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DEPARTAMENTO DE
QUÍMICA, FÍSICA Y ANALÍTICA**

TESIS DOCTORAL
**DESARROLLO DE METODOLOGÍAS
ANALÍTICAS MEDIANTE
CROMATOGRAFÍA/ESPECTROMETRÍA DE
MASAS PARA EL CONTROL DE
CONTAMINANTES ORGÁNICOS
PRIORITARIOS Y EMERGENTES EN AGUAS
RESIDUALES Y SUPERFICIALES**

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Certifican:

Que la Tesis Doctoral titulada *“Desarrollo de metodologías analíticas mediante cromatografía/espectrometría de masas para el control de contaminantes orgánicos prioritarios y emergentes en aguas residuales y superficiales”*, realizada por D. José Robles Molina, y que presenta para la obtención del grado de Doctor en Química por la Universidad de Jaén, ha sido desarrollada bajo su inmediata dirección y supervisión, en el Departamento de Química Física y Analítica de la Universidad de Jaén.

En Jaén, a 30 de Abril de 2014

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a mi madre

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*"La Tierra no es una herencia de
nuestros padres sino un préstamo de
nuestros hijos".*

Proverbio indio



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Talanta 82 (2010) 1318-1324

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AcEt	Ethyl acetate
APCI	Atmospheric Pressure Chemical Ionization
API	Atmospheric Pressure Ionization
APPI	Atmospheric Pressure Photoionization
B	Magnetic Sector
BTEXs	Benzene, Toluene, Ethylbenzene, Xylenes and Styrene
CAR	Carboxen
CE	Comunidad Europea
CEE	Comunidad Económica Europea
CI	Chemical Ionization
CIS	Centro de Investigaciones Sociológicas
CMA	Concentración Máxima Admisible
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DEET	N,N-Dietil-3-metilbenzamida
DEHP	Bis(2-ethylhexyl)phtalate
DI-SPME	Direct Immersion-Solid Phase Microextraction
DMA	Directiva Marco del Agua
DMST	N,N-dimethyl-N-tolylsulfonyldiamide
DNOC	4,6-Dinitro-o-cresol
DVB	Divinylbenzene
ECD	Electron Capture Detector
EDAR	Estaciones Depuradoras de Agua Residual
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EI	Electron Impact
EPA	Environmental Protection Agency

EPN	O-Ethyl O-(4-nitrophenyl) phenylphosphonothioate
ESA	Electric Sector Analyzer
ESI	Electrospray
FID	Flame Ionization Detector
FS	Full Scan
FT-ICR	Fourier Transform Ion Cyclotron Resonance
FT-OT	Fourier Transform Orbitrap
GC	Gas Chromatography
GC-HRMS	Gas Chromatography-High Resolution Mass Spectrometry
GC-MS	Gas Chromatography-Mass Spectrometry
GC-MS/MS	Gas Chromatography-Tandem mass spectrometry
GC-QqQMS	Gas Chromatography-Triple Quadrupole Mass Spectrometry
HCH	Hexachlorocyclohexane
HILIC	Hydrophilic Interaction Liquid Chromatography
HPLC	High Performance Liquid Chromatography
HS-SPME	Head Space-Solid Phase Microextraction
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IT	Linear Ion Trap (tridimensional)
LC	Liquid Chromatography
LC-MS	Liquid Chromatography-Mass Spectrometry
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LC-TOFMS	Liquid Chromatography-Time of Flight Mass Spectrometry
LIT	Linear Ion Trap (bidimensional)
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
MA	Media Anual

MALDI	Matrix-Assisted Laser Desorption/Ionization
MCPA	4-chloro-o-tolyloxyacetic acid
MDA	3,4-Methylenedioxyamphetamine
MDEA	N-methyl-diethanolamine
MDMA	3,4-Methylenedioxy-N-methylamphetamine
MeOH	Methanol
MIPs	Molecularly Imprinted Polymers
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
NCA	Norma de Calidad Ambiental
PA	Polyacrylate
PAHs	Polycyclic Aromatic Hydrocarbons
PBDEs	Polybrominated Diphenyl Ethers
PCBs	Polychlorinated Biphenyls
PDMS	Polydimethylsiloxane
PEG	Polyethyleneglycol
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctanesulfonic acid
Q	Quadrupole
QDs	Quantum dots
QqLIT	Hybrid Quadrupole/Linear Ion Trap (QTRAP)
QqQ	Triple Quadrupole
QTOF	Quadrupole Time of Flight
RAMs	Restricted Access Material
SBSE	Stir Bar Sorptive Extraction
SIM	Selected Ion Monitoring
SPDE	Solid Phase Dynamic Extraction

SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
SRM	Selected Reaction Monitoring
TBP	Tributylphosphate
TCB	Trichlorobenzene
TCD	Thermal Conductivity Detector
TCP	Tris (1-chloro-2-propyl) phosphate
TEP	Triethylphosphate
TOF	Time of Flight
TQ	Triple Quadrupole
UE	Unión Europea
uma	Unidad de Masa Atómica
UPLC	Ultra Performance Liquid Chromatography
UPLC-MS/MS	Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry
USA	United States of America
UV	Ultraviolet
UV-vis	Ultravioleta-Visible
VOCs	Volatile Organic Compounds
Δ -9-THC	Δ -9-tetrahydrocannabinol
WFD	Water Framework Directive
WWTP	Waste Water Treatment Plant

A large, faint watercolor splash graphic is centered on the page, tilted at a 45-degree angle. It features soft washes of pink, purple, and blue, with some darker, more saturated areas. The splash appears to be composed of several overlapping layers, creating a sense of depth and movement. The colors blend into each other, with some areas showing more intense hues than others. The overall effect is artistic and ethereal, serving as a decorative background element.

RESUMEN

RESUMEN

España presenta el mayor déficit hídrico de los países integrantes de la Unión Europea, lo que genera gran preocupación social. Según datos del CIS un 96.5% de los españoles cree que la escasez de agua parece un problema actual o de futuro. Se hace evidente que la reutilización del agua es una de las posibles actuaciones que ayudarían a paliar este problema. Aunque España es uno de los países que más reutiliza el agua residual, todavía lo hace en cantidades poco significativas (aproximadamente un 5% del volumen de aguas residuales recogidas). Para poder llevar a cabo la reutilización de esta agua de forma adecuada y en función de su uso posterior, es imprescindible conocer su estado de contaminación.

La Unión Europea estableció un Marco comunitario de actuación en el ámbito de la política de aguas, mediante la Directiva 2000/60/CE del Parlamento europeo y del Consejo del 20 de Octubre de 2000, cuyo último objetivo era alcanzar un “buen estado” ecológico y químico de todas las aguas comunitarias para 2015. A raíz de esta directiva, se establecieron unas normas de calidad ambiental (NCA) para las aguas superficiales que incluyen una lista de 33 sustancias identificadas como prioritarias. Recientemente esta lista ha sido actualizada, incrementándose a 45 el número de sustancias prioritarias.

Teniendo en cuenta que las aguas residuales y superficiales están relacionadas por los vertidos que se producen en estas últimas, y que la contaminación asociada a las mismas puede tener consecuencias muy negativas para el medioambiente y la salud humana, es necesario el desarrollo de metodologías analíticas capaces de detectar con suficiente sensibilidad los distintos tipos de contaminantes presentes en éstas.

Aunque hay una cantidad elevada de investigaciones realizadas que tratan el análisis de contaminantes en distintas matrices de agua, la mayoría se centra en familias concretas de compuestos y el número de estos rara vez supera el medio centenar. Por lo tanto, el principal objetivo de la presente Tesis es el desarrollo de metodologías analíticas basadas en la detección mediante espectrometría de masas para el análisis multi-residuo de un número elevado de contaminantes orgánicos pertenecientes a distintas clases químicas y con funciones diversas como plaguicidas, productos de higiene personal o fármacos. Se evaluaron tres

metodologías de aislamiento y preconcentración de los analitos de interés, como son la extracción líquido-líquido (LLE), la extracción en fase sólida (SPE) y la microextracción en fase sólida en la modalidad de espacio de cabeza (HS-SPME). Estos tratamientos de muestra se complementaron con análisis por cromatografía de gases acoplada a espectrometría de masas (GC-MS) y cromatografía de líquidos acoplada a espectrometría de masas (LC-MS). Los resultados derivados de estos trabajos han dado lugar a la publicación de 5 artículos en revistas científicas de difusión internacional.

En el primer trabajo (apartado IV.1. artículo científico 1) se desarrolló una metodología analítica basada en la extracción líquido-líquido (LLE) y cromatografía de gases-espectrometría de masas de alta resolución (GC-HRMS) con un analizador de sector magnético de doble enfoque, para el análisis de 14 sustancias prioritarias, entre las que se encuentran plaguicidas como el alacloro o la simazina y compuestos éteres de difenilo polibromados (PBDEs). El método se validó utilizando muestras de efluentes de agua residual fortificadas a dos niveles de concentración (0.1 y $1 \mu\text{gL}^{-1}$), obteniéndose recuperaciones comprendidas entre 80 y 120% para todos los analitos estudiados, excepto para los triclorobencenos ($\sim 50\%$). La sensibilidad conseguida hace que este método cumpla con los requisitos establecidos por la normativa vigente para el agua superficial en lo que a sustancias prioritarias se refiere. Por último, esta metodología se aplicó para la caracterización de efluentes de 5 estaciones depuradoras de agua residual (EDARs) representativas, encontrándose valores de concentración a niveles de ng L^{-1} , siendo el hexaclorobenceno el compuesto más frecuentemente detectado (100% de las muestras).

En el segundo estudio (apartado IV.2. artículo científico 2) se exponen los resultados de validación de una metodología para el análisis multi-residuo de 57 compuestos apolares volátiles, la mayoría de ellos definidos como contaminantes prioritarios, mediante LLE y espectrometría de masas en tándem con analizador de triple cuadrupolo (GC-QqQMS). En este caso se explota la potencialidad de un analizador de masas en tándem, que mediante su modo de trabajo MRM (multiple reaction monitoring) permite la identificación inequívoca de los analitos de interés, así como reducir el efecto matriz debido a su alta selectividad. Las recuperaciones obtenidas para la mayoría de compuestos estaban comprendidas entre 80 y

120% y la sensibilidad obtenida está de acuerdo con los límites establecidos por la normativa acerca de las sustancias prioritarias en aguas superficiales (para todos los compuestos el límite de cuantificación se situó por debajo de 3 ng L^{-1}). Esta metodología se aplicó al análisis de muestras reales de efluentes de agua residual tratada y no tratada procedente de distintas EDARs, siendo el clorpirifos etil el compuesto más frecuentemente detectado, con niveles de concentración por debajo de $0.5 \text{ } \mu\text{g L}^{-1}$.

En el tercer trabajo (apartado IV.3. artículo científico 3) se evaluaron tres procedimientos de extracción distintos para el análisis de contaminantes prioritarios en muestras de agua residual y su posterior análisis por GC-QqQMS. Estos procedimientos fueron LLE, extracción en fase sólida (SPE) y microextracción en fase sólida en su modalidad de espacio de cabeza (HS-SPME). Los resultados mostraron que con LLE y SPE se consiguen mejores resultados en cuanto a sensibilidad y número de compuestos medibles (57 de ambos frente a los 43 de la HS-SPME). La SPE es un proceso que consume menos tiempo y es más respetuoso con el medioambiente que la LLE, ya que utiliza una menor cantidad de disolvente, sin embargo debido a que las muestras sometidas a LLE no deben ser filtradas, con este tratamiento se consiguen mayores valores de recuperación en compuestos como los PAHs (que se quedan retenidos en la materia sólida de la muestra) que cuando se necesita filtración previa con SPE. Dependiendo de la naturaleza química de las distintas familias de compuestos seleccionadas para este trabajo, la técnica de extracción de elección sería diferente.

En el apartado IV.4. se describe el artículo científico 4 en el que se desarrolló y validó un método para el análisis de 430 contaminantes prioritarios y emergentes por extracción en fase sólida y cromatografía de líquidos-espectrometría de masas con analizador de tiempo de vuelo (LC-TOFMS). Se estudia la potencialidad del analizador tiempo de vuelo mediante la creación de una base de datos de masas exactas que es universal y reproducible independientemente del equipo de alta resolución que se emplee. Este tipo de analizador ofrece una sensibilidad muy elevada en modo *full-scan* para todo tipo de compuestos con límites de detección a nivel de picogramos (muy similar a la sensibilidad conseguida por los espectrómetros de masa en tándem). En contra de lo que ocurre con los analizadores de masas en tándem, el número de compuestos diferentes que se pueden determinar con estos

analizadores es en principio ilimitado y se puede realizar una búsqueda retrospectiva para la identificación de compuestos desconocidos o que no están incluidos en la base de datos. Por lo tanto, la LC-TOFMS ofrece unas características que encajan a la perfección con la filosofía del control de un número elevado de contaminantes orgánicos en muestras medioambientales. En general los resultados obtenidos con este método fueron satisfactorios con límites de cuantificación por debajo de 10 ng L^{-1} para el 44% de los compuestos estudiados y recuperaciones entre 50% y 130% para el 65% de los analitos (281 compuestos). Además se llevo a cabo la evaluación del efecto matriz y el método se aplicó a la determinación de los analitos seleccionados en muestras reales de agua residual y superficial.

En el artículo científico 5 del apartado IV.5. se han aplicado las metodologías de LLE seguida de GC-QqQMS y SPE seguida de LC-TOFMS, desarrolladas previamente, para evaluar la contaminación de distintitos tipos de agua superficial en la provincia de Jaén. Se analizaron un total de 83 muestras procedentes de aguas de río, pantano y humedales. La contribución más significativa a la contaminación de estas aguas proviene de la agricultura, ya que Jaén presenta la mayor superficie del mundo dedicada al cultivo del olivo. Así el perfil de contaminantes está compuesto principalmente por plaguicidas, pero también por compuestos mundialmente consumidos como la cafeína y la nicotina y en menor medida por fármacos. Los niveles de concentración en la mayoría de casos se situaban en valores de ng L^{-1} con algunas excepciones en las que se alcanzaron concentraciones de $\mu\text{g L}^{-1}$. En cuanto a la presencia de contaminantes prioritarios estudiados, se pudo concluir que las aguas analizadas presentaban un buen estado, ya que solo en dos casos puntuales se sobrepasó ligeramente el límite establecido por la normativa (Directiva 2013/39/UE). Estudios de este tipo ponen de manifiesto la necesidad de continuar con el seguimiento de la contaminación de las aguas medioambientales, desde un punto de vista tanto cuantitativo como cualitativo, ya que cada vez es mayor el número de sustancias químicas que llegan al medioambiente y de las que se desconoce aún su efecto, destino y concentración.

A large, faint watercolor splash graphic is centered on the page, tilted at a 45-degree angle. It features a mix of colors including shades of purple, pink, red, and blue, with some darker, more saturated areas and lighter, more diffused edges. The splash appears to be composed of several overlapping, irregular shapes, giving it a dynamic and artistic feel.

SUMMARY

SUMMARY

Spain is the European country with the highest water deficit, something that generates a serious social concern. According to the CIS (Centro de investigaciones sociológicas- Sociological research center), the present or future lack of water represents a problem to 96.5% of the Spanish population. Thus, water reuse is one of the alternatives that may help to solve this problem. Although Spain is within the countries with highest water reuse, its quantity is still small (only 5% of the volume of waste water is reused). For a proper reuse of this water it is mandatory to have a good knowledge of its contamination depending on his further use.

On the other hand, the European Community established a common framework for actuation in the water policy, through Directive 2000/60/CE of the European parliament and of the council of 23 October 2000, with the ultimate goal of reaching a <<good ecological and chemical state>> for all community waters by 2015. This directive fixed a list with 33 priority substances and their environmental quality standard for surface waters. Recently, the number of priority contaminants has been increased up to 45.

Given that surface and waste water are related due to the discharges from sewage treatment plant (STPs) to the environmental water, and contamination associated to them could lead to a negative impact in the environment and human health, it is necessary the development of analytical methodologies able to detect with enough sensitivity the contaminants present in those waters.

Although there is a large number of publications dealing with the analysis of contaminants in different water matrices, most of them are only focused in certain family of compounds and the number of they rarely exceed one hundred. Therefore, the main objective of the present PhD Thesis was to develop analytical methodologies based on mass spectrometry detection for multiresidue analysis of a large number of organic contaminants (500) belonging to different chemical classes with different uses such as pesticides, personal care products or pharmaceuticals. Three extraction procedures were evaluated: liquid-liquid extraction (LLE), solid phase extraction (SPE) and solid phase microextraction in head space working mode (HS-SPME). These procedures were combined with the analysis by gas chromatography coupled to

mass spectrometry (GC-MS) and liquid chromatography interfaced with mass spectrometry (LC-MS). The PhD Thesis comprises 5 articles published in international research journals, which have been discussed in the chapter IV (Resultados y discusión) after a general introduction and a detailed description of the chemicals and instruments used to develop the different methodologies.

· In the first work (section IV.1. scientific paper 1) an analytical methodology based on LLE and gas chromatography-high resolution mass spectrometry (GC-HRMS) with a double focusing magnetic sector analyzer, was developed for the analysis of 14 priority contaminants, among them there are pesticides such as alachloro or simazine and polibrominated diphenyl ethers (PBDEs). The method was validated using effluent wastewater samples spiked at 2 fortification levels (0.1 and 1 $\mu\text{g L}^{-1}$), obtaining recovery rates values between 80 and 120% for all studied compounds except for trichlorobenzenes (~50%). The sensitivity accomplished permits to fulfill the environmental quality standards for current surface water European legislation for priority substances. Finally, the methodology was applied for the characterization of effluent waste water samples of 5 different representative STPs, where priority contaminants were detected at ng L^{-1} level and hexachlorobenzene was found to be the most frequently detected compound (100% of samples).

· The second survey (section IV.2. scientific paper 2) shows the validation results of a methodology for multiresidue analysis of 57 low polar volatile compounds, most of them defined as priority substances, by LLE and mass spectrometry using a triple quadrupole analyzer (GC-QqQMS). In this case, the potential of a tandem mass spectrometer analyzer is studied, which permits through its MRM (multiple reaction monitoring) working mode unequivocal identification of the target analytes, as well as the reduction of matrix effect due to its high selectivity. The vast majority of compounds yielded recovery rates values between 80 and 120% and the achieved sensitivity is in agreement with current European legislation for priority substances (quantification limits were below 3 ng L^{-1} for all studied compounds). This methodology was used to investigate the quality of treated and untreated effluents sewage samples from different STPs. Chlorpyrifos ethyl, pyrene and γ -HCH were found to be the most frequently detected contaminants in the studied samples, although at levels below 0.6 $\mu\text{g L}^{-1}$.

Section IV.3. is dedicated to scientific paper 3 in which three extraction procedures (LLE, SPE and HS-SPME) for priority contaminants followed by GC-QqQMS analysis in waste water samples were evaluated. Results showed that LLE and SPE achieved better sensitivity and wider number of measurable compounds than HS-SPME (57 against 43). SPE is less time consuming and more environmental friendly than LLE, due to the less amount of solvent needed. However in LLE, samples are not filtered so that the recovery rate values for retained compounds in the suspend solids (for example polycyclic aromatic hydrocarbons -PAHs-) were better than those obtained when previous filtration is needed like in SPE. Nevertheless, it was demonstrated that depending on the chemical class, the treatment sample process of choice would be either SPE or LLE.

In section IV.4. scientific paper 4 is described. A methodology for the analysis of priority and emerging contaminants by SPE and liquid chromatography time of flight mass spectrometry (LC-TOFMS) was developed and validated. This work deals with the evaluation of time of flight mass analyzer by creating an accurate mass database which is universal and reproducible regardless the high resolution instrument being used. This analyzer offers a high sensitivity working in full-scan mode for a wide range of compounds with limits of detection at picogram level (similar to that obtained by tandem mass spectrometers). This sensitivity may be achieved in the entire range of masses (for example 50-1000 m/z) which means that any substance could be detected at low concentration levels without selecting certain ions (as is needed in triple quadrupole analyzers). The number of compound that could be detected in principle is unlimited and a further search could be performed to identify unknown and non-target compounds. Hence, LC-TOFMS offers specific features that match perfectly with the philosophy of control a huge number of organic contaminants that is growing dramatically in environmental samples. The overall method performance was satisfactory with limits of quantification lower than 10 ng L⁻¹ for the 44% of studied compounds. Recoveries between 50%-130% were obtained for the 65% of the analytes (281 compounds). Matrix effects occurring with wastewater matrices were also assessed. The developed method was applied to the determination of target analytes in real surface water and wastewater samples.

· The fifth study (section IV.5. scientific paper 5) contains the results of the application of the previous developed methodologies (LLE followed by GC-QqQMS and SPE followed by LC-TOFMS) to evaluate the contamination of different surface water samples at the province of Jaén. A total of 83 water samples were collected comprising 3 rivers, 5 reservoirs and 11 wetlands. The contamination of these waters is mainly due to the agricultural activity since Jaen has the largest surface in the world dedicated to olive tree cultivation. Therefore, the contaminants profile is principally composed by pesticides, and also by world-wide consumed substances such as nicotine and caffeine and to a lesser extent by pharmaceuticals. Concentration level of contaminants was in most cases at the ng L⁻¹ range, with some exceptions where µg L⁻¹ concentration was measured. Regarding the presence of studied priority pollutants it could be conclude that the water quality status was good as only 2 cases were found to slightly exceed the environmental quality standards established by regulation (Directive 2013/39/EU). This survey highlights the need of continue the monitoring of contaminants in environmental samples, from a point of view not only quantitative but also qualitative, due to the increase of chemical substances that are continuously introduced in the environment without realizing the consequences, fate and concentration, and whose negative effects may affect directly or indirectly to human health.



I. OBJETIVOS

I. OBJETIVOS

Los trabajos completados en la presente tesis están enmarcados dentro del proyecto CONSOLIDER “Tratamiento y Reutilización de Aguas Residuales para una Gestión Sostenible (TRAGUA)” (CSD2006-00044). Un total de 24 grupos de investigación de diferentes áreas se encargaron de llevar a cabo las distintas tareas necesarias para alcanzar el objetivo general del proyecto, de abordar de una manera integral la reutilización de las aguas residuales urbanas depuradas. Las actividades científicas en las que estaba implicado el grupo de investigación de Química Analítica de la Universidad de Jaén (FQM-323) y que constituyeron la base de la presente tesis doctoral eran las siguientes:

- a) Evaluación y seguimiento de la calidad de las aguas de cinco EDARs (Estaciones depuradoras de aguas residuales) representativas.
- b) Evaluación del rendimiento de distintos tratamientos avanzados de aguas residuales urbanas.
- c) Dar soporte analítico a otros grupos de investigación, realizando el tratamiento y análisis de distintos tipos de muestras de agua medioambiental
- d) Elaboración de un protocolo de técnicas de muestreo y técnicas analíticas de contaminantes prioritarios y emergentes

Como consecuencia de la participación en estas actividades se realizaron los estudios expuestos en el presente trabajo, cuyos objetivos específicos son los siguientes:

- 1) Desarrollar y validar metodologías analíticas multi-residuo para el análisis de contaminantes apolares mediante cromatografía de gases-espectrometría de masas (GC-MS), haciendo hincapié tanto en la preparación de muestra como en el análisis y detección de contaminantes principalmente prioritarios.
- 2) Evaluar distintos tipos de tratamiento de muestra (extracción líquido-líquido (LLE), extracción en fase sólida (SPE) y microextracción en fase sólida por espacio de

- cabeza (HS-SPME)) para el aislamiento y preconcentración de contaminantes orgánicos prioritarios en aguas residuales y su posterior análisis por GC-MS.
- 3) Desarrollar y validar una metodología analítica multi-residuo para el análisis de contaminantes polares mediante SPE y cromatografía de líquidos-espectrometría de masas (LC-MS). Esta metodología está basada en la realización de una base de datos que contiene un total de 430 compuestos, siendo la mayoría de ellos contaminantes emergentes, para su análisis automático en muestra reales.
 - 4) Evaluar la calidad de distintas aguas residuales y superficiales, teniendo en cuenta la normativa europea vigente referente a contaminantes prioritarios y emergentes, aplicando las metodologías desarrolladas.

A watercolor-style map of Europe is positioned in the background, tilted slightly to the right. It features soft, blended colors in shades of pink, red, and blue, with some darker, more saturated areas. The map is not a standard geographical representation but rather an artistic interpretation with a painterly texture.

I. AIM OF THE STUDY

I. AIM OF THE STUDY

The work described in the present PhD Thesis is involved in a CONSOLIDER project entitled "Treatment and Reuse of Water for a sustainable management" ("Tratamiento y Reutilización de Aguas Residuales para una Gestión Sostenible (TRAGUA)") (CSD2006-00044). A total of 24 research groups from different disciplines partnered up to fulfil the general aim of the project of addressing thoroughly the treated urban wastewater reuse. The scientific activities in which Analytical Chemistry Research Group at University of Jaén (FQM-323) was involved were the following:

- a) Evaluation and tracing of water quality of five representative Spanish STPs
- b) Evaluation of the efficiency of different advanced urban wastewater treatment
- c) Analytical support to other research groups, performing the extraction and analysis of various environmental water samples.
- d) Development of a protocol for sampling techniques and analytical methods for the analysis of priority and emerging contaminants

As a consequence of the participation in this project, the studies presented in this work were performed aiming the following specific objectives:

- 1) Develop and validate multiresidue analytical methodology for low polar contaminants analysis by gas chromatography-mass spectrometry (GC-MS) with different techniques (GC-HRMS and GC-MS/MS), emphasizing both sample treatment and detection stages.
- 2) Evaluate different sample treatments (LLE, SPE and HS-SPME) for the isolation and preconcentration of priority contaminants following by GC-MS analysis of wastewater samples.
- 3) Develop and validate a multiresidue analytical methodology for polar organic contaminants analysis by SPE and LC-MS. This methodology is based on the performance of a database comprising over 400 priority and emerging compounds for its application to real water samples.

- 4) Apply the developed methodologies to actual samples, with the aim of evaluating the quality of surface and waste water, in compliance with the current European legislation related to priority and emerging pollutants.



II. INTRODUCCIÓN Y ANTECEDENTES

II.1. Problemática general de la contaminación de las aguas

El volumen total de agua en la Tierra es de aproximadamente 1.400 millones de km³ de los cuales sólo el 2,5%, o alrededor de 35 millones de km³, corresponde a agua dulce. La mayor parte del agua dulce se presenta en estado de agregación sólido, ubicado en la región antártica y en Groenlandia, o en profundos acuíferos de aguas subterráneas. Las principales fuentes de agua para uso humano son los lagos y ríos, la humedad del suelo y las cuencas de aguas subterráneas relativamente poco profundas. La parte aprovechable proveniente de esas fuentes es aproximadamente de sólo 200.000 km³ de agua, es decir menos del 1% del total de agua dulce y sólo el 0,01% de toda el agua del planeta. La gran parte de esa agua disponible está ubicada lejos de las poblaciones humanas, lo que complica aún más las cuestiones relativas a su aprovechamiento.

Por lo que la gestión que se haga de ese agua es un factor crítico para el mantenimiento y desarrollo de la vida humana en la Tierra. Los tres principales factores que causaron un aumento en la demanda de agua en el siglo pasado fueron el crecimiento demográfico, el desarrollo industrial y la expansión del cultivo de regadío. Lo que llevó a la aparición de reglamentos y una planificación para gestionar y aprovechar el agua disponible teniendo en cuenta la demanda futura de la misma (principalmente con la construcción de infraestructuras tales como diques en los ríos). Pero el énfasis puesto en el abastecimiento de agua, combinado con una débil aplicación de los reglamentos, limitó la eficacia de la ordenación de los recursos hídricos, especialmente en las regiones en desarrollo. Por lo que actualmente los responsables de la adopción de políticas pretenden centrarse en la gestión de la demanda, recalcando la importancia de utilizar una combinación de medidas que garanticen suficiente abastecimiento de agua para diferentes sectores [1].

Teniendo en cuenta todo esto, existe un hecho preocupante y es que virtualmente no existe fuente de agua destinada al uso humano que no esté contaminada con xenobióticos o compuestos sintetizados por el hombre en el laboratorio, la mayoría de ellos introducidos en el medio ambiente en los últimos 100 años. En el mundo se conocen alrededor de 70 millones de sustancias químicas [2] de las cuales alrededor de 100.000 se usan habitualmente en la vida

diaria, tanto en hogares, industrias como agricultura [3]. Además ingresan en el mercado cada año entre 500 y 1000 sustancias nuevas, generándose entre 300 y 400 millones de toneladas de desechos peligrosos. Estos productos químicos se introdujeron sin tener en cuenta los posibles efectos adversos sobre el medio ambiente y/o la salud humana. Además en el informe de la Comisión mundial sobre el agua en 1999 se puso de manifiesto que la mitad de los principales ríos del planeta están gravemente agotados y contaminados por lo que degradan y contaminan los ecosistemas y amenazan la salud y el sustento de las personas que dependen de ellos. Por esto es de vital importancia el estudio tanto cualitativo como cuantitativo de los contaminantes que se encuentra en las aguas destinadas al uso humano y en las fuentes de contaminación de las mismas.

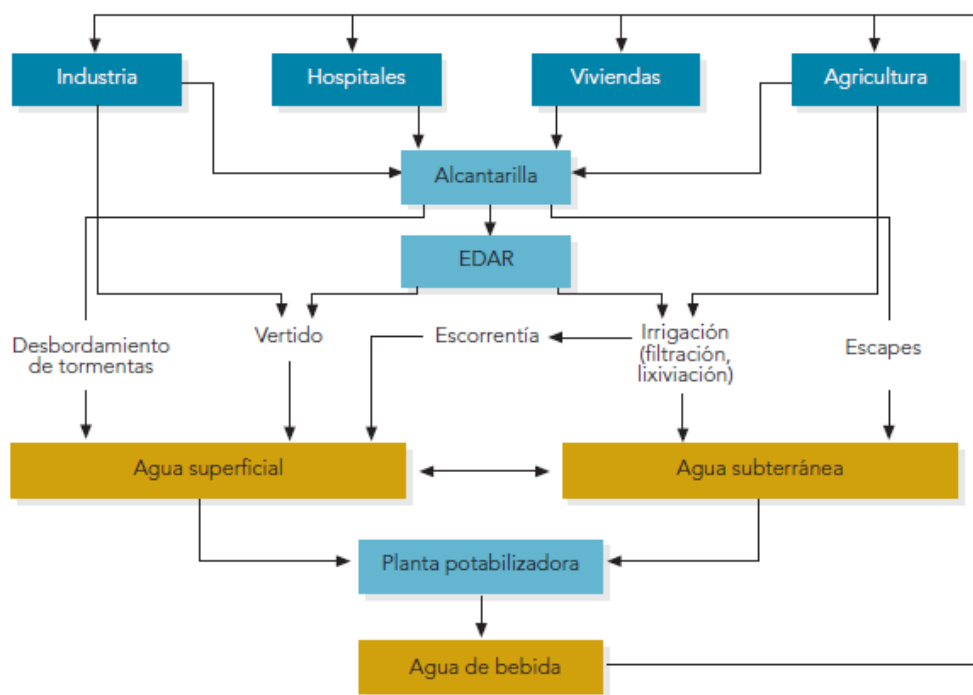


Figura II.1 Esquema de las posibles vías de incorporación de contaminantes al medio ambiente (adaptada de Ternes y Joss 2006 [4])

La incorporación de contaminantes al medio ambiente tiene lugar por diferentes vías, tanto directas, a través de descargas a las aguas superficiales desde las estaciones depuradoras de agua residual (EDAR) o la industria, como indirectas mediante filtraciones, empleo de abonos en agricultura, excrementos de animales, uso de piensos en acuicultura etc. De forma esquemática en la figura II.1. se representan las posibles rutas de entrada de los contaminantes en el medio ambiente, pudiéndose observar las fuentes de contaminación, la forma de introducción en el medio y cómo podrían aparecer en el agua de bebida.

Si nos centramos en la problemática del agua en España, es muy significativo que, según el Centro de Investigaciones Sociológicas (CIS), la escasez de agua parece un problema actual o de futuro a un 96,6 % de la población española. En un estudio más reciente a nivel europeo, la calidad del agua representa un problema serio para el 72% de los españoles encuestados [5]. Estos datos están en consonancia con la realidad que vive nuestro país, ya que como se puede comprobar en la figura II.2. España es el país europeo con mayor escasez de agua.



Figura II.2 Distribución física de la escasez de agua en las principales cuencas de los ríos más importantes del mundo (adaptada de Informe del estado mundial de los recursos de agua y tierra para la alimentación y agricultura [6])

Una de las posibles medidas que se propone para paliar los efectos negativos de esta escasez, sería la reutilización del agua ya que es una alternativa medioambientalmente necesaria frente a otros métodos que conllevan un mayor consumo energético e impacto medioambiental. Entre los métodos más rigurosos para determinar el potencial de reutilización de aguas se encuentra el elaborado por Hochstrat y col. [7] según el cual España tiene un potencial de reutilización un orden de magnitud superior al actual.

Las EDAR son instalaciones bastante estandarizadas, se diferencian unas de otras más en el tamaño que en las operaciones que en ellas se realizan. Sin duda la composición media de las aguas que reciben también es muy similar, sin embargo existen grandes diferencias en los componentes minoritarios de las mismas. Entre estos componentes se encuentran compuestos orgánicos procedentes de productos farmacéuticos, de limpieza o de cuidado personal, así como plaguicidas de diverso tipo. Teniendo en cuenta que en la actualidad solo existen normas de calidad ambiental para 45 sustancias denominadas como prioritarias por la Directiva 2013/39/EU [8] de la Unión Europea, y el número de contaminantes introducidos en el medio ambiente va aumentando de forma continua, existen todavía un gran desconocimiento acerca del resto de contaminantes que no están legislados y en cuyo interés se están centrando las investigaciones actualmente. A estos contaminantes se les denomina emergentes.

Con estos antecedentes parece claro que el desarrollo de metodologías analíticas capaces de detectar e identificar estos contaminantes orgánicos a los niveles de concentración necesarios, son de gran importancia para conocer el estado de las aguas sean o no destinadas al consumo humano así como de sus fuentes de contaminación.

II.2. Contaminantes orgánicos prioritarios

El 23 de Octubre de 2000 se aprobó la Directiva 2000/60/CE del parlamento europeo y del consejo por la que se establecía un marco comunitario en el ámbito de la política de aguas [9]. Mediante esta directiva la Unión Europea organiza la gestión de las aguas superficiales, continentales, de transición, aguas costeras y subterráneas, con el fin de prevenir y reducir su contaminación, fomentar su uso sostenible, proteger el medio acuático, mejorar la situación de

los ecosistemas acuáticos y paliar los efectos de las inundaciones y de las sequías. Para ello, entre otras medidas, se estableció una lista de 33 sustancias prioritarias mediante la Decisión número 2455/2001/CE del Parlamento Europeo y del Consejo de 20 de Noviembre de 2001 [10] cuya contaminación debe ser progresivamente reducida o eliminada. Como se puede ver en la tabla II.1. dentro de las sustancias prioritarias se pueden encontrar metales pesados y compuestos orgánicos tales como plaguicidas, hidrocarburos aromáticos policíclicos (PAHs) o surfactantes. Todas estas sustancias son de una forma u otras nocivas para el medioambiente y/o seres humanos.

Los PAHs son un grupo numeroso (entorno a unos 100) de compuestos orgánicos que químicamente son derivados poliméricos del benceno. Se introducen en el medio ambiente principalmente por procesos de combustión tanto naturales (erupciones volcánicas, incendios forestales) como antropogénicos (escape de automóviles, degradación de neumáticos, emisiones en industrias de craqueo catalítico, incineración de residuos, etc) [11,12]. Además varios de estos compuestos (especialmente los más ligeros) son solubles en agua por lo que se han encontrado en muestras de agua superficial y residual [13,14] e incluso están presentes en el aire [15] y alimentos [16,17]. La toxicidad aguda de muchos PAHs suele ser baja, sin embargo, los efectos a medio o largo plazo que producen algunos de ellos son los que hacen que estos productos despierten gran preocupación científica y pública general. Por el momento, se ha demostrado para una veintena de PAHs su actividad carcinogénica, mutagénica o inmunosupresora [18].

Por otro lado, los plaguicidas son productos químicos utilizados para prevenir, destruir o repeler plagas. Las plagas pueden ser insectos, ratones y otros animales, malas hierbas, hongos, o microorganismos tales como bacterias y virus [19]. Se pueden encontrar residuos de plaguicidas en alimentos debido a un uso legal, ya que en la mayoría de los casos los niveles no suponen ningún problema al encontrarse a muy bajas concentraciones, del orden de partes por millón (mg Kg^{-1}) o menos [20]. Pero la exposición crónica a los plaguicidas puede causar, entre otras patologías, cáncer, disrupción endocrina y problemas de fertilidad [21,22,23,24]. Además el colectivo más vulnerable a los efectos tóxicos de los plaguicidas son los niños

debido a su metabolismo menos desarrollado y la maduración continua de sus órganos [25,26,27].

El octilfenol, nonilfenol y sus correspondientes etoxilatos son compuestos utilizados principalmente en la fabricación de productos de limpieza como detergentes y, en menor medida, para otras aplicaciones como, por ejemplo, plaguicidas, resinas, etc. Son especialmente persistentes y tóxicos y se pueden acumular en organismos y sedimentos acuáticos [28,29]. Su principal efecto nocivo es que pueden actuar como disruptores endocrinos, es decir, alteran el equilibrio hormonal de una especie, bien interrumpiendo algunos procesos fisiológicos controlados por hormonas, o generando una respuesta de mayor o menor intensidad que lo habitual [30].

Formando una familia extensa de compuestos utilizados en productos industriales como retardantes de llama, se encuentran los difeniléteres bromados (PBDEs) muy utilizados debido a su bajo coste y alta eficiencia. La producción de retardantes de llama a nivel mundial es de 200.000 toneladas por año, de las cuales cerca de 47.000 corresponden a PBDEs [31]. Son compuestos para los que se han encontrado evidencias de que son tóxicos para el desarrollo neuronal, especialmente en las etapas de crecimiento cerebral (hasta los 2 años de edad) [32,33].

Por último, dentro de las sustancias prioritarias se encuentran compuestos utilizados como disolventes o para síntesis de plásticos etc, normalmente clorados (diclorometano, tetracloroetileno, triclorometano etc) que son sustancias muy nocivas para los seres humanos ya que se ha demostrado que son cancerígenas [34]. Las parafinas cloradas son formulaciones industriales consistentes en mezclas técnicas de alcanos de cadena lineal policlorados con cadenas hidrocarbonadas que varían entre 10 y 30 átomos de Carbono. Su producción anual de las aproximadamente 200 formulaciones que existen se estima en unas 300.000 toneladas por año. Una exposición prolongada a estas sustancias puede originar efectos adversos en órganos vitales como el riñón, el hígado y la glándula del tiroides. Existen evidencias claras de que puede generar cáncer en el ser humano [35]. Los cloroalcanos de cadena corta (C10-13) no son fácilmente biodegradables y son bioacumulativos, por lo que permanecen durante

mucho tiempo en el ambiente, llegando a afectar a la fauna y flora del lugar. También se han detectado en el medio marino y en los sedimentos de áreas industriales, siendo unos compuestos extremadamente tóxicos para los organismos acuáticos [36,37].

Tabla II.1. Lista de sustancias prioritarias establecida por la Decisión número 2455/2001/CE del parlamento europeo y del consejo de 20 de Noviembre de 2001

	Nombre de la sustancia prioritaria		Nombre de la sustancia prioritaria
(1)	Alacloro	(20)	Plomo y sus compuestos
(2)	Antraceno	(21)	Mercurio y sus compuestos
(3)	Atrazina	(22)	Naftaleno
(4)	Benceno	(23)	Níquel y sus compuestos
(5)	Difeniléteres bromados	(24)	Nonilfenoles (4-(para)-nonilfenol)
(6)	Cadmio y sus compuestos	(25)	Octilfenoles (para-ter-octilfenol)
(7)	Cloroalcanos C ₁₀₋₁₃	(26)	Pentaclorobenceno
(8)	Clorofenvinfos	(27)	Pentaclorofenol
(9)	Cloropirifos (clorpirifós etil)	(28)	Hidrocarburos aromáticos policíclicos
(10)	1,2-dicloroetano		(Benzo[a]pireno)
(11)	Diclorometano		(Benzo[b]fluoranteno)
(12)	Di(2-etilhexil)ftalato (DEHP)		(Benzo[g,h,i]perileno)
(13)	Diurón		(Benzo[k]fluoroanteno)
(14)	Endosulfán (alfa-endosulfan)		(Indeno[1,2,3-cd]pireno)
(15)	Fluoranteno	(29)	Simazina
(16)	Hexaclorobenceno	(30)	Compuestos del tributiltin (tributiltin catión)
(17)	Hexaclorobutadieno	(31)	Triclorobencenos (1,2,4-triclorobenceno)
(18)	Hexaclorociclohexano (gamma-isómero lindano)	(32)	Triclorometano (cloroformo)
(19)	Isoproturón	(33)	Trifluralina

Típicamente el análisis de contaminantes prioritarios se ha realizado mediante GC-MS, con analizadores de cuadrupolo simple [147,149], pero en los últimos años con al avance de la instrumentación ha pasado a realizarse mayoritariamente con GC-MS/MS [118,143,146] debido a que es más sensible y selectiva.

II.3. Contaminantes orgánicos emergentes

La Agencia para la Protección del Medioambiente de Estados Unidos (US EPA) define a los productos químicos que se han descubierto en el agua y hasta ahora no habían sido detectados o se están detectando a niveles muy diferentes a lo esperado, como contaminantes de interés emergente porque el riesgo para la salud humana y medioambiente asociados a su presencia, frecuencia de detección y fuentes de emisión pueden ser desconocidos [38]. De manera que aunque durante décadas, la comunidad científica ha centrado sus esfuerzos en el estudio de los contaminantes químicos cuya presencia en el medio ambiente ha estado o está regulada en las distintas legislaciones, en la actualidad el desarrollo de nuevos métodos de análisis más sensibles ha permitido alertar de la presencia de otros contaminantes potencialmente peligrosos denominados como emergentes [39,40,41]. Tienen la particularidad de que debido a su elevada producción y consumo, se introducen continuamente en el ambiente y por lo tanto no necesitan ser persistentes para ocasionar efectos negativos [42].

Como se puede ver en la tabla II.2., la lista de contaminantes emergentes que se han estudiado incluye una amplia variedad de productos de uso diario con aplicaciones tanto industriales como domésticas. En ella se pueden encontrar fármacos de todo tipo, productos de higiene personal, retardantes de llama, productos de limpieza, drogas de abuso etc.

Tabla II.2. Lista de los principales productos que han sido o están siendo investigados

Productos investigados como contaminantes emergentes	
- Productos farmacéuticos, como antibióticos humanos o de consumo veterinario como el trimetoprim	
- Productos analgésicos o antiinflamatorios, como ibuprofeno, paracetamol, ácido acetil-salicílico	
- Drogas psiquiátricas, como diazepam	
- Reguladores lípidos, como el bezafibrato	
- Betabloqueantes, como metropolol, propanolol o timolol	
- Contrastes para rayos X, como lopromida y opamidol diatrizoato	
- Esteroides u hormonas, como el estradiol, estrona, estriol, DES	
-Productos para la higiene y cuidado personal, como fragancias, geles o jabones.	
- Agentes para protección de la luz solar, como benzofenona, metil-benzilideno	

Productos investigados como contaminantes emergentes	
- Repelentes de insectos, como el NN-dietil-toluamida (DEET)	
- Antisépticos, como triclosan o clorofeno	
- Agentes de limpieza superficial, como alquifenoles y carboxilatos alquifenoles	
- Retardantes de llama, como los éteres polibromados, que se añaden a los plásticos especialmente a los muebles, productos electrónicos, TV, y que se encuentran en la sangre humana, leche, grasa, placenta, produciendo efectos nocivos en una disrupción endocrina	
- Agentes y aditivos industriales, como el 2-cloroetilfosfato.	
- Aditivos para la gasolina, como éteres de alquilos o el éter metil-t-butilo.	
- Subproductos de la desinfección, como bromoácidos, bromoacetnitrilos, bromatos, etc.	
- Plaguicidas polares y sus productos de degradación/transformación	

Los compuestos que han despertado mayor interés y están siendo estudiados con más detalle son los fármacos. La primera evidencia de la presencia de estos compuestos en el agua residual tuvo lugar en la década de los 70, con el análisis de ácido clofíbrico [43]. Aunque se realizaron algunos estudios en la década de los 80 en los que se detectaron fármacos como aspirina, cafeína [44] o diazepam [45] en efluentes de agua residual, y antibióticos como eritromicina, sulfametoxazol [46] o indometacina [47] en muestras de agua de río, no fue hasta la década de los 90 cuando tuvo lugar el despertar del interés científico y social por los trabajos publicados desde entonces.

El consumo de fármacos en la Unión Europea se cifra en toneladas por año, y muchos de los más usados, entre ellos los antibióticos, se emplean en cantidades similares a las de los plaguicidas [48]. En Estados Unidos la información acerca de la producción anual de fármacos no está disponible generalmente con facilidad, por lo que las estimaciones del uso y producción de antibióticos en humanos y agricultura son controvertidas [49,50]. En muchos casos, las consecuencias de su presencia en el medio ambiente no están aún claras, pero en otros el riesgo parece evidente y alarmante. Así por ejemplo, el diclofenaco se ha asociado con la desaparición de los buitres blancos en India y Pakistán [51] y se ha demostrado que afecta a la reproducción y al desarrollo de algunos organismos acuáticos especialmente en los estados iniciales de su desarrollo [52].

De todos los ingredientes activos en Europa (más de 3000) permitidos para su uso en el cuidado de la salud, sólo aproximadamente un centenar ha sido alguna vez analizado (en distintas matrices medioambientales) desde que se empezaron a encontrar en los años 70. Por lo tanto, parece evidente la necesidad de seguir trabajando en esta línea de investigación, en la que se deben incluir además los estudios de metabolitos y productos de transformación [53,54]. Hasta el momento la técnica que más se ha empleado para el análisis de contaminantes emergentes en muestras medioambientales ha sido la cromatografía de líquidos debido al carácter cada vez más polar de los nuevos contaminantes. Para su detección se ha empleado básicamente la espectrometría de masas, con analizadores de alta resolución [55,56] y analizadores de masas en tándem [57,58].

II.4. Normativa europea sobre residuos de contaminantes orgánicos en aguas

La Unión Europea ha llevado a cabo, desde los años 70 y hasta la actualidad, una expansión y reestructuración de su política en materia de agua. Inicialmente la legislación comunitaria referente al agua estuvo enfocada hacia objetivos y niveles de calidad mínimos para ciertos tipos de agua como las de baño, aguas para la cría de moluscos y las destinadas al consumo humano. Más tarde, en los años 90, se trataron de resolver los problemas de contaminación provenientes de aguas residuales urbanas, del sector agrícola y de las grandes instalaciones industriales, mediante la directiva de Aguas Residuales Urbanas (Directiva 91/271/CEE) [59]. Ésta establecía las medidas a llevar a cabo para la recolección y tratamiento de aguas residuales provenientes de asentamientos urbanos y aglomeraciones (de más de 10.000 habitantes), con la excepción de los de menor tamaño, con el fin de conseguir la mejora de la calidad de las aguas receptoras de aguas residuales tratadas.

Pero es en el año 2000, con la Directiva Marco del Agua (DMA) (Directiva 2000/60/EC) [9], cuando realmente se abre una brecha en la política de aguas europea, no solamente en lo que se refiere a su alcance en cuanto a la protección de la calidad de las aguas, sino en su aplicación e implementación. Establecía un régimen para la prevención y el control de la contaminación química de aguas superficiales y subterráneas, alcanzando a estuarios y aguas costeras. Su objetivo primordial era prevenir el deterioro del agua en términos de calidad

ecológica y química, así como promover el uso y consumo sostenible, basándose en una protección del recurso a largo plazo. Se inicia así, dentro de este proceso, la obligación de la Comisión de presentar propuestas específicas, para la identificación de sustancias de preocupación para su clasificación como prioritarias o peligrosas y por tanto, de su control ambiental.

Así en España desde la publicación de la Ley de Aguas, de 2 de Agosto de 1985 [60], y sobre todo desde su incorporación a la Unión Europea (UE), han sido muchas las medidas legislativas que se han ido adaptando progresivamente con la finalidad de proteger los recursos hídricos existentes y de armonizar nuestra legislación con la europea. En la página web del Ministerio de Agricultura Alimentación y Medioambiente [61] se puede consultar la legislación nacional acerca del estado y calidad de las aguas, sobre el dominio público hidráulico, planificación, producción de agua potable, aguas de baño, protección de la vida piscícola, vertidos, sustancias peligrosas y valoración de daños.

De manera que la normativa vigente en materia de aguas se encuentra en la actualidad dispersa en una amplia variedad de herramientas legislativas que presentan distinto nivel de competencia (europeo, nacional o autonómico), ámbito de aplicación (aguas subterráneas, aguas de consumo humano etc.) y aspectos a regular (frecuencia de muestreo, normas de calidad etc.). Sin embargo en los próximos años está previsto que muchas de estas normas queden derogadas por la Directiva 2000/60/CE por la que se establece un marco comunitario de actuación en materia de política de aguas, con el fin de alcanzar en 2015 un buen estado de las aguas superficiales continentales, de transición, costeras y subterráneas.

Así, tras la entrada en vigor de la DMA, quedaron derogadas las siguientes normativas:

- Directiva 75/440/CEE del Consejo, de 16 de Junio de 1975, relativa a la calidad requerida para las aguas superficiales destinadas a la producción de agua potable de los Estados miembros.
- Decisión 77/795/CEE del Consejo, de 12 de diciembre de 1977, por la cual se establece un procedimiento común de intercambio de informaciones relativo a la calidad de las aguas continentales superficiales en la comunidad.

- Directiva 79/869/CEE del Consejo, de 9 de octubre de 1979, relativa a los métodos de medida y a la frecuencia de los muestreos y del análisis de las aguas superficiales destinadas a la producción de agua potable en los Estados miembros.

Así mismo transcurridos catorce años desde la entrada en vigor de la DMA quedarán derogadas las siguientes Directivas:

- Directiva 78/659/CEE del Consejo, de 18 de julio de 1978, relativa a la calidad de las aguas continentales que requieren protección o mejora para ser aptas para la vida de los peces.
- Directiva 79/923/CEE del Consejo, de 30 de octubre de 1979, relativa a la calidad exigida a las aguas para la cría de moluscos.
- Directiva 80/68/CEE del Consejo, de 17 de diciembre de 1979, relativa a la protección de las aguas subterráneas contra la contaminación causada por determinadas sustancias peligrosas.
- Directiva 76/464/CEE del Consejo, de 4 de mayo de 1976, relativa a la contaminación causada por determinadas sustancias peligrosas vertidas en el medio acuático de la Comunidad (excepto su artículo 6 que quedó derogado cuando entró en vigor la DMA).

Por otro lado, a raíz de la DMA se han aprobado las siguientes Directivas a tener en cuenta por los investigadores que llevan a cabo estudios sobre la presencia, comportamiento y eliminación de contaminantes orgánicos:

- Directiva 2455/2001/CE del Parlamento Europeo y del Consejo, de 20 de noviembre de 2001, por la que se aprueba la lista de sustancias prioritarias en el ámbito de la política de aguas, y por la que se modifica la Directiva 2000/60/CE [9]. En su anexo X se aprobó la lista con 33 sustancias prioritarias (ver *tabla II.1.*) en el ámbito de la política de aguas.
- Directiva 2008/105/CE del Parlamento Europeo y del Consejo, de 16 de diciembre de 2008, relativa a las normas de calidad ambiental en el ámbito de la política de aguas, por la que se modifican y derogan ulteriormente las Directivas 82/176/CEE, 83/513/CEE, 84/156/CEE, 84/491/CEE y 86/280/CEE del Consejo, y por la que se modifica la Directiva 2000/60/CE [62].

- Directiva 2013/60/CEE del Parlamento Europeo y del Consejo, de 12 de agosto de 2013, relativa a las sustancias peligrosas en el marco de la política de aguas y que modifica las Directivas 2000/60/CEE y 2008/105/CEE [8].

En la tabla II.3., se muestran las normas de calidad (media anual y concentración máxima admisible) aplicables a las 45 sustancias prioritarias en aguas superficiales continentales y otras aguas superficiales, establecidas por la directiva anterior [10] y que se amplían o modifican por la presente.

Tabla II.3. Normas de calidad ambiental (NCA) para las sustancias prioritarias y otros contaminantes.

nº	Nombre de la sustancia	MA-NCA aguas superficiales continentales	MA-NCA otras aguas superficiales	CMA-NCA aguas superficiales continentales	CMA-NCA otras aguas superficiales
(1)	Alacloro	0.3	0.3	0.7	0.7
(2)	Antraceno	0.1	0.1	0.1	0.1
(3)	Atrazina	0.6	0.6	2.0	2.0
(4)	Benceno	10	8	50	50
(5)	Difeniléteres bromados			0.14	0.014
(6)	Cadmio y sus compuestos (en función de las clases de dureza del agua)	≤0.08 (Clase1) 0.08 (Clase2) 0.09 (Clase3) 0.15 (Clase4) 0.25 (Clase5)	0.2	≤0.45 (Clase1) 0.45 (Clase2) 0.6 (Clase3) 0.9 (Clase4) 0.1.5 (Clase5)	≤0.45 (Clase1) 0.45 (Clase2) 0.6 (Clase3) 0.9 (Clase4) 0.1.5 (Clase5)
(6a)	Tetracloruro de carbono	12	12	no aplicable	no aplicable
(7)	Cloroalcanos C ₁₀₋₁₃	0.4	0.4	1.4	1.4
(8)	Clorofenvinfos	0.1	0.1	0.3	0.3
(9)	Cloropirifos (clorpirifós etil)	0.03	0.03	0.1	0.1
(9a)	Plaguicidas ciclodieno: Aldrin Dieldrin Endrin Isodrin	Σ=0.01	Σ=0.005	no aplicable	no aplicable
(9b)	DDT total	0.025	0.025	no aplicable	no aplicable
	para-para-DDT	0.01	0.01	no aplicable	no aplicable
(10)	1,2-dicloroetano	10	10	no aplicable	no aplicable
(11)	Diclorometano	20	20	no aplicable	no aplicable
(12)	Di(2-etilhexil)ftalato (DEHP)	1.3	1.3	no aplicable	no aplicable

n°	Nombre de la sustancia	MA-NCA aguas superficiales continentales	MA-NCA otras aguas superficiales	CMA-NCA aguas superficiales continentales	CMA-NCA otras aguas superficiales
(13)	Diurón	0.2	0.2	1.8	1.8
(14)	Endosulfán (alfa-endosulfan)	0.005	0.0005	0.01	0.004
(15)	Fluoranteno	0.0063	0.0063	0.12	0.12
(16)	Hexaclorobenceno			0.05	0.05
(17)	Hexaclorobutadieno			0.6	0.6
(18)	Hexaclorociclohexano (gamma-isómero lindano)	0.02	0.002	0.04	0.02
(19)	Isoproturón	0.3	0.3	1.0	1.0
(20)	Plomo y sus compuestos	1.2	1.3	14	14
(21)	Mercurio y sus compuestos			0.07	0.07
(22)	Naftaleno	2	2	130	130
(23)	Níquel y sus compuestos	4	8.6	34	34
(24)	Nonilfenoles (4-(para)-nonilfenol)	0.3	0.3	2.0	2.0
(25)	Octilfenoles (para-ter-octilfenol)	0.1	0.01	no aplicable	no aplicable
(26)	Pentaclorobenceno	0.007	0.0007	no aplicable	no aplicable
(27)	Pentaclorofenol	0.4	0.4	1	1
(28)	Hidrocarburos aromáticos policíclicos	no aplicable	no aplicable	no aplicable	no aplicable
	(Benzo[a]pireno)	1.7×10^{-4}	1.7×10^{-4}	0.27	0.027
	(Benzo[b]fluoranteno)	Ver nota 1	Ver nota 1	0.017	0.017
	(Benzo[g,h,i]perileno)	Ver nota 1	Ver nota 1	0.017	0.017
	(Benzo[k]fluoroanteno)	Ver nota 1	Ver nota 1	8.2×10^{-3}	8.2×10^{-4}
	(Indeno[1,2,3-cd]pireno)	Ver nota 1	Ver nota 1	no aplicable	no aplicable
(29)	Simazina	1	1	4	4
(29a)	Tetracloroetileno	10	10	no aplicable	no aplicable
(29b)	Tricloroetileno	10	10	no aplicable	no aplicable
(30)	Compuestos del tributiltin (tributiltin catión)	0.0002	0.0002	0.0015	0.0015
(31)	Triclorobencenos (1,2,4-triclorobenceno)	0.4	0.4	no aplicable	no aplicable
(32)	Triclorometano (cloroformo)	.25	2.5	no aplicable	no aplicable
(33)	Trifluralina	0.03	0.03	no aplicable	no aplicable
(34)	Dicofol	1.3×10^{-3}	3.2×10^{-5}	no aplicable	no aplicable

nº	Nombre de la sustancia	MA-NCA aguas superficiales continentales	MA-NCA otras aguas superficiales	CMA-NCA aguas superficiales continentales	CMA-NCA otras aguas superficiales
(35)	Ácido perfluorooctanosulfónico y sus derivados (PFOSs)	6.5×10^{-4}	1.3×10^{-4}	36	7.2
(36)	Quinoxifen	0.15	0.015	2.7	0.54
(37)	Dioxinas y compuestos similares			no aplicable	no aplicable
(38)	Aclonifeno	0.12	0.12	0.12	0.12
(39)	Bifenox	0.012	0.0012	0.04	0.004
(40)	Cibutrina	0.0025	0.0025	0.016	0.016
(41)	Cipermetrina	8×10^{-5}	8×10^{-6}	6×10^{-4}	6×10^{-5}
(42)	Diclorvós	6×10^{-4}	6×10^{-5}	7×10^{-4}	7×10^{-5}
(43)	Hexabromociclododecano (HBCDD)	0.0016	0.0008	0.5	0.05
(44)	Heptacloro epóxido y de heptacloro	2×10^{-7}	1×10^{-8}	3×10^{-4}	3×10^{-5}
(45)	Terbutrina	0.065	0.0065	0.34	0.034

MA: media anual. CMA: concentración máxima admisible. Unidades: $\mu\text{g/l}$

Nota1: Por lo que respecta al grupo de sustancias prioritarias de hidrocarburos aromáticos policíclicos (HAP) (nº 28), las NCA de la biota y las correspondientes NCA-MA en el agua se refieren a la concentración de benzo[a]pireno, en cuya toxicidad se basan. El benzo[a]pireno puede considerarse como un marcador de los otros HAP, ya que solo tal sustancia debe ser objeto de seguimiento a efectos de comparación con las NCA de la biota o las correspondientes NCA-MA en el agua.

En esta última Directiva se amplió el número de sustancias prioritarias de 33 a 45 y cabe destacar que entre las nuevas sustancias prioritarias se han incluido lo que hasta entonces eran clasificadas como contaminantes emergentes como el ácido perfluorooctanóico y sus derivados.

II.5. Metodologías de preparación de muestra y análisis de contaminantes orgánicos en aguas

Para el análisis de residuos de contaminantes orgánicos en el medioambiente son necesarios métodos sensibles, selectivos, precisos, automáticos y aplicables a un gran número de compuestos en una amplia gama de matrices. Este análisis requiere de varias etapas que se esquematizan en la figura II.3. Todas ellas son importantes y requieren que se lleven a cabo de la forma más adecuada posible teniendo en cuenta que los errores cometidos en cada una de

ellas se van acumulando hasta el final del proceso analítico. Por lo tanto, lo ideal es que el número de etapas se reduzca, siempre que sea posible, para minimizar las posibilidades de error y disminuyan tanto el coste como el tiempo empleado.

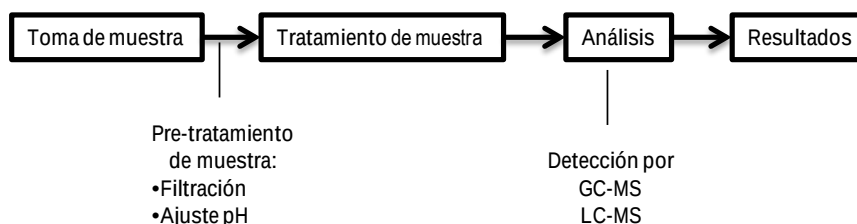


Figura II.3. Etapas fundamentales del proceso analítico

II.5.1. Preparación de la muestra

El tratamiento de muestra puede considerarse como un punto crítico dentro del proceso analítico, ya que su influencia sobre el resultado es incluso más importante que la medida en sí misma o la precisión del instrumento. Esta etapa consiste en una serie de pasos encaminados a la obtención de un extracto que contenga los analitos de interés en un disolvente adecuado para la técnica de detección a emplear. Normalmente, la falta de automatización hace que el tratamiento de muestra consuma la mayor cantidad de tiempo de todo el proceso analítico (pudiendo superar el 60%). En el caso de muestras acuosas medioambientales se lleva a cabo una etapa de extracción que, en muchos casos, lleva asociado el empleo de disolvente que bien por su toxicidad o por la gran cantidad necesaria es indeseable. El método de extracción seleccionado dependerá principalmente de la naturaleza de los compuestos a analizar, así como de sus propiedades físico-químicas y de los sistemas analíticos disponibles.

II.5.1.1. Extracción líquido-líquido (LLE)

Este tratamiento de muestra está basado en la solubilidad relativa de un analito en dos fases inmiscibles y está gobernada por el coeficiente de distribución o reparto que es la relación entre las concentraciones de analito en cada una de las fases en condiciones de equilibrio. El

procedimiento experimental consiste en mezclar en un embudo de decantación, un volumen determinado de muestra con uno o varios volúmenes de disolvente o mezcla de disolventes orgánicos de polaridad similar a la de los analitos e inmisible con la muestra. Normalmente para aumentar la cantidad de analito extraído en la fase deseada, se suele repetir la extracción varias veces. Para aumentar la selectividad de la técnica se pueden adicionar sales inorgánicas que facilitarán el paso del analito a la fase orgánica (*salting-out effect*) [63]. Otro parámetro a controlar es el pH de la muestra, de forma que la acidificaremos para que los analitos ácidos pasen a la fase orgánica más fácilmente, y la alcalinizaremos para que los analitos básicos sean extraídos. Entre los inconvenientes que presenta esta técnica de extracción se encuentran la elevada cantidad de disolvente necesaria, los bajos valores de la constante de reparto, las interferencias de matriz o la formación de emulsiones difíciles de romper. Pero presenta la ventaja que las muestras acuosas no tienen que ser filtradas previamente, por lo que los compuestos que se encuentran adsorbidos en las partículas sólidas también serán extraídos [64]. La LLE se suele aplicar a la separación de analitos no polares o semipolares en muestras acuosas empleando disolventes como n-hexano, ciclohexano, diclorometano o acetato de etilo.

II.5.1.2. Extracción en fase sólida (SPE)

Este procedimiento consiste en hacer pasar la muestra a través de un cartucho relleno con un adsorbente sólido, de forma que los analitos quedarán retenidos en él y podrán ser eluidos posteriormente con un disolvente apropiado. Se puede dividir en las siguientes etapas: 1) acondicionamiento del sorbente haciendo pasar un disolvente/s de propiedades similares a la muestra, 2) carga de la muestra en el cartucho, 3) lavado con un disolvente que no eluya los analitos, pero sí otras sustancias potencialmente interferentes presentes en la muestra, y 4) elución de los analitos con un disolvente/s apropiado.

Este proceso se utiliza a menudo para purificar extractos obtenidos mediante otras técnicas, sobre todo para el análisis de alimentos [65]. Además es muy adecuada por un lado para la eliminación de interferencias (aislamiento de los analitos) ya que se retienen los analitos de interés en la fase sólida, pasando otras especies constituyentes de la muestra que pueden actuar como interferentes. También se podría dar el caso contrario: retener los interferentes y

no los analitos. Por otro lado, también es adecuada para realizar una preconcentración de analitos que quedan retenidos en la fase sólida, pudiendo ser eluidos con pequeños volúmenes de disolvente.

La SPE se utiliza normalmente para la extracción de analitos polares o semipolares. Muchos factores influyen en la eficiencia del proceso de SPE, pero los dos más importantes son la capacidad del adsorbente y la retención de los analitos en él. Existe en el mercado una amplia gama de adsorbentes como las sílices enlazadas químicamente con cadenas alquílicas (C-18, C-8) o grupos polares (-CN, -NH₂), polímeros porosos (estireno-divinilbenceno), Florisil (silicato de magnesio activado) y carbón grafitizado. Grupos funcionales iónicos como el ácido carboxílico o los grupos amino también pueden enlazarse a sílice o polímeros para crear adsorbentes de intercambio iónico. Las interacciones analito-adsorbente pueden estar basadas, por tanto, en enlaces de hidrógeno, interacciones polares o apolares, intercambio aniónico o catiónico, y exclusión por tamaño. Esto nos permite seleccionar adsorbentes adecuados para la determinación de analitos dentro de un mayor rango de polaridades y características físico-químicas. Por último, el empleo de adsorbentes de balance hidrofílico – lipofílico, como el Oasis® HLB (Waters) está teniendo una gran aplicación. Oasis® HLB es un relleno de fase reversa, adecuado para la extracción en fase sólida de una amplia gama de compuestos, su composición consiste en una combinación de dos monómeros: N-vinilpirrolidona (hidrofílica) y divinilbenceno (lipofílica). Esta combinación proporciona una capacidad de retención superior en fase reversa, con una capacidad especial para optimizar la retención de los analitos polares.

La SPE en comparación con al LLE es más selectiva, reproducible, con un gasto bajo de disolventes orgánicos y fácilmente automatizable, pero debido a que hay que filtrar la muestra previamente a su paso por el cartucho, no se consigue extraer los compuestos absorbidos en la materia sólida de las muestras acuosas que la contengan.

II.5.1.3. Microextracción en fase sólida (SPME)

La SPME [66] utiliza una fibra de sílice fundida recubierta de un material adsorbente, en general un recubrimiento polimérico, que se utiliza para la extracción de compuestos orgánicos y/o inorgánicos volátiles derivados del mercurio, arsénico, etc. La fibra se encuentra

en el interior de un tubo hueco, de tal forma que ésta puede retraerse y extraerse de su interior, quedando así expuesta a la muestra. Su uso implica dos etapas bien diferenciadas: primero la etapa de extracción (retención de los analitos en la fase estacionaria mediante procesos de absorción o adsorción), que puede llevarse a cabo sumergiendo directamente la fibra en el interior de la muestra (*DI-SPME – direct inmersión SPME*), o bien, manteniéndola en el espacio en cabeza que está en equilibrio con la disolución (*HS-SPME – headspace-SPME*); y, segundo, la etapa de desorción, que se puede hacer térmicamente (en el inyector de un cromatógrafo de gases), o bien, utilizando disolventes orgánicos (de forma manual, o acoplado con un cromatógrafo de líquidos). Existen varios tipos de fibras disponibles comercialmente (ver tabla II.4.), que difieren en el tipo de adsorbente y en su espesor. La elección de la fibra depende de la naturaleza fisicoquímica de los compuestos a extraer.

Tabla II.4. Tipos de fibras para SPME disponibles comercialmente en el mercado.

Adsorbente	Espesor (µm)	Temperatura de operación recomendada (°C)	Usos *
Carboxen ®/Polidimetilsiloxano (Carboxen/PDMS)	75 85	250 - 310	Gases y moléculas con bajo peso molecular (30-225 MW)
Polidimetilsiloxano (PDMS)	7 30 100	220 – 320 200 – 280 200 – 280	Compuestos volátiles (60-275 MW)
Polidimetilsiloxano/Divinilbenceno (PDMS/DVB)	60 65	200 – 270	Aminas volátiles, nitroaromáticos (50-300 MW)
Poliacrilato (PA)	85	220 – 300	Compuestos polares, semivolátiles
Divinilbenceno/Carboxen/ Polidimetilsiloxano (DVB/CAR/PDMS)	50/30	230 – 270	Compuestos aromáticos, volátiles y semivolátiles, C3-C20 (40-275 MW)
Polietilenglicol (PEG)	60	200 – 250	
Carbopack® Z	15	250 – 340	
Carbowax®/Divinilbenceno (DVB)	70	200 – 240	

*MW-Peso molecular

En general, en cromatografía de gases (GC), los compuestos volátiles requieren fibras con un recubrimiento de mayor espesor, mientras para compuestos semivolátiles resulta efectivo el uso de fibras con un recubrimiento más delgado. Hay que tener en cuenta que cuanto mayor es el espesor de la fibra, mayor es el tiempo de extracción, pero también es cierto que aunque mayor es la cantidad de analito extraído, el riesgo de retención irreversible de otros

compuestos presentes en la matriz, también aumenta. Otros factores que hay que optimizar, además del tiempo de extracción son: la temperatura de extracción y desorción, el tiempo de desorción, el volumen de muestra, la velocidad de agitación de la muestra (para homogeneizarla durante el calentamiento), el pH, la fuerza iónica, el disolvente de desorción y la adición de disolventes o medios micelares.

La SPME integra extracción y preconcentración en un solo paso (la muestra es preconcentrada en la propia fibra), requiriendo mínimas cantidades de disolventes orgánicos, lo que supone una ventaja sobre la LLE y la SPE.

Desde su aparición, la SPME ha jugado un papel importante en el análisis medioambiental, dando lugar a la publicación de un elevado número de artículos científicos que tratan el análisis de muestras de aire, agua, suelo y sedimentos con este tipo de proceso de extracción. La técnica de detección más común utilizada con SPME es la GC-MS para la determinación de contaminantes medioambientales como BTEXs, compuestos orgánicos volátiles (VOCs) o plaguicidas [67].

II.5.1.4. Extracción por sorción en barra magnética agitadora (SBSE)

Es una técnica de extracción que está basada en los mismos principios que la SPME pero viene a mejorarla en algunos aspectos. Su aparición data del año 1999 cuando se publicó la información acerca de la teoría y práctica de este nuevo proceso de extracción por parte del grupo de investigación de Pat Sandra [68]. Consiste en poner en contacto la muestra acuosa directamente o por espacio de cabeza con una barra magnética que está recubierta por una película de polímero de PDMS. Seguidamente esta barra se aclara con agua, se seca y se introduce en un tubo de vidrio el cual se coloca en la unidad de desorción térmica (desarrollada comercialmente para su acoplamiento con el sistema de inyección del cromatógrafo) para la introducción de los analitos normalmente en un GC por desorción térmica. Esta desorción también se puede producir por acción de un disolvente.

Se pueden encontrar en el mercado barras agitadoras de dos tamaños distintos, 10 mm x 3.2 d.e. (diámetro externo) y 40 mm x 3.2 d.e., generalmente las de 10 mm se

recomiendan para volúmenes de muestra entre 1 y 50 ml, mientras que las de 40 mm están recomendadas para volúmenes entre 100 y 250 ml.

La diferencia fundamental que presenta esta técnica de extracción con respecto a la SPME es la cantidad de sorbente que contienen las barras magnéticas agitadoras recubiertas de PDMS, oscilando para éstas entre 55 y 300 μL dependiendo de su tamaño, frente a los 0.5 μL de polímero en las fibras de 100 μL m de SPME, de manera que la eficacia de extracción se ve aumentada de 100 a 600 veces utilizando SBSE. La desventaja frente a la SPME es que, hasta el momento solo se comercializan barras con PDMS, lo que reduce considerablemente la cantidad y variedad de compuestos que se pueden extraer en función de sus propiedades físico-químicas.

La SBSE se ha utilizado en numerosas aplicaciones medioambientales normalmente en el análisis de compuestos apolares en muestras acuosas por GC-MS [69], aunque también se ha empleado para muestras sólidas y gaseosas [70,71].

II.5.1.5. Nuevas tendencias en la preparación de muestra para matrices líquidas

Uno de los principales problemas que presentan los tratamientos de muestra en la actualidad es la posible presencia de efecto matriz (aumento o supresión de la señal debida a otros componentes presentes en la muestra) que puede afectar de forma negativa a los parámetros analíticos (límite de detección (LOD), límite de cuantificación (LOQ), linealidad, exactitud y precisión). De manera que se requiere una etapa de tratamiento de muestra para aislar los analitos de interés, purificar los extractos o preconcentrar los analitos. Este proceso consume la utilización de gran parte del total del proceso analítico. Por estas razones, se ha puesto un gran esfuerzo en la última década para desarrollar procesos de extracción más eficientes, y algunas de las tendencias y estrategias que se han desarrollado recientemente son:

- Utilización de sorbentes avanzados.
- Automatización a través del acoplamiento del módulo de preparación de muestra con el sistema de detección (extracción on-line).

-Utilización de metodologías más verdes, como técnicas que reducen la utilización de disolventes.

En el caso de muestras líquidas el proceso de extracción que está más extendido es la SPE, para la que se están desarrollando nuevos sorbentes y además se están estudiando metodologías para llevar a cabo la extracción de modo on-line, acoplando el sistema de SPE al sistema de detección, que normalmente es un cromatógrafo de líquidos acoplado a un espectrómetro de masas (LC-MS).

Alguno de los avances más recientes en cuanto al tipo de sorbente son los siguientes:

a) SPE monolítica (libre de partículas). Estos materiales tienen un gran número de potenciales ventajas como, bajo coste, robustez y alta estabilidad, no presenta volúmenes muertos con los flujos convencionales utilizados en cromatografía líquida (LC), facilidad de control de las propiedades de poro de los absorbentes mediante la variación de la composición de los monómeros para la separación de moléculas de tamaño grande y pequeño, alta permeabilidad hidráulica y la predominancia del mecanismo de convección frente al de difusión de intercambio de masa bajo condiciones dinámicas que permite la separación a flujos extremadamente altos. Aunque también tienen limitaciones como un área de superficie más pequeña que la de los materiales convencionales de SPE, mostrando una menor capacidad de retención, lo que hace que comercialmente haya pocos materiales disponibles y por consiguiente su uso está limitado. Como ejemplo de aplicación de la SPE monolítica se encuentra la determinación de drogas en orina [72].

b) Polímeros de impresión molecular (MIPs). Son polímeros sintéticos con sitios específicos que son complementarios en forma, tamaño y grupos funcionales al analito de interés. Debido a su alta especificidad por el analito, los MIPs se pueden utilizar para extraer y aislar compuestos de interés de matrices complejas como alimentos o muestras medioambientales. Se han sintetizado MIPs para algunos contaminantes emergentes, incluyendo fármacos y disruptores endocrinos. Un ejemplo de utilización de este tipo de sorbente fue el análisis de drogas de abuso en agua residual [73].

c) Materiales de acceso restringido (RAMs). El término materiales de acceso restringido fue introducido por Desilets y colaboradores [74]. Se designa así a una familia de soportes que permiten la inyección directa de fluidos biológicos mediante la limitación de la accesibilidad de los sitios de interacción dentro de los poros a sólo moléculas pequeñas. Las macromoléculas son excluidas y sólo interactúan con la superficie exterior de la partícula de soporte revestida con grupos hidrófilos, lo que minimiza la adsorción de proteínas de la matriz. En comparación con las técnicas tradicionales de extracción en modo off-line, como la LLE y SPE, estos soportes de extracción, integrados en un sistema de LC permiten la automatización, aceleración y simplificación del proceso de extracción [75]. En los últimos años estos materiales se han utilizado para el análisis de fármacos en agua medioambiental [76,77] entre otras aplicaciones.

d) Nanopartículas como material sorbente. Las nanopartículas son partículas microscópicas con (al menos) un tamaño menor de 100 nm. Hay varias formas de clasificarlas utilizando criterios complementarios como el origen (natural, artificial), naturaleza química (inorgánica, orgánica, mezcla), homogeneidad (composición simple o híbrida), etc. Pero para las aplicaciones en química analítica es muy interesante hacer una clasificación en función de sus propiedades explotadas (ver tabla II.5., adaptada de [78]). Así por ejemplo, las nanopartículas de carbono presentan propiedades químicas, eléctricas, ópticas y térmicas. Además, las posibilidades de las nanopartículas híbridas están siendo objeto de un interés creciente, bien porque las propiedades por separado de las nanopartículas son diferentes pero complementarias (como por ejemplo combinación de nanotubos de carbono con partículas de ferrita) o bien porque tienen la misma naturaleza y presentan un efecto sinérgico importante (por ejemplo, combinación de quantum dots). En la figura II.4., (adaptada de [79]) se pueden observar las nanopartículas más empleadas para el tratamiento de muestra en el análisis químico.

