Water Research 206 (2021) 117719

Contents lists available at ScienceDirect

Water Research

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

Source identification of amphetamine-like stimulants in Spanish wastewater through enantiomeric profiling

Andrea Estévez-Danta [a], Rosa Montes [a,*], Lubertus Bijlsma [b], Rafael Cela [a], Alberto Celma [b], Iria González-Mariño [a,c], Manuel Miró [d], Vanessa Gutmann [a], Unai Pérez de San Román-Landa [e], Ailette Prieto [f,g], Mireia Ventura [h], Rosario Rodil [a], José Benito Quintana [a,*]

[a] Department of Analytical Chemistry, Nutrition and Food Sciences, Institute of Research on Chemical and Biological Analysis (IAQBUS), Universidade de Santiago de Compostela, Constantino Candeira S/N, Santiago de Compostela 15782, Spain
[b] Environmental and Public Health Analytical Chemistry, Research Institute for Pesticides and Water, University Jaume I, Castellón, Spain
[c] Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemical Sciences, University of Salamanca, Salamanca, Spain
[d] FI-TRACE group, Department of Chemistry, University of the Balearic Islands, Palma de Mallorca, Spain
[e] Asociación Ai Laket!! Elkartea, Vitoria-Gasteiz, Spain
[f] Department of Analytical Chemistry, Faculty of Science and Technology, University of the Basque Country, Bilbao, Spain
[g] Research Centre for Experimental Marine Biology and Biotechnology, University of the Basque Country (PiE-UPV/EHU), Plentzia, Basque Country 48620, Spain
[h] Energy Control, Asociación Bienestar y Desarrollo, Barcelona, Spain

ARTICLE INFO

Keywords:
Drug abuse
Chiral drugs
Enantiomers
Sewage
Wastewater-based epidemiology
Prescription

ABSTRACT

Amphetamine (AMP), methamphetamine (MAMP) and 3,4-methylenedioxymethamphetamine (MDMA) occur in wastewater not only as a result of illicit consumption, but also, in some cases, from prescription drug use or by direct drug disposal into the sewage system. Enantiomeric profiling of these chiral drugs could give more insight into the origin of their occurrence. In this manuscript, a new analytical methodology for the enantiomeric analysis of amphetamine-like substances in wastewater has been developed. The method consists of a solid-phase extraction (SPE) followed by liquid chromatography-triple quadrupole-tandem mass spectrometry (LC-MS/MS), which showed low quantification limits in the 2.4–5.5 ng L^{-1} range. The LC-MS/MS method was first applied to characterize a total of 38 solid street drug samples anonymously provided by consumers. The results of these analysis showed that AMP and MDMA trafficked into Spain are synthesized as racemate, while MAMP is exclusively produced as the S(+)-enantiomer. Then, the analytical method was employed to analyse urban wastewater samples collected from the wastewater treatment plants (WWTPs) of five different cities in 2018 and 2019. Consumption estimated through normalized population loads in wastewater showed an increased pattern of AMP use in the Basque Country. Furthermore, the enantiomeric profiling of wastewater samples was contrasted to lisdexamfetamine (LIS) and selegiline (SEL) prescription figures, two pharmaceuticals which metabolize to S(+)-AMP, and to R(-)-AMP and R(-)-MAMP, respectively. From this analysis, and considering uncertainties derived from metabolism and adherence to treatment, it was concluded that LIS is a relevant source of AMP in those cases with low wastewater loads, i.e. up to a maximum of 60% of AMP detected in wastewater in some samples could originate from LIS prescription, while SEL does not represent a significant source of AMP nor MAMP. Finally, removal efficiencies could be evaluated for the WWTP (serving ca. 860,000 inhabitants) with higher AMP influent concentrations. The removal of AMP was satisfactory with rates higher than 99%, whereas MDMA showed an average removal of approximately 60%, accompanied by an enrichment of R(-)-MDMA.

1. Introduction

Amphetamine (AMP), methamphetamine (MAMP) and 3,4-methylendioxymethamphetamine (MDMA) are synthetic derivatives of phenylethylamine that were used in the past to treat narcolepsy and spastic states of the gastrointestinal tract (Myerson, 1939). However,

* Corresponding authors at: Department of Analytical Chemistry, Nutrition and Food Sciences, Institute of Research on Chemical and Biological Analysis (IAQBUS), Universidade de Santiago de Compostela, Constantino Candeira S/N, Santiago de Compostela 15782, Spain.
E-mail addresses: rosamaria.montes@usc.es (R. Montes), jb.quintana@usc.es (J.B. Quintana).

https://doi.org/10.1016/j.watres.2021.117719
Received 29 April 2021; Received in revised form 22 September 2021; Accepted 24 September 2021
Available online 29 September 2021

their stimulating effects have also been associated to a high risk of addiction (Guttmann and Sargant, 1942; Lemere 1967). Therefore, actions were taken to restrict their clandestine consumption.

Besides classical population surveys, hospital-related admissions and other epidemiological indicators, efforts to detect illicit drugs' use in a fast and non-invasive way led to the first study using wastewater-based epidemiology (WBE) in 2005 by Zuccato et al. (Zuccato et al., 2005). They estimated cocaine consumption in a specific population through the analysis of wastewater, as an complementary tool to the established epidemiological approaches. Nowadays, this methodology has been applied in many countries to get a near real-time profiling of the community-wide use of illicit drugs (Bijlsma et al., 2021; González-Mariño et al., 2020; Ort et al., 2014; Thomas et al., 2012), alcohol and tobacco (Castiglioni et al., 2015; Gao et al., 2020; López-García et al., 2020; Montes et al., 2020; Rodríguez-Álvarez et al., 2015, 2014a, 2014b; Ryu et al., 2016; Tscharke et al., 2016). Additionally, WBE has been extended to estimate (unwanted) exposure to chemicals (Senta et al., 2020), such as pesticides (Rousis et al., 2016), flame retardants (Been et al., 2018; Castro et al., 2020), bisphenol A (Lopardo et al., 2019) and plasticizers (Estévez-Danta et al., 2021; González-Mariño et al., 2021, 2017), and more recently as a useful tool to follow and predict the evolution of COVID-19 (Ahmed et al., 2020; Alygizakis et al., 2020; Medema et al., 2020).

A key factor in WBE studies is the selection of appropriate human biomarkers. However, the estimation of AMP, MAMP and MDMA consumption is sometimes troublesome, because the biomarkers usually measured in wastewater are the parent compounds (i.e. unchanged excreted fraction), which can occur in wastewater not only as a result of illicit consumption, but also from prescription drug use or direct disposal from waste of illegal drug production (Emke et al., 2014). Yet, these three drugs are chiral and contain one asymmetric carbon atom that leads to two enantiomers (R-(-) and S-(+)). In the human body, this chirality implies different biological activity, and consequently, different distribution and metabolism (Kalant 2001; Kasprzyk-Hordern et al., 2010).

Illicit AMP and MDMA are usually synthetized by the Leuckart method to yield a racemic mixture (EMCDDA, 2021a, b; Emke et al., 2018; Hauser et al., 2020; Kalant 2001; King 2009), whereas MAMP is mainly produced as pure S-enantiomer across Europe, with the only reported exception of Norway, where the synthesis facilities are different than in Central Europe, and usually synthetize MAMP as a racemate (Castrignanò et al., 2018). AMP and MAMP are used in some countries as a prescribed medication to treat attention deficit/-hyperactivity disorder (ADHD), narcolepsy, or as a dietary supplement to lose weight (Cody, 2002). In Spain, AMP is not prescribed itself, but as the prodrug lisdexamfetamine (LIS, used to treat ADHD), which is metabolized to S-(+)-AMP in the human body (Comiran et al., 2021; Krishnan et al., 2008; Pennick, 2013). MAMP and MDMA do not currently have medical applications. However, selegiline (SEL), a medication used in Parkinson treatment, metabolizes to produce the R (-)-enantiomer of AMP and MAMP (Reynolds et al., 1978). Thus, both LIS and SEL could be potential sources of AMP and MAMP in sewage, besides illicit consumption (Castrignanò et al., 2018; Lertxundi et al., 2021).

