Drogas y psicofármacos: estudio de su presencia en el medio ambiente (aguas residuales, sedimentos y mejillones) y evaluación de su consumo e impacto

Ester López García

DROGAS Y PSICOFÁRMACOS: ESTUDIO DE SU PRESENCIA EN EL MEDIO AMBIENTE (AGUAS RESIDUALES, SEDIMENTOS Y MEJILLONES) Y EVALUACIÓN DE SU CONSUMO E IMPACTO

ESTER LÓPEZ GARCÍA

UNIVERSITAT DE BARCELONA

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

2020

Tesis Doctoral

Ester López García

Programa de doctorado
"Química Analítica i Medi Ambient"

DROGAS Y PSICOFÁRMACOS: ESTUDIO DE SU PRESENCIA EN EL MEDIO AMBIENTE (AGUAS RESIDUALES, SEDIMENTOS Y MEJILLONES) Y EVALUACIÓN DE SU CONSUMO E IMPACTO

Memoria presentada para obtener el título de
Doctora por la Universidad de Barcelona

Ester López García

Directora	Co-Directora	Tutora
Dra. Miren López de Alda Villaizán	Dra. Cristina Postigo Rebollo	Dra. Mercè Granados Juan
Investigador Científico Dep. Química Ambiental IDAEA-CSIC	Investigador PhD ComFuturo Dep. Química Ambiental IDAEA-CSIC	Profesor Titular Dep. Enginyeria Química i Química Analítica Universitat de Barcelona

Barcelona, noviembre de 2020

La Dra. **Miren López de Alda Villaizán**, Investigador Científico, y la Dra. **Cristina Postigo Rebollo**, Investigadora PhDComFuturo, del Instituto de Diagnóstico Ambiental y Estudios del Agua, IDAEA, perteneciente al Consejo Superior de Investigaciones Científicas, CSIC,

Certifican:

Que la presente memoria presentada para optar al título de Doctor, titulada "***Drogas y psicofármacos: estudio de su presencia en el medio ambiente (aguas residuales, sedimentos y mejillones) y evaluación de su consumo e impacto***" ha sido realizada bajo nuestra dirección por la Sra. **Ester López García** en el Instituto de Diagnóstico Ambiental y Estudios del Agua (IDAEA), perteneciente al Consejo Superior de Investigaciones Científicas (CSIC), y que todos los resultados presentados son fruto del trabajo experimental realizado por el mencionado doctorando.

Barcelona, 26 de noviembre de 2020

Dra. Miren López de Alda Villaizán

Dra. Cristina Postigo Rebollo

Esta tesis se ha realizado con la ayuda financiera otorgada por la UE a través del proyecto SOLUTIONS (FP7; No. 603437), el Ministerio de Economía y Competitividad de España (MINECO) a través del proyecto SCARCE (Consolider-Ingenio 2010 CSD2009-00065), la Agencia Estatal de Investigación (AEI) (CTM2016-81935-REDT), y la Generalitat de Catalunya (número de expediente SA-2017-1170, SA-2018-780, SA-2019-483 y Grupo de Investigación Consolidado: 2017 SGR 01404). El trabajo desarrollado también ha sido parcialmente financiado por el Ministerio de Ciencia e Innovación (proyecto CEX2018-00794-S).

Agradecimientos

En primer lugar, me gustaría agradecer a mis directoras Miren López de Alda y Cristina Postigo. A Miren por insistirme una y otra vez para que hiciera la tesis, al final tu insistencia lo consiguió. Gracias por darme la oportunidad de hacer una tesis en este grupo de investigación y en este centro, y por toda tu ayuda y todos los consejos durante estos cinco años, he aprendido mucho de ti. A Cris, por enseñarme absolutamente todo lo que sé de química analítica, por estar siempre dispuesta a ayudar, fuera la hora que fuera, el día que fuera, y aunque tuviera muchísimas otras cosas que hacer. De verdad, muchísimas gracias. Creo que no he podido tener mejor directora que tú. Espero que Jose y tú disfrutéis muchísimo de esta nueva etapa.

A mi tutora Mercè Granados, por sus consejos durante toda la tesis.

Gracias a todos los compañeros con los que he coincidido durante estos años en el CSIC, sin duda alguna, lo mejor que me llevo de esta etapa:

- Los de la primera etapa de Garantía Juvenil: al bueno de Simón y sus bromitas a primera hora de la mañana (no sabías si tendrías el ratón del ordenador pegado al escritorio o la pantalla del ordenador bloqueada); a Carlitos que consiguió que fuera al viña (¡que viaje aquel!) desde Barcelona cuando lo tengo a escasos 20 minutos de mi pueblo, y a Dani, mi guía gastronómico y cultural de Barcelona, (quién iba a decir que íbamos a hacer tan buenas migas, eh?, ¡MADREMIA!), prometo que algún día os invitaré a la feria de Albacete y pasaremos por vuestro querido "*SIMANGO";* a Judit por esos planazos de calçotadas, barbacoas, piscina, caminatas por el Montseny (de 30 min máximo, todo hay que decirlo) y alguna que otra bandera indepe (esa foto la guardarás con especial ilusión); a Oscar, por enseñarme que hay vida más allá de la ciencia, aunque sinceramente, no creo que yo me dedique a hacer ese tipo de espectáculos; y a Mónica, por los numerosos días de playa, cenas, comidas y rutas por Barcelona.

- A los que han estado durante las dos etapas: a Mari Pau, la chica que al principio no quería relación con nadie pero que ahora se ha vuelto más blandita, seguiremos compartiendo carreras populares, partidos de pádel, y cenas, que no falten las cenas y las comidas, y si es en Ibiza mejor; a Cris por estar siempre dispuesta a ayudar en todo y con la mejor sonrisa posible; a la "bruja" Gabi por sus videos culinarios y las noches de "digestivos"; a Juanito, perdón por no seguir tu consejo, ahora ya sé que te tengo que hacer más caso jaja, gracias por todo, te tengo especial cariño; a Nico, el chico multiusos del laboratorio, espero que te valoren como te mereces; a Manu, por sus rutas "adaptadas para todo el mundo" (ya sabemos que no nos podemos fiar de tus palabras); a Gemma, queda pendiente la clase de Pole Dance y el viaje a la Garrotxa; a Berta, "mira que ets cuca", lo mismo te hace de instructora de Anigami, te enseña a subir por unas

telas u organiza unas birras, eso sí, siempre cerca de Sants; a Andrea, mírate haciendo un doctorado, la que decía que no quería hacerlo (no sé a qué me suena esto); a nuestro chef personal de las casas rurales, Massimo, gracias por los buenos ratos, sobre todo grabando el video de las Spice Girls.

- A las nuevas incorporaciones: a mi compi de lab Adrià que al principio decía que no le saludaba (con lo maja que soy yo), gracias por los numerosos viajes a Batea, a Miami Platja, los partidos de pádel y tenis, y los nuevos viernes de birras; al próximo presidente del Gobierno, como mínimo, el señor Albert Vega, espero que te acuerdes de los viejos compañeros del lab, aunque yo siempre estaré ahí con mis comentarios para que no te "subas demasiado a la parra"; a mis chicos fitness, Albert y Francesc, recuperaos pronto que echo de menos nuestras tardes de gimnasio; a Olga, Diana y Julio, ánimo con ese doctorado que acabáis de empezar.

- Por último, a mi italiana. Gracias Mavi por estos tres años, creo que no eres consciente de lo que me has ayudado. Sin ti, con quién iba yo a ensayar las presentaciones, quien me iba a dejar los documentos que yo no guardo ni escaneo, y muchas cosas más. Esto a nivel laboral, a nivel personal me llevo una amiga que es más importante. Siempre nos quedarán los recuerdos de todos los viajes post-congresos, y de las reuniones en tu súper terraza. Queda pendiente el viaje a Dublín, la ruta por Roma, una visita a Nápoles y un viaje a Albacete para que pruebes unos buenos churros con chocolate. Ya sabes de sobra que te quiero mucho, aunque no te lo diga.

Gracias a Roser, Dori y Alex, por su ayuda con los equipos de LC-MS/MS.

Fuera del CSIC, gracias a las chicas "Can Maria Rosa". A Alba, Irene y Claudia, con las que conviví durante la mayor parte del doctorado, y en especial a Lorena, que casi sin conocernos tuvo que aguantarme durante toda la cuarentena, no tuvo que ser fácil, aunque pasamos muchos ratillos buenos como nuestra "hora de la caña" y de "películas" los fines de semana, nuestra "hora de deporte" durante la semana, el puzle (lo tenemos que enmarcar) o la semana caótica de robos, e incendios. Y a la nueva incorporación, Maria Ángeles, una pena no haber coincidido más.

A los de toda la vida Myrian, Cris, Mary, y David, gracias por los ánimos a distancia.

Especial agradecimiento a mi "tío" Gonzalo y a Isabel. Gracias por abrirme vuestra casa y tratarme como una hija. Me encantaba ir a comer los domingos nuestras "gachas" manchegas y hablar sobre Minaya, aunque ahí me di cuenta, que hemos vivido en un Minaya completamente diferente. No me olvido de la invitación a Cantabria.

Y, por último, a las personas más importantes de mi vida, a mis padres, Ángel ("*El rubio de Monte Orenes*") y Suspiro ("*la de los cuatro onzas*"), a mis hermanos Álvaro, José y Alberto (mi otra mitad) ("*los cocos*"), y a Virginia ("*la de la mota*"), muchísimas gracias por el apoyo en los momentos más difíciles, pero, sobre todo, gracias papa y mama (y Álvaro), por los sacrificios que habéis hecho para que, tanto Alberto como yo, pudiéramos estudiar (creo que no ha salido mal la cosa). Papa de pequeña me dijiste: "*si no quieres trabajar en el campo, tienes que estudiar*". Pues bien, creo que ya no puedo estudiar más, así que, ¿no tengo que volver a pisarlo? Os quiero, ya lo sabéis.

SUMMARY

The use of drugs and psychoactive pharmaceuticals has continuously increased throughout the years. According to the last World Drug Report of the United Nations Office on Drugs and Crime, the number of drug users in the last year increased by 28% compared to data from 10 years ago, although in Spain consumption surveys show a stable (cannabis, hallucinogens) or lower (cocaine, amphetamine-type stimulants, heroin) consumption of illicit drugs than 20 years ago. As for psychoactive pharmaceuticals, data show that their consumption has increased in Spain since 2000, being nowadays 3 times higher in the case of antidepressants, 2 times higher in the case of analgesics, hypnotics and sedatives, and 25% greater in the case of anxiolytics. The extensive consumption of drugs and psychoactive pharmaceuticals, together with their incomplete removal in the wastewater treatment plants (WWTPs), has led to their continuous release into the environment. Therefore, these compounds and their metabolites are recognized as environmental contaminants of emerging concern.

Most studies conducted to date have shown that the levels at which these compounds are usually present in the environment are low (the lowest values in the order of ng/L in water and ng/g in solid samples). Thus, their environmental monitoring requires high-sensitivity, robust, and reliable analytical methodologies. Sample preparation is one of the critical steps in their analysis due to the complexity of the environmental matrices. Advanced extraction and purification techniques have to be applied to extract the maximum number of target compounds and minimize, at the same time, the co-extraction of other matrix components that may interfere in the analysis. Moreover, this should be done as environmentally-friendly as possible. In this context, this thesis presents three analytical methodologies for the analysis of illicit drugs, psychoactive pharmaceuticals, and their metabolites in three environmental compartments, i.e., wastewater, sediments, and biota, that are characterized by their simplicity and high-throughput (due to the use of automated systems for the extraction of the target analytes) and environmental sustainability (due to the use of small solvent amounts in the extraction process).

The methodology developed for the analysis of the target compounds in wastewater is based on on-line solid phase extraction coupled in series to liquid chromatography-and tandem mass spectrometry detection (on-line SPE-LC-MS/MS), the methodology for the analysis of sediments is based on pressurized liquid extraction (PLE) followed by solid phase extraction (SPE) purification and LC-MS/MS analysis, and the one developed for the analysis of mussels is based on “Quick, Easy, Cheap, Effective, Rugged and Safe” (QuEChERS) extraction and determination

by LC-MS/MS. All of them were validated in terms of linearity, accuracy (recovery), precision (repeatability), matrix effects, and sensitivity. Quantification in all instances based on the isotopic dilution method allowed to correct variable matrix effects and low absolute recoveries obtained for some compounds (relative recoveries obtained in the three matrices were in most cases between 78% and 120%). The quantification limits obtained (lower or equal to 16 ng/L in wastewater, 2.1 ng/g dry weight in sediments, and 6.7 ng/g fresh weight in mussels, for most of the investigated compounds) prove the suitability of the methods developed for the environmental monitoring of the substances under study. Also, method repeatability figures were satisfactory, with relative standard deviation values lower than 20%.

The analytical methodologies developed were applied to the analysis of different environmental samples. In the case of wastewater, the methodology was applied to the analysis of 7 samples collected during one week in a WWTP that serves part of Barcelona city and its metropolitan area to obtain a (first in some cases) picture of the occurrence of a total of 37 illicit drugs and psychoactive pharmaceuticals in raw wastewater of this area. Most of the investigated compounds were detected in all the samples analyzed; the exceptions were zolpidem (present in 71% of the samples), α-hydroxy-midazolam (86%), temazepam (86%), and 6-monoacetilmorphine, heroin, lysergic acid diethylamide (LSD), midazolam, α-hydroxy-alprazolam, 11-hydroxy-Δ^9-tetrahydrocannabinol, chlorpromazine, 3,4-dichloro-N-[[1-(dimethylamino) cyclohexyl]methyl]benzamide (AH-7921), methoxetamine, methylenedioxypyrovalerone (MDPV) and mephedrone, which were not detected in any sample. The concentrations found ranged from 7 ng/L (zolpidem) to 53.8 µg/L (caffeine), being the most abundant compounds, after caffeine, the antidepressants fluoxetine, sertraline, citalopram and venlafaxine (concentrations between 184 and 838 ng/L), followed by cannabis consumption indicator 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH, median concentration of 1.2 µg/L), cocaine (1.3 µg/L), its metabolite benzoylecgonine (2.2 µg/L), and ephedrine (2.3 µg/L).

In the case of sediments, the methodology was applied to the analysis of 20 drugs and metabolites in 144 samples collected from four river basins (Llobregat, Ebro, Jucar and Guadalquivir) in two sampling campaigns conducted in 2010 and 2011, being the most extensive study conducted so far on the occurrence of illicit drugs in this matrix. Significant differences on the occurrence of drugs at both spatial and temporal level were found and explained by factors such as meteorological and hydrological conditions, different WWTP removal efficiencies or consumption patterns. In general, frequency of detection (lower than 13% for all compounds except cocaine, methadone and its metabolite EDDP, that showed frequencies of detection

higher than 36%) and measured concentrations were low (median concentrations below 1.6 ng/g d.w., except for Δ^9-tetrahydrocannabinol (THC), cannabidiol and cannabinol that were measured at median values of 6.1, 15 and 25 ng/g d.w.). The most abundant drugs in sediments were those that due to their physical-chemical properties (log K_{ow} or log K_{oc} > 3) have a greater capacity to be adsorbed onto solid particles (*i.e.*, THC, cannabidiol, cannabinol, methadone and EDDP). The sediment-water distribution coefficient (log K_D) of methadone (2.79), EDDP (2.68), 3,4-methylenedioxymethamphetamine (MDMA) (1.95) and diazepam (1.79) could be estimated from experimental data for the first time showing their tendency to accumulate in sediments. Finally, according to the Hazard Quotient (HQ) approach, it could be concluded that the presence of illicit drugs and their metabolites in sediments may pose a toxicological risk for sediment-dwelling organisms in 38% of the locations investigated, being EDDP, THC and methadone, the compounds that most contributed to the overall sample toxicity.

The study of 35 psychoactive drugs and metabolites in mussels collected from the Catalan coast and in different types of commercial products revealed the absence of all the target analytes in the commercial samples and in most of the wild mussels. Only caffeine and sertraline were detected in one of the wild mussel samples collected at concentrations that, according to the HQ approach, may pose a toxicological risk to the organism.

The analysis of illicit drugs and psychoactive pharmaceuticals in wastewater, besides contributing to elucidate the environmental fate of these compounds, is used to estimate their consumption by the population through the so-called wastewater-based epidemiology (WBE) approach. The last work included in this thesis presents a nation-wide study carried out to estimate alcohol consumption in Spain. For this, the levels of ethyl sulfate, an ethanol consumption indicator, were measured throughout seven consecutive days in the raw wastewater of 17 WWTPs, covering 12.8% of the Spanish population. Ethyl sulfate was detected in all samples analyzed at concentrations between 1.4 and 74 µg/L. Ethyl sulfate concentrations were equivalent to alcohol consumption rates between 4.5 and 46 mL/day/inhabitant. Statistically significant differences in alcohol consumption were found among some of the investigated cities and their corresponding regions, and between weekdays and weekends. In 56% of the investigated populations, the alcohol consumption estimated by the WBE approach was comparable to the alcohol consumption data reported for their corresponding regions in the National Health Survey of Spain. At national level, alcohol consumption estimation was similar to that reported in the above mentioned Spanish survey as well as that reported by the Ministry of Agriculture, Fisheries and Food, although, as in many other studies carried out worldwide, WBE-derived data were lower than consumption data reported by the World Health

Organization. The results obtained showed that, despite the uncertainty associated with both WBE and the official classical consumption indicators, all approaches can be used together to improve consumption estimates of alcohol (or any other substance of abuse). Particularly, WBE is a useful tool to establish spatial and temporal patterns of consumption in a fast, objective, and economical way, as demonstrated in this work.

ESTRUCTURA

La presente memoria está estructurada en 5 capítulos. En el primer capítulo se realiza una introducción general a la temática de esta tesis doctoral: la presencia de drogas, psicofármacos y sus metabolitos en el medio ambiente. En este capítulo se presentan los compuestos objeto de estudio, se describe brevemente su consumo y producción a nivel mundial, europeo y español, se revisan los niveles medioambientales encontrados en diferentes compartimentos medioambientales, se evalúa su eliminación en las estaciones depuradoras de aguas residuales (EDARs), se explora su toxicidad ambiental tanto en estudios realizados *in vivo* como basados en modelos matemáticos, y por último, se muestran las metodologías analíticas utilizadas para su determinación en diferentes matrices ambientales. También se ha incluido una sección en la que se explican los fundamentos del análisis de aguas residuales con fines epidemiológicos.

En el segundo capítulo se describen los objetivos de esta tesis.

El tercer capítulo incluye tres artículos en los que se describen las metodologías analíticas desarrolladas y validadas para el análisis de drogas, psicofármacos y metabolitos en tres matrices (agua residual, sedimentos y biota), y su aplicación a diferentes muestras medioambientales, y un cuarto artículo en el que se ha aplicado el análisis de aguas residuales con fines epidemiológicos para la estimación del consumo de alcohol en España, todos ellos publicados en revistas científicas de alto impacto incluidas en el índice SCI (del inglés *Science Citation Index*).

En el cuarto capítulo se discuten los resultados obtenidos en los trabajos presentados en el capítulo 3. Esta discusión incluye la evaluación de los resultados obtenidos en la validación de las tres metodologías analíticas presentadas, y la comparación de dichas metodologías con otras publicadas en la literatura. También se evalúan los niveles de drogas, psicofármacos y metabolitos encontrados en las diferentes matrices ambientales investigadas, intentando justificar la presencia de los compuestos en cada una de las ellas, así como el riesgo medioambiental que su presencia puede suponer para los organismos acuáticos. Por último, se discuten los resultados de consumo de alcohol obtenidos aplicando el enfoque del análisis de aguas residuales con fines epidemiológicos y los reportados por organismos oficiales, así como la utilidad de esta metodología para determinar el consumo de alcohol y otras drogas por la población.

En el quinto capítulo se recogen las conclusiones generales obtenidas en esta tesis doctoral.

Al final de la memoria se incluye la bibliografía utilizada, así como varios anexos en los que se encuentran el índice de abreviaturas y acrónimos, el índice de tablas, el índice de figuras, una revisión de las concentraciones de drogas, psicofármacos y metabolitos en aguas. La distribución de las publicaciones incluidas en esta memoria es la siguiente:

- Capítulo 1:

  - Publicación científica #1: *The value of wastewater-based epidemiology in the estimation of alcohol consumption*. Ester López-García, Cristina Postigo, Damià Barceló, Miren López de Alda (2019) *Current Opinion in Environmental Science & Health*, 9: 19-25.
  - Publicación científica #2: *Analysis of Psychoactive Pharmaceuticals in Wastewater and Surface Water Using LC-MS*. Ester López-García, Cristina Postigo, Bozo Zonja, Damià Barceló, Miren López de Alda (2018) En: Achille Cappiello, Pierangela Palma (editores) *Advances in the Use of Liquid Chromatography-Mass Spectrometry (LC-MS): Instrumentation Developments and Applications*. Comprehensive Analytical Chemistry series, volume 79: 29-52. Elsevier B.V.

- Capítulo 3:

  - Publicación científica #3: *A fully automated approach for the analysis of 37 psychoactive substances in raw wastewater based on on-line solid phase extraction-liquid chromatography-tandem mass spectrometry*. Ester López-García, Nicola Mastroianni, Cristina Postigo, Damià Barceló, Miren López de Alda (2018) *Journal of Chromatography A,* 1576: 80-89.
  - Publicación científica #4: *Drugs of abuse and their metabolites in river sediments: Analysis, occurrence in four Spanish river basins and environmental risk assessment*. Ester López-García, Nicola Mastroianni, Nuria Ponsà-Borau, Damià Barceló, Cristina Postigo, Miren López de Alda (2021) *Journal of Hazardous Materials* 401: 123312.
  - Publicación científica #5: *Psychoactive substances in mussels: Analysis and occurrence assessment*. Ester López-García, Cristina Postigo, Miren López de Alda (2019) *Marine Pollution Bulletin* 146: 985-992.

- Publicación científica #6: *Assessing alcohol consumption through wastewater-based epidemiology: Spain as a case study*. Ester López-García, Carlos Pérez López, Cristina Postigo, Vicente Andreu, Lubertus Bijlsma, Iria González-Mariño, Félix Hernández, Rosa Maria Marcé, Rosa Montes, Yolanda Picó, Eva Pocurull, Andreu Rico, Rosario Rodil, María Rosende, Yolanda Valcárcel, Olatz Zuloaga, José Benito Quintana, Miren López de Alda (2020) *Drug and Alcohol Dependence* 215: 108241.

Tabla de contenidos

Capítulo 1.

Introducción

1.1. Drogas y psicofármacos

Las sustancias psicotrópicas también denominadas sustancias psicoactivas, son sustancias químicas, naturales o sintéticas, que afectan a la mente o a los procesos mentales produciendo una estimulación o depresión del sistema nervioso central, y cuyo uso puede producir dependencia (UNODC, 2018). En el ámbito de fiscalización de sustancias, una sustancia psicotrópica es cualquier sustancia incluida en la Lista I, II, III o IV de la "Convención de 1971 sobre Sustancias Psicotrópicas" elaborada por las Naciones Unidas y que complementa a la "Convención Única de 1961 sobre Estupefacientes" (UNODC, 1971, 1961). La inclusión de una sustancia en cada una de las listas se realiza en función de su utilidad terapéutica y su riesgo para la salud pública:

- Lista I: sustancias que presentan un alto riesgo de uso, constituyen una amenaza particularmente grave para la salud pública y no tienen un valor terapéutico.

- Lista II: sustancias que presentan un riesgo de uso indebido, constituyen una amenaza grave para la salud pública y tienen un valor terapéutico bajo o moderado.

- Lista III: sustancias que presentan un riesgo de uso indebido, constituyen una amenaza grave para la salud pública y su valor terapéutico es moderado o alto.

- Lista IV: sustancias que presentan un riesgo de uso indebido, constituyen una amenaza menor para la salud pública y su valor terapéutico es alto.

En general, las sustancias incluidas en las Listas I y II de la "Convención Única de 1961 sobre Estupefacientes" o en la Lista I de la "Convención de 1971 sobre Sustancias Psicotrópicas", engloban las sustancias coloquialmente denominadas drogas, o más concretamente drogas ilegales, es decir, aquellas sustancias utilizadas de manera ilegal que alteran el sistema nervioso central dando lugar a alucinaciones o trastornos de la función motora, del juicio, del comportamiento, de la percepción o del estado de ánimo. Las listas III y IV de la "Convención de 1971 sobre Sustancias Psicotrópicas" englobarían a los psicofármacos, es decir, medicamentos de comercio legal utilizados para aliviar el dolor, ayudar al sueño o a la lucidez y aliviar los desórdenes del estado de ánimo, entre otros, mientras que la Lista II, incluiría tanto drogas ilegales como psicofármacos.

Las drogas y psicofármacos pueden clasificarse en diferentes grupos en función de su origen y mecanismo de acción (UNODC, 2018):

- **Compuestos cocaínicos**: la cocaína es el principal alcaloide psicoactivo que se extrae de las hojas del arbusto de coca (*Erythroxilon*). Suele consumirse en forma de clorhidrato de cocaína, que se inhala (esnifa) o se inyecta, y en forma de cocaína base, que se fuma principalmente. Sus propiedades estimulantes se deben a la acción que ejerce en los sistemas neurotransmisores de la dopamina, la noradrenalina (norepinefrina) y la serotonina. La cocaína y derivados figuran en la Lista I de la "Convención Única de 1961 sobre Estupefacientes".
- **Estimulantes de tipo anfetamínico (ETAs)**: el término ETAs se utiliza para designar las sustancias sintéticas del grupo de las anfetaminas (anfetamina, metanfetamina, metcatinona y sustancias análogas) y del grupo del éxtasis (3,4-metilendioximetanfetamina (MDMA) o éxtasis y sustancias análogas, como 3,4-metilendioxianfetamina (MDA) y 3,4-metilendioxo-N-etilanfetamina (MDEA)). Al igual que en el caso de la cocaína, las propiedades estimulantes de los ETAs se deben a la acción que ejercen en los sistemas neurotransmisores de la dopamina, la noradrenalina y la serotonina, siendo el diferente grado en que se estimulen estos neurotransmisores el que determine los diferentes grupos de ETAs al que pertenecen. Así, los efectos de metanfetamina y anfetamina se deben a su influencia en los niveles de dopamina y noradrenalina, y los efectos del MDMA se deben a su influencia en los niveles de dopamina. A diferencia del MDMA cuyo uso no tiene ningún fin médico, la anfetamina y la metanfetamina se encuentran en la Lista II de la "Convención de 1971 sobre Sustancias Psicotrópicas", ya que existen productos a base de estos dos ETAs con fines terapéuticos como tratar el trastorno por déficit de atención e hiperactividad (TDAH) o tratar la narcolepsia.
- **Opiáceos y opioides**: los opiáceos son alcaloides naturales que se extraen de la planta adormidera (*Papaver somniferum*). El término opiáceos se utiliza para definir a los opiáceos naturales, como morfina y codeína, mientras que el término opioide se utiliza para denominar a los compuestos semi sintéticos (heroína), o completamente sintéticos (metadona y fentanilo). Los efectos tanto de los opiáceos como de los opioides se deben a su interacción con los neurotransmisores inhibidores y los receptores opioides. Muchos de ellos se utilizan en medicina como analgésicos, siendo los opioides sintéticos más potentes que los naturales. La morfina se utiliza para tratar el dolor crónico y para el postoperatorio, la metadona se utiliza con fines terapéuticos en la desintoxicación o tratamiento de la dependencia de los opioides, y el fentanilo es un analgésico narcótico de acción corta y con propiedades muy potentes, del que sólo 4 derivados (alfentanilo, fentanilo, remifentanilo y sufentanilo) se pueden utilizar con fines médicos. Otros 13

derivados del fentanilo, al igual que la morfina y la heroína están incluidos en la Lista I de la "Convención Única de 1961 sobre Estupefacientes".

- **Cannabinoides**: son las sustancias psicoactivas extraídas de la planta del cannabis (*Cannabis Sativa L.*). El Δ^9-tetrahidrocannabinol (THC) es la sustancia más predominante y la que posee mayor actividad psicoactiva, aunque también se puede extraer cannabinol (CBN) y cannabidiol (CBD). Los efectos psicoactivos de los cannabinoides se deben a la activación de los receptores cannabinoides del tipo 1 (CB_1). El cannabis se encuentra en la Lista I y IV de la "Convención Única de 1961 sobre Estupefacientes". Aunque el uso recreativo de esta sustancia es ilegal en la mayoría de países (sólo en Canadá, Uruguay y 11 estados de EEUU se permite la fabricación y venta de productos de cannabis con fines no médicos (UNODC, 2020)), existen algunos productos autorizados para su utilización con fines terapéuticos para tratar el dolor y la espasticidad[1] de la esclerosis múltiple, y para tratar la pérdida de apetito de los enfermos de sida, así como las náuseas y los vómitos asociados a la quimioterapia.
- **Alucinógenos o psicodélicos**: Son un grupo de drogas naturales extraídas de diversas plantas, hongos o animales, o bien sintetizadas químicamente, que provocan una distorsión de la realidad dando lugar a alucinaciones visuales y auditivas. Existen varios grupos relacionados químicamente, las triptaminas, como la (+)-lisérgida (LSD), y las fenetilaminas, como la mescalina (alucinógeno de origen vegetal extraído del peyote, *Lophophora williamsii*). Los efectos alucinógenos producidos por estos compuestos se deben a su afinidad por los receptores de la serotonina. En la actualidad los alucinógenos no están autorizados con fines médicos por lo que están presentes en la Lista I y II de la "Convención de 1971 sobre Sustancias Psicotrópicas".
- **Depresores del sistema nervioso central**: Son medicamentos utilizados para suprimir, inhibir o disminuir la actividad del cerebro. Los principales grupos son:

  (i) Benzodiazepinas: Son un grupo de depresores del sistema nervioso central que poseen estructuras químicas similares y que se utilizan en medicina como anticonvulsivos (antiepilépticos), ansiolíticos, hipnóticos, sedantes, relajantes musculares y tranquilizantes, por lo que se encuentran en las Listas III y IV del "Convenio de 1971 sobre Sustancias Psicotrópicas". Algunas de ellas también se han encontrado en el mercado ilegal, por lo que también aparecen en la Lista II. Las propiedades depresoras de las benzodiazepinas se deben a los efectos que producen en diversos

[1] Espasticidad: Trastorno del sistema nervioso central por el que algunos músculos se mantienen permanentemente contraídos.

receptores del complejo de receptores $GABA_A$ del cerebro, siendo sus efectos consecuencia de la ampliación de la acción del neurotransmisor ácido *gamma*-aminobutírico[2].

(ii) Barbitúricos: Se utilizan en medicina como anticonvulsivos o como coadyuvantes de la anestesia en procedimientos quirúrgicos y en menor medida como fármacos para la ansiedad. Actualmente se encuentran en las Lista III y IV de la "Convención de 1971 sobre Sustancias Psicotrópicas". Este tipo de compuestos están siendo sustituidos por las benzodiazepinas porque sus índices terapéuticos (relación entre la cantidad de sustancia que produce efecto terapéutico y la que produce toxicidad) son muy bajos y las sobredosis pueden ser mortales. Sus propiedades depresoras van desde la sedación leve a la anestesia general, y su mecanismo de acción es similar al de las benzodiazepinas.

(iii) Otros depresores del sistema nervioso central son la metacualona, que se utiliza como hipnótico, sedante, anticonvulsivo, antiespasmódico y anestésico local, o el ácido gamma (γ)-hidroxibutírico (GHB), que produce sedación y anestesia y se utiliza para tratar el síndrome de abstinencia del alcohol y de los opiáceos y para tratar el insomnio y la depresión clínica. Actualmente ambos se encuentran en la Lista II de la "Convención de 1971 sobre Sustancias Psicotrópicas".

- **Antidepresivos**: Medicamentos del grupo de agentes psicoactivos utilizados principalmente para tratar la depresión, aunque también se pueden utilizar para el tratamiento de los trastornos del pánico, ansiedad, o el dolor. Su mecanismo de acción está centrado en aumentar los niveles de serotonina, noradrenalina y/o dopamina en el cerebro. Los antidepresivos más comunes son los antidepresivos tricíclicos (antidepresivos heterocíclicos cuya estructura presenta una cadena con 3 anillos y que impiden la recaptación de serotonina y noradrenalina), y los inhibidores selectivos de la recaptación de serotonina (ISRS) (fluoxetina, sertralina, citalopram), de la dopamina (ISRD), de la noradrenalina (ISRN) y de la serotonina y la noradrenalina (IRSN) (venlafaxina) (Benedi y cols., 2005). A pesar de su amplio uso y propiedades psicoactivas, ninguno de los antidepresivos de uso común está sometido a fiscalización internacional, por lo que no figuran en ninguna de las listas de las convenciones.

[2] El ácido *gamma*-aminobutírico es un neurotransmisor inhibidor que ayuda a regular la actividad cerebral reduciendo la neurotransmisión, lo que produce que se ralenticen las funciones corporales normales y se provoquen efectos depresores.

- **Analgésicos**: Sustancias que reducen el dolor, con o sin propiedades psicoactivas. Actualmente se encuentran en la Lista III de la "Convención de 1971 sobre Sustancias Psicotrópicas".
- **Estimulantes**: Cualquier sustancia que activa, potencia o incrementa la actividad neuronal. Además de las anfetaminas, hay otros estimulantes en las Listas II, III y IV de la "Convención de 1971 sobre Estupefacientes".
- **Nuevas Sustancias Psicoactivas (NSPs):** Son sustancias de abuso recientemente disponibles en el mercado, bien en forma pura o en preparado, que tienen propiedades farmacológicas y/o químicas similares a las sustancias controladas internacionalmente, pero que no se encuentran bajo control internacional, por lo que constituyen un peligro para la salud. En los últimos años la junta de fiscalización de las Naciones Unidas está introduciendo en las listas de fiscalización aquellas sustancias que son altamente nocivas, siendo en el año 2019 el número de NSPs fiscalizadas de 282 (UNODC, 2020). Atendiendo a sus efectos, se dividen en varios grupos, siendo los más comunes los cannabinoides sintéticos, catinonas, fenetilaminas, triptaminas, piperazinas, aminoindanos y sustancias de tipo fenciclidina.

Las drogas, psicofármacos y metabolitos incluidos en la presente tesis pertenecen a estos grupos de compuestos. La Tabla 1 muestra los analitos investigados junto con sus propiedades físico-químicas.

Tabla 1. Propiedades físico-químicas[a] de las drogas, psicofármacos y metabolitos investigados.

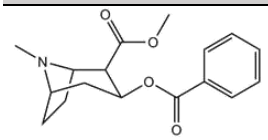
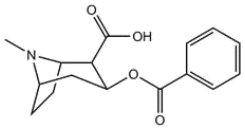
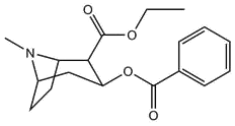
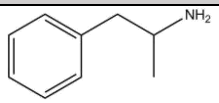
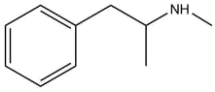
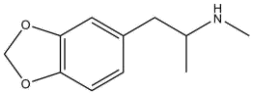
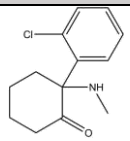
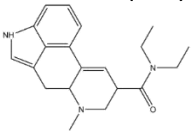
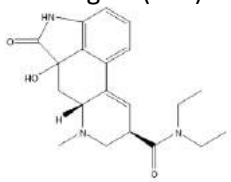
Analito	Número CAS	Masa molecular	pK_a	Log K_{ow}	Log K_{oc}	BCF (L/kg)	Presión de vapor (mm Hg)	Solubilidad (mg/L) (25 °C)
Compuestos cocaínicos								
 Cocaína (COC)	50-36-2	303,35	8,6	2,3	3,3	12	$1,9x10^{-7}$	1298
 Benzoilecgonina* (BE)	519-09-5	289,33	3,4	-1,3	2,5	3,2	$3,8x10^{-11}$	1605
 Cocaetileno* (CE)	529-38-4	317,38		2,7	3,5	22	$6,7x10^{-6}$	528
Estimulantes de tipo anfetamínico (ETAs)								
 Anfetamina (AM)	300-62-9	135,21	9,9	1,8	3,0	4,5	0,3	28.000
 Metanfetamina (MA)	537-46-2	149,23	9,9	2,1	3,2	7,8	$5,4x10^{-3}$	13290
 3,4-metilendioxi-metanfetamina (MDMA)	42542-10-9	193,25	9,9	2,3	2,7	11	$2,3x10^{-7}$	5413
Alucinógenos								
 Ketamina (KET)	1867-66-9	237,73	7,5	2,2				
 Dietilamida de ácido lisérgico (LSD)	50-37-3	323,43	7,8	3,0	5,4	37	$2,0x10^{-8}$	2,1
 Hidroxi-LSD* (OH-LSD)	111295-09-1	355,43		0,4	2,7	3,2	$1,4x10^{-14}$	2692

Tabla 1 (cont). Propiedades físico-químicasa de las drogas, psicofármacos y metabolitos investigados.

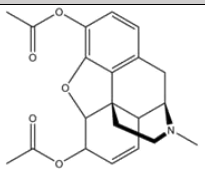
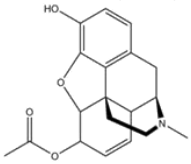
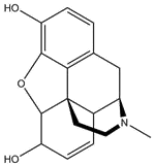
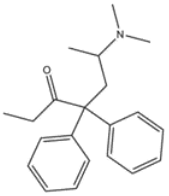
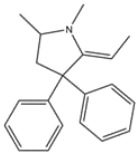
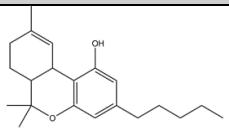
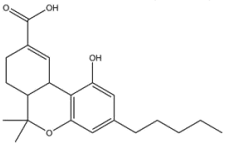
Analito	Número CAS	Masa molecular	pK_a	Log K_{ow}	Log K_{oc}	BCF (L/kg)	Presión de vapor (mm Hg)	Solubilidad (mg/L) (25 °C)
Opiáceos/opioides								
 Heroína (HER)	561-27-3	369,41	8,0	1,6	3,9	3,3	$6,2x10^{-8}$	2152
 6-monoacetil-morfina* (6ACM)	2784-73-8	327,38		1,6	4,4	3,1	$8,8x10^{-9}$	4093
 Morfina (MOR)	57-27-2	285,34	8,2	0,9	3,5	3,2	$1,9x10^{-10}$	26420
 Metadona (MET)	76-99-3	309,45	9,2	3,9	4,9	212	$1,1x10^{-6}$	49
 2-etilideno-1,5-dimetil-3,3-difenil-pirrolidina* (EDDP)	30223-73-5	277,4		4,9	5,7	1266	$3,3x10^{-6}$	10,2
Cannabinoides								
 Δ^9-Tetrahidro-cannabinol (THC)	1972-08-3	314,46	10,6	7,0	5,8	$4,6x10^4$	$4,6x10^{-8}$	0,04
 11-nor-9-carboxi-Δ^9THC* (THC-COOH)	56354-06-4	344,45		6,4	4,5	56	$1,9x10^{-10}$	0,2

Tabla 1 (cont). Propiedades físico-químicasa de las drogas, psicofármacos y metabolitos investigados.

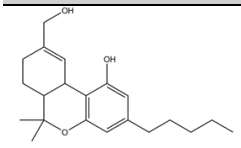
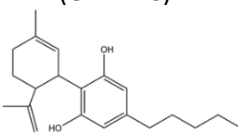
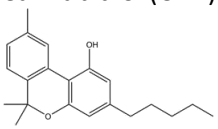
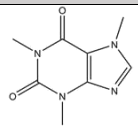
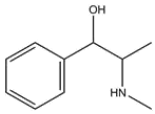
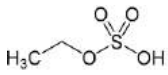
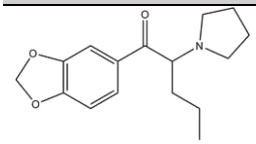
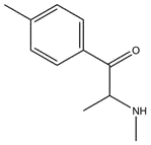
Analito	Número CAS	Masa molecular	pK_a	Log K_{ow}	Log K_{oc}	BCF (L/kg)	Presión de vapor (mm Hg)	Solubilidad (mg/L) (25 °C)
Cannabinoides								
 11-hidroxi-Δ^9THC* (OH-THC)	36557-05-8	330,46		5,3	4,6	568	$1,1x10^{-10}$	2,8
 Cannabidiol (CBD)	74219-29-7	314,46		8,0	6,4	$3,1x10^{4}$	$2,8x10^{-8}$	$5,5x10^{-3}$
 Cannabinol (CBN)	521-35-7	310,43		7,2	5,8	$2,6x10^{4}$	$7,2x10^{-8}$	$2,1x10^{-3}$
Estimulantes								
 Cafeína (CAF)	58-08-2	275,35	14,0	-0,1	1,0	3,2	$7,3x10^{-9}$	2632
 Efedrina (EPH)	299-42-3	165,24	10,3	1,1	1,9	0,3	$1,1x10^{-2}$	71480
Alcohol								
 Sulfato de etilo* (EtS)	540-82-9	126,13		-2,5	1,4	3,2	$1,4x10^{-3}$	$1,0x10^{6}$
Nuevas Sustancias Psicoactivas (NSPs)								
 3,4-metilendioxi-pirovalerona (MDPV)	687603-66-3	194,19		4,0	2,9	33	$2,4x10^{-6}$	70
 Mefedrona (MEPH)	1189805-46-6	177,25		2,4			$5,4x10^{-3}$	5211

Tabla 1 (cont). Propiedades físico-químicasa de las drogas, psicofármacos y metabolitos investigados.

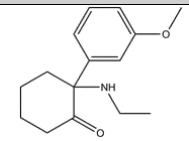
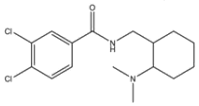
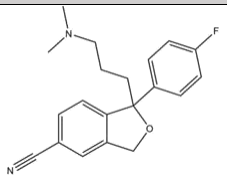
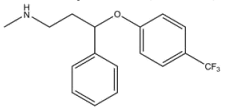
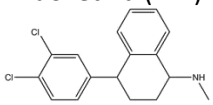
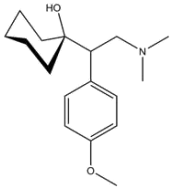
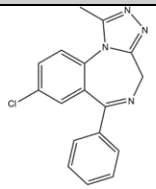
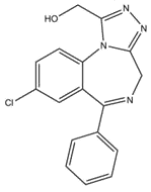
Analito	Número CAS	Masa molecular	pK_a	Log K_{ow}	Log K_{oc}	BCF (L/kg)	Presión de vapor (mm Hg)	Solubilidad (mg/L) (25 °C)
Nuevas Sustancias Psicoactivas (NSP)								
 Metoxetamina (MXE)	1239943-76-0	247,34		3,0				
 AH-7921	55154-30-8	329,27		4,4	3,5	507	$1,2x10^{-8}$	1,4
Antidepresivos								
 Citalopram (CTLP)	59729-33-8	324,4	9,8	3,7	4,4	152	$1,1x10^{-7}$	31
 Fluoxetina (FLX)	54910-89-3	309,33		4,1	5,3	262	$2,5x10^{-5}$	38
 Sertralina (STR)	79617-96-2	306,23	9,2	5,3	5,5	2347	$1,2x10^{-6}$	3,5
 Venlafaxina (VFX)	93413-69-5	277,41	10,1	3,3	3,2	67	$2,5x10^{-7}$	267
Benzodiazepinas								
 Alprazolam (ALPZ)	28981-97-7	308,77		2,1	6,3	8,6	$1,7x10^{-8}$	13
 α-hidroxi-alprazolam* (OH-ALPZ)	37115-43-8	324,77		2,4	5,1	3,2	$6,4x10^{-12}$	6,0

Tabla 1 (cont). Propiedades físico-químicasa de las drogas, psicofármacos y metabolitos investigados.

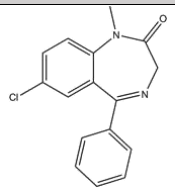
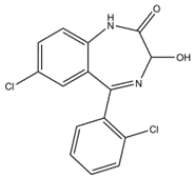
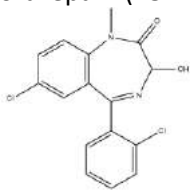
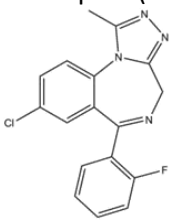
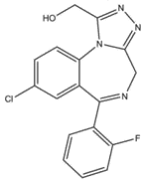
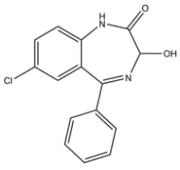
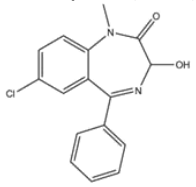
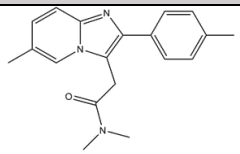
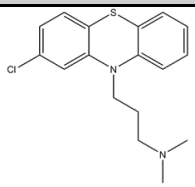
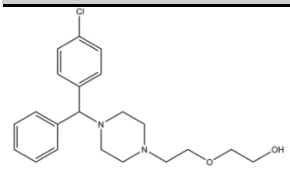
Analito	Número CAS	Masa molecular	pK_a	Log K_{ow}	Log K_{oc}	BCF (L/kg)	Presión de vapor (mm Hg)	Solubilidad (mg/L) (25 °C)
Benzodiazepinas								
 Diazepam (DIAZ)	439-14-5	284,74	3,4	2,8	4,1	30	$1,0x10^{-7}$	59
 Lorazepam (LORZ)	846-49-1	321,16	13	4,0	3,3	232	$1,3x10^{-12}$	3,7
 Lormetazepam (LRMZ)	848-75-9	335,18		2,2	3,0	11	$4,8x10^{-12}$	94
 Midazolam (MIDZ)	59467-70-8	326,76	5,5	4,3	5,6	432	$5,1x10^{-9}$	0,1
 α-hidroxi-midazolam* (OH-MIDZ)	59468-90-5	342,76		2,9	4,4	7,2	$4,3x10^{-13}$	1,9
 Oxazepam (OXA)	604-75-1	286,05	1,5	3,3	3,1	74	$6,0x10^{-12}$	21
 Temazepam (TEMZ)	846-50-4	300,74		2,2	2,7	9,7	$1,7x10^{-10}$	164

Tabla 1 (cont). Propiedades físico-químicasa de las drogas, psicofármacos y metabolitos investigados.

Analito	Número CAS	Masa molecular	pK_a	Log K_{ow}	Log K_{oc}	BCF (L/kg)	Presión de vapor (mm Hg)	Solubilidad (mg/L) (25 °C)
Depresores del sistema nerviosos central no benzodiazepínicos								
 Zolpidem (ZOLP)	82626-48-0	307,4	5,7	3,8	4,4	184	$2,3x10^{-10}$	5,7
Antipsicóticos								
 Clorpromacina (CHLOR)	50-53-3	318,86	9,3	4,3	4,4	421	$2,6x10^{-7}$	20
Antihistamínico								
 Hidroxicina (HXZ)	68-88-2	374,91	2,4	2,4	3,9	2,9	$1,2x10^{-11}$	428

[a]Datos extraídos de la base de datos ChemSpider

*Metabolito

K_a: constante de disociación acida; K_{ow}: coeficiente de reparto octanol-agua; K_{OC}: coeficiente de reparto carbono orgánico-agua; BCF: factor de bioconcentración (del inglés, *Bioconcentration Factor*).

1.2. Drogas y psicofármacos a nivel mundial: evolución y situación actual

Según el último Informe Mundial sobre las Drogas elaborado por la Oficina de las Naciones Unidas contra la Droga y el Delito (UNODC, 2020), en 2018, en torno a 269 millones de personas (5,4% de la población mundial entre 15 y 64 años de edad) consumió drogas en el último año, aunque sólo 35 millones (0,7% de la población mundial entre 15 y 64 años) fueron consumidores problemáticos o personas con drogodependencia que requirieron tratamiento. El número de muertes por consumo de drogas fue de 167.000 personas, siendo el consumo de opioides el que provocó la mayor cantidad de estas muertes.

El número de consumidores de drogas en el último año ha aumentado en torno al 28% con respecto al año 2009. Este aumento se debe en parte al crecimiento de la población (10% en la franja de edad de 15 a 64 años), pero también a un aumento del consumo de drogas en algunas regiones del mundo. El consumo de cannabis ha permanecido estable en Europa Central y Occidental, sin embargo, ha aumentado en América, África y Asia. El consumo de cocaína ha aumentado en América del Sur, en Europa Occidental y Central y en Oceanía. El consumo de opiáceos ha sido el que ha experimentado un mayor aumento. Comparado con datos de 2016, su consumo global ha aumentado un 56%, siendo África, Asia, Europa y América del Norte, las zonas en las que más ha aumentado la prevalencia de consumo. El consumo de anfetaminas se ha mantenido estable en los últimos años en Europa Central y Occidental, mientras que se ha observado un aumento del consumo de metanfetamina en América del Norte y Asia (UNODC, 2019).

En términos de prevalencia de consumo, el cannabis fue la droga más consumida en todo el mundo en 2018 (3,9% de la población mundial entre 15-64 años la consumió en el último año), seguida de los opiáceos (1,2% de la población mundial), de los estimulantes de tipo anfetamínico (0,5% de la población mundial) y de la cocaína (0,4% de la población mundial) (UNODC, 2020).

En términos de producción de drogas, la de cocaína se encuentra en niveles máximos. En 2018 se fabricaron, mayoritariamente en Colombia, 1.723 toneladas de cocaína, lo que supone un incremento del 50% con respecto a datos de 2013. Sin embargo, también ha aumentado el número de incautaciones, siendo en 2018 de 1.311 toneladas (71% más que hace 10 años). La producción de opio disminuyó un 26% con respecto a años anteriores siendo de 7.610 toneladas en 2018, y también disminuyeron las incautaciones de opiáceos en torno al 19% con respecto a años anteriores debido, principalmente, a que las incautaciones de morfina se

redujeron a la mitad en el año 2018 (morfina: 43 toneladas; heroína: 96 toneladas; opio: 704 toneladas). Estimar la producción de cannabis es difícil ya que su cultivo está extendido por todo el mundo. En este caso se utilizan tanto indicadores directos (plantas de cannabis cultivadas o erradicadas), como indirectos (número de incautaciones) para estimar su producción. En el periodo 2010-2017 se estima que se ha producido un incremento del cultivo de cannabis, mientras que el número de incautaciones ha disminuido en torno al 23% desde el año 2008, siendo en 2018 las toneladas incautadas de 5.642. Al igual que ocurre con cannabis, la producción de ETAs resulta difícil de estimar ya que la mayoría se realiza en laboratorios clandestinos, por lo que se utiliza el número de incautaciones para estimar su producción. En el periodo de 2014 a 2018 se desmantelaron en torno a 30.000 laboratorios clandestinos destinados a la producción de ETAs, de los cuales el 95% estaba destinado a la producción de metanfetamina, el 2% a la de anfetamina, el 1% a la de éxtasis y el porcentaje restante a "otros estimulantes". La cantidad de ETAs incautadas en 2018 fue de 279 toneladas, destacando el aumento de incautaciones de metanfetamina (7 veces superior) y de "otros estimulantes" (18 veces superior) con respecto a datos de 2009 (se usan en la síntesis de NSPs).

Respecto al consumo de psicofármacos, los que se encuentran en mayor proporción en el mercado legal son las benzodiazepinas, constituyendo el 63% de los psicofármacos comercializados, seguidas de barbitúricos (9,3%), anfetaminas (7%), analgésicos (4,7%) y otros psicofármacos (16,3%) (INCB, 2019). Las benzodiazepinas presentaron una mayor tasa de consumo en Europa, los barbitúricos en Europa y en América y los estimulantes de tipo anfetamínico destinados al comercio legal y los analgésicos en EEUU.

En lo que respecta a la producción, la fabricación de benzodiazepinas aumentó un 24% en 2018 con respecto a años anteriores, siendo la cantidad de benzodiazepinas fabricadas para fines médicos o científicos de 199 toneladas. La fabricación de barbitúricos se situó en 410 toneladas. Los estimulantes de tipo anfetamínico destinados al comercio legal se fabricaron principalmente en EEUU y Francia, alcanzándose en 2018 las 61 toneladas, lo que supuso un incremento del 61% con respecto a años anteriores. La fabricación de estimulantes de tipo no anfetamínico como metilfenidato y fentermina disminuyó en 2018 con respecto a años anteriores, situándose en 63 y 32 toneladas, respectivamente, mientras que la fabricación de analgésicos psicotrópicos como la buprenorfina y la pentazocina ha ido aumentando gradualmente en la última década, situándose en 2018 en las 22 toneladas.

Respecto a las nuevas sustancias psicoactivas, en 2018, el sistema de alerta temprana de la UNODC estaba vigilando 950 sustancias para incluirlas como NSPs debido a su aparición en

el mercado, aunque únicamente 541 fueron catalogadas como tal, dato que está bastante estabilizado desde el año 2015. La mayoría de NSPs son sustancias con efecto estimulante, a las que siguen los cannabinoides sintéticos y NSPs con efectos alucinógenos. Mientras que en los últimos años se ha visto una disminución del número de cannabinoides sintéticos que llegan al mercado, el número de NSPs con efectos estimulantes y sobre todo el número de opiáceos sintéticos ha aumentado notablemente. El consumo de fentanilo y sus derivados se encuentra extendido principalmente en zonas de América del Norte, donde su consumo se ha relacionado con el 67% de las muertes causadas por sobredosis de opiáceos. En Europa su uso es más limitado, aunque en los últimos años se ha observado que se está extendiendo. También preocupa el uso no médico del opioide sintético tramadol, sobre todo en África, donde las incautaciones se han multiplicado por 14 desde 2013 a 2017, aunque en 2018 disminuyeron entre el 77-84%. En cuanto al uso no médico de benzodiazepinas, en 2017, 40 países informaron que las benzodiazepinas sin receta médica fueron una de las tres sustancias más consumidas en el país (UNODC, 2019).

La situación en Europa

En concordancia con la tendencia observada a nivel mundial, en Europa el cannabis es la droga ilegal más consumida. Según el último Informe Europeo sobre Drogas (EMCDDA, 2020a), en 2019 se estima que 25,2 millones de personas adultas (7,6% de la población europea entre 15-64 años) y 18 millones de personas adultas jóvenes (15% de la población europea entre 15-34 años) consumió cannabis en el último año. En general, el consumo de cannabis está más extendido en Europa Occidental y Central, siendo los países en los que hubo mayor prevalencia de consumo entre la población adulta joven Francia, seguido de Italia, España, Países Bajos, Alemania, República Checa, Estonia y Croacia, con prevalencias de consumo superiores a la media europea (Figura 1). La forma más común en que se consume el cannabis en Europa es en forma de hierba (marihuana) y en forma de resina (hachís), siendo el contenido de THC en resina el doble que en hierba. Mientras que la hierba de cannabis se cultiva y se consume en Europa, la resina de cannabis consumida en Europa se cultiva principalmente en Marruecos.

Tras cannabis, la segunda droga más consumida en términos de prevalencia es la cocaína. En torno a 4,3 millones de personas adultas (1,3% de la población europea entre 15-64 años) y 2,9 millones de personas adultas jóvenes (2,4% de la población europea entre 15-34 años) la consumió el último año. El consumo de cocaína está más extendido en los países de Europa Occidental y Central, siendo las prevalencias de consumo en algunos de ellos (Reino

Unido, Países Bajos, Dinamarca, Francia, Irlanda, Bélgica, España, Estonia, y Suecia) superiores a la media europea (Figura 1). En general, el consumo de cocaína ha aumentado en los últimos años en Europa. La forma más común de consumo de cocaína en Europa es la inhalación de cocaína en polvo, o bien, por vía parenteral o fumada en forma de crack.

En cuanto a los estimulantes de tipo anfetamínico, su consumo también se encuentra más extendido en Europa Occidental y Central. La MDMA fue consumida por 2,7 millones de personas adultas (0,8% de la población europea entre 15-64 años) y por 2,3 millones de personas adultas jóvenes (1,9% población europea entre 15-34 años), siendo los países con mayor prevalencia de consumo entre la población adulta joven Países Bajos seguido de Irlanda, Reino Unido, Bulgaria, Alemania y Finlandia (prevalencias superiores al 2,5%) (Figura 1). La tendencia de consumo de MDMA en los últimos años es muy diversa, habiendo países que muestran un incremento del consumo (Bulgaria), otros una tendencia estable (Reino Unido) y otros una disminución (España). La forma más común de consumo de MDMA es en forma de comprimidos, bien en forma de cristal o en polvo, tanto por vía oral como nasal. Además, es frecuente su consumo junto con otras drogas, principalmente, alcohol. En el caso de anfetamina y metanfetamina, el consumo de anfetamina es muy superior al consumo de metanfetamina y en ocasiones es difícil distinguir entre una o la otra, por lo que se utiliza el término genérico de anfetaminas. En Europa en el año 2019, se estimó que 2,0 millones de personas adultas (0,6% de la población europea entre 15-64 años) y 1,4 millones de personas adultas jóvenes (1,2% de la población entre 15-34 años) consumieron anfetaminas en el último año. Los países con mayor prevalencia de consumo de anfetaminas entre población adulta joven en el último año fueron Finlandia, Alemania, Países Bajos, Croacia, Estonia, y Bulgaria con prevalencias superiores al 1,5% (Figura 1). El consumo de anfetamina se ha mantenido relativamente estable en la mayoría de países europeos. En el caso de la metanfetamina, su consumo siempre ha estado limitado a Eslovaquia y República Checa. Sin embargo, parece que su consumo se está extendiendo a más países debido a que se emplea para sintetizar NSPs.

En el caso de los opioides, en 2019 se estimó que 1,3 millones de personas adultas (0,4% de la población europea entre 15-64 años) son consumidores de opioides de alto riesgo, siendo los países con mayor prevalencia de consumo de opioides de alto riesgo Reino Unido, seguido Finlandia, Italia, Austria e Irlanda, con prevalencias superiores a 6 por cada 1000 habitantes. Al igual que a nivel mundial, los opioides están presentes en la mayoría de muertes provocadas por sobredosis en Europa, concretamente en el 82%. El opioide ilegal más consumido sigue siendo la heroína, aunque su consumo ha disminuido en la mayoría de los países europeos.

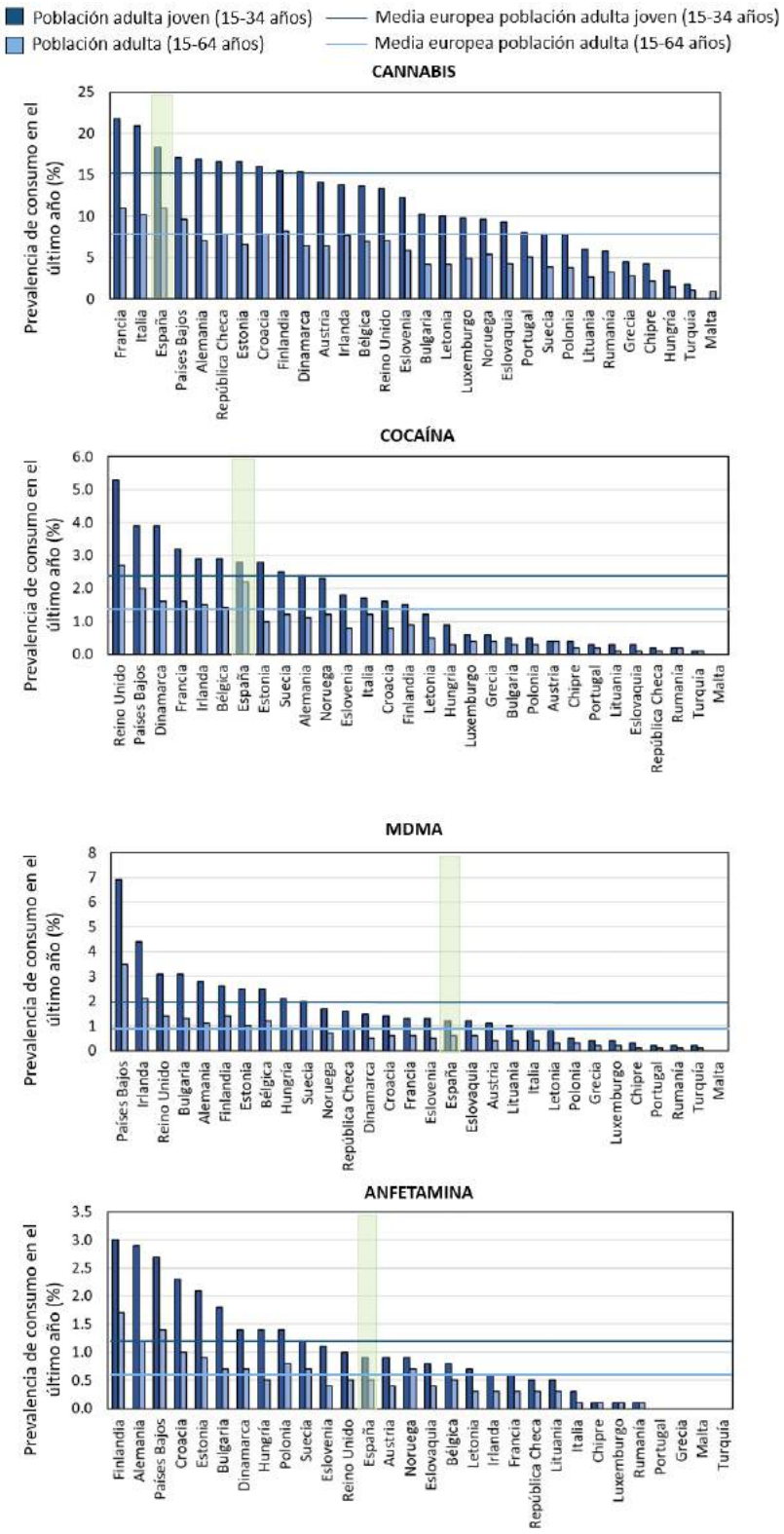


Figura 1. Prevalencia del consumo de drogas durante el último año entre la población adulta (población entre 15-64 años) y la población adulta joven (entre 15-34 años) en diferentes países europeos. Prevalencia de consumo en España resaltado en verde. Fuente: (EMCDDA, 2020a).

Además de las drogas ilegales tradicionales, en Europa se consumen otras sustancias como LSD, ketamina, hongos alucinógenos y GHB, pero su prevalencia de consumo es baja y su consumo se ha mantenido estable en los últimos años.

Respecto a las NSPs, en 2019 se notificaron al Sistema de Alerta Temprana (SAT) 53 NSPs detectadas por primera vez en el mercado, número similar al de años anteriores, aunque inferior a los máximos notificados en 2014 y 2015, sobre 100 NSPs. En total, el número de NSPs notificadas al SAT desde el año 1997 es de 790 NSPs. Aunque la prevalencia de uso es mucho menor que la de las drogas tradicionales, se estima que, entre la población adulta joven, la prevalencia de consumo se encuentra entre el 0% (Noruega) y el 1,9% (Polonia). Las que se encuentran en mayor proporción son cannabinoides sintéticos y catinonas, y al igual que ocurría a escala global, preocupa la aparición tanto de opioides sintéticos (derivados de fentanilo, principalmente), como de benzodiazepinas sintéticas (sustitutivas de alprazolam y diazepam, principalmente). Destaca el uso no médico de hipnóticos y sedativos entre los estudiantes europeos entre 15-16 años, siendo la prevalencia de uso alguna vez en la vida del 6%, alcanzándose en países como Polonia y República Checa prevalencia del 17 y 16%, respectivamente (UNODC, 2019).

En cuanto al resto de psicofármacos, en Europa los más consumidos son los antidepresivos con un consumo medio estimado en 2017 de 60 DDD_{pm} (dosis diarias definidas por cada 1000 habitantes), seguidos de los analgésicos (30 DDD_{pm}), y de los ansiolíticos, y los hipnóticos y sedantes, que presentaron, en ambos casos, un consumo en torno a 20 DDD_{pm} (Figura 2). El consumo de antidepresivos ha aumentado progresivamente desde el año 2000 en Europa (Figura 2), siendo su consumo en 2017 el doble del estimado en el año 2000. Por su parte, el consumo de analgésicos se ha mantenido bastante constante a lo largo de los últimos 17 años, mientras que el consumo tanto de ansiolíticos como de hipnóticos y sedantes ha disminuido ligeramente de 30 DDD_{pm} a 20 DDD_{pm}. El consumo de estos psicofármacos está más extendido en el norte de Europa donde países como Islandia, Suecia, o Finlandia presentan consumos superiores a la media europea. Algunos países de Europa Meridional como España y Portugal, también presentan consumos superiores a la media europea.

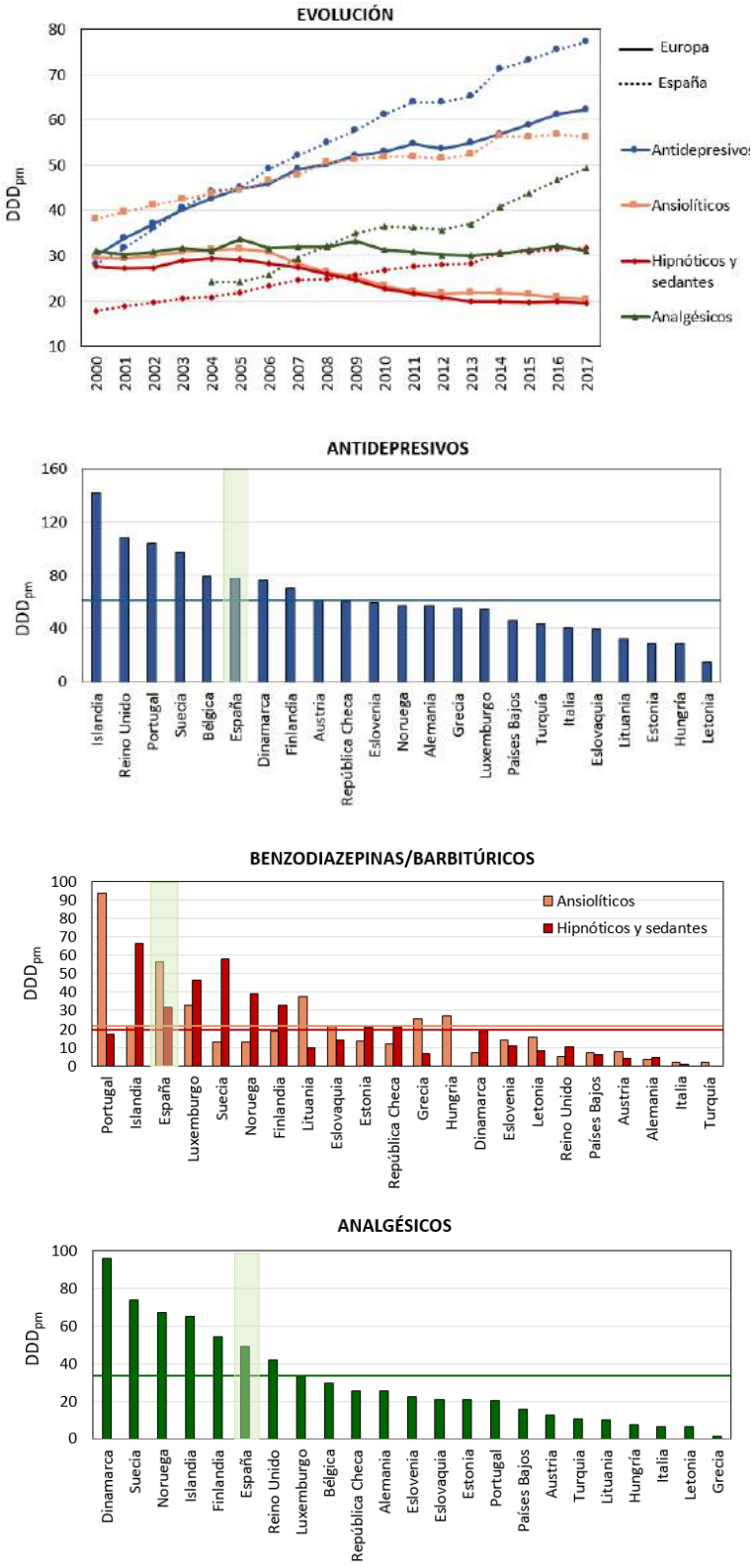


Figura 2. Evolución del consumo de psicofármacos en Europa y España desde el año 2000 y consumo de psicofármacos en diferentes países europeos expresado en dosis diarias definidas por cada mil habitantes (DDD_{pm}). (La línea horizontal indica el consumo medio de antidepresivos, ansiolíticos, hipnóticos y sedantes, y analgésicos, respectivamente, en Europa. Consumo en España resaltado en verde). Fuente: (OCDE, 2018).

La situación en España

Los últimos datos disponibles acerca del consumo de sustancias psicoactivas en España con respecto a otros países Europeos se muestran en las Figuras 1 y 2. En España, de acuerdo con el último informe publicado por el Observatorio Español de las Drogas y las Adicciones (OEDA), las sustancias psicoactivas con mayor prevalencia de consumo en el último año son el alcohol, seguido del tabaco, los hipnosedantes (con o sin receta), el cannabis y la cocaína (Figura 3) (OEDA, 2019).

Al igual que la tendencia observada mundialmente y en Europa, el cannabis fue la droga ilegal con mayor prevalencia de consumo. En 2017, la prevalencia de consumo entre la población adulta (15-64 años) fue del 11%, siendo el consumo en forma de marihuana más frecuente que el consumo en forma de hachís. El consumo de cannabis se encuentra bastante estable desde el año 2003 (Figura 3), aunque su consumo ha aumentado si se compara con datos de principios del año 2000. El cannabis es la droga ilegal que empieza a consumirse a una edad más temprana, de media a los 18 años.

Tras cannabis, la droga ilegal más consumida es la cocaína. En 2017, la prevalencia de consumo fue de 2,2%, siendo el consumo de cocaína en polvo mayor que el de cocaína en forma base. Su consumo ha disminuido ligeramente desde el año 2007, aunque en 2017, fue la droga que causó un mayor número de admisiones a tratamiento (43,1% del total), seguida del cannabis (27,6%) y los opioides (24,9%). España es considerado como el principal país de tránsito de la cocaína procedente de América del Sur hacia el resto de países europeos (UNODC, 2019).

Los ETAs constituyen la tercera sustancia psicoactiva ilegal más consumida entre los españoles, aunque presenta una prevalencia de uso residual situándose por debajo del 1%. La prevalencia de consumo varía según el grupo. Los ETAs del grupo del éxtasis o MDMA son los que presentan mayor prevalencia de consumo en el último año (0,6%), seguido del grupo de las anfetaminas (0,5%) y, por último, el grupo de la metanfetamina (0,2%). En cuanto a la evolución del consumo (Figura 3), aunque desde el año 2009 la prevalencia de consumo está bastante estabilizada, la prevalencia de consumo tanto de MDMA como de anfetaminas se ha reducido a la mitad si se compara con datos de principios de los años 2000.

Los alucinógenos presentaron una prevalencia de consumo en el último año similar a los ETAs del grupo de éxtasis y anfetamina, situándose en torno al 0,4% entre la población de 15-64 años. La prevalencia de consumo de alucinógenos (Figura 3) se ha mantenido estable en los últimos 10 años, a excepción del año 2015 en que hubo un pequeño repunte. Sin embargo, comparado con datos de principios de los 2000, su consumo se ha reducido a la mitad.

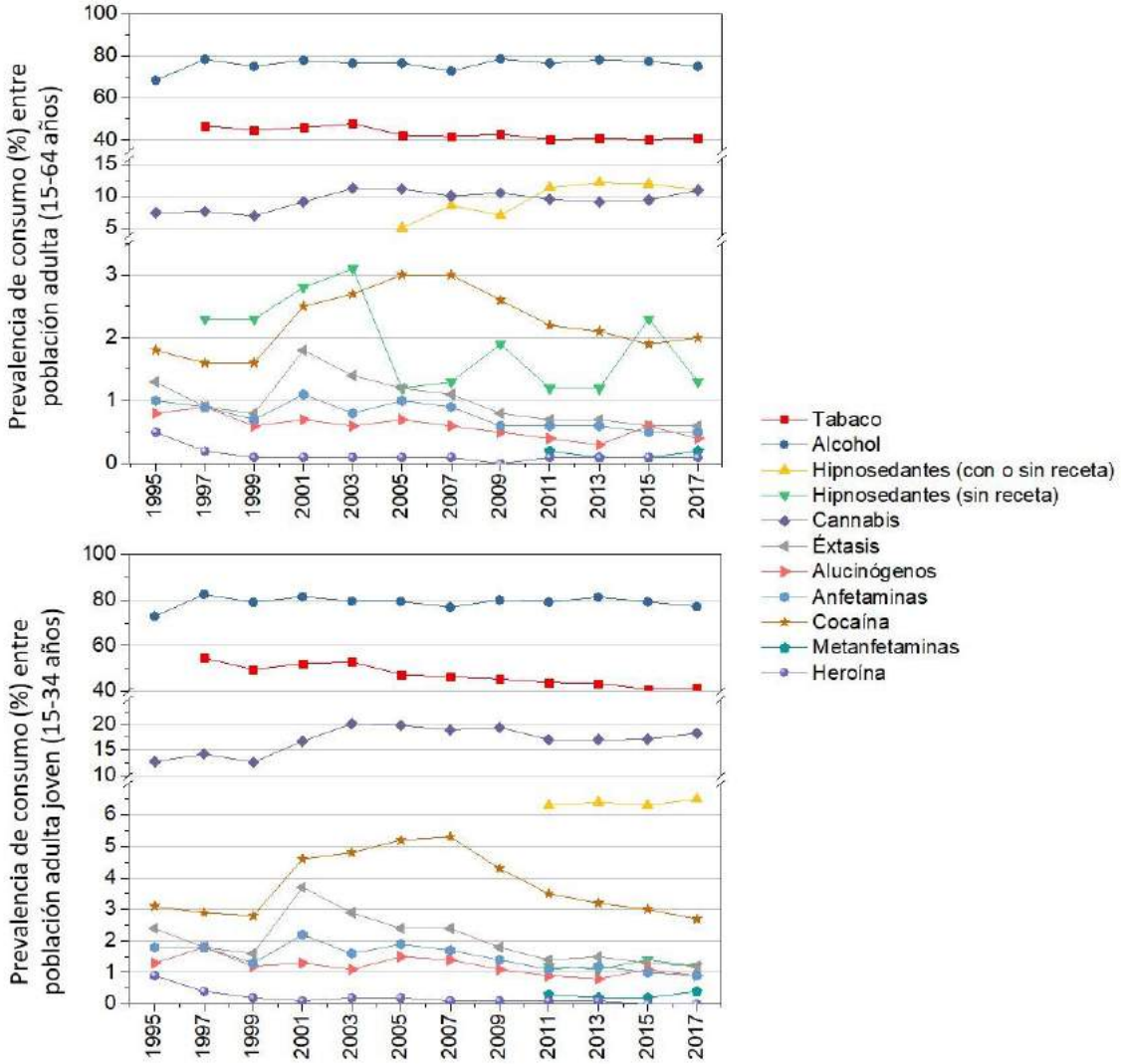


Figura 3. Prevalencia de consumo de sustancias psicoactivas en España durante el último año entre la población adulta (15-64 años) (imagen superior) y la población adulta joven (15-34 años) (imagen inferior). Fuente: (OEDA, 2019).

Por último, la heroína, es la droga ilegal que presenta menor prevalencia de consumo en el último año situándose en torno al 0,1%, valor que se ha mantenido constante desde el año 2000 (Figura 3). Aunque históricamente ha sido la droga ilegal que más problemas ha causado en España, desde mediados de los años 90, tanto su consumo, como los problemas asociados a su consumo y las admisiones a tratamiento, han disminuido, siendo éstas 3 veces inferiores a las registradas a principios de los 90. A raíz del aumento del consumo de opioides sintéticos en Europa y en el mundo en general, el OEDA incluyó en sus cuestionarios, información sobre el consumo de opioides con efectos analgésicos. En España, la prevalencia de consumo de estas sustancias es del 6,7%, siendo el rango de edad en que más se consumen entre 35-64 años. La codeína es el analgésico opioide más consumido (4,2% de prevalencia de consumo durante el

último año), seguido del tramadol (2,3%) y la morfina (0,7%). Los consumidores de analgésicos opioides manifiestan haber tomado también otras drogas ilegales como el cannabis, la cocaína, el éxtasis y las anfetaminas.

En cuanto a las sustancias psicoactivas legales, el alcohol es la que tiene mayor prevalencia de consumo en el último año, siendo en 2017 del 75,2% entre la población adulta, dato que desde principios de los años 2000 se encuentra bastante estable (Figura 3). Aunque la prevalencia de intoxicaciones etílicas sigue una tendencia descendiente desde el año 2009, en España es especialmente preocupante el consumo de alcohol en atracón o *Binge drinking* (consumo de 4 ó 5 bebidas alcohólicas seguidas en un intervalo de dos horas, para mujeres y hombres, respectivamente). Este tipo de consumo de alcohol se da principalmente entre la población entre 20 y 29 años, y desde el año 2005, su prevalencia se ha triplicado, situándose en 2017 en el 15,1%. El alcohol es el responsable del 35,5% admisiones a tratamiento, se encuentra en gran parte de los patrones de policonsumo y en la actualidad se estima que el 5,1% de la población son consumidores de riesgo.

Tras alcohol, el tabaco es la sustancia psicoactiva legal que presenta una prevalencia de consumo mayor, siendo en 2017 entre la población adulta de 40,9%, valor que se ha mantenido bastante estable desde el año 2005.

La tercera sustancia psicoactiva legal con mayor prevalencia de consumo son los hipnosedantes (tranquilizantes, sedantes y somníferos) siendo la prevalencia de consumo en el último año entre la población entre 15-64 años del 11,1%, aunque si atendemos a datos de consumo (Figura 2), los antidepresivos serían los psicofármacos más consumidos (62 DDD_{pm}), seguidos de los ansiolíticos (31 DDD_{pm}), analgésicos (20 DDD_{pm}), e hipnóticos y sedantes (19 DDD_{pm}). El consumo de psicofármacos ha aumentado desde el 2000, triplicándose el consumo de antidepresivos, duplicándose el de analgésicos, hipnóticos y sedantes y aumentando en un 25% el de ansiolíticos (Figura 2). De acuerdo con el informe de OEDA, los hipnosedantes son consumidos por mayor número de mujeres que de hombres y empiezan a consumirse a una edad media más alta (34 años). A pesar de que su uso debe estar autorizado por un médico, muchas veces son consumidos sin receta. La prevalencia de uso de hipnosedantes sin receta se sitúa en torno a 1,3%, dato que se ha mantenido estable desde el año 2005, a excepción de dos repuntes que se produjeron en 2009 y 2015. Los hipnosedantes sin receta están asociados al policonsumo, y muchos de los consumidores de hipnosedantes sin receta los han consumido junto con alcohol y drogas ilegales con el objetivo de aumentar o disminuir los efectos psicoactivos de estos.

En general, la prevalencia de consumo de drogas y psicofármacos en España es mayor en hombres que en mujeres y el rango de edad en el que su consumo es mayor es de 15-34 años (Figura 3), excepto en el caso de alcohol y tabaco, que presentan prevalencias de consumo similares, independientemente del rango de edad, y de los hipnosedantes y la heroína, cuyo uso está más extendido entre la población de mayor edad (34-65 años).

En cuanto a las NSPs en España, su uso no está muy extendido y sólo el 1,1% de la población entre 15-64 años manifiesta haberlas consumido alguna vez en la vida. Su consumo está más restringido a población comprendida entre 25-34 años, y el perfil del consumidor de NSPs es una persona que ha consumido alguna vez en la vida otras sustancias psicoactivas, principalmente cannabis, pero también cocaína, alucinógenos, anfetaminas, éxtasis y alcohol.

1.3. Drogas y psicofármacos como contaminantes emergentes ambientales

Los contaminantes emergentes son contaminantes previamente desconocidos o no reconocidos como tal, cuya presencia en el medio ambiente no es necesariamente nueva, pero sí la preocupación por las posibles consecuencias tanto para los ecosistemas como para la salud (Richardson, 2012).

Las drogas y psicofármacos, junto con sus metabolitos, constituyen uno de los grupos reconocidos como contaminantes emergentes, ya que su elevada producción y consumo y su continua introducción en el medio ambiente han dado lugar a que tengan un carácter pseudo-persistente en él.

El desarrollo en las últimas décadas de metodologías analíticas más selectivas y sensibles, basadas en cromatografía de líquidos o de gases acoplada a espectrometría de masas, ha permitido identificar y cuantificar la presencia tanto de drogas como de psicofármacos en el medio ambiente. Debido a su carácter, en general, polar o medianamente polar, la mayoría de estudios se han centrado en investigar su presencia en matrices acuosas, y menor atención se ha puesto en matrices sólidas, como pueden ser sedimentos, lodos o biota. En las siguientes secciones se muestran los trabajos en los que estos compuestos han sido estudiados tanto en matrices acuosas como sólidas, así como los efectos toxicológicos que la presencia de estos compuestos, ya sea a corto o largo plazo, puede tener sobre los ecosistemas acuáticos.

1.3.1. Presencia de drogas y psicofármacos en diferentes matrices ambientales

1.3.1.1. Drogas y psicofármacos en el medio ambiente acuático

La principal vía de introducción de las drogas y psicofármacos en el medio ambiente acuático es a través de las aguas residuales, tras su consumo y posterior excreción. Las drogas y psicofármacos, una vez consumidos, se metabolizan total o parcialmente en el cuerpo humano y se excretan a través de la orina y las heces, bien en forma del compuesto padre o en forma de metabolito. Estos productos de excreción son transportados junto con las aguas residuales urbanas a través de los sistemas de alcantarillado hasta las estaciones depuradoras de aguas residuales (EDARs), donde estos compuestos sólo son eliminados parcialmente (Devault y cols., 2017), y como resultado son liberados continuamente al medio ambiente acuático a través de los efluentes de las EDARs.

Niveles de drogas y psicofármacos en agua residual

La presencia de drogas y psicofármacos en agua residual ha sido estudiada globalmente, tal y como se refleja en las revisiones bibliográficas realizadas por Asimakopoulos y cols. (2016) y Yadav y cols. (2017). Su presencia en agua residual ha sido ampliamente investigada en numerosos países de Europa como España (Arbeláez y cols., 2014, 2015; Bijlsma y cols., 2009; González-Mariño y cols., 2010, 2012a, 2018; Huerta-Fontela y cols., 2007, 2008a; Mastroianni y cols., 2017; Postigo y cols., 2008, 2010; Racamonde y cols., 2014, 2015), Italia (Castiglioni y cols., 2006; Mari y cols., 2009; Zuccato y cols., 2008b), Bélgica (Gheorghe y cols., 2008; van der Ven y cols., 2004; van Nuijs y cols., 2011, 2009a), Croacia (Senta y cols., 2013), Francia (Karolak y cols., 2010; Nefau y cols., 2013), Alemania (Hummel y cols., 2006; Ternes, 1998), Países Bajos (Bijlsma y cols., 2012; van der Aa y cols., 2013), Suiza (Berset y cols., 2010), Eslovaquia (Mackul'ak y cols., 2016, 2014), Reino Unido (Baker y cols., 2014, 2013, 2011a; Bones y cols., 2007; Kasprzyk-Hordern y cols., 2009), República Checa (Baker y cols., 2012) y Portugal (Sousa y cols., 2011), así como en países de Oceanía (Du y cols., 2020; Irvine y cols., 2011; Kumar y cols., 2019; Lai y cols., 2013a; Yadav y cols., 2019), América (Asimakopoulos y cols., 2017; Bartelt-Hunt y cols., 2009; Chiaia y cols., 2008; Foppe y cols., 2018; Jones-Lepp y cols., 2004; Skees y cols., 2018; Snyder y cols., 2001), Asia (Deng y cols., 2020; Kim y cols., 2020; Nguyen y cols., 2018; Subedi y cols., 2015a; Xiaohan Zhang y cols., 2019) y África (Moslah y cols., 2018).

La Figura 4 muestra los rangos de concentraciones en los que estos compuestos han sido identificados en el agua residual y la Tabla AIV-1 en el Anexo IV muestra los rangos de concentraciones medidos de cada compuesto en diferentes países.

Las concentraciones de drogas y psicofármacos encontradas en agua residual suelen ir desde unos pocos nanogramos por litro (ng/L) hasta varios microgramos por litro (µg/L). En general los niveles máximos encontrados en agua residual no suelen sobrepasar los 20 µg/L, a excepción de los niveles del metabolito del alcohol, el sulfato de etilo, y del estimulante cafeína que han llegado a medirse a concentraciones máximas de 71 µg/L en Alemania (Ryu y cols., 2016) y 209 µg/L en España (Huerta-Fontela y cols., 2008a), respectivamente. También en algunas ocasiones, como es el caso de los niveles encontrados de MDMA en Castellón (España) tanto en el agua residual de entrada (27,5 µg/L) como de salida (21,2 µg/L), y de citalopram en India en agua residual de salida (820 µg/L), los niveles medidos superaron los 20 µg/L. Sin embargo, estos niveles tan elevados se debieron a la celebración de un festival de música (Bijlsma y cols., 2009) y a que la EDAR recibía las aguas residuales de varias fábricas dedicadas a la fabricación de medicamentos (Larsson y cols., 2007), respectivamente.

En general, en las aguas residuales de entrada, las drogas y psicofármacos que suelen encontrarse a mayor concentración son la cocaína y su metabolito la benzoilecgonina, los ETAs anfetamina, metanfetamina y MDMA, los opiáceos codeína, metadona y EDDP, los estimulantes cafeína y efedrina, el metabolito del alcohol, los antidepresivos citalopram, fluoxetina y su metabolito nor-fluoxetina, y las benzodiazepinas bromazepam, lorazepam y oxazepam, todos ellos encontrados a concentraciones máximas superiores a 3 µg/L.

Por su parte en las aguas residuales de salida, en general, las drogas no suelen encontrarse a niveles superiores a 1 µg/L, a excepción de los niveles de cafeína, que suelen ser mayores (desde 60 ng/L (Nguyen y cols., 2018) hasta 35 µg/L (Moslah y cols., 2018)) debido a que es un estimulante ampliamente consumido, ya sea en forma de café, de té o de bebidas de soda, y su elevada polaridad, lo cual resulta en una eliminación en planta poco eficaz. En el caso de los psicofármacos, sí que es común encontrarlos a concentraciones por encima de 1 µg/L, aunque en algunos casos, como en el de nor-fluoxetina (9810 ng/L) en Arabia Saudí (Shraim y cols., 2017), bromazepam (15542 ng/L) en España (Huerta-Fontela y cols., 2010), diazepam (4000 ng/L) en Estados Unidos (Phillips y cols., 2010), oxazepam (7434 ng/L) en Francia (Bebianno y cols., 2016), y los ya citados niveles de citalopram (820 µg/L) en India, las altas concentraciones encontradas en agua residual de salida se deben a que las aguas analizadas proceden de EDARs donde, además de agua residual doméstica, se trata agua residual procedente de industrias y/u hospitales.

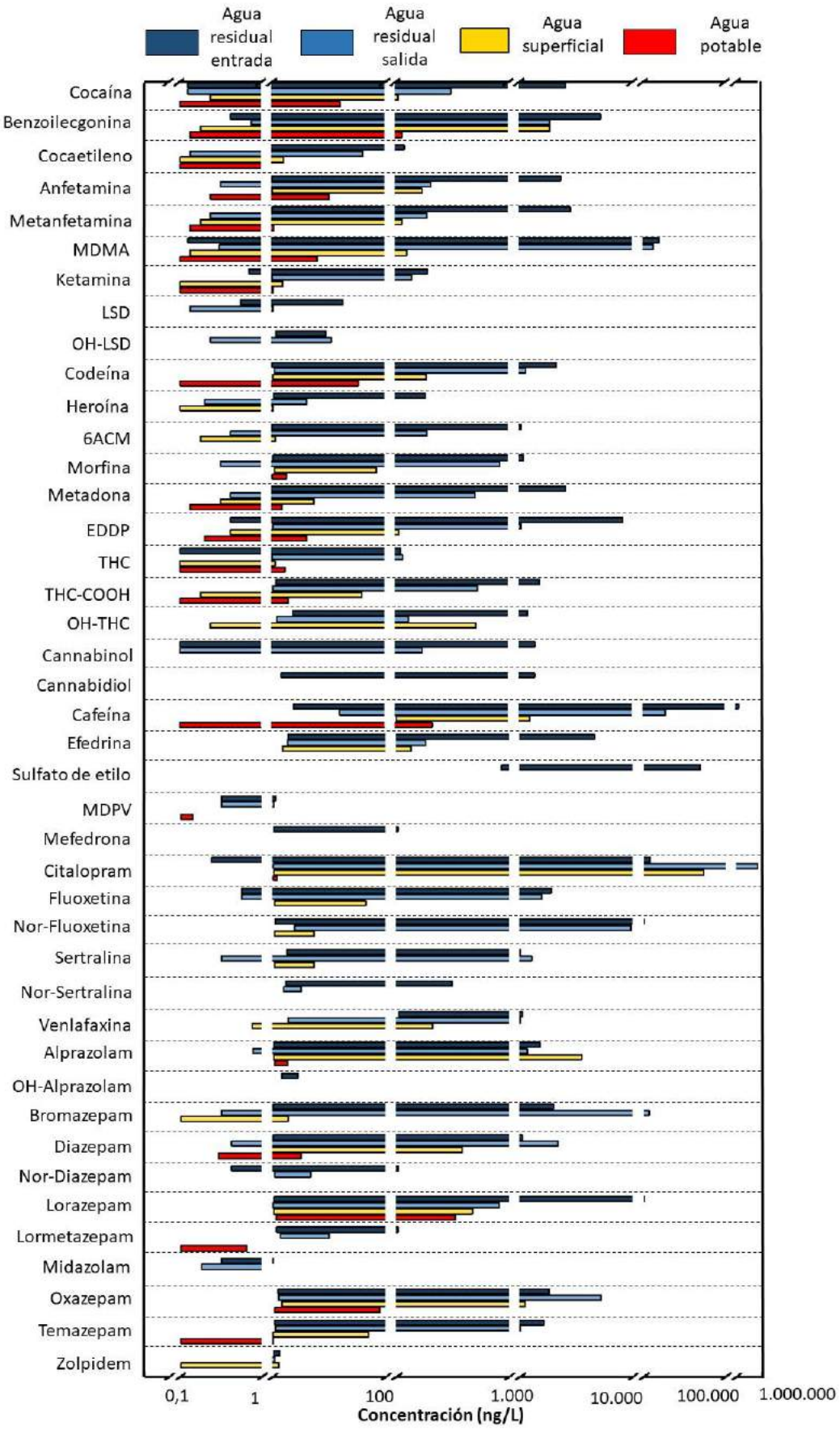


Figura 4. Concentraciones de drogas, psicofármacos y metabolitos en aguas.

Eficacia de eliminación de drogas, psicofármacos y sus metabolitos en las EDARs

La presencia de drogas, psicofármacos y sus metabolitos en agua residual de salida, indica que estos compuestos no se eliminan en su totalidad en las EDARs, ya que éstas fueron inicialmente diseñadas para la eliminación de contaminantes presentes a concentraciones más altas, del orden de mg/L. La eficacia de eliminación de estos compuestos depende de varios factores, como las propiedades físico-químicas de los compuestos y su biodegradabilidad, los tratamientos aplicados en las EDARs, la composición de las aguas residuales, e incluso las condiciones meteorológicas, como lluvias o altas temperaturas, que podrían afectar a la dilución de los efluentes o a la degradación de los compuestos, respectivamente (Devault y cols., 2017). Los tratamientos aplicados en las EDARs incluyen, según los casos, un tratamiento primario, con el que se eliminan los componentes más grandes mediante el empleo de barras, rejas o mediante decantación; un tratamiento secundario, tratamiento biológico basado en *lodos activados* o *filtros biológicos*, diseñado principalmente para reducir la demanda biológica de oxígeno, sólidos suspendidos o el exceso de nutrientes, y también reducir la concentración de contaminantes orgánicos; y en las más avanzadas un tratamiento terciario basado, tanto en tratamientos convencionales (filtración, clarificación, filtración con arena o ultra/micro filtración), como en métodos avanzados (procesos de desinfección con hipoclorito de sodio, luz UV u ozonación, adsorción con carbono activo granular y otros procesos como osmosis inversa, ultrafiltración, y reactores de membranas biológicas entre otros), que se utilizan para mejorar la eliminación de los contaminantes orgánicos (Asimakopoulos y cols., 2016).

La eficacia de eliminación de las drogas fue revisada por Devault y cols. (2017), definiendo tres grupos en función de su eliminación:

- Un primer grupo en el que se incluirían cocaína y sus metabolitos, morfina, anfetamina, THC y su metabolito OH-THC, que en la mayoría de trabajos realizados presentan porcentajes de eliminación entre el 70 y el 100%, siendo el tratamiento secundario con lodos activados más eficaz que el de los filtros biológicos (Baker y cols., 2013; Bijlsma y cols., 2012; Boleda y cols., 2009; Castiglioni y cols., 2006; Gerrity y cols., 2011; Karolak y cols., 2010; Nefau y cols., 2013; Postigo y cols., 2010; Subedi y cols., 2014).
- Un segundo grupo de compuestos que incluiría metadona y EDDP, que presentan porcentajes de eliminación mediante el empleo de lodos activados inferiores al 50% e incluso negativos (mayores concentraciones en agua residual de salida que de entrada), indicando que estos compuestos se eliminan rara vez en las EDARs (Baker y cols., 2013; Bijlsma y cols., 2012; Castiglioni y cols., 2006; Nefau y cols., 2013; Subedi y cols., 2014).

- Por último, un tercer grupo que incluiría 6-monoacetilmorfina, metanfetamina, MDMA y sus análogos (MDA y MDEA), y THC-COOH, que se caracterizarían por presentar unos porcentajes de eliminación muy variables que van desde valores negativos hasta 100% de eliminación (Andrés-Costa y cols., 2014; Baker y cols., 2013; Bartelt-Hunt y cols., 2009; Bijlsma y cols., 2012; Boleda y cols., 2009; C. Metcalfe y cols., 2010; Postigo y cols., 2010; Subedi y cols., 2014).

En lo que respecta a los psicofármacos, la eficacia de eliminación de varios antidepresivos (amitriptilina, citalopram, bupropian, sertralina y venlafaxina) se estudió en Estados Unidos en 7 EDARs equipadas con diferentes tratamientos secundarios y terciarios (Angeles y cols., 2020). Las EDARs que poseían tratamientos secundarios y terciarios convencionales (lodos activados, reactores aerobios/anaerobios) eran incapaces de eliminar los antidepresivos de las aguas residuales, variando los porcentajes de eliminación desde valores negativos hasta un máximo del 35%. Sin embargo, el uso de tratamientos terciarios avanzados, como carbón activo granulado u ozonización, permitió su eliminación completa. Similares resultados fueron encontrados en EDARs en China, donde el empleo de sistemas convencionales como lodos activados o tratamientos anaerobio-anóxico-aerobio (A^2O), mostró una eliminación ineficaz de antidepresivos, desde -4% hasta 6,5% en el caso de la venlafaxina, y del 23-69% en el caso de otros antidepresivos (paroxetina, citalopram, fluvoxamina, fluoxetina, amitriptilina, sertralina) (Cao y cols., 2020). También se han reportado porcentajes de eliminación negativos para la venlafaxina en otras EDARs equipadas con tratamientos secundarios basados en lodos activados o filtros biológicos (Kasprzyk-Hordern y cols., 2010, 2012) y tratamientos A^2O seguidos de tratamientos terciarios basados en sedimentación u ozonización (Duan y cols., 2018).

Al igual que ocurre con los antidepresivos, la eliminación de las benzodiazepinas en las plantas depuradoras varía en función de los tratamientos empleados. Así, con el empleo de métodos tradicionales, sólo se consigue una eliminación parcial (entre el 30 y el 70%), mientras que el empleo de métodos avanzados, principalmente filtración con carbón activo, permite la eliminación de la totalidad de la benzodiazepinas que llegan a las EDARs (Martin Ruel y cols., 2011).

Aunque el empleo de tratamientos convencionales y avanzados de agua residual permite reducir, al menos en parte, la presencia de contaminantes emergentes en las aguas residuales, un inconveniente de estos métodos es que durante los mismos se pueden generar subproductos o productos de transformación, de los cuales se sabe incluso menos que de los compuestos originales. En este sentido, Bijlsma y cols. (2013) identificaron 16 productos de transformación

de la cocaína y 10 productos de transformación de la benzoilecgonina después de experimentos de degradación realizados a escala de laboratorio basados en hidrólisis, cloración y fotodegradación mediante luz UV. Postigo y cols. demostraron la formación de diferentes productos de transformación de la cocaína (Postigo y cols., 2011b) y de la metadona (Postigo y cols., 2011c) durante procesos naturales (hidrólisis y fotodegradación con luz solar) y tras el tratamiento del agua con métodos avanzados (fotocatálisis heterogénea con dióxido de titanio (TiO_2) y fotocatálisis homogénea con foto-Fentón). Russo y cols. (2016) también demostraron la formación de productos de transformación de la cocaína tras aplicar un tratamiento basado en fotólisis con radiación UV en combinación con peróxido de hidrógeno. González-Mariño y cols. (2013) identificaron 7 productos de transformación del THC-COOH (González-Mariño y cols., 2013), 4 productos de transformación de cocaína (González-Mariño y cols., 2012b) y 8 productos de transformación de metadona y 3 de EDDP (González-Mariño y cols., 2015) tras el tratamiento de agua con cloro, mientras que Boix y cols. (2014) detectaron 19 productos de transformación también del THC-COOH tras la aplicación de diferentes procesos de tratamiento como hidrólisis, cloración, y fotodegradación con radiación UV y con radiación solar.

Niveles de drogas y psicofármacos en agua superficial, subterránea y agua potable

La eliminación incompleta de drogas y psicofármacos tras el tratamiento del agua residual ha dado lugar a que estos compuestos lleguen a las aguas superficiales a través de los efluentes de las EDARs. Numerosos estudios llevados a cabo en España (Acuña y cols., 2015; Boix y cols., 2015; Boleda y cols., 2009, 2007; Catalá y cols., 2015; Esteban y cols., 2012; González-Mariño y cols., 2019; González Alonso y cols., 2010; Gros y cols., 2012; Huerta-Fontela y cols., 2008a, 2007, 2011; López-Serna y cols., 2010; Martínez Bueno y cols., 2011; Mastroianni y cols., 2016; Mendoza y cols., 2014; Moreno-González y cols., 2014; Postigo y cols., 2010; Proia y cols., 2013; Racamonde y cols., 2012; Rodríguez-Gil y cols., 2010; Silva y cols., 2011; Valcárcel y cols., 2012; Vazquez-Roig y cols., 2010a), Reino Unido (Baker y cols., 2013; Jones-Lepp y cols., 2004; Kasprzyk-Hordern y cols., 2008a; Zuccato y cols., 2008a), Italia (Calamari y cols., 2003; Zuccato y cols., 2008a), Francia (Camilleri y cols., 2015; Coetsier y cols., 2009; Piel y cols., 2013), Bélgica (Gheorghe y cols., 2008; van Nuijs y cols., 2009b), Alemania (Hass y cols., 2012; Hummel y cols., 2006; Schlüsener y cols., 2015), Suiza (Berset y cols., 2010), Países Bajos (van der Aa y cols., 2013), Eslovenia (Kosjek y cols., 2012), Estados Unidos (Ferrer y cols., 2012; Schultz y cols., 2008; Skees y cols., 2018), Costa Rica (Causanilles y cols., 2017c), Canadá (Lajeunesse y cols., 2008; C. D. Metcalfe y cols., 2010), Brasil (de Almeida y cols., 2015), China (Heeb y cols., 2012; Shao y

cols., 2009) e India (Fick y cols., 2009) han mostrado la presencia de drogas, psicofármacos y metabolitos en aguas superficiales, incluyendo ríos, lagos, agua marina o arroyos. La Figura 4 muestra el rango de concentraciones entre las que se han detectado las drogas y psicofármacos en agua superficial, y la Tabla AIV-1 del Anexo IV el rango de concentraciones encontrado en función del país investigado. Estas concentraciones son más bajas que las encontradas en aguas residuales de entrada y de salida y, por lo general, no superan los 500 ng/L, a excepción de algunos compuestos como benzoilecgonina y lorazepam, encontrados en el río Llobregat (España) a 1350 ng/L (Huerta-Fontela y cols., 2008b) y 705 ng/L (Proia y cols., 2013), respectivamente, cafeína, encontrada a 1715 ng/L en un río a 1 km de la descarga de una EDAR en Inglaterra (Baker y cols., 2013), citalopram, encontrado a 76 µg/L en el arroyo Isakavagu (India) donde se descargan los efluentes de una EDAR que recibía el agua de varias industrias farmacéuticas (Fick y cols., 2009), alprazolam, encontrado en el río Cascavel (Brasil) a una concentración de 5900 ng/L (Nunes y cols., 2015), diazepam, encontrado a 625 ng/L en aguas superficiales que recibían el efluente de un hospital en Brasil, y oxazepam, encontrado a 1400 ng/L en el río Vilaine (Francia).

Las aguas superficiales contaminadas y las aguas residuales no tratadas pueden contribuir a la contaminación del agua subterránea, que en muchos países es utilizada, junto con el agua superficial, para la producción de agua potable. Jurado y cols. (2012) detectaron cocaína (máxima concentración 60 ng/L), benzoilecgonina (20 ng/L), cocaetileno (1,8 ng/L), morfina (27 ng/L), metadona (68 ng/L), EDDP (8,2 ng/L), MDMA (37 ng/L), efedrina (7,3 ng/L), diazepam (19 ng/L), alprazolam (6,4 ng/L) y lorazepam (38 ng/L) en el agua subterránea que fluye por debajo de la ciudad de Barcelona. En otros estudios se demostró la presencia de citalopram en agua subterránea a concentraciones entre 13 ng/L (Valhondo y cols., 2014) y 1400 ng/L (Fick y cols., 2009), de diazepam entre 3,9 ng/L (Cabeza y cols., 2012) y 35 ng/L (López-Serna y cols., 2013), de lorazepam entre 4 ng/L (Kovačević y cols., 2017) y 54 ng/L (López-Serna y cols., 2013) y de oxazepam entre 10 ng/L (Manamsa y cols., 2016) y 210 ng/L (Bekele y cols., 2011).

La presencia de drogas también se ha demostrado en agua potable, donde se ha detectado cocaína (máxima concentración 60 ng/L), benzoilecgonina (150 ng/L), cocaetileno (0,2 ng/L), anfetamina (50 ng/L), metanfetamina (2 ng/L), MDMA (40 ng/L), MDA (20 ng/L), morfina (12 ng/L), codeína (76 ng/L), THC-COOH (4,8 ng/L), EDDP (31 ng/L) y metadona (9 ng/L), aunque según los autores estas concentraciones no suponen un riesgo para la salud humana e incluso se podrían eliminar casi en su totalidad con tratamientos de potabilización avanzados como ultrafiltración u ósmosis inversa (Huerta-Fontela y cols., 2008b; Mendoza y cols., 2014; Rosa

Boleda y cols., 2011; Valcárcel y cols., 2012). Dado que la cloración es el método de desinfección más extendido en la producción de agua potable, la formación de productos de desinfección al reaccionar el cloro con las drogas y psicofármacos que han sobrevivido al tren de tratamientos de potabilización también es digna de estudio. En este sentido, numerosos autores han investigado los subproductos de desinfección que se forman durante la cloración de la cocaína (González-Mariño y cols., 2012b), de diferentes ETAs (Huerta-Fontela y cols., 2012), de metadona (González-Mariño y cols., 2015), de citalopram (Osawa y cols., 2019) y de diferentes benzodiazepinas como oxazepam y diazepam (Carpinteiro y cols., 2017; Yang y cols., 2018; Xin Zhang y cols., 2019).

1.3.1.2. Drogas y psicofármacos en matrices ambientales sólidas

La mayoría de los estudios ambientales de drogas, psicofármacos y sus metabolitos se han centrado en estudiar su presencia en aguas. Su estudio en matrices ambientales sólidas ha sido mucho más escaso. La Tabla 2 muestra las concentraciones de drogas y psicofármacos medidas en tres tipos de matrices sólidas (sedimentos, lodos de depuradoras y material particulado) en estudios llevados a cabo en España (Álvarez-Ruiz y cols., 2015; Arbeláez y cols., 2014; Mastroianni y cols., 2013), Estados Unidos (Jones-Lepp y cols., 2007; Klosterhaus y cols., 2013; Subedi y cols., 2015b), Reino Unido (Baker y cols., 2011b; Evans y cols., 2015; Langford y cols., 2011; Wilkinson y cols., 2018), Croacia (Senta y cols., 2013), Grecia (Gago-Ferrero y cols., 2015), China (Hu y cols., 2019), Austria (Kaleta y cols., 2006), Brasil (Beretta y cols., 2014) y Arabia Saudí (Picó y cols., 2020).

