_7
	Metoprolol	1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]-2-propanol	56392-17-7	Atenolol_d_7
	Nadolol	(2R,3S)-5-{2-Hydroxy-3-[(2-methyl-2-propanyl)amino]propoxy}-1,2,3,4-tetrahydro-2,3-naphthalenediol	42200-33-9	Atenolol_d_7
	Carazolol	1-(9H-Carbazol-4-yloxy)-3-(isopropylamino)-2-propanol	57775-29-8	Atenolol_d_7
Diuretic (3)	Hydrochlorothiazide	6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide	58-93-5	Hydrochlorothyazide_d_2
	Furosemide	4-Chloro-2-[(2-furylmethyl)amino]-5-sulfamoylbenzoic acid	54-31-9	Furosemide_d_5
	Torasemide	N-(Isopropylcarbamoyl)-4-[(3-methylphenyl)amino]-3-pyridinesulfonamide	56211-40-6	Furosemide_d_5
Antidiabetic (1)	Glibenclamide	5-Chloro-N-(2-{4-[(cyclohexylcarbamoyl)sulfamoyl]phenyl}ethyl)-2-methoxybenzamide	10238-21-8	Glibenclamide_d_3
Antihypertensives (4)	Amlodipine	3-Ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydro-3,5-pyridinedicarboxylate	111470-99-6	Amlodipine_d_4
	Losartan	(2-Butyl-4-chloro-1-{[2'-(1H-tetrazol-5-yl)-4-biphenylyl]methyl}-1H-imidazol-5-yl)methanol	124750-99-8	Valsartan_d_8
	Irbesartan	2-Butyl-3-{[2'-(1H-tetrazol-5-yl)-4-biphenylyl]methyl}-1,3-diazaspiro[4.4]non-1-en-4-one	138402-11-6	Valsartan_d_8
	Valsartan	N-Pentanoyl-N-{[2'-(1H-tetrazol-5-yl)-4-biphenylyl]methyl}-L-valine	137862-53-4	Valsartan_d_8
Antiplatelet agent (1)	Clopidogrel	Methyl (2S)-(2-chlorophenyl)(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetate	135046-48-9	Glibenclamide_d_3
Prostatic hyperplasia	Tamsulosin	5-[(2R)-2-{[2-(2-Ethoxyphenoxy)ethyl]amino}propyl]-2-methoxybenzenesulfonamide	106463-17-	Sulfamethoxazole_d_4

(1)			6	
To treat asthma (1)	Salbutamol	2-(Hydroxymethyl)-4-{1-hydroxy-2-[(2-methyl-2-propanyl)amino]ethyl}phenol	18559-94-9	Atenolol_d_7
Anticoagulant (1)	Warfarin	4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one	81-81-2	Warfarin_d5
X-ray contrast agent (1)	Iopromide	N,N'-Bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-[(methoxyacetyl)amino]-N-methylisophthalamide	73334-07-3	Sulfamethoxazole_d_4
Antihelmintics (3)	Albendazole	Methyl [5-(propylsulfanyl)-1H-benzimidazol-2-yl]carbamate	54965-21-8	Ronidazole_d_3
	Thiabendazole	2-(1,3-Thiazol-4-yl)-1H-benzimidazole	148-79-8	Ronidazole_d_3
	Levamisol	(6*S*)-6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-b][1,3]thiazole	16595-80-5	Ronidazole_d_3
Synthetic glucocorticoid (1)	Dexamethasone	(11β,16α)-9-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione	50-02-2	Dexamtehasone_d_4
Sedation and muscle relaxation (1)	Xylazine	N-(2,6-Dimethylphenyl)-5,6-dihydro-4H-1,3-thiazin-2-amine	23076-35-9	Xylazine_d_6
Tranquilizers (2)	Azaperone	1-(4-Fluorophenyl)-4-[4-(2-pyridinyl)-1-piperazinyl]-1-butanone	1649-18-9	Azaperone_d_4
	Azaperol[a]	1-(4-Fluorophenyl)-4-[4-(2-pyridinyl)-1-piperazinyl]-1-butanol	330310	Azaperone_d_4
Antibiotics (13)	Erythromycin	trahydro-2H-pyran-2-yl]oxy}-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione	59319-72-1	Erythromycin_N,N$^{13}C_2$
	Azithromycin	(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-Ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-15-oxo-11-{[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy}-1-oxa-6-azacyclopentadecan-13-yl 2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranoside	83905-01-5	Azithromycin_d_3
	Clarithromycin	3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-6-{[(2S,3R,4S,6R)-4-(Dimethylamino)-3-hydroxy-6-ethyltetrahydro-2H-pyran-2-yl]oxy}-14-ethyl-12,13-dihydroxy-4-{[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyltetra hydro-2H-pyran-2-yl]oxy}-7-methoxy-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione	81103-11-9	Azithromycin_d_3
	Tetracycline	(4S,4aS,5aS,6S,12aS)-4-(Dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-2-tetracenecarboxamide	64-75-5	Sulfamethoxazole_d_4
	Ofloxacin	9-Fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid	82419-36-1	Ofloxacine_d_3
	Ciprofloxacin	1-Cyclopropyl-6-fluoro-4-oxo-7-(1-piperazinyl)-1,4-dihydro-3-quinolinecarboxylic acid	85721-33-1	Ofloxacine_d_3
	Cefalexin	(6R,7R)-7-{[(2R)-2-Amino-2-phenylacetyl]amino}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	15686-71-2	Sulfamethoxazole_d_4
	Sulfamethoxazole	4-Amino-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide	723-46-6	Sulfamethoxazole_d_4
	Trimethoprim	5-(3,4,5-Trimethoxybenzyl)-2,4-pyrimidinediamine	738-70-5	Sulfamethoxazole_d_4
	Metronidazole	2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethanol	443-48-1	Ronidazole_d_3
	Metronidazole OH[a]	2-[2-(Hydroxymethyl)-5-nitro-1H-imidazol-1-yl]ethanol	4812-40-2	Ronidazole_d_3
	Dimetridazole	1,2-Dimethyl-5-nitro-1H-imidazole	551-92-8	Ronidazole_d_3
	Ronidazole	(1-Methyl-5-nitro-1H-imidazol-2-yl)methyl carbamate	7681-76-7	Ronidazole_d_3
Calcium channel blockers (3)	Diltiazem	(2S,3S)-5-[2-(Dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate	42399-41-7	Carbamazepine-d_{10}
	Verapamil	2-(3,4-Dimethoxyphenyl)-5-{[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino}-2-isopropylpentanenitrile	152-11-4	Verpamil_d_6

Norverapamil[a]	2-(3,4-Dimethoxyphenyl)-5-{[2-(3,4-dimethoxyphenyl)ethyl]amino}-2-isopropylpentanenitrile	67812-42-4	Verpamil_d_6

[a]Metabolites

Table S2. Sampling locations and information about three sampling campaigns.

Number of sampling site	Acronym	Sample Name	Type of water	X Coordinates*	Y Coordinates*	Autumn season October-November 2015		Winter season February-April 2016		Spring season May-June 2016	
				** WGS84*		water	sediment	water	sediment	water	sediment
1	**INF1**	Amposta WWTP IN	influent	0.608608	40.704057	✓	x	✓	x	✓	x
2	**EFF1**	Amposta WWTP OUT	effluent	0.608608	40.704057	✓	x	✓	x	✓	x
3	**INF2**	Sant Carles de la Ràpita WWTP IN	influent	0.622121	40.627036	✓	x	✓	x	✓	x
4	**EFF2**	Sant Carles de la Ràpita WWTP OUT	effluent	0.622121	40.627036	✓	x	✓	x	✓	x
5	**CSW/CSS**	Upstream of Amposta (Xerta town)	river	0.518484	40.976154	✓	✓	✓	✓	✓	✓
6	**RW/RS1**	Downstream of Amposta	river	0.689317	40.713142	✓	✓	✓	✓	✓	✓
7	**RW/RS2**	Sant Carles de la Ràpita emissary	emissary	0.622754	40.622579	✓	✓	✓	✓	✓	✓
8	**CW/CS1**	Channel A (irrigation channel)	channel	0.809254	40.706929	✓	✓	✓	✓	✓	✓
9	**CW/CS2**	Channel B (irrigation channel)	channel	0.806554	40.696697	✓	✓	x	x	✓	✓
10	**CWCS3**	Channel C (drainage channel)	channel	0.598582	40.622214	✓	✓	✓	✓	✓	✓
11	**CW/CS4**	Channel D1 (drainage channel)	channel	0.647442	40.631728	✓	✓	✓	✓	✓	✓
12	**CW/CS5**	Channel D2 (drainage channel)	channel	0.681305	40.642791	✓	✓	✓	✓	✓	✓
13	**CW/CS6**	Channel D3 (drainage channel)	channel	0.789064	40.661281	✓	✓	✓	✓	✓	✓
14	**CW/CS7**	Channel D4 (drainage channel)	channel	0.717146	40.646098	✓	✓	✓	✓	✓	✓
15	**SW/SS1**	Alfacs bay A (onshore)	seawater	0.724525	40.610015	✓	✓	✓	✓	✓	✓
16	**SW/SS2**	Alfacs bay B (onshore)	seawater	0.610270	40.601432	✓	✓	✓	✓	✓	✓
17	**SW/SS3**	Alfacs bay C (offshore)	seawater	0.579456	40.584421	✓	✓	✓	✓	✓	✓
18	**SW/SS4**	Alfacs bay D (offshore)	seawater	0.609279	40.605240	x	x	✓	✓	✓	✓
19	**SW/SS5**	Fangar bay A (onshore)	seawater	0.700231	40.800592	✓	✓	✓	✓	✓	✓
20	**SW/SS6**	Fangar bay B (onshore)	seawater	0.713269	40.079869	✓	✓	✓	✓	✓	✓
21	**SW/SS7**	Fangar bay C (offshore)	seawater	0.743906	40.815106	✓	✓	✓	✓	✓	✓
22	**SW/SS8**	Fangar bay D (offshore)	seawater	0.751861	40.817899	x	x	✓	✓	✓	✓
23	**EW/ES1**	La Tancada lagoon (onshore)	estuary	0.742459	40.645.082	✓	✓	✓	✓	✓	✓
24	**EW/ES2**	La Tancada lagoon (offshore)	estuary	0.742599	40.645.143	✓	✓	✓	✓	✓	✓

25	**EW/ES3**	Illa de Buda lagoon (onshore)	estuary	0.851054	40.695.650	✓	✓	✓	✓	✓	✓
26	**EW/ES4**	Illa de Buda lagoon (offshore)	estuary	0.841655	40.703.963	✓	✓	✓	✓	✓	✓
27	**EW/ES5**	L'Encanyissada lagoon (onshore)	estuary	0.673552	40.657.110	✓	✓	✓	✓	✓	✓
28	**EW/ES6**	L'Encanyissada lagoon (offshore)	estuary	0.670567	40.653.922	✓	✓	✓	✓	✓	✓
29	**EW/ES7**	Canal Vell lagoon (onshore)	estuary	0.788320	40.745.011	✓	✓	✓	✓	✓	✓

✓ available sampling points x- not available sampling points

Table S3. Physicochemical parameters measured for water (T, pH, O_2, conductivity, salinity, and flow) and sediment samples (TOC).

Sampling site	1st sampling campaign-Autumn							2nd sampling campaign-Winter							3rd sampling campaign-Spring						
	T (ºC)	pH	O_2 (mg/L)	Conductivity (uS/cm)	Salinity (ppt)	Flow* (m3/s)	TOC (%)	T (ºC)	pH	O_2 (mg/L)	Conductivity (uS/cm)	Salinity (ppt)	Flow* (m3/s)	TOC (%)	T (ºC)	pH	O_2 (mg/L)	Conductivity (uS/cm)	Salinity (ppt)	Flow* (m3/s)	TOC (%)
Amposta WWTP IN	21,7	7,8	9,0	1362	0,7	N/D	x	11,9	8,2	8,6	1290	0,6	N/D	x	23,5	7,9	9,6	682	0,3	N/D	x
Amposta WWTP OUT	20,3	7,6	7,8	2690	1,4	N/D	x	12,6	7,7	9,6	1344	0,6	N/D	x	24,7	8,5	N/A	1433	0,7	N/D	x
Sant Carles de la Ràpita WWTP IN	20,9	7,4	7,4	20046	11,9	N/D	x	14,6	7,8	12,9	3837	2,6	N/D	x	28,0	7,7	N/A	3940	2,1	N/D	x
Sant Carles de la Ràpita WWTP OUT	21,8	7,8	8,0	1190	0,6	N/D	x	13,7	8,4	9,1	1332	0,6	N/D	x	25,8	8,7	N/A	671	0,3	N/D	x
Upstream of Amposta (Xerta town)	21,9	7,9	9,7	1189	0,6	161,0	3,1	N/A	N/A	N/A	N/A	1,2	447,0	2,4	25,9	8,5	N/A	672	0,3	166,0	0,6
Downstream of Amposta	21,3	7,5	12,9	2933	1,5	161,0	2,5	19,0	7,6	15,1	2791	1,7	447,0	1,9	23,6	7,7	12,8	2151	1,1	166,0	1,0
Sant Carles de la Ràpita emissary	17,1	7,1	5,1	2100	1,1	N/D	3,2	15,3	7,8	10,9	2765	1,8	N/D	3,9	23,7	7,6	7,1	2595	1,4	N/D	4,8
Channel A (irrigation channel)	17,0	7,3	6,6	1800	0,9	19,0	1,6	20,1	7,9	20,9	27049	18,5	19,0	5,6	22,7	7,5	6,4	3801	2,1	19,0	5,9
Channel B (irrigation channel)	14,9	7,2	6,2	1500	0,8	25,0	1,7	12,0	7,3	10,4	8762	6,7	x	x	24,6	7,8	7,4	1451	0,7	30,0	2,1
Channel C (drainage channel)	14,9	7,4	4,2	1476	0,7	N/D	3,2	16,8	8,2	25,6	13499	9,5	N/D	4,7	25,2	7,6	13,3	17502	10,3	N/D	3,1
Channel D1 (drainage channel)	17,3	7,6	7,4	35971	22,7	N/D	4,3	N/A	N/A	N/A	N/A	29,5	N/D	5,6	31,6	8,2	7,7	65759	38,4	N/D	4,9
Channel D2 (drainage channel)	N/A	N/A	N/A	N/A	30,3	N/D	3,2	10,7	7,9	11,2	37256	33,5	N/D	3,6	28,1	8,0	6,4	59303	37,1	N/D	1,5
Channel D3 (drainage channel)	19,2	7,8	7,0	45332	29,3	N/D	4,3	12,2	7,9	10,5	39462	34,3	N/D	5,8	28,0	8,0	6,1	59344	37,2	N/D	3,3
Channel D4 (drainage channel)	N/A	N/A	N/A	N/A	29,8	N/D	2,6	12,6	7,8	10,1	40346	34,7	N/D	3,8	27,5	8,0	6,5	59939	37,9	N/D	2,9
Alfacs bay A (onshore)	20,4	7,8	8,0	47732	31,1	N/D	1,6	12,8	7,8	10,9	40073	35,1	N/D	3,1	28,0	8,0	7,2	60021	37,7	N/D	0,6
Alfacs bay B (onshore)	17,9	7,8	8,1	41647	26,7	N/D	0,7	12,0	7,8	10,5	40554	35,3	N/D	1,8	28,2	8,0	8,4	49691	30,5	N/D	0,6
Alfacs bay C (offshore)	19,9	7,8	7,9	48564	31,7	N/D	2,2	12,5	7,7	10,4	40719	35,2	N/D	1,9	28,1	8,0	7,3	50621	31,8	N/D	1,0
Alfacs bay D (offshore)	N/A	N/A	N/A	N/A	30,1	N/D	x	12,6	7,7	12,0	40892	35,3	N/D	2,3	27,9	8,0	8,0	55036	34,6	N/D	0,7
Fangar bay A (onshore)	17,3	8,3	9,1	32900	20,6	N/D	2,2	14,7	7,8	15,0	40460	33,0	N/D	1,6	29,2	8,4	12,6	47320	28,1	N/D	0,1
Fangar bay B (onshore)	17,3	8,3	10,9	32423	20,2	N/D	1,9	14,0	7,8	12,3	40227	33,4	N/D	1,5	28,9	8,1	8,3	47068	28,2	N/D	1,0
Fangar bay C (offshore)	20,7	7,7	11,6	14907	27,8	N/D	1,3	13,3	7,9	25,0	7266	34,4	N/D	1,7	27,2	7,9	7,3	5133	29,1	N/D	1,0
Fangar bay D (offshore)	20,3	7,8	10,1	14978	31,2	N/D	x	13,2	8,2	43,5	7713	34,7	N/D	1,5	25,5	8,3	9,3	8560	32,2	N/D	0,8
La Tancada lagoon (onshore)	18,7	7,5	10,5	21800	13,1	N/D	1,9	10,1	8,5	37,2	44222	33,0	N/D	2,5	24,5	8,0	5,6	35990	23,1	N/D	1,5
La Tancada lagoon (offshore)	18,7	7,9	12,6	22775	13,7	N/D	1,4	10,4	8,3	27,9	44059	32,9	N/D	3,4	23,5	8,2	8,1	35088	22,8	N/D	2,2

Illa de Buda lagoon (onshore)	17,3	8,1	14,7	27426	16,8	N/D	2,2	16,9	8,4	22,3	32431	24,2	N/D	5,1	27,5	8,1	13,9	20725	11,8	N/D	3,6
Illa de Buda lagoon (offshore)	17,7	8,2	14,3	26726	16,5	N/D	1,9	16,5	8,5	22,5	32431	25,2	N/D	4,0	25,5	8,3	15,9	20725	13,1	N/D	3,7
L'Encanyissada lagoon (onshore)	18,3	7,8	11,7	21699	13,8	N/D	2,2	12,1	7,9	25,6	43193	31,9	N/D	3,4	25,7	7,2	14,7	35990	12,8	N/D	3,3
L'Encanyissada lagoon (offshore)	18,3	7,9	11,9	22725	14,1	N/D	1,9	12,4	7,8	25,3	43245	30,9	N/D	3,4	25,5	7,4	14,2	35088	12,8	N/D	2,6
Canal Vell lagoon (onshore)	17,3	8,2	14,4	21698	17,3	N/D	2,9	11,4	7,4	26,3	32431	29,5	N/D	3,0	26,3	8,1	13,9	20725	11,9	N/D	3,6

N/A: not analysed. N/D: no data available. *Media flow values obtained from SAIH Ebrox - not available sampling point

Table S4. Target compounds and their optimized UPLC-QqLIT-MS/MS parameters by: (a) positive (PI) and (b) negative (NI) ionization mode.

a)

Compounds analyzed under PI mode	Rt (min)	Precursor ion (m/z)	Quantification		Confirmation		Ion ratio (±SD) n=5
			Q3	DP/CE/CXP	Q3	DP/CE/CXP	
Metronidazole-OH	0.96	187 $[M+H]^+$	126	51/23/18	123	51/19/16	1.2 (±0.05)
Sotalol	1.10	273 $[M+H]^+$	255	51/17/12	133	51/37/12	1.2 (±0.13)
Salbutamol	1.20	240 $[M+H]^+$	148	46/27/18	122	46/15/10	1.7 (±0.27)
Atenolol-d_7 (IS)	1.20	274 $[M+H]^+$	145	61/37/10	-	-	-
Ronidazole	1.22	201 $[M+H]^+$	140	46/17/14	-	-	-
Atenolol	1.22	267 $[M+H]^+$	145	91/27/18	190	91/37/14	1.3 (±0.03)
Ronidazole-d_3 (IS)	1.23	204 $[M+H]^+$	143	46/17/18	-	-	-
Metronidazole	1.24	172 $[M+H]^+$	128	56/35/10	82	56/21/10	1.1 (±0.09)
Ranitidine	1.24	315 $[M+H]^+$	176	66/25/24	130	66/35/12	1.1 (±0.07)
Famotidine	1.24	338 $[M+H]^+$	189	61/29/22	259	61/17/12	1.1 (±0.07)
Cimetidine-d_3 (IS)	1.26	256 $[M+H]^+$	95	81/39/14	-	-	-
Cimetidine	1.28	253 $[M+H]^+$	159	41/21/24	95	41/37/12	1.1 (±0.04)
Codeine	1.36	300 $[M+H]^+$	152	61/87/12	115	61/101/16	1.2 (±0.16)
Oxycodone	1.45	316 $[M+H]^+$	298	71/27/10	241	71/41/18	3.5 (±0.23)
Levamisol	1.46	205 $[M+H]^+$	178	41/31/14	91	41/59/14	1.1 (±0.02)
Dimetridazole	1.48	142 $[M+H]^+$	96	61/23/14	95	61/31/14	1.0 (±0.06)
Trimethoprim	1.73	291 $[M+H]^+$	230	91/33/12	261	91/35/10	1.4 (±0.05)
Cefalexin	1.74	348 $[M+H]^+$	158	31/15/24	106	31/43/12	1.7 (±0.17)
Nadolol	1.88	310 $[M+H]^+$	254	81/25/12	201	81/31/16	2.3 (±0.13)
Ofloxacin-d_3 (IS)	1.90	365 $[M+H]^+$	321	96/27/12	-	-	-
Ofloxacin	1.90	362 $[M+H]^+$	318	86/27/12	261	86/39/12	1.2 (±0.12)
Sulfamethoxazole-d_4 (IS)	1.96	258 $[M+H]^+$	160	101/23/18	-	-	-
Sulfamethoxazole	1.98	254 $[M+H]^+$	92	81/37/12	156	81/23/12	1.1 (±0.03)
Phenazone-d_3 (IS)	2.04	192 $[M+H]^+$	59	41/51/8	-	-	-
Phenazone	2.05	189 $[M+H]^+$	77	76/57/10	56	76/53/10	1.2 (±0.07)
Sulfadoxine-d_3 (surrogate)	2.06	314 $[M+H]^+$	156	51/25/12	-	-	-
Xylazine-d_6 (IS)	2.10	227 $[M+H]^+$	90	61/33/14	-	-	-
Xylazine	2.11	221 $[M+H]^+$	90	66/31/10	77	66/83/12	1.4 (±0.09)
Metoprolol	2.20	268 $[M+H]^+$	133	86/35/12	121	111/33/18	1.3 (±0.04)
Thiabendazole	2.33	202 $[M+H]^+$	175	76/37/28	131	76/45/10	1.1 (±0.05)
Tamsulosin	2.45	409 $[M+H]^+$	228	86/33/16	200	86/45/32	1.2 (±0.04)
Sulfadimethoxine-d_6 (surrogate)	2.49	317 $[M+H]^+$	162	61/33/8	-	-	-
Carazolol	2.52	299 $[M+H]^+$	116	66/27/14	222	66/29/10	2.4 (±0.21)
Trazodone	2.63	372 $[M+H]^+$	176	86/35/14	148	86/35/14	1.8 (±0.05)
Venlafaxine-d_6 (IS)	2.74	284 $[M+H]^+$	64	96/61/10	-	-	-
Venlafaxine	2.75	278 $[M+H]^+$	58	66/55/10	260	66/17/12	1.5 (±0.45)
Propranolol	2.86	260 $[M+H]^+$	116	101/25/12	183	76/25/24	1.3 (±0.05)
Citalopram-d_4 (IS)	2.89	329 $[M+H]^+$	113	21/35/14	-	-	-
Citalopram	2.90	325 $[M+H]^+$	109	56/35/16	262	56/27/12	1.4 (±0.06)
Norfluoxetine	2.93	296 $[M+H]^+$	134	61/11/12	-	-	-
Acridone	3.00	196 $[M+H]^+$	166	141/61/14	167	141/47/16	1.1 (±0.03)
Verapamil-d_6 (IS)	3.12	461 $[M+H]^+$	165	66/37/22	-	-	-
Norverapamil	3.12	441 $[M+H]^+$	165	101/35/10	150	101/57/12	3.1 (±0.46)
Verapamil	3.13	455 $[M+H]^+$	165	116/37/14	77	116/129/12	2.2 (±0.20)
Diltiazem	3.13	415 $[M+H]^+$	178	91/35/10	109	91/35/10	26.2 (±6.94)
Carbamazepine-d_{10} (IS)	3.16	247 $[M+H]^+$	204	46/31/32	-	-	-
Desloratadine	3.16	311 $[M+H]^+$	259	61/31/12	258	61/53/18	1.1 (±0.03)
Carbamazepine	3.19	237$[M+H]^+$	194	61/29/28	193	61/49/14	1.3 (±0.26)
Propyphenazone	3.20	231 $[M+H]^+$	189	151/31/8	56	151/61/8	1.4 (±0.15)
Amlodipine-d_4 (IS)	3.25	413 $[M+H]^+$	238	61/17/36	-	-	-
Paroxetine	3.26	330 $[M+H]^+$	192	106/29/26	123	101/35/10	2.7 (±0.21)

Erythromycin	3.39	734 $[M+H]^+$	576	116/27/22	158	116/39/14	1.00 (±0.06)
Erythromycin-N,$N^{13}C_2$ (IS)	3.40	736 $[M+H]^+$	578	76/29/24	-	-	-
Alprazolam	3.43	309 $[M+H]^+$	281	86/37/42	205	76/53/20	1.2 (±0.03)
Fluoxetine-d_5 (IS)	3.46	315 $[M+H]^+$	44	76/53/8	-	-	1.7 (±0.08)
Fluoxetine	3.47	310 $[M+H]^+$	44	61/61/8	148	61/13/12	7.3 (±0.35)
Amlodipine	3.53	409 $[M+H]^+$	238	41/15/12	294	41/15/12	1.7 (±0.07)
Sertraline	3.60	307 $[M+H]^+$	159	66/41/16	276	66/17/44	2.1(±0.16)
Albendazole	3.70	266 $[M+H]^+$	234	46/29/12	191	46/47/18	1.4 (±0.09)
Diazepam-d_5 (IS)	3.75	290 $[M+H]^+$	198	101/47/26	-	-	-
Diazepam	3.76	285 $[M+H]^+$	193	86/45/16	154	86/37/20	1.7 (±0.08)
Warfarin-d_5 (IS)	3.78	314 $[M+H]^+$	163	56/21/28	-	-	-
Warfarin	3.79	309 $[M+H]^+$	163	66/21/18	251	66/27/12	1.0 (±0.02)
Glibenclamide	4.00	494 $[M+H]^+$	369	116/21/14	169	116/51/26	1.2 (±0.02)
Glibenclamide-d_3 (IS)	4.00	497 $[M+H]^+$	372	131/23/12	-	-	-
Clopidogrel	4.34	322 $[M+H]^+$	212	81/23/10	184	81/31/16	1.3 (±0.06)
Loratadine	4.37	383 $[M+H]^+$	337	86/33/12	267	86/45/10	2.4 (±0.05)
Metronidazole-OH	0.96	187 $[M+H]^+$	126	51/23/18	123	51/19/16	1.2 (±0.05)
Sotalol	1.10	273 $[M+H]^+$	255	51/17/12	133	51/37/12	1.2 (±0.13)
Salbutamol	1.20	240 $[M+H]^+$	148	46/27/18	122	46/15/10	1.7 (±0.27)
Atenolol-d_7 (IS)	1.20	274 $[M+H]^+$	145	61/37/10	-	-	-
Ronidazole	1.22	201 $[M+H]^+$	140	46/17/14	-	-	-
Atenolol	1.22	267 $[M+H]^+$	145	91/27/18	190	91/37/14	1.3 (±0.03)
Ronidazole-d_3 (IS)	1.23	204 $[M+H]^+$	143	46/17/18	-	-	-
Metronidazole	1.24	172 $[M+H]^+$	128	56/35/10	82	56/21/10	1.1 (±0.09)
Ranitidine	1.24	315 $[M+H]^+$	176	66/25/24	130	66/35/12	1.1 (±0.07)
Famotidine	1.24	338 $[M+H]^+$	189	61/29/22	259	61/17/12	1.1 (±0.07)
Cimetidine-d_3 (IS)	1.26	256 $[M+H]^+$	95	81/39/14	-	-	-
Cimetidine	1.28	253 $[M+H]^+$	159	41/21/24	95	41/37/12	1.1 (±0.04)
Codeine	1.36	300 $[M+H]^+$	152	61/87/12	115	61/101/16	1.2 (±0.16)

b)

Compounds analyzed under NI mode	Rt (min)	Precursor ion (m/z)	Quantification		Confirmation		Ion ratio (±SD) n=5
			Q3	DP/CE/CXP	Q3	DP/CE/CXP	
Acetaminophen-d_4 (IS)	0.55	154 $[M-H]^-$	111	-60/ -26/-7	-	-	-
Acetaminophen	0.56	150 $[M-H]^-$	107	-45/ -24/ -15	-	-	-
Salicylic acid	0.59	137 $[M-H]^-$	93	-50/ -20/ -1	-	-	-
Tenoxicam	0.90	336 $[M-H]^-$	152	-60/ -26/ -7	272	-60/ -16/ -11	1.2 (±0.13)
Piroxicam	0.93	330 $[M-H]^-$	146	-65/ -26/ -9	266	-65/ -18/ -11	1.2 (±0.07)
Valsartan	0.95	434 $[M-H]^-$	179	-105/ -30/ -9	350	-105/ -26/ -13	1.1 (±0.17)
Valsartan-d_8 (IS)	0.95	442 $[M-H]^-$	179	-105/ -32/-11	-	-	-
Naproxen	0.96	229 $[M-H]^-$	170	-30/ -20/ -9	185	-30/ -10/ -7	1.1 (±0.47)
Furosemide-d_5 (IS)	0.96	334 $[M-H]^-$	290	-40/ -22/-11	-	-	-
Furosemide	0.97	329 $[M-H]^-$	285	-95/ -20/ -11	205	-95/ -30/ -15	1.4 (±0.03)
Ketoprofen-d_3 (Surrogate)	1.00	256 $[M-H]^-$	212	-30/ -10/ -11	-	-	-
Pravastatin	1.00	423 $[M-H]^-$	321	-100/ -20/ -13	303	-100/ -24/ -13	1.5 (±0.03)
Ketoprofen	1.01	253 $[M-H]^-$	209	-30/ -12/ -11	-	-	-
Meloxicam-d_3 (IS)	1.05	353 $[M-H]^-$	289	-60/ -20/ -13	-	-	-
Meloxicam	1.06	350 $[M-H]^-$	146	-65/ -28/ -7	286	-65/ -18/ -15	1.2 (±0.07)
Bezafibrate-d_6 (IS)	1.09	366 $[M-H]^-$	280	-15/ -24/ -11	-	-	-
Bezafibrate	1.10	360 $[M-H]^-$	274	-55/ -38/ -9	154	-55/ -22/ -11	2.2 (±0.10)
Losartan	1.17	421 $[M-H]^-$	127	-105/ -40/ -5	179	-105/ -34/ -11	1.2 (±0.04)
Ibuprofen-d_3 (IS)	1.17	208 $[M-H]^-$	164	-55/ -10/ -7	-	-	-
Ibuprofen	1.18	205 $[M-H]^-$	161	-60/ -10/ -13	-	-	-
Diclofenac	1.25	294 $[M-H]^-$	250	-65/ -16/ -11	214	-65/ -28/ -11	16.5 (±5.05)
Indomethacine-d_4 (IS)	1.26	360 $[M-H]^-$	316	-35/ -14/-13	-	-	-
Indomethacine	1.27	356 $[M-H]^-$	312	-60/ -12/ -13	297	-60/ -26/ -13	3.4 (±0.04)
Irbesartan	1.28	427 $[M-H]^-$	193	-95/ -34/ -11	399	-95/ -26/ -19	7.1 (±0.59)
Dexamethasone	1.35	451 $[M-H]^-$	361	-85/ -24/ -15	307	-85/ -46/ -13	3.8 (±0.17)
Dexamethasone-d_4 (IS)	1.34	395 $[M-H]^-$	363	-5/ -18/ -15	-	-	-
Gemfibrozil-d_6 (IS)	1.39	255 $[M-H]^-$	121	-75/ -22/ -15	-	-	-
Gemfibrozil	1.40	249 $[M-H]^-$	121	-65/ -24/ -7	127	-65/ -14/ -9	13.7 (±0.65)
Fluvastatin	1.46	410 $[M-H]^-$	210	-90/ -40/ -9	348	-90/ -22/ -9	1.5 (±0.04)
Atorvastatin	1.52	557 $[M-H]^-$	278	-65/ -42/ -5	397	-65/ -62/ -7	1.2 (±0.03)