In the case of these three amphetamine-like substances, the S (+)-enantiomer is more active and therefore metabolizes faster than the R(-)-enantiomer (Kasprzyk-Hordern, 2010; Kasprzyk-Hordern and Baker, 2012). This, consequently, results in a change of the enantiomeric ratio towards the enrichment of the R(-)-enantiomer. Thus, mainly the R-enantiomer is detected in untreated wastewater if the racemic drug is consumed. Hence, enantiomeric analyses can complement traditional WBE estimates by applying analytical methods that allow the determination of different chiral drug enantiomers, and therefore differentiate between licit (prescription) or illicit use, or direct dumping in the sewage network (Castrignanò et al., 2018; Emke et al., 2014; Gao et al., 2018).

Enantiomeric profiling has been mainly performed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) in combination with a previous sample concentration (usually a solid-phase extraction (SPE)). Kaspryzk-Horden and Baker (Kasprzyk-Hordern and Baker, 2012) were the first to address chiral analysis of these substances. In that first study, Oasis MCX cartridges were used for SPE, due to the basic nature of the illicit drugs studied. However, Oasis HLB cartridges have also been employed in some other studies (Archer et al., 2018; Castrignanò et al., 2016; Vazquez-Roig et al., 2014).

Although there are some WBE-derived studies targeting enantiomeric separation in different countries (Archer et al., 2018; Castrignanò et al., 2018; Emke et al., 2014; Gao et al., 2018; Kasprzyk-Hordern and Baker, 2012), in Spain, enantiomeric profiling has been applied only to two cities (Castellón and Valencia) of the Valencian Community region (Castrignanò et al., 2018; Vazquez-Roig et al., 2014). Moreover, in a more recent study on WBE of illicit drugs in Spain (Bijlsma et al., 2021) high levels of AMP were observed in the area of Bilbao. However, the origin of such substance could not be fully clarified (Lertxundi et al., 2021), even when a preliminary version of the enantiomeric profiling method presented here was used. Hence, the aim of this work was to delve into spatial differences by including 5 mid-to-large cities (and their metropolitan areas), located in five Spanish regions and covering around 2 million people overall. To that end, we have developed and validated a new enantiomeric analysis method and applied it to AMP, MAMP and MDMA street drug samples obtained from different locations in Spain to evaluate their enantiomeric fractions and purity. Also, wastewater samples from the above-mentioned regions were collected in 2018 and 2019 and analysed. Finally, a detailed discussion on the contribution of the prescription drugs LIS and SEL to the amounts of AMP and MAMP detected in wastewater is presented for the first time.

2. Material and methods

2.1. Chemicals and reagents

Individual solutions of 1 mg mL^{-1} of AMP, MAMP and MDMA, and of 0.1 mg mL^{-1} of their deuterated analogues (AMP-D_6, MAMP-D_5 and MDMA-D_5, used as internal standards (ISs)), were supplied by Cerilliant (Round Rock, TX, USA) as racemic mixtures. Individual solutions of 1 mg mL^{-1} of the S-(+) enantiomer of AMP, MAMP and MDMA were supplied by Merck (Darmstadt, Germany).

Ultrapure water was obtained with a Millipore Milli-Q Gradient A-10 system (Bedford, MA, USA). LC-MS grade methanol (MeOH), formic acid (95–97%), ammonium bicarbonate ($\geq$ 99.5%) and ammonia (NH_3) solution in water (25%) were supplied by Merck. Ammonia solution in MeOH (7 N) was supplied by Across Organics (Thermo Fischer Scientific, Geel, Belgium).

2.2. Drug dose samples

Street drug samples were supplied by Energy Control and Ai Laket!! as powder or crystal. These two Spanish Organizations aim to reduce risks related to recreational drug use by providing fast and anonymous information to users on the composition of the drugs they are going to consume. Hence, such drugs were submitted to the harm-reduction, drug-checking services in an anonymous way and were then shipped to Santiago de Compostela for analysis. These drug samples were diluted to a nominal concentration of 250 ng mL^{-1} of powder in MeOH, spiked with the ISs (100 ng mL^{-1} each) of and injected into the LC-MS/MS system.

2.3. Wastewater samples

Composite 24 h raw wastewater samples were collected at five wastewater treatment plants (WWTPs) located in Spain for 7 consecutive days in Spring 2018 and 2019, except in the WWTP of Palma for

which samples were only collected in 2018. Details on each location, population served by each WWTP, and sampling are displayed in Table S1.

In addition, in 2019, treated wastewater samples from the WWTP of Galindo (Bilbao and its large metropolitan area), in which high concentrations of AMP were detected, were collected with a delay of 24 h with respect to raw wastewater (June 12th-18th) in order to assess (enantioselective-)removal efficiencies, on request of the WWTP managers. This WWTP treats the wastewater from over 850,000 inhabitants, with an average flow of ca. 250,000 m^3 day^{-1} (Table S1). The WWTP is equipped with a primary treatment (flocculation and coagulation) and a secondary conventional activated sludge treatment, including anoxic/anaerobic and aerobic treatments. The average hydraulic and sludge retention times are 24 h and 22.5 days, respectively.

2.4. Wastewater samples pretreatment

Sample preparation was performed by two different analytical methods to identify and quantify chiral drugs in wastewater.

2.4.1. Method A

All samples, except those from Castellón and Madrid (see 2.4.2) were processed in Santiago de Compostela with method A, following the protocol described by González-Mariño et al. (2018) with some modifications (see discussion on 3.2). Briefly, 100 mL of samples were vacuum-filtered through 0.7 μm GF/A glass microfiber filters (Whatman, Kent, UK) and 0.45 μm cellulose acetate filters (Millipore) and spiked with the ISs (100 ng L^{-1} each). SPE was performed by mixed-mode reversed-phase strong cation-exchange cartridges (Oasis MCX-150 mg, Waters, Milford, MA, USA) previously rinsed with 5 mL of 5% NH_3 in MeOH followed by 5 mL of ultrapure water. After loading, sorbents were dried under a nitrogen stream during 30 min, washed with 4 mL of MeOH as a clean-up solution, and analytes were then eluted with 3 mL of 5% NH_3 in MeOH. Eluates were evaporated to dryness under a nitrogen stream using a Turbo-Vap II (Zymark, Hopkinton MA, USA) and a Mini-Vap (Supelco, Steinheim, Germany) concentrators. Finally, extracts were redissolved in 100 μL of MeOH, filtered through 0.22 μm PVDF syringe-driven filters (Merck) and injected into the LC-MS system.

2.4.2. Method B

Samples from Castellón and Madrid were extracted in the laboratory of the University Jaume I, following the protocol described by Bijlsma et al. (2014a), hereinafter, method B. In brief, 25 mL of sample were four-times diluted with ultrapure water, spiked with 1 ng mL^{-1} of ISs mixture and filtered through 0.45 μm cellulose filters (Millipore). Then, SPE was performed by Oasis HLB-60 mg reversed-phase cartridges (Waters) previously rinsed with 4 mL of MeOH followed by 4 mL of ultrapure water. After loading, sorbents were dried under a nitrogen stream during 30 min and analytes eluted with 5 mL of MeOH. Eluates were evaporated to dryness under nitrogen using a Turbo-Vap II and a Mini-Vap concentrators. Finally, extracts were redissolved in 1 mL of 10% MeOH in ultrapure water. These extracts were shipped frozen to Santiago de Compostela, where they were evaporated to dryness, redissolved in 100 μL of MeOH and filtered through 0.22 μm PVDF syringe-driven filters, being then ready for LC-MS/MS analysis.