Tabla II.5. Principales nanopartículas empleadas en química analítica y sus propiedades excepcionales explotadas

Propiedad explotada	Nanomateriales en Química Analítica				
	Sílice	Metálicos -Oro, plata -Óxidos metálicos -Quantum dots	Carbono -Fullerenos -Nanotubos de carbono	Polímero orgánicos - MIPs	Nanovesícula y nanomicelas
Química	√√	√	√√	√√	√√
Eléctrica	-	√√	√√	√	-
Óptica	-	√√	√√	-	-
Térmica	-	√	√	-	-
Magnética	-	√	-	-	-

Las nanopartículas de sílice se caracterizan por presentar altas áreas de superficie y tener una reactividad superficial intrínseca que les permite la posibilidad de introducir modificaciones químicas. Así por ejemplo nanopartículas de sílice modificadas con tiol han sido utilizadas para la preconcentración de drogas y plaguicidas [80]. Dentro de las nanopartículas metálicas hay un amplio rango de diferentes nanopartículas inorgánicas, de manera que los principales nanomateriales metálicos son las nanopartículas de oro y de plata, los óxidos de metales (por ejemplo el óxido de titanio, TiO_2), y los puntos cuánticos (*quantum dots*, QDs) [81]. Presentan características únicas como una gran área superficial específica, alta capacidad de absorción y baja temperatura de modificación. Entre sus desventajas se encuentra que son poco selectivos, así que para evitar este problema se ha modificado física o químicamente la superficie sorbente con compuestos orgánicos, como agentes quelantes [82]. Se han utilizado nanopartículas de Ag para el análisis de PAHs en muestras acuosas [83]. Dentro de las nanopartículas de carbono se engloban a los nanodiamantes, fullerenos, nanocarbón, nanocuernos, nanotubos y nanoconos de carbono. Las nanopartículas de carbono se emplean como sorbentes debido a que combinan una gran superficie extractiva con la afinidad por una gran variedad de analitos al poder establecer interacciones dipolo-dipolo, enlaces por puentes de hidrógeno, interacciones π - π o interacciones hidrofóbicas. Su funcionalización permite que actúen varios tipos de interacciones, incrementando la selectividad de los procesos [79]. De entre todas las nanopartículas de carbono, los nanotubos son los que más aplicaciones presentan para el análisis químico. Existen dos tipos fundamentales, los nanotubos de carbono de pared simple y los nanotubos de carbono de pared múltiple (ver figura II.4.) dependiendo de si están formados por una o más capas de grafeno. El principal problema de la utilización de

nanotubos de carbono en SPE es la formación de agregados que disminuyen la relación real entre la superficie y el área de absorción [84]. De entre las aplicaciones de este tipo de nanopartículas se puede encontrar la preconcentración de BTEX (benzeno, tolueno, etilbenzeno y xileno) [85] y disruptores endocrinos en aguas [86].

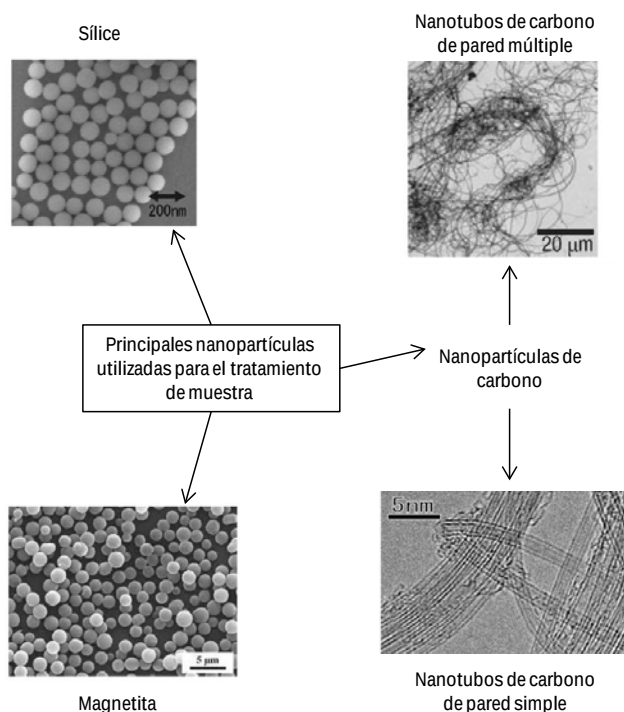


Figura II.4. Principales nanopartículas utilizadas para el tratamiento de muestra dentro del análisis químico

A parte del desarrollo de nuevos sorbentes, otra de las tendencias actuales en el terreno del tratamiento de muestra como se ha comentado anteriormente, es el acoplamiento del proceso de extracción con el sistema de detección, a lo que se conoce como extracción on-line. Este avance ha permitido la reducción de la manipulación de la muestra por lo que se incrementa la robustez del proceso así como se disminuye el tiempo empleado en llevar a cabo el mismo. También se reduce el volumen de muestra y disolventes empleados. En los últimos años han aparecido numerosas aplicaciones de la extracción on-line para el análisis de contaminantes emergentes en muestras medioambientales, humanas y de alimentos [87,88,89]. Se trata normalmente de acoplamientos de SPE con LC-MS [90]

Por último, en cuanto a técnicas que necesitan menor cantidad de disolvente se encuentra la SPME para la que han surgido variantes como la denominada extracción sobre barra agitadora (SBSE) [688] o extracción dinámica en fase sólida (SPDE) [91] que han mejorados su aplicación en distintos tipos de muestras. En general, la SBSE se considera superior a técnicas como la SPME en términos de sensibilidad y precisión, pero posee una serie de desventajas como son los largos tiempos de equilibrado, la imposibilidad de acoplarla de un modo sencillo a un inyector de un cromatógrafo de gases, la elevada manipulación que requiere y sobre todo la limitación que supone la falta de una gran gama de fases estacionarias. El único recubrimiento comercial es el PDMS, lo que limita el número de analitos con los que puede emplearse esta técnica [69]. En el caso de la SPDE, se consiguen tiempos de extracción menores que en SPME y la robustez de utilizar una fibra de acero en lugar de las que se utilizan para SPME que son más frágiles.

II.5.2. Análisis y detección de los analitos

El análisis de micro-contaminantes orgánicos y metabolitos en muestras acuosas ambientales requiere de su identificación y cuantificación en matrices complejas y, a niveles de concentración muy bajos (ultratraza). Por lo tanto, las técnicas analíticas tienen que ser específicas para la detección de estos analitos y ser lo suficientemente sensibles para permitir alcanzar bajos niveles de cuantificación. El análisis de contaminantes puede ser una tarea difícil ya que muchos son muy polares, tienen bajos pesos moleculares o son sensibles a los aumentos de temperatura. Por consiguiente, para la correcta cuantificación de este tipo de sustancias en matrices acuosas medioambientales, se necesitan técnicas analíticas avanzadas, como la cromatografía de gases o líquida (LC) y la espectrometría de masas (MS). Las investigaciones más recientes van dirigidas hacia la necesidad de analizar cada vez más analitos en un mismo método (métodos multi-analito) por lo que las metodologías analíticas multi-analito, se están convirtiendo en una de las herramientas más utilizadas y requeridas para conocer de forma fiable y más amplia, la presencia y destino de estos contaminantes en el medio ambiente.

II.5.2.1. Cromatografía de gases

En cromatografía de gases (GC), la muestra se volatiliza y se inyecta en la cabeza de una columna cromatográfica. La elución se produce por el flujo de una fase móvil de un gas inerte (generalmente helio o nitrógeno) que no interacciona con las moléculas de analito, su única función es la de transportarlo a través de la columna. El analito se distribuye entre la fase móvil gaseosa y una fase líquida inmovilizada sobre la superficie de un sólido inerte dentro de la columna cromatográfica, de manera que en función de la interacción con ambas va a presentar distinto tiempo de retención, por lo que se puede analizar cada analito por separado.

La GC se utiliza para el análisis de compuestos que presentan una volatilidad importante a temperaturas inferiores a 350-400 °C, además deben ser termoeestables a las temperaturas de trabajo y no han de degradarse ni perderse parte de los mismos a través de reacciones secundarias. La naturaleza de los analitos y su peso molecular se pueden utilizar para predecir su volatilidad, así cuanto mayor sea la polaridad y/o el peso molecular menor será ésta. De manera que, mientras que los hidrocarburos más pesados que 500 uma pueden ser analizados por medio de GC, los de peso molecular superior a 1400 umas necesitarían condiciones especiales. Si los analitos presentan grupos polares, se podrían hacer reaccionar con un reactivo adecuado (reacción de derivatización) para disminuir su volatilidad o bien rechazarlos para su análisis por GC.

Los distintos elementos que forman un cromatógrafo de gases se pueden ver en el esquema de la figura II.5.

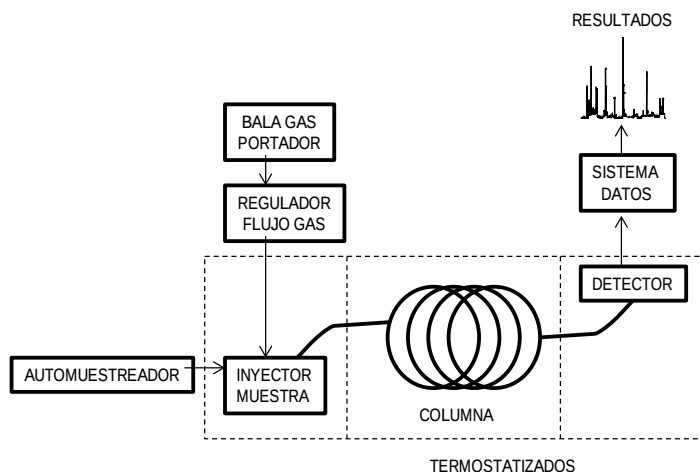


Figura II.5. Esquema de un cromatógrafo de gases

Existen distintas formas de introducción de muestra en un cromatógrafo de gases:

a) Inyección de muestras líquidas.- Es necesario preparar un extracto de la muestra en un disolvente volátil, siendo los más utilizados acetona, n-hexano y ciclohexano. El volumen de extracto inyectado está comprendido entre 1-20 μL .

b) Espacio de cabeza estático (HS).- Se inyecta un volumen del espacio de cabeza del vial, por lo que esta modalidad es muy limpia, ya que evita la acumulación de componentes no volátiles en el cromatógrafo de gases. A bajas concentraciones (< 50 ppb), el equilibrio de distribución de los analitos entre la muestra y el espacio de cabeza depende de la concentración, no del volumen de la muestra. Sin embargo, a concentraciones mayores, el volumen adquiere una mayor significación y se debe mantener constante en todas las muestras y patrones.

c) Espacio de cabeza dinámico (purga y trampa).- Los componentes volátiles de la muestra son arrastrados mediante una corriente gaseosa hasta una trampa fría o un soporte inerte, donde se produce el enriquecimiento de los analitos, que después son transferidos directamente hacia el cromatógrafo de gases mediante desorción térmica. La principal

desventaja de esta técnica es que los interferentes volátiles son preconcentrados al mismo tiempo que los analitos.

d) SPME.- Como se ha comentado previamente (Sección II.5.1.3.), los analitos pueden ser desorbidos de la fibra directamente en el inyector del cromatógrafo de gases.

Actualmente existen en el mercado muestreadores automáticos para GC muy versátiles, que permiten los análisis de muestras descritos, para lo que es necesario un pequeño ajuste de la configuración del muestreador y del cromatógrafo de gases.

Pero uno de los componentes más importantes de un cromatógrafo de gases es la columna ya que en ella se concentran la mayoría de los mecanismos necesarios para la identificación, separación y cuantificación de los componentes de la muestra. Básicamente existen dos tipos de columnas para GC, unas son las empaquetadas y las otras son las capilares que son las más utilizadas en la actualidad. Hoy en día se pueden encontrar en el mercado columnas para multitud de aplicaciones, con distintas dimensiones y fases estacionarias. [92,93].

Tradicionalmente la CG se utilizaba con detectores selectivos como el detector de conductividad térmica (TCD), el detector de ionización de llama (FID) o de captura de electrones (ECD). Era la técnica de elección para el análisis de plaguicidas en distintos tipos de matrices [94,95,96,97] ya que la mayoría presentaban las características físicas y químicas (volatilidad, polaridad, peso molecular, termoestabilidad, presencia de halógenos) necesarias para su análisis por GC. En la actualidad el acoplamiento con espectrometría de masas y sus numerosas ventajas frente a los detectores tradicionales hace que sea la técnica de elección en la mayoría de los casos para compuestos apolares o relativamente polares y volátiles.

En los últimos años el avance más significativo respecto a la GC para el análisis medioambiental se ha alcanzado con la GC multidimensional. En general, estas técnicas ofrecen un incremento en el factor de capacidad del pico, mejoran la resolución y la sensibilidad de masa [98]. Como una evolución de estas técnicas se encuentra la cromatografía de gas completa de dos-dimensiones (CG x GC), que aunque se desarrolló en los años 80, ha sido

recientemente cuando se ha extendido el uso de esta técnica, para su utilización, por ejemplo, en el análisis de contaminantes prioritarios y emergentes en muestras de agua residual y superficial [99]

II.5.2.2. Cromatografía de líquidos

La cromatografía de líquidos de alta resolución (*HPLC – High performance liquid chromatography*) es probablemente la técnica analítica de separación más utilizada actualmente, debido a su sensibilidad, su fácil adaptación a las determinaciones cuantitativas exactas y su idoneidad para la separación de especies no volátiles o termolábiles. El tipo de cromatografía líquida más utilizada es la de reparto, en la que el analito se distribuye entre la fase móvil líquida (generalmente un disolvente o mezclas de disolventes polares) y una fase líquida (fase estacionaria generalmente apolar) inmovilizada sobre la superficie de un sólido inerte según su polaridad. Generalmente, se utiliza una elución en gradiente a alta presión, de modo que se trabaja con una mezcla de dos o tres disolventes de polaridad distinta cuya proporción varía de forma programada durante el análisis. En la figura II.6., se muestra un esquema con los componentes de un cromatógrafo de líquidos.

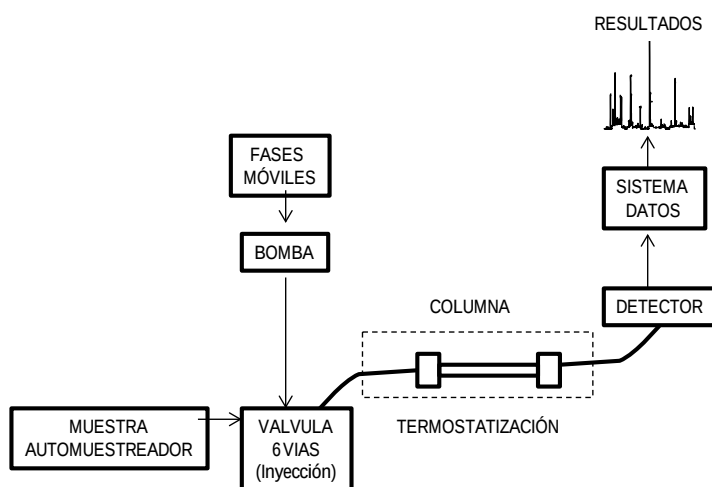


Figura II.6. Esquema de los componentes de un cromatógrafo de líquidos

El método más usado para la introducción de la muestra utiliza válvulas de 6 vías con bucles de volúmenes comprendidos entre 5 y 500 μL , que inserta ésta en la fase móvil que suele ser mezcla de disolventes que varía en función del tipo de análisis a realizar.

Los detectores más empleados en cromatografía de líquidos son ultravioleta (UV), ultravioleta visible (UV-vis) y de fluorescencia, aunque también se emplean otros como los detectores de índice de refracción diferencial, amperométricos o de conductividad. Los cromatógrafos de líquidos con detectores selectivos se han empleado para el análisis de distinto tipo de contaminantes en variadas matrices, como por ejemplo PAHs en aguas y suelos [100,101] o plaguicidas en muestras biológicas [102]. Aunque en la actualidad el acoplamiento con espectrometría de masas es la modalidad más extendida ya que ofrece potentes prestaciones en términos de número de analitos a determinar y sensibilidad, entre otros.

Hoy en día también existen una gran variedad de columnas cromatográficas para líquidos. La mayoría de ellas vienen preparadas para trabajar en fase reversa en lugar de fase normal ya que se consigue mayor versatilidad [103]. Si tenemos en cuenta el tamaño de partícula se puede hablar de HPLC si éste es igual o superior a 3,5 μm , y si contiene partículas inferiores a 2 μm , cromatografía líquida de ultra resolución (Ultra-High Performance Liquid Chromatography, UPLC). Este menor tamaño de partícula da lugar a un aumento de la resolución, con picos más estrechos e incluso puede aumentar la sensibilidad. Además se consiguen tiempos de análisis menores en comparación con los conseguidos por HPLC. La hibridación de la espectrometría de masas con UPLC se ha utilizado con éxito para desarrollar métodos para analizar contaminantes emergentes en distintos tipos de muestras de agua [104, 105, 106]. Para los compuestos polares o iónicos se ha incrementado el uso de la cromatografía líquida de interacciones hidrofílicas-lipofílicas (HILIC- Hydrophilic Interaction Liquid Chromatography) como alternativa a la fase reversa.

II.5.2.3. Espectrometría de masas como detector para cromatografía

Como ya se ha comentando tanto la GC como la LC con detectores selectivos se han empleado para el análisis medioambiental y otros tipos de muestra. Pero en la actualidad el acoplamiento de estas técnicas con la espectrometría de masas (GC-MS y LC-MS) mediante

distintas interfases, acapara gran parte de los métodos que se desarrollan para el análisis de contaminantes en muestras medioambientales (ver tabla II.8., del apartado II.5.2.4.). La espectrometría de masas es una técnica basada, previa ionización de la muestra, en la separación y registro de los iones producidos, según su relación masa-carga, en un sistema de vacío. Las ventajas que presenta la espectrometría de masas y que la convierten en técnica de referencia para el análisis de contaminantes en matrices medioambientales son las siguientes:

- ✓ Es cualitativa y cuantitativa.
- ✓ Puede analizar mezclas complejas.
- ✓ Posee gran sensibilidad.
- ✓ Es universal y específica.
- ✓ Presenta la posibilidad de determinar especies que coeluyen.
- ✓ Puede proporcionar información estructural.
- ✓ Proporciona información isotópica.
- ✓ Es una técnica rápida.

II.5.2.3.1. Interfases

El acoplamiento se produce a través de distintas interfases o fuentes de ionización, cuya elección viene determinada por el tipo de cromatografía, tipo de muestra y las características de los analitos tales como su polaridad y peso molecular, en la tabla II.6., se muestra esta información relacionada con las fuentes de ionización más comunes para su acoplamiento con MS. La idoneidad de este acoplamiento es de gran relevancia ya que una adecuada ionización de los analitos es la base para lograr un buen análisis y posterior detección de los mismos en el espectrómetro de masas.

Tabla II.6. Tipos de fuentes de ionización más comunes para acoplamiento de cromatografía con MS.

Tipo fuente	Símbolo	Muestra	Analito	Acoplamiento
Impacto electrónico	EI	Gas, líquido	Apolares y media polaridad. Bajo peso molecular	GC
Ionización química	CI	Gas, líquido	Apolares y media polaridad. Peso molecular bajo	GC
Electrospray	ESI	Líquido	Media polaridad. Peso molecular medio-alto	LC
Ionización química a presión atmosférica	APCI	Líquido, gas	Media polaridad. Peso molecular medio	LC,GC
Fotoionización a presión atmosférica	APPI	Líquido	Baja y media polaridad. Peso molecular Medio	LC
Desorción/ionización por láser asistida por una matriz	MALDI	Líquido, sólido	Baja y media polaridad. Bajo y alto peso molecular	LC

En el caso del acoplamiento GC-MS podemos encontrar las siguientes fuentes de ionización:

-Impacto electrónico (EI). La muestra llega a la fuente en forma gaseosa (o se vaporiza in situ, si se hace un análisis directo por espectrometría de masas) y allí las moléculas son ionizadas por bombardeo con un haz de electrones de elevada energía [107]. El producto primario son iones de una única carga positiva que se forman cuando los electrones de elevada energía se acercan suficientemente a las moléculas como para causarles la pérdida de electrones por repulsión electrostática (ión molecular, M^{+}). Sin embargo, la pequeña masa y la elevada energía cinética de los electrones generados en la fuente provoca pequeños aumentos en la energía cinética de las moléculas con las que chocan, de forma que éstas adquieren estados vibracionales y rotacionales excitados. La subsecuente relajación tiene lugar normalmente mediante una elevada fragmentación que da lugar a un gran número de iones positivos de diversas masas que son menores (y en ocasiones mayores) que las del ión molecular. Los complejos espectros de masas que se obtienen en la ionización por impacto de electrones son útiles para la identificación de compuestos. Existen bases de datos comerciales que contienen los espectros, obtenidos por impacto electrónico (siempre a 70 eV), de compuestos orgánicos de interés. No obstante, para cierto tipo de moléculas la fragmentación

es tan efectiva que no queda ningún ión molecular, perdiéndose por tanto la información más importante para la determinación de la masa molecular (si la molécula es desconocida).

-Ionización química (CI). El diseño de este tipo de fuente es muy similar a las de impacto electrónico, pero en este caso los electrones generados no colisionan directamente con la muestra, sino con un gas reactivo. Las moléculas gaseosas de la muestra se ionizan al colisionar con los iones producidos al bombardear con electrones un exceso de gas reactivo [107]. Los iones reactivos dan lugar a reacciones de transferencia de protones o de hidruros con las moléculas de analito, originando, respectivamente, iones moleculares protonados ($[M+H]^+$) o deprotonados ($[M-H]^-$). Normalmente se utilizan iones positivos, aunque la ionización química que origina iones negativos se utiliza ocasionalmente en aquellos analitos que contienen átomos muy electronegativos. Uno de los reactivos más comunes es el metano, aunque también se han utilizado gases como propano, isobutano y amoníaco. Cada uno de ellos produce un espectro diferente con el mismo analito, siendo en cualquier caso los espectros de ionización química más sencillos (con menor fragmentación) que los espectros de impacto de electrones.

Para el acoplamiento con LC las fuentes más utilizadas son las de presión atmosférica (API-Atmospheric Pressure Ionization). La instrumentación para todas ellas es común, excepto la sonda por donde se introduce la muestra. Se diferencian en el proceso de ionización y en las aplicaciones analíticas para las que se emplean. Las ventajas de este tipo de fuentes fueron resumidas por Voyksner [108] en cuatro aspectos:

- ✓ Permiten trabajar con volúmenes de líquido típicamente utilizados en LC
- ✓ Son apropiadas para el análisis de compuestos no volátiles, polares y térmicamente inestables
- ✓ Son sensibles, ofreciendo límites de detección comparables a los obtenidos por GC-MS
- ✓ Son robustas y relativamente fáciles de manejar

A continuación se exponen los fundamentos de las principales fuentes API como son ESI, APCI y APPI. Cada una de ellas se puede emplear para analitos con distintas características químicas y físicas como se puede ver en la figura II.7.

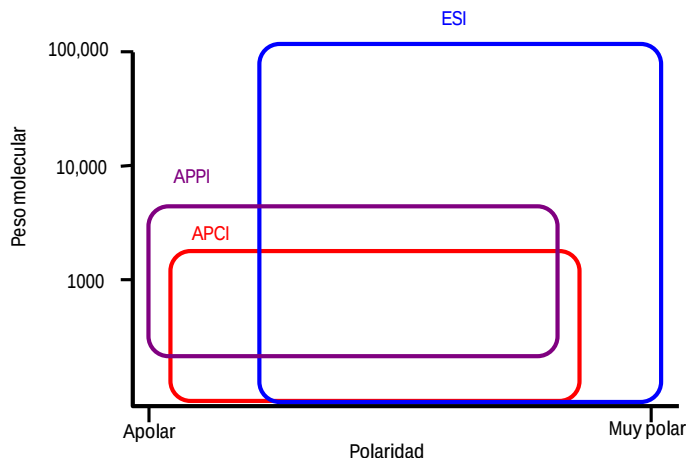


Figura II.7. Rango de aplicación de las fuentes API en función de la polaridad y el peso molecular del analito.

-Electrospray (ESI). Es la fuente de ionización más usada en LC-MS. Fue introducida en el campo de la espectrometría de masas a finales de los años 70 [109], principio de los 80 [110]. Se basa en la aplicación de un fuerte campo eléctrico a presión atmosférica a un líquido que fluye a través de un capilar metálico (aguja de nebulización) con un caudal bajo ($1 - 1000 \mu\text{L min}^{-1}$). Este campo eléctrico se genera aplicando una diferencia de potencial entre la aguja y un electrodo cilíndrico que la rodea y está separado de ella una distancia de 0.3 - 2 cm. El campo eléctrico induce una acumulación de carga en la superficie del líquido localizado al final de la aguja, de forma que éste se divide en pequeñas gotas cargadas. Un gas inyectado de forma coaxial y con un bajo caudal permite que la dispersión del spray sea limitada en el espacio. Estas gotas pasan después a través de una cortina de gas inerte (generalmente N_2) a alta temperatura para eliminar gran parte de las moléculas de disolvente, de forma que disminuye el tamaño de las gotas y aumenta la densidad de carga. Cuando la fuerza del campo en la superficie de la gota supera la energía de solvatación de los iones del analito se produce la transferencia de dichos iones a la fase gaseosa. Finalmente, el proceso se completará en el interior de un capilar que guiará a los iones del analito hacia el analizador.

La ionización se puede llevar a cabo en modo positivo o negativo. En el modo positivo, se podrán formar iones múltiplemente protonados $[M+nH]^{n+}$, donde n es el número de protones positivamente cargados en la molécula. Del mismo modo, es posible también la formación de aductos con iones de sodio, amonio, potasio, etc. En el modo negativo se observa normalmente la desprotonación de las moléculas, pudiéndose formar también iones múltiplemente desprotonados $[M-nH]^{n-}$. Esta posibilidad hace que, a diferencia de APCI, el rango de masas abaricable sea mayor.

-Ionización química a presión atmosférica (APCI) [107]. El efluente del HPLC es nebulizado y rápidamente evaporado mediante una corriente de nitrógeno coaxial en una cámara a alta temperatura (350 – 550 °C), de forma que el calor evapora el disolvente y los analitos. Aunque las elevadas temperaturas de trabajo pueden degradar los analitos, la alta velocidad de flujo del efluente del HPLC y el flujo de nitrógeno coaxial previenen la ruptura de las moléculas. Un electrodo de descarga de corona proporciona un haz de electrones que ionizan los gases de la fuente y los disolventes de la fase móvil. El plasma de iones generado será el responsable de la ionización en fase gas de los analitos. Las diferentes reacciones dependerán de la naturaleza del gas reactivo y del analito, fundamentalmente la afinidad protónica en modo positivo. En modo negativo, menos usual, el mecanismo de ionización es mediante la pérdida de un protón y/o captura de un electrón. Puesto que la formación de iones se produce en fase gas, no es apropiada para compuestos no volátiles o térmicamente lábiles. A diferencia del electrospray, APCI es una técnica que requiere trabajar con altas velocidades de flujo del efluente del HPLC (≥ 1 mL/min), ya que una baja velocidad de flujo podría generar problemas en la estabilidad del electrodo de descarga de corona. Por lo tanto, APCI es menos susceptible de miniaturización.

-Fotoionización a presión atmosférica (APPI) [111] Formalmente sería una APCI iniciada por fotoionización. El proceso de fotoionización implica la absorción de energía radiante procedente de una fuente ultravioleta donde la energía incidente es mayor que el potencial de ionización (PI) para la pérdida de un primer protón por parte de la molécula de analito. Dicho proceso de fotoionización a presión atmosférica conduce generalmente a la producción de iones moleculares (M^+), observándose escasa fragmentación. La ionización no depende de

reacciones en fase gaseosa ni de la química ácido-base, por lo que con APPI se pueden ionizar moléculas que no son ionizables por ESI o APCI o lo son con baja sensibilidad. La eficacia es relativamente pobre en algunos casos debido a la absorción de fotones por parte de los disolventes y otras especies.

II.5.2.3.2. Analizadores

Una vez que los iones han sido producidos en fase gaseosa, deben ser separados según su masa. Sin embargo, en vez de la masa, la propiedad física de los iones que miden los *analizadores de masas* es la relación masa-carga (m/z). Uno de los parámetros más característicos de los espectrómetros de masas es el poder de resolución ($R = m/\Delta m$), que se define como la habilidad de distinguir entre dos masas que difieren muy poco en su relación m/z . De forma muy general se dice que un instrumento será de alta resolución si $R > 5000$ y de baja cuando $R < 5000$. Existen varios tipos de analizadores de masas (ver tabla II.7.) [112] de manera que la separación de los iones en función de su relación masa-carga se basa en distintos principios.

Tabla II.7. Tipos de analizadores de masas

Tipo de analizador	Símbolo	Principio de separación	Llegada de los iones al detector	Resolución	Acoplamiento LC, GC
Sector eléctrico	E ó ESA	Energía cinética	Simultánea	Baja	GC
Sector magnético	B	Momento magnético	Secuencial	Baja	GC
Cuadrupolo	Q	m/z (estabilidad de la trayectoria)	Secuencial	Baja	GC, LC
Triple cuadrupolo	QqQ	m/z (frecuencia de resonancia)	Simultánea	Baja	GC, LC
Trampa de iones tridimensional	(Q)IT	m/z (frecuencia de resonancia)	Simultánea	Baja	GC, LC
Trampa de iones bidimensional o lineal	LIT	m/z (frecuencia de resonancia)	Simultánea	Baja	LC
Cuadrupolo trampa de iones lineal	QqLIT				
Tiempo de vuelo	TOF	Velocidad (tiempo de vuelo)	Simultánea	Alta	LC, GC
Cuadrupolo tiempo de vuelo	QTOF				
Resonancia de ión-ciclotrón por transformada de Fourier	FT-ICR	m/z (frecuencia de resonancia)	Simultánea	Alta	LC
Orbitrap por transformada de Fourier	FT-OT	m/z (frecuencia de resonancia)	Simultánea	Alta	LC

A parte, los modos de trabajo de cada uno de los analizadores puede variar, así instrumentos como el cuadrupolo simple o el sector magnético pueden trabajar en fullscan (FS) que es un barrido de todas las masas dentro de un rango seleccionado o en modo monitoreo de una reacción seleccionada (SIM-selected reaction monitoring) donde se filtran las masas de los compuestos deseados y el resto se envían fuera del proceso y no llegan al detector. Pero actualmente los analizadores con los que se consiguen mejores prestaciones en cuanto a sensibilidad y selectividad y son los que en general se están utilizando para la cuantificación de contaminantes orgánicos [113], son los espectrómetros de masas en tándem. Estos instrumentos fragmentan las moléculas originales de forma controlada para producir determinados iones fragmento que son los que llegan finalmente al detector. A este modo de trabajo se le conoce en general como espectrometría de masas en tándem o masas-masas (MS/MS). Se pueden hacer varias fragmentaciones consecutivas (MS^n) con analizadores como la trampa de iones o trabajar en modo monitoreo de reacciones seleccionadas (SRM-Selected Reaction Monitoring) o modo monitoreo de reacciones múltiples (MRM-multiple reaction monitoring) en el que para cada analito se van a analizar varios fragmentos (normalmente dos o tres) provenientes de la molécula original. Esto último se consigue con analizadores como el triple cuadrupolo (QqQ), el cuadrupolo tiempo de vuelo (QqTOF) o el cuadrupolo trampa de iones lineal (QqLIT). En la tabla II.8., (adaptada de [114]) se describen las características de los distintos analizadores que trabajan en MS/MS.

Tabla II.8. Comparación de las características de los analizadores en tándem

	QqQ	IT	QqLIT	QqTOF	Orbitrap
Rango de masas	√√	√√√	√√/√√	√√√	√√√√
Resolución	√	√√	√√	√√/√√√	√√√
Rango dinámico	√√√√	√√√	√√√	√√/√√√	√√√/√√√√
Precisión de masas	√	√	√	√√√√	√√√√
Coste	√√/√√	√√	√√√	√√√	√√√√/√√√√
Ventajas	·Alta selectividad y sensibilidad en modo SRM	·Sensibilidad de alta a moderada en FS ·Sensibilidad moderada en modo SIR ·(MS) ⁿ ·Tamaño compacto	·Alta sensibilidad y selectividad en modo SRM ·Sensibilidad de alta a moderada en FS · MS^3 ·Capacidad de almacenamiento de iones más	·Alta sensibilidad en FS	·Sensibilidad de alta a moderada en FS ·(MS) ⁿ ·Mayor espacio de capacidad de carga

	QqQ	IT	QqLIT	QqTOF	Orbitrap
Limitaciones	·Baja sensibilidad en modo FS	·Capacidad limitada de almacenamiento de iones	alta que QIT ·Baja precisión en la masa nominal	·Menos sensibilidad que QqQ para análisis cuantitativo	·No es completamente compatible con UPLC en alta resolución

√: bajo, √√: moderado, √√√: alto, √√√√: muy alto

A continuación, se va a hacer una descripción de los tres analizadores que se han empleado para llevar a cabo los estudios que se describen en la presente tesis, como son el sector magnético, triple cuadrupolo y tiempo de vuelo:

-Sector magnético (B). Los analizadores de sector magnético utilizan un imán permanente o un electroimán para hacer que el haz procedente de la fuente de iones se desplace con una trayectoria circular de 180, 90 ó 60 grados. Como se puede ver en la figura II.8., los iones son acelerados entre dos rendijas y salen hacia el tubo analizador metálico, que se mantiene a una presión interna alrededor de 10^{-7} torr y está sometido al campo magnético creado por un imán [107]. Se puede llevar a cabo un barrido de los iones de diferente masa a través de la rendija de salida variando la fuerza del campo del imán o el potencial de aceleración entre las rendijas de entrada (desde la fuente de iones) y salida (hacia el tubo de aceleración). Los iones que pasan a través de la rendija de salida y cruzan el tubo analizador son finalmente recogidos en un electrodo colector, dando lugar a una corriente de iones que es amplificada y registrada. Se supone que todos los iones tienen la misma energía cinética después de ser acelerados, y puesto que ésta depende de la masa, los iones más pesados se desplazan a través del sector magnético a velocidades menores.

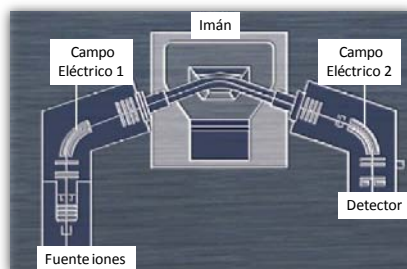


Figura II.8. Esquema de un espectrómetro con analizador de sector magnético

Los analizadores de sector magnético se denominan a menudo espectrómetros de enfoque simple. Esta terminología se utiliza porque a veces un conjunto de iones que salen de la fuente con la misma relación masa-carga tienen una distribución direccional divergente. El campo magnético es capaz de corregir estas desviaciones, haciendo converger las direcciones de los iones (“conduce los iones hacia el foco”), de forma que la energía cinética de los iones que abandonan la fuente es el factor responsable que limita en mayor medida la resolución de los instrumentos de sector magnético ($R \leq 2000$). Sin embargo, la distribución direccional de los iones no es la única limitación de este tipo de instrumentos, ya que también se puede dar una difusión de energías cinéticas que origine un ensanchamiento de haz que llega al detector, produciendo una pérdida de resolución.

Para corregir tanto las desviaciones (o aberraciones) direccionales como las de energía de los iones que dejan la fuente, se usan los espectrómetros de doble enfoque, que combinan uno o varios analizadores electrostáticos (ESA) con un analizador magnético (B). En los más simples, los iones que proceden de la fuente son acelerados a través de las rendijas y pasan a través del campo electrostático, que sirve para enfocar un haz de iones que tiene una banda estrecha de energías cinéticas sobre una rendija que conduce al campo electromagnético curvado, donde dichos iones son separados en función de su relación masa-carga. Una amplia variedad de espectrómetros de doble enfoque se encuentra disponible en el mercado, alcanzando los más sofisticados una resolución del orden de 10^5 .

-Tiempo de vuelo (TOF). Como se puede ver en el esquema de este tipo de analizador (fig. II.9.) los iones son acelerados (en la fuente de impulso o *pulser*) mediante un impulso de campo eléctrico de 10^3 a 10^4 V y una frecuencia de 10 a 50 kHz. Las partículas aceleradas pasan al tubo analizador (tubo de vuelo) de entre uno y dos metros de longitud, situado ortogonalmente a la fuente de iones y que no está sometido a ningún campo. Debido a que todos los iones que entran en el tubo idealmente tienen la misma energía cinética, sus velocidades dentro del tubo deben variar inversamente con sus masas, llegando al detector antes las partículas más ligeras que las más pesadas [107]. Los tiempos de vuelo habituales van de 1 a 100 μ s. Variaciones en la energía de los iones y en las posiciones iniciales de los mismos causan un ensanchamiento de los picos que suelen limitar la resolución obtenida.

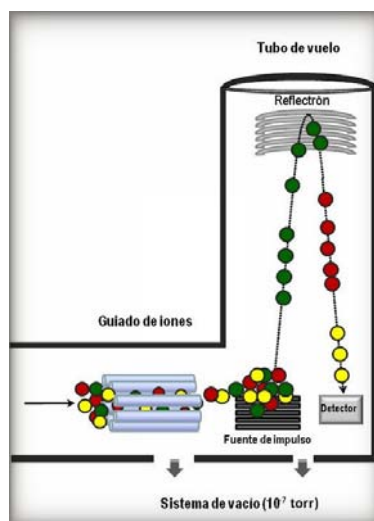


Figura II.9. Esquema de un analizador de tiempo de vuelo.

Algunas de las ventajas de este tipo de analizadores son: la sencillez, la robustez, facilidad de accesibilidad a la fuente de iones, un intervalo de masas virtualmente ilimitado y rapidez en la adquisición de datos. Otra característica interesante de los analizadores de tiempo de vuelo reside en su fácil calibración de masas (relación de tiempos de vuelo con masas) con sólo uno o dos puntos de referencia (uno o dos compuestos de masa perfectamente conocida). La resolución de masa depende del “tiempo de vuelo” y de la longitud del tubo. Para aumentar la trayectoria recorrida por los iones y, por lo tanto, su “tiempo de vuelo”, se utiliza lo que se denomina reflectrón que consiste en una serie de electrodos anulares colocados en el extremo opuesto al detector y que actúan como un “espejo” para los iones (adquisición en modo V). El reflectrón ayuda a corregir las pequeñas variaciones en la energía cinética de los iones, evitando el efecto que esto puede ocasionar en la resolución. En algunos instrumentos, para aumentar aún más la resolución, se pueden utilizar dos reflectrones (adquisición en modo W).

La principal característica de los analizadores de tiempo de vuelo es la exactitud de masa que proporcionan, de hasta cuatro cifras decimales. Esto es posible gracias a la inyección constante durante el análisis de una disolución autocalibrante compuesta por uno o dos

compuestos (dependiendo del fabricante) de masa perfectamente conocida. Recientemente se han desarrollado mejoras en el software de los instrumentos LC-MS con analizador de tiempo de vuelo que permiten la creación de bases de datos que proporcionan la identificación automática de los compuestos en base a su tiempo de retención y a su masa exacta. El acoplamiento LC-TOFMS también permite la detección de compuestos “no esperados” (*non target*) e incluso desconocidos (*unknowns*) para los cuales no se dispone de patrones analíticos a priori.

-Triple cuadrupolo (QqQ o TQ). El primer cuadrupolo (Q1) actúa como un filtro que selecciona y separa las moléculas cargadas del resto de componentes que eluyen del cromatógrafo. El tercer cuadrupolo (Q3) actúa también como filtro, pero en este caso de los fragmentos producidos por disociación que llegan del segundo cuadrupolo (Q2), dejando pasar hacia el detector solo aquellas masas de los fragmento seleccionados. El proceso de disociación que ocurre en el Q2, es inducido por un gas ionizado y acelerado, de forma que colisiona con las moléculas de analito provocando su fragmentación. Este tipo de fragmentación recibe el nombre de “disociación inducida por colisiones”. Un esquema del instrumento se puede ver en la figura II.10.

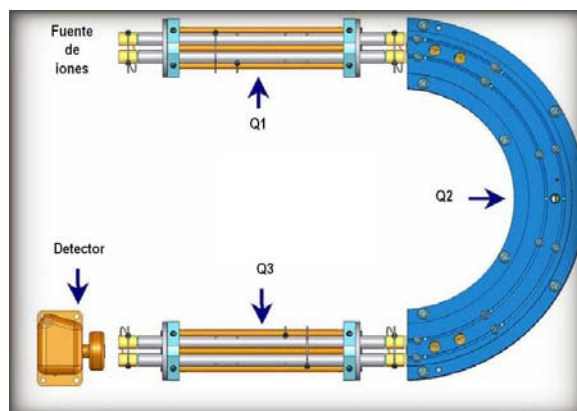


Figura II.10. Esquema de un espectrómetro de masas con analizador de triple cuadrupolo

Estos instrumentos son robustos, de relativo bajo coste, fáciles de operar y son muy selectivos, por lo que solventan de mejor forma el efecto matriz. Además, permiten monitorizar un gran número de transiciones ión precursor → ión fragmento en un único análisis. Sin embargo,

la velocidad de escaneo limita el número de transiciones que se pueden monitorizar simultáneamente con sensibilidad y precisión adecuadas. Este número de transiciones depende del instrumento (del valor de *dwell time* con el que opere el equipo). Por lo tanto, para obtener una buena resolución de los picos cromatográficos se requiere que las transiciones ión precursor → ión fragmento sean agrupadas en segmentos de tiempo (dependiendo del tiempo de retención esperado para los analitos de interés). Esto supone una desventaja a la hora de ampliar el número de compuestos que se analizan, puesto que para introducir una nueva transición en un método en uso el operador a menudo tiene que reorganizar los segmentos de tiempo establecidos. Además, si el nuevo compuesto eluye a un tiempo comprendido entre dos segmentos de tiempo, las transiciones ión precursor → ión fragmento que lo identifican deben ser incluidas en ambos segmentos. Para solventar este problema, distintos fabricantes han diseñado recientemente varios software que no requieren el establecimiento de segmentos de tiempo [115,116,117].

II.5.2.4. Aplicaciones de GC-MS y LC-MS para el análisis de contaminantes prioritarios y emergentes en muestras de aguas medioambientales

En la tabla II.9., se muestra un resumen con algunos de los métodos más representativos que se han empleado hasta el momento para el análisis multi-residuo de contaminantes prioritarios y emergentes en muestras de agua medioambiental mediante espectrometría de masas de alta resolución (HRMS), espectrometría de masas en tándem (MS/MS) y otras técnicas de detección, acopladas tanto a cromatografía de líquidos como cromatografía de gases.

Tabla II.9. Métodos para el análisis multi-residuo de contaminantes prioritarios y emergentes más representativos de los últimos años

Analitos	Tipo Agua	Tratamiento	Técnica (Interfase)	Detección	(Ref.) Año
Métodos basados en espectrometría de masas en tándem					
>50 (plaguicidas, PBDEs, PAHs, surfactantes, PCBs)	Superficial subterránea residual	SPE (C ₁₈)	GC-MS/MS (EI, CI)	QqQ	[118] 2007
46 (fenoles, fármacos, plaguicidas)	Superficial residual grifo pozo	SBSE	GC-MS/MS	LIT	[119] 2007

Analitos	Tipo Agua	Tratamiento	Técnica (Interfase)	Detección	(Ref.) Año
35 (fármacos, plaguicidas, benzotriazoles, hormonas, disruptores endocrinos, PFOS, PFOA (perfluorooctanoato))	Río	SPE (Oasis HLB)	LC-MS/MS (ESI)	QqQ	[120] 2009
11 (productos de cuidado personal)	Superficial residual	SPE (Oasis HLB) (Bond elut plexa)	LC-MS/MS (ESI)	QqQ	[121] 2009
73 (fármacos)	Ríos residual	SPE (Oasis HLB)	LC-MS/MS (ESI)	QqLIT	[122] 2009
53 (fármacos, filtros UV, retardantes de llama, pesticidas, metabolitos)	Grifo residual superficial	SPE (Oasis HLB)	LC-MS/MS (ESI)	QqQ	[123] 2009
88 (fármacos, plaguicidas, inhibidores de la corrosión, edulcorantes, metabolitos)	Subterránea superficial residual	Online SPE (mezcla Oasis HLB, Strata X-AW y Strata X-CW)	LC-MS/MS (ESI)	QqQ	[124] 2012
>200 (antibióticos, drogas, micotoxinas, plaguicidas, fármacos)	Residual	SPE (Oasis HLB)	UPLC-MS/MS (ESI)	QTOF	[125] 2012
81 (fármacos, drogas)	Bebida residual superficial marina	SPE (Oasis HLB)	UPLC-MS/MS (ESI)	QqLIT	[126] 2012
17 (fármacos, productos de cuidado personal)	Superficial residual	SPE (Oasis HLB)	UPLC-MS/MS (ESI)	QqQ	[105] 2012
55 (fármacos, metabolitos)	Residual	SPE (Oasis HLB)	UPLC-MS/MS (ESI)	QqLIT	[127] 2012
100 (fármacos, metabolitos)	Bebida residual superficial subterránea	SPE (Oasis HLB)	LC-MS/MS (ESI)	QTOF	[128] 2012
73 (fármacos)	Río	SPE (Oasis HLB)	LC-MS/MS (ESI)	QqLIT	[129] 2012
19 (fármacos y productos de cuidado personal)	Lago	SPE (Oasis HLB)	LC-MS/MS (ESI)	QqQ	[130] 2013
9 (fármacos)	Residual	US-IL-DLLME (microextracción líquida-líquida con líquidos iónicos asistida por ultrasonidos)	LC-MS/MS (ESI)	QqLIT	[131] 2013
24 (fármacos, productos de cuidado personal,	Residual subterránea superficial	Online SPE (Chromabond HR-X)	LC-MS/MS (ESI)	QqQ	[132] 2013

Analitos	Tipo Agua	Tratamiento	Técnica (Interfase)	Detección	(Ref.) Año
disruptores endocrinos, edulcorantes)					
150 (metabolitos de plaguicidas)	Subterránea superficial	Inyección directa	LC-MS/MS (ESI)	QqLIT	[133] 2013
81 (fármacos)	Residual subterránea superficial bebida	SPE (Oasis HLB)	UPLC-MS/MS (ESI)	QqLIT	[134] 2014
Métodos basados en espectrometría de masas de alta resolución (full scan)					
101 (plaguicidas)	Superficial	SPE (Seppak C18)	LC-HRMS (ESI)	TOF	[135] 2007
84 (plaguicidas, fármacos)	Residual	SPE Combinación Oasis MCX Y Strata-X	UPLC-HRMS (ESI)	TOF	[104] 2011
9 (fármacos, metabolitos)	Bebida	SPE (C ₁₈)	UPLC-HRMS	LTQ-orbitrap	[136] 2011
43 (fármacos)	Superficial residual	SPE (Oasis HLB)	LC-HRMS	Sector magnético de doble enfoque	[137] 2012
45 seleccionados + 140 sospechosos (plaguicidas y productos de transformación)	Superficial	SPE (Oasis HLB)	LC-HRMS	LTQ-orbitrap	[138] 2013
19 (antibióticos)	Residual	SPE (Oasis HLB) (Oasis MAX) (Oasis MCX)	UPLC-HRMS	TOF	[139] 2013
60 + no objetivo (plaguicidas, surfactantes, PAHs)		SPME (CW/DVB)	GC-HRMS	TOF	[140] 2014
98 + sospechosos + no objetivo (fármacos, plaguicidas, fragancias, drogas, edulcorantes, filtros UV, inhibidores de la corrosión, ácidos perfluorados, químicos industriales, productos de transformación)	Residual	SPE (Chromabond HR-X)	LC-HRMS	LTQ-orbitrap	[141] 2014
Métodos basados en la combinación de varias técnicas de detección					
66 (metales, PAHs, plaguicidas, VOCs, clorobenzenos, ftalatos, alquilfenoles)	Residual	--	GC-MS LC-MS ICP-MS LC-fluor	-- -- -- --	[142] 2008

Analitos	Tipo Agua	Tratamiento	Técnica (Interfase)	Detección	(Ref.) Año
27 (fármacos, plaguicidas, productos de cuidado personal, PBDEs, metales pesados)	Residual	SPE (Oasis HLB) LLE (n-hexano)	LC-MS/MS (ESI) GC-MS/MS (EI) GC-HRMS ICP-MS	QqLIT QqQ TOF Q	[143] 2009
170 (fármacos, productos de cuidado personal, contaminantes prioritarios)	Residual tratada y subterránea	SPE (Oasis HLB) LLE (n-hexano)	LC-MS/MS (ESI) GC-MS/MS (EI) GC-HRMS ICP-MS	QqLIT QqQ TOF Q	[144] 2010
38 (fármacos y estrógenos)	Rio	off-line SPE (Oasis HLB) on-line SPE (PLPR)	LC-MS/MS (ESI) UPLC-MS	QqQ TOF	[145] 2010
183 (fármacos, drogas de abuso, nitrosaminas, PAHs, plaguicidas, retardantes de llama, VOCs (compuestos volátiles orgánicos))	Regenerada y subterránea	LLE (n-hexano) SPE (Oasis HLB)	GC-MS/MS (EI) LC-MS (ESI)	QqQ TOF	[146] 2012
Métodos basados en la utilización de otros detectores					
109 (VOCs, plaguicidas, PAHs, PBDEs)	Superficial	SPE (Oasis)	GC-MS (EI)	Q	[147] 2000
22 (plaguicidas)	Rio	SPME	GC-MS (EI)	FID y Q	[148] 2002
35 (plaguicidas, PAHs)	Subterránea, superficial y de grifo	SBSE	GC-MS (EI)	Q	[149] 2006

La concentración de contaminantes prioritarios y emergentes encontrada en los distintos tipos de muestras de agua medioambiental normalmente se sitúa a niveles de ng L^{-1} , salvo casos puntuales en los que se alcanzan concentraciones de $\mu\text{g L}^{-1}$.

En general se puede concluir que para el análisis de contaminantes prioritarios debido a sus características físico-químicas se elige la GC-MS(MS) como técnica de análisis y detección. Por el contrario para el análisis de contaminantes emergentes que suelen ser más polares y menos volátiles se escoge la LC-MS(MS).

Hasta el momento normalmente se han desarrollado metodologías para el análisis de contaminantes por familias, aunque debido a la gran variedad de sustancias químicas presentes en el medio ambiente la tendencia va encaminada al desarrollo de métodos multi-residuo capaces de analizar compuestos con diferentes propiedades físico-químicas en un solo análisis. Esto es complicado ya que existen compuestos para los que no se encuentran materiales de referencia, como es el caso de metabolitos y productos de transformación de los contaminantes orgánicos que incluso pueden ser más nocivos que las moléculas originales. Por el momento no existe una metodología universal que se pueda aplicar para la extracción y análisis de todo tipo de contaminantes. En la actualidad se intentan salvar estos problemas con aproximaciones como la propuesta por Moschet [138] que intenta focalizar y centrar el análisis en una lista de contaminantes sospechosos de ser encontrados. Para realizar esta lista se tiene en cuenta la lista sustancias permitidas y utilizadas en la zona dónde se vaya a realizar el análisis, así como su solubilidad en agua y la posibilidad de ionización en función de su estructura química y la interfase utilizada.

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III. INSTRUMENTACIÓN Y REACTIVOS

III.1. Analitos

Los analitos seleccionados para llevar a cabo los distintos estudios que comprenden la presente tesis se seleccionaron teniendo en cuenta la lista de sustancias prioritarias de la Unión Europea [1], la lista de métodos para el monitoreo de contaminantes en agua de bebida de la Agencia para la protección del medio ambiente en EEUU (US EPA-United States Environmental Protection Agency) [2], la literatura publicada al respecto [3,4,5] y la actividad agrícola e industrial de las zonas donde se han llevado a cabo el monitoreo de las muestras de agua medioambiental.

En total son 463 contaminantes orgánicos (ver tabla III.1.) pertenecientes a diferentes categorías: fármacos, drogas de abuso, plaguicidas, nitrosaminas, perfluoroalquilos, retardantes de llama, plastificantes o PAHs y alguno de sus metabolitos más relevantes. Entre los 105 fármacos encontramos representantes de distintos grupos terapéuticos como analgésicos-antiinflamatorios (paracetamol, indomethacine, codeine, mefenamic acid, naproxen, ibuprofen, diclofenac, ketoprofen), antibióticos (metronidazole, sulfamethoxazole, trimethoprim, cefotaxime, ofloxacin, erythromycin), reguladores de lípidos (fenofibrate, bezafibrate, gemfibrozil), β -bloqueantes (atenolol, pindolol, timolol), antiepilépticos/psiquiátricos (carbamazepine, fluoxetine), antiulcerosos (ranitidine), diuréticos (furosemide, hydrochlorothiazide), y broncodilatadores (salbutamol). Debido a su relevancia y, en algunos casos, su presencia significativa un grupo de metabolitos como cotinine, clofibric acid y fenofibric acid también se incluyeron. Respecto a las 21 drogas de abuso se seleccionaron estimulantes (cocaine, ephedrine, amphetamine y compuestos pertenecientes a la familia de las anfetaminas (3,4-Methylenedioxyamphetamine -MDA-, 3,4-Methylenedioxy-N-methylamphetamine -MDMA-)), opiáceos (methadone, morphine, heroine) cannabinoides (cannabidiol, delta-9-tetrahydrocannabinol (Δ -9-THC)) y varios metabolitos como benzoylecgonine y ethylmorphine. Se seleccionaron 279 plaguicidas de distintas familias como herbicidas (prometon, terbutylazine, oxyfluorfen, anilofos) o fungicidas (imazalil, bupirimate, carbendazim) entre otros, y algunos productos de transformación y metabolitos (atrazine desethyl, desethyl terbutylazine). De la lista de sustancias prioritarias se escogieron 5 nitrosaminas, 3 PBDEs, 13 PAHs y plaguicidas como simazine, atrazine, isoproturon y hexachlorobenzene. Además se

incluyeron caffeine, nicotine y sus metabolitos theophylline, theobromine y cotinine en un grupo denominado **life-style** (5), porque son sustancias mundialmente consumidas por el ser humano. Por último debido a su toxicidad se escogieron 10 perfluoroalquilos, 2 retardantes de llama y 3 plastificantes.

Tabla III.1. Compuestos seleccionados para llevar a cabo los estudios presentados en esta tesis.

Compuestos analizados por LC-TOFMS		
279 Plaguicidas: 1-Naphtaleneacetamide 2,4-Dichlorophenoxyacetic acid 2,4-Dinitrophenol, 2,6-Dimethylaniline, 2-Hydroxybiphenyl(2-phenylphenol), 4-chloro-2-methylphenol, 4-chloro-o-tolyloxyacetic acid (MCPA), Acephate, Acetamiprid, Aclonifen*, Alachlor*, Albendazole, Aldicarb, Aldicarb sulfone, Aldicarb sulfoxide, Ametryn, Aminocarb, Amitrol, Anilofos, Asulam Atrazine*, Azinphos methyl, Azinphos-ethyl, Azoxystrobin, Benalaxyl, Benfuracarb, Bensulfuron methyl, Bensulide, Bentazon, Benzidine, Bifenazate, Bifenox, Bitertanol, Boscalid, Bromacil, Bromophos ethyl, Bromophos methyl, Bromuconazole 1, Bromuconazole 2, Bupirimate, Buprofezin, Butachlor, Butocarboxim, Butoxycarboxim, Buturon, Carbenfentazim, Carbofuran, Carbofuran 3-hydroxy, Carboxine, Carfentrazone ethyl, Chlorbromuron, Chlordimeform, Chlorfenvinfos*, Chloridazon (Pyrazon), Chloroxuron, Chlorpropham, Chlorpyrifos ethyl*, Chlorpyrifos-methyl, Chlorsulfuron, Chlortoluron, Chlothianidin, Cinosulfuron, Clomazone, Coumaphos, Cyanazine, Cycloate, Cyproconazole, Cyprodinil, Cyromazine, DEET, Deltamethrin, Desmetryn, Diafenthion, Diazinon, Dichlofenthion, Dichloran, Dichlorprop, Dichlorvos, Dicrotophos, Diethofencarb, Difenacoum, Difenoconazole, Difenoxuron, Diflubenzuron, Diflufenican, Dimethametryn, Dimethenamid, Dimethoate, Dimethomorph (E+Z), Diniconazol, Diphenylamine, Diuron*, DMST (N,N-dimethyl-N-tolylsulfonyldiamide), DNOC, Edifenphos, EPN, Eprinomectin (b1a), Ethiofencarb, Ethion, Ethiprole, Etoprofos, Etrimphos, Famphur, Fenamiphos, Fenarimol, Fenazaquin, Fenbendazol, Fenhexamid, Fenitrothion, Fenobucarb, Fenoxycarb, Fenpropidine, Fenpropimorphe, Fensulfothion, Fenthion, Fenuron, Fluazifop, Fluazifop-p-butyl, Flufenacet, Flufenoxuron, Fluomethuron, Fluquinconazole, Fluroxypyr, Flutolanil, Flutriafol, Fosthiazate, Fuberidazole, Furmecycloz, Griseofulvin, Haloxypyr, Hexaflumuron, Hexazinone, Hexythiazox, Imazalil, Imazalil metabolite, Imazamethabenz-methyl, Imazamox, Imazapyr, Imazaquin, Imidacloprid, Indoxacarb, Ioxynil, Iprodione, Irgarol (cybutryne), Isocarbophos, Isoprocarb, Isoproturon*, Isoxaben, Karbutilate, Kresoxim methyl, Lenacil, Linuron, Lufenuron, Malaoxon, Malathion, Mecarbam, Mefenacet, Mesotrion, Metalaxyl, Metamitron, Metazachlor, Methabenzthiazuron, Methamidophos, Methidathion, Methiocarb (mercaptodimethur), Methomyl, Methoxyfenozide, Metobromuron, Metolcarb, Metoxuron, Metribuzin, Mevinphos, Molinate, Monocrotophos, Monolinuron, Monuron, Myclobutanil, N,N-Diethyl-2-(1-naphthylthioxy)propanamide, Naptalam, Neburon, Nitenpyram, Norflurazon, Nuairimol, Ofurace, Omethoate, Oryzalin, Oxadiazon, Oxadixyl, Oxfendazole, Oxyfluorfen, Paclobutrazol, Parathion ethyl, Parathion-methyl, Pebulat, Penconazole, Pencycuron, Phosalone, Phosphamidon, Picloram, Picolinafen, Pirimicarb, Pirimiphos methyl, Pretilachlor, Prochloraz, Procymidone, Profenofos, Promecarb, Prometon, Prometryn, Propachlor, Propamocarb, Propanil, Propaquizafop, Propazine, Propiconazole, Propisochlor, Propoxur, Propyzamid, Proquinazid, Prosulfocarb, Prosulfuron, Pymetrozin, Pyraclostrobin, Pyranocoumarin, Pyridaben, Pyridaphenthion, Pyrimethanil, Pyriproxifen, Pyroquilon, Quinalphos, Quinmerac, Quinoclamine,		

Quinoxifen, Quizalofop-P-ethyl, Rotenone, Simazine*, Simetryn, Spinosyn A (Spinosad), Spinosyn D (Spinosad), Spiromesifen, Spiroxamine, Sulcotrione, Sulfometuron methyl, Sulfotep, Tebuconazole, Tebufenpyrad, Tebuthiuron, Teflubenzuron, Tembotrione, Temephos, Tepraloxymid, Terbutometon, Terbutylazine, Terbutryn, Tetrachlorvinfos, Thiacloprid, Thiamethoxam, Thiamphenicol, Thiocyclam (hydrogenoxalate), Thiodicarb, Triadimefon, Triadimenol, Triasulfuron, Triazophos, Triazoxid, Trichlorfon, Triclocarban, Trietazine, Trifloxystrobin, Triflumizole, Trifluriduron, Trifluralin*, Trinexapac-ethyl, Tylosin, α -Cypermethrin*, λ -Cyhalothrin, Atrazine desethyl, Terbutylazine desethyl.

21 Drogas de abuso: 11-nor-9-carboxy-THC, Acetylmorphine, Amphetamine, Bendroflumethiazide, Benzoylecgonine, Cannabidiol, Chlorothiazide, Cocaine, EDDP, Ephedrine, Ethylamphetamine, Ethylmorphine, Ketamine, MDA, MDEA, MDMA, Methadone, Methamphetamine, Morphine, Phenylephrine, Δ -9-THC.

105 fármacos: 17- α -Ethinylestradiol, 3-Methylxanthine, 4-Aminoantipyrine, 4-Androsten-3,17-dione, Ampicillin, Antipyrine, Atenolol, Atropine, Benzalkonium chloride-C10, Benzalkonium chloride-C12, Benzalkonium chloride-C14, Benzalkonium chloride-C16, Bezafibrate, Carbadox, Carbamazepine, Cefotaxime, Chloramphenicol, Chlortetracycline, Cimetidine, Ciprofloxacin, Cis-Diltiazem, Clarithromycin, Clenbuterol, Clofibrac acid, Cloxacillin, Codeine, Compactin (Mevastatin), Danofloxacin, Demeclocycline, Dexamethasone, Diclofenac, Dicloxacillin, Digoxigenin, Diphenhydramine, Doxycycline, Enalapril, Enoxacin, Enrofloxacin, Equilin, Erythromycin, Estrone, Ethoxyquin, Famotidine, Fenofibrate, Flufenamic acid, Flumequine, Fluoxetine, Furosemide, Gemfibrozil, Hydrochlorothiazide, Hydroflumethiazide, Ibuprofen, Indomethacin, Josamycin, Ketoprofen, Leucomalachite green, Lincomycin, Lomefloxacin, Malachite green, Mebendazole, Meclofenamic acid, Mefenamic acid, Metformin, Metronidazole, Miconazole, Nalidixic acid, Naproxen, Nifuroxazide, Norfloxacin, Ofloxacin, Oxacillin, Oxolinic acid, Oxytetracycline, Paracetamol (Acetaminophen), Penicillin G, Phenacetin, Phenylbutazone, Pindolol, Pipemidic acid, Pravastatin, Propranolol, Propyphenazone, Ranitidine, Roxithromycin, Salbutamol, Salicylic acid, Sarafloxacin, Spiramycin I, Sulfachloropyridazine, Sulfadiazine, Sulfadimethoxine, Sulfamerazine, Sulfamethizole, Sulfamethoxazole, Sulfanilamide, Sulfapyridine, Sulfathiazole, Testosterone, Tetracycline, Thiabendazole, Timolol, Trimethoprim, Warfarin, β -Estradiol, Estriol.

10 pefluoroalquilos: C11 Perfluoroundecanoic acid, C12 Perfluorododecanoic acid, C3 Pentafluoropropionic acid, C5 Perfluoropentanoic acid, C4 Perfluorobutyric acid, C10 Perfluorodecanoic acid, C7 Perfluoroheptanoic acid, C9 Perfluorononanoic acid, C8 Perfluorooctanoic acid, Perfluorooctanesulfonic acid* (PFOS), Heptadecafluorooctanesulfonic acid

5 Nitrosaminas: N-nitrosodiethylamine, N-nitrosodi-n-dibutylamine, N-nitrosomorpholine, N-nitrosopiperidine, N-nitrosopyrrolidine.

5 Life-style: Caffeine, Nicotine, Theophylline, Theobromine, Cotinine.

3 plastificantes: Bis(2-ethylhexyl)phthalate* (DEHP), Bisphenol A, Tris (1-chloro-2-propyl) phosphate (TCPP)

2 retardantes de llama: Triethylphosphate (TEP), tributylphosphate (TBP)

Compuestos analizados por GC-MS

50 Plaguicidas: 1,3,5-TCB*, 1,2,3-TCB*, 1,2,4-TCB*, 4,4'-DDE, 4,4'-DDT, alachlor*, aldrin, ametryn, atrazine*, atrazine desethyl, chlorfenvinphos* A, chlorfenvinphos* B, chlorotoluron, chlorpyrifos ethyl*, deltamethrin, diazinon, dieldrin, diuron*, endosulfan* sulfate, endrin, ethion, fenamiphos, fenthion, fluorene, heptachlor*, hexachloro-1,3-butadiene*, hexachlorobenzene*, iprodione, isodrin, isoproturon*, metoxychlor, oxyfluorfen, parathion ethyl, parathion methyl, pentachlorobenzene*, procymidone, prometon, propazine, simazine*, terbutylazine, terbutryn*, trifluralin*, α -cypermethrin*, α -endosulfan*, β -endosulfan*, α -HCH*, β -HCH*, γ -HCH*, δ -HCH*, λ -cyhalothrin.

13 PAHs: Acenaphthylene, Anthracene*, Benzo[a]anthracene, Benzo[a]pyrene*, Benzo[b]fluoranthene*, Benzo[ghi]perylene*, Benzo[k]fluoranthene*, Chrysene, Dibenzo[a,h]anthracene, Fluorene, Indeno[1,2,3-cd]pyrene*, Phenanthrene, Pyrene.

3 PBDEs: tetra-brominated diphenyl ether, penta-brominated diphenyl ether, hepta-brominated diphenyl ether

* Compuestos prioritarios

Los patrones de los compuestos que aparecen en la tabla III.1., fueron adquiridos a Sigma-Aldrich (Steinheim, Alemania) y Dr. Ehrenstofer (Augsburg, Alemania). El Acetato de etilo y el n-hexano de Riedel-de H  en (Seelze, Alemania), el metanol y el acetonitrilo por Sigma-Aldrich (Steinheim, Alemania) y el   cido f  rmico de Fluka (Madrid, Espa  a). El sulfato s  dico anhidro y cloruro de s  dico eran de J.T. Baker (Deventer, Holanda) y el   cido sulf  rico de Panreac (Castellar del Valles, Espa  a). Todos ellos se adquirieron con la adecuada pureza. Se prepararon disoluciones individuales de cada uno a una concentraci  n de 1000 $\mu\text{g mL}^{-1}$ utilizando como disolventes metanol y mezclas de metanol con acetonitrilo para los compuestos analizados por LC-TOFMS. Para los analitos analizados por GC-MS/MS se prepararon disoluciones individuales en n-hexano, acetato de etilo o mezclas de ambos a una concentraci  n de 1000 $\mu\text{g mL}^{-1}$. Se prepararon disoluciones de trabajo que conten  an grupos de analitos mediante la mezcla y diluci  n apropiada de las disoluciones individuales previamente preparadas, para lo que se utiliz   metanol y n-hexano seg  n el posterior an  lisis por LC y GC respectivamente.

III.2. Preparación de muestra

Para los analitos menos apolares y de carácter más volátil (compuestos GC) se realizaron tres procedimientos de extracción distintos como son LLE, SPE y HS-SPME, previos a su análisis por GC-MS(MS).

-LLE. Las muestras sin filtrar se acidifican con H_2SO_4 1M hasta pH 3 y se les añaden 250 mg de NaCl. Una alícuota de 200 ml de agua se carga en un embudo de decantación de 250 ml, se añaden 25 ml de n-hexano y se agita vigorosamente durante 3 minutos. Se separan las fases y la acuosa se vuelve a extraer con 25 ml de n-hexano otras 2 veces más. Se juntan las fases orgánicas y se secan con Na_2SO_4 anhidro. A continuación el extracto se evapora hasta casi sequedad en un rotavapor (Büchi Rotavapor R200) equipado con un baño de agua (Büchi B-490) y un controlador del vacío (Büchi V800). Finalmente el residuo se reconstituye con n-hexano hasta un volumen final de 1 ml (preconcentración 200:1). En la figura III.1., se muestra un esquema del proceso.



Figura III.1. Esquema del procedimiento de extracción líquido-líquido previo análisis del multiresiduo de sustancias prioritarias por GC-MS(MS).

-SPE. La muestra filtrada se somete al proceso de extracción con cartuchos Bond Elut C18 (6mL, 500 mg). El primer paso de la extracción es el acondicionamiento de la fase sólida dejando pasar a vacío primero 6 mL de MeOH, después 6 mL de una mezcla AcEt-diclorometano (1:1), posteriormente se vuelven a pasar 6 mL de MeOH y finalmente 6 mL de agua ultrapura. A continuación se cargan 200 mL de muestra una velocidad de 3-5 mL min⁻¹. Una vez que pasa todo el volumen de muestra, se lavan los cartuchos con 3 mL de agua

ultrapura y se secan a vacío durante 5 minutos. Seguidamente, se eluyen los compuestos retenidos en la fase sólida con dos alícuotas de 4 mL de la mezcla AcEt-diclorometano (1:1 y a continuación, se evapora el disolvente hasta casi sequedad, utilizando un Turbo Vap LV de Zymark (Hopkinton, MA), que utiliza una corriente de nitrógeno a una presión de 5 psi junto con un baño de agua a 27 °C. El extracto final se reconstituye con n-hexano hasta un volumen final de 1 mL, por lo que la preconcentración que se consigue es de 200:1. A continuación se muestra la figura III.2., con el esquema del procedimiento.

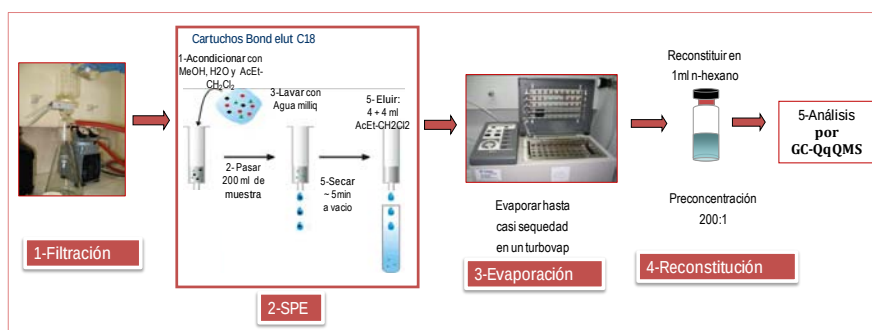


Figura III.2. Esquema del procedimiento de extracción por fase sólida previo al análisis del multiresiduo de sustancias prioritarias por GC-QqQ-MS.

-HS-SPME. En un vial de vidrio de espacio de cabeza de 20 mL se introducen 10 mL de muestra al 10% de MeOH, 1 g de NaCl y un imán para agitarla. A continuación se calienta la muestra hasta 80 °C (temperatura de incubación) y se introduce la fibra (Poliacrilato) en el vial de forma automática, dejándose transcurrir 50 minutos (tiempo de incubación) para favorecer la transferencia y adsorción de los analitos en la fibra. En estas condiciones, la cantidad de cada compuesto en el volumen de espacio de cabeza es proporcional a su concentración en la matriz. Pasado ese tiempo de incubación, la fibra se inserta en el inyector del cromatógrafo de gases donde los analitos son desorbidos a una temperatura de 260 °C. En la figura III.3., se muestra un esquema del proceso de extracción con la fibra polimérica.

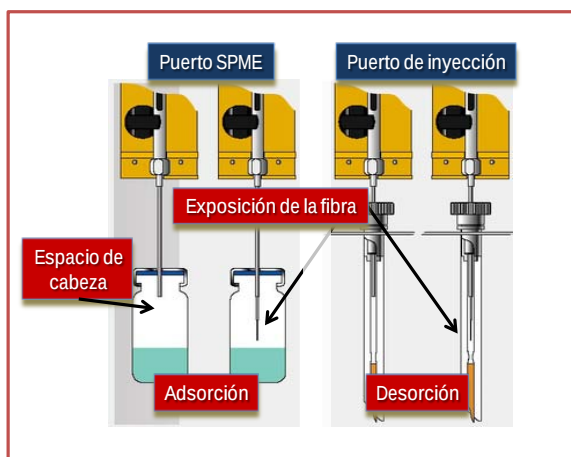


Figura III.3. Proceso de adsorción y desorción de los analitos por microextracción en fase sólida

En el caso de los analitos más polares y menos volátiles se llevó a cabo una SPE, previa al análisis por LC-TOFMS.

-SPE. Las muestras de agua se filtran en un equipo de filtración a vacío para evitar que los cartuchos de extracción se saturen. A continuación se someten a un proceso de extracción por fase sólida (SPE) utilizando cartuchos Oasis HLB (6mg). El primer paso de la extracción es el acondicionamiento de la fase sólida dejando pasar a vacío primero 4 mL de MeOH y después 4 mL de agua ultrapura; a continuación se pasan los 200 mL de muestra una velocidad de 3-5 mL min⁻¹. Una vez que pasa todo el volumen de muestra, se lavan los cartuchos con 4 mL de agua ultrapura y se secan a vacío durante 5 minutos. Seguidamente, se eluyen los compuestos retenidos en la fase sólida con dos alícuotas de 4 mL de MeOH cada una. A continuación, se evapora el disolvente hasta casi sequedad, utilizando un Turbo Vap LV, que utiliza una corriente de Nitrógeno a una presión de 15 psi junto con un baño de agua a 37 °C. El extracto final se reconstituye con una mezcla agua-metanol (90:10 v/v), hasta un volumen final de 1 mL, por lo que la preconcentración que se consigue es de 200:1. En la figura III.4., se puede observar el esquema de este procedimiento de extracción

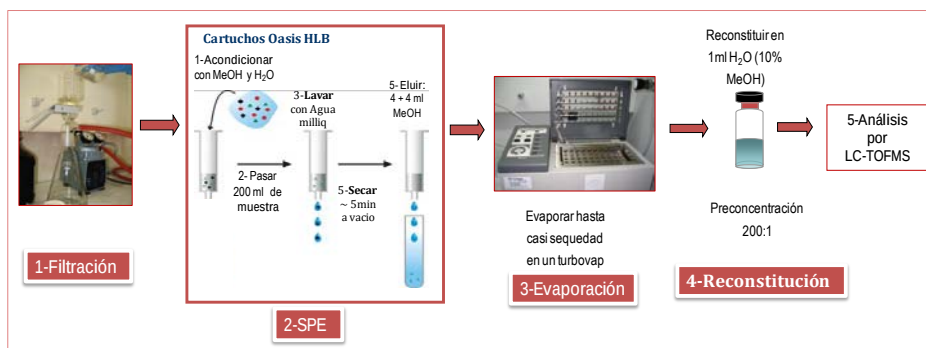


Figura III.4. Esquema del procedimiento de extracción por fase sólida previo al análisis del multiresiduo de sustancias prioritarias y emergentes por LC-TOFMS

III.3. Análisis por cromatografía-espectrometría de masas

III.3.1. Análisis por GC-HRMS

El análisis por GC-HRMS se llevó a cabo con un cromatógrafo de gases HP 6890 Series (Hewlett-Packard, Palo Alto, CA, USA) acoplado a un espectrómetro de masas de sector magnético de doble enfoque de alta resolución (Micromass AutoSpec NT (Micromass, Manchester, UK)) como se muestra en la figura III.5., operando en modo registro de iones seleccionados en alta resolución (HR SIR) utilizando los siguientes parámetros experimentales: modo de ionización por impacto electrónico (70 eV de energía); ion repeller (V) = -134 V; detector: 300 V. Para realizar la calibración se utilizó Perfluorokerosene. La adquisición de datos, así como el procesado y el control del instrumento se llevaron a cabo con el MassLynx 4.1 (Micromass, Manchester, UK). El cromatógrafo iba equipado con una columna capilar ZB-5MS (5% diphenyl/95% dimethylsiloxane), con unas dimensiones de 30m × 0.25 mm (d.i.) × 0.25 (grosor película) (Phenomenex, Torrance, CA, USA). El inyector del cromatógrafo trabajó durante la inyección en modo sin división de flujo (splitless) y la condiciones experimentales del mismo fueron: volumen de inyección de 1 µL; temperatura 250 °C; pulso inicial de presión: 30 psi (1.5 min). Se utilizó He (99.9999% de pureza) como gas portador a un flujo constante de 1 mL min⁻¹. El programa de temperatura en el horno cromatográfico fue como sigue: inicialmente se mantiene 2.0 minutos a 70 °C, se aumenta la temperatura hasta 180 °C a una velocidad de 10 °C min⁻¹ (se mantiene así durante 5 minutos), posteriormente se aumenta la temperatura

hasta 260 °C a una velocidad de 6 °C min⁻¹ y finalmente se alcanza los 300 °C utilizando una velocidad de 4 °C min⁻¹.



Figura III.5. Equipo de cromatografía de gases/espectrometría de masas con analizador de sector magnético de alta resolución (GC-HRMS) utilizado para el análisis de contaminantes prioritarios (artículo científico 1). Centro de instrumentación científico-técnica, Universidad de Jaén.