DP: Declustering Potential;

CE:Collision Energy;

CXP:Collision cell exit potential;

Table S5. Method performance parameters for the PhACs in different types of water studied: (a) recoveries (%), relative standard deviation (±RSD%, n=3).

a)

Thearpeutic group/Compound		Influent		Effluent		Estuary		River		Seawater	
		Rec	RSD	Rec	RSD	Rec	RSD	Rec	RSD	Rec	RSD
		%									
Analgesics/ant-inflammatories	Ketoprofen	83	15,4	132	13,3	65	7,4	75	5,8	93	3,8
	Naproxen	80	16,1	93	14,0	98	10,7	123	8,2	65	7,5
	Ibuprofen	66	8,8	89	7,9	167	5,8	124	3,6	130	6,9
	Indomethacine	107	12,3	108	5,6	120	2,5	111	1,8	51	4,7
	Acetaminophen	109	9,6	111	5,5	79	3,3	72	4,4	53	5,1
	Salicylic acid	110	11,9	133	2,9	126	4,0	52	2,0	54	4,9
	Diclofenac	182	17,6	84	13,3	142	3,8	59	1,2	108	8,3
	Phenazone	71	7,4	99	7,4	66	4,6	94	5,0	58	2,4
	Propylphenazone	78	8,4	95	4,7	145	2,1	98	6,3	126	1,8
	Piroxicam	88	7,7	93	8,9	147	10,1	87	7,8	87	12,4
	Meloxicam	97	7,2	103	8,6	94	6,1	80	3,7	75	4,0
	Tenoxicam	73	8,8	71	6,2	92	9,6	66	5,1	63	4,8
	Oxycodone	44	11,0	41	4,8	63	4,2	106	3,1	41	2,0
	Codeine	88	3,5	60	6,3	74	4,9	74	4,5	71	3,6
Lipid regulators and cholesterol lowering statin drugs	Bezafibrate	126	3,1	109	1,6	109	9,3	105	6,9	102	5,5
	Gemfibrozil	161	7,4	152	2,9	103	11,1	119	3,3	115	13,4
	Pravastatin	54	33,1	73	18,3	154	10,3	83	8,1	54	7,7
	Fluvastatin	77	2,6	67	4,6	46	6,1	49	5,5	36	5,6
	Atorvastatin	61	6,2	37	10,5	56	19,4	58	16,5	66	3,4
Psychiatric drugs	Carbamazepine	45	3,2	102	2,5	101	5,6	96	3,7	132	1,1
	2-HydroxyCBZ	158	9,3	107	3,9	81	7,5	97	3,8	129	1,4
	10,11-epoxyCBZ	130	7,5	113	3,2	64	6,8	99	3,4	120	2,8
	Acridone	50	6,3	55	1,7	52	5,9	77	2,6	58	1,6
	Setraline	68	5,8	68	8,7	75	9,4	68	8,8	83	6,2
	Citalopram	58	11,8	87	7,9	87	7,5	61	6,5	120	3,1
	Venlafaxine	64	3,3	68	5,0	113	5,6	101	5,4	49	1,1
	Olanzapine	91	12,6	86	9,8	92	9,3	63	7,2	83	9,0
	Trazadone	58	16,4	98	10,9	91	5,8	58	4,9	89	3,4
	Fluoxetine	69	17,3	58	8,9	67	7,6	58	5,8	86	3,9
	Norfluoxetine	64	11,2	92	10,9	87	8,2	69	7,5	115	5,5
	Paroxetine	104	12,9	107	10,0	118	10,7	97	8,3	45	9,7
	Diazepam	143	5,8	120	2,3	124	6,8	101	1,3	128	1,9
	Lorazepam	153	4,1	93	1,9	112	4,8	116	7,8	88	2,6
	Alprazolam	123	7,1	95	3,8	84	6,8	92	3,9	145	2,5
Histamine H_1 and H_2 receptor antagonist	Loratadine	79	17,6	48	13,4	67	8,2	63	7,9	60	6,1
	Desloratadine	30	16,1	65	12,5	45	10,2	59	10,5	106	8,3
	Ranitidine	94	11,5	90	7,0	73	6,2	78	6,5	136	6,9
	Famotidine	114	14,5	96	6,9	105	5,5	82	6,6	99	4,4
	Cimetidine	50	11,9	69	9,2	153	15,5	95	7,8	85	5,7
β-Blocking agents	Atenolol	32	9,1	47	5,9	65	7,6	47	4,3	51	3,5
	Sotalol	120	6,8	86	8,8	89	6,2	87	6,9	65	3,9
	Propanolol	58	11,7	59	14,7	53	10,5	69	9,3	124	4,9
	Metoprolol	65	8,0	95	8,7	61	9,1	74	7,3	137	10,1
	Nadolol	76	8,9	87	9,8	84	9,0	80	9,2	74	7,9
	Carazolol	46	12,7	60	12,4	62	8,8	76	9,3	123	3,6
Diuretic	Hydrochlorothiazide	72	2,6	81	2,0	102	5,9	87	5,1	91	1,1
	Furosemide	78	11,8	58	7,8	70	5,1	71	6,9	60	3,5
	Torasemide	46	18,2	76	11,4	39	9,5	85	3,1	78	8,2
Antidiabetic	Glibenclamide	119	6,2	66	6,5	101	9,9	95	9,1	69	1,9
Antihypertensives	Amlodipine	98	12,4	111	12,3	86	13,5	73	10,1	34	8,7

	Losartan	66	13,6	156	3,6	58	7,2	66	8,5	45	4,7
	Irbesartan	107	3,0	104	5,1	44	8,9	90	5,9	47	4,8
	Valsartan	114	10,6	122	6,6	89	3,5	122	5,0	103	4,8
Antiplatelet agent	Clopidogrel	102	16,5	31	1,4	101	5,9	58	4,8	80	2,3
Prostatic hyperplasia	Tamsulosin	36	13,8	31	2,7	79	6,6	77	7,2	58	1,7
To treat asthma	Salbutamol	34	14,7	104	7,3	138	11,1	88	9,9	32	8,7
Anticoagulant	Warfarin	119	6,3	105	5,4	94	6,8	97	5,5	136	2,5
X-ray contrast agent	Iopromide	38	9,8	59	8,9	137	11,5	127	13,4	56	11,7
Antihelmintics	Albendazole	86	6,8	102	5,3	81	11,1	78	6,3	117	3,6
	Thiabendazole	114	8,4	105	7,1	54	6,7	61	3,9	125	4,3
	Levamisol	75	6,4	61	2,5	86	1,3	66	4,6	86	3,4
Synthetic glucocorticoid	Dexamethasone	91	6,4	89	13,5	76	7,6	90	9,0	115	8,8
Sedation and muscle relaxation	Xylazine	67	7,1	84	1,7	72	5,3	98	1,1	59	2,3
Tranquilizers	Azaperone	36	21,8	108	2,9	55	21,6	48	2,9	69	4,5
	Azaperol	44	9,3	67	3,6	42	18,3	75	18,4	72	6,8
Antibiotics	Erythromycin	47	5,0	41	11,8	67	23,6	59	8,9	99	8,8
	Azithromycin	183	29,2	60	1,8	125	9,0	70	19,5	89	11,0
	Clarithromycin	56	5,5	78	4,1	51	12,7	54	18,2	134	1,8
	Tetracycline	156	4,6	128	15,5	52	8,8	83	7,2	73	3,1
	Ofloxacin	129	3,9	63	2,2	149	1,2	89	15,4	82	7,9
	Ciprofloxacin	111	3,7	72	2,1	84	8,4	50	13,7	68	5,5
	Cefalexin	65	5,3	44	7,5	76	4,2	48	7,5	64	5,6
	Sulfamethoxazole	92	9,4	55	3,3	74	2,0	98	8,8	62	1,4
	Trimethoprim	34	6,8	59	3,2	45	6,7	97	0,7	54	1,6
	Metronidazole	158	16,0	75	12,5	139	13,8	64	8,5	72	12,0
	Metronidazole OH	76	7,5	66	11,4	61	5,5	89	8,6	109	1,7
	Dimetridazole	66	15,2	48	14,3	56	3,6	94	7,3	63	3,5
	Ronidazole	122	2,8	79	2,0	60	2,9	72	3,4	82	1,4
Calcium channel blockers	Diltiazem	146	15,9	101	5,7	79	8,1	86	3,7	109	1,7
	Verapamil	61	13,6	103	3,8	98	2,6	100	4,0	68	3,5
	Norverapamil	83	9,4	127	5,6	42	3,2	59	7,3	61	5,1

Table S6. Method performance parameters for PhACs in different types of sediment studied: (a) recoveries (%) and relative standard deviation (RSD% for n=3).

a)

Thearpeutic group/Compound		River sediment		Channel sediment		Estuary sediment		Sea sediment	
		Rec	RSD	Rec	RSD	Rec	RSD	Rec	RSD
		%							
Analgesics/ant-inflammatories	Ketoprofen	58	15,5	75	2,3	51	15,4	67	22,5
	Naproxen	88	2,9	82	10,5	97	10,6	79	7,6
	Ibuprofen	43	12,9	52	9,7	60	11,6	53	3,7
	Indomethacine	56	4,9	55	12,7	73	16,4	55	11,1
	Acetaminophen	101	10,5	61	14,2	57	2,5	78	2,2
	Salicylic acid	66	8,1	74	6,3	99	6,5	111	2,8
	Diclofenac	41	5,7	33	6,0	61	7,1	41	14,9
	Phenazone	23	6,3	20	7,3	24	8,7	20	9,0
	Propylphenazone	29	2,9	28	5,4	32	9,6	28	5,4
	Piroxicam	65	9,5	55	2,3	49	3,4	42	3,8
	Meloxicam	57	1,1	65	10,9	53	6,8	49	15,6
	Tenoxicam	63	3,0	78	8,7	54	6,9	66	6,5
	Oxycodone	28	2,2	25	9,3	27	9,7	24	5,3
	Codeine	63	6,4	55	4,3	45	7,4	43	9,0
Lipid regulators and cholesterol lowering statin drugs	Bezafibrate	55	0,9	58	10,8	49	11,2	56	11,6
	Gemfibrozil	49	2,4	32	5,7	40	8,5	34	10,0
	Pravastatin	76	11,2	66	4,,4	76	6,4	37	6,5
	Fluvastatin	53	6,8	57	5,3	55	15,2	66	5,7
	Atorvastatin	73	3,6	66	8,9	54	25,6	78	15,2
Psychiatric drugs	Carbamazepine	79	2,9	60	7,0	37	9,4	36	12,3
	2-HydroxyCBZ	50	12,5	56	11,6	37	10,6	34	10,4
	10,11-epoxyCBZ	43	10,5	34	14,6	34	12,8	29	16,8
	Acridone	67	3,1	59	5,6	40	11,0	37	9,5
	Setraline	72	8,6	63	12,9	52	8,2	37	18,7
	Citalopram	33	14,5	37	11,2	31	23,0	39	10,7
	Venlafaxine	17	10,9	14	8,8	20	8,4	16	3,0
	Olanzapine	57	11,2	77	5,3	40	1,9	69	7,8
	Trazadone	25	2,0	23	12,4	22	9,7	17	8,2
	Fluoxetine	38	5,5	25	8,7	35	2,2	31	3,8
	Norfluoxetine	17	10,9	14	8,8	20	8,4	16	3,0
	Paroxetine	91	5,8	78	7,6	80	0,9	89	9,1
	Diazepam	29	3,7	29	6,0	31	8,4	29	5,5
	Lorazepam	49	10,1	66	11,9	53	5,8	57	15,3
	Alprazolam	17	7,9	24	7,3	20	12,0	21	15,4
Histamine H_1 and H_2 receptor antagonist	Loratadine	41	0,9	27	12,3	32	11,2	26	9,7
	Desloratadine	40	14,4	34	18,2	50	1,3	38	12,2
	Ranitidine	58	13,3	57	10,4	74	18,0	62	10,6
	Famotidine	73	4,2	70	3,8	49	6,0	55	12,6
	Cimetidine	83	4,9	70	7,8	84	15,8	64	17,0
β-Blocking agents	Atenolol	57	6,4	65	11,6	47	6,6	50	6,3
	Sotalol	59	7,0	56	7,5	57	9,7	51	6,3
	Propanolol	27	1,6	23	8,0	26	9,1	38	5,2
	Metoprolol	72	2,3	64	4,1	43	5,1	53	5,2
	Nadolol	64	8,1	75	9,9	68	11,6	62	7,8
	Carazolol	41	2,6	54	14,6	37	7,0	27	7,9
Diuretic	Hydrochlorothiazide	77	10,0	79	21,1	73	6,1	56	21,2
	Furosemide	77	13,2	75	1,8	52	5,7	66	18,9
	Torasemide	78	2,6	80	10,6	89	9,3	63	8,1

Antidiabetic	Glibenclamide	56	2,6	37	8,2	53	11,1	43	7,7
Antihypertensives	Amlodipine	34	1,2	31	4,0	36	2,8	34	2,9
	Losartan	58	6,1	45	8,9	65	6,5	53	14,6
	Irbesartan	63	10,3	65	8,0	69	10,9	56	10,8
	Valsartan	72	4,3	80	3,1	37	1,7	58	1,6
Antiplatelet agent	Clopidogrel	26	1,9	22	8,7	26	7,6	23	6,6
Prostatic hyperplasia	Tamsulosin	24	1,9	18	2,3	25	9,5	21	7,2
To treat asthma	Salbutamol	51	8,7	60	16,9	38	13,8	42	25,7
Anticoagulant	Warfarin	34	1,9	33	4,6	38	8,8	32	6,2
X-ray contrast agent	Iopromide	57	5,6	45	7,9	57	14,2	42	11,8
Antihelmintics	Albendazole	49	4,0	37	14,7	43	4,8	31	8,1
	Thiabendazole	40	3,2	37	21,6	32	10,7	46	10,0
	Levamisol	28	4,0	25	7,9	19	2,7	18	7,2
Synthetic glucocorticoid	Dexamethasone	59	0,8	57	12,2	46	7,3	54	21,6
Sedation and muscle relaxation	Xylazine	37	5,4	40	18,0	20	13,8	25	10,2
Tranquilizers	Azaperone	41	8,2	44	7,5	38	4,0	36	8,3
	Azaperol	37	6,4	42	3,9	20	8,6	26	3,5
Antibiotics	Erythromycin	19	7,1	17	8,2	16	15,4	11	7,9
	Azithromycin	42	2,9	40	1,0	43	5,9	44	2,6
	Clarithromycin	34	4,2	28	5,4	40	7,1	32	8,8
	Tetracycline	16	6,1	19	9,7	11	16,4	15	9,7
	Ofloxacin	50	5,2	40	2,1	46	13,7	47	5,9
	Ciprofloxacin	69	8,1	53	5,5	58	12,0	57	5,6
	Cefalexin	23	6,8	25	14,3	22	17,8	18	8,9
	Sulfamethoxazole	18	8,7	17	21,5	16	5,0	19	3,4
	Trimethoprim	26	4,8	28	8,2	21	14,5	19	12,3
	Metronidazole	21	2,4	19	15,7	19	5,4	15	9,7
	Metronidazole OH	39	3,4	35	13,7	37	5,6	32	16,9
	Dimetridazole	24	6,8	22	9,1	12	8,1	16	13,1
	Ronidazole	27	15,2	26	12,5	23	27,2	23	17,9
Calcium channel blockers	Diltiazem	36	18,1	28	15,5	27	9,2	21	12,1
	Verapamil	30	3,9	37	5,4	29	2,2	24	5,4
	Norverapamil	22	3,5	24	11,2	27	6,6	22	9,9

Table S7. Detected PhACs in influent and effluent wastewater samples from two WWTPs: (a) individual concentrations of PhACs±STD (ng L^{-1}); (b) range (minimum and maximum) and mean concentration (ng L^{-1}) in three sampling campaings.

a)

Sampling seasons		**Autumn**				**Winter**				**Spring**			
Wastewater treatment plant		**WWTP1-Amposta**		**WWTP2-Sant Carles**		**WWTP1-Amposta**		**WWTP2-Sant Carles**		**WWTP1-Amposta**		**WWTP2-Sant Carles**	
Sample acronym		**INF1**	**EFF1**	**INF2**	**EFF2**	**INF1**	**EFF1**	**INF2**	**EFF2**	**INF1**	**EFF1**	**INF2**	**EFF2**
Therapeutic gruoup	**Analyte**	**Mean±STD (n=3) (ng L^{-1})**											
Analgesics/ant-inflammatories	Ibuprofen	9157,9±174,6	n.d.	20621,4±366,9	n.d.	45319,6±856,2	blq	32434,4±623,7	blq	24214,6±462,9	n.d.	42298,1±808,7	n.d.
	Acetaminophen	41406,3±736,7	164,5±28,4	43043,7±682,4	187,5±32,8	34974,0±674,1	n.d.	28904,2±574,1	n.d.	1580,4±387,6	53,0±24,7	240,9±38,4	50,6±18,7
	Naproxen	9482,4±168,7	n.d.	12147,3±216,1	n.d.	26302,0±468,0	177,9±5,8	18401,0±327,4	148,7±4,6	2965,9±92,7	717,9±22,4	18145,7±567,5	166,6±7,2
	Salicylic acid	33180,7±590,4	285,7±13,7	35650,9±634,3	n.d.	2522,2±44,8	23,6±2,6	2889,8±35,8	19,6±7,4	130,1±21,7	63,9±14,2	1680,9±29,9	61,9±6,5
	Ketoprofen	858,0±57,6	478,8±18,4	n.d.	n.d.	1989,3±35,3	224,4±8,3	656,7±29,7	1562,1±17,4	792,0±42,9	337,1±21,7	260,2±28,5	314,8±15,7
	Diclofenac	n.d.	n.d.	n.d.	986,8±52,7	1263,9±78,9	986,4±56,7	982,9±82,3	n.d.	1363,2±112,7	637,1±53,6	854,8±87,9	362,8±31,3
	Phenazone	n.d.	n.d.	n.d.	896,9±21,3	136,7±25,6	58,9±8,9	9,9±7,8	3,6±3,2	109,1±27,8	91,7±17,4	34,0±57,8	269,8±29,7
	Codeine	164,9±32,7	112,0±21,7	175,3±21,5	30,8±8,9	230,1±36,8	9,4±7,9	412,0±47,5	131,3±21,3	70,2±32,1	27,6±17,9	141,1±97,8	n.d.
	Indomethacine	n.d.	n.d.	176,1±23,7	n.d.	222,9±18,7	49,7±9,8	92,8±25,9	n.d.	n.d.	n.d.	500,9±29,7	n.d.
	Propylphenazone	blq	n.d.	n.d.	n.d.	32,2±5,3	n.d.	6,3±2,3	n.d.	115,1±29,7	43,4±14,8	43,3±21,7	39,3±16,7
	Piroxicam	n.d.	n.d.	n.d.	n.d.	13,5±5,4	21,6±3,8	21,6±7,7	14,8±5,7	57,8±19,7	17,3±9,7	n.d.	n.d.
	Oxycodone	19,1±9,8	10,5±3,6	55,5±7,9	22,1±3,6	15,5±7,9	10,2±5,2	11,0±8,5	10,8±6,1	10,3±7,9	5,1±4,8	21,2±19,7	5,0±3,9
Lipid regulators and cholesterol lowering statin drugs	Gemfibrozil	2860,3±50,8	403,9±22,1	4788,8±29,8	407,4±12,6	9243,1±79,8	452,6±36,9	4523,0±69,9	124,1±44,7	1374,2±79,8	458,3±32,7	11823,6±210,3	718,3±22,9
	Atorvastatin	123,2±12,4	23,23±5,7	162,2±15,4	n.d.	128,1±12,3	29,8±7,5	95,4±25,9	2,4±3,2	58,7±12,6	16,8±8,5	466,0±36,7	7,0±5,4
	Bezafibrate	202,4±16,7	n.d.	165,5±6,3	45,6±2,8	36,9±19,8	23,2±5,9	176,8±15,8	39,2±4,9	251,0±59,8	64,5±32,8	225,4±63,4	25,7±12,7
	Fluvastatin	n.d.	n.d.	n.d.	n.d.	19,6±12,3	n.d.	9,2±9,5	n.d.	n.d.	n.d.	n.d.	n.d.
	Pravastatin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	28,4±12,9	12,7±5,3	540,3±34,7	7,1±3,2
Antihypertensives	Valsartan	5215,3±92,8	3787,2±34,1	5605,9±59,8	1167,1±21,3	6691,0±78,9	1838,9±55,2	5333,8±73,2	797,0±28,9	18919,1±336,1	47,5±22,9	10116,7±179,6	2032,8±36,1
	Losartan	491,7±75,8	414,9±24,7	712,5±56,9	215,6±18,9	818,0±82,6	468,0±48,9	698,6±69,9	116,5±32,3	708,8±59,7	186,3±15,9	3961,0±70,3	98,1±29,7
	Irbesartan	96,4±10,4	95,6±7,8	239,0±15,7	411,4±13,4	152,9±21,2	71,9±9,7	129,2±22,9	238,1±11,9	128,3±44,7	44,4±9,7	249,1±112,3	208,0±98,7
	Amlodipine	n.d.	n.d.	n.d.	n.d.	5,1±6,4	2,4±3,8	15,8±10,7	6,3±2,9	n.d.	n.d.	437,3±29,8	n.d.
Antihelmintics	Levamisol	152,2±16,8	142,7±12,3	153,2±22,3	185,0±19,2	4836,3±125,2	1472,1±52,6	2487,9±98,1	521,7±43,4	1377,0±114,8	940,9±87,9	39,0±23,7	890,9±29,7
	Thiabendazole	106,0±12,4	56,7±5,8	37,2±15,6	20,1±4,8	459,8±36,5	84,3±27,9	721,3±33,7	88,3±22,6	417,9±75,6	231,2±29,7	n.d.	n.d.
	Albendazole	61,7±7,8	43,4±35,2	92,5±9,7	50,1±4,9	blq	blq	blq	blq	n.d.	n.d.	n.d.	n.d.
Diuretic	Furosemide	2084,4±65,1	1442,5±20,4	2847,3±36,9	1378,0±25,3	2998,4±78,9	1213,5±15,4	2510,7±97,4	261,0±29,7	3410,3±112,7	1604,3±57,9	2247,9±87,8	410,1±32,6
	Hydrochlorothiazide	1673,6±71,5	1008,4±24,7	1829,1±28,9	1087,9±16,3	1434,8±82,4	648,9±42,3	911,6±88,7	644,8±36,7	2986,0±223,7	1594,5±97,8	3447,9±267,9	1592,4±77,3
	Torasemide	n.d.	n.d.	n.d.	n.d.	9,7±5,8	10,5±4,9	9,0±9,8	12,3±4,2	11,1±11,2	6,1±3,6	23,3±12,9	10,7±5,7
Antibiotics	Azithromycin	1064,5±33,2	864,8±18,7	331,1±26,9	615,2±15,2	2899,5±53,6	729,7±21,4	1865,0±29,8	274,2±15,6	2179,9±49,8	1069,6±49,8	1081,7±112,9	762,5±24,3
	Ofloxacin	n.d.	n.d.	n.d.	n.d.	1565,9±22,6	369,4±13,4	2153,6±39,7	524,9±12,7	494,6±25,7	317,8±10,3	1383,7±150,3	291,2±19,8
	Ciprofloxacin	n.d.	n.d.	n.d.	n.d.	697,6±37,8	296,4±8,9	1468,1±147,8	467,3±18,4	656,1±39,8	334,3±16,9	1588,7±126,7	200,0±15,4
	Sulfamethoxazole	556,3±24,1	130,0±12,9	253,8±32,8	83,8±18,9	339,2±29,8	88,4±22,7	1005,6±79,8	264,1±26,7	53,8±52,6	37,9±18,9	732,3±133,7	47,3±29,7
	Trimethoprim	259,0±11,2	89,3±9,8	74,2±7,5	16,0±3,8	323,1±7,9	63,9±5,4	685,0±77,7	107,9±27,8	52,3±44,1	39,2±17,9	108,4±78,9	4,9±4,2
	Dimetridazole	n.d.	n.d.	n.d.	n.d.	125,7±26,9	32,9±18,9	6,7±29,8	4,4±12,9	n.d.	n.d.	n.d.	n.d.
	Clarithromycin	176,4±18,9	79,4±5,1	81,3±14,6	33,7±7,8	101,9±17,2	86,0±5,9	37,3±22,3	46,0±9,8	6,4±12,9	3,2±5,6	blq	9,6±3,9
	Metronidazole-OH	n.d.	n.d.	n.d.	n.d.	27,9±12,3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Erythromycin	58,0±29,7	66,1±15,4	n.d.	n.d.	11,9±9,8	3,4±5,8	10,0±9,8	2,1±1,8	138,1±32,9	65,1±22,3	121,8±26,9	14,8±9,7
Psychiatric drugs	Venlafaxine	522,1±76,4	553,5±23,8	587,7±69,7	538,6±18,3	1461,6±87,9	720,2±44,2	648,9±59,7	421,5±36,9	882,5±67,9	702,4±41,3	864,6±59,8	1271,2±39,7
	Lorazepam	n.d.	n.d.	n.d.	n.d.	314,9±29,8	243,2±19,7	162,5±28,9	66,9±15,4	144,3±33,4	93,0±22,9	414,0±125,7	208,6±33,7
	Carbamazepine	214,0±33,4	163,3±18,5	166,7±38,9	198,3±18,5	157,4±25,6	58,6±17,9	69,0±34,7	28,9±12,4	128,8±26,7	86,8±17,9	282,6±31,7	193,2±15,4
	2-HydroxyCBZ	n.d.	61,3±10,6	n.d.	n.d.	88,6±15,8	31,5±9,8	59,9±22,9	14,5±7,7	50,9±26,7	34,8±15,7	251,6±39,7	46,7±19,7
	10,11-epoxyCBZ	93,5±18,4	105,3±12,7	53,0±19,3	126,2±16,4	95,7±19,2	34,1±9,7	54,3±22,3	14,3±12,3	73,1±25,7	47,3±10,7	274,5±79,8	75,4±9,7
	Citalopram	170,0±22,4	168,7±18,6	348,7±25,6	323,3±22,3	76,2±23,8	47,8±15,4	43,8±34,7	19,4±12,7	42,2±32,7	30,4±20,2	111,5±64,7	71,7±27,8
	Alprazolam	n.d.	15,4±4,7	18,8±9,3	22,1±5,8	10,0±5,9	7,5±4,2	blq	blq	13,1±5,9	7,6±3,7	30,2±15,7	15,1±5,9

	Fluoxetine	n.d.	n.d.	n.d.	30,3±12,6	10,0±15,8	4,8±7,9	11,5±14,7	3,1±6,8	17,1±13,9	6,5±3,8	17,2±18,9	13,1±5,7
	Diazepam	7,6±7,9	n.d.	11,4±5,6	9,9±2,8	7,4±7,6	3,4±3,9	blq	blq	19,9±5,9	9,5±4,4	14,6±17,8	11,8±8,7
	Norfluoxetine	n.d.	n.d.	n.d.	n.d.	6,4±6,2	blq	15,5±8,6	blq	n.d.	n.d.	n.d.	n.d.
	Acridone	n.d.	n.d.	n.d.	n.d.	blq	3,7±3,6	blq	blq	blq	2,4±1,9	7,2±3,8	12,0±5,6
	Setraline	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9,4±7,9	4,7±2,9	n.d.	n.d.	blq	19,0±3,8
	Olanzapine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	147,5±29,8	19,5±14,8	n.d.	n.d.	n.d.	n.d.
	Trazadone	n.d.	20,4±8,3	64,5±23,8	48,0±7,2	n.d.	n.d.	n.d.	n.d.	37,0±21,7	25,3±15,7	128,8±86,3	40,5±32,7
	Paroxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	16,6±8,7	7,1±4,6	n.d.	n.d.	47,0±25,7	9,1±12,7
β-Blocking agents	Atenolol	1690,2±34,7	224,0±21,5	1200,4±29,7	108,6±16,4	309,9±25,9	19,0±15,2	668,2±47,9	429,3±33,7	2488,8±99,7	606,2±57,9	6066,0±263,7	74,3±31,7
	Propanolol	76,4±22,7	52,6±13,6	86,3±16,7	61,6±9,7	61,4±28,8	23,0±22,3	39,5±33,4	10,4±18,7	43,9±31,7	24,3±10,8	167,0±22,7	29,8±9,7
	Sotalol	49,6±12,7	49,4±11,5	54,7±5,9	139,0±7,9	27,5±16,8	29,5±8,9	101,0±29,8	24,7±15,8	131,0±89,8	53,1±32,7	3573,5±347,8	157,7±112,7
	Metoprolol	n.d.	n.d.	n.d.	n.d.	12,0±8,6	6,2±3,7	11,3±9,8	5,7±3,7	17,3±15,7	6,5±9,8	19,3±9,8	7,1±4,3
	Nadolol	75,6±15,4	11,5±1,3	47,9±12,9	n.d.	7,3±2,9	2,1±1,8	16,0±12,3	7,0±5,8	6,7±4,7	blq	n.d.	n.d.
Histamine H1 and H2 receptor antagonist	Ranitidine	162,7±33,6	n.d.	432,9±28,7	55,3±19,6	96,0±21,3	13,7±9,7	95,8±22,7	48,3±13,7	132,6±65,7	78,5±39,7	726,7±36,7	39,4±19,7
	Desloratadine	9,8±4,7	n.d.	10,3±5,9	4,6±1,5	13,3±7,9	5,9±4,9	14,0±8,9	5,5±2,7	9,3±4,9	5,1±3,1	9,4±7,9	5,4±2,9
	Famotidine	n.d.	n.d.	n.d.	n.d.	6,5±2,9	n.d.	n.d.	n.d.	blq	blq	115,0±43,2	5,6±3,3
	Loratadine	n.d.	n.d.	n.d.	n.d.	blq	2,2±1,7	blq	2,2±2,9	n.d.	n.d.	n.d.	n.d.
	Cimetidine	n.d.	n.d.	n.d.	27,8±9,8	blq	5,2±2,9	blq	3,6±1,9	5,7±2,8	2,3±3,6	25,2±12,9	7,8±5,7
X-ray contrast agent	Iopromide	n.d.	n.d.	n.d.	n.d.	72,1±36,7	58,6±26,9	12,6±10,7	47,7±29,7	420,3±115,7	404,6±78,9	939,5±597,4	279,0±223,7
Calcium channel blockers	Diltiazem	90,8±18,6	31,0±15,7	79,3±22,3	34,5±12,3	46,1±22,7	24,6±11,3	37,0±22,7	23,2±15,9	17,1±15,7	10,5±5,9	61,4±50,7	16,3±7,9
	Verapamil	n.d.	n.d.	n.d.	n.d.	blq	1,9±2,2	blq	blq	n.d.	n.d.	n.d.	n.d.
	Norverapamil	n.d.	n.d.	n.d.	n.d.	blq	1,0±0,5	blq	blq	n.d.	n.d.	n.d.	n.d.
Antidiabetic	Glibenclamide	n.d.	31,3±12,6	n.d.	n.d.	14,2±6,3	blq	25,9±19,7	5,8±2,4	74,9±36,7	31,8±22,7	31,4±17,8	2,3±2,1
Prostatic hyperplasia	Tamsulosin	5,9±7,8	3,2±5,7	8,5±7,9	7,4±3,4	13,2±5,2	7,2±3,1	8,3±7,5	3,3±1,9	7,1±5,9	4,6±3,3	9,6±6,8	6,0±4,9
Antiplatelet agent	Clopidogrel	n.d.	n.d.	n.d.	n.d.	8,4±5,9	3,0±2,1	blq	blq	12,3±7,9	9,4±8,3	11,9±6,9	12,4±4,7
To treat asthma	Salbutamol	n.d.	n.d.	n.d.	n.d.	blq	blq	blq	blq	12,9±7,9	5,6±3,2	41,0±21,9	5,7±3,9
Anticoagulant	Warfarin	n.d.	n.d.	n.d.	n.d.	blq	blq	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total conc. (µg L^{-1})	**112,6**	**11,2**	**132,4**	**9,6**	**148,9**	**10,9**	**111,9**	**7,7**	**69,4**	**11,4**	**117,1**	**11,2**
	MIN con. (ng L^{-1})	**5,9**	**3,2**	**8,5**	**4,6**	**5,1**	**1,0**	**6,3**	**2,1**	**5,7**	**2,3**	**7,2**	**4,9**
	MAX conc. (ng L^{-1})	**41406,3**	**3787,2**	**43043,7**	**1378,0**	**45319,6**	**1838,9**	**32434,4**	**1562,1**	**24214,6**	**1604,3**	**42298,1**	**2032,8**

b)