Los compuestos que se encontraron a mayor concentración fueron opioides/opiáceos, cannabinoides y antidepresivos. Codeína y metadona se encontraron a concentraciones máximas de 685 y 602 ng/g, respectivamente, en el material particulado de una EDAR en Valencia, España (Álvarez-Ruiz y cols., 2015) mientras que EDDP se encontró a concentraciones máximas de 232 y 279 ng/g en lodos de depuradoras situadas en Valencia (Arbeláez y cols., 2014) y Barcelona (Mastroianni y cols., 2013), respectivamente. El THC se encontró a 664 ng/g en sedimentos recogidos en el Río Turia (Valencia, España) (Carmona y cols., 2014) y a 579 ng/g en lodos de depuradora en Barcelona (Mastroianni y cols., 2013), mientras que el cannabidiol se encontró a 479 ng/g en lodos de depuradora en Barcelona. En cuanto a los antidepresivos, el citalopram se encontró a 317 y 283 ng/g en lodos de EDARs situadas en Escocia (Langford y cols., 2011) y Estados Unidos (Subedi y cols., 2015b), respectivamente, mientras que la sertralina se

encontró a una concentración de hasta 1490 ng/g en lodos de una EDAR situada en Estados Unidos (Subedi y cols., 2015b). La presencia de estos compuestos en matrices ambientales sólidas no es de extrañar ya que presentan un carácter marcadamente hidrofóbico (log K_{ow} > 3) (Tabla 1), que les hacen más propensos a adsorberse en matrices sólidas. El resto de compuestos presentaron concentraciones inferiores a 200 ng/g, siendo las concentraciones más habituales del orden de unos pocos ng/g.

Tabla 2. Niveles de drogas y psicofármacos en matrices ambientales sólidas.

	País	Sedimentos	Lodos depuradora	Material particulado	Ref.
Cocaína	China	n.d.-10			[1]
	Croacia		<0,1-37	<1,2-10	[2]
	España (Cataluña)		3,8-4,7		[3]
	España (Cataluña)		0,7-23		[4]
	Estados Unidos	n.d.-2,2			[5]
	España (C. Valenciana)	<LOD-1,0	4,0-58	14-127	[6]
	Grecia		< 4,5		[7]
	Reino Unido			1,8-2,7	[8]
Benzoilecgonina	China	n.d.-3,1			[1]
	Croacia		<0,5-11	<0,5-7,4	[2]
	España (Cataluña)		3,5-19		[3]
	España (Cataluña)		0,8-32		[4]
	España (C. Valenciana)	n.d.-1,0	n.d.-4,0	3,0-258	[6]
	Grecia		<2,3		[7]
	Inglaterra	<1,0-1,3		n.d.	[9]
	Reino Unido		0,1-1,1		[8]
Cocaetileno	España (Cataluña)		n.d.		[4]
	España (C. Valenciana)	n.d.	n.d.-1,0	6,0-51	[6]
	Reino Unido			n.d.-0,2	[8]
Anfetamina	Austria		5,0-300		[10]
	China	n.d.-6,9			[1]
	Croacia		<3,5	<0,8	[2]
	Escocia	n.d.	n.d.		[11]
	España (Cataluña)		2,1-13,9		[4]
	Estados Unidos	<LOD-3,3			[5]
	Grecia		<1,5		[7]
	Inglaterra	<1,09-3,6		n.d.	[9]
	Reino Unido			n.d.-17	[8]
	Reino Unido		7,9		[12]
Metanfetamina	China	n.d.-9,1			[1]
	Estados Unidos	n.d.-4,0			[13]
	Reino Unido		3,2		[12]
MDMA	Croacia		<1,2-8,9	<0,5-5,0	[2]
	España (Cataluña)		0,4-2,7		[4]
	Grecia		<2,5		[7]
	Reino Unido			n.d.-0,7	[8]
	Reino Unido		16,3		[12]

n.d.: no detectado; <LOD: niveles por debajo del límite de detección; <LOQ: niveles por debajo del límite de cuantificación.

[1] (Hu y cols., 2019); [2] (Senta y cols., 2013); [3] (Arbeláez y cols., 2014); [4] (Mastroianni y cols., 2013); [5] (Klosterhaus y cols., 2013); [6] (Álvarez-Ruiz y cols., 2015); [7] (Gago-Ferrero y cols., 2015); [8] (Baker y cols., 2011b); [9] (Wilkinson y cols., 2018); [10] (Kaleta y cols., 2006); [11] (Langford y cols., 2011); [12] (Evans y cols., 2015); [13] (Jones-Lepp y cols., 2007).

Tabla 2 (cont). Niveles de drogas y psicofármacos en matrices ambientales sólidas.

	País	Sedimentos	Lodos depuradora	Material particulado	Ref.
Ketamina	China	n.d.-3,6			[1]
	España (C. Valenciana)	n.d.	2,0-3,0	n.d.-46	[6]
	Reino Unido			1,0-7,2	[8]
LSD	España (Cataluña)		n.d.		[4]
OH-LSD	España (Cataluña)		n.d.		[4]
Codeína	China	n.d.			[1]
	Croacia		3,3-109	20-51	[2]
	España (C. Valenciana)	n.d.	8,0-78	53-685	[6]
	España (C. Valenciana)	n.d.-3,6			[14]
	Grecia		<LOQ-21		[7]
	Reino Unido			59-240	[8]
Heroína	China	n.d.			[1]
	España (Cataluña)		n.d.		[4]
	Grecia		<0,6		[7]
6ACM	Croacia		<0,5	<0,5-2	[2]
	España (Cataluña)		n.d.		[4]
	Grecia		<4,6		[7]
	Reino Unido			n.d.	[8]
Morfina	China	n.d.-4,9			[1]
	Croacia		<2,6	6,4-24	[2]
	España (C. Valenciana)	n.d.	24-171	n.d.	[6]
	España (Cataluña)		2,2-19		[4]
	Grecia		<2,5		[7]
	Reino Unido			19-116	[8]
Metadona	China	n.d.			[1]
	Croacia		6,1-54	6,7-32	[2]
	España (C. Valenciana)	n.d.-1	24-171	21-602	[6]
	España(Cataluña)		7,7-32		[3]
	España (Cataluña)		6,7-111		[4]
	Reino Unido			19-58	[8]
EDDP	Croacia		10-125	46-190	[2]
	España (Cataluña)		9,7-232		[3]
	España (Cataluña)		8,7-279		[4]
	Reino Unido			30-194	[8]
THC	España (C. Valenciana)	n.d.-664			[15]
	España (Cataluña)		29-579		[4]
THC-COOH	Croacia		n.d.-8,5	n.d.-32	[2]
	España (C. Valenciana)	n.d.-93			[15]
OH-THC	Croacia		n.d.-10	n.d.-185	[2]
	España (Cataluña)		103-160		[4]
Cannabinol	España (Cataluña)		27-188		[4]
Cannabidiol	España (Cataluña)		32-479		[4]

Tabla 2 (cont). Niveles de drogas y psicofármacos en matrices ambientales sólidas.

	País	Sedimentos	Lodos depuradora	Material particulado	Ref.
Cafeína	Arabia Saudí	7,1-76			[16]
	Brasil	0,3-23			[17]
	Estados Unidos	<LOD-30			[5]
	Grecia		<LOQ-15		[7]
Efedrina	China	n.d.-8,4			[1]
	España (Cataluña)		0,6-44		[4]
	Grecia		<LOQ-75		[7]
	Reino Unido			n.d.	[8]
	Reino Unido		<LOD		[12]
Citalopram	Escocia	<2	<50-317		[11]
	Estados Unidos		11-283		[18]
	Grecia		110-168		[7]
Fluoxetina	Grecia		<LOQ-37		[7]
	Reino Unido			72-199	[8]
	Reino Unido		86		[12]
Sertralina	Estados Unidos		11-1490		[18]
	Grecia		20-108		[7]
Venlafaxina	Estados Unidos		1,0-129		[18]
	Grecia		<LOQ-36		[7]
	Reino Unido			2,9-15	[8]
	Reino Unido		83		[12]
Alprazolam	Arabia Saudí	n.d.-87			[16]
	España (Cataluña)		0,5-1,9		[4]
	Estados Unidos	n.d.-12			[18]
Diazepam	Brasil	<0,1-0,7			[17]
	España (Cataluña)		0,6-2,5		[4]
	España (C. Valenciana)	n.d.-1,2			[14]
	Estados Unidos		n.d.-3,3		[18]
	Reino Unido			n.d.	[8]
	Grecia		< 5,5		[7]
Lorazepam	Arabia Saudí	n.d.-126			[16]
	Estados Unidos		n.d.-12		[18]
Oxazepam	Estados Unidos		0,9-7,7		[18]
	Reino Unido			n.d.-5,2	[8]
Temazepam	Reino Unido			n.d.-5,9	[8]

n.d.: no detectado; <LOD: por debajo del límite de detección; <LOQ: por debajo del límite de cuantificación.

[1] (Hu y cols., 2019); [2] (Senta y cols., 2013); [3] (Arbeláez y cols., 2014); [4] (Mastroianni y cols., 2013); [5] (Klosterhaus y cols., 2013); [6] (Álvarez-Ruiz y cols., 2015); [7] (Gago-Ferrero y cols., 2015); [8] (Baker y cols., 2011b); [9] (Wilkinson y cols., 2018); [10] (Kaleta y cols., 2006); [11] (Langford y cols., 2011); [12] (Evans y cols., 2015); [13] (Jones-Lepp y cols., 2007); [14] (Vazquez-Roig y cols., 2012); [15] (Carmona y cols., 2014); [16] (Picó y cols., 2020); [17] (Beretta y cols., 2014); [18] (Subedi y cols., 2015b).

La presencia de estos compuestos en matrices sólidas, incluso a concentraciones bajas, indica que, por un lado, los lodos de depuradora constituyen otra ruta de entrada de este tipo de contaminantes al medio ambiente, ya que normalmente son utilizados en agricultura como fertilizantes (Mastroianni y cols., 2013), y por otro, que los sedimentos pueden actuar como reservorios de este tipo de contaminantes en el medio ambiente acuático, pudiendo ser bioacumulados por los organismos que viven o se alimentan en ellos.

La bioacumulación de drogas y psicofármacos en organismos acuáticos depende de varios factores como la tendencia del propio compuesto a bioacumularse (Log K_{ow} > 3), las tasas de metabolización de los compuestos en cada organismo, o las cinéticas de adsorción y eliminación que dependen tanto del compuesto como del organismo investigado (Huerta y cols., 2012).

Los peces constituyen uno de los organismos en los que la bioacumulación de drogas y psicofármacos ha sido más investigada, ya que estos organismos se pueden encontrar prácticamente en todas partes del medio ambiente acuático (Huerta y cols., 2012). Otro tipo de organismos ampliamente estudiado son los bivalvos, concretamente los mejillones, ya que son organismos bentónicos que se alimentan por filtración y pueden acumular los contaminantes presentes tanto en la fase acuosa como adsorbidos en las matrices sólidas (Fabbri y cols., 2016), y de hecho se utilizan como bioindicadores para la monitorización de la contaminación del agua costera (Beyer y cols., 2017). La Tabla 3 muestra las concentraciones de drogas, psicofármacos y metabolitos en estos tipos de organismos. Mientras que la bioacumulación de drogas en organismos acuáticos ha estado limitada al estudio de muy pocas drogas o metabolitos (cocaína, benzoilecgonina y anfetamina), la bioacumulación de psicofármacos, y más concretamente la de antidepresivos, ha sido más ampliamente investigada. La concentración de estos compuestos en organismos acuáticos va desde valores por debajo del límite de detección del método correspondiente, hasta valores del orden de centenas de ng/g. La mayoría de estudios publicados se han desarrollado en Estados Unidos, como el realizado en la Bahía de San Francisco (Maruya y cols., 2014), en diferentes arroyos de Colorado, Iowa, Carolina del Norte o Texas (Bringolf y cols., 2010; Schultz y cols., 2008), y en Canadá, concretamente en el Río Grande (de Solla y cols., 2016; C. D. Metcalfe y cols., 2010) o en la costa de Victoria, cerca de la desembocadura de efluentes de aguas residuales no tratadas (Krogh y cols., 2017). En Europa, diferentes estudios se han llevado a cabo en España, concretamente en el Delta del Ebro (Álvarez-Muñoz y cols., 2015) y en el Mar Menor (Moreno-González y cols., 2016), así como en Portugal (estuario del Río Tajo), Italia (Río Po) (Álvarez-Muñoz y cols., 2015), y Francia (Martínez Bueno y cols., 2014).

Por último, dado que las drogas y psicofármacos son sustancias diseñadas para actuar sobre una parte concreta del organismo, los estudios de bioacumulación deberían considerar los mecanismos de acción de estos compuestos en humanos o animales, ya que podrían indicar la parte del organismo acuático en que es más probable que se produzca su bioacumulación. Hay estudios que han investigado la bioacumulación de estos compuestos utilizando el organismo completo (C. D. Metcalfe y cols., 2010). Sin embargo, otros han demostrado que la bioacumulación de drogas y psicofármacos varía en función de la parte del organismo estudiada. Ondarza y cols. (2019) determinaron que la bioacumulación de benzoilecgonina era mayor en branquias que en músculo. En el mismo estudio los niveles encontrados de cafeína fueron mayores en branquias que en hígado y en músculo, y Brooks y cols. (2005) y Lajeunnesse y cols. (2011) determinaron que los niveles de algunos antidepresivos y sus metabolitos eran mayores en hígado y en cerebro con respecto a los medidos en el músculo.

Tabla 3. Niveles (ng/g) de drogas y psicofármacos en organismos acuáticos.

Compuesto	País	Pez		Mejillón		Ref.
		Media	Rango	Media	Rango	
Cocaína	Canadá				0,4-1,1	[1]
	Estados Unidos			0,3	n.d.-1,7	[2]
	Estados Unidos				n.d.-0,3	[3]
Benzoilecgonina	Argentina		n.d.-1,6			[4]
	Estados Unidos				n.d.	[3]
Anfetamina	Canadá				1,6-8,3	[1]
	Estados Unidos			2,3	n.d.-20	[2]
	Estados Unidos				n.d.-4,1	[3]
Codeína	Canadá				n.d.-15	[1]
	Uruguay		<LOD-1,1			[5]
Cafeína	Argentina		n.d.-13			[4]
	Estados Unidos				n.d.	[3]
	Estados Unidos				n.d.-140	[2]
	Singapur				<0,1-0,8	[6]
Citalopram	Canadá		<LOQ-2,9			[7]
	España			n.d.		[8]
	Estados Unidos		n.d.-0,2			[9]
	Italia			21		[8]
	Portugal				0,3-38	[10]
	Portugal		<LOQ	7,7		[8]
Desmetil citalopram	Canadá		n.d.-2,8			[7]
	Portugal				n.d.-50	[10]
Fluoxetina	Canadá		n.d.-1,0			[11]
	Canadá				2,6-9,0	[1]
	Canadá		<LOQ			[7]
	Estados Unidos				0,3-79,1	[12]
	Estados Unidos		0,1-1,6			[13]
	Estados Unidos		n.d.-1,6			[9]
	Portugal				0,5-63	[10]
Nor-fluoxetina	Argentina					[4]
	Canadá		n.d.-9,1		n.d.-2,0	[1]
	Canadá		<LOD-1,2			[7]
	Canadá		n.d.-1,1			[11]
	Estados Unidos		n.d.-3,5			[9]
	Estados Unidos		1,1-10,3			[13]
	Estados Unidos		n.d.-5			[14]
	Portugal				n.d.-63	[10]
Sertralina	Canadá				16-77	[1]
	Canadá				n.d.-84	[15]
	Canadá		n.d.-3,8			[7]
	Estados Unidos			1,4	n.d.-5,5	[2]

Tabla 3 (cont). Niveles (ng/g) de drogas y psicofármacos en organismos acuáticos.

Compuesto	País	Pez		Mejillón		Ref.
		Media	Rango	Media	Rango	
Sertralina	Estados Unidos				0,1-1,4	[3]
	Estados Unidos		0,3-4,3			[13]
	Estados Unidos		n.d.-4,2			[9]
	Estados Unidos		n.d.-19			[14]
Desmetil sertralina	Canadá		<LOD-1,5			[7]
	Estados Unidos		0,7-16			[13]
Nor-sertralina	Estados Unidos		n.d.-4,2			[9]
	Portugal				n.d.-13	[10]
Venlafaxina	Canadá				n.d.-25	[1]
	Canadá		n.d-1,2			[7]
	España			2,7		[8]
	España		n.d.-3,1			[16]
	Estados Unidos		n.d.-1,1			[9]
	Francia				n.d.-2,7	[17]
	Italia			36		[8]
	Portugal		<LOQ	7,7		[8]
	Uruguay		<LOD-1,6			[5]
Desmetil venlafaxina	Canadá		n.d-1,2			[7]
	España				<LOQ	[8]
	Francia				n.d.-3,7	[17]
	Italia			4,3		[8]
	Portugal			4,8		[8]
Alprazolam	España				<LOQ	[8]
	Italia				<LOQ	[8]
	Portugal				<LOQ	[8]
Diazepam	España		n.d.-15			[16]
	Estados Unidos				n.d.	[3]
	Portugal		n.d.			[8]
	Uruguay		<LOD-0,2			[5]
Lorazepam	Portugal		n.d.			[8]
	Uruguay		<LOD-0,7			[5]

n.d.: no detectado; <LOD: por debajo del límite de detección; <LOQ: por debajo del límite de cuantificación

[1] (de Solla y cols., 2016); [2] (Maruya y cols., 2014); [3] (Klosterhaus y cols., 2013); [4] (Ondarza y cols., 2019); [5] (Rojo y cols., 2019); [6] (Bayen y cols., 2016); [7] (C. D. Metcalfe y cols., 2010); [8] (Álvarez-Muñoz y cols., 2015); [9] (Schultz y cols., 2010); [10] (Silva y cols., 2017); [11] (Chu y cols., 2007); [12] (Bringolf y cols., 2010); [13] (Brooks y cols., 2005); [14] (Ramirez y cols., 2009); [15] (Krogh y cols., 2017); [16] (Moreno-González y cols., 2016); [17] (Martínez Bueno y cols., 2014).

1.3.2. Toxicidad ambiental de drogas y psicofármacos y sus metabolitos.

La presencia de drogas y psicofármacos en el agua superficial y su bioacumulación en organismos acuáticos ha provocado que en los últimos años se hayan llevado a cabo numerosos estudios para evaluar la toxicidad ocasionada por la exposición de estos compuestos biológicamente activos sobre los organismos acuáticos.

El efecto toxicológico producido por psicofármacos en organismos acuáticos se ha investigado ampliamente, siendo los antidepresivos del tipo ISRS uno de los grupos más estudiados. Trabajos recientes han demostrado que incluso a concentraciones ambientales normales, los antidepresivos tienen capacidad para afectar numerosos procesos biológicos como reproducción, crecimiento, metabolismo, inmunidad, alimentación, locomoción, aspecto físico y comportamiento (Fong y cols., 2014). Por ejemplo, la exposición de pececillos machos (*Pimephales promelas*) a 1000 ng/L de fluoxetina alteró el comportamiento de apareamiento de estos organismos (Weinberger y cols., 2014). La exposición del mejillón cebra (*D. polymorpha*) a 500 ng/L de fluoxetina, citalopram y la mezcla de estos dos antidepresivos causó, en los tres casos, alteraciones significativas del estado oxidativo de los bivalvos, mientras que la exposición únicamente a fluoxetina, indujo un ligero, pero significativo, aumento en las frecuencias celulares apoptóticas y necróticas (Magni y cols., 2017). En este sentido, la fluoxetina es considerada el antidepresivo del tipo ISRS con mayor toxicidad aguda (Silva y cols., 2015). La exposición a 300 ng/L de fluoxetina durante 96 horas y a 30 ng/L durante 48 horas provocó daños en el ADN de las branquias y de las glándulas digestivas del mejillón cebra, respectivamente (Cortez y cols., 2019). La exposición a concentraciones de fluoxetina de 1 ng/L y 100 ng/L afectó a la capacidad de camuflaje de la sepia *(Sepia officinalis*) y a su capacidad de aprendizaje y memoria (Di Poi y cols., 2014, 2013), y la exposición a 75 ng/L de fluoxetina durante dos semanas provocó la alteración de la capacidad reproductiva del mejillón cebra (*M. galloprovincialis*) (Gonzalez-Rey y cols., 2013). Además de la fluoxetina, otros estudios han demostrado los efectos toxicológicos provocados por otros antidepresivos, como la venlafaxina, que a concentraciones de 313 pg/L y 31,3 ng/L provocó el desprendimiento del sustrato de los caracoles de agua dulce *Leptoxis carinata y Stagnicola elodes* (Fong y cols., 2014), respectivamente, y la fluvoxamina, que produjo el desove del mejillón cebra cuando éste se expuso a una concentración de 318 ng/L (*D. polymorpha*) (Fong, 1998).

En lo que respecta a drogas, los estudios han sido más escasos y la mayoría de ellos han investigado los efectos toxicológicos provocados por la cocaína y sus metabolitos. Un estudio llevado a cabo en Italia, demostró que la cocaína es capaz de actuar como disruptor endocrino

modificando los niveles de dopamina cerebral y catecolaminas plasmáticas (dopamina, adrenalina y noradrenalina), así como la actividad del eje hipotalámico-hipofisario-adrenal[3] en la anguila *A. Anguilla,* tras la exposición a una concentración de 20 ng/L durante 30 días (Gay y cols., 2013). Otro trabajo estudió los efectos cito-genotóxicos producidos por la exposición a cocaína en el mejillón cebra (*Dreissena polymorpha)* a tres niveles de concentración (40, 220 y 10.000 ng/L). El estudio demostró que la exposición a corto plazo (96 horas) producía daños primarios en el ADN, y el aumento de células micro-nucleadas y la apoptosis, incluso a los niveles más bajos investigados (Binelli y cols., 2012). En otros estudios en los que también se evaluó los efectos producidos por la exposición a cocaína en el mejillón *Perna Perna* se demostró que, tras 96h de exposición a una concentración de cocaína de 2000 ng/L, se producía tanto estrés oxidativo como daños en el ADN (Dos Santos Barbosa Ortega y cols., 2018), y que la exposición del mejillón *Perna Perna* a concentraciones de 20 mg/L y 1,25 mg/L durante únicamente una hora, reducía a la mitad la tasa de fertilización y el crecimiento larval, respectivamente (Maranho y cols., 2017). En cuanto a sus metabolitos, un estudio demostró que la exposición a largo plazo (14 días) a benzoilecgonina a concentraciones de 500 y 1000 ng/L de mejillón cebra (*Dreissena polymorpha)* producía a ambos niveles estrés oxidativo (disminución de la estabilidad de la membrana lisosómica y desequilibrios de las actividades de las enzimas de defensa), y daños primarios en el ADN al nivel más alto de exposición (Parolini y cols., 2013b). Otro trabajo evaluó los efectos en el mejillón cebra (*Dreissena polymorpha)* de la exposición a 500 ng/L de ecgonina metil éster, observando también estrés oxidativo y fragmentación en el ADN primario aunque sin variaciones en el daño genético (aumento de células apoptóticas) (Parolini y cols., 2013a). Parolini y cols. demostraron que la exposición a THC (Parolini y cols., 2014) y a THC-COOH (Parolini y cols., 2017) a concentraciones ambientales (50 ng/L en el caso del THC y 100 ng/L en el de THC-COOH) no causaba efectos en el mejillón cebra (*Dreissena polymorph*). Sin embargo, la exposición a 500 ng/L durante 14 días, producía alteraciones notables en el estado oxidativo de *D. polymorpha*, dando lugar a un aumento significativo de la peroxidación lipídica y la carbonilación de *proteínas,* así como daño en el ADN. Los efectos producidos por la exposición a morfina (50 y 500 ng/L) en el mejillón cebra también se han evaluado, demostrándose que aunque a concentraciones ambientales (50 ng/L) no se producían daños, el nivel más alto de exposición inducía estrés oxidativo en los organismos, y al final del periodo, una ligera

[3] Eje hipotalámico-hipofisario-adrenal: Complejo de influencias directas e interacciones retroalimentadas entre el hipotálamo, la glándula pituitaria y la glándula suprarrenal que controla las reacciones al estrés y regula varios procesos del organismo como la digestión, el sistema inmune, las emociones, la conducta sexual y el metabolismo energético.

fragmentación de ADN primario, aunque no se observaron daños genéticos fijos ni aumento de las células apoptóticas (Magni y cols., 2016).

Además de la toxicidad provocada por las drogas individuales, Parolini y cols. evaluaron los efectos toxicológicos producidos en el mejillón cebra (*Dreissena polymorph*) por la exposición durante 14 días a una mezcla de drogas a concentraciones ambientales relevantes (cocaína (50 ng/L), benzoilecgonina (300 ng/L), morfina (100 ng/L), anfetamina (300 ng/L) y MDMA (50 ng/L)). El estudio demostró que la mezcla de drogas provocaba estrés oxidativo en el mejillón cebra, desequilibrando la actividad de las enzimas de defensa (Parolini y cols., 2015), así como un aumento significativo de la fragmentación del ADN desencadenando el proceso apoptótico y la formación de micronúcleos en los hemocitos, lo cual mostraba la potencial genotoxicidad de la mezcla hacia esta especie bivalva (Parolini y cols., 2016). Este estudió demostró que los efectos producidos por las drogas de forma individual se pueden acumular y, por tanto, se deberían tener en cuenta a la hora de evaluar la toxicidad en los organismos acuáticos.

En el caso de no disponer de datos experimentales sobre la toxicidad de una sustancia, la toxicidad se puede evaluar mediante la aplicación de modelos matemáticos. Estas herramientas predicen de forma rápida y económica la actividad biológica o química de una sustancia en base a sus propiedades físico-químicas aplicando enfoques de relación estructura-actividad (QSAR), aunque no pueden sustituir por completo a los ensayos de toxicidad *in vivo* o *in vitro.*

Uno de las herramientas utilizadas para la evaluación de los riesgos ambientales (ERA) que suponen la presencia de estos compuestos en el medio ambiente es el Índice de riesgo o HQ (del inglés, *Hazard Quotient*). El HQ se define como la ratio entre la concentración medioambiental medida (MEC, del inglés *Measured Environmental Concentration*) de un determinado compuesto y su toxicidad, normalmente expresada como la concentración a la que no se observan efectos toxicológicos (*NOEC,* del inglés *No Observed Effect Concentration*) o la concentración a la que no se esperan efectos toxicológicos (*PNEC,* del inglés *Predicted No-effect Concentration*) (Ginebreda y cols., 2010). Estos valores de PNEC, que se pueden extraer, por ejemplo, de la base de datos NORMAN, provienen del valor de toxicidad más bajo de entre los obtenidos para tres grupos de organismos modelo (algas, invertebrados y peces), dividido por un factor de corrección o de incertidumbre AF (de sus siglas en inglés *Assessment Factor*) que puede ir de 1 a 1000 dependiendo de la calidad y el tipo de datos toxicológicos disponibles (NORMAN, 2020). Para drogas los valores de AF suelen ser de 1000, ya que los datos toxicológicos se obtienen a través de modelos QSAR o de un limitado número de

concentraciones letales o efectivas medias a corto plazo ($L(E)C_{50}$), mientras que en el caso de psicofármacos los valores de AF pueden variar entre 10 (fluoxetina, diazepam), 100 (citalopram, temazepam, venlafaxina) y hasta 1000. En la base de datos NORMAN, los valores de PNEC calculados en agua, son posteriormente convertidos en PNEC correspondientes a sedimentos (mediante la aplicación de una aproximación que tiene en cuenta el coeficiente de reparto entre el carbono orgánico y el agua (K_{oc}) de la sustancia cuando el sistema está equilibrio: $PNEC_{sed}=PNEC_{agua}*2.6*(0.615 + 0.019*K_{oc})$) y a biota (mediante la aplicación del factor de bioconcentración (BCF): $PNEC_{biota/pez}=PNEC_{agua}*BCF$ y $PNEC_{biota/invertebrados}= PNEC_{biota/pez}/4$). La Tabla 4 muestras los valores de PNEC tanto en agua, como en sedimentos y biota para los compuestos incluidos en la presente tesis, calculados a partir de las ecuaciones anteriores y aplicando los valores de K_{oc} y BCF que aparecen en la Tabla 1.

Por otro lado, debido a que los contaminantes se encuentran en el medio ambiente como mezclas, el riesgo toxicológico se suele calcular como la suma de los HQ individuales de cada compuesto identificado positivamente en una muestra (Mendoza y cols., 2014), mediante la aplicación de un modelo de adicción de concentraciones (Ginebreda y cols., 2014).

En general, los valores de HQ inferiores a 1 no son considerados peligros para los organismos acuáticos, valores entre 1-10 son considerados potencialmente peligrosos y valores mayores de 10 son considerados los altamente peligrosos para los organismos acuáticos (NORMAN, 2020).

Tabla 4. Valores de PNEC en agua, sedimento y biota de las drogas y psicofármacos estudiados en esta tesis.

Compuesto	$PNEC_{agua}$[a] (µg/L)	$PNEC_{sed}$[b] (µg/kg)	$PNEC_{biota/pez}$[c] (µg/kg)	$PNEC_{biota/invertebrados}$[d] (µg/kg)
Cocaína	2,46	233	29	7,2
Benzoilecgonina	2,33	44	7,4	1,8
Cocaetileno	1,55	269	35	8,6
Anfetamina	24,8	1.399	112	28
Metanfetamina	9,74	791	76	19
MDMA	47,6	1.255	544	136
Ketamina	5,71	9,0	-	-
LSD	0,39	4.590	15	3,6
OH-LSD	-	-	-	-
Heroína	0,53	189	1,7	0,4
6ACM	5,19	6.814	16	4,0
Morfina	5,38	798	17	4,3
Metadona	0,84	3.021	178	44
EDDP	0,14	3.258	177	44
THC	0,07	2.118	3.251	813
THC-COOH	0,73	1195	41	10
OH-THC	0,28	495	159	40
Cannabinol	0,08	10.835	2.488	622
Cannabidiol	0,17	5.143	449	112
AH-7921	-	-	-	-
MDPV	3,28	141	108	27
Mefedrona	30	48	-	-
Metoxetamina	-	-	-	-
Citalopram	16	20.063	2.430	608
Fluoxetina	0,1	1.025	26	6,6
Sertralina	0,09	1.521	211	53
Venlafaxina	0,038	3	2,5	0,6
Alprazolam	0,08	8.508	0,7	0,2
OH-alprazolam	0,31	1.924	1,0	0,2
Diazepam	0,29	161	8,6	2,1
Lorazepam	0,096	10	22	5,6
Lormetazepam	0,17	8	1,8	0,4
Midazolam	0,12	2.666	60	15
OH-midazolam	0,15	167	1,1	0,3
Oxazepam	0,37	23	27	6,9
Temazepam	0,071	2	0,7	0,2
Cafeína	1,2	2,5	3,8	0,9
Efedrina	69,9	396	23	5,8
Clorpromacina	0,066	83	28	6,9
Hidroxicina	0,26	99	0,8	0,2
Zolpidem	0,18	235	33	8,3
Sulfato de etilo	92.4	250	292	73

[a] Valores de $PNEC_{agua}$ extraídos de la base de datos NORMAN.

[b] Valores calculados utilizando la fórmula $PNEC_{sed}=PNEC_{agua}*2.6*(0.615 + 0.019*K_{oc})$ y los valores de K_{oc} indicados en Tabla 1.

[c] Valores calculados utilizando la fórmula: $PNEC_{biota/pez}=PNEC_{agua}*BCF$ y los valores de BCF indicados en Tabla 1. [d] Valores calculados tras aplicar la fórmula: $PNEC_{biota/invertebrados}= PNEC_{biota/pez}/4$

1.4. Drogas y psicofármacos en aguas residuales como indicadores de consumo poblacional

Los métodos establecidos para estimar el consumo de drogas consisten en encuestas poblacionales, incautaciones de drogas, muertes relacionadas con el uso de drogas, ingresos hospitalarios, demandas de tratamiento, estadísticas de delitos relacionados con las drogas y, en el caso de drogas legales destinadas al comercio, estadísticas de ventas (Subedi y cols., 2019). Estos métodos permiten obtener información sobre la producción y el tráfico de drogas, las enfermedades asociadas al consumo de drogas, y la frecuencia, modo o patrones de consumo de drogas, pudiendo segregar el consumo en función del género y la edad. Sin embargo, presentan ciertas limitaciones, ya que cada indicador tiene sus propios desafíos metodológicos, vulnerabilidades o sesgos que alteran relativamente los resultados reales. Por ejemplo, en el caso de las encuestas los datos obtenidos no son objetivos, bien por la falta de sinceridad a la hora de responderlas por parte de la población, o por el propio desconocimiento sobre la naturaleza o pureza de las sustancias consumidas. Además, son muy costosas y requieren largos periodos de tiempo para llevarlas a cabo (Mercan y cols., 2019).

En 2001, Daughton señaló que la monitorización de los productos de excreción que llegan a una EDAR a través de los sistemas de alcantarillado podría utilizarse para estimar o recalcular la cantidad de drogas ilegales consumidas por parte de la población a la que da servicio dicha EDAR (Daughton, 2001). Cuatro años más tarde, esta metodología conocida como análisis de aguas residuales con fines epidemiológicos (a partir de ahora referida como WBE, siglas de su nombre en inglés *Wastewater-Based Epidemiology*), se aplicó por primera vez en Italia para estimar el consumo de cocaína a partir de los niveles encontrados de su metabolito, la benzoilecgonina, en agua residual sin tratar (Zuccato y cols., 2005). Actualmente, constituye una herramienta muy útil y complementaria a los métodos establecidos para estimar el consumo de drogas a nivel poblacional (EMCDDA, 2020b).

A diferencia de los métodos establecidos, la estimación del consumo de drogas mediante WBE proporciona resultados objetivos y anónimos, que se basan en evidencias y se obtienen casi a tiempo real, factor clave para identificar las tendencias de consumo de sustancias antes de que éstas cambien. Además, permite obtener patrones de consumo temporales (días, semanas, meses o años) y geográficos, así como cambios de consumo debido a eventos especiales como festivales de música (Mackul'ak y cols., 2019) o festividades (Andrés-Costa y cols., 2016b).

La estimación del consumo de drogas y en general de cualquier compuesto consumido por parte de la población mediante la aplicación de WBE, se realiza con las siguientes ecuaciones:

$$\text{mg/día/habitante} = C\left(\frac{\text{ng}}{\text{L}}\right) * 10^{-6}\left(\frac{\text{mg}}{\text{ng}}\right) * \text{Q}\left(\frac{\text{m}^3}{\text{día}}\right) * 1000\left(\frac{\text{L}}{\text{m}^3}\right) * \frac{1}{\text{P}} * \text{FC} \quad (1)$$

$$FC = \frac{PM_{compuesto}}{PM_{indicador\ de\ consumo}} * \frac{100}{\%\ excreción} \quad (2)$$

donde C es la concentración del indicador de consumo medido en el agua residual recogida a la entrada de la EDAR, Q es el caudal de agua tratado en la EDAR durante el periodo de muestreo, P es la población a la que da servicio la EDAR y FC es un factor de corrección que tiene en cuenta el porcentaje de excreción del indicador de consumo y la relación entre el peso molecular del compuesto del que se quiere determinar su consumo y su indicador de consumo.

El indicador de consumo puede ser tanto el propio compuesto del que se calcula el consumo, como un metabolito formado en el cuerpo humano tras el consumo de dicha sustancia. En general, un indicador de consumo ideal es aquel que se puede medir en el agua residual sin tratar, es liberado en los sistemas de alcantarillado únicamente como resultado de su excreción humana, tiene un perfil de excreción bien definido, no se forma por factores exógenos, tiene una adsorción limitada al material particulado y es estable tanto en las aguas residuales y en los sistemas de alcantarillado, como durante el muestreo y el almacenamiento de la muestra (Gracia-Lor y cols., 2016). En cuanto al porcentaje de excreción, se suele seleccionar el porcentaje de excreción promedio de la ruta de administración de la sustancia más frecuente (oral, intravenosa, inhalación, etc.), ya que dependiendo de ésta el porcentaje de excreción para un mismo compuesto puede variar y con ello el factor de corrección aplicado (Mercan y cols., 2019). La Tabla 5 muestra los indicadores de consumo utilizados para estimar el consumo de las drogas y psicofármacos investigados en la presente tesis (de los que se dispone de datos fármaco-cinéticos), así como el porcentaje de excreción y el factor de corrección aplicado.

Al igual que los métodos establecidos, WBE también presenta ciertas limitaciones. Castiglioni y cols. (2013) evaluaron la incertidumbre asociada a cada una de las etapas implicadas en la estimación del consumo de drogas ilegales mediante la aplicación de WBE y establecieron que la incertidumbre asociada al muestreo contribuía un 5-10% a la incertidumbre total, la asociada al análisis un 1-34%, la producida por la inestabilidad de los indicadores de consumo menos del 10%, la debida a la estimación del porcentaje de excreción del indicador de consumo seleccionado un 26% (para el caso concreto de la cocaína teniendo en cuenta todas las rutas de

administración) y la asociada a la estimación del tamaño de la población del 7-55%. Sin embargo, todas estas incertidumbres se pueden reducir si se aplican las mejores prácticas, como por ejemplo, la recogida de muestras compuestas de 24-h con muestreadores automáticos que trabajen en modo proporcional de tiempo o flujo, preservación de la muestra a 4 °C durante su recogida y a -20 °C (la muestra o el extracto obtenido tras pretratamiento y extracción de la muestra) hasta su análisis, el empleo de patrones internos para cada compuesto para corregir pérdidas durante la preparación de la muestra, y durante su análisis, la selección de los indicadores de consumo más adecuados, y la selección del mejor indicador para estimar el tamaño poblacional (EMCDDA, 2016).

El análisis de aguas residuales con fines epidemiológicos ha sido principalmente utilizado para estimar el consumo de drogas ilegales (I. González-Mariño y cols., 2020) y también el de psicofármacos como benzodiazepinas o antidepresivos (Baker y cols., 2014), cafeína (Gracia-Lor y cols., 2017), nicotina (Montes y cols., 2020), nuevas sustancias psicoactivas (Bijlsma y cols., 2019) y alcohol (publicación científica #1).

La publicación científica #1 es una revisión en la que se discute la utilidad de WBE para estimar el consumo de alcohol. En ella se discute la selección del sulfato de etilo como indicador de consumo del alcohol en base a su excreción y a su estabilidad en agua residual. Se muestran los diferentes estudios realizados hasta la fecha en que se ha estimado el consumo de alcohol, tanto a nivel local como a nivel nacional, en diferentes partes del mundo mediante el empleo de WBE, y se comparan los resultados obtenidos con WBE con aquellos obtenidos con los indicadores establecidos de consumo. Por último, se especifican las ventajas y limitaciones que presenta la estimación del consumo de alcohol mediante WBE, así como aspectos de la metodología que necesitan ser investigados o mejorados.

Tabla 5. Parámetros utilizados en la estimación del consumo de drogas y psicofármacos: indicador de consumo, porcentaje de excreción y factor de corrección.

Compuesto	Indicador de consumo	Excreción media (%)	Ratio masa molecular	Factor de corrección	Ref.
Cocaína	Benzoilecgonina	29	1,05	3,59	[1]
Anfetamina	Anfetamina	36,3	1,0	2,77	[2]
Metanfetamina	Metanfetamina[a]	22,7	1,0	4,40	[2]
	Metanfetamina[b]	40,9	1,0	2,44	[2]
MDMA	MDMA	22,5	1,0	4,40	[2]
Ketamina	Ketamina	30	1,0	3,33	[3]
	Nor-ketamina	1,6	1,1	65	[4]
LSD	LSD	< 1	1,0	>100	[5]
Heroína	6ACM	1,3	1,1	87	[5]
Metadona	EDDP	55	1,1	3,6	[6]
THC (cannabis)	THC-COOH	0,5	0,9	182	[2]
Cafeína	ácido 1,7-dimetilúrico	6,7	0,99	14,8	[7]
Efedrina	Efedrina	75	1,0	1,33	[5]
Etanol (Alcohol)	Sulfato de etilo	0,012	0,4	3047	[8]
MDPV[c]	-	-	-	-	-
Mefedrona	Mefedrona	5-15	1,0	6-20	[9]
Metoxetamina[c]	-	-	-	-	-
AH-7921[c]	-	-	-	-	-
Citalopram	Citalopram	12-20	1,0	5-8,3	[10]
	Citalopram	10,4	1,0	9,60	[11]
Fluoxetina	Fluoxetina	11	1,0	9,09	[12]
Sertralina	Sertralina	<0,2	1,0		[10]
Venlafaxina	Venlafaxina	5,0	1,0	20	[12]
Alprazolam	α-hidroxi-alprazolam	57,5	0,9	1,65	[13]
Diazepam	Diazepam	10	1,0	10	[14]
Lorazepam	Lorazepam	1,0	1,0	100	[10]
Lormetazepam[c]	-	-	-	-	-
Midazolam	α -hidroxi-midazolam	75	0,9	1,27	[15]
Oxazepam	Oxazepam	75	1,0	1,3	[14]
Temazepam	Temazepam	74,5	1,0	1,34	[12]
Zolpidem	Zolpidem	0,1-2,3	1,0	1000-43,5	[16]
Clorpromacina	Clorpromacina	0,37	1,0	270	[17]
Hidroxicina[c]	-	-	-	-	-

[a]Administración oral

[b]Administración intravenosa

[c]Para MDPV, metoxetamina, AH-7921, lormetazepam e hidroxicina no se han encontrado datos de porcentaje de excreción en orina

[1] (Castiglioni y cols., 2013); [2] (Gracia-Lor y cols., 2016); [3] (Yargeau y cols., 2014); [4] (Lai y cols., 2013b); [5] (Postigo y cols., 2011a); [6] (Thai y cols., 2016); [7] (Gracia-Lor y cols., 2017); [8] (Rodríguez-Álvarez y cols., 2015); [9] (Olesti y cols., 2019); [10] (Calisto y cols., 2009); [11] (Jose Antonio Baz-Lomba y cols., 2016); [12] (Baker y cols., 2014); [13] (Fraser y cols., 1991); [14] (Carballa y cols., 2008); [15] (Heizmann y cols., 1983); [16] (Reidy y cols., 2011); [17] (Muralidharan y cols., 1996).

Publicación científica #1

"The value of wastewater-based epidemiology in the estimation of alcohol consumption"

por:

Ester López-García, Cristina Postigo, Damià Barceló y Miren López de Alda

en Current Opinion in Environmental Science & Health 2019, 9: 19-25

https://doi.org/10.1016/j.coesh.2019.03.003

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Environmental Science & Health

The value of wastewater-based epidemiology in the estimation of alcohol consumption

Ester López-García[1], Cristina Postigo[1], Damià Barceló[1,2] and Miren López de Alda[1]

Abstract

The present review discusses the value of wastewater-based epidemiology (WBE) to estimate alcohol consumption in a given population. It gives an overview of the works conducted in this respect to date and the comparability of the WBE-derived data with official estimates provided by the World Health Organization, generated after surveys and alcohol sales data. Finally, it provides insights into the main advantages and limitations of the WBE approach over traditional methods and aspects/knowledge to be improved/investigated.

Addresses

[1] Water, Environmental and Food Chemistry (ENFOCHEM), Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona 18-26, 08034, Barcelona, Spain

[2] Catalan Institute for Water Research, H2O Building, Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003, Girona, Spain

Corresponding authors: Postigo, Cristina (cprqam@cid.csic.es); López de Alda, Miren (mlaqam@cid.csic.es)

Current Opinion in Environmental Science & Health 2019, **9**:19–25

This review comes from a themed issue on **Environmental Chemistry: Sewage Epidemiology**

Edited by **Yolanda Picó**

For a complete overview see the Issue and the Editorial

https://doi.org/10.1016/j.coesh.2019.03.003

Keywords

Sewage epidemiology, Ethyl sulfate, EtS, Alcohol consumption, Ethanol metabolism.

Introduction

Alcohol is one of the most used recreational substances in the world. According to the World Health Organization (WHO), almost half of the worldwide population (43%) consumed alcohol in 2016. Alcohol consumption increased globally between 2005 and 2010 from 5.5 L to 6.4 L per capita (older than 15 years) and has remained stable thereafter until 2016 [1]. Excessive alcohol consumption has been linked to diverse social problems (suicides, homicides, robberies, violence, traffic accidents, etc.) and health problems (digestive, infectious and cardiovascular diseases, cancer, etc.). Harmful use of alcohol was the cause of 5.3% of all deaths worldwide in 2016 [1]. Thus, the availability of tools to track alcohol consumption in time and evaluate the reliable effectiveness of adopted measures to reduce its excessive use is important.

The prevalence of alcohol use is commonly estimated through population surveys [2,3] and sales statistics [4]. However, these methods are time-consuming and expensive. Moreover, they do not always provide reliable data because of widespread underreporting in surveys [5] or unaccounted smuggled, stored, and disposed alcohol in total sales volumes. Alternatively, a novel approach known as wastewater-based epidemiology (WBE) has been applied in recent years to assess alcohol use in a given population. WBE back-calculates alcohol consumption from the concentration of an alcohol consumption indicator, most commonly ethyl sulfate (EtS), found in wastewater. Thus, WBE provides anonymous and objective information in a fast and relatively economic way. However, this approach is also subject to different sources of uncertainty. This review aims at (i) presenting the WBE approach to estimate alcohol use by a specific population, (ii) discussing its utility based on the peer-reviewed studies published so far on this topic, and (iii) highlighting its advantages and limitations.

Basis for estimation of alcohol use using WBE

WBE relies on the fact that wastewater is a diluted pooled urine sample of a specific population that provides valuable information if appropriate biomarkers are monitored. After consumption of any alcoholic beverage, most of its ethanol content (~95%) is metabolized in the liver via oxidation to acetaldehyde and acetic acid. However, a very small fraction (<0.1%) is converted to ethyl glucuronide (EtG) and EtS after conjugation with glucuronic acid and sulfate, respectively. These metabolites are excreted together with unaltered ethanol (~5%). EtG and EtS are both good indicators of recent alcohol consumption because they are excreted within a few hours after ingestion and are detectable in urine for 1 or 2 days, depending on the subject and alcohol dose [6]. However, only EtS has been used as a biomarker of

alcohol consumption because it is more stable in wastewater than EtG. Laboratory-scale experiments showed that EtS concentration in wastewater remained stable for at least 1 week at room temperature and for more than 1 month at −20 °C [7]. On the contrary, EtG concentration decreased up to 50% after 18 h at room temperature [8]. In addition, EtS is not formed in wastewater through metabolization of unaltered ethanol by endogenous bacteria, and hence, its presence in wastewater can be only attributed to alcohol consumption [8]. This makes EtS an exclusive and unequivocal biomarker to be used in WBE for back-calculation of alcohol consumption according to Eq. (1),

$$\frac{mL\ of\ EtOH}{day * person} = C_{EtS}\left[\frac{\mu g}{L}\right] * 10^{-6}\left[\frac{g}{\mu g}\right] * Q\left[\frac{m^3}{day}\right] * 10^{3}\left[\frac{L}{m^3}\right] * \frac{1}{P(persons)} * F * \frac{1}{\rho_{EtOH}\left[\frac{g}{mL}\right]} \quad (1)$$

$$F = \left[\frac{M_{EtOH}/M_{EtS}}{\%\ excretion\ rate\ EtS} * 100\right] \quad (2)$$

where C_{EtS} is the concentration of EtS in wastewater at the entrance of a wastewater treatment plant (WWTP), Q is the water flow entering the WWTP during the sampling event, P is the population connected to the WWTP, F is a correction factor that takes into account the molar mass ratio between ethanol and EtS (0.365) and the excretion rate of EtS (Table 1)(Eq. (2)), and ρ_{EtOH} is the ethanol density (0.789 g/mL).

Two approaches have been used to estimate alcohol consumption, providing fully comparable results [8]. One is the classical average mass flow method that is ideal for substances that have a long excretion half-life in urine or are consumed at a constant rate throughout the day and night by many people in the studied area. The other one is based on the EtS excretion profile. This approach may provide more accurate consumption figures for substances with short urinary excretion half-life, as it is the case for EtS (3 h).

Applicability and value of WBE to estimate alcohol consumption

The first study that applied WBE to estimate alcohol use dates from 2011 [8]. Since then, various studies, summarized in Table 1, have been conducted in this line to explore (i) the value of WBE to estimate alcohol consumption and (ii) the comparability of the data generated with this approach and traditional methods, that is, official estimates provided by the WHO and each country institutions. To date, WBE alcohol consumption figures have been derived from EtS loads entering 55 WWTPs spread in 13 different countries all over the world [8–19]. As shown in Table 1, most of the studies were conducted in major cities from European countries.

Oslo was the first city where WBE was applied to estimate alcohol consumption. For this, samples were collected daily for 25 consecutive days at the entrance of one WWTP. In this study, two calculation methods to estimate alcohol use were evaluated and compared: the classical average mass flow method and a novel method based on the EtS excretion profile. Equal alcohol consumption figures were obtained with both approaches (4900 to 7800 Kg/day). These data were in very good agreement with Norway sales statistics (6750 Kg/day), which are among the most accurate in the world because of the strict control of import and sale of alcoholic beverages by the government [8]. In areas such as the one investigated in this study, where alcohol is mostly consumed over a short period (usually during the evening), a limited number of samples can be used to derive alcohol use figures through the excretion profile integration method (morning peak of Et-S in wastewater associated with morning urination) [8].

Following this study, the average mass flow method was applied to estimate alcohol use in Milan [17], the Spanish cities Santiago de Compostela [7] and Barcelona [14], and in multicity studies conducted at international [18] and European level [10]. In all these studies, annual consumptions were derived from one-week sampling at one WWTP, except in the case of Milan where samples were collected for two weeks. The highest consumption rates (above 25 mL/day/inhabitant) were obtained for cities in Canada (Granby and Montreal), Denmark (Copenhagen), and Germany (Munich and Dresden). City-based WBE values were in good agreement (<15%) with country-based WHO data only in a few cases, that is, Dresden, Munich, Castellón, Amsterdam, and Eindhoven. Mastroianni et al. [14] also reported a good agreement between wine consumption data derived from WBE and figures reported by the Spanish Ministry of Agriculture.

Overall, the low comparability of figures provided by the WHO and WBE could be attributed to (i) the small geographic coverage of the WBE method (based on only one city or part of it) as compared with WHO data (based on nation-wide surveys and sales data) and to the fact that the area monitored by means of WBE may not be representative of country trends in alcohol consumption; (ii) the short sampling periods used to derive WBE data (usually one 'normal' week is selected to generate annual consumption data); (iii) the uncertainty associated to the WBE methodology (see further discussion in Section Limitations of WBE for estimating alcohol consumption), and (iv) imprecise estimates derived from surveys due to underreporting and sales data in areas where purchased alcohol may not be immediately consumed.

Table 1

Alcohol consumption estimates by means of the WBE approach.

Level	City or country	Alcohol consumption (mL/day/inh.)		Normalized population	Period of time	Exc. rate (%) used	Ref.
		Average	Range				
Local	Oslo (NO)	16.1		Total population	2009 (3 weeks)	0.010–−0.016	[8]
	Santiago (ES)	13.6	3.8–22.6	Total population	2012-2013-2014 (one week)	0.012	[7,17]
	Barcelona (ES)	18	11–25	>15 years	2013-2014-2015 (one week)	0.011	[14,15]
	Milan (IT)	5.1	3.2–10.5	Total population	2012-2013-2014 (two weeks)	0.012	[17]
	Valencia (ES)	4.3–17.0	1.1–56.1	>15 years	2014 (17 days in 3 WWTPs)	0.010–−0.016	[9]
	Normal week	3.3–6.2	1.1–18.31				
	Festivity	6.4–42.7	4.4–56.11				
	Canberra (AU)	14.6	9.3–22.3	Total population	2014 (one week)	0.012	[18]
	Toowoomba (AU)	9.7	6.9–14.5				
	Montreal (CA)	29.2	21.8–38.8				
	Granby (CA)	44.3	27.3–59.3				
	Lugano (CH)	6.5	4.5–8.4				
	Dortmund (DE)	23.6	18.1–34				
	Dülmen (DE)	20.3	5.5–40				
	Dresden (DE)	29.4	15.1–91.7				
	Munich (DE)	29.5	0.5–47.4				
	Berlin (DE)	16.9	13.8–22.3				
	Copenhagen (DK)	40.2	24.6–74				
	Barcelona (ES)	11.7	5.7–17.6				
	Castellón (ES)	23.4	11.6–61.6				
	Milan (IT)	6.4	5.1–8.1				
	Amsterdam (NL)	22	14.3–30.5				
	Eindhoven (NL)	21.7	13.7–30.4				
	Utrecht (NL)	12.9	7.7–20.7	Total population	2014 (one week)	0.012	[18]
	Oslo (NO)	19.2	8.8–52.9				
	Almada (PT)	14.6	8.4–24.1				
	London (UK)	21.5	10.9–36				
	Brussels (BE)	21.6		Total population	2015 (one week)	0.012	[10]
	Zurich (CH)	14.7					
	Copenhagen (DK)	29.7					
	Castellón (ES)	6.6					
	Milan (IT)	6.6					
	Utrecht (NL)	10.8					
	Oslo (NO)	18.9					
	Bristol (UK)	16.2					
	Lier (BE)			Total population	2014 (four two-week periods)	0.012	[19]
	Lesvos (GR)	5.4/3.4	2.2–11.2/1.7–7.2	Total population	2015 (one week in 2 WWTPs)	0.012	[12]
	Ho Chi Minh (Vietnam)	3.1–3.9		Total population	2015 (one week, grab samples)	0.012	[16]
	Midwestern and Northeastern region (US)	37		>15 years	2015–2016 (one weekday every month for eleven months, 3 WWTPs)	0.012	[20]
National	Belgium			>15 years	2013–2015 (one week, 8 WWTPs)	0.012	[11]
	Australia	12–13	7–24	15–79 years	2014–2015 (one week, 18 WWTPs)	0.012	[13]

WWTP, wastewater treatment plant; WBE, wastewater-based epidemiology.

A couple of studies used EtS loads entering various WWTPs to derive national alcohol consumption figures. This was carried out by Boogaerts et al. [11] in Belgium and by Lai et al. [13] in Australia, who covered 12% and 45% of the country's total population, respectively. In the case of Belgium, the WBE-derived figure was below the one reported by the WHO. However, it was in agreement with alcohol consumption figures provided by the Belgian Scientific Institute of Public Health. In the Australian study, average alcohol consumption figures obtained were very similar to those reported by the National Drug Strategy Household Survey. However, contrary to National Drug Strategy Household Survey data, WBE figures revealed large differences in alcohol consumption between rural and urban communities.

The comparability between alcohol consumption estimates produced by WBE and surveys in a medium-sized Belgian city was investigated by van Well et al. [19]. The study showed no correlation between WBE and survey data. However, authors attributed this to the small survey sample size (only 1% of survey response was obtained each study week) [19].

Besides the comparison of WBE data with survey data and official estimates, one of the main objectives of multicity studies was to investigate spatial differences in alcohol consumption. In addition to this, the collection of samples for seven consecutive days and the iteration of this exercise in consecutive years allowed investigating consumption patterns in time in each of the investigated cities throughout the week or throughout the years. As observed in the first study on this topic, conducted by Reid et al. [8], and several studies published afterward, recreational use of alcohol resulted in higher EtS loads during the weekend than during weekdays [9,11,14,15,17–19]. In contrast, such profile was not observed in Lesvos Island (Greece), which could be attributed to the fact that alcohol drinking is a cultural aspect in Southern European countries [12].

The WBE approach also allows quantifying changes in alcohol consumption associated with special events, for example, local festivities or festivals, when heavy drinking may take place. This was proved by Andrés-Costa et al. [9] during the 'Fallas' festivity in Valencia. In this study, they found almost five and three times higher alcohol consumption during this festivity than during a normal week and weekend, respectively.

Recently, Chen et al. [20] evaluated the WBE approach to estimate alcohol consumption in three different communities of the United States. This study has been the longest conducted to date because samples were collected one weekday every month for 11 months. The average alcohol consumption obtained using the WBE approach was similar to those reported by the WHO and by the National Institute of Alcohol Abuse and Alcoholism. However, consumption figures were derived from samples collected on weekdays. To take into account weekend use, when alcohol use is presumably higher than in weekdays, they applied a correction factor (1.18) based on previously published studies. After correction, only one of the three investigated communities showed good agreement between the WHO and WBE data.

Advantages of WBE for estimating alcohol consumption

The main advantage that WBE presents over traditional methods to estimate alcohol use is its capability to generate information on consumption habits in a fast, economic, objective, and anonymous way.

Contrary to traditional methods, the WBE approach allows obtaining patterns of alcohol consumption at a high spatial resolution. The coverage area of WBE-derived data is defined by the wastewater catchment (i.e. population connected to the sewage network) where the sample is collected. Thus, consumption patterns in different size populations can be well defined (see studies carried out in Australia [13], Belgium [11], and Greece [12]). For instance, alcohol use in rural areas of Australia was three to four times higher than in urban zones. On the contrary, alcohol consumption estimates for large cities in Belgium and Greece were higher than in small cities, although, in the case of Greece, differences were not significant.

Owing to the high-throughput character of the WBE approach, consumption trends in time can be rapidly defined and changes in consumption patterns during special events can be detected. For the same reason, it could be used to evaluate the effectiveness of preventive measures (educational and sensibilization campaigns in schools or specific subpopulations) or the efficacy of certain regulations.

Limitations of WBE for estimating alcohol consumption

The WBE approach to estimate alcohol use presents some limitations also, and therefore, it has to be considered as a tool that complements traditional methods. The main limitation is the uncertainty associated with this method that has to be considered when interpreting WBE results. The sources of uncertainty of the WBE approach are well described [21]. They are related to the sampling protocol, analytical measurement, stability of the selected biomarker (EtS) in wastewater and in the sewer system, metabolism of alcohol, and estimation of the population contributing to the EtS loads measured. The amount of uncertainty associated with the WBE method is specific to each

case study. This aspect has been poorly addressed in most of the studies conducted so far and needs detailed characterization and deeper understanding in future studies.

Uncertainty associated to wastewater sampling and EtS analysis can be minimized and controlled if best practice protocols are followed (collection of 24-h flow or time-proportional integrated samples, sample preservation during collection and transport in cool conditions, use of deuterated surrogate standards for analysis, etc.) [21].

Regarding stability, EtS showed little or no significant degradation in wastewater at room temperature for 18 h [8] and even one week [7] (in-sample stability). However, two recent studies conducted in both real sewer [22] and laboratory-scale sewer reactors (rising main and gravity reactors) that mimic in-sewer conditions [23] have shown that EtS is degraded to some extent in sewer systems (in-sewer stability). The study conducted in real sewer systems demonstrated that the degradation rate of EtS is approximately 8% per hour, and this could vary from catchment to catchment [22]. The experiments carried out using laboratory-scale sewer reactors showed that degradation of EtS in rising main sewer reactors is higher than in gravity reactors and that 20% of EtS could degrade after 3 and 9 h. These aspects have to be taken into consideration when estimating alcohol consumption by means of WBE and also when comparing WBE-derived alcohol consumption data among populations because they could lead to underestimating the real alcohol consumption.

Alcohol metabolism has been scarcely explored and needs further investigation to reduce the uncertainty associated. One of the studies conducted in this line showed that on a molar basis, a median of 0.011% (range: 0.010–0.016) of the alcohol consumed was excreted as EtS [24], while another study reported an EtS excretion rate of 0.022% [25]. Both studies were conducted using very few individuals (10 individuals in the first one and one individual in the second one), and consequently, they may not be representative of the general population because age, gender, race, and health conditions could also affect alcohol metabolism. WBE alcohol consumption figures reported to date are based on different EtS excretion rate values. As shown in Table 1, the median 0.011%, range 0.011–0.016%, or people-weighted mean of the aforementioned studies 0.012% has been used for this purpose. Based on this, ethanol metabolism needs to be further studied to define a single and representative excretion rate of EtS.

The size of the population that contributes to the Et-S loads measured in wastewater is an important source of uncertainty. The method selected to estimate the population size (census data, WWTP design capacity, biomarkers, or water quality parameters) was shown in a multicity study to affect illicit drug consumption rates to a different extent. Variations as low as 7% or up to 55% were found in the different cities investigated [21]. Different water quality parameters, such as biological oxygen demand, chemical oxygen demand, total nitrogen and total phosphorus, and several human metabolites and pharmaceuticals and personal care products, have been proposed and investigated as potential markers that can contribute to reduce uncertainty associated with the estimation of population size [21,26,27]. Ammonium (NH_4^+), an indirect marker of urine that can be measured online in wastewater, was reported to be an extremely useful marker to monitor fluctuations in population size in WBE studies [28]. The use of mobile device data has been recently proposed as a reliable tool to better estimate dynamic population size in a wastewater catchment [29]. However, because this type of data may be not easily accessible or may be costly, the most reliable estimation of population size has to be made in each case, after consulting WWTP experts (origin of wastewater, municipalities connected to the WWTP, and census data) and considering the population dynamics of each investigated area (presence of tourists, commuters, holiday periods, etc.). The latter also has to be taken into consideration when interpreting WBE results. For instance, the high alcohol consumption figures obtained by Chen et al. [20] in one of the communities investigated in the US between June and September reflected the impact of tourist influx rather than increased consumption of alcohol by residents living in the area. One major limitation of WBE is that, contrary to traditional methods, it does not provide alcohol consumption estimates for specific population groups, and thus, consumption patterns by age or by gender cannot be segregated.

Conclusions

The WBE approach is a very useful tool, complementary to traditional methods, to estimate the consumption of alcohol in a population. It allows obtaining timely estimates, in a fast, inexpensive, objective, and anonymous way. The uncertainty of this methodology can be reduced by selecting the best method to estimate population size and increasing the knowledge on EtS stability in the catchment and alcohol metabolism. Data comparability with WHO annual estimates for the different countries could be also improved by using longer sampling periods and covering larger populations so that estimates obtained are representative of a whole country and a year period.

With no doubt, the WBE approach is an already consolidated and valid tool to evaluate changes in alcohol consumption patterns and effectivity of control and preventive measures applied in specific locations to reduce alcohol consumption.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work has been financially supported by the Government of Catalonia (Consolidated Research Group 2017 SGR 01404-Water and Soil Quality Unit).

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. World Health Organization (WHO): *Global status report on alcohol and health 2018*. 2018. Available at: https://goo.gl/kv8FD6. December 2018.

2. Moskalewicz J, Sieroslawski J: *Drinking population surveys: guidance document for standardized approach*. Warsaw: Institute of Psychiatry and Neurology; 2010. Available at: https://goo.gl/i15bws. December 2018.

3. World Health Organization: *International guide for monitoring alcohol consumption and related harm*. 2000. Geneva. Available at: https://goo.gl/DbAjCp. December 2018.

4. Sordo L, Barrio G, Bravo MJ, Villalbí JR, Espelt A, Neira M, Regidor E: **Estimating average alcohol consumption in the population using multiple sources: the case of Spain**. *Popul Health Metrics* 2016, **14**. 21-21.

5. Stockwell T, Zhao J, Greenfield T, Li J, Livingston M, Meng Y: **Estimating under- and over-reporting of drinking in national surveys of alcohol consumption: identification of consistent biases across four English-speaking countries**. *Addiction* 2016, **111**:1203–1213.

6. Helander A, Beck O: **Ethyl sulfate: a metabolite of ethanol in humans and a potential biomarker of acute alcohol intake**. *J Anal Toxicol* 2005, **29**.

7. Rodríguez-Álvarez T, Rodil R, Cela R, Quintana JB: **Ion-pair reversed-phase liquid chromatography–quadrupole-time-of-flight and triple-quadrupole–mass spectrometry determination of ethyl sulfate in wastewater for alcohol consumption tracing**. *J Chromatogr A* 2014, **1328**:35–42.

8. * Reid MJ, Langford KH, Morland J, Thomas KV: **Analysis and interpretation of specific ethanol metabolites, ethyl sulfate, and ethyl glucuronide in sewage effluent for the quantitative measurement of regional alcohol consumption**. *Alcohol Clin Exp Res* 2011, **35**:1593–1599.
This is the first study that uses EtS concentrations in water to estimate alcohol consumption at the community level.

9. * Andrés-Costa MJ, Escrivá Ú, Andreu V, Picó Y: **Estimation of alcohol consumption during "Fallas" festivity in the wastewater of Valencia city (Spain) using ethyl sulfate as a biomarker**. *Sci Total Environ* 2016, **541**:616–622.
This study shows that the use of WBE allows detecting changes in alcohol consumption caused by a local festivity.

10. Baz-Lomba JA, Salvatore S, Gracia-Lor E, Bade R, Castiglioni S, Castrignanò E, Causanilles A, Hernandez F, Kasprzyk-Hordern B, Kinyua J, McCall A-K, van Nuijs A, Ort C, Plósz BG, Ramin P, Reid M, Rousis NI, Ryu Y, de Voogt P, Bramness J, Thomas K: **Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities**. *BMC Public Health* 2016, **16**:1035.

11. Boogaerts T, Covaci A, Kinyua J, Neels H, van Nuijs ALN: **Spatial and temporal trends in alcohol consumption in Belgian cities: a wastewater-based approach**. *Drug Alcohol Depend* 2016, **160**:170–176.

12. Gatidou G, Kinyua J, van Nuijs ALN, Gracia-Lor E, Castiglioni S, Covaci A, Stasinakis AS: **Drugs of abuse and alcohol consumption among different groups of population on the Greek Island of Lesvos through sewage-based epidemiology**. *Sci Total Environ* 2016, **563–564**:633–640.

13. ** Lai FY, Gartner C, Hall W, Carter S, O'Brien J, Tscharke BJ, Been F, Gerber C, White J, Thai P, Bruno R, Prichard J, Kirkbride KP, Mueller JF: **Measuring spatial and temporal trends of nicotine and alcohol consumption in Australia using wastewater-based epidemiology**. *Addiction* 2018, **113**:1127–1136.
This is the first study that applies the WBE approach at the national level with very large population coverage (43% of total population).

14. Mastroianni N, Lopez de Alda M, Barcelo D: **Analysis of ethyl sulfate in raw wastewater for estimation of alcohol consumption and its correlation with drugs of abuse in the city of Barcelona**. *J Chromatogr A* 2014, **1360**:93–99.

15. Mastroianni N, López-García E, Postigo C, Barceló D, López de Alda M: **Five-year monitoring of 19 illicit and legal substances of abuse at the inlet of a wastewater treatment plant in Barcelona (NE Spain) and estimation of drug consumption patterns and trends**. *Sci Total Environ* 2017, **609**:916–926.

16. Nguyen HT, Thai PK, Kaserzon SL, O'Brien JW, Eaglesham G, Mueller JF: **Assessment of drugs and personal care products biomarkers in the influent and effluent of two wastewater treatment plants in Ho Chi Minh City, Vietnam**. *Sci Total Environ* 2018, **631–632**:469–475.

17. Rodríguez-Álvarez T, Racamonde I, González-Mariño I, Borsotti A, Rodil R, Rodríguez I, Zuccato E, Quintana JB, Castiglioni S: **Alcohol and cocaine co-consumption in two European cities assessed by wastewater analysis**. *Sci Total Environ* 2015, **536**:91–98.

18. Ryu Y, Barceló D, Barron LP, Bijlsma L, Castiglioni S, de Voogt P, Emke E, Hernández F, Lai FY, Lopes A, López de Alda M, Mastroianni N, Munro K, O'Brien J, Ort C, Plósz BG, Reid MJ, Yargeau V, Thomas KV: **Comparative measurement and quantitative risk assessment of alcohol consumption through wastewater-based epidemiology: an international study in 20 cities**. *Sci Total Environ* 2016, **565**:977–983.

19. van Wel JH, Gracia-Lor E, van Nuijs AL, Kinyua J, Salvatore S, Castiglioni S, Bramness JG, Covaci A, van Hal G: **Investigation of agreement between wastewater-based epidemiology and survey data on alcohol and nicotine use in a community**. *Drug Alcohol Depend* 2016:162.

20. Chen J, Venkatesan AK, Halden RU: **Alcohol and nicotine consumption trends in three U.S. communities determined by wastewater-based epidemiology**. *Sci Total Environ* 2019, **656**:174–183.

21. ** Castiglioni S, Bijlsma L, Covaci A, Emke E, Hernández F, Reid M, Ort C, Thomas KV, van Nuijs ALN, de Voogt P, Zuccato E: **Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers**. *Environ Sci Technol* 2013, **47**:1452–1460.
Authors evaluate and quantitate the different sources of uncertainty associated with consumption estimates derived from the wastewater based epidemiology, and provide best practice protocols.

22. Gao J, Li J, Jiang G, Yuan Z, Eaglesham G, Covaci A, Mueller JF, Thai PK: **Stability of alcohol and tobacco consumption biomarkers in a real rising main sewer**. *Water Res* 2018, **138**:19–26.

23. Banks APW, Lai FY, Mueller JF, Jiang G, Carter S, Thai PK: **Potential impact of the sewer system on the applicability of alcohol and tobacco biomarkers in wastewater-based epidemiology**. *Drug Test Anal* 2018, **10**:530–538.

24. Høiseth G, Bernard JP, Stephanson N, Normann PT, Christophersen AS, Mørland J, Helander A: **Comparison between the urinary alcohol markers EtG, EtS, and GTOL/5-HIAA in a controlled drinking experiment**. *Alcohol Alcohol* 2008, **43**:187–191.

25. Wurst FM, Dresen S, Allen JP, Wiesbeck G, Graf M, Weinmann W: **Ethyl sulphate: a direct ethanol metabolite reflecting recent alcohol consumption**. *Addiction* 2006, **101**:204–211.

26. Chen C, Kostakis C, Gerber JP, Tscharke BJ, Irvine RJ, White JM: **Towards finding a population biomarker for**

wastewater epidemiology studies. *Sci Total Environ* 2014, **487**: 621–628.

27. Lai FY, Ort C, Gartner C, Carter S, Prichard J, Kirkbride P, Bruno R, Hall W, Eaglesham G, Mueller JF: **Refining the estimation of illicit drug consumptions from wastewater analysis: co-analysis of prescription pharmaceuticals and uncertainty assessment.** *Water Res* 2011, **45**:4437–4448.

28. * Been F, Rossi L, Ort C, Rudaz S, Delémont O, Esseiva P: **Population normalization with ammonium in wastewater-based epidemiology: application to illicit drug monitoring.** *Environ Sci Technol* 2014, **48**:8162–8169.

This study evaluates the use of ammonium level in wastewater as a marker of fluctuations in population size

29. * Thomas KV, Amador A, Baz-Lomba JA, Reid M: **Use of mobile device data to better estimate dynamic population size for wastewater-based epidemiology.** *Environ Sci Technol* 2017, **51**:11363–11370.

This study shows how population estimates may affect WBE-estimates and how population size can be accounted for using mobile devices data.

Además de la estimación del consumo de drogas tanto legales como ilegales, WBE puede tener muchas más aplicaciones que pueden aportar conocimientos útiles sobre la exposición a determinados contaminantes (pesticidas, retardantes de llama, plastificantes, sustancias perfluoroalquiladas), sobre la salud humana (monitoreo de biomarcadores del estrés, de la dieta o de enfermedades) o sobre los estilos de vida de la población (exposición a productos de cuidado personal, consumo de sustancias antidopantes o determinadas hormonas) como se refleja en varias revisiones publicadas recientemente (Choi y cols., 2018; Lorenzo y cols., 2019). En este sentido, un estudio llevado a cabo en España mostró niveles de exposición medios entre 2 y 1347 µg/día/habitante de varios ftalatos, superando en algunos sitios los umbrales de exposición diaria establecidos para el di-iso-butil ftalato (DiBP) y el di-n-butil ftalato (DnBP) por la Autoridad Europea de Seguridad Alimentaria (Iria González-Mariño y cols., 2020). En otro estudio realizado en Italia, se obtuvieron diferentes patrones geográficos de exposición a varios piretroides, siendo en algunas zonas los niveles de exposición superiores a la cantidad diaria aceptable (Rousis y cols., 2017), y en otro estudio realizado también en España e Italia se observó que la población estaba expuesta a varias micotoxinas, aunque los niveles de exposición encontrados no suponían un riesgo para la salud humana (Gracia-Lor y cols., 2020).

La importancia de WBE para obtener resultados casi a tiempo real se ha demostrado con los trabajos recientemente publicados con respecto al análisis de genes específicos del SARS-CoV-2 en agua residual para evaluar la incidencia de la COVID-19 en una población. Desde que a finales del año 2019 el mundo se viera sacudido por una pandemia mundial ya se han publicado numerosos estudios en los que se ha analizado la presencia de SARS-CoV-2 en agua residual. En Italia, por ejemplo, el análisis de muestras de agua residual recogidas entre febrero y abril de 2020 mostró que la mitad de las muestras analizadas eran positivas en SARS-CoV-2, correspondiendo uno de los resultados positivos obtenidos a una muestra de agua residual recogida en Milán unos días después del primer caso italiano notificado (La Rosa y cols., 2020). En otro estudio en Australia, gracias a la aplicación de WBE a muestras de aguas residuales recogidas durante los meses de marzo y abril de 2020, se estimó que el número de infectados en una determinada área estaba entre 171 y 1090, número similar al de los datos clínicos existentes (Ahmed y cols., 2020). Así pues, la aplicación de WBE a la monitorización del SARS-CoV-2, permitiría obtener información a tiempo real de la evolución de la pandemia, y establecer un sistema de alerta temprana para implementar las medidas preventivas y sanitarias correspondientes y así reducir los efectos de la misma.

1.5. Análisis de drogas y psicofármacos y metabolitos en matrices ambientales.

Las bajas concentraciones a las que se encuentran las drogas y psicofármacos y sus metabolitos en matrices ambientales, junto con la complejidad de dichas matrices, requiere del empleo de técnicas avanzadas para la extracción, pre-concentración y purificación de los analitos, así como las técnicas más sensibles y selectivas para su determinación y cuantificación fiable.

1.5.1. Metodologías analíticas para determinar drogas y psicofármacos y sus metabolitos en aguas

La publicación científica #2 revisa las metodologías analíticas desarrolladas para el análisis de psicofármacos en aguas mediante cromatografía de líquidos (LC, del inglés *Liquid Chromatography*) acoplada a espectrometría de masas (MS). En ella se muestran todos los procesos involucrados en el análisis de este tipo de compuestos en aguas, desde la recogida de muestra y su pretratamiento, hasta su extracción y purificación, y determinación final. Aunque esta publicación sólo se ha centrado en psicofármacos, los aspectos discutidos, sobre todo aquellos relacionados con el muestreo, pretratamiento y extracción de la muestra también pueden aplicarse a la determinación de drogas en agua, por lo que la siguiente sección solo estará centrada en revisar los procedimientos de LC o cromatografía de gases (GC, del inglés *Gas Chromatography*) acoplada a MS desarrollados para determinar drogas y sus metabolitos en aguas.

Separación cromatográfica

Las drogas y sus metabolitos han sido principalmente analizados utilizando LC y, en menor medida, GC acoplada a espectrometría de masas. En LC, la separación cromatográfica se ha realizado empleando principalmente columnas de fase reserva C18 rellenas de partículas que presentan un diámetro interno entre 2-5 µm en sistemas de LC de alta eficacia (o HPLC, de sus siglas en inglés *High Performance Liquid Chromatography*) (Kim y cols., 2020; Mackul'ak y cols., 2019; Xiaohan Zhang y cols., 2019) o <2 µm en sistemas de LC de ultra-alta eficacia (UPLC o UHPLC, de sus siglas en inglés *Ultra-High Performance Liquid Chromatography*), con los que se consiguen mayor eficacia de separación, sensibilidad y resolución, y tiempos de análisis menores

(Bijlsma y cols., 2009; Deng y cols., 2020; González-Mariño y cols., 2018; Skees y cols., 2018). Las fases móviles empleadas para el análisis de drogas se basan en mezclas binarias de agua con un disolvente orgánico, generalmente acetonitrilo (ACN) o metanol (MeOH). Para reducir las colas de los picos y mejorar la ionización de los compuestos, el pH de la fase móvil se puede reducir hasta un valor de 3-4 mediante la adicción de pequeñas cantidades de un ácido volátil (p.e., 0,1% de ácido fórmico o ácido acético) o algún modificador como formiato o acetato de amonio (1-150 mM) a la fase acuosa o a ambas fases (Deng y cols., 2020; Kim y cols., 2020; Mackul'ak y cols., 2019; Mercan y cols., 2019).

Aunque la LC en fase reversa es la más utilizada para el análisis de drogas y psicofármacos, en algunos casos en que las moléculas son muy polares o muy pequeñas y no se retienen en las fases reversas convencionales, la técnica de separación empleada ha sido la cromatografía de líquidos de interacción hidrofílica (o HILIC, del inglés *Hydrophilic Interaction Liquid Chromatography*) que emplea fases estacionarias polares. En este caso, a diferencia de la cromatografía de fase reserva, al principio del gradiente cromatográfico, la fase móvil presenta porcentajes altos de la fase orgánica y la elución de los analitos se lleva a cabo con la fase acuosa. Este tipo de cromatografía se ha utilizado para la separación de cocaína, benzoilecgonina, anfetamina, metanfetamina, MDMA, 6ACM, metadona y EDDP en muestras de agua residual (Gheorghe y cols., 2008; van Nuijs y cols., 2009b).

La cromatografía de intercambio iónico es una alternativa a la HILIC para la separación de compuestos muy polares e ionizables como, por ejemplo, el sulfato de etilo. En este caso, la separación se puede lograr con fases estacionarias hidrofóbicas agregando un reactivo de par iónico que sea compatible con la fase móvil, como acetato de dihexilamonio (Reid y cols., 2011), acetato de dibutilamonio (Mastroianni y cols., 2014) o bromuro de tetrabutilamonio (Rodríguez-Álvarez y cols., 2014), todos ellos utilizados en el analizar sulfato de etilo en agua residual. En un trabajo recientemente publicado, se combina la cromatografía de intercambio iónico con la cromatografía en fase reversa para el análisis de cocaína y sus metabolitos en agua residual, mostrándose como un tipo de cromatografía alternativa para el análisis de compuestos altamente polares (González-Mariño y cols., 2019).

Otro tipo de cromatografía que tiene gran importancia y que se ha desarrollado en los últimos años es la cromatografía quiral. Este tipo de cromatografía permite la separación enantiomérica de productos racémicos lo cual resulta de gran utilidad porque permite distinguir si las drogas y psicofármacos han sido consumidos ilegalmente (p.e., en el caso de la anfetamina, la forma R(-)-anfetamina indica un consumo ilegal mientras que la forma S(+)-anfetamina está

presente en medicamentos). Además, el análisis de enantiómeros permite distinguir entre consumo o la liberación directa de la droga al sistema de alcantarillado (la presencia en Holanda de la forma racémica de MDMA, indicó consumo (R(-)-MDMA) pero también liberación directa en los sistemas de alcantarillado (S-(+)-MDMA)) (Emke y cols., 2014). Este tipo de cromatografía se ha utilizado para el análisis enantiomérico de drogas ilegales como la cocaína, los estimulantes de tipo anfetamínico, efedrina, y antidepresivos en matrices acuosas (Castrignanò y cols., 2016; Coelho y cols., 2019; Kasprzyk-Hordern y cols., 2012) utilizando columnas HPLC o UPLC quirales y empleando gradientes isocráticos.

Por último, la GC también ha sido empleada para el análisis de drogas en matrices acuosas (González-Mariño y cols., 2010; Racamonde y cols., 2012; Wang y cols., 2019) y para el análisis enantiomérico (Gonçalves y cols., 2019). Sin embargo, debido a la polaridad media-alta de estos compuestos, y la baja volatilidad de la mayoría de las drogas, el uso de GC requiere llevar a cabo reacciones de derivatización que aumentan el tiempo de preparación de la muestra, puede afectar a la precisión y exactitud de los resultados, y en muchos casos limita el número detectable de compuestos que pueden ser analizados a la vez, representando una desventaja con respecto al uso de LC.

Espectrometría de masas

Las técnicas de LC y GC utilizadas para la separación de drogas y psicofármacos utilizan como detector un espectrómetro de masas que permite obtener información cualitativa y cuantitativa de la muestra, mediante la medida de la relación masa/carga (*m/z*) de los iones presentes en dicha muestra. Los espectrómetros de masas están formados por una fuente de iones y un analizador, que pueden ser de varios tipos, y cuya elección depende del tipo de compuesto a analizar o del tipo de información que se quiera obtener.

En primer lugar, los componentes de la muestra o extracto se ionizan en la fuente de ionización. La Figura 5 muestra las diferentes fuentes de ionización utilizadas, en función de la polaridad y del peso molecular de los compuestos investigados.

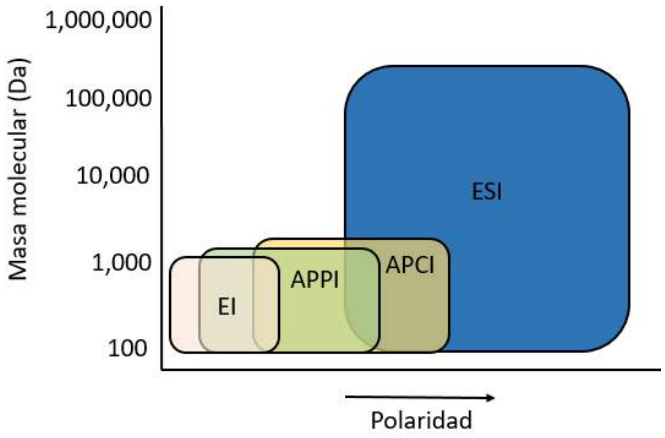


Figura 5. Fuentes de ionización utilizadas en función del peso molecular y polaridad de los compuestos investigados. EI: ionización electrónica; APPI: Fotoionización a presión atmosférica; APCI: ionización química a presión atmosférica; ESI: ionización por electrospray.

Los métodos que utilizan para la separación de drogas y psicofármacos la GC, utilizan como fuente la ionización electrónica (EI), fuente en la que la muestra es bombardeada con un haz de electrones produciendo una importante fragmentación de la misma. Por otro lado, los métodos que utilizan LC para la separación de los analitos, debido a la polaridad media-alta y al amplio rango de pesos moleculares de los mismos (100-500 Da), utilizan generalmente la fuente de electrospray (ESI), técnica de ionización más suave con la que se obtienen moléculas protonadas ($[M-H]^+$) o desprotonadas ($[M-H]^-$) en función del tipo de voltaje aplicado, positivo o negativo.

Comparada con otras fuentes de ionización, el uso de ESI presenta un inconveniente y es el aumento del efecto matriz. El efecto matriz se da cuando en el proceso de ionización de la muestra o extracto, además de los analitos, se ionizan otros componentes que coeluyen con ellos, como materia orgánica, sales, pares iónicos u otros contaminantes. Esto puede dar lugar a una disminución o aumento de la señal dando lugar a falsos negativos o falsos positivos, respectivamente. Para el análisis de drogas, el método más recomendado para corregir el efecto matriz, que además puede ser muy variable entre muestras, es el de dilución isotópica: uso de compuestos marcados isotópicamente como patrones internos (IS, del inglés *Internal Standard*). Este método consiste en añadir una concentración conocida de IS tanto a muestras como a soluciones de calibrado, soluciones control y blancos, al principio del proceso analítico, el cual nos permitirá corregir las pérdidas de analito que puedan ocurrir durante el proceso de extracción y la variación de la señal durante el proceso de ionización. El proceso de cuantificación se realiza mediante la representación de la ratio de la señal (analito/IS) frente a la ratio de la concentración (analito/IS).

Una vez los compuestos se han ionizado, se separan y detectan en el analizador de masas. El tipo de analizador utilizado determina la información obtenida, que depende del poder de resolución del mismo, así como del modo de operación utilizado. Los analizadores de tipo cuadrupolo (Q) y trampa lineal de iones (LIT) proporcionan una resolución media-baja, mientras que los analizadores tiempo de vuelo (TOF) y Orbitrap, proporcionan una resolución alta (resolución de 10,000-70,000 a la mitad de la anchura de pico (*Full width at half maximum, FWHM*) a una m/z de 200 con un error de masas inferior a 5 ppm).

La detección de drogas en aguas se ha realizado generalmente mediante espectrometría de masas en tándem (MS/MS), con instrumentos combinando diferentes analizadores, como triple cuadrupolo (QqQ) (Baker y cols., 2011a; Bijlsma y cols., 2009; Deng y cols., 2020; Foppe y cols., 2018; González-Mariño y cols., 2018; Kim y cols., 2020; Skees y cols., 2018) o cuadrupolo-trampa lineal de iones (QqLIT) (González-Mariño y cols., 2010; Irvine y cols., 2011; Jones-Lepp y cols., 2004; Mastroianni y cols., 2016; C. Metcalfe y cols., 2010; Nguyen y cols., 2018; Xiaohan Zhang y cols., 2019). Este tipo de analizadores se utiliza principalmente para realizar análisis dirigido de compuestos diana o *target analysis*, es decir, determinación de compuestos previamente seleccionados para los cuales se ha optimizado una metodología analítica. En el caso de QqLIT, el analizador LIT puede actuar como un simple cuadrupolo, y por tanto trabajar como si fuera un QqQ, o bien como una trampa de iones capaz de realizar experimentos MS^n (González-Mariño y cols., 2010; Jones-Lepp y cols., 2004). En estos espectrómetros de masas, el modo de acquisición más empleado es el registro de reacciones seleccionados (*selected reaction monitoring, SRM*) en el caso de métodos multi-analito, ya que este modo de adquisición proporciona una alta sensibilidad y selectividad, y con la adquisición de 2 transiciones SRM por compuesto se obtienen 4 puntos de identificación, cumpliendo así con los criterios establecidos en la Decisión 2002/657/CE de la Comisión Europea acerca de la identificación y confirmación de residuos de contaminantes orgánicos.

Además de QqQ y QqLIT, la hibridación de analizadores de baja resolución con los de alta resolución, como cuadrupolo-tiempo de vuelo (QqTOF) o cuadrupolo-orbitrap (Q-Exactive™-Orbitrap™), también se ha empleado para el análisis dirigido (*target analysis*) de drogas en matrices ambientales. Baz-lomba y cols. (2016) utilizaron un analizador de tipo QqTOF para el análisis de 51 drogas, NSPs y psicofármacos mientras que Fedorova y cols. (2013) utilizaron un Q-Exactive™ Orbitrap™ para el análisis de 26 drogas. Sin embargo, la gran utilidad de estos analizadores es el análisis de compuestos sospechosos (*Suspect Screening*) y desconocidos (*non-target screening*) mediante la adquisición de espectros completos de la muestra con un alto poder de resolución, tanto del ion molecular como de sus fragmentos. En

el análisis de drogas y psicofármacos, esta metodología se ha utilizado principalmente para la identificación y detección de nuevas sustancias psicoactivas en aguas, empleando instrumentos de tipo QqTOF (Andrés-Costa y cols., 2016a; Bade y cols., 2019; Causanilles y cols., 2017b), Q-Exactive™ Orbitrap™ (Fontanals y cols., 2017; Mackul'ak y cols., 2019; Salgueiro-González y cols., 2019) y LIT-Orbitrap (González-Mariño y cols., 2016a), así como de productos de transformación desconocidos (Bijlsma y cols., 2013; González-Mariño y cols., 2015; Postigo y cols., 2011c; Yang y cols., 2018). En este tipo de análisis, se realiza un barrido de masas o *Full Scan* en el que se obtiene la masa exacta del ion molecular, y posteriormente se realiza un experimento MS/MS utilizando diferentes modos de adquisición: *Data Independent Acquisition* (DIA) (Causanilles y cols., 2017b), en el que todos los iones que provienen del primer cuadrupolo se fragmentan simultáneamente; *Data Dependent Acquisition (DDA)* (González-Mariño y cols., 2016a)*, donde* sólo se fragmentan los iones más intensos o los incluidos en una lista de analitos previamente seleccionados; o el modo *Sequential Window Acquisition of all Theoretical Fragment Ion Spectra* (SWATH) (Bade y cols., 2019), que es como un DIA pero la fragmentación de los iones se realiza por grupos o ventanas de masas de entre 20 a 100 Da, lo que permite obtener espectros de MS/MS mucho más sencillos que los obtenidos con DIA.

Publicación científica #2

"Analysis of Psychoactive Pharmaceuticals in Wastewater and Surface water Using LC-MS"

por:

Ester López-García, Cristina Postigo, Bozo Zonja, Damià Barceló y Miren López de Alda

en

En Achille Cappiello, Perangela Palama (editors): Advances in the Use of liquid Chromatography Mass Spectrometry (LC-MS) Instrumentation Developments and Applications. Comprehensive Analytical Chemistry series 79 (2018), 29-52

https://doi.org/10.1016/bs.coac.2017.08.009

CHAPTER TWO

Analysis of Psychoactive Pharmaceuticals in Wastewater and Surface Water Using LC-MS

Ester López-García[1], Cristina Postigo[1,*], Bozo Zonja[1], Damià Barceló[1,2] and Miren López de Alda[1]

[1]Institute for Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain
[2]Catalan Institute for Water Research (ICRA), Girona, Spain
*Corresponding author: E-mail: cprqam@cid.csic.es

Contents

1. PHARMACEUTICALS IN THE ENVIRONMENT

There are nowadays more than 30,000 synthetic chemicals in use. Their production, use and consumption are in an exponential increase in modern society due to various social and economic factors [1]. Pharmaceutical compounds are only one of the classes of organic pollutants that are typically called emerging environmental contaminants (ECs). Other classes of ECs include, but are not limited to, industrial chemicals, nanomaterials,

Comprehensive Analytical Chemistry, Volume 79
ISSN 0166-526X
http://dx.doi.org/10.1016/bs.coac.2017.08.009

flame retardants, pesticides, personal care products, plasticizers, hormones and endocrine-disrupting compounds, perfluorinated compounds, etc.

Pharmaceuticals are principally designed to facilitate our lives and are used worldwide due to their inherent role in disease prevention and treatment for both humans and animals. According to the US Food and Drug Administration, there are currently over 1300 unique active ingredients registered and approved for usage with more than 10,000 prescriptions and over-the-counter pharmaceutical formulations [2]. On a global scale, the consumption of pharmaceuticals is expected to grow in most world regions by the year 2020 [3].

In the environment, they cannot be considered as persistent but rather pseudo-persistent organic pollutants due to their continuous discharge into water bodies via wastewater treatment plants (WWTPs). Since the WWTPs are not principally designed to eliminate these compounds, low rate of elimination in WWTPs make them ubiquitous in the aquatic environment. As a result, they can be detected even in the finished drinking water supply system [4]. Their presence can produce unforeseen adverse effects on human health and the environment. In recent years, previously not monitored chemicals have been detected in many environmental compartments because of superior sensitivity and selectivity of modern analytical instrumentation [5,6]. However, adverse ecological effects do not always correlate with high environmental concentrations detected. Anticancer drugs, for example, are often detected in the aquatic environment at sub-ng/L levels, which is low in terms of an immediate threat, but chronic exposure can have delayed toxic effects due to their interference (direct or indirect) with the DNA [7–9].

Although the presence and chronic exposure to drug residues in the environment is of general and scientific concern and interest, less attention has been paid to the evaluation of transformation of pharmaceuticals, which can be transformed in the environment by various abiotic and biotic processes. The compounds formed as a result of transformation are referred to as transformation products (TPs) [10,11]. The transformation of pharmaceuticals often starts already in the human body where they can be biotransformed by a variety of drug-metabolizing enzymes. The biodegradation can also occur via microbial degradation in WWTPs or in surface water. Other transformations that pharmaceutical compounds undergo include abiotic reactions such as hydrolysis, reduction, chemical degradation, oxidation or photolysis. However, degradation and transformation of pharmaceuticals does not necessarily mean that they will lose

their pharmacophore groups, i.e., their pharmacological activity. In the case of bioactive compounds, their effect after transformation can be retained because the pharmaceutically active part of the molecule can remain unmodified [12].

Our chapter gives an overview of recent trends in the analysis of one group of drugs – psychoactive pharmaceuticals, and their TPs in environmental aqueous samples. Emphasis is put on the analytical techniques based on liquid chromatography-mass spectrometry (LC-MS) used for detection and quantification of psychoactive substances in wastewater and surface water samples. Special attention is paid also to the use of high-resolution mass spectrometry (HRMS), not only for identification of TPs but also for target analysis. This is followed by its comparison with more common low-resolution MS methods.

Finally, the second part of the chapter gives a comprehensive summary of accumulated knowledge on psychoactive substances in the environment, which includes some recent examples of presence, transformation and fate of these drugs in the aquatic environment.

2. PSYCHOACTIVE PHARMACEUTICALS

Psychoactive substances act on the central nervous system, causing change in perception, mood, consciousness, and/or behaviour. They include prescription drugs (pharmaceuticals) and drugs of abuse legally or illicitly used (alcohol, cannabis, amphetamines, etc.). Despite the fact that several analytical approaches have been published recently to discover and quantify the presence in the environment of new psychoactive substances used only with recreational purposes and distributed on the market as 'legal highs' [13–19], the focus of this chapter is on psychoactive pharmaceuticals. These substances are also used without prescription and abusively and therefore they can lead to health issues or dependence. According to their use, psychoactive pharmaceuticals can be assembled in different groups. The most common psychoactive pharmaceuticals and their therapeutic uses are summarized in Table 1.

Like any other drug, they are metabolized in the body after their administration and excreted as the original drug and/or different metabolic byproducts in urine and faeces and, in consequence, introduced into the aquatic environment via discharged untreated/treated sewage waters. In the best-case scenario, they are partially removed during the wastewater

treatment process. Thus, the extended use of these substances, together with their continuous release into the environment, is responsible for their occurrence in different aqueous environmental matrices [20–22].

Table 1 Main psychoactive pharmaceuticals and therapeutic use

Use	Class/mode of action	Compound
Analgesic Pain reliever	Nonsteroidal antiinflammatory drugs (NSAID)	Diclofenac Ibuprofen Indomethacin Ketoprofen Mefenamic acid Naproxen
	Opioids (opioid receptor agonists)	Codeine Fentanyl Morphine Tramadol
Anesthetic Sensation loss (local) or consciousness loss (general)	NMDA receptor antagonist	Ketamine
	Amide type local anesthetic	Lidocaine
	Barbiturate	Thiopental
Antidepressant Treatment of depressive disorders	Selective serotonin reuptake inhibitors (SSRIs)/ Serotonin and norepinephrine reuptake inhibitors (SNRIs)	Citalopram Fluoxetine Paroxetine Sertraline Venlafaxine
	Tricyclic antidepressants (TCAs) — Nonselective monoamine reuptake inhibitors (NSMRIs)	Amitriptyline Clomipramine Doxepin
	Tetracyclic antidepressants (TeCAs) — Adrenergic receptor antagonist	Mirtazapine
Antiepileptic/ anticonvulsant Treatment of seizure disorders	Carboxamide antiepileptics	Carbamazepine
	Glutamate reducing agents	Lamotrigine
Antihypertensive Decrease of high blood pressure. Treatment of psychogenic polydipsia (ACE inhibitors)	β-Blockers: β-adrenergic receptor agonists/ antagonists	Atenolol Metoprolol Propranolol
	Angiotensin-converting-enzyme inhibitor (ACE inhibitors)	Captopril Enalapril

Table 1 Main psychoactive pharmaceuticals and therapeutic use—cont'd

Use	Class/mode of action	Compound
Antipsychotic Treatment of psychosis symptoms and schizophrenia, bipolar disorder, severe depression and severe anxiety	Dopamine antagonist	Clozapine Chlorpromazine Olanzapine Risperidone
Anxiolytics Treatment of anxiety and insomnia	Benzodiazepines	Alprazolam Bromazepam Diazepam Midazolam Oxazepam
	Barbiturates	Pentobarbital Phenobarbital

3. LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS OF PSYCHOACTIVE PHARMACEUTICALS IN WATER

3.1 Targeted approaches

The quantification of the occurrence of psychoactive pharmaceuticals in aqueous matrices is usually performed by LC-MS-based targeted approaches that offer maximum sensitivity and linearity range as compared to nontargeted screening. In spite of this, the detection of these substances in the environment requires their previous extraction and preconcentration from the environmental matrices so that the low levels at which they are present in the environment can be reached and undesired matrix components can be eliminated. The analytical methodologies developed to investigate the presence of psychoactive pharmaceuticals and metabolites in wastewater and surface water are summarized in Table 2 and discussed in the following subsections.

3.1.1 Sampling and sample preparation

The way in which the water samples are collected is directly related to the quality of the results obtained. Composite samples provide a more real

Table 2 Analytical methodologies used to measure psychoactive pharmaceuticals in wastewater and surface water

			Sample preparation		Liquid chromatography		Mass spectrometry		Method sensitivity LOQ (ng/L)
References	Matrix	Psychoactive pharmaceuticals	Sample pretreatment	Extraction	Type column	Mobile phase	Detector (interface)	Acq. mode	
[20]	INF (100 mL)	Analgesics (2) Anesthetics (2) Benzodiazepines (10) β-blockers (3) Opioids (2)	pH 7 SS addition	Auto SPE-DEX: HLB disks (47 mm, I.D.)	RP-UPLC Acquity HSS C18 (150 × 2.1 mm, 1.8 μm)	A: H_2O (5 mM NH_4COOH) B: ACN (0.1% FAc)	Q-ToF (ESI+)	MS[e]	0.4–187
[21]	INF EFF (50 mL)	Opiates/opioids (7) Benzodiazepines (13) Barbiturates (2) Anesthetics (6) Antiepileptics (7) Antipsychotics (6) Antidepressants (7) SSRIs (5), SNRIs (1)	Filtration (GFF, 0.7 μm) pH 2.5 (HCl) SS addition	SPE: Strata XC (200 mg, 6 mL)	RP-UPLC Kinetex PFP (50 × 2.1 mm, 1.7 μm)	ESI+ A: H_2O (0.05% FAc) B: MeOH (0.05% FAc) ESI- A: H_2O B: MeOH	QqQ (ESI+/−)	SRM	0.04–10 (87% of the compounds)
[22]	INF EFF SW (25–200 mL)	Benzodiazepines (2) SNRIs (1) SSRIs (2) TeCAs (1)	Filtration (Nylon, 0.45 μm) SS addition	MIPs (25 mg, 10 mL)	RP-UPLC Acquity BEH C18 (100 × 2.1 mm, 1.7 μm)	A: H_2O (0.1% FAc) B: ACN	QqQ (ESI+)	SRM	INF: 0.3–14 EFF: 0.1–11 SW: 0.3–12
[23,30]	INF (1 mL)	Opioids (4) Benzodiazepines (2) SNRIs (1), SSRIs (1) Antipsychotics (1) Anesthetics (1) Analgesics (2)	Filtration (Cellulose, 0.45 μm) SS addition	On-line SPE Hypersil Gold (20 × 2.1, 12 μm)	RP-HPLC Cogent bidentate (50 × 2.1 mm, 3 μm)	A: H_2O (0.1% FAc) B: ACN (0.1% FAc)	QqQ and Q-Orbitrap MS (HESI+)	2 SRM HRFS HRPS	1.3–15 0.46–13 1.7–11

[24]	INF EFF (100 mL)	Antipsychotics (4) Sedatives—hypnotics—anxiolytics (7) Antidepressants (6) Antihypertensives (6)	Centrifugation Filtration (GFF, 1 μm) SS addition	SPE Oasis HLB (200 mg/6 mL)	RP-HPLC Hypersil Gold (150 × 2.1 mm, 3 μm)	A: H_2O (0.1% FAc) B: MeOH	QqQ (ESI+)	SRM	0.1—20
[25]	DW/SW (400 mL) INF/EFF (200 mL)	Benzodiazepines (8) SSRIs (1), TCAs (2), TeCAs (1) Benzophenones (2) Antiepileptics (1)	Filtration GFF pH 8 (NH_3) MeOH (1%) SS addition	Oasis HLB (200 mg, 6 mL)	RP-HPLC Poroshell 120 EC-C18 (100 × 3 mm, 2.7 μm)	A: H_2O (2 mM NH_4CH_2COOH) B: ACN	QqQ (ESI+)	SRM	DW: 0.1—4.0 SW: 0.1—4.5 EFF: 0.1—4.1 INF: 0.1—4.8
[26]	INF (100 mL) EFF (200 mL) SW (600 mL)	Antiepileptics (1) Analgesics (1) β-blockers (2) NSAIDs (2)	Filtration (GFF) SS addition	SPE: Oasis HLB (200 mg, 6 mL)	RP-UPLC Acquity BEH C18 (100 × 2.1 mm, 1.7 μm)	A: H_2O (0.01% FAc) B: MeOH (0.01% FAc)	QqQ (ESI)	SRM	INF: 0.8—22.1 EFF: 0.1—4.3 SW: 0.1—2.9
[27]	SW	Antidepressants (9) Anxiolytics (8) Anticonvulsants (1) Antipsychotics (1)	SS addition Filtration	Auto SPE: ASPEC XL Oasis HLB (200 mg, 6 mL)	RP-HPLC Atlantis C18 (50 × 2.1 mm, 3 μm)	A: H_2O (2 mM NH_4COOH) B: ACN (0.1% FAc)	QqQ (ESI+)	SRM	10—100
[28]	SW (500 mL)	Anticonvulsants (3) Antidepressants (5) Benzodiazepines (4) Opioids (5) Antialzheimer (1) Hypnotics (2) Analgesics (1)	Filtration (GFF, 0.7 μm) EDTA (1.4 mM) pH 3 (FAc) SS addition	Oasis MCX (60 mg, 3 cc)	RP-UPLC Acquity BEH C18 (100 × 2.1 mm, 1.7 μm)	A: H_2O (0.1% FAc) B: ACN	QqQ (ESI+)	SRM	0.17—3.54

ACN, acetonitrile; *DW*, drinking water; *EDTA*, ethylenediaminetetraacetic acid; *EFF*, effluent wastewater; *ESI*, electrospray; *FAc*, formic acid; *GFF*, glass fiber filter; *HCl*, hydrochloric acid; *HESI*, heated electrospray ionization; *HRFS*, high resolution full scan; *HRPS*, high resolution product scan; *I.D.*, internal diameter; *INF*, influent wastewater; *LOQ*, limit of quantification; *MeOH*, methanol; *MIP*, molecularly imprinted polymer; *NH_4CH_2COOH*, ammonium acetate; *NH_4COOH*, ammonium formate; *NSAIDs*, nonsteroideal antiinflammatory drugs; *PFP*, pentafluorophenyl; *QqQ*, triple quadrupole; *QTOF*, quadrupole-time of flight; *RP-HPLC*, reversed-phase high performance liquid chromatography; *RP-UPCL*, reversed-phase ultra-performance liquid chromatography; *SNRIs*, serotonin and norepinephrine reuptake inhibitors; *SPE*, solid phase extraction; *SRM*, select reaction monitoring; *SS*, surrogate standard; *SSRIs*, selective serotonin reuptake inhibitors; *SW*, surface water; *TCAs*, tricyclic antidepressants; *TeCAs*, tetracyclic antidepressants.

picture than grab samples, as they may capture high concentration events that could be missed if only one grab sample is collected. Since the composition of wastewater may change throughout the day, and wastewater is a recognized major source of psychoactive pharmaceuticals, the collection of integrated samples, usually over 24-h, is the preferred sampling approach to evaluate the concentrations of these substances in this matrix [20,23–25]. The collection of composite samples can be easily accomplished by means of automatic water samplers; however, when this type of apparatus was not available, grab samples were used [21,26,27]. This is also the method conventionally used to collect surface river water since the installation of automatic water samplers unguarded in open field conditions is usually not feasible [22,26,28]. Passive samplers have also been used to overcome this limitation and obtain time-integrated concentrations (over 21 days) of psychoactive pharmaceuticals in river water [29].

Amber glass bottles have mainly been used to collect and hold the water samples [22,24,25,27,28] and, occasionally, polyethylene terephthalate (PET) bottles [21] and polypropylene (PP) bottles have also been employed [20]. After collection, samples were always maintained in cool conditions during transport and stored at -20 or 4°C until analysis.

Sample pretreatment consisted of solid suspended particles removal through vacuum filtration of the water itself or collection of the supernatant obtained after water centrifugation [24]. In the filtration process different materials have been used: glass microfiber (1 or 0.7 μm) [21,24,25,28], nylon (0.45 μm) [22], or cellulose (0.45 μm) [23]. The water samples were occasionally acidified to prevent degradation of the compounds [21,28] and the addition of a chelating agent (ethylenediaminetetraacetic acid, EDTA) has also been reported [28]. In all cases, a known amount of isotopically labelled compounds was added to the sample before [20,27] or after the filtration/centrifugation process [21–24,26,28]. These compounds are used as surrogate standards in the quantification process to correct for potential analyte losses during the extraction process and/or LC matrix effects. The addition of these compounds at the beginning of the process also helps correct for potential losses of the target analytes during sample filtration.

3.1.2 Extraction

Solid-phase extraction (SPE) has been the technique of choice to isolate target psychoactive pharmaceuticals from water samples. This is a very versatile technique that can be performed manually [21,22,24–26,28] or

with automated systems [20,27] that can even be coupled online to the LC-MS system [23]. The use of automated systems allows reducing labour and time and minimizing sample handling, which improves the reproducibility of the results. Furthermore, the sample volume is reduced and the sensibility of the method is usually improved in the case of online SPE-LC-MS approaches because the whole sample volume is transferred into the LC-MS systems instead of an aliquot of the SPE extract [30].