III.3.2. Análisis por GC-QqQ-MS

El equipo utilizado (ver figura III.6.) fue un Cromatógrafo de gases CP-3800 (Varian Inc. Walnut Creek, California, EEUU), equipado con control de flujo electrónico (EFC), un inyector capilar universal 1079 en el que se utiliza un liner fritado (Restek) y un automuestreador (Combipal autosampler, CTC Analytics). La temperatura del inyector se mantiene durante 2 minutos a 280 °C, después sube hasta 325 °C a una velocidad de 40 °C min⁻¹, manteniéndose en este valor durante 10 minutos. La relación de Split inicial es 50:1 hasta los 0.01 minutos; alcanzado este valor se cierra el Split durante 2 minutos para asegurar la transferencia de la muestra al interior de la columna y después se vuelve a abrir con un valor de 100:1 durante 13 minutos, para reducirse finalmente a 20:1. La separación de los analitos se lleva a cabo utilizando una columna capilar Varian FactorFour VF-5-ms (30m x 0.25mm i.d. x 0.25 µm) y He como gas portador a un flujo de 1.0 mL min⁻¹. La rampa de temperatura utilizada

comienza a 70 °C durante 2 minutos, después sube hasta 180 °C a una velocidad de 10 °C min⁻¹, a continuación sube hasta los 260 °C a 6 °C min⁻¹ y por último se alcanzan 300 °C a una velocidad de 4 °C min⁻¹, para un tiempo total de análisis de 43 minutos. El cromatógrafo de gases está acoplado a un espectrómetro de masas de triple cuadrupolo modelo 300-MS (Varian Inc. Walnut Creek, California, EEUU) equipado con una fuente de ionización que opera en modo de impacto electrónico (EI, 70eV) con una corriente de filamento de 50 µA y el voltaje del multiplicador se mantiene en máximo EDR. Las temperaturas de la línea de transferencia, fuente de iones y manifold son 280, 250 y 40 °C, respectivamente. Se establece un tiempo de retardo para el encendido del detector de 6.4 minutos para evitar daños en la instrumentación. El espectrómetro de masas se autocalibra cuando sea necesario con Perfluorotributylamine (PFTBA). Se utiliza Ar como gas de colisión. El modo de trabajo del espectrómetro es MRM (Multiple Reaction Monitoring) para lo que se hizo una optimización previa de las condiciones necesarias para el correcto análisis de cada analito (ver apartado IV.2.2. tabla 1). Para el control del instrumento, adquisición y evaluación de los datos se utilizó el software Varian MS Workstation (versión 6.9).



Figura III.6. Equipo de cromatografía de gases/espectrometría de masas con analizador de triple cuadrupolo (GC-QqQ-MS) utilizado para el análisis de contaminantes prioritarios (artículos científicos 2 y 3). Grupo de investigación de Química Analítica (FQM-323), Universidad de Jaén.

De los extractos procedentes de LLE y SPE se inyectaron 4 μL , mientras que los analitos procedentes del proceso HS-SPME fueron desorbidos por acción de la temperatura en el inyector del cromatógrafo. Las condiciones experimentales de GC-QqQMS para el análisis de los compuestos menos polares y volátiles para los tres métodos de extracción fueron las mismas. Lo único que hay que hacer es una pequeña modificación (cambiar el liner) para poder inyectar la fibra de SPME en el cromatógrafo.

III.3.3. Análisis por LC-TOFMS

El equipo utilizado (ver figura III.7.) es un Cromatógrafo de líquidos de alta eficacia (HPLC, Agilent Infinity 1290, Agilent Technologies, Santa Clara, California, EEUU) equipado con una columna analítica de fase reversa (4.6 mm $\times$ 50 mm and 1.8 μm). Las fases móviles empleadas fueron agua con un 0.1% de ácido fórmico (A) y acetonitrilo (B). El método cromatográfico mantiene la composición inicial de la fase móvil (10%B) durante 3 minutos, seguido de un gradiente lineal hasta el 100%B conseguido a los 25 minutos, por último se mantiene en estas condiciones 3 minutos más, para obtener un tiempo total de análisis de 28 minutos. Por último se incluyen una etapa de equilibrado con las condiciones iniciales (10%B) durante 10 minutos. El cromatógrafo de líquidos se acoplaba a un espectrómetro de masa de tiempo de vuelo (Agilent 6220 accurate mass TOF, Agilent Technologies, Santa Clara, California, EEUU) equipado con una fuente de electrospray (ESI) trabajando en modo positivo o negativo según se requiera. Los parámetros seleccionados para el espectrómetro son: voltaje del capilar, 4000 V; presión del nebulizador, 40 psig; tasa de flujo del gas de secado, 9.0 $\text{dm}^3 \text{min}^{-1}$; temperatura del gas, 325 $^{\circ}\text{C}$; voltaje del skimmer, 65 V; octapolo 1 rf, 250 V; voltaje del fragmentador, 190 V. El espectro de masa exacta se recoge en el rango de 50–1000 m/z en modo positivo y 50–1100 m/z en modo negativo. El instrumento lleva a cabo una calibración interna de masas de forma automática introduciendo la disolución de referencia que contiene TFANH_4 (ammonium trifluoroacetate, $\text{C}_2\text{O}_2\text{F}_3\text{NH}_4$, con m/z 112.985587 para modo negativo), purine ($\text{C}_5\text{H}_4\text{N}_4$, con m/z 121.050873, para modo positivo) y HP-0921 [hexakis-(1*H*,1*H*,3*H*-tetrafluoropropoxy)phosphazine, $\text{C}_{18}\text{H}_{18}\text{O}_6\text{N}_3\text{P}_3\text{F}_{24}$, con m/z 922.009798 para modo positivo y 1033.988109 para modo negativo]. El equipo proporciona una resolución típica mejor de 10000 (m/z 118) y mejor de 18000 (m/z 1522). Los datos obtenidos del full scan se registran con el

software Agilent Mass Hunter Data Acquisition (versión B.04.00) y se procesan con el software Agilent Mass Hunter Qualitative Analysis (versión B.04.00). Para realizar el análisis bajo estas condiciones se inyectan 20 μL del extracto de muestra obtenido por SPE.



Figura III.7. Equipo de cromatografía de líquidos/espectrometría de masas con analizador de tiempo de vuelo (GC-TOF-MS) utilizado para el análisis de contaminantes emergentes (artículos científicos 4 y 5). Grupo de investigación de Química Analítica (FQM-323), Universidad de Jaén.

III.4. Referencias

- [1] Directiva 2013/60/CEE del Parlamento Europeo y del Consejo, de 12 de agosto de 2013, relativa a las sustancias peligrosas en el marco de la política de aguas y que modifica las Directivas 2000/60/CEE y 2008/105/CEE.
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A faint, artistic watercolor map of Europe is positioned diagonally across the page. The map uses soft, blended colors including shades of pink, red, teal, and grey to represent different landmasses and regions. It is centered behind a dark blue horizontal band.

IV. RESULTADOS Y DISCUSIÓN

*IV.1. DETERMINACIÓN DE CONTAMINANTES ORGÁNICOS
PRIORITARIOS EN EFLUENTES DE ESTACIONES DEPURADORAS
DE AGUA RESIDUAL POR CROMATOGRAFÍA DE GASES-
ESPECTROMETRÍA DE MASAS DE ALTA RESOLUCIÓN*

V.1.1. Introducción

En las últimas décadas la preocupación por la contaminación tanto natural como antropogénica sobre el medioambiente se ha acrecentado y es un problema que no solo atañe a las autoridades políticas sino también a los ciudadanos, cada vez más preocupados por los posibles efectos nocivos de su exposición a los contaminantes presentes en el medioambiente. Por este motivo, la Comunidad Europea estableció en el año 2000 un marco comunitario de actuación en materia de política de aguas a través de su Directiva 2000/60/CE. La Directiva marco del agua responde a una petición formulada en junio de 1995 por la Comisión de Medio Ambiente del Parlamento Europeo y por el Consejo, para que se llevase a cabo una revisión a fondo de las políticas de aguas de la Comunidad. La pretensión de la Directiva es mejorar la legislación anterior creando un marco legislativo e intentando garantizar la coordinación y la adaptación de las estructuras y los principios generales de protección y usos sostenible de los recursos hídricos de la Unión Europea.

“El agua no es un bien comercial como los demás, sino un patrimonio que hay que proteger, defender y tratar como tal”

Bajo este marco legislativo se organiza la gestión de las aguas superficiales, continentales, de transición, aguas costeras y subterráneas, con el fin de prevenir y reducir su contaminación, fomentar su uso sostenible, proteger el medio acuático, mejorar la situación de los ecosistemas acuáticos y paliar los efectos de las inundaciones y de las sequías. Para ello, entre otras medidas, se estableció una lista de 33 sustancias denominadas prioritarias mediante la Decisión número 2455/2001/CE del parlamento europeo y del consejo de 20 de Noviembre de 2001, cuya contaminación debe ser progresivamente reducida o eliminada con el fin de alcanzar un “buen estado” ecológico de las aguas comunitarias a finales de 2015.

Desde la entrada en vigor de esta directiva, han sido numerosos los trabajos científicos que han tratado la evaluación de la contaminación por sustancias prioritarias en diferentes tipos de muestras medioambientales. Para su determinación se necesitan metodologías capaces de detectar de forma inequívoca la presencia de este tipo de contaminantes a los niveles de concentración tan bajos como los exigidos por la normativa. La cromatografía de gases (GC) ha

sido la técnica de elección para el análisis de analitos apolares y relativamente volátiles debido a su alta selectividad, precisión y sensibilidad, especialmente cuando se combina con la espectrometría de masas (GC-MS) como técnica de detección (Santos 2002, Santos 2003). De manera que, debido a sus ventajas, la GC-MS se ha utilizado en un número elevado de publicaciones para el análisis de contaminantes prioritarios en aguas (León 2003, Wang 2003, Cai 2004, Auesperger 2005, Tahboub 2005, Hsieh 2006, León 2006) utilizando normalmente analizadores de cuadrupolo simple.

Pero en el caso de muestras complejas como son las aguas residuales se necesita un paso más en las prestaciones de la instrumentación para llevar a cabo el análisis de forma adecuada. En este sentido espectrómetros con analizadores que permiten realizar análisis masas/masas (MS/MS), como la trampa de iones (Gonçalves 2004) o el triple cuadrupolo (Pitarch 2007) logran una mejora considerable tanto en la sensibilidad como en la selectividad (menor efecto matriz) con respecto a los espectrómetros de cuadrupolo simple. Similares prestaciones se pueden conseguir también con equipos de alta resolución como el analizador de tiempo de vuelo (Hernández 2007, Portolés 2007), logrando la cuantificación e identificación inequívoca de los analitos estudiados de acuerdo con el criterio de los puntos de identificación de la normativa europea (Decisión de la Comisión 2002/657/EC). Dentro de los analizadores de alta resolución se encuentra el doble sector magnético, pero hasta la fecha no tenemos constancia de que se haya explotado su potencial para el análisis de contaminantes orgánicos en muestras de agua residual. La desventaja que presenta este analizador es que el número de analitos que puede analizar es limitado y se ve reducido porque el tiempo que debe dedicar al análisis de cada masa (dwell time) para obtener un buen pico cromatográfico (al menos 10 puntos) es relativamente elevado en comparación por ejemplo con los analizadores de triple cuadrupolo que presentan una velocidad de scan de 2 a 3 veces mayor.

En el estudio analítico descrito en el artículo científico 1 se presenta por primera vez el desarrollo de una metodología analítica para la determinación de 14 sustancias prioritarias en efluentes de agua residual, utilizando para ello un equipo de cromatografía de gases acoplado a un espectrómetro de masas de alta resolución (GC-HRMS). Las 14 sustancias prioritarias seleccionadas fueron las siguientes: 3 isómeros del triclorobenceno (TCB), pentaclorobenceno

hexacloro-1,3-butadieno, hexaclorobenzeno, 4 isómeros del hexaclorociclohexano (HCH), alaclor y difenil éteres polibrominados (PBDEs). La metodología propuesta comprendía un tratamiento de muestra basado en extracción líquido-líquido previo a su análisis, identificación y cuantificación por GC-HRMS en su modo de trabajo de monitoreo de iones seleccionados en alta resolución. El método se aplicó para realizar la monitorización de los contaminantes prioritarios escogidos, en muestras de efluentes de agua residual procedentes de 5 estaciones depuradoras de agua residual (EDARs) españolas que recibían distintos tipos de influentes (urbanos, industriales, costeros, etc.).

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IV.1.2. Artículo científico 1

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Determination of organic priority pollutants in sewage treatment plant effluents by gas chromatography high-resolution mass spectrometry

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ABSTRACT

In this work, we report the development and validation of an analytical method for the trace level determination of 14 selected (EU-directive) priority organic pollutants (namely, 1,2,3-trichlorobenzene (1,2,3-TCB), 1,2,4-trichlorobenzene, 1,3,5-trichlorobenzene, hexachloro-1,3-butadiene, pentachlorobenzene, hexachlorobenzene, alachlor, α -hexachloro-cyclohexane (α -HCH), β -HCH, γ -HCH (lindane), δ -HCH, tetra-brominated diphenyl ether (tetra-BDE), penta-brominated diphenyl ether and hepta-brominated diphenyl ether) in wastewater samples from 5 different sewage treatment plants (STPs) located in Spain. The proposed methodology is based on liquid–liquid extraction with *n*-hexane followed by identification and confirmation of the selected pollutants by gas chromatography high-resolution mass spectrometry in selected ion recording acquisition mode. Recovery studies performed with spiked wastewater samples at two different concentration levels (0.1 and 1 $\mu\text{g L}^{-1}$) gave mean recoveries in the range 80–120% (except for trichlorobenzenes, ca. with 50%) with RSD values below 10% in most cases, thus confirming the usefulness of the proposed methodology for the analyses of this kind of complex samples. The obtained detection limits in effluent wastewater matrices were in the low nanogram per liter range, with values as low as 0.09 ng L^{-1} for tetra-BDE and 0.3 ng L^{-1} for hexachlorobenzene. Finally, the proposed methodology was successfully applied to a monitoring study intended to characterize wastewater effluents of 5 different sewage treatment plants with different major activities (Industrial, Coastal, Urban). Most of the compounds targeted were detected in the ng L^{-1} range at concentrations ranging from 0.19 ng L^{-1} to 135 ng L^{-1} (hexachlorobenzene).

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Abstract

In this work, we report the development and validation of an analytical method for the trace level determination of 14 selected (EU-directive) priority organic pollutants (namely, 1,2,3-trichlorobenzene (1,2,3-TCB), 1,2,4-trichlorobenzene, 1,3,5-trichlorobenzene, hexachloro-1,3-butadiene, pentachlorobenzene, hexachlorobenzene, alachlor, α -hexachloro-cyclohexane (α -HCH), β -HCH, γ -HCH (lindane), δ -HCH, tetra-brominated diphenyl ether (tetra-BDE), penta-brominated diphenyl ether and hepta-brominated diphenyl ether) in wastewater samples from 5 different sewage treatment plants (STPs) located in Spain. The proposed methodology is based on liquid-liquid extraction with n-hexane followed by identification and confirmation of the selected pollutants by gas chromatography high-resolution mass spectrometry in selected ion recording acquisition mode. Recovery studies performed with spiked wastewater samples at two different concentration levels (0.1 and 1 $\mu\text{g L}^{-1}$) gave mean recoveries in the range 80-120 % (except for trichlorobenzenes, ca. with 50 %) with RSD values below 10 % in most cases, thus confirming the usefulness of the proposed methodology for the analyses of this kind of complex samples. The obtained detection limits in effluent wastewater matrices were in the low nanogram per litre range, with values as low as 0.09 ng L^{-1} for tetra-BDE and 0.3 ng L^{-1} for hexachlorobenzene. Finally, the proposed methodology was successfully applied to a monitoring study intended to characterize wastewater effluents of 5 different sewage treatment plants with different major activities (Industrial, Coastal, Urban). Most of the compounds targeted were detected in the ng L^{-1} range at concentrations ranging from 0.19 ng L^{-1} to 135 ng L^{-1} (hexachlorobenzene).

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1. Introduction

The contamination of the aquatic environment by organic pollutants is of paramount concern for citizens and environmental authorities. The main tool of the European water policy to reduce chemical pollution of surface water is the water framework directive (WFD) 2000/60/EC [1,2], which establishes guidelines against pollution of water by sorting out a list of priority substances that involves a significant risk to or via the aquatic systems. In this context, urban wastewaters may have a strong contaminating effect on the natural aquatic systems. With the aim of improving the quality of the effluents by removal of potentially hazardous substances, sewage treatment plants include various stages of wastewater treatments. However, several studies have demonstrated that many organic compounds such as persistent organic pollutants, pharmaceuticals, personal care products, hormones and other disrupting compounds escape treatments becoming ubiquitous in the environment [3-9]. This impact can be reduced with enhanced wastewater treatment technologies using advanced oxidation processes [10,11]. The development of these thorough wastewater treatment technologies combined with comprehensive quality control strategies comprising chemical and microbiological markers is required in order to fully characterize these systems, with the late goal of possible wastewater reuse of the effluents for selected applications such as agriculture, to apply in regions or countries with water shortage.

For a detailed chemical characterization related to the presence and amount of priority pollutants, analytical methodologies should be able to both confirm the presence and accurately determine low levels of any pollutant detected. Gas chromatography (GC) has been the technique of choice for the analysis of non polar and relatively volatile substances in environmental samples, because of its high selectivity, precision and sensitivity, particularly when combined with mass spectrometry (GC-MS) as detection technique [12,13]. GC-MS is a powerful tool for the identification and quantitation of organic compounds in complex samples. Several methods have been developed for the analyses of organic contaminants in water samples based on GC-MS [14-23], typically using single quadrupole analyzer in selected ion monitoring acquisition mode.

Due to the high complexity of wastewater samples in comparison to surface waters a particularly sensitive and selective detection methods are required [24,25]. This extra selectivity can be provided during the detection step using GC-MS instruments operating in MS/MS acquisition mode using an ion trap [26] or a triple quadrupole instrument [27], or with high resolution capabilities [28,29]. In both cases, excellent selectivity and sensitivity is achieved enabling the simultaneous unambiguous confirmation (according to EU guidelines [30]) and quantitation of the targeted analytes.

Double focusing magnetic sector mass spectrometers are most often used for applications where high resolution and sensitivity are the primary requirements. As far as we know, it has not been explored in wastewater analysis, where the reliable quantification and confirmation of many organic pollutants is required at very low concentration levels, according to the present and future environmental regulations. The high resolution enables many chemical background masses to be eliminated and consequently allows a lower level of detection for a number of specific target compounds to be achieved. The highest sensitivity can be achieved by performing selected ion recording as this provides a better duty cycle than scanning. In the present work, an analytical methodology for the determination of 14 selected priority organic pollutants in effluent wastewater samples using gas chromatography high resolution mass spectrometry (GC-HRMS) is reported for the first time. The 14 EU priority substances included were: trichlorobenzenes isomers, pentachlorobenzene, hexachloro-1,3-butadiene, hexachlorobenzene, HCH isomers, alachlor and polybrominated diphenyl ethers (PBDEs). The proposed methodology comprised a sample treatment step based on liquid-liquid extraction followed by identification and quantitation of the target species by GC-HRMS in high resolution selected ion monitoring mode. The proposed method was applied to monitor the selected priority pollutants in different effluent wastewater samples from 5 Spanish sewage treatment plants with different profiles in terms of main activity (urban, industrial, coastal, etc).

2. Experimental section

2.1. Chemicals and Reagents

1,2,3-trichlorobenzene, 1,2,4-trichlorobenzene, 1,3,5-trichlorobenzene, hexachloro-1,3-butadiene, pentachlorobenzene, hexachlorobenzene, alachlor and mixture (1:1:1:1) of hexachloro-cyclohexane isomers (α -hexachloro-cyclohexane α -(HCH), β -HCH, γ -HCH (lindane), δ -HCH), were purchased from Riedel-de-Haën (Seelze, Germany), PESTANAL™ quality. Polybrominated diphenyl ether (PBDEs) mixture (tetra-brominated diphenyl ether (tetra-BDE), penta-brominated diphenyl ether (penta-BDE) and hepta-brominated diphenyl ether (hepta-BDE)) was obtained from Dr. Ehrenstorfer (Ausborg, Germany). Individual stock standard solutions of the target compounds were prepared in methanol (HPLC-grade, Merck, Darmstadt, Germany) at a concentration of 1 mg mL⁻¹ and stored at -20 °C. Working solutions, at different concentrations, were prepared by appropriate dilution of the stock solutions in n-hexane. n-Hexane was obtained from Riedel-de-Haën (Seelze, Germany), PESTANAL™ quality. Sodium chloride and anhydrous sodium sulfate (pesticide residue analysis quality) were ordered to J.T. Baker (Phillipsburg, NJ, USA). Sulphuric acid was purchased from Panreac (Barcelona, Spain).

2.2. Wastewater sampling

Wastewater samples used in this study were collected from 5 municipal sewage treatment plants (STPs) (Figure 1). Two were located in North Spain (Castro Urdiales and Santander (Cantabria)), both mainly with urban activity), two located in the centre (both in Alcalá de Henares, Madrid; one STP mainly with urban activity, and the other mainly with industrial activity), and one located in the southeast of Spain (El Ejido, Almería). This later plant treats urban wastewater with important contributions from greenhouses, plastic industry also related to greenhouses, and from a local hospital. Input wastewater undergoes a physical pre-treatment to remove coarse solids and greases, primary settling of particulates, and secondary treatment with activated sludge, after which the water is discharged to the sea. They are representative of different activities (urban, agricultural, and industrial). All plants apply a pretreatment for solid removal, a primary treatment to eliminate suspended material, an activated sludge biological treatment, and a final clarification. Composite samples representative of 1-day work in the STP were taken. They were taken at 3-h intervals. Sampling was carried out by an automatic device

(0.5 L/3 h). Effluent samples were collected by using pre-rinsed amber glass bottles and sent to the laboratory for sample treatment and for analysis. All samples were kept at + 4°C in the fridge and extracted within 48 h in all the cases.



Figure 1. Geographical location of the studied sewage treatment plants.

2.3. GC-HRMS system and operating conditions

GC-HRMS analyses were run on a HP 6890 Series gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) interfaced to a double focusing magnetic sector mass analyzer (Micromass AutoSpec NT (Micromass, Manchester, UK)), operating in high resolution selected ion recording mode (HR SIR) with the following operating parameters: ionization mode: electron ionization with 70 eV energy; ion repeller (V) = -134 V; detector: 300 V. Perfluorokerosene (mass-lock) were used for accurate mass calibration purposes. Data acquisition and processing, and instrumental control were performed by MassLynx 4.1 software (Micromass, Manchester, UK).

Analytes were separated in a ZB-5MS capillary column (5% diphenyl/95% dimethylsiloxane), 30 m×0.25 mm i.d., 0.25 µm film thickness (Phenomenex, Torrance, CA, USA). A split/splitless injector was used in pulse splitless mode. The injector operating conditions were as follows: injection volume 1 µL; injector temperature 250 °C; initial pulse pressure: 30 psi (1.5 min). The helium (99.9999 % purity) carrier gas flow was maintained at 1 mL min⁻¹. The oven temperature programme was 2.0 min at 70 °C, 10 °C·min⁻¹ to 180 °C (keeping 180 °C for 5 min), 6 °C·min⁻¹ to 260 °C and 4 °C·min⁻¹ to 300 °C.

2.4. Sample treatment

2.4.1. Liquid-Liquid Extraction (LLE) procedure for the isolation and preconcentration of priority organic pollutants

Samples without filtration were acidified up to pH 3 (with H₂SO₄ 1M). An aliquot of 200 mL of effluent wastewater sample were loaded in a 250-mL separatory funnel with 250 mg of NaCl in which a three-step liquid-liquid extraction was undertaken. 25 mL of n-hexane were added and the mixture was vigorously shaken for 3 minutes being then the organic phase (upper) separated from the aqueous one. Then, this extraction step was repeated three times. The organic phases were combined and water traces were removed by adding sodium sulfate anhydrous. The extract was then carefully evaporated up to near dryness using a vacuum rotary evaporator (Büchi Rotavapor R200) equipped with a heating bath (Büchi B-490) operating at 30°C and a vacuum controller (Büchi V800) operating at 150-175 mmHg. Finally, the residue was re-dissolved with 2 mL of n-hexane.

2.5. Validation of the proposed method

All the validation studies were performed by using sewage extracts taken from a mixture of the STP effluents from the 5 different STPs. Because of the impossibility to obtain blanks, the samples were previously analyzed and the presence of the target compounds considered. To minimize matrix effects, matrix-matched calibration curves were used for quantitative determinations. The linearity in the response was studied by using matrix-matched calibration solutions prepared by spiking wastewater effluent extracts at five concentration levels, ranging from the determination limit of each analyte to 200 µg L⁻¹ in the final extract (note

100:1 preconcentration factor from the sample treatment). Each point was obtained as the average of three injections. Integrated peak area data of the selected accurate masses of ions (see Table 1) were used to construct the curves. To optimize the analytical procedure, the recovery studies were carried out by spiking effluent wastewater samples at the concentration level of 0.1 and 1 $\mu\text{g L}^{-1}$ ($n = 6$), in accordance with the approximate maximum concentration levels established by EU regulations for water intended for human consumption [31]. The method detection limit (MDL) and method quantification limit (MQL) were determined experimentally from the injection of spiked wastewater samples and calculated using the minimum concentration of analyte providing signal-to-noise ratios of 3 and 10, respectively. They were estimated from the spiked extracted ion chromatograms at the lowest analyte concentration assayed. Confirmation criteria applied to the target compounds in the wastewater effluent samples were as follows: presence of two characteristic ions at the correct retention time, with the characteristic relative ion abundances and the correct targeted accurate mass. Quantification was performed by external standard calibration.

3. Results and discussion

3.1. Identification and confirmation of selected target priority pollutants by gas chromatography high resolution mass spectrometry

The aim of this article is to develop a multiresidue method for ultratrace detection of multiclass priority pollutants in wastewater treatment plants effluents. Fourteen compounds of the EU-Water Framework Directive were selected. The identification of the targeted species was performed using GC/high-resolution mass spectrometry with a double focusing magnetic instrument. The analyzer used has a three-sector, double focusing geometry of type electrostatic analyzer/magnet/electrostatic analyzer. This instrument was used in high-resolution selective ion recording HRSIR at a resolution value of 10.000 permitting the accurate identification of the targeted compounds. For identification and confirmation purposes, the accurate mass of two characteristic ions and their relative abundances were combined with retention time matching. Therefore, the proposed criteria is in compliance with the EU criteria (Commission Decision 2002/657/EC) [30] related to the confirmation of veterinary chemicals in food/feed based on identification points (IPs) (2 points gained per detection ion with high

resolution MS; 3-4 IPs required for unambiguous confirmation depending on the substance). In most cases, molecular ions were chosen for the HRMS analysis in order to enhance selectivity and sensitivity. Table 1 includes the selected ions used for identification and quantitation purposes and their corresponding elemental compositions.

Table 1. Identification of priority organic pollutants in wastewater by gas chromatography high-resolution mass spectrometry using two ions for identification and confirmation purposes.

Compound	Ret. Time (t _R)	m/z ion 1 (Q)	Elemental Composition ion 1	m/z ion 2 (q) (Relative abundance (%))	Elemental Composition ion 2
1,3,5 trichlorobenzene	5.45	179.929	C ₆ ³⁵ Cl ₃ H ₃	181.926 (96)	C ₆ ³⁵ Cl ₂ ³⁷ ClH ₃
1,2,4 trichlorobenzene	6.12	179.929	C ₆ ³⁵ Cl ₃ H ₃	181.926 (96)	C ₆ ³⁵ Cl ₂ ³⁷ ClH ₃
1,2,3 trichlorobenzene	6.66	179.929	C ₆ ³⁵ Cl ₃ H ₃	181.926 (96)	C ₆ ³⁵ Cl ₂ ³⁷ ClH ₃
Hexachloro-1,3-butadiene	6.73	224.844	C ₄ ³⁵ Cl ₅	222.841 (63)	C ₄ ³⁵ Cl ₄ ³⁷ Cl
Pentachlorobenzene	11.27	249.849	C ₆ ³⁵ Cl ₅ ³⁷ ClH	251.846 (65)	C ₆ ³⁵ Cl ₄ ³⁷ Cl ₂ H
α- HCH	13.14	180.934	C ₆ ³⁵ Cl ₃ H ₄	182.934 (96)	C ₆ ³⁵ Cl ₂ ³⁷ ClH ₄
β- HCH	13.83	180.934	C ₆ ³⁵ Cl ₃ H ₄	182.934 (96)	C ₆ ³⁵ Cl ₂ ³⁷ ClH ₄
γ- HCH	13.97	180.934	C ₆ ³⁵ Cl ₃ H ₄	182.934 (96)	C ₆ ³⁵ Cl ₂ ³⁷ ClH ₄
δ- HCH	14.71	180.934	C ₆ ³⁵ Cl ₃ H ₄	182.934 (96)	C ₆ ³⁵ Cl ₂ ³⁷ ClH ₄
Hexachlorobenzene	12.80	283.809	C ₆ ³⁵ Cl ₅ ³⁷ Cl	285.807 (80)	C ₆ ³⁵ Cl ₄ ³⁷ Cl ₂
Alachlor	16.53	160.1126	C ₁₁ H ₁₄ N	188.107 (71)	C ₁₂ H ₁₄ NO
Tetra-BDE	28.18	485.711	C ₁₂ O ⁷⁹ Br ₂ ⁸¹ Br ₂ H ₆	487.709 (65)	C ₁₂ O ⁷⁹ Br ⁸¹ Br ₃ H ₆
Penta-BDE	31.02	563.621	C ₁₂ O ⁷⁹ Br ₃ ⁸¹ Br ₂ H ₅	565.619 (98)	C ₁₂ O ⁷⁹ Br ₂ ⁸¹ Br ₃ H ₅
Hepta-BDE	31.81	563.621	C ₁₂ O ⁷⁹ Br ₃ ⁸¹ Br ₂ H ₅	565.619 (98)	C ₁₂ O ⁷⁹ Br ₂ ⁸¹ Br ₃ H ₅

Instrument dwell time was optimized with the aim of obtaining a good chromatographic peak shape (with at least 10 points recorded per peak) while maintaining satisfactory sensitivity for each compound. To obtain the maximum possible analyte signal, a 50-ms dwell time was selected for each of the monitored ions. To some extent, this might become a limitation of the method/instrument when dealing with large-scale multi-residue methods (i.e. > 80 targeted species) using GC-HRMS. The average width of the chromatographic peaks is ca. 4 seconds, which involves a maximum acquisition time per point of 0.4 seconds -assuming 10 point peak for an appropriate peak shape-. This involves that only 8 ions (4 compounds) can be screened at the same time (time segment window). In contrast, the typical dwell time of state-of-the-art GC-MS/MS (triple quadrupole) instruments is 10-25 ms (ca. 1/5 or 1/2 compared to GC-

HRMS dwell time). Therefore, the capacity of monitoring compounds is between 2-3 times higher. The main drawback associated with this issue is that a dedicated effort would be required if it is required to develop a large scale multi-residue method, including several scheduled time segments throughout the chromatographic run. On the other hand, the sensitivity and selectivity provided by high resolution are remarkable as demonstrated by the analytical features of the method.

3.2. Performance of the sample treatment procedure

Optimization of the extraction procedure was made with the aim of reaching good recoveries for the widest group of compounds in a single extraction step. To extract the targeted species from wastewater treatment plant effluents, liquid-liquid extraction (LLE) method was developed. Before selecting the LLE procedure, different procedures based on solid-phase extraction were tested. Using C₁₈ (500 mg, 6 mL, BondElutTM, Varian Inc., Palo Alto, CA, USA) cartridges and elution with 1:1 ethyl acetate/dichloromethane (v/v) or with hexane:acetone, poor recoveries (< 15 %) were obtained in the case of trichlorobenzenes, hexachlorobenzene and pentachlorobenzene. Note that the compounds included in the method have very different physicochemical properties. Therefore, it was not straightforward to obtain optimized conditions, in which all the compounds were quantitatively recovered. Finally, LLE using n-hexane was assayed with 200 mL of sample volume and three extractions with 25 mL of solvent. The effluent sample was not filtered before LLE. The pretreatment was limited to adjust the pH to 3-4. With that approach the overall recoveries for the different classes of compounds tested were satisfactory. Only for trichlorobenzenes and hexachloro-1,3-butadiene the recovery rates (shown in Table 2) are relatively lower. The recovery studies were performed at two concentration levels: 0.1 µg L⁻¹ and 1 µg·L⁻¹ level, obtaining satisfactory recovery rates in the range 39.5-117.5 % with RSD (%) values (*n* =6) between 2.1 and 15.3 %. Only trichlorobenzenes and hexachlorobutadiene displayed relatively low recovery rates probably due to their high volatility and vapor pressure [32].

Table 2. Recovery studies of the targeted priority compounds in effluent wastewater samples spiked at two concentration levels: 0.1 and 1 $\mu\text{g L}^{-1}$.

Compound	Recovery (%) ($n=6$)			
	Conc. Level. 0.1 $\mu\text{g L}^{-1}$	RSD (%)	Conc. Level. 1 $\mu\text{g L}^{-1}$	RSD (%)
1,3,5-TCB	47.7	8.2	43.1	9.6
1,2,4-TCB	53.2	10.5	58.8	9.3
1,2,3-TCB	58.7	9.7	63.6	7.1
Hexachloro-1,3-butadiene	39.5	11.9	42.0	12.5
Pentachlorobenzene	88.2	5.7	84.2	7.1
α -HCH	93.0	6.3	109.3	2.1
β -HCH	96.3	4.9	92.4	9.1
γ -HCH	91.5	6.7	108.1	6.1
δ -HCH	89.7	7.5	99.0	11.8
Hexachlorobenzene	85.2	5.5	79.7	6.6
Alachlor	109.3	6.3	117.5	4.4
Tetra-BDE	97.1	12.1	84.7	14.6
Penta-BDE	81.3	11.8	94.5	15.3
Hepta-BDE	96.5	10.6	106.8	9.5

3.3. Analytical performance of the proposed method

To evaluate the analytical performance of the proposed method based on GC-HRMS, a detailed study was carried out including the linearity, accuracy and limits of detection of the selected contaminants. The results obtained are shown in Table 3. The linearity of the proposed method was studied in the range 0.01 – 2 $\mu\text{g L}^{-1}$ (note that the sample volume used was 200 mL and the preconcentration factor 100:1). Correlation coefficients (r) > 0.99 were obtained in most cases.

Inter-day and intra-day accuracy was studied with matrix-matched standards spiked at 50 $\mu\text{g L}^{-1}$. The results obtained for intra-day study were below 15 % in most cases. Limits of detection (LODs) were estimated from the injection of matrix-matched standard solutions at 0.01 $\mu\text{g L}^{-1}$ concentration level. LODs and LOQs were assigned taking into account signal-to-noise (S/N) ratio criterion (S/N of about 3 for LOD and S/N of about 10 for LOQ) in the qualifier ion. The results obtained for each compound are included in Table 3. In preliminary studies, we also tested GC-ECD (Varian CP3800 GC) detection with the same chromatographic method, as long as it is a cheaper instrument that can be implemented in routine laboratories. Nevertheless, considering the concentration levels that are usually found in the samples, the use of a GC-ECD

may not provide enough analytical performance in terms of sensitivity to detect the species. In addition, a confirmatory MS analysis is necessary due to the complexity of the matrix (note the low preconcentration factor implemented in the method (100:1) due to the relatively complexity of the extracts). The results in terms of LOQs for GC-ECD analysis are also shown in Table 3. From the data shown in Table 3, it can be observed the low detection levels achieved by using GC-HRMS, as low as 90 pg L⁻¹ for tetra-BDE, and overall in the low ng L⁻¹ level. An example of the identification of the target species in wastewater samples by GC-HRMS is shown in Figure 2.

Table 3. Analytical parameters of the proposed method for the quantitation in effluent wastewater samples by gas chromatography high resolution mass spectrometry.

Compound	Conc. Range Tested (ng L ⁻¹)*	Regression (Correlation Coefficient) (r)	MLOD (ng L ⁻¹)	MLOQ (ng L ⁻¹)	LOQ (ng L ⁻¹) (GC-ECD)**
1,3,5-TCB	10-200	0.997	0.9	3	100
1,2,4-TCB	10-200	0.989	0.9	3	100
1,2,3-TCB	10-200	0.996	0.9	3	60
Hexachloro-1,3-butadiene	10-200	0.999	0.225	0.75	30
Pentachlorobenzene	10-200	0.995	0.3	1	30
α-HCH	10-200	0.997	1.2	4	200
β-HCH	10-200	0.999	1.8	6	250
γ-HCH	10-200	0.999	1.2	4	200
δ-HCH	10-2000	0.994	1.8	6	200
Hexachlorobenzene	10-2000	0.999	0.3	1	30
Alachlor	10-2000	0.997	3	10	250
Tetra-BDE	10-2000	0.990	0.09	0.3	100
Penta-BDE	10-2000	0.999	0.15	0.5	50
Hepta-BDE	10-2000	0.996	0.3	1	50

*Preconcentration factor (100:1) considered

**GC-ECD analyses were performed using the same chromatographic method, injection volume, column and instrument

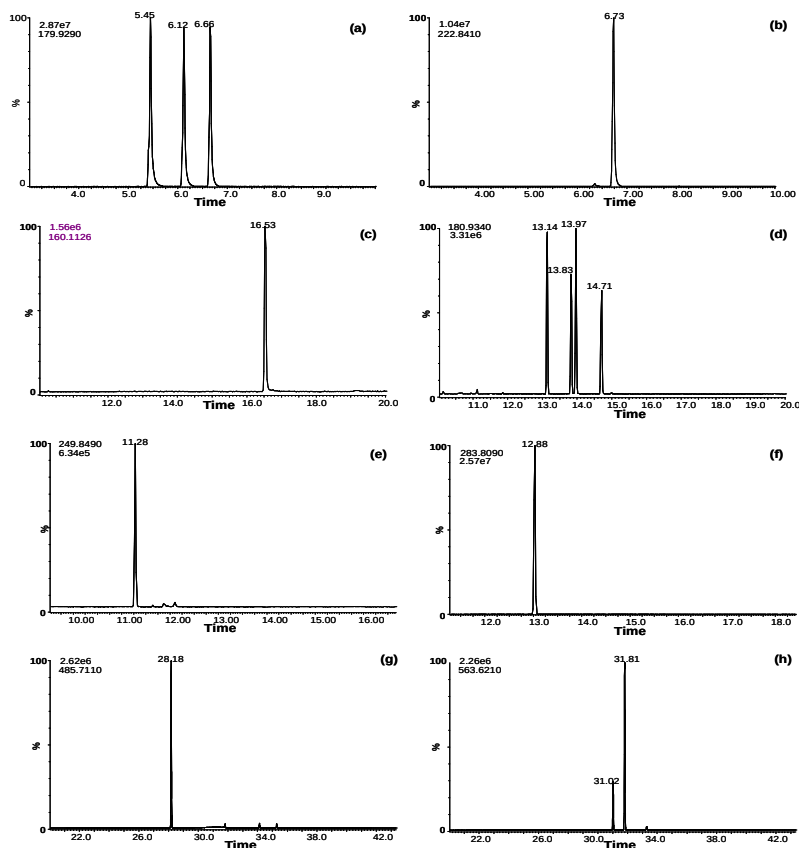


Figure 2. GC-HRMS extracted ion chromatograms of a sewage effluent matrix-matched standard mixture at $0.5 \mu\text{g L}^{-1}$ containing all target compounds: a) trichlorobenzene; b) hexachlorobutadiene; c) alachlor; d) HCHs; e) pentachlorobenzene; f) hexachlorobenzene; g) tetra-BDE; h) penta- and hepta-BDE

3.4. Application of the proposed method for the monitoring of 5 selected Spanish STPs

The proposed method was successfully tested on a monitoring program in order to control the presence of these species in sewage treatment plants effluents. Table 4 shows preliminary results obtained from the monitoring program initiated. Concentration range and mean values of the target compounds detected in 36 samples collected in the studied STPs are shown. All the compounds initially selected were detected with the exception of alachlor. The more frequently detected one was hexachlorobenzene, which was found in all the studied samples at concentrations in the range $5 - 135 \text{ ng L}^{-1}$. As an example, Figure 3 shows the chromatograms obtained in an effluent wastewater sample containing 45 ng L^{-1} of

hexachlorobenzene. From the preliminary data collected, it can be remarked that, in general, the concentration levels are relatively low, and represents a small amount when compared to the load of the so-called emerging contaminants, which can be found in the microgram per liter level according to different studies [6,33,34]. Anyhow for a comprehensive evaluation and risk analysis, the data on priority contaminants is very important, because of their high relative toxicity in comparison with emerging contaminants [6].

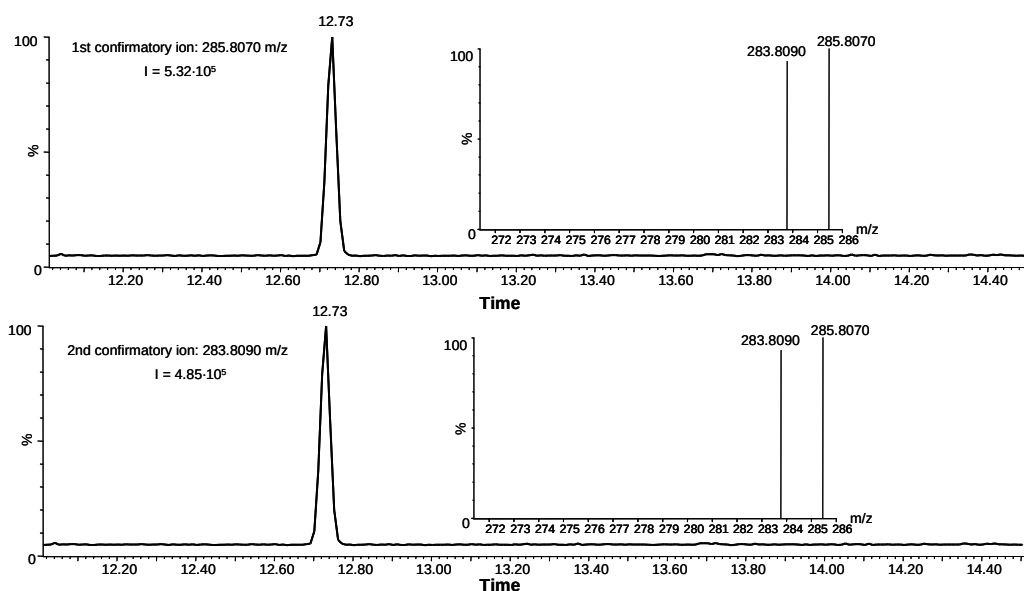


Figure 3. Determination of priority organic pollutants in wastewater treatment plants effluents by GC-HRMS. Example of a positive in an effluent WW sample (San Roman (Santander), containing 45 ng L⁻¹ of hexachlorobenzene.

Table 4. Results of the monitoring of priority pollutants from five Spanish sewage treatment plants

Location/Compound	Samples tested	% positives	Concentration range (ng L ⁻¹) of positive samples	Average concentration (ng L ⁻¹)
STP Alcalá de Henares, Madrid (mainly urban)				
Σ TCBs	15	100	2.71 – 25.6	6.96
Hexachlorobutadiene	15	20	8.3 – 109.32	14.00
Hexachlorobenzene	15	100	5.3 – 135.89	38.69
Pentachlorobenzene	15	73	0.22 – 1.80	0.63
Σ Hexachlorocyclohexane	15	66.7	0.46 – 9.28	2.30
Alachlor	15	0	-	0.00
Σ PBDEs	15	20	0.37 – 0.63	0.10
STP Alcalá de Henares, Madrid (urban-industrial)				
Σ TCBs	13	92.3	2.41 – 48.9	8.57
Hexachlorobutadiene	13	15.4	9.3 – 77.32	19.28
Hexachlorobenzene	13	100	8.15 – 81.72	41.00
Pentachlorobenzene	13	76.9	0.19 – 1.91	0.53
Σ Hexachlorocyclohexane	13	61.5	0.67 – 9.35	2.52
Alachlor	13	0	-	0.00
Σ PBDEs	13	23.1	1.73 – 11.29	1.21
STP El Ejido, Almeria (coastal-agricultural)				
Σ TCBs	4	100	5.36 – 15.31	12.16
Hexachlorobutadiene	4	0	-	0.00
Hexachlorobenzene	4	100	16.34 – 113.78	49.59
Pentachlorobenzene	4	75	1.18 – 1.82	1.10
Σ Hexachlorocyclohexane	4	100	2.03 – 33.14	18.84
Alachlor	4	0	-	0.00
Σ PBDEs	4	50	1.82 – 2.48	1.08
STP Castro Urdiales, Cantabria (mainly urban)				
Σ TCBs	2	100	5.97 – 14.05	10.01
Hexachlorobutadiene	2	0	-	0.00
Hexachlorobenzene	2	100	17.23 – 20.22	18.73
Pentachlorobenzene	2	100	0.69 – 0.70	0.70
Σ Hexachlorocyclohexane	2	100	11.31 – 17.74	14.53
Alachlor	2	0	-	0.00
Σ PBDEs	2	0	-	0.00
STP Santander, Cantabria (mainly urban)				
Σ TCBs	2	100	15.25 – 18.12	16.69
Hexachlorobutadiene	2	0	-	0.00
Hexachlorobenzene	2	100	12.58 – 17.86	15.22
Pentachlorobenzene	2	100	0.53 – 0.68	0.61
Σ Hexachlorocyclohexane	2	100	10.61 – 30.43	20.52
Alachlor	2	0	-	0.00
Σ PBDEs	2	50	0.77	0.39

4. Concluding remarks

In this work, an analytical methodology for the ultratrace detection and quantitation of priority organic pollutants in wastewater samples has been described. The data shown in the

article with the ability to determine as low as a few picograms per liter of the selected contaminants in wastewater samples reveals the usefulness of GC-HRMS as a powerful tool to monitor priority pollutants (2455/2001/CE Directive) in wastewater samples. The proposed methodology was applied to the characterization of effluents from various STPs. The concentration levels found are in most cases below the maximum concentration permitted for these species in water samples intended for human consumption [31]. Further work should be accomplished to monitor emerging contaminants since they are usually present at higher concentration levels, as they represent the major contribution to the charge of contaminants in wastewater samples ($> 25000 \text{ ng L}^{-1}$) [6,33-34]. An exhaustive knowledge and characterization of the behavior of main organic pollutants in sewage treatment plants will prompt the development of technologies for the promotion of the sustainable wastewater treatment and reuse.

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IV.2. MÉTODO DE CROMATOGRAFÍA DE GASES ACOPLADA A
ESPECTROMETRIA DE MASAS DE TRIPLE CUADRUPOLO PARA EL
MONITOREO MULTICLASE DE CONTAMINANTES ORGÁNICOS EN
EFLUENTES DE PLANTAS DE DEPURACIÓN DE AGUA RESIDUAL

IV.2.1. Introducción

La protección del medioambiente acuático de la contaminación debida a la actividad humana es un reto actual para los gobiernos europeos. Una de las medidas que se puede llevar a cabo para hacer frente a este problema, es la caracterización y monitorización de esa contaminación en los focos de origen de la misma, como por ejemplo los efluentes de descarga a la salida de las estaciones depuradoras de aguas residuales (EDARs). En España, la gran mayoría de estas estaciones están diseñadas para cumplir con los requisitos marcados por la Directiva 91/271/CEE referente al tratamiento de aguas residuales urbanas en los estados de la Unión Europea. Esta directiva establece las medidas necesarias que los estados miembros deben adoptar para garantizar que las aguas residuales urbanas reciban un tratamiento adecuado antes de su vertido para minimizar el impacto medioambiental, sin embargo no está orientada a la reutilización de las mismas. No obstante mediante los cursos de agua natural se produce una reutilización indirecta de las aguas residuales tratadas. Las descargas de agua residual tratada, exceptuando las originadas en las plantas de tratamiento de agua residual costera, se diluyen en los flujos de agua natural y son reutilizadas río abajo para nuevos usos urbanos, en la agricultura y actividades industriales (Carballa 2008). El esquema del funcionamiento de la línea de agua de una EDAR se puede observar en la figura IV.2.1.1.

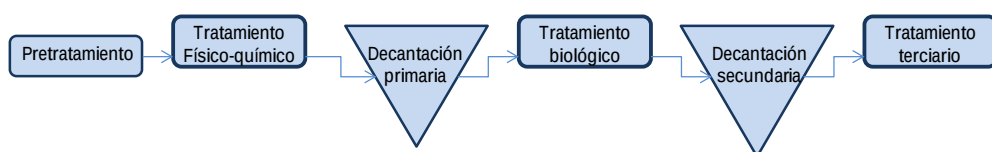


Figura IV.2.1.1. Esquema de los procesos llevados a cabo en la línea de agua de una EDAR

El pretratamiento y tratamiento primario son procesos físicos consistentes fundamentalmente en separar la contaminación presente en el agua en suspensión, flotación o arrastre. El tratamiento biológico o secundario consiste en convertir la contaminación orgánica disuelta en el agua en materia inorgánica, mediante la acción de bacterias. Por último, el tratamiento terciario engloba aquellos procesos físico-químicos destinados a afinar algunas

características del agua efluente de la depuradora con vistas a su empleo para un determinado uso. Por lo tanto, no hay un tratamiento específico para la eliminación de determinados compuestos peligrosos que pueden conllevar un impacto negativo en el medioambiente, de hecho muchos compuestos orgánicos están empezando a ser ubicuos en el mismo (Barceló 2004, Feo 2009).

Con estos antecedentes se hace evidente la necesidad del desarrollo de metodologías analíticas capaces de llevar a cabo la caracterización de los contaminantes orgánicos presentes en los efluentes de las aguas residuales, con el fin de evaluar su posible reutilización y el impacto sobre las aguas superficiales hasta donde llegan sus descargas. Por este motivo y desde la entrada en vigor de la Directiva marco del agua (2000/60/CE) que establece una política común para proteger y mejorar el estado de las aguas medioambientales europeas, se han llevado numerosos trabajos científicos a cabo para identificar la ocurrencia e impacto de sustancias denominadas como prioritarias en sistemas de agua residual (Rule 2006, Gasperi 2008, Muñoz 2008, Robles 2010, Martí 2011).

Las sustancias prioritarias normalmente son compuestos orgánicos apolares y relativamente volátiles por lo que la técnica analítica que se ha escogido para llevar a cabo su análisis ha sido la GC-MS porque ofrece las prestaciones necesarias en cuanto a precisión, sensibilidad y selectividad para detectar estos contaminantes de forma inequívoca (Santos 2003, Gómez 2009, Farré 2012). Típicamente se han utilizado analizadores de cuadrupolo simple en su modo de trabajo de monitoreo de iones seleccionados (SIM-selected ion monitoring), pero la tendencia actual es realizar análisis multiresiduo que cada vez contengan un mayor número de compuestos. Para ello se han empleado técnicas que mejoran la GC-MS para la determinación de sustancias prioritarias en muestras acuosas, como la cromatografía de gases acoplada a espectrometría de masas en tándem (GC-MS/MS) con analizador de triple cuadrupolo (Pitarch 2007), cromatografía de gases acoplada a espectrometría de alta resolución (GC-HRMS) con analizador de tiempo de vuelo (Hernández 2011) o sector magnético (Robles 2010), cromatografía de gases bidimensional acoplada a espectrometría de masas de tiempo de vuelo (Matamoros 2010) y GC-MS rápida (Cherta 2012). Para el análisis de compuestos seleccionados en muestras complejas como son las aguas residuales, el

analizador de triple cuadrupolo ofrece indudables prestaciones en cuanto a sensibilidad, selectividad y robustez. Aunque tiene limitado el número de masas medibles por ventana de tiempo, consigue barridos más rápidos que equipos como el sector magnético mejorando por tanto el número de compuestos que se pueden analizar.

Teniendo en cuenta todo lo descrito hasta ahora, se presenta en el artículo científico 2 el desarrollo de un método multiresiduo para el análisis de 57 contaminantes orgánicos (la mayoría de ellos prioritarios) por cromatografía de gases acoplada a espectrometría de masas en tándem con analizador de triple cuadrupolo (GC-QqQMS) en agua residual. La metodología consiste en una extracción líquido-líquido de la muestra sin filtrar (para evitar la pérdida de los contaminantes presentes en la materia sólida de la muestra) previa a su análisis por GC-QqQMS. El método se validó y aplicó al análisis de 33 muestras de efluentes de agua residual recolectadas en 3 estaciones depuradoras de agua residual localizadas en distintas regiones de España.

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IV.2.2. Artículo científico 2

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Gas chromatography triple quadrupole mass spectrometry method for monitoring multiclass organic pollutants in Spanish sewage treatment plants effluents

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ABSTRACT

In Spain, although more than 50% of urban wastewaters are currently being treated, only half of them are subjected to biological treatments and only 3% undergo advanced treatment technologies. Consequently, the application of more exhaustive wastewater treatment protocols, including the use of new and improved technologies, the application of wider and integrated quality control and water reuse strategies are a priority. We have used as a reference, the European Water Framework Directive (WFD; Directive 2000/60/CE), which establishes a framework for Community action in the field of water policy, setting a list of priority compounds to be monitored in water in order to evaluate their levels. The aim of the present study is to develop and validate a multi-residue method for the analysis of 57 multi-class organic contaminants in wastewater samples using gas chromatography coupled to triple quadrupole mass spectrometry and apply it to evaluate the presence of such compounds in different wastewater treatment plants. The proposed method is based on a sample treatment using liquid–liquid extraction with *n*-hexane followed by identification, confirmation and quantitation with gas chromatography tandem mass spectrometry using a triple quadrupole analyzer operating in the selected reaction monitoring mode. Three MS/MS transitions were selected for unambiguous confirmation of the target chemicals. The method was validated at two different concentration levels (15 and 150 ng L⁻¹) obtaining recovery rates in the range 70–110% in most cases. The limits of quantitation obtained for most of the compounds tested were in the low nanogram per liter range (below 3 ng L⁻¹ in all cases). Treated and untreated effluent wastewater samples of different origin (industrial, coastal and urban) provided by several sewage treatment plants (STPs) located throughout Spain were tested. Results so far showed that most of the samples assayed did not contain large amount of these contaminants. Hexachlorobenzene was found to be the more frequently detected contaminant in the studied samples, although at levels below 5 µg L⁻¹.

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Gas Chromatography Triple Quadrupole Mass Spectrometry Method for Monitoring Multiclass Organic Pollutants in Spanish Sewage Treatment Plants Effluents

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Abstract

In Spain, although more than 50% of urban wastewaters are currently being treated, only half of them are subjected to biological treatments and only 3% undergo advanced treatment technologies. Consequently, the application of more exhaustive wastewater treatment protocols, including the use of new and improved technologies, the application of wider and integrated quality control and water reuse strategies are a priority. We have used as a reference, the European Water Framework Directive (WFD; Directive 2000/60/CE), which establishes a framework for Community action in the field of water policy, setting a list of priority compounds to be monitored in water in order to evaluate their levels. The aim of the present study is to develop and validate a multi-residue method for the analysis of 57 multi-class organic contaminants in wastewater samples using gas chromatography coupled to triple quadrupole mass spectrometry and apply it to evaluate the presence of such compounds in different wastewater treatment plants. The proposed method is based on a sample treatment using liquid-liquid extraction with n-hexane followed by identification, confirmation and quantitation with gas chromatography tandem mass spectrometry using a triple quadrupole analyzer operating in the selected reaction monitoring mode. Three MS/MS transitions were selected for unambiguous confirmation of the target chemicals. The method was validated at two different concentration levels (15 and 150 ng L⁻¹) obtaining recovery rates in the range 70-110 % in most cases. The limits of quantitation obtained for most of the compounds tested were in the low nanogram per litre range (below 3 ng L⁻¹ in all cases). Treated and untreated effluent wastewater samples of different origin (industrial, coastal and urban) provided by several sewage treatment plants (STPs) located throughout Spain were tested. Results so far showed that most of the samples assayed did not contain large amount of these contaminants. Chlorpyrifos ethyl, pyrene and γ -HCH were found to be the most frequently detected contaminants in the studied samples, although at levels below 0.6 μ g L⁻¹.

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1. Introduction

Protection of aquatic environment from chemical pollution resulting from human activity is a current challenge for European governments. To cope with this issue, one of the first steps is the monitoring of sewage treatment plants (STPs) discharges. In Spain, the vast majority of urban wastewater treatment stations have been designed and operate so as to meet European Directive concerning urban wastewater treatment [1]. This regulation sets objectives for treated water quality to minimize the environmental impact on areas of discharge, but is not explicitly oriented to reuse. Nevertheless, treated wastewater is indirectly reused through natural courses. The discharged treated wastewaters (except in the case of coastal sewage treatment plants, which discharge treated wastewaters to the sea) are diluted with circulating flows and are partially reused in downstream areas for new urban uses, agriculture and industry [2]. Furthermore, secondary treatment involves biological treatment or other process designed to control the biochemical oxygen demand (BOD) and the chemical oxygen demand (COD) of the wastewater under treatment. Therefore, it is not specifically focused on the elimination of certain hazardous compounds that can impact negatively the environment, and some organic compounds are becoming ubiquitous in the environment [3,4].

In order to achieve a good chemical status of water in the EU [5], the European Water Framework Directive 2000/60/CE (WFD) [6] establishes a framework for Community action in the field of water policy, setting a list of priority compounds to be monitored in water in order to evaluate their levels. Environmental Quality Standards (EQS) have been set to control the concentration levels of these compounds in surface waters [7]. On the basis of these EQS, Member States have to establish monitoring programs in order to have a comprehensive overview of water status within each river basin district. Additionally, Member States have to identify their river basin specific pollutants, which will be the basis of further updates of the priority substances list. Consequently, improved and powerful analytical methodologies need to be available to accurately determine the very low concentration levels set [8].

Since WFD became into force, numerous studies have been undertaken to identify the occurrence and significance of priority substances in wastewater systems [9-14]. The most widely used approach in the last years for the analysis of priority organic contaminants in environmental samples is gas chromatography/mass spectrometry (GC-MS) with single quadrupole detector operating in selected ion monitoring (SIM) [15-17], not only by its cost effectiveness, sensitivity and selectivity but also by the physicochemical characteristics of most the target analytes. Considering the list of priority compounds, pesticides such as triazines (atrazine, simazine), phenylurea herbicides (diuron, isoproturon) and alachlor can be analysed by either LC-MS or GC-MS, while other of them as cyclodiene pesticides (aldrin, dieldrin, endrin, isodrin), chlorfenvinphos, chlorpyrifos ethyl, 4,4'-DDT, endosulfan, hexachlorocyclohexane (HCH) and trifluralin are only GC-amenable compounds. Polycyclic aromatic hydrocarbons (PAHs) are other group of priority pollutants that cannot be properly analyzed by LC-MS using an electrospray (ESI) interface. Numerous applications in water analysis based on GC-MS have been reported for the determination of PAHs [18-21], pesticides [22-24] and multi-residue procedures for the determination of priority and persistent organic pollutants [25,26]. As an alternative to classic GC-MS, several approaches with enhanced performance has been proposed for the sensitive and selective detection of priority organic contaminants in aqueous matrices including gas chromatography tandem mass spectrometry (GC-MS/MS) with triple quadrupole mass spectrometry [27-30], gas chromatography high resolution mass spectrometry (GC-HRMS) using either time-of flight mass spectrometry (GC-TOFMS) [29,31] or magnetic sector analyzer [10], comprehensive two dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS) [32-34] and fast GC-MS [35]. These detection methods have been accompanied by different sample preparation approaches including liquid-liquid extraction [36], solid-phase extraction (SPE) [28,33], SPME [30], liquid-liquid microextraction (LLME) [32], or stir bar sorptive extraction (SBSE) [33,37,38].

In this work, we report on the development of a multi-residue method for analysis of 57 organic pollutants (including priority substances from the European Water Framework Directive) by gas chromatography coupled to tandem mass spectrometry, and its application to the monitoring of 33 wastewaters samples at the final stage of three Spanish Sample Treatment

Plants (STP) (ready to be discharged into the environment). The proposed method is based on liquid-liquid extraction of the raw sample without previous filtration, since the WFD requires methods allowing complete extraction of organic contaminants from whole water samples (thus avoiding losses in the particulate matter). The method was validated and its performance tested with different samples from sewage treatment plant (STP) effluents collected in different regions of Spain.

2. Experimental section

2.1. Chemicals and Reagents

Organic pollutants investigated in this work are shown in table 1, being several of them regulated by European Water Framework Directive (WFD; Directive 2000/60/CE). All compounds standards were purchased from Sigma-Aldrich (Steinheim, Germany), except for procymidone, which was obtained from Dr. Ehrenstorfer (Augsburg, Germany). Ethyl acetate, n-hexane were provided from Riedel-de-Häen (Seelze, Germany), anhydrous sodium sulfate, and sodium chloride from J.T. Baker (Deventer, Netherlands), MeOH HPLC grade from Merck (Darmstadt, Germany) and sulfuric acid concentrated provided from Panreac (Castellar del Vallès, España). Individual stock standard solutions of the target compounds were prepared at a concentration level of 1000 $\mu\text{g mL}^{-1}$ by dissolving them in n-hexane, ethyl acetate or mixtures of these solvents. Purity was included in the calculation of actual concentration of each standard solution. These solutions were stored in a freezer at $-20\text{ }^{\circ}\text{C}$. The working standards mix solution was prepared by appropriate dilution of the stock solutions with n-hexane at a concentration level of 10 $\mu\text{g mL}^{-1}$.

2.2. Sample material

A total number of 33 wastewater samples of urban origin were collected from three different Sewage Treatment Plants, located in the North (N-STP), North-East (NE-STP) and South-East (SE-STP) of Spain. Wastewater samples were collected during several months after they have passed different stages of treatment, primary, secondary and tertiary. Effluents of the secondary and non-subsequent tertiary treatment stages were analyzed. Thirteen (4 from the

SE-STP, 4 from the NE-STP and 5 from the N-STP) samples corresponded to effluents of the secondary treatment stage and twenty (4 from the SE-STP, 4 from the NE-STP and 12 from the N-STP) samples corresponded to effluents of the tertiary treatment stage. Integrated samples were collected at 1-h intervals and used as representative of 24-hour work in the STPs.

2.3. Liquid-liquid extraction procedure for the isolation and preconcentration of organic pollutants

Samples (200 mL) without filtration were acidified with H_2SO_4 1M up to pH=3 was reached. Then 250 mg of NaCl were added to an aliquot of 200 mL of wastewater sample, which was subsequently loaded in a 250-mL separatory funnel to undergo a three-step liquid-liquid extraction. First, 25 mL of n-hexane were added and the mixture was vigorously shaken for 3 minutes being then the organic phase (upper) separated from the aqueous one. Then, this extraction step was repeated twice more. The organic phases were combined and water traces were removed by adding anhydrous sodium sulfate. The extract was then carefully evaporated up to near dryness using a vacuum rotary evaporator (Büchi Rotavapor R200) equipped with a heating bath (Büchi B-490) and a vacuum controller (Büchi V800). Finally, the residue was re-dissolved in 1 mL of n-hexane, obtaining thus a preconcentration factor of 200:1.

2.4. GC-MS/MS conditions

2.4.1. Gas chromatography

Determination were performed using a CP-3800 gas chromatograph (Varian Inc. Walnut Creek, California, USA) equipped with electronic flow control (EFC), a 1079 universal capillary injector and CombiPal autosampler (CTC Analytics). The injector temperature was held at 280 °C for 2.0 min during injection, then programmed at 40 °C min⁻¹ to 325 °C, which was held for 10 min. A split ratio was initially set at 50:1, at 0.01 min the split vent was closed until 2 min to ensure complete sample transfer, then, the split ratio was held at 100:1 until 13 min, and finally reduced to 20:1. Sample extracts (4 µL) in n-hexane were injected using a Frit gooseneck liner (Restek, Bellefonte, USA). Separations were performed on a Varian FactorFour Capillary Column VF-5ms analytical column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). Helium was used as a carrier gas at a flow rate of 1.0 mL min⁻¹. The oven temperature was programmed as

follows: 70 °C (held 2 min); 10 °C min⁻¹ to 180 °C (5 min); 6.0 °C min⁻¹ to 260 °C; 4 °C min⁻¹ to 300 °C (2 min).

2.4.2. Mass Spectrometry

The GC was interfaced with a model 300-MS triple quadrupole mass spectrometer (Varian Inc. Walnut Creek, California, USA) operating in an electron ionization mode (EI, 70 eV). A filament current of 50 µA and a multiplier voltage of 1300 V were used in MS/MS mode. The temperatures of the transfer line, ion source and manifold were set at 280 °C, 250 °C and 40 °C, respectively. A filament multiplier delay of 6.4 min was fixed in order to prevent instrument damages. The mass spectrometer was calibrated as needed with Perfluorotributylamine (PFTBA). For the MS/MS experiments Ar was used as a collision gas and the collision cell pressure was set at 1.80 mTorr. Multiple reaction monitoring (MRM) conditions were experimentally developed for each individual pesticide on the instrument used in this work. Precursor and product ions, collision energies and other parameters used are shown in Table 1. For instrument control, data acquisition and evaluation Varian MS Workstation, version 6.9 was used.

3. Results and discussions

3.1. GC-QqQ-MS/MS method development

As a general rule, the chromatographic separation is not a critical aspect in the development of a multi-residue method with triple quadrupole analyzers because of the possibility of monitoring co-eluted compounds in MRM mode. Optimization of triple quadrupole MS/MS was performed for each target compound in order to optimize the MRM method. Precursor ions, product ions, and collision energy optimization was carefully studied for each individual analyte for best response. The optimization was carried out by the analysis of 4 µL of mixtures of pesticide standard solutions in hexane, containing 1 mg L⁻¹ of each analyte. After obtaining the full scan spectra of each analyte, the precursor ion with the highest m/z ratio (increase of selectivity) and relative abundance (increase in sensitivity) was selected for fragmentation purposes. The same criterion was also applied to choose the more suitable product ions. Collision energies (CEs) from 5 to 50 eV were evaluated.

Three MRM transitions were used for pesticides, while for PAHs only two transitions were selected due to their difficulty to yield useful fragmentation. Most of PAHs are quantified using the same ion as precursor and as product ion, respectively. The pairs of isomers phenanthrene-anthracene, and benzo[b]fluoranthene-benzo[k]fluoranthene, use exactly the same transitions (respectively), and both compounds in each pair elute very close one to the other. Therefore for the identification and quantification of PAHs is very important to achieve a good chromatographic separation. For atrazine desethyl and diazinon the selected precursor ion for quantifier and qualifier transitions was different. The most intense transition was used as quantifier (Q), while the other transition(s) were used as qualifier (q) peak(s) for the confirmatory analysis. Precursor and product ions, collision energies and other parameters used in the developed GC-MS/MS method are shown in Table 1.

Table 1. GC-MS/MS MRM identification parameters of the studied compounds: retention time and optimized MS/MS transitions.

t_R (min)	Window (min)	Compounds	Precursor ion (m/z)	Product ion (m/z)	Type of transition Q,q ₁ ,q ₂	Dwell time (s)	Collision Energy (eV)
7.048	6.50-9.20	1,3,5-TCB	181.8	146.8	Q	0.042	20
			181.8	108.8	q ₁	0.042	30
			181.8	110.9	q ₂	0.042	25
7.834		1,2,4-TCB	181.8	108.8	Q	0.042	30
			181.8	146.8	q ₁	0.042	20
			181.8	110.9	q ₂	0.042	25
8.313		Hexachlorobutadiene	224.9	189.8	Q	0.042	20
			224.9	154.9	q ₁	0.042	25
			224.9	152.7	q ₂	0.042	45
8.372		1,2,3-TCB	181.8	108.8	Q	0.042	30
			181.8	146.8	q ₁	0.042	20
			181.8	110.9	q ₂	0.042	25
8.560		Isoproturon	161.1	146.1	Q	0.042	10
			161.1	128.0	q ₁	0.042	20
			161.1	91.0	q ₂	0.042	30
8.723		Chlorotoluron	167.0	132.0	Q	0.042	10
			167.0	77.0	q ₁	0.042	30
			167.0	104.0	q ₂	0.042	20
9.795	9.20-10.50	Diuron	186.9	123.9	Q	0.167	20
			186.9	158.8	q ₁	0.167	10
			186.9	96.8	q ₂	0.167	35
11.909	10.50-12.20	Acenaphthylene	152.0	152.1	Q	0.100	10
			152.0	151.1	q ₁	0.100	15
12.595	12.20-14.00	Pentachlorobenzene	249.8	214.8	Q	0.100	20

t_R (min)	Window (min)	Compounds	Precursor ion (m/z)	Product ion (m/z)	Type of transition Q, q ₁ , q ₂	Dwell time (s)	Collision Energy (eV)
13.682		Fluorene	249.8	142.0	q ₁	0.100	40
			249.8	107.8	q ₂	0.100	50
			166.0	165.1	Q	0.100	10
			166.0	166.0	q ₁	0.100	10
14.597	14.00- 15.10	Trifluralin	306.1	264.0	Q	0.083	10
			306.1	206.0	q ₁	0.083	15
			306.1	159.8	q ₂	0.083	25
14.715		Atrazine desethyl	172.0	105.0	Q	0.083	10
			187.0	172.0	q ₁	0.083	10
			172.0	94.0	q ₂	0.083	15
15.528	15.10- 16.00	α -HCH	218.9	182.8	Q	0.083	10
			218.9	146.9	q ₁	0.083	25
			218.9	108.8	q ₂	0.083	35
15.612		Hexachlorobenzene	283.8	248.7	Q	0.083	20
			283.8	141.8	q ₁	0.083	50
			283.8	178.7	q ₂	0.083	50
16.340	16.00- 17.40	Simazine	201.0	173.0	Q	0.033	10
			201.0	186.1	q ₁	0.033	10
			201.0	138.0	q ₂	0.033	15
16.504		Atrazine	215.1	57.9	Q	0.033	10
			215.1	200.1	q ₁	0.033	10
			215.1	138.1	q ₂	0.033	15
16.633		Propazine	229.0	58.0	Q	0.033	15
			229.0	187.1	q ₁	0.033	10
			229.0	214.1	q ₂	0.033	10
16.785		β -HCH	218.9	182.8	Q	0.033	10
			218.9	144.8	q ₁	0.033	25
			218.9	108.9	q ₂	0.033	35
16.949		γ -HCH	218.9	182.8	Q	0.033	10
			218.9	144.8	q ₁	0.033	25
			218.9	108.9	q ₂	0.033	35
17.125		Terbutylazine	214.1	132.0	Q	0.033	10
			214.1	136.0	q ₁	0.033	10
			214.1	119.0	q ₂	0.033	10
17.312		Diazinon	304.0	179.1	Q	0.033	15
			179.0	137.0	q ₁	0.033	15
			179.0	122.0	q ₂	0.033	25
17.665	17.40- 18.20	Phenantrene	178.0	178.1	Q	0.250	10
			178.0	152.1	q ₁	0.250	15
17.960		Anthracene	178.0	178.1	Q	0.250	10
			178.0	152.1	q ₁	0.250	15
18.593	18.20- 19.50	δ -HCH	218.9	182.8	Q	0.167	10
			218.9	144.8	q ₁	0.167	25
			218.9	108.9	q ₂	0.167	35
20.093	19.50- 20.80	Alachlor	188.1	160.0	Q	0.050	10

t_R (min)	Window (min)	Compounds	Precursor ion (m/z)	Product ion (m/z)	Type of transition Q,q ₁ ,q ₂	Dwell time (s)	Collision Energy (eV)
20.113		Parathion methyl	188.1	131.0	q ₁	0.050	20
			188.1	132.0	q ₂	0.050	15
			263.0	109.11	Q	0.050	10
			263.0	127.1	q ₁	0.050	10
			263.0	246.0	q ₂	0.050	5
20.357		Heptachlor	272.0	237.0	Q	0.050	15
			272.0	272.1	q ₁	0.050	5
			272.0	143.0	q ₂	0.050	35
20.549		Ametryn	227.0	185.1	Q	0.050	10
			227.0	212.1	q ₁	0.050	10
			227.0	58.0	q ₂	0.050	10
21.236	20.80- 21.50	Terbutryn	241.1	185.1	Q	0.167	10
			241.1	170.0	q ₁	0.167	15
			241.1	111.2	q ₂	0.167	25
21.875	21.50- 22.70	Chlorpyrifos ethyl	314.0	258.0	Q	0.056	15
			314.0	286.0	q ₁	0.056	10
			314.0	314.0	q ₂	0.056	5
21.917		Aldrin	262.8	192.7	Q	0.056	30
			262.8	190.8	q ₁	0.056	35
			262.8	228.0	q ₂	0.056	20
22.263		Parathion ethyl	291.0	109.0	Q	0.056	10
			291.0	81.0	q ₁	0.056	30
			291.0	142.0	q ₂	0.056	5
23.140	22.70- 24.50	Isodrin	262.8	192.7	Q	0.056	30
			262.8	190.8	q ₁	0.056	30
			262.8	228.0	q ₂	0.056	20
23.286		Chlorfenvinphos A	323.0	267.0	Q	0.056	10
			323.0	295.0	q ₁	0.056	10
			323.0	159.0	q ₂	0.056	30
23.705		Chlorfenvinphos B	323.0	267.0	Q	0.056	10
			323.0	295.0	q ₁	0.056	10
			323.0	159.0	q ₂	0.056	30
24.089		Procymidone	283.0	95.5	Q	0.056	10
			283.0	67.0	q ₁	0.056	20
			283.0	255.1	q ₂	0.056	10
24.975	24.50- 26.40	Pyrene	202.1	202.0	Q	0.037	10
			202.1	201.2	q ₁	0.037	10
24.984		α -endosulfan	338.8	160.1	Q	0.037	15
			338.8	194.9	q ₁	0.037	10
			338.8	230.9	q ₂	0.037	10
25.860		4,4'-DDE	318.0	246.0	Q	0.037	15
			318.0	248.0	q ₁	0.037	10
			318.0	283.0	q ₂	0.037	10
26.006		Dieldrin	262.8	192.7	Q	0.037	30
			262.8	190.8	q ₁	0.037	35
			262.8	227.9	q ₂	0.037	15

t_R (min)	Window (min)	Compounds	Precursor ion (m/z)	Product ion (m/z)	Type of transition Q, q ₁ , q ₂	Dwell time (s)	Collision Energy (eV)
26.152		Oxyfluorfen	361.0	300.0	Q	0.037	10
			361.0	317.0	q ₁	0.037	10
			361.0	252.0	q ₂	0.037	15
26.755	26.40- 28.85	Endrin	262.8	192.7	Q	0.033	35
			262.8	190.8	q ₁	0.033	35
			262.8	228.0	q ₂	0.033	20
27.198		β -endosulfan	338.8	266.7	Q	0.033	10
			338.8	160.0	q ₁	0.033	15
			338.8	230.8	q ₂	0.033	10
27.339		Ethion	231.0	129.0	Q	0.033	25
			231.0	157.0	q ₁	0.033	15
			231.0	185.0	q ₂	0.033	10
28.526		Endosulfan sulfate	386.8	252.9	Q	0.033	10
			386.8	288.9	q ₁	0.033	10
			386.8	205.7	q ₂	0.033	35
28.646		4,4'-DDT	235.0	164.9	Q	0.033	25
			235.0	200.0	q ₁	0.033	10
			235.0	199.0	q ₂	0.033	15
30.030	28.85- 30.70	Iprodione	314.0	245.0	Q	0.062	10
			314.0	271.0	q ₁	0.062	10
			314.0	55.9	q ₂	0.062	20
30.320		Benzo[a]anthracene	228.0	228.0	Q	0.062	10
			228.0	226.0	q ₁	0.062	25
30.466		Chrysene	228.0	228.0	Q	0.062	10
			228.0	226.0	q ₁	0.062	25
30.507		Metoxychlor	227.0	169.0	Q	0.062	25
			227.0	212.1	q ₁	0.062	10
			227.0	184.0	q ₂	0.062	15
34.698	30.70- 37.50	Benzo[b]fluoranthene	252.0	252.0	Q	0.250	10
			252.0	250.0	q ₁	0.250	30
34.810		Benzo[k]fluoranthene	252.0	252.0	Q	0.250	10
			252.0	252.0	q ₁	0.250	30
35.987		Benzo[a]pyrene	252.0	252.0	Q	0.250	10
			252.0	252.0	q ₁	0.250	30
38.378	37.50- 39.50	Deltamethrin	252.9	93.0	Q	0.167	20
			252.9	174.0	q ₁	0.167	10
			252.9	90.9	q ₂	0.167	25
40.367	39.50- 43.33	Indene[1,2,3-cd]pyrene	276.0	276.0	Q	0.125	10
			276.0	274.0	q ₁	0.125	40
40.520		Dibenzo[a,h]anthracene	278.0	278.0	Q	0.125	10
			278.0	250.0	q ₁	0.125	45
41.326		Benzo[g,h,i]perylene	276.0	276.0	Q	0.125	10
			276.0	274.0	q ₁	0.125	40

To confirm peak identity in samples, the Q/q ratio criterion was used, defined as the ratio between the intensity of the quantification transition (Q) and the confirmation transitions (q1 and q2). Firstly, the theoretical average Q/q1 and Q/q2 for each compound (only one ratio available for PAHs) were calculated as the mean value obtained from the chromatographic analysis of standard solutions. The identity of a peak was confirmed by comparison of the experimental ratio in the sample with the theoretical ratio of the reference standard, considering the percentage of variability (tolerance) established in the Decision 2002/657/EC [39], based on ion-ratio statistics for the transitions monitored. Obviously, the agreement in the retention time with standards is also required for a positive confirmation, which is particularly important for the PAHs isomers whose identification is based on the same MS/MS transitions (phenanthrene-anthracene, and benzo[b]fluoranthene- benzo[k]fluoranthene)

3.2. Liquid-liquid extraction method considerations

The main difficulty to tackle with when developing a multi-residue method is the vast array of species with very different physicochemical properties that need to be extracted with a unique set of conditions. Hence, any extraction solvent choice may eventually be biased towards the extraction of a selected class of analytes. The list of priority species included in the study (using lists from current regulations [6,7]) involved non-polar species such as PAHs and organochlorine species together with relatively polar herbicides such as diuron or simazine, whose analyses are more convenient by LC-MS. The choice of the solvent is therefore difficult given this diversity of chemicals. Besides n-hexane, other solvents such as acetone, ethyl acetate or dichloromethane might be possible choices. Considering previous literature [40], a preliminary experiment was undertaken evaluating the performance of dichloromethane and n-hexane for 11 out of the more representative organic priority species. Results obtained are shown in Table 2. From the data shown we found some problems with dichloromethane as solvent, particularly for trichlorobenzene isomers, hexachlorobenzene, pentachlorobenzene and hexachlorobutadiene. Meanwhile, the recovery rates provided by n-hexane were satisfactory in most cases -given the difficulty to find out universal conditions for all the tested species-, even for relatively polar pesticides (for GC analyses) such as triazines. Hence, the performance of n-hexane for relatively polar species was much better than the performance of dichloromethane

for non-polar species. For this reason, n-hexane was selected as extraction solvent. In addition, considering aspects such as occupational safety and health and green chemistry principles, the choice of n-hexane is more convenient (or less inconvenient) than dichloromethane.