Type of wastewater		**Influent wastewater**									**Effluent wastewater**								
Sampling season		**Autumn**			**Winter**			**Spring**			**Autumn**			**Winter**			**Spring**		
Thearpeutic groups	**Compounds**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
		(ng L^{-1})			**(ng L^{-1})**			**(ng L^{-1})**			**(ng L^{-1})**			**(ng L^{-1})**			**(ng L^{-1})**		
Analgesics/ant-inflammatories	Ibuprofen	9157,9	20621,4	14889,6	32434,4	45319,6	38877,0	24214,6	42298,1	33256,4	n.d.			<MQL			n.d.		
	Acetaminophen	41406,3	43043,7	42225,0	28904,2	34974,0	31939,1	240,9	1580,4	910,6	164,5	187,5	176,0	n.d.			50,6	53,0	51,8
	Naproxen	9482,4	12147,3	10814,9	18401,0	26302,0	22351,5	2965,9	18145,7	10555,8	n.d.			148,7	177,9	163,3	166,6	717,9	442,3
	Salicylic acid	33180,7	35650,9	34415,8	2522,2	2889,8	2706,0	130,1	1680,9	905,5	285,7	285,7	285,7	19,6	23,6	21,6	61,9	63,9	62,9
	Ketoprofen	858,0	858,0	858,0	656,7	1989,3	1323,0	260,2	792,0	526,1	478,8	478,8	478,8	224,4	1562,1	893,3	314,8	337,1	326,0
	Diclofenac	n.d.			982,9	1263,9	1123,4	854,8	1363,2	1109,0	986,8	986,8	986,8	986,4	986,4	986,4	362,8	637,1	500,0
	Phenazone	n.d.			9,9	136,7	73,3	34,0	109,1	71,6	896,9	896,9	896,9	3,6	58,9	31,3	91,7	269,8	180,8
	Codeine	164,9	175,3	170,1	230,1	412,0	321,1	70,2	141,1	105,7	30,8	112,0	71,4	9,4	131,3	70,4	27,6	27,6	27,6
	Indomethacine	176,1	176,1	176,1	92,8	222,9	157,9	500,9	500,9	500,9	n.d.			49,7	49,7	49,7	n.d.		
	Propylphenazone	<MQL			6,3	32,2	19,2	43,3	115,1	79,2	n.d.			n.d.			39,3	43,4	41,4

	Piroxicam	n.d.			13,5	21,6	17,6	57,8	57,8	57,8	n.d.			14,8	21,6	18,2	17,3	17,3	17,3
	Oxycodone	19,1	55,5	37,3	11,0	15,5	13,3	10,3	21,2	15,8	10,5	22,1	16,3	10,2	10,8	10,5	5,0	5,1	5,1
Lipid regulators and cholesterol lowering statin drugs	Gemfibrozil	2860,3	4788,8	3824,6	4523,0	9243,1	6883,1	1374,2	11823,6	6598,9	403,9	407,4	405,6	124,1	452,6	288,4	458,3	718,3	588,3
	Atorvastatin	123,2	162,2	142,7	95,4	128,1	111,8	58,7	466,0	262,4	23,3	23,3	23,3	2,4	29,8	16,1	7,0	16,8	11,9
	Bezafibrate	165,5	202,4	184,0	36,9	176,8	106,9	225,4	251,0	238,2	45,6	45,6	45,6	23,2	39,2	31,2	25,7	64,5	45,1
	Fluvastatin	n.d.			9,2	19,6	14,4	n.d.			n.d.			n.d.			n.d.		
	Pravastatin	n.d.			<MQL			28,4	540,3	284,4	n.d.			<MQL			7,1	12,7	9,9
Antihypertensives	Valsartan	5215,3	5605,9	5410,6	5333,8	6691,0	6012,4	10116,7	18919,1	14517,9	1167,1	3787,2	2477,2	797,0	1838,9	1318,0	47,5	2032,8	1040,2
	Losartan	491,7	712,5	602,1	698,6	818,0	758,3	708,8	3961,0	2334,9	215,6	414,9	315,3	116,5	468,0	292,3	98,1	186,3	142,2
	Irbesartan	96,4	239,0	167,7	129,2	152,9	141,1	128,3	249,1	188,7	95,6	411,4	253,5	71,9	238,1	155,0	44,4	208,0	126,2
	Amlodipine	n.d.			5,1	15,8	10,4	437,3	437,3	437,3	n.d.			2,4	6,3	4,4	n.d.		
Antihelmintics	Levamisol	152,2	153,2	152,7	2487,9	4836,3	3662,1	39,0	1377,0	708,0	142,7	185,0	163,9	521,7	1472,1	996,9	890,9	940,9	915,9
	Thiabendazole	37,2	106,0	71,6	459,8	721,3	590,6	417,9	417,9	417,9	20,1	56,7	38,4	84,3	88,3	86,3	231,2	231,2	231,2
	Albendazole	61,7	92,5	77,1	n.d.			n.d.			43,4	50,1	46,7	n.d.			n.d.		
Diuretic	Furosemide	2084,4	2847,3	2465,9	2510,7	2998,4	2754,6	2247,9	3410,3	2829,1	1378,0	1442,5	1410,2	261,0	1213,5	737,3	410,1	1604,3	1007,2
	Hydrochlorothiazide	1673,6	1829,1	1751,4	911,6	1434,8	1173,2	2986,0	3447,9	3217,0	1008,4	1087,9	1048,1	644,8	648,9	646,9	1592,4	1594,5	1593,5
	Torasemide	n.d.			9,0	9,7	9,4	11,1	23,3	17,2	n.d.			10,5	12,3	11,4	6,1	10,7	8,4
Antibiotics	Azithromycin	331,1	1064,5	697,8	1865,0	2899,5	2382,3	1081,7	2179,9	1630,8	615,2	864,8	740,0	274,2	729,7	501,9	762,5	1069,6	916,1
	Ofloxacin	n.d.			1565,9	2153,6	1859,8	494,6	1383,7	939,2	n.d.			369,4	524,9	447,2	291,2	317,8	304,5
	Ciprofloxacin	n.d.			697,6	1468,1	1082,8	656,1	1588,7	1122,4	n.d.			296,4	467,3	381,9	200,0	334,3	267,2
	Sulfamethoxazole	253,8	556,3	405,0	339,2	1005,6	672,4	53,8	732,3	393,1	83,8	130,0	106,9	88,4	264,1	176,2	37,9	47,3	42,6
	Trimethoprim	74,2	259,0	166,6	323,1	685,0	504,0	52,3	108,4	80,4	16,0	89,3	52,7	63,9	107,9	85,9	4,9	39,2	22,1
	Dimetridazole	n.d.			6,7	125,7	66,2	n.d.			n.d.			4,4	32,9	18,7	n.d.		
	Clarithromycin	81,3	176,4	128,9	37,3	101,9	69,6	6,4	6,4	6,4	33,7	79,4	56,6	46,0	86,0	66,0	3,2	9,6	6,4
	Metronidazole OH	<MQL			27,9	27,9	27,9	n.d.			<MQL			n.d.			n.d.		
	Erythromycin	58,0	58,0	58,0	10,0	11,9	11,0	121,8	138,1	130,0	66,1	66,1	66,1	2,1	3,4	2,8	14,8	65,1	40,0
Psychiatric drugs	Venlafaxine	522,1	587,7	554,9	648,9	1461,6	1055,3	864,6	882,5	873,6	538,6	553,5	546,0	421,5	720,2	570,9	702,4	1271,2	986,8
	Lorazepam	n.d.			162,5	314,9	238,7	144,3	414,0	279,2	n.d.			66,9	243,2	155,1	93,0	208,6	150,8
	Carbamazepine	166,7	214,0	190,4	69,0	157,4	113,2	128,8	282,6	205,7	163,3	198,3	180,8	28,9	58,6	43,8	86,8	193,2	140,0
	2-HydroxyCBZ	n.d.			59,9	88,6	74,3	50,9	251,6	151,3	61,3	61,3	61,3	14,5	31,5	23,0	34,8	46,7	40,8
	10,11-epoxyCBZ	53,0	93,5	73,2	54,3	95,7	75,0	73,1	274,5	173,8	105,3	126,2	115,8	14,3	34,1	24,2	47,3	75,4	61,4
	Citalopram	170,0	348,7	259,3	43,8	76,2	60,0	42,2	111,5	76,9	168,7	323,3	246,0	19,4	47,8	33,6	30,4	71,7	51,1
	Alprazolam	18,8	18,8	18,8	<MQL	10,0	10,0	13,1	30,2	21,7	15,4	22,1	18,8	<MQL	7,5	7,5	7,6	15,1	11,4
	Fluoxetine	n.d.			10,0	11,5	10,8	17,1	17,2	17,2	30,3	30,3	30,3	3,1	4,8	4,0	6,5	13,1	9,8
	Diazepam	7,6	11,4	9,5	<MQL	7,4	7,4	14,6	19,9	17,3	9,9	9,9	9,9	<MQL	3,4	3,4	9,5	11,8	10,7
	Norfluoxetine	n.d.			6,4	15,5	11,0	n.d.			n.d.			<MQL			n.d.		
	Acridone	n.d.			<MQL	4,7	4,7	<MQL	7,2	7,2	n.d.			<MQL	3,7	3,7	2,4	12,0	7,2
	Setraline	n.d.			9,4	9,4	9,4	n.d.			n.d.			4,7	4,7	4,7	19,0	19,0	19,0
	Olanzapine	n.d.			147,5	147,5	147,5	n.d.			n.d.			19,5	19,5	19,5	n.d.		
	Trazadone	64,5	64,5	64,5	n.d.			37,0	128,8	82,9	20,4	48,0	34,2	n.d.			25,3	40,5	32,9
	Paroxetine	n.d.			16,6	16,6	16,6	47,0	47,0	47,0	n.d.			7,1	7,1	7,1	9,1	9,1	9,1
β-Blocking agents	Atenolol	1200,4	1690,2	1445,3	309,9	668,2	489,1	2488,8	6066,0	4277,4	108,6	224,0	166,3	19,0	429,3	224,1	74,3	606,2	340,3
	Propanolol	76,4	86,3	81,4	39,5	61,4	50,5	43,9	167,0	105,5	52,6	61,6	57,1	10,4	23,0	16,7	24,3	29,8	27,1
	Sotalol	49,6	54,7	52,2	27,5	101,0	64,3	131,0	3573,5	1852,3	49,4	139,0	94,2	24,7	29,5	27,1	53,1	157,7	105,4
	Metoprolol	n.d.			11,3	12,0	11,6	17,3	19,3	18,3	n.d.			5,7	6,2	6,0	6,5	7,1	6,8
	Nadolol	47,9	75,6	61,8	7,3	16,0	11,6	6,7	6,7	6,7	11,5	11,5	11,5	2,1	7,0	4,5	<MQL		
Histamine H1 and H2	Ranitidine	162,7	432,9	297,8	95,8	96,0	95,9	132,6	726,7	429,7	55,3	55,3	55,3	13,7	48,3	31,0	39,4	78,5	59,0

receptor antagonist	Desloratadine	9,8	10,3	10,1	13,3	14,0	13,6	9,3	9,4	9,4	4,6	4,6	4,6	5,5	5,9	5,7	5,1	5,4	5,3
	Famotidine	n.d.			6,5	6,5	6,5	115,0	115,0	115,0	n.d.			n.d.			5,6	5,6	5,6
	Loratadine	n.d.			<MQL			n.d.			n.d.			2,2	2,2	2,2	n.d.		
	Cimetidine	n.d.			<MQL			5,7	25,2	15,5	27,8	27,8	27,8	3,6	5,2	4,4	2,3	7,8	5,1
X-ray contrast agent	Iopromide	n.d.			12,6	72,1	42,3	420,3	939,5	679,9	n.d.			47,7	58,6	53,2	279,0	404,6	341,8
Calcium channel blockers	Diltiazem	79,3	90,8	85,0	37,0	46,1	41,6	17,1	61,4	39,3	31,0	34,5	32,7	23,2	24,6	23,9	10,5	16,3	13,4
	Verapamil	n.d.			<MQL			n.d.			n.d.			<MQL			n.d.		
	Norverapamil	n.d.			<MQL			n.d.			n.d.			<MQL			n.d.		
Antidiabetic	Glibenclamide	n.d.			14,2	25,9	20,0	31,4	74,9	53,2	31,3	31,3	31,3	<MQL	5,8	5,8	2,3	31,8	17,1
Prostatic hyperplasia	Tamsulosin	5,9	8,5	7,2	8,3	13,2	10,7	7,1	9,6	8,4	3,2	7,4	5,3	3,3	7,2	5,2	4,6	6,0	5,3
Antiplatelet agent	Clopidogrel	n.d.			<MQL	8,4	8,4	11,9	12,3	12,1	n.d.			<MQL	3,0	3,0	9,4	12,4	10,9
To treat asthma	Salbutamol	n.d.			<MQL			12,9	41,0	27,0	n.d.			<MQL			5,6	5,7	5,7
Anticoagulant	Warfarin	n.d.			<MQL			n.d.			n.d.			<MQL			n.d.		

Table S8. Average removal efficiencies (%) in the two WWTPs, and standard deviation (±STD), calculated as averages of the efficiencies of the three sampling campaigns.

Therapeutic group	Compound	Removal efficency (%)		Average removal efficency, %
		WWTP1 (%)	WWTP2 (%)	
Analgesics/ant-inflammatories	Ketoprofen*	63	-79	-8
	Naproxen	88	99	93
	Ibuprofen	100	100	100
	Acetaminophen	98	89	94
	Salicylic acid	83	98	90
	Diclofenac	38	58	48
	Phenazone*	36	-315	-139
	Propylphenazone	32	9	21
	Piroxicam	5	31	18
	Oxycodone	43	46	45
	Codeine	63	75	69
Lipid regulators and cholesterol lowering statin drugs	Bezafibrate	56	80	68
	Gemfibrozil	83	94	88
	Pravastatin	55	83	69
	Atorvastatin	76	98	87
Psychiatric drugs	Carbamazepine	40	24	32
	2-HydroxyCBZ	48	76	62
	10,11-epoxyCBZ	29	3	16
	Acridone	22	11	16
	Setraline	-	50	50
	Citalopram	22	33	27
	Venlafaxine	22	-1	10
	Olanzapine	-	87	87
	Trazadone	32	47	39
	Fluoxetine	57	48	53
	Diazepam	53	34	43
	Lorazepam	29	54	42
	Alprazolam	33	29	31
Histamine H1 and H2 receptor antagonist	Loratadine	54	52	53
	Desloratadine	51	53	52
	Ranitidine	63	77	70
	Famotidine	-	95	95
	Cimetidine	60	69	64
β-Blocking agents	Atenolol	85	75	80
	Sotalol	26	6	16
	Propanolol	54	62	58
	Metoprolol	55	56	56
	Nadolol	78	56	67
Diuretic	Hydrochlorothiazide	51	41	46
	Furosemide	56	74	65
	Torasemide	18	9	14
Antidiabetic	Glibenclamide	78	85	81
Antihypertensives	Amlodipine	53	60	56
	Losartan	58	84	71
	Irbesartan*	59	-47	6
	Valsartan	86	81	84
Antiplatelet agent	Clopidogrel	44	27	36

Prostatic hyperplasia	Tamsulosin	40	37	39
To treat asthma	Salbutamol	74	48	61
Anticoagulant	Warfarin	37	-	37
X-ray contrast agent	Iopromide*	11	-104	-47
Antihelmintics	Albendazole	30	46	38
	Thiabendazole	58	67	62
	Levamisol*	36	-709	-336
Antibiotics	Erythromycin	37	65	51
	Azithromycin	45	18	32
	Clarithromycin	40	18	29
	Ofloxacin	56	77	67
	Ciprofloxacin	53	78	66
	Sulfamethoxazole	60	78	69
	Trimethoprim	57	85	71
	Dimetridazole	63	35	49
Calcium channel blockers	Diltiazem	50	56	53
	Verapamil*	33	-108	-37
	Norverapamil	55	53	54

*Compounds marked with asterisk were show different removal efficiency in two WWTP with STD higher than 20%;

Table S9. Individual concentrations of PhACs±STD (ng L^{-1}) in water samples during the autumn (a); the winter (b) and the spring season (c).

a)

Sampling seasons													**Autumn**											
Type of water		**River**					**Channels**							**Estuary**							**Seawater**			
Sample acronym	**CSW**	**RW1**	**RW2**	**CW1**	**CW2**	**CW3**	**CW4**	**CW5**	**CW6**	**CW7**	**EW1**	**EW2**	**EW3**	**EW4**	**EW5**	**EW6**	**EW7**	**SW1**	**SW2**	**SW3**	**SW4***	**SW5**	**SW6**	**SW7**	**SW8***
Analyte													**Therapeutic gruoup**											
Analgesics/ant-inflammatories																								
Ketoprofen	n.d.	97,9 ±14,7	n.d.	37,5 ±15,4	n.d.	n.d.	n.d.	39,2 ±4,3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Naproxen	n.d.	n.d.	n.d.	48,8 ±12,7	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.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Ibuprofen	n.d.	n.d.	18,5 ±4,7	14,0 ±8,8	n.d.	7,7 ±4,2	9,4 ±3,2	n.d.	6,7 ±3,3	n.d.	n.d.	n.d.	21,5 ±4,6	n.d.	n.d.	n.d.	n.d.	n.d.	23,9 ±12,4	n.d.	x	n.d.	n.d.	n.d.	x
Indomethacine	n.d.	n.d.	10,4 ±3,9	13,3 ±7,6	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.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Acetaminophen	n.d.	13,34 ±5,7	16,0 ±4,1	14,9 ±5,7	13,2 ±7,8	10,9 ±5,8	8,0 ±4,1	9,1 ±4,8	13,3 ±4,5	7,9 ±3,7	16,5 ±6,4	17,5 ±6,7	61,0 ±9,8	45,3 ±12,5	47,5 ±11,3	35,8 ±8,9	n.d.	2,9 ±4,4	n.d.	3,5 ±2,2	x	13,7 ±2,3	4,6 ±2,4	5,4 ±1,7	x
Salicylic acid	n.d.	n.d.	n.d.	20,5 ±11,3	19,6 ±5,3	n.d.	13,9 ±3,2	14,5 ±11,2	n.d.	15,1 ±4,3	33,4 ±9,7	29,8 ±14,6	34,6 ±4,7	n.d.	39,2 ±12,4	26,1 ±5,6	18,0 ±7,6	9,9 ±3,7	n.d.	11,5 ±3,8	x	8,7 ±1,7	13,1 ±1,9	6,0 ±2,3	x
Diclofenac	n.d.	n.d.	280,3 ±18,7	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.	23,5 ±5,5	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Phenazone	2,7 ±1,2	3,1 ±2,2	242,1 ±21,2	42,1 ±23,4	2,8 ±5,9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2,4 ±1,3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Propylphenazone	n.d.	n.d.	1,3 ±1,1	n.d.	1,2 ±1,3	1,2 ±0,9	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.	n.d.	x	n.d.	n.d.	n.d.	x
Oxycodone	n.d.	28,5 ±16,8	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.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Codeine	n.d.	49,2 ±12,8	2,0 ±0,8	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Lipid regulators and cholesterol lowering statin drugs																									
Bezafibrate	n.d.	n.d.	47,3 ±19,7	18,7 ±8,7	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.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Gemfibrozil	5,5 ±1,4	37,4 ±8,9	147,5 ±24,7	6,4 ±3,7	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.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Atorvastatin	n.d.	n.d.	n.d.	2,3 ±1,8	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.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Psychiatric drugs																									
Carbamazepine	4,0 ±2,3	112,3 ±19,5	69,6 ±13,4	3,8 ±1,7	3,6 ±2,1	3,4 ±1,7	n.d.	2,1 ±1,9	2,3 ±2,0	1,7 ±1,3	n.d.	1,1 ±1,2	1,0 ±1,1	0,7 ±0,4	0,2 ±0,2	0,2 ±0,5	n.d.	0,9 ±0,4	0,8 ±0,5	n.d.	x	1,1 ±1,0	n.d.	0,5 ±0,5	x
10,11-epoxyCBZ	n.d.	49,7 ±13,4	n.d.	41,0 ±21,6	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.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Setraline	n.d.	30,4 ±9,9	18,9 ±9,8	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Citalopram	2,1 ±1,5	46,5 ±15,7	101,8 ±26,4	n.d.	1,1 ±1,0	1,6 ±0,9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3,1 ±2,8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Venlafaxine	6,3 ±2,4	216,4 ±19,7	341,8 ±28,7	n.d.	n.d.	21,3 ±2,8	5,6 ±1,6	5,1 ±2,7	n.d.	n.d.	7,8 ±3,3	n.d.	n.d.	12,4 ±5,4	blq	blq	blq	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Trazadone	n.d.	44,5 ±11,8	12,0 ±4,6	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Fluoxetine	n.d.	n.d.	38,7 ±3,7	4,9 ±3,2	2,5 ±1,4	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.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Paroxetine	n.d.	16,2 ±8,9	6,2 ±2,4	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Diazepam	n.d.	1,3 ±1,3	3,9 ±2,1	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Alprazolam	n.d.	24,0 ±5,5	n.d.	6,8 ±1,7	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.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Histamine H1 and H2 receptor antagonist																									
Desloratadine	2,9	n.d.	2,6	1,2	1,3	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.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x

	±1,8		±1,7	±0,9	±1,1																				
Ranitidine	n.d.	n.d.	2,3 ±1,5	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Cimetidine	n.d.	36,0 ±15,7	n.d.	1,0 ±1,1	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.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
β-Blocking agents																									
Atenolol	n.d.	129,8 ±23,7	n.d.	1,6 ±1,2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2,7 ±1,8	1,7 ±1,4	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Sotalol	1,1 ±0,8	24,3 ±5,6	65,4 ±22,4	1,7 ±1,8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	15,4 ±7,8	7,8 ±4,4	n.d.	8,7 ±3,2	n.d.	n.d.	n.d.	9,8 ±3,3	n.d.	n.d.	x	n.d.	2,7 ±0,7	n.d.	x
Propanolol	n.d.	24,3 ±4,2	12,9 ±8,8	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Metoprolol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2,9 ±1,8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Carazolol	n.d.	1,0 ±0,9	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.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Diuretic																									
Hydrochlorothiazide	3,0 ±1,4	207,6 ±21,4	442,1 ±28,7	5,6 ±2,3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	19,9 ±4,4	n.d.	n.d.	2,8 ±1,1	blq	n.d.	x	n.d.	n.d.	n.d.	x
Furosemide	n.d.	n.d.	464,2 ±34,1	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Antidiabetic																									
Glibenclamide	n.d.	12,4 ±8,8	20,5 ±8,7	3,2 ±1,8	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.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Losartan	n.d.	218,7 ±16,7	71,9 ±5,7	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Irbesartan	3,3 ±2,1	39,2 ±8,5	179,7 ±9,7	12,7 ±4,9	2,0 ±1,1	1,8 ±1,2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2,6 ±2,3	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Valsartan	21,6 ±8,7	284,7 ±19,7	400,0 ±26,4	22,8 ±12,7	26,7 ±9,7	25,2 ±9,7	2,0 ±1,3	13,2 ±5,5	11,5 ±7,8	11,8 ±4,6	8,5 ±3,3	n.d.	2,6 ±1,7	4,6 ±2,1	0,8 ±0,5	1,9 ±1,2	1,6 ±1,7	4,3 ±2,2	3,3 ±1,9	2,0 ±0,8	x	3,5 ±1,7	2,1 ±0,9	2,2 ±1,1	x
Antiplatelet agent																									
Clopidogrel	n.d.	6,0 ±3,2	2,7 ±1,4	4,1 ±1,9	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.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Prostatic hyperplasia																									
Tamsulosin	n.d.	8,5 ±4,1	2,9 ±1,3	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
X-ray contrast agent																									
Iopromide	5,9 ±4,3	n.d.	n.d.	5,7 ±2,3	5,0 ±2,1	n.d.	n.d.	5,0 ±2,2	7,4 ±3,3	5,3 ±2,1	n.d.	8,4 ±4,5	n.d.	n.d.	n.d.	n.d.	n.d.	2,4 ±0,6	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Antihelmintics																									
Albendazole	n.d.	123,3 ±20,4	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.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Thiabendazole	10,0 ±3,7	34,0 ±17,3	8,3 ±2,2	2,3 ±1,5	12,4 ±7,8	8,2 ±4,3	n.d.	1,3 ±1,0	1,0 ±0,8	n.d.	5,4 ±3,4	n.d.	3,1 ±1,4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Levamisol	n.d.	56,2 ±13,4	7,1 ±3,4	2,0 ±1,7	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.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Tranquilizers																									
Azaperone	n.d.	2,9 ±1,8	3,8 ±2,4	n.d.	3,0 ±1,5	1,1 ±1,0	1,1 ±0,8	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.	x	n.d.	n.d.	n.d.	x
Antibiotics																									
Erythromycin	n.d.	25,7 ±12,0	12,3 ±3,6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	20,4 ±9,8	11,2 ±5,2	11,5 ±9,7	n.d.	n.d.	n.d.	n.d.	2,7 ±0,7	1,9 ±0,9	n.d.	x	blq	1,5 ±1,7	n.d.	x
Azithromycin	n.d.	n.d.	220,7 ±23,8	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Clarithromycin	n.d.	90,0 ±23,7	9,9 ±5,7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3,2 ±1,5	blq	blq	blq	n.d.	6,2 ±3,3	1,9 ±1,3	n.d.	x	n.d.	n.d.	n.d.	x
Tetracycline	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.	n.d.	n.d.	n.d.	n.d.	1,3 ±1,0	n.d.	3,4 ±1,3	x	1,0 ±1,1	n.d.	0,4 ±0,2	x
Ofloxacin	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Ciprofloxacin	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Sulfamethoxazole	n.d.	99,0 ±24,8	6,0 ±3,3	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Trimethoprim	n.d.	24,1	11,2	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.	n.d.	2,8	1,3	n.d.	x	n.d.	n.d.	n.d.	x

		±13,5	±4,7														±0,9	±0,5						
Calcium channel blockers																								
Diltiazem	n.d.	n.d.	n.d.	17,9 ±6,7	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.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Norverapamil	n.d.	4,7 ±2,9	3,2 ±1,9	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Total conc. (ng L^{-1})	**68,4**	**2223,1**	**3308,0**	**356,8**	**94,4**	**82,4**	**40,0**	**89,5**	**42,2**	**41,8**	**107,4**	**75,8**	**146,9**	**71,7**	**110,3**	**65,7**	**43,1**	**48,6**	**33,1**	**20,4**	**x**	**28,0**	**24,0**	**14,5**	**x**
MIN con. (ng L^{-1})	**1,1**	**1,0**	**1,3**	**1,0**	**1,1**	**1,1**	**1,1**	**1,3**	**1,0**	**1,7**	**5,4**	**1,1**	**1,0**	**0,7**	**0,2**	**0,2**	**1,6**	**0,9**	**0,8**	**2,0**	**x**	**1,0**	**1,5**	**0,4**	**x**
MAX con. (ng L^{-1})	**21,6**	**284,7**	**464,2**	**48,8**	**26,7**	**25,2**	**13,9**	**39,2**	**13,3**	**15,1**	**33,4**	**29,8**	**61,0**	**45,3**	**47,5**	**35,8**	**23,5**	**9,9**	**23,9**	**11,5**	**x**	**13,7**	**13,1**	**6,0**	**x**

* not available sampling points

b)