The retention of psychoactive pharmaceuticals onto generic polymeric reversed-phase sorbents such as Oasis HLB has been shown to be satisfactory [22,24–27]. In some cases, sorbents with cation exchange properties such as Strata XC [21] and Oasis MCX [28] have also been used. When using this type of sorbents, the sample was loaded in acidic conditions (pH ~2.5–3) so that the analytes could be retained, and the elution was performed in basic conditions (MeOH containing ammonium hydroxide or ammonia, pH ~ 10).

Automated SPE coupled to the LC-MS system has made use of a C18 column to extract and preconcentrate different psychoactive drugs including citalopram, codeine, midazolam, oxazepam, ketamine, methadone, tramadol, venlafaxine, oxycodone and some metabolites [23,30] from wastewater.

The performance of molecularly imprinted polymers (MIPs) for the extraction of psychoactive pharmaceuticals from waters has also been tested. Results showed that MIPs are highly selective and efficiently extract citalopram, paroxetine, and fluoxetine. The MIP response was consistent for these compounds up to a breakthrough volume of 200 mL. Furthermore, lower limits of detection for these compounds were obtained with MIPs than with HLB-based methods [22].

3.1.3 Liquid chromatography–mass spectrometry detection

The separation of psychoactive pharmaceuticals has been carried out with reversed-phase chromatography using high-performance liquid chromatography (HPLC) [24,25,27] or ultra-performance liquid chromatography (UPLC) [20–22,26] columns. Elution of the analytes from the chromatographic column has been achieved with an organic gradient using either ACN [20,22,23,25,27] or MeOH [21,24]. The mobile phase has been acidified with formic acid or ammonium formate to enhance the formation of molecular (protonated) ions in the positive mode [20–22,24,27]. No mobile phase modifiers have been used in the negative mode [21], although a basification of the mobile phase pH with ammonium hydroxide or ammonium bicarbonate generally favours analyte deprotonation.

Molecular ions of psychoactive pharmaceuticals amenable to mass spectrometry detection have been produced by means of electrospray ionization (ESI). This has usually been performed in the positive ESI mode. However, a few psychoactive pharmaceuticals are only amenable to negative polarity ESI ionization. This is the case of the anesthetic thiopental, the antiepileptics phenytoin and valproic acid, and the barbiturates pentobarbital and phenobarbital, which are only amenable to negative ionization [21]. The carboxylic acid–bearing compounds, such as the analgesics diclofenac, ibuprofen, mefenamic acid, naproxen, ketoprofen are amenable to both ESI polarities. Even though a better MS signal is obtained under negative ESI mode than under positive ESI mode, the latter has usually been applied, so that all target compounds could be analysed in a single chromatographic run, thereby, reducing the time and the cost of the analysis.

MS determination of the ESI-produced molecular ions has been performed with different analysers. Instruments equipped with triple quadrupole (QqQ) analysers have been the most commonly used [21,22,24–27]. They were operated in the selected reaction monitoring (SRM) mode, acquiring a minimum of two SRM transitions per compound. The recent development of benchtop HRMS instruments and the growing installation of this technology in many laboratories have boosted the creation of highly selective analytical methods for the targeted analysis of organic compounds, including psychoactive pharmaceuticals. Besides improving method selectivity due to the use of a mass tolerance window of 5 ppm, the main advantages of using HRMS hybrid analysers such as quadrupole time of flight (Q-ToF) [20] or quadrupole Orbitrap (Q-Exactive mass spectrometer) [23] compared to conventional QqQ analysers is that non-targeted full scan screening can be combined with targeted analysis in the same analytical run. Thus, a retrospective analysis of the sample can be performed any time in the future without the need of reanalysing the extract.

As for the analysis of psychoactive substances (including pharmaceuticals and illicit drugs), the quantitative performance of an HRMS Q-Exactive instrument working in targeted mode (high resolution product scan) was found to be overall better than that provided by a unit mass resolution-based tandem mass spectrometry (MS/MS) method. However, in the light of the results reported, the performance of the QqQ instrument working in SRM mode was still better than that provided by the Q-Exactive instrument working in full scan mode (HRFS) [30]. Selectivity, matrix effects and

reproducibility of the MS signal are compound-dependent parameters that need to be investigated individually for each compound.

Targeted analysis of psychoactive substances in sewage-based samples has also been performed with Q-ToF HRMS instruments using MS^e. This acquisition method allows the simultaneous registration of both precursor and product ion data during a single run. For this, full scan data were acquired after applying low (6 eV) and high (ramp from 15 to 50 eV) collision energies. Quantification of 51 target analytes was performed based on the extracted protonated precursor ion data obtained at the low collision energy value. The data gathered were also compared to a suspect screening database including mass spectra, assigned fragments and retention time for more than 1000 compounds to identify the additional analytes of interest that should be considered for target analysis [20].

3.2 Suspect screening

In recent years, a lot of attention has been dedicated to the development of suspect screening methods for ECs in environmental samples. The development of these methods has been facilitated and made possible by the introduction of HRMS instruments with both superior resolution and sensitivity. As mentioned in the previous section, the improved sensitivity of the HRMS instruments has allowed the quantification of ECs in environmental matrices at the low concentrations they are commonly present [5]. This has represented a significant shift from the use of HRMS as a tool for, almost exclusively, identification of TPs [31–33].

The main advantages of suspect screening over classical target methods is the a priori detection of suspected compounds in real environmental samples without the necessity of standards and the need to develop specific target methods [34]. Although no single report presented a comprehensive suspect screening of psychoactive pharmaceuticals, they have been readily included in large lists of environmental contaminants that were suspect screened from real-world samples [35–39]. In these studies, psychoactive pharmaceuticals like diclofenac, carbamazepine or propranolol were often detected due to the high concentrations at which they are typically found in environmental samples.

Suspect screening is already now, or will be in the future, the method of choice for screening of new psychoactive substances known as "legal highs". Since standards are not needed for screening, this cost- and time-effective solution can go a long way for rapid identification of potential new substances. This is especially important for these drugs because the list is continuously changing, and the standards can be difficult to obtain [15,20,40,41].

Moreover, the potential and applicability of suspect screening is enhanced with the idea that it can be used for screening of TPs and helps navigate through TPs generated in laboratory experiments versus real-world samples [42]. Here the biggest advantage is that, no matter the number of generated TPs in the lab, it is quite easy and efficient to screen them from environmental samples without the need of individual standards. Since the compounds are characterized by their own m/z, retention time and MS/MS fragmentation pattern and are already separated chromatographically, this information can be exported to spectral libraries and be used for suspect screening. A similar concept can also be adapted for TPs that were predicted with the use of in silico methods. There at least the m/z is certain, while the fragmentation pattern of the m/z detected should be studied for reliable confirmation. In essence, suspect screening can be applied for prior confirmation of the presence of the predicted (or lab detected) substances in the aquatic samples before their identification and characterization. On the other hand, a smart thorough suspect screening can provide a comprehensive picture of the overall contamination of samples with psychoactive pharmaceuticals.

An example of the application of suspect screening for comprehensive evaluation of TPs of drugs was that carried out by Zonja et al. for evaluation of the degradation and transformation of the antiepileptic drug lamotrigine (LMG) [43,44]. Here, the study used a suspect list made from LMG and related compounds (its human metabolites, synthetic impurities and photo-TPs) found in the literature. This suspect list was screened for in wastewater and surface water samples. The results showed the presence of significantly high concentrations of an LMG synthetic impurity (OXO-LMG) in wastewater effluent which, based on the relative MS signals, could not have been formed from LMG in the WWTP. Furthermore, once LMG was degraded in the batch reactors, OXO-LMG was not further detected. In subsequent degradation experiments carried out with other related compounds, the source of OXO-LMG was found to be the LMG human metabolite LMG-N2 glucuronide. In addition to this TP (OXO-LMG), two other TPs of LMG-N2 were detected in both laboratory and real-world samples. They were the result of deconjugation (of LMG-N2 glucuronide to LMG), oxidation of the glucuronic acid, amidine hydrolysis in combination with deconjugation (to OXO-LMG), and abiotic amidine hydrolysis. Once all the relevant compounds were identified, it was possible to assess the mass balance of LMG in three

WWTPs, which ranged from 71% to 102%. Finally, LMG-N2-G and its TPs were detected in surface water samples with median concentration ranges of 23–186 ng/L. The results of this study not only proved how a comprehensive suspect screening can lead to new insights as to transformation reactions but also that it can be used as a tool to assess the total mass balance of a compound, in this case a drug, in environmental samples.

4. OCCURRENCE

As for any other organic contaminant, concentrations of psychoactive pharmaceuticals in the aqueous environment are expected to decrease in the order influent wastewater, effluent wastewater, surface water, and drinking water. At least this is the contamination profile expected in countries where wastewater treatment is an extended practice (less common in developing countries). In the aforementioned scenario, these substances should be partially or completely removed during wastewater treatment and concentrations discharged into the water courses are further decreased due to dilution and different natural attenuation processes (photo- and biodegradation and sorption to sediments and solid particles). This cycle may be altered when the compound is not removed during water treatment processes. Furthermore, the compound may not get mineralized in the aforementioned water cycle, and it may only transform. These TPs may be more persistent than the original compound and consequently accumulate in the environment. Moreover, some of them have been proved to be more toxic than their parent compounds.

Finally, residual trace levels of these compounds and/or their TPs may also survive percolation to groundwater and drinking water production processes. In these compartments, concentrations usually in the ng/L range or lower (pg/L), or below the method limit of quantification (LOQ), have typically been reported. Examples include the concentrations of diazepam (19 ng/L), lorazepam (40 ng/L) and metadone (68 ng/L) measured in urban groundwater in the city of Barcelona (Spain) [45], or the levels of carbamazepine and amitriptyline (<LOQ), alprazolam (around 2 ng/L), and temazepam (0.2 ng/L), observed on average in drinking water in Shanghai (China) [25].

Concentrations of selected psychoactive pharmaceuticals found in wastewater and in surface water in the most recent years have been summarized in Table 3 and discussed in the next subsections.

Table 3 Average concentrations and/or concentration ranges (ng/L) reported for psychoactive pharmaceuticals in wastewater and river water

Psychoactive pharmaceutical	Country	Influent WW (ng/L)		Effluent WW (ng/L)		Surface water		References
		Range	Average	Range	Average	Range	Average	
Analgesics								
Diclofenac	China[a]	–	426	–	345	117–207	162	[26]
	France[b]		n/a		n/a	27–30	28	[28]
	Spain[c]	414–**1080**	692	189–**1150**	654		n/a	[46]
Indomethacin	China[a]	–	99	–	74		n/a	[26]
Mefenamic acid	India[d]	<LOD-**3800**	**1100**	250–750	505		n/a	[47]
Codeine	Slovakia[e]	9–75	38	n/a	n/a		n/a	[23]
	Greece[f]	85-**1632**	438	<5–112	49.7		n/a	[21]
	France[b]		n/a		n/a	8–9	8.5	[28]
	India[d]	<LOD-280	120	<LOQ-120	53.5		n/a	[47]
Morphine	Norway[g]	5.5–48	21		n/a		n/a	[20]
Tramadol	Slovakia[e]	288-**1560**	850		n/a		n/a	[23]
	France[b]		n/a		n/a	60–65	63	[28]
Antidepressants								
Citalopram	Slovakia[e]	69–151	102		n/a		n/a	[23]
	United States[h]	35–170	96	104–414	*215*		n/a	[24]
	Greece[f]	110–541	290	20–766	*367*		n/a	[21]
	Spain[i]	–	114	–	*149*	–	10	[48]
	Spain[j]		n/a		n/a	3–120	43	[27]
	Norway[g]	12–49	32		n/a		n/a	[20]
	France[b]		n/a		n/a	3–4	4	[28]

Fluoxetine	Greece[f]	0.7–20	11	0.7–24	13		n/a	[21]
	Spain[i]	–	16	–	28	–	n.d.	[48]
	Spain[j]		n/a		n/a	<10–44	14	[27]
	China[k]		3		1.4		0.4	[25]
Paroxetine	Greece[f]	<0.2–30	12	<0.2–4	1.0		n/a	[21]
	Spain[j]		n/a		n/a		n.d.	[27]
Sertraline	United States[h]	32–114	62	16–88	44		n/a	[24]
	Greece[f]	13–45	30	0.5–12	9		n/a	[21]
	Spain[i]	–	113	–	33	–	n.d.	[48]
	Spain[j]		n/a		n/a		n.d.	[27]
	India[d]	<1–91	32	<1–21	10		n/a	[47]
Venlafaxine	Slovakia[e]	142–472	256		n/a		n/a	[23]
	United States[h]	169–609	376	209–553	*410*		n/a	[24]
	Greece[f]	210–733	348	392–328	*353*		n/a	[21]
	Spain[i]	–	401	–	317	–	67	[48]
	Spain[j]		n/a		n/a	22–387	57	[27]
	France[b]		n/a		n/a	16–17	17	[28]
	India[d]	<0.1–76	22	<0.1–18	6		n/a	[47]
Amitriptyline	Greece[f]	31–98	59	<0.9–60	26		n/a	[21]
	Spain[i]	–	24	–	22	–	n.d.	[48]
	Spain[j]		n/a		n/a		n.d.	[27]
	China[k]		3		1		<1	[25]

(*Continued*)

Table 3 Average concentrations and/or concentration ranges (ng/L) reported for psychoactive pharmaceuticals in wastewater and river water—cont'd

Psychoactive pharmaceutical	Country	Influent WW (ng/L)		Effluent WW (ng/L)		Surface water		References
		Range	Average	Range	Average	Range	Average	
Antiepileptics								
Carbamazepine	United States[h]	61–588	193	91–731	*289*		n/a	[24]
	Greece[f]	10-**6822**	**1893**	21-**2200**	603		n/a	[21]
	Spain[l]	–	73	–	*181*	–	63	[48]
	Spain[l]		n/a		n/a	35–1160	82	[27]
	China[a]	–	65	–	*86*	92–120	106	[26]
	Norway[g]	35–295	138		n/a		n/a	[20]
	China[k]		45		35		25	[25]
Lamotrigine	United States[l]	–	448	–	–	–	108	[49]
	Spain[m]	13–520	184	18-**1254**		37.1–1011	189	[44]
Antihypertensives								
Atenolol	United States[h]	377-**2000**	913	299–852	510		n/a	[24]
	Norway[g]	45–166	93		n/a		n/a	[20]
	India[l]	1200-**3800**	**2150**	<LOQ-**7100**	**1045**		n/a	[47]
Metoprolol	Norway[g]	123–859	402		n/a		n/a	[20]

Propranolol	United States[h]	3–98	24	28–225	*78*		n/a	[24]
	Norway[g]	0.8–35	17		n/a		n/a	[20]
	India[d]	30–62	47	21–52	36		n/a	[47]
Anxiolytics								
Alprazolam	United States[b]	3–13	6	3–8	5		n/a	[24]
	Greece[f]	7–42	18	3–10	6		n/a	[21]
	Spain[i]	–	27	–	17	–	17	[48]
	Spain[j]		n/a		n/a		n.d.	[27]
	Norway[g]	n.d.-943	630		n/a		n/a	[20]
Diazepam	United States[h]	2–10	4	<1–4	2		n/a	[24]
	Greece[f]	<1–1	2	<1–2	*2*		n/a	[21]
	France[b]		n/a		n/a	–	0.2	[28]
	China[k]		9.5		*10*		24	[25]
	India[d]	<1–85	24	3–100	23		n/a	[47]
Lorazepam	United States[b]	6–34	18	37–114	*71*		n/a	[24]
	Greece[f]	13–30	21	8–27	17		n/a	[21]
	Spain[i]	–	**10,598**	–	589	–	167	[48]
	Spain[j]		n/a		n/a		n.d.	[27]
	China[k]		36		<4		4	[25]

(*Continued*)

Table 3 Average concentrations and/or concentration ranges (ng/L) reported for psychoactive pharmaceuticals in wastewater and river water—cont'd

Psychoactive pharmaceutical	Country	Influent WW (ng/L)		Effluent WW (ng/L)		Surface water		References
		Range	Average	Range	Average	Range	Average	
Oxazepam	Slovakia[c]	45–133	84		n/a		n/a	[23]
	United States[h]	2–17	8	4–14	9		n/a	[24]
	Greece[f]	20–63	36	6–74	37		n/a	[21]
	Spain[i]	–	83	–	84	–	n.d.	[48]
	Spain[j]	<1–210	95	<1–210	68		n/a	[27]
	France[b]		n/a		n/a	41–49	45	[28]
	China[k]		9.2		7		3	[25]
	India[d]	<1–210	95	<1–210	68		n/a	[47]

n.d., compound analysed but not detected; *n/a*, water matrix not analysed, values ≥ 1000 are highlighted in bold, average concentrations higher in the effluent than in the corresponding influent matrix are in italics. The en dash indicates that concentration ranges were not provided.

[a]Influent and effluent WW from one WWTP and SW downstream [26].

[b]Three SW sampling points [28].

[c]Influent and effluent WW samples from 10 WWTPs [46].

[d]Influent and effluent WW from one WWTP [47].

[f]Influent WW from three WWTPs collected during one week [23].

[g]Influent and effluent WW from five WWTPs [21].

[h]Influent WW from two WWTPs [20].

[i]Influent and effluent WW from two WWTPs collected during one week [24].

[j]Influent and effluent WW from five WWTPs and SW downstream [48].

[j]SW downstream 10 WWTPs [27].

[k]Locations in SW were 12, and influent and effluent WW form 5 WWTPs [25].

[l]Samples of 34 WW (not differentiated between influent and effluent), 62 SW samples [49].

[m]WW influent and effluent samples were 3, SW samples were 13 [44].

4.1 Occurrence in wastewater

As shown in Table 3, the concentrations of the individual psychoactive pharmaceuticals reviewed in influent wastewater may differ up to two orders of magnitude among countries, and also within the same country. This appears to be particularly the case for codeine and carbamazepine. This can be attributed to different consumption habits among different populations due to prescription of different drugs for the same condition or different prevalence of health conditions.

According to the literature reviewed, the most abundant psychoactive pharmaceuticals in influent wastewater, with average concentrations in the μg/L range, are the analgesic mefenamic acid (1100 ng/L), the antiepileptic carbamazepine (1893 ng/L), the antihypertensive atenolol (2150 ng/L), and the anxiolytic lorazepam (10,598 ng/L). These high concentrations were detected in India (mefenamic acid and atenolol) and in countries of Southern Europe, such as Greece and Spain. Average concentrations between 250 and 1000 ng/L have been reported also for the analgesic compounds diclofenac, codeine and tramadol, antidepressants such as citalopram and venlafaxine, the antiepileptic lamotrigine, the antihypertensive metoprolol, and the anxiolytic alprazolam in different studies. However, average concentrations within this range were only consistently detected worldwide in the case of venlafaxine; while the other compounds showed high variability among countries.

Overall, concentrations of psychoactive pharmaceuticals in effluent wastewater were lower than those measured in influent wastewater. The most abundant psychoactive pharmaceuticals in this matrix, with average concentrations above 250 ng/L, were atenolol (maximum average concentration of 1045 ng/L), diclofenac (654 ng/L), carbamazepine (603 ng/L), lorazepam (589 ng/L), mefenamic acid (505 ng/L), venlafaxine (410 ng/L) and citalopram (367 ng/L), thus coinciding to a great extent with the most abundant ones in the raw wastewater. Some compounds presented consistently higher or similar average concentrations at the outlet of the WWTP than at the inlet. This was the case for diclofenac, indomethacin, citalopram, fluoxetine, venlafaxine, carbamazepine, propranolol, diazepam, lorazepam and oxazepam. This is attributed to the poor removal of these compounds during the wastewater treatment process, which in the reviewed cases consisted of the conventional biological activated sludge process, followed occasionally by a tertiary treatment.

4.2 Occurrence in surface water

Average concentrations of individual psychoactive pharmaceuticals in the investigated river water samples were usually lower than those found in wastewater and they were always below 100 ng/L, except for diclofenac (162 ng/L) and carbamazepine (106 ng/L) in China, lamotrigine in the United States (108 ng/L) and Spain (189 ng/L), and lorazepam (167 ng/L) also in Spain (see Table 3). Maximum concentrations above 100 ng/L were recorded for the above-mentioned compounds and for the antidepressants citalopram and venlafaxine, while the antiepileptics carbamazepine and lamotrigine even reached the μg/L level. Such high concentrations were always detected downstream a WWTP outlet. Moreover, these compounds coincide with chemicals poorly removed during wastewater treatment. Taking in mind these exceptions, a few dozens of ng/L of individual psychoactive pharmaceuticals were commonly present in river waters. Despite that these low levels are not likely to cause any immediate lethal effect to aquatic organisms; the added toxicity of the mixture and its long-term effects on aquatic ecosystems are still scarcely assessed.

5. FUTURE TRENDS

The analysis of psychoactive drugs in the water cycle has attracted increasing attention in the last years, first, because of their role as emerging ECs potentially harmful for the aquatic organisms (and eventually also humans) and, second, because of their role as bioindicators of drug consumption habits, patterns, and trends. Both issues raise significant interest, each of them in their corresponding areas, and have thus prompted the development of numerous analytical methodologies with different objectives and scopes of application. Because of their physical-chemical properties and especial condition as substances subjected to illicit or abused use, LC-MS/MS appears in either case as the technique of choice for their analysis, due to the sensitivity, selectivity and reliability of results that it provides. However, the instrumentation and approach selected in each case largely depends on the main objective of the study and, of course, on the availability of the MS analysers. Whereas low resolution MS/MS instruments, operating typically in the SRM mode, remain the best option for multitargeted analysis of psychoactive drugs, high resolution MS/MS instruments are increasingly being used for suspect screening of the drugs and their metabolites and TPs. The parallel development and consolidation of large spectral databases and computer tools facilitating the identification

and confirmation of nontarget compounds has importantly contributed to this increasing acceptance and application of the suspect screening methodologies in the analytical and forensic laboratories. Since standards are not needed for screening, this cost- and time-effective solution can go a long way for rapid identification of potential new substances. However, although HRMS methods do allow for a nearly unquestionable identification and detection of new substances and TPs in qualitative analysis, authentic standards are still required for their accurate quantification in real-world samples. The years to come will likely see an increase in the development and application of both kinds of methodologies (target and suspect screening) for the analysis of psychoactive pharmaceuticals in environmental samples and the identification of many new related substances, while the assessment of their toxicity for both humans and the environment and the efficiency of new water treatments for their elimination would be desirable to obtain a comprehensive picture of their environmental occurrence, significance, and impact.

ACKNOWLEDGEMENTS

Ester López-García acknowledges the Youth Employment Initiative and the European Social Fund for the "National System of Youth Guarantee" contract within the National Operational Programme on Youth Employment 2014–2020. This work has been financially supported by the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 603437 (SOLUTIONS project) and by the Generalitat de Catalunya (Consolidated Research Groups "2014 SGR 418 - Water and Soil Quality Unit" and "2014 SGR 291 – ICRA". It reflects only the author's views. The Community is not liable for any use that may be made of the information contained therein.

REFERENCES

[1] P.H. Howard, D.C. Muir, Identifying new persistent and bioaccumulative organics among chemicals in commerce, Environ. Sci. Technol. 44 (7) (2010) 2277–2285.
[2] FDA, Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations, 2016.
[3] IMSHealth, Global Medicines Use in 2020, 2015, p. 4.
[4] A. Mendoza, B. Zonja, N. Mastroianni, N. Negreira, M. López de Alda, S. Pérez, D. Barceló, A. Gil, Y. Valcárcel, Drugs of abuse, cytostatic drugs and iodinated contrast media in tap water from the Madrid region (central Spain): a case study to analyse their occurrence and human health risk characterization, Environ. Int. 86 (2016) 107–118.
[5] B. Zonja, J. Aceña, A. Jelic, M. Petrovic, S. Pérez Solsona, D. Barceló, Transformation products of emerging contaminants: analytical challenges and future needs, in: D.A. Lambropoulou, L.M.L. Nollet (Eds.), Transformation Products of Emerging Contaminants in the Environment: Analysis, Processes, Occurrence, Effects and Risks, John Wiley and Sons Ltd, 2014, pp. 303–324.
[6] M. Petrovic, M. Farré, M.L. De Alda, S. Perez, C. Postigo, M. Köck, J. Radjenovic, M. Gros, D. Barcelo, Recent trends in the liquid chromatography–mass spectrometry

analysis of organic contaminants in environmental samples, J. Chromatogr. A 1217 (25) (2010) 4004–4017.
[7] M. Isidori, M. Lavorgna, C. Russo, M. Kundi, B. Žegura, M. Novak, M. Filipič, M. Mišík, S. Knasmueller, M.L. de Alda, D. Barceló, B. Žonja, M. Česen, J. Ščančar, T. Kosjek, E. Heath, Chemical and toxicological characterisation of anticancer drugs in hospital and municipal wastewaters from Slovenia and Spain, Environ. Pollut. 219 (2016) 275–287.
[8] T. Elersek, S. Milavec, M. Korošec, P. Brezovsek, N. Negreira, B. Zonja, M.L. de Alda, D. Barceló, E. Heath, J. Ščančar, M. Filipič, Toxicity of the mixture of selected antineoplastic drugs against aquatic primary producers, Environ. Sci. Pollut. Res. 23 (15) (2016) 14780–14790.
[9] T. Kosjek, E. Heath, Occurrence, fate and determination of cytostatic pharmaceuticals in the environment, Trends Anal. Chem. 30 (7) (2011) 1065–1087.
[10] Y. Pico, D. Barcelo, Transformation products of emerging contaminants in the environment and high-resolution mass spectrometry: a new horizon, Anal. Bioanal. Chem. 407 (21) (2015) 6257–6273.
[11] A.A. Bletsou, J. Jeon, J. Hollender, E. Archontaki, N.S. Thomaidis, Targeted and non-targeted liquid chromatography-mass spectrometric workflows for identification of transformation products of emerging pollutants in the aquatic environment, Trends Anal. Chem. 66 (2015) 32–44.
[12] B. Zhu, B. Zonja, O. Gonzalez, C. Sans, S. Pérez, D. Barceló, S. Esplugas, K. Xu, Z. Qiang, Degradation kinetics and pathways of three calcium channel blockers under UV irradiation, Water Res. 86 (2015) 9–16.
[13] V.L. Borova, P. Gago-Ferrero, C. Pistos, N.S. Thomaidis, Multi-residue determination of 10 selected new psychoactive substances in wastewater samples by liquid chromatography-tandem mass spectrometry, Talanta 144 (2015) 592–603.
[14] T. Gao, P. Du, Z. Xu, X. Li, Occurrence of new psychoactive substances in wastewater of major Chinese cities, Sci. Total Environ. 575 (2017) 963–969.
[15] I. González-Mariño, E. Gracia-Lor, R. Bagnati, C.P.B. Martins, E. Zuccato, S. Castiglioni, Screening new psychoactive substances in urban wastewater using high resolution mass spectrometry, Anal. Bioanal. Chem. 408 (16) (2016) 4297–4309.
[16] M. Ibáñez, L. Bijlsma, A.L.N. Van Nuijs, J.V. Sancho, G. Haro, A. Covaci, F. Hernández, Quadrupole-time-of-flight mass spectrometry screening for synthetic cannabinoids in herbal blends, J. Mass Spectrom. 48 (6) (2013) 685–694.
[17] M. Ibáñez, J.V. Sancho, L. Bijlsma, A.L.N. Van Nuijs, A. Covaci, F. Hernández, Comprehensive analytical strategies based on high-resolution time-of-flight mass spectrometry to identify new psychoactive substances, Trends Anal. Chem. 57 (2014) 107–117.
[18] H. Nie, X. Li, Z. Hua, W. Pan, Y. Bai, X. Fu, Rapid screening and determination of 11 new psychoactive substances by direct analysis in real time mass spectrometry and liquid chromatography/quadrupole time-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. (2016) 141–146.
[19] J.A. Weiß, M. Taschwer, O. Kunert, M.G. Schmid, Analysis of a new drug of abuse: cathinone derivative 1-(3,4-dimethoxyphenyl)-2-(ethylamino)pentan-1-one, J. Sep. Sci. 38 (5) (2015) 825–828.
[20] J.A. Baz-Lomba, M.J. Reid, K.V. Thomas, Target and suspect screening of psychoactive substances in sewage-based samples by UHPLC-QTOF, Anal. Chim. Acta 914 (2016) 81–90.
[21] V.L. Borova, N.C. Maragou, P. Gago-Ferrero, C. Pistos, N.S. Thomaidis, Highly sensitive determination of 68 psychoactive pharmaceuticals, illicit drugs, and related human metabolites in wastewater by liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 406 (17) (2014) 4273–4285.

[22] K. Demeestere, M. Petrović, M. Gros, J. Dewulf, H. Van Langenhove, D. Barceló, Trace analysis of antidepressants in environmental waters by molecularly imprinted polymer-based solid-phase extraction followed by ultra-performance liquid chromatography coupled to triple quadrupole mass spectrometry, Anal. Bioanal. Chem. 396 (2) (2010) 825–837.
[23] T. Mackuľak, L. Birošová, M. Gál, I. Bodík, R. Grabic, J. Ryba, J. Škubák, Wastewater analysis: the mean of the monitoring of frequently prescribed pharmaceuticals in Slovakia, Environ. Monit. Assess. 188 (1) (2016) 1–12.
[24] B. Subedi, K. Kannan, Occurrence and fate of select psychoactive pharmaceuticals and antihypertensives in two wastewater treatment plants in New York State, USA, Sci. Total Environ. 514 (2015) 273–280.
[25] M. Wu, J. Xiang, C. Que, F. Chen, G. Xu, Occurrence and fate of psychiatric pharmaceuticals in the urban water system of Shanghai, China, Chemosphere 138 (2015) 486–493.
[26] G. Dai, J. Huang, W. Chen, B. Wang, G. Yu, S. Deng, Major pharmaceuticals and personal care products (PPCPs) in wastewater treatment plant and receiving water in Beijing, China, and associated ecological risks, Bull. Environ. Contam. Toxicol. 92 (6) (2014) 655–661.
[27] S. González Alonso, M. Catalá, R.R. Maroto, J.L.R. Gil, Á.G de Miguel, Y. Valcárcel, Pollution by psychoactive pharmaceuticals in the Rivers of Madrid metropolitan area (Spain), Environ. Int. 36 (2) (2010) 195–201.
[28] V. Brieudes, S. Lardy-Fontan, B. Lalere, S. Vaslin-Reimann, H. Budzinski, Validation and uncertainties evaluation of an isotope dilution-SPE-LC-MS/MS for the quantification of drug residues in surface waters, Talanta 146 (2016) 138–147.
[29] G. Fedorova, T. Randak, O. Golovko, V. Kodes, K. Grabicova, R. Grabic, A passive sampling method for detecting analgesics, psycholeptics, antidepressants and illicit drugs in aquatic environments in the Czech Republic, Sci. Total Environ. 487 (1) (2014) 681–687.
[30] G. Fedorova, T. Randak, R.H. Lindberg, R. Grabic, Comparison of the quantitative performance of a Q-exactive high-resolution mass spectrometer with that of a triple quadrupole tandem mass spectrometer for the analysis of illicit drugs in wastewater, Rapid Commun. Mass Spectrom. 27 (15) (2013) 1751–1762.
[31] B. Zonja, J. Aceña, S. Pérez, D. Barceló, Chapter 16-methods for elucidation of transformation pathways: identification of intermediate products, chiral, and isotope-ratio mass spectrometry analysis, in: D.B. Mira Petrovic, P. Sandra (Eds.), Comprehensive Analytical Chemistry, Elsevier, 2013, pp. 593–610.
[32] J. Aceña, D. Rivas, B. Zonja, S. Pérez, D. Barceló, Liquid chromatography-mass spectrometry: quantification and confirmation aspects, in: Fast Liquid Chromatography-Mass Spectrometry Methods in Food and Environmental Analysis, 2015, pp. 347–377.
[33] V. Leendert, H. Van Langenhove, K. Demeestere, Trends in liquid chromatography coupled to high-resolution mass spectrometry for multi-residue analysis of organic micropollutants in aquatic environments, Trends Anal. Chem. 67 (2015) 192–208.
[34] M. Hannemann, B. Zonja, D. Barceló, S. Pérez, HRMS approaches for evaluating transformations of pharmaceuticals in the aquatic environment, in: ACS Symposium Series, 2016, pp. 25–44.
[35] L. Vergeynst, H. Langenhove, P. Joos, K. Demeestere, Suspect screening and target quantification of multi-class pharmaceuticals in surface water based on large-volume injection liquid chromatography and time-of-flight mass spectrometry, Anal. Bioanal. Chem. 406 (11) (2014) 2533–2547.

[36] A. Masia, J. Campo, C. Blasco, Y. Pico, Ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry to identify contaminants in water: an insight on environmental forensics, J. Chromatogr. A 1345 (2014) 86–97.
[37] J. Nurmi, J. Pellinen, A.-L. Rantalainen, Critical evaluation of screening techniques for emerging environmental contaminants based on accurate mass measurements with time-of-flight mass spectrometry, J. Mass Spectrom. 47 (3) (2012) 303–312.
[38] K.O. K'Oreje, K. Demeestere, P. De Wispelaere, L. Vergeynst, J. Dewulf, H. Van Langenhove, From multi-residue screening to target analysis of pharmaceuticals in water: development of a new approach based on magnetic sector mass spectrometry and application in the Nairobi River basin, Kenya, Sci. Total Environ. 437 (2012) 153–164.
[39] C. Hug, N. Ulrich, T. Schulze, W. Brack, M. Krauss, Identification of novel micropollutants in wastewater by a combination of suspect and nontarget screening, Environ. Pollut. 184 (2014) 25–32.
[40] J. Kinyua, N. Negreira, B. Miserez, A. Causanilles, E. Emke, L. Gremeaux, P. de Voogt, J. Ramsey, A. Covaci, A.L.N. van Nuijs, Qualitative screening of new psychoactive substances in pooled urine samples from Belgium and United Kingdom, Sci. Total Environ. 573 (2016) 1527–1535.
[41] M.J. Andrés-Costa, V. Andreu, Y. Picó, Analysis of psychoactive substances in water by information dependent acquisition on a hybrid quadrupole time-of-flight mass spectrometer, J. Chromatogr. A 1461 (2016) 98–106.
[42] B. Zonja, A. Delgado, S. Pérez, D. Barceló, LC-HRMS suspect screening for detection-based prioritization of iodinated contrast media photodegradates in surface waters, Environ. Sci. Technol. 49 (6) (2015) 3464–3472.
[43] B. Zonja, A. Delgado, J.L. Abad, S. Pérez, D. Barceló, Abiotic amidine and guanidine hydrolysis of lamotrigine-N2-glucuronide and related compounds in wastewater: the role of pH and N2-substitution on reaction kinetics, Water Res. 100 (2016) 466–475.
[44] B. Zonja, S. Perez, D. Barcelo, Human metabolite lamotrigine-N(2)-glucuronide is the principal source of lamotrigine-derived compounds in wastewater treatment plants and surface water, Environ. Sci. Technol. 50 (1) (2016) 154–164.
[45] A. Jurado, N. Mastroianni, E. Vàzquez-Suñé, J. Carrera, I. Tubau, E. Pujades, C. Postigo, M.L. de Alda, D. Barceló, Drugs of abuse in urban groundwater. A case study: Barcelona, Sci. Total Environ. 424 (2012) 280–288.
[46] V. Osorio, M. Imbert-Bouchard, B. Zonja, J.L. Abad, S. Perez, D. Barcelo, Simultaneous determination of diclofenac, its human metabolites and microbial nitration/nitrosation transformation products in wastewaters by liquid chromatography/quadrupole-linear ion trap mass spectrometry, J. Chromatogr. A 1347 (2014) 63–71.
[47] B. Subedi, K. Balakrishna, D.I. Joshua, K. Kannan, Mass loading and removal of pharmaceuticals and personal care products including psychoactives, antihypertensives, and antibiotics in two sewage treatment plants in southern India, Chemosphere 167 (2017) 429–437.
[48] S. Esteban, Y. Valcárcel, M. Catalá, M.G. Castromil, Psychoactive pharmaceutical residues in the watersheds of Galicia (Spain), Gac. Sanit. 26 (5) (2012) 457–459.
[49] I. Ferrer, E.M. Thurman, Identification of a new antidepressant and its glucuronide metabolite in water samples using liquid chromatography/quadrupole time-of-flight mass spectrometry, Anal. Chem. 82 (19) (2010) 8161–8168.

1.5.2. Metodologías analíticas para determinar drogas y psicofármacos y sus metabolitos en matrices sólidas

Las metodologías desarrolladas para determinar drogas y psicofármacos y metabolitos en matrices medioambientales sólidas difieren de las de aguas únicamente en el muestreo, pretratamiento y extracción de la muestra, ya que la determinación se suele realizar mediante las técnicas basadas en cromatografía de gases o de líquidos acopladas a espectrometría de masas de baja o alta resolución comentadas en el apartado anterior.

Recogida, pretratamiento y conservación de muestras sólidas

La toma de muestras ambientales sólidas como sedimentos, lodos o biota se suele realizar de forma puntual o integrando las muestras puntuales recogidas en una determinada localización. Las muestras, recogidas con dragas en el caso de lodos y sedimentos, y con redes o manualmente en el caso de biota, son depositadas en bandejas de aluminio (Carmona y cols., 2017), bolsas de plástico antimicrobianas (Gago-Ferrero y cols., 2015) o botellas de vidrio ámbar (Klosterhaus y cols., 2013), transportadas a 4 °C hasta el laboratorio, y congeladas a -20 °C hasta su extracción.

El pretratamiento de lodos o sedimentos normalmente incluye su liofilización (Evans y cols., 2015; Gago-Ferrero y cols., 2015; Langford y cols., 2011), para eliminar el contenido en agua de las muestras y facilitar la estabilidad y preservación de las mismas hasta el análisis. Posteriormente, en algunos casos, las muestras liofilizadas se tamizan hasta un tamaño de partícula que varía entre 0,1 y 2 mm para obtener una muestra homogénea (Álvarez-Ruiz y cols., 2015; Vazquez-Roig y cols., 2010b).

En el caso de biota, las muestras pueden ser diseccionadas para obtener determinados tejidos (branquias, glándulas digestivas, músculo) (Álvarez-Muñoz y cols., 2015; de Solla y cols., 2016), o bien se puede trabajar con el organismo completo (C. D. Metcalfe y cols., 2010), ya sea liofilizado (Moreno-González y cols., 2016) o fresco (Klosterhaus y cols., 2013). Las muestras de biota suelen homogeneizarse antes de su liofilización, o antes de su extracción en el caso de organismos frescos, con el uso de morteros, licuadoras (Bayen y cols., 2015) o molinillos de bolas (Moreno-González y cols., 2016).

Extracción de muestra sólidas

Las metodologías analíticas desarrolladas para el análisis de drogas, psicofármacos y sus metabolitos en muestras sólidas, como sedimentos, lodos o biota se basan en extracción con líquidos presurizados (PLE) (Álvarez-Muñoz y cols., 2015; Arbeláez y cols., 2014; Jones-Lepp y cols., 2007; Langford y cols., 2011; Mastroianni y cols., 2013; Moreno-González y cols., 2016), extracción con ultrasonidos (Álvarez-Ruiz y cols., 2015; Carmona y cols., 2017; Gago-Ferrero y cols., 2015; Klosterhaus y cols., 2013; Wilkinson y cols., 2018), extracción por agitación mecánica o manual sólido-líquido (Bayen y cols., 2015) o extracción con microondas (Evans y cols., 2015). Posteriormente el extracto obtenido se purifica con el objetivo de eliminar los componentes de la matriz que se han extraído simultáneamente con los analitos de interés, siendo la extracción en fase sólida (SPE) una de las técnicas más utilizadas (Álvarez-Muñoz y cols., 2015; Álvarez-Ruiz y cols., 2015; Carmona y cols., 2017; de Solla y cols., 2016; Evans y cols., 2015; Vazquez-Roig y cols., 2012). Para llevar a cabo la SPE, el extracto obtenido se evapora total o parcialmente con una corriente de nitrógeno, se redisuelve en agua, y se extrae utilizando el mismo tipo de cartuchos indicados en el apartado de extracción de muestras de agua (publicación #2). Además de la SPE, la cromatografía por permeación de gel (o exclusión molecular) (Álvarez-Muñoz y cols., 2015; Moreno-González y cols., 2016) también se ha empleado para purificar los extractos, mientras que en otros casos, los extractos no se purifican, únicamente se filtran (Arbeláez y cols., 2014) o centrifugan (Gago-Ferrero y cols., 2015; Langford y cols., 2011) antes de su análisis.

De los métodos de extracción anteriormente indicados, la PLE es una de las técnicas preferidas, debido a su gran eficacia de extracción, al aplicar altas temperaturas y presiones, y a su automatización, que permite obtener resultados más reproducibles, así como reducir el tiempo de extracción y la cantidad de disolvente empleado. Esta técnica requiere la optimización de varios parámetros como la cantidad de muestra, el disolvente de extracción, el número de ciclos (número secuencial de extracciones realizadas en la misma muestra), la temperatura y el tiempo de extracción (Nieto y cols., 2010). En las metodologías publicadas, entre 0,1 y 10 g de muestra se introducen en la celda de extracción, la cual se rellena posteriormente con resinas inertes, como hidromatrix, o resinas activas, como alúmina básica, neutra o ácida para llevar a cabo una primera limpieza del extracto. Las condiciones de trabajo más comúnmente utilizadas incluyen el uso de diclorometano, metanol, y diferentes mezclas de metanol/agua con o sin ácido como solventes de extracción, entre 1 y 4 ciclos de extracción, temperaturas entre 50 y 110 °C, y tiempos estáticos entre 5 y 15 minutos.

En los últimos años el uso de métodos QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) para la extracción de muestras ambientales ha tomado especial importancia. Esta metodología fue desarrollada inicialmente para el análisis de pesticidas en frutas y verduras (Anastassiades y cols., 2003) y consistía en la extracción de 10 g de muestra fresca con 10 mL de ACN y una mezcla de sales (4 mg $MgSO_4$ + 1 g NaCl) utilizadas para aumentar la fuerza iónica del medio y favorecer la extracción. El extracto obtenido se purificaba posteriormente mediante extracción en fase sólida dispersiva (*d*-SPE) con kits generalmente compuestos por sorbentes de aminas primarias y secundarias (PSA) utilizadas para la eliminación de los ácidos grasos, sulfato de magnesio para eliminar el agua y C18 para eliminar los lípidos residuales.

En los últimos años, esta metodología se ha utilizado para la extracción de diferentes compuestos en una gran variedad de matrices, empleando el método desarrollado por Anastassiades o bien introduciendo modificaciones para mejorar en cada caso la extracción de los analitos objeto de estudio (Perestrelo y cols., 2019). En el caso de drogas y psicofármacos, la mayoría de estudios investigan matrices biológicas como sangre, orina o pelo (Anzillotti y cols., 2014; Dulaurent y cols., 2016; Licata y cols., 2016). Sin embargo, también hay varios estudios que utilizaron los QuEChERS para la extracción de psicofármacos, principalmente antidepresivos y benzodiazepinas, de muestras medioambientales sólidas como sedimentos (Fernandes y cols., 2020; Santos y cols., 2016) y mejillones (Martínez Bueno y cols., 2014). En estos estudios, entre 2 y 5 gramos de muestra liofilizada, se humedecen con 10 mL de agua y posteriormente se extraen con 10 mL de ACN, o ACN con 2% de hidróxido de amonio, y diferentes mezclas de sales (4 g $MgSO_4$ + 1 g NaCl en el caso de los sedimentos, y 4 g Na_2SO_4 + 1 g NaCl + 1 g Na_3Citrato:$2H_2O$ + 0.5 g Na_2HCitrato:$3H_2O$ en el caso de mejillones), y posteriormente el extracto se purifica mediante *d-SPE* utilizando en el caso de sedimentos una mezcla de 150 mg de PSA + 150 mg C18 + 900 mg $MgSO_4$, y en el caso de mejillones un material sorbente con átomos de zirconio. Después, los extractos obtenidos se evaporan a sequedad y se reconstituyen con mezclas de ACN/agua para su posterior análisis.

Aunque este método de extracción todavía no está muy extendido para el análisis de drogas y psicofármacos en muestras ambientales sólidas, debe tenerse en consideración, ya que es un método muy fácil y rápido de usar, que no requiere instrumentación especial, proporciona extracciones robustas y fiables, y utiliza cantidades de muestra y disolventes muy reducidas, cumpliendo con los principios de la química verde

Capítulo 2.

Objetivos

Como se ha mostrado en la introducción, las drogas y psicofármacos y sus metabolitos se encuentran presentes tanto en aguas como en matrices ambientales sólidas, debido a su incompleta eliminación en las EDARs y su continúa introducción en el medioambiente. Las concentraciones a las que estos compuestos se han detectado son muy bajas (entre ng/L y µg/L), por lo que su detección requiere del empleo de metodologías analíticas muy sensibles y robustas. Hasta la fecha, numerosos trabajos han descrito el desarrollo y aplicación de metodologías analíticas, basadas principalmente en LC-MS, para caracterizar la presencia y el destino de drogas y/o psicofármacos en el medio ambiente, aunque la mayoría de ellos han analizado por separado estas dos clases de contaminantes, y en algunas matrices como sedimento o mejillones, muchos de los compuestos objeto de estudio en esta tesis no se han investigado nunca.

Por otro lado, el análisis de aguas residuales con fines epidemiológicos ha sido ampliamente utilizado en diferentes partes del mundo para estimar el consumo de drogas legales e ilegales tanto a nivel local como nacional. Sin embargo, en España, la aplicación de esta metodología ha estado limitada a estimar el consumo de drogas legales e ilegales en un escaso número de ciudades, y hasta la fecha, las estimaciones de consumo a nivel nacional se han obtenido mediante la extrapolación de los resultados obtenidos en una única ciudad.

En este contexto, el objetivo general de esta tesis fue desarrollar y validar metodologías analíticas que permitieran determinar simultáneamente la presencia de drogas y psicofármacos en diferentes matrices medioambientales, así como extender la aplicación del análisis de aguas residuales con fines epidemiológicos a un entorno más amplio en España. Los objetivos específicos de la tesis fueron:

- Validar una metodología analítica para el análisis simultáneo de drogas y psicofármacos y de algunos de sus metabolitos en agua residual y aplicar dicha metodología a la determinación de estos compuestos en agua residual recogida a la entrada de una EDAR que da servicio a parte de la ciudad de Barcelona y su área metropolitana para evaluar los niveles a los que estos compuestos están presentes en estas aguas.

- Validar una metodología analítica para el análisis de drogas y algunos de sus metabolitos en sedimentos y aplicar dicha metodología a la determinación de estos compuestos en muestras de sedimentos recogidos durante dos años consecutivos en las cuencas de los ríos Llobregat, Ebro, Júcar y Guadalquivir, con el objetivo de

evaluar la presencia y distribución (temporal y geográfica) de estos compuestos en esta matriz, apenas investigada, y el riesgo medioambiental que suponen para los organismos acuáticos expuestos.

- Desarrollar y validar una metodología analítica para el análisis de drogas, psicofármacos y algunos de sus metabolitos en mejillones y aplicar dicha metodología a la determinación de estos compuestos en muestras de mejillones salvajes recogidos en la costa catalana y de productos comerciales para evaluar su presencia en esta matriz.

- Aplicar el protocolo de análisis de aguas residuales con fines epidemiológicos a la estimación del consumo de alcohol en varias ciudades españolas para poder obtener información más completa a nivel local, autonómico y nacional.

Capítulo 3.

Resultados

Los resultados obtenidos en esta tesis se han presentado y discutido en 4 artículos científicos publicados en revistas internacionales incluidas en el SCI (del inglés *Science Citation Index*). Las publicaciones #3, #4, y #5 muestran las metodologías analíticas validadas para el análisis de drogas, psicofármacos y algunos de sus metabolitos en agua residual, sedimentos y mejillones, respectivamente, y su aplicación a distintas muestras medioambientales. La publicación #6 muestra la aplicación del análisis de aguas residuales con fines epidemiológicos para estimar el consumo de alcohol en 16 zonas geográficas de 13 ciudades de España y compara los resultados obtenidos con los datos oficiales generados a partir de los indicadores clásicos que se vienen utilizando tradicionalmente para este fin.

3.1. Análisis de drogas, psicofármacos y algunos de sus metabolitos en agua residual

Los bajos niveles a los que se encuentran las drogas y los psicofármacos, así como sus metabolitos, en el agua residual, junto con la complejidad de esta matriz, son las principales dificultades a la hora de desarrollar metodologías analíticas que sean lo suficientemente sensibles y robustas para la detección y cuantificación de estos compuestos de una manera fiable. De todas las etapas involucradas en la preparación y el análisis de una muestra, la extracción es una etapa crítica en la que se deben aplicar procedimientos de preconcentración y purificación lo suficientemente selectivos y eficaces para retener en la mayor medida posible los compuestos objeto de estudio y disminuir al máximo posible las interferencias de la matriz. La mayoría de las metodologías desarrolladas para analizar drogas y psicofármacos en aguas residuales utilizan SPE para la extracción y purificación de las muestras. Este tipo de tratamiento requiere, por lo general, un gran volumen de muestra (100-1000 mL) e implica una manipulación considerable de la muestra, que incluye normalmente un paso final de evaporación del extracto obtenido, con la consiguiente inversión de tiempo por parte del analista. Estos inconvenientes se pueden reducir con el uso de metodologías de SPE automatizadas y acopladas en línea a los sistemas de análisis (on-line SPE). Este tipo de tecnología permite reducir el volumen de muestra a extraer y de solventes utilizados en el proceso y, además, obtener resultados más reproducibles al minimizarse la manipulación de la muestra.

En lo que respecta al análisis, la LC acoplada a MS/MS es la técnica más empleada ya que, como se comentó en la sección 1.5.1, por un lado, el empleo de GC requeriría la

derivatización de los analitos, ya que son compuestos con una polaridad medio-alta y poco volátiles (Tabla 1), y por otro, el uso de MS/MS permite conseguir una gran selectividad y sensibilidad en el análisis, lo que junto con el uso del método de dilución isotópica para la cuantificación genera resultados muy fiables.

En este contexto, la publicación #3 presenta una metodología analítica multiresiduo completamente automatizada basada en SPE acoplada en línea a LC-MS/MS para la determinación simultánea de 37 drogas, psicofármacos y algunos de sus metabolitos en agua residual. Esta metodología es una variación de una metodología previamente desarrollada en el laboratorio (Postigo y cols., 2011a). Las principales diferencias de esta nueva metodología con respecto a la anterior son: (i) la inclusión de 21 compuestos nuevos, principalmente, psicofármacos y nuevas sustancias psicoactivas, (ii) la centrifugación de la muestra en lugar de su filtración, para evitar la adsorción de los compuestos más apolares en el filtro, tal y como se observó para el THC-COOH en el trabajo realizado por Causanilles y cols. (2017a), (iii) la acidificación de la fase móvil con una mezcla de formiato amónico y ácido fórmico (5 mM, pH: 3.8) para reducir la cola de los picos cromatográficos y favorecer la ionización de algunos compuestos (p.ej. los compuestos de tipo anfetamínico), (iv) el empleo de un único tipo de cartucho para la SPE (sorbente PLRP-s) y la detección de todos los compuestos en modo de ionización positivo para determinar todos los compuestos en un único análisis, en vez de en dos, como en la anterior metodología y, (v) el lavado del cartucho con sorbente PLRP-s con agua conteniendo 5% de MeOH en vez de sólo con agua, lo que mejoró la señal de algunos compuestos (aumento de la señal de fluoxetina, THC-COOH, y OH-THC en torno a 2 veces) gracias a la eliminación de componentes interferentes de la matriz que coeluyen, sin perjudicar la señal del resto de compuestos.

Durante el desarrollo de la nueva metodología, se optimizaron las condiciones de MS/MS, tanto para los nuevos compuestos incluidos en el método multiresiduo, como para los patrones internos marcados isotópicamente utilizados para su cuantificación. También se evaluaron diferentes concentraciones del tampón ácido fórmico/formiato amónico (5, 10 y 20 mM) en la fase acuosa de la fase móvil para conseguir las condiciones en que se producía una mejor separación cromatográfica y se obtenía una mejor señal de los analitos. Por último, se evaluaron diferentes porcentajes de MeOH (0%, 2,5% y 5%) en el agua empleada para lavar el cartucho con sorbente PLRP-s con el fin de eliminar al máximo las interferencias de la matriz.

Una vez obtenidas las condiciones óptimas para la extracción y el análisis, la metodología fue validada en térmicos de linealidad, recuperación, repetibilidad, efecto matriz y

sensibilidad. Todos estos parámetros se evaluaron tanto en agua de grado HPLC como en agua residual, dopadas en ambos casos con los analitos objeto de estudio a concentraciones ambientales relevantes (entre 50 y 2.500 ng/L para todos los analitos menos para la cafeína, añadida a concentraciones entre 10.050 y 102.500 ng/L, ya que como se mostró en la sección 1.3.1.1 esta sustancia suele estar presente a concentraciones superiores en el medio ambiente).

Finalmente, la metodología se aplicó al análisis de muestras de agua residual recogidas en una EDAR que da servicio a parte de la ciudad de Barcelona y su área metropolitana. La mayoría de los psicofármacos y nuevas sustancias psicoactivas incluidas en la metodología no se habían investigado previamente en estas aguas. Los resultados obtenidos mostraron que las sustancias encontradas a mayor concentración en el agua correspondían a las drogas más consumidas en España (cocaína y cannabis) según las encuestas oficiales, y a los antidepresivos también más consumidos en el país (venlafaxina, citalopram y sertralina) según la Agencia Española de Medicamentos. Esto demuestra el potencial del análisis de aguas residuales para examinar los hábitos de consumo de la población.

Publicación científica #3

"A fully automated approach for the analysis of 37 psychoactive substances in raw wastewater based on on-line solid phase extraction-liquid chromatography-tandem mass spectrometry"

por:

Ester López-García, Nicola Mastroianni, Cristina Postigo, Damià Barceló y Miren López de Alda

en

Journal of Chromatography A, 1576 (2018), 80-89

https://doi.org/10.1016/j.chroma.2018.09.038

Journal of Chromatography A, 1576 (2018) 80–89

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A fully automated approach for the analysis of 37 psychoactive substances in raw wastewater based on on-line solid phase extraction-liquid chromatography-tandem mass spectrometry

Ester López-García[a], Nicola Mastroianni[a], Cristina Postigo[a,c,*], Damià Barceló[a,b], Miren López de Alda[a,*]

[a] Water and Soil Quality Research Group, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona 18-26, 08034, Barcelona, Spain
[b] Catalan Institute for Water Research, H2O Building, Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003, Girona, Spain
[c] Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Box 7050, SE-750 07 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 15 June 2018
Received in revised form 7 September 2018
Accepted 17 September 2018
Available online 18 September 2018

Keywords:
Drugs of abuse
Psychotropic pharmaceuticals
New psychoactive substances
On-line solid phase extraction
Liquid chromatography-tandem mass spectrometry
Sewage water

ABSTRACT

This work presents a multi-residue method for the simultaneous determination of 37 legal and illicit psychoactive substances in wastewater, including the most common illicit drugs (cocaine-related compounds, amphetamine-type stimulants, hallucinogens, opiates/opioids, and cannabinoids), new psychoactive substances (two synthetic cathinones, the synthetic opioid AH-7921, and the arylcyclohexylamine methoxetamine), and legal but controlled psychoactive substances (stimulants, benzodiazepines, antidepressants, sedatives, antipsychotics, and hypnotics). To this end a fully automated analytical approach based on solid phase extraction coupled in series to liquid chromatography tandem mass spectrometry detection (on-line SPE- LC–MS/MS) was used. The methodology developed was validated in terms of linearity, recovery, repeatability, and sensitivity in wastewater. Method linearity was between 0.1 ng/L (or the analyte limit of quantification if higher) and 2,000 ng/L (10,250 ng/L in the case of caffeine). Absolute recoveries were variable (between 5% and 132%), depending on the analyte. However, the use of isotopically labeled compounds corrected for analyte losses during the extraction process and matrix effects (relative recoveries within the range of 80–120%). Repeatability of the method was satisfactory for all analytes, with RSD values lower than 13% for most compounds. Limits of detection and quantification in wastewater were below 7 and 23 ng/L, respectively, for all analytes except lormetazepam (10 and 32 ng/L), caffeine (13 and 44 ng/L), and the cannabinoids 11-nor-9-carboxy- Δ^9-tetrahydrocannabinol (18 and 61 ng/L) and 11-hydroxy-Δ^9- tetrahydrocannabinol (69 and 228 ng/L). The method was applied to the analysis of wastewater samples collected daily in Barcelona for one week. Twenty-five of the 37 analytes were detected in the samples analyzed. Average concentrations ranged from 7 ng/L in the case of zolpidem to 54 μg/L in the case of caffeine.

1. Introduction

According to the last World Drug Report of the United Nations Office on Drugs and Crime, 275 million people between 15 and 64 years used illicit drugs at least once in 2016 [29]. Although this indicates a clear increase in the number of illicit drug users since 2006, illicit drug use has remained stable in the last five years worldwide. Such trend has also been observed in Spain, with cannabis being the most consumed drug (9.5% of last year prevalence of use among the 15–64 age band in 2015), followed by cocaine (2%), ecstasy (0.6%), hallucinogens (0.6%), and amphetamines (0.5%) [1]. In recent years new psychoactive substances (NPS) or "legal highs" have appeared in the drug market. These substances produce similar effects than traditional illicit drugs; however, they are not controlled by the 1961 United Nations Single Convention on Narcotic Drugs or the 1971 United Nations Convention on Psychotropic Substances [2]. In Spain, the consumption of this type of substances is not very widespread yet with a last year prevalence of use among people aging 15–64 years of 0.9% [1]. Last year prevalence of use of hypnosedatives including tranquilizers, sedatives and somniferous has continuously increased in Spain since 2005 (ca. 10 points in

* Corresponding authors at: Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Department of Environmental Chemistry, C/ Jordi Girona 18-26, 08034, Barcelona, Spain.
E-mail addresses: cprqam@cid.csic.es (C. Postigo), mlaqam@cid.csic.es (M. López de Alda).

https://doi.org/10.1016/j.chroma.2018.09.038
0021-9673/

10 years) [1]. Although these substances are legally prescribed to relieve pain or to treat depression, seizure disorders, anxiety and/or insomnia, among others [1], their abusive use without medical supervision can lead to health issues or even dependence. In this respect, non-prescribed use of these substances has doubled in the last 10 years (last year prevalence among people aging 15–64 of 2.3%) [1].

Given the widespread use of illicit and legal psychoactive drugs, these substances in their original form or their metabolites have been introduced into the water cycle, and their presence has been reported in wastewater, surface water, groundwater, and even drinking water [3–5]. As a result, they have been recognized as a new class of emerging organic contaminants of concern in the environment. The determination of psychoactive drugs in water matrices is justified to evaluate their environmental occurrence, required for proper risk assessment, and to back-calculate their use by a specific population, which is possible from their analysis in untreated wastewater [6].

Measurement of these substances, medium to highly polar in most cases, in aqueous environmental matrices relies on the use of sensitive and selective analytical techniques, such as liquid chromatography (LC) coupled to mass spectrometry (MS) detection. While targeted LC–MS(/MS) methods have been commonly employed for their quantitative analysis [5,7–10] non-targeted approaches using sensitive high-resolution mass spectrometers have been used for the identification of NPS [11–13].

Despite the high sensitivity of MS instruments, the low concentrations at which these substances are present in the aquatic environment call for their preconcentration prior to LC–MS analysis. This has been commonly achieved by means of solid phase extraction (SPE) [14]. Fully automated methodologies based on configurations that allow coupling the SPE step to the LC–MS system allow reducing analytical lab-effort, cost, and time [15] as compared to offline SPE based methods. Furthermore, minimum sample manipulation in on-line approaches contributes to obtaining accurate, precise and reliable results. Large volume injection in modern and sensitive MS instruments has been reported to replace well the SPE preconcentration step in the analysis of clean water samples [9]. However, on-line SPE based methodologies are still appointed as the best approaches for the automated analysis of highly complex matrices such as untreated wastewater.

In this context, this work presents a multi-residue analytical method based on on-line SPE-LC-MS/MS for the simultaneous determination of 37 selected psychoactive substances in raw wastewater. The list of target psychoactive substances included the most common illicit drugs such as cocaine and related metabolites, amphetamine-type stimulants (ATS), hallucinogens, opiates, and cannabinoids; NPS, in particular, two synthetic cathinones, one synthetic opioid and one arylcyclohexylamine;the widely consumed stimulant caffeine; and the most used psychoactive pharmaceuticals in Spain, and in general worldwide, such as benzodiazepine-type anxiolytics, antidepressants, sedatives, antipsychotics and hypnotics. Despite the fact that there are already several fully automated methodologies published in the peer-reviewed literature for the analysis of illicit drugs in wastewater, prescribed psychoactive drugs and NPS are usually not included. In this regard, to the authors' knowledge this is the first time that α-hydroxy-alprazolam (OH-ALPZ), α-hydroxy-midazolam (OH-MIDZ), midazolam (MIDZ), temazepam (TEMZ), zolpidem (ZOPD), 3,4- methylenedioxypyrovalerone (MDPV), 3,4-dichloro-N-[[1-(dimethylamino)cyclohexyl]methyl]benzamide (AH-7921), mephedrone (MEPH), and methoxetamine (MXE) are analyzed in raw wastewater using a methodology based on on-line SPE. The method developed was applied to the analysis of raw wastewater of the city of Barcelona to obtain a first general picture on the occurrence of legal and illicit psychoactive substances previously not investigated in the area, e.g., NPS and most of the psychoactive pharmaceuticals included in the developed methodology.

2. Material and methods

2.1. Reagent and materials

High purity (>97%) standards of the target compounds and isotopically labeled analogs were purchased from Cerilliant (Round Rock, TX, USA) as solutions in methanol (MeOH) or acetonitrile (ACN) at a concentration of 0.1 or 1 mg/mL. The illicit drugs investigated included cocaine (COC), its major metabolite benzoylecgonine (BE) and cocaethylene (CE), a metabolite formed when cocaine and ethanol are simultaneously consumed; the ATS amphetamine (AM), methamphetamine (MA) and 3,4- methylenedioxymethamphetamine (MDMA or ecstasy); the hallucinogenic substances ketamine (KET) and lysergic acid diethylamide (LSD); the opioids/opiates morphine (MOR), heroin (HER) and its metabolite 6-acetylmorphine (6ACM), methadone (METH) and its metabolite 2-ethylidene-1,5-dimethyl-3, 3-diphenylpyrrolidine (EDDP); and the cannabinoids 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) and 11-hydroxy- Δ^9-tetrahydrocannabinol (OH-THC), which are the main THC metabolites. The NPS included were the synthetic cathinones MEPH and MDPV, the synthetic opioid AH-7921, and the synthetic arylcyclohexylamine MXE, whereas the list of prescribed psychoactive substances covered the benzodiazepine-type anxiolytics alprazolam (ALPZ), its metabolite OH-ALPZ, diazepam (DIAZ), lorazepam (LORZ), lormetazepam (LRMZ), MIDZ, its metabolite OH-MIDZ, oxazepam (OXZ) and TEMZ; the stimulants caffeine (CAF) and ephedrine (EPH); the antidepressants citalopram (CTLP), fluoxetine (FLX), sertraline (STR), and venlafaxine (VFX); the sedative hydroxyzine (HXZ), the antipsychotic chlorpromazine (CPMZ), and the hypnotic ZOPD. Table S1 in SI shows the physical-chemical properties including pKa and log K_{ow} of the target analytes.

Working standard mixture solutions were prepared in MeOH at different concentrations in the range of 0.1 and 2,000 ng/mL (10,250 ng/mL in the case of CAF) by appropriate dilution of individual stock solutions. All of them contained the isotopically labeled (surrogate) standard (SS) mixture at a concentration of 5,050 ng/mL in the case of CAF-d_3, 50 ng/mL in the case of BE-d_8, COC-d_3, CTLP-d_6, EDDP-d_3, EPH-d_3, LORZ- d_4, LRMZ-^{13}C-d_3, METH-d_3, MOR-d_3, OH-THC-d_3, OXZ-d_5, STR-d_3, THC-COOH-d_3 and VFX-d_6, and 20 ng/mL in the case of the remaining surrogate compounds. A separate solution containing only the SS at the aforementioned concentrations was also prepared for sample analysis. Working standard mixtures were used for calibration purposes and as fortification solutions in the validation studies. All standard solutions were stored in the dark at – 20 °C until use.

All solvents used were from J.T. Baker (Serviquimia, Barcelona) and were HPLC grade. Formic acid (>98%) and ammonium formate (>99%), used as mobile phase modifiers, were purchased from Merck and Fluka Analytical (Sigma Aldrich), respectively.

2.2. On-line SPE-LC-MS–/MS method

The developed automated methodology is based on a previously published method for the determination of 18 illicit drugs and metabolites and alprazolam [16] in wastewater. Main modifications included were:

1 the expansion of the list of target compounds with 21 new target substances, i.e., AH-7921, caffeine, citalopram, chlorpromazine, diazepam, fluoxetine, hydroxyzine, ketamine, lorazepam, lormetazepam, MDPV, mephedrone, midazolam, methoxetamine, α-hydroxy-alprazolam, α-hydroxy-midazolam, oxazepam, sertraline, temazepam, venlafaxine and zolpidem,

and their corresponding isotopically labeled compounds for isotope dilution quantification,
2 the centrifugation of the samples instead of their filtration in order to reduce the adsorption of the less polar compounds (e.g., cannabinoids) onto the filters,
3 the use of an acidic mobile phase (5 mM ammonium formate/formic acid, pH 3.8) to reduce chromatographic peak tailing and improve compound ionization,
4 the extraction of all target analytes onto the wettable polymeric sorbent PLRPs (cross-linked styrene- divinylbenzene polymer, 10 × 2 mm, 15–25 μm particle size) (Spark Holland, Emmen, The Netherlands),
5 a cartridge-washing step with a 5% MeOH aqueous solution to reduce the extent of matrix interferences, and
6 the MS analysis of cannabinoids (i.e., THC-COOH and OH-THC) in the positive electrospray ionization mode.

On-line SPE was performed using an automated sample processor Symbiosis™ Pico that is composed of an Alias™ autosampler equipped with a 5 mL sample loop, an HPLC binary pump, an automated cartridge exchange (ACE) module, and a high-pressure dispenser (HPD) module (Spark Holland). For extraction, five-mL of a ten-fold diluted wastewater sample containing the SS mixture was loaded onto a previously conditioned (3 mL of ACN followed by 2 mL of HPLC-grade water at a flow rate of 5 mL/min) PLRPs cartridg at a flow rate of 1 mL/min. Upon sample loading, the cartridge was washed with 1 mL of HPLC-grade water containing 5% of MeOH at a flow rate of 5 mL/min. Finally, elution of the analytes from the cartridge to the LC system was performed with the chromatographic mobile phase (water containing 5 mM of formic acid/ammonium formate buffer and ACN at a constant flow rate of 0.3 mL/min). Chromatographic elution and separation was performed with a Purospher Star RP-18 end-capped column (125 mm × 2.0 mm, particle size 5 μm) preceded by a guard column of the same packing material, both from Merck (Darmstadt, Germany) and the following organic gradient: from 5% to 40% in 12 min, then to 70% in 6 more minutes, to 80% in the next minute and to 100% in the following 9 min. Pure organic conditions were held for 5 min to clean the column. Return to initial conditions was done in 4 min, and they were maintained for 10 min for column re- equilibration before the next injection. Since SPE of a sample in a sequence is performed at the same time that the LC–MS/MS analysis of the previously extracted one, total SPE-LC–MS/MS analysis time is defined by the longest process, which in this case corresponds to the 47 min needed for the sample chromatographic separation and column cleaning and re- equilibration.

MS analysis was performed with a 4000 QTRAP hybrid triple quadrupole-linear ion trap (QqLIT) mass spectrometer equipped with a Turbo Ion Spray Source (AB-Sciex, Framingham, MA, USA) set in the positive ionization mode (ESI+). Data acquisition was performed in the selected reaction monitoring (SRM) mode recording two SRM transitions per compound and one for the corresponding SS.

2.3. Method performance

The performance of the methodology was evaluated in terms of linearity, recovery, repeatability, sensitivity, and matrix effects.

Linearity of the method was evaluated within the concentration range 0.1–2,000 ng/L (equivalent to 1 ng/L and 20 μg/L in wastewater), for all analytes except for caffeine, whose linearity was evaluated between 0.1 and 10,250 ng/L (equivalent to 1 and 102.5 μg/L in wastewater) due to the high concentrations expected in raw wastewater. For this, ten-point calibration curves were prepared in HPLC-grade water by appropriate dilution of the working standard solutions and analyzed with the optimized methodology. Calibration curves were constructed using the relation between the area of the analyte and the area of its deuterated analog through the isotope dilution approach, and weighted least-squared linear regression using $1/x^2$ as weighting factor to minimize the influence of high concentrations in the model.

Analyte recovery was evaluated in raw wastewater. For this, five replicate analysis of fortified raw wastewater was performed at three different levels (50 ng/L, 500 ng/L and 2,500 ng/L for all drugs except for caffeine that was evaluated at 10,050 ng/L, 50,500 ng/L and 102,500 ng/L). Absolute recoveries (AR) of the target and surrogate compounds were calculated by comparing the peak areas obtained after on-line SPE-LC–MS/MS analysis of fortified wastewater samples (after subtraction of the background signal (n = 3) in the wastewater sample used in the recovery study, if present) with those obtained after LC–MS/MS analysis of solutions with equivalent amounts of analytes. Relative recoveries (RR) were also evaluated. These figures corresponded to the amount of analyte recovered in relation to the recovery of its corresponding SS.

The repeatability of the method was also examined at the aforementioned three different levels as the relative standard deviation (RSD) of the signal obtained after five replicate analysis of fortified raw wastewater samples.

The sensitivity of the method was appraised through the limits of detection (LOD) and quantification (LOQ) observed for each target analyte. These limits were experimentally estimated from the analysis of raw wastewater samples as the concentration giving a signal to noise ratio of 3 and 10, respectively. Due to the matrix heterogeneity of the wastewater samples, average LODs and LOQs are provided. Method LOD and LOQ of those compounds that were not detected in the analyzed wastewater samples were extracted from the MS signals observed after analysis of wastewater samples fortified at the lowest level.

In order to investigate matrix effects (ME), analyte peak areas obtained after on-line SPE-LC–MS/MS analysis of a wastewater sample fortified with the standard mixture at 1,000 ng/L (after subtracting the amount of the analyte in the wastewater sample, if present) were compared with those obtained after on-line SPE-LC–MS/MS analysis of HPLC-grade water fortified at equal concentration (100 ng/L after wastewater dilution), according to Eq. (1):

$$\%ME : ((A_{ww-100ng/L} - A_{ww-blank})/A_{H2O-100ng/L}) * 100) - 100 \quad (1)$$

In the absence of ME, similar peak areas should be obtained in fortified wastewater and HPLC-grade water. Negative values of ME should result when the analyte signal observed in wastewater is lower than that in the HPLC-grade water and hence, they indicate ionization suppression of the signal during MS analysis. On the contrary, positive values of ME that should result when the analyte signal in wastewater is higher than that in HPLC-grade water point out to signal enhancement due to matrix components.

2.4. Sample collection and sample preparation

24-h composite wastewater samples were collected on consecutive days during one week in March 2015 at the inlet of a WWTP located in Barcelona (Northeast of Spain). Sample collection was carried out by means of an automatic ISCO 6712C compact portable sampler (Instrumentación Analítica, El Prat de Llobregat, Barcelona, Spain) programmed to sample 50 mL every 10 min. One-liter of the homogenized sample was transferred to an amber polyethylene terephthalate (PET) flask and transported in cool conditions to the laboratory. Upon arrival, the sample was 10-fold diluted with HPLC-grade water, fortified with the SS mixture and centrifuged at 3500 rpm for 10 min. Then the supernatant was transferred to a PET bottle and stored at −20 °C in the dark until analysis.

Table 1
LC–ESI–MS/MS conditions used to determine the target analytes and corresponding isotopically labeled analogs.

Analyte	Retention time (min)	Declustering Potencial (V)	SRM transition	Colision Energy (eV)	SRM ratio (SRM1/SRM2)
		0 – 12 min			
Morphine (MOR)	3.49 ± 1.65	90	286 > 152 286 > 128	75 95	1.8 ± 0.2
MOR-d_3	*3.49 ± 1.25*	*90*	*289 > 152*	*75*	
Ephedrine (EPH)	5.04 ± 0.83	40	166 > 148 166 > 133	20 30	5.2 ± 0.7
EPH-d_3	*5.03 ± 0.79*	*40*	*169 > 151*	*20*	
Amphetamine (AM)	5.86 ± 0.71	30	136 > 119 136 > 91	15 20	0.8 ± 0.1
AM-d_5	*5.78 ± 0.74*	*30*	*141 > 96*	*20*	
Caffeine (CAF)	5.92 ± 0.62	60	195 > 138 195 > 110	30 40	5.6 ± 0.2
CAF-d_3	*5.92 ± 0.59*	*60*	*198 > 140*	*35*	
6-Acetylmorphine (ACM)	6.28 ± 0.73	90	328 > 165 328 > 152	75 80	1.5 ± 0.2
6ACM-d_6	*6.25 ± 0.77*	*90*	*334 > 165*	*75*	
Methamphetamine (MA)	6.46 ± 0.92	50	150 > 91 150 > 119	20 30	3.2 ± 0.4
MA-d_{14}	*6.46 ± 0.92*	*50*	*164 > 98*	*30*	
3,4-Methylenedioxy-methamphetamine (MDMA)	6.77 ± 0.78	50	194 > 163 194 > 105	20 35	2.7 ± 0.3
MDMA-d_5	*6.75 ± 0.73*	*40*	*199 > 135*	*35*	
Benzoylecgonine (BE)	7.14 ± 0.49	80	290 > 168 290 > 77	35 100	1.8 ± 0.3
BE-d_8	*7.08 ± 0.51*	*80*	*298 > 171*	*30*	
Mephedrone (MEPH)	7.20 ± 0.63	40	178 > 160 178 > 145	20 30	1.7 ± 0.2
MEPH-d_3	*7.19 ± 0.62*	*40*	*181 > 163*	*20*	
Ketamine (KET)	7.48 ± 0.50	40	238 > 125 238 > 179	40 40	2.3 ± 0.1
KET-d_4	*7.45 ± 0.54*	*40*	*242 > 129*	*40*	
Methoxetamine (MXE)[a]	8.36 ± 0.46	50	248 > 203 248 > 121	20 40	1.3 ± 0.1
Heroin (HER)	9.08 ± 0.49	70	370 > 165 370 > 268	70 50	4.5 ± 0.5
HER-d_9	*9.03 ± 0.46*	*70*	*379 > 272*	*45*	
3,4-Methylenedioxy-pyrovalerone (MDPV)	9.50 ± 0.48	70	276 > 175 276 > 135	30 40	1.3 ± 0.1
MDPV-d_8	*9.47 ± 0.54*	*70*	*284 > 185*	*41*	
Cocaine (COC)	9.61 ± 0.51	70	304 > 182 304 > 77	30 90	4.5 ± 0.5
COC-d_3	*9.61 ± 0.50*	*70*	*307 > 185*	*25*	
Zolpidem (ZOLP)	9.92 ± 0.39	70	308 > 235 308 > 263	46 36	2.4 ± 0.4
ZOLP-d_7	*9.85 ± 0.38*	*86*	*315 > 242*	*50*	
Lysergic acid di-ethylamide (LSD)	10.14 ± 0.49	70	324 > 223 324 > 208	40 40	1.6 ± 0.3
LSD-d_3	*10.14 ± 0.49*	*60*	*327 > 226*	*35*	
Venlafaxine (VFX)	10.23 ± 0.44	54	278 > 58 278 > 260	47 17	1.4 ± 0.1
VFX-d_6	*10.23 ± 0.44*	*56*	*284 > 64*	*45*	
Cocaethylene (CE)	10.94 ± 0.45	70	318 > 196 318 > 77	95 35	3.1 ± 0.4
CE-d_3	*10.94 ± 0.45*	*70*	*321 > 199*	*30*	
		12–18.30 min			
Citalopram (CTLP)	12.65 ± 0.40	66	325 > 109 325 > 262	37 28	2.0 ± 0.2
CTLP-d_6	*12.64 ± 0.40*	*72*	*331 > 109*	*37*	
3,4-Dichloro-N-[[1-(dimethylamino)-cyclohexyl]methyl] benzamide (AH-7921)	12.29 ± 0.55	56	329 > 284 329 > 173	20 45	1.9 ± 0.2
AH-7921-d_5	*12.26 ± 0.53*	*55*	*332 > 287*	*25*	
Midazolam (MIDZ)	12.94 ± 0.39	102	326 > 291 326 > 249	38 52	4.3 ± 0.3
MIDZ-d_4	12.90 ± 0.24	107	330 > 295	38	
2-Ethylidene-1,5-dimethyl-3, 3-di-phenylpyrrolidine (EDDP)	13.36 ± 0.49	70	278 > 234 278 > 249	45 35	2.0 ± 0.1
EDDP-d_3	*13.35 ± 0.42*	*70*	*281 > 234*	*45*	
α-Hydroxy-alpra-zolam (OH-ALPZ)	13.39 ± 0.54	92	325 > 297 325 > 216	35 55	2.9 ± 0.4
OH-ALPZ-d_5	*13.33 ± 0.36*	*102*	*330 > 302*	*37*	
α-Hydroxy-mida-zolam (OH-MIDZ)	13.63 ± 0.34	92	342 > 324 342 > 203	31 37	2.4 ± 0.2

Table 1 (*Continued*)

Analyte	Retention time (min)	Declustering Potencial (V)	SRM transition	Colision Energy (eV)	SRM ratio (SRM1/SRM2)
OH-MIDZ-d_4	*13.60 ± 0.20*	*80*	*346 > 328*	*33*	
Hydroxyzine (HXZ)	13.98 ± 0.51	34	375 > 201	57	2.8 ± 0.4
			375 > 166	29	
HXZ-d_8	13.97 ± 0.54	*47*	*383 > 201*	*25*	
Oxazepam (OXA)	14.00 ± 0.18	49	287 > 241	31	1.1 ± 0.1
			287 > 269	23	
OXA-d_5	*13.92 ± 0.30*	*56*	*292 > 246*	*36*	
Alprazolam (ALPZ)	14.42 ± 0.29	70	309 > 205	60	2.2 ± 0.4
			309 > 281	35	
ALPZ-d_5	*14.38 ± 0.30*	*60*	*314 > 286*	*40*	
Lorazepam (LORZ)	14.43 ± 0.32	60	321 > 275	20	1.6 ± 0.1
			321 > 303	30	
LORZ-d_4	*14.41 ± 0.17*	*50*	*325 > 279*	*30*	
Methadone (METH)	14.55 ± 0.35	45	310 > 265	20	2.8 ± 0.3
			310 > 105	40	
METH-d_3	*14.54 ± 0.38*	*60*	*313 > 268*	*20*	
Fluoxetine (FLX)	14.86 ± 0.33	44	310 > 44	47	4.6 ± 0.4
			310 > 148	13	
FLX-d_5	*14.85 ± 0.34*	*46*	*316 > 44*	*46*	
Sertraline (STR)	15.02 ± 0.41	41	306 > 275	19	1.6 ± 0.2
			306 > 159	39	
STR-d_3	*15.02 ± 0.44*	*46*	*309 > 159*	*43*	
Chlorpromazine (CPMZ)	15.10 ± 0.49	49	319 > 86	29	1.3 ± 0.2
			319 > 58	59	
CPMZ-d_3	*15.09 ± 0.46*	*52*	*322 > 89*	*28*	
Temazepam (TEMZ)	15.50 ± 0.11	73	301 > 255	33	2.1 ± 0.3
			301 > 283	20	
TEMZ-d_5	*15.41 ± 0.19*	*56*	*306 > 260*	*32*	
Lormetazepam (LRMZ)	15.95 ± 0.32	77	335 > 289	34	8.4 ± 0.7
			335 > 177	57	
LRMZ-$^{13}CD_3$	*15.92 ± 0.27*	*56*	*339 > 293*	*31*	
Diazepam (DIAZ)	17.00 ± 0.10	75	285 > 193	40	1.4 ± 0.1
			285 > 222	40	
DIAZ-d_5	*16.91 ± 0.21*	*75*	*290 > 154*	*40*	
		18.30–47 min			
11-hydroxy-Δ^9-tetrahydrocannabinol (OH-THC)	20.43 ± 0.23	65	331 > 313	20	9.1 ± 0.3
			331 > 193	30	
OH-THC-d_3	20.52 ± 0.18	65	334 > 316	15	
11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH)	20.56 ± 0.24	50	345 > 299	20	0.43 ± 0.09
			345 > 327	29	
THC-COOH-d_3	20.52 ± 0.18	55	348 > 330	25	

[a] Ket-d4 used as surrogate standard.

3. Results

3.1. Method optimization

MS conditions for the psychoactive drugs included in the methodology were individually optimized for each compound after infusion of an acidified standard solution (0.5–1 μg/mL) with the aid of Analyst 4.2 software and manually confirmed. Optimum declustering potential was selected in full scan mode, and the two most abundant and selective SRM transitions and optimum collision energies that maximize their intensity were selected after product ion scan experiments. THC-COOH and OH-THC were the only molecules that ionized better with negative than with positive electrospray. However, the method sensitivity for THC-COOH and OH-THC was compromised in view of reducing analysis time and cost.

Despite the fast acquisition rate of the MS analyzer, the registration of the target SRM transitions (two per target analyte and one per SS) was allocated in three different time-scheduled acquisition windows to maximize method sensitivity and ensure method reproducibility.

Chromatographic separation and peak signal were evaluated using different buffer (ammonium formate/formic acid) concentrations in the aqueous mobile phase (5 mM, 10 mM, and 20 mM). For that, a solution (MeOH:HPLC water, 1:1, v/v) containing the analytes at a concentration of 125 ng/mL was injected in the LC–MS/MS system. For most compounds, less signal suppression was observed at the lowest buffer concentration (1.3–2 times lower signal at 10 mM and 2–5 times lower signal at 20 mM as compared to 5 mM); therefore 5 mM ammonium formate/formic acid was selected as optimum LC modifier. Total ion chromatograms (TIC) obtained using the different mobile phases tested are shown as supporting information in Fig. S1.

The effect of using different amounts of methanol (0%, 2.5%, and 5%) in the aqueous solution used to wash the on-line SPE cartridge on analyte breakthrough and matrix removal was also evaluated. For this, HPLC-grade water and raw wastewater (diluted 1 to 10) fortified at a working concentration of 250 ng/L were extracted in triplicate using the different washing conditions. Analysis of HPLC-grade water showed that no analyte was washed out from the cartridge using the washing solution containing the highest percentage of organic solvent tested (5%). Analyte signals observed after analysis of fortified HPLC-grade water and raw wastewater were similar for all analytes but for fluoxetine, OH-THC and THC-COOH whose signal was increased about 2 times when the cartridge was washed with HPLC-grade water containing 5% MeOH as compared to pure HPLC-grade water. Since cannabinoids were the compounds providing the lowest instrumental method sensitivity, HPLC water containing 5% of MeOH was selected as washing solvent as it improved its sensitivity due to matrix interferences removal. Fig. S2 in SI shows the extracted ion chromatograms obtained for

OH-THC, fluoxetine, and THC-COOH after on-line SPE-LC–MS/MS analysis of a fortified wastewater sample using the different evaluated washing solutions.

The chromatographic retention time, the SRM transitions and the optimum ionization conditions for the analysis of each target analyte and its SS are provided in Table 1. Extracted ion chromatograms of the target analytes obtained after on-line SPE-LC–MS/MS analysis of a wastewater sample fortified with the standard mixture at 2,500 ng/L (102,500 ng/L in the case of caffeine) are shown in Fig. 1.

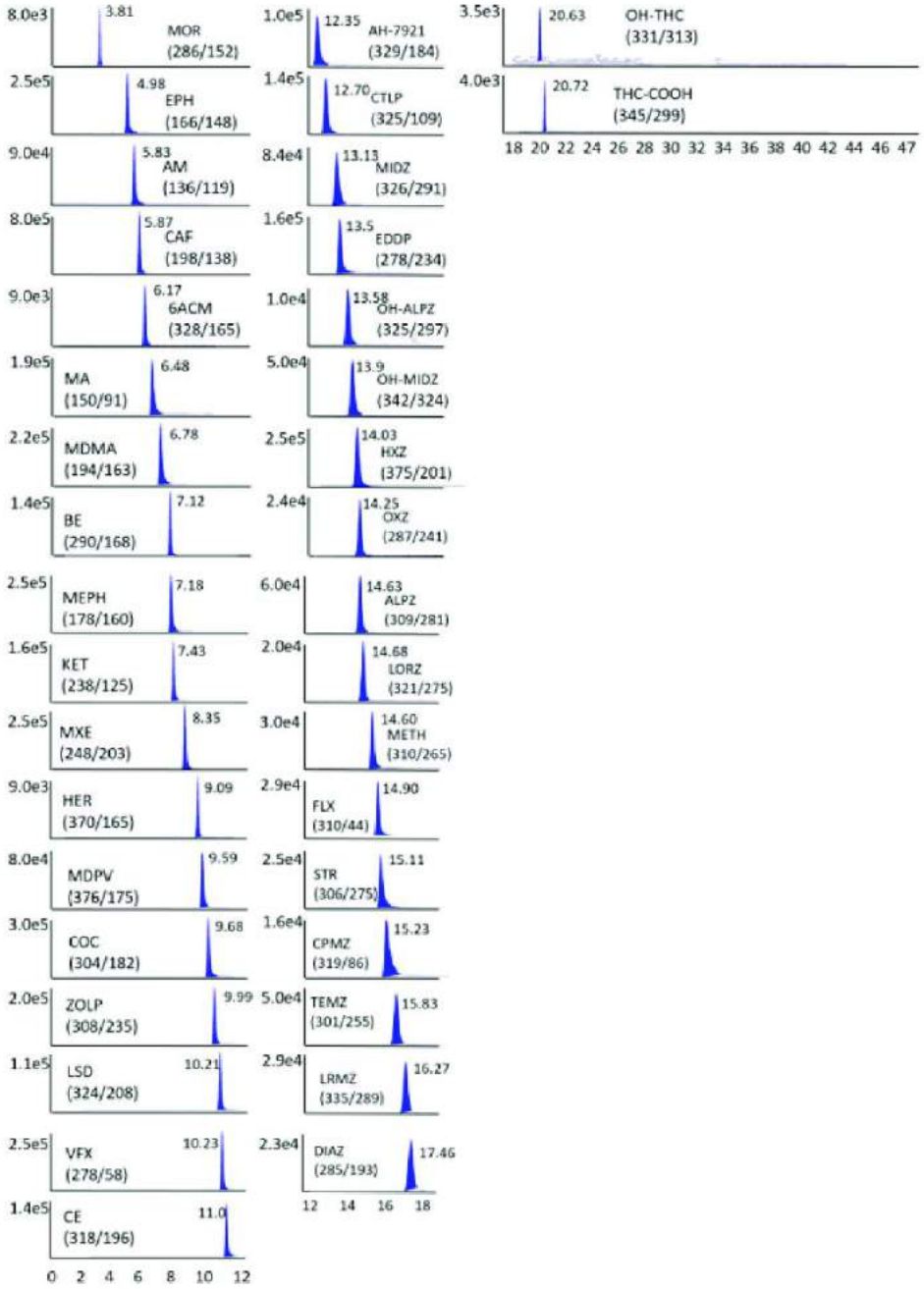


Fig. 1. Extracted ion chromatograms (XIC) of the target analytes after on-line SPE-LC–MS/MS analysis of a wastewater sample fortified with the standard compounds at a concentration of 2,500 ng/L (102,500 in the case of caffeine).

Table 2
Method performance in terms of analyte (absolute and relative) recoveries, repeatability (RSD) and sensitivity (limits of detection and quantification) in wastewater.

		Absolute recovery (n = 5)			Relative recovery (RSD) (n = 5)			LOD (ng/L)	LOQ (ng/L)
		50 ng/L	500 ng/L	2500 ng/L	50 ng/L	500 ng/L	2500 ng/L		
Cocainics	COC	n.c.	30	40	n.c.	96 (3)	97 (3)	0.2	0.8
	BE	n.c.	n.c.	70	n.c.	n.c.	98 (4)	0.5	1.5
	CE	31	33	40	108 (8)	107 (6)	105 (2)	0.5	1.4
Amphetamine-type stimulants	AM	21	50	66	106 (15)	111 (5)	112 (3)	4.0	13
	MA	37	38	44	119 (6)	110 (6)	109 (4)	2.6	8.5
	MDMA	30	36	43	98 (12)	102 (9)	98 (7)	1.0	3.4
Hallucinogens	KET	35	39	46	101 (8)	96 (5)	98 (6)	0.5	1.5
	LSD	28	32	38	96 (11)	85 (5)	99 (5)	0.8	2.8
Opioids/opiates	MOR	26	26	32	114 (20)	98 (12)	99 (8)	5.1	17
	HER	50	56	78	112 (20)	113 (7)	106 (16)	4.7	16
	6ACM	42	42	54	113 (20)	97 (9)	92 (9)	2.7	9.0
	METH	24	20	24	97 (18)	99 (7)	99 (10)	1.9	6.3
	EDDP	25	27	33	115 (6)	101 (7)	100 (2)	0.2	0.6
Cannabinoids	THC-COOH	<LOQ	102	118	< LOQ	89 (9)	94 (9)	18	61
	OH-THC	<LOQ	14	27	<LOQ	80 (8)	84 (19)	69	228
New psychoactive substances	AH-7921	36	34	37	119 (7)	103 (6)	101 (3)	1.6	5.4
	MDPV	36	34	41	109 (12)	101 (6)	98 (5)	1.3	4.3
	MEPH	43	40	47	112 (6)	101 (4)	100 (5)	3.0	10
	MXE	37	38	48	113 (9)	97 (3)	105 (6)	0.8	2.6
Benzodiazepines	ALPZ	48	62	86	112 (18)	100 (12)	93 (10)	0.6	1.9
	OH-ALPZ	80	95	117	96 (20)	110 (10)	100 (15)	3.9	13
	DIAZ	51	53	74	107 (8)	111 (8)	97 (5)	1.8	5.9
	LORZ	n.c.	99	137	n.c.	90 (5)	90 (3)	6.8	23
	LRMZ	n.c.	102	142	n.c.	118 (5)	89 (3)	10	32
	MIDZ	34	36	39	106 (8)	100 (9)	99 (5)	0.8	2.7
	OH-MIDZ	58	59	67	116 (9)	101 (9)	106 (4)	1.7	5.7
	OXZ	n.c.	81	132	n.c.	93 (8)	92 (11)	6.6	22
	TEMZ	n.c.	80	118	n.c.	100 (8)	81 (11)	2.4	7.9
Antidepressants	CTLP	n.c.	27	30	n.c.	107 (11)	104 (10)	1.9	6.4
	FLX	8	6	5	116 (18)	99 (9)	101 (9)	2.0	6.7
	STR	10	10	12	120 (13)	110 (11)	104 (13)	3.3	11
	VFX	n.c.	38	45	n.c.	103 (4)	100 (3)	0.8	2.5
Stimulants	CAF[a]	n.c.	45	58	n.c.	111 (8)	106 (7)	13	44
	EPH	n.c.	n.c.	52	n.c.	n.c.	102 (4)	4.7	16
Sedative	HXZ	37	29	32	115 (3)	110 (7)	110 (4)	0.2	0.8
Hypnotic	ZOPD	34	37	43	109 (6)	109 (3)	111 (7)	0.2	0.7
Antipsychotic	CPMZ	10	7	6	119 (20)	96 (13)	98 (18)	2.9	9.5

<LOQ: Concentration tested below the analyte limit of quantification; n.c.: not calculated, high background concentrations in the sample.
[a] Recovery studies were performed with wastewater samples spiked at 10,050, 50,500 and 102500 ng/L.

3.2. Method performance

Tables 2 and S2 in SI summarize the method performance in wastewater and HPLC- grade water, respectively, in terms of linearity, recovery, repeatability, and sensitivity.

The linearity of the method (in HPLC-grade water) expanded between 0.1 ng/L or the analyte limit of quantification (see Table S2 in SI) if higher and 2,000 ng/L for most of the compounds (10,250 ng/L in the case of caffeine). Shorter linearity ranges (see Table S2 in SI) were obtained for AH-7921, α-hydroxy-midazolam, and lorazepam. All calibration curves, constructed with a minimum of six data points, presented a coefficient of determination (r^2) higher than 0.99.

Method recovery in HPLC-grade water was evaluated at two concentration levels (50 ng/L and 250 ng/L) (see Table S2 in SI). Absolute recoveries ranged between 9 and 113%, and relative recoveries were between 82 and 120%. Analyte recoveries in raw wastewater (accounting for both extraction and matrix effects) were evaluated at three different levels: 50 ng/L, 500 ng/L, and 2,500 ng/L (10,050, 50,500 and 102,500 ng/L for caffeine). Method performance in this respect could not be assessed at the lowest tested level for cocaine, benzoylecgonine, ephedrine (not even at 500 ng/L), lorazepam, lormetazepam, oxazepam, temazepam, citalopram, venlafaxine and caffeine, due to the background levels of these substances in the wastewater used in the recovery study; and for THC-COOH and OH-THC, because of their poor method sensitivity.

Analyte absolute recoveries, in good agreement at the different levels tested, were, in general, lower than those observed in HPLC-grade water: between 30 and 60% for 55% of the analytes, between 10 and 30% for 13% of the analytes, and below 10% for 5% of the analytes. Average absolute recoveries higher than 120% were occasionally obtained for lorazepam, lormetazepam, and oxazepam. However, analyte relative recoveries, always between 80 and 120%, and in good agreement with those obtained in HPLC-grade water, proved that the use of isotopically labeled compounds allows compensating for matrix effects as well as for analyte losses during the extraction process and the suitability of HPLC-grade water based calibration solutions to quantify levels in wastewater samples.

The overall method repeatability calculated as the relative standard deviation (RSD) of n = 5 replicate analysis of fortified wastewater samples was satisfactory with RSD values always below 20%.

Regarding method sensitivity, average LODs and LOQs in raw wastewater were between 0.2 and 69 ng/L and from 0.6 to 228 ng/L, respectively. Compounds presenting the lowest sensitivity were OH-THC, THC-COOH, lormetazepam, and caffeine with LOQs ranging between 32 and 228 ng/L. Overall, most of the target analytes could be detected in wastewater with the described methodology at 5 ng/L (80% of the target compounds) and quantified at concentrations below 16 ng/L (78% of the compounds). This high sensitivity, considering the low sample volume used (5 mL), is possible thanks to the complete transfer of the sample through the on-line SPE-LS-MS/MS instrument.

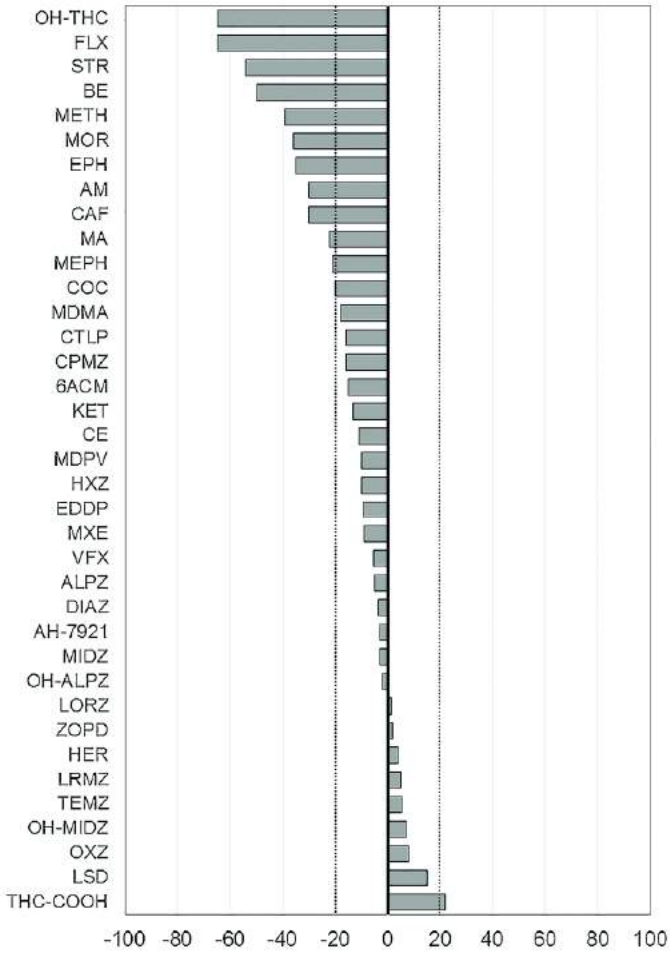


Fig. 2. Matrix effects (%) observed for the on-line SPE-LC-MS/MS analysis of psychoactive substances in raw wastewater (fortified at 1000 ng/L).

3.3. Matrix effects

The extent of matrix effects in the analysis of psychoactive substances in wastewater is shown in Fig. 2. In general, low matrix effects, within ±20%, were observed for most compounds. MS signal suppression greater than 20% was observed in the case of fluoxetine (-65%), sertraline (-54%), benzoylecgonine (-50%), methadone (-39%), morphine (-36%), ephedrine (-35%), caffeine (-30%), and amphetamine (-30%). This could explain the low absolute recoveries obtained for these compounds, particularly for sertraline and fluoxetine (AR < 10%). On the contrary, MS analysis of THC-COOH was affected by matrix components that enhanced its signal more than 20%. However, as previously mentioned, relative recoveries in the range of 80–120% for all compounds demonstrate how useful surrogate standards are at compensating matrix effects (in addition to extraction losses).

3.4. Occurrence of psychoactive substances in wastewater

The validated methodology was applied to the analysis of raw wastewater samples collected at a WWTP located in Barcelona, Spain. Fig. 3 shows the levels of psychoactive substances measured in the influent wastewater samples analyzed.

Most of the investigated compounds were present in all wastewater samples analyzed. The exceptions were zolpidem, α-hydroxy-midazolam, and temazepam that were detected in 71, 86 and 86% of the analyzed samples, respectively, and 6-acetylmorphine, heroin, LSD, midazolam, α-hydroxy- alprazolam, OH-THC, chlorpromazine, AH-7921, methoxetamine, MDPV and mephedrone that were not detected in any sample. Alprazolam was detected in all samples; however, it could not be quantified because levels measured were below its LOQ (1.9 ng/L).

The most abundant compounds in the wastewater samples analyzed presented average concentrations in the μg/L range and were the stimulants caffeine (53.8 μg/L) and ephedrine (2.3 μg/L), and indicators related to the most consumed illicit drugs in the area [1]: benzoylecgonine (2.2 μg/L), cocaine (1.3 μg/L), and THC-COOH (1.1 μg/L). The high levels of caffeine measured are in agreement with wastewater concentrations reported for this stimulant in other developed countries [14,17]. This is attributed to the high consumption of beverages such as coffee, tea or soda drinks that contain this psychoactive substance, e.g., 1.8 cups of coffee or tea were consumed per capita per day in Spain in 2015–2016 [18]. The

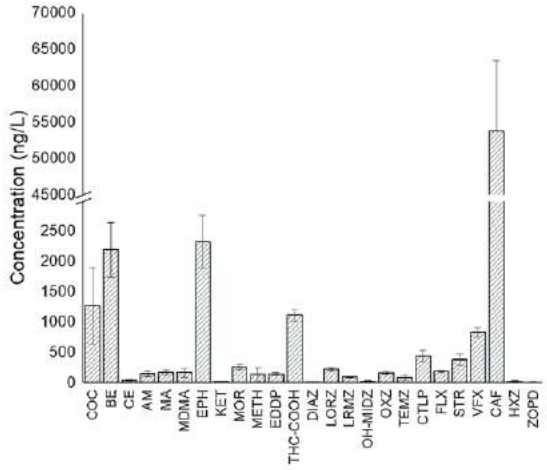


Fig. 3. Concentration (ng/L) of psychoactive substances (mean ± SD) in raw wastewater (n = 7).

extended use of ephedrine could be attributed to the therapeutic use of this substance to treat asthma, bronchitis, and hypotension, in addition to its illegal use as an amphetamine-type stimulant.

The antidepressants venlafaxine, with 838 ng/L, citalopram, with 442 ng/L and sertraline, with 381 ng/L, were the most abundant psychoactive pharmaceuticals. According to the Spanish Agency of Medicines and Medical Devices [19], consumption of antidepressants has continuously increased in Spain since 2000, being selective serotonin uptake inhibitors (SSRIs), i.e., paroxetine, fluoxetine, sertraline, citalopram and its S-stereoisomer escitalopram, and selective serotonin and norepinephrine reuptake inhibitors (SSNRI), i.e., venlafaxine and duloxetine, the most consumed drugs [19]. Such an increase in the use of antidepressants is mainly attributed to an increasing incidence of mood disorders among the population as well as to the prescription of these substances for additional therapeutic uses. Antidepressant levels measured in Barcelona were overall higher than those measured in raw wastewaters of Slovakian cities (256 ng/L in the case of venlafaxine and 102 ng/L in the case of citalopram) [20], in wastewaters of the city of New York (376 ng/L for venlafaxine, 62 ng/L for sertraline, and 96 ng/L for citalopram on average) [21], in wastewaters of the Santorini island in Greece (11 ng/L for fluoxetine, 348 ng/L for venlafaxine, 290 ng/L for citalopram, and 30 ng/L for sertraline on average) [8] and in wastewaters of other Spanish regions, e.g. Galicia (northern Spain) (16 ng/L of fluoxetine, 401 ng/L of venlafaxine, and 114 ng/L of citalopram on average) [22].

Illicit ATS amphetamine, methamphetamine and MDMA, and the opiate morphine and the synthetic opioid methadone and its metabolite EDDP presented similar concentrations in the wastewaters analyzed (between 145 and 255 ng/L). The presence of morphine in wastewater may origin from its therapeutic use but also from heroin consumption. Despite the fact that neither heroin nor its exclusive metabolite 6-acetylmorphine were detected in the investigated samples, heroin use as a source of morphine cannot be discarded because heroin is highly metabolized after consumption and 6-acetylmorphine, excreted at a very low rate, is unstable in wastewater [23]. Levels of amphetamine and morphine measured in Barcelona were lower than those measured, for example, in England (830 ng/L for amphetamine and 481 ng/L for morphine); however, higher concentrations of methamphetamine and methadone were measured in Barcelona compared with England (2 ng/L and 87 ng/L on average) [7] while similar levels of EDDP were measured in both places.

As for benzodiazepines, average concentrations decreased in the order lorazepam (227 ng/L), oxazepam (163 ng/L), lormetazepam (99 ng/L), temazepam (93 ng/L), and alprazolam (<1.9 ng/L). The benzodiazepine metabolites analyzed were either below the method LOD (α-hydroxy-alprazolam) or measured at very low concentrations (24 ng/L for α-hydroxy-midazolam). Except for alprazolam, concentrations of benzodiazepines found in wastewaters of Barcelona were higher than in wastewaters analyzed in Greece (2 ng/L of diazepam, 21 ng/L of lorazepam, and 36 ng/L of oxazepam on average) [8], in Slovakia (84 ng/L of oxazepam on average) [20] and in the city of New York (4 ng/L of diazepam, 18 ng/L of lorazepam and 8 ng/L of oxazepam on average) [21].

Finally, the lowest average concentrations were observed for cocaethylene (42 ng/L), ketamine (20 ng/L), the sedative hydroxyzine (20 ng/L), diazepam (16 ng/L), and the hypnotic zolpidem (7 ng/L), which could be attributed to a low consumption of these substances in the investigated area and their likely high metabolization before excretion (ketamine and zolpidem are excreted after consumption as unchanged drugs in urine in 2.3% and < 1%, respectively) [24,25]. This is in agreement with concentrations of these substances reported in wastewater in other investigated locations, e.g., levels of zolpidem measured in influent wastewater in the Northwest of Spain ranged from 2.9 to 3.9 ng/L [26] and were below the method LOQ in another study performed in the Northeast of Spain [27] while levels of ketamine in wastewater from Colombia were between < LOQ and 32 ng/L [28].

From the water management point of view, it may be worth noting that the results obtained correspond exclusively to influent raw waters, and that treated effluent wastewaters usually contain much lower concentrations of substances not representing risk for the environment or human health.

4. Conclusions

A fully automated method has been developed and validated for the simultaneous analysis of 37 psychoactive substances in wastewater. The method, based on on-line SPE-LC-MS/MS, allows determining most of the target compounds in the low ng/L range in a fast and simple way. The use of isotopically labeled compounds compensates potential analyte losses during the extraction process as well as matrix effects.