One the other hand, with regards to the sample volume used a 200-mL of wastewater were preconcentrated to a final volume of 1 mL. This involved a relative high consumption of hexane (75 mL). This sample size was selected considering the case of highly dirty samples (eg. from untreated effluents) which were difficult to filter before GC-MS. The extracts obtained were so dirty that most of the sample extracts were lost during the filtration step, and eventually the entire extract from the extraction could be lost. Hence, a final volume of 1-mL was selected for the stuffy. Keeping this in mind, in the case of less complex samples (i.e. effluents), the procedure could be re-sized so that less organic solvent was consumed, for instance, 3 replicates with 10-15 mL with half of sample volume (eg. 100 mL), obtaining a final volume of 0.5 mL which would enable the same preconcentration factor. This selection may vary depending on the type of samples tested.

Table 2. Preliminary evaluation of different solvents (n-hexane and dichloromethane) for the liquid-liquid extraction (LLE) of multiclass organic contaminants (fortification level: 100 ng L⁻¹)

	LLE with n-hexane ^(a)		LLE with dichloromethane ^(b)	
	Recovery (%)	R.S.D (%) (n = 3)	Recovery (%)	R.S.D (%) (n = 3)
1,3,5-TCB	42.2	9.7	34.5	16.7
1,2,4-TCB	43.3	9.2	35.6	13.7
1,2,3-TCB	49.4	6.0	36.6	13.5
Hexachlorobutadiene	45.4	10.8	27.2	20.4
Pentachlorobenzene	56.0	1.6	43.2	18.0
α-HCH	75.3	2.8	59.6	19.9
β-HCH	71.6	5.0	48.2	21.1
γ-HCH	78.1	3.5	60.1	17.8
δ-HCH	67.3	5.5	46.2	22.4
Hexachlorobenzene	58.5	2.1	51.7	23.1
Alachlor	83.4	5.8	63.9	15.9

^(a) Proposed method [this work]. ^(b) Adapted from ref. [40]. Briefly, 200 ml water sample + 500 mg NaCl, add 30 mL dichloromethane (DCM); 5 min vortex (separate organic phase). Add HCl to change aqueous phase to pH =2, add an additional aliquot of 30 mL DCM, extract and separate DCM phase; change pH of aqueous phase to 11 with NaOH, add 30 mL DCM. Combine DCM extracts and evaporate with rotavapor and finally taken up with 2-mL hexane.

3.3. Analytical performance

The linearity of the calibration curves was studied using matrix-matched target compounds standard solutions at seven concentrations ranging between 0.1 and 100 $\mu\text{g L}^{-1}$ which corresponded to 0.5 and 500 ng L^{-1} in the wastewater samples using 7 calibration solutions prepared in n-hexane and in extract of wastewater obtained by LLE extraction procedure as any other sample. The response function in matrix-matched standards was found to be linear with a regression coefficient (r^2) higher than 0.993 in the tested range for all organic pollutants.

Limit of detection (LOD) was estimated as the analyte concentration that produces a peak signal of three times the background noise from the chromatogram at the lowest matrix-matched calibration level as low as it was possible. Limit of quantitation (LOQ) was calculated as 3.3 times the LOD. As can be seen in table 3, LOQs were in the low range 0.03 – 5.00 ng L^{-1} , improving those previously reported by using the same technique for the determination of a similar mixture of compounds [28]. Taking into account that EQS for the studied compounds are in the range 2 - 1000 ng L^{-1} , the proposed method fulfills by far the performance criterion “LOQ less than 30% of EQS” proposed by the Chemical Monitoring Activity (CMA) working group of the European Commission [7].

Table 3. Analytical parameters of the studied compounds: equation, lineal dynamic range, regression coefficient, limits of detection and quantification.

Compounds	Equation	Linearity (r^2)	LOD (ng L^{-1})	LOQ (ng L^{-1})	LDR (ng L^{-1})
Acenaphthylene	$y = 33.97 x + 3.18$	0.999	0.07	0.23	0.5-500
Alachlor	$y = 3.04 x + 0.87$	0.998	0.81	2.69	2.5-500
Aldrin	$y = 0.10 x + 0.002$	0.998	0.10	0.33	2.5-500
Ametryn	$y = 1.92 x + 0.48$	0.997	0.83	2.78	0.5-500
Anthracene	$y = 57.36 x + 9.84$	0.998	0.30	1.00	0.5-500
Atrazine	$y = 1.21 x + 0.24$	0.997	1.36	4.55	2.5-500
Atrazine desethyl	$y = 1.32 x + 0.21$	0.998	0.35	1.17	2.5-500
Benzo[a]anthracene	$y = 9.51 x + 0.86$	0.999	0.20	0.67	0.5-500
Benzo[a]pyrene	$y = 9.05 x + 0.39$	0.999	0.06	0.20	0.5-500
Benzo[b]fluoranthene	$y = 10.33 x + 0.54$	0.999	0.17	0.58	2.5-500
Benzo[ghi]perylene	$y = 10.27 x + 0.59$	0.998	0.13	0.42	0.5-500
Benzo[k]fluoranthene	$y = 10.75 x + 0.62$	0.998	0.09	0.30	2.5-500

Compounds	Equation	Linearity (r^2)	LOD (ng L ⁻¹)	LOQ (ng L ⁻¹)	LDR (ng L ⁻¹)
Chlorfenvinphos A	$y = 0.13 x + 0.01$	0.999	0.66	2.19	2.5-500
Chlorfenvinphos B	$y = 1.08 x + 0.07$	0.999	0.96	3.21	0.5-500
Chlorotoluron	$y = 1.36 x + 0.0008$	0.998	0.38	1.25	2.5-500
Chlorpyrifos ethyl	$y = 1.03 x + 0.21$	0.998	0.27	0.90	0.5-500
Chrysene	$y = 10.16 x + 1.37$	0.999	0.50	1.67	0.5-500
Deltamethrin	$y = 0.29 x + 0.01$	0.998	0.82	2.75	2.5-500
Diazinon	$y = 1.10 x + 0.71$	0.998	0.01	0.03	0.5-500
Dibenzo[a,h]anthracene	$y = 8.64 x + 0.10$	0.999	1.01	3.38	2.5-500
4,4'-DDE	$y = 0.38 x + 0.02$	0.999	0.33	1.12	0.5-500
4,4'-DDT	$y = 1.32 x + 0.09$	0.999	0.18	0.59	0.5-500
Dieldrin	$y = 0.05 x + 0.001$	0.996	0.10	0.34	5-500
Diuron	$y = 0.94 x + 0.57$	0.998	0.10	0.33	0.5-500
α -Endosulfan	$y = 0.07 x + 0.01$	0.999	0.27	0.89	5-500
β -Endosulfan	$y = 0.02 x + 0.006$	0.998	0.09	0.29	50-500
Endosulfan sulfate	$y = 0.04 x + 0.002$	0.998	0.23	0.78	3-500
Endrin	$y = 0.06 x + 0.006$	0.998	1.50	5.00	2.5-500
Ethion	$y = 0.80 x + 0.01$	0.999	1.50	5.00	0.5-500
Fluorene	$y = 30.39 x + 3.05$	0.999	0.05	0.17	0.5-500
Heptachlor	$y = 2.41 x + 0.31$	0.998	0.01	0.03	0.5-500
Hexachlorobenzene	$y = 1.18 x + 0.07$	0.999	0.07	0.24	0.5-500
Hexachloro-1,3-butadiene	$y = 1.41 x + 0.10$	0.999	0.09	0.29	0.5-500
α -HCH	$y = 1.00 x + 0.08$	0.999	0.09	0.28	0.5-500
β -HCH	$y = 1.59 x + 0.13$	0.999	0.16	0.55	2.5-500
δ -HCH	$y = 1.02 x + 0.09$	0.999	0.11	0.37	0.5-500
γ -HCH	$y = 0.71 x + 0.32$	0.997	0.14	0.46	0.5-500
Indene[1,2,3-cd]pyrene	$y = 8.73 x + 0.10$	0.998	0.11	0.36	2-500
Iprodione	$y = 0.04 x + 0.01$	0.998	0.07	0.25	3.5-500
Isodrin	$y = 0.03 x + 0.002$	0.998	0.60	1.98	2.5-500
Isoproturon	$y = 0.71 x + 0.11$	0.993	0.67	2.23	2.5-500
Metoxychlor	$y = 0.82 x + 0.10$	0.998	0.07	0.23	0.5-500
Oxyfluorfen	$y = 0.09 x + 0.005$	0.999	0.11	0.37	0.5-500
Parathion ethyl	$y = 0.80 x + 0.04$	0.999	0.82	2.72	2.5-500
Parathion methyl	$y = 1.06 x + 0.04$	0.998	0.75	2.50	2.5-500
Pentachlorobenzene	$y = 1.00 x + 0.09$	0.999	0.01	0.03	0.5-500
Phenanthrene	$y = 58.71 x + 16.16$	0.998	0.28	0.94	0.5-500
Procymidone	$y = 0.39 x + 0.02$	0.998	0.90	3.01	0.5-500
Propazine	$y = 1.26 x + 0.19$	0.998	0.95	3.16	2.5-500
Pyrene	$y = 10.10 x + 6.78$	0.997	0.13	0.45	0.5-500
Simazine	$y = 0.94 x + 0.22$	0.999	1.53	5.10	0.5-500

Compounds	Equation	Linearity (r^2)	LOD (ng L ⁻¹)	LOQ (ng L ⁻¹)	LDR (ng L ⁻¹)
Terbutryn	$y = 2.42 x + 0.83$	0.997	0.42	1.41	0.5-500
Terbutylazine	$y = 1.33 x + 0.77$	0.999	0.60	2.00	0.5-500
1,2,3-TCB	$y = 0.94 x + 0.02$	0.999	0.51	1.70	0.5-500
1,2,4-TCB	$y = 0.98 x + 0.05$	0.999	0.67	2.23	0.5-500
1,3,5-TCB	$y = 1.03 x + 0.05$	0.999	0.53	1.77	0.5-500
Trifluralin	$y = 3.05 x + 0.29$	0.999	0.04	0.15	0.5-500

It should be also emphasized the satisfactory performance achieved with GC-MS/MS for relatively polar compounds such as diuron, isoproturon, chlortoluron, or desethyl atrazine, whose analyses fit better with LC-MS. The idea of the proposed GC-MS/MS method was to explore the potential use of the selected technique for a suite of representative multiclass compounds. For a comprehensive evaluation of the total amount of priority and emerging contaminants, the combination of GC-MS and LC-MS is mandatory. However, we focused our efforts in the highest number of compounds included in EU regulations [6,7] -amongst them atrazine, diuron, isoproturon and simazine- whose analysis could be also suitable by GC-MS. For these relatively polar species, LC-MS may eventually be more convenient, but however those more apolar species such as PAHs or organochlorinated species must be tested with GC-MS. Hence, in this case dealing with priority substances targeted in this work, if only one technique should be selected, GC-MS should be the choice.

The accuracy and precision of the method was estimated by means of recovery experiments, analyzing wastewater sample fortified ($n = 7$) at two concentration levels, 15 ng L⁻¹ and 150 ng L⁻¹. The results are detailed in Table 4, showing recoveries in the range 70-120% for about 60% of the studied analytes (with some differences between concentration levels). Particularly outstanding is the fact that all studied PAHs showed good recoveries, taking into account that these chemicals tend to accumulate in the suspended solids.

Table 4. Average recovery (%) and R.S.D. (in parenthesis) after the application of the GC-(EI)MS/MS procedure to wastewater sample fortified (n=7) at two concentration levels, 15 and 150 ng L⁻¹.

Compounds	Fortification levels and relative standard deviation			
	15 ng L ⁻¹	R.S.D. (%)	150 ng L ⁻¹	R.S.D. (%)
Acenaphthylene	102	4	88	7
Alachlor	137	3	93	12
Aldrin	102	7	62	18
Ametryn	47	19	33	12
Anthracene	141	4	84	8
Atrazine	74	6	54	10
Atrazine desethyl	52	17	44	20
Benzo[a]anthracene	85	7	62	16
Benzo[a]pyrene	71	7	57	15
Benzo[b]fluoranthene	62	7	64	20
Benzo[ghi]perylene	132	2	52	7
Benzo[k]fluoranthene	63	7	64	15
Chlorfenvinphos A	154	9	69	8
Chlorfenvinphos B	100	6	97	9
Chlorotoluron	55	7	20	17
Chlorpyrifos ethyl	105	16	74	18
Chrysene	99	6	86	13
Deltamethrin	--	--	32	22
Diazinon	112	5	90	10
Dibenzo[a,h]anthracene	77	4	54	12
4,4'-DDE	104	7	69	19
4,4'-DDT	94	9	69	18
Dieldrin	89	8	76	7
Diuron	54	13	17	21
α-Endosulfan	175	7	86	9
β-Endosulfan	120	9	75	10
Endosulfan sulfate	111	6	69	18
Endrin	99	14	81	12
Ethion	74	5	67	22
Fluorene	123	3	79	13
Heptachlor	115	6	67	12
Hexachlorobenzene	96	3	74	12
Hexachloro-1,3-butadiene	52	15	32	19
α-HCH	107	6	93	5
β-HCH	164	8	113	15
δ-HCH	95	11	101	12
γ-HCH	134	4	94	13

Compounds	Fortification levels and relative standard deviation			
	15 ng L ⁻¹	R.S.D. (%)	150 ng L ⁻¹	R.S.D. (%)
Indene[1,2,3-cd]pyrene	76	5	54	12
Iprodione	52	14	56	8
Isodrin	104	5	73	12
Isoproturon	42	8	30	14
Metoxychlor	94	1	79	18
Oxyfluorfen	142	7	90	18
Parathion ethyl	112	5	101	8
Parathion methyl	102	4	102	10
Pentachlorobenzene	105	7	73	9
Phenanthrene	108	6	75	14
Procymidone	125	5	84	9
Propazine	120	3	82	7
Pyrene	43	20	71	13
Simazine	--	--	25	15
Terbutylazine	139	4	89	9
Terbutryn	104	5	89	15
1,2,3, TCB	76	11	52	10
1,2,4, TCB	88	10	46	9
1,3,5, TCB	77	8	43	16
Trifluralin	103	5	83	1

3.4. Application to analysis of real (STP) samples

The optimized MRM procedure was applied to the analysis of 33 wastewater samples of urban origin, collected as previously described (see section 2.2) at different months from 3 STP located in the North (N-STP), North-East (NE-STP) and South-East (SE-STP) of Spain (see Figure 1). Results are summarized in Tables 5 and 6. Fourteen of the studied compounds were found in more than 50% of the analyzed samples. Amongst them, the sum of hexachlorocyclohexane (HCH) isomers and the sum of benzo[ghi]perylene and indeno[1,2,3-cd]pyrene are identified as priority hazardous substances and environmental quality standards (EQS) have been established in the European Union regarding the concentration of these hazardous contaminants in surface waters [7]. Besides, priority hazardous compounds, EQS have also been set for pesticides as chlorpyrifos ethyl and diuron, frequently detected in the present survey. Chlorpyrifos ethyl was found in all analyzed samples, and except in the case of

effluents of the secondary treatment stage of the SE-STP, the average concentration level of this compound was below its EU annual EQS, established at 30 ng L⁻¹ for inland surface waters [7].

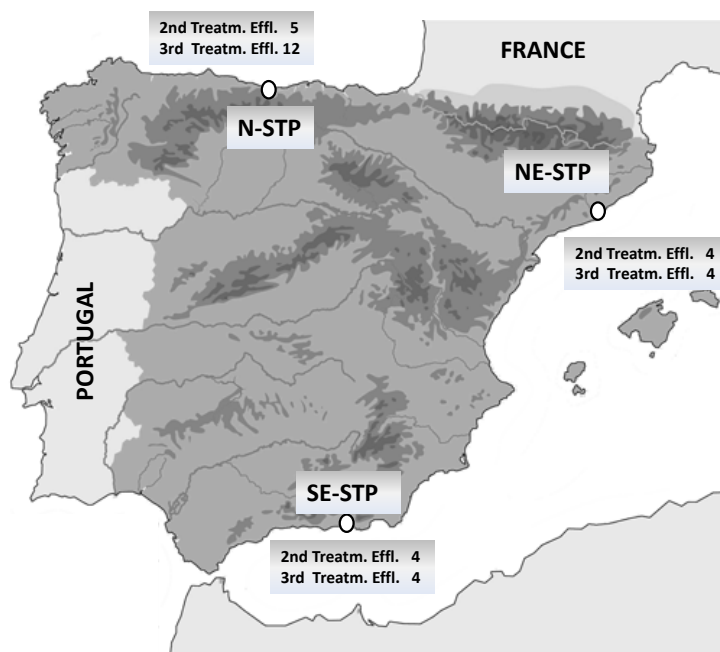


Figure 1. Geographical location of the monitored sewage treatment plants, including the number of effluents of the secondary and tertiary treatments collected, respectively.

With regards to effluents of the secondary treatment stage of the SE-STP, samples collected from March to May contained concentrations of chlorpyrifos ethyl between 1.6 and 5.6 times higher than the maximum allowable concentration for this compound, established at 100 ng L⁻¹ [7]. Therefore, the necessity of tertiary treatment to keep the contaminants at low levels is pointed out. Similarly, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene, identified as priority hazardous compounds, were frequently detected at concentration levels below the 2 ng L⁻¹ EU EQS, except in the case of effluents of the secondary treatment stage of the N-STP, enforcing the necessity of tertiary treatment to keep the contaminants at low levels. As an example, Figure 2 shows the positive findings of chlorpyrifos ethyl and pyrene in a wastewater effluent of the secondary treatment of the NE-STP.

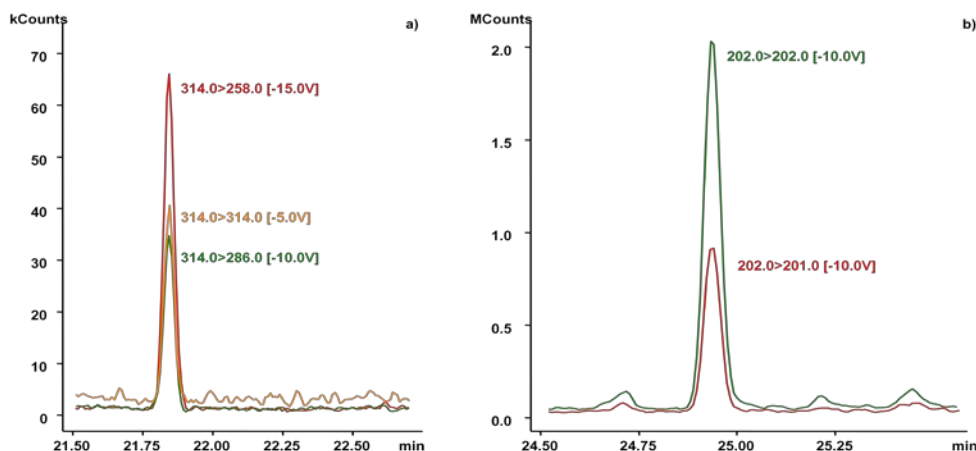


Figure 2. Positive findings of a) chlorpyrifos ethyl (6 ng L⁻¹) and b) pyrene (12.4 ng L⁻¹) in an effluent wastewater sample corresponding to the secondary treatment stage of the STP located in the North-East of Spain.

Lindane (γ -HCH isomer) was detected in the 97% of the analyzed effluent samples and diuron was found in the 82% of the studied samples. The average value of the sum of hexachlorocyclohexane isomers individual concentrations and the average concentration level of diuron were below their corresponding EU environmental quality standards, set at 20 ng L⁻¹ and 200 ng L⁻¹, respectively.

Table 5. Results from the analyses of wastewater samples from secondary treatment STP effluents. Percentage of positive samples and concentration ranges of the most frequently detected analytes.

Compound	Secondary treatment STP effluents (13 samples)			Priority hazardous substances regulation [7]	
	% positive samples	Concentration range (ng L ⁻¹)	Average concentration (ng L ⁻¹)	AA-EQS ⁽¹⁾ (ng L ⁻¹)	MAC-EQS ⁽²⁾ (ng L ⁻¹)
Acenaphthylene	76.9	1.0 - 89.1	13.1	--	--
Benzo[a]anthracene	76.9	0.3 - 15.1	5.7	--	--
Benzo[ghi]perylene	84.6	0.3 - 6.9	2.4	$\Sigma = 2$	
Indeno[1,2,3-cd] pyrene	38.5	0.4 - 8.0	2.6		
Chlorpyrifos ethyl	100.0	4.7 - 590.0	87.2	30	100
Diazinon	61.5	1.5 - 92.6	27.9	--	--
Diuron	92.3	0.2 - 68.5	15.6	200	1800
Fluorene	92.3	1.2 - 80.7	21.0	--	--
Hexachlorobenzene	23.1	0.4 - 1.8	0.9	10	50
α -HCH	23.1	0.2 - 0.8	0.5	$\Sigma = 20$	
β -HCH	15.4	0.8 - 1.3	1.1		
γ -HCH	92.3	1.8 - 29.1	7.4		
Phenanthrene	92.3	2.6 - 141.0	35.0	--	--
Procymidone	46.2	1.2 - 27.5	7.9	--	--

Compound	Secondary treatment STP effluents (13 samples)			Priority hazardous substances regulation [7]	
	% positive samples	Concentration range (ng L ⁻¹)	Average concentration (ng L ⁻¹)	AA-EQS ⁽¹⁾ (ng L ⁻¹)	MAC-EQS ⁽²⁾ (ng L ⁻¹)
Pyrene	100.0	4.9 - 73.9	27.2	--	--
Terbutryn	30.8	4.4 - 46.8	20.1	--	--

(¹) AA-EQS: Environmental Quality Standard established for the annual average concentration of the corresponding contaminant in inland surface waters. Directive 2008/105/EC.

(²) MAC-EQS: Environmental Quality Standard established for the maximum allowable concentration of the corresponding contaminant in inland surface waters. Directive 2008/105/EC.

Table 6. Results from the analyses of wastewater samples from tertiary treatment STP effluents. Percentage of positive samples and concentration ranges of the most frequently detected analytes.

Compound	Tertiary treatment STP effluents (20 samples)			Priority hazardous substances regulation [7]	
	% positive samples	Concentration range (ng L ⁻¹)	Average concentration (ng L ⁻¹)	AA-EQS ⁽¹⁾ (ng L ⁻¹)	MAC-EQS ⁽²⁾ (ng L ⁻¹)
Acenaphthylene	70.0	1.1 - 7.9	2.7	--	--
Benzo[a]anthracene	45.0	0.3 - 2.7	1.1	--	--
Benzo[ghi]perylene	55.0	0.2 - 2.9	1.0	$\Sigma = 2$	
Indeno[1,2,3-cd] pyrene	25.0	0.4 - 2.2	1.1		
Chlorpyrifos ethyl	100.0	0.6 - 50.3	11.9	30	100
Diazinon	70.0	1.1 - 43.4	9.0	--	--
Diuron	80.0	1.1 - 46.1	11.1	200	1800
Fluorene	90.0	0.7 - 17.6	4.5	--	--
Hexachlorobenzene	40.0	0.2 - 3.3	1.2	10	50
α -HCH	35.0	0.1 - 0.7	0.4	$\Sigma = 20$	
β -HCH	10.0	0.4 - 1.4	0.9		
γ -HCH	100.0	0.8 - 37.3	7.1		
Phenanthrene	80.0	1.4 - 41.0	11.1	--	--
Procymidone	65.0	0.8 - 15.4	6.6	--	--
Pyrene	75.0	0.2 - 121.4	23.2	--	--
Terbutryn	85.0	0.6 - 19.0	10.0	--	--

(¹) AA-EQS: Environmental Quality Standard established for the annual average concentration of the corresponding contaminant in inland surface waters. Directive 2008/105/EC.

(²) MAC-EQS: Environmental Quality Standard established for the maximum allowable concentration of the corresponding contaminant in inland surface waters. Directive 2008/105/EC.

Hexachlorobenzene was detected in the 35% of the studied samples, corresponding mainly to the N-STP, while it was detected in the 98% of the samples in a previous study [10] comprising samples from 5 STPs. Interestingly, fluorene, phenanthrene and pyrene were frequently detected and several samples presented concentration levels of these compounds

higher than 40 ng L⁻¹, even higher than 100 ng L⁻¹, but no EQS have been set for these compounds. Twenty six compounds were not detected in the studied samples. In this group are included priority compounds as anthracene (the only one not detected amongst the studied PAHs), cyclodiene pesticides, endosulfan, simazine, atrazine, or δ -HCH.

4. Concluding remarks

In this study, an analytical method based on liquid-liquid extraction followed by GC-MS/MS has been developed for the detection of 57 priority organic contaminants in wastewater samples. The proposed LLE method, which comprises the analysis of both liquid phase and particulate matter of wastewater samples -as required by the European WFD-, used n-hexane as it was found the more appropriate solvent for the extraction of such variety of species. This LLE step was followed by determination with the proposed GC-MS/MS method, which was found to provide the excellent selectivity and sensitivity attained, enabling the unambiguous identification of the target species by the determination of three MRM transitions (when available). It should be also emphasized the satisfactory performance achieved with GC-MS/MS for relatively polar compounds such as diuron, isoproturon, chlortoluron, or desethyl atrazine, whose analyses fit better with LC-MS. In contrast, for PAHs only two transitions were selected due to their difficulty to yield useful fragmentation. Most of PAHs were quantified using, respectively, the same ion as precursor and as product ion. In particular, the identification of isomers phenanthrene-anthracene, and benzo[b]fluoranthene-benzo[k]fluoranthene, which used the same MS/MS transitions, relied on the chromatographic separation. Finally, the results obtained from the monitoring study of these organic contaminants in wastewater samples as provided by the developed procedure, demonstrated their ubiquitous presence which emphasizes the need to control such group of contaminants. Thus, it has been demonstrated that the proposed GC-MS/MS method for multiclass organic pollutants is useful in order to know concentration levels of pollutants in waste water sewage treatment plant effluents, and accordingly to proceed with appropriate tertiary treatments when necessary.

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*IV.3. COMPARACIÓN DE EXTRACCIÓN LÍQUIDO-LÍQUIDO,
EXTRACCIÓN EN FASE SÓLIDA Y MICROEXTRACCIÓN EN FASE
SÓLIDA PARA LA DETERMINACIÓN MULTICLASE DE CONTAMINANTES
ORGÁNICOS PRIORITARIOS POR CROMATOGRFÍA DE GASES-
ESPECTROMETRÍA DE MASAS EN AGUAS RESIDUALES*

V.2.1. Introducción

Dentro del proceso analítico, el tratamiento de muestra puede considerarse como un punto crítico, ya que su influencia sobre el resultado es incluso más importante que la medida en sí misma o la precisión del instrumento. Esta etapa consiste en una serie de pasos encaminados a la obtención de un extracto que contenga los analitos de interés en un disolvente adecuado para la técnica de detección a emplear. En el caso de muestras acuosas medioambientales se lleva a cabo una etapa de extracción que, en muchos casos, lleva asociado el empleo de disolvente que bien por su toxicidad o por la gran cantidad necesaria es indeseable.

En los últimos años se ha hecho un gran esfuerzo para desarrollar procesos de extracción más eficientes, y la tendencia en la actualidad va encaminada a conseguir 3 hitos principales, que son los siguientes:

- Desarrollo y utilización de sorbentes avanzados.
- Automatización a través del acoplamiento del módulo de preparación de muestra con el sistema de detección (extracción on-line).
- Utilización de metodologías más verdes, como técnicas que reducen la utilización de disolventes.

A pesar de las nuevas tendencias, hoy en día los procedimientos de extracción más extendidos para el análisis de muestras acuosas son la LLE y la SPE (Quintana 2006). La LLE tradicional normalmente requiere de la utilización de una cantidad de disolvente elevada, por lo que no está en consonancia con los requerimientos actuales de la química verde, sin embargo permite la extracción de los contaminantes absorbidos en la materia sólida de muestras de agua residual ya que no requiere de una filtración previa (Ligon 2008, Robles-Molina 2010). Por otro lado, la SPE se presenta como una alternativa más rápida y menos nociva para el medioambiente por lo que ha sido muy utilizada para la determinación de contaminantes orgánicos en aguas (Pichon 2000, Barrek 2009, Lacorte 2010, Prieto 2010, Cherta 2012).

Como una alternativa a ambos procesos de extracción se desarrolló en el año 1990 la microextracción en fase sólida (SPME) por el grupo del Prof. Pawliszyn, caracterizada por ser una técnica de extracción fácilmente automatizable, rápida y sin consumo de disolvente en la mayoría de los casos (Beltrán 2000, Lord 2000, Sakamoto 2004, Guimarães 2008, Tankiewicz 2013). Las desventajas que presenta la SPME es que en el caso de muestras complejas se debe realizar en su modalidad de espacio de cabeza (HS-SPME) porque de lo contrario la fibra se deterioraría rápidamente, además con las fibras que hay actualmente en el mercado sólo se puede aplicar a un grupo reducido de compuestos con características físico-químicas similares.

Posterior al tratamiento de muestra tiene lugar la etapa de análisis, que en el caso de las sustancias prioritarias debido a su escasa polaridad se realiza normalmente mediante GC-MS en lugar de LC-MS (Alder 2006). La mayoría de estudios publicados están dedicados al análisis de contaminantes en muestras de agua superficial y solo un pequeño porcentaje se dedica al análisis de muestras complejas de agua residual como son los influentes que llegan a la EDARs (Cortada 2009).

Por todo lo explicado hasta ahora, en el artículo científico 3 se compararon tres métodos de preparación de muestra para la determinación de contaminantes orgánicos multiclase en agua residual mediante GC-MS/MS. Se seleccionaron un total de 57 contaminantes (44 plaguicidas y 13 PAHs) la mayoría de ellos incluidos en la lista de prioritarios de la Directiva marco del agua (2000/60/CE). Las metodologías ensayadas fueron: a) LLE con n-hexano, b) SPE con cartuchos C18 y elución con mezcla 1:1 de acetato de etilo y diclorometano y c) HS-SPME utilizando dos tipos de fibras: poliacrilato (PA) y polidimetilsiloxano/carboxen/divinilbenceno (PDMS/CAR/DVB). La identificación y confirmación de los 57 compuestos se realizó utilizando un cromatógrafo de gases acoplado a un espectrómetro de masas con un analizador de triple cuadrupolo, en su modo de trabajo de monitoreo de reacciones múltiples (MRM).

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IV.3.2. Artículo científico 3

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Comparative evaluation of liquid–liquid extraction, solid-phase extraction and solid-phase microextraction for the gas chromatography–mass spectrometry determination of multiclass priority organic contaminants in wastewater



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ABSTRACT

The European Water Framework Directive (WFD) 2000/60/EC establishes guidelines to control the pollution of surface water by sorting out a list of priority substances that involves a significant risk to or via the aquatic systems. In this article, the analytical performance of three different sample preparation methodologies for the GC–MS/MS determination of multiclass organic contaminants—including priority compounds from the WFD—in wastewater samples using gas chromatography–mass spectrometry was evaluated. The methodologies tested were: (a) liquid–liquid extraction (LLE) with *n*-hexane; (b) solid-phase extraction (SPE) with C_{18} cartridges and elution with ethyl acetate:dichloromethane (1:1 (v/v)), and (c) headspace solid-phase microextraction (HS-SPME) using two different fibers: polyacrylate and polydimethylsiloxane/carboxen/divinylbenzene. Identification and confirmation of the selected 57 compounds included in the study (comprising polycyclic aromatic hydrocarbons (PAHs), pesticides and other contaminants) were accomplished using gas chromatography tandem mass spectrometry (GC–MS/MS) with a triple quadrupole instrument operated in the multiple reaction monitoring (MRM) mode. Three MS/MS transitions were selected for unambiguous confirmation of the target chemicals. The different advantages and pitfalls of each method were discussed. In the case of both LLE and SPE procedures, the method was validated at two different concentration levels (15 and 150 ng L^{−1}) obtaining recovery rates in the range 70–120% for most of the target compounds. In terms of analyte coverage, results with HS-SPME were not satisfactory, since 14 of the compounds tested were not properly recovered and the overall performance was worse than the other two methods tested. LLE, SPE and HS-SPME (using polyacrylate fiber) procedures also showed good linearity and precision. Using any of the three methodologies tested, limits of quantitation obtained for most of the detected compounds were in the low nanogram per liter range.

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Comparative Evaluation of Liquid-Liquid Extraction, Solid-Phase Extraction and Solid-Phase Microextraction for the Gas Chromatography-Mass Spectrometry Determination of Multi-Class Priority Organic Contaminants in Wastewater

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Abstract

The European Water Framework Directive (WFD) 2000/60/EC establishes guidelines to control the pollution of surface water by sorting out a list of priority substances that involves a significant risk to or via the aquatic systems. In this article, the analytical performance of three different sample preparation methodologies for the GC-MS/MS determination of multi-class organic contaminants -including priority compounds from the WFD- in wastewater samples using gas chromatography-mass spectrometry was evaluated. The methodologies tested were: a) liquid-liquid extraction (LLE) with n-hexane; b) solid-phase extraction (SPE) with C₁₈ cartridges and elution with ethyl acetate:dichloromethane (1:1 (v/v)), and c) headspace solid-phase microextraction (HS-SPME) using two different fibers: polyacrylate and Polydimethylsiloxane/Carboxen/Divinilbenzene. Identification and confirmation of the selected 57 compounds included in the study (comprising polycyclic aromatic hydrocarbons (PAHs), pesticides and other contaminants) was accomplished using gas chromatography tandem mass spectrometry (GC-MS/MS) with a triple quadrupole instrument operated in the multiple reaction monitoring (MRM) mode. Three MS/MS transitions were selected for unambiguous confirmation of the target chemicals. The different advantages and pitfalls of each method were discussed. In the case of both LLE and SPE procedures, the method was validated at two different concentration levels (15 and 150 ng L⁻¹) obtaining recovery rates in the range 70-120 % for most of the target compounds. In terms of analyte coverage, results with HS-SPME were not satisfactory, since 14 of the compounds tested were not properly recovered and the overall performance was worse than the other two methods tested. LLE, SPE and HS-SPME (using polyacrylate fiber) procedures also showed good linearity and precision. Using any of the three methodologies tested, limits of quantitation obtained for most of the detected compounds were in the low nanogram per liter range.

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1. Introduction

WFD [1] includes a list of priority compounds to be monitored in surface water to whom Environmental Quality Standards (EQS) are set to control their concentration levels [2]. Several analytical methodologies have been reported in the literature for the analysis of priority organic contaminants compounds in natural water and wastewater [3]. The developed methods are generally based on gas chromatography-mass spectrometry (GC-MS) rather than liquid chromatography-mass spectrometry (LC-MS), because of the physicochemical properties particularly due to their low polarity and solubility in water [4].

Despite current trends in sample handling focus on the development of faster, safer, and more environmentally friendly extraction techniques, both (LLE) and solid-phase extraction (SPE) yet are useful and widely accepted techniques for the exhaustive extraction of organic contaminants from water matrices [5]. Traditional liquid-liquid extraction does not fulfill the current requirements of green analytical chemistry. However, it allows the application of the procedure to the raw wastewater sample without filtration, thus offering the possibility to extract organic contaminants that tend to accumulate in the suspended solids [6,7]. SPE is an alternative less time-consuming and more environmentally friendly. Hence, it has been widely used for the determination of organic pollutants in natural waters [8-13]. The need to filter sample prior to load the sample on the SPE cartridge, makes this technique less suitable for the thorough extraction of contaminants from suspended solids.

As an alternative to these two widely accepted sample treatment methodologies, Solid-phase microextraction (SPME) is also an established technique for volatile compounds and many applications in environmental analysis have been reported, since it is rapid, easily automated, and solvent-free [14-18]. Further advanced microextraction techniques described in the literature for trace analysis of organic contaminants in water [18] include stir-bar sorptive extraction [20-23], single-drop microextraction [24-26], dispersive liquid-liquid microextraction [27,28] and membrane assisted microextraction [29,30]. However, most of the studies so far are usually devoted to (surface) water samples. Only a little percentage of the abundant literature deals with complex wastewater samples such as untreated influents [31]. The inherent

complexity of this matrix may originate several problems. For instance, SPME must be accomplished in headspace mode instead of by direct immersion of the SPME fiber. In addition, most of the methods developed are usually focused on a selected class of species rather than comprising a vast array of chemicals with different physicochemical properties, which is the preferred situation, so that all the species that are usually included in by water quality regulations could eventually be covered by a single method. For these demanding analytical tasks, LLE, SPE and HS-SPME are yet well established and reliable sample treatment techniques. Finally, wastewater matrices usually contained insoluble particles that retain part of the targeted contaminants, particularly those apolar species with high K_{ow} values. Depending upon the type of technique used, the analytes retained in the suspended particles may produce underestimation errors on the total amount of contaminants.

In this article, the analytical performance of three established sample preparation methodologies for the GC-MS/MS determination of multi-class organic contaminants in wastewater samples using gas chromatography-mass spectrometry is evaluated. A total of 57 multiclass organic pollutants (44 pesticides and 13 PAHs), including priority substances from the WFD were examined. The methodologies tested were: a) LLE with n-hexane; b) SPE with C_{18} cartridges and elution with ethyl acetate:dichloromethane (1:1 (v/v)), and c) HS-SPME using two different fibers: polyacrylate (PA) and Polydimethylsiloxane/Carboxen/Divinilbenzene (PDMS/CAR/DVB). Identification and confirmation of the selected 57 compounds was accomplished using gas chromatography tandem mass spectrometry with a triple quadrupole instrument operated in the multiple reaction monitoring mode.

2. Experimental

2.1. Chemical and reagents

The selected multiclass organic contaminants included in this work are shown in Table 1, being most of them regulated by WFD (Directive 2000/60/CE). All standards were purchased from Sigma-Aldrich (Steinheim, Germany), except procymidone, which was obtained from Dr. Ehrenstofer (Augsburg, Germany). Ethyl acetate, dichloromethane (DCM) and n-hexane were obtained from Riedel-de-Häen (Seelze, Germany). Anhydrous sodium sulfate and

sodium chloride were from J.T. Baker (Deventer, Netherlands). MeOH HPLC grade was from Merck (Darmstadt, Germany) and sulfuric acid was provided by Panreac (Castellar del Vallès, España). Individual stock standard solutions of the target compounds were prepared at a concentration level of 1000 mg L⁻¹. These solutions were stored in a freezer at -20 °C. The working standard solution of multi compounds was prepared by appropriate dilution of the stock solutions with n-hexane at a concentration level of 10 mg L⁻¹. SPME fibers (85 µm PA and 50/30 µm DVB/CAR/PDMS), 20 mL glass flat-bottomed vials (22.7 mm OD x 75 mm) as well as magnetic PTFE-silicone seals (3.0 mm i.d.) were purchased from Supelco (Madrid, Spain). PTFE-encapsulated magnetic stirring bars (6 mm x 12 mm) and SPE Bond Elut C₁₈ cartridges (500 mg, 6 mL) were purchased from Varian Inc. (Walnut Creek, California, USA).

2.2. Sample treatment methodologies evaluated

The sample used for the study corresponds to the effluent of an urban waste water sample treatment plant from the south-east of Spain with appropriate spiked amounts of analytes.

2.2.1. LLE procedure for the isolation and preconcentration of organic pollutants

The tested procedure was adapted from a previous work [32]. Samples without filtration were acidified with H₂SO₄ 1M up to pH=3 was reached. Then 250 mg of NaCl were added to an aliquot of 200 mL of wastewater sample, which was subsequently loaded in a 250-mL separatory funnel to undergo a three-step LLE. First, 25 ml of n-hexane were added and the mixture was vigorously shaken for 3 minutes being then the organic phase (upper) separated from the aqueous one. Then, this extraction step was repeated two times more. The three organic phases were combined and water traces were removed by adding anhydrous sodium sulfate. The extract was then carefully evaporated up to near dryness using a vacuum rotary evaporator (Büchi Rotavapor R200) equipped with a heating bath (Büchi B-490) operating at 34°C and a vacuum controller (Büchi V800). Finally, the residue was redissolved in 1 mL of n-hexane, obtaining a preconcentration factor of 200:1.

2.2.2. SPE procedure

The selected procedure was adapted from Pitarch *et al.* [33] and consisted of passing 200 mL of filtered wastewater sample through the C₁₈ SPE cartridge previously conditioned by passing 6 mL methanol, 6 mL ethyl acetate:DCM (50:50), 6 mL methanol and 6 mL ultrapure water, avoiding dryness. After loading the sample, cartridges were washed with 3 mL of ultrapure water. The cartridge was dried by passing air, using vacuum for at least 15 min, and then the elution was performed by passing 5 mL of the mixture ethyl acetate:DCM (50:50). The extract collected was evaporated under a gentle nitrogen stream using a Turbo Vap LV from Zymark (Hopkinton, MA), with a water bath temperature of 27 °C and a N₂ pressure of 5 psi. Finally, the residue was redissolved in 1 mL of n-hexane, obtaining a preconcentration factor of 200:1.

2.2.3. Automated HS-SPME procedure

The first step of the analysis consisted of the introduction of 10 mL of unfiltered wastewater sample in a 20-mL HS glass vial with a PTFE-encapsulated magnetic stirring bar. Then, 1 mL of MeOH (10% volume) and 1 g of NaCl were added and the vial was immediately sealed with a PTFE-silicone septum. It was placed in the CombiPAL heated module for heating with mechanical stirring (600 rpm) during 50 minutes at 80 °C in order to assess the equilibration between gas-phase and sample. During this incubation time, the PA fiber (85 µm thickness) was exposed to the headspace of the vial. After the exposition to the analytes, the fiber was automatically inserted in the injection port of the GC-MS system for 10 minutes (at a desorption temperature of 260 °C). During GC-MS/MS analysis, specific MRM transitions were recorded for each target compound.

2.3. GC-MS/MS conditions

2.3.1. Gas chromatography

Determination was performed using a CP-3800 gas chromatograph (Varian Inc. Walnut Creek, California, USA) equipped with electronic flow control (EFC) and a 1079 universal capillary injector that allows programmed temperature injection (a PTV injection port). The gas chromatograph was also equipped with an autosampler (CombiPAL autosampler, CTC

Analytics) with capacity for 98 2-mL vials and a robotic arm. The injector temperature was held at 280 °C for 2.0 min during injection, then programmed at 40 °C min⁻¹ to 325 °C, which was held for 10 min. Separations were performed on a Varian FactorFour Capillary Column VF-5ms analytical column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). Helium (99.9999%) was used as a carrier gas at a flow rate of 1.0 mL min⁻¹. The oven temperature was programmed as follows: 70 °C (held 2 min); 10 °C min⁻¹ to 180 °C (5 min); 6.0 °C min⁻¹ to 260 °C; 4 °C min⁻¹ to 300 °C (2 min). Sample extracts obtained after LLE or SPE sample treatments were injected (4 µL) using a Frit gooseneck liner (Restek, Bellefonte, USA).

2.3.2. Gas chromatography conditions for automated HS-SPME analysis Mass Spectrometry

The gas chromatograph described in section 2.3.1 was also equipped with an autosampler (CombiPAL autosampler, CTC Analytics) with capacity for 32 20-mL headspace vials composed of an oven for sample heating/headspace generation and a robotic arm where the headspace syringe was placed. For the fiber injection a split open deactivated insert liner of 5 mm OD x 54 mm x 3.4 mm ID (Varian Inc., Walnut Creek, California, USA) was placed inside the GC injection port. The column oven temperature program is described in 2.3.1.

2.3.3. Mass Spectrometry

The GC was interfaced with a model 300-MS triple quadrupole mass spectrometer (Varian Inc. Walnut Creek, California, USA) operating in an electron ionization mode (EI, 70 eV). A filament current of 50 µA and a multiplier voltage of 1300 V were used in MS/MS mode. The temperatures of the transfer line, ion source and manifold were set at 280 °C, 250 °C and 40 °C, respectively. A filament multiplier delay of 6.4 min was fixed in order to prevent instrument damages. The mass spectrometer was calibrated as needed with Perfluorotributylamine (PFTBA). For the MS/MS experiments Ar was used as a collision gas and the collision cell pressure was set at 1.80 mTorr. Multiple reaction monitoring (MRM) conditions were experimentally developed for each individual pesticide on the instrument used in this work. Precursor and product ions, collision energies and other parameters used are shown in Table 1.

For instrument control, data acquisition and evaluation Varian MS Workstation, version 6.9 was used.

2.4. Optimization of MS/MS method

To set the MRM method, precursor ions, product ions, and collision energy were studied for each individual analyte. The most intense transition was used as quantifier (Q), while the other transition(s) were used as qualifier (q) peak(s) for the confirmatory analysis. Precursor and product ions, collision energies and other parameters used in the developed GC-MS/MS method are shown in Table 1. Three MRM transitions were used for pesticides, while for PAHs only two transitions were selected due to their difficulty to yield useful fragmentation. For atrazine desethyl and diazinon the selected precursor ions for quantifier and qualifier transitions were different.

Table 1. Optimized MS/MS transitions of the target compounds, including retention time, dwell time and collision energy.

t _R (min)	Window (min)	Compounds	Precursor ion (m/z)	Product ion (m/z)	Type of transition Q, q ₁ , q ₂	Dwell time (s)	Collision Energy (eV)
7.048	6.50-9.20	1,3,5-TCB	181.8	146.8	Q	0.042	20
			181.8	108.8	q ₁	0.042	30
			181.8	110.9	q ₂	0.042	25
7.834		1,2,4-TCB	181.8	108.8	Q	0.042	30
			181.8	146.8	q ₁	0.042	20
			181.8	110.9	q ₂	0.042	25
8.313		Hexachlorobutadiene	224.9	189.8	Q	0.042	20
			224.9	154.9	q ₁	0.042	25
			224.9	152.7	q ₂	0.042	45
8.372		1,2,3-TCB	181.8	108.8	Q	0.042	30
			181.8	146.8	q ₁	0.042	20
			181.8	110.9	q ₂	0.042	25
8.560		Isoproturon	161.1	146.1	Q	0.042	10
			161.1	128.0	q ₁	0.042	20
			161.1	91.0	q ₂	0.042	30
8.723		Chlorlitoluron	167.0	132.0	Q	0.042	10
			167.0	77.0	q ₁	0.042	30
			167.0	104.0	q ₂	0.042	20
9.795	9.20-10.50	Diuron	186.9	123.9	Q	0.167	20
			186.9	158.8	q ₁	0.167	10
			186.9	96.8	q ₂	0.167	35
11.909	10.50-12.20	Acenaphthylene	152.0	152.1	Q	0.100	10
			152.0	151.1	q ₁	0.100	15
12.595	12.20-	Pentachlorobenzene	249.8	214.8	Q	0.100	20

t_R (min)	Window (min)	Compounds	Precursor ion (m/z)	Product ion (m/z)	Type of transition Q, q ₁ , q ₂	Dwell time (s)	Collision Energy (eV)
	14.00		249.8	142.0	q ₁	0.100	40
			249.8	107.8	q ₂	0.100	50
13.682		Fluorene	166.0	165.1	Q	0.100	10
			166.0	166.0	q ₁	0.100	10
14.597	14.00- 15.10	Trifluralin	306.1	264.0	Q	0.083	10
			306.1	206.0	q ₁	0.083	15
			306.1	159.8	q ₂	0.083	25
14.715		Atrazine desethyl	172.0	105.0	Q	0.083	10
			187.0	172.0	q ₁	0.083	10
			172.0	94.0	q ₂	0.083	15
15.528	15.10- 16.00	α -HCH	218.9	182.8	Q	0.083	10
			218.9	146.9	q ₁	0.083	25
			218.9	108.8	q ₂	0.083	35
15.612		Hexachlorobenzene	283.8	248.7	Q	0.083	20
			283.8	141.8	q ₁	0.083	50
			283.8	178.7	q ₂	0.083	50
16.340	16.00- 17.40	Simazine	201.0	173.0	Q	0.033	10
			201.0	186.1	q ₁	0.033	10
			201.0	138.0	q ₂	0.033	15
16.504		Atrazine	215.1	57.9	Q	0.033	10
			215.1	200.1	q ₁	0.033	10
			215.1	138.1	q ₂	0.033	15
16.633		Propazine	229.0	58.0	Q	0.033	15
			229.0	187.1	q ₁	0.033	10
			229.0	214.1	q ₂	0.033	10
16.785		β -HCH	218.9	182.8	Q	0.033	10
			218.9	144.8	q ₁	0.033	25
			218.9	108.9	q ₂	0.033	35
16.949		γ -HCH	218.9	182.8	Q	0.033	10
			218.9	144.8	q ₁	0.033	25
			218.9	108.9	q ₂	0.033	35
17.125		Terbutylazine	214.1	132.0	Q	0.033	10
			214.1	136.0	q ₁	0.033	10
			214.1	119.0	q ₂	0.033	10
17.312		Diazinon	304.0	179.1	Q	0.033	15
			179.0	137.0	q ₁	0.033	15
			179.0	122.0	q ₂	0.033	25
17.665	17.40- 18.20	Phenantrene	178.0	178.1	Q	0.250	10
			178.0	152.1	q ₁	0.250	15
17.960		Anthracene	178.0	178.1	Q	0.250	10
			178.0	152.1	q ₁	0.250	15
18.593	18.20- 19.50	δ -HCH	218.9	182.8	Q	0.167	10
			218.9	144.8	q ₁	0.167	25
			218.9	108.9	q ₂	0.167	35
20.093	19.50-	Alachlor	188.1	160.0	Q	0.050	10

t _R (min)	Window (min)	Compounds	Precursor ion (m/z)	Product ion (m/z)	Type of transition Q,q ₁ ,q ₂	Dwell time (s)	Collision Energy (eV)
20.80			188.1	131.0	q ₁	0.050	20
			188.1	132.0	q ₂	0.050	15
20.113		Parathion methyl	263.0	109.11	Q	0.050	10
			263.0	127.1	q ₁	0.050	10
			263.0	246.0	q ₂	0.050	5
20.357		Heptachlor	272.0	237.0	Q	0.050	15
			272.0	272.1	q ₁	0.050	5
			272.0	143.0	q ₂	0.050	35
20.549		Ametryn	227.0	185.1	Q	0.050	10
			227.0	212.1	q ₁	0.050	10
			227.0	58.0	q ₂	0.050	10
21.236	20.80- 21.50	Terbutryn	241.1	185.1	Q	0.167	10
			241.1	170.0	q ₁	0.167	15
			241.1	111.2	q ₂	0.167	25
21.875	21.50- 22.70	Chlorpyrifos ethyl	314.0	258.0	Q	0.056	15
			314.0	286.0	q ₁	0.056	10
			314.0	314.0	q ₂	0.056	5
21.917		Aldrin	262.8	192.7	Q	0.056	30
			262.8	190.8	q ₁	0.056	35
			262.8	228.0	q ₂	0.056	20
22.263		Parathion ethyl	291.0	109.0	Q	0.056	10
			291.0	81.0	q ₁	0.056	30
			291.0	142.0	q ₂	0.056	5
23.140	22.70- 24.50	Isodrin	262.8	192.7	Q	0.056	30
			262.8	190.8	q ₁	0.056	30
			262.8	228.0	q ₂	0.056	20
23.286		Chlorfenvinphos A	323.0	267.0	Q	0.056	10
			323.0	295.0	q ₁	0.056	10
			323.0	159.0	q ₂	0.056	30
23.705		Chlorfenvinphos B	323.0	267.0	Q	0.056	10
			323.0	295.0	q ₁	0.056	10
			323.0	159.0	q ₂	0.056	30
24.089		Procymidone	283.0	95.5	Q	0.056	10
			283.0	67.0	q ₁	0.056	20
			283.0	255.1	q ₂	0.056	10
24.975	24.50- 26.40	Pyrene	202.1	202.0	Q	0.037	10
			202.1	201.2	q ₁	0.037	10
24.984		α-endosulfan	338.8	160.1	Q	0.037	15
			338.8	194.9	q ₁	0.037	10
			338.8	230.9	q ₂	0.037	10
25.860		4,4'-DDE	318.0	246.0	Q	0.037	15
			318.0	248.0	q ₁	0.037	10
			318.0	283.0	q ₂	0.037	10
26.006		Dieldrin	262.8	192.7	Q	0.037	30
			262.8	190.8	q ₁	0.037	35

t_R (min)	Window (min)	Compounds	Precursor ion (m/z)	Product ion (m/z)	Type of transition Q, q ₁ , q ₂	Dwell time (s)	Collision Energy (eV)
26.152		Oxyfluorfen	262.8	227.9	q ₂	0.037	15
			361.0	300.0	Q	0.037	10
			361.0	317.0	q ₁	0.037	10
			361.0	252.0	q ₂	0.037	15
26.755	26.40- 28.85	Endrin	262.8	192.7	Q	0.033	35
			262.8	190.8	q ₁	0.033	35
			262.8	228.0	q ₂	0.033	20
27.198		β -endosulfan	338.8	266.7	Q	0.033	10
			338.8	160.0	q ₁	0.033	15
			338.8	230.8	q ₂	0.033	10
27.339		Ethion	231.0	129.0	Q	0.033	25
			231.0	157.0	q ₁	0.033	15
			231.0	185.0	q ₂	0.033	10
28.526		Endosulfan sulfate	386.8	252.9	Q	0.033	10
			386.8	288.9	q ₁	0.033	10
			386.8	205.7	q ₂	0.033	35
28.646		4,4'-DDT	235.0	164.9	Q	0.033	25
			235.0	200.0	q ₁	0.033	10
			235.0	199.0	q ₂	0.033	15
30.030	28.85- 30.70	Iprodione	314.0	245.0	Q	0.062	10
			314.0	271.0	q ₁	0.062	10
			314.0	55.9	q ₂	0.062	20
30.320		Benzo[a]anthracene	228.0	228.0	Q	0.062	10
			228.0	226.0	q ₁	0.062	25
30.466		Chrysene	228.0	228.0	Q	0.062	10
			228.0	226.0	q ₁	0.062	25
30.507		Metoxychlor	227.0	169.0	Q	0.062	25
			227.0	212.1	q ₁	0.062	10
			227.0	184.0	q ₂	0.062	15
34.698	30.70- 37.50	Benzo[b]fluoranthene	252.0	252.0	Q	0.250	10
			252.0	250.0	q ₁	0.250	30
34.810		Benzo[k]fluoranthene	252.0	252.0	Q	0.250	10
			252.0	252.0	q ₁	0.250	30
35.987		Benzo[a]pyrene	252.0	252.0	Q	0.250	10
			252.0	252.0	q ₁	0.250	30
38.378	37.50- 39.50	Deltamethrin	252.9	93.0	Q	0.167	20
			252.9	174.0	q ₁	0.167	10
			252.9	90.9	q ₂	0.167	25
40.367	39.50- 43.33	Indene[1,2,3-cd]pyrene	276.0	276.0	Q	0.125	10
			276.0	274.0	q ₁	0.125	40
40.520		Dibenzo[a,h]anthracene	278.0	278.0	Q	0.125	10
			278.0	250.0	q ₁	0.125	45
41.326		Benzo[g,h,i]perylene	276.0	276.0	Q	0.125	10
			276.0	274.0	q ₁	0.125	40

To confirm peak identity in samples, retention time and the Q/q ratio criterion were used, defined as the ratio between the intensity of the quantification transition (Q) and the confirmation transitions (q1 and q2). Firstly, the theoretical average Q/q1 and Q/q2 for each compound (only one ratio available for PAHs) were calculated as the mean value obtained from the chromatographic analysis of standard solutions. The identity of a peak was confirmed by comparison of the experimental ratio in the sample with the theoretical ratio of the reference standard, considering the percentage of variability (tolerance) established in the Decision 2002/657/EC [34], based on ion-ratio statistics for the transitions monitored. As an example, Figure 1 shows the identification of hexachlorobenzene in a wastewater sample spiked at a concentration level of 50 ng L⁻¹ for the different sample treatment methods tested.

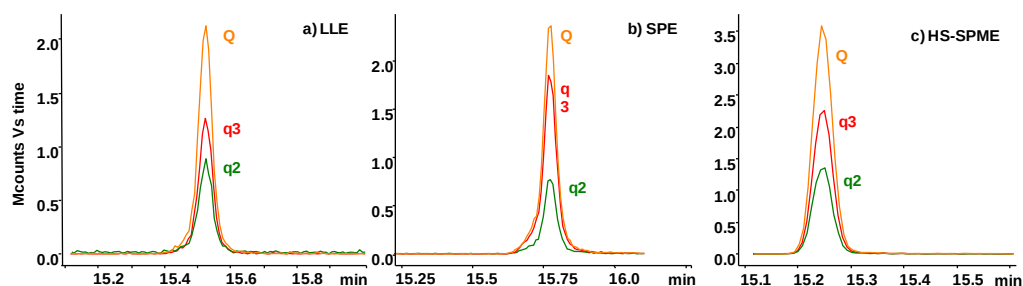


Figure 1. Identification of hexachlorobenzene by GC-MS/MS in a wastewater sample spiked at a concentration level of 50 ng L⁻¹ a) after LLE with n-hexane; b) after the application of the proposed SPE procedure; c) HS-SPME analysis.

3. Results and discussions

3.1. Evaluation of the selected extraction procedures

The three sample treatment methodologies tested were: a) LLE with n-hexane; b) SPE with C₁₈ cartridges and elution with ethyl acetate:dichloromethane (1:1 (v/v)), and c) HS-SPME using two different fibers: PA and (DVB/CAR/PDMS). The comparative study is summarized in Table 2.

Table 2. Comparative study of sample treatment methodologies for the determination of 57 organic pollutants by GC-MS/MS.

	LLE	SPE	HS-SPME (PA Fiber Results)
Number of measurable compounds	57	57	43
Treatment + Analysis time per sample	≈ 75 min	≈ 55 min	≈ 60 min*
Suspended particulate matter subject to analysis?	<u>Yes</u>	<u>Not</u>	<u>Yes (although only for relatively volatile species)</u>
Solvents, consume per sample	76 mL n-hexane	12 mL MeOH 14 mL EtAc+DCM (1:1)	1mL MeOH
LOQs	0.03 – 5.10 ng L ⁻¹	0.03 – 5.00 ng L ⁻¹	0.10 – 150.00 ng L ⁻¹
Accuracy (Recovery rates)	70-110 %	70-120%	73-119%
Precision (RSD %)	3-15 %	1-10 % in most cases	7-20%
Linearity (r ²)	1-500 ng L ⁻¹ (At least 0.999 for most compounds)	1-500 ng L ⁻¹ (At least 0.999 for most compounds)	5-50 ng L ⁻¹ (At least 0.991 for most compounds)

EtAc : ethyl acetate; DMC : dichloromethane

* Overlapped mode: injection of sample “n” + time incubation of sample “n+1”

3.1.1. Method scope

The main goal of the proposed study was to evaluate which of these three methodologies was more convenient for the large-scale testing of multiclass organic contaminants in wastewater samples. First, it should be noted that wastewater influents are usually composed by two phases: aqueous phase and suspended solids. A relevant aspect to consider in an extraction method is the ability to extract contaminants from both the whole water, not only the dissolved fraction but also the suspended solids fraction. However, most of the analytical methods described in the literature for the analysis of organic contaminants in wastewater rely on the examination of the aqueous phase obtained after sample filtration, disregarding thus the solid particles retained on the filters [35]. Depending on the physicochemical properties of the analytes (Water solubility, octanol/water partition coefficient ($\log K_{ow}$)), each analyte exhibits a specific partitioning behaviour between the two phases. This fact made clear the relevance of the analysis of both phases in wastewater samples. Otherwise, an important contribution of selected contaminants may be underestimated. According to the

current regulations (EQS) [2], solid suspended particles should be considered since the whole water (dissolved + suspended solids bound fraction) must be included in the analyses.

In this sense, while LLE with solvent (hexane) permits the thorough extraction of the entire water sample (including solid suspended material), SPE usually require a filtration prior to sample load in order to prevent clogging of cartridge, this fact being particularly important for high preconcentration factors or with complex matrices. Thus, part of the contaminants present in wastewater may be underestimated. In the case of HS-SPME, due to the complexity of the matrix and because of the suspended particulate solids, the method of choice must be HS-SPME instead of direct immersion SPME. This means that the entire unfiltered sample is subjected to analyses. In contrast, the use of HS-SPME probably along with the low volatility and behavior according to Henry's law of selected analytes offers severe drawback in terms of analyte coverage (14 of the studied compounds could not be recovered and detected). Given these data, HS-SPME was found as less versatile or at least not suited to cover such a wide variety of chemicals.

From the point of view of analyte coverage, both LLE and SPE are definitely more versatile than HS-SPME. Either with the choice of an apolar solvent (such as hexane) or a combination of two solvents to tune the extraction towards more polar compounds are effective and straightforward approaches. On the other hand, SPE with C₁₈ and a careful selection of eluting solvents enabled similar recovery rates that LLE, but with the clear advantages of higher automation and lower solvent consumption. The main drawback associated with SPE is the limitation related to solid particles and the need of filtering the sample, which makes that part of the contaminants present in wastewater may be underestimated.

With regards to HS-SPME method, the choice of fiber was done considering that different chemical classes should be analyzed within a run. Under optimized values for HS-SPME variables (sample volume, heating temperature, incubation time, magnetic stirring rate, desorption time and the addition of modifiers were studied), higher signals was obtained in most cases using PA fiber. In addition, PAHs and organochlorine pesticides were strongly retained in the DVB/CAR/PDMS fiber, causing carry-over/memory effects, cross contamination and

reducing the lifetime of the fiber. Therefore, PA fiber was selected as optimum for the target analysis of this application. The main advantages of HS-SPME are the low consumption of organic solvents (only 1 mL of MeOH used as additive in each sample) and the low required volume of sample (10 mL instead of 200 mL for either LLE or SPE). The main disadvantage compared to the other studied methodologies is the limited number of species detected. Fourteen compounds were not conveniently detected: alachlor, ametryn, atrazine, atrazine desethyl, chlorotoluron, deltamethrin, endosulfan sulphate, heptachlor, iprodione, isoproturon, parathion methyl, propazine, simazine, and terbutryn. In contrast, all target compounds were effectively extracted using either LLE or SPE, and consequently detected by GC-MS/MS.

3.1.2. Extraction efficiency: recovery rates

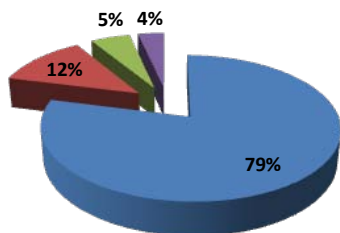
To evaluate the effectiveness of the extraction using the studied LLE and SPE extraction procedures, recovery studies ($n = 6$) were carried out after spiking wastewater samples at two concentration levels, 15 and 150 ng L⁻¹. Results are detailed in Table 2, showing that both LLE and SPE in general showed similar results. At both concentration levels tested, about 60% of the studied analytes showed recoveries in the range 70-120%, and over 85% of the studied analytes showed recoveries at least of 50% (see Figure 2). However, if the discussion is focused on the results by chemical class (see table 3), it is possible to notice that a proper extraction process choice make the difference.

It is clear that SPE is the extraction process of choice for the triazine group, it showed recoveries above 90 % for all compounds in both fortification levels. In contrast, 4 out of 7 triazines gave recovery values below 60% when LLE process was applied. The extraction of these compounds is not affected by the filtration of the sample, but by the more polar solvents employed in SPE process. Similar behavior showed the phenylurea pesticides, with poor recoveries in all cases when LLE process is used. Regarding PAH compounds, at the lowest fortification level, LLE process presented in all cases better results than SPE. While recoveries values are very similar (slightly better for LLE) when comparing results at the highest fortification level. This behavior suggests that part of the PAHs is bonded to the suspended solid and this fact is of more importance at low concentration levels. Similar conclusion can be achieved for

OCP, with better results for LLE at the lowest fortification level for most compounds, with recovery values below 70% for almost all of them. While slight differences are observed at the highest fortification level among both extraction processes. In the case of OPP good recovery rates (above 70% in most cases) are obtained with both extraction processes.

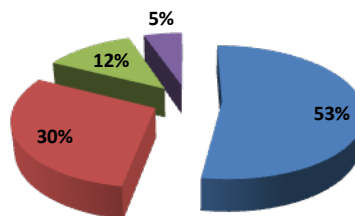
Liquid-Liquid Extraction (15 ng L⁻¹)

■ >70 % ■ 50-70 % ■ 30-50 % ■ <30 %



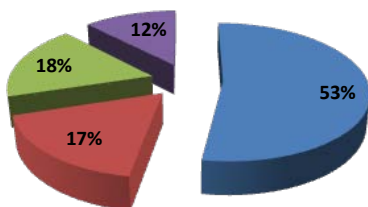
Liquid-Liquid Extraction (150 ng L⁻¹)

■ >70 % ■ 50-70 % ■ 30-50 % ■ <30 %



Solid-Phase Extraction (15 ng L⁻¹)

■ >70 % ■ 50-70 % ■ 30-50 % ■ <30 %



Solid-Phase Extraction (150 ng L⁻¹)

■ >70 % ■ 50-70 % ■ 30-50 % ■ <30 %

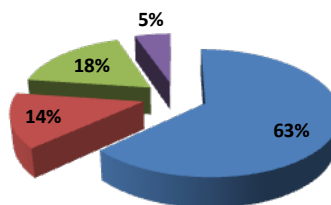


Figure 2. Comparison between recovery studies obtained by LLE and SPE methods at both 15 and 150 ng/L spiking levels.

In the case of HS-SPME, the study was performed at 25 ng L⁻¹ (n = 3). In this case, mean recoveries ranging between 73% and 119% were obtained for the (43) detected analytes. However, it should be emphasized that in this type of microextraction techniques only a fraction of the analytes contained in the sample is recovered. SPME is a non-exhaustive extraction technique unlike SPE or LLE. For this reason, relative recoveries (using spiked-water standards as reference) were used instead for HS-SPME, since absolute recoveries are more appropriately used for exhaustive extraction techniques.

Table 3. Recovery studies of LLE (n=6), SPE (n=6) and HS-SPME (n=3) methods.

Compound	Chem. class*	LLE Fortification levels (ng L ⁻¹) and RSD(%)				SPE Fortification levels (ng L ⁻¹) and RSD(%)				HS-SPME Fortification level (ng L ⁻¹) and RSD(%)	
		15	RSD (%)	150	RSD (%)	15	RSD (%)	150	RSD (%)	25	RSD (%)
Ametryn	TrzP	47.2	19.4	33.5	11.6	90	13.3	93	4.9	--	--
Atrazine	TrzP	74.5	6.1	53.9	9.7	108	3.9	100	8.2	--	--
Atrazine desethyl	TrzP	52.1	17	44.4	19.6	95	0.3	117	5.8	--	--
Propazine	TrzP	120.1	3	82.1	7.3	94	6	101	5.8	--	--
Simazine	TrzP	--	--	25.2	15.4	--	--	94	9.9	--	--
Terbutylazine	TrzP	139	3.8	88.8	9.2	93	9	103	8.6	86.25 ^a	4.32
Terbutryn	TrzP	104.2	5.1	89.1	15.4	97	14.3	94	5.6	--	--
Chlorotoluron	PhUP	54.9	7.3	20	17.5	98	7.5	120	3.6	--	--
Diuron	PhUP	54	12.6	17.5	21.1	85	13.1	130	5.1	83.06	14.89
Isoproturon	PhUP	42	8.5	29.8	13.8	97	6.9	118	2.7	--	--
Deltamethrin	PEP	--	--	32.1	22.4	50	14.1	54	4.8	--	--
Chlorfenvinphos A	OPP	154.3	8.7	68.8	8.4	99	7	118	11.7	79.75	12.26
Chlorfenvinphos B	OPP	100.1	5.7	96.6	9.4	98	6	107	2.7	73.32	2.08
Chloropyrifos ethyl	OPP	104.6	16.4	73.8	18.1	73	11.3	74	9	118.68	6.72
Diazinon	OPP	112	5.2	90.5	10.4	89	12.7	88	9.2	123.82	5.38
Ethion	OPP	74.5	4.8	66.6	22.6	70	10	77	8.3	100.84	3.09
Parathion ethyl	OPP	112.4	5.1	100.9	8.4	112	9.8	100	2.6	89.04	12.55
Parathion methyl	OPP	101.7	3.7	102.4	10.1	79	15.3	97	5.7	--	--
Aldrin	OCP	102.1	7.3	62.1	17.8	37	14.4	30	14.3	102.57	9.42
4,4'-DDE	OCP	103.7	6.9	69.4	19.2	36	6.3	47	11.6	105.81	7.8
4,4'-DDT	OCP	93.9	9.3	69.4	18.5	48	5.9	68	7.2	102.86	6.89
Dieldrin	OCP	88.8	8.2	76.3	7.1	48	7.9	70	6.1	100.93	4.76
α-Endosulfan	OCP	175.1	7.5	85.6	8.7	--	--	74	7.2	98.71	6.94
β-Endosulfan	OCP	119.8	8.8	75.6	9.9	89	6.2	82	21.5	91.35	7.5
Endosulfan sulfate	OCP	111.3	6	69.2	18.2	110	9.8	96	6.6	--	--
Endrin	OCP	98.9	13.7	81.1	11.6	75	10	82	8.3	97.38	8.83
Heptachlor	OCP	115	5.7	67.1	11.6	49	15	37	6.3	--	--
Hexachloro-1,3-butadiene	OCP	52	15.3	31.6	19	16	6.3	18	14	104.07	1.39
Hexachlorobenzene	OCP	96.5	3.1	73.9	12.4	40	9.4	40	13.7	107.98	4.55
α-HCH	OCP	107	5.8	93	5.2	71	7	81	10.4	101.19	1.74
β-HCH	OCP	164.1	7.8	112.7	14.8	99	7.4	120	8.1	99.75	8.56
γ-HCH	OCP	133.8	3.7	93.7	13	90	8.7	91	9.9	95.53	13.92
δ-HCH	OCP	94.9	10.8	101.2	12.3	80	1.8	91	8.6	93.25 ^a	8.65
Isodrin	OCP	103.9	5.2	73	12.5	38	11.4	39	8.8	101.87	7.13
Metoxychlor	OCP	94.2	1.4	78.9	17.6	74	7	89	6.1	100.04	5.28
Pentachlorobenzene	OCP	105.4	7.1	72.9	9.2	30	1.6	36	10.8	106.46	2.08
1,2,3-TCB	OCP	76.1	10.9	52.5	9.9	27	10	33	16.3	102.34	4.34
1,2,4-TCB	OCP	88.2	9.9	45.8	9.5	30	10.2	27	10.9	104.27	3.55
1,3,5-TCB	OCP	77.3	8.1	43	15.6	26	6.4	20	15.5	116.29	4.17
Oxyfluorfen	NPhEP	142.3	7.4	90.1	18	111	3.1	88	9.6	82.35	8.13
Trifluralin	DnaP	102.6	4.6	82.7	1	57	6.6	57	7.7	101.39	8.38
Iprodione	DcDP	51.7	13.9	56.5	7.7	100	13.6	120	6.1	--	--
Procymidone	DcDP	125.4	5	84.4	9.1	76	8.5	93	5.7	95.62	5.36
Alachlor	ChaP	136.6	2.7	92.7	12.2	82	8	96	9.7	--	--

Compound	Chem. class*	LLE Fortification levels (ng L ⁻¹) and RSD(%)				SPE Fortification levels (ng L ⁻¹) and RSD(%)				HS-SPME Fortification level (ng L ⁻¹) and RSD(%)	
		15	RSD (%)	150	RSD (%)	15	RSD (%)	150	RSD (%)	25	RSD (%)
Acenaphthylene	PAH	102	4.1	88.2	6.7	39	8.5	50	9.5	103.52	0.18
Anthracene	PAH	140.7	3.7	84.3	7.9	63	7.8	70	6.1	101.07	5.91
Benzo[a]anthracene	PAH	85.3	7.4	62.2	16.2	64	6.3	77	5.5	105.1	1.29
Benzo[a]pyrene	PAH	71.3	7.4	57.6	15.4	59	6.2	53	4.1	107.23	5.89
Benzo[b]fluoranthene	PAH	61.6	7.4	64.4	20.4	58	11	66	5.5	93.34	7.38
Benzo[ghi]perylene	PAH	132.4	1.6	52.1	6.8	54	15.1	49	4.5	100.89	6.68
Benzo[k]fluoranthene	PAH	63	7.1	63.7	14.6	45	14.5	63	5.3	98.49	2.3
Chrysene	PAH	98.9	6.1	86.5	13.5	60	10.1	75	4.6	105.31	0.98
Dibenzo[a,h]anthracene	PAH	76.8	4.1	54.5	11.9	50	18.1	48	4.1	97.51	7.94
Fluorene	PAH	123.4	3	79.4	12.8	48	12.8	57	9.1	106.07	0.97
Indene[1,2,3-cd]pyrene	PAH	75.7	4.8	54	11.6	63	3.6	46	2.8	105.07	18.09
Phenanthrene	PAH	108.1	5.8	74.9	14.1	70	6.5	71	10.3	111.32	7.19
Pyrene	PAH	43	20.2	70.9	13.4	73	11.2	75	8.9	104.21	4.89

^a Recovery studies carried out at a concentration level of 150 ng L⁻¹ (n=3)

*Description of the acronyms: TrzP-Triazines pesticides, PhuP-Phenylureas pesticides, PEP-Pyrethroid ester pesticides, OPP- Organophosphate pesticides, OCP-Organochlorine pesticides, NPhEP-Nitrophenyl ether pesticides, DnaP-Dinitroaniline pesticides, DnaP- Dinitroaniline pesticides, DcDP- Dichlorophenyl dicarboximides pesticides, ChAP-Chloroacetanilide pesticides, PAH- Polycyclic aromatic hydrocarbon.

3.1.3. Automation, solvent consumption and waste generation

As included in Table 2, from the point of view of solvent consumption and waste generation, HS-SPME offers clear advantages against both SPE and LLE as being the more environmentally friendly approach [36], using only 1 mL of MeOH as chemical modifier. The solvent consumption is negligible compared to SPE and especially to LLE. In the two latter techniques, the selected sample volume was 200 mL. Considering a preconcentration factor of 200:1, the final volume of the extract is 1 mL. Working with lower volumes (ej. 0.5 mL with 100 mL of loaded sample) was discarded because some of the matrices obtained were so complex than sometimes emulsions were obtained and a dedicated filtration of the final extracts through membrane filters was necessary. The use of SPE involves 34% reduction in organic solvent consumption compared to LLE, and is less time-consuming than both LLE and HS-SPME. Finally, the sensitivity offered by both LLE and SPE is pretty similar (36 analytes showed LOQs below 1 ng L⁻¹ in the case of SPE, while 32 analytes showed LOQs below 1 ng L⁻¹ in the case of LLE).

3.2. Analytical performance of GC-MS/MS methods

The linearity of the calibration curves was studied using matrix-matched standard solutions containing the target compounds at six concentration levels in the range from 1 to 500 ng L⁻¹. The response was found to be linear with a regression coefficient (R^2) higher than 0.999 in the range tested for most of studied organic pollutants when either LLE or SPE procedures were used. In the case of HS-SPME, results were not as satisfactory with calibration curves only linear in the range 5 – 50 ng L⁻¹ for most of studied compounds, although several analytes were linear up to 150 ng L⁻¹, obtaining values of at least 0.991 for regression coefficients.

Table 4. Limits of quantitation for the determination of 57 organic pollutants by GC-MS/MS using the three studied sample treatment methodologies.