Sampling seasons													**Winter**											
Type of water		**River**				**Channels**								**Estuary**									**Seawater**	
Sample acronym	**CSW**	**RW1**	**RW2**	**CW1**	**CW2***	**CW3**	**CW4**	**CW5**	**CW6**	**CW7**	**EW1**	**EW2**	**EW3**	**EW4**	**EW5**	**EW6**	**EW7**	**SW1**	**SW2**	**SW3**	**SW4**	**SW5**	**SW6**	**SW7**	**SW8**
Analyte												**Therapeutic gruoup**												
Analgesics/ant-inflammatories																								
Ketoprofen	n.d.	n.d.	215,1 ±4,7	n.d.	x	21,4 ±2,4	n.d.	17,8 ±1,8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	34,7 ±7,9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Naproxen	n.d.	35,9 ±3,7	53,2 ±3,4	30,2 ±2,5	x	n.d.	7,3 ±1,6	n.d.	40,5 ±5,7	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.	n.d.	n.d.	n.d.
Ibuprofen	4,9 ±1,2	28,9 ±4,1	n.d.	15,3 ±3,7	x	n.d.	n.d.	21,4 ±8,9	45,3 ±4,3	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.	n.d.	n.d.	4,2 ±1,7
Indomethacine	n.d.	n.d.	16,1 ±2,1	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetaminophen	n.d.	n.d.	134,8 ±8,9	n.d.	x	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	23,2 ±5,7	n.d.	16,5 ±3,4	n.d.	n.d.	5,4 ±2,7	2,6 ±1,9	n.d.	7,7 ±2,2	n.d.
Salicylic acid	11,6 ±2,6	23,1 ±0,9	12,1 ±5,9	17,4 ±5,4	x	n.d.	28,4 ±6,4	8,4 ±2,7	9,1 ±2,8	13,1 ±5,4	43,6 ±2,7	24,7 ±3,1	37,1 ±9,7	43,9 ±6,4	36,7 ±3,8	n.d.	14,2 ±6,7	14,5 ±2,8	8,2 ±3,0	5,6 ±1,5	n.d.	12,2 ±5,7	12,4 ±3,4	n.d.	4,8 ±2,1
Diclofenac	n.d.	169,7 ±6,1	n.d.	n.d.	x	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5,7 ±2,6	blq	n.d.	n.d.	n.d.	blq	n.d.	n.d.	n.d.
Phenazone	n.d.	29,9 ±2,3	55,4 ±4,7	1,6 ±1,3	x	n.d.	n.d.	n.d.	1,5 ±24,7	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.	n.d.	n.d.	n.d.
Propylphenazone	n.d.	1,01 ±0,7	n.d.	0,9 ±0,7	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Piroxicam	n.d.	n.d.	11,4 ±2,4	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Oxycodone	n.d.	n.d.	n.d.	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Codeine	n.d.	2,7 ±1,3	7,9 ±3,4	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lipid regulators and cholesterol lowering statin drugs																									
Bezafibrate	n.d.	1,8 ±2,1	28,3 ±8,9	1,7 ±2,5	x	n.d.	n.d.	n.d.	blq	1,0 ±0,9	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.	n.d.	n.d.
Gemfibrozil	n.d.	8,2 ±4,0	87,5 ±14,5	8,5 ±1,7	x	n.d.	n.d.	n.d.	10,8 ±2,7	14,8 ±2,6	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.	n.d.	n.d.
Atorvastatin	n.d.	0,9 ±2,8	1,7 ±3,2	n.d.	x	n.d.	n.d.	n.d.	blq	n.d.	n.d.	n.d.	n.d.	0,8 ±1,1	0,9 ±1,4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs																									
Carbamazepine	3,1 ±2,4	26,5 ±5,9	36,8 ±7,5	1,9 ±1,5	x	n.d.	blq	blq	blq	blq	blq	blq	1,9 ±1,2	1,8 ±2,4	0,6 ±0,8	0,6 ±0,7	0,3 ±0,5	1,1 ±2,3	0,9 ±0,7	blq	0,5 ±0,7	blq	blq	blq	1,4 ±2,1
2-HydroxyCBZ	n.d.	1,3 ±1,7	21,2 ±4,8	0,5 ±0,8	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10,11-epoxyCBZ	n.d.	1,8 ±1,6	21,6 ±3,7	1,6 ±2,1	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acridone	blq	blq	1,8 ±0,4	blq	x	blq	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Setraline	n.d.	n.d.	4,7 ±3,6	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Citalopram	1,7 ±1,3	0,9 ±1,4	42,8 ±13,5	n.d.	x	n.d.	n.d.	n.d.	0,8 ±0,5	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.	n.d.	n.d.
Venlafaxine	4,7 ±3,8	n.d.	144,2 ±18,7	3,6 ±1,8	x	n.d.	n.d.	n.d.	1,5 ±0,7	n.d.	n.d.	12,7 ±2,6	n.d.	7,8 ±1,7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fluoxetine	n.d.	n.d.	5,8 ±2,7	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diazepam	n.d.	0,5 ±0,7	2,5 ±3,2	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lorazepam	n.d.	n.d.	214,7 ±58,5	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Alprazolam	n.d.	n.d.	4,3 ±2,2	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Histamine H1 and H2 receptor antagonist																								
Loratadine	2,5 ±2,2	1,3 ±1,7	1,2 ±2,4	1,3 ±1,2	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Desloratadine	n.d.	n.d.	3,6 ±2,9	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ranitidine	n.d.	1,3 ±0,8	14,5 ±4,1	n.d.	x	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,9 ±0,5	1,7 ±1,5	0,9 ±0,5	2,1 ±2,4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cimetidine	n.d.	1,4 ±0,6	4,4 ±3,1	1,4 ±1,4	x	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,4 ±1,2	0,9 ±0,9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
β-Blocking agents																								
Atenolol	n.d.	10,8 ±5,7	24,6 ±15,7	1,2 ±0,4	x	n.d.	n.d.	n.d.	7,3 ±4,3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,9 ±2,1	0,7 ±0,4	n.d.	n.d.	n.d.	n.d.	n.d.
Sotalol	n.d.	36,2 ±8,9	28,8 ±4,9	n.d.	x	n.d.	n.d.	n.d.	20,1 ±12,3	n.d.	n.d.	n.d.	11,3 ±5,2	9,8 ±3,7	n.d.	n.d.	n.d.	3,4 ±3,6	n.d.	n.d.	n.d.	1,3 ±0,9	n.d.	n.d.	n.d.
Propanolol	n.d.	0,7 ±0,7	19,1 ±3,4	0,6 ±0,5	x	n.d.	n.d.	n.d.	blq	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.	n.d.	n.d.	n.d.
Metoprolol	blq	0,9 ±0,9	4,3 ±2,1	n.d.	x	n.d.	n.d.	n.d.	3,4 ±2,3	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.	n.d.	n.d.	n.d.
Nadolol	blq	1,2 ±3,6	1,3 ±0,8	0,3 ±0,2	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diuretic																								
Hydrochlorothiazide	2,9 ±1,4	124,5 ±15,8	305,1 ±19,7	0,5 ±0,4	x	n.d.	n.d.	n.d.	6,8 ±2,6	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.	n.d.	n.d.	n.d.
Furosemide	n.d.	248,6 ±24,3	n.d.	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Torasemide	n.d.	n.d.	9,8 ±4,6	0,5 ±0,3	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antidiabetic																								
Glibenclamide	n.d.	n.d.	1,3 ±1,2	n.d.	x	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,2 ±0,4	0,1 ±0,5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antihypertensives																								
Amlodipine	n.d.	n.d.	1,8 ±1,3	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Losartan	n.d.	255,3 ±16,8	n.d.	n.d.	x	n.d.	n.d.	n.d.	15,8 ±9,8	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.	n.d.	n.d.	n.d.
Irbesartan	n.d.	4,5 ±2,2	289,6 ±22,3	4,3 ±3,6	x	n.d.	n.d.	n.d.	3,5 ±2,5	n.d.	n.d.	n.d.	n.d.	0,3 ±0,5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Valsartan	n.d.	234,2 ±21,3	323,2 ±25,6	40,8 ±10,7	x	n.d.	25,3 ±3,9	n.d.	n.d.	n.d.	n.d.	2,4 ±1,8	9,9 ±7,9	10,7 ±4,9	4,7 ±2,9	5,8 ±3,7	1,6 ±1,3	2,7 ±2,4	2,4 ±1,8	1,3 ±0,9	1,1 ±0,7	0,6 ±0,7	n.d.	n.d.	n.d.
Antiplatelet agent																								
Clopidogrel	blq	blq	4,8 ±3,4	blq	x	n.d.	n.d.	n.d.	blq	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.	n.d.	n.d.	n.d.
Prostatic hyperplasia																								
Tamsulosin	n.d.	n.d.	5,6 ±4,3	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
To treat asthma																								
Salbutamol	n.d.	n.d.	blq	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Anticoagulant																								

Warfarin	blq	n.d.	blq	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
X-ray contrast agent																								
Iopromide	2,3 ±1,7	18,3 ±7,9	27,7 ±12,3	n.d.	x	12,3 ±9,7	n.d.	n.d.	n.d.	n.d.	3,9 ±4,3	4,1 ±2,3	n.d.	2,7 ±1,8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antihelmintics																								
Albendazole	n.d.	blq	blq	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Thiabendazole	n.d.	n.d.	3,5 ±2,2	4,1 ±2,8	x	7,2 ±7,6	n.d.	2,7 ±1,7	1,4 ±0,7	n.d.	3,6 ±1,8	n.d.	4,7 ±2,2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Levamisol	n.d.	102,1 ±14,8	147,4 ±18,7	33,3 ±7,9	x	n.d.	n.d.	n.d.	27,0 ±18,9	4,1 ±2,3	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.	n.d.
Antibiotics																								
Erythromycin	n.d.	n.d.	1,4 ±2,8	n.d.	x	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.	n.d.	n.d.	n.d.	1,2 ±0,8	0,9 ±0,6	n.d.	2,1 ±1,8
Azithromycin	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	1,1 ±1,5	4,1 ±2,3	n.d.	4,4 ±2,6	n.d.	n.d.	n.d.	n.d.	3,7 ±1,3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Clarithromycin	n.d.	1,3 ±1,2	36,2 ±9,9	0,3 ±0,3	x	blq	blq	0,6 ±0,3	0,6 ±0,9	blq	n.d.	blq	1,2 ±0,8	1,1 ±1,3	1,1 ±1,7	1,2 ±0,8	blq	blq	blq	1,3 ±0,8	blq	1,9 ±0,9	n.d.	1,2 ±2,3	n.d.
Tetracycline	n.d.	n.d.	n.d.	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ofloxacin	n.d.	8,0 ±5,6	224,0 ±18,7	6,7 ±2,8	x	7,5 ±3,7	8,5 ±4,3	n.d.	7,4 ±4,7	n.d.	n.d.	6,5 ±4,7	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.
Ciprofloxacin	n.d.	n.d.	162,6 ±15,4	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfamethoxazole	4,7 ±2,4	16,0 ±9,7	37,2 ±6,4	13,9 ±4,7	x	n.d.	0,9 ±0,8	n.d.	n.d.	2,7 ±1,7	n.d.	n.d.	2,7 ±1,3	1,5 ±0,7	n.d.	n.d.	n.d.	n.d.	1,6 ±0,7	n.d.	2,2 ±0,7	2,4 ±3,2	0,5 ±0,5	0,9 ±0,7	n.d.
Trimethoprim	n.d.	1,8 ±2,1	17,4 ±3,8	1,1 ±0,9	x	n.d.	n.d.	n.d.	0,5 ±0,5	1,0 ±1,0	n.d.	n.d.	1,4 ±0,9	0,8 ±0,5	n.d.	2,2 ±1,2	n.d.	2,2 ±0,8	2,2 ±1,4	3,2 ±2,1	1,9 ±1,7	n.d.	n.d.	n.d.	blq
Total conc. (ng L^{-1})	**38,4**	**1401,5**	**2824,3**	**193,5**	**x**	**48,4**	**70,4**	**50,9**	**204,4**	**40,8**	**51,1**	**54,8**	**72,7**	**83,9**	**44,9**	**35,1**	**60,2**	**41,3**	**16,0**	**11,4**	**11,1**	**22,2**	**13,8**	**9,8**	**12,5**
MIN con. (ng L^{-1})	**1,7**	**0,5**	**1,2**	**0,3**	**x**	**7,2**	**0,9**	**0,6**	**0,5**	**1,0**	**3,6**	**2,4**	**0,2**	**0,1**	**0,6**	**0,6**	**0,3**	**0,9**	**0,7**	**1,3**	**0,5**	**0,6**	**0,5**	**0,9**	**1,4**
MAX con. (ng L^{-1})	**11,6**	**255,3**	**323,2**	**40,8**	**x**	**21,4**	**28,4**	**21,4**	**45,3**	**14,8**	**43,6**	**24,7**	**37,1**	**43,9**	**36,7**	**23,2**	**34,7**	**16,5**	**8,2**	**5,6**	**5,4**	**12,2**	**12,4**	**7,7**	**4,8**

* not available sampling point.

c)

Sampling seasons	**Spring**																							
Type of water	**River**			**Channels**							**Estuary**							**Seawater**						
Sample acronym	**CSW**	**RW1**	**RW2**	**CW1**	**CW2**	**CW3**	**CW4**	**CW5**	**CW6**	**CW7**	**EW1**	**EW2**	**EW3**	**EW4**	**EW5**	**EW6**	**EW7**	**SW1**	**SW2**	**SW3**	**SW4**	**SW5**	**SW6**	**SW7**	**SW8**
Analyte	**Therapeutic gruoup**																							
Analgesics/ant-inflammatories																								
Ketoprofen	n.d.	n.d.	198,2 ±16,7	n.d.	n.d.	n.d.	34,5 ±7,5	22,6 ±8,8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	44,2 ±16,7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Naproxen	n.d.	n.d.	96,6 ±12,3	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ibuprofen	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.	n.d.	n.d.	n.d.	n.d.	15,4 ±5,7	n.d.	n.d.	n.d.	1,2 ±1,2	n.d.	n.d.	n.d.
Acetaminophen	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	35,2 ±9,7	n.d.	n.d.	n.d.	n.d.	59,0 ±13,7	n.d.	n.d.	n.d.	2,9 ±1,3	4,4 ±2,1	n.d.	7,1 ±4,1	1,7 ±0,7	1,4 ±0,8	2,5 ±1,4	n.d.
Salicylic acid	53,6 ±9,8	44,8 ±6,7	25,4 ±9,7	35,6 ±12,4	35,7 ±9,7	38,8 ±13,4	34,3 ±6,7	29,5 ±6,7	38,0 ±11,3	32,5 ±8,7	49,5 ±7,7	47,3 ±9,8	38,6 ±16,7	n.d.	41,0 ±14,8	53,2 ±17,3	38,2 ±14,6	16,5 ±8,9	15,8 ±12,7	5,7 ±1,4	n.d.	8,9 ±2,4	7,7 ±2,7	n.d.	n.d.
Diclofenac	n.d.	n.d.	319 ±19,7	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.	18,4 ±12,7	n.d.	n.d.	n.d.	n.d.	8,4 ±1,8	n.d.	n.d.	4,5 ±1,4
Phenazone	n.d.	n.d.	132 ±9,6	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Propylphenazone	0,5 ±0,5	7,9 ±3,2	68,7 ±13,4	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lipid regulators and cholesterol lowering statin drugs																								
Bezafibrate	n.d.	n.d.	12,6 ±7,8	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gemfibrozil	3,9 ±2,1	3,2 ±1,5	5,9 ±6,4	n.d.	3,3 ±1,7	0,7 ±0,7	1,2 ±0,7	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.	n.d.	n.d.	n.d.	n.d.
Atorvastatin	0,9 ±0,8	0,9 ±0,9	5,1 ±3,4	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs																								
Carbamazepine	2,1 ±1,3	2,2 ±1,6	111,0 ±14,7	2,2 ±0,9	2,1 ±2,0	0,6 ±0,3	1,4 ±0,9	1,6 ±1,4	1,7 ±0,8	1,6 ±1,3	0,4 ±0,2	0,3 ±0,7	1,6 ±0,8	1,6 ±0,8	0,2 ±0,2	0,1 ±0,3	5,0 ±3,8	1,2 ±1,1	0,2 ±0,1	0,3 ±0,1	n.d.	n.d.	0,1 ±0,1	0,7 ±0,2	0,3 ±0,4
2-HydroxyCBZ	n.d.	n.d.	33,8 ±9,3	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10,11-epoxyCBZ	n.d.	n.d.	45,2 ±12,8	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acridone	n.d.	n.d.	5,3 ±2,1	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Setraline	n.d.	n.d.	13,7 ±5,4	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Citalopram	n.d.	n.d.	34,3 ±7,7	n.d.	n.d.	n.d.	n.d.	n.d.	0,8 ±1,3	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.	n.d.	n.d.	n.d.
Venlafaxine	2,2 ±1,8	226,5 ±15,8	348,7 ±21,2	3,6 ±1,3	n.d.	n.d.	n.d.	n.d.	1,5 ±0,9	n.d.	n.d.	14,7 ±8,5	n.d.	6,7 ±3,2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Olanzapine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,2 ±0,4	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.	n.d.
Trazadone	n.d.	n.d.	23,2 ±6,7	3,6 ±1,7	3,7 ±1,7	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fluoxetine	n.d.	n.d.	8,3 ±5,1	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Paroxetine	n.d.	n.d.	6,9 ±3,7	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diazepam	n.d.	n.d.	7,0 ±2,7	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lorazepam	n.d.	n.d.	95,2 ±16,9	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Alprazolam	n.d.	n.d.	8,9 ±5,7	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Histamine H1 and H2 receptor antagonis																								
Desloratadine	n.d.	n.d.	2,8 ±1,4	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ranitidine	n.d.	n.d.	20,1 ±12,7	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Famotidine	n.d.	blq	3,7 ±2,4	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cimetidine	1,0	1,2	4,7	1,1	1,3	1,2	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

	±1,2	±0,8	±3,9	±0,9	±1,7	±0,7																		
β-Blocking agents																								
Atenolol	0,6 ±0,3	0,7 ±0,5	24,7 ±4,7	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.	n.d.	0,9 ±1,5	n.d.	0,5 ±0,1	0,4 ±0,2	0,1 ±0,1	0,2 ±0,1	0,1 ±0,1	n.d.
Sotalol	n.d.	n.d.	82,7 ±9,8	n.d.	n.d.	n.d.	n.d.	n.d.	1,3 ±1,1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2,9 ±1,4	n.d.	n.d.	n.d.	3,2 ±0,8	n.d.	n.d.	n.d.
Propanolol	n.d.	n.d.	17,9 ±2,9	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Metoprolol	n.d.	n.d.	3,8 ±2,4	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diuretic																								
Hydrochlorothiazide	2,1 ±1,3	289,7 ±20,6	458,3 ±18,4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,3 ±0,8	n.d.	n.d.	blq	n.d.	n.d.	n.d.	4,2 ±1,7	1,9 ±0,6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Furosemide	n.d.	125,6 ±16,7	426,4 ±9,3	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Torasemide	n.d.	n.d.	7,5 ±4,2	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antidiabetic																								
Glibenclamide	n.d.	n.d.	3,7 ±2,7	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Losartan	1,8 ±0,9	n.d.	57,4 ±6,9	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Irbesartan	0,7 ±0,5	n.d.	143,6 ±13,7	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Valsartan	12,2 ±4,6	67,9 ±9,7	346,9 ±19,4	15,6 ±3,9	15,0 ±6,7	n.d.	n.d.	n.d.	n.d.	n.d.	2,9 ±2,4	3,3 ±2,1	n.d.	n.d.	n.d.	6,4 ±2,4	4,4 ±2,7	n.d.	n.d.	n.d.	0,3 ±0,1	n.d.	0,4 ±0,3	0,4 ±0,2	0,3 ±0,1
Antiplatelet agent																								
Clopidogrel	n.d.	n.d.	9,7 ±3,7	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Prostatic hyperplasia																								
Tamsulosin	n.d.	n.d.	3,5 ±2,1	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
To treat asthma																								
Salbutamol	blq	blq	2,4 ±1,7	blq	blq	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
X-ray contrast agent																								
Iopromide	n.d.	69,3 ±7,9	60,3 ±3,4	2,6 ±2,2	4,3 ±2,4	n.d.	n.d.	n.d.	n.d.	n.d.	60,1 ±13,4	72,0 ±17,8	19,0 ±9,7	n.d.	n.d.	45,5 ±13,4	16,8 ±7,4	1,7 ±0,7	0,8 ±0,3	n.d.	n.d.	2,1 ±1,4	n.d.	n.d.	n.d.
Antihelmintics																								
Thiabendazole	n.d.	n.d.	n.d.	4,1 ±1,7	n.d.	7,2 ±3,2	n.d.	2,7 ±2,1	1,4 ±0,7	n.d.	3,6 ±1,7	n.d.	4,7 ±2,3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Levamisol	1,9 ±1,3	225,6 ±15,7	571,3 ±6,7	1,8 ±1,2	1,8 ±1,3	0,8 ±0,7	n.d.	n.d.	2,2 ±1,5	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.	n.d.	n.d.	n.d.
Antibiotics																								
Erythromycin	n.d.	n.d.	29,8 ±3,4	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Azithromycin	n.d.	blq	123,4 ±7,7	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Clarithromycin	0,7 ±0,6	0,6 ±0,9	4,6 ±3,2	1,7 ±0,8	1,8 ±1,7	1,3 ±1,1	1,4 ±1,1	1,3 ±1,1	1,5 ±0,8	1,2 ±0,7	1,2 ±0,6	2,4 ±2,7	1,3 ±0,8	1,1 ±0,7	1,1 ±1,4	1,1 ±0,7	1,2 ±0,7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tetracycline	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.	n.d.	n.d.	n.d.	n.d.	5,8 ±2,1	2,3 ±1,5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ofloxacin	n.d.	n.d.	132,3 ±5,7	n.d.	2,8 ±1,9	n.d.	4,3 ±2,0	n.d.	n.d.	n.d.	6,6 ±2,4	0,2 ±0,1	blq	1,1 ±0,8	blq	blq	blq	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ciprofloxacin	n.d.	n.d.	119,1 ±9,7	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.	n.d.	3,8 ±1,5	6,4 ±2,4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfamethoxazole	3,2 ±1,8	3,5 ±1,7	20 ±7,8	8,5 ±3,4	3,6 ±2,2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3,6 ±1,5	2,1 ±1,3	n.d.	n.d.	n.d.	5,3 ±2,1	n.d.	1,3 ±2,1	1,2 ±0,8	3,3 ±0,7	4,6 ±1,7	n.d.	n.d.
Trimethoprim	n.d.	blq	3,6 ±2,2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5,4 ±2,3	1,1 ±0,5	n.d.	7,2 ±3,4	n.d.	0,9 ±0,5	1,2 ±0,7	3,1 ±3,2	0,4 ±0,2	0,4 ±0,1	0,7 ±0,3	2,3 ±1,8	0,4 ±0,2
Total conc. (ng L^{-1})	**87,4**	**1069,6**	**4289,2**	**80,4**	**75,4**	**50,6**	**77,1**	**57,7**	**83,6**	**35,3**	**126,8**	**140,2**	**74,2**	**72,7**	**42,3**	**106,3**	**128,2**	**61,5**	**33,0**	**10,9**	**9,4**	**28,9**	**15,1**	**6,0**	**5,5**
MIN con. (ng L^{-1})	**0,5**	**0,6**	**2,4**	**1,1**	**1,3**	**0,6**	**1,2**	**1,3**	**0,8**	**1,2**	**0,4**	**0,2**	**1,3**	**1,1**	**0,2**	**0,1**	**1,2**	**0,9**	**0,2**	**0,3**	**0,3**	**0,1**	**0,1**	**0,1**	**0,3**
MAX con. (ng L^{-1})	**53,6**	**289,7**	**571,3**	**35,6**	**35,7**	**38,8**	**34,5**	**29,5**	**38,0**	**32,5**	**60,1**	**72,0**	**38,6**	**59,0**	**41,0**	**53,2**	**44,2**	**16,5**	**15,8**	**5,7**	**7,1**	**8,9**	**7,7**	**2,5**	**4,5**

Tabela 10. Frequency of detection (%), range of concentration (min-max) and mean value in ng L^{-1} measured for the target analytes in downstream sites and irrigation and drainage channels during three sampling campaigns.

Sampling season	**Autumn**								**Winter**								**Spring**							
Type of water	**Downstream sites (n=2)**				**Channels (n=7)**				**Downstream sites (n=2)**				**Channels (n=6)**				**Downstream sites (n=2)**				**Channels (n=7)**			
Compounds	**Freq (%)**	**Min**	**Max**	**Mean**	**Freq (%)**	**Min**	**Max**	**Mean**	**Freq (%)**	**Min**	**Max**	**Mean**	**Freq (%)**	**Min**	**Max**	**Mean**	**Freq (%)**	**Min**	**Max**	**Mean**	**Freq (%)**	**Min**	**Max**	**Mean**
		(ng L^{-1})				**(ng L^{-1})**				**(ng L^{-1})**				**(ng L^{-1})**				**(ng L^{-1})**				**(ng L^{-1})**		
Ketoprofen	50	n.d.	97,9	97,9	29	37,5	39,2	38,4	50	n.d.	215,1	215,1	33	17,8	21,4	19,6	50	n.d.	198,2	198,2	29	22,6	34,5	28,6
Naproxen	n.d.				14	n.d.	48,8	48,8	100	35,9	53,2	44,6	50	7,3	40,5	26	50	n.d.	96,6	96,6	n.d.			
Ibuprofen	50	n.d.	18,5	18,5	57	6,7	14,0	9,4	50	n.d.	28,9	28,9	50	15,3	45,3	27,3	n.d.				n.d.			
Indomethacine	50	n.d.	10,4	10,4	14	n.d.	13,3	13,3	50	n.d.	16,1	16,1	n.d.				n.d.				n.d.			
Acetaminophen	100	13,4	16,0	14,7	100	7,9	14,9	11,0	50	n.d.	135	135	n.d.				n.d.				14	n.d.	35,2	35,2
Salicylic acid	n.d.				71	13,9	20,5	16,7	100	12,1	23,1	17,6	83	8,4	28,4	15,3	100	25,4	44,8	35,1	100	29,5	38,8	34,9
Diclofenac	50	n.d.	280,3	280,3	n.d.				50	n.d.	169,7	169,7	n.d.				50	n.d.	319,0	319,0	n.d.			
Phenazone	100	3,1	242,1	122,6	29	2,8	42,1	22,5	100	29,9	55,4	42,7	33	1,5	1,6	1,5	50	n.d.	132,0	132,0	n.d.			
Propylphenazone	50	n.d.	1,3	1,3	29	1,2	1,2	1,2	50	n.d.	1,1	1,1	17	<blq	0,9	0,9	100	7,9	68,7	38,3	n.d.			
Piroxicam	n.d.				n.d.				50	n.d.	11,4	11,4	n.d.				n.d.				n.d.			
Oxycodone	50	n.d.	28,5	28,5	n.d.				n.d.				n.d.				n.d.				n.d.			
Codeine	100	2	49,2	25,6	n.d.				100	2,7	7,9	5,3	n.d.				n.d.				n.d.			
Bezafibrate	50	n.d.	47,3	47,3	14	<blq	18,7	18,7	100	1,8	28,3	15,1	33	1,0	1,7	1,4	50	n.d.	12,6	12,6	n.d.			
Gemfibrozil	100	37,4	147,5	92,5	14	<blq	6,4	6,4	100	8,2	87,5	47,9	50	8,5	14,8	11,4	100	3,2	5,9	4,6	43	0,7	3,3	1,7
Atorvastatin	n.d.				14	n.d.	2,3	2,3	100	0,9	1,7	1,3	n.d.				100	0,9	5,1	3,0	n.d.			

Carbamazepine	100	69,6	112,3	91,0	86	1,7	3,8	2,8	100	26,5	36,8	31,6	17	n.d.	1,9	1,9	100	2,2	111,0	56,6	100	0,6	2,2	1,6
2-HydroxyCBZ	<blq				<blq				100	1,3	21,2	11,3	17	n.d.	0,5	0,5	50	n.d.	33,8	33,8	<blq			
10,11-epoxyCBZ	50	n.d.	49,7	49,7	14	n.d.	41,0	41,0	100	1,8	21,6	11,7	17	n.d.	1,6	1,6	50	n.d.	45,2	45,2	n.d.			
Acridone	n.d.				n.d.				50	n.d.	1,8	1,8	n.d.				50	n.d.	5,3	5,3	n.d.			
Setraline	100	18,9	30,4	24,7	n.d.				50	n.d.	4,7	4,7	n.d.				50	n.d.	13,7	13,7	n.d.			
Citalopram	100	46,5	101,8	74,1	43	1,1	52,8	18,5	100	0,9	42,8	21,8	17	<blq	0,8	0,8	50	n.d.	34,3	34,3	14	n.d.	0,8	0,8
Venlafaxine	100	216,4	341,8	279,1	43	5,1	21,3	10,7	50	n.d.	144,2	144,2	33	1,5	3,6	2,6	100	227	349	288	29	1,5	3,6	2,6
Trazadone	100	12,0	44,5	28,2	n.d.				n.d.				n.d.				50	n.d.	23,2	23,2	29	3,6	3,7	3,7
Fluoxetine	50	n.d.	38,7	38,7	29	2,5	4,9	3,7	50	n.d.	5,8	5,8	n.d.				50	n.d.	8,3	8,3	n.d.			
Paroxetine	100	6,2	16,2	11,2	n.d.				n.d.				n.d.				50	n.d.	6,9	6,9	n.d.			
Diazepam	100	1,3	3,9	2,6	n.d.				100	0,5	2,5	1,5	n.d.				50	n.d.	7,0	7,0	n.d.			
Lorazepam	n.d.				n.d.				50	n.d.	215	215	n.d.				50	n.d.	95,2	95,2	n.d.			
Alprazolam	50	n.d.	24,0	24,0	14	n.d.	6,8	6,8	50	n.d.	4,3	4,3	n.d.				50	n.d.	8,9	8,9	n.d.			
Loratadine	n.d.				n.d.				100	1,2	1,3	1,3	17	n.d.	1,3	1,3	n.d.				n.d.			
Desloratadine	50	n.d.	2,6	2,6	29	1,2	1,3	1,2	50	n.d.	3,6	3,6	n.d.				50	n.d.	2,8	2,8	n.d.			
Ranitidine	50	n.d.	2,3	2,3	n.d.				100	1,3	14,5	7,9	n.d.				50	n.d.	20,1	20,1	n.d.			
Famotidine	n.d.				n.d.				n.d.				n.d.				50	n.d.	3,7	3,7	n.d.			
Cimetidine	50	n.d.	36,0	36,0	14	n.d.	1,0	1,0	100	1,4	4,4	2,9	17	<blq	1,4	1,4	100	1,2	4,7	3,0	43	1,1	1,3	1,2
Atenolol	50	n.d.	129,8	129.8	14	<blq	1,6	1,6	100	10,8	24,6	17,7	33	1,2	7,3	4,3	100	0,7	24,7	12,7	n.d.			
Sotalol	100	24,3	65,4	44,9	14	n.d.	1,7	1,7	100	28,8	36,2	32,5	17	n.d.	20,1	20,1	50	n.d.	82,7	82,7	14	n.d.	1,3	1,3
Propanolol	100	12,9	34,1	23,5	n.d.				100	0,7	19,1	9,9	17	n.d.	0,6	0,6	50	n.d.	17,9	17,9	n.d.			
Metoprolol	n.d.				n.d.				100	0,9	4,3	2,6	17	n.d.	3,4	3,4	50	n.d.	3,8	3,8	n.d.			
Nadolol	n.d.				n.d.				100	1,2	1,3	1,2	17	n.d.	0,3	0,3	n.d.				n.d.			
Carazolol	50	n.d.	1,0	1,0	n.d.				n.d.				n.d.				n.d.				n.d.			
Hydrochlorothiazide	100	207,6	442,1	324,9	14	n.d.	5,6	5,6	100	124,5	305,1	214,8	33	0,5	6,8	3,7	100	289,7	458,3	374,0	n.d.			
Furosemide	50	n.d.	464,2	464,2	n.d.				50	n.d.	248,6	248,6	n.d.				100	125,6	426,4	276,0	n.d.			
Torasemide	n.d.				n.d.				50	n.d.	9,8	9,8	17	<blq	0,5	0,5	50	n.d.	7,5	7,5	n.d.			
Glibenclamide	100	12,4	20,5	16,4	14	n.d.	3,2	3,2	50	n.d.	1,3	1,3	n.d.				50	n.d.	3,7	3,7	n.d.			
Amlodipine	n.d.				n.d.				50	n.d.	1,8	1,8	n.d.				n.d.				n.d.			
Losartan	100	71,9	218,7	145,3	n.d.				50	n.d.	255,3	255,3	17	n.d.	15,8	15,8	50	n.d.	57,4	57,4	n.d.			
Irbesartan	100	39,2	189,7	109,4	43	1,8	12,7	5,5	100	4,5	289,6	147,1	33	3,5	4,3	3,9	50	n.d.	143,6	143,6	n.d.			
Valsartan	100	284,7	400,0	342,4	100	2,0	26,7	16,2	100	234,2	323,2	278,7	33	25,3	40,8	33,1	100	67,9	346,9	207,4	29	15	15,6	15,3
Clopidogrel	100	2,7	6,0	4,4	14	n.d.	4,1	4,1	50	n.d.	4,8	4,8	n.d.				50	n.d.	9,7	9,7	n.d.			
Tamsulosin	100	2,9	8,5	5,7	n.d.				50	n.d.	5,6	5,6	n.d.				50	n.d.	3,5	3,5	n.d.			
Salbutamol	n.d.				n.d.				n.d.				n.d.				50	n.d.	2,4	2,4	n.d.			
Iopromide	n.d.				71	5,0	7,4	5,7	100	18,3	27,7	23	17	n.d.	12,3	12,3	100	60,3	69,3	64,8	29	2,6	4,3	3,5
Albendazole	50	n.d.	123,3	123,3	n.d.				n.d.				n.d.				n.d.				n.d.			
Thiabendazole	100	8,3	34,0	21,1	71	1,1	12,4	5,1	50	n.d.	3,5	3,5	67	1,4	7,2	3,9	n.d.				57	1,4	7,2	3,9

Levamisol	100	7,1	56,2	31,7	14	<blq	2,0	2,0	100	102	147,4	124,8	50	4,1	33,3	21,5	100	225,6	571,3	398,5	57	0,8	2,2	1,7
Azaperone	100	2,9	3,8	3,4	43	1,1	3,0	1,7	n.d.				n.d.				n.d.				n.d.			
Erythromycin	100	12,3	25,7	19,0	n.d.				50	n.d.	1,4	1,4	n.d.				50	n.d.	29,8	29,8	n.d.			
Azithromycin	50	n.d.	220,7	220,7	n.d.				n.d.				33	1,1	4,1	2,6	50	n.d.	123,4	123,4	n.d.			
Clarithromycin	100	9,9	90,0	49,9	n.d.				100	1,3	36,2	18,7	50	0,3	0,6	0,5	100	0,6	4,6	2,6	100	1,2	1,8	1,5
Ofloxacin	n.d.				n.d.				100	8,0	224,0	116,0	67	6,7	8,5	7,5	50	n.d.	132,3	132,3	29	2,8	4,3	3,6
Ciprofloxacin	n.d.				n.d.				50	n.d.	162,6	162,6	n.d.				50	n.d.	119,1	119,1	n.d.			
Sulfamethoxazole	100	6,0	99,0	52,5	n.d.				100	16	37,2	26,6	50	0,9	13,9	5,8	100	3,5	20,0	11,8	29	3,6	8,5	6,1
Trimethoprim	100	11,2	24,1	17,6	n.d.				100	1,8	17,4	9,6	50	0,5	1,1	0,9	50	n.d.	3,6	3,6	n.d.			
Diltiazem	n.d.				14	n.d.	17,9	17,9	n.d.				n.d.				n.d.				n.d.			
Norverapamil	100	3,2	4,7	4,0	n.d.				<blq				n.d.				<blq				n.d.			