Additional advantages include (i) the use of low sample volumes, which facilitates sample collection, transport/shipment, and storage, (ii) minimum manipulation of the sample, which allows to improve the reproducibility of the results, as well as reduce cross contamination processes, (iii) improvement of the sensitivity due to the introduction of the whole sample volume (instead of an aliquot of a sample extract) in the analytical system, and (iv) the provision of more reliable quantitative results because samples and calibration curves are processed in exactly the same way. In contrast, the methodology presents as disadvantages: (i) the Symbiosis™ Pico system cannot be used with ultra-high performance liquid chromatography columns due to the high back pressure generated in the system, (ii) only one SPE cartridge (instead of e.g. various cartridges in tandem) can be used at a time, and thus the extraction and sensitivity of specific analytes included in a multi-residue method can be compromised, and (iii) solvents used in the on-line SPE process (e.g. in the washing step) must be compatible with the LC mobile phase.

The application of the method to influent wastewater samples from a WWTP located in Barcelona revealed the ubiquitous presence of 68% of the investigated analytes in this matrix. The most abundant compounds were the stimulant caffeine, analytes

related to the illicit drugs most consumed in the investigated area (THC-COOH, cocaine, and benzoylecgonine), and psychoactive pharmaceuticals such as ephedrine and the antidepressants venlafaxine, citalopram and sertraline. This methodology will be applied to the analysis of larger sets of samples to assess in more depth the presence of these compounds in wastewaters and to explore the use of psychoactive pharmaceutical concentrations in wastewater as potential indicators of population's health.

Acknowledgments

This work has been financially supported by the EU through the EU FP7 project SOLUTIONS (603437), and by the Government of Catalonia (Consolidated Research Groups: 2017 SGR 1404 - Water and Soil Quality Unit). It reflects only the author's views. The Community is not liable for any use that may be made of the information contained therein. Merck is acknowledged for the gift of LC columns. Thanks to the support provided by the JAE Program (CSIC-European Social Funds) to N. Mastroianni, the "National System of Youth Guarantee" contract within the National Operational programme on Youth Employment 2014-2020 (Youth Employment Initiative and the European Social Fund) to E. López García, and the Swedish University of Agricultural Sciences (August T Larsson Guest Researcher Programme) to C. Postigo . Begoña Martínez López and Cristian Mesa García from Aguas de Barcelona are acknowledged for their assistance in the collection of wastewater samples.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.chroma.2018.09.038.

References

[1] OEDA, Observatorio Español de las Drogas y las Adicciones. Informe anual 2017. Alcohol, tabaco y drogas ilegales en España. Available at https://goo.gl/jh46g9. Accessed January 2018.
[2] EMCDDA, European monitoring centre for drugs and drug addiction. European drug report, in: Trends and Developments, 2015, Available at https://goo.gl/d2Ejzx, Accessed January 2018.
[3] V. Calisto, V.I. Esteves, Psychiatric pharmaceuticals in the environment, Chemosphere 77 (2009) 1257–1274.
[4] R. Pal, M. Megharaj, K.P. Kirkbride, R. Naidu, Illicit drugs and the environment — a review, Sci. Total Environ. 463–464 (2013) 1079–1092.
[5] E. López-García, C. Postigo, B. Zonja, D. Barceló, M. López de Alda, Analysis of psychoactive pharmaceuticals in wastewater and surface water using LC-MS, in: Comprehensive Analytical Chemistry, Elsevier, 2017.
[6] S. Castiglioni, K.V. Thomas, B. Kasprzyk-Hordern, L. Vandam, P. Griffiths, Testing wastewater to detect illicit drugs: state of the art, potential and research needs, Sci. Total Environ. 487 (2014) 613–620.
[7] D.R. Baker, B. Kasprzyk-Hordern, Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography–positive electrospray ionisation tandem mass spectrometry, J. Chromatogr. A 1218 (2011) 1620–1631.
[8] V.L. Borova, N.C. Maragou, P. Gago-Ferrero, C. Pistos, N.S. Thomaidis, Highly sensitive determination of 68 psychoactive pharmaceuticals, illicit drugs, and related human metabolites in wastewater by liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 406 (2014) 4273–4285.
[9] C. Boix, M. Ibáñez, J.V. Sancho, J. Rambla, J.L. Aranda, S. Ballester, F. Hernández, Fast determination of 40 drugs in water using large volume direct injection liquid chromatography–tandem mass spectrometry, Talanta 131 (2015) 719–727.
[10] K. Li, P. Du, Z. Xu, T. Gao, X. Li, Occurrence of illicit drugs in surface waters in China, Environ. Pollut. 213 (2016) 395–402.
[11] M.J. Andrés-Costa, V. Andreu, Y. Picó, Analysis of psychoactive substances in water by information dependent acquisition on a hybrid quadrupole time-of-flight mass spectrometer, J. Chromatogr. A 1461 (2016) 98–106.
[12] J.A. Baz-Lomba, M.J. Reid, K.V. Thomas, Target and suspect screening of psychoactive substances in sewage-based samples by UHPLC-QTOF, Anal. Chim. Acta 914 (2016) 81–90.
[13] A. Causanilles, J. Kinyua, C. Ruttkies, A.L.N. van Nuijs, E. Emke, A. Covaci, P. de Voogt, Qualitative screening for new psychoactive substances in wastewater collected during a city festival using liquid chromatography coupled to high-resolution mass spectrometry, Chemosphere 184 (2017) 1186–1193.
[14] M.L. Hedgespeth, Y. Sapozhnikova, P. Pennington, A. Clum, A. Fairey, E. Wirth, Pharmaceuticals and personal care products (PPCPs) in treated wastewater discharges into Charleston Harbor, South Carolina, Sci. Total Environ. 437 (2012) 1–9.
[15] A. Rubirola, M.R. Boleda, M.T. Galceran, Multiresidue analysis of 24 Water Framework Directive priority substances by on-line solid phase extraction-liquid chromatography tandem mass spectrometry in environmental waters, J. Chromatogr. A 1493 (2017) 64–75.
[16] C. Postigo, M.L. de Alda, D. Barceló, Evaluation of drugs of abuse use and trends in a prison through wastewater analysis, Environ. Int. 37 (2011) 49–55.
[17] V. de Jesus Gaffney, V.V. Cardoso, E. Cardoso, A.P. Teixeira, J. Martins, M.J. Benoliel, C.M.M. Almeida, Occurrence and behavior of pharmaceutical compounds in a Portuguese wastewater treatment plant: removal efficiency through conventional treatment processes, Environ. Sci. Pollut. Res. Int. 24 (2017) 14717–14734.
[18] ForumCafe, Consumo de café en España, Available at, 2018, Accessed January 2018 https://goo.gl/XtWdWA.
[19] AEMPS, Agencia Española de Medicamentos y Productos Sanitarios, Utilización de medicamentos antidepresivos en España durante el periodo 2000-2013. Informe de utilización de medicamentos U/AD/V1/14012015, Available at, 2018, Accessed January 2018 https://goo.gl/jdHXt5.
[20] T. Mackuľak, L. Birošová, M. Gál, I. Bodík, R. Grabic, J. Ryba, J. Škubák, Wastewater analysis: the mean of the monitoring of frequently prescribed pharmaceuticals in Slovakia, Environ. Monit. Assess. 188 (2016) 18.
[21] B. Subedi, K. Kannan, Occurrence and fate of select psychoactive pharmaceuticals and antihypertensives in two wastewater treatment plants in New York State, USA, Sci. Total Environ. 514 (2015) 273–280.
[22] S. Esteban, Y. Valcárcel, M. Catalá, M.G. Castromil, Psychoactive pharmaceutical residues in the watersheds of Galicia (Spain), Gac. Sanit. 26 (2012) 457–459.
[23] E. Gracia-Lor, E. Zuccato, S. Castiglioni, Refining correction factors for back-calculation of illicit drug use, Sci. Total Environ. 573 (2016) 1648–1659.
[24] D.R. Baker, L. Barron, B. Kasprzyk-Hordern, Illicit and pharmaceutical drug consumption estimated via wastewater analysis. Part A: chemical analysis and drug use estimates, Sci. Total Environ. 487 (2014) 629–641.
[25] D.M. Schwope, A. DePriest, D.L. Black, Y.H. Caplan, E.J. Cone, R. Heltsley, Determining zolpidem compliance: urinary metabolite detection and prevalence in chronic pain patients, J. Anal. Toxicol. 38 (2014) 513–518.
[26] I. Racamonde, R. Rodil, J.B. Quintana, E. Villaverde-de-Sáa, R. Cela, Determination of benzodiazepines, related pharmaceuticals and metabolites in water by solid-phase extraction and liquid-chromatography–tandem mass spectrometry, J. Chromatogr. A 1352 (2014) 69–79.
[27] M. Huerta-Fontela, M.T. Galceran, F. Ventura, Fast liquid chromatography–quadrupole-linear ion trap mass spectrometry for the analysis of pharmaceuticals and hormones in water resources, J. Chromatogr. A 1217 (2010) 4212–4222.
[28] L. Bijlsma, A.M. Botero-Coy, R.J. Rincón, G.A. Peñuela, F. Hernández, Estimation of illicit drug use in the main cities of Colombia by means of urban wastewater analysis, Sci. Total Environ. 565 (2016) 984–993.
[29] UNODC,United Nations Office on Drug and Crime. World Drug Report 2018, Executive Summary. Available at https://goo.gl/bKqasi. Accessed July 2018.

Supplementary Material

A fully automated approach for the analysis of 37 psychoactive substances in raw wastewater based on on-line solid phase extraction-liquid chromatography-tandem mass spectrometry

Ester López-García[1], Nicola Mastroianni[1], Cristina Postigo[1*], Damià Barceló[1,2], Miren López de Alda[1*]

[1] Water and Soil Quality Research Group, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain.

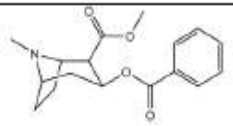
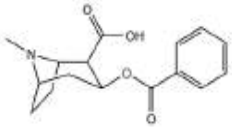
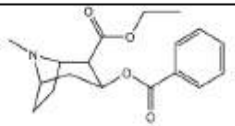
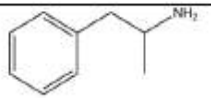
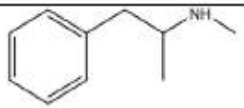
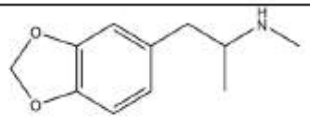
[2] Catalan Institute for Water Research, Girona, H2O Building, Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003 Girona, Spain.

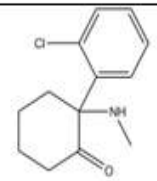
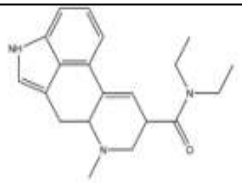
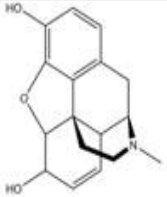
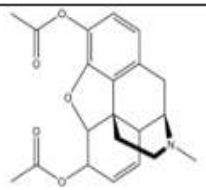
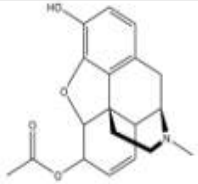
***Corresponding Authors**: Cristina Postigo, Miren López de Alda
Institute of Environmental Assessment and Water Research (IDAEA-CSIC),
Department of Environmental Chemistry
C/ Jordi Girona 18-26, 08034 Barcelona, Spain.
cprqam@cid.csic.es, mlaqam@cid.csic.es
Tel: +34-934-006-100, Fax: +34-932-045-904

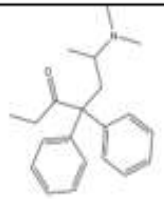
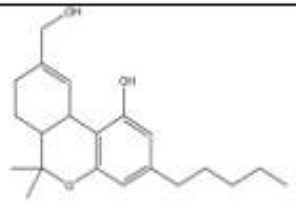
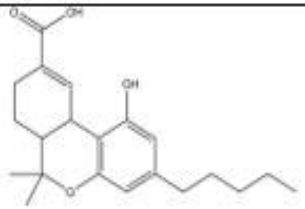
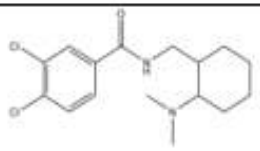
List of Tables:

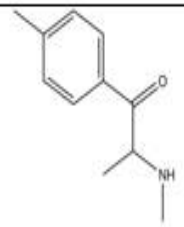
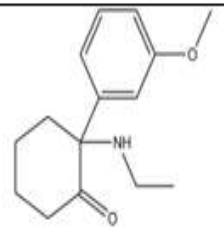
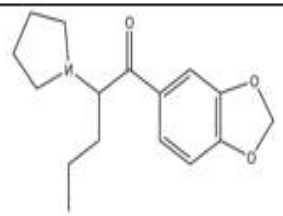
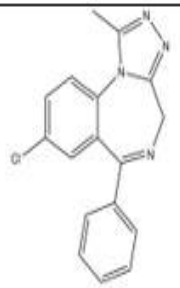
List of Figures:

Table S1. Physical-chemical properties of target compounds: molecular structure, CAS number, molecular weight, octanol water partition coefficient (log Kow) and acid dissociation constant (pKa).

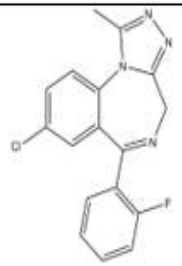
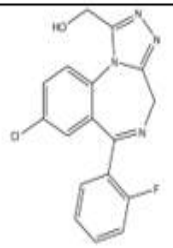
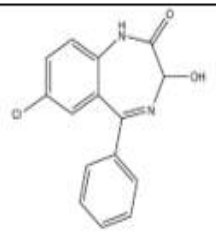
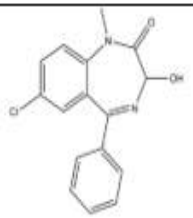
Class of compounds	Target analyte	Molecular structure	CAS number	Molecular weight	Log Kow [a]	pKa
Cocaine compounds	Cocaine (COC)		50-36-2	303.36	2.30	8.61
	Benzoylecgonine (BE)		519-09-5	289.33	-1.32*	Strongest Acidic: 3.15** Strongest Basic: 9.54**
	Cocaethylene (CE)		529-38-4	317.39	2.66*	Strongest Basic: 8.77**
Amphetamine-type stimulants	Amphetamine (AM)		300-62-9	135.21	1.76	10.1
	Methamphetamine (MA)		4846-07-5	149.24	2.07	9.87
	3,4-Methylenedioxy methamphetamine (MDMA)		42542-10-9	193.25	2.15	Strongest Basic: 10.14**

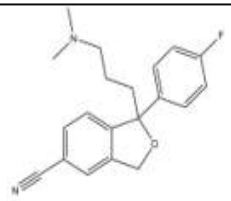
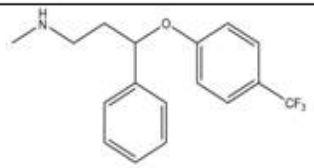
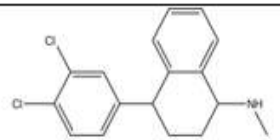
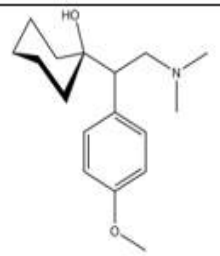
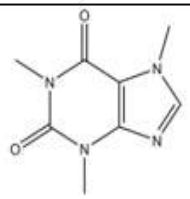
Hallucinogens	Ketamine (KET)		1867-66-9	237.73	2.18	7.5
	Lysergic acid diethylamide (LSD)		50-37-3	323.44	2.44	7.8
Opioids/opiates	Morphine (MOR)		57-27-2	285.34	0.89	8.21
	Heroin (HER)		561-27-3	369.42	1.58	7.95
	6-Monoacetylmorphine (6 ACM)		2784-73-8	327.38	0.40*	Strongest Acidic: 10.19 ** Strongest Basic: 9.08 **

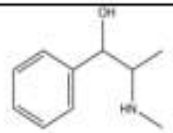
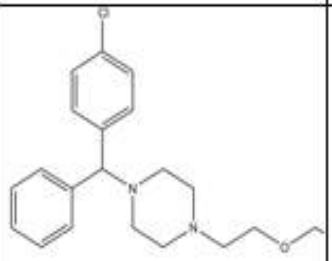
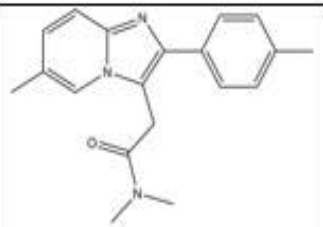
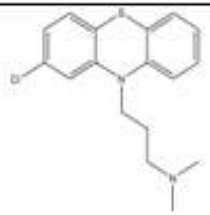
	Methadone (METH)		76-99-3	309.45	3.93	8.94
	2-Ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium (EDDP)		66729-78-0	277.41	4.94*	Strongest Basic: 9.64**
Cannabinoids	11-Hydroxy-Δ⁹THC (OH-THC)		36557-05-8	330.47	5.33*	Strongest Acidic: 9.34** Strogest Basic: -2.7**
	11-Nor-9-carboxy-Δ⁹THC (THC-COOH)		56354-06-4	344.45	6.36*	Strongest Acidic: 4.21** Strogest Basic: -4.9**
New Psychoactive substances (NPS)	3,4-Dichloro-N-[[1-(dimethylamino)cyclohexyl]methyl]benzamide (AH-7921)		55154-30-8	329.27	4.39*	

	Mephedrone (MEPH)		1189805-46-6	177.25	2.39*	Strongest Acidic: 18.66 ** Strongest Basic: 8.05**
	Methoxetamine (MXE)		1239943-76-0	247.34	3.05*	Strongest Acidic: 13.61** Strogest Basic: 9.28**
	3,4-Methylenedioxy pyrovalerone (MDPV)		687603-66-3	194.19	3.97*	8.4
Benzodiazepines	Alprazolam (ALPZ)		28981-97-7	308.77	2.12	Strongest Acidic: 18.3** Strongest Basic: 5.08**

α-Hydroxy-alprazolam (OH-ALPZ)		37115-43-8	324.77	2.41*		Strongest Acidic: 13.72** Strongest Basic: 4.97**
Diazepam (DIAZ)		439-14-5	284.74	2.82	3.4	
Lorazepam (LORZ)		846-49-1	321.16	2.39	13	
Lormetazepam (LRMZ)		848-75-9	335.18	2.23*		Strongest Acidic: 10.68** Strongest Basic: -2.2**

	Midazolam (MIDZ)		59467-70-8	326.76	4.33*	5.5
	α-Hydroxy-midazolam (OH-MIDZ)		59468-90-5	342.76	2.87*	Strongest Acidic: 13.95** Strongest Basic: 4.99**
	Oxazepam (OXZ)		604-75-1	286.05	2.24	1.55
	Temazepam (TEMZ)		846-50-4	300.74	2.19	Strongest Acidic: 10.68** Strongest Basic: -1.4**

Antidepressants	Citalopram (CTLP)		59729-33-8	324.40	3.74*	Strongest Basic: 9.78**
	Fluoxetine (FLX)		54910-89-3	309.33	4.05	Strongest Basic: 9.8**
	Sertraline (STR)		79617-96-2	306.23	5.29*	Strongest Basic: 9.85**
	Venlafaxine (VFX)		93413-69-5	277.41	3.28*	10.09
Stimulants	Caffeine (CAF)		58-08-2	275.35	-0.07	10.4

	Ephedrine (EPH)		321-97-1	165.24	0.89	9.65
Sedative	Hydroxyzine (HXZ)		68-88-2	374.91	2.36*	2.47
Hypnotic	Zolpidem (ZOPD)		82626-48-0	307.40	3.85*	6.2
Antipsychotic	Chlorpromazine (CPMZ)		50-53-3	318.86	5.41	9.3

[a] Data extracted from EPI Suite ™. Available at: https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface . Experimental data and/or estimations (*) provided by KOWWIN v1.68 software.

** Data extracted from https://www.drugbank.ca/. Estimations (**) provided by ChemAxon.

Table S2. Linearity, analyte (absolute and relative) recovery, repeatability and limit of detection and quantification of the method in HPLC-grade water

		Linearity		Absolute recovery (n=5)		Relative recovery (RSD) (n=5)		LOD (ng/L)	LOQ (ng/L)
		r^2	Range (ng/L)	50 ng/L	250 ng/L	50 ng/L	250 ng/L		
Cocainics	COC	0.9908	0.1-2000	38	42	93 (3)	98 (3)	< 0.1	0.1
	BE	0.9916	0.1-2000	62	65	92 (4)	96 (2)	0.1	0.5
	CE	0.9920	0.5-2000	40	40	116 (3)	105 (4)	0.1	0.5
Amphetamine-type stimulants	AM	0.9930	1-2000	74	70	108 (6)	107 (3)	0.5	1.0
	MA	0.9930	1-2000	53	55	107 (6)	115 (5)	0.5	1.0
	MDMA	0.9922	0.1-2000	45	49	105 (11)	101 (13)	< 0.1	0.1
Hallucinogens	KET	0.9908	0.5-2000	49	51	98 (5)	93 (5)	0.1	0.5
	LSD	0.9914	0.5-2000	28	34	92 (11)	103 (12)	0.1	0.5
Opioids/ opiates	MOR	0.9954	5-2000	54	59	97 (3)	104 (4)	1.0	5.0
	HER	0.9930	1-2000	56	60	87 (6)	97 (14)	0.5	1.0
	6ACM	0.9908	1-2000	57	64	101 (5)	93 (6)	0.5	1.0
	METH	0.9908	0.5-2000	33	33	100 (2)	105 (3)	0.1	0.5
	EDDP	0.9908	0.5-2000	30	33	97 (5)	100 (5)	0.1	0.5
Cannabinoids	THC-COOH	0.9902	10-2000	100	67	101 (8)	107 (8)	7.0	10
	OH-THC	0.9974	10-2000	34	12	94 (13)	82 (9)	7.0	10
New psychoactive substances	AH-7921	0.9912	0.1-100	35	36	98 (5)	98 (5)	< 0.1	0.1
	MDPV	0.9966	1-2000	38	39	104 (6)	94 (7)	0.1	1.0
	MEPH	0.9908	0.5-2000	55	54	101 (4)	99 (5)	0.1	0.5
	MXE	0.9902	0.5-2000	44	51	89 (3)	99 (8)	0.1	0.5
Benzo-diazepines	ALPZ	0.9928	0.5-2000	70	68	103 (4)	102 (4)	0.1	0.5
	OH-ALPZ	0.9970	5-2000	92	87	98 (9)	106 (6)	2.0	5.0
	DIAZ	0.9914	1-2000	60	62	99 (3)	103 (8)	0.5	1.0
	LORZ	0.9932	5-500	113	96	92 (5)	88 (3)	1.0	5.0
	LRMZ	0.9934	1-2000	98	92	96 (5)	91 (2)	0.5	1.0
	MIDZ	0.9936	1-2000	38	38	101 (6)	98 (7)	0.5	1.0
	OH-MIDZ	0.9942	1-1000	68	67	103 (7)	105 (3)	0.5	1.0
	OXZ	0.9930	1-2000	110	104	103 (3)	98 (4)	0.5	1.0
	TEMZ	0.9960	0.5-2000	87	83	95 (5)	93 (3)	0.1	0.5
Anti-depressants	CTLP	0.9934	0.5-2000	32	35	101 (4)	102 (4)	0.1	0.5
	FLX	0.9970	1-2000	12	13	90 (3)	90 (12)	0.5	1.0
	STR	0.9920	1-2000	14	18	109 (4)	109 (3)	0.5	1.0
	VFX	0.9928	0.1-2000	42	45	93 (5)	103 (3)	< 0.1	0.1
Stimulants	CAF*	0.9918	0.1-10250	78	77	120 (10)	119 (8)	<0.1	0.1
	EPH	0.9908	1-2000	65	65	108 (4)	103 (1)	0.5	1.0
Sedative	HXZ	0.9900	0.5-2000	35	36	108 (8)	109 (4)	0.1	0.5
Hypnotic	ZOPD	0.9920	0.5-2000	36	38	106 (3)	113 (3)	0.1	0.5
Antipsychotic	CPMZ	0.9924	1-2000	9	12	98 (16)	105 (2)	0.5	1.0

* Recovery studies were performed with HPLC-grade water spiked at 1005, 5050 and 10250 ng/L

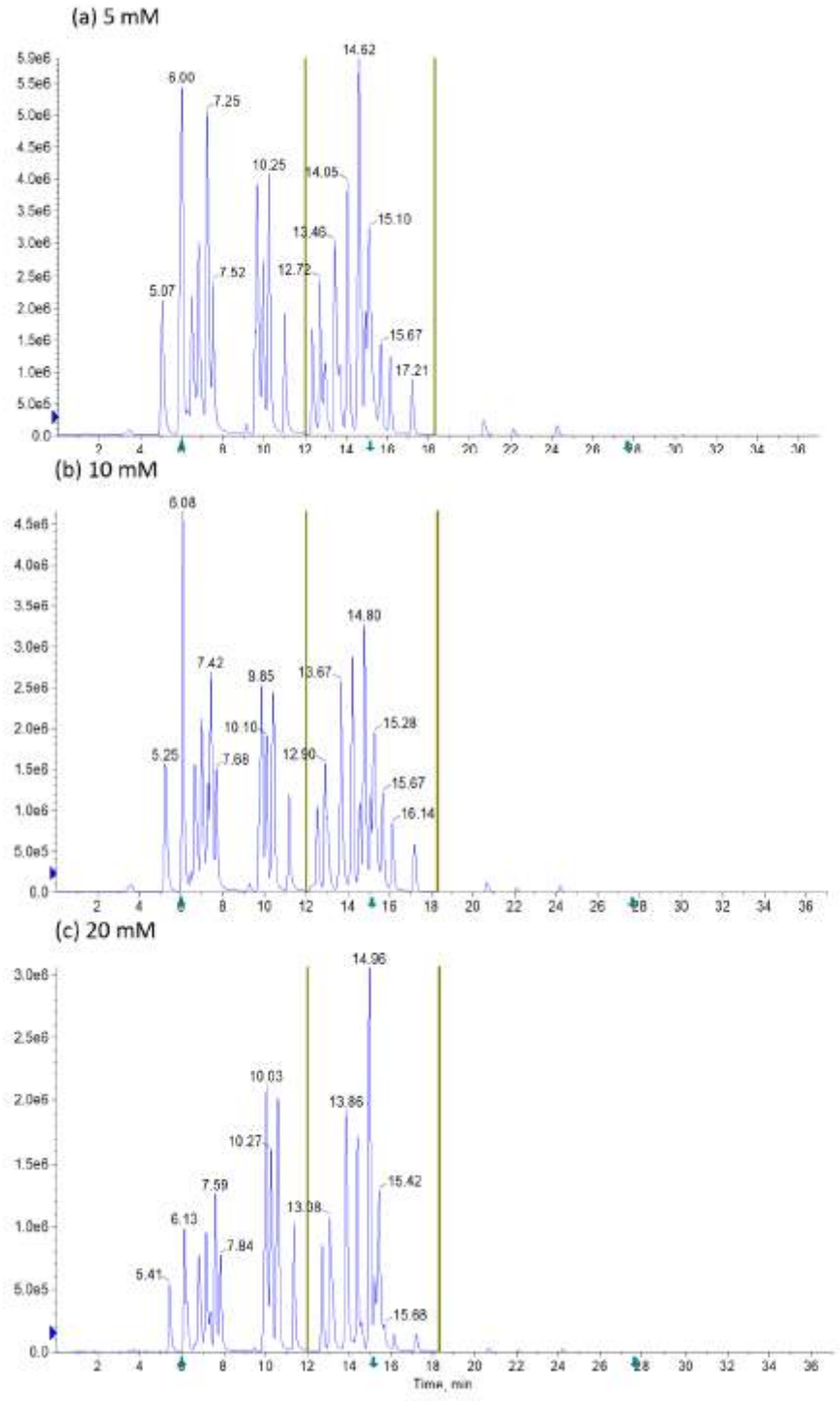


Figure S1. Total ion chromatogram (TIC) obtained after LC-MS/MS analysis of a standard solution containing 125 ng/mL of the target analytes using different buffer (ammonium formate/formic acid) concentrations in the aqueous solutions of the mobile phase: (a) 5 mM; (b) 10 mM; (c) 20 mM

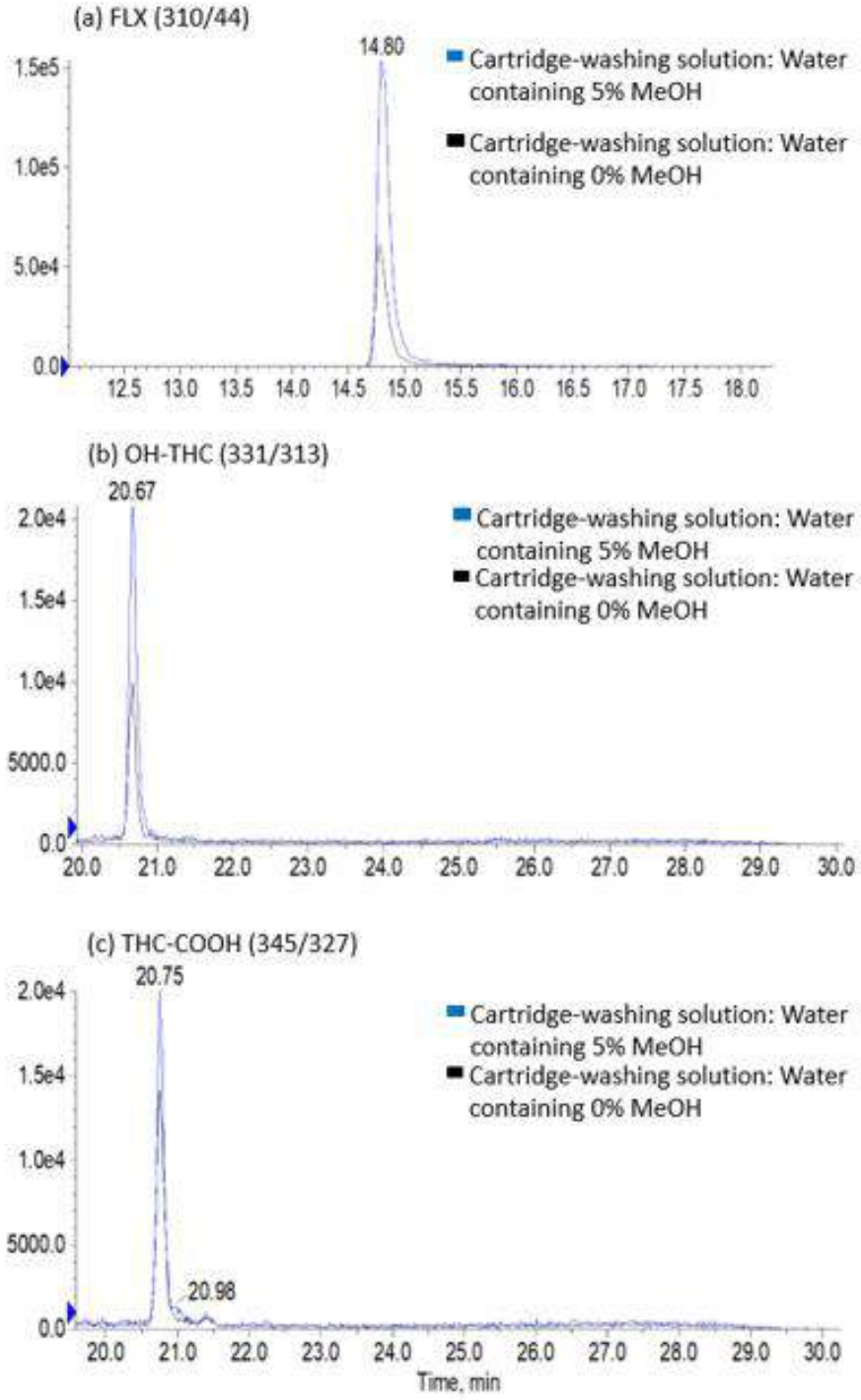


Figure S2. Extracted ion chromatograms of (a) FLX, (b) OH-THC, and (c) THC-COOH obtained after on-line SPE-LC-MS/MS analysis of wastewater fortified at a concentration of 2500 ng/L using different cartridge-washing solutions.

3.2. Análisis de drogas, psicofármacos y algunos de sus metabolitos en sedimentos

Como se mostró en la sección 1.3.1.2., a pesar de su carácter polar o medianamente polar, las drogas y psicofármacos junto con sus metabolitos, son capaces de acumularse en sedimentos, siendo las concentraciones a las que se han determinado del orden de ng/g. Aunque en la literatura existen numerosos procedimientos para la extracción de muestras sólidas (sección 1.5.2), las escasas metodologías analíticas desarrolladas para el análisis de este tipo de compuestos en sedimentos se basan en la extracción y purificación de la muestra con PLE y SPE, respectivamente, y su posterior análisis mediante LC-MS/MS, ya que estas tecnologías permiten obtener la sensibilidad y selectividad requerida para analizar este tipo de matriz y, además, proporcionan una alta reproducibilidad debido a la automatización del proceso de extracción.

El objetivo de la publicación #4 fue validar una metodología basada en PLE-SPE-LC-MS/MS, previamente desarrollada para el análisis de drogas, unos pocos psicofármacos y algunos de sus metabolitos en lodos de depuradora (Mastroianni y cols., 2013), para la determinación de los mismos compuestos en sedimentos. La metodología se validó en términos de linealidad, recuperación, repetibilidad, efecto matriz, y sensibilidad, obteniéndose resultados satisfactorios en todos los parámetros evaluados.

Esta metodología se aplicó al análisis de 144 muestras de sedimentos recogidos durante dos años consecutivos en las cuencas de los ríos Llobregat, Ebro, Júcar, y Guadalquivir con el objetivo de:

- Estudiar la presencia de drogas, psicofármacos y algunos de sus metabolitos en los sedimentos recogidos en las cuatro cuencas y comparar los resultados obtenidos con los niveles reportados para estos compuestos en otros estudios.
- Estudiar la distribución geográfica y temporal de los analitos estudiados en las cuatro cuencas y evaluar si existen diferencias significativas mediante la aplicación de pruebas estadísticas.
- Investigar la distribución preferente de las drogas, psicofármacos y metabolitos estudiados entre el sedimento y el agua, matriz previamente investigada por Mastroianni y cols. (2016) en los mismos puntos de muestreo, y determinar experimentalmente, en los casos en que hubiera suficiente número de datos, el coeficiente de distribución sedimento-agua (K_D).

- Evaluar el riesgo ambiental que supone la presencia de estos compuestos en los sedimentos para los organismos acuáticos y los compuestos que más contribuyen al mismo mediante el cálculo del índice HQ en cada muestra.

Los resultados obtenidos mostraron que la cocaína, la metadona y su metabolito EDDP eran los compuestos más ubicuos (con frecuencias de detección superiores al 36%), y que el cannabinol, el THC y la metadona eran los compuestos más abundantes (con concentraciones máximas de 44, 37 y 33 ng/g peso seco (p.s.), respectivamente). En general, las concentraciones encontradas en sedimentos fueron bajas, concentraciones medianas inferiores a 1,6 ng/g (p.s.), excepto para los cannabinoides THC, cannabidiol y cannabinol que se encontraron a concentraciones medianas de 6,1, 15, y 28 ng/g (p.s.), respectivamente.

En cuanto a la distribución geográfica y temporal, la cuenca con mayor acumulación de drogas fue la del Ebro con niveles acumulados de 91 ng/g (p.s.), seguida de la del Llobregat (65 ng/g (p.s.)), el Guadalquivir (47 ng/g (p.s.)) y el Júcar (14 ng/g (p.s.)). Los niveles acumulados de drogas en 2010 fueron superiores en las cuencas del Llobregat y del Ebro, inferiores en la del Guadalquivir, y similares en la del Júcar, con respecto a los medidos en 2011. Se encontraron diferencias significativas en la presencia de drogas entre las diferentes cuencas investigadas y entre las dos campañas de muestreo, debidas en la mayoría de casos, a una mezcla de factores como son las condiciones hidrológicas y meteorológicas de cada cuenca, la eficacia de eliminación de los contaminantes de cada EDAR, y los diferentes patrones de consumo de drogas en cada una de las zonas investigadas.

El K_D obtenido experimentalmente para varios analitos mostró que los compuestos con mayor tendencia a adsorberse en los sedimentos son la metadona y su metabolito EDDP (log K_D ≥ 2,68), seguidos de la cocaína, el MDMA y el diazepam (log K_D entre 2,45 y 1,79, respectivamente). Esta es la primera vez que se reportan valores experimentales de K_D para MDMA, diazepam, metadona y EDDP. También se observó una correlación significativa en la presencia de EDDP y metadona entre agua y sedimento, sugiriendo un buen equilibrio de estos compuestos entre ambos compartimentos.

Por último, la evaluación del riesgo medioambiental reveló que el 28% de las muestras analizadas presentaban valores de ∑HQ > 1, lo que sugiere que la presencia de drogas y psicofármacos en sedimentos puede suponer un riesgo para los organismos acuáticos que viven en, o se alimentan de ellos, siendo EDDP, THC y metadona los compuestos que más contribuían a este riesgo.

Publicación científica #4

"Drugs of abuse and their metabolites in river sediments: Analysis, occurrence in four Spanish river basins and environmental risk assessment"

por:

Ester López-García, Nicola Mastroianni, Nuria Ponsà-Borau,

Damià Barceló, Cristina Postigo, y Miren López de Alda

en

Journal of Hazardous Material 401 (2021) 123312

https://doi.org/10.1016/j.jhazmat.2020.123312

Journal of Hazardous Materials 401 (2021) 123312

Contents lists available at ScienceDirect

Journal of Hazardous Materials

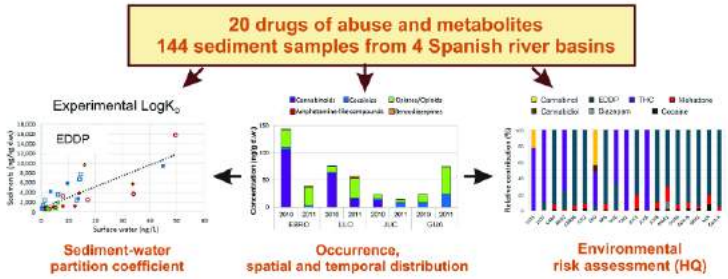
journal homepage: www.elsevier.com/locate/jhazmat

Drugs of abuse and their metabolites in river sediments: Analysis, occurrence in four Spanish river basins and environmental risk assessment

Ester López-García[a], Nicola Mastroianni[a], Núria Ponsà-Borau[b], Damià Barceló[a,c], Cristina Postigo[a,*], Miren López de Alda[a,*]

[a] Water, Environmental, and Food Chemistry Unit (ENFOCHEM), Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain
[b] Universitat Oberta de Catalunya, Barcelona, Spain
[c] Catalan Institute for Water Research, H2O Building, Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003, Girona, Spain

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: R Teresa

Keywords:
Illicit drugs
Benzodiazepines
Liquid chromatography-mass spectrometry
Pressurized liquid extraction
Hazard quotients

ABSTRACT

The environmental impact produced by the presence of drugs of abuse in sediments has been scarcely studied to date, even though many of them may adsorb onto particulate matter due to their physical-chemical properties. This study presents an analytical method for the determination of 20 drugs of abuse and metabolites in sediments. The validated method was satisfactory in terms of linearity ($r^2 > 0.99$), recovery (90–135 %), repeatability (relative standard deviations < 15 %), sensitivity (limits of quantification < 2.1 ng/g d.w, except for cannabinoids), and matrix effects (ionization suppression < 40 %). The method was applied to the analysis of 144 sediments collected in four Spanish river basins. Cocaine, methadone, and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) were the most ubiquitous compounds (detection frequencies > 36 %), whereas cannabinol, Δ^9-tetrahydrocannabinol (THC), and methadone were the most abundant compounds (up to 44, 37, and 33 ng/g d.w, respectively). The presence of EDDP, THC, and methadone in the sediments of 28 locations may pose a risk to sediment-dwelling organisms. To the author's knowledge, this is the most extensive study conducted so far on the occurrence of drugs of abuse in sediments, and the first time that sediment-water distribution coefficients for EDDP, methadone, MDMA, and diazepam are reported from field observations.

* Corresponding authors at: Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Department of Environmental Chemistry, C/ Jordi Girona 18-26, 08034, Barcelona, Spain.
E-mail addresses: cprqam@cid.csic.es (C. Postigo), mlaqam@cid.csic.es (M. López de Alda).

https://doi.org/10.1016/j.jhazmat.2020.123312
Received 16 April 2020; Received in revised form 22 June 2020; Accepted 23 June 2020
Available online 25 June 2020

1. Introduction

In recent years, the number of studies conducted to assess the consumption of drugs of abuse has increased worldwide due to the effects that these compounds produce to health (e.g., 585,000 people died as a consequence of illicit drug use in 2017 (UNODC, 2018)). Likewise, their occurrence and fate in the environment have increasingly become a matter of scientific concern. The release of these substances into the aquatic environment is directly related to their consumption, production, and direct disposal into the sewage system or other water compartments. In the best-case scenario, these substances and/or their metabolites are collected in the sewage network and conducted to a wastewater treatment plant (WWTP) where they are only partially eliminated (Baker and Kasprzyk-Hordern, 2013). In the receiving water bodies, the concentrations present in the WWTP effluents are diluted to different extents, and with that the negative effects that they may cause on the aquatic organisms (Postigo et al., 2012). However, in areas that suffer water scarcity, the dilution factor is very low, and during drought or low-flow periods WWTP discharges represent the largest fraction of the total river flow, and thus, the effects of these substances on the aquatic ecosystem functions may be of relevance (Navarro-Ortega et al., 2012).

The occurrence of illicit drugs and their metabolites in surface water, including rivers, streams, lakes, and creeks has been extensively studied worldwide (Pal et al., 2013; Yadav et al., 2017). From these studies, it can be concluded that the illicit drugs and metabolites most commonly detected in surface waters are benzoylecgonine, cocaine, norcocaine, norbenzoylecgonine, cocaethylene, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), morphine, cannabis, codeine, methadone, and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). All of them are usually measured at concentrations below 100 ng/L, except for benzoylecgonine, amphetamine, and codeine that have been found at concentrations up to 316 ng/L (González-Mariño et al., 2010), 309 ng/L (Martínez Bueno et al., 2011), and 341 ng/L (Baker and Kasprzyk-Hordern, 2013), respectively. The occurrence of legal drugs of abuse, like benzodiazepines and the antidepressant citalopram, in water was reviewed by Cunha et al. (2017). Benzodiazepines, the most commonly prescribed psychoactive pharmaceuticals in 2018 (INCB, 2019), are overall more abundant in surface water than illicit drugs, reaching occasionally the μg/L level, as it was the case for alprazolam in the Cascavel River, Brazil (5900 ng/L) (Nunes et al., 2015), and oxazepam (1400 ng/L) in the Vilaine River basin, France (Piel et al., 2013). Given their overall medium to high polarity, it is expected that these compounds remain in the aqueous phase and for this reason, most of the studies conducted so far have focused on environmental water matrices. However, some of these substances, such as cannabinoids and the opioid methadone and its main metabolite EDDP, present hydrophobic properties (log $K_{ow} > 3$) that make them susceptible to adsorb onto organic-rich solid matrices (Postigo et al., 2010).

Sediments can accumulate a large variety of organic contaminants and consequently, they become contaminant sources during re-suspension processes (Matić Bujagić et al., 2019). To date, very few studies have investigated the occurrence of drugs of abuse in sediments, and overall, they were multi-residue studies that included a limited number of drugs of abuse. The illicit drugs and metabolites most commonly investigated in sediments so far are cocaine, benzoylecgonine, amphetamine, methamphetamine, methadone, and Δ^9-tetrahydrocannabinol (THC). They were found at concentrations ranging from not detectable to 200 ng/g (Álvarez-Ruiz et al., 2015; Carmona et al., 2017; Klosterhaus et al., 2013; Langford et al., 2011; Wilkinson et al., 2018). Similar concentrations were also measured in sediments for the benzodiazepines alprazolam, diazepam, and lorazepam (Beretta et al., 2014; Matić Bujagić et al., 2019; Picó et al., 2020; Vazquez-Roig et al., 2012).

Given the low concentrations of this type of compounds in sediments, it is necessary to apply highly sensitive and selective analytical methodologies for their determination. Extraction of these substances from solid matrices has been achieved with pressurized liquid extraction (PLE) (Arbeláez et al., 2014; Baker and Kasprzyk-Hordern, 2011; Langford et al., 2011; Mastroianni et al., 2013; Senta et al., 2013), ultrasonic-assisted extraction (UAE) (Álvarez-Ruiz et al., 2015; Carmona et al., 2017; Gago-Ferrero et al., 2015; Wilkinson et al., 2018) or solid-liquid extraction (SLE) (Klosterhaus et al., 2013). PLE was the preferred technique because of its high extraction efficiency, due to the application of high temperature and pressure, and automation, which leads to highly reproducible results and allows saving time and solvent consumption (Álvarez-Ruiz et al., 2015; Biel-Maeso et al., 2017; Montesano et al., 2017). The extracts obtained need to be cleaned-up before their analysis. Extract clean-up has been accomplished using solid-phase extraction (SPE), while analyte determination has been commonly done with liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS).

The objective of this work was to validate an analytical methodology based on PLE extraction, SPE clean-up and LC–MS/MS determination for the simultaneous analysis of 20 drugs of abuse and metabolites in sediment samples and to apply this method to the analysis of 144 river sediment samples collected along four Spanish rivers in two sampling campaigns to (i) study the occurrence and distinct geographical and temporal distribution of the target drugs of abuse among river basins and between sampling campaigns, as well as between the water and sediment compartments, and (ii) assess the environmental risk that they pose to aquatic organisms, as well as the compounds of highest concern, by applying the hazard quotient (HQ) approach. The sampled areas were selected because of their Mediterranean character, which makes them subject to water scarcity periods and prone to a greater accumulation of emerging pollutants. Moreover, a previous study had revealed the presence of up to 80 % of the targeted drugs of abuse in the water of these river basins at maximum concentrations of 144 ng/L (Mastroianni et al., 2016).

2. Material and methods

2.1. Reagents and materials

The compounds investigated included cocaine-related compounds (cocaine (COC) and its metabolites benzoylecgonine (BE) and cocaethylene (CE)), amphetamine-type stimulants (ATS) (amphetamine (AM), methamphetamine (MA), 3,4-methylenedioxymethamphetamine (MDMA), ephedrine (EPH)), opiates/opioids (morphine (MOR), heroin (HER) and its exclusive metabolite 6-acetylmorphine (6ACM), methadone (METH) and its metabolite EDDP), hallucinogens (lysergic acid diethylamide (LSD) and its metabolite 2-oxo-3-hydroxy-LSD (OH-LSD)), cannabinoids (THC, its metabolite 11-hydroxy-Δ^9-THC (OH-THC), cannabidiol (CBD), and cannabinol (CBN)), and benzodiazepines (alprazolam (ALP) and diazepam (DIA)). Unfortunately, 11-nor-9-carboxy-Δ^9-THC (THC − COOH), the THC metabolite most investigated in environmental samples, was not included in the method because the analytical standard was not available in the lab at the time of the study. The main physical-chemical properties of these compounds are provided in Table S1 in Supplementary Material (SM).

High-purity (> 97 %) standard solutions of the above-mentioned target compounds and isotopically labeled analogs were purchased from Cerilliant (Round Rock, TX, USA) as solutions in methanol (MeOH) or acetonitrile (ACN) at a concentration of 1 mg/mL or 0.1 mg/mL.

Working standard mixture solutions were prepared in MeOH at different concentrations in the range of 0.1 and 1000 ng/mL by appropriate dilution of individual stock solutions. All of them contained the isotopically labeled compounds at a fixed concentration so that they could be used as surrogate standards (SS) in the quantification process. 6ACM-d_6, AM-d_5, CE-d_3, EDDP-d_3, EPH-d_3, HER-d_9, LSD-d_3, MA-d_{14}

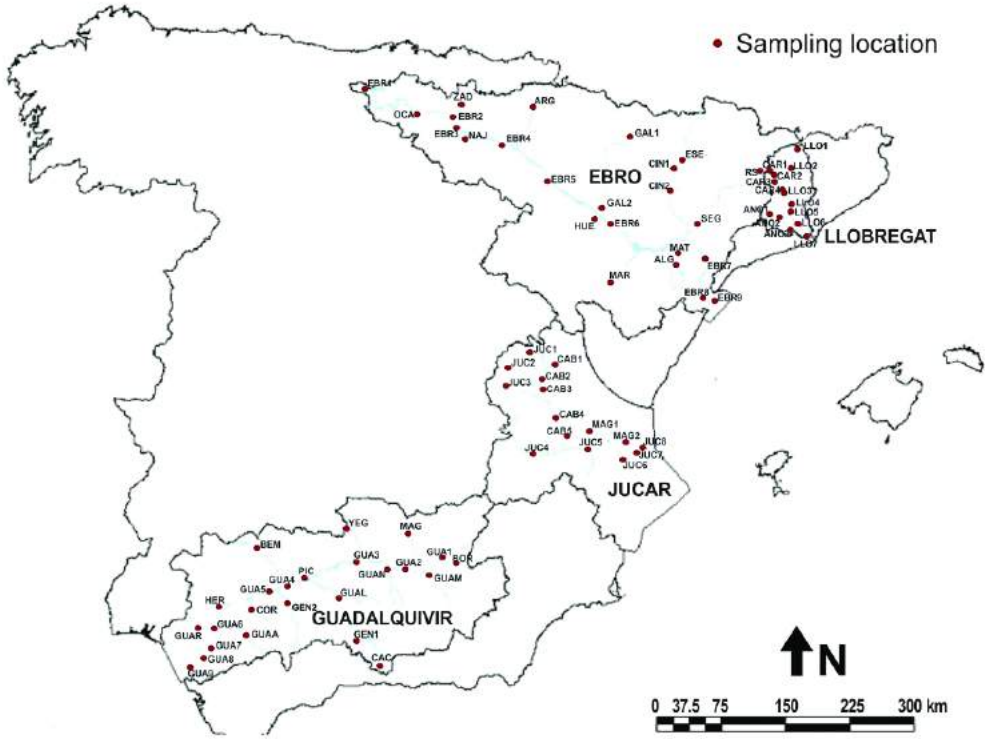


Fig. 1. Map showing the location of the sediment sampling stations in each river basin.

and MDMA-d_5 were added at a final concentration of 20 ng/mL, whereas ALP-d_5, BE-d_3, CBD-d_3, COC-d_3, DIA-d_5, METH-d_5, MOR-d_3, OH-THC-d_3 and THC-d_3 were added at 50 ng/mL. All standard solutions were stored in the dark at – 20 °C until use.

All solvents used were HPLC-grade and were supplied by Merck (Darmstadt, Germany), as well as formic acid (> 98 %) and activated neutral aluminum oxide (Al_2O_3) (99 % purity). Ammonium formate (> 99 %) used as a mobile phase modifier was purchased from Fluka Analytical (Sigma Aldrich).

Cellulose filters (0.45 µm pore size) placed in PLE cells to prevent the transfer of fine particles into the extract and plugging of the PLE system were purchased from Dionex Corporation (Sunnyvale, CA, USA). Evolute ABN cartridges (50 µm, 200 mg, 6 mL) used for SPE clean-up were provided by Biotage (Uppsala, Sweden).

Nitrogen gas (99.995 %) used for extract evaporation was produced by a nitrogen generator system (Centralair, San Sebastian, Spain).

2.2. Sample collection

A total of 144 river sediment samples were collected as grab samples from 75 different locations (Fig. 1) along four Spanish river basins, namely, Llobregat, Ebro, Jucar, and Guadalquivir, during two sampling campaigns conducted in September, October and November 2010 and 2011. Sediments were collected with a van Veen drag and placed in an aluminum tray that was wrapped with aluminum foil. They were kept at 4 °C during transport to the laboratory. Once in the laboratory, sediments were freeze-dried using a LyoAlfa 6–50 freeze-drier (Telstar S.A., Barcelona, Spain), finely ground with a mortar, and sieved through 125 µm mesh to obtain a homogeneous sediment sample. Finally, samples were stored at -20 °C until analysis. Table S2 in SM shows the total organic carbon (%) content of the sediment samples collected during 2011.

2.3. Sample preparation

Preparation of sediment samples was performed following an analytical methodology previously described for the determination of drugs of abuse in sewage sludge samples (Mastroianni et al., 2013).

Briefly, 1 g of freeze-dried river sediment was transferred into 11 mL stainless extraction cells containing a cellulose filter at the bottom of the cell and partially filled with activated Al_2O_3 (approximately 5 g, activated at 350 °C during 15 min). Then, the SS mixture was added (10 ng and 25 ng of the SS present in the calibration curve at a concentration of 20 ng/mL and 50 ng/mL, respectively (see section 2.1), to ensure the same SS concentration in the final extract and the calibration curve). Cells were left overnight under a fume hood at room temperature to allow interaction of the SS with the matrix and methanol evaporation. The next day, cells were filled up with activated Al_2O_3 and covered with another cellulose filter. PLE was done using an ASE 200 (Dionex Corporation, Sunnyvale, CA, USA). The PLE conditions applied to the extraction process were: pressure, 1250 psi; temperature, 50 °C; preheating time, heating time and static time, 5 min each, number of static cycles, one; flush volume, 60 %; and purge time, 1 min. The extraction solvent used was a mixture of MeOH/H_2O (9:1, v/v).

The PLE extract obtained (about 14 mL) was evaporated to an approximate volume of 1.5 mL under a gentle stream of N_2 with a TurboVap LV evaporator (Zymark, Hopkinton, MA, USA), diluted with HPLC-grade water to a final volume of 25 mL, and purified through SPE with an SPE vacuum manifold (J.T. Baker, The Netherlands) using a polymeric Evolute ABN cartridge (200 mg, 6 mL), and a gravity-assisted flow. Before extract loading, the cartridge was sequentially conditioned and equilibrated with 6 mL of MeOH and 6 mL of H_2O. After loading the extract, the sorbent was washed with 3 mL of H_2O followed by 3 mL of a mixture of H_2O/MeOH (95:5, v/v), to remove undesired matrix components in the final extract. Then, the sorbent was vacuum dried for 15

min and after dryness, analytes were eluted with 3 mL of MeOH followed by 3 mL of a mixture of MeOH/formic acid (99:1, v/v). Finally, the combined eluted fractions were evaporated under nitrogen to dryness with a PIERCE ReactiTherm III evaporator (Rockford, IL, USA) and reconstituted with 0.5 mL of MeOH for LC–MS/MS analysis.

2.4. LC–MS/MS analysis

Analysis of the extracts was performed with an HPLC Symbiosis™ Pico System (Spark Holland, Emmen, The Netherlands) connected in series with a 4000 QTRAP hybrid triple quadrupole-linear ion trap (QqLIT) mass spectrometer (Applied Biosystem-Sciex, Foster City, CA, USA). LC separation was achieved with a Purospher Star RP-18 end-capped column (125 mm × 2.0 mm, 5 µm) (Merk, Darmstadt, Germany) and a mobile phase of formic acid/ammonium formate buffer (20 mM) and ACN. The ionization of the compounds was achieved with a Turbo Ion Spray source operating in the positive ionization mode (ESI +). Mass acquisition was performed in the selected reaction monitoring mode (SRM) recording two SRM transitions per compound and one per SS. The conditions used for the LC–MS/MS determination of the target compounds are described in detail elsewhere (Mastroianni et al., 2013).

2.5. Method performance

The performance of the methodology in sediments was evaluated in terms of linearity, sensitivity, recovery, repeatability, and matrix effects.

The linearity of the method was evaluated between 0.1 and 1000 ng/mL (equivalent to 0.05 and 500 ng/g d.w.) through the analysis of eleven methanolic standard solutions at different concentrations covering the aforementioned range. A calibration curve was constructed for each analyte using the internal standard approach by plotting the area ratio between the analyte and its corresponding surrogate standard and applying weighted least-squares linear regression. A weighting factor of $1/x^2$ was used to reduce the influence of the high concentrations in the linear model.

Analyte recoveries were calculated from replicate analysis (n = 6) of river sediment samples fortified at two levels, 10 ng/g d.w. and 25 ng/g d.w. Absolute recoveries were calculated by comparing the analyte peak areas obtained in fortified samples (after subtracting the peak area corresponding to the amount of analyte in the blank if present) and in standard solutions at equivalent concentrations. Relative recoveries were calculated by comparing the absolute recoveries obtained for each analyte and its corresponding surrogate standard.

The method repeatability was calculated as the relative standard deviation (RSD) of the response (analyte/surrogate standard) after the replicate analysis (n = 6) of river sediment samples fortified at 10 ng/g d.w. and 25 ng/g d.w.

The sensitivity of the method was evaluated through the limit of detection (LOD) and limit of quantification (LOQ) observed for each analyte. Average LODs and LOQs were experimentally estimated from the analysis of river sediment samples as the concentration of the analyte giving a signal to noise ratio of 3 and 10, respectively. In the case that the target compounds were not detected in any sample, LODs and LOQs were estimated from the signal observed in river sediment samples fortified at the lower level (10 ng/g d.w., n = 6).

Matrix effects (ME) were evaluated by comparing the peak area obtained for each analyte in the river sediment extract fortified (25 ng/g d.w.) at the end of the sample treatment procedure, i.e., after the PLE and SPE steps ($A_{sediment}$) (after subtracting the peak area corresponding to the amount of the analyte in the blank if present (A_{blank})), and a standard solution at an equivalent concentration ($A_{standard}$) (50 ng/mL), according to the following equation:

$$ME\,(\%) = \left[\frac{(A_{sediment} - A_{blank}) - (A_{standard})}{A_{standard}}\right] * 100$$

Negative values indicate MS signal suppression by matrix components, whereas positive values indicate signal enhancement. Values close to 0 indicate the absence of matrix effects.

2.6. Statistical analysis

Data were statistically analyzed to compare the occurrence of drugs of abuse among river basins and between sampling campaigns. Since data were not normally distributed, non-parametric tests were applied. The Wilcoxon Rank-Sum test was used to compare compound distribution between sampling campaigns (two independent samples). Then, a multivariate analysis consisting of the adjustment of the Quantile Regression Models (Median Regression Models) was used to predict the median concentration of each drug of abuse in each basin in the two sampling campaigns. The differences between the sampling campaigns median predictions and their 95 % confidence interval were estimated.

The Kruskal-Wallis test was used to compare the distribution of each compound (present in at least three basins) among the four basins. If significant differences among groups were obtained, they were subsequently investigated by applying the Wilcoxon Rank-Sum test to each pair of basins. False Discovery Rate (FDR) correction for multiple testing was applied to reduce the number of "false positives".

The relationship between the concentrations of a specific drug found in the sediment and water compartments of the different investigated sampling stations was evaluated using the Spearman's correlation test.

All the statistical analyses were done using the software R and considering a confidence level of 95 % (α = 0.05).

3. Results and discussion

3.1. Method performance

Table 1 shows the method performance in terms of linearity, recovery, repeatability, and sensitivity. Fig. 2 shows the total ion chromatogram (TIC) and the extracted ion chromatograms (XIC) of the target analytes after PLE-SPE-LC–MS/MS analysis of a sediment sample fortified at a concentration of 25 ng/g d.w.

The linearity of the method was satisfactory for all analytes. Coefficients of determination obtained for at least six-point calibration curves were higher than 0.99.

As for method precision and accuracy, absolute recoveries were in good agreement at the two concentration levels tested for all compounds. Most of the compounds presented absolute recoveries between 40 and 78 % except amphetamine, morphine, EDDP, THC, cannabidiol, cannabinol, and alprazolam that presented absolute recoveries below 32 %. Despite this, the relative recoveries obtained for all compounds (between 90 and 113 %), except OH-THC at the low concentration level (135 %) and cannabinol at both levels (141 %), indicate that the use of isotopically labeled analogs as surrogate standards allows correcting analyte losses during sample preparation as well as matrix effects. In the case of OH-THC and cannabinol, the high relative recoveries obtained can be attributed to their presence in the matrix used for validation at concentrations close to the levels tested in the validation approach.

The repeatability of the method was satisfactory with RSD values (n = 6) below 15 % for all the compounds at the two levels tested. Such good repeatability of the method can be attributed to the partial automation of the sample treatment process by using PLE.

Regarding sensitivity, LODs and LOQs were below 1.1 and 2.1 ng/g d.w., respectively, except in the case of the cannabinoids that presented LODs between 0.84 and 2.3 ng/g d.w. and LOQs between 3.2 and 13 ng/g d.w. The comparatively lower sensitivity observed for cannabinoids can be explained by several factors: low absolute recovery, high matrix suppression ionization effects, and/or low signal response

Table 1
Method performance in terms of linearity, analyte recoveries (absolute and relative), repeatability (RSD), matrix effects, and sensitivity (limits of detection and quantification) in river sediments.

	Linearity	Absolute recovery [%], (n = 6)		Relative recovery (repeatability), [%, (RSD)] (n = 6)		Matrix effect [%], (n = 3)	Sensitivity	
	r^2	10 ng/g d.w.	25 ng/g d.w.	10 ng/g d.w.	25 ng/g d.w.	25 ng/g d.w.	LOD (ng/g d.w.)	LOQ (ng/g d.w.)
COC	0.9968	62	70	101 (3.0)	105 (5.3)	−11	0.11	0.16
BE	0.9986	71	68	109 (5.0)	97 (4.8)	−20	0.02	0.04
CE	0.9952	72	78	102 (4.1)	99 (4.6)	−8.3	0.01	0.08
AM	0.9958	26	31	105 (4.8)	103 (3.6)	−16	1.1	2.1
MA	0.9944	61	64	95 (4.1)	99 (4.9)	−12	0.01	0.03
MDMA	0.9916	67	67	95 (4.1)	101 (4.9)	−6.9	0.03	0.06
EPH	0.9984	51	48	102 (3.7)	95 (5.3)	−28	0.07	0.21
MOR	0.9976	15	16	113 (8.8)	104 (12)	−14	0.13	0.70
6ACM	0.9978	62	67	106 (7.3)	106 (9.4)	−23	0.04	0.11
HER	0.9994	59	62	97 (5.1)	104 (11)	−17	0.13	0.35
METH	0.9924	71	77	97 (2.8)	102 (6.2)	−12	0.12	0.16
EDDP	0.9982	31	28	102 (3.2)	98 (9.0)	−4.7	0.16	0.41
LSD	0.9996	58	66	90 (8.7)	98 (5.4)	−29	0.02	0.08
OH-LSD	0.9972	66	63	106 (14)	94 (5.7)	−34	0.05	0.24
THC	0.9978	18	24	92 (4.6)	105 (4.9)	−16	0.84	3.2
OH-THC	0.9900	44	46	135 (6.5)	112 (6.8)	−29	1.9	5.1
CBD	0.9997	24	29	100 (5.0)	99 (8.8)	−24	2.2	5.9
CBN	0.9943	28	32	141 (6.8)	141 (3.8)	−40	2.3	13
ALP	0.9984	25	52	97 (2.3)	101 (4.4)	−29	0.12	0.35
DIA	0.9998	58	66	91 (6.8)	99 (6.9)	−15	0.04	0.10

RSD: Relative standard deviation; LOD: Limit of detection; LOQ: Limit of quantification.

provided by the instrumentation under positive ionization. The analysis of cannabinoids under favorable negative electrospray ionization conditions would have required an additional chromatographic run with a basic mobile phase. In this context, the simultaneous analysis of all target analytes, in detriment of cannabinoids sensitivity, was prioritized to save chemicals, reagents, and time.

Table 1 also summarizes the matrix effects observed during sediment analysis. For most of the compounds, matrix effects were negligible (ME ≤ 20 %). Only nine compounds, namely, benzoylecgonine, ephedrine, 6-acetylmorphine, LSD, its metabolite OH-LSD, OH-THC, cannabidiol, cannabinol, and alprazolam, were affected by matrix components to a higher extent (> 20 %), but ionization suppression of their MS signal did not surpass 40 %.

3.2. Occurrence of drugs of abuse and their metabolites in river sediments

The frequency of detection and median and maximum concentrations of the target drugs of abuse and metabolites in all sediment samples, as well as in each river basin and sampling campaign are summarized in Table 2. Cumulative levels of all investigated compounds in each sample are shown in Figs. 3 and 4.

Cocaethylene, amphetamine, morphine, 6-acetylmorphine, heroin, LSD and its metabolite (OH-LSD), and OH-THC were not detected in any sample. The most ubiquitous compounds were cocaine, found in 74 % of the analyzed samples, followed by methadone and its metabolite EDDP, present in 51 and 36 % of the analyzed samples, respectively. MDMA was found in 13 % of the samples and the remaining compounds, *viz.*, benzoylecgonine, methamphetamine, ephedrine, THC, cannabidiol, cannabinol, alprazolam, and diazepam were detected in less than 7 % of the samples. In general, concentrations were very low. The maximum concentrations were measured for cannabinol (44 ng/g d.w.), THC (37 ng/g d.w.), and methadone (33 ng/g d.w.). In terms of median concentrations, calculated only with values above the method LOQ, all quantified compounds were measured at median concentrations in all basins below 1.6 ng/g d.w., except the cannabinoids THC, cannabidiol, and cannabinol that were found at median concentrations of 6.1, 15 and 28 ng/g d.w, respectively. However, it should be pointed out that these high median concentrations are obtained from the detection of THC in only 5.6 % of the 144 samples analyzed (three samples in both the Ebro and the Llobregat basins, and one sample in the Jucar basin), only one sample in the Ebro basin in the case of cannabidiol, and two samples in the Ebro and the Llobregat basin in the case of cannabinol.

As for each family of compounds, cocaine was more ubiquitous and abundant than its human metabolite, benzoylecgonine, which is more polar and is usually present at a higher concentration in surface water (Mastroianni et al., 2016). Cocaine and benzoylecgonine were detected at maximum concentrations of 5.0 ng/g d.w. (GUA7) and 0.81 ng/g d.w. (LLO7), respectively, in sediment samples collected in 2011 in the main rivers of the Guadalquivir and the Llobregat basins. These concentrations were higher than those found in sediments of the San Francisco Bay (USA) (benzoylecgonine was not detected and cocaine was detected at 2.2 ng/g) (Klosterhaus et al., 2013), and lower than those found in the Beiyunhe River, China (benzoylecgonine: 3.1 ng/g d.w.; cocaine: 10 ng/g d.w.) (Hu et al., 2019). In a study previously conducted in Spain, similar concentrations of benzoylecgonine (0.95 ng/g) and higher concentrations of cocaine (30 ng/g) were measured in sediments of the Turia River basin, Valencia (Álvarez-Ruiz et al., 2015).

Within the amphetamine-type stimulants class, MDMA was the most ubiquitous compound with a frequency of detection of 13 %, while ephedrine and methamphetamine were only detected in 3.5 and 2.1 % of the samples, respectively, and amphetamine was not detected. Maximum levels of MDMA (0.83 ng/g d.w.) were found in a tributary river of the Llobregat basin, Anoia River (ANO2), in 2011. Maximum levels of ephedrine (0.48 ng/g d.w.) and methamphetamine (0.63 ng/g d.w.) were found in a tributary river of the Ebro basin, Zadorra River (ZAD), in 2011 and the main Guadalquivir River in 2011 (GUA4), respectively. As compared with this study, higher concentrations of amphetamine (6.9 ng/g d.w.) and methamphetamine (9.1 ng/g d.w.) were found in sediments of China (Hu et al., 2019) and also in the San Francisco Bay (USA) where amphetamine was detected at maximum levels of 3.3 ng/g (Klosterhaus et al., 2013).

As for opiates/opioids, neither morphine nor heroin or its metabolite 6ACM was positively identified in the investigated samples. Methadone and EDDP were found at maximum concentrations of 33 ng/g d.w. (GUA4) and 16 ng/g d.w. (ANO2), respectively, in samples collected in 2011 in the main river of the Guadalquivir basin and a tributary river of the Llobregat basin. The maximum methadone

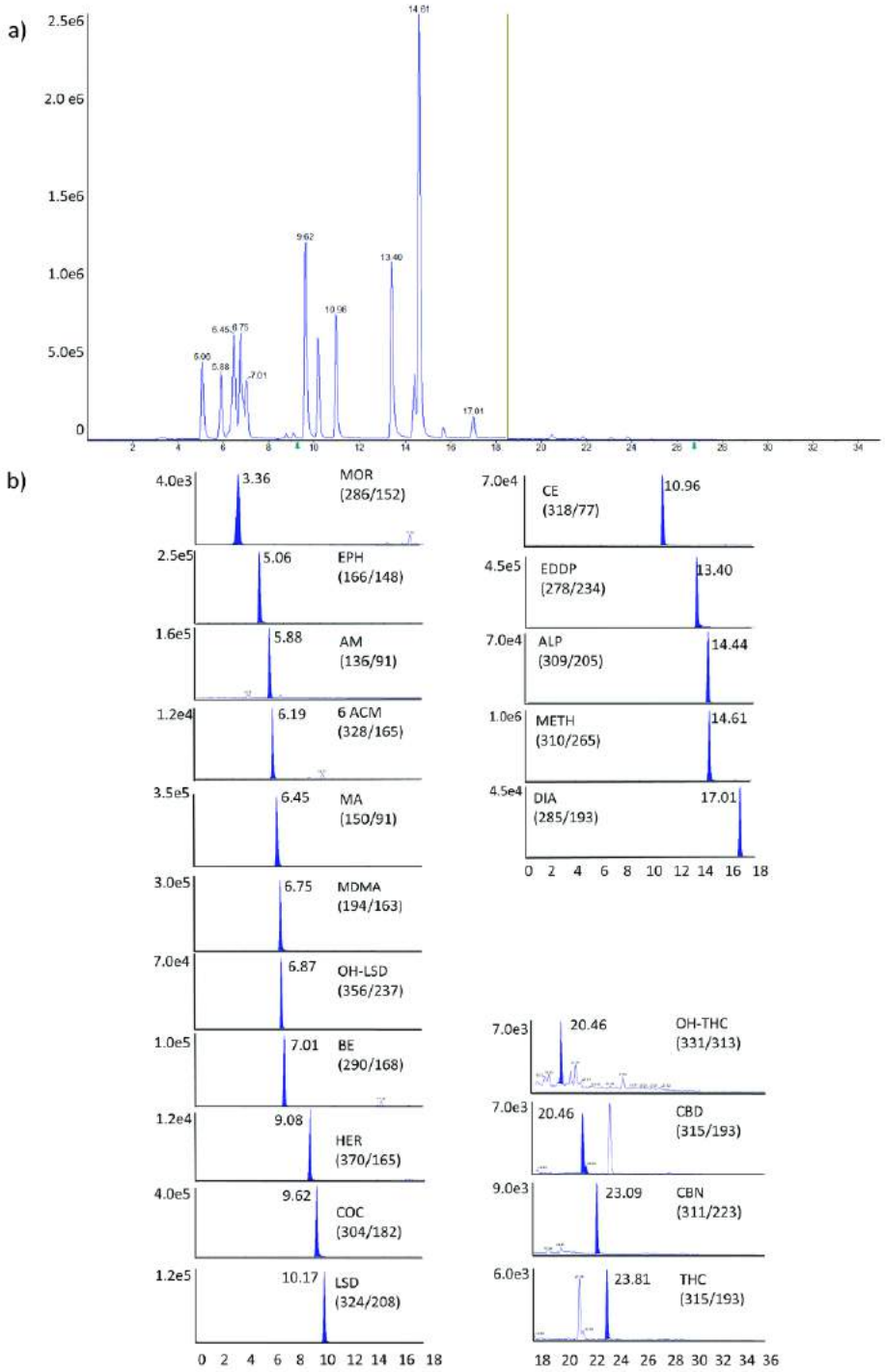


Fig. 2. Total ion chromatogram (TIC) (a) and extracted ion chromatograms (XIC) (b) of the target analytes after PLE-SPE-LC–MS/MS analysis of a sediment sample fortified at a concentration of 25 ng/g d.w.

Table 2
Frequency of detection (%), and median (ng/g d.w.) and maximum (ng/g d.w.) concentrations of the target drugs of abuse and metabolites found in all the samples analyzed during the two sampling campaigns ("All basins"), and in each river basin in 2010 and 2011 (values separated by "/").

	All basins			Ebro basin			Llobregat basin			Jucar basin			Guadalquivir basin		
	Freq. (%) (n = 144)	Median[a] (ng/g d.w.)	Max. (ng/g d.w.)	Freq. (%) (n = 19/23)	Median[a] (ng/g d.w.)	Max. (ng/g d.w.)	Freq. (%) (n = 14/14)	Median[a] (ng/g d.w.)	Max. (ng/g d.w.)	Freq. (%) (n = 12/15)	Median[a] (ng/g d.w.)	Max. (ng/g d.w.)	Freq. (%) (n = 24/23)	Median[a] (ng/g d.w.)	Max. (ng/g d.w.)
COC	74	0.34	5.0	100/35	0.31/0.38	1.0/0.44	93/50	0.25/0.40	0.47/0.72	100/47	0.27/0.53	1.2/4.6	100/74	0.30/0.75	0.75/5.0
BE	2.1	0.46	0.81	-/-	-/-	-/-	-/7.1	-/0.81	-/0.81	-/13	-/0.37	-/0.46	-/-	-/-	-/-
CE	–	–	–	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
AM	–	–	–	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
MA	2.1	0.25	0.63	-/4.3	-/0.18	-/0.18	-/-	-/-	-/-	-/-	-/-	-/-	-/8.7	-/0.44	-/0.63
MDMA	13	0.17	0.83	26/13	0.17/0.19	0.31/0.43	21/21	0.17/0.17	0.20/0.83	25/-	< LOQ/-	-/-	4.1/-	< LOQ	-/-
EPH	3.5	0.48	0.48	5.3/13	0.40/0.48	0.40/0.48	-/7.1	-/0.48	-/0.48	-/-	-/-	-/-	-/-	-/-	-/-
MOR	–	–	–	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
6ACM	–	–	–	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
HER	–	–	–	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
METH	51	0.30	33	42/39	0.70/0.65	3.7/2.7	43/32	0.37/0.89	1.1/5.7	100/33	0.21/0.49	1.1/0.56	75/43	0.25/0.37	1.7/33
EDDP	36	1.6	16	42/52	5.1/2.4	9.5/7.8	43/57	1.2/2.9	3.8/16	33/42	0.64/0.91	0.76/1.9	29/8.3	0.89/5.3	5.8/9.7
LSD	–	–	–	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
OH-LSD	–	–	–	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
THC	5.6	6.1	37	16/-	6.1/-	36/-	21/7.1	6.3/14	37/14	8.3/-	3.9/-	3.9/-	-/-	-/-	-/-
OH-THC	–	–	–	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	–	-/-	-/-
CBD	0.7	15	15	5.3/-	15/-	15/-	-/-	-/-	-/-	8.3/-	< LOQ/-	-/-	-/-	-/-	-/-
CBN	2.1	28	44	5.3/-	44/-	44/-	7.1/-	13/-	13/-	8.3/-	< LOQ/-	-/-	-/-	-/-	-/-
ALP	0.7	< LOQ	–	-/-	-/-	-/-	-/6.7	-/ < LOQ	-/-	-/-	-/-	-/-	-/-	-/-	-/-
DIA	6.9	0.24	1.3	11/-	0.24/-	0.24/-	21/21	0.19/0.80	0.25/1.3	8.3/6.7	0.20/0.13	0.20/0.13	-/-	-/-	-/-

-: not detected; < LOQ: below limit of quantification.
[a] Only values above the limit of quantification (LOQ) were considered in the calculation of the median concentration.

concentration measured in this study was higher than the maximum concentration found in sediments collected in the Turia River basin, Valencia (Spain) (0.53 ng/g) (Álvarez-Ruiz et al., 2015).

As for cannabinoids, cannabinol and cannabidiol were found at maximum concentrations of 44 and 15 ng/g d.w., respectively, in a tributary river of the Ebro basin (ZAD) in 2010, and THC was found at a maximum concentration of 37 ng/g d.w. in the main river of the Llobregat basin (LLO3) in 2010. Despite this, THC levels found were not as high as those found in the Turia River, Valencia (Spain), where the THC concentration in sediments reached 200 ng/g (Carmona et al., 2017).

Alprazolam was detected in only one sample but at levels below the method LOQ. Higher levels of alprazolam (maximum concentrations of 87 ng/g d.w.) were found in sediment samples collected in the lakes Al-Hufuf and Al-Oyun in Saudi Arabia (Picó et al., 2020). The other investigated benzodiazepine, diazepam, was detected in 6.9 % of the analyzed samples at maximum concentrations of 1.3 ng/g d.w. in the main river of the Llobregat basin (LLO7) in 2011. The maximum diazepam concentration found in the present study was lower than that found in the Danube River (Serbia) (48 ng/g) (Radović et al., 2015), and higher than the maximum concentrations found in sediment samples collected in the Salvador Bay (Brazil) (0.71 ng/g d.w.) (Beretta et al., 2014). In the Turia River (Spain) (Carmona et al., 2017), and the Douro and the Lima Rivers (Portugal) (Santos et al., 2016), diazepam was either not present or at levels below the LOD of the corresponding method.

3.3. Spatial and temporal variability of drugs of abuse in river sediments

Fig. 3 shows the cumulative levels of the different classes of drugs of abuse and metabolites investigated along the four main rivers in the two sampling campaigns. The concentrations measured in tributary rivers are depicted in Fig. 4.

The most polluted river basin in terms of drugs of abuse and taking into account both, concentrations found in the main river and its tributaries, and the two sampling campaigns conducted was the Ebro River basin, with a total cumulative level of drugs of abuse on average of 91 ng/g d.w. The Ebro River basin was followed by the Llobregat River basin (65 ng/g d.w.), the Guadalquivir River basin (47 ng/g d.w.), and the Jucar River basin (14 ng/g d.w.). The Llobregat and the Ebro River basins presented higher cumulative levels in 2010 than in 2011 (74 *vs* 56 ng/g d.w. in the case of the Llobregat basin, and 143 *vs* 38 ng/g d.w. in the case of the Ebro basin), while the Guadalquivir River basin presented higher cumulative levels during 2011 than during 2010 (72 *vs* 22 ng/g d.w, respectively). In the case of the Jucar River similar cumulative levels were found in both years (15 and 13 ng/g d.w. respectively).

The most polluted sampling locations were ZAD in 2010 (the Ebro River basin) and LLO3 in 2010 (the Llobregat River basin), with cumulative levels of 109 and 50 ng/g d.w., respectively, followed by GUA4 (the Guadalquivir River basin) in 2011 with a cumulative level of 38 ng/g d.w. Cannabinoids was the chemical class that contributed the most to ZAD and LLO3 total concentrations, whereas opioids was the most abundant class in GUA4. The samples ANO2 (the Llobregat River basin) in 2011, LLO7 (the Llobregat River basin) in 2011, GUA-A (the Guadalquivir River basin) in 2011, HUE (the Ebro River basin) in 2010, ANO2 (the Llobregat River basin) in 2010 and ZAD (the Ebro River basin) in 2011, presented cumulative levels of drugs of abuse between 23 and 12 ng/g d.w., while the rest of sediment samples contained cumulative levels below 10 ng/g d.w. Similar to the results obtained for surface water collected in the same sampling locations (Mastroianni et al., 2016), the highest accumulation of drugs of abuse was found in small tributary rivers located downstream of medium- (40.000 inhabitants) to large-size (2 M inhabitants) urban areas, like ZAD

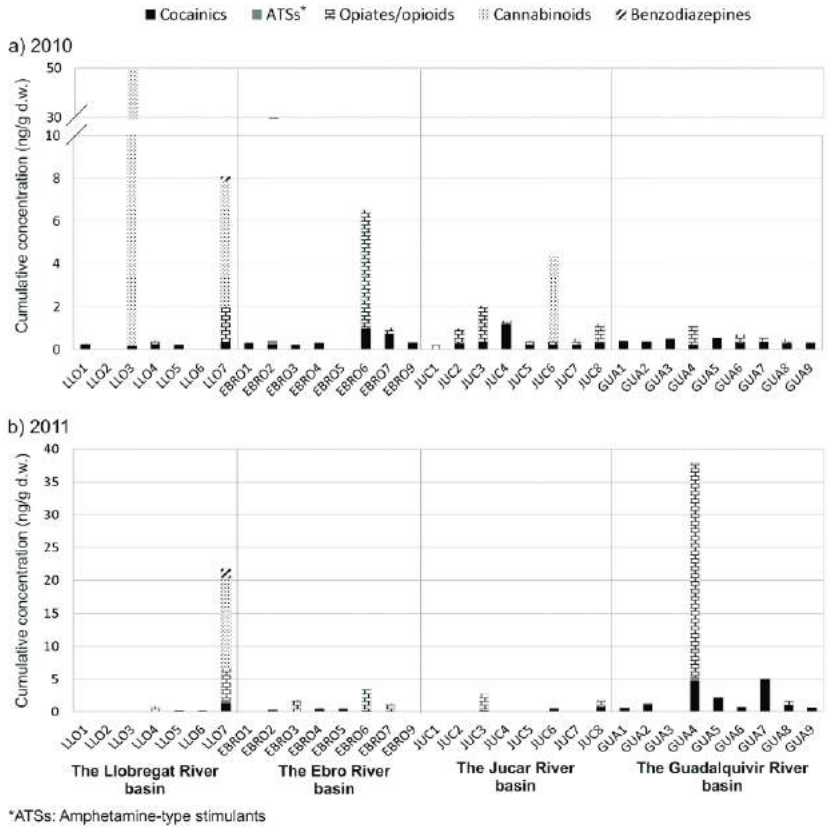
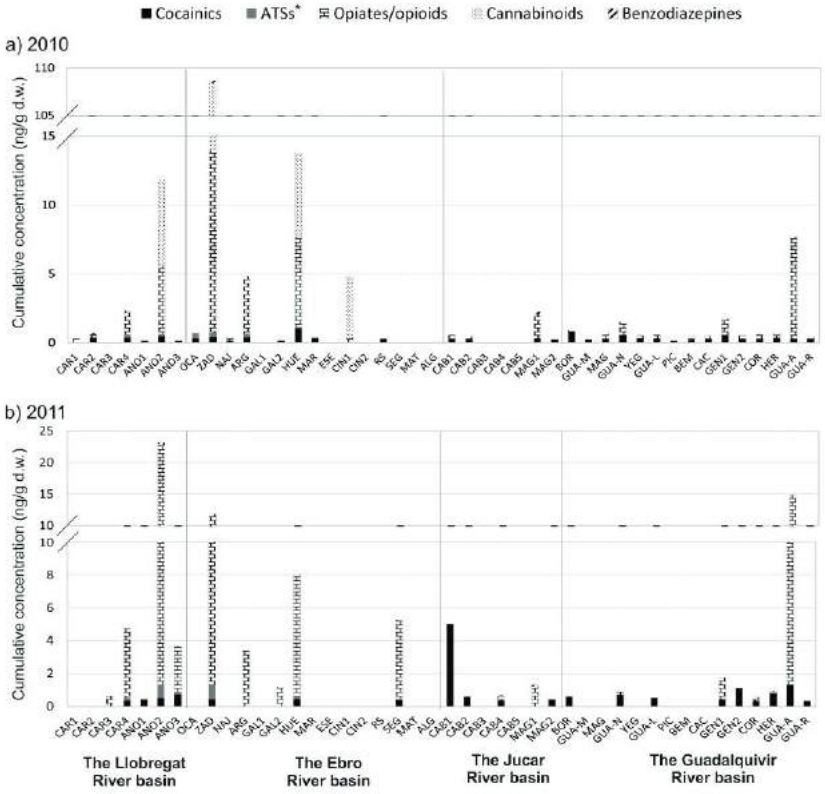


Fig. 3. Cumulative concentration (ng/g, d.w.) of drugs of abuse classes in the main rivers in 2010 (a) and 2011 (b).

(Gasteiz), ANO2 (Igualada), GUA-A (Sevilla) or HUE (Zaragoza) or close to WWTP discharge points like GUA4 (WWTP from Córdoba). The lower dilution capacity of tributaries as compared to the main rivers of the wastewater effluent discharges may favor the accumulation of drugs of abuse and/or their metabolites in sediments. High cumulative levels were also found in main river locations like LLO3 and LLO7, which correspond to the middle and the final section of the Llobregat River. Unlike other river basins, where the pollution gradient decreases downstream the main river due to its increasing flow and hence dilution capacity, in the Llobregat River pollution could increase from its head to its mouth due to a growing population density and number of WWTPs downstream. WWTP discharges may even represent almost 100 % of the Llobregat River flow in drought periods (Boleda et al., 2009; Osorio et al., 2012). Our findings are in agreement with other studies conducted in this basin, that reported the highest levels of organic micropollutants like pharmaceuticals (Osorio et al., 2016) and endocrine disruptors (Gorga et al., 2015) in sediments samples collected near the mouth of the river.

To study differences in the occurrence of drugs of abuse between river basins and sampling campaigns, the most detected compounds (cocaine, methadone, EDDP, and MDMA), as well as the sum of all detected compounds ("Sum") and the sum of all detected compounds excluding cannabinoids ("Sum No Cannabinoids"), were statistically evaluated. Table 3 shows the p-values obtained after applying the Wilcoxon Rank-Sum test to assess differences in the distribution of drugs of abuse between sampling campaigns stratifying by river basin. Concentrations of drugs of abuse detected in sediment samples collected in 2011 were statistically significantly different than those collected in 2010 for cocaine in the Ebro and the Guadalquivir basins, methadone in the Jucar and the Guadalquivir basins, and EDDP in the Guadalquivir basin. The whole set of data obtained in this study was then employed for a multivariate analysis based on the quantile regression model (median regression model) to predict in both years the median concentration of drugs of abuse in the investigated basins (see Table S3a and Table S3b in SM). Compared to 2010, in 2011 statistically significant (α = 0.05) lower median concentrations of cocaine in the Ebro basin (Δ median -0.25, 95 % CI -0.41; 0.09) and methadone in the Jucar (Δ median -0.14, 95 % CI -0.23; -0.04) and the Guadalquivir (Δ median -0.14, 95 % CI -0.21; -0.06) basins were predicted, while a higher median concentration of cocaine in the Guadalquivir (Δ median 0.32, 95 % CI 0.17; 0.47) basin was predicted in 2011 compared to 2010. There was a reduced flow, and hence lower dilution factor and higher diffusion rates of the water concentrations into the sediments, in most of the sampling locations investigated in 2011 compared to 2010 (data not shown). This could explain the statistically significant higher cocaine concentrations found in the Guadalquivir basin found in 2011. However, hydrological conditions cannot explain the larger concentrations of cocaine in the Ebro river basin and methadone in the Jucar and the Guadalquivir river basins found in 2010. Storm events may also play a relevant role in the desorption of organic pollutants from sediments. Additionally, other factors such as the patterns of consumption of drugs of abuse by the surrounding population, the efficiency of the WWTPs to remove the drugs of abuse, or natural attenuation processes (like photodegradation and biodegradation) may be responsible for the overall high concentrations of drugs of abuse in the water and consequently in the sediments observed in 2010.



*ATSs: Amphetamine-type stimulants

Fig. 4. Cumulative concentration (ng/g, d.w.) of drugs of abuse classes in the tributary rivers in 2010 (a) and 2011 (b).

Table 3
Comparsion of compound distribution between 2010 and 2011 stratifying by river basin (Wilcoxon Rank-Sum test p-value).

Compound	Llobregat	Ebro	Jucar	Guadalquivir
Cocaine	0.52	< 0.01*	0.92	0.04*
MDMA	0.83	0.34	0.08†	0.35
Methadone	0.79	0.64	0.02*	0.05*
EDDP	0.40	0.69	0.72	0.04*
Sum	0.80	0.35	0.21	0.54
Sum No Cannabinoids	0.87	0.50	0.87	0.54

* $p < 0.05$.
† $p < 0.10$.

Differences in the distribution of drugs of abuse between basins stratifying by year were also studied. The results are shown in Table 4. Statistically significant different distributions were only found for cocaine and EDDP in 2011. To find the basins where there was a difference in the distribution of these compounds, a posthoc analysis of each pair of basins was done (Wilcoxon Sum Rank test) and the p-value obtained was corrected by a multiple comparison method (False Discovery Rate, FDR). For both compounds, EDDP and cocaine, statistically different distributions were obtained between the Ebro and the Guadalquivir basins, and between the Llobregat and the Guadalquivir basins in 2011. These differences are due to the remarkably high concentrations of cocaine found in the Guadalquivir basin and EDDP in the Ebro and the Llobregat basins in 2011 (Table S3a, Table S3b), and could be associated with a different consumption pattern of cocaine and methadone by the population living in these areas, provided that EDDP comes mainly from methadone consumption and to a minor extent from methadone photolysis (Postigo et al., 2011). However, since official data on the annual consumption of drugs is only available for the whole Spanish territory, without distinguishing among regions (except in the single case of cannabis) (OEDA, 2019), a solid association of these results with distinct human consumption habits is not possible. Moreover, other factors such as drug trafficking, apart from those aforementioned, could also play a role (the Guadalquivir River is a trafficking route used to introduce drugs, particularly cannabis, in Spain and hence, in the European markets).

3.4. Distribution of drugs of abuse in the sediment and water compartments

The distribution of drugs of abuse and metabolites between the sediment and the aqueous phase in the various sampling sites investigated was evaluated through the experimental determination of the sediment-water distribution coefficient (K_D). This coefficient corresponds with the average value of the ratios between the sediment and water concentrations obtained in each sampling location. K_D was calculated only for compounds that were found to be present in more than 6 % of the sediment samples analyzed, *viz.*, cocaine, MDMA, diazepam, methadone, and EDDP. The source data used, reported in this study for sediments and elsewhere for water (Mastroianni et al., 2016), and the experimental K_D estimated for these compounds are summarized in Table S4 as SM. The compounds presenting the greatest tendency to become absorbed into the sediments rather than to remain in the

Table 4
Comparison of compound distribution between the four basins stratifying by year (Kruskal-Wallis test p-values). If statistical differences were shown, pairwise basin comparisons were performed (Wilcoxon Rank-Sum test p-values). False Discovery Rate (FDR) correction for multiple testing was applied.

Compound	All basins[a]		Ebro-Llo[b]		Ebro-Juc[b]		Ebro-Gua[b]		Llo-Juc[b]		Llo-Gua[b]		Juc-Gua[b]		FDR[b]	
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
Cocaine	0.06[†]	< 0.01[*]		0.41		0.31		< 0.01[*]		0.78		0.04[*]		0.06[†]		Ebro-Gua, Llo-Gua
MDMA	0.13	0.06[†]														
Methadone	0.36	0.79														
EDDP	0.58	0.01[*]		0.70		0.22		0.01[*]		0.20		0.01[*]		0.19		Ebro-Gua, Llo-Gua
Sum	0.51	0.65														
Sum No Cannabinoids	0.26	0.66														

Ebro = the Ebro River basin; Llo = the Llobregat River basin; Juc = the Jucar River basin; Gua = the Guadalquivir River basin.
[*] $p < 0.05$.
[†] $p < 0.10$.
[a] Null hypothesis in Kruskal-Wallis test (H_0: the compound distribution in the four independent basins are equal) was rejected if corresponding p-value < 0.05.
[b] Null hypothesis in Wilcoxon Sum-Rank test (H_0: the compound distribution in the independent pair of basins tested are equal) was rejected if corresponding p-value was lower than the corresponding corrected significance level $\alpha_{corrected}$ obtained after applying the False Discovery Rate correction due to Multiple Testing (six multiple pairs compared). Overall significance level was = 0.05.

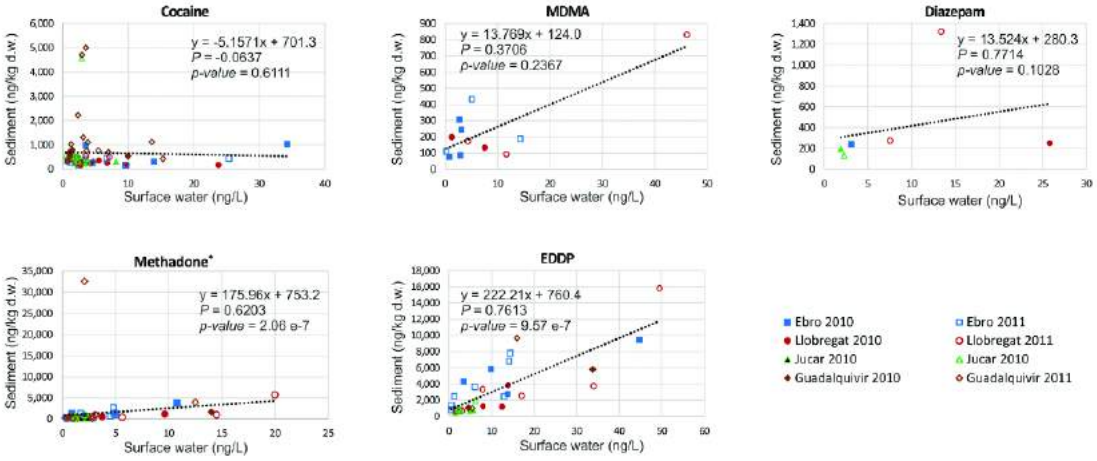


Fig. 5. Correlation between drugs of abuse distribution in surface water and sediment. (*P*: Spearman correlation coefficient; **p-value* < 0.05 were considered statistically significant).

aqueous phase were methadone (K_D: 619 L/kg, log K_D: 2.79) and EDDP (K_D: 474 L/kg, log K_D: 2.68), followed by cocaine (K_D: 281 L/kg, log K_D: 2.45), MDMA (K_D: 88 L/kg, log K_D: 1.95) and diazepam (K_D: 64 L/kg, log K_D: 1.79). Additionally, the Spearman's correlation test carried out with the data set (Fig. 5) showed a significant correlation (Spearman p-value < 0.05) for methadone and EDDP, suggesting a good equilibrium of these compounds between both compartments. The K_D value reported for cocaine differs from those obtained in previous studies (K_D: 840 L/kg (Plósz et al., 2013); 469.5 L/kg (Hu et al., 2019)), possibly due to the different physical-chemical characteristics of the sediment samples investigated in each work, or exceptional events altering surface water concentrations and normal diffusion rates of this compound (e.g. delivered disposal of cocaine into the water). K_D values for amphetamine, methamphetamine, ketamine, ephedrine, benzoylecgonine, and morphine have been previously reported (Hu et al., 2019), but, to the author's knowledge, this is the first time that K_D values for EDDP, methadone, MDMA, and diazepam are reported from field observations.

3.5. Environmental risk assessment

The accumulation of drugs of abuse and their metabolites in sediments may pose a toxicological risk for aquatic organisms living or feeding on/in river sediments since these substances are biologically active and their chronic effects are relatively unknown (Ginebreda et al., 2014). To assess the environmental risk, the Hazard Quotient (HQ) approach, where the measured environmental concentration (MEC) of a given compound is compared with its predicted non-effect concentration (PNEC), at which no toxic effects are expected, was applied. The PNEC values in sediments ($PNEC_{sed}$) (Table S1 in SM) were extracted from the NORMAN Ecotoxicology Database for all compounds except for cocaine, as it was not covered by the database. In this case, the $PNEC_{sed}$ was calculated from the $PNEC_{water}$ value reported in Mendoza et al. (2014) by applying the equilibrium partitioning approach that uses the NORMAN database to convert $PNEC_{water}$ values (predicted by QSAR models or obtained experimentally) into $PNEC_{sed}$ values (Table S1 in SM) (NORMAN, 2020).

To jointly consider the effects produced by the mixture of the drugs of abuse investigated, the toxicological risk caused by their presence in each sample was evaluated by applying a concentration addition model (Ginebreda et al., 2010), i.e., in each sample, the total HQ was calculated as the sum of the individual HQ of each drug or metabolite positively identified in the sample. When ΣHQ < 1, sampling sites were not considered hazardous, whereas ΣHQ values between 1 and 10 indicated potentially hazardous sites, and ΣHQ values > 10 pointed out the most hazardous sites for aquatic organisms living or feeding on/in sediments. Tables S5 and S6 in SM show HQ values obtained for each

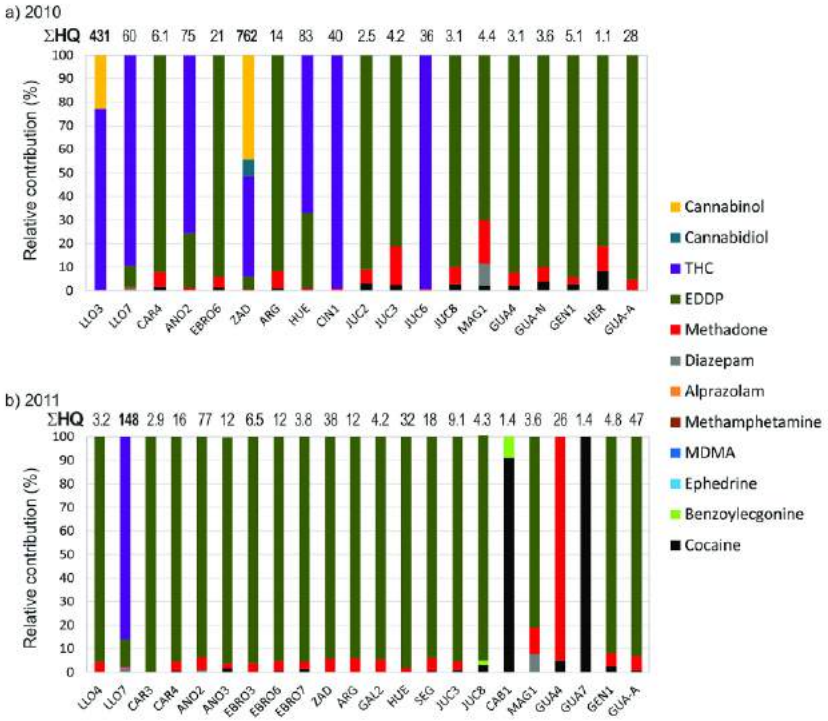


Fig. 6. Relative contribution (%) of different drugs of abuse and metabolites to the hazard quotient obtained in each sample showing a toxicological risk (ΣHQ > 1).

sediment sample collected in 2010 and 2011 sampling campaigns, respectively. The relative contribution weight of each compound to the overall HQ value in those locations where ΣHQ > 1 is depicted in Fig. 6.

ΣHQ values < 1 were obtained for 72.5 % (2010) and 70.6 % (2011) of the sampling locations, indicating low or no potential risk for sediment-dwelling organisms. On the contrary, ΣHQ values between 1 and 10 were obtained for 13.0 % (2010) and 14.7 % (2011) of the samples, and ΣHQ > 10 were obtained for 14.5 % (2010) and 14.7 % (2011) of the investigated sediments, indicating risk for the aquatic organisms living or feeding on/in sediments in those sampling locations. However, it should be noted that, in most cases, these high values of HQ are due to the low $PNEC_{sed}$ values of specific target analytes (Table S1 in SM) rather than to a high accumulation of drugs of abuse.

The maximum ΣHQ values were obtained for the sample collected in ZAD in 2010 (ΣHQ: 762) due to the contribution of cannabinol (HQ: 338), THC (HQ: 324), cannabidiol (HQ: 55), and EDDP (HQ: 43), the sample collected in LLO3 in 2010 (ΣHQ: 431) mainly due to the contribution of THC (HQ: 333) and cannabinol (HQ: 98), and the sample collected in LLO7 in 2011 (ΣHQ: 148) due to the main contribution of THC (HQ: 127). The remaining sampling locations presented ΣHQ < 83 (Tables S5 and S6 in SM). Overall, the compounds that contributed the most to the toxicity of the samples were EDDP and THC in 2010 and EDDP in 2011 (Fig. 6). Methadone contributed also to the toxicity of many samples but its relative contribution was low (below 20 % in all samples except in GUA4 in 2011).

In both sampling campaigns, the sampling locations LLO7, CAR4, and ANO2 in the Llobregat River basin; EBRO6, ZAD, ARG, and HUE in the Ebro River basin; JUC3, JUC8, and MAG1 in the Jucar River basin, and GUA4, GEN1, and GUA-A in the Guadalquivir River basin presented ΣHQ values > 1 so they could be considered sites with certain toxicological risk. Nevertheless, it is important to stress that these results correspond to the analysis of grab samples and hence they are not necessarily representative of a long-term exposure scenario.

4. Conclusions

An analytical methodology based on PLE extraction and SPE clean up followed by LC–MS/MS determination has been validated and applied to assess the occurrence of 20 drugs of abuse and their metabolites in 144 sediment samples collected in four Spanish river basins. Overall, concentrations in river sediment samples were in the low ng/g d.w, being the most polluted samples those collected in tributary rivers and locations downstream urban areas or impacted by WWTP effluents. Statistically significant different distributions of some drugs of abuse and metabolites were observed between sampling campaigns and among river basins. However, the observed changes could not be related to a single factor, but a mixture of them (e.g., hydrological conditions, storm events and consumption patterns of drugs of abuse in the investigated areas). Only in the case of EDDP, which is mainly formed after methadone consumption, its significant different distribution among river basins may be more solidly associated with different consumption patterns of methadone in those areas.

The sediment-water distribution coefficient (K_D) of EDDP, methadone, MDMA, diazepam, and cocaine were experimentally calculated by studying the relationship between their concentrations in water and sediment in each investigated location. EDDP and methadone were the drugs that showed the greatest tendency to become adsorbed onto the sediments (Log $K_D \geq 2.68$).

Finally, the risk assessment study showed that the drugs present in some sampling sites may pose a high risk for the aquatic organisms living or feeding on/in their sediments. However, this assessment is based on grab samples. Further studies including composite samples and extended in time would be required to assess the long-term

exposure of sediment-dwelling organisms to drugs of abuse.

CRediT authorship contribution statement

Ester López-García: Data curation, Formal analysis, Writing - original draft, Visualization. **Nicola Mastroianni:** Investigation. **Núria Ponsà-Borau:** Formal analysis. **Damià Barceló:** Funding acquisition. **Cristina Postigo:** Supervision, Visualization, Writing - review & editing. **Miren López de Alda:** Conceptualization, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

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

Acknowledgments

This work has been financially supported by the EU through the EU FP7 project SOLUTIONS (603437), the Spanish Ministry of Economy and Competitiveness through the project SCARCE (Consolider-Ingenio 2010 CSD2009-00065), the Spanish Ministry of Science and Innovation (Project CEX2018-000794-S), and the Generalitat de Catalunya (Consolidated Research Group 2017 SGR 01404-Water and Soil Quality Unit). Biotage is acknowledged for the gift of SPE cartridges.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2020.123312.

References

Álvarez-Ruiz, R., Andrés-Costa, M.J., Andreu, V., Picó, Y., 2015. Simultaneous determination of traditional and emerging illicit drugs in sediments, sludges and particulate matter. J. Chromatogr. A 1405, 103–115. https://doi.org/10.1016/j.chroma.2015.05.062.

Arbeláez, P., Borrull, F., María Marcé, R., Pocurull, E., 2014. Simultaneous determination of drugs of abuse and their main metabolites using pressurized liquid extraction and liquid chromatography-tandem mass spectrometry. Talanta 125, 65–71. https://doi.org/10.1016/j.talanta.2014.02.053.

Baker, D.R., Kasprzyk-Hordern, B., 2011. Multi-residue determination of the sorption of illicit drugs and pharmaceuticals to wastewater suspended particulate matter using pressurised liquid extraction, solid phase extraction and liquid chromatography coupled with tandem mass spectrometry. J. Chromatogr. A 1218, 7901–7913. https://doi.org/10.1016/j.chroma.2011.08.092.

Baker, D.R., Kasprzyk-Hordern, B., 2013. Spatial and temporal occurrence of pharmaceuticals and illicit drugs in the aqueous environment and during wastewater treatment: new developments. Sci. Total Environ. 454–455, 442–456. https://doi.org/10.1016/j.scitotenv.2013.03.043.

Beretta, M., Britto, V., Tavares, T.M., da Silva, S.M.T., Pletsch, A.L., 2014. Occurrence of pharmaceutical and personal care products (PPCPs) in marine sediments in the Todos os Santos Bay and the north coast of Salvador, Bahia, Brazil. J. Soils Sediments 14, 1278–1286. https://doi.org/10.1007/s11368-014-0884-6.

Biel-Maeso, M., Corada-Fernández, C., Lara-Martín, P.A., 2017. Determining the distribution of pharmaceutically active compounds (PhACs) in soils and sediments by pressurized hot water extraction (PHWE). Chemosphere 185, 1001–1010. https://doi.org/10.1016/j.chemosphere.2017.07.094.

Boleda, M.R., Galceran, M.T., Ventura, F., 2009. Monitoring of opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain. Water Res. 43, 1126–1136. https://doi.org/10.1016/j.watres.2008.11.056.

Carmona, E., Andreu, V., Picó, Y., 2017. Multi-residue determination of 47 organic compounds in water, soil, sediment and fish—turia river as case study. J. Pharm. Biomed. Anal. 146, 117–125. https://doi.org/10.1016/j.jpba.2017.08.014.

Cunha, D.L., de Araujo, F.G., Marques, M., 2017. Psychoactive drugs: occurrence in aquatic environment, analytical methods, and ecotoxicity—a review. Environ. Sci. Pollut. Res. 24, 24076–24091. https://doi.org/10.1007/s11356-017-0170-4.

Gago-Ferrero, P., Borova, V., Dasenaki, M.E., Thomaidis, N.S., 2015. Simultaneous determination of 148 pharmaceuticals and illicit drugs in sewage sludge based on ultrasound-assisted extraction and liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 407, 4287–4297. https://doi.org/10.1007/s00216-015-8540-6.