2.5. Instrumental analysis

Instrumental analysis was performed with a Waters Acquity UPLC® H-class system equipped with a quaternary solvent pump, a thermostated LC column compartment, and a sample manager. The UPLC system was interfaced to a triple quadrupole mass spectrometer Xevo TQD from Waters.

The chromatographic separation was performed at 40 °C on a Lux AMP chiral column (150 × 3 mm I.D., 3 μm particle size) from Phenomenex (Torrance, CA, USA). Under final working conditions, a dual eluent system consisting of (A) ultrapure water with 50 mM NH_3 and (B) MeOH was used at a flow rate of 0.4 mL min^{-1}. The linear gradient consisted of the following stages: 0 min (60% B), 15 min (60% B), 20 min (95% B), 25 min (95% B), 25.1 min (60% B) and 30 min (60% B). Injection volume was set at 10 μL.

The interface between the UPLC system and the Xevo TQD mass spectrometer was an electrospray ionization (ESI) source operating in positive mode at a fixed capillary voltage of 3 kV and a temperature of 150 °C. Nitrogen, provided by a nitrogen generator from Peak Scientific (Barcelona, Spain), was used as desolvation gas at 600 L h^{-1} and 450 °C, and as cone gas at 10 L h^{-1}. Analyses were performed by MS/MS in Selected Reaction Monitoring (SRM) mode acquiring one precursor/product ion transitions per IS and two transitions per analyte (one of them used for quantification and the second one for confirmatory purposes). Argon was used as collision gas. Table S2 compiles chemical formulae, retention times (RT), transitions (Q) and optimal cone voltages (CV) and collision energies (CE) for every analyte.

2.6. Method performance and quality assurance

Instrumental detection and quantification limits (IDLs and IQLs) were estimated from the lowest concentration level of the calibration curve providing a signal-to-noise ratio (S/N) of 3 and 10, respectively. Calibration curves were prepared in MeOH and ranged from the IQL to 2500 ng mL^{-1} for AMP enantiomers and from the IQL to 500 ng mL^{-1} for the enantiomers of the remaining compounds (spiked IS concentration, referred to the final extract and each enantiomer: 100 ng mL^{-1} for method A and 10 ng mL^{-1} for method B). Intra-day and inter-day instrumental precision were assessed by the relative standard deviation (RSD%) of seven injections of two calibration standards, containing 5 ng mL^{-1} and 50 ng mL^{-1} of all analytes and 100 ng mL^{-1} of IS. Injections were performed within the same day (intra-day precision) and in four different days within a month (inter-day precision).

Trueness and precision of the whole SPE-LC-MS/MS method A were assessed by recovery studies in ultrapure water and wastewater spiked with 12.5 ng L^{-1} and 125 ng L^{-1}, respectively, of all the analytes (100 ng L^{-1} of IS). Wastewater aliquots spiked only with ISs were also analysed to account for analyte levels in this matrix. Matrix effects (MEs) were calculated as the signal (analyte peak area) percentage in a 125 ng mL^{-1} spiked wastewater extract, after non-spiked sample signal subtraction and referred to the signal of a 125 ng mL^{-1} standard. Method detection limits (MDLs) and method quantification limits (MQLs) were calculated from non-spiked wastewater samples for a signal-to-noise ratio (S/N) of 3 and 10, respectively.

In the case of method B, quality of data was assured by analysing additional extracts provided by the University Jaume I, which had been previously already analysed in such University and were used as quality controls (QC). Thus, four samples of wastewater samples were spiked at two concentration levels (two of them with 50 ng L^{-1} and the remaining two with 400 ng L^{-1}, referring to each enantiomer) were extracted at the University Jaume I, following sample pretreatment method B. The resulting extracts were shipped to the University of Santiago de Compostela, where they were analysed to assess method's trueness and precision, and to calculate the MDLs and MQLs (extrapolated from the lowest level spiked sample).

2.7. Calculations of enantiomeric fractions

The elution order of enantiomers was confirmed by the analysis of S (+)-enantiomerically pure standards. The concentration of each enantiomer was calculated by the internal standard calibration method. Then, the concentration of each enantiomer (C_R for the R(-)-enantiomer and C_S for S(+)-enantiomer) was used to obtain the enantiomeric fraction (EF). In this work, EF is presented as EF_R, i.e. the ratio between the concentrations of the R(-)-enantiomer and the sum of both enantiomers,

as shown in Eq. (1).

$$EF_R = \frac{C_R}{C_R + C_S} \quad (1)$$

2.8. Estimation of human illicit drugs consumption

Drug concentration (sum of both enantiomers) in 24 h composite influent samples were used to estimate population-normalized daily load levels (Eq. (2)) of each drug and, eventually, consumption (Eq. (3)):

$$\text{Daily loads} = \frac{\text{Concentration} \times \text{Flow rate}}{\text{Population}} \times 1000 \quad (2)$$

$$\text{Consumption} = \text{Daily loads} \times \text{CF} \quad (3)$$

The correction factor (CF) values, which consider the fraction of drug excreted after human metabolism, were: 2.77 (AMP), 2.3 (MAMP), and 4.4 (MDMA), as proposed in (Gracia-Lor et al., 2016).

3. Results and discussion

3.1. Liquid chromatography-tandem mass spectrometry

Separation was performed on a Lux AMP column. A dual eluent system consisting of (A) ultrapure water and (B) MeOH was used, with the addition of three different modifiers to the aqueous phase: 5 mM of ammonium bicarbonate at pH 11 (recommended by the column supplier), 50 mM of NH_3 at pH 11, and 5 mM of ammonium acetate at pH 9. An adequate separation of enantiomers was observed at pH 11, independently of the modifier used (ammonium bicarbonate and NH_3). Conversely, ammonium acetate at pH 9 could not resolve the chromatographic peaks of the enantiomers, likely due to the incomplete neutralization of the target species at this pH (Fig. S1). Finally, 50 mM of NH_3 at pH 11 was selected as aqueous mobile phase additive due to the higher signal intensity and lower noise observed as compared to the addition of ammonium bicarbonate (Fig. S1).

Instrumental parameters investigated include linearity, IDLs, IQLs, and intra- and inter-day precision (Table 1). The representation of the analyte area/IS area (response) versus spiked analyte concentration (IQL-2500 ng mL^{-1} range for each AMP enantiomer and IQL-500 ng mL^{-1} range for the remaining enantiomers) fitted a linear model with determination coefficients (R^2) higher than 0.997. IDL and IQL values varied between 0.2 ng mL^{-1} and 0.4 ng mL^{-1}, and between 0.6 ng mL^{-1} and 1.4 ng mL^{-1}, respectively. RSD values from the intra-day precision varied between 0.7% and 4.3% for the 5 ng mL^{-1} standard and between 0.5% and 3.3% for the 50 ng mL^{-1} level. RSD from the inter-day precision was < 8.6% at 50 ng mL^{-1}, and < 4.8% at 5 ng mL^{-1} except for S (+)-MAMP, for which it was 12%.