Table S11. Frequency of detection (%), range of concentration (min-max) and mean value in ng L^{-1} measured for the target analytes in eatuarine and seawater samples during three sampling campaign.

Sampling season	**Autumn**								**Winter**								**Spring**							
Type of water	**Estuary (n=7)**				**Seawater (n=6)**				**Estuary (n=7)**				**Seawater (n=6)**				**Estuary (n=7)**				**Seawater (n=6)**			
Compounds	Freq (%)	Min	Max	Mean	Freq (%)	Min	Max	Mean	Freq (%)	Min	Max	Mean	Freq (%)	Min	Max	Mean	Freq (%)	Min	Max	Mean	Freq (%)	Min	Max	Mean
		(ng L^{-1})				(ng L^{-1})				(ng L^{-1})				(ng L^{-1})				(ng L^{-1})				(ng L^{-1})		
Ketoprofen	n.d.				n.d.				14	n.d.	34,7	34,7	n.d.				14	n.d.	44,2	44,2	n.d.			
Ibuprofen	14	n.d.	21,5	21,5	17	n.d.	23,9	23,9	n.d.				13	n.d.	4,2	4,2	n.d.				25	1,2	15,4	8,3
Acetaminophen	86	16,5	61,0	37,3	83	2,9	13,7	6,0	14	n.d.	23,2	23,2	50	2,6	16,5	8,1	14	n.d.	59,0	59,0	75	1,4	7,1	3,3
Salicylic acid	86	18,0	39,2	30,2	83	6,0	13,1	9,8	86	14,2	43,9	33,4	75	4,8	14,5	9,6	86	38,2	53,2	44,6	63	5,7	16,5	10,9
Diclofenac	14	n.d.	23,5	23,5	n.d.				14	n.d.	5,7	5,7	n.d.				14	n.d.	18,4	18,4	25	4,5	8,4	6,5
Phenazone	14	n.d.	2,4	2,4	n.d.				n.d.				n.d.				n.d.				n.d.			
Atorvastatin	n.d.				n.d.				29	0,8	0,9	0,9	n.d.				n.d.				n.d.			
Carbamazepine	71	0,2	1,1	0,6	67	0,5	1,1	0,8	71	0,3	1,9	1,0	50	0,5	1,4	1,0	100	0,1	5,0	1,3	75	0,1	1,2	0,5
Citalopram	14	n.d.	3,1	3,1	n.d.				n.d.				n.d.				n.d.				n.d.			
Venlafaxine	29	7,8	12,4	10,1	n.d.				29	7,8	12,7	10,3	n.d.				29	6,7	14,7	10,7	n.d.			
Olanzapine	n.d.				n.d.				n.d.				n.d.				14	n.d.	1,2	1,2	n.d.			
Ranitidine	n.d.				n.d.				57	0,9	2,1	1,4	n.d.				n.d.				n.d.			

Cimetidine	n.d.				n.d.				29	0,9	1,4	1,2	n.d.				n.d.				n.d.			
Atenolol	29	1,7	2,7	2,2	n.d.				n.d.				25	0,7	0,9	0,8	n.d.				75	0,1	0,9	0,4
Sotalol	43	7,8	15,4	10,6	33	2,7	9,8	6,3	29	9,8	11,3	10,6	25	1,3	3,4	2,4	n.d.				25	2,9	3,2	3,0
Metoprolol	14	n.d.	2,9	2,9	n.d.				n.d.				n.d.				n.d.				n.d.			
Hydrochlorothiazide	14	n.d.	19,9	19,9	17	n.d.	2,8	2,8	n.d.				<blq				14	n.d.	1,3	1,3	25	1,9	4,2	3,1
Glibenclamide	n.d.				n.d.				29	0,1	0,2	0,2	n.d.				n.d.				n.d.			
Irbesartan	n.d.				17	n.d.	2,6	2,6	14	<blq	0,3	0,3	n.d.				n.d.				n.d.			
Valsartan	86	0,8	8,5	3,3	100	2,0	4,3	2,9	86	1,6	10,7	5,9	63	0,6	2,7	1,6	57	2,9	6,4	4,2	50	0,3	0,4	0,4
Iopromide	14	n.d.	8,4	8,4	17	n.d.	2,4	2,4	43	2,7	4,1	3,6	n.d.				71	16,8	72,0	42,7	38	0,8	2,1	1,5
Thiabendazole	29	3,1	5,4	4,2	n.d.				29	3,6	4,7	4,2	n.d.				29	3,6	4,7	4,2	n.d.			
Erythromycin	43	11,2	20,4	14,4	50	1,5	2,7	2,0	n.d.				38	0,9	2,1	1,4	n.d.				<blq			
Azithromycin	n.d.				n.d.				29	3,7	4,4	4,1	n.d.				n.d.				<blq			
Clarithromycin	14	n.d.	3,2	3,2	33	1,9	6,2	4,1	57	1,1	1,2	1,2	38	1,2	1,9	1,5	100	1,1	2,4	1,3	n.d.			
Tetracycline	n.d.				67	0,4	3,4	1,5	n.d.				n.d.				n.d.				25	2,3	5,8	4,1
Ofloxacin	n.d.				n.d.				14	n.d.	6,5	6,5	n.d.				43	0,2	6,6	2,6	n.d.			
Ciprofloxacin	n.d.				n.d.				n.d.				n.d.				n.d.				25	3,8	6,4	5,1
Sulfamethoxazole	n.d.				n.d.				29	1,5	2,7	2,1	63	0,5	2,4	1,5	29	2,1	3,6	2,9	63	1,2	5,3	3,1
Trimethoprim	n.d.				33	1,3	2,8	2,1	43	0,8	2,2	1,5	50	1,9	3,2	2,4	43	1,1	7,2	4,6	100	0,4	3,1	1,2

Table S12. Pearson's correlation between the salinity and total PhACs concentrations for estuary and seawater.

*estuary and seawater				
Correlations				
		Total_con_phac_water	**Num_of_compounds_water**	**Salinity**
Salinity	**Pearson's correlation**	-,090	,170	**1**
	Sig. (2-tailed)	,557	,264	
	N	**45**	**45**	**45**
***. Correlation is significant at the 0.05 level (2-tailed).**				

Table S13. Individual concentrations of PhACs±STD (ng L^{-1}) in sediment samples during the autumn (a); the winter (b) and spring season (c).

a)

Sampling seasons													**Autumn**										
Type of water		**River**				**Channels**								**Estuary**							**Seawater**			
Sample acronym	**CSS**	**RS1**	**RS2**	**CS1**	**CS2**	**CS3**	**CS4**	**CS5**	**CS6**	**CS7**	**ES1**	**ES2**	**ES3**	**ES4**	**ES5**	**ES6**	**ES7**	**SS1**	**SS2**	**SS3**	**SS4***	**SS5**	**SS6**	**SS7**	**SS8***
Analyte													**Therapeutic gruoup**												
Analgesics/ant-inflammatories																									
Ketoprofen	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Ibuprofen	n.d.	n.d.	n.d.	10,3±4,5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2,1±1,4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	1,1±0,8	n.d.	n.d.	x
Diclofenac	n.d.	n.d.	6,8±2,2	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Phenazone	n.d.	n.d.	2,8±1,2	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.	n.d.	n.d.	n.d.	n.d.	x.	n.d.	n.d.	n.d.	x.
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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Lipid regulators and cholesterol lowering statin drugs																									
Bezafibrate	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Gemfibrozil	n.d.	n.d.	11,3±3,2	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Pravastatin	n.d.	n.d.	n.d.	1,8±1,1	1,4±1,2	n.d.	n.d.	n.d.	n.d.	8,2±4,5	2,5±1,3	n.d.	n.d.	n.d.	n.d.	n.d.	3,2±2,4	n.d.	1,1±3,2	n.d.	x	n.d.	n.d.	n.d.	x
Psychiatric drugs																									
Carbamazepine	0,1±0,5	1,2±0,8	2,0±0,9	n.d.	n.d.	n.d.	n.d.	blq	blq	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	blq	n.d.	n.d.	x	blq	n.d.	n.d.	x
Citalopram	blq	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Fluoxetine	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
β-Blocking agents																									
Sotalol	n.d.	n.d.	blq	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Propanolol	n.d.	1,7±0,7	2,4±1,2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,7±0,5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Diuretic																									
Hydrochlorothiazide	n.d.	n.d.	2,6±2,1	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.	n.d.	n.d.	n.d.	n.d.	x	blq	n.d.	n.d.	x
Furosemide	n.d.	n.d.	1,4±1,9	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.	n.d.	blq	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Antihypertensives																									
Irbesartan	n.d.	n.d.	3,5±2,4	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Valsartan	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Antihelmintics																									
Thiabendazole	blq	n.d.	3,4±1,8	2,2±1,5	1,3±1,1	n.d.	n.d.	n.d.	n.d.	n.d.	4,8±2,1	n.d.	n.d.	n.d.	n.d.	n.d.	2,8±1,4	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Levamisol	n.d.	n.d.	blq	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.	blq	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Antibiotics																									
Erythromycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,5±0,8	1,5±1,2	2,8±3,2	n.d.	n.d.	n.d.	2,5±1,8	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Azithromycin	n.d.	n.d.	2,3±1,5	1,9±0,8	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.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Clarithromycin	n.d.	n.d.	1,1±0,9	n.d.	n.d.	n.d.	n.d.	blq	blq	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,1±1,2	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Calcium channel blockers																									
Verapamil	n.d.	n.d.	1,8±0,5	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.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Norverapamil	n.d.	n.d.	2,8±2,0	n.d.	n.d.	n.d.	n.d.	n.d.	blq	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	x
Total PhACs conc (ng g^{-1})	**0,1**	**2,9**	**44,2**	**16,2**	**2,7**	n.d.	n.d.	n.d.	blq	**9,9**	**7,3**	**1,5**	**3,6**	**2,8**	n.d.	n.d.	**6**	**3,6**	**1,1**	n.d.	**x**	**1,1**	n.d.	n.d.	**x**
MIN conc. (ng g^{-1})	**0,1**	**1,2**	**1,1**	**1,8**	**1,3**	n.d.	n.d.	n.d.	blq	**1,7**	**2,5**	**1,5**	**1,5**	**2,8**	n.d.	n.d.	**2,8**	**1,1**	**1,1**	n.d.	**x**	**1,1**	n.d.	n.d.	**x**
MAX conc. (ng g^{-1})	**0,1**	**1,7**	**11,3**	**10,3**	**1,4**	n.d.	n.d.	n.d.	blq	**8,2**	**4,8**	**1,5**	**2,1**	**2,8**	n.d.	n.d.	**3,2**	**2,5**	**1,1**	n.d.	**x**	**1,1**	n.d.	n.d.	**x**

* not available sampling points.

b)

Sampling seasons														**Winter**											
Type of water		**River**				**Channels**									**Estuary**						**Seawater**				
Sample acronym	**CSS**	**RS1**	**RS2**	**CS1**	**CS2***	**CS3**	**CS4**	**CS5**	**CS6**	**CS7**	**ES1**	**ES2**	**ES3**	**ES4**	**ES5**	**ES6**	**ES7**	**SS1**	**SS2**	**SS3**	**SS4**	**SS5**	**SS6**	**SS7**	**SS8**
Analyte														**Therapeutic gruoup**											
Analgesics/ant-inflammatories																									
Ketoprofen	n.d.	blq	12,5±3,4	n.d.	x	6,5±1,9	n.d.	7,2±2,1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8,8±3,5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ibuprofen	n.d.	n.d.	n.d.	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diclofenac	n.d.	n.d.	2,4±2,1	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phenazone	n.d.	n.d.	1,3±1,9	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Codeine	blq	blq	blq	0,9±0,5	x	n.d.	n.d.	n.d.	0,8±0,5	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.	n.d.
Lipid regulators and cholesterol lowering statin drugs																							
Bezafibrate	n.d.	n.d.	1,2±0,7	0,4±0,1	x	0,5±0,3	0,6±0,4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,8±1,2	1,2±0,7	n.d.	n.d.	blq	n.d.	n.d.	n.d.	blq	blq	n.d.
Gemfibrozil	n.d.	n.d.	1,6±2,3	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pravastatin	n.d.	n.d.	1,5±1,1	5,0±2,0	x	n.d.	n.d.	n.d.	8,5±3,6	n.d.	1,9±1,8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,5±1,0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs																							
Carbamazepine	n.d.	1,2±0,9	1,7±0,9	n.d.	x	n.d.	0,8±0,2	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.	n.d.	n.d.	n.d.	n.d.
Citalopram	blq	1,1±1,3	7,8±1,3	n.d.	x	n.d.	n.d.	n.d.	3,0±2,8	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.	n.d.	n.d.
Fluoxetine	n.d.	n.d.	blq	n.d.	x	n.d.	n.d.	n.d.	4,4±3,3	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.	n.d.	n.d.
β-Blocking agents																							
Sotalol	n.d.	n.d.	blq	n.d.	x	n.d.	n.d.	n.d.	0,7±0,4	0,6±0,5	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.	n.d.
Propanolol	n.d.	n.d.	2,8±0,9	n.d.	x	n.d.	n.d.	n.d.	0,4±0,2	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.	n.d.	n.d.
Diuretic																							
Hydrochlorothiazide	n.d.	n.d.	8,6±3,2	n.d.	x	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.	n.d.	n.d.	n.d.	blq	n.d.	n.d.	n.d.
Furosemide	n.d.	n.d.	n.d.	n.d.	x	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	blq	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antihypertensives																							
Irbesartan	n.d.	n.d.	blq	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Valsartan	n.d.	n.d.	1,89±1,1	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antihelmintics																							
Thiabendazole	1,1±0,8	1,7±2,2	7,5±2,1	n.d.	x	n.d.	1,2±0,5	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.	n.d.	n.d.	n.d.	n.d.	n.d.
Levamisol	0,2±0,1	0,1±0,5	3,2±1,6	0,1±0,1	x	0,1±0,2	n.d.	n.d.	0,3±0,8	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.	n.d.	n.d.	n.d.
Antibiotics																							
Erythromycin	n.d.	blq	blq	n.d.	x	blq	blq	blq	n.d.	blq	blq	blq	n.d.	blq	blq	blq	n.d.	n.d.	blq	n.d.	blq	blq	blq	blq	blq
Azithromycin	blq	1,2±0,7	2,2±0,8	1,3±0,4	x	n.d.	5,23±1,8	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.	n.d.	n.d.	n.d.	n.d.	n.d.
Clarithromycin	blq	blq	blq	blq	x	blq	blq	blq	blq	blq	blq	blq	blq	blq	blq	blq	blq	blq	blq	blq	blq	blq	blq	blq	blq
Calcium channel blockers																							
Verapamil	n.d.	n.d.	2,4±2,4	n.d.	x	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Norverapamil	blq	blq	3,2±1,7	n.d.	x	n.d.	blq	n.d.	blq	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.	n.d.	n.d.	n.d.
Total PhACs conc (ng g^{-1})	**1,3**	**5,3**	**61,8**	**7,7**	**x**	**7,1**	**7,8**	**7,2**	**18,1**	**0,6**	**1,9**	blq	blq	blq	**1,8**	**1,2**	**8,8**	**1,5**	blq	blq	blq	blq	blq	blq	blq
MIN conc. (ng g^{-1})	**0,2**	**0,1**	**1,2**	**0,1**	**x**	**0,1**	**0,6**	**7,2**	**0,3**	**0,6**	**1,9**	blq	blq	blq	**1,8**	**1,2**	**8,8**	**1,5**	blq	blq	blq	blq	blq	blq	blq
MAX conc. (ng g^{-1})	**1,1**	**1,7**	**12,5**	**5,0**	**x**	**6,5**	**5,2**	**7,2**	**8,5**	**0,6**	**1,9**	blq	blq	blq	**1,8**	**1,2**	**8,8**	**1,5**	blq	blq	blq	blq	blq	blq	blq

* not available sampling point.

c)

Sampling seasons													**Spring**											
Type of water		**River**					**Channels**							**Estuary**								**Seawater**		
Sample acronym	**CSS**	**RS1**	**RS2**	**CS1**	**CS2**	**CS3**	**CS4**	**CS5**	**CS6**	**CS7**	**ES1**	**ES2**	**ES3**	**ES4**	**ES5**	**ES6**	**ES7**	**SS1**	**SS2**	**SS3**	**SS4**	**SS5**	**SS6**	**SS7**	**SS8**
Analyte													**Therapeutic gruoup**											
Analgesics/ant-inflammatories																								
Ketoprofen	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ibuprofen	n.d.	n.d.	n.d.	3,3±2,4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,8±1,2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,1±1,6	n.d.	n.d.	n.d.
Diclofenac	n.d.	n.d.	3,7±1,3	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phenazone	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Codeine	n.d.	blq	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lipid regulators and cholesterol lowering statin drugs																									
Bezafibrate	n.d.	n.d.	1,2±0,9	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gemfibrozil	n.d.	n.d.	1,6±2,1	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pravastatin	n.d.	n.d.	n.d.	1,2±1,7	1,1±0,8	n.d.	n.d.	n.d.	2,4±1,3	3,2±1,5	1,5±0,8	n.d.	n.d.	n.d.	n.d.	n.d.	1,5±0,7	n.d.	1,1±0,5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs																									
Carbamazepine	n.d.	blq	n.d.	n.d.	n.d.	n.d.	0,8±0,2	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.	n.d.	n.d.	n.d.	n.d.	n.d.
Citalopram	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3,0±2,8	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.	n.d.	n.d.	n.d.
Fluoxetine	n.d.	blq	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,0±0,8	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.	n.d.	n.d.	n.d.
β-Blocking agents																									
Sotalol	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Propanolol	n.d.	blq	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,7±0,7	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.	n.d.	n.d.
Diuretic																									
Hydrochlorothiazide	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	blq	n.d.	n.d.	n.d.
Furosemide	n.d.	n.d.	1,8±1,4	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.	n.d.	blq	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antihypertensives																									

Irbesartan	n.d.	n.d.	blq	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Valsartan	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antihelmintics																									
Thiabendazole	n.d.	blq	7,8±4,4	2,2±1,1	1,3±0,4	n.d.	n.d.	n.d.	n.d.	n.d.	5,1±2,1	n.d.	n.d.	n.d.	n.d.	n.d.	2,8±0,8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Levamisol	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.	n.d.	n.d.	n.d.	blq	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antibiotics																									
Erythromycin	n.d.	blq	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,9±1,0	2,5±3,2	2,8±1,7	n.d.	n.d.	n.d.	2,5±2,4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Azithromycin	n.d.	n.d.	n.d.	3,9±2,4	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Clarithromycin	n.d.	blq	n.d.	n.d.	n.d.	n.d.	n.d.	blq	blq	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,1±0,9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Calcium channel blockers																									
Verapamil	n.d.	n.d.	2,6±4,8	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Norverapamil	n.d.	blq	3,8±1,9	n.d.	n.d.	n.d.	n.d.	n.d.	blq	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.	n.d.	n.d.	n.d.
Total PhACs conc (ng g^{-1})	**n.d.**	**blq**	**22,5**	**10,6**	**2,4**	**n.d.**	**0,8**	**blq**	**6,4**	**4,9**	**6,6**	**1,9**	**4,3**	**2,8**	**n.d.**	**n.d.**	**4,3**	**3,6**	**1,1**	**n.d.**	**n.d.**	**1,1**	**n.d.**	**n.d.**	**n.d.**
MIN conc. (ng g^{-1})	**n.d.**	**blq**	**1,2**	**1,2**	**1,1**	**n.d.**	**0,8**	**blq**	**1,0**	**1,7**	**1,5**	**1,9**	**1,8**	**2,8**	**n.d.**	**n.d.**	**1,5**	**1,1**	**1,1**	**n.d.**	**n.d.**	**1,1**	**n.d.**	**n.d.**	**n.d.**
MAX conc. (ng g^{-1})	**n.d.**	**blq**	**7,8**	**3,9**	**1,3**	**n.d.**	**0,8**	**blq**	**3,0**	**3,2**	**5,1**	**1,9**	**2,5**	**2,8**	**n.d.**	**n.d.**	**2,8**	**2,5**	**1,1**	**n.d.**	**n.d.**	**1,1**	**n.d.**	**n.d.**	**n.d.**

Table S14. Frequency of detection (%), range of concentration (min-max) and mean value in ng g^{-1} measured for the target analytes in sediment samples taken from: a) downstream sites and irrigation channels and b) estuarie and sea, during three sampling campaigns.

a)

Sampling season	**Autumn**								**Winter**								**Spring**							
Type of water	**Downstream sites (n=2)**				**Channel (n=7)**				**Downstream sites (n=2)**				**Channel (n=6)**				**Downstream sites (n=2)**				**Channel (n=7)**			
Compounds	**Freq. (%)**	**Min**	**Max**	**Mean**	**Freq. (%)**	**Min**	**Max**	**Mean**	**Freq. (%)**	**Min**	**Max**	**Mean**	**Freq. (%)**	**Min**	**Max**	**Mean**	**Freq. (%)**	**Min**	**Max**	**Mean**	**Freq. (%)**	**Min**	**Max**	**Mean**
		(ng g^{-1})				**(ng g^{-1})**				**(ng g^{-1})**				**(ng g^{-1})**				**(ng g^{-1})**				**(ng g^{-1})**		
Ketoprofen	<blq				n.d.				50	n.d.	12,5	12,5	29	6,5	7,2	6,9	<blq				n.d.			
Ibuprofen	n.d.				14	n.d.	10,3	10,3	n.d.				n.d.				n.d.				14	n.d.	3,3	3,3
Diclofenac	50	n.d.	6,8	6,8	n.d.				50	n.d.	2,4	2,4	n.d.				50	n.d.	3,7	3,7	n.d.			
Phenazone	50	n.d.	2,8	2,8	n.d.				50	n.d.	1,3	1,3	n.d.				n.d.				n.d.			
Codeine	n.d.				n.d.				n.d.				14	n.d.	0,8	0,8	n.d.				n.d.			
Bezafibrate	n.d.				n.d.				50	n.d.	1,2	1,2	29	0,5	0,6	0,6	50	n.d.	1,2	1,2	n.d.			
Gemfibrozil	50	<blq	11,3	11,3	n.d.				50	<blq	1,6	1,6	n.d.				50	<blq	1,6	1,6	n.d.			
Pravastatin	n.d.				43	1,4	8,2	3,8	50	n.d.	1,5	1,5	14	n.d.	8,5	8,5	n.d.				57	1,1	3,2	2,0
Carbamazepine	100	1,2	2,0	1,6	n.d.				100	1,2	1,7	1,4	14	n.d.	0,8	0,8	n.d.				14	n.d.	0,8	0,8
Citalopram	n.d.				n.d.				100	1,1	7,8	4,5	14	n.d.	3,0	3,0	n.d.				14	n.d.	3,0	3,0
Fluoxetine	n.d.				n.d.				n.d.				14	n.d.	4,4	4,4	n.d.				14	n.d.	1,0	1,0
Sotalol	n.d.				n.d.				n.d.				29	0,6	0,7	0,6	n.d.				n.d.			
Propanolol	100	1,7	2,4	2,0	14	n.d.	1,7	1,7	50	n.d.	2,8	2,8	14	n.d.	0,4	0,4	n.d.				14	n.d.	1,7	1,7
Hydrochlorothiazide	50	<blq	2,6	2,6	n.d.				50	<blq	8,6	8,6	n.d.				n.d.				n.d.			
Furosemide	50	n.d.	1,4	1,4	n.d.				n.d.				n.d.				50	n.d.	1,8	1,8	n.d.			
Irbesartan	50	n.d.	3,5	3,5	n.d.				n.d.				n.d.				n.d.				n.d.			

Valsartan	n.d.				n.d.				50	n.d.	1,9	1,9	n.d.				n.d.				n.d.			
Thiabendazole	50	n.d.	3,4	3,4	29	1,3	2,2	1,8	100	1,7	7,5	4,6	14	n.d.	1,2	1,2	50	<blq	7,8	7,8	29	1,3	2,2	1,8
Levamisol	n.d.				n.d.				100	0,1	3,2	1,7	29	0,1	0,3	0,2	n.d.				n.d.			
Erythromycin	<blq				<blq				<blq				<blq				<blq				<blq			
Azithromycin	50	n.d.	2,3	2,3	14	n.d.	1,9	1,9	100	1,2	2,2	1,7	14	n.d.	5,3	5,3	n.d.				14	n.d.	3,9	3,9
Clarithromycin	50	n.d.	1,1	1,1	n.d.				n.d.				n.d.				n.d.				n.d.			
Verapamil	50	n.d.	1,8	1,8	n.d.				50	n.d.	2,4	2,4	n.d.				50	<blq	2,6	2,6	n.d.			

b)

Sampling season	**Autumn**								**Winter**								**Spring**							
Type of water	**Estuary (n=7)**				**Seawater (n=6)**				**Estuary (n=7)**				**Seawater (n=8)**				**Estuary (n=7)**				**Seawater (n=8)**			
Compounds	**Freq. (%)**	**Min**	**Max**	**Mean**	**Freq. (%)**	**Min**	**Max**	**Mean**	**Freq. (%)**	**Min**	**Max**	**Mean**	**Freq. (%)**	**Min**	**Max**	**Mean**	**Freq. (%)**	**Min**	**Max**	**Mean**	**Freq. (%)**	**Min**	**Max**	**Mean**
		(ng g-1)				**(ng g-1)**				**(ng g-1)**				**(ng g-1)**				**(ng g-1)**				**(ng g-1)**		
Ketoprofen	<blq				n.d.				14	n.d.	8,8	8,8	n.d.				<blq				n.d.			
Ibuprofen	14	n.d.	2,1	2,1	17	n.d.	1,1	1,1	n.d.				n.d.				14	n.d.	1,8	1,8	13	n.d.	1,1	1,1
Bezafibrate	n.d.				n.d.				29	1,2	1,8	1,5	n.d.				n.d.				n.d.			
Pravastatin	29	2,5	3,2	2,9	17	n.d.	1,1	1,1	n.d.				13	n.d.	1,5	1,5	29	1,5	1,5	1,5	13	n.d.	1,1	1,1
Fluoxetine	<blq				n.d.				<blq				n.d.				<blq				n.d.			
Propanolol	<blq				n.d.				<blq				n.d.				<blq				n.d.			
Furosemide	<blq				n.d.				<blq				n.d.				<blq				n.d.			
Thiabendazole	29	2,8	4,8	3,8	n.d.				n.d.				n.d.				29	2,8	5,1	4,0	n.d.			
Erythromycin	43	1,5	2,8	1,9	17	n.d.	2,5	2,5	<blq				<blq				43	1,9	2,8	2,4	13	n.d.	2,5	2,5
Azithromycin	<blq				<blq				<blq				n.d.				<blq				n.d.			
Clarithromycin	<blq				17	n.d.	1,1	1,1	n.d.				n.d.				n.d.				13	n.d.	1,1	1,1

Table S15. Pearson's correlation between TOC and total PhACs concentrations/number of compounds in sediment.

Correlations				
Sediment samples		**Total_con_PhACs_sediment**	**Num_of_compounds_sediment**	**TOC**
TOC	**Pearson's correlation**	,356**	,314**	**1**
	Sig. (2-tailed)	,002	,006	

	N	**75**	**75**	**75**
****. Correlation is significant at the 0.01 level (2-tailed).**				

TOC=total organic carbon;

Table S16. Pearson's correlation between the physic.chemical properties and total PhACs concentrations for sediment.