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

Ginebreda, A., Kuzmanovic, M., Guasch, H., López de Alda, M., López-Doval, J.C., Muñoz, I., Ricart, M., Romaní, A.M., Sabater, S., Barceló, D., 2014. Assessment of multi-chemical pollution in aquatic ecosystems using toxic units: Compound prioritization, mixture characterization and relationships with biological descriptors. Sci. Total Environ. 468–469, 715–723. https://doi.org/10.1016/j.scitotenv.2013.08.086.

González-Mariño, I., Quintana, J.B., Rodríguez, I., Cela, R., 2010. Determination of drugs of abuse in water by solid-phase extraction, derivatisation and gas chromatography-ion trap-tandem mass spectrometry. J. Chromatogr. A 1217, 1748–1760. https://doi.org/10.1016/j.chroma.2010.01.046.

Gorga, M., Insa, S., Petrovic, M., Barceló, D., 2015. Occurrence and spatial distribution of EDCs and related compounds in waters and sediments of Iberian rivers. Sci. Total Environ. 503–504, 69–86. https://doi.org/10.1016/j.scitotenv.2014.06.037.

Hu, P., Guo, C., Zhang, Yan, Lv, J., Zhang, Yuan, Xu, J., 2019. Occurrence, distribution and risk assessment of abused drugs and their metabolites in a typical urban river in north China. Front. Environ. Sci. Eng. 13, 1–11. https://doi.org/10.1007/s11783-019-1140-5.

International Narcotic Control Board, (INCB 2019), 2019. Psychotropic Substances. Statistics for 2018. Assessments of Annual Medical and Scientific Requirements. Available at. https://www.incb.org/documents/Psychotropics/technical-publications/2019/PSY_Technical_Publication_2019.pdf.

Klosterhaus, S.L., Grace, R., Hamilton, M.C., Yee, D., 2013. Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. Environ. Int. 54, 92–99. https://doi.org/10.1016/j.envint.2013.01.009.

Langford, K.H., Reid, M., Thomas, K.V., 2011. Multi-residue screening of prioritised human pharmaceuticals, illicit drugs and bactericides in sediments and sludge. J. Environ. Monit. 13, 2284–2291. https://doi.org/10.1039/c1em10260e.

Martínez Bueno, M.J., Uclés, S., Hernando, M.D., Fernández-Alba, A.R., 2011. Development of a solvent-free method for the simultaneous identification/quantification of drugs of abuse and their metabolites in environmental water by LC-MS/MS. Talanta 85, 157–166. https://doi.org/10.1016/j.talanta.2011.03.051.

Mastroianni, N., Postigo, C., De Alda, M.L., Barcelo, D., 2013. Illicit and abused drugs in sewage sludge: method optimization and occurrence. J. Chromatogr. A 1322, 29–37. https://doi.org/10.1016/j.chroma.2013.10.078.

Mastroianni, N., Bleda, M.J., López de Alda, M., Barceló, D., 2016. Occurrence of drugs of abuse in surface water from four Spanish river basins: spatial and temporal variations and environmental risk assessment. J. Hazard. Mater. 316, 134–142. https://doi.org/10.1016/j.jhazmat.2016.05.025.

Matić Bujagić, I., Grujić, S., Laušević, M., Hofmann, T., Micić, V., 2019. Emerging contaminants in sediment core from the Iron gate I Reservoir on the Danube River. Sci. Total Environ. 662, 77–87. https://doi.org/10.1016/j.scitotenv.2019.01.205.

Mendoza, A., Rodríguez-Gil, J.L., González-Alonso, S., Mastroianni, N., López de Alda, M., Barceló, D., Valcárcel, Y., 2014. Drugs of abuse and benzodiazepines in the Madrid Region (Central Spain): seasonal variation in river waters, occurrence in tap water and potential environmental and human risk. Environ. Int. 70, 76–87. https://doi.org/10.1016/j.envint.2014.05.009.

Montesano, C., Vannutelli, G., Massa, M., Simeoni, M.C., Gregori, A., Ripani, L., Compagnone, D., Curini, R., Sergi, M., 2017. Multi-class analysis of new psychoactive substances and metabolites in hair by pressurized liquid extraction coupled to HPLC-HRMS. Drug Test. Anal. 9, 798–807. https://doi.org/10.1002/dta.2043.

Navarro-Ortega, A., Acuña, V., Batalla, R.J., Blasco, J., Conde, C., Elorza, F.J., Elosegi, A., Francés, F., La-Roca, F., Muñoz, I., Petrovic, M., Picó, Y., Sabater, S., Sanchez-Vila, X., Schuhmacher, M., Barceló, D., 2012. Assessing and forecasting the impacts of global change on Mediterranean rivers. The SCARCE Consolider project on Iberian basins. Environ. Sci. Pollut. Res. 19, 918–933. https://doi.org/10.1007/s11356-011-0566-5.

NORMAN, 2020. NORMAN Ecotoxicology Database. (accessed 5.26.20). https://www.norman-network.com/nds/ecotox/.

Nunes, C.N., Pauluk, L.E., Dos Anjos, V.E., Lopes, M.C., Quináia, S.P., 2015. New approach to the determination of contaminants of emerging concern in natural water: study of alprazolam employing adsorptive cathodic stripping voltammetry. Anal. Bioanal. Chem. 407, 6171–6179. https://doi.org/10.1007/s00216-015-8792-1.

Observatorio Español de las Drogas y las Adicciones, (OEDA, 2019), 2019. Informe 2019 Alcohol, Tabaco Y Drogas Ilegales En España. Available at:. https://pnsd.sanidad.gob.es/profesionales/sistemasInformacion/informesEstadisticas/pdf/2019OEDA-INFORME.pdf.

Osorio, V., Pérez, S., Ginebreda, A., Barceló, D., 2012. Pharmaceuticals on a sewage impacted section of a Mediterranean River (Llobregat River, NE Spain) and their relationship with hydrological conditions. Environ. Sci. Pollut. Res. 19, 1013–1025. https://doi.org/10.1007/s11356-011-0603-4.

Osorio, V., Larrañaga, A., Aceña, J., Pérez, S., Barceló, D., 2016. Concentration and risk of pharmaceuticals in freshwater systems are related to the population density and the livestock units in Iberian Rivers. Sci. Total Environ. 540, 267–277. https://doi.org/10.1016/j.scitotenv.2015.06.143.

Pal, R., Megharaj, M., Kirkbride, K.P., Naidu, R., 2013. Illicit drugs and the environment - A review. Sci. Total Environ. 463–464, 1079–1092. https://doi.org/10.1016/j.scitotenv.2012.05.086.

Picó, Y., Alvarez-Ruiz, R., Alfarhan, A.H., El-Sheikh, M.A., Alshahrani, H.O., Barceló, D., 2020. Pharmaceuticals, pesticides, personal care products and microplastics contamination assessment of Al-Hassa irrigation network (Saudi Arabia) and its shallow lakes. Sci. Total Environ. 701, 135021. https://doi.org/10.1016/j.scitotenv.2019.135021.

Piel, S., Blondeau, S., Pérot, J., Baurès, E., Thomas, O., 2013. Pharmaceutical removal by the activated carbon process. Water Qual J Canada 48, 121–132. https://doi.org/10.2166/wqrjc.2013.138.

Plósz, B.G., Reid, M.J., Borup, M., Langford, K.H., Thomas, K.V., 2013. Biotransformation kinetics and sorption of cocaine and its metabolites and the factors influencing their estimation in wastewater. Water Res. 47, 2129–2140. https://doi.org/10.1016/j.watres.2012.12.034.

Postigo, C., López de Alda, M.J., Barceló, D., 2010. Drugs of abuse and their metabolites in the Ebro River basin: occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation. Environ. Int. 36, 75–84. https://doi.org/10.1016/j.envint.2009.10.004.

Postigo, C., Sirtori, C., Oller, I., Malato, S., Maldonado, M.I., López de Alda, M., Barceló, D., 2011. Photolytic and photocatalytic transformation of methadone in aqueous solutions under solar irradiation: kinetics, characterization of major intermediate products and toxicity evaluation. Water Res. 45, 4815–4826. https://doi.org/10.1016/j.watres.2011.06.027.

Postigo, C., Mastroianni, N., López de Alda, M., Barceló, D., 2012. Illicit drugs and metabolites in the Llobregat River Basin. Handbook of Environmental Chemistry. Springer Verlag, pp. 239–261. https://doi.org/10.1007/698_2012_146.

Radović, T., Grujić, S., Petković, A., Dimkić, M., Laušević, M., 2015. Determination of pharmaceuticals and pesticides in river sediments and corresponding surface and ground water in the Danube River and tributaries in Serbia. Environ. Monit. Assess. 187, 1–17. https://doi.org/10.1007/s10661-014-4092-z.

Santos, L.H.M.L.M., Ramalhosa, M.J., Ferreira, M., Delerue-Matos, C., 2016. Development of a modified acetonitrile-based extraction procedure followed by ultra-high performance liquid chromatography-tandem mass spectrometry for the analysis of psychiatric drugs in sediments. J. Chromatogr. A 1437, 37–48. https://doi.org/10.1016/j.chroma.2016.01.079.

Senta, I., Krizman, I., Ahel, M., Terzic, S., 2013. Integrated procedure for multiresidue analysis of dissolved and particulate drugs in municipal wastewater by liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 405, 3255–3268. https://doi.org/10.1007/s00216-013-6720-9.

United Nations Office on Drugs and Crime, (UNODC, 2018), 2018. Annual Report: Covering Activities During 2018. Available at: . https://www.unodc.org/documents/AnnualReport/Annual-Report_2018.pdf.

Vazquez-Roig, P., Andreu, V., Blasco, C., Picó, Y., 2012. Risk assessment on the presence of pharmaceuticals in sediments, soils and waters of the Pego-Oliva Marshlands (Valencia, eastern Spain). Sci. Total Environ. 440, 24–32. https://doi.org/10.1016/j.scitotenv.2012.08.036.

Wilkinson, J.L., Hooda, P.S., Swinden, J., Barker, J., Barton, S., 2018. Spatial (bio)accumulation of pharmaceuticals, illicit drugs, plasticisers, perfluorinated compounds and metabolites in river sediment, aquatic plants and benthic organisms. Environ. Pollut. 234, 864–875. https://doi.org/10.1016/j.envpol.2017.11.090.

Yadav, M.K., Short, M.D., Aryal, R., Gerber, C., van den Akker, B., Saint, C.P., 2017. Occurrence of illicit drugs in water and wastewater and their removal during wastewater treatment. Water Res. 124, 713–727. https://doi.org/10.1016/j.watres.2017.07.068.

Supplementary Material

Drugs of abuse and their metabolites in river sediments: Analysis, occurrence in four Spanish river basins and environmental risk assessment

Ester López-García[1], Nicola Mastroianni[1], Núria Ponsà-Borau[2], Damià Barceló[1,3], Cristina Postigo[1*], Miren López de Alda[1*]

[1]Water, Environmental, and Food Chemistry Unit (ENFOCHEM), Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain
[2]Universitat Oberta de Catalunya, Barcelona, Spain
[3]Catalan Institute for Water Research, H2O Buiding, Scientific and Technological Park of the University of Grona, Emili Grahit 101, 17003, Girona, Spain

*__Corresponding Authors__: Cristina Postigo; Miren López de Alda
Institute of Environmental Assessment and Water Research (IDAEA-CSIC)
Department of Environmental Chemistry
C/ Jordi Girona 18-26, 08034 Barcelona, Spain.
cprqam@cid.csic.es; mlaqam@cid.csic.es
Tel: +34-934-006-134, Fax: +34-932-045-904

List of Tables

Table S1. CAS number, main physical-chemical properties, and predicted no-effect concentration (PNEC) of the target analytes in water and sediments.

Compound	CAS number	Molecular formula	Molecular weight	Log K_{ow} [a]	Log K_{oc} [a]	$PNEC_{water}$ (µg/L) [b]	$PNEC_{sed}$ (ng/g) [b]
Cocaine (COC)	50-36-2	$C_{17}H_{21}NO_4$	303.35	2.30	3.28	2.28 [c]	3.65 [c]
Benzoylecgonine (BE)	519-09-5	$C_{16}H_{19}NO_4$	289.33	-1.32*	2.55	2.33	3.73
Cocaethylene (CE)	529-38-4	$C_{18}H_{23}NO_4$	317.38	2.66*	3.54	1.55	2.48
Amphetamine (AM)	300-62-9	$C_9H_{13}N$	135.21	1.76	3.05	24.80	39.66
Methamphetamine (MA)	537-46-2	$C_{10}H_{15}N$	149.23	2.07	3.21	9.74	15.57
3,4-methylenedioxymethamphetamine (MDMA)	537-46-2	$C_{11}H_{15}NO_2$	193.24	2.28	2.70	47.60	76.11
Ephedrine (EPH)	299-42-3	$C_{10}H_{15}NO$	165.23	1.13	1.92	69.90	111.77
Morphine (MOR)	57-27-2	$C_{17}H_{19}NO_3$	285.34	0.89	3.47	5.38	8.60
6-acetylmorphine (6ACM)	2784-73-8	$C_{19}H_{21}NO_4$	327.37	1.55	4.42	3.33	5.32
Heroin (HER)	561-27-3	$C_{21}H_{23}NO_5$	369.41	1.58	3.86	0.53	0.85
Methadone (METH)	76-99-3	$C_{21}H_{27}NO$	309.45	3.93	4.86	0.84	1.34
2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	30223-73-5	$C_{20}H_{23}N$	277.40	4.94*	5.67	0.14	0.22
Lysergic acid diethylamide (LSD)	50-37-3	$C_{20}H_{25}N_3O$	323.43	2.95	5.38	0.39	0.62
2-oxo-3-hydroxy-LSD (OH-LSD)	111295-09-1	$C_{20}H_{25}N_3O_3$	355.43	0.39*	2.68	-	-
Δ^9-tetrahydrocannabinol (THC)	1972-08-3	$C_{21}H_{30}O_2$	314.46	6.97	5.79	0.07	0.12
11-hydroxy-Δ^9-tetrahydrocannabinol (OH-THC)	36557-05-8	$C_{21}H_{30}O_3$	330.46	5.33	4.55	0.28	0.45
Cannabidiol (CBD)	74219-29-7	$C_{21}H_{30}O_2$	314.46	8.01*	6.44	0.17	0.27
Cannabinol (CBN)	521-35-7	$C_{21}H_{26}O_2$	310.43	7.23*	5.79	0.08	0.13
Alprazolam (ALP)	28981-97-7	$C_{17}H_{13}ClN_4$	308.77	2.12	6.33	0.08	0.12
Diazepam (DIA)	439-14-5	$C_{16}H_{13}ClN_2O$	284.74	2.82	4.05	0.29	0.46

[a] Data were obtained from the ChemSpider database. Predicted data were generated using the US Environmental Protection Agency`s EPISuite™ (*Estimated)

[b] Data were obtained from NORMAN Ecotoxicology DataBase

[c] $PNEC_{water}$ obtained from Mendoza et al. (2014) and $PNEC_{sed}$ by applying the following equation: $PNEC_{sed} = PNEC_{water} * 2.6 * (0.615 + 0.019 * K_{oc})$

Table S2. Total organic carbon (TOC, % C) content of the sediment samples collected during the 2011 sampling campaign.

	Main River	TOC (% C)	Tributary River	TOC (% C)	Tributary River	TOC (% C)
Llobregat basin	LLO1	1.33	CAR1	2.1		
	LLO2	2.24	CAR2	2.81		
	LLO3	1.25	CAR3	1.31		
	LLO4	2.03	CAR4	1.82		
	LLO5	0.56	ANO1	1.06		
	LLO6	0.65	ANO2	4.79		
	LLO7	2.14	ANO3	1.23		
Ebro basin	EBRO1	2.78	OCA	1.98	ESE	0.34
	EBRO2	2.58	ZAD	5.22	CIN1	0.84
	EBRO3	3.95	NAJ	2.85	CIN2	1.83
	EBRO4	1.35	ARG	1.14	RS	2.98
	EBRO5	2.27	GAL1	0.42	SEG	4.86
	EBRO6	3.77	GAL2	2.53	MAT	2.42
	EBRO7	3.05	HUE	1.23	ALG	0.56
	EBRO9	0.71	MAR	2.82		
Jucar basin	JUC1	1.20	CAB1	3.83		
	JUC2	0.65	CAB2	1.89		
	JUC3	1.99	CAB3	1.41		
	JUC4	0.96	CAB4	1.95		
	JUC5	3.43	CAB5	0.85		
	JUC6	0.51	MAG1	2.94		
	JUC7	2.43	MAG2	1.92		
	JUC8	2.55				
Guadalquivir basin	GUA1	0.79	BOR	2.19	GUA-A	1.07
	GUA2	0.68	GUA-M	0.63	GUA-R	1.87
	GUA3	0.69	MAG	0.77		
	GUA4	1.20	GUA-N	0.63		
	GUA5	0.98	YEG	3.43		
	GUA6	0.67	GUA-L	0.84		
	GUA7	0.39	PIC	0.50		
	GUA8	1.14	BEM	0.41		
	GUA9	0.88	CAC	1.00		
			GEN1	0.68		
			GEN2	1.52		
			COR	0.59		
			HER	1.07		

Table S3a. Predicted median concentration (ng/g d.w.) in 2010 and 2011 obtained with the Quantile Regression Models (Median Regression Models) in the Ebro and the Llobregat River basins. Difference between 2011 and 2010 predicted medians and its 95% confidence interval.

Compounds	Year	Ebro basin				Llobregat basin			
		Predicted median conc. (ng/g d.w.)	95%Ci[a]	Δ median (ng/g d.w.)	95%Ci[a]	Predicted median conc. (ng/g d.w.)	95%Ci[a]	Δ median (ng/g d.w.)	95%Ci[a]
Cocaine	2010	0.3	(0.19;0.42)	**(-0.25*)**	(-0.41;0.09)	0.22	(0.09;0.36)	(-0.01)	(-0.20;0.19)
	2011	0.05	(-0.54;0.16)			0.22	(0.08;0.35)		
MDMA	2010	0.08	(0.03;0.13)	(-0.06)		0.03	(-0.03;0.09)	(-0.02)	
	2011	0.02	(-0.03;0.06)			0.01	(-0.04;0.07)		
METH	2010	0.06	(0.00;0.12)	0		0.11	(0.04;0.17)	(-0.05)	(-0.14;0.49)
	2011	0.06	(0.01;0.11)			0.06	(-0.01;0.13)		
EDDP	2010	0.03	(-0.13;0.18)	0.06	(-0.15;0.28)	0.15	(-0.04;0.33)	0.06	(-0.20;0.32)
	2011	0.09	(-0.05;0.23)			0.21	(0.26;0.39)		
Sum	2010	0.41	(-0.03;0.85)	(-0.10)	(-0.70;0.50)	0.32	(-0.19;0.83)	0.27	(-0.46;1.00)
	2011	0.31	(-0.09;0.71)			0.59	(0.08;1.10)		
Sum No Cannabinoids	2010	0.39	(0.03;0.76)	(-0.08)	(-0.58;0.42)	0.32	(-0.11;0.74)	0.27	(-0.34;0.88)
	2011	0.31	(-0.02;0.64)			0.59	(0.16;1.01)		

*Statistically significant difference between predicted median in 2011 and 2010 concentrations (p-value ≤ 0.05)

[a] 95% Confidence Interval

Table S3b. Predicted median concentration (ng/g d.w.) in 2010 and 2011 obtained with the Quantile Regression Models (Median Regression Models) in the Jucar and the Guadalquivir River basins. Difference between 2011 and 2010 predicted medians and its 95% confidence interval.

Compounds	Year	Jucar basin				Guadalquivir basin			
		Predicted median conc. (ng/g d.w.)	95%Ci[a]	Δ median (ng/g d.w.)	95%Cia	Predicted median conc. (ng/g d.w.)	95%Ci[a]	Δ median (ng/g d.w.)	95%Ci[a]
Cocaine	2010	0.24	(0.11;0.37)	(-0.13)	(-0.32;0.05)	0.31	(0.20;0.41)	**(0.32*)**	(0.17;0.47)
	2011	0.11	(-0.03;0.24)			0.63	(0.52;0.74)		
MDMA	2010	0.01	(-0.04;0.07)	0		0.01	(-0.03;0.06)	0	
	2011	0.01	(-0.04;0.07)			0.01	(-0.03;0.06)		
METH	2010	0.20	(0.13;0.26)	**(-0.14*)**	(-0.23;-0.04)	0.20	(0.15;0.25)	**(-0.14*)**	(-0.21;0.06)
	2011	0.06	(-0.01;0.13)			0.06	(0.01;0.11)		
EDDP	2010	0.03	(-0.15;0.20)	0		0.03	(-0.12;0.17)	0	
	2011	0.03	(-0.15;0.20)			0.03	(-0.12;0.17)		
Sum	2010	1.01	(0.46;1.56)	(-0.49)	(-1.23;0.26)	0.56	(0.16;0.95)	0.13	(-0.44;0.69)
	2011	0.53	(0.03;1.02)			0.68	(0.28;1.08)		
Sum No Cannabinoids	2010	0.47	(0.06;0.89)	0.05	(-0.54;0.64)	0.56	(0.23;0.88)	0.13	(-0.34;0.59)
	2011	0.53	(0.11;0.94)			0.68	(0.35;1.01)		

*Statistically significant difference between predicted median in 2011 and 2010 concentrations (p-value ≤ 0.05)

[a] 95% Confidence Interval

Table S4. Concentrations of cocaine, MDMA, diazepam, methadone, and EDDP in the sampling stations where they were positively identified in both the water and sediment compartments and experimental K_D obtained.

			Cocaine (n=65)			MDMA (n=11)			Diazepam (n=6)			Methadone (n=58)			EDDP (n=34)		
			Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)
Llobregat basin	2010	LLO3	24	171	7.2												
		LLO4	6.8	252	37							1.7	164	96			
		LLO5	4.2	223	53												
		LLO7	5.6	353	63				26	249	9.7	3.8	472	126	13	1180	94
		CAR1										0.57	276	483			
		CAR2	0.89	380	426							0.46	194	422			
		CAR4	0.81	374	461							2.3	510	221	8.0	1235	154
		ANO1	2.8	170	60												
		ANO2	1.6	474	298	7.6	133	18				9.6	1100	114	14	3835	276
		ANO3	9.8	170	17												
	2011	LLO4										1.1	191	176	3.1	680	219
		LLO5	2.2	244	110												
		LLO6	2.7	216	80												
		LLO7	3.6	540	151				13	1320	99	15	945	65	34	3715	109
		CAR3													1.8	635	359
		CAR4	1.9	330	178	4.3	174	40				3.1	885	286	7.9	3345	422
		ANO1	1.2	403	342												
		ANO2	7.1	498	70	46	830	18	7.6	274	36	20	5700	285	50	15800	319
		ANO3				12	93	8				5.6	333	59	17	2535	148
Ebro basin	2010	EBRO4	14	310	22												
		EBRO6	3.5	975	280							0.92	1260	1370	3.4	4260	1249
		EBRO9	0.73	333	455												

Table S4. (continued)

			Cocaine (n=65)			MDMA (n=11)			Diazepam (n=6)			Methadone (n=58)			EDDP (n=34)		
			Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)
	2010	ZAD				2.7	306	113	3.1	243		11	3740	346	45	9450	211
		NAJ	2.5	173	70							0.67	162	241			
		ARG				3.1	245	78				4.9	1370	281	14	2760	200
		HUE	34	1040	30	3.0	87	29				2.0	700	345	9.9	5850	593
		MAR	2.8	321	114	0.84	76	90									
		CIN1										2.0	391	193			
		RS	3.4	276	80												
	2011	EBRO2	1.1	281	260	0.27	111	419									
Ebro basin		EBRO3										0.37	338	915	0.62	1375	2236
		EBRO4	1.3	405	307							0.59	171	291			
		EBRO5	3.5	391	113												
		EBRO6	4.6	243	52							0.89	685	771	1.3	2460	1937
		EBRO7	9.7	166	17							2.9	1666	57	0.47	795	1688
		ZAD	6.7	437	65	5.1	432	84				4.8	2685	561	14	7800	542
		ARG										5.0	945	188	13	2430	188
		GAL2										1.0	302	299	1.6	865	554
		HUE	25	444	17	14	188	13				4.5	650	145	14	6800	482
		SEG	1.9	363	196							1.7	1305	759	6.0	3615	599
	2010	JUC1										2.4	197	81			
Jucar basin		JUC2	2.2	276	125							1.8	198	110	1.4	491	343
		JUC3	2.8	381	137							2.2	925	420	5.8	755	131
		JUC5	1.9	227	117							0.77	174	224			
		JUC6	2.5	234	92							1.2	167	136			

Table S4. (continued)

			Cocaine (n=65)			MDMA (n=11)			Diazepam (n=6)			Methadone (n=58)			EDDP (n=34)		
			Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)
Jucar basin	2010	JUC7	3.4	225								1.1	289	255			
		JUC8	8.1	316								1.2	298	244	2.6	605	237
		CAB1										1.0	277	279			
		CAB2	1.8	268								0.41	207	499			
		MAG1	3.4	307					1.9	197		2.1	1075	524	4.8	675	140
	2011	JUC3	3.8	287								1.3	486	379	6.3	1915	306
		JUC6	2.4	525													
		JUC8	1.8	525											2.2	905	404
		CAB1	2.9	4560													
		CAB2	0.54	595													
		CAB4	2.4	346													
		MAG1							2.3	131		1.2	555	474	2.7	645	237
		MAG2	1.5	399													
Guadalquivir basin	2010	GUA4										0.79	236	300	2.4	625	256
		GUA6	4.6	307								1.0	381	369			
		GUA7										0.45	186	415			
		GUA8										0.42	235	554			
		BOR	1.5	790								0.31	165	532			
		GUA-N	2.3	500								1.1	305	274	2.5	720	286
		YEG										0.24	198	818			
		GUA-L										0.38	251	668			

Table S4. (continued)

			Cocaine (n=65)			MDMA (n=11)			Diazepam (n=6)			Methadone (n=58)			EDDP (n=34)		
			Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)	Water (ng/L)	Sed. (ng/kg d.w.)	K_D (L/kg)
Guadalquivir basin	2010	GEN1	9.9	535								1.4	185	128	4.5	1050	230
		GEN2	2.9	269								0.69	249	359			
		HER										0.43	165	379			
		GUA-A										14	1660	119	34	5800	172
		GUA-R										0.43	193	446			
	2011	GUA1	1.0	630													
		GUA2	3.9	1100													
		GUA4	3.0	4695								2.1	32600	15673			
		GUA5	2.4	2215													
		GUA6	3.7	750													
		GUA7	3.6	4990													
		GUA8	1.3	1020								0.65	620	957			
		GUA9	0.94	680													
		BOR	1.2	600													
		GUA-N	7.0	700								2.8	200	72			
		GUA-L	1.1	510													
		GEN1	15	424								2.9	368	126	5.5	960	175
		COR	0.81	322								0.50	236	468			
		HER	5.5	760								0.98	194	198			
		GUA-A	3.1	1305								13	3910	313	16	9650	603
Average all basins					**281**			**88**			**64**			**619**			**474**

Table S5. Hazard Quotient (HQ) values calculated for each compound at each sampling point during the 2010 sampling campaign.

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	*HQ (max)*	*∑HQ*
Llobregat basin (main river)	LLO1	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	LLO2	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	LLO3	<0.1	0	0	0	0	0	0	0	0	333	0	98	***333***	***431***
	LLO4	<0.1	0	0	0	0	0	0	0.1	0	0	0	0	*0.1*	*0.2*
	LLO5	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	LLO6	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	LLO7	<0.1	0	0	0	0	0	0.5	0.4	5.4	53	0	0	***53***	***60***
Ebro basin (main river	EBRO1	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	EBRO2	<0.1	0	0	<0.1	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	EBRO3	<0.1	0	0	0	0	0	0	<0.1	0.7	0	0	0	*0.7*	*0.9*
	EBRO4	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	EBRO5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EBRO6	0.3	0	0	0	0	0	0	0.9	19	0	0	0	***19***	***21***
	EBRO7	0.2	0	0	0	0	0	0	0.2	0	0	0	0	*0.2*	*0.4*
	EBRO8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EBRO9	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
Jucar basin (main river)	JUC1	0	0	0	0	0	0	0	0.1	0	0	0	0	*0.1*	*0.1*
	JUC2	<0.1	0	0	0	0	0	0	0.1	2.2	0	0	0	***2.2***	***2.5***
	JUC3	0.1	0	0	0	0	0	0	0.7	3.4	0	0	0	***3.4***	***4.2***
	JUC4	0.3	0	0	0	0	0	0	0.1	0	0	0	0	*0.3*	*0.5*
	JUC5	<0.1	0	0	0	0	0	0	0.1	0	0	0	0	*0.1*	*0.2*
	JUC6	<0.1	0	0	0	0	0	0	0.1	0	36	0	0	***36***	***36***
	JUC7	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	*0.2*	*0.3*
	JUC8	<0.1	0	0	0	0	0	0	0.2	2.8	0	0	0	***2.8***	***3.1***

Table S5. (continued)

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	*HQ (max)*	*∑HQ*
Guadalquivir basin (main river)	GUA1	0.1	0	0	0	0	0	0	0	0	0	0	0	*0.1*	*0.1*
	GUA2	0.1	0	0	0	0	0	0	0	0	0	0	0	*0.1*	*0.1*
	GUA3	0.1	0	0	0	0	0	0	0	0	0	0	0	*0.1*	*0.1*
	GUA4	<0.1	0	0	0	0	0	0	0.2	2.8	0	0	0	***2.8***	***3.1***
	GUA5	0.2	0	0	0	0	0	0	0	0	0	0	0	*0.2*	*0.2*
	GUA6	<0.1	0	0	0	0	0	0	0.3	0	0	0	0	*0.3*	*0.4*
	GUA7	0.1	0	0	0	0	0	0	0.1	0	0	0	0	*0.1*	*0.2*
	GUA8	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	*0.2*	*0.3*
	GUA9	<0.1	0	0	0	0	0	0	0.0	0	0	0	0	*<0.1*	*<0.1*
Llobregat basin (tributaries)	CAR1	0	0	0	0	0	0	0	0.2	0	0	0	0	*0.2*	*0.2*
	CAR2	0.1	0	0	0	0	0	0.3	0.1	0	0	0	0	*0.3*	*0.5*
	CAR3	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	CAR4	0.1	0	0	<0.1	0	0	0	0.4	5.6	0	0	0	***5.6***	***6.1***
	ANO1	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	ANO2	0.1	0	0	<0.1	0	0	0	0.8	17.4	57	0	0	***57***	***75***
	ANO3	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
Ebro basin (tributaries)	OCA	<0.1	0	<0.1	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	ZAD	0.1	0	0	<0.1	0	0	0.5	2.8	43	324	55	338	***338***	***762***
	NAJ	<0.1	0	0	0	0	0	0	0.1	0	0	0	0	*0.1*	*0.2*
	ARG	0.1	0	0	<0.1	0	0	0	1.0	13	0	0	0	***13***	***14***
	GAL1	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	GAL2	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	HUE	0.3	0	0	<0.1	0	0	0	0.5	27	55	0	0	***55***	***83***
	MAR	<0.1	0	0	<0.1	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*

Table S5. (continued)

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	*HQ (max)*	*∑HQ*
	ESE	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	CIN1	0	0	0	0	0	0	0	0.3	0.0	40	0	0	***40***	***40***
	CIN2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	RS	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	SEG	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MAT	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ALG	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
Jucar basin (tributaries)	CAB1	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	*0.2*	*0.3*
	CAB2	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	*0.2*	*0.2*
	CAB3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CAB4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CAB5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MAG1	<0.1	0	0	0	0	0	0.4	0.8	3.1	0	0	0	***3.1***	***4.4***
	MAG2	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
Guadalquivir basin (tributaries)	BOR	0.2	0	0	0	0	0	0	0.1	0	0	0	0	*0.2*	*0.3*
	GUA-M	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	MAG	<0.1	0	0	0	0	0	0	0.3	0	0	0	0	*0.3*	*0.4*
	GUA-N	0.1	0	0	0	0	0	0	0.2	3.3	0	0	0	***3.3***	***3.6***
	YEG	<0.1	0	0	0	0	0	0	0.1	0	0	0	0	*0.2*	*0.2*
	GUA-L	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	*0.2*	*0.3*
	PIC	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	BEM	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	*0.2*	*0.3*
	CAC	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	*0.2*	*0.3*
	GEN1	0.1	0	0	0	0	0	0	0.1	4.8	0	0	0	***4.8***	***5.1***

Table S5. (continued)

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	*HQ (max)*	*∑HQ*
	GEN2	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	*0.2*	*0.3*
	COR	<0.1	0	0	0	0	0	0	0.3	0	0	0	0	*0.3*	*0.3*
	HER	<0.1	0	0	0	0	0	0	0.1	0.9	0	0	0	*0.9*	***1.1***
	GUA-A	<0.1	0	0	0	0	0	0	1.2	26	0	0	0	*26*	*28*
	GUA-R	<0.1	0	0	0	0	0	0	0.1	0	0	0		*0.1*	*0.2*
HQ (max)		*0.3*	*0*	*0*	*0*	*0*	*0*	*0.5*	***2.8***	*43*	*333*	*55*	*338*		

∑HQ values between 1 and 10 are indicated in bold, and ∑HQ>10 in red. - Sampling stations where sediments could not be collected

Table S6. Hazard Quotients (HQ) values calculated for each compound at each sampling point during the 2011 sampling campaign.

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	*HQ (max)*	*∑HQ*
Llobregat basin (main river)	LLO1	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	LLO2	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	LLO3	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	LLO4	0	0	0	0	0	0	0	0.1	3.1	0	0	0	***3.1***	***3.2***
	LLO5	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	LLO6	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	LLO7	0.1	0.2	<0.1	0	0	0	2.8	0.7	17	127	0	0	***127***	***148***
Ebro basin (main river)	EBRO1	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	EBRO2	<0.1	0	0	<0.1	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
	EBRO3	0	0	0	0	0	0	0	0.3	6.3	0	0	0	***6.3***	***6.5***
	EBRO4	0.1	0	0	0	0	0	0	0.1	0	0	0	0	*0.1*	*0.2*
	EBRO5	0.1	0	0	0	<0.1	0	0	0	0	0	0	0	*0.1*	*0.1*
	EBRO6	<0.1	0	0	0	0	0	0	0.5	11	0	0	0	***11***	***12***
	EBRO7	<0.1	0	0	0	0	0	0	0.1	3.6	0	0	0	***3.6***	***3.8***
	EBRO8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EBRO9	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
Jucar basin (main river)	JUC1	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	JUC2	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	JUC3	<0.1	0	0	0	0	0	0	0.4	8.7	0	0	0	***8.7***	***9.1***
	JUC4	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	JUC5	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	JUC6	0.1	0	0	0	0	0	0	0	0	0	0	0	*0.1*	*0.1*
	JUC7	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*

Table S6. (continued)

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	*HQ (max)*	*∑HQ*
	JUC8	0.1	<0.1	0	0	0	0	0	0	4.1	0	0	0	***4.1***	***4.3***
Guadalquivir basin (main river)	GUA1	0.2	0	0	0	0	0	0	0	0	0	0	0	*0.2*	*0.2*
	GUA2	0.3	0	0	0	<0.1	0	0	0	0	0	0	0	*0.3*	*0.3*
	GUA3	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	GUA4	1.3	0	0	0	<0.1	0	0	24	0	0	0	0	***24***	***26***
	GUA5	0.6	0	0	0	<0.1	0	0	0	0	0	0	0	*0.6*	*0.6*
	GUA6	0.2	0	0	0	0	0	0	0	0	0	0	0	*0.2*	*0.2*
	GUA7	1.4	0	0	0	0	0	0	0	0	0	0	0	***1.4***	***1.4***
	GUA8	0.3	0	0	0	0	0	0	0.5	0	0	0	0	*0.5*	*0.7*
	GUA9	0.2	0	0	0	0	0	0	0	0	0	0	0	*0.2*	*0.2*
Llobregat basin (tributaries)	CAR1	0.0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	CAR2	0.0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	CAR3	0.0	0	0	0	0	0	0	0	2.9	0	0	0	***2.9***	***2.9***
	CAR4	<0.1	0	0	<0.1	0	0	0	0.7	15	0	0	0	***15***	***16***
	ANO1	0.1	0	0	0	0	0	0	0	0	0	0	0	*0.1*	*0.1*
	ANO2	0.1	0	0	<0.1	0	0	0.6	4.3	72	0	0	0	***72***	***77***
	ANO3	0.2	0	0	<0.1	0	0	0	0.2	12	0	0	0	***12***	***12***
Ebro basin (tributaries)	OCA	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	ZAD	0.1	0	<0.1	<0.1	0	0	0	2.0	35	0	0	0	***35***	***38***
	NAJ	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	ARG	0	0	0	0	0	0	0	0.7	11	0	0	0	***11***	***12***
	GAL1	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	GAL2	0	0	0	0	0	0	0	0.2	3.9	0	0	0	***3.9***	***4.2***
	HUE	0.1	0	0	<0.1	0	0	0	0.5	31	0	0	0	***31***	***32***

Table S6. (continued)

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	*HQ (max)*	*∑HQ*
	MAR	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	ESE	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	CIN1	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	CIN2	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	RS	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	SEG	<0.1	0	0	0	0	0	0	1.0	16	0	0	0	***16***	***18***
	MAT	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	ALG	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
Jucar basin (tributaries)	CAB1	1.2	0.1	0	0	0	0	0	0	0	0	0	0	***1.2***	***1.4***
	CAB2	0.2	0	0	0	0	0	0	0	0	0	0	0	*0.2*	*0.2*
	CAB3	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	CAB4	<0.1	0	0	0	0	0	0	0.3	0	0	0	0	*0.3*	*0.3*
	CAB5	0	0	0	0	0	0	0	0.0	0	0	0	0	*<0.1*	*<0.1*
	MAG1	0	0	0	0	0	0	0.3	0.4	2.9	0	0	0	***2.9***	***3.6***
	MAG2	0.1	0	0	0	0	0	0	0	0	0	0	0	*0.1*	*0.1*
Guadalquivir basin (tributaries)	BOR	0.2	0	0	0	0	0	0	0	0	0	0	0	*0.2*	*0.2*
	GUA-M	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	MAG	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	GUA-N	0.2	0	0	0	0	0	0	0.1	0	0	0	0	*0.2*	*0.3*
	YEG	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	GUA-L	0.1	0	0	0	0	0	0	0	0	0	0	0	*0.1*	*0.1*
	PIC	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*
	BEM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CAC	0	0	0	0	0	0	0	0	0	0	0	0	*0*	*0*

Table S6. (continued)

		COC	BE	EPH	MDMA	MA	ALP	DIA	METH	EDDP	THC	CBD	CBN	*HQ (max)*	*∑HQ*
	GEN1	0.1	0	0	0	0	0	0	0.3	4.4	0	0	0	***4.4***	***4.8***
	GEN2	0.3	0	0	0	0	0	0	0	0	0	0	0	*0.3*	*0.3*
	COR	<0.1	0	0	0	0	0	0	0.2	0	0	0	0	*0.2*	*0.3*
	HER	0.2	0	0	0	0	0	0	0.1	0	0	0	0	*0.2*	*0.4*
	GUA-A	0.4	0	0	0	0	0	0	2.9	44	0	0	0	***44***	***47***
	GUA-R	<0.1	0	0	0	0	0	0	0	0	0	0	0	*<0.1*	*<0.1*
HQ (max)		*1.4*	*0.2*	*0*	*0*	*0*	*0*	*2.8*	***24***	***72***	***127***	*0*	*0*		

∑HQ values between 1 and 10 are indicated in bold, and ∑HQ>10 in red. - Sampling stations where sediments could not be collected

3.3. Análisis de drogas, psicofármacos y algunos de sus metabolitos en mejillones

La continua introducción de drogas y psicofármacos en el medio ambiente acuático puede dar lugar a su bioacumulación en los organismos acuáticos, como han demostrado varios estudios publicados en la literatura (Tabla 3, sección 1.3.1.2.). Por ello, disponer de metodologías analíticas capaces de determinar esta bioacumulación puede ser de gran utilidad, ya que, como se mostró en la sección 1.3.2., la exposición a concentraciones ambientales de algunos de estos compuestos en agua puede tener diferentes efectos toxicológicos en los organismos expuestos y, en el caso de organismos comestibles como los mejillones, representar también un peligro para la salud pública. Al igual que en el caso de las matrices ambientales anteriores, la etapa de extracción es crítica en el análisis de drogas y fármacos en biota. Con el fin de reducir el coste, el tiempo de preparación de la muestra, minimizar la cantidad de disolventes utilizados, y evitar el empleo de instrumentación especial, se evaluó el uso de QuEChERS para este fin, frente a otras técnicas de extracción comúnmente utilizadas para el análisis de muestras medioambientales sólidas (p.ej. la PLE) (Perestrelo y cols., 2019). Como organismo diana para estudiar la contaminación del medio marino y la bioacumulación de este tipo de compuestos, se seleccionó el mejillón, ya que al alimentarse por filtración tiene una alta capacidad para bioacumular contaminantes, razón por la cual se utiliza frecuentemente como bioindicador (Dodder y cols., 2014).

En este contexto, los objetivos de la publicación #5 fueron:

- Desarrollar una metodología analítica basada en extracción con QuEChERS y análisis mediante LC-MS/MS para determinar drogas, psicofármacos y algunos de sus metabolitos en mejillones.
- Validar la metodología en términos de linealidad, recuperación, repetibilidad, efectos de matriz, y sensibilidad.
- Aplicar la metodología a muestras de mejillones salvajes y a diferentes productos comerciales de mejillón.
- Comparar los resultados obtenidos con los niveles de drogas, psicofármacos y metabolitos reportados en mejillones en otros trabajos publicados en la literatura.

En el método desarrollado, 10 g de mejillón fresco se extraen con QuEChERS utilizando ACN como disolvente de extracción y una mezcla de 4 g de $MgSO_4$, 1g de NaCl, 1 g de citrato de sodio y 0,5 g de citrato de disodio sesquihidratado como sales de extracción, y el extracto obtenido se

purifica posteriormente mediante *d*-SPE utilizando una mezcla de 40 mg de PSA, 400 mg de C18 y 1.200 mg de $MgSO_4$.

Esta metodología analítica se validó en términos de linealidad, recuperación, repetibilidad, efectos de matriz y sensibilidad, con resultados satisfactorios en todos los casos, y su aplicación posterior al análisis de varias muestras de mejillones, mostró la presencia únicamente de dos compuestos, cafeína y sertralina, en una muestra de mejillones salvajes a concentraciones comparables a las obtenidas en otros estudios internacionales.

Publicación científica #5

"Psychoactive substances in mussels: Analysis and occurrence assessment"

por:

Ester López-García, Cristina Postigo, y Miren López de Alda

en

Marine Pollution Bulletin 146 (2019) 985-992

https://doi.org/10.1016/j.marpolbul.2019.07.042

Marine Pollution Bulletin 146 (2019) 985–992

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Psychoactive substances in mussels: Analysis and occurrence assessment

Ester López-García, Cristina Postigo*, Miren López de Alda

Water, Environmental and Food Chemistry (ENFOCHEM) Research Group, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain

ARTICLE INFO

Keywords:
Biota
Drugs of abuse
Antidepressants
Benzodiazepines
QuEChERS
Liquid chromatography-tandem mass spectrometry

ABSTRACT

This work presents an analytical methodology based on a "Quick, easy, cheap, effective, rugged, and safe" (QuEChERS) extraction and liquid chromatography-tandem mass spectrometry detection (LC-MS/MS) for the simultaneous determination of 35 psychoactive substances in fresh mussel samples. The compounds investigated include illicit drugs, new psychoactive substances, commonly prescribed psychiatric pharmaceuticals, and caffeine. The methodology was validated in terms of recovery (relative recoveries 77–118%), repeatability (relative standard deviation values < 20%), and sensitivity (limits of detection and quantification < 2 ng/g fresh weight (f.w.) and < 6.7 ng/g f.w., respectively, for most compounds). The method was applied to the analysis of 15 samples, covering both commercially available mussels purchased from local food markets and wild fresh mussels collected in the Northeast coast of Spain in the Mediterranean Sea. Only one sample corresponding to wild mussels was found to contain 2 of the target analytes, namely, sertraline (1.5 ng/g f.w.) and caffeine, (12.8 ng/g f.w.).

1. Introduction

Psychoactive substances are substances that act on the central nervous system. They include psychoactive pharmaceuticals legally used to treat mental disorders such as depression, anxiety or seizure disorders, among others, but also drugs of abuse illegally used to alter the state of mind, consciousness or both. Given the extensive use of these substances by the population as well as their incomplete removal in the wastewater treatment plants (WWTPs), these compounds and/or their metabolic byproducts are continuously released into the water cycle (Calisto and Esteves, 2009; Pal et al., 2013; Peng et al., 2016). Although levels found in the aquatic environment are in general low, concentrations discharged by wastewater treatment plants can reach the high μg/L range (Cunha et al., 2017; Yadav et al., 2017). Fabbri and Franzellitti (2016) have recently reviewed the concentrations of pharmaceuticals in seawater worldwide. Concentrations of analgesics in seawater were between 0.4 and 1952 ng/L, antidepressants were between 0.6 and 596 ng/L, antiepileptics were between 0.3 and 1400 ng/L, and antianxiety drugs were between 0.5 and 12.7 ng/L. Illicit drugs were also found in marine environments at concentrations in the range 2.4–9.7 ng/L.

The continuous introduction of these compounds into the aquatic ecosystems may result in their bioaccumulation in aquatic organisms (Ebele et al., 2017; Fabbri and Franzellitti, 2016; Gaw et al., 2014; Huerta et al., 2012; Mezzelani et al., 2018; Rodríguez-Mozaz et al., 2016). Mussels are able to bioaccumulate organic and inorganic pollutants dissolved in water or attached to sediments because they are benthic filter-feeders. They have been used as sentinel organisms for coastal pollution monitoring (Beyer et al., 2017). Several studies have already investigated the capacity of mussels to bioaccumulate psychoactive substances. For instance, several illicit drugs, stimulants, and antidepressants have been analyzed in mussels collected along the coast of California (167 chemicals) (Maruya et al., 2014a) and in a wastewater-impacted river from Ontario (Canada) (118 pharmaceuticals and personal care products) (de Solla et al., 2016). Among other compounds, the marine mussel samples investigated were found to contain the illicit drugs cocaine and amphetamine, and the antidepressant sertraline at concentrations up to 1.7, 20, and 5.5 ng/g dry weight (d.w.), respectively (Maruya et al., 2014b). Meanwhile, the freshwater mussels from Ontario also presented residues of cocaine (0.1–0.6 ng/g fresh weight (f.w)), amphetamine (2.3–8.3 ng/g f.w), and various antidepressants, including sertraline (6.1–77 ng/g f.w), fluoxetine (1.3–8.9 ng/g f.w), venlafaxine (5.1–25 ng/g f.w) and citalopram (6.9–37 ng/g f.w) (de Solla et al., 2016). The fate of fluoxetine in freshwater mussels downstream from a WWTP discharge and its potential toxic effects in these organisms were specifically investigated by

* Corresponding author at: Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Department of Environmental Chemistry, C/Jordi Girona 18-26, 08034 Barcelona, Spain.
E-mail address: cprqam@cid.csic.es (C. Postigo).

https://doi.org/10.1016/j.marpolbul.2019.07.042
Received 4 April 2019; Received in revised form 16 July 2019; Accepted 18 July 2019
Available online 31 July 2019
0025-326X/

Table 1
Method performance in terms of linearity, analyte recoveries (absolute and relative), repeatability (RSD), and sensitivity (limits of detection and quantification) in fresh mussel.

		Linearity		Absolute recovery $n = 3$		Relative recovery, (RSD[e]) n = 3		Sensitivity	
		Range (ng/mL)	r^2	25 ng/g f.w.	100 ng/g f.w.	25 ng/g f.w.	100 ng/g f.w.	LOD (ng/g f.w.)	LOQ (ng/g f.w.)
Cocaine compounds	Cocaine (COC)	0.25–500	0.9952	60	58	94 (1)	86 (1)	0.2	0.5
	Benzoylecgonine (BE)	0.25–500	0.9930	42	45	84 (5)	93 (3)	1.8	6.0
	Cocaethylene (CE)	0.25–500	0.9916	56	56	89 (9)	84 (2)	0.7	2.4
Amphetamine-type stimulants	Amphetamine (AM)	0.5–500	0.9958	32	39	100 (1)	102 (1)	0.5	1.7
	Methamphetamine (MA)	0.25–500	0.9982	44	51	111 (18)	99 (18)	0.2	0.5
	MDMA[a]	0.25–500	0.9978	49	52	97 (4)	99 (3)	0.3	1.0
Opioids	Morphine (MOR)	2.5–500	0.9908	16	30	114 (2)	115 (3)	1.5	5.0
	6-monoacetylmorphine (6ACM)	2.5–500	0.9956	18	32	86 (7)	80 (5)	5.0	15.0
	Methadone (METH)	0.05–500	0.9962	56	54	118 (5)	96 (6)	0.1	0.3
	EDDP[b]	0.25–500	0.9934	67	58	80 (4)	99 (4)	0.1	0.3
Hallucinogens	Ketamine (KET)	0.25–500	0.9962	51	52	92 (5)	96 (10)	0.1	0.3
	Lysergic acid diethylamide (LSD)	0.25–500	0.9952	43	41	90 (13)	94 (5)	0.2	0.8
Cannabinoids	Δ^9Tetrahydrocannabinol (THC)	2.5–500	0.9920	4	2	84 (12)	80 (14)	10.0	25.0
	11-hydroxy-Δ^9THC (OH-THC)	2.5–500	0.9908	–	28	–	115 (20)	10.0	30.0
	11-nor-9-carboxy-Δ^9THC (THC-COOH)	5–500	0.9930	–	7	–	84 (6)	10.0	30.0
New psychoactive compounds	AH-7921[c]	0.25–25	0.9916	47	47	95 (4)	95 (4)	0.3	1.0
	Mephedrone (MEPH)	0.25–500	0.9986	40	47	81 (5)	90 (2)	0.3	1.0
	MDPV[d]	0.5–500	0.9928	54	55	84 (14)	90 (12)	0.3	1.0
Stimulants	Caffeine (CAF)	2.5–500	0.9924	41	45	99 (8)	80 (8)	1.2	3.9
	Ephedrine (EPH)	0.05–500	0.9920	35	42	95 (4)	94 (4)	0.1	0.4
Benzodiazepines	Alprazolam (ALPZ)	0.05–500	0.9916	58	55	87 (1)	89 (2)	0.5	1.6
	α-hydroxy-ALPZ (OH-ALPZ)	2.5–500	0.9904	81	73	81 (9)	83 (4)	1.0	3.0
	Midazolam (MIDZ)	0.25–500	0.9908	38	36	80 (7)	81 (5)	0.3	1.0
	α-hydroxy-MIDZ (OH-MIDZ)	0.25–500	0.9910	47	46	86 (2)	83 (5)	2.0	6.7
	Lormetazepam (LRMZ)	0.25–500	0.9938	–	82	–	93 (1)	0.1	0.3
	Diazepam (DIAZ)	0.25–500	0.9910	46	44	80 (3)	82 (7)	1.4	4.6
	Oxazepam (OXZ)	2.5–500	0.9922	40	39	110 (10)	83 (9)	1.2	3.9
	Temazepam (TEMZ)	0.5–500	0.9980	101	89	81 (2)	81 (1)	1.4	4.7
Antidepressants	Citalopram (CTLP)	0.5–500	0.9950	64	51	83 (5)	101 (9)	0.2	0.5
	Fluoxetine (FLX)	0.05–500	0.9970	45	42	80 (2)	86 (6)	0.1	0.4
	Sertraline (STR)	0.25–500	0.9924	18	18	100 (18)	105 (10)	0.1	0.3
	Venlafaxine (VFX)	0.05–500	0.9936	44	39	95 (13)	89 (8)	0.1	0.3
Hypnotic	Zolpidem (ZOPD)	0.05–500	0.9938	53	52	80 (7)	82 (2)	0.1	0.3
Antipsychotic	Chlorpromazine (CPMZ)	0.25–500	0.9984	33	30	82 (1)	85 (3)	0.1	0.5
Sedative	Hydroxyzine (HXZ)	0.05–500	0.9936	43	45	77 (7)	82 (2)	0.1	0.4

[a] MDMA: 3,4-methylenedioxy methamphetamine.
[b] EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
[c] AH-7921: 3,4-dichloro-N-[[1-(dimethylamino)cyclohexyl]methyl]benzamide.
[d] MDPV: 3,4-methylenedioxy pyrovalerone.
[e] RSD: relative standard deviation of triplicate analysis of fortified fresh mussel samples.

Bringolf et al. (2010). In this study, native freshwater mussels were found to contain up to 79 ng/g f.w of fluoxetine. The bioaccumulation of venlafaxine and different venlafaxine metabolites was investigated in mussels from the Southeast of France (Martínez Bueno et al., 2014). In this case, trace amounts of venlafaxine (up to 2.7 ng/g d.w.) and its metabolites (up to 3.8 ng/g d.w.) were measured. The occurrence of antidepressants in mussels has also been investigated in specimens collected at different locations in Europe: the Portuguese coast (Silva et al., 2017), the Ebro River Delta (Spain), the Tagus River Estuary (Portugal), and the Po River Delta (Italy) (Álvarez-Muñoz et al., 2015). Average fluoxetine and citalopram concentrations in marine mussels from the Portuguese coast were 4.83 ng/g d.w. and 3.26 ng/g d.w., respectively (Silva et al., 2017). Specimens collected from the Po River Delta contained 21 ng/g d.w. of citalopram and 36 ng/g d.w. of venlafaxine, whereas lower concentrations were observed in mussels collected from the Tagus River Estuary (7.7 ng/g d.w. of venlafaxine and no detectable levels of citalopram) and the Ebro River Delta (2.7 ng/g d.w. of venlafaxine and 1.9 ng/g d.w. of citalopram) (Álvarez-Muñoz et al., 2015).

Methodologies commonly used to determine psychoactive substances in mussels consist of a sample extraction and clean-up step followed by liquid chromatography-mass spectrometry (LC-MS) detection. Such approaches allow obtaining enough selectivity and sensitivity to detect the low concentrations at which these compounds can be present in these organisms. Pressurized liquid extraction (PLE) (Alvarez-Muñoz et al., 2015), solid-liquid extraction (Bayen et al., 2015) or ultrasonic assisted extraction (USE) (Dodder et al., 2014) followed by solid phase extraction (SPE) are the techniques of choice for extraction of the analytes and subsequent clean-up of the extract, respectively. However, some of them are time-consuming and labor-intensive (SLE, USE and SPE) and, in the case of PLE, expensive and complex instrumentation is needed. Alternatively, QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction represents a simple, attractive approach for mussel sample preparation. This method was initially developed for extraction of pesticides from vegetables and fruits (Anastassiades et al., 2003). However, in recent years, it has been also used for extraction of different chemicals from a variety of matrices. As far as psychoactive substances are concerned, QuEChERS

methods have been used for extraction of illicit drugs and psychiatric pharmaceuticals like benzodiazepines and antidepressants from human biological matrices, such as blood and urine (Alves et al., 2017; Anzillotti et al., 2014; Dulaurent et al., 2016). Still, target methodologies that apply QuEChERS to extract this type of compounds from environmental matrices, and in particular mussels, are scarce, and to the authors' knowledge, they cover only psychoactive pharmaceuticals, but not illicit drugs or the widely consumed stimulant caffeine (Martínez Bueno et al., 2013; Martínez Bueno et al., 2014; Núñez et al., 2015).

In this context, the aim of this work was to develop an analytical methodology based on QuEChERS extraction combined with LC-MS/MS detection for the simultaneous determination of 37 psychoactive substances (during method optimization the number of target compounds was reduced to 35 substances due to analytical restrictions in the case of heroin and lorazepam) in fresh mussel samples. The compounds investigated were selected on the basis of their extensive use by the population and their occurrence in the aquatic environment and include the most consumed illicit drugs, new psychoactive substances, and pharmaceuticals commonly prescribed such as benzodiazepines, stimulants, antidepressants, sedatives, hypnotics, and antipsychotics. The method developed was applied to the analysis of these substances in wild mussel samples and mussels commercially available at local food markets. To the author's knowledge, this is the first time that illicit drugs other than cocaine, benzoylecgonine, and amphetamine, new psychoactive substances and some psychoactive pharmaceuticals (α-hydroxy-alprazolam, midazolam, α-hydroxy-midazolam, ephedrine, temazepam, lormetazepam, oxazepam, hydroxyzine, chlorpromazine and zolpidem) have been investigated in mussel samples.

2. Material and methods

2.1. Chemicals

The list of target compounds considered for analysis is shown in Table 1. The illicit drugs investigated included cocaine (COC) and its metabolites benzoylecgonine (BE) and cocaethylene (CE); the amphetamine-type stimulants amphetamine (AM), methamphetamine (MA), and 3,4-methylenedioxymethamphetamine (MDMA or ecstasy); the opioids/opiates morphine (MOR), 6-monoacetylmorphine (6ACM), heroin (HER), methadone (METH) and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenyl pyrrolidine (EDDP); the hallucinogens ketamine (KET) and lysergic acid diethylamide (LSD); the cannabinoids Δ^9-tetrahydrocannabinol (THC) and its main metabolites 11-hydroxy-Δ^9-THC (OH-THC) and 11-nor-9-carboxy-Δ^9-THC (THC-COOH), and the new psychoactive substances 3,4-dichloro-N-[[1-(dimethylamino)cyclohexyl]methyl]benzamide (AH-7921), mephedrone (MEPH), and methylenedioxypyrovalerone (MDPV). The psychoactive pharmaceuticals selected were the stimulants caffeine (CAF) and ephedrine (EPH); the benzodiazepine-type anxiolytics alprazolam (ALPZ), its metabolite α-hydroxy-alprazolam (OH-ALPZ), midazolam (MIDZ), its metabolite α-hydroxy-midazolam (OH-MIDZ), lorazepam (LORZ), lormetazepam (LRMZ), diazepam (DIAZ), oxazepam (OXZ), and temazepam (TEMZ); the antidepressants citalopram (CTLP), fluoxetine (FLX), sertraline (STR), and venlafaxine (VFX); the hypnotic zolpidem (ZOPD); the antipsychotic chlorpromazine (CPMZ), and the sedative hydroxyzine (HXZ).

Standards of the target compounds and their isotopically labeled analogs (listed in Table S1) (purity > 97%, concentration of 0.1 or 1 mg/mL) were purchased from Cerilliant (Round Rock, TX, USA) as solutions in methanol (MeOH) or acetonitrile (ACN).

Standard mixtures were prepared in MeOH at different concentrations in the range 0.1 to 1000 ng/mL by appropriate dilution of individual stock solutions (0.1 mg/mL). All of them contained the isotopically labeled (surrogate) standard (SS) mixture at a concentration of 50 ng/mL in the case of BE-d_8, CAF-d_3, COC-d_3, CTLP-d_6, EDDP-d_3, EPH-d_3, LORZ-d_4, LRMZ-$^{13}Cd_3$, METH-d_3, MOR-d_3, OH-THC-d_3, OXZ-d_5, STR-d_3, THC-d_3, THC-COOH-d_3 and VFX-d_6, and 20 ng/mL in the case of the remaining surrogate compounds (see Table S1). Two solutions, one containing only the target compounds at a concentration of 1 μg/mL and another one containing only the SS at 1 μg/mL were prepared in MeOH for sample fortification during the validation study. The solution containing only the SS at 1 μg/mL was also used for sample analysis. All standard solutions were stored in the dark at −20 °C.

All solvents used were from J.T. Baker (Serviquimia, Barcelona) and were HPLC grade. Formic acid (> 98%) and ammonium formate (> 99%), used as mobile phase modifiers, were purchased from Merck and Fluka Analytical (Sigma Aldrich), respectively.

Magnesium sulfate ($MgSO_4$), sodium citrate (NaCitrate), sodium chloride (NaCl) and disodium citrate sesquihydrate (DCS), used as extraction salts, and primary and secondary amine (PSA), C18e and $MgSO_4$, used for clean-up, were obtained from Bekolut GmbH & Co (Hauptstuhl, Germany) as CITRATE-KIT-01 and PSA-KIT-04A, respectively.

2.2. Sample collection

Wild mussel samples (*Mytilus galloprovincialis*) were collected in the Catalan coast (NE Spain) at two different locations: Sant Feliu de Guixols and the Ebro River Delta (see Fig. S1). They were collected as grab samples and were transported to the laboratory in cold conditions (4 °C). Once in the laboratory, they were prepared in < 24 h following the procedure described in Section 2.3. Also, different types of mussels (*Mytilus galloprovincialis*) commercially available in local food markets, including fresh mussels, boiled mussels, and natural canned mussels, were analyzed. One of these types of mussels was also used in the validation study.

2.3. Sample extraction

Ten grams of homogeneous fresh composite mussel sample, prepared by blending various individuals with a mixer, was weighted into a 50 mL polypropylene (PP) centrifuge tube, spiked with the SS at a concentration of 25 ng/g in the case of BE-d_8, CAF-d_3, COC-d_3, CTLP-d_6, EDDP-d_3, EPH-d_3, LORZ-d_4, LRMZ-$^{13}Cd_3$, METH-d_3, MOR-d_3, OH-THC-d_3, OXZ-d_5, STR-d_3, THC-d_3, THC-COOH-d_3 and VFX-d_6 and 10 ng/g in the case of the remaining surrogate compounds, vortexed at 2500 rpm for 1 min and stored during 12 h at 4 °C to allow methanol evaporation and interaction of the compounds with the matrix. For extraction, 10 mL of ACN, used as the extraction solvent, was added to the centrifuge tube, manually shaken, and immediately afterwards the extraction salts (4 g $MgSO_4$, 1 g NaCl, 1 g NaCitrate and 0.5 g DCS) were also added. After vortex-mixing at 2500 rpm for 1 min and centrifugation at 3500 rpm for 5 min, the supernatant was transferred to a PP centrifuge tube containing the clean-up sorbents (40 mg of PSA, 400 mg of C18e and 1200 mg $MgSO_4$). The mixture was vortexed at 2500 rpm for 1 min and centrifuged at 3500 rpm for 5 min. Then the supernatant was transferred into a vial and stored at −20 °C for 4 h and centrifuged again at 3500 rpm for 5 min to precipitate the remaining salts. One mL of the resulting supernatant was transferred to a 1 mL-volumetric flask, evaporated to 0.5 mL with a PIERCE ReactiTherm III evaporator (Rockford, IL; USA), and made up to the initial 1 mL volume with water, so that the final extract consisted of a mixture of ACN-H_2O (1:1, v/v). The reconstituted extract was transferred to a vial and stored at −20 °C until analysis.

2.4. LC-MS/MS analysis

Extract analysis was performed with an HPLC Symbiosis™ Pico System (Spark Holland, Emmen, The Netherlands) connected in series with a 4000 QTRAP hybrid quadrupole-linear ion trap (QqLIT) mass

spectrometer (Applied Biosystem-Sciex, Foster City, CA, USA).

For LC, 5 μL of the extract was injected onto a Purospher Star RP-18 end-capped column (125 mm × 2.0 mm, 5 μm) preceded by a guard column of the same packing material, both from Merk (Darmstadt, Germany). Analyte separation was achieved by applying a linear organic gradient of a binary mobile phase (water with 20 mM of a buffer formic acid/ammonium formate (pH: 3.8) and ACN) at a constant flow rate of 0.3 mL/min. The organic composition of the mobile phase was: 5% at 0 min, 40% at 12 min, 70% at 18 min, 80% at 19 min, and 100% at 26 min. Pure organic conditions were maintained for 2 min (28 min), and initial composition of 5% was achieved in the next 2 min (30 min) and maintained for 10 min for column re-equilibration (40 min).

Ionization of the compounds was achieved with a Turbo Ion Spray source operating in the positive ionization mode (ESI+). Mass acquisition was done in the selected reaction monitoring (SRM) mode, recording two SRM transitions per analyte and one per SS. Optimum ESI-MS/MS conditions used for analysis of the target compounds are listed in Table S1.

2.5. Method validation

The methodology was validated in terms of linearity, sensitivity, recovery, repeatability, and matrix effects.

The linearity of the method was evaluated in the range 0.05–500 ng/mL constructing nine-point calibration curves by diluting the methanolic standard mixtures (see Section 2.1) with HPLC-grade water (1:1, v/v). Quantification was performed by the isotope dilution approach, comparing the analyte peak area of each compound with that corresponding to its surrogate standard, and weighted least-squared linear regression, using $1/x^2$ as the weighting factor, was used to build the calibration curves to minimize the influence of high concentrations in the linear model.

Recovery studies were performed with a fresh mussel sample fortified at two different levels (25 ng/g f.w. and 100 ng/g f.w.) in triplicate. Absolute recoveries were calculated by comparing the analyte peak areas obtained after the LC-MS/MS analysis of the fortified mussel samples vs. standard solutions at equivalent concentrations. Relative recoveries were calculated by comparing the absolute recoveries of each compound and its corresponding surrogate standard.

The repeatability of the method was also evaluated at 25 ng/g f.w. and 100 ng/g f.w by calculating the relative standard deviation (RSD) of the triplicate analysis of fortified fresh mussel samples.

The sensitivity of the method was assessed through the limits of detection (LOD) and quantification (LOQ) observed for each analyte. Average LODs and LOQs were experimentally estimated from the analysis in triplicate of fresh mussel samples fortified at low concentrations (5 ng/g f.w. or 10 ng/g f.w.) with the target analytes. The LODs and LOQs were calculated as the concentration of analyte giving a signal to noise ratio of 3 and 10, respectively.

Matrix effects were evaluated by comparing the peak areas obtained after LC-MS/MS analysis of a mussel sample fortified at the end of the QuEChERs extraction protocol (Section 2.3) (i.e., the final mussel extract obtained after the clean-up step was evaporated until dryness and it was reconstituted with the standard mixture at a concentration of 25 ng/mL) and a standard solution at equivalent concentration (25 ng/mL) according to the following equation: $ME\% = 100^{*} (A_{mussels}-A_{blank})/A_{standard})-100$. The sample used for method validation was free from all target compounds. Therefore, subtraction of A_{blank} (signal in the non-fortified sample) was unnecessary.

3. Results

3.1. Method performance

Fig. 1 shows extracted ion chromatograms of the target analytes after QuEChERS extraction and LC-MS/MS analysis of a fresh mussel sample fortified at 100 ng/g f.w. Table 1 summarizes the performance of the method for the analysis of the 35 selected psychoactive substances in mussels in terms of linearity, recovery, repeatability, and sensitivity.

The linearity of the method was satisfactory between the analyte LOQ and 500 ng/mL for all target compounds except AH-7921, for which the method provided a linear response only between its LOQ and 25 ng/mL. The coefficients of determination obtained for calibration curves, constructed with at least 6 data points, were higher than 0.99.

Analyte absolute recoveries, in good agreement at the two levels tested, expanded in most cases between 30% and 101%. Lower absolute recoveries (< 30%) were observed for morphine, 6-monoacetylmorphine, and highly apolar substances, i.e., the cannabinoids (Log K_{ow} 5.33–6.97) and sertraline (log K_{ow} 5.29). Despite this, relative recoveries for all compounds were satisfactory. They were in the range between 80 and 118% at both levels tested for all compounds, except for hydroxyzine (77% at the low level tested). This indicates that poor recoveries or potential losses during the extraction procedure as well as potential matrix effects are well compensated by the use of isotopically labeled analogs.

The method was also shown to provide very good repeatability for all compounds. RSD values of triplicate analyses of fortified fresh mussel samples were below 20% in all cases.

Regarding the sensitivity of the method, LODs were between 0.1 and 2.0 ng/g f.w. and LOQs were between 0.3 and 6.7 ng/g f.w. for all compounds, except for 6-monoacetylmorphine and the cannabinoids, that presented LODs and LOQs between 5 and 10 ng/g f.w. and between 15 and 30 ng/g f.w., respectively. The lower sensitivity observed for these compounds is due to some extent to their low absolute recovery and/or high matrix suppression ionization effects, and to some other extent, especially in the case of cannabinoids, to the low signal response provided by the instrumentation (ESI-MS/MS) under positive ionization. Sensitivity for these compounds could be improved under negative ionization, but this requires the performance of an additional analysis. Thus to save analytical resources (chemicals and materials used and analysis time), this option was discarded in detriment of a better method sensitivity for cannabinoids.

Fig. 2 shows how the matrix affects the analysis of the target compound. Negative values indicate ionization suppression of the MS signal and positive values indicate MS signal enhancement. As can be observed, 43% of the psychoactive compounds under study were not significantly affected by components of the matrix (matrix effects within ± 20%). However, when matrix effects were relevant, most compounds were affected by ionization suppression rather than enhancement. In this regard, the compounds that showed the highest signal suppression (percentages above 40%) were those for which the lowest absolute recoveries were obtained (morphine, the cannabinoids, and sertraline), and also fluoxetine, and chlorpromazine. Ionization enhancement above 40% due to matrix components was mainly observed for the benzodiazepines α-hydroxy-alprazolam, lormetazepam, and temazepam.

Lorazepam and heroin were also attempted to be analyzed with the present method. However, they could not be validated due to extremely poor recovery or, what is more likely, matrix interferences, that resulted in no signal in the case of the two SRM transitions of heroin and in the case of the confirmation SRM transition of lorazepam in fortified mussel samples.

3.2. Occurrence of psychoactive substances in mussel samples

The methodology developed was applied to the analysis of 15 mussel samples, 5 of them corresponding to wild mussels collected in nearby areas and 10 to commercial products purchased in local food markets. Two criteria were adopted for the positive identification of the

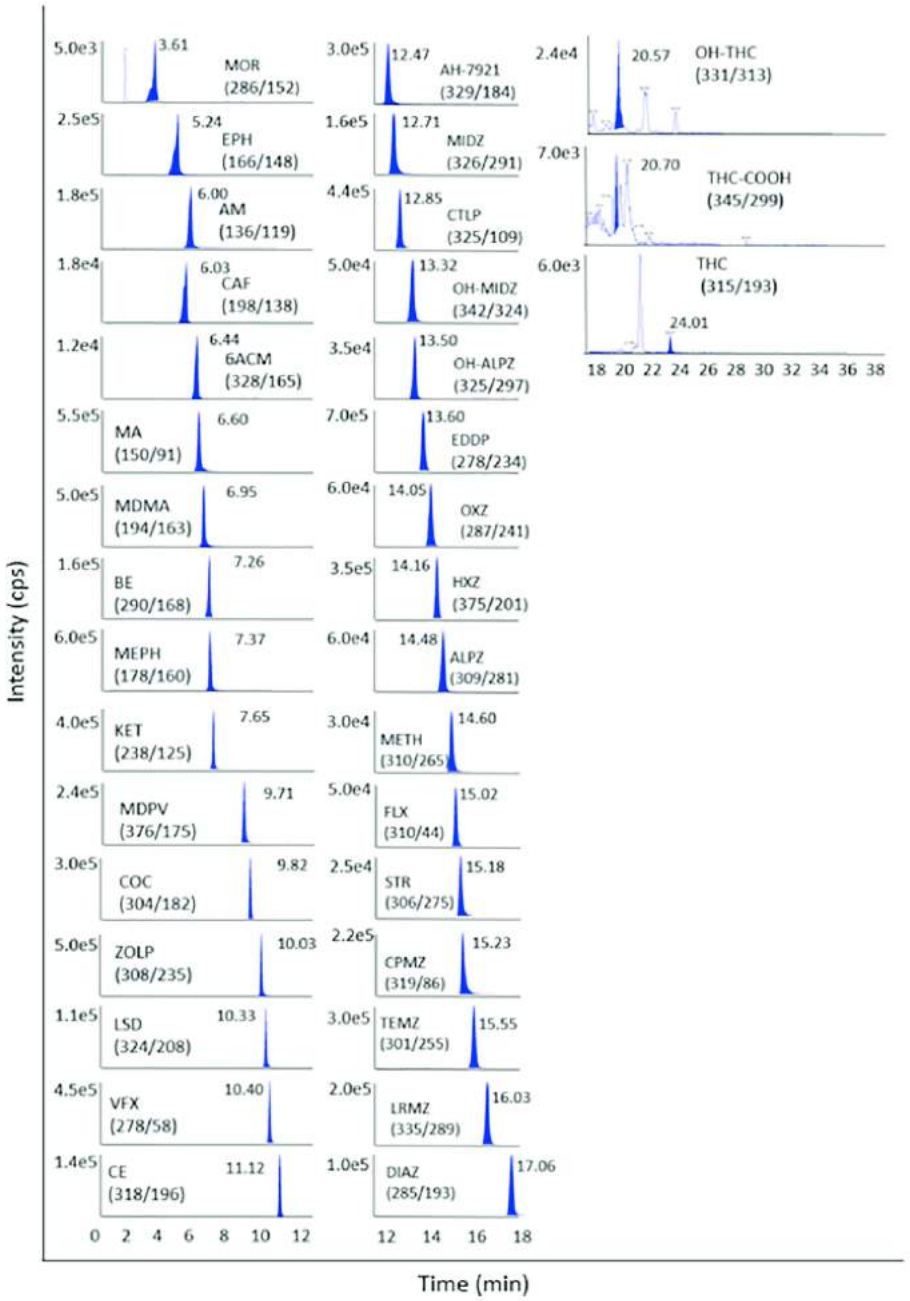


Fig. 1. Extracted ion chromatograms (XIC) after QuEChERS extraction and LC-MS/MS analysis of a mussel sample spiked with the target analytes at a concentration of 100 ng/g f.w.

analytes in the samples: i) the analyte SRM ratio between the quantification transition and the confirmation transition in the sample and in the calibration standards had to be similar (in this sense, when the average analyte SRM ratio in the standards was between 1–2, 2–5, and 5–10, a deviation of the SRM ratio observed in the sample within ± 20%, ± 25%, or ± 30%, respectively, was accepted), and, ii) the retention time of the analyte in the sample and in the standard solution had to be comparable (± 2%) (higher deviations were accepted when the surrogate standard was also affected) (see Table S1).

All commercial mussel samples analyzed were free of the target

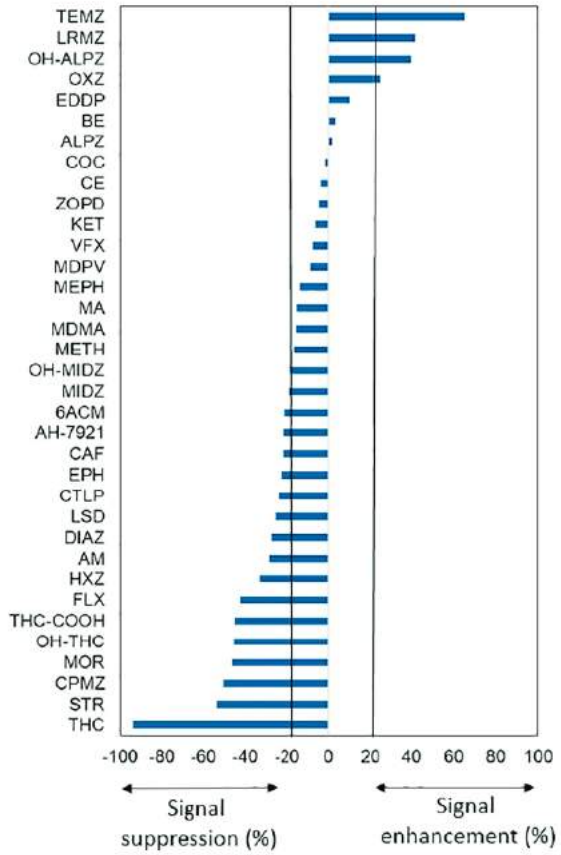


Fig. 2. Matrix effects (%) observed for QuEChERS extracted and LC-MS/MS analyzed psychoactive substances in fresh mussel samples fortified at 25 ng/g f.w. Vertical lines at −20% and 20% delimit the cases where matrix effects in the form of signal suppression and enhancement, respectively, are relevant.

psychoactive substances. However, in one of the wild mussel samples analyzed, two psychoactive substances, caffeine and sertraline, were found at concentrations of 12.8 and 1.5 ng/g f.w., respectively. Fig. 3 shows the extracted ion chromatogram for the quantification and the confirmation SRM transitions of sertraline and caffeine in the mussel sample where they were positively identified.

The caffeine concentration found in this study was similar to those found in California (USA) (14 ng/g d.w.) (Maruya et al., 2014b), Portugal (11.1 ng/g d.w.), and Italy (11.7 ng/g d.w.) (Álvarez-Muñoz et al., 2015), and higher than those found in Singapore (mean concentration 1.55 ng/g wet weight (w.w)) (Bayen et al., 2015) and San Francisco Bay, where analyzed mussels were free of caffeine (Klosterhaus et al., 2013). Despite its high polarity (Log Kow of −0.07), meaning low capacity of partition into lipids, the widespread presence of this compound in the aquatic environment (Dafouz et al., 2018) may result in its bioaccumulation in marine organisms. Regarding the presence of caffeine in mussels and the effects that this psychoactive substance can cause in this organism, a recent study demonstrated that the exposure of mussels to environmentally relevant concentrations of caffeine (between 50 and 500 ng/L) triggered the appearance of stress syndrome in this organism, although no toxic effects were induced (Capolupo et al., 2016). The only previous investigation of the presence of caffeine in marine water in the studied area ($n = 2$ samples collected at the Ebro River Delta and at the coast of Barcelona) reported concentrations of 8.4 and 37 ng/L (Nödler et al., 2014). However, considerably higher concentrations, up to 857 ng/L, have been measured in coastal waters in the NW of Spain, while maximum levels worldwide have reached 11 μg/L (in Australia) (Dafouz et al., 2018).

In the case of sertraline, the level found in this study was in agreement with the concentration of sertraline reported in mussels collected along the coast of California (USA) (1 ng/g f.w and 1.4 ng/g d.w. in San Francisco Bay) (Klosterhaus et al., 2013; Maruya et al., 2014b) and lower than those found in Grand River, in Ontario (Canada) (wild mussel: 29–76.9 ng/g f.w; caged mussel: 6.1–26.3 ng/g f.w) (de Solla et al., 2016), and in the coast of Victoria (Canada) close to a sewage outfall (< 1.07–84.1 ng/g d.w.) (Krogh et al., 2017). It is not surprising that sertraline can bioaccumulate in mussels because it has a log K_{ow} of 5.29 and presents a bioaccumulation factor (BAF) (measured in caged mussels) of 32,022 (de Solla et al., 2016). In spite of this, no toxic effects have been observed at environmental relevant concentrations of sertraline in peer-reviewed published studies (LC50 and EC50: 0.02–0.04 mg/L) (Gilroy et al., 2017).

4. Conclusions

An analytical methodology based on QuEChERS extraction combined with LC-ESI-MS/MS detection has been developed and validated for the determination of 35 psychoactive substances in mussels. The use of QuEChERS allows sample preparation in a very rapid, inexpensive and simple way reducing the time, the effort and the amount of solvents used compared with other methods traditionally used for the preparation of biota samples, such as Soxhlet extraction or ultrasonic solvent extraction. Other advantages are the no need for costly, specialized instrumentation (as for example in the case of pressurized liquid extraction (PLE) or microwave assisted extraction (MAE)), and limited evaporation of the extract (approximately 0.5 mL of ACN), without reaching dryness, which in other procedures represents a tedious and time consuming step that, in addition, can compromise the analysis of volatile compounds like amphetamine and methamphetamine (vapor pressure equal to 0.31 and 0.15 mmHg, respectively), thanks to the extraction of an initial large amount of sample (10 g). In terms of analytical performance, satisfactory results were obtained during validation for 35 out of the 37 initially considered compounds (all but heroin and lorazepam), allowing their detection in the low ng/g f.w. range. In addition, the use of isotopically labeled compounds corrects potential losses during the extraction process as well as matrix effects, that may vary among individuals.

The application of the method to commercial and wild mussels revealed the bioaccumulation of two psychoactive substances, caffeine and sertraline, in one wild fresh mussel sample collected in the Catalan coast. In view of the results obtained, the psychoactive substances present in the aquatic environment are able to bioaccumulate in aquatic organisms and, therefore, it is useful to have analytical methodologies that can determine this bioaccumulation, especially, because some works have already shown that the presence of psychoactive substances at environmental concentrations pose a risk for these organisms. For instance, the antidepressant fluoxetine induced endocrine disruption effects in mussels (reproduction fitness alterations due to alkali-labile phosphates downregulation in both sex-differentiated gonads) at concentrations of 75 ng/L (Gonzalez-Rey and Bebianno, 2013), and a mixture of illicit drugs formed by cocaine, benzoylecgonine, amphetamine, morphine, and MDMA at concentrations between 50 and 300 ng/L impaired the oxidative status of zebra mussels (Parolini et al., 2015).

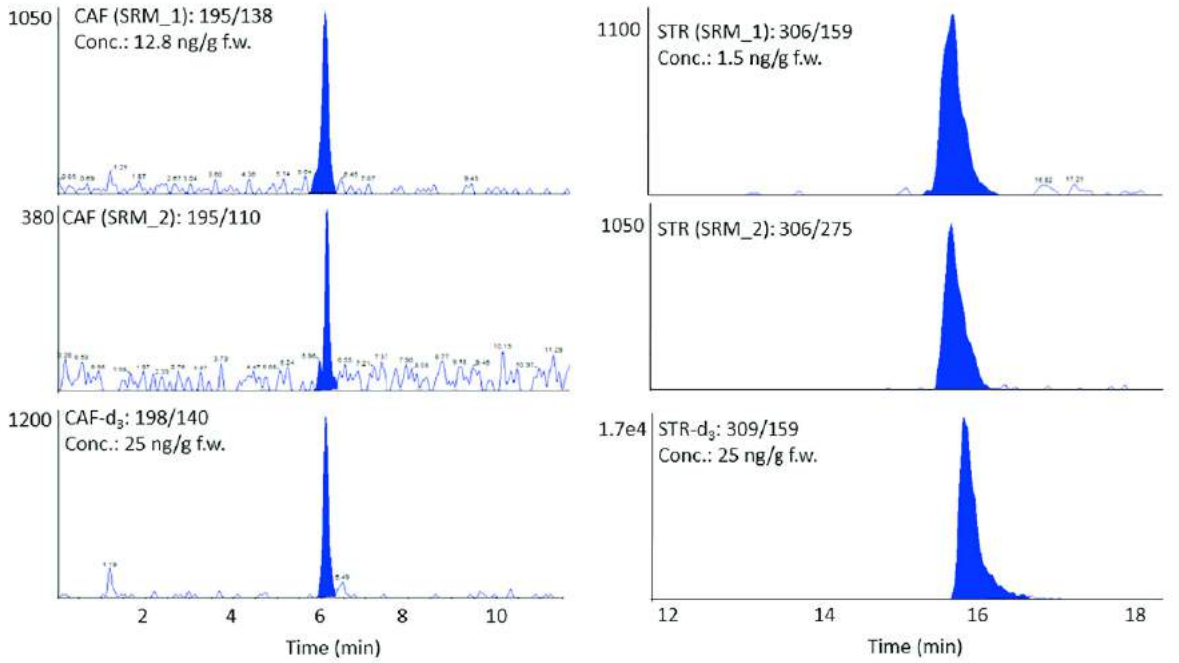


Fig. 3. Extracted ion chromatogram of quantification (SRM_1) and confirmation (SRM_2) transitions of caffeine (left) and sertraline (right) positively identified in a wild fresh mussel sample. MS signals obtained for the corresponding deuterated analogs are also shown.

Acknowledgments

This work has been financially supported by the European Union Seventh Framework Programme through the project SOLUTIONS under grant agreement no. 603437 and by the Government of Catalonia (Consolidated Research Group 2017 SGR 01404-Water and Soil Quality Unit). It reflects only the author's views. The Community is not liable for any use that may be made of the information contained therein. Bekolut is acknowledged for the gift of QuEChERS kits. Marinel·la Farré Urgell from IDAEA-CSIC and Maria Rambla from Institute of Agrifood Research and Technology (IRTA) are acknowledged for the collection of the wild mussel samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2019.07.042.

References

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

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

Alves, V., Conceição, C., Gonçalves, J., Teixeira, H.M., Câmara, J.S., 2017. Improved analytical approach based on QuECHERS/UHPLC-PDA for quantification of fluoxetine, clomipramine and their active metabolites in human urine samples. J. Anal. Toxicol. 41, 45–53.

Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F.J., 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J. AOAC Int. 86, 412–431.

Anzillotti, L., Odoardi, S., Strano-Rossi, S., 2014. Cleaning up blood samples using a modified "QuEChERS" procedure for the determination of drugs of abuse and benzodiazepines by UPLC–MSMS. Forensic Sci. Int. 243, 99–106.

Bayen, S., Estrada, E.S., Juhel, G., Kelly, B.C., 2015. Direct injection of tissue extracts in liquid chromatography/tandem mass spectrometry for the determination of pharmaceuticals and other contaminants of emerging concern in mollusks. Anal. Bioanal. Chem. 407, 5553–5558.

Beyer, J., Green, N.W., Brooks, S., Allan, I.J., Ruus, A., Gomes, T., et al., 2017. Blue mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution monitoring: a review. Mar. Environ. Res. 130, 338–365.

Bringolf, R.B., Heltsley, R.M., Newton, T.J., Eads, C.B., Fraley, S.J., Shea, D., et al., 2010. Environmental occurrence and reproductive effects of the pharmaceutical fluoxetine in native freshwater mussels. Environ. Toxicol. Chem. 29, 1311–1318.

Calisto, V., Esteves, V.I., 2009. Psychiatric pharmaceuticals in the environment. Chemosphere 77, 1257–1274.

Capolupo, M., Valbonesi, P., Kiwan, A., Buratti, S., Franzellitti, S., Fabbri, E., 2016. Use of an integrated biomarker-based strategy to evaluate physiological stress responses induced by environmental concentrations of caffeine in the Mediterranean mussel *Mytilus galloprovincialis*. Sci. Total Environ. 563-564, 538–548.

Cunha, D.L., de Arnujo, F.G., Marques, M., 2017. Psychoactive drugs: occurrence in aquatic environment, analytical methods, and ecotoxicity—a review. Environ. Sci. Pollut. Res. 24, 24076–24091.

Dafouz, R., Cáceres, N., Rodríguez-Gil, J.L., Mastroianni, N., López de Alda, M., Barceló, D., et al., 2018. Does the presence of caffeine in the marine environment represent an environmental risk? A regional and global study. Sci. Total Environ. 615, 632–642.

de Solla, S.R., Gilroy, È.A.M., Klinck, J.S., King, L.E., McInnis, R., Struger, J., et al., 2016. Bioaccumulation of pharmaceuticals and personal care products in the unionid mussel *Lasmigona costata* in a river receiving wastewater effluent. Chemosphere 146, 486–496.

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

Dulaurent, S., El Balkhi, S., Poncelet, L., Gaulier, J.-M., Marquet, P., Saint-Marcoux, F., 2016. QuEChERS sample preparation prior to LC-MS/MS determination of opiates, amphetamines, and cocaine metabolites in whole blood. Anal. Bioanal. Chem. 408, 1467–1474.

Ebele, A.J., Abou-Elwafa Abdallah, M., Harrad, S., 2017. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerg. Contamin. 3, 1–16.

Fabbri, E., Franzellitti, S., 2016. Human pharmaceuticals in the marine environment: focus on exposure and biological effects in animal species. Environ. Toxicol. Chem. 35, 799–812.

Gaw, S., Thomas, K.V., Hutchinson, T.H., 2014. Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. Philos. Trans. R. Soc., B 369.

Gilroy, È.A.M., Gillis, P.L., King, L.E., Bendo, N.A., Salerno, J., Giacomin, M., et al., 2017. The effects of pharmaceuticals on a unionid mussel (*Lampsilis siliquoidea*): an examination of acute and chronic endpoints of toxicity across life stages. Environ.

Toxicol. Chem. 36, 1572–1583.
Gonzalez-Rey, M., Bebianno, M.J., 2013. Does selective serotonin reuptake inhibitor (SSRI) fluoxetine affects mussel *Mytilus galloprovincialis*? Environ. Pollut. 173, 200–209.
Huerta, B., Rodríguez-Mozaz, S., Barceló, D., 2012. Pharmaceuticals in biota in the aquatic environment: analytical methods and environmental implications. Anal. Bioanal. Chem. 404, 2611–2624.
Klosterhaus, S.L., Grace, R., Hamilton, M.C., Yee, D., 2013. Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. Environ. Int. 54, 92–99.
Krogh, J., Lyons, S., Lowe, C.J., 2017. Pharmaceuticals and personal care products in municipal wastewater and the marine receiving environment near Victoria Canada. Front. Mar. Sci. 4.
Martínez Bueno, M.J., Boillot, C., Fenet, H., Chiron, S., Casellas, C., Gómez, E., 2013. Fast and easy extraction combined with high resolution-mass spectrometry for residue analysis of two anticonvulsants and their transformation products in marine mussels. J. Chromatogr. A 1305, 27–34.
Martínez Bueno, M.J., Boillot, C., Munaron, D., Fenet, H., Casellas, C., Gómez, E., 2014. Occurrence of venlafaxine residues and its metabolites in marine mussels at trace levels: development of analytical method and a monitoring program. Anal. Bioanal. Chem. 406, 601–610.
Maruya, K.A., Dodder, N.G., Schaffner, R.A., Weisberg, S.B., Gregorio, D., Klosterhaus, S., et al., 2014a. Refocusing Mussel Watch on contaminants of emerging concern (CECs): the California pilot study (2009–10). Mar. Pollut. Bull. 81, 334–339.
Maruya, K.A., Dodder, N.G., Weisberg, S.B., Gregorio, D., Bishop, J.S., Klosterhaus, S., et al., 2014b. The Mussel Watch California pilot study on contaminants of emerging concern (CECs): synthesis and next steps. Mar. Pollut. Bull. 81, 355–363.
Mezzelani, M., Gorbi, S., Regoli, F., 2018. Pharmaceuticals in the aquatic environments: evidence of emerged threat and future challenges for marine organisms. Mar. Environ. Res. 140, 41–60.
Nödler, K., Voutsa, D., Licha, T., 2014. Polar organic micropollutants in the coastal environment of different marine systems. Mar. Pollut. Bull. 85, 50–59.
Núñez, M., Borrull, F., Fontanals, N., Pocurull, E., 2015. Determination of pharmaceuticals in bivalves using QuEChERS extraction and liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 407, 3841–3849.
Pal, R., Megharaj, M., Kirkbride, K.P., Naidu, R., 2013. Illicit drugs and the environment — a review. Sci. Total Environ. 463-464, 1079–1092.
Parolini, M., Magni, S., Castiglioni, S., Zuccato, E., Binelli, A., 2015. Realistic mixture of illicit drugs impaired the oxidative status of the zebra mussel (*Dreissena polymorpha*). Chemosphere 128, 96–102.
Peng, Y., Hall, S., Gautam, L., 2016. Drugs of abuse in drinking water – a review of current detection methods, occurrence, elimination and health risks. TrAC Trends Anal. Chem. 85, 232–240.
Rodríguez-Mozaz, S., Huerta, B., Barceló, D., 2016. Bioaccumulation of emerging contaminants in aquatic biota: patterns of pharmaceuticals in mediterranean river networks. In: Petrovic, M., Sabater, S., Elosegi, A., Barceló, D. (Eds.), Emerging Contaminants in River Ecosystems: Occurrence and Effects Under Multiple Stress Conditions. The Handbook of Environmental Chemistry, vol. 46. Springer, Cham, pp. 121–141.
Silva, L.J.G., Pereira, A.M.P.T., Rodrigues, H., Meisel, L.M., Lino, C.M., Pena, A., 2017. SSRIs antidepressants in marine mussels from Atlantic coastal areas and human risk assessment. Sci. Total Environ. 603-604, 118–125.
Yadav, M.K., Short, M.D., Aryal, R., Gerber, C., van den Akker, B., Saint, C.P., 2017. Occurrence of illicit drugs in water and wastewater and their removal during wastewater treatment. Water Res. 124, 713–727.

Supporting Information

Psychoactive substances in mussels: Analysis and occurrence assessment

Ester López-García, Cristina Postigo*, Miren López de Alda

Water, Environmental and Food Chemistry (ENFOCHEM) Research Group, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona 18-26, 08034, Barcelona, Spain.

*__Corresponding Author__: Cristina Postigo

Institute of Environmental Assessment and Water Research (IDAEA-CSIC)
Department of Environmental Chemistry
C/ Jordi Girona 18-26, 08034 Barcelona, Spain.
cprqam@cid.csic.es
Tel: +34-934-006-100, Fax: +34-932-045-904

List of Tables:

List of Figures:

Table S1. LC-ESI-MS/MS analysis of the target compounds and surrogate standards (QI –quantification, CI – confirmation ion).

Analyte	t_R (min)	Parent ion (m/z)	QI (m/z) (CE(eV))	CI (m/z) (CE(eV))	Ratio (SRM_{QI}/SRM_{CI})
Time window 1 (from 0 to 12 min)					
Morphine (MOR)	3.5	286	152 (75)	128 (75)	1.8
MOR-d_3		289	152 (75)		
Ephedrine (EPH)	5.0	166	148 (20)	133 (30)	5.2
EPH-d_3		169	151 (20)		
Amphetamine (AM)	5.9	136	119 (15)	91 (20)	0.8
AM-d_5		141	96 (20)		
Caffeine (CAF)	5.9	195	138 (30)	110 (40)	5.6
CAF-d_3		198	140 (35)		
6-monoacetylmorphine (ACM)	6.3	328	165 (75)	152 (80)	1.5
6ACM-d_6		334	165 (75)		
Methamphetamine (MA)	6.5	150	91 (20)	119 (30)	3.2
MA-d_{14}		164	98 (30)		
MDMA[†]	6.8	194	163 (20)	105 (35)	2.7
MDMA-d_5		199	135 (35)		
Benzoylecgonine (BE)	7.1	290	168 (35)	77 (100)	1.8
BE-d_8		298	171 (30)		
Mephedrone (MEPH)	7.2	178	160 (20)	145 (30)	1.7
MEPH-d_3		181	163 (20)		
Ketamine (KET)	7.5	238	125 (40)	179 (40)	2.3
KET-d_4		242	129 (40)		
MDPV°	9.5	276	175 (30)	135 (40)	1.3
MDPV-d_8		284	185 (41)		
Cocaine (COC)	9.6	304	182 (30)	77 (90)	4.5
COC-d_3		307	185 (25)		
Zolpidem (ZOLP)	9.9	308	235 (46)	263 (36)	2.4
ZOLP-d_7		315	242 (50)		
Lysergic acid diethylamide (LSD)	10.1	324	223 (40)	208 (40)	1.6
LSD-d_3		327	226 (35)		
Venlafaxine (VFX)	10.2	278	58 (47)	260 (17)	1.4
VFX-d_6		284	64 (45)		
Cocaethylene (CE)	10.9	318	196 (95)	77 (35)	3.1
CE-d_3		321	199 (30)		
Time window 2 (from 12 to 18.30 min)					
Citalopram (CTLP)	12.7	325	109 (37)	262 (28)	2.0
CTLP-d_6		331	109 (37)		
AH-7921[*]	12.3	329	284 (20)	173 (45)	1.9
AH-7921-d_5		332	287 (25)		
Midazolam (MIDZ)	12.9	326	291 (38)	249 (52)	4.3
MIDZ-d_4		330	295 (38)		
EDDP[**]	13.4	278	234 (45)	249 (35)	2.0
EDDP-d_3		281	234 (45)		
α-hydroxy-alprazolam (OH-ALPZ	13.4	325	297 (35)	216 (55)	2.9
OH-ALPZ-d_5		330	302 (37)		
α-hydroxy-midazolam (OH-MIDZ	13.6	342	324 (31)	203 (37)	2.4
OH-MIDZ-d_4		346	328 (33)		
Hydroxyzine (HXZ)	14.0	375	201 (57)	166 (29)	2.8
HXZ-d_8		383	201 (25)		

Analyte	tR (min)	Parent ion (m/z)	QI (m/z) (CE(eV))	CI (m/z) (CE(eV))	Ratio (SRM_{QI}/SRM_{CI})
Oxazepam (OXA)	14.0	287	241 (31)	269 (23)	1.1
OXA-d_5		292	246 (36)		
Alprazolam (ALPZ)	14.4	309	205 (60)	281 (35)	2.2
ALPZ-d_5		314	286 (40)		
Methadone (METH)	14.6	310	265 (20)	105 (40)	2.8
METH-d_3		313	268 (20)		
Fluoxetine (FLX)	14.9	310	44 (47)	148 (13)	4.6
FLX-d_6		316	44 (46)		
Sertraline (STR)	15.0	306	275 (19)	159 (39)	1.6
STR-d_3		309	159 (43)		
Chlorpromazine (CPMZ)	15.1	319	86 (29)	58 (59)	1.3
CPMZ-d_3		322	89 (28)		
Temazepam (TEMZ)	15.5	301	255 (33)	283 (20)	2.1
TEMZ-d_5		306	260 (32)		
Lormetazepam (LRMZ)	16.0	335	289 (34)	177 (57)	8.4
LRMZ-$^{13}Cd_3$		339	293 (31)		
Diazepam (DIAZ)	17.0	285	193 (40)	222 (40)	1.4
DIAZ-d_5		290	154 (40)		
Time window 3 (from 18.30 to 30 min)					
11-hydroxy-Δ^9THC (OH-THC)	20.4	331	313 (20)	193 (30)	9.1
OH-THC-d_3		334	316 (15)		
11-nor-9-carboxy-Δ^9THC (THC-COOH)	20.6	345	299 (20)	327 (29)	0.4
THC-COOH-d_3		348	330 (25)		
Δ^9Tetrahydroxycannabinol (THC)	24.0	315	193 (31)	123 (47)	1.4
THC-d_3		318	196 (31)		

† MDMA - 3,4-methylenedioxy methamphetamine

° MDPV - 3,4-Methylenedioxy pyrovalerone

*AH7921 - 3,4-dichloro-N-[[1-(dimethylamino)cyclohexyl]methyl]benzamide

** EDDP - 2-ethylidene-1,5-dimethyl-3, 3-diphenylpyrrolidine

Figure S1. Sampling locations of wild mussel samples collected: A) Sant Feliu de Guixols, B) Ebro River Delta.

3.4. Estimación del consumo de alcohol en España mediante el análisis de aguas residuales con fines epidemiológicos

En España la aplicación de WBE para estimar el consumo de drogas y psicofármacos está limitado a pocas ciudades. Únicamente, Barcelona, Castellón, Santiago de Compostela y Valencia reportan anualmente desde 2011 (2013 en el caso de Valencia) los niveles de cocaína, anfetamina, metanfetamina, MDMA, 6ACM y THC-COOH consumidos a la red europea SCORE (del inglés Sewage Analysis CORe group Europe), en un ejercicio que se realiza a nivel internacional para estimar el consumo de las drogas anteriormente mencionadas en diferentes ciudades del mundo aplicando WBE (I. González-Mariño y cols., 2020). Estos datos se facilitan anualmente al Observatorio Europeo de las Drogas y las Toxicomanías, (EMCDDA del inglés, *European Monitoring Center for Drugs and Drug Addiction*), que considera WBE como una herramienta complementaria a los métodos establecidos para estimar el consumo de drogas en Europa. Aparte de los estudios realizados en las cuatro ciudades anteriormente citadas de forma sistemática para analizar el consumo de drogas ilegales (cocaína, anfetamina, metanfetamina, MDMA, heroína y cannabis), y en menor medida legales (alcohol y tabaco), también se han desarrollado trabajos puntuales en otras zonas de la geografía española, como la cuenca del Ebro, donde se estudiaron las principales ciudades en los márgenes del mismo (Postigo y cols., 2010), Talavera de la Reina (Toledo) (Chicharro y cols., 2014) y varias ciudades de Cataluña (Huerta-Fontela y cols., 2008a).

Con el objetivo de expandir la aplicación de WBE en España y contribuir a su desarrollo científico, en 2017 se creó la "Red española de análisis de aguas residuales con fines epidemiológicos" (ESAR-NET, www.esarnet.es). La red está formada por 6 grupos de investigación españoles, con más de 20 investigadores expertos en química analítica y epidemiología, y cuenta con el EMCDDA, Energy Control, Socidrogalcohol y la Delegación del Gobierno para el Plan Nacional Sobre Drogas como entidades asociadas. Hasta la fecha, gracias a la creación de la red, se ha conseguido expandir la aplicación del análisis de aguas residuales con fines epidemiológicos a 13 ciudades españolas, para determinar el consumo de drogas legales como nicotina (Montes y cols., 2020), drogas ilegales (manuscrito en preparación), y evaluar la exposición de las poblaciones investigadas a ftalatos (Iria González-Mariño y cols., 2020).

En este contexto, la publicación #6 describe la aplicación de WBE para estimar el consumo de alcohol en España, a través del análisis del sulfato de etilo, metabolito formado tras

el consumo de alcohol (etanol), en aguas residuales recogidas en 17 EDARs que dan servicio a 13 ciudades, en algunos casos con sus áreas metropolitanas, de 7 comunidades autónomas, con el objetivo de:

- Evaluar los niveles a los que se encuentra el sulfato de etilo en las aguas residuales de las zonas investigadas, y estimar el consumo de alcohol en cada una de ellas a partir de los niveles encontrados.
- Evaluar si existen diferencias significativas de consumo, tanto a nivel local como autonómico, mediante la aplicación de pruebas estadísticas.
- Evaluar si existen diferencias significativas de consumo entre los días laborables y el fin de semana.
- Extrapolar el consumo de alcohol obtenido en las zonas investigadas (12,8% de la población española) a la población total del país, y comparar estos resultados con los datos oficiales proporcionados tanto por instituciones nacionales como por la Organización Mundial de la Salud (OMS).

Los resultados obtenidos mostraron que en todas las EDARs investigadas se detectó el indicador de consumo del alcohol a concentraciones que variaban entre 1,4 y 74 µg/L. Las concentraciones de sulfato de etilo encontradas se correspondían con un consumo de alcohol que oscilaba entre 4,5 y 46 mL/día/habitante. Tarragona, Bilbao y Móstoles fueron las ciudades en las que se estimó un mayor consumo de alcohol, y Toledo, Santiago de Compostela, Lleida, Madrid-Centro, Castellón y una zona de Valencia, en las que menos. Hay que señalar que este es el primer estudio en el que se ha estimado el consumo de alcohol en ciudades como Madrid, ciudad con mayor población de España, y Bilbao o Palma de Mallorca, ciudades también importantes por su tamaño o su actividad social.

En lo que respecta a los patrones geográficos de consumo, se obtuvieron diferencias significativas tanto a nivel local como a nivel autonómico. A nivel local, Bilbao mostró un consumo de alcohol significativamente superior (entre 1,5 y 3 veces) al obtenido en 9 ciudades, y Palma de Mallorca y Móstoles también presentaron un consumo significativamente mayor que el obtenido en Toledo y Castellón, respectivamente. A nivel autonómico, el consumo en el País Vasco fue significativamente superior al del resto de comunidades autónomas investigadas (Castilla-La Mancha, Comunidad de Madrid, Comunidad Valenciana, Galicia, e Islas Baleares) excepto en Cataluña, y el consumo en las Islas Baleares también fue significativamente superior al obtenido en Galicia y Castilla-La Mancha.

En cuanto a las tendencias semanales de consumo, el consumo durante el fin de semana fue significativamente superior (aproximadamente el doble) al calculado para los los días laborables.

Por último, la extrapolación de los datos a nivel nacional dio resultados comparables tanto con el dato reportado en la Encuesta Nacional de Salud como con el reportado por el Ministerio de Agricultura, Pesca y Alimentación. Sin embargo, como en muchos otros trabajos, fue inferior que el reportado por la OMS.