3.2. Solid-phase extraction

The extraction protocol applied in method A was based on a previous study (González-Mariño et al., 2018) but modified in order to improve its selectivity, by including a clean-up step, and optimizing the elution solvent volume. First, absolute recoveries for samples extracted with Oasis MCX (125 ng L^{-1} spike level) were compared to the sample preparation recoveries obtained when introducing a clean-up step with 4 mL of MeOH before the elution (performed with 5% NH_3 in MeOH in both cases) (Fig. S2a). Both protocols showed good and comparable recoveries; thus, no significant losses were observed due to the clean-up. Also, MEs were tested for both protocols, since previous studies had reported improvements in this regard after the introduction of a clean-up step (González-Mariño et al., 2012, 2009; Senta et al., 2013). Significantly lower matrix effects were observed (i.e. values of %ME close to 100%) when the clean-up step was included (Fig. S2b). Finally, the elution volume was optimized by collecting three consecutive fractions of 3 mL of 5% NH_3 in MeOH, which were analysed independently. More than 94% of all analytes eluted in the first fraction (data not shown), and, consequently, the elution volume was reduced from 10 mL in the former method (González-Mariño et al., 2018), to only 3 mL.

3.3. Method performance

Method A was validated in terms of trueness, precision, MDLs and MQLs (Table 1). Percentages of recovery (%R) for triplicate analyses of ultrapure water samples, spiked with 12.5 ng L^{-1} of all analytes and 100 ng L^{-1} of IS, varied between 90% and 105%, with RSDs between 1% and 6%. In raw wastewater samples spiked with 125 ng L^{-1} of all enantiomers and 100 ng L^{-1} of IS,%R varied between 82% and 116%, and RSD between 4% and 15%. MDLs ranged from 0.7 ng L^{-1} to 1.8 ng L^{-1}, and MQLs from 2.4 ng L^{-1} to 5.5 ng L^{-1}. Table S3 compares the performance of the proposed method versus other analytical methods developed for the determination of chiral amphetamine-like substances in raw wastewater. IQLs and MQLs were at the same order of magnitude than those reported in other methodologies (Archer et al., 2018; Castrignanò et al., 2016, Castrignanò et al., 2018; Gao et al., 2018; Kasprzyk-Hordern et al., 2010). However, all those methods rely on the application of reversed-phase Oasis HLB cartridges, which can perform well in terms of trueness (see also below), but do not offer the same degree of selectivity as obtained by mixed-mode SPE. A further advantage of the method proposed here is that the chromatographic separation is performed under gradient conditions, which increases column lifetime when a complex matrix, as is the case of wastewater, is analysed. Finally, the run time of the chromatographic method developed here is 30 min, considerably lower than over 140 min required for other chiral separations (Kasprzyk-Hordern, 2010; Vazquez-Roig et al., 2014).

Since the samples from Castellón and Madrid had already been extracted by another SPE protocol based on Oasis HLB cartridges

Table 1
Instrumental validation and method performance.

Compound	R^2 [a]	Instrumental precision				IDL [b]	IQL [c]	Trueness and Precision %R (RSD)		MDL [b]	MQL [c]
		Intra-day (%RSD, $n = 7$)		Inter-day (%RSD, $n = 7$)		ng mL^{-1}	ng mL^{-1}	Ultrapure water [d]	Wastewater [e]	ng L^{-1}	ng L^{-1}
		5 ng mL^{-1}	50 ng mL^{-1}	5 ng mL^{-1}	50 ng mL^{-1}						
R(-)- AMP	0.9991	2.1	1.5	3.8	5.4	0.3	0.8	95 (6)	95 (6)	1.8	5.5
S(+)- AMP	0.9995	4.3	3.3	3.3	8.5	0.4	1.0	90 (6)	100 (7)	1.7	5.1
R(-)- MAMP	0.9972	2.9	1.2	4.8	2.1	0.4	1.4	99 (3)	82 (15)	0.7	2.4
S(+)- MAMP	0.9988	1.2	1.8	12	8.6	0.3	1.0	105 (1)	94 (9)	0.7	2.4
R(-)- MDMA	0.9999	1.1	1.1	2.8	2.8	0.2	0.6	100 (5)	116 (4)	1.5	4.9
S(+)- MDMA	0.9990	0.72	0.51	0.92	1.2	0.2	0.6	100 (3)	111 (4)	1.4	3.5

[a] IQL-500 ng mL^{-1}, except AMP enantiomers: IQL-2500 ng mL^{-1}.
[b] Calculated for a S/N = 3 for the qualifier transition.
[c] Calculated for a S/N = 10 for the quantifier transition and S/N ≥ 3 for the qualifier transition.
[d] Samples spiked with 12.5 ng L^{-1} of each enantiomer, $n = 4$.
[e] Samples spiked with 125 ng L^{-1} of each enantiomer, $n = 4$.

(method B) and the enrichment increased by further evaporation of the extracts (see 2.4.2), the performance of this protocol was evaluated through recovery studies with the QC samples. As it is displayed in Table S4, recoveries varied between 66% and 125% and RSD < 17%; thus, this SPE protocol was acceptable in terms of trueness and precision. MQL values, estimated from the lowest concentration QC samples, ranged from 3.8 to 8.3 ng L^{-1}.

3.4. Drug samples characterization

Sample code, main drug, origin, purity and EF_R of each drug sample herein analysed are listed in Table S5. The 38 drug dose samples were submitted to the two drug-checking services (Energy Control and Ai Laket!!) by anonymous drug consumers. Consumers labelled them as the drugs they expected them to contain: 18 were labelled as AMP, 6 as MAMP, and 14 as MDMA. Sample purity (evaluated as explained in *2.2*) is summarized in Table 2 (details in Table S5). AMP samples presented a variable purity ranging from 2.7% to 103%. MAMP purity was more consistent and ranged between 54% and 76%, while MDMA purity varied between 0% and 107%. No MDMA was found in the sample coded MDMA-4, collected in Andalucía (Table S5). The results as regards EF_R show a concordance with the reported synthesis route of these drugs in Europe (Castrignanò et al., 2018; King 2009). Thus, AMP and MDMA samples were all racemate mixtures, while MAMP samples were all the pure S(+)-enantiomer (Table 2 summarizes also the results shown in Table S5). The limited number of samples does not allow us to address regional patterns.

3.5. Wastewater analysis

EF_R of the amphetamine-like substances found in wastewater were calculated from the concentrations measured in this matrix (see Eq. (1)). Following WBE calculations, loads (Eq. (2)) and human consumption (Eq. (3)) were subsequently estimated. The total concentration (sum of the two enantiomers) measured is summarized in Table 3 (detailed results are provided in Table S6). Excretion loads and estimated consumption values per city, substance and year are summarized in Table 3 (further details in Tables S7 and S8). Weekend peaks in loads/consumption (Tables S7 and S8) were observed in most locations for MDMA, while this was not so clear in the case of AMP or MAMP. A potential explanation is that these two last substances may originate either from daily abusers or prescription patients, particularly in the case of AMP, as further discussed below. Similar results have been observed in several other countries (Castrignanò et al., 2018; Thomas et al., 2012).

3.5.1. Amphetamine

AMP was positively detected in all the wastewater samples. The high concentration levels found in Bilbao and its metropolitan area in 2018 (mean 663 ng L^{-1}) (Bijlsma et al., 2021) were confirmed in 2019 (mean: 1375 ng L^{-1}) (Table 3). Although considerably lower, the second highest concentrations were detected in Palma (mean values: 106 ng L^{-1}, only samples from 2018 available). The highest loads were observed in Bilbao in 2019 (mean 277 mg day^{-1} 1000 $inhabitant^{-1}$), even higher than the loads reported in 2018 (mean 203 mg day^{-1} 1000 $inhabitant^{-1}$). In the remaining cities, the estimated loads of AMP was lower than 45 mg day^{-1} 1000 $inhabitant^{-1}$ (Table 3). These results match former observations in Spain and confirms the distinct pattern of consumption in the area of Bilbao, which is closer to the patterns observed in other countries such as Belgium, Western Germany (Been et al., 2016) or some Nordic countries, where AMP is one of the most prevalent drugs (González-Mariño et al., 2020).