Correlations						
		Mean_KD	**KOW**	**Solubility**	**log_D**	**p_Ka**
Mean_KD	**Pearson's correlation**	**1**	.424**	**-,082**	**,144**	**-,041**
	Sig. (2-tailed)		,010	**,536**	**,276**	**,757**
	N	**60**	**59**	**59**	**56**	**60**
****. Correlation is significant at the 0.01 level (2-tailed).**						
***. Correlation is significant at the 0.05 level (2-tailed).**						

logKOW = log of octanol-water partition coefficient;

logKOC = log of organic carbon partition coefficient;

logD =log of partition of a chemical compound between the lipid and aqueous phases;

pKa=the negative base-10 logarithm of the acid dissociation constant (Ka) of a solution, pKa = -log10Ka;

Table S17. Pysicochemical parametres for PhACs detected in marine water.

Therapeutic groups	Compounds	Type of water						Pysicochemical parametres				
		Averge freq (%)	estuary		Averge freq (%)	seawater		LogKow	Solubility (mg L^{-1})	$t_{1/2}$ in water (days)	K_d (L kg^{-1})	BCF
			Min (ng L^{-1})	Max (ng L^{-1})		Min (ng L^{-1})	Max (ng L^{-1})					
Analgesics/ant-inflammatories	Ketoprofen	10	34,7	44,2	-			3,1	120,4	15	253,6	3,162
	Ibuprofen	5	21,5	21,5	18	1,2	23,9	3,5	41,05	15	96,8-916,7	3,162
	Acetaminophen	38	16,5	61,0	68	1,4	16,5	0,3	30350	15	-	3,162
	Salicylic acid	86	14,2	53,2	73	4,8	16,5	2,3	3808	15	-	3,162
	Diclofenac	14	5,7	23,5	9	4,5	8,4	4,5	4,518	37,5	-	3,162
	Phenazone	5	2,4	2,4	-	n.d.	n.d.	0,4	23760	15	-	3,162
Lipid regulators	Atorvastatin	10	0,8	0,9	-	n.d.	n.d.	-	-	-	-	-
Psychiatric drugs	Carbamazepine	81	0,1	5,0	64	0,1	1,4	2,3	17,66	37,5	-	15,36
	Citalopram	5	3,1	3,1	-	n.d.	n.d.	3,7	31,09	180	-	151,9
	Venlafaxine	29	6,7	14,7	-	n.d.	n.d.	3,3	266,7	60	-	67
	Olanzapine	5	1,2	1,2	-	n.d.	n.d.	2,6	53,33	60	-	18,71
Histamine H_1 and H_2 receptor antagonist	Ranitidine	19	0,9	2,1	-	n.d.	n.d.	0,3	24660	37,5	-	3,162
	Cimetidine	10	0,9	1,4	-	n.d.	n.d.	0,6	7426	37,5	-	3,162
β-Blocking agents	Atenolol	10	1,7	2,7	36	0,1	0,9	(-)0,03	685,2	37,5	-	3,162
	Sotalol	24	7,8	15,4	27	1,3	9,8	0,2	5513	15	-	3,162
	Metoprolol	5	2,9	2,9	-	n.d.	n.d.	1,9	4777	37,5	-	1,252
Diuretic	Hydrochlorothiazide	10	1,3	19,9	14	1,9	4,2	(-)0,2	1292	60	-	3,162
Antidiabetic	Glibenclamide	10	0,1	0,2	-	n.d.	n.d.	2,4	0,063456	180	-	973,8
Antihypertensives	Irbesartan	5	0,3	0,3	5	2,6	2,6	5,3	0,05991	37,5	-	2427
	Valsartan	76	0,8	10,7	68	0,3	4,3	4,0	1,406	15	-	3,162
X-ray contrast agent	Iopromide	43	2,7	72,0	18	0,8	2,4	-	-	-	-	-
Antihelmintics	Thiabendazole	29	3,1	5,4	-	n.d.	n.d.	2,5	339,2	15	88,9-1416,7	15,92
Antibiotics	Erythromycin	14	11,2	20,4	27	0,9	2,7	2,5	<1	-	126,1-911,1	-
	Azithromycin	10	3,7	4,4	-	n.d.	n.d.	0,9	slight	-	-	-
	Clarithromycin	57	1,1	3,2	23	1,2	6,2	3,2	0,33	-	177,4	-
	Tetracycline	-	n.d.	n.d.	27	0,4	5,8	(-)1,3	3887	60	-	3,162
	Ofloxacin	19	0,2	6,6	-	n.d.	n.d.	(-)2,0	676200	60	-	3,162
	Ciprofloxacin	-	n.d.	n.d.	9	3,8	6,4	0,3	11480	60	-	3,162
	Sulfamethoxazole	19	1,5	3,6	45	0,5	5,3	0,9	3942	37,5	-	3,162
	Trimethoprim	29	0,8	7,2	64	0,4	3,2	0,9	2334	60	-	3,162

Table S18. Priority chemical markers with physicho-chemical properties (see **Table 2**, main manuscript).

Therapeutic groups	Compounds	Max freq of detcetion (%)	Max detected con. (estuary and seawater)	HQ=MEC/PNEC	BCF	WWTP1	WWTP2	$t_{1/2}$ in water (days)	Score
						Average removal, %			
Analgesics/ant-inflammatories	Ketoprofen	14	44.2	0,0001	3,162	63	-79	15	**13**
	Ibuprofen	25	23,9	0,0004	3,162	100	100	15	**11**
	Acetaminophen	86	61,0	0,0003	3,162	98	89	15	**14**
	Salicylic acid	86	53,2	0,0000	3,162	83	98	15	**14**
	Diclofenac	25	23,5	0,0005	3,162	38	58	37,5	**15**
	Phenazone	14	2,4	0,0002	3,162	36	-315	15	**11**
Lipid regulators	Atorvastatin	29	0,9	0,0001	-	76	98	-	**6**
Psychiatric drugs	Carbamazepine	100	5,0	0,0005	15,36	40	24	37,5	**16**
	Citalopram	14	3,1	0,0002	151,9	22	33	180	**15**
	Venlafaxine	29	14,7	0,0006	67	22	-1	60	**17**
	Olanzapine	14	1,2	-	18,71		87	60	**13**
Histamine H_1 and H_2 receptor antagonist	Ranitidine	57	2,1	0,000001	3,162	63	77	37,5	**13**
	Cimetidine	29	1,4	-	3,162	60	69	37,5	**12**
β-Blocking agents	Atenolol	75	2,7	0,00002	3,162	85	75	37,5	**13**
	Sotalol	43	15,4	0,0002	3,162	26	6	15	**14**
	Metoprolol	14	2,9	0,00001	1,252	55	56	37,5	**12**
Diuretic	Hydrochlorothiazide	25	19,9	0,00001	3,162	51	41	60	**16**
Antidiabetic	Glibenclamide	29	0,2	-	973,8	78	85	180	**12**
Antihypertensives	Irbesartan	17	2,6	0,0491	2427	59	-47	37,5	**15**
	Valsartan	100	10,7	0,0005	3,162	86	81	15	**14**
X-ray contrast agent	Iopromide	71	72,0	0,000001	-	11	-104	-	**12**
Antihelmintics	Thiabendazole	29	5,4	0,0004	15,92	58	67	15	**14**
Antibiotics	Erythromycin	50	20,4	-	-	37	83	-	**10**
	Azithromycin	29	4,4	-	-	48	10	-	**8**
	Clarithromycin	100	6,2	-	-	40	18	-	**11**
	Tetracycline	67	5,8	-	3,162	-	-	60	**11**
	Ofloxacin	43	6,6	-	3,162	56	77	60	**13**
	Ciprofloxacin	25	6,4	-	3,162	53	78	60	**13**
	Sulfamethoxazole	63	5,3	0,0001	3,162	60	78	37,5	**14**
	Trimethoprim	100	7,2	0,0001	3,162	57	86	60	**16**

Fig. S1. Average removal efficiencies (%) calculated as averages of the efficiencies of three sampling campaigns in two selected WWTPs.

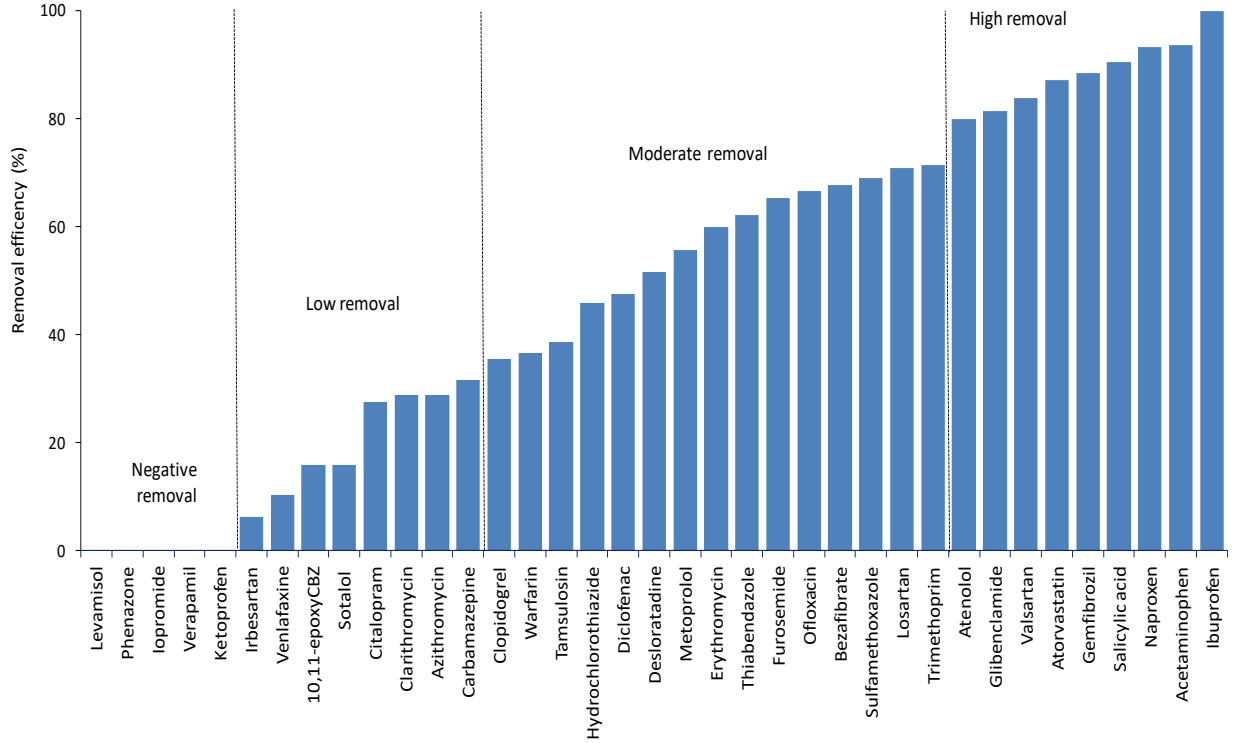
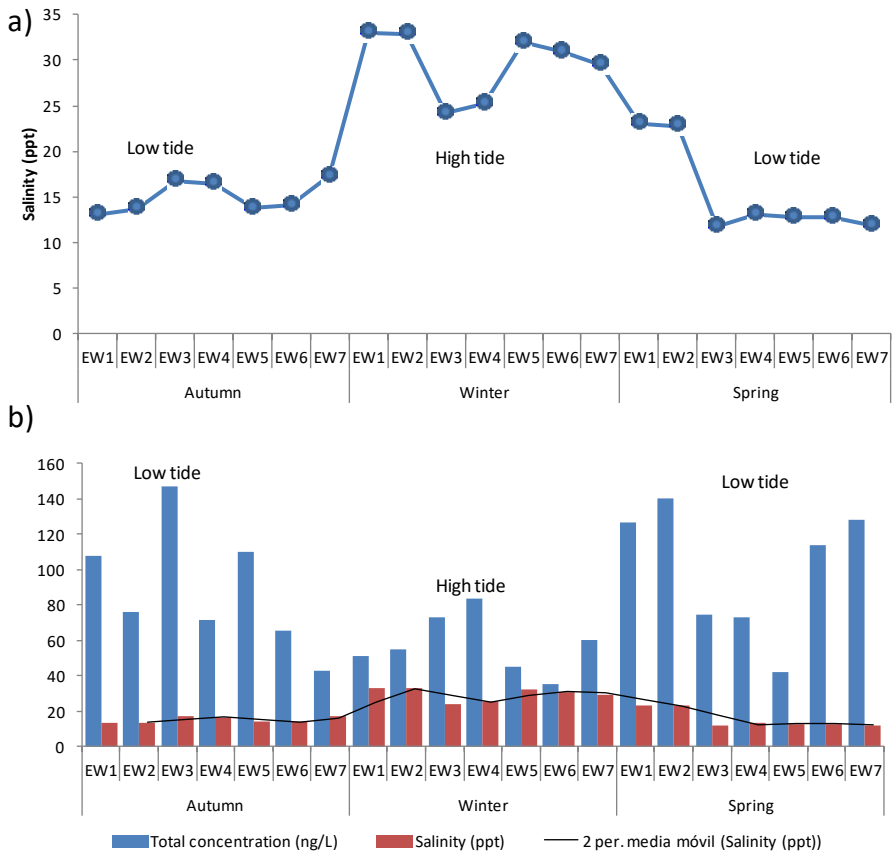


Fig. S2 a) Salinity (ppt) during three sampling campaings at different sampling points in estuaries; b) Total concentrations of PhACs during the tidal event at different sampling points in estuarine waters.



Supplementary material of Chapter 3

Article Nº4:

Mira Čelić, Adrián Jaén-Gil, Susana Briceño-Guevara, Sara Rodriguez-Mozaz, Meritxell Gros, and Mira Petrović

Extended suspect screening to identify emerging organic contaminants in riverine and coastal ecosystems and assessment of environmental risks

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Supplementary Material has been reformed to match the style of the thesis.

Extended suspect screening to identify emerging organic contaminants in riverine and coastal ecosystems and assessment of environmental risks

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Table S1. Suspect list provided by the Catalan Water Agency (ACA) that contained information about the 360 emerging organic contaminants (EOCs), their transformation products (TPs), and metabolites.

Aplication	Compound	CAS number	Chem formula	Exact mass	$[M+H]^+$	$[M-H]^-$
Pharmaceuticals and metabolites (130)						
Analgesics / ant-inflammatories	Salycilic acid	69-72-7	C7H6O3	138.0316940	139.0389706	137.0244174
	5-Aminosalicylic acid (Mesalamine)	89-57-6	C7H7NO3	153.0425930	154.0498696	152.0353164
	Diclofenac	15307-86-5	C14H11Cl2NO2	295.0166839	296.0239606	294.0094073
	4′-Hydroxydiclofenac	64118-84-9	C14H11Cl2NO3	311.0115985	312.0188752	310.0043219
	Ibuprofen	15687-27-1	C13H18O2	206.1306797	207.1379564	205.1234031
	1-Hydroxyibuprofen	53949-53-4	C13H18O3	222.1255943	223.1328710	221.1183177
	2-Hydroxyibuprofen	51146-55-5	C13H18O3	222.1255943	223.1328710	221.1183177
	Carboxy-Ibuprofen	15935-54-3	C13H16O4	236.1048589	237.1121355	235.0975823
	Naproxen	22204-53-1	C14H14O3	230.0942942	231.1015708	229.0870176
	Acetaminophen (Paracetamol)	103-90-2	C8H9NO2	151.0633285	152.0706051	150.0560518
	Propyphenazone	479-92-5	C14H18N2O	230.1419131	231.1491898	229.1346365
	Phenazone	60-80-0	C11H12N2O	188.0949630	189.1022396	187.0876863
	4-Acetamidoantipyrine	83-15-8	C13H15N3O2	245.1164266	246.1237033	244.1091500
	4-Formylaminoantipyrine	1672-58-8	C12H13N3O2	231.1007766	232.1080532	230.0935000
	Codeine	76-57-3	C18H21NO3	299.1521434	300.1594201	298.1448668
	Ketoprofen	22071-15-4	C16H14O3	254.0942942	255.1015708	253.0870176
	Indomethacine	53-86-1	C19H16ClNO4	357.0767856	358.0840622	356.0695090
	Piroxicam	36322-90-4	C15H13N3O4S	331.0626765	332.0699531	330.0553999
	Meloxicam	71125-38-7	C14H13N3O4S2	351.0347472	352.0420238	350.0274706
	Tenoxicam	59804-37-4	C13H11N3O4S2	337.0190971	338.0263738	336.0118205
	Oxycodone	76-42-6	C18H21NO4	315.1470580	316.1543347	314.1397814
	Flunixin	38677-85-9	C14H11N2O2	239.0820525	240.0893292	238.0747759
Lipid regulators and cholesterol lowering statin drugs	Bezafibrate	41859-67-0	C19H20ClNO4	361.1080857	362.1153623	360.1008091
	Gemfibrozil	25812-30-0	C15H22O3	250.1568945	251.1641711	249.1496178
	Simvastatin Hydroxy Acid Sodium Salt	101314-97-0	C25H39NaO6	458.2644335	459.2717101	457.2571568
	Simvastatin	79902-63-9	C25H38O5	418.2719241	419.2792008	417.2646475
	Pravastatin	81093-37-0	C23H36O7	424.2461033	425.2533799	423.2388267
	Fluvastatin	93957-54-1	C24H26FNO4	411.1845864	412.1918630	410.1773098
	Atorvastatin	134523-00-5	C33H35FN2O5	558.2530003	559.2602769	557.2457236
	Ortiatrovastatin	214217-86-4	C33H38FN2NaO8	632.2509888	633.2582655	631.2437122
Psychiatric drugs	Acridone	578-95-0	C13H9NO	195.0684139	196.0756905	194.0611372
	Setraline	79617-96-2	C17H17Cl2N	305.0738049	306.0810815	304.0665283
	Olanzapine	132539-06-1	C17H20N4S	312.1408673	313.1481439	311.1335907
	Trazadone	19794-93-5	C19H22ClN5O	371.1512880	372.1585646	370.1440113
	Paroxetine	61869-08-7	C19H20FNO3	329.1427216	330.1499982	328.1354450
	Alprazolam	28981-97-7	C17H13Cl4	356.9771362	357.9844128	355.9698596
	Carbamazepine	298-46-4	C15H12N2O	236.0949630	237.1022396	235.0876863
	Carbamazepine 10,11-epoxide	36507-30-9	C15H12N2O2	252.0898776	253.0971542	251.0826009
	2-Hydroxycarbamazepine	68011-66-5	C15H12N2O2	252.0898776	253.0971542	251.0826009
	Citalopram	59729-33-8	C20H21FN2O	324.1637914	325.1710681	323.1565148
	Desmethylcitalopram	62498-67-3	C19H19FN2O	310.1481414	311.1554180	309.1408647
	Didemethylcitalopram	62498-69-5	C18H17FN2O	296.1324913	297.1397679	295.1252147
	Citalopram N-Oxide	63284-72-0	C20H21FN2O2	340.1587060	341.1659827	339.1514294
	Diazepam	439-14-5	C16H13ClN2O	284.0716407	285.0789173	283.0643641
	Nordiazepam (metabolit diazepam)	1088-11-5	C15H11ClN2O	270.0559906	271.0632673	269.0487140
	Oxazepam	604-75-1	C15H11ClN2O2	286.0509052	287.0581819	285.0436286
	Lorazepam	846-49-1	C15H10Cl2N2O2	320.0119329	321.0192095	319.0046563
	Lormetazepam	848-75-9	C16H12Cl2N2O2	334.0275830	335.0348596	333.0203063
	Temazepam	846-50-4	C16H13ClN2O2	300.0665553	301.0738319	299.0592787
	Fluoxetine	54910-89-3	C17H18F3NO	309.1340487	310.1413254	308.1267721
	Fluoxetine Hydrochloride	54910-89-3	C17H19ClF3NO	345.1107265	346.1180031	344.1034498
	Norfluoxetine	83891-03-6	C16H17F3NO	296.1262237	297.1335003	295.1189471
	Venlafaxine	93413-69-5	C17H27NO2	277.2041790	278.2114556	276.1969024
	N-Desmethylvenlafaxine	93413-62-8	C16H25NO2	263.1885289	264.1958056	262.1812523
	O-Desmethylvenlafaxine	149289-30-5	C16H25NO2	263.1885289	264.1958056	262.1812523
	Diidesmethylvenlafaxine	93413-77-55	C15H23NO2	249.1728789	250.1801555	248.1656023
Opioid analgesics	Tramadol	27203-92-5	C16H25NO2	263.1885289	264.1958056	262.1812523
	O-Desmethyltramadol	80456-81-1	C15H23NO2	249.1728789	250.1801555	248.1656023
	N-Desmethyltramadol	73806-55-0	C15H23NO2	249.1728789	250.1801555	248.1656023
	O-Desmethyltramadol	73806-55-0	C15H23NO2	249.1728789	250.1801555	248.1656023
	Morphine	57-27-2	C17H19NO3	285.1364934	286.1437700	284.1292167
	Methadone	76-99-3	C21H27NO	309.2092644	310.2165410	308.2019878
	EDDP perchlorate (CRM)	31161-17-8	C20H24ClNO4	377.1393858	378.1466624	376.1321092
	Methadone hydrochloride	1095-90-5	C21H28ClNO	345.1859421	346.1932188	344.1786655
β-Blocking agents	Atenolol	29122-68-7	C14H22N2O3	266.1630425	267.1703191	265.1557658
	Metoprolol	37350-58-6	C15H25NO3	267.1834435	268.1907202	266.1761669
	Metoprolol acid (Atenolol acid)	56392-14-4	C14H21NO4	267.1470580	268.1543347	266.1397814

	a-Hydroxy metroprolol acid	56392-16-6	C15H25NO4	283.1783582	284.1856348	282.1710815
	Sotalol	3930-20-9	C12H20N2O3S	272.1194631	273.1267397	271.1121865
	Propanolol	525-66-6	C16H21NO2	259.1572288	260.1645055	258.1499522
	Nadolol	42200-33-9	C17H27NO4	309.1940082	310.2012848	308.1867316
	Carazolol	57775-29-8	C18H22N2O2	298.1681279	299.1754045	297.1608512
	Bisoprolol	66722-44-9	C18H31NO4	325.2253083	326.2325850	324.2180317
Diuretics	Furosemide	54-31-9	C12H11ClN2O5S	330.0077197	331.0149964	329.0004431
	Torasemide	56211-40-6	C16H20N4O3S	348.1256111	349.1328877	347.1183345
	Hydrochlorothiazide	58-93-5	C7H8ClN3O4S2	296.9644747	297.9717514	295.9571981
Cardiovascular drugs	Lisinopril	83915-83-7	C21H35N3O7	441.2475002	442.2547769	440.2402236
	Lidocaine	91484-71-8	C14H22N2O	234.1732133	235.1804899	233.1659366
	Enalapril	75847-73-3	C20H28N2O5	376.1998218	377.2070985	375.1925452
	Enalaprilat	76420-72-9	C18H24N2O5	348.1685217	349.1757983	347.1612451
	Verapamil	52-53-9	C27H38N2O	454.2831575	455.2904342	453.2758809
Anticonvulsants and metabolites	Primidone	125-33-7	C12H14N2O2	218.1055276	219.1128042	217.0982510
	Lamotrigine	84057-84-1	C9H7Cl2N5	255.0078506	256.0151272	254.0005740
	Valproic acid	99-66-1	C8H16O2	144.1150297	145.1223063	143.1077531
	Gabapentin	60142-96-3	C9H17NO2	171.1259287	172.1332053	170.1186521
	Gabapentin Lactam	64744-50-9	C9H15NO	153.1153640	154.1226407	152.1080874
	Phenytoin	57-41-0	C15H12N2O2	252.0898776	253.0971542	251.0826009
Antihypertensives	Valsartan	137862-53-4	C24H29N5O3	435.2270397	436.2343163	434.2197630
	Losartan	114798-26-4	C22H23ClN6O	422.1621870	423.1694636	421.1549104
	5-Carboxilosartan (Losartan Carboxylic Acid)	124750-92-1	C22H21ClN6O2	436.1414515	437.1487282	435.1341749
	Telmisartan	144701-48-4	C33H30N4O2	514.2368761	515.2441527	513.2295995
	Irbesartan	138402-11-6	C25H28N6O	428.2324594	429.2397361	427.2251828
	Amlodipine	88150-42-9	C20H25ClN2O5	408.1451994	409.1524761	407.1379228
Histamine H1 and H2 receptor antagonist	Cimetidine	51481-61-9	C10H16N6S	252.1157152	253.1229918	251.1084386
	Loratadine	79794-75-5	C22H23ClN2O2	382.1448056	383.1520822	381.1375290
	Desloratadine	100643-71-8	C19H19ClN2	311.1309529	310.1236763	309.1163996
	Famotidine	76824-35-6	C8H15N7O2S3	337.0449347	338.0522114	336.0376581
	Ranitidine	66357-35-5	C13H22N4O3S	314.1412612	315.1485378	313.1339845
Contrast agents and metabolites	Diatrizoic Acid	117-96-4	C11H9I3N2O4	613.7696357	614.7769123	612.7623590
	Iohexol	66108-95-0	C19H26I3N3O9	820.8803082	821.8875848	819.8730315
	Iomeprol	78649-41-9	C17H22I3N3O8	776.8540935	777.8613701	775.8468168
	Iopamidol	60166-93-0	C17H22I3N3O8	776.8540935	777.8613701	775.8468168
	Iopromide	73334-07-3	C18H24I3N3O8	790.8697435	791.8770202	789.8624669
Antihypertensive Agents	Diltiazem	42399-41-7	C22H26N2O4S	414.1613279	415.1686045	413.1540513
Calcium channel blockers	Norverapamil	67018-85-3	C26H36N2O4	440.2675075	441.2747841	439.2602309
Antifungal agents	Clotrimazole	23593-75-1	C22H17ClN2	344.1080262	345.1153028	343.1007496
Immunosuppressant drug	Mycophenolic acid	24280-93-1	C17H20O6	320.1259882	321.1332648	319.1187116
Corticosteroids	Prednisolone	50-24-8	C21H28O5	360.1936738	361.2009505	359.1863972
	Dexamethasone	50-02-2	C22H29FO5	392.1999021	393.2071787	391.1926254
Anti-androgen agent	Finasteride	98319-26-7	C23H36N2O2	372.2776783	373.2849549	371.2704017
Aromatase inhibitors	Letrozole	112809-51-5	C17H11N5	285.1014453	286.1087220	284.0941687
Antidiabetic	Metformin	657-24-9	C4H11N5	129.1014453	130.1087220	128.0941687
	Glibenclamide (Glyburide)	10238-21-8	C23H28ClN3O5S	493.1438192	494.1510959	492.1365426
PDE5 inhibitors	Sildenafil	139755-83-2	C22H30N6O4S	474.2049240	475.2122006	473.1976474
Angiotensin 2 receptor blocker	Candesartan	139481-59-7	C24H20N6O3	440.1596884	441.1669650	439.1524118
Adrenergic beta-2 Receptor Agonists	Salbutamol	18559-94-9	C13H21NO3	239.1521434	240.1594201	238.1448668
	Xylazine	7361-61-7	C12H16N2S	220.1034192	221.1106958	219.0961426
Antineoplastic Agents, Alkylating Agents	Cyclophosphamide	50-18-0	C7H15Cl2N2O2P	260.0248195	261.0320962	259.0175429
Sympathomimetics	Apophedrin (Phenylethanolamine)	7568-93-6	C8H11NO	107.0860753	108.0933520	106.0787987
	Ephedrine	299-42-3	C10H15NO	165.1153640	166.1226407	164.1080874
Proton Pump Inhibitors and metabolites	5-Hydroxyomeprazole	92340-57-3	C17H19N3O4S	362.1169033	361.1096267	360.1023500
Antiplatelet inhibidor	Clopidogrel	113665-84-2	C16H16ClNO2S	321.0590271	322.0663037	320.0517505
Prostatic hyperplasia	Tamsulosin	106133-20-4	C20H28N2O5S	408.1718925	409.1791692	407.1646159
Anticoagulant	Warfarin	81-81-2	C19H16O4	308.1048589	309.1121355	307.0975823
Antihelmintic Agents	Albendazole	54965-21-8	C12H15N3O2S	265.0884974	266.0957740	264.0812207
	Thiabendazole	148-79-8	C10H7N3S	201.0360679	202.0433445	200.0287913
	Flubendazole	31430-15-6	C16H12FN3O3	313.0862694	314.0935460	312.0789927
	Levamisol	14769-73-4	C11H12N2S	204.0721191	205.0793957	203.0648424
Tranquilizers	Azaperone	1649-18-9	C19H22FN3O	327.1746905	328.1819671	326.1674138
	Azaperol	2804-05-9.	C19H24FN3O	329.1903405	330.1976171	328.1830639
Antibiotics and metabolites (42)						
Cephalosporins	Cefalexin	15686-71-2	C16H17N3O4S	347.0939766	348.1012532	346.0867000
	Cefuroxime	55268-75-2	C16H16N4O8S	424.0688840	425.0761606	423.0616073