Publicación científica #6

"Assessing alcohol consumption through wastewater-based epidemiology: Spain as a case study"

por:

Ester López-García, Carlos Pérez-López, Cristina Postigo, Vicente Andreu, Lubertus Bijlsma, Iria González-Mariño, Félix Hernández, Rosa María Marcé, Rosa Montes, Yolanda Picó, Eva Pocurull, Andreu Rico, Rosario Rodil, María Rosende, Yolanda Valcárcel, Olatz Zuloaga, José Benito Quintana, y Miren López de Alda

en

Drug and Alcohol Dependence 215 (2020) 108241

https://doi.org/10.1016/j.drugalcdep.2020.108241

Drug and Alcohol Dependence 215 (2020) 108241

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Full length article

Assessing alcohol consumption through wastewater-based epidemiology: Spain as a case study

Ester López-García[a], Carlos Pérez-López[a], Cristina Postigo[a,*], Vicente Andreu[b], Lubertus Bijlsma[c], Iria González-Mariño[d,e], Félix Hernández[c], Rosa Maria Marcé[f], Rosa Montes[g], Yolanda Picó[b], Eva Pocurull[f], Andreu Rico[g], Rosario Rodil[d], María Rosende[h], Yolanda Valcárcel[i], Olatz Zuloaga[j], José Benito Quintana[d], Miren López de Alda[a,*]

[a] Water, Environmental, and Food Chemistry Unit (ENFOCHEM), Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain
[b] Food and Environmental Safety Research Group (SAMA-UV), Desertification Research Centre (CIDE), CSIC-Generalitat Valenciana-University of Valencia, Valencia, Spain
[c] Environmental and Public Health Analytical Chemistry, Research Institute for Pesticides and Water, University Jaume I, Castellón, Spain
[d] Department of Analytical Chemistry, Nutrition and Food Sciences, Institute of Research on Chemical and Biological Analysis (IAQBUS), Universidade de Santiago de Compostela, Santiago de Compostela, Spain
[e] Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemical Sciences, University of Salamanca, Salamanca, Spain
[f] Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira i Virgili, Tarragona, Spain
[g] IMDEA Water Institute, Science and Technology Campus of the University of Alcalá, Alcalá de Henares, Spain
[h] FI-TRACE Group, Department of Chemistry, University of the Balearic Islands, Palma de Mallorca, Spain
[i] Research Group in Environmental Toxicology and Risk Assessment (TAyER), Medical Specialties and Public Health, Faculty of Health Sciences, Rey Juan Carlos University, Madrid, Spain
[j] Department of Analytical Chemistry, Faculty of Science and Technology (UPV/EHU) & Plentzia Marine Station of Basque Country University (EHU/UPV), Basque Country, Spain

ARTICLE INFO

Keywords:
Sewage epidemiology
Alcohol abuse
Liquid chromatography-mass spectrometry
Consumption patterns

ABSTRACT

Background: In this study, an alternative and complementary method to those approaches currently used to estimate alcohol consumption by the population is described. This method, known as wastewater-based epidemiology (WBE), allows back-calculating the alcohol consumption rate in a given population from the concentrations of a selected biomarker measured in wastewater.

Methods: Composite (24-h) wastewater samples were collected at the inlet of 17 wastewater treatment plants located in 13 Spanish cities for seven consecutive days in 2018. The sampled area covered 12.8% of the Spanish population. Wastewater samples were analyzed to determine the concentration of ethyl sulfate, the biomarker used to back-calculate alcohol consumption.

Results: Alcohol consumption ranged from 4.5 to 46 mL/day/inhabitant. Differences in consumption were statistically significant among the investigated cities and between weekdays and weekends. WBE-derived estimates of alcohol consumption were comparable to those reported by its corresponding region in the Spanish National Health Survey in most cases. At the national level, comparable results were obtained between the WBE-derived annual consumption rate (5.7 ± 1.2 L ethanol per capita (aged 15+)) and that reported by the National Health Survey (4.7 L ethanol per capita (aged 15+)).

Conclusions: This is the largest WBE study carried out to date in Spain to estimate alcohol consumption rates. It confirms that this approach is useful for establishing spatial and temporal patterns of alcohol consumption, which could contribute to the development of health care management plans and policies. Contrary to established methods, it allows obtaining information in a fast and relatively economical way.

* Corresponding authors at: Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Department of Environmental Chemistry, C/ Jordi Girona 18-26, 08034 Barcelona, Spain.
E-mail addresses: cprqam@cid.csic.es (C. Postigo), miren.lopezdealda@idaea.csic.es (M. López de Alda).

https://doi.org/10.1016/j.drugalcdep.2020.108241
Received 15 April 2020; Received in revised form 1 July 2020; Accepted 12 August 2020
Available online 19 August 2020

1. Introduction

In 2016, the consumption of alcohol was responsible for 3 million deaths worldwide and it became one of the main health risk factors for the population, being more harmful than digestive diseases, road injuries, diabetes, or violence (WHO, 2018). In Spain, alcohol is the psychoactive substance most consumed (OEDA, 2019). In 2017 (last reported year), 91% of the Spanish population aged 15–64 years had consumed alcohol at some point in their lifetime, while 75% had consumed alcohol in the last year, and 63% did it in the last month. Overall, the consumption by men is higher than by women and the average age at which alcohol begins to be consumed is 16.6 years (OEDA, 2019). According to the 2018 Global status report on alcohol and health provided by the WHO, the annual intake of alcohol in Spain was 10 L of pure alcohol per capita (aged 15+) in 2016, which is similar to the European average (9.8 L) (WHO, 2018). These estimates are traditionally obtained from population surveys, recorded alcohol data (alcohol taxation or sales), and unrecorded alcohol data (homemade or informally produced alcohol, smuggled alcohol, alcohol for industrial or medical uses, alcohol obtained through cross-border shopping, or surrogate alcohol) (WHO, 2018). Through surveys, consumption figures can be disaggregated for specific population groups by age or gender. However, the use of these tools/data to derive alcohol consumption figures is time-consuming and relatively expensive, and consequently, it does not allow obtaining real-time estimates (i.e., consumption data in Spain are provided with a delay of two years). Furthermore, the data obtained by surveys may not be representative of actual population consumption due to misreporting of alcohol consumption by survey participants (Stockwell et al., 2016; van Wel et al., 2016) or to inaccurate estimates of unrecorded alcohol (Probst et al., 2019). Therefore, it is necessary to propose alternative approaches that provide quick and precise information and that, together with the traditional ones, can help to obtain a more reliable picture of alcohol consumption rates.

Wastewater-based epidemiology (WBE) is a novel approach that has been applied in the last decade to estimate illicit drug use at the city level (González-Mariño et al., 2020). The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has adopted it, indeed, as a complementary indicator to established methods for illicit drug use estimation (EMCDDA, 2016). The WBE approach is based on the fact that, after consumption, the substances are excreted via urine and feces, either unaltered or as a metabolite, and conducted through the sewage network to a wastewater treatment plant (WWTP). Thus, a raw wastewater sample contains specific biomarkers of the drugs that can be used to back-calculate the amount of substance that has been consumed. In the case of alcohol, after human consumption, about 95% is metabolized in the liver via oxidation to acetaldehyde and acetic acid, about 5% is excreted unaltered, and a small part (< 0.1%) is excreted as ethyl sulfate (EtS) and ethyl glucuronide (EtG) after conjugation with sulfate and glucuronic acid, respectively. EtS and EtG can be detected in urine after 1 h of alcohol intake (Helander and Beck, 2005), so they have been proposed as good indicators for recent alcohol consumption. However, only EtS is stable in wastewater (Rodríguez-Álvarez et al., 2014) and its occurrence in wastewater is exclusively due to alcohol consumption and not to the metabolism of unaltered alcohol by endogenous bacteria (Reid et al., 2011). Thus, EtS has been pointed out as the best biomarker to estimate alcohol consumption through WBE.

WBE was first applied to estimate alcohol consumption in 2011 in Oslo (Norway) (Reid et al., 2011) and, since then, many studies have been carried out in cities from other European countries (Andrés-Costa et al., 2016; Baz-Lomba et al., 2016; Gatidou et al., 2016; Mastroianni et al., 2014, 2017; Rodríguez-Álvarez et al., 2014, 2015; van Wel et al., 2016), Vietnam (Nguyen et al., 2018), China (Gao et al., 2020), United States (Chen et al., 2019), Canada (Ryu et al., 2016), and Australia (Zheng et al., 2020). The main objective of these studies was not only to investigate spatial differences of alcohol consumption between populations or to assess changes in alcohol consumption due to special events (Andrés-Costa et al., 2016) but also, to compare WBE-derived alcohol estimates with alcohol consumption figures obtained using traditional methods, such as official data provided by the WHO or by national surveying institutions. In these studies, the alcohol consumption rates were estimated from data gathered from a single WWTP, which only serves a city or part of it, after a sampling period of one week in most of the cases, except for Milan (Italy) and Santiago de Compostela (Spain) (Rodríguez-Álvarez et al., 2015), Oslo (Norway) (Reid et al., 2011), Lied (Belgium) (van Wel et al., 2016), three U.S communities (Chen et al., 2019) and one Australian regional city (Zheng et al., 2020), for which longer sampling periods were used (namely, 2 weeks, 3 weeks, four-two weeks periods, one weekday every month during eleven months, and one week every two months during 6 years, respectively). To date, only three studies have conducted nation-wide investigations by collecting samples from different WWTPs: a study conducted in Australia, in which 18 WWTPs were sampled, covering 45% of the whole country population (Lai et al., 2018); one carried out in Belgium, which covered 8 WWTPs and 12.8% of the total population (Boogaerts et al., 2016); and another one in China, which included 48 WWTPs and 3.3% of the Chinese population (Gao et al., 2020).

The present study is one of the few nation-wide applications of WBE to estimate alcohol consumption rates, and the largest conducted so far in Spain. Wastewater samples were analyzed from 17 WWTPs, covering 12.8% of the Spanish population. The specific objectives of this work were: i) to assess spatial differences in alcohol consumption between the different investigated areas in Spain, ii) to assess weekly consumption patterns, and iii) to extrapolate the estimated alcohol consumption in the studied areas to the whole Spanish population, and to compare it with official data reported by the WHO and national institutions.

2. Material and methods

2.1. Reagents

Analytical standards of ethyl sulfate (EtS) and its isotopically labeled compound, EtS-d_5, were obtained as EtS sodium salt and ethyl-d_5 sulfate salt from Cerilliant (Round Rock, TX, USA) as solutions in methanol (MeOH) at a concentration of 1 mg/mL. Water and MeOH, both HPLC-grade, and acetic acid (98% purity) used as a mobile phase modifier, were purchased from Merck (Darmstadt, Germany). Dibutylamine (> 99.5% purity) to prepare dibutylammonium acetate (DBAA), used as a mobile phase modifier, was obtained from Sigma Aldrich (Steinheim, Germany).

2.2. Standard solutions

Stock standard solutions were prepared at different concentrations in the range of 10–20,000 µg/L by appropriate dilution of the commercial EtS standard in MeOH, with a constant concentration of EtS-d_5 of 2500 µg/L, and were stored in the dark at −20 °C until analysis. Before analysis, working standard solutions were freshly prepared by dilution of these stock standard solutions in HPLC-grade water (1:100, v/v).

2.3. Sample collection and preparation

Influent wastewater samples were collected from 17 WWTPs located in 13 Spanish cities that belong to 7 out of the 17 regions of Spain. Figure S1 in the Supporting Information shows the location of the sampled WWTPs. The sampling covers populations of various sizes (i.e, between 47,961 and 1,163,154 inhabitants). In total, the population reached with the sampling was 5,981,848 inhabitants, which corresponds to 12.8% of the Spanish population. The cities sampled were

Barcelona, Bilbao, Castellón, Guadalajara, Lleida, Madrid, Móstoles, Palma de Mallorca, Reus, Santiago de Compostela, Tarragona, Toledo, and Valencia, including in some cases part of their metropolitan area. Except for Barcelona, Madrid, and Móstoles, where the WWTPs investigated only covered 35, 30, and 90% of their total population, respectively, in all the remaining cities the population was fully covered (100% of their population). Table S1 shows the populations served by each WWTP as well as the sampling protocol conducted in each location.

From each WWTP, 24-h composite influent wastewater samples were collected during seven consecutive days in the spring of 2018 using time or flow proportional techniques (Table S1). The sampling was conducted during a "normal week" so that special events such as holidays or festivals were avoided. After collection, samples were immediately stored at −20 °C. They were sent frozen by courier in less than 24 h to the laboratory in Barcelona, where all samples were analyzed. Once in the laboratory, an aliquot of 10 mL was spiked with EtS-d_5 at a concentration of 25 µg/L and 1 mL of this sample was transferred to a 1.5 mL microcentrifuge tube and centrifuged at 10,000 rpm for 10 min at a temperature of 4 °C (Eppendorf 5810R, Hamburg, Germany). Then, the supernatant was transferred to a glass vial and stored at −20 °C in the darkness until its analysis by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS).

2.4. Sample analysis

The analysis of EtS was performed with a previously described and validated methodology based on ion-pair LC–MS/MS (Mastroianni et al., 2014) using a Symbiosis™ Pico System (Spark Holland, Emmen, The Netherlands) equipped with a 100 µL sample loop. The LC system was coupled to a 4000QTRAP hybrid triple quadrupole-linear ion trap (QqLIT) mass spectrometer provided with a Turbo Ion Spray source (AB-Sciex, Foster City, CA, USA) that was operated in the negative ionization mode (ESI-). Chromatographic separation was performed with a Purospher Star RP-18 end-capped column (125 mm × 2 mm, particle size 5 µm) preceded by a guard column of the same packing material and particle size, both from Merck (Darmstadt, Germany) and a mobile phase consisting of MeOH and water both containing 5 mM of DBAA at a constant flow rate of 0.3 mL/min. MS/MS detection was performed in selected reaction monitoring mode (SRM) recording 2 SRM transitions for EtS (125 → 97, 125 → 80) and one for EtS-d_5 (130 → 98). Data acquisition and evaluation was performed with Analyst 1.5 software (AB-Sciex, Foster City, CA, USA). Quantification of the samples was based on the isotope dilution method.

2.5. Quality control and quality assurance

A calibration curve was freshly prepared in water for the analysis of each batch of samples in the range 0.1–200 µg/L. For this, appropriate amounts of stock standard solutions were fortified in water and processed following the sample treatment protocol. The calibration curve was injected at the beginning and the end of each batch of samples, and calibration curves were constructed with the average response, using weighted least square regression models ($1/x^2$ as weight) to reduce the effect of high concentrations in the model. Only those calibration solutions that did not deviate more than 20% from the theoretical concentration were used to construct the model.

Quality controls, i.e., a standard solution containing EtS and EtS-d5 at concentrations of 5 µg/L and 25 µg/L, respectively, were injected every 6 samples to check the correct operation of the instrument. MS signals for EtS were absent in solvent blanks (HPLC-grade water injected every 3 samples) and method blanks (HPLC-grade water processed following the sample treatment protocol and thus, fortified with EtS-d5 at a concentration of 25 µg/L). Therefore, analyte carryover between injections and cross-contamination during sample preparation could be discarded.

2.6. Alcohol consumption estimates

Back calculation of alcohol consumption was made according to the following equation:

$$\frac{mL\ EtOH}{day * inhabitant} = C_{EtS}\left[\frac{\mu g}{L}\right] * 10^{-6}\left[\frac{g}{\mu g}\right] * Q\left[\frac{m^3}{day}\right] * 10^{3}\left[\frac{L}{m^3}\right] * \frac{1}{P} * 3047 * \frac{1}{\rho_{EtOH}\left(\frac{g}{mL}\right)}$$

where C_{EtS} is the concentration of EtS measured in the wastewater sample, Q is the water flow entering the WWTP, P is the total population served by the WWTP (Table S1), 3047 is the correction factor applied which takes into account the molar mass ratio between ethanol (MW: 46.07 g/mol) and EtS (MW: 126.13 g/mol) and the excretion rate of EtS in urine (0.012%) (Rodríguez-Álvarez et al., 2015), and ρ_{EtOH} is ethanol density (0.789 g/mL).

2.7. Statistical data analysis

Data were statistically analyzed to compare alcohol consumption rates between populations, regions, weekdays, and weekends, and between populations grouped according to their size (above or below 300,000 inhabitants). Since data were not normally distributed (after Shapiro Wilk test, *p-value* < 0.05) and/or the sample size was too small ($n < 10$) in some cases, non-parametric tests were applied. The Mann-Whitney U test was used to compare two independent samples, whereas the Kruskal-Wallis test was used to compare three or more individual groups. If the latter revealed significant differences among groups, they were subsequently investigated after applying the Mann-Whitney U test to every two populations. False Discovery Rate (FDR) correction for multiple testing was applied to reduce the number of "false positives". Spearman correlation test was also applied to assess the correlation between WBE-derived data and those reported by established indicators. All the analyses were done using the software R (version R 3.5.3) and considering a 95% confidence level ($\alpha = 0.05$).

3. Results

3.1. Occurrence of EtS in wastewater samples and alcohol consumption estimations

Table 1 shows the concentrations of EtS, the mass loads of EtS that reached each WWTP and the estimated alcohol consumption in each investigated area, expressed as average, median and range; whereas Fig. 1 depicts alcohol consumption in the form of boxplots by each investigated population in the various considered regions. EtS was found in all samples above LOQ (0.07 µg/L) at concentrations ranging from 1.4 µg/L (Santiago de Compostela) to 74 µg/L (Tarragona). The average weekly concentrations of EtS ranged from 2.9 to 43 µg/L, with the lowest values being found in the WWTPs that serve the cities of Santiago de Compostela, Lleida, and Guadalajara (below 10 µg/L) and the highest values in the WWTPs that serve Móstoles (31 µg/L) and Tarragona (43 µg/L). The average weekly levels of EtS measured in the remaining WWTPs were between 11 (Toledo) and 21 µg/L (Reus).

The alcohol consumption estimated from levels of EtS in the analyzed samples ranged from 4.5 (Santiago de Compostela) to 46 mL/day/inhabitant (Tarragona). The cities with the highest average alcohol consumption were Tarragona, Bilbao, and Móstoles, with average weekly consumption of 27, 20, and 17 mL/day/inhabitant, respectively. The lowest average alcohol consumptions (< 10 mL/day/inhabitant) were estimated in Toledo (7.4), Santiago de Compostela (8.4), Lleida (8.5), Madrid-Centre (8.9), Castellón (9.0), and Valencia-QB (9.4). In the remaining investigated areas (Guadalajara, Barcelona, Reus, Madrid-North, Valencia-PI, Valencia-PII, and Palma de Mallorca), average alcohol consumption was between 11 and 14 mL/day/inhabitant.

Comparing with previous studies conducted in Spain, similar

Table 1
Frequency of detection of EtS (%), EtS concentration (μg/L), EtS load (mg/day/inhabitant) and alcohol consumption (mL/day/inhabitant) in the investigated cities (expressed as average, median and range).

		Concentration (μg/L)			EtS load (mg/day/inhabitant)			Alcohol (mL/day/inhabitant)				
	Freq. (%)	Average	Median	Range	Average	Median	Range	Average	Median	Range	Average weekdays	Average weekend
Palma I	100	15	15	11 – 21	–	–	–	–	–	–	–	–
Palma II	100	18	16	14 – 26	–	–	–	–	–	–	–	–
Palma de Mallorca[a]		–	–	–	3492	3221	2581 – 4702	14	12	10 – 18	12	17
Bilbao	100	17	16	18 – 29	5133	4867	3906 – 7632	20	19	15 – 30	19	23
Guadalajara	100	9.3	7.8	6.5 – 15	2857	2499	2051 – 4417	11	9.7	7.9 – 17	9.0	16
Toledo	100	11	9.1	7.8 – 19	1926	1555	1426 – 3007	7.4	6.0	5.5 – 12	5.8	11
Barcelona	100	16	14	5.9 – 25	3455	3021	2030 – 5352	13	12	7.8 – 21	11	20
Lleida	100	7.4	6.9	5.6 – 10	2208	1807	1663 – 3333	8.5	7.0	6.4 – 13	7.2	12
Reus	100	21	13	12 – 39	3081	2036	1814 – 5363	12	7.9	7.0 – 21	8.8	20
Tarragona	100	43	50	11 – 74	7091	8597	1935 – 11906	27	33	7.5 – 46	27	28
Madrid-Centre	100	15	15	9.4 – 23	2301	2175	1381 – 3431	8.9	8.4	5.3 – 13	7.6	12
Madrid-North	100	18	17	9.4 – 26	3375	3342	1719 – 5327	13	13	6.6 – 21	13	14
Móstoles	100	31	28	18 – 50	4430	4147	2592 – 7520	17	16	10 – 29	15	22
Santiago de Compostela	100	2.9	2.7	1.4 – 4.4	2178	2197	1173 – 3124	8.4	8.5	4.5 – 12	7.0	12
Castellón	100	12	11	7.3 – 23	2325	1964	1635 – 4101	9.0	7.6	6.3 – 16	7.4	13
Valencia-PI	100	13	13	7.5 – 19	2977	2829	1722 – 4364	12	11	6.6 – 17	9.6	16
Valencia-PII	100	12	11	6.9 – 19	2957	3282	2168 – 3483	11	13	8.4 – 13	11	13
Valencia-QB	100	14	11	10 – 22	2438	2339	1693 – 3770	9.4	9.0	6.5 – 15	8.0	13

[a] During sampling period Palma I derived part of its water flow to Palma II, and therefore to calculate EtS loads and estimate alcohol consumption, Palma I and Palma II were jointly treated as Palma de Mallorca.

alcohol consumption rates were reported in Barcelona (18 mL/day/inhabitant) (Mastroianni et al., 2014) and Castellón (6.6 mL/day /inhabitant) (Baz-Lomba et al., 2016), and higher in Santiago de Compostela (13.6–16.3 mL/day/inhabitant) (Rodríguez-Álvarez et al., 2015, 2014). On the contrary, the alcohol consumption estimated during a normal week in Valencia (Valencia-PI (6.2 mL/day/inhabitant (aged 15+)), Valencia-PII (3.3 mL/day/inhabitant (aged 15+)) and Valencia-QB (5.9 mL/day/inhabitant (aged 15+)) was lower than that estimated in the present study, even though consumption figures in that study were obtained considering only the population aged 15+ (Andrés-Costa et al., 2016).

Comparing with other international studies, the estimated rates in the investigated Spanish populations (average alcohol consumption 7.4–27 mL/day/inhabitant), were similar to those reported by other investigated cities (Table S2) except in Ho Chin Minh (Vietnam) (Nguyen et al., 2018), Lesvos (Greece) (Gatidou et al., 2016), Milan (Italy) (Baz-Lomba et al., 2016; Rodríguez-Álvarez et al., 2015) and Lugano (Switzerland) (Ryu et al., 2016), where alcohol consumption rates (from 3.4–6.6 mL/day/inhabitant) were lower than those estimated for Spanish populations. On the contrary, Copenhagen (Denmark) and Granby (Canada) (Ryu et al., 2016), showed higher alcohol consumption rates, 40 and 44 mL/day/inhabitant, respectively.

3.2. Spatial variation in alcohol consumption

The statistical test applied to evaluate spatial variation in alcohol consumption among different populations showed that populations belonging to the same region showed no statistically significant differences in alcohol consumption (*p-value* > 0.05, Mann-Whitney U test) (Table S3) while, statistically significant differences between populations belonging to different regions were found (*p-value* < 0.05, Mann-Whitney U test) (Table S3). In particular, alcohol consumption estimated for the population served by Bilbao WWTP was different to that observed in 9 other populations, namely, Castellón, Guadalajara, Lleida, Madrid-Centre, Santiago de Compostela, Toledo, Valencia-PI, Valencia-PII, and Valencia-QB, with median alcohol consumption in Bilbao between 1.5 (Valencia-PII) and 3 (Toledo) times higher than in the aforementioned cities. Also, statistically significant differences were

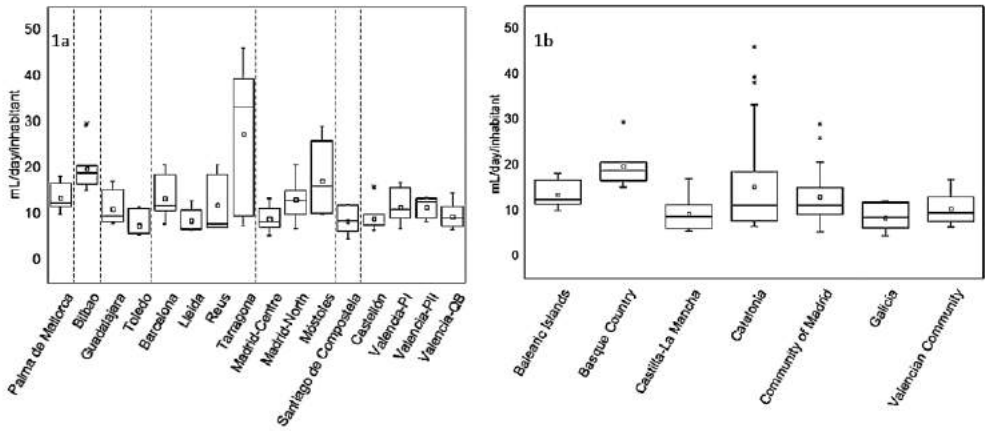


Fig. 1. Distribution of alcohol consumption among investigated populations (Fig. 1a) and regions (Fig. 1b). (In Fig. 1a, populations belonging to the same region are shown between vertical lines; * Outlier).

observed between Palma de Mallorca and Toledo (consumption in Palma de Mallorca 2 times higher than in Toledo) and between Móstoles and Castellón (consumption in Móstoles 1.7 times higher than in Castellón) (Table 1 and S3).

At the regional level (Fig. 1b, Table S4) differences of alcohol consumption were statistically significant (*p-value* < 0.05, Mann-Whitney U test) between the Basque Country and all the other investigated regions, except Catalonia, and between the Balearic Islands and the region of Castilla-La Mancha and Galicia (Table S4). The median consumption of alcohol in the Basque Country (19 mL/day/inhabitant) was between 1.5 and 2.2 times higher than the median consumption observed in the Balearic Islands (12), Community of Madrid (11), Valencian Community (9.5), Castilla-La Mancha (8.7) and Galicia (8.5 mL/day/inhabitant). The Balearic Islands presented a median figure of alcohol consumption 1.5 times higher than those obtained in Castilla-La Mancha and Galicia.

As for the city size, small cities, i.e., those with official census populations < 300,000 inhabitants (Toledo, Guadalajara, Santiago de Compostela, Reus, Tarragona, Lleida, Castellón and Móstoles), showed significantly lower alcohol consumption rates per capita than large cities, i.e., those with official census population > 300,000 (*p-value* < 0.05, Mann Whitney U).

3.3. Weekly patterns

Fig. 2 shows the daily alcohol consumption expressed as mL/day/inhabitant or as the contribution of each day to the total weekly consumption observed in each population. The difference in the amount of alcohol consumed during the weekend (Saturday and Sunday) (median = 15 mL/day/inhabitant) and during the weekdays (Monday to Friday) (median = 9.0 mL/day/inhabitant) was found to be statistically significant (*p-value* < 0.05, Mann Whitney U).

Figure S2 shows the weekly trends of alcohol consumption in the investigated populations. The strongest differences in alcohol consumption between weekdays and weekends were observed in Reus and Toledo (with average consumption figures 2.2 and 2.0 times higher, respectively, during the weekend than during weekdays), and the weakest in Madrid-North (where Monday is the day of the highest consumption) and Tarragona (where, in fact, large variations in alcohol consumption were observed throughout the week (Figure S2)).

Fig. 2 also shows a general high contribution of Mondays to total weekly alcohol consumption figures when compared with the other weekdays. According to Høiseth et al., EtS can remain in urine for several hours (between 25 and 48 h) depending on the dose of ethanol ingested (Høiseth et al., 2008). Therefore, the high value of alcohol consumption estimated on Monday could be attributed to the presence of EtS in wastewater from its consumption during the weekend.

3.4. Nationwide extrapolation

The total daily alcohol load (kg/day) that arrived at each WWTP was used to back-calculate alcohol consumption at the national level. Data were extrapolated taking into account that the population covered by the study was about 6.0 million inhabitants (12.8% of the Spanish population) and the total population of Spain in 2018 accounted for 46.7 million inhabitants (INE, 2018). The extrapolation resulted in annual consumption of 4.8 ± 1.1 L of pure ethanol per capita in Spain, which increases to 5.7 ± 1.2 L or 5.9 ± 1.3 L of pure ethanol when only population above 15 years (aged 15+) or adult population (aged 18+) is considered, respectively (Table S5). This value is in line with the official data reported by the National Health Survey (INE) (Table S6), i.e., an average weekly consumption of 13 mL/day/inhabitant (aged 15+) equivalent to an average annual consumption of 4.7 L of pure ethanol per capita (aged 15+), and also with the official data published by the Spanish Ministry of Agriculture, Fishing and Food, which indicates consumption of 51.8 L of beer per capita (+18) (MAPA, 2018), equivalent to 4.3 L of pure ethanol per capita (aged 18+) taking into account that alcohol consumption by type of alcoholic beverage is distributed as 54% beer, 18% wine and 28% spirits and the alcohol content in each one is 4.5, 12 and 40%, respectively (WHO, 2018). On the contrary, a higher alcohol consumption rate (10 L of pure ethanol per capita (aged 15+)) was reported for Spain in the WHO report (WHO, 2018).

4. Discussion

In this study, alcohol consumption in different populations of Spain was estimated through WBE. The population investigated covers 12.8% of the total Spanish population and is distributed around 13 main cities and 7 different regions. Results showed spatial variations in alcohol consumption among specific populations and regions. Although Tarragona, Bilbao, and Móstoles were the cities with the highest average alcohol consumption figures, Bilbao was the only one where alcohol consumption was significantly different from several other populations (see Table S3 and Fig. 1). Also, alcohol consumption in Palma de Mallorca and Móstoles was significantly higher than in Toledo and Castellón, respectively. WBE-derived alcohol consumption figures were compared with the latest data reported by the National Health Survey carried out by the Spanish Ministry of Health, Consumption and Social Welfare in collaboration with the National Institute of Statistics (INE) (INE, 2017) and with prevalence data reported in the Annual Report of the Spanish Observatory on Drugs and Drugs Addiction

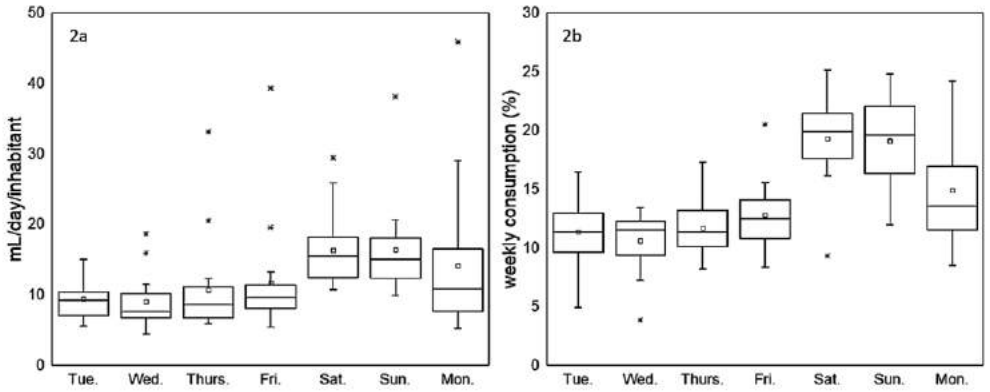


Fig. 2. Distribution of alcohol consumption throughout the week expressed as mL/day/inhabitant (Fig. 2a) and the contribution of each day to the total weekly consumption (%) (Fig. 2b). (*Outlier).

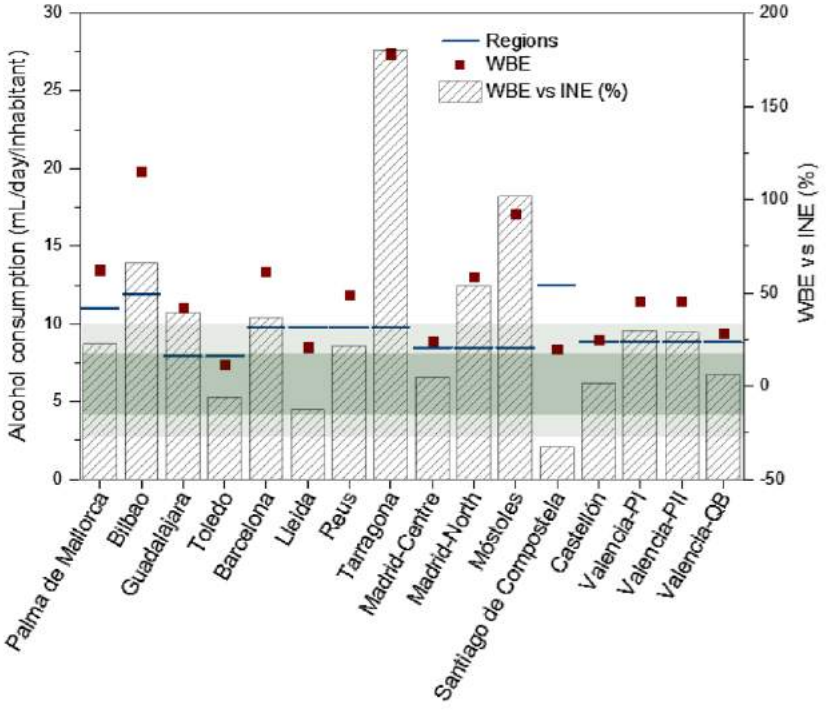


Fig. 3. Alcohol consumption estimated in the investigated populations through WBE (red square), data reported for the corresponding region in the INE National Health Survey (blue line), and differences of consumption between WBE data and survey data (grated bars) (%). (The bars within the dark green zone delimit consumption differences between both methodologies below 15% and those within the light green zone below 30%).

(OEDA, 2019). Since official data are only provided at the level of regions, the average alcohol consumption obtained in each investigated population was compared with consumption data reported for its corresponding region. Fig. 3 compares WBE data and INE National Health Survey data. WBE-derived alcohol consumption figures in five of the investigated populations (Toledo, Lleida, Madrid-Centre, Castellón, and Valencia-QB) showed good correlation with INE official data at the region level, being the differences of consumption figures lower than 13%, whereas a weaker correlation (differences of consumption between 22 and 30%) was observed in 4 populations (Palma de Mallorca, Reus, Valencia-PI, and Valencia-PII). WBE-derived data in the remaining populations (Bilbao, Guadalajara, Barcelona, Tarragona, Madrid-North, Móstoles, and Santiago de Compostela) showed large differences with official INE data.

The comparison of WBE-data with prevalence data of alcohol consumption reported for each region, showed poor correlation when all investigated populations were considered (see Figure S3). However, as shown in Fig. 4, when the data from the 7 populations that did not correlate with official INE consumption figures (Bilbao, Guadalajara, Barcelona, Tarragona, Madrid-North, Móstoles, and Santiago de Compostela) were removed, a significant correlation was observed (r^2 "Lifetime prevalence": 0.4499, *p-value* < 0.05; r^2 "Last year prevalence": 0.5407, *p-value* < 0.05). According to WBE-data the population belonging to the Basque Country presented a significantly higher consumption than populations belonging to the other regions (except Catalonia), and alcohol consumption in the Balearic Islands was significantly higher than in Castilla-La Mancha and Galicia (Fig. 1b, Table S4). Compared to prevalence data reported by the Spanish Annual Report (Figure S4), WBE results are in agreement with prevalence data only in the case of the Balearic Islands, since the Balearic Islands show a higher prevalence of consumption than Castilla-La Mancha and Galicia. On the contrary, in the case of the Basque Country, the prevalence of alcohol consumption, although above the Spanish average, is similar to that reported for the Valencian Community or Galicia (Figure S4).

The differences observed between WBE-derived alcohol consumption figures and established indicators could have different explanations. On the one hand, data reported by established methods may not represent the actual consumption by the population since they are affected by a degree of uncertainty. The two established indicators used to compare the WBE-derived estimates, provided indeed different results, in the sense that the highest prevalence data was reported for the Balearic Islands (see Figure S4) whereas the highest alcohol consumption rate was reported for Galicia in the INE National Health Survey (Table S6). On the other hand, the populations sampled may not be representative of alcohol consumption in the whole region. As previously demonstrated, significant differences in alcohol consumption were observed between small and large populations (section 3.2). In some regions, only one municipality was sampled (i.e., the Balearic Islands and Galicia) which may not adjust to the alcohol consumption patterns of the whole region. This hypothesis is supported by the fact that within the same region, WBE-data derived from some populations correlated well with the INE survey data, whereas others did not (see Castilla-La Mancha, Catalonia, and Community of Madrid in Fig. 3). Despite this, at the national level, the annual alcohol consumption rate obtained through WBE was comparable to that reported by the National Health Survey, which may indicate that the sampled population is quite representative of the whole country.

Unlike the Spanish National Health Survey, the national WBE-derived data show a low correlation to those reported by the WHO. This fact was also observed in the nation-wide study carried out in Belgium (Boogaerts et al., 2016) in which the national alcohol consumption rate estimated by the WBE approach was half that reported by the WHO. Such differences could be attributed to the fact that WHO data may not appropriately represent the actual consumption of alcohol by the population. WHO data are derived from production, import, export and sale data, which in countries where there is not a strict control, like Spain, can lead to an overestimation of consumption, since alcohol can be stored and not consumed shortly after purchase. In countries like

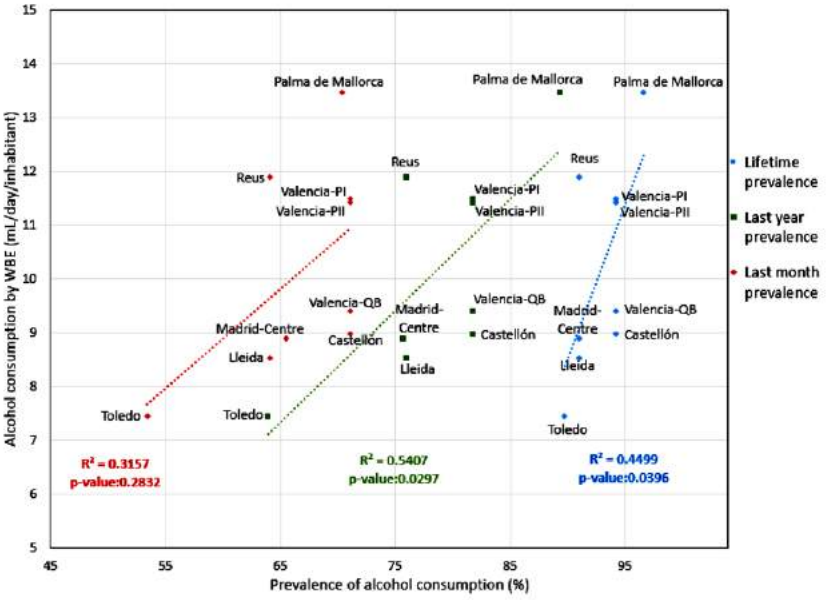


Fig. 4. Correlation between average alcohol consumption estimated in each city by WBE (mL/day/inhabitant) and prevalence data ("Lifetime prevalence", "Last year prevalence" and "Last month prevalence") reported by its region in the Annual Report of the Spanish Observatory on Drugs and Drugs Addiction 2019. (Data from Guadalajara, Barcelona, Tarragona, Madrid-North, Móstoles, Santiago de Compostela, and Bilbao were excluded; Spearman correlation p-value < 0.05 were considered statistically significant).

Norway, where sales statistics are among the most accurate in the world, a good correlation was obtained between WBE and WHO data (Reid et al., 2011).

As expected, the weekly consumption patterns in most populations showed an increase in alcohol consumption during the weekend. Saturday and Sunday were the days when alcohol consumption contributed the most to the total weekly consumption, with a median contribution of 20%, while the remaining days of the week contributed between 11% (Tuesday) and 14% (Monday) (Fig. 2b). Similar results were obtained in Australia, where each weekend day contributed with 20% to the weekly consumption rate, while the rest of the days of the week varied between 11% and 13% (Lai et al., 2018). The increase in alcohol consumption during the weekend was also reported in an international study conducted in 11 different countries worldwide (Baz-Lomba et al., 2016), in Norway (Reid et al., 2011), Belgium (Boogaerts et al., 2016; van Wel et al., 2016), and in Spain, where previous studies, far less ambitious than the present study, were done in Barcelona (Mastroianni et al., 2017, 2014), Santiago de Compostela (Rodríguez-Álvarez et al., 2015, 2014), and Valencia (Andrés-Costa et al., 2016). The increase of alcohol consumption during the weekend was also reported by the INE National Health Survey for all regions investigated in the present study in terms of consumption rate (see Table S6) (INE, 2017), so again, a good correlation was obtained between WBE approach and established indicators.

Despite the good correlation mostly obtained between WBE-derived data and those obtained with established indicators, the estimates of alcohol consumption through WBE are affected by some degree of uncertainty that should be taken into consideration. On the one hand, it has been shown that EtS is stable in wastewater (one week at room temperature and more than 1 month at −20 °C) (Rodríguez-Álvarez et al., 2014); however, EtS could degrade to some extent in sewage systems (Banks et al., 2018; Gao et al., 2018). This could lead to an underestimation of the real alcohol consumption, which could (partially) explain the lower consumption estimates obtained through WBE compared to those reported by the WHO. However, degradation can be corrected by applying a correction factor, as demonstrated in a recent study conducted in Australia (Zheng et al., 2020). On the other hand, the excretion rate used to back-calculate alcohol consumption was obtained from two studies in which only 10 men (Høiseth et al., 2008) and one man (Wurst et al., 2006) were investigated. Further studies involving more volunteers of different ages, gender, and race, or studying the excretion rate among the Spanish population could help to obtain a more representative excretion rate which would increase the accuracy of the back-calculations. An additional source of uncertainty may come from the sampling (collection of a not representative sample). In this study, WBE data have been obtained from samples collected during only one week, which may not be representative of alcohol consumption throughout the entire year. Increasing the sampling period, several times a year or during consecutive years could also be used to obtain temporal trends in alcohol consumption within one year and throughout the years. Furthermore, unlike the estimates at the national level, the differences observed in some regions between WBE-derived data and those reported by established indicators could indicate that population sampled are not representative of the whole region. Increasing the population sampled or sampling populations of different sizes within one region could lead to a more representative picture of the habits of consumption of the whole region. Finally, other sources of uncertainty may come from inaccurate measurement of the water volume entering the plant, and the calculation of the size of the population that contributes to the total EtS load measured in wastewater (Castiglioni et al., 2013). In the present study, the latter was assessed using different methods (census data, population connected to the WWTP, water quality parameters), following the recommendations provided by the WWTP experts to obtain the value that best reflects the population served by each WWTP.

Regardless of the aforementioned limitations, the WBE approach appears as a promising, convenient tool for alcohol consumption assessment, which surely needs to be refined in the next few years. WBE is much useful to establish spatial and temporal variations in alcohol consumption in a fast, objective, and inexpensive way, providing data in nearly real-time. WBE can complement in this way the information gained with the established methodologies which are also affected by some uncertainties. In this sense, the use of different indicators and sources of information would improve the alcohol consumption estimates and hence, contribute to the better development and evaluation of health care management plans and policies.

5. Conclusions

The present work represents the first nation-wide study conducted in Spain to evaluate alcohol consumption through the application of the WBE approach and is one of the very few nation-wide assessments available worldwide. The study has covered 13 main cities (in some cases including surrounding towns) that represent 12.8% of the Spanish total population. The results show that WBE is a useful tool to define spatial and temporal variations in alcohol consumption in a fast, objective, and inexpensive way, providing complementary data to the information gained with the established methodologies. The WBE-derived alcohol consumption data correlated well (within ± 15%) with official data reported by conventional methods at the regional level in 5 out of the 16 populations investigated (31% of the total population examined), and satisfactorily (within ± 30%) in 9 of the populations studied (accounting for 56% of the scrutinized population). Also, extrapolation of WBE-derived alcohol consumption estimates to the national territory led to an annual consumption of alcohol in Spain comparable to that reported for the country by the National Health Survey, although, lower than that reported by the WHO. The comparison of WBE data with those obtained with established consumption indicators should be done with caution because both methodologies are subject to some uncertainties. Increasing the sampling period, the sampled population, and conducting further studies on alcohol metabolism to establish appropriate correction factors would help to reduce the main uncertainties associated with WBE and, therefore, to improve the accuracy of the consumption estimates.

Contributors

Ester López-García – Sample and data collection and handling in Barcelona and Lleida wastewater treatment plants (WWTPs), analysis of ethyl sulfate in all the samples, writing of the first draft of the manuscript

Carlos Pérez-López – statistical data treatment

Cristina Postigo - Sample collection and handling in Barcelona and Lleida WWTPs, detailed revision of the first draft of the manuscript and submission of the manuscript

Miren López de Alda - Sample collection and handling in Barcelona and Lleida WWTPs, detailed revision of the first draft of the manuscript

Vicente Andreu and Yolanda Picó - Sample and data collection and handling in Valencia and its metropolitan area WWTPs, provision of critical feedback of the manuscript

Lubertus Bijlsma, and Felix Hernández - Sample and data collection and handling in Castellón and Madrid North WWTPs, provision of critical feedback of the manuscript

Iria González-Mariño, Rosa Montes, Rosario Rodil, José Benito Quintana - Sample and data collection and handling in Santiago the Compostela WWTP, provision of critical feedback of the manuscript

Rosa Maria Marcé and Eva Pocurull - Sample and data collection and handling in Tarragona and Reus WWTPs, provision of critical feedback of the manuscript

Andreu Rico and Yolanda Valcárcel - Sample and data collection and handling in Mostoles, Madrid South, Guadalajara, and Toledo WWTPs, provision of critical feedback of the manuscript

Olatz Zuloaga - Sample and data collection and handling in Basque Country WWTP and provision of critical feedback of the manuscript

María Rosende - Sample and data collection and handling in Palma de Mallorca WWTPs

All authors have read and approved the final version of the article.

The contributions of all authors have been declared in the contributor statement.

Funding

This work has been supported by the Spanish State Research Agency (AEI) (CTM2016-81935-REDT, CTM2017-84763-C3-1-R, CTM2017-84763-C3-3-R and CTM2017-84763-C3-2-R), Generalitat de Catalunya (expedient number SA-2018-780 and Consolidated Research Group 2017 SGR 01404-Water and Soil Quality Unit), and Galician Council of Culture, Education and Universities (ED481D 2017/003 and EM2014/004). Several of these projects are cofounded by the European Regional Development Fund.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Declaration of Competing Interest

None.

Acknowledgments

The authors would like to acknowledge all the city councils of the cities participating in this study for their collaboration as well as the operators of the WWTPs for their assistance in the collection of the wastewater samples, especially to: Cristian Mesa and Angela Vidal from Aigües de Barcelona (Barcelona WWTP), Jordi Palatsi from Aqualia (Lleida WWTP), María Dolores Mateo and Víctor Fernández from EMAYA (Palma de Mallorca WWTPs), Iñigo González Canal (Consorcio de Aguas de Bilbao Bizkaia), Viaqua and Concello de Santiago de Compostela, the Public Entity of Wastewater Treatment (EPSAR) of the Generalitat Valenciana and especially to Fernando Llavador, Luis Aceiton, Enrique Albors, Angel Jiménez, Maria José Tarrega, Sonia Tristante and all the personnel of the WWTPs (Aguas de Valencia, Spain), Sociedad de Fomento Agrícola Castellonense (FACSA, Castellón), and especially WWTP operators Santiago Querol and Sara Gargallo for providing wastewater samples from Castellón, Subdirección General de Gestión del Agua from Ayuntamiento de Madrid, for allowing the collection of samples at Madrid WWTPs, and the Metropolitan Area of Barcelona (AMB) as owner of the facilities of origin of the data of the Barcelona WWTP.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.drugalcdep.2020.108241.

References

Andrés-Costa, M.J., Escrivá, Ú., Andreu, V., Picó, Y., 2016. Estimation of alcohol consumption during "Fallas" festivity in the wastewater of Valencia city (Spain) using ethyl sulfate as a biomarker. Sci. Total Environ. 541, 616–622. https://doi.org/10.1016/j.scitotenv.2015.09.126.

Banks, A.P.W., Lai, F.Y., Mueller, J.F., Jiang, G., Carter, S., Thai, P.K., 2018. Potential impact of the sewer system on the applicability of alcohol and tobacco biomarkers in wastewater-based epidemiology. Drug Test. Anal. 10, 530–538. https://doi.org/10.1002/dta.2246.

Baz-Lomba, J.A., Salvatore, S., Gracia-Lor, E., Bade, R., Castiglioni, S., Castrignanò, E., Causanilles, A., Hernandez, F., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.-K., van Nuijs, A., Ort, C., Plósz, B.G., Ramin, P., Reid, M., Rousis, N.I., Ryu, Y., de Voogt, P., Bramness, J., Thomas, K., 2016. Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities. BMC Public Health 16, 1035. https://doi.org/10.1186/s12889-016-3686-5.

Boogaerts, T., Covaci, A., Kinyua, J., Neels, H., Van Nuijs, A.L.N., 2016. Spatial and temporal trends in alcohol consumption in Belgian cities: a wastewater-based approach. Drug Alcohol Depend. 160, 170–176. https://doi.org/10.1016/j.drugalcdep.2016.01.002.

Castiglioni, S., Bijlsma, L., Covaci, A., Emke, E., Hernández, F., Reid, M., Ort, C., Thomas, K.V., Van Nuijs, A.L.N., De Voogt, P., Zuccato, E., 2013. Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. Environ. Sci. Technol. 47, 1452–1460. https://doi.org/10.1021/es302722f.

Chen, J., Venkatesan, A.K., Halden, R.U., 2019. Alcohol and nicotine consumption trends in three U.S. Communities determined by wastewater-based epidemiology. Sci. Total Environ. 656, 174–183. https://doi.org/10.1016/j.scitotenv.2018.11.350.

EMCDDA - European Monitoring Centre for Drugs and Drug Addiction, 2016. Assessing

Illicit Drugs in Wastewater: Advances in Wastewater-based Drug Epidemiology. http://www.emcdda.europa.eu/system/files/publications/2273/TDXD16022ENC_4.pdf (Accessed 3.9.20).

Gao, J., Li, J., Jiang, G., Yuan, Z., Eaglesham, G., Covaci, A., Mueller, J.F., Thai, P.K., 2018. Stability of alcohol and tobacco consumption biomarkers in a real rising main sewer. Water Res. 138, 19–26. https://doi.org/10.1016/j.watres.2018.03.036.

Gao, J., Zheng, Q., Lai, F.Y., Gartner, C., Du, P., Ren, Y., Li, X., Wang, D., Mueller, J.F., Thai, P.K., 2020. Using wastewater-based epidemiology to estimate consumption of alcohol and nicotine in major cities of China in 2014 and 2016. Environ. Int. 136, 105492. https://doi.org/10.1016/j.envint.2020.105492.

Gatidou, G., Kinyua, J., van Nuijs, A.L.N., Gracia-Lor, E., Castiglioni, S., Covaci, A., Stasinakis, A.S., 2016. Drugs of abuse and alcohol consumption among different groups of population on the Greek Island of Lesvos through sewage-based epidemiology. Sci. Total Environ. 563–564, 633–640. https://doi.org/10.1016/j.scitotenv.2016.04.130.

González-Mariño, I., Baz-Lomba, J.A., Alygizakis, N.A., Andrés-Costa, M.J., Bade, R., Bannwarth, A., Barron, L.P., Been, F., Benaglia, L., Berset, J.-D., Bijlsma, L., Bodík, I., Brenner, A., Brock, A.L., Burgard, D.A., Castrignanò, E., Celma, A., Christophoridis, C.E., Covaci, A., Delémont, O., de Voogt, P., Devault, D.A., Dias, M.J., Emke, E., Esseiva, P., Fatta-Kassinos, D., Fedorova, G., Fytianos, K., Gerber, C., Grabic, R., Gracia-Lor, E., Grüner, S., Gunnar, T., Hapeshi, E., Heath, E., Helm, B., Hernández, F., Kankaanpaa, A., Karolak, S., Kasprzyk-Hordern, B., Krizman-Matasic, I., Lai, F.Y., Lechowicz, W., Lopes, A., López de Alda, M., López-García, E., Löve, A.S.C., Mastroianni, N., McEneff, G.L., Montes, R., Munro, K., Nefau, T., Oberacher, H., O'Brien, J.W., Oertel, R., Olafsdottir, K., Picó, Y., Plósz, B.G., Polesel, F., Postigo, C., Quintana, J.B., Ramin, P., Reid, M.J., Rice, J., Rodil, R., Salgueiro-González, N., Schubert, S., Senta, I., Simões, S.M., Sremacki, M.M., Styszko, K., Terzic, S., Thomaidis, N.S., Thomas, K.V., Tscharke, B.J., Udrisard, R., van Nuijs, A.L.N., Yargeau, V., Zuccato, E., Castiglioni, S., Ort, C., 2020. Spatio-temporal assessment of illicit drug use at large scale: evidence from 7 years of international wastewater monitoring. Addiction 115, 109–120. https://doi.org/10.1111/add.14767.

Helander, A., Beck, O., 2005. Ethyl sulfate: a metabolite of ethanol in humans and a potential biomarker of acute alcohol intake. J. Anal. Toxicol. 29, 270–274. https://doi.org/10.1093/jat/29.5.270.

Høiseth, G., Bernard, J.P., Stephanson, N., Normann, P.T., Christophersen, A.S., Mørland, J., Helander, A., 2008. Comparison between the urinary alcohol markers EtG, EtS, and GTOL/5-HIAA in a controlled drinking experiment. Alcohol Alcohol. 43, 187–191. https://doi.org/10.1093/alcalc/agm175.

INE - Instituto Nacional de Estadística, 2017. Consumo Medio Diario de Alcohol (en Gramos de Alcohol Puro) según Sexo y Comunidad Autónoma. Media y Desviación Típica. Población de 15 y más Años que Consume Bebidas Alcohólicas uno o más Días a la Semana. https://www.ine.es/jaxi/Tabla.htm?path=/t15/p419/a2017/p03/l0/&file=03011.px&L=0 (Accessed 1.23.20).

INE - Instituto Nacional de Estadística, 2018. Población Residente por Fecha, Sexo y Edad. https://www.ine.es/jaxiT3/Datos.htm?t=9663 (Accessed 3.3.20).

Lai, F.Y., Gartner, C., Hall, W., Carter, S., O'Brien, J., Tscharke, B.J., Been, F., Gerber, C., White, J., Thai, P., Bruno, R., Prichard, J., Kirkbride, K.P., Mueller, J.F., 2018. Measuring spatial and temporal trends of nicotine and alcohol consumption in Australia using wastewater-based epidemiology. Addiction 113, 1127–1136. https://doi.org/10.1111/add.14157.

MAPA - Ministerio de Agricultura, Pesca y Alimentación, 2018. Informe Socioeconómico del Sector de la Cerveza en España, 2018. https://cerveceros.org/uploads/5d1b32241b8be_InformeSocioeconomicoCerveza_2018.pdf (Accessed 11.28.19).

Mastroianni, N., López de Alda, M., Barcelo, D., 2014. Analysis of ethyl sulfate in raw wastewater for estimation of alcohol consumption and its correlation with drugs of abuse in the city of Barcelona. J. Chromatogr. A 1360, 93–99. https://doi.org/10.1016/j.chroma.2014.07.051.

Mastroianni, N., López-García, E., Postigo, C., Barceló, D., López de Alda, M., 2017. Five-year monitoring of 19 illicit and legal substances of abuse at the inlet of a wastewater treatment plant in Barcelona (NE Spain) and estimation of drug consumption patterns and trends. Sci. Total Environ. 609, 916–926. https://doi.org/10.1016/J.SCITOTENV.2017.07.126.

Nguyen, H.T., Thai, P.K., Kaserzon, S.L., O'Brien, J.W., Eaglesham, G., Mueller, J.F., 2018. Assessment of drugs and personal care products biomarkers in the influent and effluent of two wastewater treatment plants in Ho Chi Minh City, Vietnam. Sci. Total Environ. 631–632, 469–475. https://doi.org/10.1016/j.scitotenv.2018.02.309.

OEDA - Observatorio Español de las Drogas y las Adicciones, 2019. Informe 2019 Alcohol, Tabaco Y Drogas Ilegales En España. https://pnsd.sanidad.gob.es/profesionales/sistemasInformacion/informesEstadisticas/pdf/2019OEDA-INFORME.pdf. (Accessed 3.3.20).

Probst, C., Fleischmann, A., Gmel, G., Poznyak, V., Rekve, D., Riley, L., Rylett, M., Shield, K.D., Rehm, J., 2019. The global proportion and volume of unrecorded alcohol in 2015. J. Glob. Health 9. https://doi.org/10.7189/jogh.09.010421.

Reid, M.J., Langford, K.H., Mørland, J., Thomas, K.V., 2011. Analysis and interpretation of specific ethanol metabolites, ethyl sulfate, and ethyl glucuronide in sewage effluent for the quantitative measurement of regional alcohol consumption. Alcohol. Clin. Exp. Res. 35 (9), 1593–1599. https://doi.org/10.1111/j.1530-0277.2011.01505.x.

Rodríguez-Álvarez, T., Rodil, R., Cela, R., Quintana, J.B., 2014. Ion-pair reversed-phase liquid chromatography-quadrupole-time-of-flight and triple-quadrupole-mass spectrometry determination of ethyl sulfate in wastewater for alcohol consumption tracing. J. Chromatogr. A 1328, 35–42. https://doi.org/10.1016/j.chroma.2013.12.076.

Rodríguez-Álvarez, T., Racamonde, I., González-Mariño, I., Borsotti, A., Rodil, R., Rodríguez, I., Zuccato, E., Quintana, J.B., Castiglioni, S., 2015. Alcohol and cocaine co-consumption in two European cities assessed by wastewater analysis. Sci. Total Environ. 536, 91–98. https://doi.org/10.1016/j.scitotenv.2015.07.016.

Ryu, Y., Barceló, D., Barron, L.P., Bijlsma, L., Castiglioni, S., de Voogt, P., Emke, E., Hernández, F., Lai, F.Y., Lopes, A., López de Alda, M., Mastroianni, N., Munro, K., O'Brien, J., Ort, C., Plósz, B.G., Reid, M.J., Yargeau, V., Thomas, K.V., 2016. Comparative measurement and quantitative risk assessment of alcohol consumption through wastewater-based epidemiology: an international study in 20 cities. Sci. Total Environ. 565, 977–983. https://doi.org/10.1016/j.scitotenv.2016.04.138.

Stockwell, T., Zhao, J., Greenfield, T., Li, J., Livingston, M., Meng, Y., 2016. Estimating under- and over-reporting of drinking in national surveys of alcohol consumption: identification of consistent biases across four English-speaking countries. Addiction 111, 1203–1213. https://doi.org/10.1111/add.13373.

van Wel, J.H.P., Gracia-Lor, E., van Nuijs, A.L.N., Kinyua, J., Salvatore, S., Castiglioni, S., Bramness, J.G., Covaci, A., Van Hal, G., 2016. Investigation of agreement between wastewater-based epidemiology and survey data on alcohol and nicotine use in a community. Drug Alcohol Depend. 162, 170–175. https://doi.org/10.1016/j.drugalcdep.2016.03.002.

WHO - World Health Organization, 2018. Global status report on alcohol and health 2018. https://apps.who.int/iris/bitstream/handle/10665/274603/9789241565639-eng.pdf?ua=1 (Accessed 11.15.19).

Wurst, F.M., Dresen, S., Allen, J.P., Wiesbeck, G., Graf, M., Weinmann, W., 2006. Ethyl sulphate: a direct ethanol metabolite reflecting recent alcohol consumption. Addiction 101, 204–211. https://doi.org/10.1111/j.1360-0443.2005.01245.x.

Zheng, Q., Tscharke, B.J., Krapp, C., O'Brien, J.W., Mackie, R.S., Connor, J., Mueller, J.F., Thomas, K.V., Thai, P.K., 2020. New approach for the measurement of long-term alcohol consumption trends: application of wastewater-based epidemiology in an Australian regional city. Drug Alcohol Depend. 207. https://doi.org/10.1016/j.drugalcdep.2019.107795.

Supporting Information

Assessing alcohol consumption through wastewater-based epidemiology: Spain as case study

Ester López-García[1], Carlos Pérez López[1], Cristina Postigo[1*], Vicente Andreu[2], Lubertus Bijlsma[3], Iria González-Mariño[4,5], Félix Hernández[3], Rosa Maria Marcé[6], Rosa Montes[4], Yolanda Picó[2], Eva Pocurull[6], Andreu Rico[7], Rosario Rodil[4], María Rosende[8], Yolanda Valcárcel[9], Olatz Zuloaga[10], José Benito Quintana[4], Miren López de Alda[1*]

[1]Water, Environmental, and Food Chemistry Unit (ENFOCHEM), Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona
[2]Food and Environmental Safety Research Group (SAMA-UV), Desertification Research Centre (CIDE), CSIC-Generalitat Valenciana-University of Valencia, Valencia
[3]Environmental and Public Health Analytical Chemistry, Research Institute for Pesticides and Water, University Jaume I, Castellón
[4]Department of Analytical Chemistry, Nutrition and Food Sciences, Institute of Research on Chemical and Biological Analysis (IAQBUS), Universidade de Santiago de Compostela, Santiago de Compostela
[5]Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemical Sciences, University of Salamanca, Salamanca.
[6]Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira i Virgili, Tarragona
[7]IMDEA Water Institute, Science and Technology Campus of the University of Alcalá, Alcalá de Henares
[8]FI-TRACE group, Department of Chemistry, University of the Balearic Islands, Palma de Mallorca
[9]Research Group in Environmental Toxicology and Risk Assessment (TAyER), Medical specialties and Public Health, Faculty of Health Sciences, Rey Juan Carlos University, Madrid
[10]Department of Analytical Chemistry, Faculty of Science and Technology (UPV/EHU) & Plentzia Marine Station of Basque Country University (EHU/UPV), Basque Country

*__Corresponding Authors__: Cristina Postigo; Miren López de Alda
Institute of Environmental Assessment and Water Research (IDAEA-CSIC)
Department of Environmental Chemistry
C/ Jordi Girona 18-26, 08034 Barcelona, Spain.
cprqam@cid.csic.es; miren.lopezdealda@idaea.csic.es
Tel: +34-934-006-100, Fax: +34-932-045-904

Number of pages: 16

Number of tables: 6

Number of figures: 4

List of Tables

List of Figures

Table S1. Description of sampled WWTPs (name, population served, and locations/districts covered with main city in bold) and the sampling protocol carried out (location of autosampler, sampling mode, start time and period).

Regions	City[a]	WWTP name	Population served by the WWTPs	Method used to estimate the population served[b]	Locations/districts served by the WWTPs	Percentage of the main city covered by the WWTP[c]	Location of autosampler	Sampling mode[d]	Sampling start time	Sampling period
Balearic Islands	**Palma de Mallorca**	Palma I	406,492	Census 2017	Palma beach, Sant Jordi, El Pil·lari, Son Sant Joan airport, part of Palma de Mallorca	100	After fine screen	T (100 mL/ 15 min)	10:00	10/04/2018-16/04/2018
		Palma II	47,961	Census 2017	Palma de Mallorca (main part), Marratxí, Esporles, Bunyola and Son Castelló, Can Valero, Son Rosinyol industrial states		After fine screen	T (100 mL/ 15 min)	10:00	18/04/2018-24/04/2018
Basque Country	**Bilbao**	Galindo	860,237	Census 2016	Abanto-Zierbena, Alontsotegi, Arrigorriaga, Barakaldo, Barrika, Basauri, Berango, Bilbao, Derio, Erandio, Etxebarri, Galdakao, Getxo, Leioa, Lezama, Loiu, Ortuella, Portugalete, Santurtzi, Sestao, Sondika, Sopelana, Trapagaran, Ugao-Miravalles, Urduliz, Zamudio, Zaratamo, Zeberio	100	After coarse screens and pumping	T (100mL/ 60 min)	8:00	17/04/2018-23/04/2018

Castilla-La Mancha	**Toledo**	Estiviel	79,793	Average BOD April-May 2018	Toledo	100	After sieving	T (100 mL/ 15 min)	8:00	17/04/2018-23/04/2018
	Guadalajara	Guadalajara	94,755	Average BOD Jan-April 2018	Guadalajara	100	Before fine screen	T (200 mL/ 60 min)	10:00	02/05/2018-08/05/2018
Catalonia	**Barcelona**	Baix Llobregat	1,163,154	Census 2017	Barcelona, Cervelló, Cornellà de Llobregat, Esplugues de Llobregat, Hospitalet de Llobregat, El Prat de Llobregat, Sant Boi de Llobregat, San Joan Despí, San Just Desvern	35	Mechanical bar screens	T (50 mL/ 10min)	9:00	14/03/2018-20/03/2018
	Lleida	Lleida	143,612	Census 2017	Lleida , Alpicat	100	Before fine screen	T (200 mL/ 60 min)	6:00	07/03/2018-13/03/2018
	Reus	Reus	115,000	Census 2017	Reus , Castellvell, Almoster	100	After fine screen	F	20:00	17/04/2018-23/04/2018
	Tarragona	Tarragona	142,635	Census 2017	Tarragona , La Canonja, Els Pallaresos	100	Before fine screen	T (450 mL/ 60 min)	8:00-9:00	17/04/2018-23/04/2018
Community of Madrid	**Madrid**	Madrid-Centre	727,176	Average COD for the sampling period	Madrid-Center (Neighborhoods: Chamartín, Tetuán, Moncloa-Aravaca, Chamberí, Centro, Arganzuela, Retiro, Ciudad Lineal, Salamanca, Moratalaz, Puente de Vallecas).	30	After sieving	T (400 mL/ 30 min)	8:00	16/05/2018-22/05/2018

	Madrid	Madrid-North	227,869	Average BOD 2016 (with 60 g BOD/d)	Pozuelo y Madrid-North: (Neighborhoods: Chamartín, Tetuán, Moncloa, Aravaca, Fuencarral, El Pardo, Las Rozas, Majadahonda)		After fine screen	T (100 mL/ 60 min)	8:00	20/06/2018-26/06/2018
	Móstoles	El Soto	187,281	H x 3.5 (WWTP recomm.)	Móstoles, Alcorcón, Fuenlabrada	90	After fine screen	T (100 mL/ 60 min)	8:00	17/05/2018-23/05/2018
Galicia	**Santiago de Compostela**	Silvouta	136,500	H x 2.5 (WWTP recomm.)	Santiago de Compostela	100	After fine screen	T (150 mL/ 10 min)	9:00	13/03/2018-19/03/2018
Community of Valencia	**Castellón**	Castellón de la Plana	171,669	Census 2015	Castellón	100	Before fine screen	T (100 mL/ 15 min)	8:30	11/04/2018-17/04/2018
	Valencia	Pinedo I (Valencia-PI)	527,222	COD	Valencia (main part)	100	After fine screen	T (100 mL/ 60 min)	8:00	10/04/2018-16/04/2018
	Valencia	Pinedo II (Valencia-PII)	788,242	COD	Albal, Alcàsser, Alfafar, Benetúser, Beniparrell, Burjassot, Catarrojja, Llocnou de la Corona, Massanassa, Mislata, Paiporta, Paterna, Picanya, Picassent, Sedaví, Silla, Torrent, part of Valencia		After fine screen	T (100 mL/ 60 min)	8:00	10/04/2018-16/04/2018
	Valencia	Quart-Benager (Valencia-QB)	162,249	COD	Alaquàs, Aldaia, Manises, Mislata, Quart de Poblet, Xirivella		After fine screen	F	8:00	10/04/2018-16/04/2018

[a]Name of the main city served by the WWTPs (some WWTPs receive wastewater from other towns included in the capital metropolitan area). [b]BOD: Biochemical Oxygen Demand; COD: Chemical Oxygen Demand; H: Number of homes connected to the sewage system. WWTP recomm: following WWTP recommendations. [c]WWTPs serving parts of the same main city were considered all together for this calculation. [d]T: time-proportional (volume sampled/frequency of sampling); F: Flow-proportional

Table S2. Alcohol consumption rates estimated by means of WBE approach in different cities worldwide.

City (Country)	Alcohol consumption (mL/day/inhabitant)		Year	Reference
	Average	Range		
Ho Chi Minh (Vietnam)	3.1-3.9		2015	(Nguyen et al., 2018)
Lesvos (Greece)	3.4/5.4	1.7-7.2/2.2-11.2	2015	(Gatidou et al., 2016)
Valencia-PII (Spain)	3.3[a]	1.1-6.4[a]	2014	(Andrés-Costa et al., 2016)
Milan (Italy)	5.1	3.2-10.5	2012-2014	(Rodríguez-Álvarez et al., 2015)
	6.4	5.1-8.1	2014	(Ryu et al., 2016)
	6.6		2015	(Baz-Lomba et al., 2016)
Valencia-QB (Spain)	5.9[a]	3.3-12.8[a]	2014	(Andrés-Costa et al., 2016)
Valencia-PII[b] (Spain)	6.1[a]	4.3-9.1[a]	2014	(Andrés-Costa et al., 2016)
Valencia-PI (Spain)	6.2[a]	1.1-18.31[a]	2014	(Andrés-Costa et al., 2016)
Lugano (Switzerland)	6.5	4.5-8.4	2014	(Ryu et al., 2016)
Toowoomba (Australia)	9.7	6.9-14.5	2014	(Ryu et al., 2016)
Utrecht (The Netherlands)	10.8		2015	(Baz-Lomba et al., 2016)
	12.9	7.7-20.7	2014	(Ryu et al., 2016)
Santiago de Compostela (Spain)	13.6	3.8-22.6	2012-2014	(Rodríguez-Álvarez et al., 2015)
	16.3	9.3-23.5	2012	(Rodríguez-Álvarez et al., 2014)
Valencia-PII[b]	14.4[a]	4.9-23.8[a]	2014	(Andrés-Costa et al., 2016)
Almada (Portugal)	14.6	8.4-24.1	2014	(Ryu et al., 2016)
Canberra (Australia)	14.6	9.3-22.3	2014	(Ryu et al., 2016)
Zurich (Switzerland)	14.7		2015	(Baz-Lomba et al., 2016)
Bristol (The United Kingdom)	16.2		2015	(Baz-Lomba et al., 2016)
Berlin (Germany)	16.9	13.8-22.3	2014	(Ryu et al., 2016)
Oslo (Norway)	16.1		2009	(Reid et al., 2016)
	18.9		2015	(Baz-Lomba et al., 2016)
	19.2	8.8-52.9	2014	(Ryu et al., 2016)
Barcelona (Spain)	18[a]	7-31[a]	2011-2015	(Mastroianni et al., 2017)
Dülmen (Germany)	20.3	5.5-40	2014	(Ryu et al., 2016)
London (United Kingdom)	21.5	10.9-36	2014	(Ryu et al., 2016)
Brussels (Belgium)	21.6		2015	(Baz-Lomba et al., 2016)
Eindhoven (The Netherlands)	21.7	13.7-30.4	2014	(Ryu et al., 2016)
Amsterdam (The Netherlands)	22	14.3-30.5	2014	(Ryu et al., 2016)

Castellón (Spain)	23.4	11.6-61.6	2014	(Ryu et al., 2016)
Dortmund (Germany)	23.6	18.1-34	2014	(Ryu et al., 2016)
Munich (Germany)	29.5	0.5-47.4	2014	(Ryu et al., 2016)
Dresden (Germany)	29.4	15.1-91.7	2014	(Ryu et al., 2016)
Montreal (Canada)	29.2	21.8-38.8	2014	(Ryu et al., 2016)
Copenhagen (Denmark)	29.7		2015	(Baz-Lomba et al., 2016)
	40.2	24.6-74	2014	(Ryu et al., 2016)
Granby (Canada)	44.3	27.3-59.3	2014	(Ryu et al., 2016)
Valencia-QB[b]	40.9[a]	27.0-56.1[a]	2014	(Andrés-Costa et al., 2016)

[a]Alcohol consumption expressed in mL/day/inhabitant (aged 15+)
[b]Alcohol consumption rate during “Fallas festivity”

Table S3. Comparison of alcohol consumption between pairs of investigated populations (U Mann Whitney test p-values)[a].

	Barcelona	Bilbao	Castellón	Guadalajara	Lleida	Madrid-La China	Madrid-Viveros	Móstoles	Palma de Mallorca	Reus	Santiago de Compostela	Tarragona	Toledo	Valencia-PI	Valencia-PII
Bilbao	0.114														
Castellón	0.095	0.020*													
Guadalajara	0.389	0.045*	0.209												
Lleida	0.114	0.012*	0.789	0.287											
Madrid-La China	0.148	0.012*	0.855	0.389	0.729										
Madrid-Viveros	1.000	0.075	0.237	0.601	0.114	0.171									
Móstoles	0.855	0.389	0.045*	0.095	0.075	0.075	0.534								
Palma de Mallorca	0.925	0.060	0.075	0.237	0.070	0.070	0.855	0.789							
Reus	0.729	0.114	0.662	0.662	0.237	0.729	0.789	0.287	0.662						
Santiago de Compostela	0.171	0.012*	0.925	0.348	1.000	0.855	0.148	0.075	0.060	0.601					
Tarragona	0.389	0.662	0.114	0.209	0.070	0.095	0.287	0.534	0.389	0.171	0.075				
Toledo	0.070	0.012*	0.237	0.171	0.237	0.534	0.070	0.075	0.045*	0.114	0.389	0.060			
Valencia-PI	0.459	0.045*	0.237	0.662	0.237	0.389	0.729	0.389	0.348	0.925	0.287	0.209	0.171		
Valencia-PII	0.925	0.012*	0.171	0.729	0.148	0.171	0.601	0.237	0.601	0.789	0.114	0.237	0.075	1.000	
Valencia-QB	0.171	0.012*	0.789	0.459	0.601	0.855	0.209	0.075	0.095	0.789	0.662	0.095	0.209	0.459	0.348

[a]Firstly, a non-parametric test (Kruskal Wallis test) was applied in order to compare alcohol consumption among all investigated populations since the number of data per city was $n < 10$. Since $p < 0.05$, (Kruskal Wallis p-value = 0.0003887), the null hypothesis (H_0: alcohol consumption among all investigated populations is equal) was rejected and a U Mann Whitney test was applied to compare alcohol consumption between pairs of populations. False Discovery Rate (FDR) correction for multiple testing was applied to reduce the number of "false positive".

*$p < 0.05$, null hypothesis in U Mann Whitney test (H_0: alcohol consumption between pairs of populations is equal) is rejected.

Table S4. Comparison of alcohol consumption between pairs of regions (U Mann Whitney test p-values)[a].

	Castilla-La Mancha	Catalonia	Community of Madrid	Valencian Community	Galicia	Balearic Islands
Catalonia	0.088					
Community of Madrid	0.088	1.000				
Valencian Community	0.286	0.335	0.200			
Galicia	1.000	0.169	0.096	0.221		
Balearic Islands	0.029*	0.558	0.406	0.073	0.025*	
Basque Country	0.001*	0.073	0.020*	<0.001*	0.004*	0.025*

[a]Firstly, a Kruskal Wallis test was applied in order to compare alcohol consumption among all investigated regions since for 3 regions (Galicia, Balearic Islands and Basque Country), $n < 10$. As p-value < 0.05 (Kruskal Wallis p-value = 0.000588), the null hypothesis (H_0: alcohol consumption among all regions is equal) was rejected and a U Mann Whitney test was applied to compare alcohol consumption between pairs of regions. False Discovery Rate (FDR) correction for multiple testing was applied to reduce the number of "false positive".

*$p < 0.05$ and null hypothesis in U Mann Whitney (H_0: alcohol consumption between pairs of regions is equal) is rejected.

Table S5. Average alcohol consumption estimated in Spain through WBE.

	Alcohol consumption in the investigated populations	Alcohol consumption in Spain				
	Kg/day	Kg/day	L/day	L/year/ inhabitants	L/year/ inhabitants (aged 15+)	L/year/ inhabitants (aged 18+)
Tuesday	48187	376424	477090	3.7	4.4	4.6
Wednesday	50115	391487	496181	3.9	4.6	4.8
Thursday	55403	432792	548532	4.3	5.1	5.3
Friday	57734	451005	571616	4.5	5.3	5.5
Saturday	84030	656420	831965	6.5	7.7	8.0
Sunday	77172	602852	764071	6.0	7.1	7.3
Monday	62306	486721	616884	4.8	5.7	5.9
Average	*62135*	*485386*	*615191*	*4.8*	*5.7*	*5.9*
SD	*13597*	*106216*	*134621*	*1.1*	*1.2*	*1.3*

Table S6. Average alcohol consumption (mL/day/inhabitant (aged 15+)) in the investigated regions in this study and Spain reported by the National Health Survey (INE).

	Week (Mon-Sun)		Weekdays (Mon-Thurs)		Weekend (Frid-Sun)	
	Average	sd	Average	sd	Average	sd
Balearic Island	18	14	15	14	22	17
Basque Country	19	14	11	15	30	19
Castilla-La Mancha	13	13	7.5	13	20	17
Catalonia	16	13	10	13	23	17
Community of Madrid	14	16	8.0	16	21	18
Galicia	20	12	16	13	25	13
Valencian Community	14	11	8.5	12	22	15
Spain	*13*	*12*	*8.4*	*12*	*19*	*16*

Source: National Health Survey (INE, 2017).
https://www.ine.es/jaxi/Tabla.htm?path=/t15/p419/a2017/p03/l0/&file=03011.px&L=0

Figure S1. Map of Spain with the location of the samples WWTPs (regions are indicated in different colors).

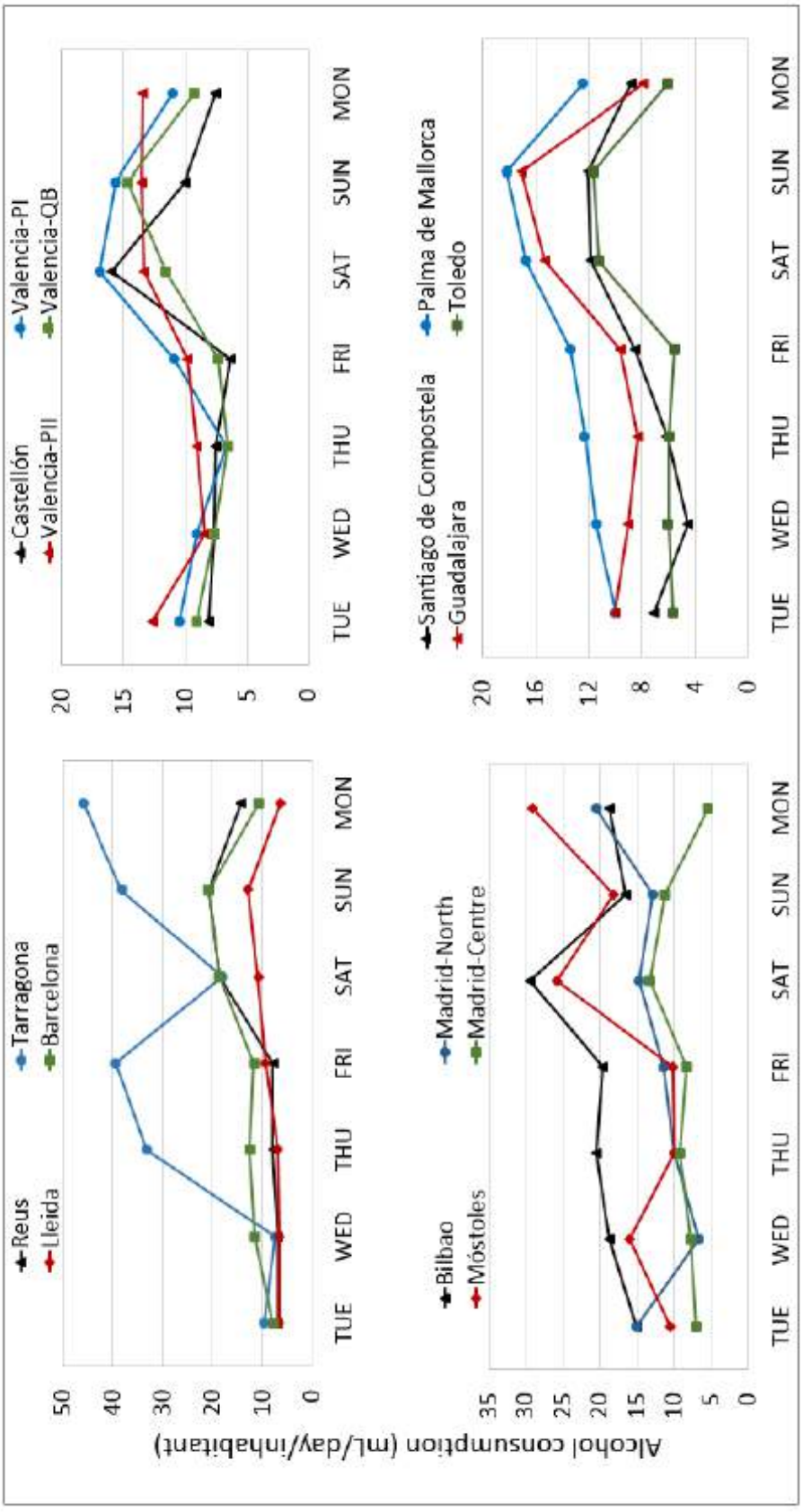


Figure S2. Weekly trends of alcohol consumption in the investigated populations.

13

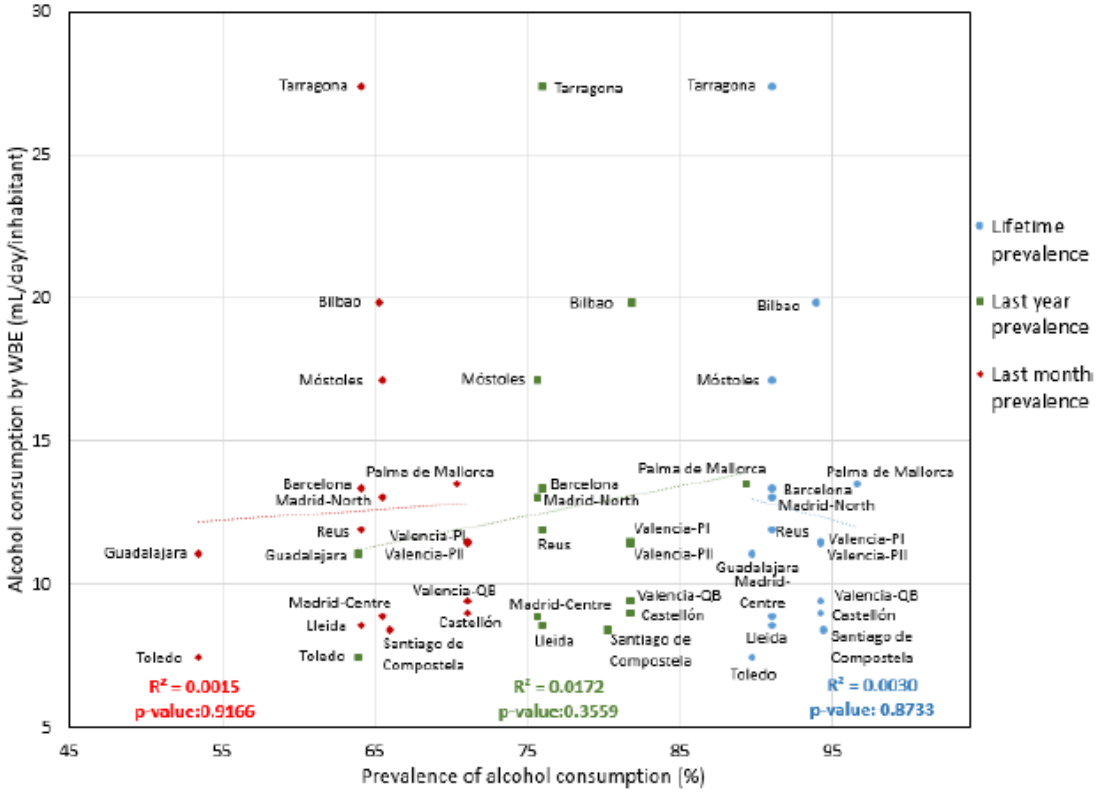


Figure S3. Correlation between average alcohol consumption estimated in each city by WBE (mL/day/inhabitant) and prevalence data ("Lifetime prevalence", "Last year prevalence" and "Last month prevalence") reported by its region in the annual Report of the Spanish Observatory on Drugs and Drugs Addiction 2019. (Data from all investigated populations are shown; Spearman correlation p-values < 0.05 were considered statistically significant).

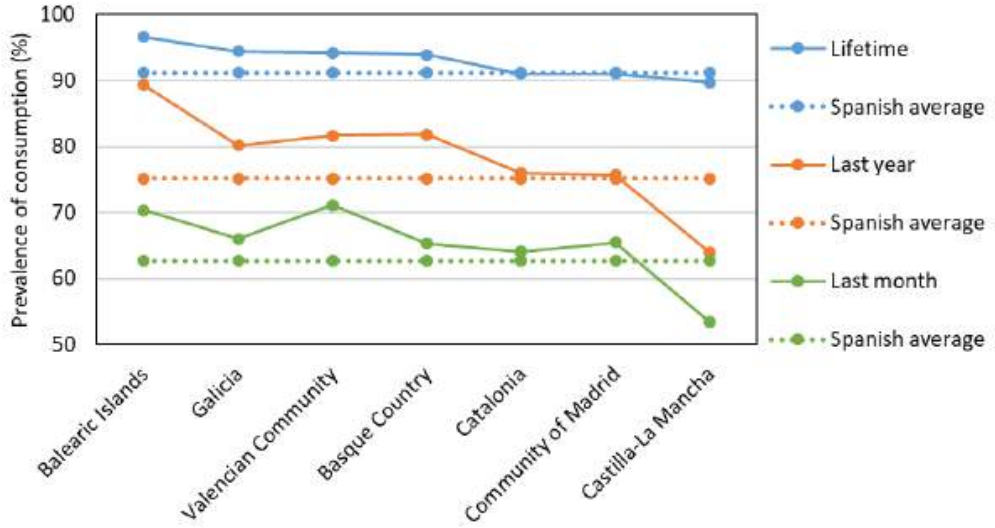


Figure S4. Prevalence data of alcohol consumption in the investigated regions and Spain reported in the Annual Report of the Spanish Observatory on Drugs and Drugs Addiction 2019.

References

Andrés-Costa, M.J., Escrivá, Ú., Andreu, V., Picó, Y., 2016. Estimation of alcohol consumption during "Fallas" festivity in the wastewater of Valencia city (Spain) using ethyl sulfate as a biomarker. Sci. Total Environ. 541, 616–622. https://doi.org/10.1016/j.scitotenv.2015.09.126

Baz-Lomba, J.A., Salvatore, S., Gracia-Lor, E., Bade, R., Castiglioni, S., Castrignanò, E., Causanilles, A., Hernandez, F., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.-K., van Nuijs, A., Ort, C., Plósz, B.G., Ramin, P., Reid, M., Rousis, N.I., Ryu, Y., de Voogt, P., Bramness, J., Thomas, K., 2016. Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities. BMC Public Health 16, 1035. https://doi.org/10.1186/s12889-016-3686-5

Gatidou, G., Kinyua, J., van Nuijs, A.L.N., Gracia-Lor, E., Castiglioni, S., Covaci, A., Stasinakis, A.S., 2016. Drugs of abuse and alcohol consumption among different groups of population on the Greek Island of Lesvos through sewage-based epidemiology. Sci. Total Environ. 563–564, 633–640. https://doi.org/10.1016/j.scitotenv.2016.04.130

Mastroianni, N., López-García, E., Postigo, C., Barceló, D., López de Alda, M., 2017. Five-year monitoring of 19 illicit and legal substances of abuse at the inlet of a wastewater treatment plant in Barcelona (NE Spain) and estimation of drug consumption patterns and trends. Sci. Total Environ. 609, 916–926. https://doi.org/10.1016/j.scitotenv.2017.07.126

Nguyen, H.T., Thai, P.K., Kaserzon, S.L., O'Brien, J.W., Eaglesham, G., Mueller, J.F., 2018. Assessment of drugs and personal care products biomarkers in the influent and effluent of two wastewater treatment plants in Ho Chi Minh City, Vietnam. Sci. Total Environ. 631–632, 469–475. https://doi.org/10.1016/j.scitotenv.2018.02.309

Reid, M.J., Langford, K.H., Mørland, J., Thomas, K. V., 2011. Analysis and Interpretation of Specific Ethanol Metabolites, Ethyl Sulfate, and Ethyl Glucuronide in Sewage Effluent for the Quantitative Measurement of Regional Alcohol Consumption. Alcohol. Clin. Exp. Res. no-no. https://doi.org/10.1111/j.1530-0277.2011.01505.x

Rodríguez-Álvarez, T., Racamonde, I., González-Mariño, I., Borsotti, A., Rodil, R., Rodríguez, I., Zuccato, E., Quintana, J.B., Castiglioni, S., 2015. Alcohol and cocaine co-consumption in two European cities assessed by wastewater analysis. Sci. Total Environ. 536, 91–98. https://doi.org/10.1016/j.scitotenv.2015.07.016

Rodríguez-Álvarez, T., Rodil, R., Cela, R., Quintana, J.B., 2014. Ion-pair reversed-phase liquid chromatography-quadrupole-time-of-flight and triple-quadrupole-mass spectrometry determination of ethyl sulfate in wastewater for alcohol consumption tracing. J. Chromatogr. A 1328, 35–42. https://doi.org/10.1016/j.chroma.2013.12.076

Ryu, Y., Barceló, D., Barron, L.P., Bijlsma, L., Castiglioni, S., de Voogt, P., Emke, E., Hernández, F., Lai, F.Y., Lopes, A., López de Alda, M., Mastroianni, N., Munro, K., O'Brien, J., Ort, C., Plósz, B.G., Reid, M.J., Yargeau, V., Thomas, K. V., 2016. Comparative measurement and quantitative risk assessment of alcohol consumption through wastewater-based epidemiology: An international study in 20 cities. Sci. Total Environ. 565, 977–983. https://doi.org/10.1016/j.scitotenv.2016.04.138

Capítulo 4.

Discusión de resultados

4.1. Metodologías analíticas

En esta tesis se han validado tres metodologías analíticas para el análisis de drogas, psicofármacos y algunos de sus metabolitos en agua residual, sedimentos y mejillones. La Tabla 6 muestra los analitos investigados en cada una de las matrices estudiadas.

Tabla 6. Analitos incluidos en cada una de las metodologías analíticas desarrolladas.

Analito	Agua residual	Sedimento	Biota (mejillón)
Cocaína	✓	✓	✓
Benzoilecgonina	✓	✓	✓
Cocaetileno	✓	✓	✓
Anfetamina	✓	✓	✓
Metanfetamina	✓	✓	✓
MDMA	✓	✓	✓
Ketamina	✓	✗	✓
LSD	✓	✓	✓
OH-LSD	✗	✓	✗
Heroína	✓	✓	✗
6-monoacetilmorfina	✓	✓	✓
Morfina	✓	✓	✓
Metadona	✓	✓	✓
EDDP	✓	✓	✓
THC	✗	✓	✓
THC-COOH	✓	✗	✓
OH-THC	✓	✓	✓
Cannabidiol	✗	✓	✗
Cannabinol	✗	✓	✗
Cafeína	✓	✗	✓
Efedrina	✓	✓	✓
MDPV	✓	✗	✓
Mefedrona	✓	✗	✓
Metoxetamina	✓	✗	✗
AH-7921	✓	✗	✓
Citalopram	✓	✗	✓
Fluoxetina	✓	✗	✓
Sertralina	✓	✗	✓
Venlafaxina	✓	✗	✓
Alprazolam	✓	✓	✓
OH-alprazolam	✓	✗	✓
Diazepam	✓	✓	✓
Lorazepam	✓	✗	✗
Lormetazepam	✓	✗	✓

Tabla 6 (cont). Analitos incluidos en cada una de las metodologías analíticas desarrolladas.

Analito	Agua residual	Sedimento	Biota (mejillón)
Midazolam	✓	✗	✓
OH-midazolam	✓	✗	✓
Oxazepam	✓	✗	✓
Temazepam	✓	✗	✓
Zolpidem	✓	✗	✓
Clorpromacina	✓	✗	✓
Hidroxicina	✓	✗	✓

La inclusión o no de un compuesto en una metodología tiene que ver, aparte de con las características del compuesto y de la matriz, que permiten su análisis o no en la mismas, con la época en la que se desarrolló el estudio, ya que con el tiempo se ha ido ampliando progresivamente el número de compuestos investigados.

La metodología desarrollada para agua (publicación #3) mostró ser lineal (coeficientes de determinación superiores a 0,99) entre 0,1 ng/L o el límite de cuantificación (Tabla S2, publicación #3) y 2 µg/L, extendiéndose hasta 10,25 µg/L en el caso de la cafeína. El 22% de los compuestos (anfetamina, THC-COOH, OH-alprazolam, lorazepam, lormetazepam, oxazepam, temazepam y cafeína) presentaron recuperaciones absolutas en agua de grado HPLC superiores al 70% (Figura 6). El resto de compuestos mostraron recuperaciones más bajas, entre el 30 y 70%, el 68% de los compuestos investigados, e inferiores al 30% el resto de compuestos (OH-THC, fluoxetina, sertralina y clorpromacina). En agua residual, las recuperaciones absolutas fueron comparativamente inferiores, con solo el 19% de los compuestos mostrando recuperaciones absolutas por encima del 70% (benzoilecgonina, THC-COOH, OH-alprazolam, lorazepam, lormetazepam, oxazepam y temazepam), el 59% entre 30 y 70%, y el 26% por debajo de 30% (morfina, metadona, EDDP, OH-THC, citalopram, fluoxetina, sertralina y clorpromacina) (Figura 6). Las recuperaciones absolutas en agua residual están afectadas tanto por la extracción como por el efecto matriz. Sin embargo, las recuperaciones absolutas obtenidas en agua de grado HPLC sólo están afectadas por la eficacia de la extracción. En base a esto, los compuestos peor extraídos con el cartucho seleccionado (PLRP-s: sorbente polimérico de estireno-divinilbenceno reticulado) fueron OH-THC, fluoxetina, sertralina y clorpromacina. Estos compuestos, presentan un carácter marcadamente hidrofóbico (log K_{ow} entre 4,1 y 9,3, ver Tabla 1, sección 1.1.), por lo que su recuperación se podría favorecer con el empleo de cartuchos más lipofílicos (por ejemplo, con relleno C8 o C18). Sin embargo, dado que el sistema de extracción para realizar la SPE on-line (Symbiosis™ Pico) no permite utilizar dos cartuchos en serie (en SPE

off-line se pueden hacer extracciones en tándem con varios cartuchos), para mejorar la extracción de estos compuestos sería necesario llevar a cabo dos análisis por muestra (uno con el cartucho PLRP-s y otro con el cartucho C8/C18). La determinación simultánea de todos los analitos objeto de estudio en un único análisis justifica sin embargo comprometer la sensibilidad de estos compuestos. En cuanto al efecto matriz, se observó que los compuestos más afectados (ordenados de mayor a menor) fueron OH-THC, seguido de fluoxetina, sertralina, benzoilecgonina, metadona, morfina, efedrina, anfetamina, cafeína, metanfetamina y mefedrona, que vieron suprimida su señal entre -65% y -21%, respectivamente, y el THC-COOH, cuya señal aumentó un 22% por la composición de la matriz (Figura 6).

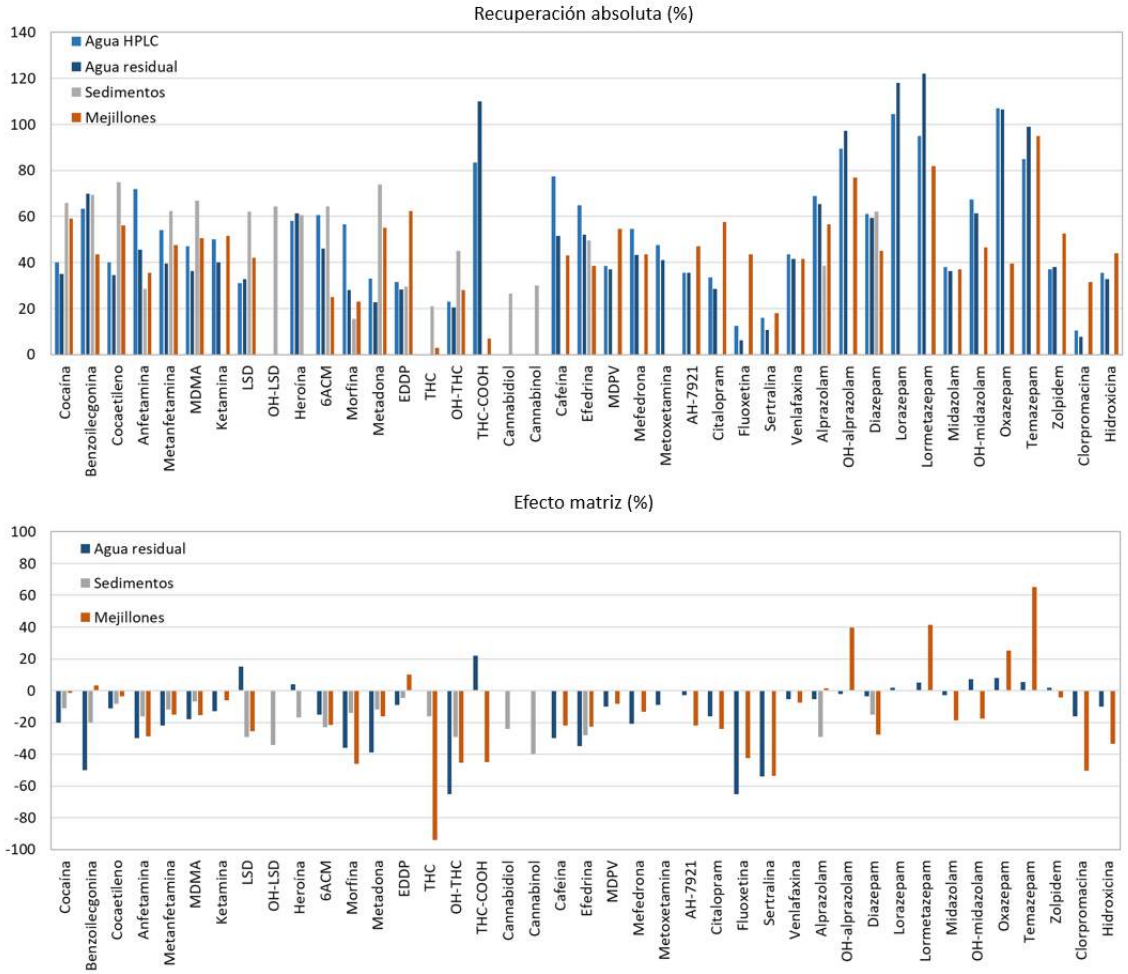


Figura 6. Recuperación absoluta media (%) y efecto matriz (%) obtenidos para drogas, psicofármacos y algunos de sus metabolitos en las tres matrices ambientales investigadas.

A pesar de que las recuperaciones absolutas no fueron satisfactorias para todos los compuestos y que hubo efecto matriz para muchos de ellos, las recuperaciones relativas obtenidas tanto en agua de grado HPLC (88-120%) como en agua residual (80-120%), indican que el empleo de patrones internos marcados isotópicamente permitió corregir ambos aspectos y, que la metodología analítica desarrollada es, por tanto, adecuada para el análisis de estos compuestos en agua. Esto último, se ha confirmado con los buenos resultados obtenidos en los ejercicios inter-laboratorio a los que se somete la metodología analítica anualmente desde el año 2011 (van Nuijs y cols., 2018) en el marco de la red internacional SCORE. En estos ejercicios se analiza cocaína, benzoilecgonina, anfetamina, MDMA, metanfetamina, 6ACM y THC-COOH en soluciones de metanol y en muestras de agua dopadas con los analitos a distintas concentraciones. La Figura 7 muestra los resultados obtenidos en agua para el caso concreto de cocaína en los ejercicios inter-laboratorio realizados en el periodo 2013-2016.

En cuanto a la sensibilidad, los límites de detección (LODs) y cuantificación (LOQs) del método en agua de grado HPLC fueron inferiores o iguales a 0,5 ng/L (81% de los compuestos) y 1 ng/L (86% de los compuestos), respectivamente. Únicamente lorazepam, morfina (LODs y LOQs de 1 y 5 ng/L, respectivamente), OH-alprazolam (LOD y LOQ de 2 y 5 ng/L, respectivamente) y THC-COOH y OH-THC (LODs y LOQs de 7 y 10 ng/L, respectivamente) mostraron LODs y LOQs superiores. En el caso del OH-THC, la baja sensibilidad se puede deber principalmente a la poca recuperación absoluta (inferior al 30%). Sin embargo, en el resto de casos, dado que las recuperaciones absolutas fueron elevadas, entre 87 y 113%, a excepción de morfina y THC-COOH, que variaron entre 54 y 100%, la baja sensibilidad de estos compuestos se puede atribuir principalmente a una ionización deficiente de los compuestos en la fuente. En agua residual debido a la composición de la matriz, los LODs y LOQs, como cabría esperar, fueron superiores. La mayoría de compuestos presentaron LODs inferiores o iguales a 5,1 ng/L, y LOQs inferiores o iguales a 16 ng/L, a excepción del oxazepam (LOD y LOQ 6,6 y 22 ng/L, respectivamente), lorazepam (LOD y LOQ de 6,8 y 23 ng/L, respectivamente), lormetazepam (LOD y LOQ de 10 y 32 ng/L, respectivamente), cafeína (LOD y LOQ de 13 y 44 ng/L, respectivamente), THC-COOH (LOD y LOQ de 18 y 61 ng/L, respectivamente) y OH-THC (LOD y LOQ de 69 y 228 ng/L, respectivamente). Comparando con otras metodologías multi-residuo utilizadas para el análisis de drogas, psicofármacos y metabolitos en agua residual (Tabla 7), esta metodología permite la determinación de los compuestos objeto de estudio a niveles similares, pero con la ventaja de necesitar solamente un volumen de muestra de 10 mL, frente a los hasta 100 mL o más que se utilizan en otras metodologías. Esto es así gracias al acoplamiento on-line SPE-LC-MS/MS en donde toda la muestra extraída (5 mL) es analizada, mientras que, en el resto

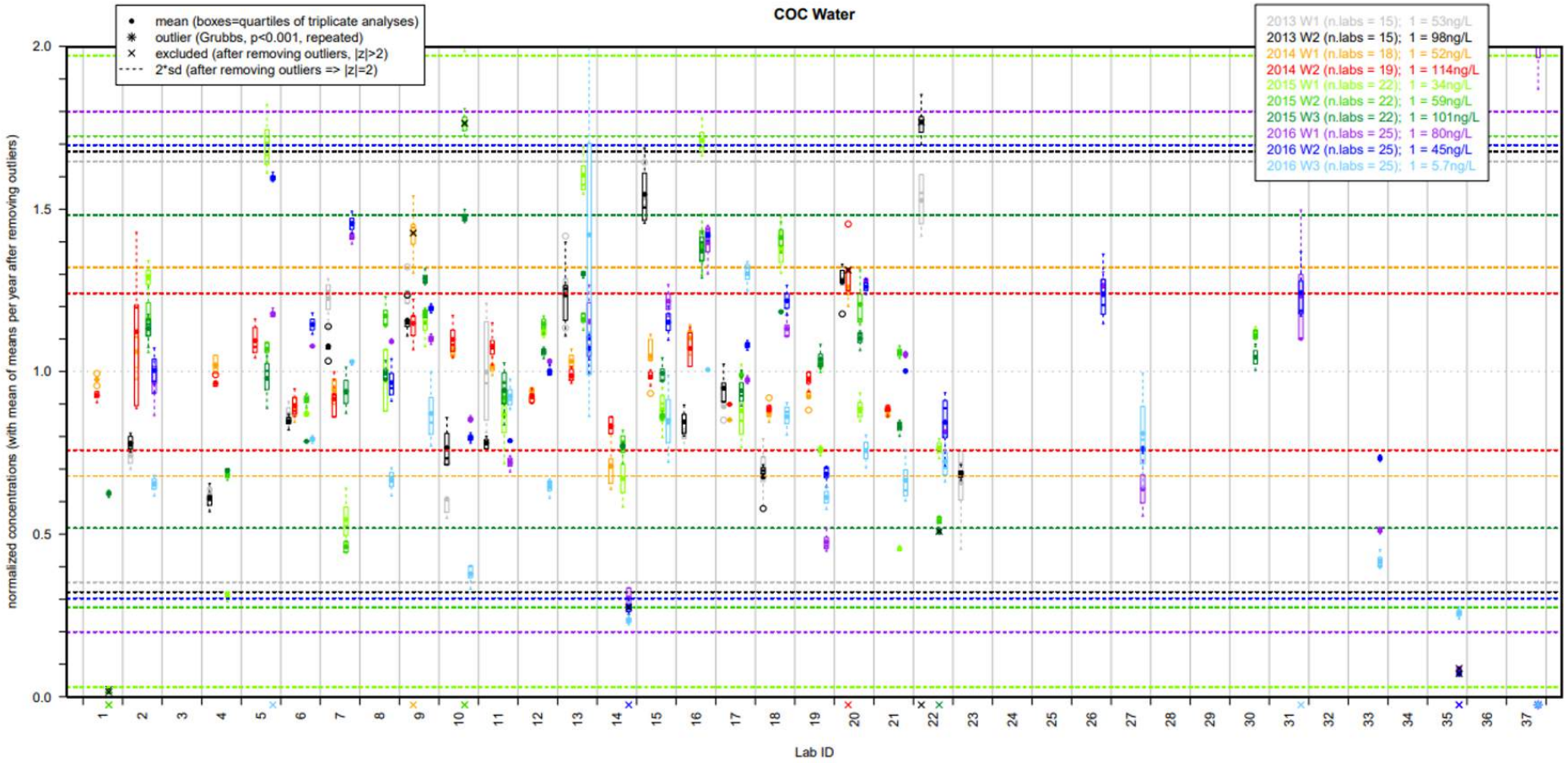


Figura 7. Resultados obtenidos para cocaína en el ejercicio inter-laboratorio realizado entre 2013 y 2016 en una muestra de agua (código de identificación 9). Fuente: (van Nuijs y cols., 2018).

de metodologías únicamente una porción del extracto obtenido (entre 1,5 y 20 µL) se inyecta en el sistema de análisis. El requerimiento de un volumen de muestra menor facilita tanto su recogida, como su transporte y almacenamiento. Otra ventaja de esta metodología es que todo el proceso, incluida la extracción y el análisis, se realiza en 47 minutos, mientras que, en otras metodologías únicamente el análisis LC-MS/MS ya necesita un tiempo superior. Además, la completa automatización del proceso favorece la obtención de resultados más precisos (RSD < 20%).