The enantiomeric analysis showed a slight enrichment of R(-)-AMP in the wastewater of most cities, with EF_R higher than 0.5 (Table 3, details in Table S9). This matches the data obtained from the analysis of urine of 165 abusers with provided and average EF_R of 0.508 (George and Braithwaite, 2000) and highlights the fact that enrichment of R(-)-AMP (due to faster metabolization of S(+)-AMP) in the human body is not as high as in the case of MDMA (see 3.5.3), therefore making it difficult to differentiate illicit consumption from dumping events (that would lead to racemic AMP) on the basis of enantiomeric analysis only, as described by Emke et al. (2014). Despites this fact, dumping it is unlikely to play a major role in our study since, even in the case of Bilbao, where very high loads would point to direct disposal, no abnormal lead peak was detected on any singular day and associated to a change in the value of EF_R (Tables S6–S9), as already observed, for instance, for AMP and MDMA dumping events in The Netherlands (Emke et al., 2014).

Yet, the EF_R obtained here are similar to those reported in other cities across Europe (Castrignanò et al., 2018), including Valencia (Vazquez-Roig et al., 2014). In the case of Castellón, the EF_R laid between 0.49±0.03 and 0.50±0.02 (Table 3), which is equivalent to a racemic mixture. Samples from Castellón were also measured in 2015 by Castrignanò et al. (Castrignanò et al., 2018), yet AMP was not detected that study. Conversely, an enrichment of S(+)-AMP was detected in Madrid (Northern area) in 2018 with an average EF_R of 0.41±0.03, while only the S(+) isomer was detected above the MQL in 2019 (Table 3). Such observation could be partly related to a high contribution of LIS prescription in that area. In order to evaluate the potential contribution of medical prescription of LIS to WBE-derived consumption estimations, these data were compared with the available LIS prescription data from four of the five studied regions (Galicia, Basque Country, Balearic Islands and Community of Madrid; data from the Community of Valencia was not available). These data (as defined daily doses (DDD) day^{-1}1,000 $inhabitants^{-1}$) were obtained on a month basis for the province or municipality (see details in Table S10). Prescription data were converted into excretion loads of AMP, considering the DDD of LIS (30 mg, https://www.whocc.no/atc_ddd_index/), the average excretion of S(+)-AMP from LIS (44.75%), and the molecular weights of both drugs. The excretion value of 44.75% was derived as the average from two studies, performed with 7 individuals each, where S(+)-AMP accounted for 48.5% and 41% of the LIS dose, respectively (Comiran et al., 2021; Krishnan et al., 2008) (Table S11).

As it is displayed in Table 4, the expected loads of S(+)-AMP from LIS prescription range from 1.0 to 7.0 mg day^{-1} 1000 $inhabitant^{-1}$. When compared to the loads of AMP (sum of both enantiomers) actually found in wastewater, the prescription of LIS would account for less than 1% of the AMP consumption estimated in Bilbao and its metropolitan area, clearly pointing to illicit drug use. Conversely, in Madrid (Northern area) about 58% of AMP consumption in 2018 could be explained by prescription, which, together with EF_R results (< 0.50 in 2018, R(+)-AMP below MQL in 2019) could confirm a mixed origin (illicit use and LIS prescription). In Santiago de Compostela, medical prescription contribution is expected to be relatively high (over 37–44%, Table 4), but the EF_R was above 0.53 (Table S9) in all samples, which could then indicate that illicit consumption would be more relevant than LIS prescription. This disagreement may be explained by the fact that the external psychology consultations at the Santiago's hospital cover a larger healthcare area, thus, many of the patients do not live in this area and thus do no contribute to the wastewater samples. Further factors contributing to the uncertainty of the estimations made are non-adherence to prescription, which has been calculated to be a 30% in Spain (Siffel et al., 2020). Therefore, data presented in Table 4 would

Table 2
Summary of the results obtained from the analysis of drug samples.

Drug	N	EF_R Mean ± SD	Purity (%)			
			Mean	Median	SD	Range
AMP	18	0.506 ± 0.006	41	39	29	2.7–103
MAMP	6	0	65	63	7	54–76
MDMA	14	0.505 ± 0.006	70	55	33	0–107

N: number of samples.

Table 3
Summary of the results obtained for the drugs measured in the various wastewater treatment plants.

Substance	Location	Year	Concentration(ng L^{-1})		Loads (mg day^{-1} 1000 $inhabitant^{-1}$)		Consumption (mg day^{-1} 1000 $inhabitant^{-1}$)		EF_R	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
AMP	Bilbao and metropolitan area	2018	663	118	203	36	561	101	0.54	0.03
		2019	1375	745	277	155	766	428	0.56	0.01
	Castellón	2018	17	3	3.2	0.4	9	1	0.50	0.02
		2019	23	5	5	1	14	3	0.49	0.03
	Madrid (North)	2018	28	5	5	1	15	3	0.41	0.03
		2019	9	1	1.8	0.2	5	1	<0.45 [a]	NC [a]
	Santiago de Compostela	2018	17	5	13	3	37	10	0.57	0.04
		2019	47	10	16	3	44	9	0.73	0.05
	Palma	2018	106	27	13	3	35	8	0.56	0.03
MAMP	Bilbao and metropolitan area	2018	23	14	7	5	16	11	0	0
		2019	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	Castellón	2018	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
		2019	6	4	1.3	0.8	4	2	0	0
	Madrid (North)	2018	43	7	8	2	19	4	0	0
		2019	21	5	4	1	10	3	0	0
	Santiago de Compostela	2018	15	9	12	6	27	14	0	0
		2019	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	Palma	2018	24	15	2.9	1.9	7.1	4.6	0	0
MDMA	Bilbao and metropolitan area	2018	80	32	24	10	108	46	0.54	0.04
		2019	93	110	19	23	83	100	0.68	0.07
	Castellón	2018	12	2	2.2	0.3	8.4	2.8	0.65	0.01
		2019	41	31	9	7	39	30	0.59	0.01
	Madrid (North)	2018	78	62	18	10	80	44	0.66	0.04
		2019	36	21	7	4	33	20	0.64	0.04
	Santiago de Compostela	2018	50	21	38	14	168	60	0.57	0.03
		2019	45	24	15	8	67	35	0.64	0.03
	Palma	2018	173	104	20	11	91	51	0.55	0.02

[a] R(-)-AMP was below MQL in all samples from Madrid in 2019. Thus, an average EF_R but would be lower than 0.45 taking into consideration the MQL value.

Table 4
Comparison of AMP loads (in mg day^{-1} 1000 $inhabitant^{-1}$) expected from LIS prescription and actually found in wastewater in three of the monitored cities. See details on loads from prescription of LIS calculation in Table S10.

City	Loads of AMP measured by WBE		Expected loads from LIS prescription		AMP from prescription of LIS (%)	
	2018	2019	2018	2019	2018	2019
Bilbao and metropolitan area	203	277	1.52	1.03	0.75	0.37
Madrid	5.4	NC [a]	3.10	4.14	58	NC [a]
Palma	13	–	2.62	NC [a]	20	NC [a]
Santiago de Compostela	13	16	4.96	7.03	37	44

[a] NC: not calculated because: R(-)-AMP was below MQL in all samples from Madrid in 2019 and samples from Palma were only collected in 2018.

likely represent an overestimating scenario (maximum contribution of prescription) and the real contribution of LIS would be lower. Even with these data in mind some EF_R values would be higher than 0.5 in locations where prescription should be a relevant source. This is further limited by the fact that when pure (George and Braithwaite, 2000) or enriched enantiomer (Cody et al., 2003) medications are prescribed, a certain degree of interconversion occurs over time, which would lead to excretion of some R(-)-AMP and not only pure S(+)-AMP.