	Cetiofur	80370-57-6	C19H17N5O7S3	523.0290098	524.0362864	522.0217332
	Azitromycin	83905-01-5	C38H72N2O12	748.5085254	749.5158020	747.5012487
	Clarithromycin	81103-11-9	C38H69NO13	747.4768909	748.4841675	746.4696142
	Erythromycin	114-07-8	C37H67NO13	733.4612408	734.4685174	732.4539642
Macrolides	Anhydroerythromycin A	23893-13-2	C37H65NO12	715.4506761	716.4579528	714.4433995
	Roxithromycin	80214-83-1	C41H76N2O15	836.5245693	837.5318459	835.5172926
	Tilmicosin	108050-54-0	C46H80N2O13	868.5660402	869.5733168	867.5587636
	Tylosin	1401-69-0	C46H77NO17	915.5191495	916.5264261	914.5118729
Lincosamides	Clindamycin	21462-39-5	C18H33ClN2O5S	424.1798704	425.1871470	423.1725938
	Lincomycin	154-21-2	C18H34N2O6S	406.2137573	407.2210339	407.2210339
	Ciprofloxacin	85721-33-1	C17H18FN3O3	331.1332195	332.1404962	330.1259429
	Desethylene ciprofloxacin	103222-12-4	C15H16FN3O3	305.1175695	306.1248461	304.1102929
	Enrofloxacin	93106-60-6	C19H22FN3O3	359.1645197	360.1717963	358.1572430
Quinolones	Marbofloxacin	115550-35-1	C17H19FN4O4	362.1390332	363.1463098	361.1317565
	Norfloxacin	70458-96-7	C16H18FN3O3	319.1332195	320.1404962	318.1259429
	Ofloxacin	82419-36-1	C18H20FN3O4	361.1437842	362.1510608	360.1365076
	Oxolonic acid	14698-29-4	C13H11NO5	261.0637223	262.0709990	260.0564457
	Sulfametoxazole	723-46-6	C10H11N3O3S	253.0521118	254.0593885	252.0448352
Sulfonamides & Trimethoprim	N4-Acetylsulfamethoxazole	21312-10-7	C12H13N3O4S	295.0626765	296.0699531	294.0553999
	Sulfadiazine	68-35-9	C10H10N4O2S	250.0524462	251.0597228	249.0451696
	Trimethoprim	738-70-5	C14H18N4O3	290.1378903	291.1451670	289.1306137
	3-Desmethyl trimethoprim	27653-69-6	C13H16N4O3	276.1222403	277.1295169	275.1149636
Tetracyclines	Doxycycline	564-25-0	C22H24N2O8	444.1532655	445.1605422	443.1459889
	Tetracycline	60-54-8	C22H24N2O8	444.1532655	445.1605422	443.1459889
	Amoxicillin	26787-78-0	C16H19N3O5S	365.1045413	366.1118179	364.0972646
	Ampicilin	69-53-4	C16H19N3O4S	349.1096267	350.1169033	348.1023500
	Metronidazole	443-48-1	C6H9N3O3	171.0643911	172.0716677	170.0571144
Penicillins	Metronidazole OH	4812-40-2	C6H9N3O4	187.0593057	188.0665823	186.0520290
	Dimetridazole	551-92-8	C5H7N3O2	141.0538264	142.0611030	140.0465498
	Penicilin	61-33-6	C16H18N2O4S	334.0987276	335.1060043	333.0914510
	Ronidazole	7681-76-7	C6H8N4O4	200.0545546	201.0618313	199.0472780
Peptidyl transferase/Amphenicols	Chloramphenicol	56-75-7	C11H12Cl2N2O5	322.0123268	323.0196034	321.0050501
	Florfenicol	73231-34-2	C12H14Cl2FNO4S	357.0004621	358.0077387	355.9931855
Sulfonamide antibacterial and metabolites	Sulfadoxine	2447-57-6	C12H14N4O4S	310.0735755	311.0808522	309.0662989
	N4-Acetyl Sulfadoxine	5018-54-2	C14H16N4O5S	352.0841402	353.0914168	351.0768636
	Sulfamethazine (Sulfadimidine)	57-68-1	C12H14N4O2S	278.0837463	279.0910229	277.0764697
	Sulfapyridine	144-83-2	C11H11N3O2S	249.0571972	250.0644739	248.0499206
	N-Acetyl Sulfapyridine	19077-98-6	C13H13N3O3S	291.0677619	292.0750385	290.0604853
	Sulfadimethoxine	122-11-2	C12H14N4O4S	310.0735755	311.0808522	309.0662989
	Prostaglandin E2	363-24-6	C20H32O5	352.2249740	353.2322506	351.2176973
Psychoactive drugs and metabolites (11)	Aminorex	2207-50-3	C9H10N2O	162.0793129	163.0865895	161.0720363
	Amphetamine	300-62-9	C9H13N	135.1047994	136.1120760	134.0975228
	Benzoylecgonine (BECG)	519-09-5	C16H19NO4	289.1314080	290.1386846	288.1241313
	Cocaine (COC)	50-36-2	C17H21NO4	303.1470580	304.1543347	302.1397814
	Dimefline	1165-48-6	C20H21NO3	323.1521434	324.1594201	322.1448668
	Lysergic acid diethylamide (LSD)	50-37-3	C20H25N3O	323.1997624	324.2070390	322.1924857
	Methamphetamine (MA)	537-46-2	C10H15N	149.1204495	150.1277261	148.1131728
	DL-Methamphetamine	7632-10-02	C9H13ClFN	189.0720553	190.0793319	188.0647787
	Δ9-Tetrahydrocannabinol (THC)	1972-08-03	C21H30O2	314.2245801	315.2318567	313.2173035
	11-Nor-9-carboxy-THC	64280-14-4	C21H28O4	344.1987592	345.2060359	343.1914826
	MDMA hydrochloride	64057-70-1	C11H16ClNO2	229.0869564	230.0942330	228.0796798
Stimulant and metabolites (8)	Nicotine	54-11-5	C10H14N2	162.1156984	163.1229750	161.1084218
	4-Aminonicotinic acid	7418-65-7	C6H6N2O2	138.0429274	139.0502040	137.0356508
	Cotinine	486-56-6	C10H12N2O	176.0949630	177.1022396	175.0876863
	Caffeine	58-08-2	C8H10N4O2	194.0803755	195.0876521	193.0730989
	Xanthine	69-89-6	C5H4N4O2	152.0334253	153.0407019	151.0261487
	1-Methylxanthine	6136-37-4	C6H6N4O2	166.0490754	167.0563520	165.0417988
	1,3-Dimethylxanthine (Theophylline)	58-55-9	C7H8N4O2	180.0647254	181.0720021	179.0574488
	1,7-Dimethylxanthine	611-59-6	C7H8N4O2	180.0647254	181.0720021	179.0574488
Hormones and metabolites (9)	17α-estradiol	57-91-0	C18H24O2	272.1776299	273.1849065	271.1703533
	17β-estradiol	50-28-2	C18H24O2	272.1776299	273.1849065	271.1703533
	Estrone	19973-76-3	C18H22O2	270.1619799	271.1692565	269.1547032
	Estriol	50-27-1	C18H24O3	288.1725445	289.1798212	287.1652679
	17α-etinilestradiol	57-63-6	C20H24O2	296.1776299	297.1849065	295.1703533
	Estrona-3-sulphate	481-97-0	C18H22O5S	350.1187944	351.1260710	349.1115177
	Testosterone	58-22-0	C19H28O2	288.2089300	289.2162067	287.2016534
	6β-Hydroxytestosterone	62-99-7	C19H28O3	304.2038446	305.2111213	303.1965680
	Progesterone	57-83-0	C21H30O2	314.2245801	315.2318567	313.2173035
Personal care products (6)	Methylparaben	99-76-3	C8H8O3	152.0473440	153.0546207	151.0400674
	Ethylparaben	120-47-8	C9H10O3	166.0629941	167.0702707	165.0557175
	Propylparaben	94-13-3	C10H12O3	180.0786442	181.0859208	179.0713675
	Benzylparaben	94-18-8	C14H12O3	228.0786442	229.0859208	227.0713675
	Panthenol	81-13-0	C9H19NO4	205.1314080	206.1386846	204.1241313

	Triethanolamine (TEA)	102-71-6	C6H15NO3	149.1051933	150.1124699	148.0979166
Artificial sweeteners (4)	Acesulfame Potassium	55589-62-3	C4H4KNO4S	200.9498101	201.9570867	199.9425335
	Cyclamate	139-05-9	C6H11NHSO3Na	201.0435586	202.0508352	200.0362819
	Saccharin	81-07-2	C7H5NO3S	182.9990136	184.0062903	181.9917370
	Sucralose	56038-13-2	C12H19Cl3O8	396.0145505	397.0218271	395.0072738
Phenols (3)	Nonylphenol	84852-15-3	C15H24O	220.1827153	221.1899919	219.1754387
	Octylphenol	27193-28-8	C14H22O	206.1670653	207.1743419	205.1597886
	4-tert-Octylphenol	140-66-9	C14H22O	206.1670653	207.1743419	205.1597886
Plasticizer (2)	Bisphenol A	80-05-7	C15H16O2	228.1150297	229.1223063	227.1077531
	Tris(2-isopropylphenyl) Phosphate	64532-95-2	C27H33O4P	452.2116459	453.2189225	451.2043693
Antibacterial agent (2)	Triclocarban	101-20-2	C13H9Cl3N2O	313.9780460	314.9853226	312.9707693
	Triclosan	3380-34-5	C12H7Cl3O2	287.9511625	288.9584391	286.9438859
Others (18)						
UV filter (1)	2-Ethylhexyl 4-methoxycinnamate (EHMC)	5466-77-3	C18H26O3	290.1881946	291.1954712	289.1809180
Phthalates (1)	Bis(2-ethylhexyl) phthalate (DEHP)	117-81-7	C24H38O4	390.2770095	391.2842862	389.2697329
Dietary supplement (1)	L-Phenylalanine	63-91-2	C9H11NO2	165.0789785	166.0862552	164.0717019
Corrosion inhibitor (1)	Tolyltriazole	29385-43-1	C9H9N3	159.0796473	160.0869239	158.0723706
Surfactant (1)	Dodecanedioic acid	693-23-2	C12H22O4	230.1518091	231.1590857	229.1445324
Additive (1)	Benzotriazole	95-14-7	C6H5N3	119.0483472	120.0556238	118.0410705
Synthetic musk and its metabolite (2)	Galaxolide	1222-05-5	C18H26O	258.1983654	259.2056420	257.1910888
	Galaxolidone	507442-49-1	C18H24O2	272.1776299	273.1849065	271.1703533
Metabolites/ Antioxidant (10)	3,4-Dihydroxybenzenesulfonic acid	7134-09-0	C6H6O5S	189.9935939	191.0008705	188.9863173
	Ethylmalonic acid	601-75-2	C5H8O4	132.0422586	133.0495353	131.0349820
	Tetradecanedioic acid	821-38-5	C14H26O4	258.1831092	259.1903858	257.1758326
	1,7-Dimethyluric acid	33868-03-0	C7H8N4O3	196.0596400	197.0669167	195.0523634
	3,7-Dimethyluric acid	13087-49-5	C7H8N4O3	196.0596400	197.0669167	195.0523634
	3-Hydroxyphenylacetic acid	621-37-4	C8H8O3	152.0473440	153.0546207	151.0400674
	3-Phenyllactic acid	828-01-3	C9H10O3	166.0629941	167.0702707	165.0557175
	2,6-Dichlorobenzamide	2008-58-4	C7H5Cl2NO	188.9748192	189.9820958	187.9675425
	2,4,5-Trichlorophenoxyacetic acid	93-76-5	C8H5Cl3O3	253.9304270	254.9377037	252.9231504
	2,4-Dichlorophenoxyacetic acid (2,4-D)	94-75-7	C8H6Cl2O3	219.9693994	220.9766760	218.9621227
Pesticides (125)						
Herbicide and metabolites	Acetachlor	34256-82-1	C14H20ClNO2	269.1182565	270.1255331	268.1109799
	Aclonifen	74070-46-5	C12H9ClN2O3	264.0301698	265.0374464	263.0228931
	Alachlor	15972-60-8	C14H20ClNO2	269.1182565	270.1255331	268.1109799
	Atrazine	1912-24-9	C8H14ClN5	215.0937731	216.1010497	214.0864965
	Atrazine-desethyl-desisopropyl-2-hydroxy	645-92-1	C3H5N5O	127.0494098	128.0566864	126.0421331
	Atrazine-desisopropyl	1007-28-9	C5H8ClN5	173.0468229	174.0540996	172.0395463
	Bentazon	25057-89-0	C10H12N2O3S	240.0568629	241.0641395	239.0495862
	Bifenox	42576-02-3	C14H9Cl2NO5	340.9857777	341.9930543	339.9785010
	Bromacil	314-40-9	C9H13BrN2O2	260.0160402	261.0233168	259.0087636
	Bromoxynil	1689-84-5	C7H3Br2NO	274.8581389	275.8654155	273.8508623
	Chloridazon	1698-60-8	C10H8ClN3O	221.0355895	222.0428662	220.0283129
	Chlortoluron	15545-48-9	C10H13ClN2O	212.0716407	213.0789173	211.0643641
	Desethylatrazine	6190-65-4	C6H10ClN5	187.0624730	188.0697496	186.0551964
	Desethylterbuthylazine	30125-63-4	C7H12ClN5	201.0781231	202.0853997	200.0708464
	Desisopropylatrazine	1007-28-9	C6H8ClN5	185.0468229	186.0540996	184.0395463
	Dichlorprop (2,4-DP)	120-36-5	C9H8Cl2O3	233.9850494	234.9923261	232.9777728
	Diuron	330-54-1	C9H10Cl2N2O	232.0170183	233.0242949	231.0097417
	Ethofumesate	26225-79-6	C13H18O5S	286.0874942	287.0947709	285.0802176
	Glyphosate	1071-83-6	C3H8NO5P	169.0140087	170.0212854	168.0067321
	Aminomethylphosphonic acid (AMPA)	1066-51-9	CH6NO3P	111.0085295	112.0158061	110.0012529
	Isoproturon	34123-59-6	C12H18N2O	206.1419131	207.1491898	205.1346365
	Lenacil	2164-08-01	C13H18N2O2	234.1368277	235.1441044	233.1295511
	2-methyl-4-chlorophenoxyacetic acid (MCPA)	94-74-6	C9H9ClO3	200.0240218	201.0312984	199.0167451
	Mecoprop (MCPP)	7085-19-0	C10H11ClO3	214.0396718	215.0469485	213.0323952
	Mecoprop-P (MCPP)	16484-77-8	C10H11ClO3	214.0396718	215.0469485	213.0323952
	Metamitron	41394-05-2	C10H10N4O	202.0854609	203.0927375	201.0781843
	Metazachlor	67129-08-2	C14H16ClN3O	277.0981898	278.1054664	276.0909132
	Chloridazon-methyl-desphenyl	17254-80-7	C5H6ClN3O	159.0199395	160.0272161	158.0126629
	Metolachlor	51218-45-2	C15H22ClNO2	283.1339066	284.1411832	282.1266299
	Metribuzin	21087-64-9	C8H14N4OS	214.0888317	215.0961083	213.0815551
	Simazine	122-34-9	C7H12ClN5	201.0781231	202.0853997	200.0708464
	Terbuthylazine	5915-41-3	C9H16ClN5	229.1094232	230.1166998	228.1021466
	Terbutryn	886-50-0	C10H19N5S	241.1361163	242.1433929	240.1288396
	Trifluralin	1582-09-8	C13H16F3N3O4	335.1092905	336.1165671	334.1020138
	Clofibric acid	882-09-7	C10H11ClO3	214.0396718	215.0469485	213.0323952
	2,4,5-TP (Silvex®)	93-72-1	C9H7Cl3O3	267.9460771	268.9533537	266.9388005
	2,4-DB	94-82-6	C10H10Cl2O3	248.0006995	249.0079761	246.9934229
	Ametryn	834-12-8	C9H17N5S	227.1204662	228.1277428	226.1131896
	Bromoxynil	1689-84-5	C7H3Br2NO	274.8581389	275.8654155	273.8508623

	Cyanazine	21725-46-2	C9H13ClN6	240.0890221	241.0962987	239.0817455
	Dichlobenil	1194-65-6	C7H3Cl2N	170.9642545	171.9715311	169.9569779
	Fenuron	101-42-8	C9H12N2O	164.0949630	165.1022396	163.0876863
	Fluroxypyr	69377-81-7	C7H5Cl2FN2O3	253.9661255	254.9734022	252.9588489
	Ioxynil	1689-83-4	C7H3I2NO	370.8303997	371.8376763	369.8231231
	Linuron	330-55-2	C9H10Cl2N2O2	248.0119329	249.0192095	247.0046563
	MCPB	94-81-5	C11H13ClO3	228.0553219	229.0625985	227.0480453
	Methabenzthiazuron	18691-97-9	C10H11N3OS	221.0622826	222.0695593	220.0550060
	Metolachlor ESA	171118-09-5	C15H23NO5S	329.1296934	330.1369700	328.1224168
	Molinate	2212-67-1	C9H17NOS	187.1030848	188.1103614	186.0958082
	Oxadiazon	19666-30-9	C15H18Cl2N2O3	344.0694477	345.0767244	343.0621711
	Pendimethalin	40487-42-1	C13H19N3O4	281.1375560	282.1448326	280.1302793
	Prometryn	7287-19-6	C10H19N5S	241.1361163	242.1433929	240.1288396
	Propanil	709-98-8	C9H9Cl2NO	217.0061193	218.0133959	215.9988426
	Propazine	139-40-2	C9H16ClN5	229.1094232	230.1166998	228.1021466
	Prosulfocarb	52888-80-9	C14H21NOS	251.1343849	252.1416616	250.1271083
	Terbuthylazine-2-hydroxy	66753-07-9	C9H17N5O	211.1433101	212.1505867	210.1360335
	Tiobencarb	28249-77-6	C12H16ClNOS	257.0641125	258.0713891	256.0568359
	Tri-allate	2303-17-5	C10H16Cl3NOS	303.0018179	304.0090945	301.9945413
	Triclopyr	55335-06-3	C7H4Cl3NO3	254.9256760	255.9329526	253.9183994
	Trifluralin	1582-09-8	C13H16F3N3O4	335.1092905	336.1165671	334.1020138
	Glyphosate	213-997-4	C3H8NO5P	169.0140087	170.0212854	168.0067321
	(Aminomethyl)phosphonic acid (AMPA)	1066-51-9	CH6NO3P	111.0085295	112.0158061	110.0012529
	Azoxystrobin	131860-33-8	C22H17N3O5	403.1168205	404.1240971	402.1095439
	Azoxystrobin acid	1185255-09-7	C21H15N3O5	389.1011704	390.1084471	388.0938938
	Carbendazim	10605-21-7	C9H9N3O2	191.0694765	192.0767531	190.0621998
	Carboxin	5234-68-4	C12H13NO2S	235.0666993	236.0739759	234.0594227
	Metalaxyl	57837-19-1	C15H21NO4	279.1470580	280.1543347	278.1397814
	Quinoxyfen	124495-18-7	C15H8Cl2FNO	306.9966974	308.0039741	305.9894208
	Hexachlorobenzene (HCB)	118-74-1	C6Cl6	281.8131162	282.8203928	281.8136646
	Prochloraz	67747-09-5	C15H16Cl3N3O2	375.0308098	376.0380864	374.0235331
	Propiconazole	60207-90-1	C15H17Cl2N3O2	341.0697821	342.0770587	340.0625055
	Tebuconazole	107534-96-3	C16H22ClN3O	307.1451400	308.1524166	306.1378633
	Thiabendazole	148-79-8	C10H7N3S	201.0360679	202.0433445	200.0287913
	Imazalil	35554-44-0	C14H14Cl2N2O	296.0483184	297.0555951	295.0410418
Insecticide	Aldrin	309-00-2	C12H8Cl6	361.8757164	362.8829931	360.8684398
	Bromophos-ethyl	4824-78-6	C10H12BrCl2O3PS	391.8805194	392.8877960	390.8732427
	Clothianidin	210880-92-5	C6H8ClN5O2S	249.0087228	250.0159995	248.0014462
	Chlorfenvinphos	470-90-6	C12H14Cl3O4P	357.9695284	358.9768051	356.9622518
	Chlorpyrifos	2921-88-2	C9H11Cl3NO3PS	348.9262834	349.9335601	347.9190068
	Chlorpyrifos-methyl	5598-13-0	C7H7Cl3NO3PS	320.8949833	321.9022599	319.8877067
	Cypermethrin	52315-07-8	C22H19Cl2NO3	415.0741988	416.0814754	414.0669221
	Demeton (Systox)	8065-48-3	C16H38O6P2S4	516.1026445	517.1099212	515.0953679
	Diazinon	333-41-5	C12H21N2O3PS	304.1010496	305.1083263	303.0937730
	Dichlorvos	62-73-7	C4H7Cl2O4P	219.9459005	220.9531771	218.9386239
	Dieldrin	60-57-1	C12H8Cl6O	377.8706310	378.8779077	376.8633544
	Endosulfan sulphate	1031-07-8	C9H6Cl6O4S	419.8117955	420.8190721	418.8045189
	Endrin	72-20-8	C12H8Cl6O	377.8706310	378.8779077	376.8633544
	Ethion	563-12-2	C9H22O4P2S4	383.9876149	384.9948915	382.9803382
	Ethoprophos	13194-48-4	C8H19O2PS2	242.0564077	243.0636843	241.0491310
	Fenthion	55-38-9	C10H15O3PS2	278.0200221	279.0272988	277.0127455
	Hexachlorocyclohexane (HCH)	608-73-1	C6H6Cl6	287.8600664	288.8673430	286.8527898
	Lindane (g-HCH)	58-89-9	C6H6Cl6	287.8600664	288.8673430	286.8527898
	Heptachlor	76-44-8	C10H5Cl7	369.8210940	370.8283707	368.8138174
	Heptachlor epoxide	1024-57-3	C10H5Cl7O	385.8160087	386.8232853	384.8087320
	Isodrin	465-73-6	C12H8Cl6	361.8757164	362.8829931	360.8684398
	Malathion	121-75-5	C10H19O6PS2	330.0360661	331.0433427	329.0287894
	Pentachlorophenol	87-86-5	C6Cl5O	262.8391781	263.8464547	262.8397265
	(Z)-Chlorfenvinphos	18708-86-6	C12H14Cl3O4P	357.9695284	358.9768051	356.9622518
	Acetamiprid	135410-20-7	C10H11ClN4	222.0672240	223.0745007	221.0599474
	Aldicarb	116-06-3	C7H14N2O2S	190.0775983	191.0848749	189.0703217
	Aldicarb-sulfoxide	1646-87-3	C7H14N2O3S	206.0725129	207.0797895	205.0652363
	Carbaryl	63-25-2	C12H11NO2	201.0789785	202.0862552	200.0717019
	Carbofuran	1563-66-2	C12H15NO3	221.1051933	222.1124699	220.0979166
	Carbofuran-3-hydroxy	16655-82-6	C12H15NO4	237.1001079	238.1073845	236.0928312
	Clothianidin	210880-92-5	C6H8ClN5O2S	249.0087228	250.0159995	248.0014462
	Fenitrothion	122-14-5	C9H12NO5PS	277.0173796	278.0246562	276.0101029
	Imidacloprid	105827-78-9	C9H10ClN5O2	255.0523022	256.0595788	254.0450256
	Isoprocarb	2631-40-5	C11H15NO2	193.1102787	194.1175553	192.1030020
	Methiocarb	2032-65-7	C11H15NO2S	225.0823494	226.0896260	224.0750727
	Methomyl	16752-77-5	C5H10N2O2S	162.0462982	163.0535748	161.0390216
	Pirimicarb	23103-98-2	C11H18N4O2	238.1429757	239.1502524	237.1356991
	Propoxur	114-26-1	C11H15NO3	209.1051933	210.1124699	208.0979166

	Thiacloprid	111988-49-9	C10H9ClN4S	252.0236447	253.0309213	251.0163680
	Tebufenozide	412-850-3	C22H28N2O2	352.2150780	353.2223547	351.2078014
	Thiamethoxam	153719-23-4	C8H10ClN5O3S	291.0192875	292.0265641	290.0120109
	Metaflumizone	139968-49-3	C24H16F6N4O2	506.1177449	507.1250215	505.1104683
Rodenticide	Brodifacoum	56073-10-0	C31H23BrO3	522.0830571	523.0903337	521.0757805
	Difenacoum	56073-07-5	C31H24O3	444.1725445	445.1798212	443.1652679
	Difethialone	104653-34-1	C31H23BrO2S	538.0602132	539.0674898	537.0529366
	Bromadiolon	28772-56-7	C30H23BrO4	526.0779717	527.0852483	525.0706951
Molluscicide	Metaldehyde	108-62-3	C8H16O4	176.1048589	177.1121355	175.0975823
Insect repellents	Diethyltoluamide (DEET)	134-62-3	C12H17NO	191.1310141	192.1382907	190.1237375
Acaricide and	Dicofol	115-32-2	C14H9Cl5O	367.9096034	368.9168800	366.9023267
metabolite	4,4′-Dichlorobenzophenone	90-98-2	C13H8Cl2O	249.9952202	251.0024969	248.9879436
Algistat	Cybutryne	28159-98-0	C11H19N5S	253.1361163	254.1433929	252.1288396

Table S2. List of 26 isotopically labelled internal standards (ILIs) used for semi-quantification.

Internal Standards	RT [min]	CAS number	Chemical Formula	Recoveries (%) (±SD)	HESI mode
Pharmaceuticals					
Acetaminophen d_4	6.7	64315-36-2	$C_8D_4H_5NO_2$	61 (±1.8)	PI (+)
Antipyrine d_3	7.8	65566-62-3	$C_{11}H_9D_3N_2O$	89 (±2.3)	PI (+)
Atenolol d_7	6.2	1202864-50-3	$C_{14}D_7H_{15}N_2O_3$	90 (±3.2)	PI (+)
Carbamazepine d_{10}	9.6	132183-78-9	$C_{15}D_{10}H_2N_2O$	96 (±2.5)	PI (+)
Citalopram d_4	8.8	390817-87-5	$C_{19}H_{18}D_4FNO$	75 (±4.2)	PI (+)
Hydrochlorothiazide d_2	7.7	1219798-89-6	$C_7H_6D_2ClN_3O_4S_2$	87 (±1.5)	NI (-)
Lamotrigine-13C3	7.7	1188265-38-4	$13C_3C_6H_7Cl_2N_5$	84 (±2.8)	PI (+)
Lidocaine-(diethyl d_{10})	7.5	851528-09-1	$C_{14}D_{10}H_{12}N_2O$	68 (±1.9)	PI (+)
Metformin-(dimethyl d_6) hydrochloride	2.9	1185166-01-1	$C_4D_6H_6N_5Cl$	72 (±3.0)	PI (+)
Tramadol-^{13}C, d_3 hydrochloride	7.4	-	$13CC_{15}D_3H_{23}NO_2Cl$	65 (±2.2)	PI (+)
Valsartan d_3	10.7	1331908-02-1	$C_{24}H_{26}D_3N_5O_3$	93 (±1.2)	NI (-)
Venlafaxine d_6 hydrochloride	8.2	1062606-12-5	$C_{17}D_6H_{22}NO_2Cl$	75 (±4.1)	PI (+)
Caffeine-(3-methyl-13C)	6.9	202282-98-2	$13CC_7H_{10}N_4O_2$	98 (±5.2)	PI (+)
DL-Nicotine-(methyl d_3)	4.4	69980-24-1	$C_{10}D_3H_{11}N_2$	89 (±3.2)	PI (+)
Drug of abuse					
Cocaine d_5	8.1	259526-49-3	$C_{17}H_{16}D_5NO_4$	92 (±2.1)	PI (+)
Herbicide					
Metolachlor-(2-ethyl-6-methylphenyl d_{11})	13.1	1632119-30-2	$C_{15}D_{11}H_{11}ClNO_2$	86 (±3.8)	PI (+)
Terbuthylazine-(ethyl d_5)	11.9	222986-60-9	$C_9D_5H_{11}ClN_5$	90 (±2.7)	PI (+)
Terbutryn-(ethyl d_5)	12.5	1219804-47-3	$C_{10}D_5H_{14}N_5S$	89 (±1.8)	PI (+)
Fungicide					
Azoxystrobin-(cyanophenoxy d_4)	11.8	-	$C_{22}D_4H_{13}N_3O_5$	92 (±1.8)	PI (+)
Metalaxyl-(phenyl-13C6)	10.8	1356199-69-3	$13C_6C_9H_{21}NO_4$	95 (±2.5)	PI (+)
Prochloraz-(ethylene d_4)	13.1	-	$C_{15}D_4H_{12}Cl_3N_3O_2$	98 (±4.3)	PI (+)
Propiconazole-(phenyl d_3)	13.1	-	$C_{15}D_3H_{14}Cl_2N_3O_2$	83 (±3.0)	PI (+)
Tebuconazole-(tert-butyl d_9)	12.4	1246818-83-6	$C_{16}D_9H_{13}ClN_3O$	90 (±2.5)	PI (+)
Insect repellents					
DEET-(diethyl d_{10})	10.8	1215576-01-4	$C_{12}D_{10}H_7NO$	92 (±4.2)	PI (+)
Personal care products					
Triclosan-methyl-ether d_3	7.8	1020720-00-6	$C_{13}H_6D_3Cl_3O_2$	53 (±7.2)	NI (-)
Oxybenzone -(phenyl d_5)	13.0	1219798-54-5	$C_{14}D_5H_7O_3$	70 (±3.3)	NI (-)

Table S3. Sampling information: coordinates (X, Y) of sampling sites and physicochemical parameters measured in each site.

N	Acronym	Sample Name	Type of water	X Coordinates*	Y Coordinates*	Physicochemical parameters				
				** WGS84*		T (ºC)	pH	O_2 (mg/L)	Conductivity (uS/cm)	Salinity (ppt)
1	**INF1**	Amposta WWTP IN	influent	0.608608	40.704057	24.8	8.1	10.5	2200	2.9
2	**EFF1**	Amposta WWTP OUT	effluent	0.608608	40.704057	22.2	7.6	5.7	1916	1.8
3	**INF2**	Sant Carles de la Ràpita WWTP IN	influent	0.622121	40.627036	26.4	8.3	4.1	6580	3.6
4	**EFF2**	Sant Carles de la Ràpita WWTP OUT	effluent	0.622121	40.627036	23.8	7.8	4.4	6000	3.2
5	**ER1**	Upstream	Ebro River	0.608565	41.032962	24.5	7.1	9.2	758	0.3
6	**ER2**	Upstream	Ebro River	0.518484	40.976154	25.0	8.2	9.4	767	0.3
7	**ER3**	Upstream	Ebro River	0.516012	40.97485	25.1	8.1	9.6	772	0.4
8	**ER4**	Downstream of WWTP1	Ebro River	0.581698	40.715022	27.6	8.0	6.7	783	0.3
9	**ER5**	Downstream	Ebro River	0.689317	40.713142	26.7	8.2	6.4	1121	0.4
10	**ER6**	Downstream	Ebro River	0.717407	40.712942	27.3	8.5	6.2	1294	0.6
11	**ER7**	Downstream	Ebro River	0.716723	40.713967	27.2	7.68	7.1	1026	0.5
12	**SCR**	Discharge WWTP2 Sant Carles de la Ràpita	emissary	0.622754	40.622579	31.7	8.0	4.8	1590	0.8
13	**CW1**	Channel A (irrigation channel)	channel	0.809254	40.706929	25.0	8.3	12.7	759	0.3
14	**CW2**	Channel B (irrigation channel)	channel	0.806554	40.696697	25.0	8.5	13.0	752	0.3
15	**CW3**	Channel C (irrigation channel)	channel	0.598582	40.622214	29.5	7.8	N/D	1624	0.8
16	**CW4**	Channel D1 (drainage channel)	channel	0.647442	40.631728	28.9	8.3	5.5	1449	0.7
17	**CW5**	Channel D2 (drainage channel)	channel	0.681305	40.642791	30.0	9.1	10.2	1392	1.2
18	**CW6**	Channel D3 (drainage channel)	channel	0.789064	40.661281	29.3	7.8	5.5	1464	0.7
19	**CW7**	Channel D4 (drainage channel)	channel	0.717146	40.646098	31.3	7.8	5.29	8080	4.6
20	**SW1**	Alfacs bay A (onshore)	seawater	0.605632	40.614640	29.8	7.7	10.4	51707	26.7
21	**SW2**	Alfacs bay B (onshore)	seawater	0.661707	40.622999	29.1	7.8	10.3	51104	30.8
22	**SW3**	Alfacs bay C (offshore)	seawater	0.652181	40.600416	29.3	7.9	12.3	50946	30.8
23	**SW4**	Alfacs bay D (offshore)	seawater	0.656485	40.596650	28.1	7.4	13.7	51207	29.8
24	**SW5**	Fangar bay A (onshore)	seawater	0.737865	40.766660	32.8	8.12	13..3	46286	25.5
25	**SW6**	Fangar bay B (onshore)	seawater	0.710630	40.803269	26.7	7.8	12.6	41752	28.7
26	**SW7**	Fangar bay C (offshore)	seawater	0.728952	40.795664	26.7	7.5	11.7	45335	31.1
27	**SW8**	Fangar bay D (offshore)	seawater	0.743862	40.814594	25.9	7.4	13.2	50119	31.7
28	**EW1**	La Tancada lagoon	estuary	0.742459	40.645.082	30.8	8.8	5.3	45300	29.6
29	**EW2**	Illa de Buda lagoon	estuary	0.851054	40.695.650	30.5	8.1	5.6	43300	21.3
30	**EW3**	L'Encanyissada lagoon	estuary	0.673552	40.657.110	30.8	8.3	6.1	10210	18.8
31	**EW4**	Canal Vell lagoon	estuary	0.788320	40.745.011	29.1	8.8	9.2	N/D	25.2

Table S4. Data processing parameters selected to perform and reproduce the integrated suspect screening methodology in Compounds Discoverer 2.1.