Compound	LOQ (ng L ⁻¹)		
	LLE	SPE	HS-SPME
Acenaphthylene	0.2	0.3	5.0
Alachlor	2.7	2.6	--
Aldrin	0.3	0.2	5.0
Ametryn	2.7	2.7	--
Anthracene	1.0	1.0	24.8
Atrazine desethyl	1.2	1.4	--
Atrazine	4.5	2.5	--
Benzo(a)anthracene	0.7	0.2	24.8
Benzo(a)pyrene	0.2	0.4	24.8
Benzo(b)fluoranthene	0.6	0.3	24.8
Benzo(ghi)perylene	0.4	0.3	5.0
Benzo(k)fluoranthene	0.3	0.2	24.8
Chlorfenvinfos A	2.2	2.4	2.6
Chlorfenvinfos B	3.2	2.7	14.9
Chloropyrifos ethyl	0.9	0.8	0.7
Chlorotoluron	1.3	2.6	--
Chrysene	1.6	0.2	24.8
Deltamethrin	2.8	4.1	--
Diazinon	0.03	0.03	0.1
Dibenzo(a,h)anthracene	3.4	0.5	5.0
4,4'-DDE	1.1	0.3	24.8
4,4'-DDT	0.6	0.6	5.0
Dieldrin	0.4	5.0	5.0
Diuron	0.3	0.3	24.8
α -Endosulfan	0.9	99.0	24.8
β -Endosulfan	0.3	9.9	24.8
Endosulfan sulfate	0.8	0.2	--
Endrin	5.0	4.1	5.0
Ethion	5.0	0.6	1.0

Compound	LOQ (ng L ⁻¹)		
	LLE	SPE	HS-SPME
Fluorene	0.2	0.2	5.0
Heptachlor	0.03	0.03	--
Hexachloro-1,3-butadiene	0.3	0.7	1.0
Hexachlorobenzene	0.2	0.4	5.0
α -HCH	0.3	0.3	1.0
β -HCH*	0.5	0.8	5.0
γ -HCH	0.4	0.6	5.0
δ -HCH	0.4	0.6	148.5
Indene(1,2,3-cd)pyrene	0.4	0.3	5.0
Iprodione	0.2	1.5	--
Isodrin	2.0	2.7	24.8
Isoproturon	2.2	2.7	--
Metoxychlor	0.2	0.6	5.0
Oxyfluorfen	0.4	0.0	0.7
Parathion ethyl	2.7	3.1	24.8
Parathion methyl	2.5	2.5	--
Pentachlorobenzene	0.03	0.1	5.0
Phenanthrene	0.9	0.7	24.8
Procymidone	3.0	0.4	24.8
Propazine	3.2	2.1	--
Pyrene	0.4	0.6	24.8
Simazine	5.1	5.1	--
Terbutylazine	2.0	1.1	148.5
Terbutryn	1.4	1.4	--
1,2,3-TCB	1.7	1.9	5.0
1,2,4-TCB	2.2	2.7	5.0
1,3,5, TCB	1.8	2.7	5.0
Trifluralin	0.2	0.2	0.7

Limit of quantitation (LOQ) was estimated as the analyte concentration that produces a peak signal of ten times the background noise from the chromatogram at the lowest calibration

level as low as it was possible using the q_2 transition. The proposed method showed LOQs in the low ng L⁻¹ range, although the results were better for both SPE and LLE when compared with HS-SPME. Results are detailed in Table 4. In terms of sensitivity, LLE is similar to SPE, obtaining LOQs in the range 0.03 – 5.00 ng L⁻¹ for both methodologies. However, 14 compounds were not effectively detected by HS-SPME. The main reasons are their low volatility and affinity towards the selected SPME fiber. For the rest of compounds detected, HS-SPME offered LOQs in the range 0.1 – 25 ng L⁻¹, except for terbuthylazine and δ -HCH, which showed LOQ of 150 ng L⁻¹.

A precision study was carried out by calculating the relative standard deviation (RSD) from the analysis of six replicates of fortified wastewater at 15 and 150 ng L⁻¹ concentration levels for LLE and SPE. Nevertheless, in HS-SPME, 25 ng L⁻¹ (three replicates) was the selected level for HS-SPME because all extractable compounds, except terbuthylazine and δ -HCH, could be measured at this level. No higher concentration was chosen for this method, due to the lack of linearity for most compounds beyond 150 ng L⁻¹. Relative standard deviation values were in most cases in the range 3-15% when LLE was used, while using SPE procedure RSD (%) was ranging 1-10% and values between 7 and 15% were found when HS-SPME methodology was applied (Table 3).

4. Conclusions

In the present work, three sample treatment methodologies have been evaluated and compared for analysis by GC-MS/MS of organic pollutants included in the list of priority substances of WFD. LLE with n-hexane and SPE using with C₁₈ cartridges and ethyl acetate:dichloromethane (1:1 (v/v)) as eluent showed recoveries in the range 70-120% for the majority of the studied compounds. Linearity and precision were evaluated, obtaining satisfactory results for all the three sample treatment methodologies tested. The limits of quantitation obtained for most of the target compounds were in the low nanogram per litre range, using any of the three procedures tested. LLE and SPE were revealed as the most sensitive methods, but SPE is less time consuming and more environmentally friendly than LLE in terms of solvent consumption, although species that may be retained in suspended solids would be out

of the scope of the method. Nevertheless, it has been demonstrated that depending on the chemical class, the treatment sample process of choice would be either SPE or LLE.

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*IV.4. DESARROLLO DE UNA BASE DE DATOS DE MASA EXACTA
PARA EL MONITOREO DE CONTAMINANTES PRIORITARIOS Y
EMERGENTES EN AGUAS MEDIANTE LC-TOFMS*

V.2.1. Introducción

Sin duda alguna el avance tecnológico ha hecho que la vida para los humanos sea más segura, duradera y confortable. Pero también ha dado lugar a otros problemas como un aumento considerable de la contaminación. En el mundo se conocen alrededor de 70 millones de sustancias químicas de las cuales alrededor de 100.000 se usan habitualmente en la vida diaria, tanto en hogares, industrias como agricultura (Primel 2012). Estos químicos se han introducido principalmente en los últimos 100 años sin tener en cuenta los posibles efectos adversos sobre el medio ambiente y/o la salud humana. Actualmente el foco de atención de la investigación en muestras medioambientales está centrado en los denominados contaminantes emergentes, que se llaman así porque no se conoce su presencia, concentración o destino y además pueden producir efectos perjudiciales tanto para el medio como para la salud humana.

Hablamos de sustancias como fármacos, productos de higiene personal o productos de degradación de pesticidas. Los medicamentos ingeridos no son totalmente asimilados por el organismo, se excretan y por último acaban en las aguas residuales que se encargan de distribuirlos por el medioambiente. El problema se agrava cuando nos deshacemos de los medicamentos no utilizados o caducados vertiéndolos en el inodoro o depositándolos en el cubo de la basura. Asimismo, los medicamentos administrados a animales o los químicos utilizados en el medio rural, son arrastrados por la lluvia hacia ríos y lagos. Las depuradoras actuales, tanto las convencionales como las avanzadas (con reactores biológicos de membranas), no están diseñadas en general para eliminar este tipo de contaminantes por lo que su destino será ir fluyendo por los distintos sistemas acuáticos con una gran probabilidad de entrar en la cadena alimentaria.

Hasta el momento sólo existen normas de calidad ambiental para un número relativamente bajo de compuestos químicos, conocidos como contaminantes prioritarios, en las aguas superficiales europeas (Directiva 2013/39/UE). Por lo que es de esperar que esta lista se vaya actualizando e incrementando el número y tipo de contaminantes a los que habrá que prestar atención, para que la calidad de las aguas comunitarias sea buena y se asegure que no va a haber efectos nocivos sobre la biota y los seres humanos.

La relevancia creciente del problema abre distintas líneas de investigación, por un lado en cuanto a la actuación en los sistemas de tratamiento de agua residual para conseguir una mayor y más eficiente eliminación de los contaminantes y por otro en lo referente al desarrollo de metodologías analíticas seguras que permitan analizar un elevado número de compuestos de forma sensible y selectiva en muestras medioambientales (Hernández 2012, Farré 2012, Lebedev 2013). En cuanto a las metodologías analíticas, se han desarrollado varias para el análisis de determinadas clases de plaguicidas (**Petrović** 2005, Marín 2009, **Petrović** 2010) y fármacos (Gros 2009, Kuster 2009, Cahill 2011) en aguas tanto superficiales como residuales.

El enfoque analítico a la hora de abordar el análisis de los contaminantes emergentes puede ser diverso. Hay metodologías cuyo desarrollo está basado en la presencia y toxicidad de los mismos (Rodil 2009, Huerta-Fontela 2010). La mayoría de estos métodos utilizan LC-MS/MS como técnica de análisis y monitorizan transiciones MS/MS seleccionadas (molécula original-fragmento) de los compuestos de interés. Aunque es con mucho la modalidad más robusta y sensible para lograr una adecuada cuantificación de los contaminantes, presenta también limitaciones. Por un lado, el número de especies que se pueden incluir en el método es limitado, normalmente en un rango comprendido entre 50 y 150 especies (Huntscha 2012, Gros 2012, **Petrović** 2014), y por otro este tipo de métodos no detectan aquellas sustancias que están en la muestra pero no se han seleccionado para el análisis, por lo que no se puede realizar un análisis retrospectivo de compuestos inesperados o sospechosos de encontrarse en la muestra.

Como alternativa se utilizan los espectrómetros de alta resolución (LC-HRMS) que permiten la adquisición en teoría de un número ilimitado de compuestos en función de su masa exacta ya que realizan medidas en full-scan. Entre las distintas metodologías que se han descrito para el *screening* de contaminantes emergentes se puede hacer una clara distinción entre aquellas que proporcionan datos experimentales utilizando materiales de referencia (m/z y tiempo de retención) y aquellas que utilizan librerías que compilan la información teórica de composiciones elementales de los analitos de interés (Broeker 2011). En la literatura hay descritas metodologías para llevar a cabo el *screening* de cientos hasta miles de especies utilizando tecnología Orbitrap (Pinhancos 2011, Moschet 2013) y QTOF (Martínez-Bueno

2007, Nurmi 2011, Ferrer 2012). Pero desafortunadamente ninguno de estos trabajos lleva a cabo un estudio a fondo del procedimiento de preparación de muestra, como por ejemplo estudios de recuperación.

Por todo lo anterior, en el artículo científico 4 se describe el desarrollo de un método basado en el uso de LC-HRMS para la determinación de aproximadamente 400 contaminantes entre los que se encuentran sustancias prioritarias y emergentes, así como varios de sus productos de degradación. De entre los analitos seleccionados se pueden encontrar varios grupos de interés, como son plaguicidas, fármacos, compuestos perfluorados, drogas de abuso y sustancias mundialmente consumidas por el ser humano como son la cafeína y la nicotina. Se empleó y validó un tratamiento de muestra genérico basado en una SPE, de manera que se ofrecen valores de efecto matriz así como de recuperación para los 430 compuestos evaluados en agua residual.

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IV.4.2. Artículo científico 4

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Multi-residue method for the determination of over 400 priority and emerging pollutants in water and wastewater by solid-phase extraction and liquid chromatography-time-of-flight mass spectrometry

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ABSTRACT

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This article describes the development and validation of a liquid chromatography high-resolution mass spectrometry method for the simultaneous determination of over 400 multi-class priority and emerging pollutants with different physicochemical properties in environmental waters (surface water and wastewater). The proposed approach is based on the use of a database consisting of retention time/exact mass (of selected ions) pairs implemented with specific software for data analysis. The targeted list comprises 430 contaminants belonging to different compound categories, including 105 multiclass pharmaceuticals (analgesics/anti-inflammatories, antibiotics, lipid regulators, β -blockers, antiepileptic/psychiatric ulcer healings, diuretics, hormones and bronchodilators), life-style products (caffeine, nicotine), 21 drugs of abuse and their metabolites, 279 pesticides and some of their more relevant metabolites, nitrosamines, flame retardants, plasticizers and perfluorinated compounds. The proposed approach included a simple offline solid phase extraction (SPE) step using polymeric cartridges (Oasis HLB) with 200 mL of water sample loaded, followed by analysis by rapid resolution liquid chromatography electrospray time-of-flight mass spectrometry (LC-TOFMS) in both positive and negative modes. The identification of the positive findings is accomplished with the data from accurate masses of the target ions along with retention time data and characteristic in-source fragment ions. The overall method performance was satisfactory with limits of quantification lower than 10 ng L⁻¹ for the 44% of studied compounds. Recoveries between 50%–130% were obtained for the 65% of the analytes (281 compounds). Matrix effects occurring with wastewater matrices were also assessed. The developed method was applied to the determination of target analytes in real surface water and wastewater samples.

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Multi-residue method for the determination of over 400 priority and emerging pollutants in water and wastewater by solid-phase extraction and liquid chromatography-time-of-flight mass spectrometry

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Abstract

This article describes the development and validation of a liquid chromatography high-resolution mass spectrometry method for the simultaneous determination of over 400 multi-class priority and emerging pollutants with different physicochemical properties in environmental waters (surface water and wastewater). The proposed approach is based on the use of a database consisting of retention time/exact mass (of selected ions) pairs implemented with specific software for data analysis. The targeted list comprises 430 contaminants belonging to different compound categories, including 105 multiclass pharmaceuticals (*analgesics/anti-inflammatories, antibiotics, lipid regulators, β -blockers, antiepileptic/psychiatrics ulcer healings, diuretics, hormones and bronchodilatadors*), life-style products (caffeine, nicotine), 21 drugs of abuse and their metabolites, 279 pesticides and some of their more relevant metabolites, nitrosamines, flame retardants, plasticizers and perfluorinated compounds. The proposed approach included a simple offline solid phase extraction (SPE) step using polymeric cartridges (Oasis HLB) with 200 mL of water sample loaded, followed by analysis by rapid resolution liquid chromatography electrospray time-of-flight mass spectrometry (LC-TOFMS) in both positive and negative modes. The identification of the positive findings is accomplished with the data from accurate masses of the target ions along with retention time data and characteristic in-source fragment ions. The overall method performance was satisfactory with limits of quantification lower than 10 ng L⁻¹ for the 44% of studied compounds. Recoveries between 50%-130% were obtained for the 65% of the analytes (281 compounds). Matrix effects occurring with wastewater matrices were also assessed. The developed method was applied to the determination of target analytes in real surface water and wastewater samples.

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1. Introduction

The 20th century introduced more than 10⁵ chemicals that are still being used in our everyday life, either in households, industries or agriculture [1]. It is important to notice that although the number of restricted chemicals is increasing constantly, new chemicals are synthesized and introduced to the market at the same time. Thus, it is very probable that new contaminants are constantly ending up into the environment. Thus, the chemical status of the environment is one issue of growing public concern. Particularly, the status of water resources is in the spotlight since the European Water Framework Directive (WFD) entered into force one decade ago [2]. This regulation commits the Member States to establish monitoring programs to identify surface water pollutants and to keep them under control in order to prevent environmental and human health damage. Up to now, EU has set a list of 45 priority compounds to be monitored and kept under certain levels in surface waters [3]. It is a dynamic list changing with the awareness of the persistence in the water cycle -and therefore the possibility of negative effects- of chemical compounds used in our daily life. The list of priority pollutants consists of heavy metals, organohalogenated compounds (mainly pesticides), and polycyclic aromatic compounds (PAHs). On the other hand, the so-called emerging contaminants, which are yet to be regulated, can enter the list of priority substances in future updates. The interest is currently devoted mainly to pharmaceuticals, polar pesticides and personal care products. For instance, the proposal COM (2001) 876 of the EU contained three of the target compounds, diclofenac, 17- α -ethynylestradiol and β -estradiol, but they were finally not included in the Directive 2013/39/EU as priority substances.

The current scenario fosters the development of reliable analytical methods, which enable a wide-scope straightforward, sensitive and selective determination of these priority and emerging pollutants in environmental samples [4-12]. Several methods for the analysis of selected classes of pesticides [13-15] and pharmaceuticals [16-18] in surface waters and wastewater have been developed up to now. There are methods of analysis described in the literature that cover different groups of emerging contaminants, which are chosen principally on the basis of their occurrence and toxicity [19-22]. Most of these methods are developed by LC-

MS/MS [4-29] by monitoring selected MS/MS transitions (precursor-product) of the compounds of interest. This approach is by far the most sensitive and rugged for quantitation purposes of environmental samples at ultratrace levels, but it has two main limitations: the number of compounds usually included in the methods is limited typically to 50-150 species [13-28]. Exceptionally, methods for higher number of species are accomplished at the expense of the number of MS/MS transitions used and the sensitivity/signal intensity [29]. In addition, these LC-MS/MS methods are blind to all species present in the sample except those strictly targeted, so that untargeted analysis or retrospective analysis of unexpected or suspected compounds on formerly analyzed samples is not feasible.

As an alternative, the use of high-resolution mass spectrometers (LC-HRMS) enables the acquisition of a theoretically unlimited number of species by means of accurate mass measurements in full-scan mode. This allows to obtain the elemental composition of acquired ions, useful for identification of targeted and untargeted compounds, metabolites, or transformation products [30,31]. The use of LC-HRMS for screening contaminants in water samples has been extensively reported in the literature, including methods addressing the detection of nearly one hundred to thousands of species [30-44] using either Orbitrap [32-34] or (Q-)TOF technology [35-44]. From these methods described in the literature, a clear distinction should be made between those which provide experimental data with reference standards (actual m/z measurements, retention time) and those which are based on theoretically compiled libraries of elemental compositions without experimental accurate mass data (eg. ion detected, fragment ions, adducts) and retention information included [42]. On the other hand, unfortunately most of these reported screening methods lacks of a thorough sample preparation step performance evaluation (eg. recovery studies) as they are specifically focused on qualitative analysis. In this article, a method based on LC-HRMS is described for the determination of over 400 priority and emerging contaminants, and some of their degradation products in water samples. The proposed approach is based on the use of an empiric database of exact mass measurements/retention time pairs. It comprises 430 priority and emerging compounds of several groups, including pesticides and degradation products, pharmaceuticals and metabolites, perfluorinated compounds (PFCs), drugs of abuse and life-style products (LS) such

as caffeine and nicotine. A comprehensive assessment of matrix effects and recovery studies were carried out for all the 430 tested species in wastewater effluent samples using a generic SPE procedure with a polymeric solid phase.

2. Experimental

2.1. Chemical and reagents

The selected multiclass organic contaminants targeted in this work are shown in Table 1, including priority pollutants regulated by the European WFD. All standards (of quality grade suitable for trace analysis) were purchased from Sigma-Aldrich (Madrid, Spain) and Dr. Ehrenstorfer (Augsburg, Germany). Methanol HPLC grade was from Sigma-Aldrich (Madrid, Spain). Individual stock standard solutions of the targeted compounds were prepared at a default concentration level of 500 mg L⁻¹ in different solvents depending upon compound solubility and stability (MeOH isopropanol, acetonitrile, and/or water in basic or acidic media).. These solutions were stored in a freezer at -20 °C. Working solutions containing between 30-50 compounds were prepared by appropriate dilution of the stock solutions with MeOH at a concentration level of 10 mg L⁻¹. Formic acid was obtained from Fluka (Buchs, Switzerland). A Milli-Q-Plus ultra-pure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain the HPLC-grade water used during the analyses. Oasis HLB™ SPE cartridges (200 mg, 6 mL) were purchased from Waters (Milford, MA, USA). A Supelco Visiprep™ (Bellefonte, PA, USA) SPE vacuum system was also used.

Table 1. Identification of the studied multiclass contaminants tested by LC-TOFMS.

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Acephate	1.86	C ₄ H ₁₀ NO ₃ PS	[M+Na] ⁺	206.0011	123.8	Pesticide
Acetamiprid	8.79	C ₁₀ H ₁₁ ClN ₄	[M+H] ⁺	223.0745	12.9	Pesticide
Acetylmorphine	4.94	C ₁₉ H ₂₁ NO ₄	[M+H] ⁺	328.1543	23.8	Drug
Aclonifen	17.90	C ₁₂ H ₉ ClN ₂ O ₃	[M+H] ⁺	265.0374	11.1	Pesticide
Alachlor	17.20	C ₁₄ H ₂₀ ClNO ₂	[M+H] ⁺	270.1255	2.5	Pesticide
Albendazole	10.51	C ₁₂ H ₁₅ N ₃ O ₂ S	[M+H] ⁺	266.0957	5.1	Pesticide
Aldicarb	10.18	C ₇ H ₁₄ N ₂ O ₂ S	[M+Na] ⁺	213.0668	82.5	Pesticide

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Aldicarb sulfone	4.81	C ₇ H ₁₄ N ₂ O ₄ S	[M+H] ⁺	223.0747	61.5	Pesticide
Aldicarb sulfoxide	2.70	C ₇ H ₁₄ N ₂ O ₃ S	[M+H] ⁺	207.0798	131.1	Pesticide
Ametryn	10.38	C ₉ H ₁₇ N ₅ S	[M+H] ⁺	228.1277	3.8	Pesticide
4-Aminoantipyrine	2.39	C ₁₁ H ₁₃ N ₃ O	[M+H] ⁺	204.1131	0.7	Pharmaceutical
Aminocarb	1.85	C ₁₁ H ₁₆ N ₂ O ₂	[M+H] ⁺	209.1285	58.5	Pesticide
Amitrol	1.36	C ₂ H ₄ N ₄	[M+H] ⁺	85.0509	213.9	Pesticide
Amphetamine	3.82	C ₉ H ₁₃ N	[M+H] ⁺	136.1121	74.4	Drug
Ampicillin	7.93	C ₁₆ H ₁₉ N ₃ O ₄ S	[M+H] ⁺	350.1169	267.1	Pharmaceutical
4-Androsten-3,17-dione	14.79	C ₁₉ H ₂₆ O ₂	[M+H] ⁺	287.2006	16.2	Pharmaceutical
Anilofos	18.49	C ₁₃ H ₁₉ ClNO ₃ PS ₂	[M+H] ⁺	368.0305	1.6	Pesticide
Antipyrine	6.97	C ₁₁ H ₁₂ N ₂ O	[M+H] ⁺	189.1022	9.1	Pharmaceutical
Asulam	5.43	C ₈ H ₁₀ N ₂ O ₄ S	[M+H] ⁺	231.0434	34.4	Pesticide
Atenolol	1.76	C ₁₄ H ₂₂ N ₂ O ₃	[M+H] ⁺	267.1703	5.8	Pharmaceutical
Atrazine	12.66	C ₈ H ₁₄ ClN ₅	[M+H] ⁺	216.1010	1.3	Pesticide
Atrazine desethyl	7.97	C ₆ H ₁₀ ClN ₅	[M+H] ⁺	188.0697	6.6	Pesticide metabolite
Atropine	6.29	C ₁₇ H ₂₃ NO ₃	[M+H] ⁺	290.1751	1.3	Pharmaceutical
Azinphos-ethyl	17.45	C ₁₂ H ₁₆ N ₃ O ₃ PS ₃	[M+Na] ⁺	346.0443	48.1	Pesticide
Azinphos methyl	15.42	C ₁₀ H ₁₂ N ₃ O ₃ PS ₂	[M+Na] ⁺	339.9950	165.9	Pesticide
Azoxystrobin	15.81	C ₂₂ H ₁₇ N ₃ O ₅	[M+H] ⁺	404.1241	0.7	Pesticide
Benalaxyl	18.09	C ₂₀ H ₂₃ NO ₃	[M+H] ⁺	326.1751	1.8	Pesticide
Bendroflumethiazide	15.00	C ₁₅ H ₁₄ F ₃ N ₃ O ₄ S ₂	[M+H] ⁺	422.0451	58.9	Drug
Benfuracarb	20.80	C ₂₀ H ₃₀ N ₂ O ₅ S	[M+H] ⁺	411.1948	17.0	Pesticide
Bensulfuron methyl	14.20	C ₁₆ H ₁₈ N ₄ O ₇ S	[M+H] ⁺	411.0969	4.5	Pesticide
Bensulide	18.59	C ₁₄ H ₂₄ NO ₄ PS ₃	[M+H] ⁺	398.0678	39.4	Pesticide
Bentazon	12.67	C ₁₀ H ₁₂ N ₂ O ₃ S	[M+H] ⁺	241.0641	854.5 ⁽¹⁾	Pesticide
Benzalkonium chloride-C10 (**)	14.29	C ₁₉ H ₃₃ N	[M+H] ⁺	276.2686	0.6 ⁽¹⁾	Pharmaceutical
Benzalkonium chloride-C12 (**)	16.24	C ₂₁ H ₃₇ N	[M+H] ⁺	304.2999	40.2	Pharmaceutical
Benzalkonium chloride-C14 (**)	18.35	C ₂₃ H ₄₁ N	[M+H] ⁺	332.3312	12.9	Pharmaceutical
Benzalkonium chloride-C16 (**)	20.63	C ₂₅ H ₄₅ N	[M+H] ⁺	360.3625	0.2	Pharmaceutical

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Benzidine	1.36	C ₁₂ H ₁₂ N ₂	[M+H] ⁺	185.1073	183.2	Pesticide
Benzoylcegonine	6.61	C ₁₆ H ₁₉ NO ₄	[M+H] ⁺	290.1387	4.3	Drug
Bezafibrate	13.92	C ₁₉ H ₁₉ ClNO ₄	[M+H] ⁺	362.1154	7.3	Pharmaceutical
Bifenazate	16.61	C ₁₇ H ₂₀ N ₂ O ₃	[M+H] ⁺	301.1547	NR	Pesticide
Bifenox	19.59	C ₁₄ H ₉ Cl ₂ NO ₅	[M+H] ⁺	341.9931	200.5	Pesticide
Bis (2-ethylhexyl)phtalate (DEHP)	27.48	C ₂₄ H ₃₈ O ₄	[M+H] ⁺	391.2843	96.5 ⁽²⁾	Plasticizer
Bisphenol A	13.10	C ₁₅ H ₁₆ O ₂	[M-H] ⁻	227.1078	NR	Plasticizer
Bitertanol	16.59	C ₂₀ H ₂₃ N ₃ O ₂	[M+H] ⁺	338.1863	9.2	Pesticide
Boscalid	16.10	C ₁₈ H ₁₂ Cl ₂ N ₂ O	[M+H] ⁺	343.0399	6.9	Pesticide
Bromacil	10.53	C ₉ H ₁₃ BrN ₂ O ₂	[M+H] ⁺	261.0233	30.1	Pesticide
Bromophos ethyl	23.20	C ₁₀ H ₁₂ BrCl ₂ O ₃ PS	[M+H] ⁺	392.8878	NR	Pesticide
Bromophos methyl	21.12	C ₈ H ₈ BrCl ₂ O ₃ PS	[M+H] ⁺	364.8565	264.6	Pesticide
Bromuconazole 1	15.45	C ₁₃ H ₁₂ BrCl ₂ N ₃ O	[M+H] ⁺	375.9613	2.7	Pesticide
Bromuconazole 2	16.16	C ₁₃ H ₁₂ BrCl ₂ N ₃ O	[M+H] ⁺	375.9613	3.2	Pesticide
Bupirimate	14.43	C ₁₃ H ₂₄ N ₄ O ₃ S	[M+H] ⁺	317.1641	5.3	Pesticide
Buprofezin	18.30	C ₁₆ H ₂₃ N ₃ OS	[M+H] ⁺	306.1635	4.0	Pesticide
Butachlor	21.34	C ₁₇ H ₂₆ ClNO ₂	[M+H] ⁺	312.1725	29.0	Pesticide
Butocarboxim	9.65	C ₇ H ₁₄ N ₂ O ₂ S	[M+Na] ⁺	213.0668	46.3	Pesticide
Butoxycarboxim	4.36	C ₇ H ₁₄ N ₂ O ₄ S	[M+H] ⁺	223.0747	45.7	Pesticide
Buturon	14.49	C ₁₂ H ₁₃ ClN ₂ O	[M+H] ⁺	237.0789	8.6	Pesticide
Caffeine	5.08	C ₈ H ₁₀ N ₄ O ₂	[M+H] ⁺	195.0877	0.6	Life-style compound (LS)
Cannabidiol	21.21	C ₂₁ H ₃₀ O ₂	[M+H] ⁺	315.2319	146.9	Drug
Carbadox	6.32	C ₁₁ H ₁₀ N ₄ O ₄	[M+H] ⁺	263.0775	2.2	Pharmaceutical
Carbamazepine	11.21	C ₁₅ H ₁₂ N ₂ O	[M+H] ⁺	237.1022	2.5	Pharmaceutical
Carbendazim	3.27	C ₉ H ₉ N ₃ O ₂	[M+H] ⁺	192.0768	1.0	Pesticide
Carbofuran	12.09	C ₁₂ H ₁₅ NO ₃	[M+H] ⁺	222.1125	64.5	Pesticide
Carbofuran 3-hydroxy	7.87	C ₁₂ H ₁₅ NO ₄	[M+H] ⁺	238.1074	55.2	Pesticide
Carboxine	13.14	C ₁₂ H ₁₃ NO ₂ S	[M+H] ⁺	236.0740	9.2	Pesticide
Carfentrazone ethyl	17.96	C ₁₅ H ₁₄ Cl ₂ F ₃ N ₃ O ₃	[M+H] ⁺	412.0437	19.2	Pesticide

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Cefotaxime	6.29	C ₁₆ H ₁₇ N ₅ O ₇ S ₂	[M+H] ⁺	456.0642	2.7	Pharmaceutical
Chloramphenicol	9.50	C ₁₁ H ₁₂ Cl ₂ N ₂ O ₅	[M-H] ⁻	321.0051	3.2	Pharmaceutical
Chlorbromuron	15.71	C ₉ H ₁₀ BrClN ₂ O ₂	[M+H] ⁺	292.9686	7.9	Pesticide
Chlordimeform	6.56	C ₁₀ H ₁₃ ClN ₂	[M+H] ⁺	197.0840	11.0	Pesticide
Chlorfenvinfos	17.61	C ₁₂ H ₁₄ Cl ₃ O ₄ P	[M+H] ⁺	358.9768	4.8	Pesticide
Chloridazon (Pyrazon)	8.14	C ₁₀ H ₈ ClN ₃ O	[M+H] ⁺	222.0429	2.9	Pesticide
4-chloro-2-methylphenol	13.15	C ₇ H ₇ ClO	[M-H] ⁻	141.0113	1.0	Pesticide
4-chloro-o-tolyloxyacetic acid (MCPA)	13.17	C ₉ H ₉ ClO ₃	[M-H] ⁻	199.0167	3.5	Pesticide
Chlorothiazide	4.72	C ₇ H ₆ ClN ₃ O ₄ S ₂	[M-H] ⁻	293.9415	1.4	Drug
Chloroxuron	15.32	C ₁₅ H ₁₅ ClN ₂ O ₂	[M+H] ⁺	291.0894	3.6	Pesticide
Chlorpropham	16.50	C ₁₀ H ₁₂ ClNO ₂	[M+Na] ⁺	236.0449	1872 ⁽¹⁾	Pesticide
Chlorpyrifos ethyl	21.63	C ₉ H ₁₁ Cl ₃ NO ₃ PS	[M+H] ⁺	349.9336	30.0	Pesticide
Chlorpyrifos-methyl	19.50	C ₇ H ₇ Cl ₃ NO ₃ PS	[M+H] ⁺	321.9023	22.7	Pesticide
Chlorsulfuron	12.50	C ₁₂ H ₁₂ ClN ₅ O ₄ S	[M+H] ⁺	358.0371	4.9	Pesticide
Chlortetracycline	8.16	C ₂₂ H ₂₃ ClN ₂ O ₈	[M+H] ⁺	479.1216	34.5	Pharmaceutical
Chlortoluron	12.38	C ₁₀ H ₁₃ ClN ₂ O	[M+H] ⁺	213.0789	29.5	Pesticide
Chlothianidin	7.84	C ₆ H ₈ ClN ₅ O ₂ S	[M+H] ⁺	250.0160	58.7	Pesticide
Cimetidine	1.83	C ₁₀ H ₁₆ N ₆ S	[M+H] ⁺	253.1230	1.9	Pharmaceutical
Cinosulfuron	11.92	C ₁₅ H ₁₉ N ₅ O ₇ S	[M+H] ⁺	414.1078	3.2	Pesticide
Ciprofloxacin	6.39	C ₁₇ H ₁₈ FN ₃ O ₃	[M+H] ⁺	332.1405	134.6	Pharmaceutical
Cis-Diltiazem	10.14	C ₂₂ H ₂₆ N ₂ O ₄ S	[M+H] ⁺	415.1686	0.4	Pharmaceutical
Clarithromycin	11.10	C ₃₈ H ₆₉ NO ₁₃	[M+H] ⁺	748.4842	1.6	Pharmaceutical
Clenbuterol	7.15	C ₁₂ H ₁₈ Cl ₂ N ₂ O	[M+H] ⁺	277.0869	13.1	Pharmaceutical
Clofibric acid	16.25	C ₁₀ H ₁₁ ClO ₃	[M+H] ⁺	215.0469	9.1	Pharmaceutical
Clomazone	14.38	C ₁₂ H ₁₄ ClNO ₂	[M+H] ⁺	240.0786	9.2	Pesticide
Cloxacillin	13.34	C ₁₉ H ₁₈ ClN ₃ O ₅ S	[M+H] ⁺	436.0728	9.8	Pharmaceutical
Cocaine	7.30	C ₁₇ H ₂₁ NO ₄	[M+H] ⁺	304.1543	17.2	Drug
Codeine	3.16	C ₁₈ H ₂₁ NO ₃	[M+H] ⁺	300.1594	6.7	Pharmaceutical
Compactin (Mevastatin)	15.71	C ₂₃ H ₃₄ O ₅	[M+H] ⁺	391.2479	221.0	Pharmaceutical

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Cotinine	1.35	C ₁₀ H ₁₂ N ₂ O	[M+H] ⁺	177.1025	13.2	LS - Nicotine metab.
Coumaphos	19.06	C ₁₄ H ₁₆ ClO ₅ PS	[M+H] ⁺	363.0217	5.5	Pesticide
Cyanazine	11.24	C ₉ H ₁₃ ClN ₆	[M+H] ⁺	241.0963	12.1	Pesticide
Cycloate	19.78	C ₁₁ H ₂₁ NOS	[M+H] ⁺	216.1417	11.9	Pesticide
λ-Cyhalothrin	23.08	C ₂₃ H ₁₉ ClF ₃ NO ₃	[M+H] ⁺	450.1078	1179.5	Pesticide
α-Cypermethrin	23.20	C ₂₂ H ₁₉ Cl ₂ NO ₃	[M+Na] ⁺	438.0634	7090 ⁽¹⁾	Pesticide
Cyproconazole	14.89	C ₁₅ H ₁₈ ClN ₃ O	[M+H] ⁺	292.1211	3.4	Pesticide
Cyprodinil	14.28	C ₁₄ H ₁₅ N ₃	[M+H] ⁺	226.1339	1.6	Pesticide
Cyromazine	1.36	C ₆ H ₁₀ N ₆	[M+H] ⁺	167.1039	13.6	Pesticide
Danofloxacin	6.74	C ₁₉ H ₂₀ FN ₃ O ₃	[M+H] ⁺	358.1561	8.1	Pharmaceutical
DEET	12.82	C ₁₂ H ₁₇ NO	[M+H] ⁺	192.1383	0.2	Pesticide
Deltamethrin	23.31	C ₂₂ H ₁₉ Br ₂ NO ₃	[M+H] ⁺	503.9804	12658 ⁽¹⁾	Pesticide
Demeclocycline	7.36	C ₂₁ H ₂₁ ClN ₂ O ₈	[M+H] ⁺	465.1059	36.0	Pharmaceutical
Desmetryn	8.80	C ₈ H ₁₅ N ₅ S	[M+H] ⁺	214.1121	4.9	Pesticide
Dexamethasone	11.51	C ₂₂ H ₂₉ FO ₅	[M+H] ⁺	393.2072	32.3	Pharmaceutical
Diafenthuron	22.30	C ₂₃ H ₃₂ N ₂ OS	[M+H] ⁺	385.2308	NR	Pesticide
Diazinon	18.82	C ₁₂ H ₂₁ N ₂ O ₃ PS	[M+H] ⁺	305.1083	1.1	Pesticide
Diclofenac	16.24	C ₁₄ H ₁₁ Cl ₂ NO ₂	[M+H] ⁺	296.0240	19.4	Pharmaceutical
Dichlofenthion	21.46	C ₁₀ H ₁₃ Cl ₂ O ₃ PS	[M+H] ⁺	314.9773	34.5	Pesticide
Dichloran	14.59	C ₆ H ₄ Cl ₂ N ₂ O ₂	[M+H] ⁺	206.9723	168.5	Pesticide
2,4-Dichlorophenoxyacetic acid	13.10	C ₈ H ₆ Cl ₂ O ₃	[M-H] ⁻	218.9621	57.3	Pesticide
Dichlorprop (racemate mix)	14.37	C ₉ H ₈ Cl ₂ O ₃	[M-H] ⁻	232.9778	20.9	Pesticide
Dichlorvos	11.15	C ₄ H ₇ Cl ₂ O ₄ P	[M+H] ⁺	220.9532	5.3	Pesticide
Dicloxacillin	14.39	C ₁₉ H ₁₇ Cl ₂ N ₃ O ₅ S	[M+H] ⁺	470.0339	12.8	Pharmaceutical
Dicrotophos	6.41	C ₈ H ₁₆ NO ₅ P	[M+H] ⁺	238.0839	3.8	Pesticide
Diethofencarb	15.31	C ₁₄ H ₂₁ NO ₄	[M+H] ⁺	268.1543	147.7	Pesticide
Difenacoum	21.71	C ₃₁ H ₂₄ O ₃	[M+H] ⁺	445.1798	10.9	Pesticide
Difenoconazole	18.03	C ₁₉ H ₁₇ Cl ₂ N ₃ O ₃	[M+H] ⁺	406.0720	2.5	Pesticide
Difenoxyuron	13.30	C ₁₆ H ₁₈ N ₂ O ₃	[M+H] ⁺	287.1390	5.7	Pesticide

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Diflubenuron	16.71	C ₁₄ H ₉ ClF ₂ N ₂ O ₂	[M+H] ⁺	311.0393	56.1	Pesticide
Diflufenican	19.68	C ₁₉ H ₁₁ F ₅ N ₂ O ₂	[M+H] ⁺	395.0813	4.1	Pesticide
Digoxigenin	8.14	C ₂₃ H ₃₄ O ₅	[M+H] ⁺	391.2479	40.7	Pharmaceutical
Dimethametryn	13.13	C ₁₁ H ₂₁ N ₅ S	[M+H] ⁺	256.1590	0.9	Pesticide
Dimethenamid	15.51	C ₁₂ H ₁₈ ClNO ₂ S	[M+H] ⁺	276.0820	10.1	Pesticide
Dimethoate	9.00	C ₅ H ₁₂ NO ₃ PS ₂	[M-H] ⁻	227.9923	17.8	Pesticide
Dimethomorph isomer 1	14.14	C ₂₁ H ₂₂ ClNO ₄	[M+H] ⁺	388.1310	13.4	Pesticide
Dimethomorph isomer 2	14.40	C ₂₁ H ₂₂ ClNO ₄	[M+H] ⁺	388.1310	9.0	Pesticide
2,6-Dimethylaniline	5.94	C ₈ H ₁₁ N	[M+H] ⁺	122.0964	29.7	Pesticide
Diniconazol	17.19	C ₁₅ H ₁₇ Cl ₂ N ₃ O	[M+H] ⁺	326.0821	1.3	Pesticide
2,4-Dinitrophenol	11.51	C ₆ H ₄ N ₂ O ₅	[M-H] ⁻	183.0047	NR	Pesticide
Diphenhydramine	9.81	C ₁₇ H ₂₁ NO	[M+H] ⁺	256.1696	9.6	Pharmaceutical
Diphenylamine	17.19	C ₁₂ H ₁₁ N	[M+H] ⁺	170.0964	9.1	Pesticide
Diuron	13.12	C ₉ H ₁₀ Cl ₂ N ₂ O	[M+H] ⁺	233.0243	1.1	Pesticide
DMST (N,N-dimethyl-N-tolylsulfonyldiamide)	12.95	C ₉ H ₁₄ N ₂ O ₂ S	[M+H] ⁺	215.0849	400.7	Pesticide
DNOC	14.21	C ₇ H ₆ N ₂ O ₅	[M-H] ⁻	197.0204	3.9	Pesticide
Doxycycline	8.75	C ₂₂ H ₂₄ N ₂ O ₈	[M+H] ⁺	445.1605	578.5	Pharmaceutical
EDDP	10.51	C ₂₀ H ₂₄ N	[M+H] ⁺	278.1903	2.9	Drug
Edifenphos	17.62	C ₁₄ H ₁₅ O ₂ PS ₂	[M+H] ⁺	311.0324	65.6	Pesticide
Enalapril	9.21	C ₂₀ H ₂₈ N ₂ O ₅	[M+H] ⁺	377.2071	19.5	Pharmaceutical
Enoxacin	6.01	C ₁₅ H ₁₇ FN ₄ O ₃	[M+H] ⁺	321.1357	25.0	Pharmaceutical
Enrofloxacin	6.91	C ₁₉ H ₂₂ FN ₃ O ₃	[M+H] ⁺	360.1718	27.7	Pharmaceutical
Ephedrine	2.78	C ₁₀ H ₁₅ NO	[M+H] ⁺	166.1226	1.7	Drug
EPN	19.97	C ₁₄ H ₁₄ NO ₄ PS	[M+H] ⁺	324.0454	111.0	Pesticide
Eprinomectin (b1a)	21.17	C ₅₀ H ₇₅ NO ₁₄	[M+H] ⁺	914.5260	31.1	Pesticide
Equilin	14.41	C ₁₈ H ₂₀ O ₂	[M+H] ⁺	269.1536	139.7	Pharmaceutical
Erythromycin	9.73	C ₃₇ H ₆₇ NO ₁₃	[M+H] ⁺	734.4685	49.0	Pharmaceutical
β-Estradiol	13.38	C ₁₈ H ₂₄ O ₂	[M+H] ⁺	273.1849	592.4 ⁽¹⁾	Pharmaceutical
Estriol	9.36	C ₁₈ H ₂₄ O ₃	[M+H] ⁺	289.1798	66.6	Pharmaceutical (Hormone metab.)

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Estrone	14.60	C ₁₈ H ₂₂ O ₂	[M+H] ⁺	271.1693	777.9	Pharmaceutical
Ethiofencarb	13.07	C ₁₁ H ₁₅ NO ₂ S	[M+Na] ⁺	248.0716	360.8	Pesticide
Ethion	21.70	C ₉ H ₂₂ O ₄ P ₂ S ₄	[M+H] ⁺	384.9949	183.8	Pesticide
Ethiprole	14.87	C ₁₃ H ₉ Cl ₂ F ₃ N ₄ OS	[M+H] ⁺	396.9899	3.9	Pesticide
Ethoxyquin	11.83	C ₁₄ H ₁₉ NO	[M+H] ⁺	218.1539	107.8	Pharmaceutical
Ethylamphetamine	5.85	C ₁₁ H ₁₇ N	[M+H] ⁺	164.1434	100.0	Drug
Ethylmorphine	5.83	C ₁₉ H ₂₃ NO ₃	[M+H] ⁺	314.1751	6.8	Drug
17- α -Ethinylestradiol	14.28	C ₂₀ H ₂₄ O ₂	[M+H] ⁺	297.1849	550.4 ⁽¹⁾	Pharmaceutical
Etoprofos	16.05	C ₈ H ₁₉ O ₂ PS ₂	[M+H] ⁺	243.0637	3.4	Pesticide
Etrimphos	18.81	C ₁₀ H ₁₇ N ₂ O ₄ PS	[M+H] ⁺	293.0719	1.4	Pesticide
Famotidine	1.81	C ₈ H ₁₅ N ₇ O ₂ S ₃	[M+H] ⁺	338.0522	8.4	Pharmaceutical
Famphur	15.29	C ₁₀ H ₁₆ NO ₅ PS ₂	[M+H] ⁺	326.0280	11.9	Pesticide
Fenamiphos	15.41	C ₁₃ H ₂₂ NO ₃ PS	[M+H] ⁺	304.1131	4.3	Pesticide
Fenarimol	15.33	C ₁₇ H ₁₂ Cl ₂ N ₂ O	[M+H] ⁺	331.0399	3.0	Pesticide
Fenazaquin	22.00	C ₂₀ H ₂₂ N ₂ O	[M+H] ⁺	307.1805	9.6	Pesticide
Fenbendazol	12.59	C ₁₅ H ₁₃ N ₃ O ₂ S	[M+H] ⁺	300.0801	16.0	Pesticide
Fenhexamid	15.96	C ₁₄ H ₁₇ Cl ₂ NO ₂	[M+H] ⁺	302.0709	13.9	Pesticide
Fenitrothion	17.20	C ₉ H ₁₂ NO ₅ PS	[M+H] ⁺	278.0247	20.2	Pesticide
Fenobucarb	15.02	C ₁₂ H ₁₇ NO ₂	[M+H] ⁺	208.1332	25.3	Pesticide
Fenofibrate	21.54	C ₂₀ H ₂₁ ClO ₄	[M+H] ⁺	361.1201	16.0	Pharmaceutical
Fenoxycarb	17.00	C ₁₇ H ₁₉ NO ₄	[M+H] ⁺	302.1386	11.3	Pesticide
Fenpropidine	12.48	C ₁₉ H ₃₁ N	[M+H] ⁺	274.2529	1.1	Pesticide
Fenpropimorphe	12.37	C ₂₀ H ₃₃ NO	[M+H] ⁺	304.2635	11.9	Pesticide
Fensulfothion	13.52	C ₁₁ H ₁₇ O ₄ PS ₂	[M+H] ⁺	309.0379	3.1	Pesticide
Fenthion	18.54	C ₁₀ H ₁₅ O ₃ PS ₂	[M+H] ⁺	279.0273	16.5	Pesticide
Fenuron	7.72	C ₉ H ₁₂ N ₂ O	[M+H] ⁺	165.1022	20.7	Pesticide
Fluazifop	15.10	C ₁₅ H ₁₂ F ₃ NO ₄	[M+H] ⁺	328.0791	5.0	Pesticide
Fluazifop-p-butyl	21.15	C ₁₉ H ₂₀ F ₃ NO ₄	[M+H] ⁺	384.1417	2.8	Pesticide
Flufenacet	17.24	C ₁₄ H ₁₃ F ₄ N ₃ O ₂ S	[M+H] ⁺	364.0737	13.0	Pesticide

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Flufenamic acid	17.54	C ₁₄ H ₁₀ F ₃ NO ₂	[M+H] ⁺	282.0736	2.8	Pharmaceutical
Flufenoxuron	21.25	C ₂₁ H ₁₁ ClF ₆ N ₂ O ₃	[M+H] ⁺	489.0435	37.2	Pesticide
Flumequine	11.88	C ₁₄ H ₁₂ FNO ₃	[M+H] ⁺	262.0874	4.4	Pharmaceutical
Fluometuron	12.61	C ₁₀ H ₁₁ F ₃ N ₂ O	[M+H] ⁺	233.0896	3.5	Pesticide
Fluoxetine	11.44	C ₁₇ H ₁₈ F ₃ NO	[M+H] ⁺	310.1413	18.4	Pharmaceutical
Fluquinconazole	16.24	C ₁₆ H ₈ Cl ₂ FN ₅ O	[M+H] ⁺	376.0163	3.9	Pesticide
Fluroxypyr	11.07	C ₇ H ₅ Cl ₂ FN ₂ O ₃	[M+H] ⁺	254.9734	27.1	Pesticide
Flutolanil	17.03	C ₁₇ H ₁₆ F ₃ NO ₂	[M+H] ⁺	324.1206	3.9	Pesticide
Flutriafol	12.56	C ₁₆ H ₁₃ F ₂ N ₃ O	[M+H] ⁺	302.1099	2.9	Pesticide
Fosthiazate	12.68	C ₉ H ₁₈ NO ₃ PS ₂	[M+H] ⁺	284.0538	12.6	Pesticide
Fuberidazole	5.19	C ₁₁ H ₈ N ₂ O	[M+H] ⁺	185.0709	3.9	Pesticide
Furmecyclox	17.68	C ₁₄ H ₂₁ NO ₃	[M+H] ⁺	252.1594	3.2	Pesticide
Furosemide	11.56	C ₁₂ H ₁₁ ClN ₂ O ₅ S	[M-H] ⁻	329.0004	4.0	Pharmaceutical
Gemfibrozil	18.07	C ₁₅ H ₂₂ O ₃	[M+Na] ⁺	273.1461	4.7	Pharmaceutical
Griseofulvin	13.29	C ₁₇ H ₁₇ ClO ₆	[M+H] ⁺	353.0786	5.4	Pesticide
Haloxypop	16.75	C ₁₅ H ₁₁ ClF ₃ NO ₄	[M+H] ⁺	362.0401	1.8	Pesticide
Hexaflumuron	19.08	C ₁₆ H ₈ Cl ₂ F ₆ N ₂ O ₃	[M+H] ⁺	460.9888	104.1	Pesticide
Hexazinone	10.09	C ₁₂ H ₂₀ N ₄ O ₂	[M+H] ⁺	253.1659	5.6	Pesticide
Hexythiazox	21.66	C ₁₇ H ₂₁ ClN ₂ O ₂ S	[M+H] ⁺	353.1085	49.1	Pesticide
Hydrochlorothiazide	5.57	C ₇ H ₈ ClN ₃ O ₄ S ₂	[M-H] ⁻	295.9572	1.8	Pharmaceutical
Hydroflumethiazide	7.79	C ₈ H ₈ F ₃ N ₃ O ₄ S ₂	[M-H] ⁻	329.9836	4.9	Pharmaceutical
2-Hydroxybiphenyl (2-phenylphenol)	16.30	C ₁₂ H ₁₀ O	[M+H] ⁺	171.0804	61.7	Pesticide
Ibuprofen	16.58	C ₁₃ H ₁₈ O ₂	[M+H] ⁺	207.1380	15.2	Pharmaceutical
Imazalil	10.91	C ₁₄ H ₁₄ Cl ₂ N ₂ O	[M+H] ⁺	297.0556	4.0	Pesticide
Imazalil metabolite	7.72	C ₁₁ H ₁₀ Cl ₂ N ₂ O	[M+H] ⁺	257.0243	1.3	Pesticide
Imazamethabenz-methyl	9.12	C ₁₆ H ₂₀ N ₂ O ₃	[M+H] ⁺	289.1547	3.3	Pesticide
Imazamox	8.04	C ₁₅ H ₁₉ N ₃ O ₄	[M+H] ⁺	306.1448	13.6	Pesticide
Imazapyr	16.48	C ₁₃ H ₁₅ N ₃ O ₃	[M+H] ⁺	262.1186	28.0	Pesticide
Imazaquin	11.04	C ₁₇ H ₁₇ N ₃ O ₃	[M+H] ⁺	312.1343	1.3	Pesticide

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Imidacloprid	8.22	C ₉ H ₁₀ ClN ₅ O ₂	[M+H] ⁺	256.0596	14.4	Pesticide
Indomethacine	16.23	C ₁₉ H ₁₆ ClNO ₄	[M+H] ⁺	358.0841	11.2	Pharmaceutical
Indoxacarb	19.68	C ₂₂ H ₁₇ ClF ₃ N ₃ O ₇	[M+H] ⁺	528.0779	100.8	Pesticide
Ioxynil	14.40	C ₇ H ₃ I ₂ NO	[M-H] ⁻	369.8231	1.4	Pesticide
Iprodione	19.13	C ₁₃ H ₁₃ Cl ₂ N ₃ O ₃	[M+H] ⁺	330.0407	71.3	Pesticide
Irgarol (cybutryne)	12.56	C ₁₁ H ₁₉ N ₅ S	[M+H] ⁺	254.1434	1.7	Pesticide
Isocarbophos	14.94	C ₁₁ H ₁₆ NO ₄ PS	[M+Na] ⁺	312.0430	35.8	Pesticide
Isoprocarb	13.62	C ₁₁ H ₁₅ NO ₂	[M+H] ⁺	194.1175	84.1	Pesticide
Isoproturon	12.94	C ₁₂ H ₁₈ N ₂ O	[M+H] ⁺	207.1492	1.7	Pesticide
Isoxaben	16.48	C ₁₈ H ₂₄ N ₂ O ₄	[M+H] ⁺	333.1809	7.0	Pesticide
Josamycin	12.02	C ₄₂ H ₆₉ NO ₁₅	[M+H] ⁺	828.4740	0.9	Pharmaceutical
Karbutilate	11.57	C ₁₄ H ₂₁ N ₃ O ₃	[M+H] ⁺	280.1656	6.9	Pesticide
Ketamine	6.31	C ₁₃ H ₁₆ ClNO	[M+H] ⁺	238.0993	7.8	Drug
Ketoprofen	13.69	C ₁₆ H ₁₄ O ₃	[M+H] ⁺	255.1016	2.9	Pharmaceutical
Kresoxim methyl	18.02	C ₁₈ H ₁₉ NO ₄	[M+H] ⁺	314.1387	31.5	Pesticide
Lenacil	11.30	C ₁₃ H ₁₈ N ₂ O ₂	[M+H] ⁺	235.1441	33.3	Pesticide
Leucomalachite green	12.60	C ₂₃ H ₂₆ N ₂	[M+H] ⁺	331.2169	531.6	Pharmaceutical
Lincomycin	3.66	C ₁₈ H ₃₄ N ₂ O ₆ S	[M+H] ⁺	407.2210	4.5	Pharmaceutical
Linuron	15.30	C ₉ H ₁₀ Cl ₂ N ₂ O ₂	[M+H] ⁺	249.0192	11.0	Pesticide
Lomefloxacin	6.60	C ₁₇ H ₁₉ F ₂ N ₃ O ₃	[M+H] ⁺	352.1467	19.1	Pharmaceutical
Lufenuron	21.66	C ₁₇ H ₈ Cl ₂ F ₃ N ₂ O ₄	[M+H] ⁺	510.9857	61.6	Pesticide
Malachite green	12.82	C ₂₃ H ₂₄ N ₂	[M+H] ⁺	329.2012	6.3	Pharmaceutical
Malaoxon	11.82	C ₁₀ H ₁₉ O ₇ PS	[M+H] ⁺	315.0662	26.8	Pesticide
Malathion	17.02	C ₁₀ H ₁₉ O ₆ PS ₂	[M+H] ⁺	331.0433	12.6	Pesticide
MDA	4.74	C ₁₀ H ₁₃ NO ₂	[M+H] ⁺	180.1019	45.1	Drug
MDEA	6.16	C ₁₂ H ₁₇ NO ₂	[M+H] ⁺	208.1332	21.3	Drug
MDMA	5.39	C ₁₁ H ₁₅ NO ₂	[M+H] ⁺	194.1176	27.7	Drug
Mebendazole	10.70	C ₁₆ H ₁₃ N ₃ O ₃	[M+H] ⁺	296.1030	1.0	Pharmaceutical
Mecarbam	17.78	C ₁₀ H ₂₀ NO ₅ PS ₂	[M+Na] ⁺	352.0413	57.2	Pesticide

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Meclofenamic acid	17.74	C ₁₄ H ₁₁ Cl ₂ NO ₂	[M+H] ⁺	296.0240	2.1	Pharmaceutical
Mefenacet	16.01	C ₁₆ H ₁₄ N ₂ O ₂ S	[M+H] ⁺	299.0849	3.2	Pesticide
Mefenamic acid	17.69	C ₁₅ H ₁₅ NO ₂	[M+H] ⁺	242.1176	3.3	Pharmaceutical
Mesotrion	12.02	C ₁₄ H ₁₃ NO ₇ S	[M+H] ⁺	340.0485	2.2	Pesticide
Metalaxyl	12.94	C ₁₅ H ₂₁ NO ₄	[M+H] ⁺	280.1543	4.3	Pesticide
Metamitron	7.57	C ₁₀ H ₁₀ N ₄ O	[M+H] ⁺	203.0927	15.9	Pesticide
Metazachlor	14.06	C ₁₄ H ₁₆ ClN ₃ O	[M+H] ⁺	278.1055	29.9	Pesticide
Metformin	0.92	C ₄ H ₁₁ N ₅	[M+H] ⁺	130.1087	59.8	Pharmaceutical
Methabenzthiazuron	12.08	C ₁₀ H ₁₁ N ₃ OS	[M+H] ⁺	222.0696	4.5	Pesticide
Methadone	11.39	C ₂₁ H ₂₇ NO	[M+H] ⁺	310.2165	3.5	Drug
Methamidophos	1.61	C ₂ H ₆ NO ₂ PS	[M+H] ⁺	142.0086	284.8	Pesticide
Methamphetamine	4.88	C ₁₀ H ₁₅ N	[M+H] ⁺	150.1277	56.4	Drug
Methidathion	15.33	C ₆ H ₁₁ N ₂ O ₄ PS ₃	[M+Na] ⁺	324.9511	60.1	Pesticide
Methiocarb (mercaptodimethur)	15.02	C ₁₁ H ₁₅ NO ₂ S	[M+Na] ⁺	248.0716	847.6	Pesticide
Methomyl	5.39	C ₅ H ₁₀ N ₂ O ₂ S	[M+Na] ⁺	163.0536	298.1	Pesticide
Methoxyfenozide	16.58	C ₂₂ H ₂₈ N ₂ O ₃	[M+H] ⁺	369.2173	4.7	Pesticide
3-Methylxanthine	1.47	C ₆ H ₆ N ₄ O ₂	[M+H] ⁺	167.0564	1.4	Pharmaceutical
Metobromuron	13.78	C ₉ H ₁₁ BrN ₂ O ₂	[M+H] ⁺	259.0076	21.4	Pesticide
Metolcarb	11.02	C ₉ H ₁₁ NO ₂	[M+H] ⁺	166.0863	1186.7	Pesticide
Metoxuron	10.16	C ₁₀ H ₁₃ ClN ₂ O ₂	[M+H] ⁺	229.0738	6.3	Pesticide
Metribuzin	11.46	C ₈ H ₁₄ N ₄ OS	[M+H] ⁺	215.0961	5.5	Pesticide
Metronidazole	2.77	C ₆ H ₉ N ₃ O ₃	[M+H] ⁺	172.0717	6.5	Pharmaceutical
Mevinphos	9.10	C ₇ H ₁₃ O ₆ P	[M+H] ⁺	225.0523	31.7	Pesticide
Miconazole	13.99	C ₁₈ H ₁₄ Cl ₄ N ₂ O	[M+H] ⁺	414.9933	1.1	Pharmaceutical
Molinate	16.03	C ₉ H ₁₇ NOS	[M+H] ⁺	188.1104	19.9	Pesticide
Monocrotophos	5.55	C ₇ H ₁₄ NO ₅ P	[M+H] ⁺	224.0682	17.5	Pesticide
Monolinuron	13.25	C ₉ H ₁₁ ClN ₂ O ₂	[M+H] ⁺	215.0582	25.0	Pesticide
Monuron	10.81	C ₉ H ₁₁ ClN ₂ O	[M+H] ⁺	199.0633	8.5	Pesticide
Morphine	1.47	C ₁₇ H ₁₉ NO ₃	[M+H] ⁺	286.1438	1.0	Drug

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Myclobutanil	15.60	C ₁₅ H ₁₇ ClN ₄	[M+H] ⁺	289.1215	4.5	Pesticide
Nalidixic acid	11.49	C ₁₂ H ₁₂ N ₂ O ₃	[M+H] ⁺	233.0921	39.8	Pharmaceutical
1-Naphtaleneacetamide	10.00	C ₁₂ H ₁₁ NO	[M+H] ⁺	186.0913	14.4	Pesticide
Naproxen	13.82	C ₁₄ H ₁₄ O ₃	[M+H] ⁺	231.1016	23.2	Pharmaceutical
Naptalam	11.72	C ₁₈ H ₁₃ NO ₃	[M+H] ⁺	292.0968	59.9	Pesticide
Neburon	17.45	C ₁₂ H ₁₆ Cl ₂ N ₂ O	[M+H] ⁺	275.0712	4.9	Pesticide
Nicotine	1.36	C ₁₀ H ₁₄ N ₂	[M+H] ⁺	163.1230	11.8	Life-style compound (LS)
Nifuroxazide	9.51	C ₁₂ H ₉ N ₃ O ₅	[M+H] ⁺	276.0615	4.3	Pharmaceutical
Nitenpyram	5.22	C ₁₁ H ₁₅ ClN ₄ O ₂	[M+H] ⁺	271.0956	8.6	Pesticide
N,N-Diethyl-2-(1-naphthoxy) propanamide	16.31	C ₁₇ H ₂₁ NO ₂	[M+H] ⁺	272.1645	4.2	Pesticide
N-nitrosodiethylamine	15.87	C ₄ H ₁₀ N ₂ O	[M+H] ⁺	103.0866	94.0	Nitrosamines
N-nitrosodi-n-dibutylamine	15.87	C ₈ H ₁₈ N ₂ O	[M+H] ⁺	159.1493	3.1	Nitrosamines
N-nitrosomorpholine	2.21	C ₄ H ₈ N ₂ O ₂	[M+H] ⁺	117.0658	49.3	Nitrosamines
N-nitrosopiperidine	6.57	C ₆ H ₁₀ N ₂ O	[M+H] ⁺	115.0865	54.5	Nitrosamines
N-nitrosopyrrolidine	2.66	C ₄ H ₈ N ₂ O	[M+H] ⁺	101.0710	46.6	Nitrosamines
Norfloxacin	13.46	C ₁₆ H ₁₈ N ₃ FO ₄	[M+H] ⁺	320.1405	119.4	Pharmaceutical
Norflurazon	13.46	C ₁₂ H ₉ ClF ₃ N ₃ O	[M+H] ⁺	304.0459	2.7	Pesticide
Nuarimol	14.15	C ₁₇ H ₁₂ ClFN ₂ O	[M+H] ⁺	315.0695	3.7	Pesticide
Ofloxacin	6.24	C ₁₈ H ₂₀ FN ₃ O ₄	[M+H] ⁺	362.1511	3.1	Pharmaceutical
Ofurace	13.29	C ₁₄ H ₁₆ ClNO ₃	[M+H] ⁺	282.0891	6.0	Pesticide
Omethoate	2.22	C ₅ H ₁₂ NO ₄ PS	[M+H] ⁺	214.0297	84.3	Pesticide
Oryzalin	17.02	C ₁₂ H ₁₈ N ₄ O ₆ S	[M+H] ⁺	347.1020	52.7	Pesticide
Oxacillin	12.85	C ₁₉ H ₁₉ N ₃ O ₅ S	[M+H] ⁺	402.1118	3.7	Pharmaceutical
Oxadiazon	21.38	C ₁₅ H ₁₈ Cl ₂ N ₂ O ₃	[M+H] ⁺	345.0767	34.6	Pesticide
Oxadixyl	10.89	C ₁₄ H ₁₈ N ₂ O ₄	[M+H] ⁺	279.1339	27.2	Pesticide
Oxfendazole	8.64	C ₁₅ H ₁₃ N ₃ O ₃ S	[M+H] ⁺	316.0750	2.8	Pesticide
Oxolinic acid	9.71	C ₁₃ H ₁₁ NO ₅	[M+H] ⁺	262.0710	2.5	Pharmaceutical
Oxyfluorfen	21.00	C ₁₅ H ₁₁ ClF ₃ NO ₄	[M+H] ⁺	362.0401	83.9	Pesticide
Oxytetracycline	6.11	C ₂₂ H ₂₄ N ₂ O ₉	[M+H] ⁺	461.1555	195.3	Pharmaceutical

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Paclobutrazol	14.57	C ₁₅ H ₂₀ ClN ₃ O	[M+H] ⁺	294.1368	4.0	Pesticide
Paracetamol (Acetaminophen)	2.66	C ₈ H ₉ NO ₂	[M+H] ⁺	152.0706	8.6	Pharmaceutical
Parathion ethyl	18.50	C ₁₀ H ₁₄ NO ₅ PS	[M+H] ⁺	292.0403	93.0	Pesticide
Parathion-methyl	16.34	C ₈ H ₁₀ NO ₅ PS	[M+H] ⁺	264.0090	26.7	Pesticide
Pebulat	19.64	C ₁₀ H ₂₁ NOS	[M+H] ⁺	204.1417	13.3	Pesticide
Penconazole	16.70	C ₁₃ H ₁₅ Cl ₂ N ₃	[M+H] ⁺	284.0715	2.6	Pesticide
Pencycuron	19.21	C ₁₉ H ₂₁ ClN ₂ O	[M+H] ⁺	329.1415	3.1	Pesticide
Penicillin G	10.77	C ₁₆ H ₁₈ N ₂ O ₄ S	[M+H] ⁺	335.1064	59.3	Pharmaceutical
C3 Pentafluoropropionic acid fragment 1	12.73	C ₂ HF ₅	[M-H] ⁻	118.9926	51.7	PFAA
C4 Perfluorobutyric acid (PFBA) fragment 1	6.68	C ₃ HF ₇	[M-H] ⁻	168.9894	185.8	PFAA (PFBA)
C5 Perfluoropentanoic acid fragment 1	9.36	C ₄ HF ₉	[M-H] ⁻	218.9862	65.9	PFAA
C7 Perfluoroheptanoic acid (PFHpA)	5.16	C ₇ HO ₂ F ₁₃	[M-H] ⁻	362.9696	28.8	PFAA (PFHpA)
C8 Perfluorooctanoic acid (PFOA)	5.67	C ₈ HO ₂ F ₁₅	[M-H] ⁻	412.9664	19.5	PFAA (PFOA)
Perfluorooctanesulfonic acid (PFOS)	7.43	C ₈ HO ₃ F ₁₇ S	[M-H] ⁻	498.9302	13.7	PFAA (PFOS)
C9 Perfluorononanoic acid (PFNA)	6.17	C ₈ HO ₂ CF ₁₇	[M-H] ⁻	462.9632	13.3	PFAA (PFNA)
C10 Perfluorodecanoic acid (PFDA)	6.65	C ₁₀ HO ₂ F ₁₉	[M-H] ⁻	512.9600	8.4	PFAA (PFDA)
C11 Perfluoroundecanoic acid	7.46	C ₁₁ HO ₂ F ₂₁	[M-H] ⁻	562.9568	22.6	PFAA
C12 Perfluorododecanoic acid fragment 1	21.34	C ₁₁ HF ₂₃	[M-H] ⁻	568.9638	2.8	PFAA
Phenacetin	9.38	C ₁₀ H ₁₃ NO ₂	[M+H] ⁺	180.1019	24.4	Pharmaceutical
Phenylbuthazone	17.16	C ₁₉ H ₂₀ N ₂ O ₂	[M+H] ⁺	309.1598	42.5	Pharmaceutical
Phenylephrine	1.36	C ₉ H ₁₃ NO ₂	[M+H] ⁺	168.1019	16.2	Drug
Phosalone	19.43	C ₁₂ H ₁₅ ClNO ₄ PS ₂	[M+H] ⁺	367.9941	59.7	Pesticide
Phosphamidon	10.20	C ₁₀ H ₁₉ ClNO ₅ P	[M+H] ⁺	300.0762	1.8	Pesticide
Picloram	6.84	C ₆ H ₃ Cl ₃ N ₂ O ₂	[M+H] ⁺	240.9333	242.1	Pesticide
Picolinafen	20.52	C ₁₉ H ₁₂ F ₄ N ₂ O ₂	[M+H] ⁺	377.0908	43.4	Pesticide
Pindolol	5.55	C ₁₄ H ₂₀ N ₂ O ₂	[M+H] ⁺	249.1598	0.1	Pharmaceutical
Pipemidic acid	4.78	C ₁₄ H ₁₇ N ₅ O ₃	[M+H] ⁺	304.1404	265.8	Pharmaceutical
Pirimicarb	6.86	C ₁₁ H ₁₈ N ₄ O ₂	[M+H] ⁺	239.1502	10.7	Pesticide
Pirimiphos methyl	18.98	C ₁₁ H ₂₀ N ₃ O ₃ PS	[M+H] ⁺	306.1036	0.6	Pesticide

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Pravastatin	10.73	C ₂₃ H ₃₆ O ₇	[M+Na] ⁺	447.2353	0.4	Pharmaceutical
Pretilachlor	19.93	C ₁₇ H ₂₆ ClNO ₂	[M+H] ⁺	312.1725	7.4	Pesticide
Prochloraz	14.90	C ₁₅ H ₁₆ Cl ₃ N ₃ O ₂	[M+H] ⁺	376.0380	8.0	Pesticide
Procymidone	17.12	C ₁₃ H ₁₁ Cl ₂ NO ₂	[M+H] ⁺	284.0240	93.9	Pesticide
Profenofos	19.91	C ₁₁ H ₁₅ BrClO ₃ PS	[M+H] ⁺	372.9424	6.6	Pesticide
Promecarb	15.02	C ₁₂ H ₁₇ NO ₂	[M+H] ⁺	208.1332	54.0	Pesticide
Prometon	9.02	C ₁₀ H ₁₉ N ₅ O	[M+H] ⁺	226.1662	1.1	Pesticide
Prometryn	12.06	C ₁₀ H ₁₉ N ₅ S	[M+H] ⁺	242.1434	2.6	Pesticide
Propachlor	13.94	C ₁₁ H ₁₄ ClNO	[M+H] ⁺	212.0837	8.3	Pesticide
Propamocarb	2.30	C ₉ H ₂₀ N ₂ O ₂	[M+H] ⁺	189.1598	2.7	Pesticide
Propanil	14.62	C ₉ H ₉ Cl ₂ NO	[M+H] ⁺	218.0134	15.5	Pesticide
Propaquizafop	20.42	C ₂₂ H ₂₂ ClN ₃ O ₅	[M+H] ⁺	444.1321	39.5	Pesticide
Propazine	14.46	C ₉ H ₁₆ ClN ₅	[M+H] ⁺	230.1167	2.0	Pesticide
Propiconazole	17.26	C ₁₅ H ₁₇ Cl ₂ N ₃ O ₂	[M+H] ⁺	342.0771	0.9	Pesticide
Propisochlor	18.46	C ₁₅ H ₂₂ ClNO ₂	[M+H] ⁺	284.1412	218.5	Pesticide
Propoxur	11.90	C ₁₁ H ₁₅ NO ₃	[M+H] ⁺	210.1125	174.3	Pesticide
Propranolol	9.19	C ₁₆ H ₂₁ NO ₂	[M+H] ⁺	260.1645	0.8	Pharmaceutical
Propyphenazone	11.92	C ₁₄ H ₁₈ N ₂ O	[M+H] ⁺	231.1492	0.1	Pharmaceutical
Propyzamid	16.27	C ₁₂ H ₁₁ Cl ₂ NO	[M+H] ⁺	256.0290	7.7	Pesticide
Proquinazid	22.97	C ₁₄ H ₁₇ IN ₂ O ₂	[M+H] ⁺	373.0407	6.3	Pesticide
Prosulfocarb	20.40	C ₁₄ H ₂₁ NOS	[M+H] ⁺	252.1417	12.9	Pesticide
Prosulfuron	15.18	C ₁₅ H ₁₆ F ₃ N ₅ O ₄ S	[M+H] ⁺	420.0948	6.8	Pesticide
Pymetrozin	1.40	C ₁₀ H ₁₁ N ₅ O	[M+H] ⁺	218.1036	13.1	Pesticide
Pyraclostrobin	18.99	C ₁₉ H ₁₈ ClN ₃ O ₄	[M+H] ⁺	388.1059	2.2	Pesticide
Pyranocoumarin	17.91	C ₂₀ H ₁₈ O	[M+H] ⁺	323.1278	14.9	Pesticide
Pyridaben	23.13	C ₁₉ H ₂₅ ClN ₂ OS	[M+H] ⁺	365.1448	175.4	Pesticide
Pyridaphenthion	16.12	C ₁₄ H ₁₇ N ₂ O ₄ PS	[M+H] ⁺	341.0719	1.7	Pesticide
Pyrimethanil	11.63	C ₁₂ H ₁₃ N ₃	[M+H] ⁺	200.1182	3.1	Pesticide
Pyriproxifen	21.22	C ₂₀ H ₁₉ NO ₃	[M+H] ⁺	322.2220	601.4	Pesticide

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Pyroquilon	10.15	C ₁₁ H ₁₁ NO	[M+H] ⁺	174.0913	5.1	Pesticide
Quinalphos	18.16	C ₁₂ H ₁₅ N ₂ O ₃ PS	[M+H] ⁺	299.0613	5.2	Pesticide
Quinmerac	7.78	C ₁₁ H ₈ ClNO ₂	[M+H] ⁺	222.0316	9.9	Pesticide
Quinoclamine	11.37	C ₁₀ H ₆ ClNO ₂	[M+H] ⁺	208.0160	19.8	Pesticide
Quinoxifen	20.26	C ₁₅ H ₈ Cl ₂ FNO	[M+H] ⁺	308.0039	5.1	Pesticide
Quizalofop-P-ethyl	20.19	C ₁₉ H ₁₇ ClN ₂ O ₄	[M+H] ⁺	373.0950	167.8	Pesticide
Ranitidine	2.03	C ₁₃ H ₂₂ N ₄ O ₃ S	[M+H] ⁺	315.1485	2.9	Pharmaceutical
Rotenone	17.18	C ₂₃ H ₂₂ O ₆	[M+H] ⁺	395.1489	22.6	Pesticide
Roxithromycin	11.28	C ₄₁ H ₇₆ N ₂ O ₁₅	[M+H] ⁺	837.5318	2.9	Pharmaceutical
Salbutamol	1.63	C ₁₃ H ₂₁ NO ₃	[M+H] ⁺	240.1594	7.1	Pharmaceutical
Salicylic acid	14.18	C ₇ H ₆ O ₃	[M-H] ⁻	137.0244	143.5	Pharmaceutical
Sarafloxacin	7.50	C ₂₀ H ₁₇ F ₂ N ₃ O ₃	[M+H] ⁺	386.1311	61.4	Pharmaceutical
Simazine	10.66	C ₇ H ₁₂ ClN ₅	[M+H] ⁺	202.0854	0.7	Pesticide
Simetryn	8.74	C ₈ H ₁₅ N ₅ S	[M+H] ⁺	214.1121	2.2	Pesticide
Spinosyn A (Spinosad)	14.94	C ₄₂ H ₆₇ NO ₁₀	[M+H] ⁺	732.4681	117.0	Pesticide
Spinosyn D (Spinosad)	14.21	C ₄₁ H ₆₅ NO ₁₀	[M+H] ⁺	746.4838	14.9	Pesticide
Spiramycin I	7.59	C ₄₃ H ₇₄ N ₂ O ₁₄	[M+H] ⁺	843.5213	14.5	Pharmaceutical
Spiromesifen	23.07	C ₂₃ H ₃₀ O ₄	[M+H] ⁺	371.2216	449.7	Pesticide
Spiroxamine	12.53	C ₁₈ H ₃₅ NO ₂	[M+H] ⁺	298.2740	3.5	Pesticide
Sulcotrione	12.25	C ₁₄ H ₁₃ ClO ₅ S	[M+H] ⁺	329.0245	8.6	Pesticide
Sulfachloropyridazine	8.42	C ₁₀ H ₉ ClN ₄ O ₂ S	[M+H] ⁺	285.0208	3.2	Pharmaceutical
Sulfadiazine	4.16	C ₁₀ H ₁₀ N ₄ O ₂ S	[M+H] ⁺	251.0597	7.9	Pharmaceutical
Sulfadimethoxine	10.41	C ₁₂ H ₁₄ N ₄ O ₄ S	[M+H] ⁺	311.0809	1.6	Pharmaceutical
Sulfamerazine	5.73	C ₁₁ H ₁₂ N ₄ O ₂ S	[M+H] ⁺	265.0754	4.5	Pharmaceutical
Sulfamethizole	7.12	C ₉ H ₁₀ N ₄ O ₂ S ₂	[M+H] ⁺	271.0318	4.0	Pharmaceutical
Sulfamethoxazole	9.06	C ₁₀ H ₁₁ N ₃ O ₃ S	[M+H] ⁺	254.0594	15.0	Pharmaceutical
Sulfanilamide	1.82	C ₆ H ₈ N ₂ O ₂ S	[M+H] ⁺	173.0379	7.6	Pharmaceutical
Sulfapyridine	5.27	C ₁₁ H ₁₁ N ₃ O ₂ S	[M+H] ⁺	250.0645	2.8	Pharmaceutical
Sulfathiazole	5.20	C ₉ H ₉ N ₃ O ₂ S ₂	[M+H] ⁺	256.0209	14.2	Pharmaceutical

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Sulfometuron methyl	12.16	C ₁₅ H ₁₆ N ₄ O ₅ S	[M+H] ⁺	365.0914	6.0	Pesticide
Sulfotep	19.28	C ₈ H ₂₀ O ₅ P ₂ S ₂	[M+H] ⁺	323.0300	34.9	Pesticide
Tebuconazole	16.16	C ₁₆ H ₂₂ ClN ₃ O	[M+H] ⁺	308.1524	2.6	Pesticide
Tebufenpyrad	20.15	C ₁₈ H ₂₄ ClN ₃ O	[M+H] ⁺	334.1680	10.7	Pesticide
Tebuthiuron	9.87	C ₉ H ₁₆ N ₄ OS	[M+H] ⁺	229.1118	4.2	Pesticide
TCP	15.20	C ₉ H ₁₈ Cl ₃ O ₄ P	[M+H] ⁺	327.0081	8.3	Plasticizer
Teflurbenzuron	19.43	C ₁₄ H ₆ Cl ₂ F ₄ N ₂ O ₂	[M+H] ⁺	380.9815	132.8	Pesticide
Tembotrione	15.70	C ₁₇ H ₁₆ ClF ₃ O ₆ S	[M+H] ⁺	441.0381	10.7	Pesticide
Temephos	21.19	C ₁₆ H ₂₀ O ₆ P ₂ S ₃	[M+H] ⁺	466.9970	324.7	Pesticide
TEP (Triethyl phosphate)	9.03	C ₆ H ₁₅ O ₄ P	[M+H] ⁺	183.0781	6.8	Flame retardant
Tepraloxymid	16.18	C ₁₇ H ₂₄ ClNO ₄	[M+H] ⁺	342.1467	19.5	Pesticide
Terbumeton	9.32	C ₁₀ H ₁₉ N ₅ O	[M+H] ⁺	226.1662	1.2	Pesticide
Terbutylazine	14.94	C ₉ H ₁₆ ClN ₅	[M+H] ⁺	230.1167	2.1	Pesticide
Terbutylazine desethyl	11.18	C ₇ H ₁₂ ClN ₅	[M+H] ⁺	202.0853	1.1	Pesticide metabolite
Terbutryn	18.28	C ₁₀ H ₁₉ N ₅ S	[M+H] ⁺	242.1434	1.7	Pesticide
Testosterone	13.71	C ₁₉ H ₂₈ O ₂	[M+H] ⁺	289.2162	6.1	Pharmaceutical
Tetrachlorvinfos	16.94	C ₁₀ H ₉ Cl ₄ O ₄ P	[M+H] ⁺	364.9065	5.4	Pesticide
Tetracycline	6.62	C ₂₂ H ₂₄ N ₂ O ₈	[M+H] ⁺	445.1605	35.0	Pharmaceutical
11-nor-9-carboxy-THC	19.06	C ₂₁ H ₂₈ O ₄	[M+H] ⁺	345.2060	13.4	Drug
Δ-9-THC	23.61	C ₂₁ H ₃₀ O ₂	[M+H] ⁺	315.2319	321.5	Drug
Theobromine	1.96	C ₇ H ₈ N ₄ O ₂	[M+H] ⁺	181.0720	21.6	LS - Caffeine metab.
Theophylline	2.80	C ₇ H ₈ N ₄ O ₂	[M+H] ⁺	181.0720	21.4	LS - Caffeine metab.
Thiabendazole	4.54	C ₁₀ H ₇ N ₃ S	[M+H] ⁺	202.0433	7.0	Pharmaceutical
Thiacloprid	10.12	C ₁₀ H ₉ ClN ₄ S	[M+H] ⁺	253.0309	4.3	Pesticide
Thiamethoxam	6.79	C ₈ H ₁₀ ClN ₅ O ₃ S	[M+H] ⁺	292.0266	42.6	Pesticide
Thiamphenicol	6.60	C ₁₂ H ₁₅ Cl ₂ NO ₅ S	[M-H] ⁻	353.9975	27.1	Pesticide
Thiocyclam (hydrogenoxalate)	2.02	C ₅ H ₁₁ NS ₃	[M+H] ⁺	182.0126	19.8	Pesticide
Thiodicarb	11.80	C ₁₀ H ₁₈ N ₄ O ₄ S ₃	[M+H] ⁺	355.0563	31.8	Pesticide
Timolol	6.87	C ₁₃ H ₂₄ N ₄ O ₃ S	[M+H] ⁺	317.1642	7.9	Pharmaceutical

COMPOUND	RT (min)	ELEMENTAL COMPOSITION	ION	m/z	LOQ (ng L ⁻¹)	POLLUTANT FAMILY
Triadimefon	15.96	C ₁₄ H ₁₆ ClN ₃ O ₂	[M+H] ⁺	294.1004	4.7	Pesticide
Triadimenol	14.38	C ₁₄ H ₁₈ ClN ₃ O ₂	[M+H] ⁺	296.1160	2.6	Pesticide
Triasulfuron	12.39	C ₁₄ H ₁₆ ClN ₃ O ₅ S	[M+H] ⁺	402.0633	5.6	Pesticide
Triazophos	17.07	C ₁₂ H ₁₉ N ₃ O ₃ PS	[M+H] ⁺	314.0722	2.4	Pesticide
Triazoxid	9.64	C ₁₀ H ₆ ClN ₅ O	[M+H] ⁺	248.0334	2.6	Pesticide
Tributyl phosphate (TBP)	18.43	C ₁₂ H ₂₇ PO ₄	[M+H] ⁺	267.1720	10.7	Flame retardant
Trichlorfon	7.12	C ₄ H ₆ Cl ₃ O ₄ P	[M+H] ⁺	256.9299	119.9	Pesticide
Triclocarban	19.12	C ₁₃ H ₉ Cl ₃ N ₂ O	[M+H] ⁺	314.9853	27.0	Pesticide
Trietazine	16.57	C ₉ H ₁₆ ClN ₅	[M+H] ⁺	230.1167	1.8	Pesticide
Trifloxystrobin	19.84	C ₂₀ H ₁₉ F ₃ N ₂ O ₄	[M+H] ⁺	409.1370	23.3	Pesticide
Triflumizole	16.93	C ₁₅ H ₁₅ ClF ₃ N ₃ O	[M+H] ⁺	346.0929	26.0	Pesticide
Triflurmon	18.19	C ₁₅ H ₁₀ ClF ₃ N ₂ O ₃	[M+H] ⁺	359.0405	17.6	Pesticide
Trifluralin	21.80	C ₁₃ H ₁₆ F ₃ N ₃ O ₄	[M+H] ⁺	336.1166	295.5	Pesticide
Trimethoprim	5.60	C ₁₄ H ₁₈ N ₄ O ₃	[M+H] ⁺	291.1452	7.8	Pharmaceutical
Trinexapac-ethyl	14.24	C ₁₃ H ₁₆ O ₅	[M+H] ⁺	253.1071	41.3	Pesticide
Tylosin	10.14	C ₄₆ H ₇₇ NO ₁₇	[M+H] ⁺	916.5264	6.1	Pesticide
Warfarin	14.95	C ₁₉ H ₁₆ O ₄	[M+H] ⁺	309.1121	10.3	Pharmaceutical

(**) The percentage of each compound in the technical mixture have been considered to calculate the analytical parameters:

Benzalkonium chloride-C10 (0.06%)

Benzalkonium chloride-C12 (69.07%)

Benzalkonium chloride-C14 (30.79%)

Benzalkonium chloride-C16 (0.17%)

(1) Recovery study performed at 10 ug L⁻¹ concentration level.