Considering these limitations, it seems still evident that the contribution of LIS prescription should be taken into account in future studies, particularly in those areas where the amount of AMP measured in wastewater is rather low.

As regards the contribution of SEL prescription to R(-)-AMP in wastewater, the expectable loads would range from 0.0028 to 0.022 mg day^{-1} 1000 $inhabitant^{-1}$ (Table S12). Those data were obtained from SEL prescription figures, considering a 15.4% excretion rate, as the weighted average of 4 different studies with a total of 21 individuals (detailed in Table S13) (Elsworth et al., 1978; Heinonen et al., 1989; Liebowitz et al., 1985; Reynolds et al., 1978) and the corresponding molecular weights. Such loads can be considered as negligible as they are two orders of magnitude lower than those from LIS, therefore representing less than 0.2% of the total AMP in any of the WWTPs. Actually, even in Palma, where the prescription of SEL was higher and with a dry precipitation regime (thus lower WWTP inflows) such loads will translate into ca. 0.2 ng L^{-1} concentrations of R(-)-AMP, i.e. below the MDL of the method.

3.5.2. *Methamphetamine*

Only S(+)-MAMP was detected in wastewater and the concentrations were low in all cities, with average values below 45 ng L^{-1} (Table 3) and maximum values up to 54 ng L^{-1} (Table S6). S(+)-MAMP average loads were lower than 13 mg day^{-1} 1000 $inhabitants^{-1}$ (Tables 3 and S7), confirming previous observations in Spain, with Barcelona, not analysed here, being the exception (Bijlsma et al., 2021; González-Mariño et al., 2020).

The prescription of SEL would be equivalent to loads in the 0.0090–0.072 mg day^{-1} 1000 $inhabitant^{-1}$ (Table S12), after considering an average excretion of R(-)-MAMP of 45.5% from SEL, according to the metabolism data compiled in Table S13 (Elsworth et al., 1978; Heinonen et al., 1989; Liebowitz et al., 1985; Reynolds et al., 1978). Again, as in the case of AMP, considering Palma as the place where the highest contribution of SEL prescription towards R(-)-MAMP is expected, this would result into concentrations of ca. 0.6 ng L^{-1}, which is below the MDL.

Thus, as regards the enantiomeric profiling of MAMP, EF_R was always 0, i.e. R(-)-MAMP was below MDL in all samples (Table S9). These data reinforces the results observed in *3.4* that indicated that MAMP consumed in Spain is synthesized as pure S(+)-MAMP, as in most parts of Europe, while the contribution of SEL is negligible. This would prevent from detecting any direct dumping event, but since no particularly high concentrations could be detected, this was not expected to have occurred in any of the WWTPs investigated during the sampling period.

3.5.3. *MDMA*

As for AMP, the two enantiomers of MDMA were detected in wastewater. The mean MDMA concentration (as sum of both isomers)

ranged from <MQL up to 374 ng L^{-1} (Table S6). The highest concentrations were found in the wastewater from Palma, but once corrected for population and flows, this does not translate into higher loads. Average loads varied between 2 and 38 mg day^{-1} 1000 $inhabitant^{-1}$ (Table 3), which is in the range of the already estimated MDMA loads in Spain in former studies (González-Mariño et al., 2020).

The EF_R average for MDMA was 0.61±0.05, being above 0.5 in all samples (Table S9) thus indicating the predominance of R(-)-MDMA in wastewater. These values match with urinary data, where 6 volunteers administered 100 mg of racemic MDMA leaded to an average EF_R of 0.657 over 24 h (Pizarro et al., 2002). Furthermore, an autopsy study revealed an EF_R of 0.57 (Moore et al., 1996). As MDMA is trafficked as racemate (see 3.4) and the S(+)-enantiomer is metabolized faster in the human body, the observed enrichment of R(-)-MDMA corroborates illicit consumption as the main source of MDMA in wastewater (Castrignanò et al., 2018). No event of drug disposal in the sewage network was detected.

3.5.4. Treated wastewater

Given the high concentrations of AMP detected in the WWTP of Bilbao and its metropolitan area in both 2018 and 2019, treated wastewater samples were also collected in 2019 and in the same way as raw wastewater, but with a delay of 24 h to account for the hydraulic residence time in the plant. AMP and MAMP levels were below the MQL in all treated wastewater samples, whereas MDMA was detected in all samples. As detailed in Table S14, MDMA levels ranged from 31 to 99 ng L^{-1} (average 57 ng L^{-1}), which is similar to the median value of 56 ng L^{-1} measured in the effluents of 42 WWTPs from the region of Catalonia (Spain) collected during 2006 and 2007 (Huerta-Fontela et al., 2008). Little is known about the ecotoxicological effects of MDMA, but the anticipated value of predicted non-effect concentration for this substance is 220 ng L^{-1} (Fernández-Rubio et al., 2019), hence emissions from the WWTP are not expected to generate ecotoxicological effects. Yet, there is a clear need for further experimental data, particularly considering the co-occurrence of different enantiomers (Sanganyado et al., 2017).

These data imply that the removal of AMP in the WWTP, considering the MQL, was higher than 99%, while MDMA nominal removal was 61% when considering the average concentrations measured in the effluent and influent (MAMP was below the MQL in both types of wastewater). Similar good removal rates for AMP but a higher variability in the case of MDMA have been reported in the literature (Bijlsma et al., 2012, 2014b; Huerta-Fontela et al., 2008; Yadav et al., 2017).

Furthermore, the EF_R of MDMA shifted from 0.68±0.07 in the influent to 0.88±0.04 in the effluent, which implies a further prevalence of the less biologically active enantiomer, R(-)-MDMA, after the wastewater treatment and a clear distinct elimination profile of both enantiomers, i.e.: average removals of 21% and 77%, for R(-)-MDMA and S(+)-MDMA, respectively. Such enantioselective elimination has already been observed in a WWTP in Valencia studied in 2012 (Vazquez-Roig et al., 2014) but, as mentioned, ecotoxicological implications remain unknown.

4. Conclusions

An analytical methodology based on SPE and LC-MS/MS has been successfully developed for the determination of three chiral amphetamine-like substances in urban wastewater and street drug samples. The analyses of consumer-donated street drugs clearly indicated that AMP and MDMA are produced as racemic mixtures for the Spanish illicit market, while MAMP is produced as the pure active S(+)-enantiomer. The enantiomeric profiling from wastewater analyses indicated that much higher levels of AMP occur in the metropolitan area of Bilbao compared to other Spanish cities, which, combined with LIS and SEL prescription data indicate that its origin can be attributed mainly to illicit consumption. Conversely, in the remaining Spanish cities investigated, where AMP levels are low, the prescription of LIS may become a relevant source of AMP, whereas the contribution of the prescription of the pharmaceutical SEL to AMP and MAMP loads is negligible. Finally, the analysis of effluent samples in the area of Bilbao showed that AMP is well removed in a WWTP equipped with conventional biological water treatments, while the removal of MDMA is relatively high for the S(+)-enantiomer, but much more limited for R(-)-MDMA.

CRediT authorship contribution statement

Andrea Estévez-Danta: Investigation, Methodology, Formal analysis, Visualization, Writing – original draft. **Rosa Montes:** Methodology, Supervision, Formal analysis, Writing – original draft. **Lubertus Bijlsma:** Investigation, Resources, Methodology, Writing – review & editing. **Rafael Cela:** Resources, Funding acquisition. **Alberto Celma:** Investigation, Resources, Writing – review & editing. **Iria González-Mariño:** Methodology, Supervision, Writing – review & editing. **Manuel Miró:** Resources, Writing – review & editing. **Vanessa Gutmann:** Investigation, Methodology. **Unai Pérez de San Román-Landa:** Resources, Writing – review & editing. **Ailette Prieto:** Resources, Writing – review & editing. **Mireia Ventura:** Resources, Writing – review & editing. **Rosario Rodil:** Resources, Supervision, Funding acquisition, Formal analysis, Writing – review & editing. **José Benito Quintana:** Resources, Visualization, Supervision, Funding acquisition, 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.