Peak filtering of candidates

Processing node 7: **Select Spectra**

Spectrum Properties Filter:

- Lower RT Limit: 0.5
- Upper RT Limit: 17
- Min. Precursor Mass: 100 Da
- Max. Precursor Mass: 1000 Da
- Polarity Mode: + or -

Peak Filters:

- S/N Threshold (FT-only): 10

Processing node 26: **Align Retention Times**

- Alignment Model: Adaptive curve
- Maximum RT Shift [min]: 0.3
- Mass Tolerance: ±5 ppm

Processing node 9: **Detect Unknown Compounds**

General Settings:

- Mass Tolerance [ppm]: ±5 ppm
- Intensity Tolerance [%]: 30
- S/N Threshold: 10
- Min. Peak Intensity: 100000
- Ions: $[M-H]^{-1}$/ $[M+H]^{+1}$
- Min. Element Counts: C H
- Max. Element Counts: C_{90} H_{190} Br_3 Cl_4 K_2 N_{10} Na_2 O_{15} P_3 S_5 F_3

Peak Detection:

- Max. Peak Width [min]: 0.3
- Min. # Scans per Peak: 5
- Min. # Isotopes: 2

Processing node 31: **Group Unknown Compounds**

Compound Consolidation:

- Mass Tolerance: ±5 ppm
- RT Tolerance [min]: 0.3

Identification strategies

Processing node 22: **Search mzCloud**

Search Settings:

- Compound Classes: All
- Match Ion Activation Type: True
- Match Ion Activation Energy: Match with Tolerance
- Ion Activation Energy Tolerance: 20
- Precursor Mass Tolerance: ±5 ppm
- FT Fragment Mass Tolerance: ±5 ppm
- Identity Search: HighChem/HighRes
- Match Factor Threshold: 50
- Max. # Results: 10

Processing node 32: **Search mzVault**

Search Settings:

- mzVault Library: mzVault February 2017.db
- Compound Classes: All
- Match Ion Activation Type: True
- Match Ion Activation Energy: Match with Tolerance
- Ion Activation Energy Tolerance: 20
- Precursor Mass Tolerance: ±5 ppm
- FT Fragment Mass Tolerance: ±5 ppm
- Search Algorithm: HighChem/HighRes
- Match Factor Threshold: 50
- Max. # Results: 100

Processing node 23: **Search ChemSpider**

Search Settings:

- Mass Tolerance: ±5 ppm
- Database(s): MassBank
- Max. # of results per compound: 10
- Max. # of Predicted Compositions to be searched per Compound: 3
- Result Order (for Max. # of results per compound): Order By Reference Count (DESC)

Processing node 29: **Predict Compositions**

Prediction Settings:

- Mass Tolerance: ±5 ppm
- Min. Element Counts: C H
- Max. Element Counts: C_{90} H_{190} Br_3 Cl_4 N_{10} O_{18} P_3 S_5
- Max. # Candidates: 10
- Max. # Internal Candidates: 200

Pattern Matching:

- Intensity Tolerance [%]: 30
- Intensity Threshold [%]: 0.1
- S/N Threshold: 3

Fragments Matching:

- Use Fragments Matching: True
- Mass Tolerance: ±5 ppm
- S/N Threshold: 3

Table S5. Compounds with their lowest PNEC values (µg/L) obtained from NORMAN Database.

Compound class	Compound name	CAS	PNEC type	Biotest used					Justification		Data source link
				Scientific name	Endpoint/ Duration/ Effect	AF	Experimental / predicted	Lowest PNEC fresh water (µg/L)	Data source name		
Pharmaceuticals and metabolites (16)	Mesalamine (5-Aminosalicylic acid)	CAS_RN: 89-57-6	PNEC aqua (freshwater)	n.r.	n.r/n.r./n.r.	10	Deterministic	570	ECHA website of registered substances	Long-term results (e.g. EC10 or NOECs) from at least three species (normally fish, Daphnia and algae) representing three trophic levels	https://echa.europa.eu/information-on-chemicals/registered-substances
	Acetaminophen (Paracetamol)	CAS_RN: 103-90-2	PNEC aqua (freshwater)	n.r.	n.r/n.r./n.r.	1000	Deterministic	134	ECHA website of registered substances	At least one short-term L(E)C50 from each of three trophic levels (fish, invertebrates (preferred Daphnia) and algae) (i.e. base set)	https://echa.europa.eu/information-on-chemicals/registered-substances
	4-Acetamidoantipyrine	CAS_RN: 83-15-8	JD-UQN proposal	Daphnia magna	EC50/n.r.	1000	Deterministic	100	ETOX: Information System Ecotoxicology and Environmental Quality Targets	n.r.	https://webetox.uba.de/webETOX/public/search/ziel/open.do
	4-Formylaminoantipyrine	CAS_RN: 1672-58-8	JD-UQN proposal	Daphnia magna	n.r/n.r./n.r.	10	Deterministic	1000	ETOX: Information System Ecotoxicology and Environmental Quality Targets	Long-term results (e.g. EC10 or NOECs) from at least three species (normally fish, Daphnia and algae) representing three trophic levels	https://webetox.uba.de/webETOX/public/search/ziel/open.do
	Tramadol	CAS_RN: 27203-92-5	P-PNEC pred	Selenastrum capricornutum	IC50/72h	1000	Deterministic	8.65383	NORMAN SusDat: Suspect List Exchang	One predicted short-term L(E)C50 from each of three trophic levels (i.e. base set)	https://46.229.226.228/nds/susdat/
	Carbamazepine	CAS_RN: 298-46-4	PNEC chronic	Daphnia magna	n.r/n.r./n.r.	10	Deterministic	0.05	n.r.	Long-term results (e.g. EC10 or NOECs) from at least three species (normally fish, Daphnia and algae) representing three trophic levels	n.r.
	Citalopram	CAS_RN: 59729-32-7	PNEC chronic	Poecilia reticulata	n.r/n.r./n.r.	100	Deterministic	10	Ecotox Knowledgebase	One long-term EC10 or NOEC (either fish or Daphnia)	https://cfpub.epa.gov/ecotox/search.cfm
	Venlafaxine	CAS_RN: 93413-69-5	EQS-proposal	Oryzias latipes	n.r/n.r./n.r.	100	Deterministic	0.038	n.r.	One long-term EC10 or NOEC (either fish or Daphnia)	n.r.
	Didesmethylvenlafaxine	CAS_RN: 135308-74-6	P-PNEC pred	Daphnia magna	EC50/48h	1000	Deterministic	11.85232	NORMAN SusDat: Suspect List Exchang	One predicted short-term L(E)C50 from each of three trophic levels (i.e. base set)	https://46.229.226.228/nds/susdat/
	Atenolol acid	CAS_RN: 56392-14-4	P-PNEC pred	Pimephales promelas	IC50/96h	1000	Deterministic	49.90096	NORMAN SusDat: Suspect List Exchang	One predicted short-term L(E)C50 from each of three trophic levels (i.e. base set)	https://46.229.226.228/nds/susdat/
	Lidocaine	CAS_RN: 137-58-6	P-PNEC pred	Selenastrum capricornutum	IC50/72h	1000	Deterministic	4.67202	NORMAN SusDat: Suspect List Exchang	One predicted short-term L(E)C50 from each of three trophic levels (i.e. base set)	https://46.229.226.228/nds/susdat/
	Lamotrigine	CAS_RN: 84057-84-1	PNEC chronic	Desmodesmus subspicatus	n.r/n.r./n.r.	50	Deterministic	10	ETOX: Information System Ecotoxicology and Environmental Quality Targets	Two long-term results (e.g. EC10 or NOECs) from species representing two trophic levels (fish and/or Daphnia and/or algae)	https://webetox.uba.de/webETOX/public/search/ziel/open.do
	Valsartan	CAS_RN: 137862-53-4	AA-EQS	Daphnia magna	n.r/n.r./n.r.	10	Deterministic	560	Proposals for Acute and Chronic Quality Standards	Long-term results (e.g. EC10 or NOECs) from at least three species (normally fish, Daphnia and algae) representing three trophic levels	http://www.ecotoxcentre.ch/expert-service/quality-standards/proposals-for-acute-and-chronic-quality-standards
	Telmisartan	CAS_RN: 144701-48-4	P-PNEC pred	Pimephales promelas	IC50/72h	1000	Deterministic	0.00055	NORMAN SusDat: Suspect List Exchang	One predicted short-term L(E)C50 from each of three trophic levels (i.e. base set)	https://46.229.226.228/nds/susdat/
	Metformin	CAS_RN:		n.r.	n.r/n.r./n.r.	n.r.	n.r.	156	Proposals for Acute and	n.r.	http://www.ecotoxcentre.ch/expert-

		657-24-9	AA-EQS						Chronic Quality Standards		service/quality-standards/proposals-for-acute-and-chronic-quality-standards
	Hydrochlorothiazide	CAS_RN: 58-93-5	P-PNEC pred	Selenastrum capricornutum	IC50/72h	100 0	Deterministic	8.38089	NORMAN SusDat: Suspect List Exchang	One predicted short-term L(E)C50 from each of three trophic levels (i.e. base set)	https://46.229.226.228/nds/susdat/
Stimulants and metabolites (6)											
	Xanthine	CAS_RN: 69-89-6	P-PNEC pred	Selenastrum capricornutum	IC50/72h	100 0	Deterministic	19.33541	NORMAN SusDat: Suspect List Exchang	One predicted short-term L(E)C50 from each of three trophic levels (i.e. base set)	https://46.229.226.228/nds/susdat/
	Caffeine	CAS_RN: 58-08-2	JD-UQN proposal	Daphnia magna	n.r/n.r./n.r.	100	Deterministic	1.2	ETOX: Information System Ecotoxicology and Environmental Quality Targets	One long-term EC10 or NOEC (either fish or Daphnia)	https://webetox.uba.de/webETOX/public/search/ziel/open.do
	Methylxanthine	CAS_RN: 6136-37-4	P-PNEC pred	Selenastrum capricornutum	IC50/72h	100 0	Deterministic	19.84635	NORMAN SusDat: Suspect List Exchang	One predicted short-term L(E)C50 from each of three trophic levels (i.e. base set)	https://46.229.226.228/nds/susdat/
	Theophylline	CAS_RN: 58-55-9	P-PNEC pred	Selenastrum capricornutum	IC50/72h	100 1	Deterministic	14.82427	NORMAN SusDat: Suspect List Exchang	One predicted short-term L(E)C50 from each of three trophic levels (i.e. base set)	https://46.229.226.228/nds/susdat/
	Nicotine	CAS_RN: 54-11-5	PNEC acute	Lepomis macrochirus	n.r/n.r./n.r.	100 0	Deterministic	5.45	Ecotox Knowledgebase	At least one short-term L(E)C50 from each of three trophic levels (fish, invertebrates (preferred Daphnia) and algae) (i.e. base set)	https://cfpub.epa.gov/ecotox/search.cfm
	Cotinine	CAS_RN: 486-56-6	PNEC chronic	Lemna gibba	n.r/n.r./n.r.	100	Deterministic	10	Ecotox Knowledgebase	One long-term EC10 or NOEC (either fish or Daphnia)	https://cfpub.epa.gov/ecotox/search.cfm
Drug of abuse											
	Cocaine	CAS_RN: 71387-58-1	P-PNEC pred	Pimephales promelas	LC50/96h/	100 0	Deterministic	3.45	NORMAN SusDat: Suspect List Exchang	One predicted short-term L(E)C50 from each of three trophic levels (i.e. base set)	https://46.229.226.228/nds/susdat/
Personal care products (3)											
	Panthenol	CAS_RN: 81-13-0	P-PNEC pred	Selenastrum capricornutum	IC50/72h	100 0	Deterministic	80.33955	NORMAN SusDat: Suspect List Exchang	One predicted short-term L(E)C50 from each of three trophic levels (i.e. base set)	https://46.229.226.228/nds/susdat/
	Galaxolidone	CAS_RN: 507442-49-1	P-PNEC pred	Daphnia magna	EC50/48h	100 0	Deterministic	0.10062	NORMAN SusDat: Suspect List Exchang	One predicted short-term L(E)C50 from each of three trophic levels (i.e. base set)	https://46.229.226.228/nds/susdat/ http://www.ecotoxcentre.ch/expert-service/quality-standards/proposals-for-acute-and-chronic-quality-standards
	Diethyltoluamide (DEET)	CAS_RN: 134-62-3	AA-EQS	n.r.	n.r/n.r./n.r.	n.r.	n.r.	88	Proposals for Acute and Chronic Quality Standards	n.r.	
Pesticides and metabolites (10)											
	Metolachlor	CAS_RN: 51218-45-2	JD-UQN	Scenedesmus subspicatus	n.r/n.r./n.r.	10	Deterministic	0.2	ETOX: Information System Ecotoxicology and Environmental Quality Targets	Long-term results (e.g. EC10 or NOECs) from at least three species (normally fish, Daphnia and algae) representing three trophic levels	https://webetox.uba.de/webETOX/public/search/ziel/open.do
	Terbutylazine	CAS_RN: 5915-41-3	AA-QSwater_eco	Selenastrum capricornutum	n.r/n.r./n.r.	10	Deterministic	0.06	Portail Substances Chemique	Long-term results (e.g. EC10 or NOECs) from at least three species (normally fish, Daphnia and algae) representing three trophic levels	https://substances.ineris.fr/
	Desethylterbutylazin	CAS_RN: 30125-63-4	JG-MKN (total)	n.r.	n.r/n.r./n.r.	n.r.	n.r.	0.25	Zoeksysteem Risico's van stoffen	n.r.	https://rvszoeksysteem.rivm.nl/
	Terbutryn	CAS_RN: 886-50-0	EQS chronic water (=AA-EQS)	n.r.	n.r/n.r./n.r.	n.r.	n.r.	0.065	CIRCA web server of the EC	n.r.	https://circabc.europa.eu
	Azoxystrobin	CAS_RN: 131860-33-8	JG-MKN (opgelost)	n.r.	n.r/n.r./n.r.	n.r.	n.r.	0.2	Zoeksysteem Risico's van stoffen	n.r.	https://rvszoeksysteem.rivm.nl/
	Metalaxyl	CAS_RN:			n.r/n.r./n.r.	50		20	Portail Substances Chemique	Two long-term results (e.g. EC10 or NOECs) from	https://substances.ineris.fr/

	57837-19-1	AA-QSwater_eco	invertebrates			Deterministic			species representing two trophic levels (fish and/or Daphnia and/or algae)	
Prochloraz	CAS_RN: 67747-09-5	PNEC chronic	algae	n.r/n.r./n.r.	50	Deterministic	0.2	Footprint pesticide database	Two long-term results (e.g. EC10 or NOECs) from species representing two trophic levels (fish and/or Daphnia and/or algae)	https://sitem.herts.ac.uk/aeru/ppdb/en/search.htm
Propiconazole	CAS_RN: 60207-90-1	JD-UQN	n.r.	n.r/n.r./n.r.	n.r.	n.r.	1	ETOX: Information System Ecotoxicology and Environmental Quality Targets	n.r.	https://webetox.uba.de/webETOX/public/search/ziel/open.do
Tebuconazole	CAS_RN: 107534-96-3	AA-EQS	n.r.	n.r/n.r./n.r.	n.r.	n.r.	0.24	Proposals for Acute and Chronic Quality Standards	n.r.	http://www.ecotoxcentre.ch/expert-service/quality-standards/proposals-for-acute-and-chronic-quality-standards
Tebufenozide	CAS_RN: 112410-23-8	PNEC chronic	Daphnia magna	n.r/n.r./n.r.	10	Deterministic	0.29	Footprint pesticide database	Long-term results (e.g. EC10 or NOECs) from at least three species (normally fish, Daphnia and algae) representing three trophic levels	https://sitem.herts.ac.uk/aeru/ppdb/en/search.htm

Table S6. Total number of suspects (48) based on search in three databases of Compound Discoverer 2.1.

Compound name	Formula	m/z expected	m/z measured	Δppm	RT [min]	Results (ChemSpider/mzCloud/mzVault)			mzCloud Best	mzVault Best	ChemSpider #ref. number*
PI (34)											
Nicotine	C10H14N2	162.1156984	162.1156247	2.928	4.5	37	12	12	95.8	97.6	#14690
Caffeine	C8H10N4O2	194.0803755	194.0801518	1.673	7.0	1	2	2	87.1	89.6	#13024
Lidocaine	C14H22N2O	234.1732133	234.1729747	1.323	7.6	2	9	9	83.8	97.1	#12321
Acetaminophen	C8H9NO2	151.0633285	151.0634021	4.118	4.1	10	2	4	54.4	56.9	#9678
Carbamazepine	C15H12N2O	236.0949630	236.0948962	2.040	8.1	2	3	3	91.1	99.5	#7400
Citalopram	C20H21FN2O	324.1637914	324.1637085	1.436	8.9	2	4	4	90.5	97.2	#2164
Lamotrigine	C9H7Cl2N5	255.0078506	255.0075148	0.834	7.7	1	1	1	77.5	75.6	#2068
Venlafaxine	C17H27NO2	277.2041790	277.2041255	1.785	8.2	1	8	8	88.0	90.3	#1476
Tramadol	C16H25NO2	263.1885289	263.1882385	0.980	7.4	4	20	20	92.2	98.4	#1265
Panthenol	C9H19NO4	205.1314080	205.1314741	2.996	6.1	7	2	1	81.8	91.7	#1247
Valsartan	C24H29N5O3	435.2270397	435.2265712	0.184	10.7	1	3	3	95.2	95.7	#1159
Triethanolamine	C6H15NO3	149.1051933	149.1049914	2.324	2.8	3	5	5	95.0	98.6	#771
DEET	C12H17NO	191.1310141	191.1308429	1.973	11.0	83	12	12	99.1	97.7	#691
Telmisartan	C33H30N4O2	514.2368761	514.2366309	0.590	11.4	1	6	6	62.6	90.5	#672
Prochloraz	C15H16Cl3N3O2	375.0308098	375.0305063	0.653	13.1	1	1	1	88.7	92.0	#566
Metolachlor	C15H22ClNO2	283.1339066	283.1340051	2.285	13.1	1	1	1	81.9	96.5	#437
Tebuconazole	C16H22ClN3O	307.1451400	307.1450911	1.626	12.5	3	11	12	92.4	93.7	#387
Metformin	C4H11N5	129.1014453	129.1015230	4.849	3.0	1	2	2	94.2	94.3	#385
Metalaxyl	C15H21NO4	279.1470580	279.1472232	2.556	10.9	3	6	6	87.9	98.0	#349
Propiconazole	C15H17Cl2N3O2	341.0697821	341.0695230	0.848	13.1	1	2	2	93.5	98.7	#232
Azoxystrobin	C22H17N3O5	403.1168205	403.1166983	1.057	11.9	2	2	2	93.6	93.7	#139
Cotinine	C10H12N2O	176.0949630	176.0950294	3.492	5.6	4	2	2	89.6	84.9	#1959
Mesalamine	C7H7NO3	153.0425930	153.0426504	3.958	5.4	11	4	2	85.8	94.9	#1772
Cocaine	C17H21NO4	303.1470580	303.1468221	1.031	8.1	5	2	2	80.4	85.0	#312
Terbutryn	C10H19N5S	241.1361163	241.1358993	1.374	12.6	2	4	4	90.4	97.9	#311
Terbuthylazine	C9H16ClN5	229.1094232	229.1095695	3.032	12.0	4	22	16	87.0	98.6	#266

4-Aminonicotinic acid	C6H6N2O2	138.0429274	138.0428231	4.665	4.0	52	21	18	92.6	99.5	#229
4-Acetamidoantipyrine	C13H15N3O2	245.1164266	245.1163026	1.731	7.0	2	3	3	88.2	98.1	#165
4-Formylaminoantipyrine	C12H13N3O2	231.1007766	231.1004498	0.959	7.0	4	1	1	84.1	96.8	#135
Desethylterbuthylazine	C7H12ClN5	201.0781231	201.0780047	2.139	10.1	5	8	4	76.5	92.7	#104
6ß-Hydroxytestosterone	C19H28O3	304.2038446	304.2041242	2.722	12.9	1	6	6	64.9	71.0	#94
Didesmethylvenlafaxine	C15H23NO2	249.1728789	249.1726930	1.455	7.0	4	2	3	59.0	62.9	#76
Atenolol acid	C14H21NO4	267.1470580	267.1468723	1.358	6.8	1	1	1	69.2	94.3	#74
Galaxolidone	C18H24O2	272.1776299	272.1774995	1.536	14.8	5	39	26	94.2	99.6	#24
NI (14)											
Xanthine	C5H4N4O2	152.0334253	152.0332074	2.174	3.6	2	6	6	88.5	92.0	#7630
Hydrochlorothiazide	C7H8ClN3O4S2	296.9644747	296.9645520	2.107	7.7	1	1	1	83.0	99.6	#4020
Succinic acid	C4H6O4	118.0266086	118.0263184	2.188	2.7	3	1	1	86.9	89.9	#1926
2-Naphthalenesulfonic acid	C10H8O3S	208.0194147	208.0192437	1.814	7.0	1	2	2	78.3	81.5	#1863
Ferulic acid	C10H10O4	194.0579087	194.0577985	2.258	5.9	16	2	2	87.6	94.4	#1354
Azelaic acid	C9H16O4	188.1048589	188.1047498	2.336	4.0	11	4	6	84.8	96.3	#526
Cyclamic acid	C6H13NO3S	179.0616139	179.0614207	1.984	6.3	1	4	2	84.8	60.8	#243
Tebufenozide	C22H28N2O2	352.2150780	352.2153695	2.385	12.8	2	4	4	88.1	91.7	#189
Hydrocinnamic acid	C9H10O2	150.0680795	150.0678072	1.840	7.0	25	1	1	82.6	89.2	-
Theophylline	C7H8N4O2	180.0647254	180.0645497	2.070	6.5	3	3	3	90.2	90.3	#11885
1-Methylxanthine	C6H6N4O2	166.0490754	166.0490757	3.305	5.8	2	23	3	92.0	84.4	#783
Caffeic acid	C9H8O4	180.0422586	180.0421118	2.230	6.6	8	2	2	81.1	84.0	#57
3,7-Dimethyluric acid	C7H8N4O3	196.0596400	196.0595227	2.199	3.1	2	6	6	91.3	98.0	-
1,7-Dimethyluric acid	C7H8N4O3	196.0596400	196.0595019	2.093	4.0	2	12	12	87.5	96.7	-

*Sorting the hit list in descending order by the number of associated #references in ChemSpider

Table S7. Summary table of results of semi-quantitative analysis.

Group of compounds	Sampling sites	Ebro River (n=7)				Emissary SCR (n=1)	Channels (n=7)				Estuary (n=4)				Seawater (n=8)				Summary for freshwater		Effluent (n=2)			Influent (n=2)		
	Compound name	N*	Min (ng/L)	Max (ng/L)	Mean (ng/L)	C (ng/L)	N*	Min (ng/L)	Max (ng/L)	Mean (ng/L)	N*	Min (ng/L)	Max (ng/L)	Mean (ng/L)	N*	Min (ng/L)	Max (ng/L)	Mean (ng/L)	Freq (%)	Max (ng/L)	Min (ng/L)	Max (ng/L)	Mean (ng/L)	Min (ng/L)	Max (ng/L)	Mean (ng/L)
Pharmaceuticals and metabolites (16)	Mesalamine	5	58.6	114.5	76.7	n.d.	6	57.6	266	118.2	2	70.7	98.4	84.6	7	59.6	82.1	76.3	74%	266	89.2	123	106.1	n.d.		
	Acetaminophen	n.d.				n.d.	n.d.				n.d.				n.d.				n.d.		n.d.			442.3	1759	1100
	4-Acetamidoantipyrine	7	28.9	65.4	46.4	92.7	6	16.3	64.4	39.2	2	19.9	26.3	23.1	n.d.				59%	92.7	293.2	2190	1242	2756	7831	5293
	4-Formylaminoantipyrine	3	4.1	143.9	54.8	1.2	1	1.7	1.7	1.7	n.d.				1	0.5	0.5	0.5	22%	143.9	3.2	228	116.1	48.5	110.9	79.7
	Tramadol	1	1.5	1.5	1.5	70.5	n.d.				n.d.				n.d.				7%	70.5	1228	1237	1233	243.4	844.4	543.9
	Carbamazepine	7	0.9	2.0	1.3	170.4	2	4.8	166.2	85.5	n.d.				4	0.2	0.5	0.3	52%	170.4	109.3	175.1	142.2	102.7	177.7	140.2
	Citalopram	n.d.				76	n.d.				n.d.				n.d.				4%	76	25.8	53.6	39.7	26.8	393.4	210.1
	Venlafaxine	7	47.7	144.1	78.9	78.1	1	59.2	59.2	59.2	n.d.				2	1.4	5.8	3.6	41%	144.1	300.3	576.6	438.5	845.6	2342	1594
	Didesmethylvenlafaxine	6	23.7	106.3	58.0	143.6	n.d.				n.d.				n.d.				26%	143.6	308.6	484.8	396.7	167.7	270	218.8
	Atenolol acid	7	3.5	10.8	6.0	182.8	2	4.2	8.4	6.3	n.d.				n.d.				37%	182.8	261.6	983	622.3	275.5	398.1	336.8
	Lidocaine	n.d.				126.3	n.d.				n.d.				n.d.				4%	126.3	12.6	98.8	55.7	359.5	518.1	438.8
	Lamotrigine	6	0.3	43.7	7.7	78.6	5	0.2	0.4	0.4	n.d.				n.d.				44%	78.6	45.1	63.1	54.1	6.3	17.8	12.1
	Valsartan	7	3.8	11.1	5.2	457.8	3	3.6	13.3	7.1	n.d.				n.d.				41%	457.8	201.7	3915	2058	5499	9959	7729
	Telmisartan	7	15.6	237.9	81.4	534.7	6	33.0	249.1	118.1	1	87.7	87.7	87.7	n.d.				56%	534.7	2870	3852	3361	n.d.		
	Metformin	7	89.4	188.6	134.2	89.3	7	8.5	192.3	78.7	2	6.4	27.1	16.8	8	2.8	24.6	7.3	93%	192.3	151.6	618.5	385	763.6	1115	939.5
	Hydrochlorothiazide	2	0.8	3.9	2.4	85.0	1	4.0	4.0	4.0	n.d.				n.d.				15%	85	176.5	379.6	278.1	287.1	287.1	287.1
Pesticides and meatbolites (10)	Metolachlor	7	23.9	44.6	31.7	20.9	6	0.6	118.3	33.3	1	8.3	8.3	8.3	4	0.3	4	1.4	70%	118.3	n.d.			n.d.		
	Terbuthylazine	7	14.6	30.6	21.4		5	17.2	145.7	47	2	160.6	339.4	250	1	5	5	5	56%	339.4	15.4	97.9	56.6	n.d.		
	Desethylterbuthylazine	7	2.2	3.2	2.6		6	0.7	6.9	2.9	2	43.7	48.4	46.1	8	0.4	1.5	0.7	85%	48.4	10.3	33.4	21.9	n.d.		
	Terbutryn	6	1	2.2	1.6	76.7	6	1.5	3	2.5	2	2.2	9.3	5.8	2	0.7	1.5	1.1	63%	76.7	269.5	269.5	269.5	n.d.		
	Azoxystrobin	7	9	33.7	20	42	7	8.9	479.9	142.9	4	21.4	116.2	62.4	8	2.8	64.2	23.2	100%	479.9	3.6	3.6	3.6	n.d.		
	Metalaxyl	7	7.3	42	26.9	3.9	6	3.6	58.1	22.5	2	3.7	7.4	5.6	4	0.4	9.1	2.8	74%	58.1	n.d.			n.d.		
	Prochloraz	1	16.7	16.7	16.7		4	0.5	23.8	12	2	0.9	17.2	9.1	5	0.5	74.7	29.4	44%	74.7	10.6	10.6	10.6	n.d.		
	Propiconazole	7	1.2	2.3	1.9	3.9	7	1.4	599.1	203	4	4.4	52	21.2	8	1.8	85.9	25.3	100%	599.1	4	8.5	6.3	n.d.		
	Tebuconazole	7	4.3	30.5	15.3	87.7	7	15.5	408.8	188.8	4	97.3	355.6	219.6	8	5.4	88.4	25.7	100%	408.8	n.d.			n.d.		
	Tebufenozide	6	3.4	24	10.6	131	2	8.8	9.5	9.2	4	75.8	393.4	220.3	3	5.4	24.8	24.8	59%	393.4	121.1	555.5	338.3	576.8	646.7	611.7
Stimulants and metabolites (7)	Caffeine	3	0.6	2.7	1.5	n.d.	3	14.1	108.9	51.6	n.d.				2	9.4	11	10.2	30%	108.9	10.9	10.9	10.9	1325	1588	1456
	Xanthine	n.d.				n.d.	5	0.9	79.4	22.6	2	8.2	23.1	15.7	n.d.				26%	79.4	109.8	299.7	204.8	1594	1594	1594
	1-Methylxanthine	n.d.				n.d.	1	22.4	22.4	22.4	n.d.				n.d.				4%	22.4	36	36	36	1751	1751	1751
	Theophylline	n.d.				n.d.	2	15.5	35.4	25.5	n.d.				n.d.				7%	35.4	n.d.			430.8	514.2	472.5
	1,7-dimethyluric acid	n.d.				n.d.	n.d.				n.d.				n.d.				n.d.		16	212.6	114.3	n.d.		
	Nicotine	5	2.7	11.8	7.5	47.6	1	18.8	18.8	18.8	n.d.				6	12.6	76.5	40.3	48%	76.5	17.7	1453	735.3	2871	3672	3272
	Cotinine	7	8.3	16.6	11.1	115.2	7	6.5	34.7	12.3	3	6.2	8.7	7.8	5	4.7	86.1	26.7	85%	115.2	201.7	3915	2058	1784	3131	2458
Drug of abuse	Cocaine	n.d.				n.d.	3	6.9	13.1	9.3	n.d.				1	1.2	1.2	1.2	15%	13.1	14.8	14.8	14.8	985.2	1515	1250
Personal care	Panthenol	7	25.5	94.9	73.5	54.0	6	31.1	332.3	127.3	n.d.				8	13.3	87.3	42.7	81%	332.3	671.4	2150	1411	n.d.		

products (3)	Galaxolidone	2	3.5	15.7	9.6	32.3	n.d.				n.d.				n.d.				11%	32.3	1781	2131	1956	1060	2228	1644
	Diethyltoluamide (DEET)	7	40.9	126.3	74.2	253.9	7	108.1	343.4	207.4	4	64.3	114.6	88	8	12.6	76.5	36.9	100%	343.4	0.6	0.6	0.6	n.d.		

*N- number of positive findings in each simple type

Fig. S1. Map of the sampling area.