Además de la metodología descrita en la publicación #3, la metodología publicada por Fedorova y cols. (2013) para la determinación de drogas, psicofármacos y metabolitos en agua también se basa en on-line SPE-LC-MS. En dicha metodología en lugar de cartuchos desechables, se utiliza una columna de extracción reutilizable. Este tipo de metodología, se diferencia de la metodología descrita en la publicación #3, en que los blancos, las soluciones de calibrado, las muestras y las soluciones control se extraen usando la misma columna, lo que podría generar un efecto memoria o contaminación de las muestras y dar lugar a falsos positivos. Para minimizar esto, una vez han eluido todos los compuestos de la columna de extracción, la fase móvil (compuesta mayoritariamente por disolvente orgánico) se pasa a través de la columna de extracción para aumentar su lavado. Otra desventaja de esta metodología comparada con la metodología descrita en la publicación #3, es que la cantidad máxima de muestra que se puede extraer con el sistema utilizado es de 1 mL. Esto puede explicar la menor sensibilidad del método. De hecho, de los 15 compuestos que se investigan en común con ambas metodologías, más de la mitad (cocaína, benzoilecgonina, anfetamina, ketamina, LSD, EDDP, midazolam, y venlafaxina) presentan mejores LOQs con la metodología descrita en la publicación #3.

Tabla 7. Metodologías analíticas desarrolladas recientemente para el análisis de drogas, psicofármacos y algunos de sus metabolitos en agua residual.

Compuestos investigados[a]	Preparación de la muestra		Análisis			LOQ[b]		Ref.
	Pretratamiento	Extracción	LC-MS	V (µL) inyección	Tiempo (min)	Agua HPLC	Agua residual	
37 drogas, psicofármacos y metabolitos	10 mL muestra + agua HPLC (1:10, v/v) + IS + centrifugación	online SPE (PLRP-s)	HPLC-(ESI+)-MS/MS (QqLIT)	5.000	47 min[c]	0,1-10	0,6-61[d]	**Publicación #3**
32 drogas, psicofármacos y metabolitos (21)	100 mL + centrifugación + filtración (FV: 1,2 µm) + IS	SPE (Oasis HLB)	HPLC-(ESI+)-MS/MS (QqQ)	1,5	21	0,50-16		(Skees y cols., 2018)
38 drogas, psicofármacos y metabolitos (23)	100 mL muestra + IS + filtración (0,7 µm FV + lavado filtro 2 x 5 mL MeOH)	SPE (Oasis MCX)	UPLC-(ESI+)-MS/MS (QqQ)	2	8 min	0,2-22	0,3-83	(González-Mariño y cols., 2018)
89 drogas, psicofármacos y metabolitos (28)	**A**: 25 mL muestra + 25 mL MeOH + evaporación (2-5 mL) + 10 mL AcEt + evaporación (0,5 mL) + dilución (1 mL); **B**: 15 mL muestra + extracción líquido-líquido (60 min agitación, 3 x 30 mL AcEt) + evaporación (2-5 mL) + 10 mL AcEt + 1 mL agua + centrifugación + evaporación (casi sequedad) + dilución hasta 0,5 mL (MeOH:agua (1:1 v/v)) + filtración		HPLC-(ESI+)-MS/MS (QqQ)	10	40		**A**: 0,4-800 **B**: 0,3-667	(Asimakopoulos y cols., 2017)

Tabla 7 (cont). Metodologías analíticas desarrolladas recientemente para el análisis de drogas, psicofármacos y algunos de sus metabolitos en agua residual.

Compuestos investigados[a]	Preparación de la muestra		Análisis			LOQ[b]		Ref.
	Pretratamiento	Extracción	LC-MS	V (µL) inyección	Tiempo (min)	Agua HPLC	Agua residual	
148 drogas, psicofármacos y metabolitos (26)	50 mL muestra + IS + filtración (FV: 0,7 µm) + acidificación (pH: 2,5)	SPE (Strata-X)	UHPLC-(ESI+/-)-MS/MS (QqQ)	10	ESI+: 45 min ESI-: 37 min		2,6-263	(Thomaidis y cols., 2016)
51 drogas, psicofármacos y metabolitos (24)	100 mL muestra + IS	Auto SPE (discos HLB, 47 mm d.i.)	UPLC-(ESI+)-MS (QTOF)	5	15 min		0,4-85,6	(J. A. Baz-Lomba y cols., 2016)
68 drogas, psicofármacos y metabolitos (24)	50 mL muestra + filtración (FV: 0,7 µm) + acidificación (pH: 2,5) + IS	SPE (Strata-XC)	UPLC-(ESI+/-)-MS/MS (QqQ)	10	ESI+: 65 min ESI-: 32 min		0,24-189	(Borova y cols., 2014)
27 drogas, psicofármacos y metabolitos (15)	10 mL muestra + filtración + IS	on-line SPE: (columna C18: Hypersil Gold, 20 mm x 2,1 mm, d.i., 12 µm)	HPLC-(ESI+)-MS/MS (QqQ) HPLC-(ESI+)-MS (Q-Exactive, operando tanto en FS y PS)	1.000	15 min[c]	QqQ: 2,2-24 HRFS: 0,5-54 HRPS: 1,5-8,6		(Fedorova y cols., 2013; Mackul'ak y cols., 2016)

Tabla 7 (cont). Metodologías analíticas desarrolladas recientemente para el análisis de drogas, psicofármacos y algunos de sus metabolitos en agua residual.

Compuestos investigados[a]	Preparación de la muestra		Análisis			LOQ[b]		Ref.
	Pretratamiento	Extracción	LC-MS	V (µL) inyección	Tiempo (min)	Agua HPLC	Agua residual	
65 drogas, psicofármacos y metabolitos (20)	100 mL muestra + filtración (FV 2,7 µm + 0,7 µm) + acidificación (pH 1,8-1,9) + IS	SPE (Oasis MCX)	UPLC-(ESI+)-MS/MS (QqQ)	20	34 min		0,6-140	(Baker y cols., 2011a)

[a] Entre paréntesis se indica el número de compuestos investigados en la presente tesis que también se analizan en las otras metodologías multi-residuo

[b] Los límites de cuantificación (LOQs) indicados corresponden únicamente a los compuestos investigados en la presente tesis

[c] Incluye tanto el tiempo de extracción de la muestra como el del análisis LC-MS

[d] Rango de LOQs de todos los analitos investigados excepto OH-THC que tiene un LOQ de 228 ng/L

AcEt: acetato de etilo; d.i.: diámetro interno; FS: barrido completo (del inglés *Full Scan*); FV: fibra de vidrio; HLB: sorbente polimérico de equilibrio hidrofílico-lipofílico (del inglés *Hydrophilic-Lipophilic balanced*); IS: patrón interno (del inglés I*nternal Standard*); MCX: sorbente polimérico mixto de intercambio catiónico-fase reversa (del inglés *Mixed mode Cation eXchange-reversed phase*); MeOH: metanol; MS: espectrometría de masas (de inglés *Mass Spectrometry*); PS: barrido de iones producto (del inglés *Product Ion Scan)*; QqQ: triple cuadrupolo; SPE: extracción en fase sólida (del inglés *Solid Phase Extraction);* Strata-X: cartucho polimérico de fase reversa; Strata-XC: cartucho polimérico de fase reversa-intercambio catiónico; UHPLC o UPLC: cromatografía líquida de ultra-alta eficacia (del inglés *Ultra-High Performance Liquid Chromatography*).

En cuanto a las metodologías desarrolladas para muestras ambientales sólidas, se desarrollaron dos, una basada en PLE-SPE y análisis con LC-MS/MS, y otra basada en extracción con QuEChERS y análisis con LC-MS/MS para la determinación de drogas, psicofármacos y metabolitos en sedimentos y mejillones, respectivamente. Ambas metodologías se validaron en términos de linealidad, recuperación, repetibilidad, efecto matriz, y sensibilidad.

Al igual que en agua, las recuperaciones absolutas de los analitos fueron relativamente bajas. En el caso de sedimentos, la mayoría de compuestos presentaron recuperaciones absolutas entre 30 y 78%, siendo los compuestos con peor recuperación (inferior al 32%) anfetamina, morfina, EDDP, THC, cannabinol y cannabidiol (Figura 6). En el caso de mejillones, los compuestos que presentaron peor recuperación (inferior al 30%) fueron 6-monoacetilmorfina, morfina (al igual que en agua residual y sedimentos), THC (al igual que en sedimentos, en agua no se analizó), OH-THC (al igual que en agua), THC-COOH, y sertralina (al igual que en agua, en sedimentos no se analizó). El resto de compuestos presentaron recuperaciones entre 30 y 67%, y únicamente OH-alprazolam, lormetazepam y temazepam presentaron recuperaciones absolutas superiores al 70% (al igual que en agua). Las bajas recuperaciones de morfina en las tres matrices investigadas se pueden atribuir a su elevada polaridad y, en consecuencia, a su escasa retención en la columna analítica utilizada (C18); el empleo de columnas con partículas de sílica modificadas para incrementar la retención de compuestos polares o incluso de columnas HILIC podría favorecer su análisis. En el caso de la metodología en aguas, esta misma circunstancia sería la que llevaría a una pérdida del analito en el proceso de extracción consecuencia de su baja afinidad con el sorbente utilizado.

Al igual que en agua residual, el análisis de drogas, psicofármacos y metabolitos en sedimentos y mejillones estuvo afectado por la matriz. En sedimentos, 45% de los compuestos (ordenados de menor a mayor efecto matriz: benzoilecgonina, 6-monoacetilmorfina, cannabidiol, efedrina, LSD, alprazolam, OH-THC, OH-LSD, y cannabinol) presentaron supresión de la señal por la matriz (entre -20% y -40%). En mejillones también el 46% de los compuestos (ordenados de menor a mayor: 6-monoacetilmorfina, AH-7921, cafeína, efedrina, citalopram, LSD, diazepam, anfetamina, hidroxicina, fluoxetina, THC-COOH, OH-THC, morfina, clorpromacina, sertralina, y THC) presentaron supresión de la señal por la matriz, en algún caso de hasta el 94% (THC), y el 11% de los compuestos investigados (oxazepam, OH-alprazolam, lormetazepam, y temazepam) presentaron incremento de la señal por la matriz (entre 25 y 65%). Esto último podría explicar el hecho de que estos tres compuestos presentaran recuperaciones absolutas en mejillones superiores al 70%. Aunque no es estrictamente correcto comparar el efecto matriz obtenido con las técnicas de PLE+SPE y QuEChERS debido a que se han extraído matrices diferentes, en otros estudios en que se comparó la extracción de fármacos mediante

PLE y QuEChERS utilizando la misma matriz también se observó que el efecto matriz era superior con el empleo de QuEChERS (Alvarez-Muñoz y cols., 2015; Carmona y cols., 2017; Huerta y cols., 2013). En la PLE el uso de temperaturas y presiones altas puede favorecer la extracción de los analitos, aunque a su vez de otros componentes de la matriz. Sin embargo, la realización de dos etapas de purificación (una con alúmina en la propia celda de extracción durante la PLE y otra del extracto obtenido mediante SPE), puede dar lugar a mejores recuperaciones y extractos más limpios. A pesar de ello, el uso de QuEChERS constituye una alternativa atractiva a la PLE para la extracción de muestras ambientales, ya que no se requieren equipos especiales y es una técnica de extracción más rápida y que utiliza menor cantidad de disolventes. Por ello, en los últimos años, se han incluido varias modificaciones al método original con el objetivo de mejorar la extracción de los analitos y disminuir el efecto matriz, por ejemplo, la sustitución de las sales originales NaCl y $MgSO_4$ por sales más volátiles como NH_4Cl, NH_4HCO_2 o $NH_4CH_3CO_2$, para evitar que las sales precipiten en la fuente de ionización y disminuya la sensibilidad del instrumento, o el uso de otros sorbentes para la purificación de los extractos mediante *d*-SPE, como polímeros de impresión molecular, nanotubos de carbono, nanopartículas de dióxido de zirconio, o sorbentes magnéticos (Perestrelo y cols., 2019). En este sentido, el uso de $NH_4CH_3CO_2$ en lugar de NaCl como sal de extracción permitió disminuir el ruido de fondo y obtener mejores recuperaciones y límites de detección en el análisis de fármacos en leche (Rúbies y cols., 2016), y el empleo de sorbentes de sílice con nanopartículas de zirconio para la purificación de muestras de mejillones extraídas con QuEChERS dio lugar a que únicamente 2 de los 7 antidepresivos analizados presentaran efectos de matriz, siendo éstos inferiores al 31% (Martínez Bueno y cols., 2014).

Las bajas recuperaciones absolutas y los efectos de matriz observados en las diferentes metodologías se pueden corregir en gran medida con la adicción de patrones internos (los compuestos objeto de estudio marcados isotópicamente) a la muestra al principio del proceso analítico. Las recuperaciones relativas obtenidas tanto en sedimentos (90-113%, a excepción de OH-THC y cannabinol, que presentaron recuperaciones relativas superiores a 120% debido, probablemente, a su presencia en la muestra utilizada para la validación a concentraciones similares a las dopadas) como en mejillones (77-118%), demuestran que ambos métodos son adecuados para el análisis de drogas, psicofármacos y metabolitos en ambas matrices. Es cierto que el uso de compuestos marcados isotópicamente encarece en principio el análisis pero, a la larga, si el método desarrollado se va a utilizar con frecuencia o de forma rutinaria, resulta beneficioso ya que su uso permite conocer para cada muestra si el análisis se ha desarrollado correctamente (obviando así en general la necesidad de realizar triplicados) y corregir los variables efectos de matriz (tanto en la extracción como en el análisis) que podemos

encontrarnos en las distintas muestras a analizar, incluso aunque pertenezcan a una misma zona y campaña de muestreo. Por otro lado, este método asegura la obtención de resultados fiables de una manera rápida si se compara por ejemplo con el uso de rectas de calibrado realizadas en la propia matriz o el método de la adición de patrones. Uno de los aspectos claves a tener en cuenta en el desarrollo de metodologías de dilución isotópica para LC-MS/MS es que las SRM de los patrones marcados isotópicamente no interfieran con la señal de los analitos objeto de estudio con los que coeluyan. Para ello se recomienda la adquisición de patrones marcados en los que se haya sustituido un elevado número de átomos por sus respectivos isótopos (p.ej. H por D y ^{12}C por ^{13}C), con el fin de aumentar la diferencia de masa entre un compuesto específico y su correspondiente compuesto marcado isotópicamente y poder obtener fragmentos específicos para cada uno.

Respecto a la sensibilidad, en sedimentos los LOQs variaron entre 0,03 y 2,1 ng/g (p.s.) para todos los compuestos excepto para los cannabinoides (THC, OH-THC, cannabidiol y cannabinol) cuyos LOQs estuvieron entre 3,2 y 13 ng/g (p.s.). En el caso de mejillones, también los cannabinoides (THC, OH-THC y THC-COOH) presentaron LOQs mayores (entre 25 y 30 ng/g peso fresco (p.f.)), mientras que el resto de compuestos presentaron LOQs entre 0,3 y 6,7 ng/g (p.f.). Al igual que en el análisis de agua, estos mayores LOQs obtenidos para cannabinoides se pueden justificar debido a su baja recuperación, a la supresión de la señal por el efecto matriz, y a su análisis en condiciones de ionización positiva, las cuales no son las más favorables para estos compuestos. A pesar de ello, para el resto de compuestos los LOQs obtenidos en ambas matrices son comparables a los obtenidos con otras metodologías multi-residuo utilizadas para la determinación de diferentes contaminantes emergentes (incluyendo drogas, psicofármacos y metabolitos) en sedimentos y mejillones (Tabla 8).

Tabla 8. Metodologías multi-residuo para el análisis de drogas, psicofármacos y metabolitos (entre otros contaminantes) en sedimentos y mejillones.

Compuestos investigados[a]	Matriz	Preparación de la muestra			Análisis	LOQ[b]	Ref.
		Cantidad de muestra	Extracción	Purificación	LC-MS		
20 drogas, psicofármacos y metabolitos	Sedimentos	1 g (p.s.)	PLE ($MeOH:H_2O$, 9:1, v/v)	SPE (ABN)	HPLC-(ESI+)-LC-MS/MS (QqLIT)	0,03-13	**publicación #4**
11 drogas y metabolitos (8)	Sedimentos	1 g (p.s.)	US (tampón McIlvaine-MeOH, 1:1, v/v)	SPE (Oasis MCX)	UPLC-(ESI+)-MS/MS (QqQ)	1,5-5,4	(Hu y cols., 2019)
47 contaminantes orgánicos (incluyendo drogas, psicofármacos y metabolitos) (4)	Sedimentos	1 g (p.s.)	US (MeOH: H_2O; tampón McIlvaine-EDTA, 1:1:1, v/v/v)	SPE (Strata-X)	UPLC-(ESI+/-)-MS/MS (QqQ)	5-10	(Carmona y cols., 2017)
41 drogas y metabolitos (12)	Sedimentos	1 g (p.s.)	US (tampón McIlvaine: MeOH, 1:1, v/v)	SPE (Strata-X)	UPLC-(ESI+)-MS/MS (QqQ)	0,12-3,75	(Álvarez-Ruiz y cols., 2015)
74 fármacos y productos de cuidado personal (5)	Sedimentos	1 g (p.s.)	US (extracción ácida: tampón fosfato + ACN; extracción básica: tampón NH_4OH + ACN)	SPE (Oasis HLB)	HPLC-(ESI+/-)-MS/MS (QqQ)	0,04-0,4[c]	(Klosterhaus y cols., 2013)
14 fármacos, drogas, psicofármacos y metabolitos (4)	Sedimentos	10 g (p.s.)	PLE (MeOH/A.F., 100:0,1 v/v)	Centrifugación	UPLC-(ESI+/-)-MS/MS (QqQ)	2-20[d]	(Langford y cols., 2011)
17 fármacos (1)	Sedimentos	3 g (p.s.)	PLE (100% H_2O)	SPE (SAX + HLB)	HPLC-(ESI+/-)-MS/MS (QqQ)	2,3[e]	(Vazquez-Roig y cols., 2010b)
35 drogas, psicofármacos y metabolitos	Mejillón	10 g (p.f.)	QuEChERS (ACN)	*d*-SPE	HPLC-(ESI+)-LC-MS/MS (QqLIT)	0,3-30	**publicación #5**
118 fármacos y productos de cuidado personal (7)	Mejillón	n.i.	n.i.	n.i.	LC-MS/MS	0,2-15	(Krogh y cols., 2017)

Tabla 8 (cont). Metodologías multi-residuo para el análisis de drogas, psicofármacos y metabolitos (entre otros contaminantes) en sedimentos y mejillones.

Compuestos investigados[a]	Matriz	Preparación de la muestra			Análisis	LOQ[b]	Ref.
		Cantidad de muestra	Extracción	Purificación	LC-MS		
7 antidepresivos (3)	Mejillón	1 g (p.s.)	US (ACN: 0,1% A.F.)	SPE (Oasis MCX)	HPLC-(ESI+)-MS/MS (QqLIT)	1,7-3,4	(Silva y cols., 2017)
145 fármacos y productos de cuidado personal (6)	Mejillón	1-2,5 g (p.f.)	US (extracción ácida: ACN + tampón fosfato; extracción básica: ACN + de tampón NH_4OH)	SPE (Oasis HLB)	HPLC-(ESI+/-)-MS/MS (QqQ)	0,2-1,8	(de Solla y cols., 2016)
23 fármacos (3)	Mejillón	0,5 g (p.s.)	PLE ($MeOH:H_2O$, 1:2, v/v)	SPE (Oasis HLB)	UPLC-(ESI+/-)-MS/MS (QqLIT)	0,03-0,21	(Alvarez-Muñoz y cols., 2015)
6 antidepresivos (1)	Mejillón	2 g (p.s.)	QuEChERs (ACN/H_2O, 1:1, v/v)	*d*-SPE (Z-Sep)	HPLC-(ESI+)-MS (Q-Exactive)	0,5[f]	(Martínez Bueno y cols., 2014)
166 contaminantes emergentes (7)	Mejillón	1-2,5 g (p.f.)	US (extracción ácida: ACN + tampón fosfato; extracción básica: ACN + tampón NH_4OH)	SPE (Oasis HLB)	HPLC-(ESI+/-)-MS/MS (QqQ)	0,06-5,95	(Dodder y cols., 2014)

[a] Entre paréntesis se indica el número de compuestos investigados en la presente tesis que también se analizan en las otras metodologías multi-residuo;
[b] Los límites de cuantificación (LOQs) indicados corresponden únicamente a los compuestos investigados en la presente tesis;
[c] Punto más bajo de la recta de calibrado (ng/mL);
[d] LODs;
[e] LOQ de diazepam, único analito analizado en común en esta metodología y la metodología descrita en la publicación #4;
[f] LOQ de venlafaxina, único analito analizado en común en esta metodología y la metodología descrita en la publicación #5.
ABN: sorbente polimérico para la extracción de compuestos ácidos, básicos y neutros (del inglés *Acidic, Basic and Neutral Analytes*); ACN: acetonitrilo; A.F.: ácido fórmico; *d*-SPE: extracción en fase sólida dispersiva (del inglés *dispersive Solid Phase Extraction*); EDTA: ácido etilendiaminotetraacético; Strata-X: cartucho polimérico de fase reversa; HLB: sorbente polimérico de equilibrio hidrofílico-lipofílico (del inglés *Hydrophilic-Lipophilic balanced*); H_2O: agua; n.i.: no indicado; McIlvaine: tampón citrato-fosfato; MCX: sorbente polimérico mixto de intercambio catiónico-fase reversa (del inglés *Mixed mode Cation eXchange-reversed phase*); MeOH: metanol; NH_4OH: hidróxido amónico; p.f.: peso fresco; PLE: extracción con líquidos presurizados (del inglés *Pressurized Liquid Extraction*); p.s.: peso seco; SAX: sorbente de intercambio aniónico (del inglés: Strong Anionic eXchange); SPE: extracción en fase sólida (del inglés *Solid Phase Extraction*); US: ultrasonidos; Z-Sep: material sorbente con átomos de zirconio.

Aspectos a destacar de las metodologías descritas en esta tesis son: (i) la metodología descrita en la publicación #3 demuestra las ventajas del empleo de sistemas automatizados de SPE acoplados en línea al sistema de análisis por LC-MS/MS (on-line SPE) con respecto a los métodos que se basan en SPE off-line para el análisis de aguas, siendo hasta la fecha la única metodología basada en on-line SPE-LC-MS/MS con la que se han analizado OH-alprazolam, OH-midazolam, temazepam, zolpidem, MDPV, AH-7921, mefedrona y metoxetamina en agua residual; (ii) la metodología descrita en la publicación #4 permite la determinación de las drogas y psicofármacos que más se han investigado en sedimentos hasta la fecha (cocaína, benzoilecgonina, anfetamina, metanfetamina, metadona, THC, alprazolam y diazepam) y la amplia a otros compuestos importantes tanto por su consumo, como MDMA, como por su capacidad de adsorberse en esta matriz, como cannabidiol, y (iii) la metodología descrita en la publicación #5 ha permitido ampliar la lista de drogas que se han determinado en mejillones, ya que hasta la fecha sólo se había investigado la acumulación de cocaína, benzoilecgonina y anfetamina, e incluye tanto psicofármacos previamente estudiados en mejillones (citalopram, fluoxetina, sertralina, venlafaxina, alprazolam, diazepam), como psicofármacos investigados por primera vez en esta matriz (OH-alprazolam, midazolam, OH-midazolam, temazepam, lormetazepam, oxazepam, hidroxicina, clorpromacina y zolpidem).

A pesar de investigar un amplio rango de drogas y psicofármacos, las metodologías analíticas se podrían mejorar incluyendo otros compuestos relevantes tanto por su consumo como por su presencia en el medio ambiente. Así, se podrían incluir compuestos como nicotina o sus metabolitos (tras alcohol es la sustancia psicoactiva más consumida en España), los opiáceos codeína (previamente detectado en agua potable, superficial y residual a concentraciones ambientales relevantes, Figura 4, sección 1.3.1.1.), tramadol y fentanilo (el consumo de opiáceos sintéticos ha aumentado en los últimos años, siendo los principales responsables de las muertes causadas por sobredosis, sección 1.2.), y metabolitos de los psicofármacos, como nor-fluoxetina, nor-sertralina, desmetil sertralina, nor-diazepam, desmetil citalopram, desmetil venlafaxina (compuestos que se han detectado tanto en biota (Tabla 3, sección 1.3.1.2.) como en agua (Figura 4, sección 1.3.1.1.) e incluso a concentraciones superiores que las detectadas para el propio psicofármaco).

Por último, el uso de metodologías analíticas basadas en LC-MS/MS es útil para determinar con la necesaria sensibilidad las NSPs más relevantes o más conocidas en el mercado, a pesar de los bajos niveles esperados en el medio ambiente para estos compuestos debido a su baja prevalencia de uso. Sin embargo, dada la continua aparición de NSPs (como se comentó en la sección 1.2., en 2018 se estaban vigilando 950 sustancias), el empleo de

metodologías analíticas basadas en alta resolución sería más conveniente ya que, por un lado, no se disponen de todos los patrones comerciales de dichas sustancias, y por otro, la compra de todos ellos y sus análogos deuterados daría lugar a metodologías analíticas muy costosas.

4.2. Presencia e impacto ambiental de drogas, psicofármacos y metabolitos

La Figura 8 muestra la frecuencia de detección y las concentraciones de drogas, psicofármacos y metabolitos medidas en las muestras de agua residual, sedimentos y biota analizadas en las publicaciones #3, #4 y #5, respectivamente.

Los compuestos que no se detectaron en ninguna de las 3 matrices analizadas fueron la heroína y su metabolito 6-monoacetilmorfina, LSD, OH-LSD, y OH-THC. La no detección de heroína y LSD en ninguna de las tres matrices es probablemente debido a su menor consumo (como se vio en la introducción, la prevalencia de consumo en el último año de LSD y heroína en España era inferior al 0,4% y 0,1%, respetivamente (OEDA, 2019)) y, en el caso del LSD, además, a las bajas dosis utilizadas debido a su elevada potencia psicoactiva (µg *vs* mg). Por otro lado, estos compuestos se metabolizan intensamente después de su administración. En el caso de la heroína, la dosis consumida se excreta en un 0,025% en forma de heroína inalterada (Baker y cols., 2014), un 42% en forma de morfina y un 1,3% en forma de 6-monoacetilmorfina, que es además inestable en agua residual (Gracia-Lor y cols., 2016). En el caso de LSD, <1% de la dosis consumida se excreta como LSD inalterado, <1,2% como nor-LSD y entre 2 y 25% como OH-LSD (Postigo y cols., 2010). Todo esto hace que su presencia en el medio ambiente sea menor, y por lo tanto difícil de detectar. Del mismo modo, solo el 2% del cannabis consumido se excreta como OH-THC que además se metaboliza rápidamente a THC-COOH (Postigo y cols., 2011a). Tampoco se detectaron ni en agua residual ni en mejillones (en sedimentos estos compuestos no se analizaron, Tabla 6), las 4 NSPs analizadas, MDPV, mefedrona, metoxetamina y AH-7921, ni los psicofármacos OH-alprazolam, midazolam, y clorpromacina. En el caso de las NSPs, dado que su consumo es muy inferior al del resto de las drogas "clásicas" (prevalencia de consumo alguna vez en la vida de 1,1% (OEDA, 2019)), no es de extrañar que no se hayan detectado en el agua residual. En cuanto a las benzodiazepinas, el midazolam, como se muestra en la Figura 4 (sección 1.3.1.1), se ha encontrado en otros estudios a concentraciones muy bajas, del orden de 1 ng/L. Por otro lado, el alprazolam, a pesar de ser el segundo ansiolítico más consumido en España en 2019 (AEMPS, 2020), de su baja tasa de eliminación en las EDARs (<50%) (Estrada-Arriaga y cols.,

2016), y de su estabilidad en agua residual (hasta 1 semana a temperatura ambiente (Racamonde y cols., 2014)), es administrado en dosis muy bajas (1 mg) (INCB, 2019), razón por la cual no es de extrañar que sólo se haya detectado en el agua residual investigada a niveles inferiores al LOQ (1,9 ng/L), y por lo tanto, es también normal que no se haya detectado su metabolito, el cual se excreta en un porcentaje mayor que el padre (57,5 vs 21,4%) (Fraser y cols., 1991). En otro estudio llevado a cabo en España, tanto alprazolam como su metabolito (OH-alprazolam) se detectaron en agua residual, pero a concentraciones muy bajas entre 2 y 14 ng/L (González-Mariño y cols., 2018).

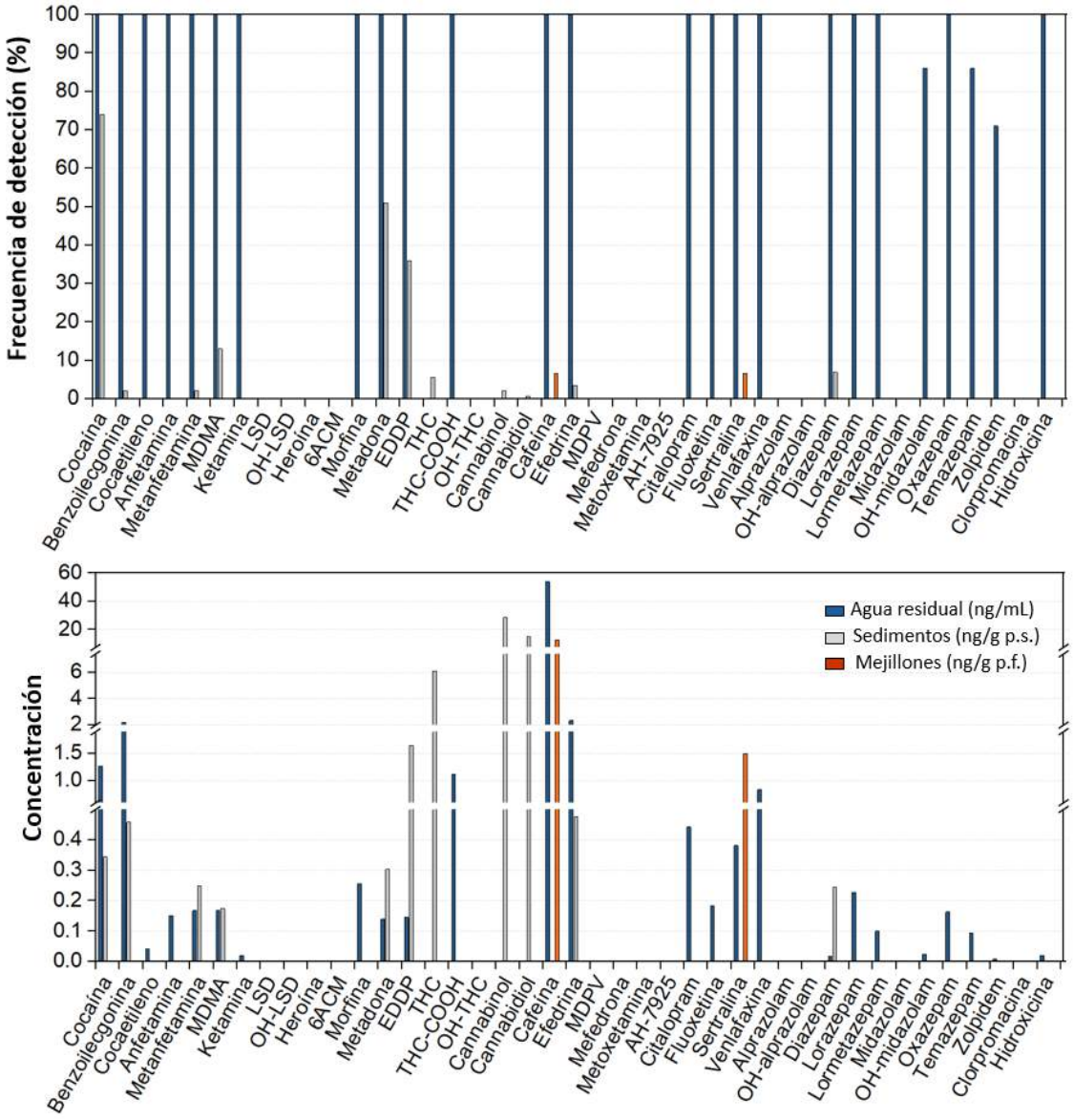


Figura 8. Frecuencia de detección (%) y concentración media de drogas, psicofármacos y metabolitos en agua residual, sedimentos y mejillones.

El resto de compuestos se detectaron en alguna de las matrices investigadas, siendo el agua residual la matriz donde mayor número de compuestos se detectó (68% de los 37 compuestos investigados), seguida de sedimentos (60% de los 20 compuestos investigados) y de mejillones (5,7% de los 35 compuestos investigados). En agua residual las frecuencias de detección de los compuestos también fueron mayores, la mayoría de compuestos se detectaron en el 100% de las muestras analizadas (n=7), mientras que en sedimentos la mayoría de compuestos presentaron frecuencias de detección (n=144) inferiores al 13% y únicamente 2 compuestos, cocaína y metadona, presentaron frecuencias de detección superiores al 50%, sin llegar en ningún caso a detectarse en el 100% de las muestras. En mejillones, únicamente se detectaron dos compuestos (sertralina y cafeína) y con frecuencias de detección bajas, 6,6% en ambos casos. La presencia de menor número de compuestos en sedimentos y biota no es de extrañar ya que como se vio en la introducción, este tipo de compuestos pueden eliminarse parcialmente en las EDARs (Devault y cols., 2017), siendo las concentraciones liberadas al medio mucho más bajas que las detectadas en aguas residuales sin tratar (como las analizadas en la publicación #3). Además, la descarga de los efluentes de las EDARs ya sea en cauces fluviales o directamente en el mar resulta en la dilución de sus concentraciones. Por otro lado, su carácter polar o medianamente polar, los hacen más propensos a permanecer en la fase acuosa que a adsorberse en matrices sólidas.

Los compuestos encontrados a concentraciones medias más altas fueron la cafeína, los cannabinoides THC, THC-COOH, cannabinol y cannabidiol, la efedrina, la cocaína y su metabolito benzoilecgonina, y los antidepresivos citalopram, sertralina y venlafaxina. La cafeína es el compuesto que se ha encontrado a mayor concentración tanto en agua como en mejillones (en sedimentos no se analizó, ver Tabla 6). El consumo extensivo de este estimulante en bebidas a base de café, té o bebidas carbonatadas hace que los niveles encontrados en el medio ambiente sean muy elevados, tal y como se observa en la Figura 4 (ver sección 1.3.1.1), hecho por el cual, a pesar de su alta polaridad (log K_{ow} -0.1, ver Tabla 1, sección 1.1.) se ha podido incluso cuantificar en mejillones. El resto de los compuestos detectados a mayor concentración coinciden con las drogas (Figura 3, sección 1.2.) y psicofármacos (Figura 2, sección 1.2.) que, de acuerdo con las encuestas o los datos oficiales, se consumen más en España.

Aunque las aguas residuales y los sedimentos investigados en esta tesis no se corresponden en tiempo y espacio, y por tanto, no es del todo correcto comparar los niveles encontrados en ambas matrices, cabe mencionar que las concentraciones tanto de benzoilecgonina como de cocaína en aguas residuales fueron superiores a las encontradas en sedimentos, por lo que se podría decir que, debido a su carácter polar (log K_{ow} de 2,3 y -1,3,

respectivamente, ver Tabla 1, sección 1.1.), ambos compuestos presentan una mayor tendencia a permanecer en la fase acuosa. Por el contrario, compuestos como la metadona y su metabolito EDDP (log K_{ow} y log K_{oc} > 3,9, Tabla 1, sección 1.1.), los cannabinoides (log K_{ow} y log K_{oc} > 4,5, Tabla 1, sección 1.1.), y la sertralina (log K_{ow} y log K_{oc} > 5,3, Tabla 1, sección 1.1.), compuestos todos ellos con un marcado carácter hidrofóbico, presentaron niveles superiores en sedimentos y/o en mejillones que en las aguas. Como se mostró en la publicación #4 con el cálculo del coeficiente de distribución (K_D), las propiedades físico-químicas de metadona y EDDP los hacen más propensos a ser adsorbidos en matrices sólidas. En el caso de los cannabinoides, estudios previamente publicados en la literatura han mostrado que estos compuestos tienden a adsorberse en matrices sólidas (Mastroianni y cols., 2013; Senta y cols., 2013) y, en el caso de la sertralina, su elevado factor de bioacumulación estimado experimentalmente en mejillones (32.000) (de Solla y cols., 2016) indica su alto potencial para bioacumularse en estos organismos. Los niveles de benzoilecgonina fueron superiores a los de cocaína tanto en agua residual como en sedimentos porque después de su consumo, la mayor parte de cocaína (entre 29-45%) se metaboliza y se excreta en forma de benzoilecgonina, y únicamente el 7% se excreta en forma de cocaína inalterada (Gracia-Lor y cols., 2016). A esto cabe añadir el hecho de que la cocaína en el medio ambiente también se puede transformar en benzoilecgonina por fotólisis e hidrólisis (Postigo y cols., 2011b). En el caso de los cannabinoides, los niveles más altos de cannabinol y cannabidiol con respecto al THC en sedimentos podrían justificarse porque ambos presentan valores de log K_{ow} ligeramente superiores a los del THC (ver Tabla 1, sección 1.1.), además, el THC se metaboliza mucho y apenas se excreta inalterado (Boleda y cols., 2009; Gracia-Lor y cols., 2016). En cuanto al THC-COOH, metabolito del THC, sólo se detectó en agua residual (en sedimentos no se analizó), donde estudios previos han mostrado que es muy estable (Causanilles y cols., 2017a; González-Mariño y cols., 2012a).

Como se mostró en la publicación #4 con el cálculo del índice de riesgo (HQ), los niveles encontrados de drogas y psicofármacos, en este caso en sedimentos, constituyen un riesgo toxicológico para los organismos acuáticos que viven o se alimentan en los sedimentos. Concretamente en el 14% de las muestras analizadas (n=144) los valores de ∑HQ estuvieron entre 1 y 10, y en el 15% de las muestras los valores de ∑HQ fueron superiores a 10, lo que implica riesgo, siendo el EDDP, el THC y la metadona los compuestos que más contribuyen a la toxicidad. El cálculo del HQ se realizó comparando los niveles de drogas y psicofármacos medidos en las muestras de sedimentos con los valores de $PNEC_{sed}$ que aparecían en la base de datos NORMAN para cada uno de los compuestos investigados (ver Tabla S1, publicación #4). Los valores de $PNEC_{sed}$ reportados en la base de datos NORMAN no se obtuvieron de estudios

de toxicidad, sino mediante la aplicación del principio de reparto en equilibrio (ecuación 3, derivada de la combinación de las ecuaciones 4, 5 y 6), el cual estima la concentración de una sustancia en sedimentos a partir de la concentración de dicha sustancia en el agua contenida en los poros del sedimento cuando el sistema está en equilibrio (NORMAN, 2013; US EPA, 2012).

$$PNEC_{sed}\ (\text{peso seco})\ (\mu g/kg) = PNEC_{agua} \times 2{,}6 \times (0{,}615 + 0{,}019 \times K_{oc}) \quad (3)$$

$$PNEC_{sed}\ (\text{peso fresco})\ (\mu g/kg) = (K_{sed\text{-}agua}/RHO_{sed}) \times PNEC_{agua} \times 1000 \quad (4)$$

$$K_{sed\text{-}agua} = (F_{aire\text{-}sed} \times K_{aire\text{-}agua}) + F_{agua\text{-}sed} + (F_{sólido\text{-}sedimento} \times (K\rho_{sed}/1000) \times RHO_{sólido}) \quad (5)$$

$$K\rho_{sed} = K_{oc} \times F_{oc.sed} \quad (6)$$

donde,

$PNEC_{agua}$ es el valor de PNEC en agua (ver Tabla 4, sección 1.3.2.); K_{oc} es el coeficiente de reparto entre el carbono orgánico y el agua; $K_{sed\text{-}agua}$ es el coeficiente de reparto sedimento-agua; RHO_{sed} es la densidad del sedimento (1300 kg/m^3); $F_{aire\text{-}sed}$ es la fracción de aire en sedimento (despreciable); $K_{aire\text{-}agua}$ es el coeficiente de reparto aire-agua; $F_{agua\text{-}sed}$ es la fracción de agua en sedimento (0,8); $F_{sólido\text{-}sedimento}$ es la fracción de sólidos en el sedimento (0,2); $K\rho_{sed}$ es el coeficiente de reparto sólido-agua en sedimentos; $RHO_{sólido}$ es la densidad de la fracción sólida (2500 kg kg/m^3); $F_{oc.sed}$ es la fracción de carbono orgánico en el sedimento (0,05); y 2,6 es el factor de conversión de la concentración en sedimento en peso fresco a peso seco.

El principio de reparto en equilibrio permite predecir la adsorción de un compuesto a partir de los valores de K_{oc} de dicho compuesto. En la base de datos NORMAN se selecciona el mínimo valor de K_{oc} disponible para calcular el $PNEC_{sed}$ para considerar el escenario más conservador posible. Cuando se preparó la publicación #4, la base de datos NORMAN aplicaba para los compuestos investigados un valor de K_{oc} de 0. Sin embargo, si en lugar de 0 se aplicaran los valores de K_{oc} de cada compuesto (ver Tabla 1, sección 1.1.) para calcular el $PNEC_{sed}$ de acuerdo con el principio de reparto en equilibrio (los valores de $PNEC_{sed}$ calculados aparecen en la Tabla 4, sección 1.3.2.), y posteriormente se calculara el ∑HQ de las muestras analizadas, se obtendría que todas las muestras presentan un ∑HQ inferior a 1, y por tanto, las concentraciones de estos compuestos en sedimentos no supondrían un riesgo ecotoxicológico, al contrario de lo reportado en la publicación #4. El riesgo reportado en la publicación #4 se puede atribuir a los bajos valores de $PNEC_{sed}$ registrados en la base de datos NORMAN (por ejemplo, en el caso concreto de EDDP, metadona y THC, que eran los compuestos que más contribuían a la

toxicidad, los $PNEC_{sed}$ eran de 0,22, 1,34 y 0,12 ng/g, respectivamente, ver Tabla S1, publicación #4), más que a la acumulación de altos niveles de drogas y psicofármacos en los sedimentos. El uso del valor de K_{oc} de cada compuesto, en lugar de un valor 0, para estimar los valores de $PNEC_{sed}$ puede conducir a un escenario más realista, ya que se tienen en cuenta las propiedades físico-químicas de los compuestos (K_{oc}). Los compuestos que tienen un log $K_{oc} \geq 3$ son los que tienen mayor capacidad para adsorberse en matrices sólidas y encontrarse a mayores concentraciones en sedimentos. Por tanto, es fundamental disponer de un valor de $PNEC_{sed}$ que sea representativo de la toxicidad de dichos compuestos en esta matriz para no sobreestimar la toxicidad asociada a la presencia de los contaminantes presentes en los sedimentos, como ocurrió con el cálculo del HQ en la publicación #4.

Si aplicamos el principio de reparto en equilibrio (ecuaciones 7 y 8) para las muestras de biota analizadas en la publicación #5, los $PNEC_{biota\text{-}invertebrados}$ calculados para cafeína y sertralina serían de 0,9 y 53 ng/g (ver Tabla 4, sección 1.3.2.), respectivamente. Considerando las concentraciones detectadas de estos compuestos en una de las muestras de mejillones analizadas (12,8 ng/g de cafeína y 1,5 ng/g de sertralina), el ∑HQ sería de 14 y, por tanto, la bioacumulación de cafeína y sertralina en esta muestra, podría suponer un riesgo toxicológico para el organismo.

$$PNEC_{biota/pez}\,(\text{peso fresco})\,(\mu g/kg) = PNEC_{agua} \times 2{,}6 \times BCF \quad (7)$$

$$PNEC_{biota/invertebrado}\,(\text{peso fresco})\,(\mu g/kg) = PNEC_{biota/pez} / 4 \quad (8)$$

donde BCF es el factor de bioconcentración (Tabla 1, sección 1.1.).

La ventaja del principio de reparto en equilibrio es que permite evaluar el riesgo toxicológico en diferentes matrices (sedimentos y biota) a partir de estudios de toxicidad realizados en agua, ya que para este tipo de compuestos los datos de toxicidad en sedimentos y biota son escasos y en algunos casos inexistentes. Además, a diferencia de los enfoques empíricos, que evalúan la concentración de sustancia que produce efectos biológicos en los organismos, el principio de reparto en equilibrio tiene en cuenta procesos químicos y biológicos para predecir la toxicidad, los cuales pueden ayudar a elucidar la causa de los efectos toxicológicos (Birch, 2018).

4.3. Estimación del consumo de sustancias psicoactivas mediante el análisis de aguas residuales

En la publicación #6 se estimó el consumo de alcohol a nivel local, autonómico y nacional a partir de los datos obtenidos en 16 zonas geográficas de 13 ciudades españolas. Este trabajo describe por primera vez la aplicación de WBE a nivel nacional en España para estimar el consumo de alcohol, y complementa los pocos estudios realizados con anterioridad a nivel nacional (Boogaerts y cols., 2016; Gao y cols., 2020; Lai y cols., 2018).

Los datos de consumo de alcohol estimados con WBE a los tres niveles se compararon con varios indicadores oficiales de consumo: los datos de consumo reportados en la Encuesta Nacional de Salud de España (ENSE), las prevalencias de consumo reportadas en la Encuesta sobre Alcohol y otras Drogas en España (EDADES) realizada por el Observatorio Español de las Drogas y las Adicciones (OEDA), los datos de consumo proporcionados por el Ministerio de Agricultura, Pesca y Alimentación, y los datos de consumo proporcionados por la Organización Mundial de la Salud (OMS). En general, se obtuvo una buena comparabilidad de datos entre los derivados de WBE y los indicadores oficiales.

A nivel local, los resultados obtenidos con WBE en el 56% de las zonas geográficas investigadas estaban en línea con los datos de consumo (expresados en mL/día/habitante) reportados en la ENSE para sus respectivas comunidades autónomas, siendo en el 31% de los casos (Toledo, Lleida, Madrid-Centro, Castellón, y Valencia-QB) las diferencias entre ambas metodologías inferiores al 15%, y en el 25% de los casos (Palma de Mallorca, Reus, Valencia-PI, y Valencia-PII) inferiores al 30%. Además, estas zonas geográficas también mostraron una correlación significativa (p-valor < 0,05, correlación de Spearman) (Figura 4, publicación #6) con los datos de prevalencia de consumo de alcohol (alguna vez en la vida y último año) reportados en la EDADES del OEDA. Las estimaciones de alcohol derivadas del análisis de aguas residuales para el resto de las zonas geográficas investigadas (Bilbao, Guadalajara, Barcelona, Tarragona, Madrid-Norte, Móstoles, y Santiago de Compostela), mostraron diferencias superiores al 30%.

A nivel autonómico, mediante la aplicación de WBE se observó que el consumo en las Islas Baleares era significativamente superior al de Castilla-La Mancha y Galicia. Dicha observación encaja con los datos de prevalencia de consumo en estas comunidades autónomas (prevalencia de consumo alguna vez en la vida, en el último año y en el último mes en las Islas Baleares de 97, 89 y 70%, respectivamente, mientras que en Castilla-La Mancha fue de 90, 64 y

53% y en Galicia de 94, 80 y 66%, respectivamente) (Figura S4, publicación #6), y también con los datos de consumo publicados en la ENSE (consumo en Islas Baleares de 18 mL/día/habitante), aunque sólo en el caso de Castilla-La Mancha (13 mL/día/habitante), ya que en Galicia se reportó un consumo de alcohol ligeramente superior (20 mL/día/habitante) (Tabla S6, publicación #6). También, con WBE se estimó un consumo de alcohol en el País Vasco significativamente superior al de Galicia, Castilla-La Mancha, Comunidad Valenciana, Comunidad de Madrid e Islas Baleares. Sin embargo, dicha estimación sólo se corroboró con los datos de prevalencia en el caso de Castilla-La Mancha (prevalencias de consumo alguna vez en la vida, último año y último mes en el País Vasco de 94, 82 y 65%, respectivamente, y en Castilla-La Mancha de 90, 64 y 53%, respectivamente) y la Comunidad de Madrid (prevalencias de consumo alguna vez en la vida, último año y último mes de 91, 76 y 66%, respectivamente), ya que según la EDADES las prevalencia de consumo en el País Vasco fueron similares a las de la Comunidad Valenciana (94, 82, y 71%) y Galicia (94, 80 y 66%), e inferiores a las de las Islas Baleares (97, 89, 70%) (Figura S6, publicación #6). En comparación con los datos de consumo reportados en la ENSE, el consumo significativamente superior estimado en el País Vasco con WBE (19 mL/día/habitante) sólo se corroboró en el caso de Castilla-La Mancha (13 mL/día/habitante), Comunidad Valenciana y Comunidad de Madrid (ambas 14 mL/día/habitante), ya que en Galicia (20 mL/día/habitante) e Islas Baleares (18 mL/día/habitante) el consumo reportado por la ENSE fue similar al del País Vasco.

Las diferencias observadas en algunas de las zonas geográficas estudiadas entre los datos estimados con WBE y los reportados por fuentes oficiales, como los derivados de encuestas poblacionales, se deben a la incertidumbre que presentan ambas metodologías. Con ambas metodologías, la población muestreada puede no ser representativa de los hábitos de consumo de la población en general. En el trabajo descrito en la publicación #6, se estimó un consumo significativamente mayor en las zonas geográficas de mayor tamaño (mayor de 300.000 habitantes) que en las de tamaño inferior. Esto también se observó en varios estudios en los que se aplicó WBE a poblaciones urbanas y rurales, obteniéndose diferencias significativas de consumo entre los dos tipos de población. En Australia (Lai y cols., 2018) y en China (Gao y cols., 2020), se estimó un consumo mayor en las zonas rurales, mientras que en Bélgica (Boogaerts y cols., 2016) se estimó un consumo mayor en las zonas urbanas. Por tanto, incluir diferentes ciudades o municipios dentro de una misma región (por ejemplo, las estimaciones realizadas en Galicia provienen del muestreo en una única ciudad (Tabla S1, publicación #6)), así como con tamaños de población y tipo de actividad económica diferenciados, permitiría obtener una muestra más representativa de la región en cuestión. En el caso de las encuestas, la

población encuestada también puede no ser representativa de la población en general, ya que las encuestas poblacionales, aunque incluyen zonas rurales y urbanas, se realizan a la población residente en hogares familiares, quedando fuera de la población entrevistada la que reside en instituciones (cárceles, cuarteles, residencias de estudiantes), o en establecimientos colectivos (hoteles, pensiones, etc.) (OEDA, 2019). Además, hay que tener en cuenta que los resultados de consumo obtenidos mediante las encuestas pueden no ser muy exactos ya que se puede subestimar la cantidad de alcohol consumida, ya sea por falta de sinceridad a la hora de responder las preguntas o por infravalorar la cantidad de alcohol consumida. Esta incertidumbre asociada a los indicadores oficiales queda reflejada en los resultados proporcionados por las dos encuestas realizadas a nivel nacional (ENSE y EDADES), ya que según la ENSE la región con mayor consumo sería Galicia, mientras que, atendiendo a los datos de prevalencia de consumo reportados en la EDADES, la región con mayor consumo sería Islas Baleares.

A pesar de la incertidumbre asociada a ambas metodologías, los datos de consumo estimados mediante WBE a nivel nacional (4,8 ± 1,1 L alcohol/año/habitante, o 5,7 ± 1,2 L/año/habitante (mayor de 15 años) o 5,9 ± 1,3 L/año/habitante (mayor de 18 años)), fueron comparables tanto con los datos reportados para España en la ENSE (4,7 L/año/habitante (mayor de 15 años)) como con los datos de consumo reportados por el Ministerio de Agricultura, Pesca y Alimentación (4,3 L/año /habitante (mayor de 18 años)). La similitud de los resultados obtenidos con ambas metodologías parece indicar que tanto la población muestreada como la semana en que se realizó el estudio, serían representativos de la población en general y del consumo de alcohol durante todo el año, respectivamente. Una buena similitud entre los resultados derivados de WBE y los datos oficiales también se ha reportado en otros dos estudios realizados a nivel nacional llevados a cabo en Bélgica, donde la aplicación de WBE (muestras recogidas durante una semana y cubriendo el 12% de la población) estimó un consumo medio de alcohol de 1,2 bebidas estándares/día/habitante (mayor de 15 años), muy similar al reportado por el Instituto Científico Belga de Salud Pública de 1,4 bebidas estándares/día/habitante (mayor de 15 años), y en Australia (muestras recogidas durante una semana y cubriendo el 45% de la población), donde se estimó mediante WBE un consumo medio de alcohol entre 16 y 25 mL de alcohol/día/habitante (entre 15-79 años), similar al reportado por la Oficina Estadística Australiana, 26 mL de alcohol/día/habitante (mayor de 15 años). A diferencia de la buena comparabilidad con las encuestas poblacionales a nivel nacional, los resultados estimados con WBE fueron muy inferiores a los datos de consumo reportados para España por la OMS (10 L/año/habitante (mayor de 15 años)). La misma observación (datos de consumo de alcohol derivados mediante WBE < datos de consumo proporcionados por la OMS)

se ha dado también en otros estudios realizados a nivel nacional en otros países (Boogaerts y cols., 2016; Gao y cols., 2020), lo cual se puede atribuir a que en países donde los datos de ventas de alcohol, importación o exportación (indicadores que utiliza la OMS para calcular el consumo de alcohol) no están estrictamente controlados, se puede sobreestimar la cantidad de alcohol consumida ya que no se tiene en cuenta el alcohol que se almacena, se desecha o se utiliza para otros fines diferentes al consumo (por ejemplo, cocinar) (Lai y cols., 2018). Esta hipótesis se vio reforzada por el hecho de que en países donde sí existe este control, como Noruega, los datos estimados con WBE son comparables a los reportados por la OMS (Reid y cols., 2011).

En vista de los resultados obtenidos, WBE se podría utilizar, junto con los indicadores tradicionales de consumo, para estimar de una manera más exacta el consumo o la tendencia de consumo de alcohol en una población. En este sentido, en el año 2019, la Generalitat de Cataluña, incorporó WBE como una herramienta complementaria a los indicadores oficiales (solicitudes a tratamiento, urgencias hospitalarias, incautaciones, etc.) para estimar el consumo de alcohol, drogas ilegales y psicofármacos, incluyendo los resultados obtenidos con WBE en el Informe Anual de 2019 sobre Drogodependencias de Catalunya publicado por la Agencia de Salud Pública de Cataluña (ASPC) (ASPC, 2020). En este informe se muestran los resultados obtenidos tras aplicar las metodologías descritas en las publicaciones #3 y #6 para determinar los niveles de indicadores de consumo de drogas y alcohol, respectivamente, en el agua residual recogida en las EDARs que dan servicio a las ciudades de Barcelona y Lleida, y estimar mediante WBE el consumo de drogas y alcohol en ambas ciudades (en la Tabla 5, sección 1.4, se indican los indicadores de consumo así como el factor de corrección aplicado en cada caso). Estos datos, existentes desde 2016, se han generado en el marco de la presente tesis doctoral.

A modo de ejemplo, la Figura 9 muestra el consumo estimado de alcohol, cocaína, anfetamina, MDMA, cannabis y metadona mediante la aplicación de WBE en Barcelona (desde 2011) y Lleida (desde el año 2016), y el número (en porcentaje) de admisiones a tratamiento en Cataluña según la droga principal que los motiva, ambos resultados publicados en el Informe Anual 2019 de la ASPC. Comparando ambas metodologías, se observa que el número de admisiones a tratamiento por uso de cocaína en Cataluña aumentó por cuarto año consecutivo, mientras que, según WBE, el consumo de cocaína en Barcelona durante los últimos 4 años también aumentó ligeramente. En el caso de alcohol, se observó un descenso en las admisiones a tratamiento en los 4 últimos años, mientras que, según WBE el consumo de alcohol tanto en Barcelona como en Lleida fue estable. En el caso del cannabis, las admisiones a tratamiento se mantuvieron estables en los últimos años, mientras que según WBE se observó un ligero descenso de su consumo desde el año 2017 cuando se alcanzó un consumo máximo. Por último,

las admisiones a tratamiento por consumo de heroína se mantuvieron estables durante los últimos años, mientras que tanto en Barcelona como en Lleida se observó una ligera disminución del consumo de metadona (sustancia que se utiliza para tratar la adicción a opioides).

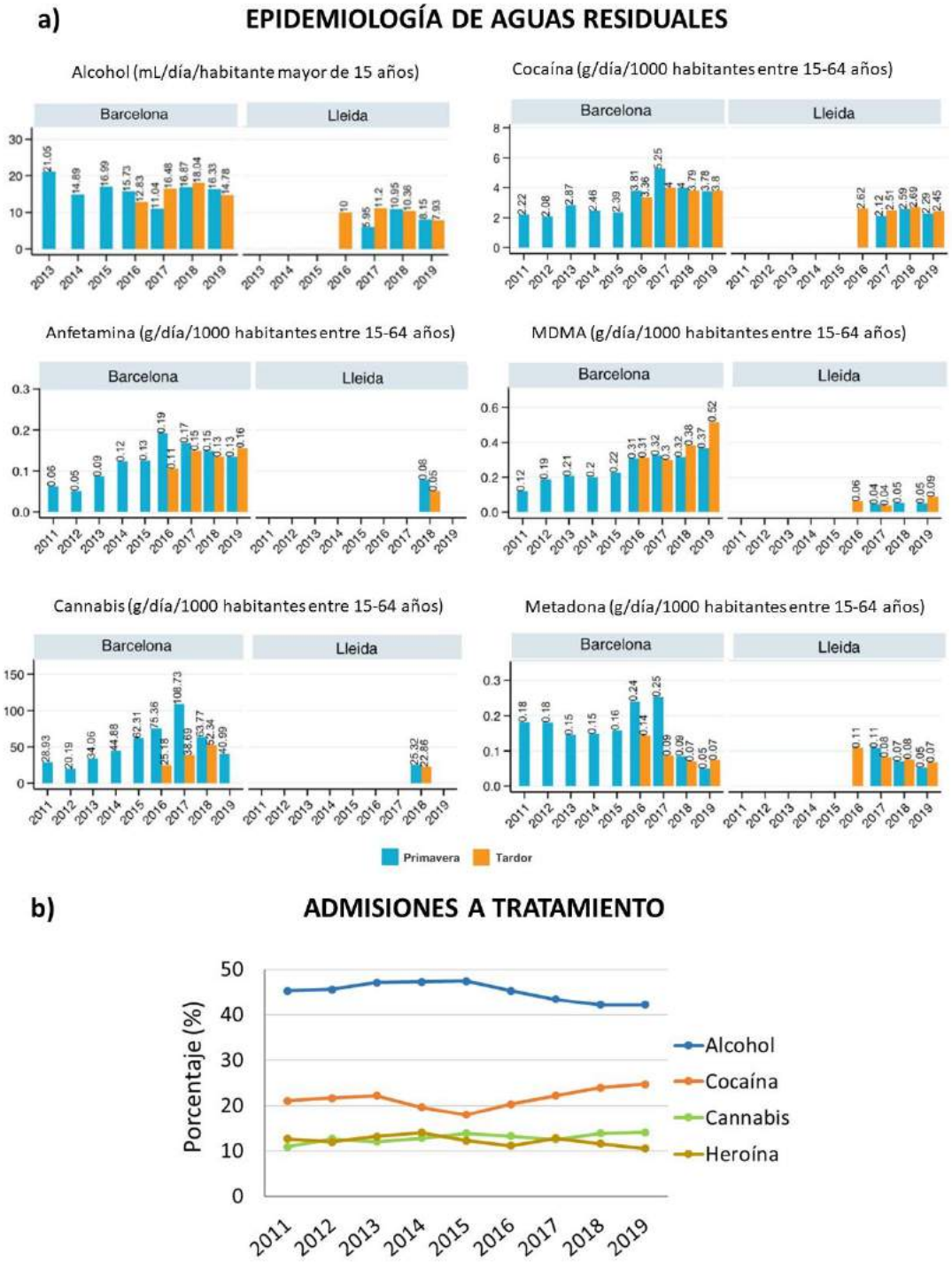


Figura 9. a) Consumo de alcohol, cocaína, anfetamina, metanfetamina, cannabis y metadona en Barcelona y Lleida estimado mediante WBE durante primavera (azul) y otoño (naranja) de 2011 (2016 en Lleida) a 2019; b) evolución anual (%) de las admisiones a tratamiento según la droga principal que los motiva en Cataluña en el mismo periodo. Fuente: (ASPC, 2020).

Los resultados de consumo de cocaína obtenidos en Barcelona con WBE fueron similares a los observados en Cataluña con las admisiones a tratamiento, por lo que se podría decir que hay una buena comparabilidad entre los resultados obtenidos en Barcelona con ambos indicadores. Sin embargo, Barcelona y Lleida son sólo dos ciudades de Cataluña, que pueden no ser representativas del consumo de drogas en toda la comunidad, en tanto que las admisiones a tratamiento son también sólo uno de los indicadores de consumo utilizados por la ASPC para evaluar el consumo de drogas ilegales en Cataluña, razón por la cual para el resto de sustancias pueden no tenerse resultados tan comparables.

Los datos de consumo de drogas en Barcelona, generados en el marco de esta tesis doctoral desde el año 2016, también se publican anualmente en el Informe Europeo sobre Drogas (EMCDDA, 2020a) y en la página web del EMCDDA (EMCDDA, 2020b), ya que este organismo utiliza WBE como una herramienta complementaria a las encuestas poblacionales y otros indicadores. La Figura 10 muestra la prevalencia de consumo de cocaína, anfetamina, metanfetamina y MDMA en el último año en los países europeos y el consumo medio de dichas drogas estimado mediante WBE en diferentes ciudades europeas. En comparación con otras ciudades europeas, Barcelona fue una de las ciudades incluidas en el estudio donde se reporta un mayor consumo de cocaína. También cabría destacar el consumo elevado estimado de metanfetamina, estimulante cuyo uso estaba históricamente concentrado en la República Checa y Eslovaquia, pero que en los últimos años se ha extendido también a otros países de Europa occidental. Por el contrario, los niveles de anfetamina y MDMA estimados en Barcelona fueron inferiores a los obtenidos en ciudades del norte, este y centro de Europa donde está más extendido el consumo de estas sustancias. En general, los resultados obtenidos con WBE en las ciudades europeas fueron comparables a los datos de prevalencia de consumo estimados para cada país a partir de otros indicadores. Según WBE las ciudades donde se estimó mayor consumo de cocaína pertenecían a Bélgica, España, Holanda y Reino Unido, países que también eran de los que presentaban mayores prevalencias de consumo en el último año. El consumo de anfetaminas fue mayor en ciudades de Suecia, Finlandia, Bélgica y Croacia, estando Finlandia y Croacia entre los países con mayor prevalencia de consumo de anfetamina en el último año. El mayor consumo de metanfetamina se estimó en ciudades de Alemania y España, siendo Alemania uno de los países con mayor prevalencia de consumo de esta droga en el último año, y el mayor consumo de MDMA se estimó en ciudades de Bélgica, Alemania y Países Bajos, tres de los países que presentan las prevalencias de consumo de MDMA en el último año más elevadas.

El análisis de aguas residuales con fines epidemiológicos constituye una herramienta que permite obtener patrones de consumo de una forma rápida, objetiva y económica. Además, su alta resolución temporal (resultados en el término de días) y espacial (resultados a nivel de ciudades e incluso lugares específicos (Postigo y cols., 2011a)) la convierten en una herramienta ideal para determinar cambios de consumo que se podrían utilizar para desarrollar e implementar medidas de prevención o acción contra el uso abusivo de determinadas sustancias.

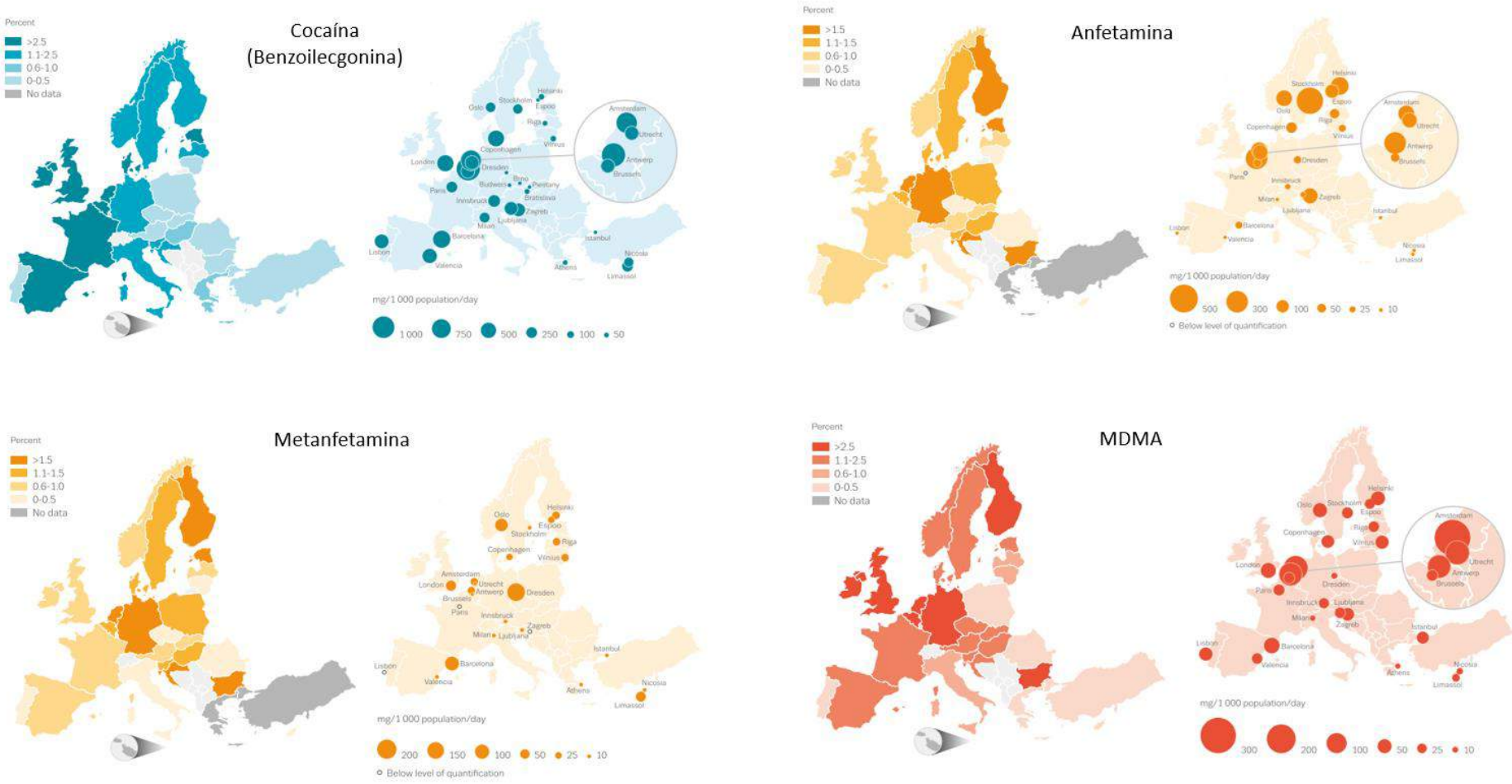


Figura 10. Prevalencia de consumo de cocaína, anfetamina, metanfetamina y MDMA durante el último año en países europeos (mapa sombreado) y consumo medio de estas drogas en ciudades europeas estimado mediante WBE (mapa con círculos) en el año 2019. Fuente: (EMCDDA, 2020a).

Capítulo 5.

Conclusiones

En esta tesis se han validado tres metodologías analíticas que, junto con una metodología previamente desarrollada en el grupo de investigación para la determinación de sulfato de etilo, se han utilizado para la determinación de drogas, psicofármacos y algunos de sus metabolitos en 3 compartimentos medioambientales, incluyendo agua residual, sedimentos y biota, con el objetivo de estudiar la presencia, destino e impacto de estos compuestos en el medio ambiente, y evaluar su consumo por la población.

Los resultados de estos estudios se han presentado y discutido en 4 artículos científicos publicados en revistas internacionales incluidas en el SCI, así como en diferentes congresos mediante presentaciones orales y en formato de póster.

Las principales conclusiones obtenidas de estos trabajos son:

- Las metodologías analíticas desarrolladas para el análisis de drogas, psicofármacos y metabolitos en agua residual (basada en *on-line* SPE-LC-MS/MS), sedimentos (basada en extracción mediante PLE, purificación mediante SPE y análisis con LC-MS/MS) y mejillones (extracción y purificación con QuEChERS y análisis con LC-MS/MS), presentan una elevada sensibilidad (límites de cuantificación inferiores o iguales a 16 ng/L en agua residual, 2,1 ng/g (p.s.) en sedimentos, y 6,7 ng/g (p.f.) en mejillones, para la mayoría de compuestos), selectividad (adquisición de dos transiciones SRM por compuesto), repetibilidad (RSD < 20% en las tres matrices investigadas) y fiabilidad (debido al uso del método de dilución isotópica para la cuantificación, utilizando compuestos marcados isotópicamente para el 97% de los analitos investigados, y además, en el caso de las muestras de agua, debido al procesamiento de las muestras y las soluciones de calibrado de la misma forma). Otras ventajas de las metodologías desarrolladas en comparación con otras descritas en la literatura, son su simplicidad (debido al uso de equipos automatizados para la preparación y/o el análisis de las muestras, en el caso de sedimentos y agua) y su rapidez y sostenibilidad (debido al uso de volúmenes pequeños de solventes).

- La detección de numerosas drogas, psicofármacos y metabolitos las aguas residuales, sedimentos y mejillones indica que en las zonas investigadas hay un consumo relevante de las mismas y que, además, algunas de ellas presentan tendencia a adsorberse o bioacumularse en matrices sólidas con el consiguiente riesgo ambiental que esto supone.

- Los niveles de drogas, psicofármacos y metabolitos (n = 37) medidos en el agua residual recogida a la entrada de la EDAR que da servicio a parte de la ciudad de Barcelona (n = 7) muestran que los compuestos más ubicuos y abundantes son los relacionados con las drogas que según los datos de prevalencia se consumen más en España, como la cocaína y su metabolito benzoilecgonina, y el metabolito del cannabis THC-COOH, además de los estimulantes ampliamente utilizados por la población cafeína y efedrina. Todos ellos se han detectado en el 100% de las muestras y a niveles que alcanzan los µg/L. Por el contrario, la heroína y su metabolito 6-monoacetilmorfina, LSD y las NSPs, que según datos de prevalencia se consumen en menor proporción que el resto de drogas ilegales, no se han detectado en las muestras analizadas, al igual que los psicofármacos midazolam, clorpromacina, y algunos metabolitos de drogas y psicofármacos como el OH-THC, y el OH-alprazolam. El resto de compuestos investigados se han encontrado a concentraciones que varían entre 7 ng/L (zolpidem) y 838 ng/L (venlafaxina) y con frecuencias de detección para la mayoría de ellos del 100%.

- Los niveles de drogas (n = 20) medidos en muestras de sedimentos (n = 144) recogidas en 4 cuencas españolas (Llobregat, Ebro, Júcar y Guadalquivir) durante dos años consecutivos muestran una frecuencia de detección baja (inferior al 13% para la mayoría de los compuestos investigados) y concentraciones medianas también bajas (inferiores a 1,6 ng/g (p.s.) en la mayor parte de los casos). Los compuestos más ubicuos y abundantes son los relacionados con las drogas de mayor consumo, como la cocaína (encontrada en el 74% de las muestras y a una concentración máxima de 5 ng/g (p.s.)), y los que, por sus propiedades físico-químicas presentan mayor tendencia a adsorberse en el material sólido, como la metadona (encontrada en el 51% de las muestras y a una concentración máxima de 33 ng/g (p.s.)), el EDDP (encontrado en el 36% de las muestras y a una concentración máxima de 16 ng/g (p.s.)), el THC (encontrado a una concentración máxima de 37 ng/g (p.s.), aunque sólo se detectó en el 5,6% de las muestras), y el cannabinol (encontrado a una concentración máxima de 44 ng/g (p.s.), aunque únicamente en el 2,1% de las muestras investigadas).

- El análisis de sedimentos ha permitido establecer diferencias significativas en la distribución de drogas tanto a nivel geográfico como temporal, gobernadas por una mezcla de factores (condiciones hidrológicas, meteorológicas, eficacia de eliminación en las EDARs, y patrones de consumo). En general, los sedimentos recogidos en afluentes situados cerca de ciudades de tamaño medio o grande, y en localizaciones cercanas a

los sitios de descarga de las EDARs son los que presentan una mayor acumulación de compuestos.

- Los compuestos con mayor tendencia a acumularse en sedimentos son la metadona y el EDDP, de acuerdo con los valores del coeficiente de distribución sedimento-agua (K_D) calculados experimentalmente para estos dos compuestos (log K_D metadona: 2,79; log K_D EDDP: 2,68). Además de para la metadona y el EDDP, el estudio realizado permitió reportar por primera vez datos experimentales de K_D para MDMA y diazepam.

- El riesgo medioambiental causado por la presencia de drogas en sedimentos puede ser diferente en función de los escenarios considerados. En el escenario más conservador, el utilizado por la base de datos NORMAN en la que el valor de $PNEC_{sed}$ se estima a partir del PNEC en agua utilizando un valor de K_{oc} de 0, la acumulación de drogas en sedimentos constituye en la mayoría de los casos investigados un riesgo toxicológico para los organismos acuáticos que se alimentan o viven en estos sedimentos, siendo los compuestos que más contribuyen a dicha toxicidad el EDDP, el THC y la metadona. Por el contrario, en un escenario más realista, en el que se utiliza el K_{oc} de cada compuesto para estimar el valor de $PNEC_{sed}$ y por tanto se tienen en cuenta sus propiedades físico-químicas, la presencia de drogas en sedimentos observada en nuestro estudio no supondría un riesgo toxicológico para los organismos acuáticos.

- La presencia de drogas, psicofármacos y algunos de sus metabolitos en el medio ambiente acuático, incluyendo el medio marino, puede conducir a la bioacumulación de estos compuestos en los organismos acuáticos. En el estudio realizado, se encontraron cafeína y sertralina a concentraciones de 12,8 y 1,5 ng/g (p.f.), respectivamente, en una muestra de mejillones salvajes recogida en la costa catalana, lo que supone un riesgo toxicológico para los organismos ($\sum HQ > 10$).

- La aplicación del análisis de aguas residuales con fines epidemiológicos en 16 zonas geográficas de 13 ciudades localizadas en 7 comunidades autónomas para estimar el consumo de alcohol a nivel local, autonómico y nacional, en el estudio más extenso de este tipo llevado a cabo en España hasta la fecha, ha mostrado diferencias significativas en los patrones de consumo de alcohol tanto a nivel local como autonómico y a lo largo de la semana.

- La estimación del consumo de alcohol en España a partir de los resultados obtenidos en el 12,8% de la población muestreada mediante el análisis de aguas residuales con fines

epidemiológicos es comparable a los datos de consumo publicados por varias fuentes oficiales como la Encuesta Nacional de Salud de España, realizada por el Instituto Nacional de Estadística, y los datos de consumo de alcohol reportados por el Ministerio de Agricultura, Pesca y Alimentación, aunque inferiores a los datos de consumo reportados por la OMS, tal y como ya se había observado en otros estudios llevados a cabo a nivel nacional en otros países.

- La comparación de los datos de consumo obtenidos con los métodos oficiales y con WBE se debe hacer con precaución ya que ambas metodologías presentan incertidumbres asociadas. En el caso de WBE, esta incertidumbre se puede reducir aumentando la población y el periodo de muestreo y realizando más estudios de metabolismo y de estabilidad tanto de drogas como de psicofármacos, para mejorar la exactitud de las estimaciones.

- El análisis de aguas residuales con fines epidemiológicos es una herramienta muy útil para evaluar de una forma rápida, objetiva y económica patrones geográficos y tendencias temporales de consumo de drogas y psicofármacos a nivel poblacional, aportando información complementaria a la obtenida con los métodos clásicos oficiales, tal y como se hace ya en el Observatorio Europeo de Drogas y Toxicomanías y en la Agencia de Salud Pública de Cataluña en España.

Bibliografía

Acuña, V., von Schiller, D., García-Galán, M.J., Rodríguez-Mozaz, S., Corominas, L., Petrovic, M., Poch, M., Barceló, D., Sabater, S., 2015. Occurrence and in-stream attenuation of wastewater-derived pharmaceuticals in Iberian rivers. Sci. Total Environ. 503-504, 133-141. https://doi.org/10.1016/j.scitotenv.2014.05.067

AEMPS, 2020. Agencia Española de Medicamentos y Productos Sanitarios. Utilización de medicamentos ansiolíticos e hipnóticos en España durante el periodo 2010-2018 [WWW Document]. URL https://www.aemps.gob.es/medicamentos-de-uso-humano/observatorio-de-uso-de-medicamentos/informes-ansioliticos-hipnoticos-espana-2010-2018/ (accedido 10.8.20).

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., Choi, P.M., Kitajima, M., Simpson, S.L., Li, J., Tscharke, B., Verhagen, R., Smith, W.J.M., Zaugg, J., Dierens, L., Hugenholtz, P., Thomas, K. V., Mueller, J.F., 2020. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total Environ. 728, 138764. https://doi.org/10.1016/j.scitotenv.2020.138764

Alvarez-Muñoz, D., Huerta, B., Fernandez-Tejedor, M., Rodríguez-Mozaz, S., Barceló, D., 2015. Multi-residue method for the analysis of pharmaceuticals and some of their metabolites in bivalves. Talanta 136, 174-182. https://doi.org/10.1016/j.talanta.2014.12.035

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

Álvarez-Ruiz, R., Andrés-Costa, M.J., Andreu, V., Picó, Y., 2015. Simultaneous determination of traditional and emerging illicit drugs in sediments, sludges and particulate matter. J. Chromatogr. A 1405, 103-115. https://doi.org/10.1016/j.chroma.2015.05.062

Anastassiades, M., Lehotay, S.J., Štajnbaher, D., Schenck, F.J., 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and. J. AOAC Int. 86, 412-431. https://doi.org/10.2478/s11687-011-0011-9

Andrés-Costa, M.J., Andreu, V., Picó, Y., 2016a. Analysis of psychoactive substances in water by information dependent acquisition on a hybrid quadrupole time-of-flight mass spectrometer. J. Chromatogr. A 1461, 98-106.

https://doi.org/10.1016/j.chroma.2016.07.062

Andrés-Costa, M.J., Escrivá, Ú., Andreu, V., Picó, Y., 2016b. Estimation of alcohol consumption during «Fallas» festivity in the wastewater of Valencia city (Spain) using ethyl sulfate as a biomarker. Sci. Total Environ. 541, 616-622. https://doi.org/10.1016/j.scitotenv.2015.09.126

Andrés-Costa, M.J., Rubio-López, N., Morales Suárez-Varela, M., Pico, Y., 2014. Occurrence and removal of drugs of abuse in Wastewater Treatment Plants of Valencia (Spain). Environ. Pollut. 194, 152-162. https://doi.org/10.1016/j.envpol.2014.07.019

Angeles, L.F., Mullen, R.A., Huang, I.J., Wilson, C., Khunjar, W., Sirotkin, H.I., McElroy, A.E., Aga, D.S., 2020. Assessing pharmaceutical removal and reduction in toxicity provided by advanced wastewater treatment systems. Environ. Sci. Water Res. Technol. 6, 62-77. https://doi.org/10.1039/c9ew00559e

Anzillotti, L., Odoardi, S., Strano-Rossi, S., 2014. Cleaning up blood samples using a modified «QuEChERS» procedure for the determination of drugs of abuse and benzodiazepines by UPLC-MSMS{star, open}. Forensic Sci. Int. 243, 99-106. https://doi.org/10.1016/j.forsciint.2014.05.005

Arbeláez, P., Borrull, F., Maria Marcé, R., Pocurull, E., 2014. Simultaneous determination of drugs of abuse and their main metabolites using pressurized liquid extraction and liquid chromatography-tandem mass spectrometry. Talanta 125, 65-71. https://doi.org/10.1016/j.talanta.2014.02.053

Arbeláez, P., Borrull, F., Pocurull, E., Marcé, R.M., 2015. Liquid chromatography-tandem mass spectrometry to determine sedative hypnotic drugs in river water and wastewater. Int. J. Environ. Anal. Chem. 95, 669-684. https://doi.org/10.1080/03067319.2015.1055474

Asimakopoulos, A.G., Kannan, K., 2016. Neuropsychiatric pharmaceuticals and illicit drugs in wastewater treatment plants: A review. Environ. Chem. https://doi.org/10.1071/EN15202

Asimakopoulos, A.G., Kannan, P., Higgins, S., Kannan, K., 2017. Determination of 89 drugs and other micropollutants in unfiltered wastewater and freshwater by LC-MS/MS: an alternative sample preparation approach. Anal. Bioanal. Chem. 409, 6205-6225. https://doi.org/10.1007/s00216-017-0561-x

ASPC, 2020. Agència de Salut Pública de Catalunya (ASPC). Sistema d'Informació sobre Drogodependències de Catalunya. Informe anual 2019 [WWW Document]. URL

http://drogues.gencat.cat/web/.content/minisite/drogues/professionals/epidemiologia/docs/INFORME-SIDC-2019_ok.pdf (accedido 10.5.20).

Bade, R., Bijlsma, L., Sancho, J. V., Baz-Lomba, J.A., Castiglioni, S., Castrignanò, E., Causanilles, A., Gracia-Lor, E., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.K., van Nuijs, A.L.N., Ort, C., Plósz, B.G., Ramin, P., Rousis, N.I., Ryu, Y., Thomas, K. V., de Voogt, P., Zuccato, E., Hernández, F., 2017. Liquid chromatography-tandem mass spectrometry determination of synthetic cathinones and phenethylamines in influent wastewater of eight European cities. Chemosphere 168, 1032-1041. https://doi.org/10.1016/j.chemosphere.2016.10.107

Bade, R., Tscharke, B.J., White, J.M., Grant, S., Mueller, J.F., O'Brien, J., Thomas, K. V., Gerber, C., 2019. LC-HRMS suspect screening to show spatial patterns of New Psychoactive Substances use in Australia. Sci. Total Environ. 650, 2181-2187. https://doi.org/10.1016/j.scitotenv.2018.09.348

Baker, D.R., Barron, L., Kasprzyk-Hordern, B., 2014. Illicit and pharmaceutical drug consumption estimated via wastewater analysis. Part A: Chemical analysis and drug use estimates. Sci. Total Environ. 487, 629-641. https://doi.org/10.1016/j.scitotenv.2013.11.107

Baker, D.R., Kasprzyk-Hordern, B., 2013. Spatial and temporal occurrence of pharmaceuticals and illicit drugs in the aqueous environment and during wastewater treatment: New developments. Sci. Total Environ. 454-455, 442-456. https://doi.org/10.1016/j.scitotenv.2013.03.043

Baker, D.R., Kasprzyk-Hordern, B., 2011a. Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography-positive electrospray ionisation tandem mass spectrometry. J. Chromatogr. A 1218, 1620-1631. https://doi.org/10.1016/j.chroma.2011.01.060

Baker, D.R., Kasprzyk-Hordern, B., 2011b. Multi-residue determination of the sorption of illicit drugs and pharmaceuticals to wastewater suspended particulate matter using pressurised liquid extraction, solid phase extraction and liquid chromatography coupled with tandem mass spectrometry. J. Chromatogr. A 1218, 7901-7913. https://doi.org/10.1016/j.chroma.2011.08.092

Baker, D.R., Očenášková, V., Kvicalova, M., Kasprzyk-Hordern, B., 2012. Drugs of abuse in wastewater and suspended particulate matter - Further developments in sewage epidemiology. Environ. Int. 48, 28-38. https://doi.org/10.1016/j.envint.2012.06.014

Bartelt-Hunt, S.L., Snow, D.D., Damon, T., Shockley, J., Hoagland, K., 2009. The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska. Environ. Pollut. 157, 786-791. https://doi.org/10.1016/j.envpol.2008.11.025

Bayen, S., Estrada, E.S. egovi., Juhel, G., Kelly, B.C., 2015. Direct injection of tissue extracts in liquid chromatography/tandem mass spectrometry for the determination of pharmaceuticals and other contaminants of emerging concern in mollusks. Anal. Bioanal. Chem. 407, 5553-5558. https://doi.org/10.1007/s00216-015-8760-9

Bayen, S., Estrada, E.S., Juhel, G., Kit, L.W., Kelly, B.C., 2016. Pharmaceutically active compounds and endocrine disrupting chemicals in water, sediments and mollusks in mangrove ecosystems from Singapore. Mar. Pollut. Bull. 109, 716-722. https://doi.org/10.1016/j.marpolbul.2016.06.105

Baz-Lomba, J. A., Reid, M.J., Thomas, K. V., 2016. Target and suspect screening of psychoactive substances in sewage-based samples by UHPLC-QTOF. Anal. Chim. Acta 914, 81-90. https://doi.org/10.1016/j.aca.2016.01.056

Baz-Lomba, Jose Antonio, Salvatore, S., Gracia-Lor, E., Bade, R., Castiglioni, S., Castrignanò, E., Causanilles, A., Hernandez, F., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.-K., van Nuijs, A., Ort, C., Plósz, B.G., Ramin, P., Reid, M., Rousis, N.I., Ryu, Y., de Voogt, P., Bramness, J., Thomas, K., 2016. Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities. BMC Public Health 16, 1035. https://doi.org/10.1186/s12889-016-3686-5

Bebianno, M.J., Sroda, S., Gomes, T., Chan, P., Bonnafe, E., Budzinski, H., Geret, F., 2016. Proteomic changes in Corbicula fluminea exposed to wastewater from a psychiatric hospital. Environ. Sci. Pollut. Res. 23, 5046-5055. https://doi.org/10.1007/s11356-015-5395-5

Bekele, E., Toze, S., Patterson, B., Higginson, S., 2011. Managed aquifer recharge of treated wastewater: Water quality changes resulting from infiltration through the vadose zone. Water Res. 45, 5764-5772. https://doi.org/10.1016/j.watres.2011.08.058

Benedi, J., Romero, C., 2005. Antidepresivos [WWW Document]. URL https://www.elsevier.es/es-revista-farmacia-profesional-3-pdf-13081504 (accedido 4.28.20).

Beretta, M., Britto, V., Tavares, T.M., da Silva, S.M.T., Pletsch, A.L., 2014. Occurrence of

pharmaceutical and personal care products (PPCPs) in marine sediments in the Todos os Santos Bay and the north coast of Salvador, Bahia, Brazil. J. Soils Sediments 14, 1278-1286. https://doi.org/10.1007/s11368-014-0884-6

Berset, J.D., Brenneisen, R., Mathieu, C., 2010. Analysis of llicit and illicit drugs in waste, surface and lake water samples using large volume direct injection high performance liquid chromatography - Electrospray tandem mass spectrometry (HPLC-MS/MS). Chemosphere 81, 859-866. https://doi.org/10.1016/j.chemosphere.2010.08.011

Beyer, J., Green, N.W., Brooks, S., Allan, I.J., Ruus, A., Gomes, T., Bråte, I.L.N., Schøyen, M., 2017. Blue mussels (Mytilus edulis spp.) as sentinel organisms in coastal pollution monitoring: A review. Mar. Environ. Res. https://doi.org/10.1016/j.marenvres.2017.07.024

Bijlsma, L., Boix, C., Niessen, W.M.A., Ibáñez, M., Sancho, J. V., Hernández, F., 2013. Investigation of degradation products of cocaine and benzoylecgonine in the aquatic environment. Sci. Total Environ. 443, 200-208. https://doi.org/10.1016/j.scitotenv.2012.11.006

Bijlsma, L., Celma, A., López, F.J., Hernández, F., 2019. Monitoring new psychoactive substances use through wastewater analysis: current situation, challenges and limitations. Curr. Opin. Environ. Sci. Heal. 9, 1-12. https://doi.org/10.1016/j.coesh.2019.03.002

Bijlsma, L., Emke, E., Hernández, F., De Voogt, P., 2012. Investigation of drugs of abuse and relevant metabolites in Dutch sewage water by liquid chromatography coupled to high resolution mass spectrometry. Chemosphere 89, 1399-1406. https://doi.org/10.1016/j.chemosphere.2012.05.110

Bijlsma, L., Sancho, J. V., Pitarch, E., Ibáñez, M., Hernández, F., 2009. Simultaneous ultra-high-pressure liquid chromatography-tandem mass spectrometry determination of amphetamine and amphetamine-like stimulants, cocaine and its metabolites, and a cannabis metabolite in surface water and urban wastewater. J. Chromatogr. A 1216, 3078-3089. https://doi.org/10.1016/j.chroma.2009.01.067

Binelli, A., Pedriali, A., Riva, C., Parolini, M., 2012. Illicit drugs as new environmental pollutants: Cyto-genotoxic effects of cocaine on the biological model Dreissena polymorpha. Chemosphere 86, 906-911. https://doi.org/10.1016/j.chemosphere.2011.10.056

Birch, G.F., 2018. A review of chemical-based sediment quality assessment methodologies for the marine environment. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2018.05.039

Boix, C., Ibáñez, M., Bijlsma, L., Sancho, J. V., Hernández, F., 2014. Investigation of cannabis biomarkers and transformation products in waters by liquid chromatography coupled to time of flight and triple quadrupole mass spectrometry. Chemosphere 99, 64-71. https://doi.org/10.1016/j.chemosphere.2013.10.007

Boix, C., Ibáñez, M., Sancho, J. V., Rambla, J., Aranda, J.L., Ballester, S., Hernández, F., 2015. Fast determination of 40 drugs in water using large volume direct injection liquid chromatography-tandem mass spectrometry. Talanta 131, 719-727. https://doi.org/10.1016/j.talanta.2014.08.005

Boleda, M.R., Galceran, M.T., Ventura, F., 2009. Monitoring of opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain. Water Res. 43, 1126-1136. https://doi.org/10.1016/j.watres.2008.11.056

Boleda, M.R., Galceran, M.T., Ventura, F., 2007. Trace determination of cannabinoids and opiates in wastewater and surface waters by ultra-performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1175, 38-48. https://doi.org/10.1016/j.chroma.2007.10.029

Bones, J., Thomas, K. V., Paull, B., 2007. Using environmental analytical data to estimate levels of community consumption of illicit drugs and abused pharmaceuticals. J. Environ. Monit. 9, 701-707. https://doi.org/10.1039/b702799k

Boogaerts, T., Covaci, A., Kinyua, J., Neels, H., Van Nuijs, A.L.N., 2016. Spatial and temporal trends in alcohol consumption in Belgian cities: A wastewater-based approach. Drug Alcohol Depend. 160, 170-176. https://doi.org/10.1016/j.drugalcdep.2016.01.002

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

Brandeburová, P., Bodík, I., Horáková, I., Žabka, D., Castiglioni, S., Salgueiro-González, N., Zuccato, E., Špalková, V., Mackuľak, T., 2020. Wastewater-based epidemiology to assess the occurrence of new psychoactive substances and alcohol consumption in Slovakia. Ecotoxicol. Environ. Saf. 200, 110762. https://doi.org/10.1016/j.ecoenv.2020.110762

Brieudes, V., Lardy-Fontan, S., Lalere, B., Vaslin-Reimann, S., Budzinski, H., 2016. Validation and uncertainties evaluation of an isotope dilution-SPE-LC-MS/MS for the quantification of

drug residues in surface waters. Talanta 146, 138-147. https://doi.org/10.1016/j.talanta.2015.06.073

Bringolf, R.B., Heltsley, R.M., Newton, T.J., Eads, C.B., Fraley, S.J., Shea, D., Cope, W.G., 2010. Environmental occurrence and reproductive effects of the pharmaceutical fluoxetine in native freshwater mussels. Environ. Toxicol. Chem. 29, n/a-n/a. https://doi.org/10.1002/etc.157

Brooks, B.W., Chambliss, C.K., Stanley, J.K., Ramirez, A., Banks, K.E., Johnson, R.D., Lewis, R.J., 2005. Determination of select antidepressants in fish from an effluent-dominated stream. Environ. Toxicol. Chem. 24, 464-469. https://doi.org/10.1897/04-081R.1

Cabeza, Y., Candela, L., Ronen, D., Teijon, G., 2012. Monitoring the occurrence of emerging contaminants in treated wastewater and groundwater between 2008 and 2010. The Baix Llobregat (Barcelona, Spain). J. Hazard. Mater. 239-240, 32-39. https://doi.org/10.1016/j.jhazmat.2012.07.032

Calamari, D., Zuccato, E., Castiglioni, S., Bagnati, R., Fanelli, R., 2003. Strategic survey of therapeutic drugs in the rivers Po and lambro in Northern Italy. Environ. Sci. Technol. 37, 1241-1248. https://doi.org/10.1021/es020158e

Calisto, V., Esteves, V.I., 2009. Psychiatric pharmaceuticals in the environment. Chemosphere. https://doi.org/10.1016/j.chemosphere.2009.09.021

Camilleri, J., Baudot, R., Wiest, L., Vulliet, E., Cren-Olivé, C., Daniele, G., 2015. Multiresidue fully automated online SPE-HPLC-MS/MS method for the quantification of endocrine-disrupting and pharmaceutical compounds at trace level in surface water. Int. J. Environ. Anal. Chem. 95, 67-81. https://doi.org/10.1080/03067319.2014.983494

Cao, J., Fu, B., Zhang, T., Wu, Y., Zhou, Z., Zhao, J., Yang, E., Qian, T., Luo, J., 2020. Fate of typical endocrine active compounds in full-scale wastewater treatment plants: Distribution, removal efficiency and potential risks. Bioresour. Technol. 310, 123436. https://doi.org/10.1016/j.biortech.2020.123436

Carballa, M., Omil, F., Lema, J.M., 2008. Comparison of predicted and measured concentrations of selected pharmaceuticals, fragrances and hormones in Spanish sewage. Chemosphere 72, 1118-1123. https://doi.org/10.1016/j.chemosphere.2008.04.034

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

117-125. https://doi.org/10.1016/j.jpba.2017.08.014

Carmona, E., Andreu, V., Picó, Y., 2014. Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: From waste to drinking water. Sci. Total Environ. 484, 53-63. https://doi.org/10.1016/j.scitotenv.2014.02.085

Carpinteiro, I., Rodil, R., Quintana, J.B., Cela, R., 2017. Reaction of diazepam and related benzodiazepines with chlorine. Kinetics, transformation products and in-silico toxicological assessment. Water Res. 120, 280-289. https://doi.org/10.1016/j.watres.2017.04.063

Castiglioni, S., Bijlsma, L., Covaci, A., Emke, E., Hernández, F., Reid, M., Ort, C., Thomas, K. V., Van Nuijs, A.L.N., De Voogt, P., Zuccato, E., 2013. Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. Environ. Sci. Technol. 47, 1452-1460. https://doi.org/10.1021/es302722f

Castiglioni, S., Zuccato, E., Crisci, E., Chiabrando, C., Fanelli, R., Bagnati, R., 2006. Identification and Measurement of Illicit Drugs and Their Metabolites in Urban Wastewater by Liquid Chromatography-Tandem Mass Spectrometry. https://doi.org/10.1021/ac061095b

Castrignanò, E., Lubben, A., Kasprzyk-Hordern, B., 2016. Enantiomeric profiling of chiral drug biomarkers in wastewater with the usage of chiral liquid chromatography coupled with tandem mass spectrometry. J. Chromatogr. A 1438, 84-99. https://doi.org/10.1016/j.chroma.2016.02.015

Catalá, M., Domínguez-Morueco, N., Migens, A., Molina, R., Martínez, F., Valcárcel, Y., Mastroianni, N., López de Alda, M., Barceló, D., Segura, Y., 2015. Elimination of drugs of abuse and their toxicity from natural waters by photo-Fenton treatment. Sci. Total Environ. 520, 198-205. https://doi.org/10.1016/j.scitotenv.2015.03.042

Causanilles, A., Baz-Lomba, J.A., Burgard, D.A., Emke, E., González-Mariño, I., Krizman-Matasic, I., Li, A., Löve, A.S.C., McCall, A.K., Montes, R., van Nuijs, A.L.N., Ort, C., Quintana, J.B., Senta, I., Terzic, S., Hernandez, F., de Voogt, P., Bijlsma, L., 2017a. Improving wastewater-based epidemiology to estimate cannabis use: focus on the initial aspects of the analytical procedure. Anal. Chim. Acta 988, 27-33. https://doi.org/10.1016/j.aca.2017.08.011

Causanilles, A., Kinyua, J., Ruttkies, C., van Nuijs, A.L.N., Emke, E., Covaci, A., de Voogt, P., 2017b. Qualitative screening for new psychoactive substances in wastewater collected during a city festival using liquid chromatography coupled to high-resolution mass spectrometry. Chemosphere 184, 1186-1193. https://doi.org/10.1016/j.chemosphere.2017.06.101

Causanilles, A., Ruepert, C., Ibáñez, M., Emke, E., Hernández, F., de Voogt, P., 2017c. Occurrence and fate of illicit drugs and pharmaceuticals in wastewater from two wastewater treatment plants in Costa Rica. Sci. Total Environ. 599-600, 98-107. https://doi.org/10.1016/j.scitotenv.2017.04.202

Chen, J., Venkatesan, A.K., Halden, R.U., 2019. Alcohol and nicotine consumption trends in three U.S. communities determined by wastewater-based epidemiology. Sci. Total Environ. 656, 174-183. https://doi.org/10.1016/j.scitotenv.2018.11.350

Chiaia, A.C., Banta-Green, C., Field, J., 2008. Eliminating solid phase extraction with large-volume injection LC/MS/MS: Analysis of illicit and legal drugs and human urine indicators in US wastewaters. Environ. Sci. Technol. 42, 8841-8848. https://doi.org/10.1021/es802309v

Chicharro, A.F., González-Alonso, S., Rubio, J.C.M., Rivera, Y.V., 2014. Estimación del consumo de drogas de abuso y sus metabolitos a partir de su presencia en el agua residual de Talavera de la reina y en el Río Tajo. Rev. Esp. Salud Publica 88, 289-299. https://doi.org/10.4321/S1135-57272014000200011

Choi, P.M., Tscharke, B.J., Donner, E., O'Brien, J.W., Grant, S.C., Kaserzon, S.L., Mackie, R., O'Malley, E., Crosbie, N.D., Thomas, K. V., Mueller, J.F., 2018. Wastewater-based epidemiology biomarkers: Past, present and future. TrAC - Trends Anal. Chem. https://doi.org/10.1016/j.trac.2018.06.004

Chu, S., Metcalfe, C.D., 2007. Analysis of paroxetine, fluoxetine and norfluoxetine in fish tissues using pressurized liquid extraction, mixed mode solid phase extraction cleanup and liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1163, 112-118. https://doi.org/10.1016/j.chroma.2007.06.014

Coelho, M.M., Lado Ribeiro, A.R., Sousa, J.C.G., Ribeiro, C., Fernandes, C., Silva, A.M.T., Tiritan, M.E., 2019. Dual enantioselective LC–MS/MS method to analyse chiral drugs in surface water: Monitoring in Douro River estuary. J. Pharm. Biomed. Anal. 170, 89-101. https://doi.org/10.1016/j.jpba.2019.03.032

Coetsier, C.M., Spinelli, S., Lin, L., Roig, B., Touraud, E., 2009. Discharge of pharmaceutical products (PPs) through a conventional biological sewage treatment plant: MECs vs PECs? Environ. Int. 35, 787-792. https://doi.org/10.1016/j.envint.2009.01.008

Cortez, F.S., Souza, L. da S., Guimarães, L.L., Pusceddu, F.H., Maranho, L.A., Fontes, M.K., Moreno, B.B., Nobre, C.R., Abessa, D.M. de S., Cesar, A., Pereira, C.D.S., 2019. Marine

contamination and cytogenotoxic effects of fluoxetine in the tropical brown mussel Perna perna. Mar. Pollut. Bull. 141, 366-372. https://doi.org/10.1016/j.marpolbul.2019.02.065

Cunha, D.L., de Araujo, F.G., Marques, M., 2017. Psychoactive drugs: occurrence in aquatic environment, analytical methods, and ecotoxicity—a review. Environ. Sci. Pollut. Res. 24, 24076-24091. https://doi.org/10.1007/s11356-017-0170-4

Daglioglu, N., Guzel, E.Y., Kilercioglu, S., 2019. Assessment of illicit drugs in wastewater and estimation of drugs of abuse in Adana Province, Turkey. Forensic Sci. Int. 294, 132-139. https://doi.org/10.1016/j.forsciint.2018.11.012

Daughton, C.G., 2001. Illicit Drugs in Municipal Sewage, en: ACS Symposium Series. pp. 348-364. https://doi.org/10.1021/bk-2001-0791.ch020

de Almeida, C.A.A., Oliveira, M.S., Mallmann, C.A., Martins, A.F., 2015. Determination of the psychoactive drugs carbamazepine and diazepam in hospital effluent and identification of their metabolites. Environ. Sci. Pollut. Res. 22, 17192-17201. https://doi.org/10.1007/s11356-015-4948-y

de Solla, S.R., Gilroy, A.M., Klinck, J.S., King, L.E., McInnis, R., Struger, J., Backus, S.M., Gillis, P.L., 2016. Bioaccumulation of pharmaceuticals and personal care products in the unionid mussel Lasmigona costata in a river receiving wastewater effluent. Chemosphere 146, 486-496. https://doi.org/10.1016/j.chemosphere.2015.12.022

Deng, Y., Guo, C., Zhang, H., Yin, X., Chen, L., Wu, D., Xu, J., 2020. Occurrence and removal of illicit drugs in different wastewater treatment plants with different treatment techniques. Environ. Sci. Eur. 32, 28. https://doi.org/10.1186/s12302-020-00304-x

Devault, D.A., Néfau, T., Levi, Y., Karolak, S., 2017. The removal of illicit drugs and morphine in two waste water treatment plants (WWTPs) under tropical conditions. Environ. Sci. Pollut. Res. 24, 25645-25655. https://doi.org/10.1007/s11356-015-6032-z

Di Poi, C., Bidel, F., Dickel, L., Bellanger, C., 2014. Cryptic and biochemical responses of young cuttlefish Sepia officinalis exposed to environmentally relevant concentrations of fluoxetine. Aquat. Toxicol. 151, 36-45. https://doi.org/10.1016/j.aquatox.2013.12.026

Di Poi, C., Darmaillacq, A.S., Dickel, L., Boulouard, M., Bellanger, C., 2013. Effects of perinatal exposure to waterborne fluoxetine on memory processing in the cuttlefish Sepia officinalis. Aquat. Toxicol. 132-133, 84-91. https://doi.org/10.1016/j.aquatox.2013.02.004

Dodder, N.G., Maruya, K.A., Lee Ferguson, P., Grace, R., Klosterhaus, S., La Guardia, M.J.,

Lauenstein, G.G., Ramirez, J., 2014. Occurrence of contaminants of emerging concern in mussels (Mytilus spp.) along the California coast and the influence of land use, storm water discharge, and treated wastewater effluent. Mar. Pollut. Bull. 81, 340-346. https://doi.org/10.1016/j.marpolbul.2013.06.041

Dos Santos Barbosa Ortega, A., Maranho, L.A., Nobre, C.R., Moreno, B.B., Guimarães, R.S., Lebre, D.T., de Souza Abessa, D.M., Ribeiro, D.A., Pereira, C.D.S., 2018. Detoxification, oxidative stress, and cytogenotoxicity of crack cocaine in the brown mussel Perna perna. Environ. Sci. Pollut. Res. 1-10. https://doi.org/10.1007/s11356-018-1600-7

Du, P., Liu, X., Zhong, G., Zhou, Z., Thomes, M.W., Lee, C.W., Bong, C.W., Zhang, X., Hao, F., Li, X., Zhang, G., Thai, P.K., 2020. Monitoring Consumption of Common Illicit Drugs in Kuala Lumpur, Malaysia, by Wastewater-Cased Epidemiology. Int. J. Environ. Res. Public Health 17, 889. https://doi.org/10.3390/ijerph17030889

Duan, L., Zhang, Y., Wang, B., Deng, S., Huang, J., Wang, Y., Yu, G., 2018. Occurrence, elimination, enantiomeric distribution and intra-day variations of chiral pharmaceuticals in major wastewater treatment plants in Beijing, China. Environ. Pollut. 239, 473-482. https://doi.org/10.1016/j.envpol.2018.04.014

Dulaurent, S., El Balkhi, S., Poncelet, L., Gaulier, J.M., Marquet, P., Saint-Marcoux, F., 2016. QuEChERS sample preparation prior to LC-MS/MS determination of opiates, amphetamines, and cocaine metabolites in whole blood. Anal. Bioanal. Chem. 408, 1-8. https://doi.org/10.1007/s00216-015-9248-3

EMCDDA, 2020a. European Monitoring Centre for Drugs and Drug Addiction, 2020. European Drug Report. Trends and Developments. https://doi.org/10.2810/123451

EMCDDA, 2020b. Wastewater-based epidemiology and drugs topic page | www.emcdda.europa.eu [WWW Document]. URL https://www.emcdda.europa.eu/topics/wastewater_en (accedido 7.6.20).