(2) Bis (2-ethylhexyl)phthalate (DEHP) . LOQ calculated with solvent standards.

2.2. Selection of Analytes

Substances included in this study were selected on the basis of legislation [1-3], previous experience [35] and other relevant literature [15-46]. They comprise 430 pollutants belonging to different compound classes such as pharmaceuticals, life-style products (LS), drugs of abuse, pesticides and some of their more relevant metabolites. Among the 105 selected pharmaceuticals, there are representatives of different therapeutical groups, such as

analgesics/anti-inflammatories (paracetamol, indomethacine, codeine, mefenamic acid, naproxen, ibuprofen, diclofenac, ketoprofen), *antibiotics* (metronidazole, sulfamethoxazole, trimethoprim, cefotaxime, ofloxacin, erythromycin), *lipid regulators* (fenofibrate, bezafibrate, gemfibrozil), β -*blockers* (atenolol, pindolol, timolol), *antiepileptic/psychiatric* (carbamazepine, fluoxetine), *ulcer healings* (ranitidine), *diuretics* (furosemide, hydrochlorothiazide), *hormones* (estrone, testosterone) and *bronchodilators* (salbutamol). Because of their relevance and, in some cases, significant presence, a group of metabolites such as clofibric acid and fenofibric acid were also included. Caffeine and nicotine are worldwide-used substances consumed in large quantities. Therefore they were included with their metabolites in a group called *life-style compounds* (LS). The selected list of 21 drugs of abuse comprises *stimulants* (cocaine, ephedrine, amphetamine and some of the compounds that are referred as amphetamine family [3,4-Methylenedioxyamphetamine -MDA-, 3,4-Methylenedioxy-N-methylamphetamine -MDMA-]), *opiate analgesic drugs* (methadone, morphine, heroine) *cannabinoids* [cannabidiol, delta-9-tetrahydrocannabinol (Δ -9-THC)] and some metabolites of the selected drugs such as benzoylecgonine, ethylmorphine. In addition, 279 pesticides and two of their metabolites (atrazine desethyl, terbutylazine desethyl) were selected, including *herbicides* (prometon, terbutylazine, oxyfluorfen, anilofos) *insecticides* (chlorpyrifos ethyl, aldicarb, fenthion) or *fungicides* (imazalil, bupirimate, carbendazim) amongst others. Due to their risk to human health, a group of 5 nitrosamines were selected. Additionally, 10 perfluorinated compounds (PFCs) included in the group of perfluoroalkyl acids PFAAs (C8 perfluorooctanoic acid (PFOA), C9 perfluorononanoic acid (PFNA), perfluorooctanesulfonic acid (PFOS)), were also included in the study, together with 2 flame retardants (triethylphosphate (TEP) and tributylphosphate (TBP)) and 3 plasticizers (Bisphenol A, DEHP and TCPP). Nineteen of the target analytes are considered in the EPA Contaminant Candidate List 3 (CCL3) for drinking water [47], 17 are included in the Directive 2013/39/EU for surface water [3] and 11 in the EPA Unregulated Contaminant Monitoring Rule 3 (UCMR3) for public water systems [48]. The proposal COM (2001) 876 of the EU contained three of the target compounds, diclofenac, 17- α -ethynylestradiol and β -estradiol, but they were finally disregarded from the Directive 2013/39/EU. All the regulated compounds are listed in Table S2 (Supplementary Information).

2.3. Water and wastewater samples

Surface waters and effluent wastewater samples, collected from different sampling points (WWTPs and river or reservoirs) described elsewhere [49-51], were used for method development and performance evaluation. A pool of different WWTPs samples from these studies was used to prepare wastewater extracts for matrix-matched calibration and the evaluation of matrix effects.

2.4. Sample treatment

The selected generic offline solid phase extraction (SPE) procedure for the extraction and preconcentration of polar and semi-polar compounds was adapted partially from previous literature [35,39,52]. 200 mL of surface or wastewater sample (pH 7) were passed through an Oasis HLB SPE cartridge (200 mg) previously conditioned with 4 mL of methanol and 8 mL of Milli-Q water. After loading the sample, the elution was performed with two aliquots of 4 mL of MeOH. The extract collected was evaporated under a gentle nitrogen stream using a Turbo Vap LV from Zymark (Hopkinton, MA), with a water bath temperature of 37 °C and a N₂ pressure of 15 psi. Finally, the residue was re-dissolved in 1 mL of MeOH/water (20:80), achieving a final preconcentration factor of 200:1.

2.5. LC-TOFMS analysis

Chromatographic analysis was carried out using an Agilent 1290 Infinity HPLC system consisting of a vacuum degasser, an autosampler and a binary pump (Agilent Technologies, Santa Clara, CA) equipped with a C-18 analytical column (Agilent ZORBAX Eclipse Plus C18, 50 mm × 4.6 mm, 1.8 µm particle size). 20 µL of the sample extract was injected in each run. Mobile phases A and B were mili-Q water and acetonitrile, both with 0.1% (v/v) formic acid. The chromatographic method held the initial mobile phase composition (10% B) constant for 3 min, followed by a linear gradient to 100% B up to 25 min, and kept for 3 min at 100% B. After each run, a 10-min equilibration was performed with the initial mobile phase composition. The flow rate was 0.5 mL min⁻¹. The HPLC system was connected to an time-of flight mass spectrometer (Agilent 6220 accurate mass TOF, Agilent Technologies, Santa Clara, CA) equipped with an

electrospray interface operated in positive or negative ionization mode, using the following operation parameters: capillary voltage, 4000 V; nebulizer pressure, 40 psi; drying gas flow rate, 9 L min⁻¹; gas temperature, 325 °C; skimmer voltage, 65 V; octopole rf, 250 V; fragmentor voltage: 190 V. LC–MS accurate mass spectra were recorded across the range of 50–1000 m/z in positive ionization mode, and in the range 60–1050 m/z in negative ionization mode. Although the instrument features fast polarity-switching acquisition, dedicated runs in each ionization mode were performed. The instrument provides a mass resolution of ca. 19000 for 922 m/z and of ca. 12000–14000 for most of the masses analyzed, which permits to distinguish coeluting isobaric species with a mass difference between 20 and 30 mDa. Accurate mass measurements of each peak from the total ion chromatograms were obtained using an automated calibrant delivery system to provide mass correction. The instrument performed the internal mass calibration automatically, using a dual-nebulizer electrospray source with an automated calibrant delivery system, which introduces the flow from the outlet of the chromatograph together with a low flow (approximately 10 µL min⁻¹) of a calibrating solution containing the internal reference masses purine (C₅H₅N₄/[M+H]⁺/at m/z 121.050 873) and HP-0921 ([hexakis-(1H,1H,3H-tetrafluoropentoxo)-phosphazene] (C₁₈H₁₉O₆N₃P₃F₂₄)/[M+H]⁺/at m/z 922.009798). The full-scan data recorded was processed with Agilent MassHunter Software (version B.04.00).

2.6. Analytical parameters and method validation

To evaluate the analytical features of the proposed method, calibration curves of the target compounds were constructed at different concentrations, in the range 1–500 µg L⁻¹ using wastewater extracts spiked with selected compounds, to prepare matrix-matched standards at 6 concentration levels (1, 10, 50, 100, 200 and 500 µg L⁻¹). Working with matrix-matched standard in environmental samples could lead to representativeness problems, therefore a pool of different effluent wastewater samples from different WWTPs were used to obtain a representative extract. To assess the impact of the matrix on the ionization suppression/enhancement on the analytes (compared to neat standards), the slopes obtained in the calibration with effluent wastewater matrix-matched standards were compared with those obtained with solvent-based standards, calculating slope ratios matrix/solvent for each of the 430 target compounds. Ideally a matrix-matched standard should not contain any of the selected

compounds, but in practice this is not always possible, thus the areas of the compounds which are present in the blank extract has to be subtracted to the area obtained from the matrix-matched standards to calculate the LOQs and to construct the matrix calibration curves. These compounds often found in blank matrices were usually at low concentration levels (eg. distinctly lower than the lowest calibration level used for LOQ calculation) so that the uncertainty associated subtracting blank peak areas and assuming a even behavior of the S/N ratio is probably lower than the uncertainty associated to the entire method including composition of matrix extracts. Only for DEHP, the concentration level found in the sample was too high to enable an estimation of LOQ. In this case, the LOQ was calculated from solvent standards and it is appropriately annotated in the text (Table 1).

On the other hand, the effectiveness of the extraction method was evaluated by means of recovery studies using spiked wastewater from the same pool of effluent wastewater samples from different plants described elsewhere [49,50]. Several water aliquots were spiked at two different concentration levels (50 and 500 ng L⁻¹) with the working standard solution. An additional recovery study at 10 µg L⁻¹ was carried out for those compounds (Table 1) with LOQs above the highest recovery level (500 ng L⁻¹). The spiked samples (n=6) were extracted with the SPE method described in the experimental section and subsequently analyzed with the developed LC–MS method.

2.7. Experimental accurate-mass database of water priority and emerging pollutants

Mixtures Mixtures containing ca. 30-50 compounds, at individual concentrations of 200 µg L⁻¹ each were injected in the LC–TOFMS system to collect retention time (t_R) data and the accurate masses of target ions together with the elemental composition. For confirmatory purposes of the compounds detected in real samples, the mass spectrum was carefully investigated to identify characteristic fragment ions produced by collision induced dissociation (CID) in ion transportation region (in-source CID). In some cases, individual standards of priority and emerging compounds were used for further confirmation of diagnostic fragment ions. The database comprises 430 species (426 detected as parent molecules and 4 detected as fragment ions). For the screening method step, an Excel spreadsheet was constructed containing for each

analyte the compound name, molecular formula, theoretical exact mass, fragment ions and retention time. This file was converted into csv format for use by the Agilent Mass Hunter Data Acquisition software (version B.04.00). When a sample run is finished and the corresponding data acquired, the information contained in the csv file is searched automatically by the Mass Hunter software taking into account a defined tolerance for mass and retention time deviations ($t_R \pm 0.2$ min and ion exact mass ± 20 ppm), and a report is generated with the compounds tentatively found in the analyzed sample data file.

3. Results and discussion

3.1. Development of an accurate-mass database for automated screening of contaminants in water samples

In order to build the database, solvent-based standards with mixtures of the different studied contaminants were analyzed. These runs are used as a reference for setting the retention time and to select the ions used for identification purposes. The accurate-mass database (Table 1) includes the exact monoisotopic masses for 430 multiclass pollutants. The vast majority of the target compounds were detected in positive ionization mode, and the protonated molecule $[M+H]^+$ was used in most cases (389 compounds) from identification purposes. Sodium adducts ($[M+Na]^+$) were used instead for 15 compounds because the relative intensity of the sodium adduct ions was distinctly higher than the corresponding protonated molecule. Only 26 compounds were detected in negative ionization mode, selecting the deprotonated molecule $[M-H]^-$ as identification criterion for most of them except for perfluoropropionic acid, perfluorobutyric acid, perfluoropentanoic acid and perfluorododecanoic acid, which were identified with characteristic fragments.

Once the database is built with experimental data, parameters related to data processing (compound detection and filtering) were optimized. From previous experience, a 0.4-min retention time window ($t_R \pm 0.2$ min) was used together with a 40-ppm mass tolerance window (ion exact mass ± 20 ppm –relative mass error-). An additional filter based on a minimum intensity value of 5000 (ca. the typical height corresponding to approximately the LOD

of a large number of the studied compounds) was also applied. This threshold value was chosen only to minimize false positives coming from the low intensity background signals.

An issue associated to methods covering such a large number of analytes might be the eventual resolution of compounds that have the same nominal mass (isobars if they present different composition but the same nominal mass and isomers if they have same exact mass and composition). The use of LC-HRMS provides four different parameters to resolve and distinguish these compounds: (a) by retention time –separation by HPLC-; (b) by using the resolving power of the instrument –separated by significantly different accurate mass-; (c) by comparing isotopic patterns; (d) by using characteristic fragmentation by measuring different fragment ion for each of the compounds affected. From the first aspect, the chromatography is a powerful tool and from preliminary experiments with short or “ultrafast” chromatographic methods (eg. less than 10-min), we found that wastewater extracts were highly complex and with short gradients the likelihood of suffering interferences coming from isobaric species from the matrix was relatively high (Figure S1, Supplementary Information). In addition, strong matrix effects (signal suppression/enhancement) were found with these short methods. In addition, there are several isomers (viz. triazine compounds) that need a relatively dedicated gradient to obtain appropriate separation. For these reasons we considered the use of a 1.8- μm particle size C18 column with a long 28 minutes gradient as the most rugged approach. With this choice, minimized matrix effects were encountered so resulting in increased matrix separation and the presence of huge signals in the TIC chromatogram was avoided and the matrix effects associated thereof. Probably, this method could be shortened without a dramatic decrease in overall ruggedness, but the throughput requested in our application does not require such level, so we did not push the limit even further than this 28-min method. In addition, considering the possibility of retrospective analyses of new compounds and the extension of the method to new compounds, this method would be more appropriate and less subjected to matrix interferences than faster gradients.

A selectivity study of the different compounds included in the database was performed to assess the resolution of the method, even keeping in mind that the possibility of having

interferences between two targeted compounds is much lower than the chance of having a (wastewater) matrix-induced interference.

Interestingly, to clearly establish the crucial role of the chromatographic separation and therefore to include the retention time data in the database, 288 (66.9%) out of the 430 studied compounds were involved in isobaric groups (110 sets from 2 to 7 compounds each one). The vast majority of cases (260 compounds) were clearly resolved by retention time as detailed in Table S3 (Supplementary information). In the remaining cases with isobaric species involved, at least two compounds eluted relatively close (with calculated chromatographic resolution (R_s) less than 1.5), which may eventually lead to interferences, so it is necessary to use additional criteria to resolve them. The main features available with LC-HRMS approaches are as follows: (i) the use of mass spectrometer resolving power/ narrow accurate mass windows; (ii) isotope pattern matching information and (iii) the use of characteristic diagnostic ions coming from CID experiments.

The resolution of the instrument on the m/z axis could be used to distinguish between some of the isobaric coeluting species. The TOF instrument used displayed a resolution of ca. 12000 for the mass range common to most of our target compounds, which can resolve interferences in the range of ca. 25-30 mDa (Δm). Thus, using this feature, 8 pairs of compounds could be distinguished (see Table S3) besides the small retention time differences that still may be used in some cases. Advances in instrumentation recently introduced, *i.e.* the use of a longer flight path and/or enhanced electronics are supposed to provide a resolution of 50000 or above for small molecules (< 500 Da). As an alternative to TOF, the use of Orbitrap provides resolutions > 140000 for small molecules, with scan speeds fitting HPLC separation. Although this resolving power is not enough for resolving isomers with the same elemental composition and exact mass, it provides enhanced selectivity by means of using narrower extraction mass windows.

Finally, additional information was included in the database to differentiate between the isobaric species that cannot be resolved by chromatographic or accurate masses of parent ions. For this reason, fragment ions obtained from in-source CID fragmentation were included for the

14 compounds that could not be elucidated by retention time or resolving power (Table S5, Supplementary information). These fragmentation data were provided for the species, which gave values of R_s and Δm below or close to the cut-off levels, 1.5 and 30 mDa respectively (Table S4, Supplementary information). All of the cases are illustrated in Figure S2 (Supplementary information), where the chromatogram and mass spectrum of each set of isobaric compounds are superposed. Although in some cases, such as the pair desmetryn-simetryn presented even some similar fragments, elucidation of all 14 compounds was successfully achieved by fragmentation pattern differences. In conclusion, all the analytes included in this study could be distinguished between themselves by retention time, mass resolving power or characteristic fragmentation. Finally, an additional tool to further confirm the identity of a positive is the isotope pattern matching of experimental data with the theoretical value assigned to the elemental composition of the tentative positive. The used software includes in the results report a score coefficient (scale up to 100), which considers the relative abundance of the different isotope signal of the detected species, the space (m/z gap) between these signals and the relative mass error [39].

3.2. Sample treatment and recovery studies

Considering the wide array of species with different physicochemical properties, a generic sample treatment procedure was selected, based on polymeric Oasis HLB SPE cartridge. This polymer-based sorbent enables simultaneously various types of interactions, hydrophilic and lipophilic, thus being more suitable than specific SPE sorbents relying on a simple retention mechanism. Moreover, the use of a generic procedure may eventually enable the extraction of non-target or unknown compounds that can be further investigated in retrospective analyses of the samples.

The results of the effectiveness of the extraction method study are summarized in Table S1 (supplementary information) and Figure 1. To provide a general overview of the results obtained with SPE extraction, the recovery percentages obtained for all the compounds are plotted against retention time in figure 1b, which shows that there is not a clear tendency that can lead to a statement on the correlation between the polarity of the compounds and the

recovery values reached. This can be attributed partially due to the different coexisting mechanisms of the used SPE cartridge (both hydrophobic and hydrophilic). In general, the results are satisfactory as most of the analytes tested are recovered appropriately. Only 1.2% of the studied compounds (5 compounds) were not recovered at all (e.g., 2,4-dinitrophenol, bifentazate). Another 14.9% of the studied analytes (64 compounds) showed recoveries below the 30% of the spiked concentration, e.g. acephate, ethoxyquin, oxytetracycline. 35% (153 compounds) displayed recovery values between 70 and 128%. Although the majority of the compounds included in the database, that is 49% of the analytes (209 compounds), showed recoveries between 30-70%, out of the normally accepted range of 70-120%, this does not prevent the proper application of this procedure to compounds of interest such as acetonitrile, irgarol, PFOS (regulated by Directive 2013/39/EU) and emerging contaminants (eg. 4-aminoantipyrine, cis-Diltiazem, clarithromycin etc.) since the precision of the method is satisfactory with RSD% values below 10% for most of analytes and the sensitivity reached allowed the identification of regulated compounds below the limits established by normative. The relative standard deviations (RSD%) values (n=6) were in the range 1-15% for 83% of the target compounds (357 compounds), as can be seen in Table S1. These results show the feasibility of the studied extraction method for multi-residue pollutants analysis in wastewater. Matrix effects were also assessed and discussed in the following section.

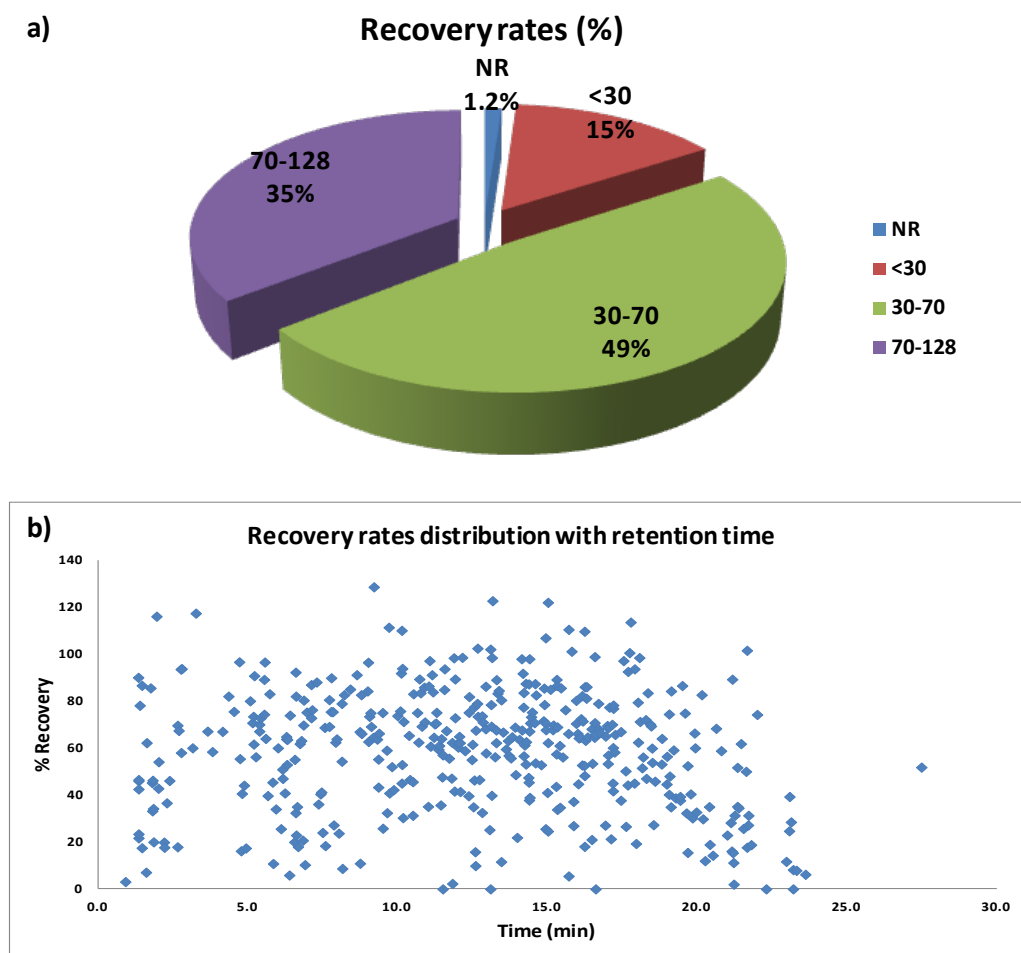


Figure 1. Percentage of pollutants according their recoveries at a concentration level of 500 ng L⁻¹ (a). 2D Plot representing the recovery percentages for all selected compounds over the entire LC run.

3.3. Analytical performance of the proposed method

The linearity of the analytical response across the studied range is excellent, taking into account that the calibration curves of the analyzed pollutants showed correlation coefficients higher than 0.999 in most cases. The limits of quantification (LOQs) (Table 1) were calculated as the minimum concentration of analyte corresponding to a signal-to-noise ratio (S/N) = 10:1. LOQs were calculated using the data acquired from matrix-matched extracts obtained during recovery experiments (at 50 and 500 ng L⁻¹) or by subsequent dilutions of these extracts with

non-spiked matrix. The more abundant ion for each compound based on the signal from high-resolution extracted ion chromatograms with narrow mass window was used. For those compounds already present in the matrix extract, the area obtained in the non-spiked matrix extract was subtracted for that obtained in the spiked matrix-matched standard. Therefore, for these compounds was also possible to calculate the LOQs assuming an even behavior of the S/N ratio, indeed at the lowest concentration level ($1 \mu\text{g L}^{-1}$) the signal offered by the matrix-matched standard was always distinctly higher than that obtained in the blank matrix, except for DEHP. In figure 2 XICs for some representative compounds at the lowest matrix-matched calibration level are shown.

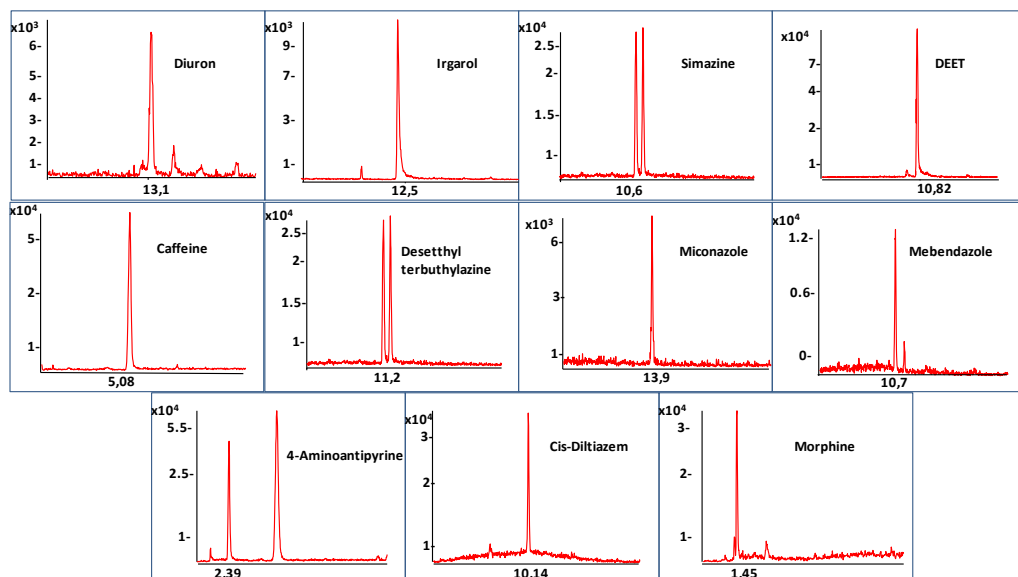


Figure 2. Extracted ion chromatograms XICs for some representative selected pollutants in wastewater extract spiked with $1 \mu\text{g L}^{-1}$ of each compound. Counts Vs retention time are represented.

As mentioned before, 1.2% of the studied compounds (5) were not recovered at all, and therefore they appear in Table 1 as non-detectable compounds. Only five analytes showed LOQs higher than one microgram per liter, λ -cyhalothrin, metolcarb, chlorpropham, α -cypermethrin, and deltamethrin. The vast majority compounds included in the database showed limits of quantification in the low nanogram per liter concentration level. Briefly, 3.7% of the target compounds showed LOQs below 1 ng L^{-1} , 44% of the studied analytes showed LOQs

between 1 – 10 ng L⁻¹, 38.6% showed LOQs in the range 10 – 100 ng L⁻¹ and 12.6% of the analytes showed LOQs above 100 ng L⁻¹. These data are represented graphically in Figure 3.

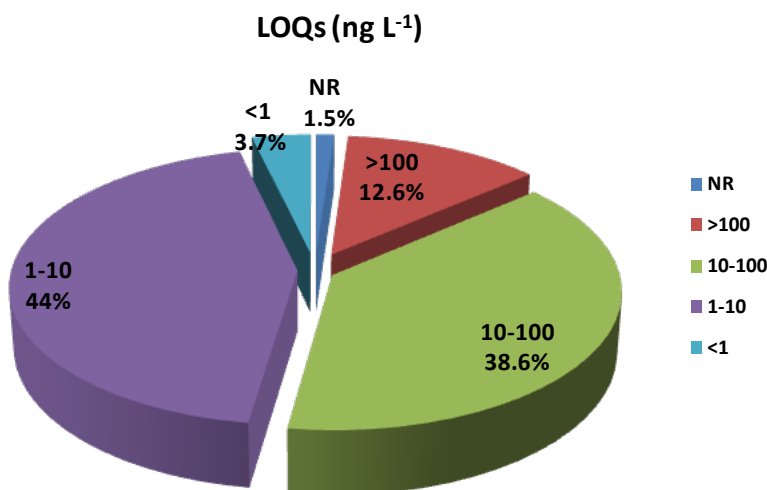


Figure 3. Percentages of target compounds according their LOQ (ng L⁻¹).

It should be emphasized that the results are in most cases appropriate for the targeted application according to the current regulations, even considering that the TOF instrument is far from being the state-of-the-art of its class. Actually, current TOF instruments from the same or other manufacturers easily offer a dramatic 10 to 50-fold sensitivity increase, which definitely assure the feasibility of the proposed approach for the screening of such amount of compounds with a single method.

Matrix components can both reduce or enhance the signal displayed by the analytes when they are subjected to ionization step in LC-MS, particularly with electrospray interfaces. For this reason, a thorough study of matrix effects was undertaken by comparing the slopes of calibration curves in the presence and absence of matrix components. Slope ratios significantly above 1 correspond to signal enhancement, while slope ratios significantly below 1 correspond to signal suppression. The results are summarized in the Figure 4, expressing the matrix effects as a percentage of signal variation. As can be concluded in figure 4b when matrix effects are plotted against LC run time, most of compounds are subjected to matrix suppression.

Reasonably positive results were obtained for over 75 % of the compounds. Actually, 55.8% of the analytes (240 compounds) showed low matrix effects, below the 25% of signal suppression/enhancement, while 20.7 % (89 compounds) displayed moderate matrix effects (25-50 % of suppression/enhancement). On the other hand, 21.9% of the compounds studied (94 compounds) showed strong matrix effects, with signal suppression or enhancement higher than 50%. Moreover, it was not possible to calculate the matrix effect value for 7 compounds (noted as N/A -not available- in table S1), 5 of them (bisphenol A, chlorpropham, deltamethrin, bentazon, α -cypermethrin) displayed LOQs above 100 $\mu\text{g L}^{-1}$. On the other hand, it was not possible to construct the matrix matched standard calibration curve for DEHP and estriol, the first one due to its high concentration in the unspiked matrix extract (blank) and the second one because suffered total signal suppression of matrix matched standard.

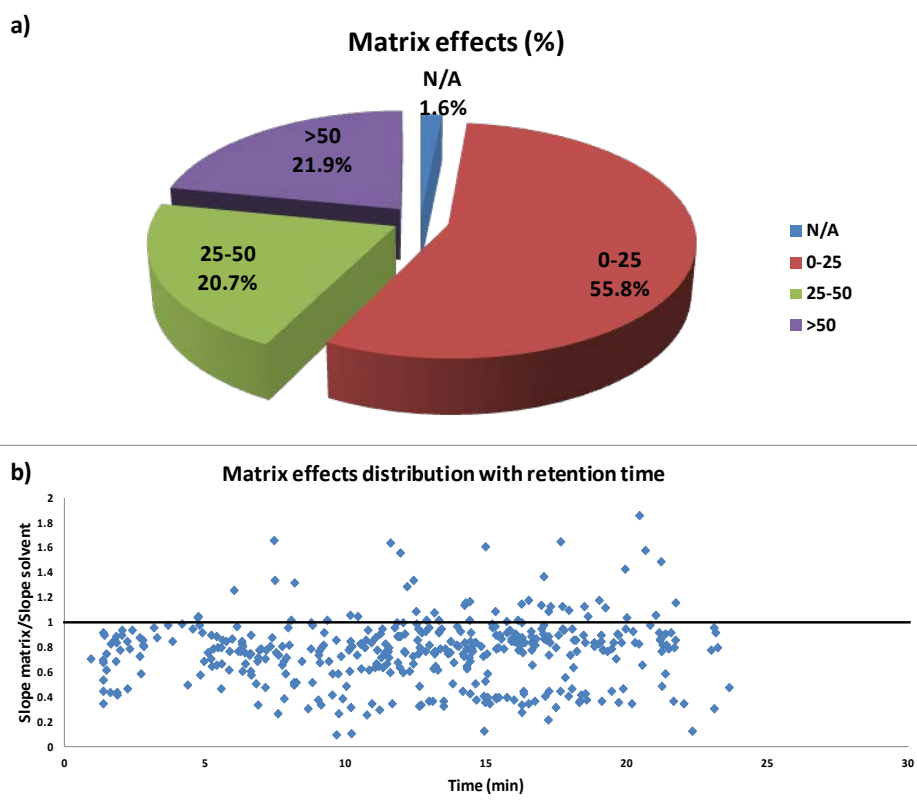


Figure 4. (a) Percentage of contaminants according their matrix effects (ME%); (b) 2D Plot of matrix effects displayed by each analyte vs retention time.

The proposed method has been applied to the analysis of surface waters of the province of Jaén (Spain) and also to different effluent wastewater samples. As an example, Figure 5 shows the positive findings of fluometuron and caffeine in a surface water sample from a previous study [51] and the positive detection of nicotine and ephedrine in an effluent wastewater sample. The identification criteria for real sample positives followed for this methodology were mass accurate measurement, isotopic pattern, characteristic fragments ions and retention time.

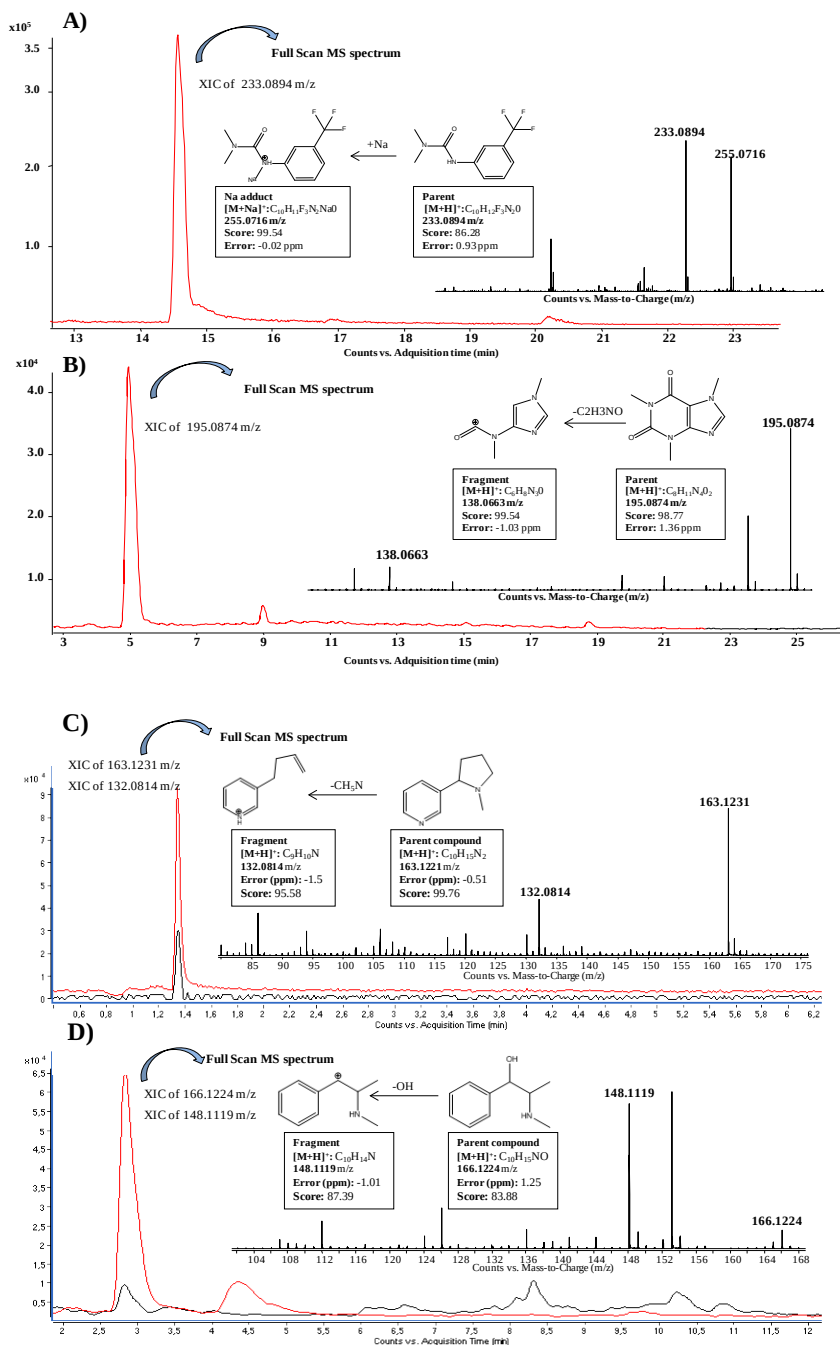


Figure 5. Positive finding of fluometuron (79 ng L⁻¹) (a) and caffeine (35 ng L⁻¹) (b) in surface water. Positive finding of nicotine (47 ng L⁻¹) (c) and ephedrine (27 ng L⁻¹) (d) in effluent wastewater sample after application of automated search database.

4. Concluding remarks

In this work, an analytical methodology using LC-TOFMS for the ultratrace detection and quantification of over 400 priority and emerging organic pollutants in environmental water samples has been described and successfully applied to the analysis of surface water and wastewater. Accurate mass measurements combined with retention time information and fragmentation -when required- were used for appropriate analyte detection and identification by means of an automated and straightforward procedure with available software. It should be emphasized that, unlike in previous related studies, in the present study, sample preparation step performance (recovery rates) was thoroughly evaluated along with the analytical features and matrix effects experimental data provided for all the 430 tested compounds. The overall performance of the method was satisfactory for most of the compounds targeted. Limits of quantification varied from compound to compound, but were in the low nanogram per litre range ($< 100 \text{ ng L}^{-1}$) for 372 of the studied compounds. The use of SPE with Oasis HLB permitted reasonable recovery rates in most cases, which were higher than 30 % for 84 % of the compounds. In addition, the use of such generic sample treatment combined with sensitive full-scan acquisition would allow performing both non-target and retrospective analysis of the samples.

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IV.4.3. Material suplementario

Figure S1. Overlapped LC-TOFMS (+) Total ion chromatogram of a wastewater sample with a 10-min gradient method and the selected 28-min method.

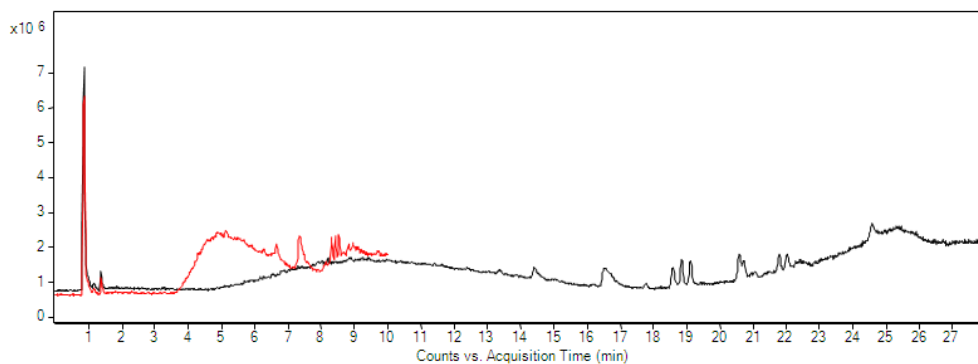
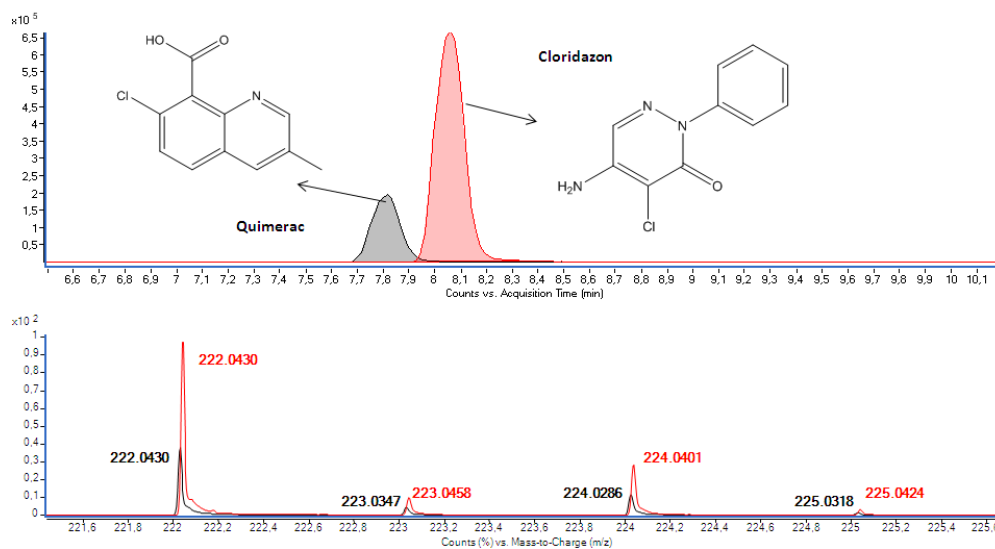
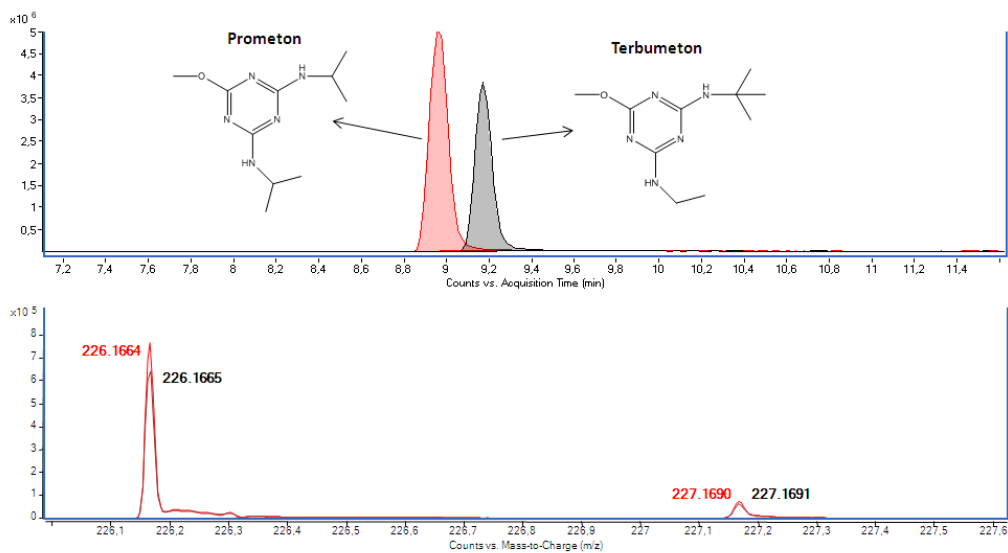


Figure S2 (A to G). Overlapped extracted ion chromatograms and mass spectrums for the coeluted isobaric/isomeric compounds.

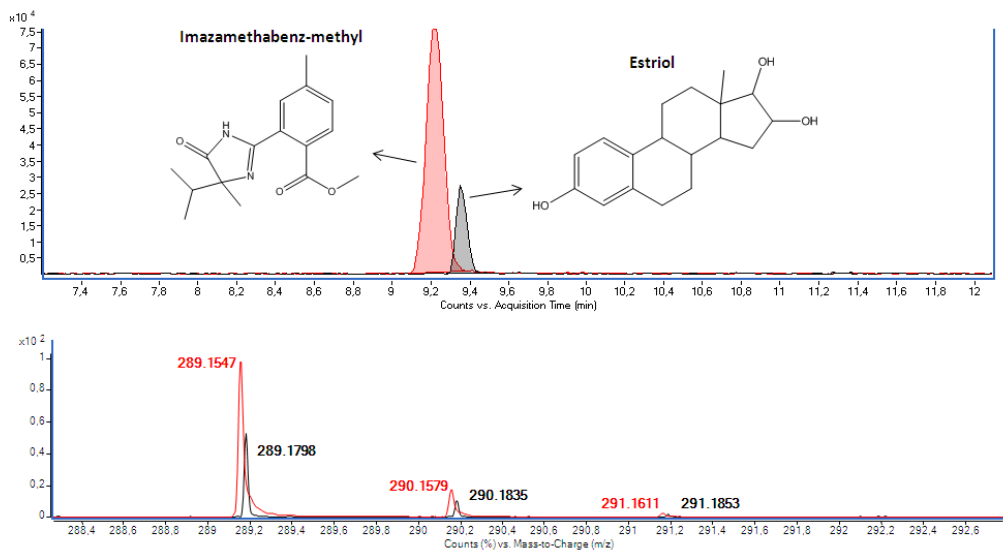
A)



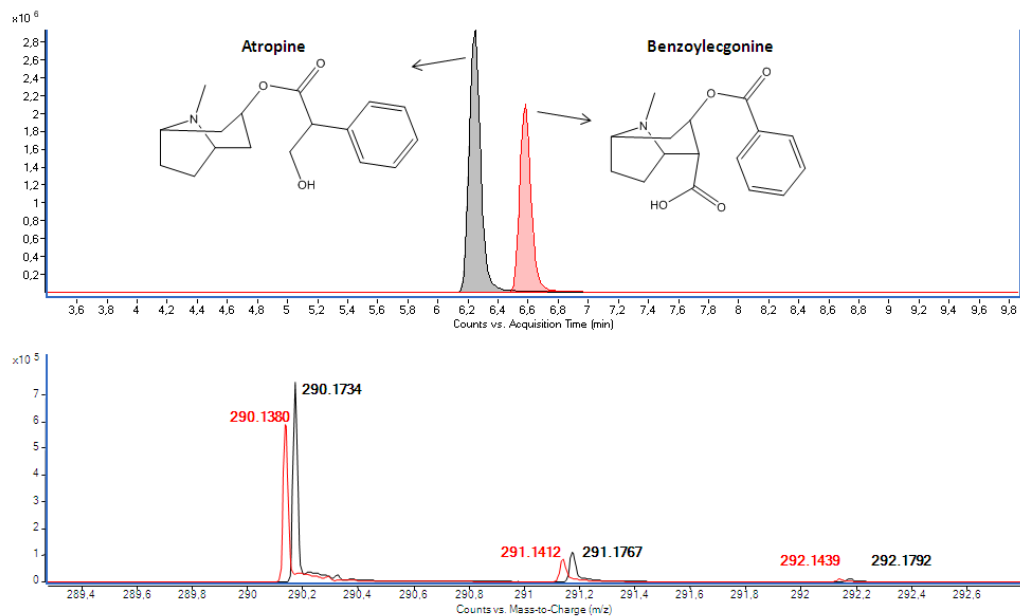
B)



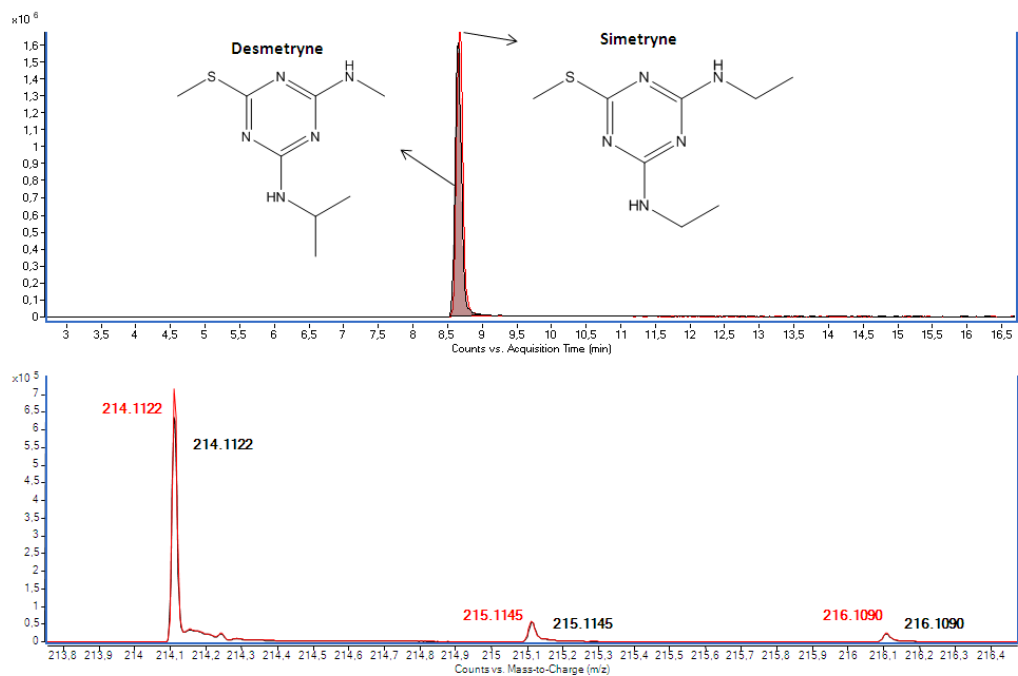
C)



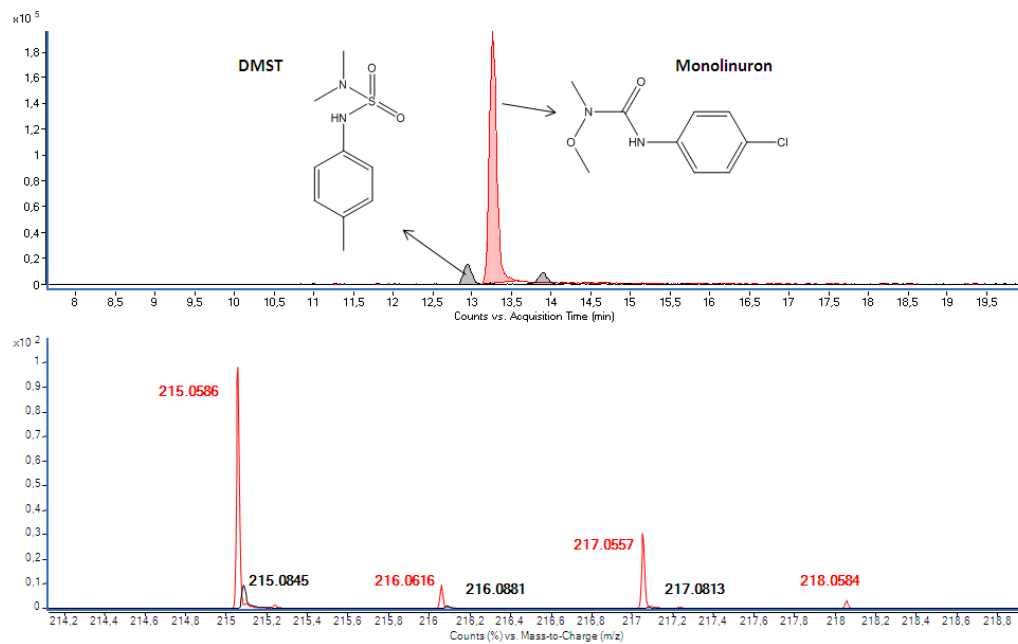
D)



E)



F)



G)

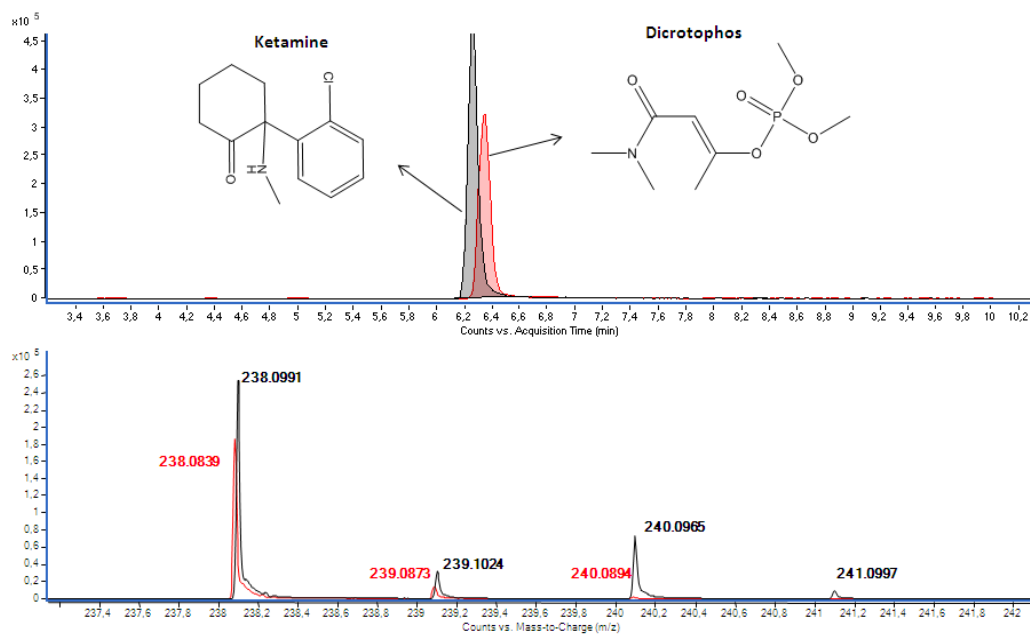


Table S1. Validation data of the analytical method: Recoveries and matrix effects.

Compound	Rec (%) 50 ng L ⁻¹	RSD (%) 50 ng L ⁻¹	Rec (%) ⁽⁴⁾ 500 ng L ⁻¹	RSD (%) 500 ng L ⁻¹	Matrix effect ⁽¹⁾
Acephate	15.0	10.0	20.0	4.2	0.42
Acetamiprid	102.7	10.6	82.6	6.1	0.52
Acetylmorphine	43.6	13.3	17.4	75.9	0.69
Acronifen	42.0	12.0	45.0	8.9	0.41
Alachlor	77.0	7.5	78.2	5.9	0.45
Albendazole	22.2	23.4	45.6	9.7	0.83
Aldicarb	33.5	12.2	30.3	6.8	0.11
Aldicarb sulfone	79.2	8.8	40.6	6.8	0.58
Aldicarb sulfoxide	FL < LOQ ⁽²⁾	-	67.4	7.8	0.59
Ametryn	69.1	5.3	65.3	4.3	0.74
4-Aminoantipyrine	6.7	25.8	46.1	6.1	0.94
Aminocarb	67.7	7.9	34.2	9.7	0.44
Amitrol	FL < LOQ	-	23.4	10.0	0.54
Amphetamine	44.6	46.8	58.4	1.6	0.85
Ampicillin	FL < LOQ	-	62.4	16.7	0.96
4-Androsten-3,17-dione	58.7	6.2	52.8	9.9	0.81
Anilofos	103.1	5.5	59.9	5.7	0.84
Antipyrine	76.6	9.6	75.2	5.9	0.79
Asulam	59.7	6.2	72.7	7.0	0.80
Atenolol	79.1	5.7	85.5	2.8	0.86
Atrazine	78.9	6.1	78.9	5.6	0.34
Atrazine desethyl	72.5	9.3	63.8	5.5	0.82
Atropine	63.8	7.0	63.8	5.6	0.83
Azinphos methyl	FL < LOQ	-	37.7	15.0	0.32
Azinphos-ethyl	36.8	7.6	34.7	5.8	0.40
Azoxystrobin	127.4	8.7	101.1	4.6	0.94
Benalaxyl	85.3	5.0	71.2	3.7	0.86
Bendroflumethiazide	38.4	13.4	41.0	12.0	0.75
Benfuracarb	57.3	3.2	58.8	6.8	0.98
Bensulfuron methyl	82.2	5.9	77.3	3.9	1.14
Bensulide	36.4	16.5	45.8	10.8	1.05
Bentazon	FL < LOQ	-	102.4 ⁽⁴⁾	1.5	N/A
Benzalkonium chloride-C10	FL < LOQ	-	62.6 ⁽⁴⁾	9.2	0.63
Benzalkonium chloride-C12	74.4	16.1	48.2	15.2	0.34
Benzalkonium chloride-C14	83.4	16.9	83.4	17.9	0.42

Compound	Rec (%) 50 ng L ⁻¹	RSD (%) 50 ng L ⁻¹	Rec (%) ⁽⁴⁾ 500 ng L ⁻¹	RSD (%) 500 ng L ⁻¹	Matrix effect ⁽¹⁾
Benzalkonium chloride-C16	FL < LOQ	-	68.3	16.5	1.58
Benzidine	17.9	12.6	21.7	17.3	0.35
Benzoylecgonine	82.0	10.6	82.0	11.8	0.90
Bezafibrate	94.4	4.9	68.8	3.5	0.82
Bifenazate	NR ⁽³⁾	-	NR	-	0.66
Bifenox	FL < LOQ	-	74.8	8.97	0.71
Bis (2-ethylhexyl) phtalate (DEHP)	FL < LOQ	-	51.8	35.4	N/A
Bisphenol A	NR	-	NR	-	N/A
Bitertanol	68.6	8.0	65.8	3.6	0.78
Boscalid	68.6	7.1	64.5	7.0	1.02
Bromacil	85.8	4.1	83.0	1.9	0.63
Bromophos ethyl	NR	-	NR	-	0.80
Bromophos methyl	FL < LOQ	-	28.2	6.0	0.92
Bromuconazole 1	84.2	9.1	85.5	2.6	0.90
Bromuconazole 2	82.6	9.2	82.2	4.6	0.88
Bupirimate	67.0	5.9	70.6	3.7	0.92
Buprofezin	48.4	7.1	47.0	5.8	0.37
Butachlor	36.3	6.0	51.7	5.0	0.91
Butocarboxim	36.3	17.1	32.4	10.0	0.10
Butoxycarboxim	79.4	9.4	82.0	3.3	0.50
Buturon	96.4	6.0	75.2	2.3	0.77
Caffeine	84.8	8.6	80.0	2.8	0.76
Cannabidiol	FL < LOQ	-	11.2	22.9	0.77
Carbadox	59.2	11.2	63.6	5.1	0.81
Carbamazepine	64.5	3.6	64.5	3.0	0.91
Carbendazim	108.8	7.8	117.3	9.6	0.88
Carbofuran (2,3-Dihydro-2,2-dimethyl-7-benzo-furanol N-methylcarbamate)	34.8	26.3	41.4	17.3	0.76
Carbofuran 3-hydroxy	42.0	17.2	27.2	10.6	0.72
Carboxine	35.8	9.9	39.8	5.8	0.75
Carfentrazone ethyl	FL < LOQ	-	19.3	15.1	0.74
Cefotaxime	63.1	19.7	64.8	9.4	0.85
Chloramphenicol	71.0	12.5	75.0	10.4	0.42
Chlorbromuron	63.4	15.0	110.4	8.3	0.40
Chlordimeform	9.2	8.0	20.0	9.0	0.74
Chlorfenvinfos	74.4	8.2	50.2	9.2	0.45
Chloridazon (Pyrazon)	56.6	2.3	78.9	3.0	0.52

Compound	Rec (%) 50 ng L ⁻¹	RSD (%) 50 ng L ⁻¹	Rec (%) ⁽⁴⁾ 500 ng L ⁻¹	RSD (%) 500 ng L ⁻¹	Matrix effect ⁽¹⁾
4-chloro-2-methylphenol	102.5	5.2	98.4	3.4	0.77
4-chloro-o-tolxyloxyacetic acid (MCPA)	113.0	10.5	122.6	7.6	0.65
Chlorothiazide	71.7	8.4	96.6	8.4	1.04
Chloroxuron	84.2	4.0	86.5	2.6	0.90
Chlorpropham	FL < LOQ	-	53.4 ⁽⁴⁾	14.2	N/A
Chlorpyrifos ethyl	38.5	7.5	50.0	3.5	0.37
Chlorpyrifos-methyl	25.8	13.8	86.5	2.6	0.84
Chlorsulfuron	60.5	6.4	61.5	6.4	0.99
Chlortetracycline	9.6	25.6	8.7	23.89	1.32
Chlortoluron	84.6	6.2	75.1	2.6	0.67
Chlothianidin	67.1	11.1	75.4	6.7	0.59
Cimetidine	25.4	13.5	46.2	9.4	0.84
Cinosulfuron	102.3	9.4	89.3	7.7	1.56
Ciprofloxacin	8.5	24.7	5.9	35.9	0.76
Cis-Diltiazem	41.0	25.0	45.0	7.2	0.80
Clarithromycin	60.7	9.9	60.7	5.8	0.78
Clenbuterol	77.6	10.2	76.2	6.6	0.78
Clofibric acid	77.5	8.6	86.0	5.9	0.88
Clomazone	47.7	12.0	45.4	14.7	1.02
Cloxacillin	74.3	9.3	83.2	5.4	0.79
Cocaine	81.0	13.0	88.0	8.0	0.81
Codeine	58.4	8.2	60.0	4.2	0.96
Compactin (Mevastatin)	8.7	113.5	5.5	72.5	0.92
Cotinine	104.6	11.4	90.0	7.8	0.70
Coumaphos	43.7	5.5	40.2	8.7	0.85
Cyanazine	69.6	6.6	70.2	4.3	0.72
Cycloate	40.5	8.4	40.4	9.5	0.84
λ-Cyhalothrin	FL < LOQ	-	39.3	11.6	0.31
α-Cypermethrin	FL < LOQ	-	8.20 ⁽⁴⁾	22.4	N/A
Cyproconazole	85.4	4.6	85.4	3.5	0.41
Cyprodinil	41.3	6.9	53.1	5.7	0.79
Cyromazine	56.2	5.0	46.5	9.4	0.68
Danofloxacin	45.6	15.0	61.8	18.8	2.01
DEET	73.6	1.7	73.6	4.6	0.77
Deltamethrin	FL < LOQ	-	7.9 ⁽⁴⁾	24.9	N/A
Demeclocycline	39.0	15.0	36.1	27.4	0.88

Compound	Rec (%) 50 ng L ⁻¹	RSD (%) 50 ng L ⁻¹	Rec (%) ⁽⁴⁾ 500 ng L ⁻¹	RSD (%) 500 ng L ⁻¹	Matrix effect ⁽¹⁾
Desmetryn	58.3	6.9	66.1	8.5	0.98
Dexamethasone	55.7	6.3	57.0	8.2	0.80
Diafenthion	NR	-	NR	-	0.13
Diazinon	59.3	4.1	64.0	4.5	0.81
Dichlofenthion	FL < LOQ	-	18.1	16.5	1.00
Dichloran	FL < LOQ	-	61.8	6.6	0.79
2,4-Dichlorophenoxyacetic acid	FL < LOQ	-	87.3	13.9	0.51
Dichlorprop (racemate mix)	119.2	11.1	102.0	4.8	0.63
Dichlorvos	48.5	7.8	47.4	9.5	0.72
Diclofenac	86.2	5.9	91.1	4.4	0.65
Dicloxacillin	52.2	9.8	37.8	24.6	1.17
Dicrotophos	68.2	2.3	73.9	2.9	0.67
Diethofencarb	FL < LOQ	-	89.1	7.4	0.40
Difenacoum	37.7	11.0	31.3	13.9	1.16
Difenoconazole	68.0	2.4	62.4	2.6	0.95
Difenoxyuron	86.0	3.2	89.0	3.1	0.93
Diflubenzuron	78.2	13.6	68.0	15.3	0.82
Diflufenican	52.4	6.4	52.4	17.0	0.90
Digoxigenin	41.6	6.3	54.3	7.2	0.50
Dimethametryn	55.3	7.5	69.2	5.1	1.08
Dimethenamid	52.9	4.8	56.1	4.2	0.87
Dimethoate	84.2	6.7	84.2	6.0	0.38
Dimethomorph isomer 1	100.5	2.9	97.9	5.0	0.45
Dimethomorph isomer 2	100.5	2.9	97.9	5.0	0.43
2,6-Dimethylaniline	34.0	12.8	34.0	16.6	0.87
Diniconazol	61.4	6.0	60.1	8.9	1.09
2,4-Dinitrophenol	NR	-	NR	-	0.72
Diphenhydramine	52.5	8.4	52.0	5.0	0.73
Diphenylamine	39.6	5.1	41.7	8.6	0.89
Diuron	86.0	5.8	78.4	2.9	0.76
DMST solution	FL < LOQ	-	57.8	7.1	0.82
DNOC	94.3	4.3	83.5	9.9	0.84
Doxycycline	FL < LOQ	-	10.9	12.2	1.00
EDDP	26.3	27.3	31.3	28.2	0.83
Edifenphos	9.4	16.2	26.6	18.1	1.65
Enalapril	89.5	11.1	128.5	10.7	0.92

Compound	Rec (%) 50 ng L ⁻¹	RSD (%) 50 ng L ⁻¹	Rec (%) ⁽⁴⁾ 500 ng L ⁻¹	RSD (%) 500 ng L ⁻¹	Matrix effect ⁽¹⁾
Enoxacin	33.5	17.9	60.0	21.2	1.26
Enrofloxacin	12.2	16.8	10.3	18.8	0.78
Ephedrine	58.4	9.3	58.0	11.8	0.82
EPN	25.6	13.4	32.7	13.6	1.04
Eprinomectin (b1a)	75.4	10.9	89.2	11.4	0.87
Equilin	132.7	9.3	38.9	31.8	0.85
Erythromycin	44.9	11.9	40.8	8.8	0.27
β-Estradiol	FL < LOQ	-	84.4 ⁽⁴⁾	8.6	0.95
Estriol	FL < LOQ	-	55.2	12.8	N/A
Estrone	FL < LOQ	-	53.6	12.4	0.84
Ethiofencarb	FL < LOQ	-	25.2	8.3	0.37
Ethion	FL < LOQ	-	27.2	11.6	0.86
Ethiprole	84.0	6.3	71.5	2.0	0.73
Ethoxyquin	FL < LOQ	-	2.3	11.5	0.68
Ethylamphetamine	51.6	2.6	10.8	14.9	0.77
Ethylmorphine	49.2	9.2	45.4	8.4	0.62
17-α-Ethynylestradiol	FL < LOQ	-	87.4 ⁽⁴⁾	8.1	0.65
Etoprofos	70.2	16.2	64.2	14.4	0.84
Etrimphos	42.3	8.0	53.1	3.5	0.84
Famotidine	19.5	19.8	45.2	6.7	0.69
Famphur	60.5	6.6	43.6	12.1	0.97
Fenamiphos	73.9	5.5	61.0	6.8	0.80
Fenarimol	84.7	5.5	68.9	2.8	0.79
Fenazaquin	14.9	19.2	74.2	3.0	0.35
Fenbendazol	36.2	25.6	15.8	10.1	0.33
Fenhexamid	80.2	4.5	80.2	9.6	0.38
Fenitrothion	61.0	8.2	76.8	5.0	0.74
Fenobucarb	121.9	12.2	121.9	12.2	0.40
Fenofibrate	29.8	9.7	25.7	11.4	0.92
Fenoxycarb	73.0	3.5	69.5	4.2	0.91
Fenpropidine	50.8	10.2	55.0	8.6	1.09
Fenpropimorphe	36.3	18.1	39.6	8.6	0.73
Fensulfothion	68.4	4.1	66.7	4.4	0.79
Fenthion	31.0	12.0	27.3	10.4	0.38
Fenuron	64.7	10.8	69.1	7.0	0.66
Fluazifop	90.5	7.4	84.9	4.3	0.35

Compound	Rec (%) 50 ng L ⁻¹	RSD (%) 50 ng L ⁻¹	Rec (%) ⁽⁴⁾ 500 ng L ⁻¹	RSD (%) 500 ng L ⁻¹	Matrix effect ⁽¹⁾
Fluazifop-p-butyl	26.1	8.9	16.1	11.5	0.86
Flufenacet	57.7	4.2	58.2	2.5	0.84
Flufenamic acid	104.7	6.3	97.1	7.9	0.90
Flufenoxuron	35.2	11.8	31.3	13.5	0.82
Flumequine	35.8	12.2	98.3	5.7	0.89
Fluometuron	77.5	7.9	67.5	5.3	0.80
Fluoxetine	28.0	12.2	35.7	10.8	0.67
Fluquinconazole	102.3	14.2	109.6	15.9	0.28
Fluroxypyr	81.0	8.5	97.1	4.9	0.67
Flutolanil	55.1	6.1	56.9	7.6	0.87
Flutriafol	95.6	4.5	77.5	3.6	0.75
Fosthiazate	67.7	3.8	73.4	2.9	0.92
Fuberidazol	85.5	5.5	73.4	3.3	0.71
Furmecyclox	44.3	8.9	44.2	14.1	1.13
Furosemide	102.8	3.8	84.8	6.6	0.60
Gemfibrozil	74.8	10.5	98.4	9.1	0.64
Griseofulvin	65.8	8.5	67.6	7.2	1.02
Haloxypop	69.9	5.5	66.5	4.2	0.87
Hexaflumuron	FL < LOQ	-	48.0	9.8	0.37
Hexazinone	70.7	5.2	75.7	3.5	0.78
Hexythiazox	15.0	9.5	17.7	4.7	0.80
Hydrochlorothiazide	86.9	6.8	96.5	3.8	0.67
Hydroflumethiazide	96.7	6.2	89.8	6.2	0.39
2-Hydroxybiphenyl (2-phenylphenol)	91.0	6.1	81.1	7.3	0.45
Ibuprofen	85.8	5.6	98.8	2.3	0.84
Imazalil	68.0	10.8	71.0	5.2	0.35
Imazalil metabolite	52.2	4.4	80.5	2.9	0.81
Imazamethabenz-methyl	56.3	3.7	69.1	3.5	0.80
Imazamox	11.6	39.3	23.7	50.8	1.02
Imazapyr	11.9	53.0	21.0	80.2	0.35
Imazaquin	86.3	5.4	86.3	3.3	0.88
Imidacloprid	96.8	5.8	82.6	5.5	0.52
Indomethacine	52.1	7.9	52.5	9.2	1.15
Indoxacarb	44.5	8.4	15.5	55.0	0.36
Ioxynil	80.5	4.9	67.1	8.8	0.69
Iprodione	FL < LOQ	-	84.2	7.8	0.78

Compound	Rec (%) 50 ng L ⁻¹	RSD (%) 50 ng L ⁻¹	Rec (%) ⁽⁴⁾ 500 ng L ⁻¹	RSD (%) 500 ng L ⁻¹	Matrix effect ⁽¹⁾
Irgarol (cybutryne)	47.8	12.3	46.3	16.7	0.95
Isocarbophos	15.5	20.6	25.6	20.8	0.36
Isoprocarb	FL < LOQ	-	59.5	7.6	0.75
Isoproturon	89.4	7.5	86.2	5.2	0.37
Isoxaben	61.8	10.5	68.1	5.8	1.18
Josamycin	51.2	12.4	62.1	7.8	1.00
Karbutilate	57.5	14.8	93.6	10.1	1.64
Ketamine	65.9	13.2	52.9	2.8	0.61
Ketoprofen	64.4	6.9	63.1	6.0	0.76
Kresoxim methyl	72.9	9.2	79.4	7.1	0.47
Lenacil	105.1	9.2	75.1	3.3	0.64
Leucomalachite green	FL < LOQ	-	9.9	62.8	0.49
Lincomycin	67.1	6.4	67.1	7.3	0.98
Linuron	66.9	11.7	57.4	11.9	1.09
Lomefloxacin	17.7	20.0	32.4	17.2	0.75
Lufenuron	FL < LOQ	-	101.5	23.8	3.74
Malachite green	25.3	13.8	32.5	15.2	0.93
Malaoxon	45.6	8.5	62.3	7.1	0.89
Malathion	51.6	12.3	68.5	8.5	1.37
MDA	82.0	6.9	55.4	15.7	1.05
MDEA	74.3	11.4	50.9	6.3	0.77
MDMA	70.0	5.8	70.0	13.3	0.89
Mebendazole	81.2	5.8	62.4	1.6	0.86
Mecarbam	FL < LOQ	-	113.5	13.4	0.56
Meclofenamic acid	101.9	7.8	100.5	6.1	0.94
Mefenacet	63.1	7.8	66.1	5.9	0.91
Mefenamic acid	96.0	5.7	92.5	7.7	0.95
Mesotrion	57.7	6.9	60.3	5.8	0.60
Metalaxyl	88.7	6.3	68.1	4.6	0.96
Metamitron	74.2	5.9	68.7	3.2	0.27
Metazachlor	51.4	13.6	63.6	7.7	0.93
Metformin	FL < LOQ	-	3.1	11.7	0.71
Methabenzthiazuron	58.4	7.6	62.2	8.6	0.91
Methadone	61.6	16.6	61.0	9.3	0.83
Methamidophos	FL < LOQ	-	7.1	11.2	0.44
Methamphetamine	33.6	14.7	44.1	10.9	0.92