Acknowledgement

Financial support: This study was supported by MINECO/MICINN/AEI projects (CTM2016-81935-REDT, CTM2017-84763-C3-2-R, CTM2017-84763-C3-1-R, CTM2017-84763-C3-3-R, PID2020-117686RB-C32, PID2020-117686RB-C31, PID2020-117686RB-C33), Galician Council of Culture, Education and Universities (ED481D-2017/003, ED431C-2017/36, ED481A-2020/258 and ED431C 2021/06), cofounded by FEDER/ERDF. UJI authors acknowledge the financial support of Generalitat Valenciana (Excellence Research Group, Prometeo 2019/040). Alberto Celma acknowledges the Spanish Ministry of Economy and Competitiveness for his predoctoral Grant (BES-2016-076914). Vanessa Gutmann acknowledges the support of the ERASMUS+ program.

Sampling and analytical support: Viaqua (Marta Clemente) and Concello de Santiago, Santiago Querol and Sara Gargallo from Sociedad de Fomento Agrícola Castellonense (FACSA, Castellón), Dr. María Dolores Mateo, Mr. Víctor Fernández and technicians from EMAYA (Palma), Iñigo González Canal (Consorcio de Aguas de Bilbao Bizkaia), Subdirección General de Gestión del Agua, Ayuntamiento de Madrid, Canal de Isabel II.

LIS prescription data: Dirección de Farmacia, Departamento de Salud - Gobierno Vasco (Basque Country), Subdirección General de Farmacia y Productos Farmacéuticos - Comunidad de Madrid (Community of Madrid), Subdirección Xeral de Farmacia - Xunta de Galicia (Galicia) and Conselleria de Salut i Consum (Dr. Gemma Melero & Mrs. Ángela Aguiló) – Govern de les Illes Balears (Balearic Islands).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117719.

References

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.

Alygizakis, N., Markou, A.N., Rousis, N.I., Galani, A., Avgeris, M., Adamopoulos, P.G., Scorilas, A., Lianidou, E.S., Paraskevis, D., Tsiodras, S., Tsakris, A., Dimopoulos, M.-A., Thomaidis, N.S., 2020. Analytical methodologies for the detection of SARS-CoV-2 in wastewater: protocols and future perspectives. TrAC Trends Anal. Chem. 134, 116125.

Archer, E., Castrignanò, E., Kasprzyk-Hordern, B., Wolfaardt, G.M., 2018. Wastewater-based epidemiology and enantiomeric profiling for drugs of abuse in South African wastewaters. Sci. Total Environ. 625, 792–800.

Been, F., Bastiaensen, M., Lai, F.Y., Libousi, K., Thomaidis, N.S., Benaglia, L., Esseiva, P., Delémont, O., van Nuijs, A.L.N., Covaci, A., 2018. Mining the chemical information on urban wastewater: monitoring human exposure to phosphorus flame retardants and plasticizers. Environ. Sci. Technol. 52 (12), 6996–7005.

Been, F., Bijlsma, L., Benaglia, L., Berset, J.-.D., Botero-Coy, A.M., Castiglioni, S., Kraus, L., Zobel, F., Schaub, M.P., Bücheli, A., Hernández, F., Delémont, O., Esseiva, P., Ort, C., 2016. Assessing geographical differences in illicit drug consumption—a comparison of results from epidemiological and wastewater data in Germany and Switzerland. Drug Alcohol Depend. 161, 189–199.

Bijlsma, L., Beltrán, E., Boix, C., Sancho, J.V., Hernández, F., 2014a. Improvements in analytical methodology for the determination of frequently consumed illicit drugs in urban wastewater. Anal. Bioanal. Chem. 406 (17), 4261–4272.

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 (11), 1399–1406.

Bijlsma, L., Picó, Y., Andreu, V., Celma, A., Estévez-Danta, A., González-Mariño, I., Hernández, F., López de Alda, M., López-García, E., Marcé, R.M., Miró, M., Montes, R., Pérez de Sanromán-Landa, U., Pitarch, E., Pocurull, E., Postigo, C., Prieto, A., Rico, A., Rodil, R., Valcárcel, Y., Ventura, M., Quintana, J.B., 2021. The embodiment of wastewater data for the estimation of illicit drug consumption in Spain. Sci. Total Environ. 772, 144794.

Bijlsma, L., Serrano, R., Ferrer, C., Tormos, I., Hernández, F., 2014b. Occurrence and behavior of illicit drugs and metabolites in sewage water from the Spanish Mediterranean coast (Valencia region). Sci. Total Environ. 487, 703–709.

Castiglioni, S., Senta, I., Borsotti, A., Davoli, E., Zuccato, E., 2015. A novel approach for monitoring tobacco use in local communities by wastewater analysis. Tob. Control 24 (1), 38.

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.

Castrignanò, E., Yang, Z., Bade, R., Baz-Lomba, J.A., Castiglioni, S., Causanilles, A., Covaci, A., Gracia-Lor, E., Hernandez, F., 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., Kasprzyk-Hordern, B., 2018. Enantiomeric profiling of chiral illicit drugs in a pan-European study. Water Res. 130, 151–160.

Castro, V., Montes, R., Quintana, J.B., Rodil, R., Cela, R., 2020. Determination of 18 organophosphorus flame retardants/plasticizers in mussel samples by matrix solid-phase dispersion combined to liquid chromatography-tandem mass spectrometry. Talanta 208, 120470.

Cody, J.T., 2002. Precursor medications as a source of methamphetamine and/or amphetamine positive drug testing results. J. Occup. Environ. Med. 44 (5), 435–450.

Cody, J.T., Valtier, S., Nelson, S.L., 2003. Amphetamine enantiomer excretion profile following administration of adderall*. J. Anal. Toxicol. 27 (7), 485–492.

Comiran, E., Carlos, G., Barreto, F., Pechanksy, F., Fröehlich, P.E., Limberger, R.P., 2021. Lisdexamfetamine and amphetamine pharmacokinetics in oral fluid, plasma, and urine after controlled oral administration of lisdexamfetamine. Biopharm. Drug Dispos. 42 (1), 3–11.

Elsworth, J.D., Glover, V., Reynolds, G.P., Sandler, M., Lees, A.J., Phuapradit, P., Shaw, K.M., Stern, G.M., Kumar, P., 1978. Deprenyl administration in man: a selective monoamine oxidase B inhibitor without the 'cheese effect. Psychopharmacology 57 (1), 33–38.

EMCDDA (2021a) Amphetamine drug profile, European Monitoring Centre for Drugs and Drug Addiction.

EMCDDA (2021b) MDMA ('Ecstasy') drug profile, European Monitoring Centre for Drugs and Drug Addiction.

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 (1), 666–672.

Emke, E., Vughs, D., Kolkman, A., de Voogt, P., 2018. Wastewater-based epidemiology generated forensic information: amphetamine synthesis waste and its impact on a small sewage treatment plant. Forensic Sci. Int. 286, e1–e7.

Estévez-Danta, A., Rodil, R., Pérez-Castaño, B., Cela, R., Quintana, J.B., González-Mariño, I., 2021. Comprehensive determination of phthalate, terephthalate and di-iso-nonyl cyclohexane-1,2-dicarboxylate metabolites in wastewater by solid-phase extraction and ultra(high)-performance liquid chromatography-tandem mass spectrometry. Talanta 224, 121912.