EMCDDA, 2016. Assessing illicit drugs in wastewater: Advances in wastewater-based drug epidemiology [WWW Document]. URL https://www.emcdda.europa.eu/system/files/publications/2273/TDXD16022ENC_4.pdf (accedido 7.7.20).

Emke, E., Evans, S., Kasprzyk-Hordern, B., de Voogt, P., 2014. Enantiomer profiling of high loads of amphetamine and MDMA in communal sewage: A Dutch perspective. Sci. Total Environ.

487, 666-672. https://doi.org/10.1016/j.scitotenv.2013.11.043

Esteban, S., Valcárcel, Y., Catalá, M., Castromil, M.G., 2012. Psychoactive pharmaceutical residues in the watersheds of Galicia (Spain). Gac. Sanit. 26, 457-459. https://doi.org/10.1016/j.gaceta.2012.01.018

Estrada-Arriaga, E.B., Cortés-Muñoz, J.E., González-Herrera, A., Calderón-Mólgora, C.G., de Lourdes Rivera-Huerta, M., Ramírez-Camperos, E., Montellano-Palacios, L., Gelover-Santiago, S.L., Pérez-Castrejón, S., Cardoso-Vigueros, L., Martín-Domínguez, A., García-Sánchez, L., 2016. Assessment of full-scale biological nutrient removal systems upgraded with physico-chemical processes for the removal of emerging pollutants present in wastewaters from Mexico. Sci. Total Environ. 571, 1172-1182. https://doi.org/10.1016/j.scitotenv.2016.07.118

Evans, S.E., Davies, P., Lubben, A., Kasprzyk-Hordern, B., 2015. Determination of chiral pharmaceuticals and illicit drugs in wastewater and sludge using microwave assisted extraction, solid-phase extraction and chiral liquid chromatography coupled with tandem mass spectrometry. Anal. Chim. Acta 882, 112-126. https://doi.org/10.1016/j.aca.2015.03.039

Fabbri, E., Franzellitti, S., 2016. Human pharmaceuticals in the marine environment: Focus on exposure and biological effects in animal species. Environ. Toxicol. Chem. 35, 799-812. https://doi.org/10.1002/etc.3131

Fedorova, G., Randak, T., Lindberg, R.H., Grabic, R., 2013. Comparison of the quantitative performance of a Q-Exactive high-resolution mass spectrometer with that of a triple quadrupole tandem mass spectrometer for the analysis of illicit drugs in wastewater. Rapid Commun. Mass Spectrom. 27, 1751-1762. https://doi.org/10.1002/rcm.6628

Fernandes, M.J., Paíga, P., Silva, A., Llaguno, C.P., Carvalho, M., Vázquez, F.M., Delerue-Matos, C., 2020. Antibiotics and antidepressants occurrence in surface waters and sediments collected in the north of Portugal. Chemosphere 239, 124729. https://doi.org/10.1016/j.chemosphere.2019.124729

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

Fick, J., Söderström, H., Lindberg, R.H., Phan, C., Tysklind, M., Larsson, D.G.J., 2009.

Contamination of surface, ground, and drinking water from pharmaceutical production. Environ. Toxicol. Chem. 28, 2522-2527. https://doi.org/10.1897/09-073.1

Fong, P.P., 1998. Zebra Mussel Spawning Is Induced in Low Concentrations of Putative Serotonin Reuptake Inhibitors. Biol. Bull. 194, 143-149. https://doi.org/10.2307/1543044

Fong, P.P., Ford, A.T., 2014. The biological effects of antidepressants on the molluscs and crustaceans: A review. Aquat. Toxicol. https://doi.org/10.1016/j.aquatox.2013.12.003

Fontanals, N., Marcé, R.M., Borrull, F., 2017. Solid-phase extraction followed by liquid chromatography-high resolution mass spectrometry to determine synthetic cathinones in different types of environmental water samples. J. Chromatogr. A 1524, 66-73. https://doi.org/10.1016/j.chroma.2017.10.002

Foppe, K.S., Hammond-Weinberger, D.R., Subedi, B., 2018. Estimation of the consumption of illicit drugs during special events in two communities in Western Kentucky, USA using sewage epidemiology. Sci. Total Environ. 633, 249-256. https://doi.org/10.1016/j.scitotenv.2018.03.175

Fraser, A.D., Bryan, W., Isner, A.F., 1991. Urinary screening for alprazolam and its major metabolites by the Abbott ADx and TDx analyzers with confirmation by GC/MS. J. Anal. Toxicol. 15, 25-29. https://doi.org/10.1093/jat/15.1.25

Gago-Ferrero, P., Borova, V., Dasenaki, M.E., Thomaidis, N.S., 2015. Simultaneous determination of 148 pharmaceuticals and illicit drugs in sewage sludge based on ultrasound-assisted extraction and liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 407, 4287-4297. https://doi.org/10.1007/s00216-015-8540-6

Gao, J., O'Brien, J., Du, P., Li, X., Ort, C., Mueller, J.F., Thai, P.K., 2016. Measuring selected PPCPs in wastewater to estimate the population in different cities in China. Sci. Total Environ. 568, 164-170. https://doi.org/10.1016/j.scitotenv.2016.05.216

Gao, J., Zheng, Q., Lai, F.Y., Gartner, C., Du, P., Ren, Y., Li, X., Wang, D., Mueller, J.F., Thai, P.K., 2020. Using wastewater-based epidemiology to estimate consumption of alcohol and nicotine in major cities of China in 2014 and 2016. Environ. Int. 136, 105492. https://doi.org/10.1016/j.envint.2020.105492

Gao, T., Du, P., Xu, Z., Li, X., 2017. Occurrence of new psychoactive substances in wastewater of major Chinese cities. Sci. Total Environ. 575, 963-969. https://doi.org/10.1016/j.scitotenv.2016.09.152

Gatidou, G., Kinyua, J., van Nuijs, A.L.N., Gracia-Lor, E., Castiglioni, S., Covaci, A., Stasinakis, A.S., 2016. Drugs of abuse and alcohol consumption among different groups of population on the Greek Island of Lesvos through sewage-based epidemiology. Sci. Total Environ. 563-564, 633-640. https://doi.org/10.1016/j.scitotenv.2016.04.130

Gay, F., Maddaloni, M., Valiante, S., Laforgia, V., Capaldo, A., 2013. Endocrine disruption in the european eel, anguilla anguilla, exposed to an environmental cocaine concentration. Water. Air. Soil Pollut. 224. https://doi.org/10.1007/s11270-013-1579-0

Gerrity, D., Trenholm, R.A., Snyder, S.A., 2011. Temporal variability of pharmaceuticals and illicit drugs in wastewater and the effects of a major sporting event. Water Res. 45, 5399-5411. https://doi.org/10.1016/j.watres.2011.07.020

Gheorghe, A., Van Nuijs, A., Pecceu, B., Bervoets, L., Jorens, P.G., Blust, R., Neels, H., Covaci, A., 2008. Analysis of cocaine and its principal metabolites in waste and surface water using solid-phase extraction and liquid chromatography-ion trap tandem mass spectrometry, en: Analytical and Bioanalytical Chemistry. Springer Verlag, pp. 1309-1319. https://doi.org/10.1007/s00216-007-1754-5

Ginebreda, A., Kuzmanovic, M., Guasch, H., de Alda, M.L., López-Doval, J.C., Muñoz, I., Ricart, M., Romaní, A.M., Sabater, S., Barceló, D., 2014. Assessment of multi-chemical pollution in aquatic ecosystems using toxic units: Compound prioritization, mixture characterization and relationships with biological descriptors. Sci. Total Environ. 468-469, 715-723. https://doi.org/10.1016/j.scitotenv.2013.08.086

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

Gonçalves, R., Ribeiro, C., Cravo, S., Cunha, S.C., Pereira, J.A., Fernandes, J.O., Afonso, C., Tiritan, M.E., 2019. Multi-residue method for enantioseparation of psychoactive substances and beta blockers by gas chromatography–mass spectrometry. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1125, 121731. https://doi.org/10.1016/j.jchromb.2019.121731

González-Mariño, Iria, Ares, L., Montes, R., Rodil, R., Cela, R., López-García, E., Postigo, C., de Alda, M.L., Pocurull, E., Marcé, R.M., Bijlsma, L., Hernández, F., Picó, Y., Andreu, V., Rico, A., Valcárcel, Y., Miró, M., Etxebarria, N., Quintana, J.B., 2020. Assessing population exposure to phthalate plasticizers in thirteen Spanish cities through the analysis of

wastewater. J. Hazard. Mater. 123272. https://doi.org/10.1016/j.jhazmat.2020.123272

González-Mariño, I., Baz-Lomba, J.A., Alygizakis, N.A., Andrés-Costa, M.J., Bade, R., Barron, L.P., Been, F., Berset, J.-D., Bijlsma, L., Bodík, I., Brenner, A., Brock, A.L., Burgard, D.A., Castrignanò, E., Christophoridis, C.E., Covaci, A., de Voogt, P., Devault, D.A., Dias, M.J., Emke, E., Fatta-Kassinos, D., Fedorova, G., Fytianos, K., Gerber, C., Grabic, R., Grüner, S., Gunnar, T., Hapeshi, E., Heath, E., Helm, B., Hernández, F., Kankaanpaa, A., Karolak, S., Kasprzyk-Hordern, B., Krizman-Matasic, I., Lai, F.Y., Lechowicz, W., Lopes, A., López de Alda, M., López-García, E., Löve, A.S.C., Mastroianni, N., McEneff, G.L., Montes, R., Munro, K., Nefau, T., Oberacher, H., O'Brien, J.W., Olafsdottir, K., Picó, Y., Plósz, B.G., Polesel, F., Postigo, C., Quintana, J.B., Ramin, P., Reid, M.J., Rice, J., Rodil, R., Senta, I., Simões, S.M., Sremacki, M.M., Styszko, K., Terzic, S., Thomaidis, N.S., Thomas, K.V., Tscharke, B.J., van Nuijs, A.L.N., Yargeau, V., Zuccato, E., Castiglioni, S., Ort, C., 2020. Spatio-temporal assessment of illicit drug use at large scale: evidence from 7 years of international wastewater monitoring. Addiction 115, 109-120. https://doi.org/10.1111/add.14767

González-Mariño, I., Castro, V., Montes, R., Rodil, R., Lores, A., Cela, R., Quintana, J.B., 2018. Multi-residue determination of psychoactive pharmaceuticals, illicit drugs and related metabolites in wastewater by ultra-high performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1569, 91-100. https://doi.org/10.1016/j.chroma.2018.07.045

González-Mariño, I., Gracia-Lor, E., Bagnati, R., Martins, C.P.B., Zuccato, E., Castiglioni, S., 2016a. Screening new psychoactive substances in urban wastewater using high resolution mass spectrometry. Anal. Bioanal. Chem. 408, 4297-4309. https://doi.org/10.1007/s00216-016-9521-0

González-Mariño, I., Gracia-Lor, E., Rousis, N.I., Castrignanò, E., Thomas, K. V., Quintana, J.B., Kasprzyk-Hordern, B., Zuccato, E., Castiglioni, S., 2016b. Wastewater-Based Epidemiology to Monitor Synthetic Cathinones Use in Different European Countries. Environ. Sci. Technol. 50, 10089-10096. https://doi.org/10.1021/acs.est.6b02644

González-Mariño, I., Quintana, J.B., Rodríguez, I., Cela, R., 2010. Determination of drugs of abuse in water by solid-phase extraction, derivatisation and gas chromatography-ion trap-tandem mass spectrometry. J. Chromatogr. A 1217, 1748-1760. https://doi.org/10.1016/j.chroma.2010.01.046

González-Mariño, I., Quintana, J.B., Rodríguez, I., Cores, M., Cela, R., 2015. Transformation of

methadone and its main human metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), during water chlorination. Water Res. 68, 759-770. https://doi.org/10.1016/j.watres.2014.10.058

González-Mariño, I., Quintana, J.B., Rodríguez, I., Gonzáez-Díez, M., Cela, R., 2012a. Screening and selective quantification of illicit drugs in wastewater by mixed-mode solid-phase extraction and quadrupole-time-of-flight liquid chromatography-mass spectrometry. Anal. Chem. 84, 1708-1717. https://doi.org/10.1021/ac202989e

González-Mariño, I., Quintana, J.B., Rodríguez, I., Sánchez-Méndez, N., Cela, R., 2012b. Transformation of cocaine during water chlorination. Anal. Bioanal. Chem. 404, 3135-3144. https://doi.org/10.1007/s00216-012-6428-2

González-Mariño, I., Rodríguez, I., Quintana, J.B., Cela, R., 2013. Investigation of the transformation of 11-nor-9-carboxy-δ9-tetrahydrocannabinol during water chlorination by liquid chromatography-quadrupole-time-of-flight-mass spectrometry. J. Hazard. Mater. 261, 628-636. https://doi.org/10.1016/j.jhazmat.2013.08.006

Gonzalez-Rey, M., Bebianno, M.J., 2013. Does selective serotonin reuptake inhibitor (SSRI) fluoxetine affects mussel Mytilus galloprovincialis? Environ. Pollut. 173, 200-209. https://doi.org/10.1016/j.envpol.2012.10.018

González-Mariño, I., Estévez-Danta, A., Rodil, R., Da Silva, K.M., Sodré, F.F., Cela, R., Quintana, J.B., 2019. Profiling cocaine residues and pyrolytic products in wastewater by mixed-mode liquid chromatography–tandem mass spectrometry. Drug Test. Anal. 11, 1018-1027. https://doi.org/10.1002/dta.2590

González Alonso, S., Catalá, M., Maroto, R.R., Gil, J.L.R., de Miguel, Á.G., Valcárcel, Y., 2010. Pollution by psychoactive pharmaceuticals in the Rivers of Madrid metropolitan area (Spain). Environ. Int. 36, 195-201. https://doi.org/10.1016/j.envint.2009.11.004

Gracia-Lor, E., Rousis, N.I., Zuccato, E., Bade, R., Baz-Lomba, J.A., Castrignanò, E., Causanilles, A., Hernández, F., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.K., van Nuijs, A.L.N., Plósz, B.G., Ramin, P., Ryu, Y., Santos, M.M., Thomas, K., de Voogt, P., Yang, Z., Castiglioni, S., 2017. Estimation of caffeine intake from analysis of caffeine metabolites in wastewater. Sci. Total Environ. 609, 1582-1588. https://doi.org/10.1016/j.scitotenv.2017.07.258

Gracia-Lor, E., Zuccato, E., Castiglioni, S., 2016. Refining correction factors for back-calculation of illicit drug use. Sci. Total Environ. 573, 1648-1659.

https://doi.org/10.1016/j.scitotenv.2016.09.179

Gracia-Lor, E., Zuccato, E., Hernández, F., Castiglioni, S., 2020. Wastewater-based epidemiology for tracking human exposure to mycotoxins. J. Hazard. Mater. 382, 121108. https://doi.org/10.1016/j.jhazmat.2019.121108

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

Hass, U., Duennbier, U., Massmann, G., 2012. Occurrence and distribution of psychoactive compounds and their metabolites in the urban water cycle of Berlin (Germany). Water Res. 46, 6013-6022. https://doi.org/10.1016/j.watres.2012.08.025

Heeb, F., Singer, H., Pernet-Coudrier, B., Qi, W., Liu, H., Longrée, P., Müller, B., Berg, M., 2012. Organic micropollutants in rivers downstream of the megacity Beijing: Sources and mass fluxes in a large-scale wastewater irrigation system. Environ. Sci. Technol. 46, 8680-8688. https://doi.org/10.1021/es301912q

Heizmann, P., Eckert, M., Ziegler, W., 1983. Pharmacokinetics and bioavailability of midazolam in man. Br. J. Clin. Pharmacol. 16, 43S-49S. https://doi.org/10.1111/j.1365-2125.1983.tb02270.x

Hu, P., Guo, C., Zhang, Yan, Lv, J., Zhang, Yuan, Xu, J., 2019. Occurrence, distribution and risk assessment of abused drugs and their metabolites in a typical urban river in north China. Front. Environ. Sci. Eng. 13, 1-11. https://doi.org/10.1007/s11783-019-1140-5

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

Huerta-Fontela, M., Galceran, M.T., Ventura, F., 2011. Occurrence and removal of pharmaceuticals and hormones through drinking water treatment. Water Res. 45, 1432-1442. https://doi.org/10.1016/j.watres.2010.10.036

Huerta-Fontela, M., Galceran, M.T., Ventura, F., 2010. Fast liquid chromatography-quadrupole-linear ion trap mass spectrometry for the analysis of pharmaceuticals and hormones in water resources. J. Chromatogr. A 1217, 4212-4222.

https://doi.org/10.1016/j.chroma.2009.11.007

Huerta-Fontela, M., Galceran, M.T., Ventura, F., 2008b. Stimulatory drugs of abuse in surface waters and their removal in a conventional drinking water treatment plant. Environ. Sci. Technol. 42, 6809-6816. https://doi.org/10.1021/es800768h

Huerta-Fontela, M., Galceran, M.T., Ventura, F., 2007. Ultraperformance liquid chromatography tandem mass spectrometry analysis of stimulatory drugs of abuse in wastewater and surface waters. Anal. Chem. 79, 3821-3829. https://doi.org/10.1021/ac062370x

Huerta-Fontela, M., Pineda, O., Ventura, F., Galceran, M.T., 2012. New chlorinated amphetamine-type-stimulants disinfection-by-products formed during drinking water treatment. Water Res. 46, 3304-3314. https://doi.org/10.1016/j.watres.2012.03.029

Huerta, B., Jakimska, A., Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2013. Analysis of multi-class pharmaceuticals in fish tissues by ultra-high-performance liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1288, 63-72. https://doi.org/10.1016/j.chroma.2013.03.001

Huerta, B., Rodríguez-Mozaz, S., Barceló, D., 2012. Pharmaceuticals in biota in the aquatic environment: Analytical methods and environmental implications. Anal. Bioanal. Chem. 404, 2611-2624. https://doi.org/10.1007/s00216-012-6144-y

Hummel, D., Löffler, D., Fink, G., Ternes, T.A., 2006. Simultaneous determination of psychoactive drugs and their metabolites in aqueous matrices by liquid chromatography mass spectrometry. Environ. Sci. Technol. 40, 7321-7328. https://doi.org/10.1021/es061740w

INCB, 2019. International Narcotics Control Board, 2019. Psychotropic Substances. Statistic for 2018: Assessments of Annual Medical and Scientific Requeriments.

Irvine, R.J., Kostakis, C., Felgate, P.D., Jaehne, E.J., Chen, C., White, J.M., 2011. Population drug use in Australia: A wastewater analysis. Forensic Sci. Int. 210, 69-73. https://doi.org/10.1016/j.forsciint.2011.01.037

Jones-Lepp, T.L., Alvarez, D.A., Petty, J.D., Huckins, J.N., 2004. Polar organic chemical integrative sampling and liquid chromatography- electrospray/ion-trap mass spectrometry for assessing selected prescription and illicit drugs in treated sewage effluents. Arch. Environ. Contam. Toxicol. 47, 427-439. https://doi.org/10.1007/s00244-004-3146-6

Jones-Lepp, T.L., Stevens, R., 2007. Pharmaceuticals and personal care products in biosolids/sewage sludge: The interface between analytical chemistry and regulation, en:

Analytical and Bioanalytical Chemistry. Anal Bioanal Chem, pp. 1173-1183. https://doi.org/10.1007/s00216-006-0942-z

Jurado, A., Mastroianni, N., Vàzquez-Suñé, E., Carrera, J., Tubau, I., Pujades, E., Postigo, C., de Alda, M.L., Barceló, D., 2012. Drugs of abuse in urban groundwater. A case study: Barcelona. Sci. Total Environ. 424, 280-288. https://doi.org/10.1016/j.scitotenv.2012.02.074

Kaleta, A., Ferdig, M., Buchberger, W., 2006. Semiquantitative determination of residues of amphetamine in sewage sludge samples. J. Sep. Sci. 29, 1662-1666. https://doi.org/10.1002/jssc.200500465

Karolak, S., Nefau, T., Bailly, E., Solgadi, A., Levi, Y., 2010. Estimation of illicit drugs consumption by wastewater analysis in Paris area (France). Forensic Sci. Int. 200, 153-160. https://doi.org/10.1016/j.forsciint.2010.04.007

Kasprzyk-Hordern, B., Baker, D.R., 2012. Enantiomeric profiling of chiral drugs in wastewater and receiving waters. Environ. Sci. Technol. 46, 1681-1691. https://doi.org/10.1021/es203113y

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

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008a. Multiresidue methods for the analysis of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra performance liquid chromatography-electrospray tandem mass spectrometry, en: Analytical and Bioanalytical Chemistry. Springer, pp. 1293-1308. https://doi.org/10.1007/s00216-008-1854-x

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

Kasprzyk-Hordern, B., Kondakal, V.V.R., Baker, D.R., 2010. Enantiomeric analysis of drugs of abuse in wastewater by chiral liquid chromatography coupled with tandem mass spectrometry. J. Chromatogr. A 1217, 4575-4586. https://doi.org/10.1016/j.chroma.2010.04.073

Kim, K.Y., Lai, F.Y., Kim, H.Y., Thai, P.K., Mueller, J.F., Oh, J.E., 2015. The first application of

wastewater-based drug epidemiology in five South Korean cities. Sci. Total Environ. 524-525, 440-446. https://doi.org/10.1016/j.scitotenv.2015.04.065

Kim, K.Y., Oh, J.-E., 2020. Evaluation of pharmaceutical abuse and illicit drug use in South Korea by wastewater-based epidemiology. J. Hazard. Mater. 396, 122622. https://doi.org/10.1016/j.jhazmat.2020.122622

Klosterhaus, S.L., Grace, R., Hamilton, M.C., Yee, D., 2013. Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. Environ. Int. 54, 92-99. https://doi.org/10.1016/j.envint.2013.01.009

Kosjek, T., Perko, S., Zupanc, M., ZanoŠki Hren, M., Landeka Dragičević, T., Žigon, D., Kompare, B., Heath, E., 2012. Environmental occurrence, fate and transformation of benzodiazepines in water treatment. Water Res. 46, 355-368. https://doi.org/10.1016/j.watres.2011.10.056

Kovačević, S., Radišić, M., Laušević, M., Dimkić, M., 2017. Occurrence and behavior of selected pharmaceuticals during riverbank filtration in The Republic of Serbia. Environ. Sci. Pollut. Res. 24, 2075-2088. https://doi.org/10.1007/s11356-016-7959-4

Krogh, J., Lyons, S., Lowe, C.J., 2017. Pharmaceuticals and Personal Care Products in Municipal Wastewater and the Marine Receiving Environment Near Victoria Canada. Front. Mar. Sci. 4, 1-13. https://doi.org/10.3389/fmars.2017.00415

Kumar, R., Tscharke, B., O'Brien, J., Mueller, J.F., Wilkins, C., Padhye, L.P., 2019. Assessment of drugs of abuse in a wastewater treatment plant with parallel secondary wastewater treatment train. Sci. Total Environ. 658, 947-957. https://doi.org/10.1016/j.scitotenv.2018.12.167

La Rosa, G., Iaconelli, M., Mancini, P., Bonanno Ferraro, G., Veneri, C., Bonadonna, L., Lucentini, L., Suffredini, E., 2020. First detection of SARS-CoV-2 in untreated wastewaters in Italy. Sci. Total Environ. 736, 139652. https://doi.org/10.1016/j.scitotenv.2020.139652

Lai, F.Y., Bruno, R., Hall, W., Gartner, C., Ort, C., Kirkbride, P., Prichard, J., Thai, P.K., Carter, S., Mueller, J.F., 2013a. Profiles of illicit drug use during annual key holiday and control periods in Australia: wastewater analysis in an urban, a semi-rural and a vacation area. Addiction 108, 556-565. https://doi.org/10.1111/add.12006

Lai, F.Y., Bruno, R., Leung, H.W., Thai, P.K., Ort, C., Carter, S., Thompson, K., Lam, P.K.S., Mueller,

J.F., 2013b. Estimating daily and diurnal variations of illicit drug use in Hong Kong: A pilot study of using wastewater analysis in an Asian metropolitan city. Forensic Sci. Int. 233, 126-132. https://doi.org/10.1016/j.forsciint.2013.09.003

Lai, F.Y., Gartner, C., Hall, W., Carter, S., O'Brien, J., Tscharke, B.J., Been, F., Gerber, C., White, J., Thai, P., Bruno, R., Prichard, J., Kirkbride, K.P., Mueller, J.F., 2018. Measuring spatial and temporal trends of nicotine and alcohol consumption in Australia using wastewater-based epidemiology. Addiction 113, 1127-1136. https://doi.org/10.1111/add.14157

Lajeunesse, A., Gagnon, C., Gagné, F., Louis, S., Čejka, P., Sauvé, S., 2011. Distribution of antidepressants and their metabolites in brook trout exposed to municipal wastewaters before and after ozone treatment - Evidence of biological effects. Chemosphere 83, 564-571. https://doi.org/10.1016/j.chemosphere.2010.12.026

Lajeunesse, A., Gagnon, C., Sauvé, S., 2008. Determination of basic antidepressants and their N-desmethyl metabolites in raw sewage and wastewater using solid-phase extraction and liquid chromatography-tandem mass spectrometry. Anal. Chem. 80, 5325-5333. https://doi.org/10.1021/ac800162q

Langford, K.H., Reid, M., Thomas, K. V., 2011. Multi-residue screening of prioritised human pharmaceuticals, illicit drugs and bactericides in sediments and sludge. J. Environ. Monit. 13, 2284-2291. https://doi.org/10.1039/c1em10260e

Larsson, D.G.J., de Pedro, C., Paxeus, N., 2007. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J. Hazard. Mater. 148, 751-755. https://doi.org/10.1016/j.jhazmat.2007.07.008

Licata, M., Rustichelli, C., Palazzoli, F., Ferrari, A., Baraldi, C., Vandelli, D., Verri, P., Marchesi, F., Silingardi, E., 2016. Hair testing in clinical setting: Simultaneous determination of 50 psychoactive drugs and metabolites in headache patients by LC tandem MS. J. Pharm. Biomed. Anal. 126, 14-25. https://doi.org/10.1016/j.jpba.2016.04.015

López-Serna, R., Jurado, A., Vázquez-Suñé, E., Carrera, J., Petrović, M., Barceló, D., 2013. Occurrence of 95 pharmaceuticals and transformation products in urban groundwaters underlying the metropolis of Barcelona, Spain. Environ. Pollut. 174, 305-315. https://doi.org/10.1016/j.envpol.2012.11.022

López-Serna, R., Pérez, S., Ginebreda, A., Petrović, M., Barceló, D., 2010. Fully automated determination of 74 pharmaceuticals in environmental and waste waters by online solid

phase extraction-liquid chromatography- electrospray-tandem mass spectrometry. Talanta 83, 410-424. https://doi.org/10.1016/j.talanta.2010.09.046

Lorenzo, M., Picó, Y., 2019. Wastewater-based epidemiology: current status and future prospects. Curr. Opin. Environ. Sci. Heal. https://doi.org/10.1016/j.coesh.2019.05.007

Mackul'ak, T., Birošová, L., Gál, M., Bodík, I., Grabic, R., Ryba, J., Škubák, J., 2016. Wastewater analysis: the mean of the monitoring of frequently prescribed pharmaceuticals in Slovakia. Environ. Monit. Assess. 188, 1-12. https://doi.org/10.1007/s10661-015-5011-7

Mackul'ak, T., Brandeburová, P., Grenčíková, A., Bodík, I., Staňová, A.V., Golovko, O., Koba, O., Mackuľaková, M., Špalková, V., Gál, M., Grabic, R., 2019. Music festivals and drugs: Wastewater analysis. Sci. Total Environ. 659, 326-334. https://doi.org/10.1016/j.scitotenv.2018.12.275

Mackul'ak, T., S˘kubák, J., Grabic, R., Ryba, J., Birošová, L., Fedorova, G., S˘palková, V., Bodík, I., 2014. National study of illicit drug use in Slovakia based on wastewater analysis. Sci. Total Environ. 494-495, 158-165. https://doi.org/10.1016/j.scitotenv.2014.06.089

Magni, S., Parolini, M., Binelli, A., 2016. Sublethal effects induced by morphine to the freshwater biological model *D reissena polymorpha*. Environ. Toxicol. 31, 58-67. https://doi.org/10.1002/tox.22021

Magni, S., Parolini, M., Della Torre, C., de Oliveira, L.F., Catani, M., Guzzinati, R., Cavazzini, A., Binelli, A., 2017. Multi-biomarker investigation to assess toxicity induced by two antidepressants on Dreissena polymorpha. Sci. Total Environ. 578, 452-459. https://doi.org/10.1016/j.scitotenv.2016.10.208

Manamsa, K., Lapworth, D.J., Stuart, M.E., 2016. Temporal variability of micro-organic contaminants in lowland chalk catchments: New insights into contaminant sources and hydrological processes. Sci. Total Environ. 568, 566-577. https://doi.org/10.1016/j.scitotenv.2016.01.146

Maranho, L.A., Fontes, M.K., Kamimura, A.S.S., Nobre, C.R., Moreno, B.B., Pusceddu, F.H., Cortez, F.S., Lebre, D.T., Marques, J.R., Abessa, D.M.S., Ribeiro, D.A., Pereira, C.D.S., 2017. Exposure to crack cocaine causes adverse effects on marine mussels Perna perna. Mar. Pollut. Bull. 123, 410-414. https://doi.org/10.1016/j.marpolbul.2017.08.043

Mari, F., Politi, L., Biggeri, A., Accetta, G., Trignano, C., Di Padua, M., Bertol, E., 2009. Cocaine and heroin in waste water plants: A 1-year study in the city of Florence, Italy. Forensic Sci.

Int. 189, 88-92. https://doi.org/10.1016/j.forsciint.2009.04.018

Martin Ruel, S., Choubert, J.M., Esperanza, M., Miège, C., Navalón Madrigal, P., Budzinski, H., Le Ménach, K., Lazarova, V., Coquery, M., 2011. On-site evaluation of the removal of 100 micro-pollutants through advanced wastewater treatment processes for reuse applications. Water Sci. Technol. 63, 2486-2497. https://doi.org/10.2166/wst.2011.470

Martínez Bueno, M.J., Boillot, C., Munaron, D., Fenet, H., Casellas, C., Gómez, E., 2014. Occurrence of venlafaxine residues and its metabolites in marine mussels at trace levels: Development of analytical method and a monitoring program. Anal. Bioanal. Chem. 406, 601-610. https://doi.org/10.1007/s00216-013-7477-x

Martínez Bueno, M.J., Uclés, S., Hernando, M.D., Fernández-Alba, A.R., 2011. Development of a solvent-free method for the simultaneous identification/quantification of drugs of abuse and their metabolites in environmental water by LC-MS/MS. Talanta 85, 157-166. https://doi.org/10.1016/j.talanta.2011.03.051

Maruya, K.A., Dodder, N.G., Weisberg, S.B., Gregorio, D., Bishop, J.S., Klosterhaus, S., Alvarez, D.A., Furlong, E.T., Bricker, S., Kimbrough, K.L., Lauenstein, G.G., 2014. The mussel watch California pilot study on contaminants of emerging concern (CECs): Synthesis and next steps. Mar. Pollut. Bull. 81, 355-363. https://doi.org/10.1016/j.marpolbul.2013.04.023

Mastroianni, N., Bleda, M.J., López de Alda, M., Barceló, D., 2016. Occurrence of drugs of abuse in surface water from four Spanish river basins: Spatial and temporal variations and environmental risk assessment. J. Hazard. Mater. 316, 134-142. https://doi.org/10.1016/j.jhazmat.2016.05.025

Mastroianni, N., López-García, E., Postigo, C., Barceló, D., López de Alda, M., 2017. Five-year monitoring of 19 illicit and legal substances of abuse at the inlet of a wastewater treatment plant in Barcelona (NE Spain) and estimation of drug consumption patterns and trends. Sci. Total Environ. 609, 916-926. https://doi.org/10.1016/J.SCITOTENV.2017.07.126

Mastroianni, N., Lopez de Alda, M., Barcelo, D., 2014. Analysis of ethyl sulfate in raw wastewater for estimation of alcohol consumption and its correlation with drugs of abuse in the city of Barcelona. J. Chromatogr. A 1360, 93-99. https://doi.org/10.1016/J.CHROMA.2014.07.051

Mastroianni, N., Postigo, C., De Alda, M.L., Barcelo, D., 2013. Illicit and abused drugs in sewage sludge: Method optimization and occurrence. J. Chromatogr. A 1322, 29-37. https://doi.org/10.1016/j.chroma.2013.10.078

Mendoza, A., Rodríguez-Gil, J.L., González-Alonso, S., Mastroianni, N., López de Alda, M., Barceló, D., Valcárcel, Y., 2014. Drugs of abuse and benzodiazepines in the Madrid Region (Central Spain): Seasonal variation in river waters, occurrence in tap water and potential environmental and human risk. Environ. Int. 70, 76-87. https://doi.org/10.1016/j.envint.2014.05.009

Mercan, S., Kuloglu, M., Asicioglu, F., 2019. Monitoring of illicit drug consumption via wastewater: development, challenges, and future aspects. Curr. Opin. Environ. Sci. Heal. https://doi.org/10.1016/j.coesh.2019.05.002

Metcalfe, C., Tindale, K., Li, H., Rodayan, A., Yargeau, V., 2010. Illicit drugs in Canadian municipal wastewater and estimates of community drug use. Environ. Pollut. 158, 3179-3185. https://doi.org/10.1016/j.envpol.2010.07.002

Metcalfe, C.D., Chu, S., Judt, C., Li, H., Oakes, K.D., Servos, M.R., Andrews, D.M., 2010. Antidepressants and their metabolites in municipal wastewater, and downstream exposure in an urban watershed. Environ. Toxicol. Chem. 29, 79-89. https://doi.org/10.1002/etc.27

Mole, R.A., Brooks, B.W., 2019. Global scanning of selective serotonin reuptake inhibitors: occurrence, wastewater treatment and hazards in aquatic systems. Environ. Pollut. https://doi.org/10.1016/j.envpol.2019.04.118

Montes, R., Rodil, R., Rico, A., Cela, R., González-Mariño, I., Hernández, F., Bijlsma, L., Celma, A., Picó, Y., Andreu, V., de Alda, M.L., López-García, E., Postigo, C., Pocurull, E., Marcé, R.M., Rosende, M., Olivares, M., Valcárcel, Y., Quintana, J.B., 2020. First nation-wide estimation of tobacco consumption in Spain using wastewater-based epidemiology. Sci. Total Environ. 741, 140384. https://doi.org/10.1016/j.scitotenv.2020.140384

Moreno-González, R., Rodríguez-Mozaz, S., Gros, M., Pérez-Cánovas, E., Barceló, D., León, V.M., 2014. Input of pharmaceuticals through coastal surface watercourses into a Mediterranean lagoon (Mar Menor, SE Spain): Sources and seasonal variations. Sci. Total Environ. 490, 59-72. https://doi.org/10.1016/j.scitotenv.2014.04.097

Moreno-González, R., Rodríguez-Mozaz, S., Huerta, B., Barceló, D., León, V.M., 2016. Do pharmaceuticals bioaccumulate in marine molluscs and fish from a coastal lagoon? Environ. Res. 146, 282-298. https://doi.org/10.1016/j.envres.2016.01.001

Moslah, B., Hapeshi, E., Jrad, A., Fatta-Kassinos, D., Hedhili, A., 2018. Pharmaceuticals and illicit

drugs in wastewater samples in north-eastern Tunisia. Environ. Sci. Pollut. Res. 25, 18226-18241. https://doi.org/10.1007/s11356-017-8902-z

Munro, K., Martins, C.P.B., Loewenthal, M., Comber, S., Cowan, D.A., Pereira, L., Barron, L.P., 2019. Evaluation of combined sewer overflow impacts on short-term pharmaceutical and illicit drug occurrence in a heavily urbanised tidal river catchment (London, UK). Sci. Total Environ. 657, 1099-1111. https://doi.org/10.1016/j.scitotenv.2018.12.108

Muralidharan, G., Cooper, J.K., Hawes, E.M., Korchinski, E.D., Midha, K.K., 1996. Quinidine inhibits the 7-hydroxylation of chlorpromazine in extensive metabolisers of debrisoquine. Eur. J. Clin. Pharmacol. 50, 121-128. https://doi.org/10.1007/s002280050079

Nefau, T., Karolak, S., Castillo, L., Boireau, V., Levi, Y., 2013. Presence of illicit drugs and metabolites in influents and effluents of 25 sewage water treatment plants and map of drug consumption in France. Sci. Total Environ. 461-462, 712-722. https://doi.org/10.1016/j.scitotenv.2013.05.038

Nguyen, H.T., Thai, P.K., Kaserzon, S.L., O'Brien, J.W., Eaglesham, G., Mueller, J.F., 2018. Assessment of drugs and personal care products biomarkers in the influent and effluent of two wastewater treatment plants in Ho Chi Minh City, Vietnam. Sci. Total Environ. 631-632, 469-475. https://doi.org/10.1016/j.scitotenv.2018.02.309

Nieto, A., Borrull, F., Pocurull, E., Marcé, R.M., 2010. Pressurized liquid extraction: A useful technique to extract pharmaceuticals and personal-care products from sewage sludge. TrAC - Trends Anal. Chem. https://doi.org/10.1016/j.trac.2010.03.014

NORMAN, 2020. NORMAN Ecotoxicology Database [WWW Document]. URL https://www.norman-network.com/nds/ecotox/ (accedido 5.26.20).

NORMAN, 2013. NORMAN Association Network of reference laboratories and related organisations for monitoring and bio-monitoring of emerging environmental substances Working Group on Prioritisation of Emerging Substances NORMAN Prioritisation framework for emerging subst.

Nunes, C.N., Pauluk, L.E., Dos Anjos, V.E., Lopes, M.C., Quináia, S.P., 2015. New approach to the determination of contaminants of emerging concern in natural water: study of alprazolam employing adsorptive cathodic stripping voltammetry. Anal. Bioanal. Chem. 407, 6171-6179. https://doi.org/10.1007/s00216-015-8792-1

O'Brien, J.W., Thai, P.K., Eaglesham, G., Ort, C., Scheidegger, A., Carter, S., Lai, F.Y., Mueller, J.F.,

2014. A model to estimate the population contributing to the wastewater using samples collected on census day. Environ. Sci. Technol. 48, 517-525. https://doi.org/10.1021/es403251g

OCDE, 2018. Organización para la Cooperación y el Desarrollo Económicos, 2018. Health Status [WWW Document]. URL https://stats.oecd.org/Index.aspx?DatasetCode=HEALTH_STAT (accedido 5.12.20).

OEDA, 2019. Informe 2019. Alcohol, tabaco y drogas ilegales en España [WWW Document]. URL https://pnsd.sanidad.gob.es/profesionales/sistemasInformacion/informesEstadisticas/pdf/2019OEDA-INFORME.pdf (accedido 8.13.20).

Olesti, E., Farré, M., Carbó, M., Papaseit, E., Perez-Mañá, C., Torrens, M., Yubero-Lahoz, S., Pujadas, M., Pozo, Ó.J., de la Torre, R., 2019. Dose-Response Pharmacological Study of Mephedrone and Its Metabolites: Pharmacokinetics, Serotoninergic Effects, and Impact of CYP2D6 Genetic Variation. Clin. Pharmacol. Ther. 106, 596-604. https://doi.org/10.1002/cpt.1417

Ondarza, P.M., Haddad, S.P., Avigliano, E., Miglioranza, K.S.B., Brooks, B.W., 2019. Pharmaceuticals, illicit drugs and their metabolites in fish from Argentina: Implications for protected areas influenced by urbanization. Sci. Total Environ. 649, 1029-1037. https://doi.org/10.1016/j.scitotenv.2018.08.383

Osawa, R.A., Carvalho, A.P., Monteiro, O.C., Oliveira, M.C., Florêncio, M.H., 2019. Transformation products of citalopram: Identification, wastewater analysis and in silico toxicological assessment. Chemosphere 217, 858-868. https://doi.org/10.1016/j.chemosphere.2018.11.027

Pandopulos, A.J., Bade, R., O'Brien, J.W., Tscharke, B.J., Mueller, J.F., Thomas, K., White, J.M., Gerber, C., 2020. Towards an efficient method for the extraction and analysis of cannabinoids in wastewater. Talanta 217, 121034. https://doi.org/10.1016/j.talanta.2020.121034

Parolini, M., Binelli, A., 2014. Oxidative and genetic responses induced by δ-9-tetrahydrocannabinol (δ-9-THC) to Dreissena polymorpha. Sci. Total Environ. 468-469, 68-76. https://doi.org/10.1016/j.scitotenv.2013.08.024

Parolini, M., Binelli, A., 2013a. Adverse effects induced by ecgonine methyl ester to the zebra mussel: A comparison with the benzoylecgonine. Environ. Pollut. 182, 371-378.

https://doi.org/10.1016/j.envpol.2013.07.038

Parolini, M., Castiglioni, S., Magni, S., Della Torre, C., Binelli, A., 2017. Increase in cannabis use may indirectly affect the health status of a freshwater species. Environ. Toxicol. Chem. 36, 472-479. https://doi.org/10.1002/etc.3575

Parolini, M., Magni, S., Castiglioni, S., Binelli, A., 2016. Genotoxic effects induced by the exposure to an environmental mixture of illicit drugs to the zebra mussel. Ecotoxicol. Environ. Saf. 132, 26-30. https://doi.org/10.1016/j.ecoenv.2016.05.022

Parolini, M., Magni, S., Castiglioni, S., Zuccato, E., Binelli, A., 2015. Realistic mixture of illicit drugs impaired the oxidative status of the zebra mussel (Dreissena polymorpha). Chemosphere 128, 96-102. https://doi.org/10.1016/j.chemosphere.2014.12.092

Parolini, M., Pedriali, A., Riva, C., Binelli, A., 2013b. Sub-lethal effects caused by the cocaine metabolite benzoylecgonine to the freshwater mussel Dreissena polymorpha. Sci. Total Environ. 444, 43-50. https://doi.org/10.1016/j.scitotenv.2012.11.076

Perestrelo, R., Silva, P., Porto-Figueira, P., Pereira, J.A.M., Silva, C., Medina, S., Câmara, J.S., 2019. QuEChERS - Fundamentals, relevant improvements, applications and future trends. Anal. Chim. Acta. https://doi.org/10.1016/j.aca.2019.02.036

Phillips, P.J., Smith, S.G., Kolpin, D.W., Zaugg, S.D., Buxton, H.T., Furlong, E.T., Esposito, K., Stinson, B., 2010. Pharmaceutical formulation facilities as sources of opioids and other pharmaceuticals to wastewater treatment plant effluents. Environ. Sci. Technol. 44, 4910-4916. https://doi.org/10.1021/es100356f

Picó, Y., Alvarez-Ruiz, R., Alfarhan, A.H., El-Sheikh, M.A., Alshahrani, H.O., Barceló, D., 2020. Pharmaceuticals, pesticides, personal care products and microplastics contamination assessment of Al-Hassa irrigation network (Saudi Arabia) and its shallow lakes. Sci. Total Environ. 701, 135021. https://doi.org/10.1016/j.scitotenv.2019.135021

Piel, S., Blondeau, S., Pérot, J., Baurès, E., Thomas, O., 2013. Pharmaceutical removal by the activated carbon process. https://doi.org/10.2166/wqrjc.2013.138

Postigo, C., de Alda, M.L., Barceló, D., 2011a. Evaluation of drugs of abuse use and trends in a prison through wastewater analysis. Environ. Int. 37, 49-55. https://doi.org/10.1016/j.envint.2010.06.012

Postigo, C., Lopez De Alda, M.J., Barceló, D., 2008. Fully Automated Determination in the Low Nanogram per Liter Level of Different Classes of Drugs of Abuse in Sewage Water by On-

Line Solid-Phase Extraction-Liquid Chromatography-Electrospray-Tandem Mass Spectrometry. https://doi.org/10.1021/ac702060j

Postigo, C., López de Alda, M.J., Barceló, D., 2010. Drugs of abuse and their metabolites in the Ebro River basin: Occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation. Environ. Int. 36, 75-84. https://doi.org/10.1016/j.envint.2009.10.004

Postigo, C., Sirtori, C., Oller, I., Malato, S., Maldonado, M.I., López de Alda, M., Barceló, D., 2011b. Solar transformation and photocatalytic treatment of cocaine in water: Kinetics, characterization of major intermediate products and toxicity evaluation. Appl. Catal. B Environ. 104, 37-48. https://doi.org/10.1016/j.apcatb.2011.02.030

Postigo, C., Sirtori, C., Oller, I., Malato, S., Maldonado, M.I., López de Alda, M., Barceló, D., 2011c. Photolytic and photocatalytic transformation of methadone in aqueous solutions under solar irradiation: Kinetics, characterization of major intermediate products and toxicity evaluation. Water Res. 45, 4815-4826. https://doi.org/10.1016/j.watres.2011.06.027

Proia, L., Osorio, V., Soley, S., Köck-Schulmeyer, M., Pérez, S., Barceló, D., Romaní, A.M., Sabater, S., 2013. Effects of pesticides and pharmaceuticals on biofilms in a highly impacted river. Environ. Pollut. 178, 220-228. https://doi.org/10.1016/j.envpol.2013.02.022

Racamonde, I., Quintana, J.B., Rodil, R., Cela, R., 2015. Application of polypropylene tubes as single-use and low-cost sorptive extraction materials for the determination of benzodiazepines and zolpidem in water samples. Microchem. J. 119, 58-65. https://doi.org/10.1016/j.microc.2014.10.011

Racamonde, I., Rodil, R., Quintana, J.B., Villaverde-de-Sáa, E., Cela, R., 2014. Determination of benzodiazepines, related pharmaceuticals and metabolites in water by solid-phase extraction and liquid-chromatography-tandem mass spectrometry. J. Chromatogr. A 1352, 69-79. https://doi.org/10.1016/j.chroma.2014.05.064

Racamonde, I., Villaverde-de-Sáa, E., Rodil, R., Quintana, J.B., Cela, R., 2012. Determination of Δ9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in water samples by solid-phase microextraction with on-fiber derivatization and gas chromatography-mass spectrometry. J. Chromatogr. A 1245, 167-174. https://doi.org/10.1016/j.chroma.2012.05.017

Ramirez, A.J., Brain, R.A., Usenko, S., Mottaleb, M.A., O'Donnell, J.G., Stahl, L.L., Wathen, J.B.,

Snyder, B.D., Pitt, J.L., Perez-Hurtado, P., Dobbins, L.L., Brooks, B.W., Chambliss, C.K., 2009. Occurrence of pharmaceuticals and personal care products in fish: Results of a national pilot study in the United States. Environ. Toxicol. Chem. 28, 2587-2597. https://doi.org/10.1897/08-561.1

Reid, M.J., Langford, K.H., Mørland, J., Thomas, K. V., 2011. Analysis and Interpretation of Specific Ethanol Metabolites, Ethyl Sulfate, and Ethyl Glucuronide in Sewage Effluent for the Quantitative Measurement of Regional Alcohol Consumption. Alcohol. Clin. Exp. Res. no-no. https://doi.org/10.1111/j.1530-0277.2011.01505.x

Reidy, L., Nolan, B., Ramos, A.R., Walls, H.C., Steele, B.W., 2011. Zolpidem urine excretion profiles and cross-reactivity with ELISA® kits in subjects using zolpidem or ambien® CR as a prescription sleep aid. J. Anal. Toxicol. 35, 294-301. https://doi.org/10.1093/anatox/35.5.294

Richardson, S.D., 2012. Environmental mass spectrometry: Emerging contaminants and current issues. Anal. Chem. https://doi.org/10.1021/ac202903d

Rodríguez-Álvarez, T., Racamonde, I., González-Mariño, I., Borsotti, A., Rodil, R., Rodríguez, I., Zuccato, E., Quintana, J.B., Castiglioni, S., 2015. Alcohol and cocaine co-consumption in two European cities assessed by wastewater analysis. Sci. Total Environ. 536, 91-98. https://doi.org/10.1016/j.scitotenv.2015.07.016

Rodríguez-Álvarez, T., Rodil, R., Cela, R., Quintana, J.B., 2014. Ion-pair reversed-phase liquid chromatography-quadrupole-time-of-flight and triple-quadrupole-mass spectrometry determination of ethyl sulfate in wastewater for alcohol consumption tracing. J. Chromatogr. A 1328, 35-42. https://doi.org/10.1016/j.chroma.2013.12.076

Rodríguez-Gil, J.L., Catalá, M., Alonso, S.G., Maroto, R.R., Valcárcel, Y., Segura, Y., Molina, R., Melero, J.A., Martínez, F., 2010. Heterogeneous photo-Fenton treatment for the reduction of pharmaceutical contamination in Madrid rivers and ecotoxicological evaluation by a miniaturized fern spores bioassay. Chemosphere 80, 381-388. https://doi.org/10.1016/j.chemosphere.2010.04.045

Rojo, M., Álvarez-Muñoz, D., Dománico, A., Foti, R., Rodriguez-Mozaz, S., Barceló, D., Carriquiriborde, P., 2019. Human pharmaceuticals in three major fish species from the Uruguay River (South America) with different feeding habits. Environ. Pollut. 252, 146-154. https://doi.org/10.1016/j.envpol.2019.05.099

Rosa Boleda, M., Huerta-Fontela, M., Ventura, F., Galceran, M.T., 2011. Evaluation of the presence of drugs of abuse in tap waters. Chemosphere 84, 1601-1607. https://doi.org/10.1016/j.chemosphere.2011.05.033

Rousis, N.I., Zuccato, E., Castiglioni, S., 2017. Wastewater-based epidemiology to assess human exposure to pyrethroid pesticides. Environ. Int. 99, 213-220. https://doi.org/10.1016/j.envint.2016.11.020

Rúbies, A., Guo, L., Centrich, F., Granados, M., 2016. Analysis of non-steroidal anti-inflammatory drugs in milk using QuEChERS and liquid chromatography coupled to mass spectrometry: triple quadrupole versus Q-Orbitrap mass analyzers. Anal. Bioanal. Chem. 408, 5769-5778. https://doi.org/10.1007/s00216-016-9679-5

Russo, D., Spasiano, D., Vaccaro, M., Cochran, K.H., Richardson, S.D., Andreozzi, R., Li Puma, G., Reis, N.M., Marotta, R., 2016. Investigation on the removal of the major cocaine metabolite (benzoylecgonine) inwater matrices by UV254/H2O2process by using a flow microcapillary film array photoreactor as an efficient experimental tool. Water Res. 89, 375-383. https://doi.org/10.1016/j.watres.2015.11.059

Ryu, Y., Barceló, D., Barron, L.P., Bijlsma, L., Castiglioni, S., de Voogt, P., Emke, E., Hernández, F., Lai, F.Y., Lopes, A., de Alda, M.L., Mastroianni, N., Munro, K., O'Brien, J., Ort, C., Plósz, B.G., Reid, M.J., Yargeau, V., Thomas, K. V., 2016. Comparative measurement and quantitative risk assessment of alcohol consumption through wastewater-based epidemiology: An international study in 20 cities. Sci. Total Environ. 565, 977-983. https://doi.org/10.1016/j.scitotenv.2016.04.138

Salgueiro-González, N., Castiglioni, S., Gracia-Lor, E., Bijlsma, L., Celma, A., Bagnati, R., Hernández, F., Zuccato, E., 2019. Flexible high resolution-mass spectrometry approach for screening new psychoactive substances in urban wastewater. Sci. Total Environ. 689, 679-690. https://doi.org/10.1016/j.scitotenv.2019.06.336

Santos, L.H.M.L.M., Ramalhosa, M.J., Ferreira, M., Delerue-Matos, C., 2016. Development of a modified acetonitrile-based extraction procedure followed by ultra-high performance liquid chromatography-tandem mass spectrometry for the analysis of psychiatric drugs in sediments. J. Chromatogr. A 1437, 37-48. https://doi.org/10.1016/j.chroma.2016.01.079

Schlüsener, M.P., Hardenbicker, P., Nilson, E., Schulz, M., Viergutz, C., Ternes, T.A., 2015. Occurrence of venlafaxine, other antidepressants and selected metabolites in the Rhine catchment in the face of climate change. Environ. Pollut. 196, 247-256.

https://doi.org/10.1016/j.envpol.2014.09.019

Schultz, M.M., Furlong, E.T., 2008. Trace analysis of antidepressant pharmaceuticals and their select degradates in aquatic matrixes by LC/ESI/MS/MS. Anal. Chem. 80, 1756-1762. https://doi.org/10.1021/ac702154e

Schultz, M.M., Furlong, E.T., Kolpin, D.W., Werner, S.L., Schoenfuss, H.L., Barber, L.B., Blazer, V.S., Norris, D.O., Vajda, A.M., 2010. Antidepressant pharmaceuticals in two U.S. effluent-impacted streams: Occurrence and fate in water and sediment and selective uptake in fish neural tissue. Environ. Sci. Technol. 44, 1918-1925. https://doi.org/10.1021/es9022706

Senta, I., Krizman, I., Ahel, M., Terzic, S., 2013. Integrated procedure for multiresidue analysis of dissolved and particulate drugs in municipal wastewater by liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 405, 3255-3268. https://doi.org/10.1007/s00216-013-6720-9

Shao, B., Chen, D., Zhang, J., Wu, Y., Sun, C., 2009. Determination of 76 pharmaceutical drugs by liquid chromatography-tandem mass spectrometry in slaughterhouse wastewater. J. Chromatogr. A 1216, 8312-8318. https://doi.org/10.1016/j.chroma.2009.08.038

Shraim, A., Diab, A., Alsuhaimi, A., Niazy, E., Metwally, M., Amad, M., Sioud, S., Dawoud, A., 2017. Analysis of some pharmaceuticals in municipal wastewater of Almadinah Almunawarah. Arab. J. Chem. 10, S719-S729. https://doi.org/10.1016/j.arabjc.2012.11.014

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

Silva, L.J.G., Pereira, A.M.P.T., Meisel, L.M., Lino, C.M., Pena, A., 2015. Reviewing the serotonin reuptake inhibitors (SSRIs) footprint in the aquatic biota: Uptake, bioaccumulation and ecotoxicology. Environ. Pollut. https://doi.org/10.1016/j.envpol.2014.12.002

Silva, L.J.G., Pereira, A.M.P.T., Rodrigues, H., Meisel, L.M., Lino, C.M., Pena, A., 2017. SSRIs antidepressants in marine mussels from Atlantic coastal areas and human risk assessment. Sci. Total Environ. 603-604, 118-125. https://doi.org/10.1016/j.scitotenv.2017.06.076

Skees, A.J., Foppe, K.S., Loganathan, B., Subedi, B., 2018. Contamination profiles, mass loadings, and sewage epidemiology of neuropsychiatric and illicit drugs in wastewater and river

waters from a community in the Midwestern United States. Sci. Total Environ. 631-632, 1457-1464. https://doi.org/10.1016/j.scitotenv.2018.03.060

Snyder, S.A., Kelly, K.L., Grange, A.H., Sovocool, G.W., Snyder, E.M., Giesy, J.P., 2001. Pharmaceuticals and personal care products in the waters of Lake Mead, Nevada. ACS Symp. Ser. 791, 116-139. https://doi.org/10.1021/bk-2001-0791.ch007

Sousa, M.A., Gonçalves, C., Cunha, E., Hajšlová, J., Alpendurada, M.F., 2011. Cleanup strategies and advantages in the determination of several therapeutic classes of pharmaceuticals in wastewater samples by SPE-LC-MS/MS. Anal. Bioanal. Chem. 399, 807-822. https://doi.org/10.1007/s00216-010-4297-0

Subedi, B., Balakrishna, K., Sinha, R.K., Yamashita, N., Balasubramanian, V.G., Kannan, K., 2015a. Mass loading and removal of pharmaceuticals and personal care products, including psychoactive and illicit drugs and artificial sweeteners, in five sewage treatment plants in India. J. Environ. Chem. Eng. 3, 2882-2891. https://doi.org/10.1016/j.jece.2015.09.031

Subedi, B., Burgard, D., 2019. Wastewater-Based Epidemiology as a Complementary Approach to the Conventional Survey-Based Approach for the Estimation of Community Consumption of Drugs Drugs of Abuse-A Global Concern, Wastewater-Based Epidemiology: Estimation of Community Consumption of Drugs and Diets, Part 1 - Wastewater-Based Epidemiology as a Complementary Approach to the Conventional Survey-Based Approach for the Estimation of Community Consumption of Drugs. https://doi.org/10.1021/bk-2019-1319.ch001

Subedi, B., Kannan, K., 2015b. Occurrence and fate of select psychoactive pharmaceuticals and antihypertensives in two wastewater treatment plants in New York State, USA. Sci. Total Environ. 514, 273-280. https://doi.org/10.1016/j.scitotenv.2015.01.098

Subedi, B., Kannan, K., 2014. Mass loading and removal of select illicit drugs in two wastewater treatment plants in New York State and estimation of illicit drug usage in communities through wastewater analysis. Environ. Sci. Technol. 48, 6661-6670. https://doi.org/10.1021/es501709a

Sulej-Suchomska, A.M., Klupczynska, A., Dereziński, P., Matysiak, J., Przybyłowski, P., Kokot, Z.J., 2020. Urban wastewater analysis as an effective tool for monitoring illegal drugs, including new psychoactive substances, in the Eastern European region. Sci. Rep. 10, 1-12. https://doi.org/10.1038/s41598-020-61628-5

Ternes, T.A., 1998. Occurrence of drugs in German sewage treatment plants and rivers. Water Res. 32, 3245-3260. https://doi.org/10.1016/S0043-1354(98)00099-2

Terzic, S., Senta, I., Ahel, M., 2010. Illicit drugs in wastewater of the city of Zagreb (Croatia) - Estimation of drug abuse in a transition country. Environ. Pollut. 158, 2686-2693. https://doi.org/10.1016/j.envpol.2010.04.020

Thai, P.K., Lai, F.Y., Bruno, R., van Dyken, E., Hall, W., O'Brien, J., Prichard, J., Mueller, J.F., 2016. Refining the excretion factors of methadone and codeine for wastewater analysis - Combining data from pharmacokinetic and wastewater studies. Environ. Int. 94, 307-314. https://doi.org/10.1016/j.envint.2016.05.033

Thomaidis, N.S., Gago-Ferrero, P., Ort, C., Maragou, N.C., Alygizakis, N.A., Borova, V.L., Dasenaki, M.E., 2016. Reflection of Socioeconomic Changes in Wastewater: Licit and Illicit Drug Use Patterns. Environ. Sci. Technol. 50, 10065-10072. https://doi.org/10.1021/acs.est.6b02417

UNODC, 2020. World Drug Report 2020 [WWW Document]. URL https://wdr.unodc.org/wdr2020/index.html (accedido 8.13.20).

UNODC, 2019. World Drug Report 2019 [WWW Document]. URL https://wdr.unodc.org/wdr2019/ (accedido 8.13.20).

UNODC, 2018. Terminología e Información sobre Drogas. Tercera edición [WWW Document]. URL https://www.unodc.org/documents/scientific/Terminology_and_Information_on_Drugs_S_3rd_Edition.pdf (accedido 8.13.20).

UNODC, 1971. Convention on Psychotropics Substances of 1971 [WWW Document]. URL https://www.incb.org/incb/en/psychotropics/1971_convention.html (accedido 8.13.20).

UNODC, 1961. Single Convention on Narcotic Drugs of 1961 [WWW Document]. URL https://www.incb.org/incb/en/narcotic-drugs/1961_Convention.html (accedido 8.13.20).

US EPA, 2012. Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: Procedures for the Determination of the Freely Dissolved Interstitial Water Concentrations of Nonionic Organics.

Valcárcel, Y., Martínez, F., González-Alonso, S., Segura, Y., Catalá, M., Molina, R., Montero-Rubio, J.C., Mastroianni, N., López de Alda, M., Postigo, C., Barceló, D., 2012. Drugs of abuse in surface and tap waters of the Tagus River basin: Heterogeneous photo-Fenton process is effective in their degradation. Environ. Int. 41, 35-43.

https://doi.org/10.1016/j.envint.2011.12.006

Valhondo, C., Carrera, J., Ayora, C., Barbieri, M., Nödler, K., Licha, T., Huerta, M., 2014. Behavior of nine selected emerging trace organic contaminants in an artificial recharge system supplemented with a reactive barrier. Environ. Sci. Pollut. Res. 21, 11832-11843. https://doi.org/10.1007/s11356-014-2834-7

van der Aa, M., Bijlsma, L., Emke, E., Dijkman, E., van Nuijs, A.L.N., van de Ven, B., Hernández, F., Versteegh, A., de Voogt, P., 2013. Risk assessment for drugs of abuse in the Dutch watercycle. Water Res. 47, 1848-1857. https://doi.org/10.1016/j.watres.2013.01.013

van der Ven, K., Van Dongen, W., Maes, B.U.W., Esmans, E.L., Blust, R., De Coen, W.M., 2004. Determination of diazepam in aquatic samples by capillary liquid chromatography-electrospray tandem mass spectrometry. Chemosphere 57, 967-973. https://doi.org/10.1016/j.chemosphere.2004.08.062

van Nuijs, A.L.N., Lai, F.Y., Been, F., Andres-Costa, M.J., Barron, L., Baz-Lomba, J.A., Berset, J.D., Benaglia, L., Bijlsma, L., Burgard, D., Castiglioni, S., Christophoridis, C., Covaci, A., de Voogt, P., Emke, E., Fatta-Kassinos, D., Fick, J., Hernandez, F., Gerber, C., González-Mariño, I., Grabic, R., Gunnar, T., Kannan, K., Karolak, S., Kasprzyk-Hordern, B., Kokot, Z., Krizman-Matasic, I., Li, A., Li, X., Löve, A.S.C., Lopez de Alda, M., McCall, A.K., Meyer, M.R., Oberacher, H., O'Brien, J., Quintana, J.B., Reid, M., Schneider, S., Simoes, S.S., Thomaidis, N.S., Thomas, K., Yargeau, V., Ort, C., 2018. Multi-year inter-laboratory exercises for the analysis of illicit drugs and metabolites in wastewater: Development of a quality control system. TrAC - Trends Anal. Chem. https://doi.org/10.1016/j.trac.2018.03.009

van Nuijs, A.L.N., Mougel, J.F., Tarcomnicu, I., Bervoets, L., Blust, R., Jorens, P.G., Neels, H., Covaci, A., 2011. Sewage epidemiology - A real-time approach to estimate the consumption of illicit drugs in Brussels, Belgium. Environ. Int. 37, 612-621. https://doi.org/10.1016/j.envint.2010.12.006

van Nuijs, A.L.N., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P.G., Bervoets, L., Blust, R., Neels, H., Covaci, A., 2009a. Spatial and temporal variations in the occurrence of cocaine and benzoylecgonine in waste- and surface water from Belgium and removal during wastewater treatment. Water Res. 43, 1341-1349. https://doi.org/10.1016/j.watres.2008.12.020

van Nuijs, A.L.N., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P.G., Bervoets, L., Blust, R., Neels, H., Covaci, A., 2009b. Cocaine and metabolites in waste and surface water across

Belgium. Environ. Pollut. 157, 123-129. https://doi.org/10.1016/j.envpol.2008.07.020

Vazquez-Roig, P., Andreu, V., Blasco, C., Picó, Y., 2012. Risk assessment on the presence of pharmaceuticals in sediments, soils and waters of the Pego-Oliva Marshlands (Valencia, eastern Spain). Sci. Total Environ. 440, 24-32. https://doi.org/10.1016/j.scitotenv.2012.08.036

Vazquez-Roig, P., Andreu, V., Blasco, C., Picó, Y., 2010a. SPE and LC-MS/MS determination of 14 illicit drugs in surface waters from the Natural Park of L'Albufera (València, Spain), en: Analytical and Bioanalytical Chemistry. pp. 2851-2864. https://doi.org/10.1007/s00216-010-3720-x

Vazquez-Roig, P., Segarra, R., Blasco, C., Andreu, V., Picó, Y., 2010b. Determination of pharmaceuticals in soils and sediments by pressurized liquid extraction and liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1217, 2471-2483. https://doi.org/10.1016/j.chroma.2009.11.033

Wang, Z., Gao, S., Dai, Q., Zhao, M., Yang, F., 2020. Occurrence and risk assessment of psychoactive substances in tap water from China. Environ. Pollut. 261. https://doi.org/10.1016/j.envpol.2020.114163

Wang, Zhe, Shao, X.T., Tan, D.Q., Yan, J.H., Xiao, Y., Zheng, Q. Da, Pei, W., Wang, Zhuang, Wang, D.G., 2019. Reduction in methamphetamine consumption trends from 2015 to 2018 detected by wastewater-based epidemiology in Dalian, China. Drug Alcohol Depend. 194, 302-309. https://doi.org/10.1016/j.drugalcdep.2018.10.023

Weinberger, J., Klaper, R., 2014. Environmental concentrations of the selective serotonin reuptake inhibitor fluoxetine impact specific behaviors involved in reproduction, feeding and predator avoidance in the fish Pimephales promelas (fathead minnow). Aquat. Toxicol. 151, 77-83. https://doi.org/10.1016/j.aquatox.2013.10.012

Wilkinson, J.L., Hooda, P.S., Swinden, J., Barker, J., Barton, S., 2018. Spatial (bio)accumulation of pharmaceuticals, illicit drugs, plasticisers, perfluorinated compounds and metabolites in river sediment, aquatic plants and benthic organisms. Environ. Pollut. 234, 864-875. https://doi.org/10.1016/j.envpol.2017.11.090

Wu, M., Xiang, J., Que, C., Chen, F., Xu, G., 2015. Occurrence and fate of psychiatric pharmaceuticals in the urban water system of Shanghai, China. Chemosphere 138, 486-493. https://doi.org/10.1016/j.chemosphere.2015.07.002

Yadav, M.K., Short, M.D., Aryal, R., Gerber, C., van den Akker, B., Saint, C.P., 2017. Occurrence of illicit drugs in water and wastewater and their removal during wastewater treatment. Water Res. 124, 713-727. https://doi.org/10.1016/j.watres.2017.07.068

Yadav, M.K., Short, M.D., Gerber, C., van den Akker, B., Aryal, R., Saint, C.P., 2019. Occurrence, removal and environmental risk of markers of five drugs of abuse in urban wastewater systems in South Australia. Environ. Sci. Pollut. Res. 26, 33816-33826. https://doi.org/10.1007/s11356-018-2464-6

Yang, B., Xu, C., Kookana, R.S., Williams, M., Du, J., Ying, G., Gu, F., 2018. Aqueous chlorination of benzodiazepines diazepam and oxazepam: Kinetics, transformation products and reaction pathways. Chem. Eng. J. 354, 1100-1109. https://doi.org/10.1016/j.cej.2018.08.082

Yargeau, V., Taylor, B., Li, H., Rodayan, A., Metcalfe, C.D., 2014. Analysis of drugs of abuse in wastewater from two Canadian cities. Sci. Total Environ. 487, 722-730. https://doi.org/10.1016/j.scitotenv.2013.11.094

Zhang, Xiaohan, Huang, R., Li, P., Ren, Y., Gao, J., Mueller, J.F., Thai, P.K., 2019. Temporal profile of illicit drug consumption in Guangzhou, China monitored by wastewater-based epidemiology. Environ. Sci. Pollut. Res. 26, 23593-23602. https://doi.org/10.1007/s11356-019-05575-3

Zhang, Xin, Yang, Yunjia, Zhang, J., Yang, Yi, Shen, F., Shen, J., Shao, B., 2019. Determination of emerging chlorinated byproducts of diazepam in drinking water. Chemosphere 218, 223-231. https://doi.org/10.1016/j.chemosphere.2018.11.076

Zheng, Q., Tscharke, B.J., Krapp, C., O'Brien, J.W., Mackie, R.S., Connor, J., Mueller, J.F., Thomas, K. V., Thai, P.K., 2020. New approach for the measurement of long-term alcohol consumption trends: Application of wastewater-based epidemiology in an Australian regional city. Drug Alcohol Depend. 207. https://doi.org/10.1016/j.drugalcdep.2019.107795

Zuccato, E., Castiglioni, S., Bagnati, R., Chiabrando, C., Grassi, P., Fanelli, R., 2008a. Illicit drugs, a novel group of environmental contaminants. Water Res. 42, 961-968. https://doi.org/10.1016/j.watres.2007.09.010

Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R., Fanelli, R., 2008b. Estimating community drug abuse by wastewater analysis. Environ. Health Perspect. 116, 1027-1032.

https://doi.org/10.1289/ehp.11022

Zuccato, E., Chiabrando, C., Castiglioni, S., Calamari, D., Bagnati, R., Schiarea, S., Fanelli, R., 2005. Cocaine in surface waters: A new evidence-based tool to monitor community drug abuse. Environ. Heal. A Glob. Access Sci. Source 4. https://doi.org/10.1186/1476-069X-4-14

Anexos

Anexo I. Abreviaciones y acrónimos

6ACM	6-Monoacetilmorfina
A.F.	Ácido fórmico
ABN	Sorbente polimérico para la extracción de compuestos ácidos, básicos y neutros (del inglés, *Acidic Basic and Neutral Analytes*)
AcEt	Acetato de etilo
ACN	Acetonitrilo
ADN	Ácido desoxirribonucleico
AEMPS	Agencia Española de Medicamentos y Productos Sanitarios
AH-7921	3,4-dicloro-N-[(1-(dimetilamino)ciclohexil)metil]benzamida
ALPZ	Alprazolam
AM	Anfetamina
APCI	Ionización química a presión atmosférica
APPI	Fotoionización a presión atmosférica
ASPC	Agencia de Salud Pública de Cataluña
BCF	Factor de bioconcentración (del inglés, *Bioconcentration Factor*)
BE	Benzoilecgonina
CAF	Cafeína
CBD	Cannabidiol
CBN	Cannabinol
CE	Cocaetileno
CG	Cromatografía de gases
CL	Cromatografía de líquidos
CHLOR	Clorpromacina
COC	Cocaína
CTLP	Citalopram
d.i.	Diámetro interno
DDD_{pm}	Dosis Diarias Definidas (por cada mil habitantes)
DIAZ	Diazepam
DiBP	Di-iso-butil ftalato
DnBP	Di-n-butil ftalato
d-SPE	Extracción en fase sólida dispersiva (del inglés, *dispersive Solid Phase Extraction*)

EC_{50}	Concentración efectiva media (del inglés, *half maximal Effective Concentration*)
EDADES	Encuesta sobre Alcohol y otras Drogas en España
EDAR	Estación depuradora de aguas residuales
EDDP	2-Etilideno-1,5-dimetil-3,3-difenil-pirrolidina
EDTA	Ácido etilendiaminotetraacético (del inglés, E*thyleneDiamineTetraAcetic acid*)
EI	Ionización electrónica
EMCDDA	Observatorio Europeo de las Drogas y las Toxicomanías (del inglés, *European Monitoring Center for Drugs and Drug Addition*)
ENSE	Encuesta Nacional de Salud de España
EPH	Efedrina
ERA	Evaluación de Riesgo Ambiental
ESAR-NET	Red española de análisis de aguas residuales con fines epidemiológicos
ESI (+/-)	Ionización por electrospray (modo positivo/negativo)
ETAs	Estimulantes de tipo anfetamínico
EtS	Sulfato de etilo (del inglés, *Ethyl Sulfate*)
$F_{agua-sed}$	Fracción de agua en sedimento
$F_{aire-sed}$	Fracción de aire en sedimento
FLX	Fluoxetina
$F_{oc.sed}$	Fracción de carbono orgánico en sedimento
FS	Barrido completo (del inglés, *Full Scan*)
$F_{sólido-sedimento}$	Fracción de sólido en el sedimento
FV	Fibra de vidrio
GABA	Ácido gamma (γ)-aminobutírico (del inglés, *Gamma-AminoButyric Acid*)
GC	Cromatografía de gases (del inglés, *Gas Chromatography*)
GHB	Ácido (γ)-hidroxibutírico (del inglés, *Gamma-HydroxyButyric acid*)
HER	Heroína
HILIC	Cromatografía de líquidos de interacción hidrofílica (del inglés, *Hydrophilic Interaction Liquid Chromatography*)
HLB	Sorbente polimérico de equilibrio hidrofílico-lipofílico (del inglés, *Hydrophilic-Lipophilic balanced*)
HPLC	Cromatografía de líquidos de alta eficacia (del inglés, *High Performance Liquid Chromatography)*

HQ	Índice de riesgo (del inglés, *Hazard Quotient*)
HXZ	Hidroxicina
INE	Instituto Nacional de Estadística
IRSN	Inhibidor de la Recaptación de Serotonina y Noradrenalina
IS	Patrón interno (del inglés, *Internal Standard*)
ISRD	Inhibidor Selectivo de la Recaptación de Dopamina
ISRN	Inhibidor Selectivo de la Recaptación de Noradrenalina
ISRS	Inhibidor Selectivo de la Recaptación de Serotonina
$K_{aire\text{-}agua}$	Coeficiente de reparto aire-agua
KET	Ketamina
K_D	Coeficiente de distribución sedimento-agua
K_{oc}	Coeficiente de reparto carbono orgánico-agua
K_{ow}	Coeficiente de reparto octanol-agua
$K_{sed\text{-}agua}$	Coeficiente de reparto sedimento-agua
$K\rho_{sed}$	Coeficiente de reparto sólido-agua en sedimentos
LC	Cromatografía líquida (del inglés, *Liquid Chromatography*)
LC_{50}	Concentración letal media (del inglés, *half maximum Lethal Concentration*)
LIT	Analizador de trampa lineal de iones (del inglés, *Linear Ion Trap*)
LODs	Límites de detección (del inglés, *Limits Of Detection*)
LOQs	Límites de cuantificación (del inglés, *Limits Of Quantification*)
LORZ	Lorazepam
LRMZ	Lormetazepam
LSD	Dietilamida de ácido lisérgico (del alemán, *LysergSäure-Diethylamid)*
m/z	Relación masa-carga
MA	Metanfetamina
McIlvaine	Tampón citrato-fosfato
MCX	Sorbente polimérico mixto de intercambio catiónico-fase reversa (del inglés, *Mixed-mode Cation-eXchange reversed phase*)
MDA	3,4-Metilendioxianfetamina
MDEA	3,4-Metilendioxo-N-etilanfetamina
MDMA	3,4-Metilendioximetanfetamina
MDPV	3,4-Metilendioxi-pirovalerona
MEC	Concentración medioambiental medida (del inglés, *Measured Environmental Concentration)*

MeOH	Metanol
MEPH	Mefedrona
MET	Metadona
$MgSO_4$	Sulfato de magnesio
MIDZ	Midazolam
MOR	Morfina
MS	Espectrometría de masas (de inglés, *Mass Spectrometry*)
MS/MS	Espectrometría de masas en tándem
MXE	Metoxetamina
n.d.	No detectado
n.i.	No indicado
NaCl	Cloruro de sodio
Na_2SO_4	Sulfato de sodio
$NH_4CH_3CO_2$	Acetato de amonio
NH_4Cl	Cloruro de amonio
NH_4HCO_2	Formiato de amonio
NH_4OH	Hidróxido amónico
NOEC	Concentración a la que no se observan efectos toxicológicos (del inglés, *No Observed Effect Concentration*)
NSPs	Nuevas Sustancias Psicoactivas
OCDE	Organización para la Cooperación y el Desarrollo Económicos
OEDA	Observatorio Español de las Drogas y las Adicciones
OH-ALPZ	α-hidroxi-alprazolam
OH-LSD	Hidroxi-LSD
OH-MIDZ	α-hidroxi-midazolam
OH-THC	11-hidroxi-Δ^9THC
OMS	Organización Mundial de la Salud
OXA	Oxazepam
p.f.	Peso fresco
p.s.	Peso seco
PLE	Extracción con líquidos presurizados (del inglés, *Pressurized Liquid Extraction*)
PLRP-s	Sorbente polimérico de estireno-divinilbenceno reticulado

PNEC	Concentración más baja a la que no se esperan efectos toxicológicos (del inglés, *Predicted No-effect Concentration*)
PS	Barrido de iones producto (del inglés, *Product ion Scan)*
PSA	Sorbente de aminas primarias y secundarias (del inglés, Primary Secondary Amine)
QqLIT	Analizador híbrido de cuadrupolo-trampa lineal de iones
QqOrbitrap	Analizador híbrido de cuadrupolo-trampa orbital de iones
QqQ	Analizador de triple cuadrupolo
QqTOF	Analizador híbrido de cuadrupolo-tiempo de vuelo
QSAR	Enfoques de relación estructura-actividad (del inglés, *Quantitative Structure-Activity Relationship*)
RHO_{sed}	Densidad del sedimento
$RHO_{sólido}$	Densidad de la fracción sólida
SAT	Sistema de Alerta Temprana
SAX	Sorbente de intercambio aniónico (del inglés, Strong *Anionic eXchange*)
SCI	Índice de citación en ciencia (del inglés*, Science Citation Index)*
SCORE	Red europea de análisis de aguas residuales (del inglés, *Sewage analysis CORe group Europe*)
SPE	Extracción en fase sólida (del inglés, *Solid Phase Extraction*)
SRM	Registro de reacciones seleccionadas (del inglés, *Selected Reaction Monitoring*)
STR	Sertralina
Strata-X	Cartucho polimérico de fase reversa
Strata-XC	Cartucho polimérico de fase reversa-intercambio catiónico
TDAH	Trastorno por déficit de atención e hiperactividad
TEMZ	Temazepam
THC	Δ^9-tetrahidrocannabinol
THC-COOH	11-nor-9-carboxi-Δ^9THC
TiO_2	Dióxido de titanio
TOF	Analizador de alta resolución de tiempo de vuelo (del inglés, *Time of Flight*)
UHPLC o UPLC	Cromatografía líquida de ultra-alta eficacia (del inglés, *Ultra-High Performance Liquid Chromatography*).
UNODC	Oficina de las Naciones Unidas contra la Droga y el Delito (del inglés, *United Nations Office on Drug and Crime*)

US	Ultrasonidos
UV	Ultravioleta
Valencia-PI	Valencia Pinedo I
Valencia-PII	Valencia-Pinedo II
Valencia-QB	Valencia-Quart Benàger
VFX	Venlafaxina
WBE	Análisis de aguas residuales con fines epidemiológicos (del inglés, *Wastewater-Based Epidemiology)*
ZOLP	Zolpidem
Z-Sep	Material sorbente con átomos de zirconio

Anexo II. Índice de tablas

Anexo III. Índice de figuras

Anexo IV. Revisión de niveles de drogas de abuso, psicofármacos y metabolitos en aguas

Tabla AIV- 1. Concentración de drogas, psicofármacos y metabolitos (ng/L) en aguas.

	País	Agua residual		Agua Superficial	Agua potable	Referencias
		Entrada	Salida			
COC	Bélgica	22-678		n.d.-115		[1-4]
	Canadá	209-823	<LOQ-530			[5]
	China	0,2-562	0,2-0,5		<LOD-0,02	[6,7]
	Costa Rica	525-2710	29-62	<LOQ-10		[8]
	Croacia	0,7-114	0,7-70			[9,10]
	Eslovaquia	<LOD-193				[11,12]
	España	4-4700	1-540	n,d-120	0,1-60	[13-27]
	EEUU	10-860	<LOD-27	2,3-4,2		[28-31]
	Francia	4,8-1532	1,2-335			[32,33]
	Grecia	4,4-49	3,2-11,4			[31]
	Irlanda	489	47-138	25-33		[35]
	Italia	42-421		0,5-44		[36-40]
	Malasia	<LOD-11				[41]
	N. Zelanda	<LOD-167	<LOD - 286			[42]
	Países Bajos	87-957	10-235	2		[43,44]
	Reino Unido	5,1-1575	0,6-149	n.d.-17		[40,45-51]
	R. Checa	55-155				[52]
	Suiza	218-248	10,7-15	0,4		[37,38,53]
	Túnez	25-450	27-234			[54]
	Turquía	0,6-3,7				[55]
BE	Alemania	78	49	3		[56]
	Australia	10-800	25-300			[57]
	Bélgica	82-1898		n.d.-520		[1-4]
	Canadá	287-2624	62-775			[5]
	China	<LOD-1190	<LOD			[6,58]
	Costa Rica	2100-4500	340-1450	4-3440		[8]
	Croacia	5-325	0,8-174			[9,10]
	Eslovaquia	24-219				[11]
	España	9-7500	1-3425	n.d.-1350	0,2-150	[13-27]
	EEUU	0,6-1590	0,8-2800	2,3-24		[28-31]
	Francia	21-3050	7,9-910			[32-33]
	Grecia	9-105	3,3-77			[34]
	Irlanda	290	22-31			[35]
	Italia	117-1132		3,7-183		[36-40]
	Malasia	<LOD-35				[41]
	N. Zelanda	<LOD-184	<LOD-85			[42]
	Países Bajos	260-3701	10-351	5		[43,44]
	Reino Unido	992-2544	13-1597	0,3-123		[40,45-51]

Tabla AIV-1 (cont). Concentración de drogas, psicofármacos y metabolitos (ng/L) en aguas.

	País	Agua Residual		Agua Superficial	Agua potable	Referencias
		Entrada	Salida			
	R. Checa	116-309				[52]
	Suiza	10,7-604	96-100	0,4-16		[37,38,53]
	Túnez	<LOQ-102				[54]
	Turquía	3,2-99				[55]
CE	España	<LOD-190	<LOD-80	<LOD-11	<LOD-0,9	[17-22, 25-27]
	EEUU	0,9-44,7	0,5-7,9			[28-31]
	Francia	10-167	5-25			[32,33]
	Italia	11,5		n,d-1,3		[36,38,40]
	Reino Unido	5,4-45	5,4	0,1-0,3		[40,45,48,50,51]
	R. Checa	1,8-10,7				[52]
	Suiza	5,9	0,2			[37,38,53]
AM	Canadá	<LOQ-25	<LOQ-14			[5]
	China	<LOD-101	<LOD-2,8			[6,58]
	Corea del Sur	2,6-6,6	<LOQ			[59]
	Croacia	3,6-31	0,9-54			[10]
	Eslovaquia	<LOD-90				[11,12]
	España	3-1400	0,5-225	n.d.-309	0,4-50	[13-27]
	EEUU	80-550	<LOD-12,8	2,3-7,7		[28-31]
	Francia	52-82				[32,33]
	Grecia	5-10				[34]
	Italia	2,7-14,7		n.d.		[37,38,40]
	N. Zelanda	<LOD-137	<LOD-383			[42]
	Países Bajos	40-1779	4,5-12			[43]
	Reino Unido	77-4310	2-201	n.d.-21		[40,45-48,50,51]
	R. Checa	33-119				[52]
	Suiza			n.d.-1,2		[53]
	Turquía	1,2-203				[55]
MA	Australia	17-5064				[57,60,61]
	Canadá	5-65	3-95			[5]
	China	1,2-1153	<LOD-22		<LOD-2,6	[6,7,58]
	Corea del Sur	<LOQ-43	<LOQ-2,7			[59]
	Eslovaquia	79-889				[11,12]
	España	3-614	1,3-90	n.d.-5	<0,2-2	[13-27]
	EEUU	10-2000	2-350	n.d.-151		[28-31]
	Francia	51	24			[32,33]
	Grecia	<LOD	<LOD			[34]
	Italia	4,5-16	3,5	n.d.-2,1		[36,38,40]
	Malasia	47-1640				[41]
	N. Zelanda	421-1268	<LOD-59			[42]
	Países Bajos	16-17				[43]
	Reino Unido	18-40	0,4-1,3	n.d.-0,3		[40,45,48,50,51]

Tabla AIV-1 (cont). Concentración de drogas, psicofármacos y metabolitos (ng/L) en aguas.

	País	Agua residual		Agua Superficial	Agua potable	Referencias
		Entrada	Salida			
	R. Checa	393-823				[52]
	Turquía	1,4-1115				[55]
MDMA	Australia	50-450	25-175			[57]
	Bélgica	10,5-35				[1-3]
	Canadá	9-35	<LOD-32			[5]
	China	0,2-0,6	<LOD-0,5		<LOD-0,03	[6,7,58]
	Croacia	2,2-35	<0,3-34			[9,10]
	Eslovaquia	<LOD-330				[11,12]
	España	2-27500	2-21200	n,d-190	1-40	[13-27]
	EEUU	3-108	0,5-38	5,8-6,4		[28-31]
	Francia	2,6-136	<0,7-165			[32,33]
	Grecia	1-2,2	0,9-7,8			[34]
	Italia	4,2-14,2	4,4	0,2-1,7		[36,38,40]
	Malasia	5-1296				[41]
	N. Zelanda	<LOD-26	<LOD-20			[42]
	Países Bajos	12-241	19-222	2		[43,44]
	Reino Unido	0,7-455	0,6-178	3,6-25		[40,45,48,50,51]
	R. Checa	14-102				[52]
	Suiza	14-26	5,1-11			[37,38,53]
	Túnez	<LOQ-151	<LOQ			[54]
	Turquía	154-6037				[55]
KET	China	0,8-2,5	<LOD-1,2		<LOD-1,7	[6,7,58]
	España	<LOD-50	<LOD-49	<LOD-10		[13,14,22]
	Grecia	6,1				[34]
	Malasia	188-354				[41]
	Reino Unido	145-277	50-228			[45,48]
LSD	España	1,1-4,7	0,2-1,6			[16]
	EEUU	5-62				[28]
	Grecia	0,7-1,5	0,3-0,5			[34]
OH-LSD	España	<LOD-5,6	<LOD-0,8			[16]
	Grecia	4,8-43	23-52			[34]
COD	Alemania			35,8		[56]
	China	<LOD-11	<LOD-0,6		<LOD-0,07	[6,7,58]
	Corea del Sur	1,3-21				[62]
	Costa Rica	448-538	11-665	<LOD-252		[8]
	Croacia	146-364	4,4-382			[9,10]
	Eslovaquia	9-541				[12]
	España	5,7-120	3-397	8,9-232	14-76	[15,20,25,63-65]
	EEUU			3,9-46		[30]
	Italia			1,8-51		[40]
	Malasia	<LOD-45				[41]

Tabla AIV-1 (cont). Concentración de drogas, psicofármacos y metabolitos (ng/L) en aguas.

	País	Agua residual		Agua Superficial	Agua potable	Referencias
		Entrada	Salida			
COD	N. Zelanda	317-1140	80-145			[42]
	Países Bajos	73-894	89-599	7		[43]
	Reino Unido	236-3973	9,7-1502	29-341		[40,45-47,50]
	Suiza	228	204	2,85		[37,38,53]
	Turquía	35-146				[55]
	Vietnam	40-180				[66]
HER	España	<LOD-2,4	1,2	n,d-1,6		[16,27]
	EEUU	<LOD-337	<LOD-8,4			[29,31]
	China	<LOD-7,0	0,3-1,8			[6]
	Francia	25-194	31			[33]
6ACM	Croacia	1,4-28	<0,2-7,7			[9,10]
	España	2,7-63	2-3,6	n,d-3,4		[13,15-21,23-27]
	EEUU	1,2-1126	<LOD-7,5			[29,31]
	Francia	49-756	30-352			[33]
	Italia	3,3-20		n.d.		[37-40]
	Malasia	<LOD-13				[41]
	Países Bajos	27-73	7,3-30			[43]
	Reino Unido	3-224	0,6-7,7	0,3-0,4		[38,45,48,50]
	Suiza	10,4-38	<LOD	n,d-1,2		[37,38,53]
MOR	Alemania	<LOD-820	<LOD-110	30		[56]
	Costa Rica	16-77	15-61	<LOD-36		[8]
	Croacia	45-476	0,5-157			[9,10]
	EEUU	192-768	<LD-167	5,1-7,3		[30,31]
	España	17-1346	5-102	n.d.-89	1,5-12	[13-21,23-27,65]
	Francia	28-83	29-152			[33]
	Grecia	1,8-20	<LOD-2,6			[34]
	Italia	11-83		3-38		[37-40]
	Países Bajos			7		[44]
	Reino Unido	66-986	13-874	n.d.-42		[40,45,48,50]
	R. Checa	81,7				[52]
	Suiza	102-1007	55-929	n.d.-14		[37,38,53]
	Turquía	5,5-58				[55]
METH	Croacia	12-100	7-60			[9,10]
	España	3,4-4704	3,4-732	n,d-37	0,2-9	[14,16-27,65]
	EEUU	5-62	13-26	2,2-21,1		[28,30,31]
	Francia	11-234	10-118			[33]
	Grecia	0,9-5,6	0,6-4,4			[34]
	Italia	12-50	9,1-33	0,5-8,6		[37-40]
	Países Bajos	<LOD	6-58	2		[43,44]
	Reino Unido	2,6-171	1,4-91	0,6-24		[45,48,50]
	R. Checa	13-19				[52]

Tabla AIV-1 (cont). Concentración de drogas, psicofármacos y metabolitos (ng/L) en aguas.

	País	Agua residual		Agua Superficial	Agua potable	Referencias
		Entrada	Salida			
EDDP	Bélgica	37-109				[1-3]
	China	0,6-2,9				[58]
	Croacia	27-232	23-219			[10]
	Costa Rica			<LOD-127		[8]
	España	3,3-9262	2,7-1150	2-112	0,3-31	[14,16-27,65]
	EEUU	84-208	17-34			[29,31]
	Francia	6-260	10-246			[33]
	Grecia	1,2-4,4	1,6-6,5			[34]
	Italia	20-91	23-72	1-18		[37-40]
	Malasia	3-56				[41]
	N. Zelanda	32-71	35-50			[42]
	Países Bajos		9-206			[43]
	Reino Unido	3,7-342	2,6-162	1,2-15		[45,48,50]
	R. Checa	16-28				[52]
	Suiza	91-315	72-294	0,6-12		[37,38,53]
THC	España	11-127	13-21	<LOD-3,6	<LOD-12	[16,17,19,20,23, 25-27,63-65,67]
	EEUU	0,1-140	<LOD-155			[29,31]
	Grecia	2,8-32	1,2-22			[34]
	Italia	63				[37]
	Suiza	91	7,2			[37]
THC-COOH	Croacia	15-128	<LOD-5,8			[9,10]
	Costa Rica	124-502	10-37			[8]
	Eslovaquia	42-412	10-24			[11,12]
	España	4,3-2591	1,8-753	<LOD-79	<LOD-15	[16-20,22,23,25-27,63-65,67]
	EEUU	30-2413	99			[29,31]
	Francia	44-1196	6-161			[33]
	Italia	20-63		0,3-3,7		[37,38,40]
	Países Bajos	73-489	11-22			[43]
	Reino Unido			<LOD-1		[40]
	Suiza	43-91	7,2			[37,38,53]
	Turquía	<LOD-440				[55]
OH-THC	Costa Rica	26-192	20-200	<LOD-458		[8]
	Croacia	23-66	<LOD-5,2			[10]
	España	19-62		<LOD-0,4		[17,22,25,26]
	EEUU	34-1620		32-742		[29,30]
CBN	EEUU	<LOD-2270	<LOD-313			[31]
CBD	Australia	9-2243				[68]
	EEUU	<LOD	<LOD			[31]

Tabla AIV-1 (cont). Concentración de drogas, psicofármacos y metabolitos (ng/L) en aguas.

	País	Agua residual		Agua Superficial	Agua potable	Referencias
		Entrada	Salida			
CAF	Australia	27100 - 136000				[69]
	China	20 – 28600				[70]
	España	700-209000			<LOD-392	[14,65]
	EEUU	31900-97300	359-1140			[31]
	Reino Unido	1000-150000	7137	107-1715		[45,48,50]
	Túnez	2444-59069	1054-34578			[54]
	Vietnam	12140-41000	60-1600			[66]
EPH	Corea del Sur	881-2351	15-83			[59]
	España	360-3257	92-266	13-206		[13,14,16,17,20, 21[24-27]
	EEUU	580-6900	14-27			[28,31]
	Grecia	962-6784	41-320			[34]
	Reino Unido	14-1032	127	9,4-15		[45,48,50,71]
	R. Checa	819-1368				[52]
EtS	Alemania	10300-70700				[72]
	Australia	9900-24400				[72,73]
	Bélgica	1700-32700				[74]
	Canadá	5400-20000				[72]
	China	500-12100				[75]
	España	1460-69300				[72,76-78]
	EEUU	1600-25100				[79]
	Dinamarca	22700-60300				[72]
	Grecia	2190-12243				[80]
	Italia	2300-5700				[72]
	Noruega	4200-32000				[72]
	Países Bajos	13100-36900				[72]
	Vietnam	940-5940				[66]
MDPV	China	0,5-2,5	0,5-1,6		<LOD-0,1	[7,81]
	Europa	0,8-2,6				[82,83]
MEPH	Eslovaquia	2,2-7,6				[84]
	Europa	1,7-110				[82,83]
	Polonia	2,4-8,9				[85]
	Reino Unido	14-67				[86]
CTLP		0,4-2040	1,5-840000		1,5-3,4	[87]
		<LOD-17100	<LOD-9200			[88]
	Alemania			1,8-96		[89]
	Canadá			3,4-206		[90]
	Eslovaquia	11-309				[91]
	España	110-183		1,9-140		[22,92-94]
	EEUU	90-173	<LOD-107	3,7-139		[30,31,95]

Tabla AIV-1 (cont). Concentración de drogas, psicofármacos y metabolitos (ng/L) en aguas.

	País	Agua residual		Agua Superficial	Agua potable	Referencias
		Entrada	Salida			
CTLP	Grecia	110-541	20-766			[34]
	India			400-76000		[96]
FLX		<LOD-3465	<LOD-2700			[88]
	China	2,6				[97]
	España	<LOD-64		<LOQ-44		[22,98,99]
	EEUU	81-122	41-81	2,6-13		[30,31]
	Grecia	0,7-20	0,7-24			[34]
	Reino Unido	14-176	43	n.d.-79		[45,48,50]
	R. Checa	3,1-6,3				[52]
Nor-FLX		<LOD-10400	<LOD-9810			[88]
	España	<LOD-43				[22,98]
	EEUU			2,3-34,4		[30,31]
	Reino Unido	3,3-118	20,1			[34]
STR		<0,1-997	<LOD-1930			[88]
	España	<LOD-455	<LOD	<LOD-12		[22,98]
	EEUU	<LOD-44,9	<LOD-49	2,3-34,4		[30,31]
	Grecia	13-45	0,5-12,4			[34]
Nor-STR		<LOD-386				[88]
	España	209-531				[22]
	Grecia	13-24	10,4-15			[34]
VFX	Eslovaquia	74-852				[91]
	España	<LOD-1063	14-372	<LOD-387		[22,98-100]
	EEUU	342-451	261-1000	2-340		[30,95]
	Francia			10-17		[101]
	Grecia	210-733	328-393			[34]
	Reino Unido	120-344	270	0,8-85		[48,50]
	R. Checa	253-391				[52]
ALPZ		1,7-2580	0,8-176		2,3-11	[87]
	Brasil			5900		[102]
	China	7,6			<LOD-0,06	[7,97]
	España	<LOD-414	<LOD-1554	<LOD-14		[22,98,100,110]
	EEUU	<LOD-5,8	<LOD-4,3	1,6-8,5		[30,95]
	Grecia	6,6-42	2,6-10,3			[34]
OH-ALPZ	España	9-14				[22]
	EEUU	<LOD-8,5	<LOD			[31]
BROMZ		1,3-3662	0,5-15542			[87]
	China	2,3				[97]
	Eslovenia	<LOD	6-158			[103]
	España	<LOD-3662	<LOD-1554	<LOD-14		[98,100]
	EEUU	<LOD-25	<LOD			[31]
	Grecia	52-316	34-87			[24]

Tabla AIV-1 (cont). Concentración de drogas, psicofármacos y metabolitos (ng/L) en aguas.

	País	Agua residual		Agua Superficial	Agua potable	Referencias
		Entrada	Salida			
DIAZ		1,7-1180	0,6-4000			[104]
					0,5-24	[87]
	Bélgica	10-1180				[105]
	Brasil			524-625		[106]
	China	9,5		1-140	<LOD-25	[7,97,107,108]
	Eslovenia	21-25	17-111	6-69		[103]
	España	<LOD-69	<LOD-64	<LOD-569		[22,25,98,109-112]
	EEUU	6,4-18	<LOD-14	2,4-8		[30,31]
	Grecia	<LOD-1,4	<LOD-1,8			[34]
	Italia			n.d.-2,1		[113]
	Países Bajos	<LOD	2-5			[44,50]
	Reino Unido	<LOD-8	<LOD-7	n.d.-1,1		[45,48,50]
Nor-DIAZ	China	<LOQ				[97]
	España	5-56	5-25	<LOD		[22,109,110]
	EEUU	<LOD	<LOD			[31]
	Grecia	0,6-3,1	2,6-4,7			[34]
	Países Bajos	<LOD-21	5-31			[43,44]
	Reino Unido	5-64	<1-16			[45,48]
	R. Checa	33-115				[52]
LORZ		2,1-10598	1,2-920	1,6-706	4-562	[87]
	China	36			<LOD-0,04	[7,97]
	España	<LOD-502	<LOD-532	6,4-706		[98,110]
	EEUU	<LOD	<LOD	12-20		[30,31]
	Francia			1,6-40		[115]
	Grecia	13-30	8,3-27			[34]
LRMZ	España	4-108	7,7-42			[22,110]
	China				<LOD-0,7	[7]
MIDZ	Grecia	0,5-0,9	0,3-0,6			[34]
OXZ		5,6-3300	6,3-7434		2,5-91	[87]
	Alemania			30-400		[56]
	China	9,2			<LOD-0,1	[7,97]
	Eslovaquia	13-133				[91]
	Eslovenia	54-58	11-133	11-31		[103]
	España	9-1218	<LOD-532	<LOD-129		[22,98-100,110]
	EEUU	<LOD	<LOD	18-43		[30-31]
	Francia			9,1-1400		[116,117]
	Grecia	20-63	5,8-74			[34]
	Países Bajos	109-2020	237-1746			[43,44]
	Reino Unido	6-155	83	n.d.-20,6		[45,48,50]
	R. Checa	106-122				[52]

Tabla AIV-1 (cont). Concentración de drogas, psicofármacos y metabolitos (ng/L) en aguas.

	País	Agua residual		Agua Superficial	Agua potable	Referencias
		Entrada	Salida			
TEMZ	China	10,6			<LOD-1,01	[7,97]
	España	5,8-24	12-36			[22,110]
	EEUU	<LOD-108	<LOD-51	2,4-83		[30,31]
	Grecia	2,8-152	3,4-23			[34]
	Países Bajos	92-813	133-1016			[43,44]
	Reino Unido	12-278	17-251	1,1-78		[45,48,50]
	R. Checa	<LOD				[52]
	Vietnam	520-2900				[66]
ZOLP	Corea del Sur	2,6-4,9	0,9-2			[59]
	España	<LOD-3,9	1,5-1,9	<LOD-6		[98,100,110]
	Reino Unido	<LOD-2				[86]

n.d.: no detectado; <LOD: por debajo del límite de detección; <LOQ: por debajo del límite de cuantificación; COC: cocaína; BE: benzoilecgonina; CE: cocaetileno; AM: anfetamina; MA: metanfetamina; KET: ketamina; COD: codeína; HER: heroína; 6ACM: 6-monoacetilmorfina; MOR: morfina; METH: metadona; CBN: cannabinol; CBD: cannabidiol; EPH: efedrina; EtS: sulfato de etilo; MEPH: mefedrona; CTLP: citalopram; FLX: fluoxetina; Nor-FLX: nor-fluoxetina; STR: sertralina: nor-STR: nor-sertralina; VFX: venlafaxina; ALPZ: alprazolam; BROMZ: bromazepam; DIAZ: diazepam; nor-DIAZ: nor-diazepam; LORZ: lorazepam; LRMZ; lormetazepam; MIDZ: midazolam; OXZ: oxazepam; TEMZ: temazepam; ZOLP: Zolpidem.

[1] (van Nuijs y cols., 2011); [2] (Gheorghe y cols., 2008); [3] (van Nuijs y cols., 2009a); [4] (van Nuijs y cols., 2009b); [5] (C. Metcalfe y cols., 2010); [6] (Deng y cols., 2020); [7] (Wang y cols., 2020); [8] (Causanilles y cols., 2017c); [9] (Terzic y cols., 2010); [10] (Senta y cols., 2013); [11] (Mackul'ak y cols., 2014); [12] (Mackul'ak y cols., 2019); [13] (Huerta-Fontela y cols., 2007); [14] (Huerta-Fontela y cols., 2008a); [15] (Huerta-Fontela y cols., 2008b); [16] (Postigo y cols., 2008); [17] (Postigo y cols., 2010); [18] (Bijlsma y cols., 2009); [19] (González-Mariño y cols., 2010); [20] (González-Mariño y cols., 2012a); [21] (Mastroianni y cols., 2017); [22] (González-Mariño y cols., 2018); [23] (Vazquez-Roig y cols., 2010a); [24] (Martínez Bueno y cols., 2011); [25] (Valcárcel y cols., 2012); [26] (Mendoza y cols., 2014); [27] (Mastroianni y cols., 2016); [28] (Chiaia y cols., 2008); [29] (Foppe y cols., 2018); [30] (Skees y cols., 2018); [31] (Asimakopoulos y cols., 2017); [32] (Karolak y cols., 2010); [33] (Nefau y cols., 2013); [34] (Borova y cols., 2014); [35] (Bones y cols., 2007); [36] (Zuccato y cols., 2005); [37] (Castiglioni y cols., 2006); [38] (Zuccato y cols., 2008b); [39] (Mari y cols., 2009); [40] (Zuccato y cols., 2008a); [41] (Du y cols., 2020); [42] (Kumar y cols., 2019); [43] (Bijlsma y cols., 2012); [44] (van der Aa y cols., 2013); [45] (Baker y cols., 2014); [46] (Kasprzyk-Hordern y cols., 2008a); [47] (Kasprzyk-Hordern y cols., 2009); [48] (Baker y cols., 2011a); [49] (Munro y cols., 2019); [50] (Baker y cols., 2013); [51] (Kasprzyk-Hordern y cols., 2008b); [52] (Baker y cols., 2012); [53] (Berset y cols., 2010); [54] (Moslah y cols., 2018); [55] (Daglioglu y cols., 2019); [56] (Hummel y cols., 2006); [57] (Yadav y cols., 2019); [58] (Xiaohan Zhang y cols., 2019); [59] (Kim y cols., 2020); [60] (Irvine y cols., 2011); [61] (Lai y cols., 2013a); [62] (Kim y cols., 2015); [63] (Boleda y cols., 2007); [64] (Boleda y cols., 2009); [65] (Boleda y cols., 2011); [66] (Nguyen y cols., 2018); [67] (Racamonde y cols., 2012); [68] (Pandopulos y cols., 2020); [69] (O'Brien y cols., 2014); [70] (Gao y cols., 2016); [71] (Kasprzyk-Hordern y cols., 2010); [72] (Ryu y cols., 2016); [73] (Zheng y cols., 2020); [74] (Boogaerts y cols., 2016); [75] (Gao y cols., 2020); [76] (Mastroianni y cols., 2014); [77] (Rodríguez-Álvarez y cols., 2014); [78] (Andrés-Costa y cols., 2016b); [79] (Chen y cols., 2019); [80] (Gatidou y cols., 2016);

[81] (Gao y cols., 2017); [82](González-Mariño y cols., 2016b); [83] (Bade y cols., 2017); [84] (Brandeburová y cols., 2020); [85] (Sulej-Suchomska y cols., 2020); [86] (Castrignanò y cols., 2016); [87] (Cunha y cols., 2017); [88] (Mole y cols., 2019); [89] (Schlüsener y cols., 2015); [90] (Lajeunesse y cols., 2008); [91] (Mackul'ak y cols., 2016); [92] (Rodríguez-Gil y cols., 2010); [93] (Moreno-González y cols., 2014); [94] (Acuña y cols., 2015); [95] (Schultz y cols., 2008); [96] (Fick y cols., 2009); [97] (Wu y cols., 2015); [98] (Huerta-Fontela y cols., 2010); [99] (González Alonso y cols., 2010); [100] (Huerta-Fontela y cols., 2011); [101] (Brieudes y cols., 2016); [102] (Nunes y cols., 2015); [103] (Kosjek y cols., 2012); [104] (Calisto y cols., 2009); [105] (van der Ven y cols., 2004); [106] (de Almeida y cols., 2015); [107] (Shao y cols., 2009); [108] (Heeb y cols., 2012); [109] (Arbeláez y cols., 2015); [110] (Racamonde y cols., 2014); [111] (Silva y cols., 2011); [112] (López-Serna y cols., 2010); [113] (Calamari y cols., 2003); [114] (Proia y cols., 2013); [115] (Coetsier y cols., 2009); [116] (Piel y cols., 2013); [117] (Camilleri y cols., 2015).