Compound	Rec (%) 50 ng L ⁻¹	RSD (%) 50 ng L ⁻¹	Rec (%) ⁽⁴⁾ 500 ng L ⁻¹	RSD (%) 500 ng L ⁻¹	Matrix effect ⁽¹⁾
Methidathion	37.8	6.4	33.8	9.4	0.39
Methiocarb (mercaptodimethur)	FL < LOQ	-	24.6	6.9	0.59
Methomyl	FL < LOQ	-	67.1	8.2	0.66
Methoxyfenozide	80.0	10.0	70.7	4.8	0.89
3-Methylxanthine	18.5	11.7	17.5	12.7	0.62
Metobromuron	68.0	6.7	55.7	7.4	0.51
Metolcarb	FL < LOQ	-	35.1	15.8	0.64
Metoxuron	96.6	6.3	93.8	5.5	0.32
Metribuzin	56.8	4.9	63.9	1.6	0.76
Metronidazole	83.6	2.9	93.5	4.5	0.86
Mevinphos	71.5	8.4	74.9	4.7	0.34
Miconazole	41.3	19.7	21.9	4.2	0.81
Molinate	23.0	12.3	44.7	17.1	0.90
Monocrotophos	87.6	12.3	89.1	5.5	0.47
Monolinuron	57.3	7.5	56.2	8.2	0.62
Monuron	79.3	6.5	69.2	3.2	0.62
Morphine	86.5	8.9	86.6	5.2	0.75
Myclobutanil	88.9	5.8	76.2	4.0	0.79
Nalidixic acid	42.1	16.1	47.6	12.2	0.77
1-Naphtaleneacetamide	75.4	8.0	73.7	5.7	0.49
Naproxen	69.7	11.4	64.7	5.7	0.68
Naptalam	97.2	5.8	55.6	6.3	0.96
Neburon	76.9	3.0	66.9	3.4	0.80
Nicotine	66.1	19.3	42.5	11.7	0.45
Nifuroxazide	14.3	8.7	25.8	32.1	0.59
Nitenpyram	89.4	7.7	90.7	3.8	0.65
N,N-Diethyl-2-(1-naphthoxy)propanamide	81.3	3.6	86.0	4.1	0.43
N-nitrosodiethylamine	39.9	10.8	37.2	10.3	0.85
N-nitrosodi-n-dibutylamine	3.5	16.0	26.9	12.9	0.87
N-nitrosomorpholine	28.6	8.9	19.9	8.5	0.85
N-nitrosopiperidine	72.8	17.6	55.1	6.3	0.75
N-nitrosopyrrolidine	15.0	22.7	17.9	10.5	0.88
Norfloxacin	6.6	17.1	11.6	37.5	0.33
Norflurazon	85.1	7.0	80.4	5.0	0.37
Nuarimol	68.4	4.7	67.6	4.0	0.75
Ofloxacin	18.2	13.1	40.8	19.8	0.77

Compound	Rec (%) 50 ng L ⁻¹	RSD (%) 50 ng L ⁻¹	Rec (%) ⁽⁴⁾ 500 ng L ⁻¹	RSD (%) 500 ng L ⁻¹	Matrix effect ⁽¹⁾
Ofurace	49.2	3.8	62.2	5.8	0.92
Omethoate	52.9	9.4	17.7	20.6	0.47
Oryzalin	48.5	3.5	53.3	8.6	0.98
Oxacillin	59.1	8.1	70.9	10.3	1.02
Oxadiazon	48.2	5.5	34.5	6.6	0.82
Oxadixyl	106.0	6.8	85.8	10.1	0.84
Oxfendazole	108.3	8.1	91.1	6.1	0.31
Oxolinic acid	39.9	9.5	111.3	8.6	0.59
Oxyfluorfen	FL < LOQ	-	22.9	23.0	1.06
Oxytetracycline	12.7	46.9	25.6	16.5	0.97
Paclobutrazol	69.9	4.4	70.7	3.3	0.84
Paracetamol (Acetaminophen)	69.6	4.1	69.6	4.5	0.73
Parathion ethyl	FL < LOQ	-	53.8	7.0	0.43
Parathion-methyl	63.0	12.4	64.6	7.0	0.84
Pebulat	27.7	11.5	32.3	9.6	0.91
Penconazol	77.3	10.9	78.9	4.9	0.37
Pencycuron	56.4	8.8	59.1	6.6	1.12
Penicillin G	57.2	20.0	83.4	7.1	0.94
C3 Pentafluoropropionic acid fragment 1	51.0	5.0	46.5	19.1	2.48
C4 Perfluorobutyric acid (PFBA) fragment 1	21.0	7.8	18.0	26.0	2.83
C5 Perfluoropentanoic acid fragment 1	58.7	20.1	43.3	24.2	2.49
C7 Perfluoroheptanoic acid (PFHpA)	107.5	23.9	70.7	24.0	4.37
C8 Perfluorooctanoic acid (PFOA)	89.2	18.4	39.7	27.5	3.01
Perfluorooctanesulfonic acid (PFOS)	47.8	1.6	40.4	10.1	1.66
C9 Perfluorononanoic acid (PFNA)	68.0	5.4	47.0	22.1	2.93
C10 Perfluorodecanoic acid (PFDA)	49.7	15.4	35.0	27.5	2.28
C11 Perfluoroundecanoic acid	18.9	5.9	41.2	23.2	1.34
C12 Perfluorododecanoic acid fragment 1	38.0	9.2	35.1	23.8	0.59
Phenacetin	57.0	3.6	66.2	3.6	0.69
Phenylbuthazone	33.5	11.2	70.0	7.1	0.86
Phenylephrine	46.4	15.2	46.0	6.5	0.92
Phosalone	43.7	10.9	39.3	8.8	0.45
Phosphamidon	62.2	3.5	71.2	3.7	0.81
Picloram	FL < LOQ	-	20.7	17.3	0.69
Picolinafen	20.3	3.5	14.3	4.5	0.66
Pindolol	83.6	3.9	74.3	4.4	0.80

Compound	Rec (%) 50 ng L ⁻¹	RSD (%) 50 ng L ⁻¹	Rec (%) ⁽⁴⁾ 500 ng L ⁻¹	RSD (%) 500 ng L ⁻¹	Matrix effect ⁽¹⁾
Pipemidic acid	FL < LOQ	-	16.2	19.2	0.98
Pirimicarb	73.7	7.0	69.7	8.4	0.34
Pirimiphos methyl	47.1	3.5	44.6	4.0	0.83
Pravastatin	231.1	12.6	89.3	7.1	0.26
Pretilachlor	65.0	9.3	60.0	2.7	0.95
Prochloraz	76.4	12.3	78.4	8.2	0.13
Procymidone	FL < LOQ	-	21.3	8.3	0.86
Profenofos	22.1	10.5	66.3	12.2	1.43
Promecarb	84.6	9.0	62.2	4.4	0.40
Prometon	96.4	2.8	96.4	5.7	0.74
Prometryn	73.2	4.8	64.9	4.7	0.77
Propachlor	45.2	9.4	48.6	9.1	0.97
Propamocarb	40.5	14.9	36.6	4.8	0.79
Propanil	82.2	6.6	82.5	6.0	0.38
Propaquizafop	14.7	14.3	18.9	10.0	1.86
Propazine	73.6	4.2	73.2	4.3	0.79
Propiconazole	71.4	5.2	65.7	7.6	1.13
Propisochlor	FL < LOQ	-	69.3	9.2	1.13
Propoxur	FL < LOQ	-	41.6	10.2	0.64
Propranolol	62.6	3.7	64.5	3.8	0.82
Propyphenazone	72.8	3.9	72.8	3.7	0.89
Propyzamid	89.2	11.7	63.2	3.1	0.76
Proquinazid	11.1	22.2	11.7	13.1	0.78
Prosulfocarb	32.6	18.1	35.0	19.0	0.83
Prosulfuron	66.4	4.2	67.7	4.5	0.99
Pymetrozin	89.2	5.1	78.1	8.2	0.90
Pyraclostrobin	53.0	5.8	56.4	6.5	1.18
Pyranocoumarin	83.6	8.7	93.6	7.8	1.10
Pyridaben	FL < LOQ	-	28.5	6.3	0.92
Pyridaphenthion	85.0	6.3	72.4	3.7	0.78
Pyrimethanil	67.3	7.3	67.3	5.2	0.35
Pyriproxifen	2.4	15.7	2.0	30.7	0.49
Pyroquilon	49.2	9.8	52.9	7.9	0.85
Quinalphos	59.3	4.2	51.5	5.4	0.86
Quinmerac	93.6	7.0	75.4	6.9	0.69
Quinoclamine	56.2	9.5	59.3	6.8	0.76

Compound	Rec (%) 50 ng L ⁻¹	RSD (%) 50 ng L ⁻¹	Rec (%) ⁽⁴⁾ 500 ng L ⁻¹	RSD (%) 500 ng L ⁻¹	Matrix effect ⁽¹⁾
Quinoxifen	14.1	4.8	12.0	11.9	0.84
Quizalofop-P-ethyl	FL < LOQ	-	29.8	11.9	0.93
Ranitidine	48.3	9.2	54.1	6.3	0.94
Rotenone	35.1	11.4	45.0	9.0	0.22
Roxithromycin	59.0	12.8	70.5	4.4	0.95
Salbutamol	64.2	7.0	62.2	4.5	0.83
Salicylic acid	FL < LOQ	-	91.7	9.2	0.73
Sarafloxacin	8.9	15.2	24.0	25.2	0.76
Simazine	70.6	6.2	75.0	3.9	0.80
Simetryn	62.3	7.0	66.9	5.2	0.79
Spinosyn A (Spinosad)	FL < LOQ	-	106.8	14.6	6.72
Spinosyn D (Spinosad)	53.1	30.6	56.6	10.7	3.63
Spiramycin I	7.4	11.5	18.4	14.2	0.74
Spiromesifen	FL < LOQ	-	24.7	13.8	0.96
Spiroxamine	28.5	11.2	34.9	14.0	0.86
Sulcotrione	57.4	5.9	58.8	5.5	0.61
Sulfachloropyridazine	94.2	13.0	85.0	4.8	0.69
Sulfadiazine	63.7	8.5	67.0	8.8	0.99
Sulfadimethoxine	46.5	9.1	46.5	6.6	1.05
Sulfamerazine	74.4	4.2	83.0	3.7	0.85
Sulfamethizole	72.8	14.1	72.8	6.4	0.71
Sulfamethoxazole	63.9	3.5	73.4	4.1	0.68
Sulfanilamide	38.3	8.8	33.2	5.9	0.89
Sulfapyridine	54.6	10.0	56.2	7.1	0.79
Sulfathiazole	56.2	6.5	61.6	7.2	0.90
Sulfometuron methyl	116.5	7.5	98.5	3.2	1.29
Sulfotep	41.5	3.0	38.7	8.7	0.77
TCPP	66.1	9.2	52.7	8.1	0.82
Tebuconazole	75.9	12.7	82.6	8.8	0.35
Tebufenpyrad	46.8	4.9	42.2	5.6	0.39
Tebuthiuron	64.9	6.5	69.7	5.8	0.96
Teflubenzuron	FL < LOQ	-	37.7	17.9	0.79
Tembotrione	69.7	7.1	66.1	6.9	1.01
Temephos	FL < LOQ	-	15.4	11.4	1.49
TEP (Triethyl phosphate)	68.2	14.3	62.7	8.1	0.77
Tepraloxydim	85.3	7.5	71.4	4.8	0.82

Compound	Rec (%) 50 ng L ⁻¹	RSD (%) 50 ng L ⁻¹	Rec (%) ⁽⁴⁾ 500 ng L ⁻¹	RSD (%) 500 ng L ⁻¹	Matrix effect ⁽¹⁾
Terbumeton	55.0	7.7	63.7	7.1	1.02
Terbuthylazine	70.2	3.8	70.5	4.6	0.53
Terbuthylazine desethyl	83.8	5.0	83.8	5.8	0.30
Terbutryn	71.9	6.2	72.0	4.4	0.36
Testosterone	49.8	25.9	56.8	3.6	0.85
Tetrachlorinfos	71.2	5.2	65.0	3.6	0.95
Tetracycline	14.1	18.5	22.9	27.8	0.58
11-nor-9-carboxy-THC	50.8	12.3	74.4	6.3	0.90
Δ-9-THC	FL < LOQ	-	6.2	18.0	0.48
Theobromine	116.7	5.7	116.0	6.0	0.78
Theophylline	91.5	7.1	93.7	5.2	0.81
Thiabendazole	62.6	7.1	75.5	2.7	0.95
Thiacloprid	95.8	3.9	91.8	1.9	0.61
Thiamethoxam	78.1	12.8	63.1	5.1	0.51
Thiamphenicol	75.6	9.1	92.2	8.2	0.61
Thiocyclam (hydrogenoxalate)	43.5	6.9	42.8	5.9	0.90
Thiodicarb	29.1	15.9	47.1	18.5	1.00
Timolol	70.1	6.6	80.3	6.9	0.72
Triadimefon	73.2	4.1	70.4	5.0	0.85
Triadimenol	65.3	11.4	87.2	3.6	0.79
Triasulfuron	85.3	8.1	81.8	6.8	1.34
Triazophos	78.6	9.7	77.3	10.8	0.41
Triazoxid	44.1	14.6	59.0	13.9	0.86
Tributyl phosphate	103.1	15.3	70.1	6.6	0.90
Trichlorfon	FL < LOQ	-	86.9	45.6	0.48
Triclocarban	31.5	15.8	34.9	16.9	0.78
Trietazina	65.0	6.7	64.5	5.4	0.93
Trifloxystrobin	50.5	5.8	30.2	15.9	0.47
Triflumizole	14.8	5.3	27.1	5.6	1.14
Triflumuron	53.7	13.1	56.1	11.2	0.77
Trifluralin	FL < LOQ	-	18.8	15.4	6.09
Trimethoprim	64.4	11.9	64.0	5.2	0.86
Trinexapac-ethyl	71.3	13.9	61.2	5.3	1.16
Tylosin	88.1	5.8	110.0	7.5	1.06
Warfarin	53.3	6.0	67.7	5.6	1.61

(1) N/A - Not Available. See section 3.3.

- (2) FL - Fortification level. FL<LOQ indicates those compounds having a LOQ above the lowest fortification level (50 ng L⁻¹)
- (3) NR - Not recovered
- (4) Recovery study performed at 10 µg L⁻¹ concentration level.

Table S2. List of contaminants targeted in specific official regulations or monitoring lists (besides the general default 0.1 µg L⁻¹ (Directive 98/83 EC) set of pesticides in water).

COMPOUND	LOQ (ng L ⁻¹)	Pollutant Family	Contaminant list	MAC-EQS ¹ (ng L ⁻¹)	AA-EQS ¹ (ng L ⁻¹)	MRL ² (ng L ⁻¹)
17-α-Ethynylestradiol	592.4	Pharma	COM (2011) 876, CCL3, UCMR-3	not applicable	0.035	0.9
4-Androsten-3,17-dione	16.2	Pharma	UCMR-3			0.3
Acephate	123.8	Pesticide	CCL3			
Aclonifen	11.1	Pesticide	Dir. 2013/39/EU	120	120	
Alachlor	2.5	Pesticide	Dir. 2013/39/EU	700	300	
Atrazine	2.0	Pesticide	Dir. 2013/39/EU	2000	600	
Bifenoxy	200.5	Pesticide	Dir. 2013/39/EU	40	12	
Bis (2-ethylhexyl) phthalate (DEHP)	96.5	Plasticizer	Dir. 2013/39/EU	not applicable	1300	
C7 Perfluoroheptanoic acid	28.8	PFAA (PFHpA)	UCMR-3			10
C8 Perfluorooctanoic acid	19.5	PFAA (PFOA)	CCL3, UCMR-3			20
C9 Perfluorononanoic acid	13.3	PFAA (PFNA)	UCMR-3			20
Carbofuran 3-hydroxy	55.2	Pesticide	CCL3			
Chlorfenvinfos	4.8	Pesticide	Dir. 2013/39/EU	300	100	
Chlorpyrifos ethyl	30.0	Pesticide	Dir. 2013/39/EU	100	30	
Dichlorvos	5.3	Pesticide	Dir. 2013/39/EU	0.7	0.6	
Diclofenac	19.4	Pharma	COM (2011) 876	not applicable	100	
Dimethoate	25.4	Pesticide	CCL3			
Diuron	1.1	Pesticide	Dir. 2013/39/EU, CCL3	1800	200	
Equilin	139.7	Pharma	CCL3, UCMR-3			4
Erythromycin	39.7	Pharma	CCL3			
Estrilol	66.6	Pharma. (Hormone metab.)	CCL3, UCMR-3			0.8
Estrone	777.9	Pharma	CCL3, UCMR-3			
Fenamiphos	4.3	Pesticide	CCL3			

COMPOUND	LOQ (ng L ⁻¹)	Pollutant Family	Contaminant list	MAC-EQS ¹ (ng L ⁻¹)	AA-EQS ¹ (ng L ⁻¹)	MRL ² (ng L ⁻¹)
Irgarol (cybutryne)	1.7	Pesticide	Dir. 2013/39/EU	16	2.5	
Isoproturon	5.4	Pesticide	Dir. 2013/39/EU	1000	300	
Methamidophos	284.8	Pesticide	CCL3			
Molinate	19.9	Pesticide	CCL3			
Oxyfluorfen	83.9	Pesticide	CCL3			
PFOS Heptadecafluorooctanesulfonic acid	13.7	PFAA (PFOS)	Dir. 2013/39/EU, CCL3, UCMR-3	36000	0.65	40
Profenofos	6.6	Pesticide	CCL3			
Quinoxifen	5.1	Pesticide	Dir. 2013/39/EU	2700	150	
Simazine	1.0	Pesticide	Dir. 2013/39/EU	4000	1000	
Tebuconazole	2.6	Pesticide	CCL3			
Terbutryn	2.4	Pesticide	Dir. 2013/39/EU	340	65	
Testosterone	6.1	Pharma	UCMR-3			0.1
Thiodicarb	31.8	Pesticide	CCL3			
Trifluralin	295.5	Pesticide	Dir. 2013/39/EU	not applicable	30	
α-Cypermethrin	7090.5	Pesticide	Dir. 2013/39/EU	0.6	0.08	
β-Estradiol	592.4	Pharma	COM (2011) 876, CCL3, UCMR-3	not applicable	0.4	0.4

1: EU directive/proposal. Environmental Quality Standard for Inland surface waters

2: UCMR-3 list MRL in drinking water

Table S3. List of isobaric compounds (same nominal mass) of this study and criterion for their elucidation.

Compound	Elemental composition	Retention time (min)	m/z	Elucidation criterion
Metolcarb	C ₉ H ₁₁ NO ₂	11.02	166.0863	Distinguished by retention time
Ephedrine	C ₁₀ H ₁₅ NO	2.78	166.1226	
MDA	C ₁₀ H ₁₃ NO ₂	4.74	180.1019	Distinguished by retention time
Phenacetin	C ₁₀ H ₁₃ NO ₂	9.38	180.1019	
Theobromine	C ₇ H ₈ N ₄ O ₂	1.96	181.072	Distinguished by retention time
Theophylline	C ₇ H ₈ N ₄ O ₂	2.80	181.072	
Fuberidazole	C ₁₁ H ₈ N ₂ O	5.19	185.0709	Distinguished by retention time
Benzidine	C ₁₂ H ₁₂ N ₂	1.36	185.1073	
Atrazine desethyl	C ₈ H ₁₀ ClN ₅	7.97	188.0697	Distinguished by retention time
Molinate	C ₉ H ₁₇ NOS	16.03	188.1104	
Antipyrine	C ₁₁ H ₁₂ N ₂ O	6.97	189.1022	Distinguished by retention time
Propamocarb	C ₉ H ₂₀ N ₂ O ₂	2.30	189.1598	
Carbendazim	C ₉ H ₉ N ₃ O ₂	3.27	192.0768	Distinguished by retention time
DEET	C ₁₂ H ₁₇ NO	12.82	192.1383	
Isoprocarb	C ₁₁ H ₁₅ NO ₂	13.62	194.1175	Distinguished by retention time
MDMA	C ₁₁ H ₁₅ NO ₂	5.39	194.1176	

Compound	Elemental composition	Retention time (min)	m/z	Elucidation criterion
Thiabendazole	C ₁₀ H ₇ N ₃ S	4.54	202.0433	Distinguished by retention time
Terbuthylazine desethyl	C ₇ H ₁₂ ClN ₅	11.18	202.0853	
Simazine	C ₇ H ₁₂ ClN ₅	10.66	202.0854	
4-Aminoantipyrine	C ₁₁ H ₁₃ N ₃ O	2.39	204.1131	Distinguished by retention time
Pebulat	C ₁₀ H ₂₁ NOS	19.64	204.1417	
Dichloran	C ₆ H ₄ Cl ₂ N ₂ O ₂	14.59	206.9723	Distinguished by retention time
Aldicarb sulfoxide	C ₇ H ₁₄ N ₂ O ₃ S	2.70	207.0798	
Ibuprofen	C ₁₃ H ₁₈ O ₂	16.58	207.138	
Isoproturon	C ₁₂ H ₁₈ N ₂ O	12.94	207.1492	Distinguished by retention time
Quinoclamine	C ₁₀ H ₆ ClNO ₂	11.37	208.016	
MDEA	C ₁₂ H ₁₇ NO ₂	6.16	208.1332	
Fenobucarb	C ₁₂ H ₁₇ NO ₂	15.0	208.1332	
Promecarb	C ₁₂ H ₁₇ NO ₂	15.5	208.1332	
Aldicarb	C ₇ H ₁₄ N ₂ O ₂ S	10.18	213.0668	Distinguished by retention time
Butocarboxim	C ₇ H ₁₄ N ₂ O ₂ S	9.65	213.0668	
Chlortoluron	C ₁₀ H ₁₃ ClN ₂ O	12.38	213.0789	
Omethoate	C ₈ H ₁₂ NO ₄ PS	2.22	214.0297	Distinguished by retention time from Simetryn and desmetryn
Clofibrilic acid	C ₁₀ H ₁₁ ClO ₃	16.25	215.0469	
Metribuzin	C ₈ H ₁₄ N ₄ OS	11.46	215.0961	Distinguished by retention time from Monolinuron and DMST
Atrazine	C ₆ H ₁₄ ClN ₅	12.66	216.101	
Cycloate	C ₁₁ H ₂₁ NOS	19.78	216.1417	
Propanil	C ₉ H ₉ Cl ₂ NO	14.62	218.0134	Distinguished by retention time
Pymetrozin	C ₁₀ H ₁₁ N ₅ O	1.40	218.1036	
Ethoxyquin	C ₁₄ H ₁₉ NO	11.83	218.1539	
2,4-Dichlorophenoxyacetic acid	C ₈ H ₆ Cl ₂ O ₃	13.10	218.9621	Distinguished by retention time
C5 Perfluoropentanoic acid (F1)	C ₄ HF ₉	9.36	218.9862	
Acetamiprid	C ₁₀ H ₁₁ ClN ₄	8.79	223.0745	Distinguished by retention time
Aldicarb sulfone	C ₇ H ₁₄ N ₂ O ₄ S	4.81	223.0747	
Butoxycarboxim	C ₇ H ₁₄ N ₂ O ₄ S	4.36	223.0747	
Cyprodinil	C ₁₄ H ₁₅ N ₃	14.28	226.1339	Distinguished by retention time from prometon and terbumeton
Propazine	C ₉ H ₁₆ ClN ₅	14.46	230.1167	
Terbuthylazine	C ₉ H ₁₆ ClN ₅	14.94	230.1167	Distinguished by retention time
Trietazine	C ₉ H ₁₆ ClN ₅	16.57	230.1167	
Asulam	C ₈ H ₁₀ N ₂ O ₄ S	5.43	231.0434	Distinguished by retention time
Naproxen	C ₁₄ H ₁₄ O ₃	13.82	231.1016	
Propyphenazone	C ₁₄ H ₁₈ N ₂ O	11.92	231.1492	
Diuron	C ₉ H ₁₀ Cl ₂ N ₂ O	13.12	233.0243	Distinguished by retention time
Fluometuron	C ₁₀ H ₁₁ F ₃ N ₂ O	12.61	233.0896	
Nalidixic acid	C ₁₂ H ₁₂ N ₂ O ₃	11.49	233.0921	
Chlorpropham	C ₁₀ H ₁₂ ClNO ₂	16.50	236.0449	Distinguished by retention time
Carboxine	C ₁₂ H ₁₃ NO ₂ S	13.14	236.074	
Buturon	C ₁₂ H ₁₃ ClN ₂ O	14.49	237.0789	Distinguished by retention time
Carbamazepine	C ₁₅ H ₁₂ N ₂ O	11.21	237.1022	
Carbofuran 3-hydroxy	C ₁₂ H ₁₅ NO ₄	7.87	238.1074	Distinguished by retention time from Dicrotophos and ketamine
Clomazone	C ₁₂ H ₁₄ ClNO ₂	14.38	240.0786	
Salbutamol	C ₁₃ H ₂₁ NO ₃	1.63	240.1594	Distinguished by retention time
Picloram	C ₆ H ₃ Cl ₃ N ₂ O ₂	6.84	240.9333	
Bentazon	C ₁₀ H ₁₂ N ₂ O ₃ S	12.67	241.0641	
Cyanazine	C ₉ H ₁₃ ClN ₆	11.24	241.0963	
Mefenamic acid	C ₁₅ H ₁₅ NO ₂	17.69	242.1176	
Prometryn	C ₁₀ H ₁₉ N ₅ S	12.06	242.1434	Distinguished by retention time
Terbutryn	C ₁₀ H ₁₉ N ₅ S	18.28	242.1434	
Triazoxid	C ₁₀ H ₆ ClN ₅ O	9.64	248.0334	Distinguished by retention time
Ethiofencarb	C ₁₁ H ₁₅ NO ₂ S	13.07	248.0716	
Methiocarb (mercaptodimethur)	C ₁₁ H ₁₅ NO ₂ S	15.02	248.0716	

Compound	Elemental composition	Retention time (min)	m/z	Elucidation criterion
Linuron	C ₉ H ₁₀ Cl ₂ N ₂ O ₂	15.30	249.0192	Distinguished by retention time
Pindolol	C ₁₄ H ₂₀ N ₂ O ₂	5.55	249.1598	
Chlothianidin	C ₈ H ₈ ClN ₅ O ₂ S	7.84	250.016	Distinguished by retention time
Sulfapyridine	C ₁₁ H ₁₁ N ₃ O ₂ S	5.27	250.0645	
Prosulfocarb	C ₁₄ H ₂₁ NOS	20.40	252.1417	Distinguished by retention time
Furmecyclox	C ₁₄ H ₂₁ NO ₃	17.68	252.1594	
Trinexapac-ethyl	C ₁₃ H ₁₆ O ₅	14.24	253.1071	Distinguished by retention time from Thiachlopid and Hexazinone
Cimetidine	C ₁₀ H ₁₆ N ₆ S	1.83	253.123	
Sulfamethoxazole	C ₁₀ H ₁₁ N ₃ O ₃ S	9.06	254.0594	Distinguished by retention time
Irgarol (cybutryne)	C ₁₁ H ₁₉ N ₅ S	12.56	254.1434	
Fluroxypyr	C ₇ H ₅ Cl ₂ FN ₂ O ₃	11.07	254.9734	Distinguished by retention time
Ketoprofen	C ₁₆ H ₁₄ O ₃	13.69	255.1016	
Sulfathiazole	C ₉ H ₉ N ₃ O ₂ S ₂	5.20	256.0209	Distinguished by retention time
Propyzamid	C ₁₂ H ₁₁ Cl ₂ NO	16.27	256.029	
Imidacloprid	C ₉ H ₁₀ ClN ₅ O ₂	8.22	256.0596	
Dimethametryn	C ₁₁ H ₂₁ N ₅ S	13.13	256.159	
Diphenhydramine	C ₁₇ H ₂₁ NO	9.81	256.1696	
Trichlorfon	C ₄ H ₈ Cl ₃ O ₄ P	7.12	256.9299	Distinguished by retention time
Imazalil metabolite	C ₁₁ H ₁₀ Cl ₂ N ₂ O	7.72	257.0243	
Oxolinic acid	C ₁₃ H ₁₁ NO ₅	9.71	262.071	Distinguished by retention time
Flumequine	C ₁₄ H ₁₂ FNO ₃	11.88	262.0874	
Imazapyr	C ₁₃ H ₁₅ N ₃ O ₃	16.48	262.1186	
Aclonifen	C ₁₂ H ₉ ClN ₂ O ₃	17.90	265.0374	Distinguished by retention time
Sulfamerazine	C ₁₁ H ₁₂ N ₄ O ₂ S	5.73	265.0754	
Atenolol	C ₁₄ H ₂₂ N ₂ O ₃	1.76	267.1703	Distinguished by retention time
Tributyl phosphate (TBP)	C ₁₂ H ₂₇ PO ₄	18.43	267.172	
Sulfamethizole	C ₉ H ₁₀ N ₄ O ₂ S ₂	7.12	271.0318	Distinguished by retention time
Nitenpyram	C ₁₁ H ₁₅ ClN ₄ O ₂	5.22	271.0956	
Estrone	C ₁₈ H ₂₂ O ₂	14.60	271.1693	Distinguished by retention time
Gemfibrozil	C ₁₅ H ₂₂ O ₃	18.07	273.1461	
β-Estradiol	C ₁₈ H ₂₄ O ₂	13.38	273.1849	Distinguished by retention time
Nifuroxazide	C ₁₂ H ₉ N ₃ O ₅	9.51	276.0615	
Dimethenamid	C ₁₂ H ₁₈ ClNO ₂ S	15.51	276.082	
Benzalkonium chloride-C10	C ₁₉ H ₃₃ N	14.29	276.2686	
Fenitrothion	C ₉ H ₁₂ NO ₃ PS	17.20	278.0247	
Metazachlor	C ₁₄ H ₁₆ ClN ₃ O	14.06	278.1055	Distinguished by retention time
EDDP	C ₂₀ H ₂₄ N	10.51	278.1903	
Fenthion	C ₁₀ H ₁₅ O ₃ PS ₂	18.54	279.0273	Distinguished by retention time
Oxadixyl	C ₁₄ H ₁₈ N ₂ O ₄	10.89	279.1339	
Metalaxyl	C ₁₅ H ₂₁ NO ₄	12.94	280.1543	Distinguished by retention time
Karbutilate	C ₁₄ H ₂₁ N ₃ O ₃	11.57	280.1656	
Flufenamic acid	C ₁₄ H ₁₀ F ₃ NO ₂	17.54	282.0736	Distinguished by retention time
Ofurace	C ₁₄ H ₁₆ ClNO ₃	13.29	282.0891	
Procymidone	C ₁₃ H ₁₁ Cl ₂ NO ₂	17.12	284.024	Distinguished by retention time
Fosthiazate	C ₉ H ₁₈ NO ₃ PS ₂	12.68	284.0538	
Penconazole	C ₁₃ H ₁₅ Cl ₂ N ₃	16.70	284.0715	
Propisochlor	C ₁₅ H ₂₂ ClNO ₂	18.46	284.1412	
Difenoxuron	C ₁₆ H ₁₈ N ₂ O ₃	13.30	287.139	Distinguished by retention time
4-Androsten-3,17-dione	C ₁₉ H ₂₆ O ₂	14.79	287.2006	
Myclobutanil	C ₁₅ H ₁₇ ClN ₄	15.60	289.1215	Distinguished by retention time from Imazamethabenz-methyl and Estriol
Testosterone	C ₁₉ H ₂₈ O ₂	13.71	289.2162	
Chloroxuron	C ₁₅ H ₁₅ ClN ₂ O ₂	15.32	291.0894	Distinguished by retention time
Trimethoprim	C ₁₄ H ₁₈ N ₄ O ₃	5.60	291.1452	
Thiamethoxam	C ₈ H ₁₀ ClN ₅ O ₃ S	6.79	292.0266	Distinguished by retention time
Parathion ethyl	C ₁₀ H ₁₄ NO ₅ PS	18.50	292.0403	
Naptalam	C ₁₈ H ₁₃ NO ₃	11.72	292.0968	

Compound	Elemental composition	Retention time (min)	m/z	Elucidation criterion
Cyproconazole	C ₁₅ H ₁₈ ClN ₃ O	14.89	292.1211	Distinguished by retention time
Chlorbromuron	C ₉ H ₁₀ BrClN ₂ O ₂	15.71	292.9686	
Etrimpfos	C ₁₀ H ₁₇ N ₂ O ₄ PS	18.81	293.0719	
Triadimefon	C ₁₄ H ₁₆ ClN ₃ O ₂	15.96	294.1004	Distinguished by retention time
Paclobutrazol	C ₁₅ H ₂₀ ClN ₃ O	14.57	294.1368	
Diclofenac	C ₁₄ H ₁₁ Cl ₂ NO ₂	16.24	296.024	
Meclofenamic acid	C ₁₄ H ₁₁ Cl ₂ NO ₂	17.74	296.024	Distinguished by retention time
Mebendazole	C ₁₆ H ₁₃ N ₃ O ₃	10.70	296.103	
Triadimenol	C ₁₄ H ₁₈ ClN ₃ O ₂	14.38	296.116	
Imazalil	C ₁₄ H ₁₄ Cl ₂ N ₂ O	10.91	297.0556	Distinguished by retention time
17- α -Ethinylestradiol	C ₂₀ H ₂₄ O ₂	14.28	297.1849	
Quinalphos	C ₁₂ H ₁₅ N ₂ O ₃ PS	18.16	299.0613	Distinguished by retention time
Mefenacet	C ₁₆ H ₁₄ N ₂ O ₂ S	16.01	299.0849	
Phosphamidon	C ₁₀ H ₁₉ ClNO ₅ P	10.20	300.0762	Distinguished by retention time
Fenbendazol	C ₁₅ H ₁₃ N ₃ O ₂ S	12.59	300.0801	
Codeine	C ₁₈ H ₂₁ NO ₃	3.16	300.1594	Distinguished by retention time
Fenhexamid	C ₁₄ H ₁₇ Cl ₂ NO ₂	15.96	302.0709	
Flutriafol	C ₁₆ H ₁₃ F ₂ N ₃ O	12.56	302.1099	
Fenoxycarb	C ₁₇ H ₁₉ NO ₄	17.00	302.1386	Distinguished by retention time
Norflurazon	C ₁₂ H ₉ ClF ₃ N ₃ O	13.46	304.0459	
Fenamiphos	C ₁₃ H ₂₂ NO ₃ PS	15.41	304.1131	
Pipemidic acid	C ₁₄ H ₁₇ N ₅ O ₃	4.78	304.1404	Distinguished by retention time
Cocaine	C ₁₇ H ₂₁ NO ₄	7.30	304.1543	
Fenpropimorphe	C ₂₀ H ₃₃ NO	12.37	304.2635	
Benzalkonium chloride-C12	C ₂₁ H ₃₇ N	16.24	304.2999	Distinguished by retention time
Pirimiphos methyl	C ₁₁ H ₂₀ N ₃ O ₃ PS	18.98	306.1036	
Imazamox	C ₁₅ H ₁₉ N ₃ O ₄	8.04	306.1448	
Buprofezin	C ₁₆ H ₂₃ N ₃ OS	18.30	306.1635	Distinguished by retention time
Quinoxifen	C ₁₅ H ₈ Cl ₂ FNO	20.26	308.0039	
Tebuconazole	C ₁₆ H ₂₂ ClN ₃ O	16.16	308.1524	
Fensulfothion	C ₁₁ H ₁₇ O ₄ PS ₂	13.52	309.0379	Distinguished by retention time
Warfarin	C ₁₉ H ₁₆ O ₄	14.95	309.1121	
Phenylbuthazone	C ₁₉ H ₂₀ N ₂ O ₂	17.16	309.1598	
Edifenphos	C ₁₄ H ₁₅ O ₂ PS ₂	17.62	311.0324	Distinguished by retention time
Diflubenzuron	C ₁₄ H ₉ ClF ₂ N ₂ O ₂	16.71	311.0393	
Sulfadimethoxine	C ₁₂ H ₁₄ N ₄ O ₄ S	10.41	311.0809	
Isocarbophos	C ₁₁ H ₁₆ NO ₄ PS	14.94	312.043	Distinguished by retention time
Imazaquin	C ₁₇ H ₁₇ N ₃ O ₃	11.04	312.1343	
Butachlor	C ₁₇ H ₂₆ ClNO ₂	21.34	312.1725	
Pretilachlor	C ₁₇ H ₂₆ ClNO ₂	19.93	312.1725	Distinguished by retention time
Triazophos	C ₁₂ H ₁₆ N ₃ O ₃ PS	17.07	314.0722	
Kresoxim methyl	C ₁₈ H ₁₉ NO ₄	18.02	314.1387	
Ethylmorphine	C ₁₉ H ₂₃ NO ₃	5.83	314.1751	Distinguished by retention time
Triclocarban	C ₁₃ H ₉ Cl ₃ N ₂ O	19.12	314.9853	
Malaaxon	C ₁₀ H ₁₉ O ₇ PS	11.82	315.0662	
Nuarimol	C ₁₇ H ₁₂ ClFN ₂ O	14.15	315.0695	Distinguished by retention time
Ranitidine	C ₁₃ H ₂₂ N ₄ O ₃ S	2.03	315.1485	
Δ -9-THC	C ₂₁ H ₃₀ O ₂	23.61	315.2319	
Bupirimate	C ₁₃ H ₂₄ N ₄ O ₃ S	14.43	317.1641	Distinguished by retention time
Timolol	C ₁₃ H ₂₄ N ₄ O ₃ S	6.87	317.1642	
Chlorpyrifos-methyl	C ₇ H ₇ Cl ₃ NO ₃ PS	19.50	321.9023	Distinguished by retention time
Pyriproxifen	C ₂₀ H ₁₉ NO ₃	21.22	322.222	
Sulfotep	C ₈ H ₂₀ O ₅ P ₂ S ₂	19.28	323.03	Distinguished by retention time
Pyranocoumarin	C ₂₀ H ₁₈ O	17.91	323.1278	
EPN	C ₁₄ H ₁₄ NO ₄ PS	19.97	324.0454	Distinguished by retention time
Flutolanil	C ₁₇ H ₁₆ F ₃ NO ₂	17.03	324.1206	
Famphur	C ₁₀ H ₁₆ NO ₅ PS ₂	15.29	326.028	Distinguished by retention time
Diniconazol	C ₁₅ H ₁₇ Cl ₂ N ₃ O	17.19	326.0821	
Benalaxyl	C ₂₀ H ₂₃ NO ₃	18.09	326.1751	

Compound	Elemental composition	Retention time (min)	m/z	Elucidation criterion
Fluazifop	C ₁₅ H ₁₂ F ₃ NO ₄	15.10	328.0791	Distinguished by retention time
Acetylmorphine	C ₁₉ H ₂₁ NO ₄	4.94	328.1543	
Sulcotrione	C ₁₄ H ₁₃ ClO ₅ S	12.25	329.0245	Distinguished by retention time
Pencycuron	C ₁₉ H ₂₁ ClN ₂ O	19.21	329.1415	
Malachite green	C ₂₃ H ₂₄ N ₂	12.82	329.2012	
Fenarimol	C ₁₇ H ₁₂ Cl ₂ N ₂ O	15.33	331.0399	Distinguished by retention time
Malathion	C ₁₀ H ₁₉ O ₆ PS ₂	17.02	331.0433	
Leucomalachite green	C ₂₃ H ₂₆ N ₂	12.60	331.2169	
Ciprofloxacin	C ₁₇ H ₁₈ FN ₃ O ₃	6.39	332.1405	Distinguished by retention time
Benzalkonium chloride-C14	C ₂₃ H ₄₁ N	18.35	332.3312	
Famotidine	C ₈ H ₁₅ N ₇ O ₂ S ₃	1.81	338.0522	Distinguished by retention time
Bitertanol	C ₂₀ H ₂₃ N ₃ O ₂	16.59	338.1863	
Azinphos methyl	C ₁₀ H ₁₂ N ₃ O ₃ PS ₂	15.42	339.995	Distinguished by retention time
Mesotrion	C ₁₄ H ₁₃ NO ₇ S	12.02	340.0485	
Bifenox	C ₁₄ H ₉ Cl ₂ NO ₅	19.59	341.9931	Distinguished by retention time
Propiconazole	C ₁₅ H ₁₇ Cl ₂ N ₃ O ₂	17.26	342.0771	
Tepraloxymid	C ₁₇ H ₂₄ ClNO ₄	16.18	342.1467	
Oxadiazon	C ₁₅ H ₁₈ Cl ₂ N ₂ O ₃	21.38	345.0767	Distinguished by retention time
11-nor-9-carboxy-THC	C ₂₁ H ₂₈ O ₄	19.06	345.206	
Azinphos-ethyl	C ₁₂ H ₁₆ N ₃ O ₃ PS ₃	17.45	346.0443	Distinguished by retention time
Triflumizole	C ₁₅ H ₁₅ ClF ₃ N ₃ O	16.93	346.0929	
Chlorpyrifos ethyl	C ₉ H ₁₁ Cl ₃ NO ₃ PS	21.63	349.9336	Distinguished by retention time
Ampicillin	C ₁₆ H ₁₉ N ₃ O ₄ S	7.93	350.1169	
Mecarbam	C ₁₀ H ₂₀ NO ₃ PS ₂	17.78	352.0413	Distinguished by retention time
Lomefloxacin	C ₁₇ H ₁₉ F ₂ N ₃ O ₃	6.60	352.1467	
Griseofulvin	C ₁₇ H ₁₇ ClO ₆	13.29	353.0786	Distinguished by retention time
Hexythiazox	C ₁₇ H ₂₁ ClN ₂ O ₂ S	21.66	353.1085	
Chlorsulfuron	C ₁₂ H ₁₂ ClN ₅ O ₄ S	12.50	358.0371	Distinguished by retention time
Indomethacine	C ₁₉ H ₁₆ ClNO ₄	16.23	358.0841	
Danofloxacin	C ₁₉ H ₂₀ FN ₃ O ₃	6.74	358.1561	
Chlorfenvinfos	C ₁₂ H ₁₄ Cl ₃ O ₄ P	17.61	358.9768	Distinguished by retention time
Triflumuron	C ₁₅ H ₁₀ ClF ₃ N ₂ O ₃	18.19	359.0405	
Enrofloxacin	C ₁₉ H ₂₂ FN ₃ O ₃	6.91	360.1718	Distinguished by retention time
Benzalkonium chloride-C16	C ₂₅ H ₄₅ N	20.63	360.3625	
Haloxfop	C ₁₅ H ₁₁ ClF ₃ NO ₄	16.75	362.0401	Distinguished by retention time
Oxyfluorfen	C ₁₅ H ₁₁ ClF ₃ NO ₄	21.00	362.0401	
Bezaflibrate	C ₁₉ H ₂₀ ClNO ₄	13.92	362.1154	
Ofloxacin	C ₁₈ H ₂₀ FN ₃ O ₄	6.24	362.1511	
Bromophos methyl	C ₈ H ₈ BrCl ₂ O ₃ PS	21.12	364.8565	Distinguished by retention time
Tetrachlorinfos	C ₁₀ H ₉ Cl ₄ O ₄ P	16.94	364.9065	
Sulfometuron methyl	C ₁₅ H ₁₆ N ₄ O ₅ S	12.16	365.0914	
Pyridaben	C ₁₉ H ₂₅ ClN ₂ OS	23.13	365.1448	
Phosalone	C ₁₂ H ₁₅ ClNO ₄ PS ₂	19.43	367.9941	Distinguished by retention time
Anilofos	C ₁₃ H ₁₉ ClNO ₃ PS ₂	18.49	368.0305	
Proquinazid	C ₁₄ H ₁₇ IN ₂ O ₂	22.97	373.0407	Distinguished by retention time from Quizalofop-P-ethyl and Profenofos
Prochloraz	C ₁₅ H ₁₆ Cl ₃ N ₃ O ₂	14.90	376.038	Distinguished by retention time from bromuconazole 2 and Fluquinconazole
Bromuconazole 1	C ₁₃ H ₁₂ BrCl ₂ N ₃ O	15.45	375.9613	
Picolinafen	C ₁₉ H ₁₂ F ₄ N ₂ O ₂	20.52	377.0908	Distinguished by retention time
Enalapril	C ₂₀ H ₂₈ N ₂ O ₅	9.21	377.2071	
Teflubenzuron	C ₁₄ H ₆ Cl ₂ F ₄ N ₂ O ₂	19.43	380.9815	Distinguished by retention time
Fluazifop-p-butyl	C ₁₉ H ₂₀ F ₃ NO ₄	21.15	384.1417	
Ethion	C ₉ H ₂₂ O ₄ P ₂ S ₄	21.70	384.9949	Distinguished by retention time
Diafenthiuron	C ₂₃ H ₃₂ N ₂ OS	22.30	385.2308	
Pyraclostrobin	C ₁₉ H ₁₈ ClN ₃ O ₄	18.99	388.1059	Distinguished by retention time
Dimethomorph (E+Z)	C ₂₁ H ₂₂ ClNO ₄	14.40	388.131	
Compactin (Mevastatin)	C ₂₃ H ₃₄ O ₅	15.71	391.2479	Distinguished by retention time

Compound	Elemental composition	Retention time (min)	m/z	Elucidation criterion
Digoxigenin	C ₂₃ H ₃₄ O ₅	8.14	391.2479	
Bis (2-ethylhexyl) phthalate (DEHP)	C ₂₄ H ₃₈ O ₄	27.48	391.2843	
Bromophos ethyl	C ₁₀ H ₁₂ BrCl ₂ O ₃ PS	23.20	392.8878	Distinguished by retention time
Dexamethasone	C ₂₂ H ₂₈ FO ₅	11.51	393.2072	
Diffufenican	C ₁₉ H ₁₁ F ₅ N ₂ O ₂	19.68	395.0813	Distinguished by retention time
Rotenone	C ₂₃ H ₂₂ O ₆	17.18	395.1489	
Triasulfuron	C ₁₄ H ₁₆ ClN ₅ O ₅ S	12.39	402.0633	Distinguished by retention time
Oxacillin	C ₁₉ H ₁₉ N ₃ O ₅ S	12.85	402.1118	
Bensulfuron methyl	C ₁₆ H ₁₈ N ₄ O ₇ S	14.20	411.0969	Distinguished by retention time
Benfuracarb	C ₂₀ H ₃₀ N ₂ O ₅ S	20.80	411.1948	
Doxycycline	C ₂₂ H ₂₄ N ₂ O ₈	8.75	445.1605	Distinguished by retention time
Tetracycline	C ₂₂ H ₂₄ N ₂ O ₈	6.62	445.1605	
Difenacoum	C ₃₁ H ₂₄ O ₃	21.71	445.1798	
Hexaflumuron	C ₁₆ H ₈ Cl ₂ F ₆ N ₂ O ₃	19.08	460.9888	Distinguished by retention time
Oxytetracycline	C ₂₂ H ₂₄ N ₂ O ₉	6.11	461.1555	
Fluoxetine	C ₁₇ H ₁₈ F ₃ NO	11.44	310.1413	Distinguished by resolving power
Methadone	C ₂₁ H ₂₇ NO	11.39	310.2165	
Dichlofenthion	C ₁₀ H ₁₃ Cl ₂ O ₃ PS	21.46	314.9773	Distinguished by resolving power
Cannabidiol	C ₂₁ H ₃₀ O ₂	21.21	315.2319	
Profenofos	C ₁₁ H ₁₅ BrClO ₃ PS	19.91	372.9424	Distinguished by resolving power
Quizalofop-P-ethyl	C ₁₉ H ₁₇ ClN ₂ O ₄	20.19	373.095	
Bromuconazole 2	C ₁₃ H ₁₂ BrCl ₂ N ₃ O	16.16	375.9613	Distinguished by resolving power
Fluquinconazole	C ₁₆ H ₈ Cl ₂ FN ₅ O	16.24	376.0163	
3-Methylxanthine	C ₈ H ₈ N ₄ O ₂	1.47	167.0564	Distinguished by resolving power
Cyromazine	C ₈ H ₁₀ N ₆	1.36	167.1039	
Thiacloprid	C ₁₀ H ₉ ClN ₄ S	10.12	253.0309	Distinguished by resolving power
Hexazinone	C ₁₂ H ₂₀ N ₄ O ₂	10.09	253.1659	
Metoxuron	C ₁₀ H ₁₃ ClN ₂ O ₂	10.16	229.0738	Distinguished by resolving power and isotopic pattern
Tebuthiuron	C ₉ H ₁₆ N ₄ OS	9.87	229.1118	
Methabenzthiazuron	C ₁₀ H ₁₁ N ₃ OS	12.08	222.0696	Distinguished by resolving power
Carbofuran	C ₁₂ H ₁₅ NO ₃	12.08	222.1125	
Quinmerac	C ₁₁ H ₈ ClNO ₂	7.81	222.0316	Distinguished by fragmentation pattern
Chloridazon (Pyrazon)	C ₁₀ H ₈ ClN ₃ O	8.06	222.0429	
Prometon	C ₁₀ H ₁₉ N ₅ O	9.02	226.1662	Distinguished by fragmentation pattern
Terbumeton	C ₁₀ H ₁₉ N ₅ O	9.32	226.1662	
Imazamethabenz-methyl	C ₁₆ H ₂₀ N ₂ O ₃	9.12	289.1547	Distinguished by fragmentation pattern
Estriol	C ₁₈ H ₂₄ O ₃	9.36	289.1798	
Benzoylcegonine	C ₁₆ H ₁₉ NO ₄	6.61	290.1387	Distinguished by fragmentation pattern
Atropine	C ₁₇ H ₂₃ NO ₃	6.29	290.1751	
Simetryn	C ₈ H ₁₅ N ₅ S	8.7	214.1121	Distinguished by fragmentation pattern
Desmetryn	C ₈ H ₁₅ N ₅ S	8.8	214.1121	
Monolinuron	C ₉ H ₁₁ ClN ₂ O ₂	13.25	215.0582	Distinguished by fragmentation pattern
DMST (*)	C ₉ H ₁₄ N ₂ O ₂ S	12.95	215.0849	
Dicrotophos	C ₈ H ₁₆ NO ₅ P	6.41	238.0839	Distinguished by fragmentation pattern
Ketamine	C ₁₃ H ₁₆ ClNO	6.31	238.0993	

Table S4. List of compounds with values of chromatographic resolution (Rs) and Δm below or close to the cut-off levels (Rs=1.5 and $\Delta m=0.030$ Da)

Compound	Retention time (min)	m/z	Rs (*)	Δm (mDa)
Quinmerac	7.81	222.0316	1.25	0.0113
Chloridazon (Pyrazon)	8.06	222.0429		
Prometon	9.02	226.1662	1.50	0.0000
Terbumeton	9.32	226.1662		
Imazamethabenz-methyl	9.12	289.1547	1.20	0.0251
Estriol	9.36	289.1798		
Benzoylecgonine	6.61	290.1387	1.60	0.0364
Atropine	6.29	290.1751		
Simetryn	8.7	214.1121	0.30	0.0000
Desmetryn	8.8	214.1121		
Monolinuron	13.25	215.0582	1.50	0.0267
DMST	12.95	215.0849		
Dicrotophos	6.41	238.0839	0.50	0.0154
Ketamine	6.31	238.0993		

(*)Rs: Chromatographic resolution defined as the difference in retention times between the two peaks, divided by the combined widths of the elution peaks, $Rs = 2[(tR)_B - (tR)_A]/(W_B + W_A)$.

Table S5. Fragmentation study of the isobaric compounds which present values of Rs and Δm below or close to the cut-off levels

Compounds	m/z	Ion formula	Error (ppm)	Score	DBE (*)
Quimerac	222.0312	C ₁₁ H ₉ ClNO ₂	2.03	96.97	8
Quimerac-F1	204.0206	C ₁₁ H ₇ ClNO	2.14	96.55	9
Quimerac-F2	176.0257	C ₁₀ H ₇ CIN	2.43	97.48	7
Quimerac-F3	149.0147	C ₉ H ₆ Cl	3.67	79.17	7
Chloridazon	222.043	C ₁₀ H ₉ ClN ₃ O	-0.52	97.85	8
Chloridazon-Na adduct	244.0248	C ₁₀ H ₈ ClN ₃ NaO	-0.25	96.06	8
Prometon	226.1665	C ₁₀ H ₁₉ N ₅ O	-1.12	96.87	4
Prometon-F1	184.1194	C ₇ H ₁₄ N ₅ O	-0.38	99.86	4
Prometon-F2	142.0725	C ₄ H ₈ N ₅ O	-1.21	87.39	4
Terbumeton	226.1664	C ₁₀ H ₁₉ N ₅ O	-0.62	96.56	4
Terbumeton-F1	170.1038	C ₆ H ₁₁ N ₅ O	-0.77	86.62	4
Imazamethabenz-methyl	289.1541	C ₁₆ H ₂₁ N ₂ O ₃	1.86	95.38	8
Imazamethabenz-methyl-F1	257.1278	C ₁₅ H ₁₇ N ₂ O ₂	2.57	97.41	9
Imazamethabenz-methyl-F2	229.1331	C ₁₄ H ₁₇ N ₂ O	1.96	98.17	8
Imazamethabenz-methyl-F3	215.0809	C ₁₂ H ₁₁ N ₂ O ₂	2.99	97.54	9
Imazamethabenz-methyl-F4	176.0701	C ₁₀ H ₁₀ NO ₂	2.89	95.76	7
Imazamethabenz-methyl-F5	161.0709	C ₉ H ₉ N ₂ O	0.63	98.25	7
Imazamethabenz-methyl-F6	144.0444	C ₉ H ₈ NO	0.4	74.98	8
Estriol	289.1798	C ₁₈ H ₂₄ O ₃	0.23	99.39	7
Estriol-Na adduct	311.1619	C ₁₈ H ₂₄ NaO ₃	-0.49	98.8	7
Estriol-F2	271.1688	C ₁₈ H ₂₃ O ₂	1.54	85.16	8
Estriol-F3	253.1582	C ₁₈ H ₂₁ O	2.16	85.69	9
Estriol-F4	227.143	C ₁₆ H ₁₉ O	0.24	95.61	8
Estriol	289.1798	C ₁₈ H ₂₄ O ₃	0.23	99.39	7
Atropine	290.1734	C ₁₇ H ₂₄ NO ₃	5.78	87.46	7
Atropine-F1	124.1106	C ₈ H ₁₄ N	11.72	84.06	3
Benzoylecgonine	290.138	C ₁₆ H ₂₀ NO ₄	1.32	94.49	8
Benzoylecgonine-F1	272.1272	C ₁₆ H ₁₈ NO ₃	3.35	96.4	9

Compounds	m/z	Ion formula	Error (ppm)	Score	DBE (*)
Benzoylecgonine-F2	168.1015	C ₉ H ₁₄ NO ₂	2.4	98.87	4
Desmetryn	214.1122	C ₈ H ₁₅ N ₅ S	-0.6	96.69	4
Desmetryn-F1	172.0652	C ₅ H ₁₀ N ₅ S	-0.26	99.41	4
Desmetryn-F2	130.0432	C ₄ H ₈ N ₃ S	0.78	98.47	3
Desmetryn-F3	124.0617	C ₄ H ₈ N ₅	0.78	87.65	5
Desmetryn-F4	116.0279	C ₃ H ₆ N ₃ S	-2.16	98.91	3
Simetryn	214.1122	C ₈ H ₁₅ N ₅ S	-0.73	96.4	4
Simetryn-F1	186.0811	C ₆ H ₁₂ N ₅ S	-1.39	61.42	4
Simetryn-F2	166.1086	C ₇ H ₁₂ N ₅	0.76	85.09	5
Simetryn-F3	144.0597	C ₅ H ₁₀ N ₃ S	-4.82	97.07	3
Simetryn-F4	124.0869	C ₆ H ₁₀ N ₃	0.35	85.98	4
Simetryn-F5	116.0276	C ₃ H ₆ N ₃ S	0.63	99.41	3
Monolinuron	215.0586	C ₉ H ₁₂ ClN ₂ O ₂	-1.93	98.63	5
Monolinuron-Na adduct	237.0403	C ₉ H ₁₁ ClNaN ₂ O ₃	-0.27	98.04	5
Monolinuron-F1	183.0322	C ₈ H ₈ ClN ₂ O	-1.5	97.23	6
Monolinuron-F2	126.011	C ₆ H ₅ ClN	-3.94	96.07	5
DMST	215.0845	C ₉ H ₁₅ N ₂ O ₂ S	1.07	97.77	4
DMST-Na adduct	237.0665	C ₉ H ₁₄ NaN ₂ O ₂ S	1.38	98.62	4
DMST-F1	151.1231	C ₉ H ₁₅ N ₂	-0.73	99.73	4
Ketamine	238.0991	C ₁₃ H ₁₇ ClNO	0.75	97.06	6
Ketamine-F1	220.0886	C ₁₃ H ₁₅ ClN	0.73	98.82	7
Ketamine-F2	207.0569	C ₁₂ H ₁₂ ClO	1.27	98.62	7
Ketamine-F3	179.0619	C ₁₁ H ₁₂ Cl	1.78	99.22	6
Ketamine-F4	125.0151	C ₇ H ₈ Cl	1.38	98.91	5
Dicotrophos	238.0839	C ₈ H ₁₇ NO ₅ P	0.04	99.08	2
Dicotrophos-Na	260.0659	C ₈ H ₁₆ NaNO ₅ P	-0.17	98.56	2
Dicotrophos-F1	193.0259	C ₆ H ₁₀ O ₅ P	0.48	99.89	3
Dicotrophos-F2	112.0759	C ₆ H ₁₀ O ₅ P	-1.56	99.33	3

(*)DBE: Double bond equivalent.

*IV.5. MONITOREO DE CONTAMINANTES PRIORITARIOS Y
EMERGENTES EN EL RÍO GUADALQUIVIR Y OTRAS
AGUAS SUPERFICIALES EN LA PROVINCIA DE JAÉN*

IV.5.1. Introducción

La gestión de las aguas superficiales europeas se organiza a través de la Directiva Marco del agua (2000/60/CE) con la que se pretende prevenir y reducir la contaminación de las mismas. Para ello, entre otras medidas, estableció una lista que contenía 33 sustancias prioritarias, a las que se le debía prestar un especial interés, mediante la Decisión número 2455/2001/CE del parlamento europeo y del consejo de 20 de Noviembre de 2001, con el fin de alcanzar un "buen estado" ecológico de las aguas comunitarias a finales de 2015. Pero debido a que el número sustancias químicas potencialmente contaminantes que se introducen en el medioambiente es cada vez mayor, esta lista no es cerrada. De hecho, a través de la Directiva 2013/39/EU se incrementó el número de sustancias prioritarias a 45. Por lo tanto, son los propios estados miembros los responsables de identificar los contaminantes de sus cuencas hidrográficas para seguir actualizando esta lista.

A parte de presentar una alta toxicidad, las sustancias prioritarias presentan la característica de que son persistentes en el medioambiente y son lipofílicas por lo que tienden a acumularse en el tejido adiposo de animales y a través de la cadena alimentaria se pueden acumular además en el de los seres humanos (Barron 1990, Kelly 2004). Una gran parte de estos contaminantes se incorporará a los sedimentos por lo que estos mismos actúan como una fuente de sustancias prioritarias a largo plazo debido a su estabilidad y persistencia. De manera que se han llevado a cabo numerosos trabajos para estudiar la presencia de estas sustancias en distintos tipos de aguas, como residuales (Pitarch 2007, Robles-Molina 2010, Martí 2011, Barco-Bonilla 2013) de bebida (Auersperger 2005, León 2006, Tillner 2013) y superficiales (Erger 2012, Olujimi 2012, Esteban 2014, Vorkamp 2014). En general, el nivel de concentración de estos contaminantes se sitúa en el orden de los ng L^{-1} .

Sin embargo, en la actualidad la atención en cuanto a la contaminación del medioambiente se centra en la investigación de los llamados contaminantes emergentes, para los que no existe legislación y no se conoce de forma precisa su efecto y presencia en el medioambiente. Dentro de estas sustancias se pueden encontrar fármacos, drogas de abuso, productos de higiene personal o pesticidas. Al contrario de lo que sucede con los prioritarios,

estos compuestos no necesitan ser persistentes para producir efectos adversos en el medioambiente, ya que su rápida degradación se compensa por su continua introducción en el medio (**Daughton 2004, Petrović 2003**). Los principales focos de contaminación de estos compuestos son las descargas de agua residual urbanas (hogares, hospitales), industriales (procesos de producción y limpieza industrial) y agrícolas (uso de plaguicidas). Así que son muchos los estudios acerca de este tipo de contaminantes y su aparición en el medioambiente (Loos 2009, Estévez 2012, Gros 2012, Reemtsma 2013, **Petrović 2014**).

Por otro lado, una zona que se presenta a priori como apropiada para llevar a cabo el estudio de la presencia y concentración de contaminantes prioritarios y emergentes es la provincia de Jaén. Numerosos son los ríos, pantanos y humedales que se sitúan a lo largo de toda su extensión (13.496 Km²), en la que se ubican hasta 4 parques naturales. El río Guadalquivir, uno de los más importantes de la península ibérica, recorre esta provincia de este a oeste y se ve sometido a una gran presión contaminante principalmente debida a la extensión del cultivo del olivar, única en el mundo, ya que es el lugar con mayor producción de aceite de oliva del mundo. Además, aunque en menor extensión, se llevan a cabo actividades industriales de la madera, metalurgia o el ladrillo, y recorre varios municipios y ciudades, por lo que va a presentar también contribuciones de contaminación debida a descargas de aguas residuales urbanas e industriales. A pesar de su importancia, pocos han sido los trabajos dedicados al estudio de la contaminación por compuestos orgánicos prioritarios y emergentes en las aguas del río Guadalquivir y otras aguas superficiales relacionadas con el mismo, como afluentes o pantanos. Estos se han dedicado principalmente al análisis de plaguicidas (Belmonte-Vega 2005, Campo 2013, Masiá 2013) y sólo unos pocos al de fármacos (Martín 2011, López-Serna 2013). Teniendo en cuenta estos antecedentes parece claro que es necesario un estudio más completo en esta zona, acerca de la contaminación debida tanto a plaguicidas como compuestos denominados como emergentes, es decir, fármacos, drogas de abuso, productos de higiene personal, etc y sus productos de transformación ya que a su vez pueden ser más tóxicos que los compuestos de los que proceden.

Teniendo en cuenta todo lo anterior, en el artículo científico 5 se realizó la monitorización de contaminantes orgánicos tanto prioritarios como emergentes en aguas superficiales de la

provincia de Jaén. Para asegurarse una amplia cobertura geográfica se seleccionaron un total de 19 puntos de muestreo. Durante un periodo de 20 meses se recogieron un total de 83 muestras de agua correspondientes a 3 ríos, entre ellos el Guadalquivir, 5 pantanos y 11 humedales. Las muestras se analizaron siguiendo las 2 metodologías multiresiduo descritas en los apartados IV.2 y IV.4 de la presente tesis. De manera que por un lado se realizó una LLE seguida del análisis por GC-QqQ-MS para compuestos apolares y por otro una SPE seguida del análisis por HPLC-TOFMS para compuestos más polares. Con la primera metodología se conseguían analizar 63 compuestos y con la segunda 340, de manera que el número total de contaminantes estudiados asciende hasta los 373. Se abarca así un amplio rango de contaminantes, como son PAHs, plaguicidas, fármacos, drogas de abuso, nitrosaminas y sus productos de transformación. Además se han incluido en el estudio sustancias mundialmente consumidas por el ser humano en grandes cantidades, como son la cafeína y la nicotina junto con sus principales productos de transformación.

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IV.5.2. Artículo científico 5

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Monitoring of selected priority and emerging contaminants in the Guadalquivir River and other related surface waters in the province of Jaén, South East Spain

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HIGHLIGHTS

- 373 pollutants monitored in surface waters from the province of Jaén (S.E. Spain)
- Combination of an experimental LC-TOFMS database together with a GC-MS/MS method
- 83 samples analyzed from different water bodies (rivers, reservoirs and wetlands)
- Concentrations found of studied priority compounds were below the WFD standards.
- Olive grove pesticides and ubiquitous pharmaceuticals were the main species found.

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ABSTRACT

The province of Jaén counts with four natural parks, numerous rivers, reservoirs and wetlands; moreover, it is probably the region with higher olive oil production in the world, which makes this zone a proper target to be studied based on the European Water Framework Directive 2000/60/CE. The aim of this survey is to monitor a total number of 373 compounds belonging to different families (pesticides, PAHs, nitrosamines, drugs of abuse, pharmaceuticals and life-style compounds) in surface waters located at different points of the province of Jaén. Among these compounds some priority organic substances (regulated by the EU Directive 2008/105/EC) and pollutants of emerging concern (not regulated yet) can be found. A liquid chromatography electrospray time-of-flight mass spectrometry (LC-TOFMS) method covering 340 compounds was developed and applied, together with a gas chromatography triple-quadrupole mass spectrometry (GC-MS/MS) method which enabled the analysis of 63 organic contaminants (30 of these compounds are analyzed by LC-TOFMS as well). From April 2009 to November 2010 a total of 83 surface water samples were collected (rivers, reservoirs and wetlands). In this period numerous organic contaminants were detected, most of them at the ng L⁻¹ level. The most frequently priority substances found were chlorpyrifos ethyl, diuron and hexachlorobenzene. Within the other groups, the most frequently detected compounds were: terbutylazine, oxyfluorfen, desethyl terbutylazine, diphenylamine (pesticide family); fluorene, phenanthrene, pyrene (PAHs group), codeine, paracetamol (pharmaceuticals compounds) and caffeine, nicotine (life-style compounds). As is could be expected, the total concentration of emerging contaminants is distinctly larger than that of priority pollutants, highlighting the importance of continuing with the study of their presence, fate and effects in aquatic environments. However, concentration levels (at the ng per liter level) are low in general for both kinds of contaminants which minimizes the possible harmful effect on the environment.

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Monitoring of selected priority and emerging contaminants in the Guadalquivir river and other related surface waters in the province of Jaén, South East Spain

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Abstract

The European Water Framework Directive (WFD; Directive 2000/60/CE) establishes a framework for Community action in the field of water policy, highlighting the necessity of improving the ecological quality of surface water in the European Community. Consequently, the application of wider and integrated quality control applying the adequate analytical technique is a priority. The province of Jaén counts with four natural parks, numerous rivers, reservoirs and wetlands; this fact together with the extensive agricultural activity (Jaén is probably the region with higher olive oil production in the world) make this zone a proper target to be studied taking into account the European Water Framework Directive. With this background, the aim of this survey is to monitor a total number of 373 compounds belonging to different families (pesticides, PAHs, nitrosamines, drugs of abuse, pharmaceuticals and life-style compounds) in surface waters located at different points of the province of Jaén. Among these compounds some priority organic substances (regulated by the EU Directive 2008/105/EC) and pollutants of emerging concern (not regulated yet) can be found. For this purpose, a liquid chromatography electrospray time-of-flight mass spectrometry (LC-TOFMS) method covering 340 compounds was developed and applied, together with a gas chromatography triple-quadrupole mass spectrometry (GC-MS/MS) method which enabled the analysis of 63 organic contaminants (30 of these compounds are analyzed by LC-TOFMS as well). From April 2009 to November 2010 a total of 83 surface water samples were collected (rivers, reservoirs and wetlands). In this period numerous organic contaminants have been detected, most of them at the ng L^{-1} level. The most frequently priority substances found were chlorpyrifos ethyl, diuron and hexachlorobenzene. With respect to the other groups the most frequently detected compounds were: terbuthylazine, oxyfluorfen, desethyl terbuthylazine, diphenylamine for the pesticide family; fluorene, phenanthrene, pyrene for the PAHs group, codeine, paracetamol within the pharmaceuticals

compounds and caffeine, nicotine as life-style compounds. As is could be expected, the total concentration of emerging contaminants is distinctly larger than that of priority pollutants, highlighting the importance of continue with the study of their presence, fate and effects in aquatic environments. However, concentration levels (at the ng per liter level) are low in general for both kinds of contaminants which minimizes the possible harmful effect on the environment.

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1. Introduction

Water quality is an important issue in the European Union. Particularly, there is a growing concern related to the presence of emerging and priority substances in surface water. For this reason, the European Union Water Framework Directive (WFD) 2000/60/EC [1] was set, committing European Union member states to achieve good ecological and chemical status of all water bodies. The WFD first established a list of 33 priority compounds (Decision 2455/2001 EC) -that represented a significant risk to or via the aquatic environment- to be monitored in water in order to evaluate their levels. Environmental Quality Standards (EQS) have been set to control the concentration levels of these compounds in surface waters (Directive 2008/105/EC [2]). On the basis of these EQS, Member States have to establish monitoring programs in order to have a comprehensive overview of water status within each river basin district. Additionally, Member States have to identify their river basin specific pollutants, which will be the basis of further updates of the priority substances list. Recently, the European Union has approved a directive (Directive 2013/39/EU [3]) that increases the number of priority substances up to 45, including aconitine, cypermethrin, heptachlor and terbutryn, among others.

The hazardous nature of priority pollutants is caused by their toxicity in combination with high chemical and biological stability, and a high lipophilicity. They accumulate in the adipose tissues of fishes, wildlife and humans through dietary and non-dietary routes [4,5]. A major part of these contaminants that are released into aquatic environments will be incorporated in sediments [6]. These sediments might later act as the major source of contaminants to water and biota. Hence, different studies have been undertaken to measure the occurrence and significance of priority substances in different types of water samples, such as wastewater [7,8,9,10,11,12], drinking water [13,14,15,16] and surface water [17,18,19,20,21,22]. In general, the concentration of the detected compounds in these reports was at the ng L⁻¹ level.

However, the focus for water pollution research has recently been shifted from the conventional organic priority pollutants to the so-called emerging contaminants, many of them not being regulated yet. They include pharmaceuticals, personal care products and disinfectants, among others. It is not necessary for these contaminants to persist in the environment to cause negative effects since their high transformation/removal rates can be

compensated by their continuous introduction into the environment [23,24]. Pharmaceuticals and personal care products (PPCPs) enter surface water mainly through insufficiently treated wastewater effluent. The level of their concentration and fate in the aqueous environment varied and depended on several parameters such as geographical position, effectiveness of wastewater treatment, proximity to wastewater plants and meteorological conditions, mainly rainfall [25]. For these reasons, several surveys of their appearance in the environment have been carried out in the last years [26,27,28,29,30,31,32]. From these studies, the most comprehensive in terms of sampling area was carried out by the Joint Research Centre -Institute for Environment and Sustainability (JRC-IES), monitoring 35 selected polar organic pollutants in 122 samples of European rivers [33].

The most widely used analytical methodologies for the analysis of priority and emerging contaminants in environmental waters are based on solid phase extraction (SPE) followed by either liquid chromatography coupled to mass spectrometry (LC-MS) or gas chromatography coupled to mass spectrometry (GC-MS) [34,35,36]. The analysis of selected contaminants by tandem mass spectrometry (MS/MS) is the most common approach. However, to characterize the chemical contamination associated with a river basin, it is very convenient the development of screening methods based on high sensitive full scan acquisition, which permits retrospective analysis and detection of “non-target” or unexpected compounds. To this aim, high resolution mass analyzers, such as time-of-flight or orbitrap (LC-TOFMS) have the ability to record an unlimited number of compounds because of operating in full-scan mode, being this approach really convenient for the development of screening strategies based on using accurate-mass databases [37,38,39,40,41,42].

With a length of 657 km, the Guadalquivir river is one of the most important rivers in Spain, it flows southwest through the region of Andalusia, with a drainage area of 57 527 km², affecting population regions of more than 4 million inhabitants. In order to evaluate the organic contamination of this river, several studies have been carried out to monitor pesticides [43,44,45] or pharmaceuticals [46,47], the later often related to sewage treatment plants (STP) discharges. In the present work we perform a study the occurrence of 373 organic compounds, comprising a LC-TOFMS database (340 compounds) and a GC-MS/MS method (63

compounds). The target area covers surface waters related to the beginning of the Guadalquivir river basin in the province of Jaen, which is highly affected by olive harvesting, and also by wastewater discharges.

2. Experimental

2.1. Chemical and reagents

The list of organic compounds studied is included in table 1. Standards were purchased from Sigma-Aldrich (Steinheim, Germany) and Dr. Ehrenstofer (Augsburg, Germany). Ethyl acetate and n-hexane were obtained from Riedel-de H  en (Seelze, Germany), methanol and acetonitrile from Sigma-Aldrich (Steinheim, Germany) and formic acid from Fluka (Madrid, Spain). Anhydrous sodium sulfate and sodium chloride were purchased from J.T. Baker (Deventer, Holland) and sulfuric acid from Panreac (Castellar del Valles, Spain). All of them were provided with the adequate purity. Individual stock standard solutions for LC-TOFMS were prepared at a concentration level of 1000 $\mu\text{g mL}^{-1}$ by dissolving the analytes in methanol and mixtures of acetonitrile and methanol depending on the compound solubility. For GC-MS/MS analysis individual stock standard solutions were prepared in n-hexane or ethyl acetate and mixtures containing both solvents, at a concentration level of 1000 $\mu\text{g mL}^{-1}$. The working standard solutions with the different mixtures of compounds were prepared by appropriate dilutions of the stock solutions with methanol or n-hexane for LC and GC injection respectively.

Table 1. Classification of the target compounds measured by LC-TOFMS and GC-MS/MS.

Tested compounds by LC-TOFMS
209 Pesticides: 1-naphtalene-acetamide, 2,4-dichlorophenoxyacetic acid, 2,4-dimethylphenol, 2,4-dinitrophenol, 2-hydroxybiphenyl, 4-chloro-2-methylphenol, 4-chloro-o-tolylxyacetic acid, acephate, acetamiprid, acclonifen*, alachlor*, albendazole, aldicarb, aldicarb sulfone, aldicarb sulfoxide, ametryn, aminocarb, amitrol, AMPA, anilofos, atrazine*, atrazine desethyl, azinphos methyl, azinphos ethyl, azoxystrobin, benalaxyl, bendiocarb, bentazon, bifeno, biteranol, bromacil, bromophos ethyl, bromophos methyl, bromuconazole (2 isomers), bupirimate, buprofezin, butocarboxim, butoxycarboxim, buturon, carbaryl, carbendazim, carbofuran, carfentrazone ethyl, chlorbromuron, chlorfenvinphos*, chloridazon, chloroprotham, chlorotoluron, chloroxuron, chlorpyrifos ethyl*, chlorpyrifos methyl, cymoxanil, cyprodinil, cyromazine, cyproconazole, DEET, deltamethrin, demeton-s-methyl, desethyl terbutylazine, diafenthion, diazinon, dicamba, dichlofenthion, dichloran, dichlorprop, diethofencarb, difenoconazole, difenoxuron, diflubenuron, diflufenican, dimethoate, dimethomorph (isomers E+Z), diphenylamine, diuron*, DNOC, edifenphos, endosulfan* sulfate, ethiofencarb, ethion, ethiprole, ethoxyquin, etofenprox, fenamiphos, fenarimol, fenazaquin, febedazol, fenhexamid, fenitrothion, fenobucarb, fenoxycarb, fenthion, fenuron, fenvalerate, fluzazifop, fluzazifop-p-butyl, flufenoxuron, fluomethuron, fluquinconazole, fluroxypyr, flutriafol, fuberidazol, glyphosate, hexaflumuron,

hexythiazox, imazalil, imazalil metabolite, imazapyr, imazaquin, imidacloprid, indoxacarb, iprodione, isocarbophos, isoprocarb, isoproturon*, kresoxim methyl, lenacil, lufenuron, malaoxon, malathion, MCPA sodium, mecarbam, mecoprop, metalaxyl, metamitron, methamidophos, methidathion, methiocarb, methiocarb sulfoxide, methomyl, metobromuron, metolcarb, metoxuron, mevinphos, miconazole, monocrotophos, monuron, myclobutanil, N,N-diethyl-2-(1-naphthoxy)propamide (NNDP), naptalam, neburon, nitenpyram, norflurazon, omethoate, oxadiazon, oxadixyl, oxamyl, oxfendazole, oxyfluorfen, parathion ethyl, parathion methyl, penconazole, pentachlorophenol*, phosalone, phosmet, pirimicarb, pirimiphos methyl, prochloraz, procymidone, promecarb, prometon, prometryn, propamocarb, propanil, propargite, propazine, propoxur, propyzamid, pyridaben, pyridaphenthion, pyrimethanil, pyriproxifen, quinalphos, quinmerac, quinoxifen, quizalofop-p-ethyl, rotenone (2 isomers), simazine*, simetryn, spinosad (spinosyn A+D), spiromesifen, spiroxamine, sulfometuron methyl, tebuconazole, tebufenpyrad, teflubenzuron, terbuthylazine, terbutryn, tetrachlorvinphos, thiabendazole, thiacloprid, thiamethoxam, thiocyclam, thiophanate methyl, triadimefon, triadimenol, triazophos, trichlorfon, triclocarban, tridemorph, trietazine, TEP (triethyl phosphate), trifloxystrobin, triflumizole, triflururon, trifluralin*, XMC (3,5-xylylmethylcarbamate), α -cypermethrin*, λ -cyhalothrin.

24 drugs of abuse: 11-nor-9-carboxy-THC, acetylmorphine, amphetamine, bendroflumethiazide, benzoylecgonine, cannabidiol, chlorothiazide, cocaine, δ -9-THC, EDDP, ephedrine, ethylamphetamine, ethylmorphine, heroin, hydrochlorthiazide, hydroflumethiazide, ketamine, MDA, MDEA, MDMA, methadone, methamphetamine, morphine, phenylephrine.

97 Pharmaceuticals: 3-methylxanthine, 4-aminoantipyrine, antipyrine, atenolol, atropine, benzalkonium chloride (4 isomers), bezafibrate, carbadox, carbamazepine, cefotaxime, chloramphenicol, chlorotetracycline, cimetidine, cisdiltiazem, clarithromycin, clenbuterol, clofibrate, cloxacillin, codeine, compactin, danofloxacin, demeclocycline, diclofenac, dicloxacillin, digoxigenin, digoxin, diphenhydramine, doxycycline, enalapril, enoxacin, enrofloxacin, erythromycin, famotidine, fenofibrate, flufenamic acid, flumenique, fluoxetine, furosemide, gemfibrozil, hydrochlorthiazide, hydroflumethiazide, ibuprofen, indomethacine, josamycin, ketoprofen, leucomalachite green, lincomycin, lomefloxacin, malachite green, mebendazole, meclofenamic acid, mefenamic acid, metformin, metronidazole, miconazole, minocycline, naproxen, nifuroxazide, norfloxacin, ofloxacin, oxacillin, oxolinic acid, oxytetracycline, paracetamol, penicillin G, phenacetin, phenylbutazone, pindolol, pipemidic acid, pravastatin, propyphenazone, ranitidine, roxithromycin, salbutamol, salicylic acid, sarafloxacin, spiramycin I, sulfachloropyridazine, sulfadiazine, sulfadimethoxin, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfathiazole, tetracycline, thiamphenicol, timolol, trimethoprim, tylosine, warfarin.

5 nitrosamines: N-nitrosodiethylamine, N-nitrosodi-n-dibutylamine, N-nitrosomorpholine,

N-nitrosopiperidine, N-nitrosopyrrolidine.

5 Life-style compounds: caffeine, nicotine and their metabolites cotinine, theobromine and theophylline.

Tested compounds by GC-MS/MS

50 pesticides: 1,3,5-TCB*, 1,2,3-TCB*, 1,2,4-TCB*, 4,4'-DDE, 4,4'-DDT, alachlor*, aldrin, ametryn, atrazine*, atrazine desethyl, chlorfenvinphos* A, chlorfenvinphos* B, chlorotoluron, chlorpyrifos ethyl*, deltamethrin, diazinon, dieldrin, diuron*, endosulfan* sulfate, endrin, ethion, fenamiphos, fenthion, fluorene, heptachlor*, hexachloro-1,3-butadiene*, hexachlorobenzene*, iprodione, isodrin, isoproturon*, metoxychlor, oxyfluorfen, parathion ethyl, parathion methyl, pentachlorobenzene*, procymidone, prometon, propazine, simazine*, terbuthylazine, terbutryn*, trifluralin*, α -cypermethrin*, α -endosulfan*, β -endosulfan*, α -HCH*, β -HCH*, γ -HCH*, δ -HCH*, λ -cyhalothrin.

13 polycyclic aromatic hydrocarbons (PAHs): Acenaphthylene, Anthracene*, Benzo(a)anthracene, Benzo(a)pyrene*, Benzo(b)fluoranthene*, Benzo(ghi)perylene*, Benzo(k)fluoranthene*, Chrysene,

Dibenzo(a,h)anthracene, Fluorene, Indeno(1,2,3-cd)pyrene*, Phenanthrene, Pyrene.

*Priority compound (Directive 2013/39/EU)

2.2. Analytes selected

Analytes included in this study were selected taking into account the agricultural activity of sampling area, the European priority substances list [3], the United States Environmental Protection Agency methods for monitoring drinking water (www.epa.gov/safewater/methods/analyticalmethods.html) and published literature [48,49,50,51]. They comprise a group of 373 organic pollutants belonging to different compound categories: Pharmaceuticals, life-style compounds (LS), drugs of abuse, pesticides, nitrosamines, polycyclic aromatic hydrocarbons (PAHs) and some of their more relevant metabolites. Among the pharmaceuticals, there are representatives of different therapeutic groups, such as *analgesics/anti-inflammatories* (paracetamol, indomethacin, codeine, mefenamic acid, naproxen, ibuprofen, diclofenac, ketoprofen), *antibiotics* (metronidazole, sulfamethoxazole, trimethoprim, cefotaxime, ofloxacin, erythromycin), *lipid regulators* (fenofibrate, bezafibrate, gemfibrozil), *β -blockers* (atenolol, pindolol, timolol), *antiepileptic/psychiatric* (carbamazepine, fluoxetine), *ulcer healings* (ranitidine), *diuretics* (furosemide, hydrochlorothiazide), and *bronchodilators* (salbutamol). Because of their relevance, metabolites such as, clofibric acid and fenofibric acid were also included. Regarding drugs of abuse selected, they comprise *stimulants* (cocaine, ephedrine, amphetamine and some of the compounds that are referred as amphetamine family (3,4-Methylenedioxyamphetamine -MDA-, 3,4-Methylenedioxy-N-methylamphetamine -MDMA-)), *opiate analgesic drugs* (methadone, morphine, heroin) *cannabinoids* (cannabidiol, delta-9-tetrahydrocannabinol (Δ -9-THC)) and several metabolites from some of the selected drugs such as benzoylecgonine, ethylmorphine. Because of the sample sites are located in a zone where an intensive agricultural activity is carried out, a large number of pesticides and some of their metabolites (atrazine desethyl, desethyl terbutylazine) were selected, such as *herbicides* (prometon, terbutylazine, oxyfluorfen, anilofos) *insecticides* (chlorpyrifos ethyl, aldicarb, fenthion) or *fungicides* (imazalil, bupirimate, carbendazim). On the other hand, caffeine and nicotine are consumed worldwide substances by human in large quantities, therefore we have included

them, together with their metabolites cotinine, theophylline and theobromine in a group called life-style compounds (LS). Finally, from the priority list, 5 nitrosamines, PAHs group and several pesticides such as simazine, atrazine, isoproturon, hexachlorobenzene were included.

2.3. Sampling

The province of Jaén with a surface of 13.496 km² is one of the largest in Spain. The Guadalquivir river crosses Jaén from east to west, holding a significant contaminant pressure due to the industrial and mainly to the agricultural activity since Jaen is the main olive oil producer in the world. In Spain the cultivated olive tree surface is 2280451 ha which 570000 approximately belong to the province of Jaén, representing the 25 % of the total Spanish surface dedicated to olive cultivation (<http://www.magrama.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/>). Therefore, the agricultural activity is the main source of richness in this province, and to a lesser extent are wood, metallurgy and brick industries.

On the other hand, these activities can lead to water pollution due to the intensive use of pesticides for pest control. The most extensively applied agrochemicals in olive plantations of Mediterranean countries are insecticides, herbicides and fungicides. Those more extensively used in Spain belong to the class of organophosphorus (OP) insecticides and are mainly dimethoate, diazinon, and parathion-methyl; the OP chlorpyrifos was formerly used in combination with the organochlorine endosulfan, but the latter was globally banned by its inclusion in the Annex A of the Stockholm Convention on Persistent Organic Pollutants (fifth conference of parties (COP5), Friday April 29th 2011 [52]).

In order to ensure extensive geographic coverage of the province, a total of 19 sampling points were selected which are shown in Figure 1. Representative samples of each point were collected in amber glass bottles with Teflon caps (1L), and then were transported to the laboratory where they were stored at 4 °C for a maximum of 48 h before extraction. During a period of 20 months, 83 surface water samples were collected comprising 3 rivers, 5 reservoirs and 11 wetlands within the province of Jaén.

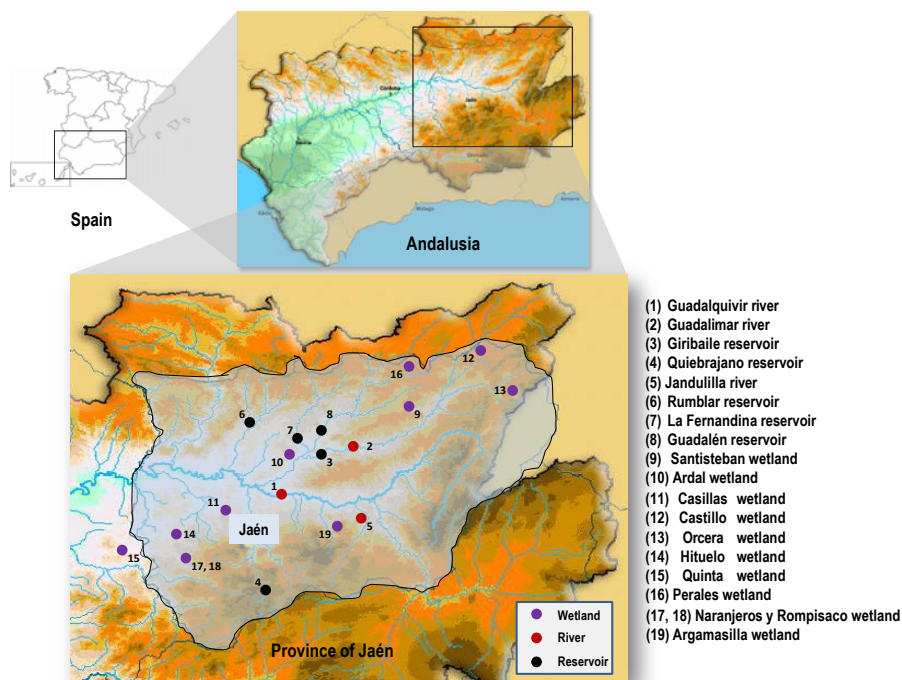


Figure 1. The 19 sampling sites of the province of Jaén: 3 rivers, 5 reservoirs and 11 wetlands.

2.4. Methods of analysis

The analysis of the samples was carried out by using two different analytical methods depending on the vast array of different physicochemical features of the pollutants tested. The sample treatment applied for the isolation and pre-concentration of non-polar and semi-polar volatile compounds was a liquid–liquid extraction (LLE) with n-hexane followed by gas chromatography coupled to mass spectrometry analysis. The instrument used was a CP-3800 gas chromatograph coupled with a 300-MS triple quadrupole mass spectrometer (Varian Inc. Walnut Creek, California, USA). The details of the validated GC-MS/MS method are described elsewhere [11]. On the other hand, a solid phase extraction (SPE) procedure with Oasis HLB™ SPE cartridges (200 mg, 6 mL), partially adapted from previous literature [53,50] was performed for the isolation and preconcentration of polar and semi-polar compounds, followed by liquid chromatography coupled with mass spectrometry analysis. For this purpose, a high-performance

liquid chromatograph (Agilent Infinity 1290, Agilent Technologies, Santa Clara, California) connected to a time-of-flight mass spectrometer (Agilent 6220 accurate mass TOF, Agilent Technologies, Santa Clara, California) was employed. The liquid chromatograph was equipped with a reversed-phase XDB-C18 analytical column of 4.6 mm x 50 mm and 1.8 μm particle size (Agilent Technologies, Santa Clara, CA). The chromatographic method held the initial mobile phase composition (10% acetonitrile, 90% ultrapure water with 0.1% of formic acid) constant for 3 min, followed by a linear gradient to 100% of acetonitrile up to 25 min, and then was held constant up to 28 min. Both methodologies were satisfactorily validated and obtained good recovery rates, as well as the RSD (%) for most compounds. The LC-TOFMS method analytical parameters of the compounds detected are provided in Table S1 (Supplementary Information).

2.5. LC-TOFMS accurate-mass database

The compounds selected for this study are listed in Table 1 grouped into different families, as pesticides, PAHs, drugs of abuse, nitrosamines, pharmaceuticals and life-style compounds. These compounds were divided into mixtures (containing from 30 to 70 analytes each at a concentration of 500 ng mL⁻¹), which were injected in the LC-TOFMS system. The next step was to compile in an Excel file the retention time, theoretical accurate mass, elemental composition and name of compounds and their selected fragment ions (with relative abundance above 10%) for the development of the database. This file was put into csv format for its use by the Agilent Mass Hunter Data Acquisition software (version B.04.00). The csv file permits to perform an automatic search of the target compounds by the Agilent software, which provides a list with the requested information such as retention time, mass error and experimental accurate mass of the selected masses among others, to unambiguously identify the compounds found during the search. As a result for the application of the database, the software shows the extracted ion chromatogram as well as the mass spectrum of each analyte. The developed LC-TOFMS accurate mass database and the GC-MS/MS method were used to investigate the surface water quality at the province of Jaén.

3. Results and discussions

3.1. Guadalquivir and tributary rivers

The list of contaminants found in the Guadalquivir River samples is detailed in Table 2, as well as their prevalence, the average concentration between April 2009 – November 2010 and the maximum concentration detected.

Table 2. Summary of results for the compounds detected in the Guadalquivir river (11 samples).

Compound	Family	Method	n° positives	Freq (%)	Average (ng L ⁻¹)	C max (ng L ⁻¹)
Ephedrine	Drugs of abuse	LC-MS	1	9.1	7.2	7.2
Phenylephrine	Drugs of abuse	LC-MS	3	27.3	27.9	47.8
Benzoyllecgonine	Cocaine metabolite	LC-MS	4	36.4	16.3	32.3
Caffeine	LS	LC-MS	6	54.5	91.9	233
Theobromine	LS (Caffeine metabolite)	LC-MS	7	63.6	260.3	827.9
Theophylline	LS (Caffeine metabolite)	LC-MS	8	72.7	164.1	455.8
Nicotine	LS	LC-MS	9	81.8	24.4	44.9
Cotinine	LS (Nicotine metabolite)	LC-MS	5	45.5	44.8	113
Acenaphthylene	PAH	GC-MS	4	36.4	16.4	45.4
Fluorene	PAH	GC-MS	11	100.0	1.7	4.6
Phenanthrene	PAH	GC-MS	10	90.9	10.1	25.3
Pyrene	PAH	GC-MS	11	100.0	8.9	27.2
4,4'-DDE	Pesticides	GC-MS	10	90.9	1.5	2.8
DEET	Pesticides	LC-MS	2	18.2	4.0	5.7
Diazinon	Pesticides	GC-MS	4	36.4	61.5	234.5
Diflufenican	Pesticides	LC-MS	1	9.1	15.8	15.8
Dimethoate	Pesticides	LC-MS	3	27.3	1908.6	5166.9
Diphenylamine	Pesticides	LC-MS	8	72.7	148.9	220.4
Fenhexamid	Pesticides	LC-MS	1	9.1	27.7	27.7
Fluomethuron	Pesticides	LC-MS	7	63.6	79.9	272.1
alfa-HCH	Pesticides	GC-MS	1	9.1	15.1	15.1
beta-HCH	Pesticides	GC-MS	1	9.1	2.8	2.8
gamma-HCH	Pesticides	GC-MS	1	9.1	1.9	1.9
Metoxychlor	Pesticides	GC-MS	1	9.1	1.0	1
Oxyfluorfen	Pesticides	GC-MS	11	100.0	13.7	51.1
Procymidone	Pesticides	GC-MS	1	9.1	1.2	1.2
Tebuconazole	Pesticides	LC-MS	6	54.5	5.6	14.2
Terbutylazine	Pesticides	LC-MS	11	100.0	102.2	534
Desethyl terbutylazine	Terbutylazine metabolite	LC-MS	8	72.7	54.3	172
Antipyrine	Pharmaceuticals	LC-MS	1	9.1	3.6	3.6
Atenolol	Pharmaceuticals	LC-MS	3	27.3	6.2	10.2
Codeine	Pharmaceuticals	LC-MS	5	45.5	40.4	96.9
Lyncomycin	Pharmaceuticals	LC-MS	2	18.2	9.9	13.9
Paracetamol	Pharmaceuticals	LC-MS	5	45.5	979.6	2694.5
Anthracene	PAH Priority	GC-MS	1	9.1	4.2	4.2
1,2,4, TCB	Pesticides priority	GC-MS	3	27.3	1.0	1.5
Atrazine	Pesticides priority	LC-MS	1	9.1	2.2	2.2
Chlorfenvinfos B	Pesticides priority	GC-MS	3	27.3	1.4	1.8
Chloropyrifos ethyl	Pesticides priority	GC-MS	8	72.7	13.8	41.3
Diuron	Pesticides priority	GC-MS	10	90.9	6.4	19.4
Hexachlorobutadiene	Pesticides priority	GC-MS	1	9.1	1.3	1.3
Hexachlorobenzene	Pesticides priority	GC-MS	3	27.3	20.1	79.6
Pentachlorobenzene	Pesticides priority	GC-MS	1	9.1	4.7	4.7
Simazine	Pesticides Priority	LC-MS	8	72.7	56.3	195.2

The most frequently detected compounds in the Guadalquivir River belonging to the pesticide group were oxyfluorfen and terbuthylazine, which appeared in all the samples tested. Other pesticides frequently detected were 4,4'-DDE in 91% samples, diphenylamine and desethyl terbuthylazine in 73% samples, fluomethuron and tebuconazole in 64% and 54% of studied samples, respectively. The high frequency of detection of the metabolite 4,4'-DDE could be strange taking into account that his parent compound DDT is supposed not to be used from many years ago, however it is rather persistent, hydrophobic and tend to be accumulated in sediments [54], therefore the concentration found is very low with an average of 1.5 ng L^{-1} and maximum concentration of 2.8 ng L^{-1} . Regarding pesticides grouped as priority compounds, diuron was detected in the 91% of the studied samples, chlorpyrifos ethyl and simazine were detected in the 73% of samples and hexachlorobenzene was found in the 27% of samples. Except hexachlorobenzene, all priority pesticides detected in Guadalquivir river were found at average concentration levels below their respective Environmental Quality Standard (EQS) set by the EU [3]. With regards to other (non-priority) pesticides, the single compounds that gave the highest concentration were dimethoate with $0.5 \text{ } \mu\text{g L}^{-1}$ and $5.2 \text{ } \mu\text{g L}^{-1}$ in April and October 2009 respectively and terbuthylazine with $0.5 \text{ } \mu\text{g L}^{-1}$ in October 2009.

Concerning other contaminants present in the samples, codeine and paracetamol were the most detected pharmaceuticals (both 45.5% samples) with a maximum concentration of 96.9 ng L^{-1} and $2.7 \text{ } \mu\text{g L}^{-1}$ respectively. Belonging to the LS group, the stimulants nicotine (with its metabolite cotinine) and caffeine (with its metabolites theobromine and theophylline) were detected in at least 45% of studied samples, with concentrations at the ng L^{-1} level. Finally, in the group of PAHs the most detected contaminants were fluorene and pyrene, which appeared in all samples and phenanthrene with 91% of frequency of detection. The concentration of PAHs ranged between 0.1 and 45.4 ng L^{-1} , low values that are consistent with their low solubility in water [56]. An example of the identification of two contaminants detected in the Guadalquivir river samples, paracetamol (LC-TOFMS) and hexachlorobenzene (GC-MS/MS), is shown in Figure 2.

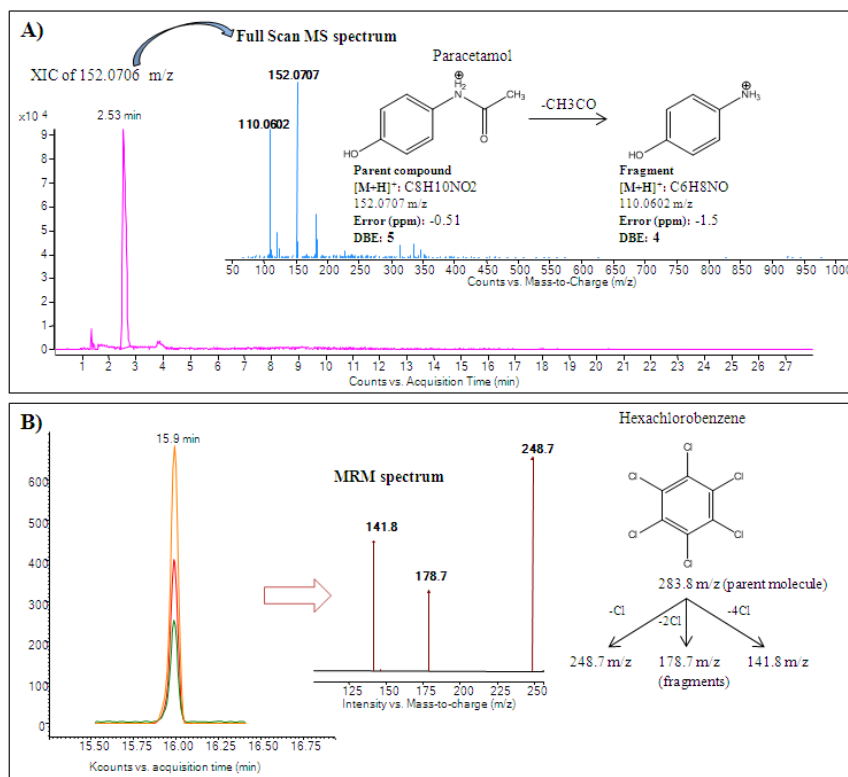


Figure 2. Examples of positive findings in two different samples of the Guadalquivir river by the two methods used. A) LC-TOFMS extracted ion chromatogram and full scan spectrum of paracetamol. B) GC-MS/MS chromatogram and MRM spectrum of the three selected transitions for hexachlorobenzene. The identification criteria for LC-TOFMS were mass exact measurement, isotopic pattern, characteristic fragments and retention time, whereas for GC-MS/MS the ratio between the intensity of the quantitation transition (Q) and 2 (for PAHs) or 3 qualifier transitions (q) was chosen as identification criterion, together with retention time.

The application of the two methods to the Guadalimar river samples gave a profile of detected compounds similar to that found in the Guadalquivir river, 38 out of 40 contaminants detected in the tributary river were also found in the main river, where a total number of 45 contaminants were detected. In the case of the other sampled tributary river, Jandulilla, results showed that only 28 contaminants were present, from which 22 were coincident with both Guadalquivir and Guadalimar rivers.

There were two priority contaminants that exceeded the limits (EQS) established by the Directive 2013/39/EU [3]. On one hand, hexachlorobenzene slightly exceeded the maximum

allowable concentration (MAC) set at 50 ng L⁻¹ in one sample with a value of 79.6 ng L⁻¹ in the Guadalquivir river. On the other hand, in the Guadalimar river the MAC limit (100 ng L⁻¹) for chlorpyrifos was slightly exceeded in one sample with a concentration of 119.0 ng L⁻¹. The concentrations found on the rest of priority substances analyzed were always below the thresholds set in this directive. The results of the priority contaminants found in Guadalimar river are shown in Table S2 in the Supplementary Information.

The profile of pesticides found is consistent with the current and former olive grove practices (triazines, diuron, dimethoate,...). Therefore, the main chemical pollution of the studied rivers could be associated to the agricultural activity in the zone, as it was described in previous works [43,45]. Moreover, the concentrations of pharmaceuticals and LS compounds make an important contribution to the total contamination of Guadalquivir river water, as shown in Figure 3. These compounds can derive from upstream wastewater discharges. Roughly, pesticides used in olive harvesting together with pharmaceuticals are the main contaminants in the studied area. The concentration of priority compounds comprises only between 2 and 9% of the total concentration of contaminants detected in the survey. Despite of the importance of this river there are few works dealing with the appearance of emerging contaminants in its waters [8,47,55]. The sampling of those investigations was mainly focused in the lower course of Guadalquivir river, only covering 18 compounds though. For these reasons it was not possible to establish relationships between the profiles of emerging pollutants detected within all the studies.

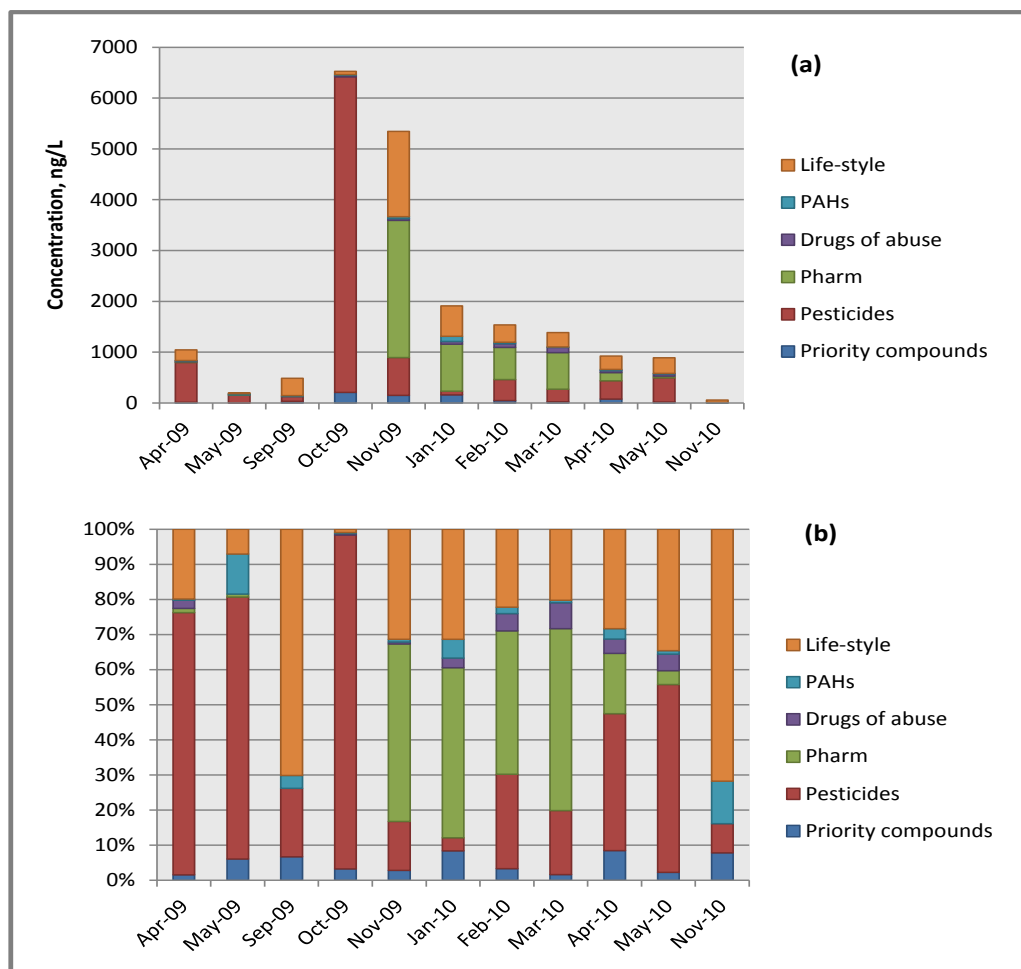


Figure 3. a) Total concentration of contaminants found in the Guadalquivir river samples and b) Percent contribution of different families of compounds to total concentration of contaminants found.

3.2. Wetlands

The Ramsar Convention (February 2, 1971) is an international treaty for the conservation and sustainable utilization of wetlands, especially as waterfowl habitat. Wetlands play a number of roles in the environment, principally water purification, flood control, and shoreline stability [57]. These functions can easily be overloaded in areas of heavy human development and activity. Therefore, it is necessary to explore the contamination due to the human activity that can reach the wetlands.

Samples from 11 different wetlands were collected during the same period (May 2010), therefore to discuss the results we have considered all the samples as a batch. The results are shown in Table 3, where it can be observed that fluorene, pyrene (PAHs), terbutylazine and chlorpyrifos (pesticides) were always detected. Other frequently detected (more than 4 positives) compounds were the pesticides simazine, tebuconazole, fluomethuron, oxyfluorfen, desethyl terbutylazine (terbutylazine metabolite), diuron and pentachlorobenzene, as well as the PAH phenanthrene. From the other groups, the stimulants nicotine, caffeine, cotinine (nicotine metabolite) and phenylephrine were the most frequently detected. Table 3 summarizes the detected contaminants and their concentrations.

Table 3. Summary of results for the compounds detected in the studied wetlands.

Compound	Family	Method anal.	Freq (%)	Cmax (ng L ⁻¹)	(wetland)	Average (ng L ⁻¹)
Phenylephrine	Drugs of abuse	LC-MS	60	45.7	(Naranjeros)	29.8
Caffeine	LS	LC-MS	50	185.4	(Argamasilla)	105.5
Nicotine	LS	LC-MS	90	2529.2	(Argamasilla)	432.6
Cotinine	LS (Nicotine metabolite)	LC-MS	90	153.3	(Argamasilla)	71.7
Acenaphthylene	PAH	LC-MS	20	3.9	(Orcera)	2.2
Fluorene	PAH	GC-MS	100	2.7	(Argamasilla)	1.1
Phenanthrene	PAH	GC-MS	90	9.1	(Argamasilla)	3.7
Pyrene	PAH	GC-MS	100	34.6	(Rompisaco)	7.6
4,4'-DDE	Pesticides	GC-MS	20	1	(Argamasilla)	1.0
Diflufenican	Pesticides	LC-MS	30	9	(Rompisaco)	6.9
Dimethoate	Pesticides	LC-MS	20	229.9	(Rompisaco)	170.9
Diphenylamine	Pesticides	LC-MS	20	195.5	(Santisteban)	178.9
Fluomethuron	Pesticides	LC-MS	50	419.8	(Rompisaco)	149.5
beta-HCH	Pesticides	GC-MS	10	18.2	(Perales)	7.8
Oxyfluorfen	Pesticides	GC-MS	50	54.9	(Perales)	19.0
Procymidone	Pesticides	GC-MS	20	9.3	(Naranjeros)	7.3
Tebuconazole	Pesticides	LC-MS	60	77.4	(Hituelo)	22.8
Terbutylazine	Pesticides	LC-MS	100	1167.0	(Hituelo)	431.5
Desethyl terbutylazine	Terbutylazine metabolite	LC-MS	90	658.8	(Hituelo)	235.4
Anthracene	PAH Priority	GC-MS	20	207	(Hituelo)	124.6
Chlorfenvinfos B	Pesticides priority	GC-MS	30	2.4	(Naranjeros)	1.6
Chlorpyrifos ethyl	Pesticides priority	GC-MS	100	75.3	(Naranjeros)	14.0
Diuron	Pesticides priority	GC-MS	90	16.9	(Rompisaco)	5.0
Hexachlorobenzene	Pesticides priority	GC-MS	20	3.6	(Perales)	3.1
Pentachlorobenzene	Pesticides priority	GC-MS	60	2	(Argamasilla)	1.2
Simazine	Pesticides priority	LC-MS	30	57.6	(Casillas)	30.4

The concentration of the priority substances present in these samples was always below the limits established by the Directive 2013/39/EU [3]. Terbutylazine (1.2 µg L⁻¹ at

Hituelo wetland) and its metabolite (desethyl) ($0.7 \mu\text{g L}^{-1}$ at Argamasilla wetland), and nicotine ($2.5 \mu\text{g L}^{-1}$ at Argamasilla) were the contaminants detected at higher concentrations in this study. One of the application of terbuthylazine to the olive grove occurs at the beginning of the spring, so the location of this sampling site surrounded by olives (Andalucía wetland inventory, <http://www.juntadeandalucia.es/medioambiente/web>) can be the cause of this contamination. On the other hand, the presence of nicotine, caffeine and cotinine in Argamasilla wetland maybe attributed to the fact that this wetland presents influence by other water bodies such as groundwater from a local aquifer, surface and subsurface runoff and the excess of irrigation (Andalucía wetland inventory, <http://www.juntadeandalucia.es/medioambiente/web>). However, no pharmaceuticals were detected in any of the wetlands, which suggests that the appearance of unexpected compound such as caffeine or nicotine could be associated to recreational use.

3.3. Reservoirs

Five reservoirs were monitored. In summary, the samples showed between 23 and 39 positives and among them between 5 and 8 were priority substances. From the data shown in Table 4, it can be firstly conclude that the Quiebrajano reservoir is the least polluted of the monitored reservoirs. This is consistent with its location relatively far with respect to the other sampling points and also because of the relatively scarce number of olive agricultural exploitations in its surrounding environment.

Secondly, according with the frequency of detection, it can be concluded that all the sampling sites (except Quiebrajano due to the reason above mentioned) presented a similar profile of compounds that are more frequently detected, these are fluorene, phenanthrene, pyrene (PAHs), terbuthylazine, chlorpyrifos ethyl, oxyfluorfen, hexachlorobenzene, diuron, simazine and desethyl terbuthylazine (pesticides), nicotine, caffeine and cotinine (LS), codeine (pharmaceutical). In general, the concentration of the pollutants is at the ng L^{-1} level, none of the priority substances exceeded the limits established by the Directive 2013/39/EU [3]. The results of the detected compounds for the five reservoirs are summarized in Table 4.

Table 4. Summary of the results for the compounds detected in the five reservoirs studied in this work.

Compound	Family	Method ana.	Gibraltar (9 samples)			Quetorjano (5 samples)			Ramblor (10 samples)			La Fernández (11 samples)			Gaditan (11 samples)		
			Freq (%)	Average (ng L ⁻¹)	Cmax (ng L ⁻¹)	Freq (%)	Average (ng L ⁻¹)	Cmax (ng L ⁻¹)	Freq (%)	Average (ng L ⁻¹)	Cmax (ng L ⁻¹)	Freq (%)	Average (ng L ⁻¹)	Cmax (ng L ⁻¹)	Freq (%)	Average (ng L ⁻¹)	Cmax (ng L ⁻¹)
Ephedrine	Drugs of abuse	LC-MS	22.2	1.4	1.7	--	--	--	--	--	--	--	--	--	--	--	--
Benzoyllecgonine	Drugs of abuse	LC-MS	22.2	8.9	10.4	--	--	--	--	--	--	--	--	--	--	--	--
Caffeine	LS	LC-MS	33.3	78.0	97.8	--	--	--	50.0	1061.4	4874.8	54.5	372.2	1794.8	63.6	83.5	155.8
Theobromine	LS (Caffeine metabolite)	LC-MS	44.4	107.1	177.5	40.0	75.7	117.6	20.0	189.4	189.4	27.3	683.9	1805.7	27.3	127.2	163.1
Theophylline	LS (Caffeine metabolite)	LC-MS	44.4	114.0	161.7	--	--	--	20.0	104.4	120.3	36.4	819.6	2688.4	45.5	118.0	166.8
Nicotine	LS	LC-MS	77.8	30.7	125.2	80.0	36.9	131	80.0	131	24.2	81.8	45.7	166.1	54.5	12.3	17.1
Cotinine	LS (Nicotine metabolite)	LC-MS	55.6	24.8	32.3	60.0	15.3	18.6	30.0	15.9	18.2	36.4	14.8	27.9	36.4	35.5	40.2
Acenaphthylene	PAH	GC-MS	22.2	4.3	8.4	40.0	0.2	0.3	20.0	9.2	18.3	27.3	12.1	23.8	27.3	11.5	25.0
Fluorene	PAH	GC-MS	88.9	1.5	6.0	100.0	1.0	1.3	100.0	1.4	3.2	100.0	1.0	2.5	100.0	1.2	2.7
Phenanthrene	PAH	GC-MS	88.9	8.8	17.5	80.0	6.8	16.3	100.0	7.9	13.2	100.0	6.6	19.3	100.0	7.0	18.1
Pyrene	PAH	GC-MS	100.0	7.1	23.3	100.0	4.5	8.3	100.0	8.3	22.8	100.0	14.2	109.0	100.0	9.7	45.3
Alachlor	Pesticides	GC-MS	11.1	3.4	3.4	--	--	--	--	--	--	--	--	--	--	--	--
Cyromazine	Pesticides	LC-MS	--	--	--	--	--	--	10.0	det	det	--	--	--	--	--	--
4,4'-DDE	Pesticides	GC-MS	66.7	2.1	4.6	--	--	--	10.0	0.5	0.5	9.1	0.6	0.6	27.3	1.0	1.4
4,4'-DDT	Pesticides	GC-MS	22.2	0.6	0.7	--	--	--	10.0	3.4	3.4	--	--	--	--	--	--
DEET	Pesticides	LC-MS	--	--	--	--	--	--	30.0	16.2	43.5	18.2	4.0	4.2	18.2	2.0	3.0
Deltamethrin	Pesticides	GC-MS	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Diazinon	Pesticides	GC-MS	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Disulfoton	Pesticides	LC-MS	33.3	8.4	11.8	--	--	--	--	--	--	--	--	--	--	--	--
Dimethoate	Pesticides	LC-MS	22.2	68.0	68.2	--	--	--	--	--	--	--	--	--	--	--	--
Diphenylamine	Pesticides	LC-MS	66.7	113.0	176.2	40.0	57.8	64.2	30.0	128.7	163.6	45.5	141.1	203.2	36.4	125.7	181.2
β -Endosulfan	Pesticides	GC-MS	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Fenhexamid	Pesticides	LC-MS	--	--	--	--	--	--	--	--	--	9.1	36.6	36.6	--	--	--
Fenitrothion	Pesticides	GC-MS	--	--	--	--	--	--	--	--	--	9.1	10.6	10.6	9.1	131.2	131.2
Fluomethuron	Pesticides	GC-MS	66.7	20.2	71.6	--	--	--	--	--	--	--	--	--	54.5	10.4	17.9
α -HCH	Pesticides	GC-MS	22.2	4.6	7.6	--	--	--	--	--	--	--	--	--	--	--	--
β -HCH	Pesticides	GC-MS	--	--	--	--	--	--	10.0	1.7	1.7	27.3	2.1	2.7	27.3	1.9	2.4
δ -HCH	Pesticides	GC-MS	--	--	--	--	--	--	10.0	4.5	4.5	--	--	--	--	--	--
Inazaphen	Pesticides	LC-MS	11.1	1.3	1.3	--	--	--	--	--	--	--	--	--	--	--	--
Methoxychlor	Pesticides	GC-MS	11.1	9.9	9.9	20.0	0.7	0.7	10.0	0.5	0.5	9.1	15.4	15.4	--	--	--
NNDP	Pesticides	LC-MS	11.1	122.8	122.8	--	--	--	--	--	--	--	--	--	9.1	68.0	68.0
Oxyfluorfen	Pesticides	GC-MS	66.7	26.9	79.1	20.0	5.1	5.1	70.0	4.1	12.3	54.5	2.8	6.1	72.7	30.2	91.5
Procymidone	Pesticides	GC-MS	11.1	0.9	0.9	20.0	0.6	0.6	--	--	--	9.1	1.3	1.3	--	--	--
Prometon	Pesticides	LC-MS	--	--	--	20.0	2731.1	2731.1	10.0	1409.9	1409.9	9.1	4805.5	4805.5	--	--	--
Propazine	Pesticides	LC-MS	--	--	--	20.0	48.8	48.8	--	--	--	9.1	79.1	79.1	--	--	--
Tebuconazole	Pesticides	LC-MS	44.4	4.6	10.7	--	--	--	10.0	det	det	--	--	--	27.3	2.2	5.0
Tebuhyalazine	Pesticides	LC-MS	100.0	227.9	894.0	80.0	2.6	6.3	90.0	13.0	40.7	100.0	30.9	89.2	100.0	53.3	147.0
Tebuhyalazine	Pesticides	LC-MS	11.1	5.3	5.3	--	--	--	--	--	--	--	--	--	9.1	0.9	0.9
Desethyl	Tebuhyalazine metabolite	LC-MS	88.9	78.6	175.6	20.0	16.1	16.1	--	--	--	90.9	21.8	44.5	100.0	33.3	108.6
Atenolol	Pharmaceuticals	LC-MS	11.1	9.1	9.1	20.0	1.2	1.2	10.0	6.2	6.2	9.1	3.3	3.3	9.1	5.0	5.0
Codine	Pharmaceuticals	LC-MS	44.4	35.9	71.9	20.0	18.2	18.2	60.0	18.2	37.2	45.5	25.0	71.6	54.5	25.5	54.0
Clenbuterol	Pharmaceuticals	LC-MS	11.1	13.9	13.9	20.0	35.8	35.8	--	--	--	9.1	28.6	28.6	--	--	--
Erythromycin	Pharmaceuticals	LC-MS	--	--	--	--	--	--	--	--	--	9.1	249.1	249.1	--	--	--
Lincosynin	Pharmaceuticals	LC-MS	--	--	--	--	--	--	10.0	13.4	13.4	27.3	5.6	6.9	36.4	8.6	15.9
Paracetamol	Pharmaceuticals	LC-MS	--	--	--	--	--	--	10.0	72.7	72.7	9.1	402.7	402.7	--	--	--
TEP	Several uses	LC-MS	11.1	86.1	86.1	--	--	--	--	--	--	9.1	173.0	173.0	--	--	--
Anthracene	PAH priority	GC-MS	--	--	--	20.0	11.9	11.9	10.0	119.0	119.0	--	--	--	--	--	--
1,2,4-TCB	Pesticides priority	GC-MS	33.3	0.9	1.3	20.0	0.7	0.7	20.0	1.0	1.1	18.2	1.7	2.6	27.3	0.9	1.1
Chlorofenox B	Pesticides priority	GC-MS	22.2	1.3	1.7	--	--	--	50.0	1.0	1.6	18.2	0.5	0.6	18.2	1.5	1.7
Chlorpyrifos ethyl	Pesticides priority	GC-MS	100.0	13.6	66.9	80.0	6.5	12.5	90.0	10.2	39.5	90.9	6.0	15.5	90.9	8.4	36.3
Duron	Pesticides priority	GC-MS	100.0	7.8	15.3	40.0	4.1	7.3	40.0	1.7	2.7	54.5	1.5	1.3	81.8	4.2	11.7
Hexachlorobenzene	Pesticides priority	GC-MS	44.4	8.8	20.3	40.0	2.4	4.2	70.0	11.6	26.4	45.5	9.6	26.8	45.5	12.0	36.8
Hexachlorobutadiene	Pesticides priority	GC-MS	--	--	--	--	--	--	10.0	0.6	0.6	9.1	0.5	0.5	9.1	0.9	0.9
Pentachlorobenzene	Pesticides priority	GC-MS	33.3	2.4	5.4	--	--	--	20.0	3.2	4.3	9.1	2.7	2.7	9.1	2.8	2.8

Regarding single compounds with the highest concentration, it was interestingly observed that prometon was detected only one time but at relative high concentrations in the same month (October 2009) in three different reservoirs Quiebrajano ($2.7 \mu\text{g L}^{-1}$), Rumblar ($1.4 \mu\text{g L}^{-1}$) and La Fernandina ($4.8 \mu\text{g L}^{-1}$). This herbicide belongs to triazines family which are usually applied to the olive groves in two periods (October-December and February-April) in order to eliminate undesired plants that hinder the harvesting of the crop (between November and February). Caffeine and its metabolites theobromine and theophylline were found at concentration of 1.7 , 1.8 and $2.7 \mu\text{g L}^{-1}$, respectively in La Fernandina reservoir during February 2010. Finally, in October 2010 a concentration of $4.9 \mu\text{g L}^{-1}$ of caffeine was detected in the Rumblar reservoir.

This profile of pesticides is typical of olive groves practices; therefore the contamination of the reservoirs could be associated to the agricultural activity in the zone. Moreover, the concentrations of pharmaceuticals and drugs were in most cases in the nanogram per liter level, relatively low to address them as environmentally relevant.

4. Conclusions

In this study, the pollution mainly due to the agricultural activity, other industries and wastewater discharges from Guadalquivir river and other related surface waters at the province of Jaén was evaluated. A LC-TOFMS accurate mass database along with a GC-MS/MS method were used to investigate the surface water quality at the province of Jaén. An approach based on the use of a database of exact masses of ions comprising up to 340 compounds was developed and implemented, using high resolution LC-MS with a time of flight mass spectrometer (LC-TOFMS). Additionally, a method for the analysis of 63 compounds by GC-MS/MS (with triple quadrupole) prior LLE was used, so the total number of monitored compounds was 373.

During the period between April 2009 and November 2010, a total of 83 samples of surface water were processed, comprising three rivers (Guadalquivir river and two of its tributary rivers, Guadalimar and Jandulilla), five reservoirs and eleven wetlands at the province of Jaén. During this period, several organic pollutants both priority and emerging were detected at

concentrations in the nanogram per liter range, except in some cases where the concentration was at the low microgram per liter level.

The concentrations of priority compounds were below the limits established by the Directive 2013/39/EU [3] in mostly all cases, except for one sample that exceeded the maximum allowable concentration of hexachlorobenzene in Guadalquivir river and for the concentration of chlorpyrifos in one sample of Guadalimar river. Therefore, it can be concluded that the quality of the surface waters is good, on the basis of the concentration levels of studied priority compounds, because only two samples presented concentration slightly over the limits established by the European WFD for hexachlorobenzene and chlorpyrifos-ethyl in the Guadalquivir and Guadalimar rivers, respectively.

The profile of pesticides found (terbuthylazine in all studied samples and the frequent detection of diuron, oxyfluorfen and simazine (priority compound)) is typical of olive grove practices. Therefore, the contamination of the waters can be associated mainly to the agricultural activity in the zone, clearly the driving economic force of the region rather than other minor industrial activity.

On the other hand, the concentrations of pharmaceuticals and drugs were in most cases of the nanograms per liter level, which rules out any potential harmful effect on the environment. This prevalence has been previously described in several rivers of Europe and the United States. The most frequently detected emerging (non-regulated) compounds in the surface waters analyzed were codeine, paracetamol, the alkaloids and caffeine metabolites theobromine and theophylline, and cocaine metabolite benzoylecgonine. Belonging to the PAHs family fluorene, phenanthrene, pyrene were the most frequently detected as well as terbuthylazine, chlorpyrifos ethyl, oxyfluorfen, hexachlorobenzene, diuron, simazine, desethyl terbuthylazine withing the pesticides groups. Their ubiquitous presence maybe linked to abundant use or inefficient removal from sewage treatment plants as it is the case of fluorene, phenanthrene and pyrene frequently detected in a previous report comprising wastewater samples located in different places of Spain [11]. Regarding pesticides, some studies [11,26,43,44,45] dealing with their determination in Spanish waters presented similar results

related to the high frequency of detection of chlorpyrifos ethyl, simazine, terbutylazine, desethyl terbutylazine, oxyfluorfen or diuron. In the case of caffeine, nicotine and their metabolites, the high frequency of detection could be associated to their extended use as “life-style” products. Finally, codeine was also frequently detected. This compound is included in the group of pharmaceuticals since is mostly used as a muscle relaxant, instead of as a drug of abuse because is highly inefficient and therefore scarcely used.

Many different water body types were analyzed in this survey: rivers, reservoirs and wetlands of different size and environment. Therefore, although the results of this study cannot be seen as a final statement of the water quality in the province of Jaén, the sample set can be actually considered to be somewhat representative of the surface waters in the region. Future studies will be convenient to further control the prevalence and evolution of the contaminants detected.

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IV.5.3. Material suplementario

Table S1. Analytical parameters of detected compounds obtained for both methodologies (LLE/GC-MS/MS and SPE/LC-TOF MS).

Name	Group	LOQ (ng L ⁻¹)	LOD (ng L ⁻¹)	Recovery rate (%)	RSD (%) (n=7)
Compounds analyzed by LLE followed by GC-MS/MS					
1,2,4-TCB*	Pesticides	0.6	0.2	88.1	9.8
4,4'-DDE	Pesticides	0.2	0.06	103.7	6.9
4,4'-DDT	Pesticides	0.09	0.03	93.8	9.3
Alachlor	Pesticides	2.7	0.8	93.0	12.0
Chlorfenvinfos* B	Pesticides	1.0	0.3	100.1	5.6
Chlorpyrifos ethyl*	Pesticides	0.5	0.15	104.6	16.3
Deltamethrin	Pesticides	2.8	0.8	32.0	22
Diazinon	Pesticides	0.03	0.01	111.9	5.2
Diuron*	Pesticides	0.3	0.1	54.0	12.5
β-Endosulfan	Pesticides	0.3	0.09	120.0	9.0
α-HCH	Pesticides	0.3	0.09	93.0	5.0
β-HCH	Pesticides	0.6	0.2	113.0	15.0
γ-HCH	Pesticides	0.5	0.1	93.6	12.9
δ-HCH	Pesticides	0.4	0.1	101.0	12.0
Hexachlorobenzene*	Pesticides	0.2	0.07	96.5	3.0
Hexachlorobutadiene*	Pesticides	0.3	0.09	51.9	15.2
Metoxychlor	Pesticides	0.3	0.09	94.1	1.4
Oxyfluorfen	Pesticides	0.1	0.03	112.3	7.3
Pentachlorobenzene*	Pesticides	0.03	0.01	105.3	7.1
Procymidone	Pesticides	3.0	0.9	84.0	9.0
Acenaphthylene	PAH	0.2	0.07	101.9	4.0
Anthracene*	PAH	1.0	0.3	84.0	8.0
Fluorene	PAH	0.2	0.05	79.3	12.0
Phenanthrene	PAH	0.9	0.3	108.1	5.7
Pyrene	PAH	0.9	0.3	70.8	13.4
Compounds analyzed by SPE followed by LC-TOFMS					
Benzoylcegonine	Cocaine metabolite	3.5	1.1	82.0	10.6
Codeine	Pharmaceuticals	4.0	1.2	60.0	8.3
Ephedrine	Drugs of abuse	1.0	0.3	58.0	9.3
Phenylephrine	Drugs of abuse	5.0	1.5	46.0	15.2
Antipyrine	Pharmaceuticals	6.9	2.1	75.2	7.0
Atenolol	Pharmaceuticals	5.0	1.5	85.5	5.7
Caffeine	Life-Style	0.5	0.2	80.0	9.1
Clenbuterol	Pharmaceuticals	13.0	4.0	76.2	6.7
Cotinine	Nicotine metab.(LS)	13.0	4.0	90.0	7.8
Erythromycin	Pharmaceuticals	20.0	6.0	40.9	15.1
Lyncomycin	Pharmaceuticals	3.0	0.9	67.1	6.4
Nicotine	Life-Style	5.0	1.5	41.0	15.6
Paracetamol	Pharmaceuticals	6.0	1.8	69.6	4.1
Theobromine	Caffeine metabolite (LS)	40.0	12.0	116.0	5.7
Theophylline	Caffeine metabolite (LS)	20.0	6.0	91.0	7.1
Trimethoprim	Pharmaceuticals	5.7	1.7	64.0	6.5
Atrazine*	Pesticides	1.0	0.3	78.9	6.1
Cyromazine	Pesticides	7.9	2.4	61.5	5.4
DEET	Pesticides	0.3	0.09	73.6	1.7
Desethyl terbutylazine	Terbutylazine metabolite	1.1	0.3	83.8	5.0
Diflufenican	Pesticides	2.2	0.7	52.4	6.4

Dimethoate	Pesticides	25.4	7.7	84.2	6.8
Diphenylamine	Pesticides	3.8	1.2	41.7	8.6
Fenhexamid	Pesticides	10.1	3.1	80.2	4.6
Fluomethuron	Pesticides	3.5	1.1	77.5	7.9
Imazaquin	Pesticides	1.1	0.3	86.3	5.4
NNDP ¹	Pesticides	4.3	1.3	85.9	4.1
Prometon	Pesticides	1.1	0.3	96.4	2.8
Propazine	Pesticides	2.0	0.6	73.2	5.1
Simazine	Pesticides	0.5	0.2	75.0	7.8
Tebuconazole	Pesticides	2.2	0.7	82.6	8.8
Terbutylazine	Pesticides	2.0	0.6	70.5	6.1
Terbutryn	Pesticides	1.3	0.4	72.0	5.9
TEP	Several uses	4.0	1.2	68.0	14.3

* Priority compounds

¹ NNDP = N,N-diethyl-2-(1-naphthylxy)propamide

Table S2. Priority compounds found in Guadalimar river samples (n=8). Concentration levels are compared to Environmental Quality Standards established by Directive 2013/39/EU.

Priority compound	% positive samples	Concentration range (ng L ⁻¹)	AA (ng L ⁻¹)	AA-EQS (ng L ⁻¹)	MC (ng L ⁻¹)	MAC-EQS (ng L ⁻¹)
1,2,4-TCB	25.0	<LOQ	<LOQ	400.0	<LOQ	not applicable
Hexachlorobutadiene	12.5	0.3	0.3	-	0.3	600.0
Diuron	100.0	2.1 - 65.9	17.2	200.0	65.9	1800.0
Chlorpyrifos ethyl	100.0	1.0 - 119.0	21.1	30.0	119.0	100.0
Chlorfenvinphos	75.0	<LOQ - 23.8	23.8	100.0	23.8	300.0
Simazine	100.0	<LOQ - 31.3	17.5	1000.0	31.3	4000.0
Pentachlorobenzene	37.5	1.2 - 11.9	5.4	7.0	11.9	not applicable
Hexachlorobenzene	37.5	4.1 - 43.7	18.6	-	43.7	50.0

MC: Maximum concentration found in studied samples

AA-EQS: Environmental Quality Standard established for the annual average concentration of the corresponding contaminant in inland surface waters. Directive 2013/39/EU.

MAC-EQS: Environmental Quality Standard established for the maximum allowable concentration of the corresponding contaminant in inland surface waters. Directive 2013/39/EU.

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V. CONCLUSIONES

V. CONCLUSIONES

La conclusión general que se puede extraer de la presente tesis es que se ha demostrado la potencialidad de los analizadores de masas en tándem (MS/MS) y de alta resolución (HRMS) para realizar la detección, identificación y cuantificación de un número elevado de contaminantes orgánicos en muestras medioambientales. Gracias al acoplamiento con GC y LC se ha podido cubrir un amplio rango de compuestos con características físico-químicas distintas, sobre todo en lo que respecta a la volatilidad y polaridad. Por un lado, se abordó por primera vez la evaluación del acoplamiento GC-HRMS con analizador de doble sector magnético para el análisis de sustancias prioritarias en muestras de agua residual, obteniéndose límites de cuantificación que permitían cumplir sin problemas con la normativa vigente. Por otro lado el acoplamiento GC-QqQMS, se ha podido aplicar al análisis de un mayor número de contaminantes prioritarios alcanzando niveles de detección, en la mayoría de los casos, muy por debajo de las normas de calidad establecidas por las autoridades europeas, todo ello previa evaluación y validación de 3 tratamientos de muestra distintos (LLE, SPE y HS-SPME), lo que permite escoger el más apropiado en función de los analitos a determinar. Por último en cuanto al acoplamiento LC-TOFMS se puede concluir que es una potente herramienta cuando se quiere abordar el monitoreo de un elevado número de compuestos, ofreciendo valiosa información estructural y de composición elemental mediante medidas de masa exacta, además puede realizar en teoría el análisis de un número ilimitado de analitos por su modo de trabajo en full-scan y permite el análisis retrospectivo. Por lo que su uso en la actualidad es esencial ya que cada vez es mayor el número de químicos que se introducen en el medioambiente y que hay que tener bajo control.

A continuación se pasan a describir las conclusiones específicas:

1-Se desarrolló y validó por primera vez un método basado en la extracción LLE seguida del análisis por GC-HRMS con analizador de doble sector magnético, para un grupo de 14 contaminantes prioritarios en muestras de agua residual. Aunque los límites de detección alcanzados cumplen con holgura los establecidos por la normativa europea, este acoplamiento presenta la desventaja de una baja velocidad de scan con respecto a otros analizadores, como

el triple cuadrupolo, por lo que el número de compuestos que puede analizar es limitado. No obstante esta metodología se empleó con éxito para la caracterización de muestras de efluentes de agua residual procedentes de 5 estaciones depuradoras españolas distintas.

2-Se desarrolló una metodología basada en LLE seguida de GC-QqQMS para el análisis de 57 contaminantes prioritarios (PAHs, plaguicidas y productos de transformación) en muestras de agua residual. Los resultados de validación fueron satisfactorios, obteniéndose valores de recuperación entre 70 y 110 % para la mayoría de compuestos y límites de detección que cumplían con creces los límites establecidos por la Directiva 2008/105/CE. Aunque el triple cuadrupolo presenta la desventaja de que no se puede utilizar para llevar a cabo análisis retrospectivos en su modalidad de trabajo full-scan (baja resolución), sí que es uno de los mejores analizadores disponibles para llevar a cabo la cuantificación de compuestos seleccionados de una forma robusta, sensible y selectiva, minimizando el efecto matriz en su modo de trabajo MRM.

3-Se llevó a cabo la evaluación de tres tratamientos de muestra distintos como son la LLE, SPE y HS-SPME para el análisis de sustancias prioritarias por GC-QqQMS en aguas residuales. Se obtuvieron similares resultados (valores de recuperación y LODs) para SPE y LLE, sin embargo ésta última aunque necesita de la utilización de una mayor cantidad de disolvente, permite la extracción de los contaminantes adsorbidos en la materia particulada de la muestra, ya que no es necesaria una filtración previa como ocurre con la SPE. Por otro lado la HS-SPME se presenta como una alternativa medioambientalmente más respetuosa ya que requiere la utilización de una cantidad mínima de disolvente, pero no logra una cobertura tan amplia de compuestos analizables como la alcanzada con LLE y SPE.

4-Se desarrolló una metodología basada en SPE seguida de LC-TOFMS para la detección y cuantificación de alrededor de 400 contaminantes prioritarios y emergentes en agua residual. Para ello se utilizó el potencial del analizador de tiempo vuelo, que permite trabajar con bases de datos creadas experimentalmente y que contenía los valores de masa exacta, tiempo de retención, de los analitos seleccionados así como de algunos de sus aductos y fragmentos típicos. En esta modalidad de trabajo se consigue en apenas unos segundos el barrido de todas las masas

seleccionadas en una muestra real. Hasta donde llega nuestro conocimiento, es la primera vez que se valida un tratamiento de muestra genérico para un número tan elevado de compuestos con un analizador de alta resolución.

5-Se llevó a cabo de forma satisfactoria el monitoreo de 373 contaminantes prioritarios y emergentes en muestras de agua superficial recogidas en la provincia de Jaén. Para ello se aplicaron dos de las metodologías previamente desarrolladas como son LLE seguida de GC-QqQ y SPE seguida de LC-TOFMS. Con lo que se consiguió abarcar un número importante de contaminantes con distintas características físico-químicas. Los resultados recogieron un perfil de contaminantes propios de la actividad agrícola única en el mundo que se realiza en esta provincia, y en menor medida propios de otras actividades industriales y contribuciones de contaminación procedente de vertidos de agua residual. Así los plaguicidas son los contaminantes que contribuyen en mayor medida a la contaminación de las aguas superficiales jienenses, seguida de fármacos y productos mundialmente consumidos como la cafeína y la nicotina. Por último, en la gran mayoría de los casos los niveles de concentración de los contaminantes prioritarios detectados estaban por debajo de lo establecido por las norma de calidad europeas (Directiva 2013/39EU).

A faint, artistic watercolor map of Europe is centered on the page. The map uses soft, blended colors including shades of blue, green, yellow, and red to represent different landmasses and regions. The style is painterly and abstract, with visible brushstrokes and a soft, ethereal quality. It is positioned behind a solid blue horizontal band that contains the section header.

V.CONCLUSIONS

V. CONCLUSIONS

The main conclusion derived from the different studies presented in this Doctoral Thesis is the great potential suitability of both high resolution mass spectrometry and tandem mass spectrometry for the determination (detection, identification and quantification) of a large number of organic contaminants in environmental samples. LC-HRMS analyzers has been demonstrated as a powerful analytical tool for the development of methods based on accurate-mass databases which allow the qualitative and quantitative measurement of an outstanding number of compounds in a single run combining accurate mass measurements and retention time data. On the other hand, gas chromatography/triple quadrupole mass spectrometry is highly appropriate for the quantitative determination and confirmation of nonpolar organic contaminants in environmental samples. Thanks to the combined use of both GC-MS and LC-MS, a wide range of compounds with different physicochemical properties has been covered using different standard sample treatments (LLE, SPE and SPME). In general, the limits of detection exhibited for the vast majority of priority contaminants are in compliance with current European Union standards.

The specific conclusions derived from the five research articles presented in this Thesis are detailed below:

1- The use of GC-HRMS (with double focusing magnetic sector mass analyzer) is a powerful analytical tool to monitor priority organic pollutants (2455/2001/CE Directive) in complex wastewater matrices at levels as low as a few pictograms per liter. In contrast, the number of species that could be analyzed (only 14 with the present method) in a run is distinctly lower than GC-MS/MS with triple quadrupole mass analyzer. The developed method was applied to the characterization of effluents from various STPs. The concentration levels found are in most cases below the maximum concentration permitted for these species in water samples intended for human consumption.

2- The use of GC-MS/MS permits the development of multi-residue methods intended for the monitoring of up to 100 priority contaminants. A method based on liquid-liquid extraction (LLE) with hexane, followed by GC-MS/MS was developed for the detection of 57 priority

organic contaminants in wastewater samples. The proposed method, which comprises the analysis of both liquid phase and particulate matter of wastewater samples -as required by the European WFD-, displayed excellent selectivity and sensitivity, enabling the unambiguous identification of the target species by the determination of three MRM transitions (when available). A satisfactory performance was achieved for relatively polar compounds such as diuron, isoproturon, chlortoluron, or desethyl atrazine, whose analyses fit better with LC-MS. In contrast, only two transitions were selected for PAHs due to their difficulty to yield useful fragmentation. Most of PAHs were quantified using, respectively, the same ion as precursor and as product ion. In particular, the identification of isomers phenanthrene-anthracene, and benzo[b]fluoranthene-benzo[k]fluoranthene, which used the same MS/MS transitions, relied on the chromatographic separation. Finally, the method was applied to a monitoring study of these organic contaminants in wastewater samples, with a high percentage of positives, showing the ubiquitous presence of these compounds, which emphasizes the need to control them.

3- Three sample treatment methodologies were evaluated and compared for analysis by GC-MS/MS of organic pollutants included in the list of priority substances of WFD: SPE, LLE and HS-SPME. From the results obtained both SPE and LLE were found more suitable the targeted application. LLE with n-hexane and SPE using with C₁₈ cartridges and ethyl acetate:dichloromethane (1:1 (v/v)) as eluent showed recoveries in the range 70-120% for the majority of the studied compounds. HS-SPME showed poor performance for 14 out of the 57 compounds tested. Linearity and precision were evaluated, obtaining satisfactory results for all the three sample treatment methodologies tested. The limits of quantitation obtained for most of the target compounds were in the low nanogram per litre range, using any of the three procedures tested. LLE and SPE were revealed as the most sensitive methods, but SPE is less time consuming and more environmentally friendly than LLE in terms of solvent consumption, although species that may be retained in suspended solids would be out of the scope of the method. Nevertheless, it has been demonstrated that depending on the chemical class, the treatment sample process of choice would be either SPE or LLE.

4- An analytical methodology using LC-TOFMS was developed for the ultratrace detection and quantification of over 400 priority and emerging organic pollutants. It was

successfully applied to both surface water and wastewater. Accurate mass measurements combined with retention time information and fragmentation -when required- were used for appropriate analyte detection and identification by means of an automated and straightforward procedure with available software. It should be emphasized that, unlike in previous related studies, in the present study, sample preparation step performance (recovery rates) was thoroughly evaluated along with the analytical features and matrix effects experimental data provided for all the 430 tested compounds. The overall performance of the method was satisfactory for most of the compounds targeted. Limits of quantification varied from compound to compound, but were in the low nanogram per litre range ($< 100 \text{ ng L}^{-1}$) for 372 of the studied compounds. The use of SPE with Oasis HLB permitted appropriate recovery rates in most cases, which were higher than 30 % for 84 % of the compounds. In addition, the use of such generic sample treatment combined with sensitive full-scan acquisition allows performing both non-target and retrospective analysis of the samples.

5- The developed GC-MS/MS and LC-TOFMS approaches were satisfactorily applied to the monitoring of 373 priority and emerging contaminants in the Guadalquivir river and other surface waters in the province of Jaén. For this purpose, the previous developed methodologies, LLE followed by GC-QqQMS analysis and SPE followed by LC-TOFMS were applied, which allowed studying a large number of compounds with different physicochemical properties. A total of 83 river, reservoir and wetland samples were collected and analyzed. Results showed that the main contribution to the surface water pollution came from pesticides and life-style compounds such as caffeine and nicotine which is consistent with the olive grove practices carried out in this province and with the wastewater discharges from WWTP. In general, the concentration of the contaminants is in the low ng L^{-1} level which minimizes the possible harmful effect on the environment. Besides, the concentration of most of the priority contaminants was below the limit established by the Directive 2013/39/EU.



VI. CONTRIBUCIONES CIENTÍFICAS

ARTÍCULOS CIENTÍFICOS QUE COMPONEN LA TESIS DOCTORAL

Artículo científico 1

Determination of organic priority pollutants in sewage treatment plant effluents by gas chromatography high-resolution mass spectrometry

José Robles-Molina, Bienvenida Gilbert-López, Juan F. García-Reyes, Antonio Molina-Díaz.

Talanta 80 (2010) 1318-1324

Artículo científico 2

Gas chromatography triple quadrupole mass spectrometry method for monitoring multiclass organic pollutants in Spanish sewage treatment plants effluents.

José Robles-Molina, Bienvenida Gilbert-López, Juan F. García-Reyes, Antonio Molina-Díaz.

Talanta 111 (2013) 196-205

Artículo científico 3

Comparative evaluation of liquid–liquid extraction, solid-phase extraction and solid-phase microextraction for the gas chromatography–mass spectrometry determination of multiclass priority organic contaminants in wastewater

José Robles-Molina, Bienvenida Gilbert-López, Juan F. García-Reyes, Antonio Molina-Díaz.

Talanta 117 (2013) 382-391

Artículo científico 4

Multi-residue method for the determination of over 400 priority and emerging pollutants in water and wastewater by solid-phase extraction and liquid chromatography-time-of-flight mass spectrometry

José Robles-Molina, Felipe J. Lara-Ortega, Bienvenida Gilbert-López, Juan F. García-Reyes, Antonio Molina-Díaz.

Journal of Chromatography A (aceptado 01/05/2014). DOI: 10.1016/j.chroma.2014.05.003

Artículo científico 5

Monitoring of selected priority and emerging contaminants in the Guadalquivir River and other related surface waters in the province of Jaén, South East Spain

José Robles-Molina, Bienvenida Gilbert-López, Juan F. García-Reyes, Antonio Molina-Díaz.

Science of the Total Environment 479-448 (2014) 247-257

OTRAS CONTRIBUCIONES CIENTÍFICAS RELACIONADAS CON LA TESIS DOCTORAL

Además de los artículos científicos descritos en la presente tesis, se han realizado otras aportaciones científicas relacionadas con la misma.

Dentro de éstas se encuentran 4 artículos científicos:

- Screening of emerging contaminants and priority substances (2008/105/EC) in reclaimed water for irrigation and groundwater in a volcanic aquifer (Gran Canaria, Canary Islands, Spain).

Esmeralda Estévez, María del Carmen Cabrera, Antonio Molina-Díaz, José Robles-Molina, María del Pino Palacios-Díaz

Science of the Total Environment 433 (2012) 538-546

- Removal of sulfamethoxazole from waters and wastewaters by conductive diamond electrochemical oxidation

De Vidales, Maria Jose; Robles-Molina, Jose; Domínguez-Romero, Juan Carlos; Cañizares-cañizares, Pablo; Sáez-jiménez, Cristina; Molina-Díaz, Antonio; Rodrigo-Rodrigo, Manuel Andrés

Journal of chemical technology and biotechnology 87 (2012) 1441-1449

- Conductive-diamond electrochemical oxidation of chlorpyrifos in wastewater and identification of its main degradation products by LC–TOFMS

José Robles-Molina, María J. Martín de Vidales, Juan F. García-Reyes, Pablo Cañizares, Cristina Sáez, Manuel A. Rodrigo, Antonio Molina-Díaz

Chemosphere 89 (2012) 1169–1176

- Degradation of caffeine by conductive diamond electrochemical oxidation

Chloe Indermuhle, María J. Martín de Vidales, Cristina Sáez, José Robles-Molina, Pablo Cañizares, Juan F. García-Reyes, Antonio Molina-Díaz, Christos Comninellis, Manuel A. Rodrigo

Chemosphere 93 (2013) 1720-1725

Por otro lado se han presentado 24 comunicaciones a congresos de distinto ámbito:

10 comunicaciones a congresos internacionales:

- Use of accurate mass databases of molecules and diagnostic ions for LC/MS-based targeted and untargeted screening of emerging contaminants in the environment.

García-Reyes, Juan Francisco; Robles-Molina, Jose; Domínguez-Romero, Juan Carlos; Gilbert-López, Bienvenida; Molina-Díaz, Antonio; Agüera-López, Ana; Gómez-Ramos, María Del Mar; Mezcuá-Peral, Milagros; Rodríguez-Fernández-Alba, Amadeo

58 th ASMS Conference on Mass Spectrometry and Allied Topics

- Identification of transformation products of organic pollutants in water using liquid chromatography electrospray time-of-flight mass spectrometry

Domínguez-Romero, Juan Carlos; Robles-Molina, Jose; Gilbert-López, Bienvenida; García-Reyes, Juan Francisco; Ramos-Martos, Natividad; Molina-Díaz, Antonio

ANQUE international congress (VII)

- Development of data processing workflows for the automated detection of transformation products of emerging contaminants in the environment

Agüera-López, Ana; Gómez-Ramos, María Del Mar; Rodríguez-Fernández-Alba, Amadeo; Robles-Molina, Jose; Domínguez-Romero, Juan Carlos; Gilbert-López, Bienvenida; García-Reyes, Juan Francisco; Molina-Díaz, Antonio

ANQUE international congress (VII)

- Development of an accurate-mass database for the screening of emerging contaminants in wastewater samples using LC-TOFMS

Robles-Molina, Jose; Domínguez-Romero, Juan Carlos; Gilbert-López, Bienvenida; García-Reyes, Juan Francisco; Molina-Díaz, Antonio

ANQUE international congress (VII)

- Evaluation of different sample treatment methodologies for the determination of priority organic pollutants in effluent wastewater samples

Robles-Molina, Jose; Gilbert-López, Bienvenida; García-Reyes, Juan Francisco; Molina-Díaz, Antonio

ANQUE international congress (VII)

- Development of data processing workflows for the automated detection of transformation products of emerging contaminants in the environment

García-Reyes, Juan Francisco; Pérez-Parada-,Andrés; Gómez-Ramos, María Del Mar; Rodríguez-Fernández-Alba, Amadeo; Robles-Molina, Jose; Domínguez-Romero, Juan Carlos; Gilbert-López, Bienvenida; Molina-Díaz, Antonio; Agüera-López, Ana

28th International Symposium on Chromatography

- Use of Accurate-Mass databases of molecules and diagnostic ions for LC/MS-Based targeted and untargeted screening of emerging contaminants in environment

García-Reyes, Juan Francisco; Robles-Molina, Jose; Domínguez-moreno, Juan ; Gilbert-López, Bienvenida; Molina-Díaz, Antonio; Agüera-López, Ana; Gómez-Ramos, María José; Gómez-Ramos, María Del Mar; Mezcua-Peral, Milagros; Rodríguez-Fernández-Alba, Amadeo

58 th ASMS Conference on Mass Spectrometry and Allied Topics

- Multi-residue screening of emerging pollutants in effluent wastewater based on accurate mass databases with LC-TOFMS

Robles-Molina, Jose; Gilevska, T; Gilbert-López, Bienvenida; Domínguez-Romero, Juan Carlos; García-Reyes, Juan Francisco; Molina-Díaz, Antonio

34th International Symposium on High Performance Liquid Phase Separations and Related Techniques

- Multiclass Determination of 60 Organic Pollutants in Wastewater Samples by Gas Chromatography Triple Quadrupole Spectrometry

Molina-Díaz, Antonio; Robles-Molina, Jose; Gilbert-López, Bienvenida; García-Reyes, Juan Francisco

PITTCON 2009

- Temporal evolution of emerging contaminants, priority substances and heavy metals most frequently detected in reclaimed water and groundwater in a volcanic aquifer

E. Estévez, M.C. Cabrera, J.R. Fernández-Vera, A. Molina-Díaz, J.R. Robles-Molina, M.P. Palacios-Díaz

XIII International Conference on Environmental Science and Technology

7 contribuciones a congresos nacionales:

- Monitoring of priority and emerging contaminants in effluent wastewaters from spanish wastewater treatment plants

Robles-Molina, Jose; Gilbert-López, Bienvenida; Domínguez-Romero, Juan Carlos; García-Reyes, Juan Francisco; Gómez-Ramos, María José; Agüera-López, Ana; Rodríguez-Fernández-Alba, Amadeo; Molina-Díaz, Antonio

XV Reunión de la Sociedad Española de Química Analítica (XV SEQA)

- Electrochemical oxidation of chlorpyrifos in water samples: transformation products assessment by LC-TOF-MS

Robles-Molina, Jose; García-Reyes, Juan Francisco; Molina-Díaz, Antonio; Martin De Vidales-calvo, Maria Jose; Saez-jimenez, Cristina

V Reunión Sociedad Española de Espectrometría de Masas

- Multiclass determination of 60 organic pollutants in wastewater samples by gas chromatography triple quadrupole mass spectrometry

Molina-Díaz, Antonio; Robles-Molina, Jose; Gilbert-López, Bienvenida; García-Reyes, Juan Francisco

XV Reunión de la Sociedad Española de Química Analítica (XV SEQA)

- Evaluation of fragmentation coverage of in-source CID fragmentation in single-stage MS compared to dedicated CID MS/MS fragmentation for small-molecule applications: a case study of multiclass emerging contaminants

Juan F. García-Reyes, José Robles-Molina, Antonio Molina-Díaz, Andrés Pérez-Parada, M.M. Gómez-Ramos, Ana Agüera, Amadeo R. Fernández-Alba

XVIII Reunión SEQA

- Removal of sulfamethoxazole from waters and wastewater by conductive-diamond electrochemical oxidation

J. Robles-Molina, M.J. Martín de Vidales, J.C. Domínguez-Romero, P. Cañizares, Cristina Sáez, A. Molina-Díaz, M.A. Rodrigo

XIII Jornadas de Análisis Instrumental (JAI)

- Resultados preliminares de los análisis de contaminantes emergentes y sustancias prioritarias (2008/105/CE) en la red de control del campo de golf de Bandama

Estévez, E, Cabrera, MC, Molina-Díaz, A, Robles, J y Palacios-Díaz, MP

VIII Jornadas de investigación de la zona no saturada del suelo

- Evaluación de la afección al acuífero del NE de Gran Canaria por el riego con aguas regeneradas en un campo de golf

Esmeralda Estévez, M Pino Palacios, Tatiana Cruz, Annetty Benavides, M Davinia Rodríguez-Díaz, Juan Ramón Fernández-Vera, Antonio Molina-Díaz, José Robles-Molina y M Carmen Cabrera

Congreso ibérico sobre las aguas subterráneas: desafíos de la gestión para el siglo XXI

7 contribuciones a congresos de ámbito regional:

- Respuesta hidrodinámica e hidroquímica del acuífero de la Vega de Granada en el entorno de una chopera regada con aguas residuales urbanas depuradas

Robles-Arenas, Virginia María; Rojas-Hita, Diego; Benedetti, Iacopo; Sánchez-Díaz, Luis Manuel; Molina-Díaz, Antonio; Robles-Molina, José; García-Reyes, Juan Francisco; Castillo-Martin, Antonio; Benavente-Herrera, José

VIII Simposio del Agua en Andalucía

- Degradación electroquímica de sulfametoxazol y cafeína e identificación de los principales intermedios mediante LC-TOFMS

Molina-Díaz, Antonio; Robles-Molina, José; Martín -de Vidales, María José ; Cañizares, Pablo; Sáez, Cristina; Rodrigo, Manuel A

XIII Reunión GRASEQA

- Desarrollo de un método multiresiduo para la detección de contaminantes orgánicos prioritarios y emergentes en aguas superficiales mediante LC-TOFMS

Robles-Molina, José; García-Reyes, Juan Francisco; Molina-Díaz, Antonio

XIII Reunión GRASEQA

- Desarrollo de flujos de trabajo automatizados de procesamiento de datos para la detección de productos de transformación de contaminantes orgánicos en muestras medioambientales

Agüera-López, Ana; Gómez-Ramos, María Del Mar; Robles-Molina, Jose; Domínguez-Romero, Juan Carlos; Gilbert-López, Bienvenida; García-Reyes, Juan Francisco; Molina-Díaz, Antonio; Rodríguez-Fernández-Alba, Amadeo

XII Reunión GRASEQA

- Desarrollo de una base de datos de masas exactas de iones para la detección automatizada de contaminantes orgánicos emergentes en muestras medioambientales

Robles-Molina, Jose; Domínguez-Romero, Juan Carlos; García-Reyes, Juan Francisco; Gilbert-López, Bienvenida; Molina-Díaz, Antonio

XII Reunión GRASEQA

- Evaluación del contenido de microcontaminantes en efluentes de estaciones depuradoras de aguas residuales mediante espectrometría de masas avanzada

Robles-Molina, Jose; Gilbert-López, Bienvenida; García-Reyes, Juan Francisco; Llorent-Martínez, Eulogio José; Ortega-Barrales, María Del Pilar; Molina-Díaz, Antonio

Símpoio del Agua en Andalucía (SIAGA)

- Monitorización de contaminantes prioritarios en EDARS: metodologías analíticas basadas en espectrometría de masas avanzada

Robles-Molina, Jose; Gilbert-López, Bienvenida; García-Reyes, Juan Francisco; Llorent-Martínez, Eulogio José; Ortega-Barrales, María Del Pilar; Molina-Díaz, Antonio

XI Reunión GRASEQA

Por último, se participó en la creación de un protocolo para el análisis de contaminantes emergentes y prioritarios:

Título: Protocolo de técnicas de muestreo y técnicas analíticas de contaminantes emergentes y prioritarios

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