Fernández-Rubio, J., Rodríguez-Gil, J.L., Postigo, C., Mastroianni, N., López de Alda, M., Barceló, D., Valcárcel, Y., 2019. Psychoactive pharmaceuticals and illicit drugs in coastal waters of North-Western Spain: environmental exposure and risk assessment. Chemosphere 224, 379–389.

Gao, J., Xu, Z., Li, X., O'Brien, J.W., Culshaw, P.N., Thomas, K.V., Tscharke, B.J., Mueller, J.F., Thai, P.K., 2018. Enantiomeric profiling of amphetamine and methamphetamine in wastewater: a 7-year study in regional and urban Queensland, Australia. Sci. Total Environ. 643, 827–834.

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.

George, S., Braithwaite, R.A., 2000. Using amphetamine isomer ratios to determine the compliance of amphetamine abusers prescribed dexedrine. J. Anal. Toxicol. 24 (3), 223–227.

González-Mariño, I., Ares, L., Montes, R., Rodil, R., Cela, R., López-García, E., Postigo, C., López de Alda, M., Pocurull, E., Marcé, R.M., Bijlsma, L., Hernández, F., Picó, Y., Andreu, V., Rico, A., Valcárcel, Y., Miró, M., Etxebarria, N., Quintana, J.B., 2021. Assessing population exposure to phthalate plasticizers in thirteen Spanish cities through the analysis of wastewater. J. Hazard. Mater. 401, 123272.

González-Mariño, I., Baz-Lomba, J.A., Alygizakis, N.A., Andrés-Costa, M.J., Bade, R., 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., de Voogt, P., Devault, D.A., Dias, M.J., 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., de Alda, M.L., 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-Gonzàlez, 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 (1), 109–120.

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.

González-Mariño, I., Quintana, J.B., Rodríguez, I., González-Díez, M., Cela, R., 2012. 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 (3), 1708–1717.

González-Mariño, I., Quintana, J.B., Rodríguez, I., Rodil, R., González-Peñas, J. and Cela, R. (2009) Comparison of molecularly imprinted, mixed-mode and hydrophilic balance sorbents performance in the solid-phase extraction of amphetamine drugs from wastewater samples for liquid chromatography – tandem mass spectrometry determination. 1216, 8435–8441.

González-Mariño, I., Rodil, R., Barrio, I., Cela, R., Quintana, J.B., 2017. Wastewater-based epidemiology as a new tool for estimating population exposure to phthalate plasticizers. Environ. Sci. Technol. 51 (7), 3902–3910.

Gracia-Lor, E., Zuccato, E., Castiglioni, S., 2016. Refining correction factors for back-calculation of illicit drug use. Sci. Total Environ. 573, 1648–1659.

Guttmann, E., Sargant, W., 1942. Addiction to Benzedrine. Br. Med. J. 1 (4243), 564. -564.

Hauser, F.M., Pütz, M., Rößler, T., Hulshof, J.W., 2020. Identification of specific markers for amphetamines synthesized from glycidic acid pre-precursors and retrospective search in German profiling database. Drug Test. Anal. 12 (1), 41–52.

Heinonen, H.E., Myllylä, V., Sotaniemi, K., Lammintausta, R., Salonen, S.J., Anttila, M., Savijärvi, M., Kotila, M., Rinne, K.U., 1989. Pharmacokinetics and metabolism of selegiline. Acta Neurol. Scand. 80 (s126), 93–99.

Huerta-Fontela, M., Galceran, M.T., Martin-Alonso, J., Ventura, F., 2008. Occurrence of psychoactive stimulatory drugs in wastewaters in north-eastern Spain. Sci. Total Environ. 397 (1), 31–40.

Kalant, H., 2001. The pharmacology and toxicology of "ecstasy" (MDMA) and related drugs. CMAJ 165 (7), 917–928.

Kasprzyk-Hordern, B., 2010. Pharmacologically active compounds in the environment and their chirality. Chem. Soc. Rev. 39 (11), 4466–4503.

Kasprzyk-Hordern, B., Baker, D.R., 2012. Enantiomeric profiling of chiral drugs in wastewater and receiving waters. Environ. Sci. Technol. 46 (3), 1681–1691.

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 (27), 4575–4586.

King, L.A. (2009) Forensic chemistry of substance misuse: a guide to drug control.

Krishnan, S.M., Pennick, M., Stark, J.G., 2008. Metabolism, distribution and elimination of lisdesxamfetamine dimesylate. Clin. Drug Investig. 28 (12), 745–755.

Lemere, F., 1967. The danger of amphetamine dependency. Obstet. Gynecol. Surv. 22 (3), 404–405.

Lertxundi, U., Domingo-Echaburu, S., Orive, G., 2021. Estimation of illicit amphetamine consumption using wastewater based epidemiology: a tiny contribution. Sci. Total Environ. 779, 146349.

Liebowitz, M.R., Karoum, F., Quitkin, F.M., Davies, S.O., Schwartz, D., Levitt, M., Linnoila, M., 1985. Biochemical effects of L-deprenyl in atypical depressives. Biol. Psychiatry 20 (5), 558–565.

Lopardo, L., Petrie, B., Proctor, K., Youdan, J., Barden, R., Kasprzyk-Hordern, B., 2019. Estimation of community-wide exposure to bisphenol A via water fingerprinting. Environ. Int. 125, 1–8.

López-García, E., Pérez-López, C., Postigo, C., Andreu, V., Bijlsma, L., González-Mariño, I., Hernández, F., Marcé, R.M., Montes, R., Picó, Y., Pocurull, E., Rico, A.,

Rodil, R., Rosende, M., Valcárcel, Y., Zuloaga, O., Quintana, J.B., López de Alda, M., 2020. Assessing alcohol consumption through wastewater-based epidemiology: spain as a case study. Drug Alcohol Depend. 215, 108241.
Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., Brouwer, A., 2020. Presence of SARS-coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. Environ. Sci. Technol. Lett. 7 (7), 511–516.
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.
Moore, K.A., Mozayani, A., Fierro, M.F., Poklis, A., 1996. Distribution of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) stereoisomers in a fatal poisoning. Forensic Sci. Int. 83 (2), 111–119.
Myerson, A., 1939. The reciprocal pharmacologic effects of amphetamine (benzedrine) sulfate and the barbiturates. Eng. J. Med. 221, 561–564.
Ort, C., van Nuijs, A.L.N., Berset, J.D., Bijlsma, L., Castiglioni, S., Covaci, A., de Voogt, P., Emke, E., Fatta-Kassinos, D., Griffiths, P., Hernández, F., González-Mariño, I., Grabic, R., Kasprzyk-Hordern, B., Mastroianni, N., Meierjohann, A., Nefau, T., Östman, M., Pico, Y., Racamonde, I., Reid, M., Slobodnik, J., Terzic, S., Thomaidis, N., Thomas, K.V., 2014. Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis. Addiction 109 (8), 1338–1352.
Pennick, M., 2013. Metabolism of the prodrug lisdexamfetamine dimesylate in human red blood cells from normal and sickle cell disease donors. J. Drug Assess. 2 (1), 17–20.
Pizarro, N., Ortuño, J., Farré, M., Hernández-López, C., Pujadas, M., Llebaria, A., Joglar, J., Roset, P.N., Mas, M., Segura, J., Camí, J., de la Torre, R., 2002. Determination of MDMA and its metabolites in blood and urine by gas chromatography-mass spectrometry and analysis of enantiomers by capillary electrophoresis. J. Anal. Toxicol. 26 (3), 157–165.
Reynolds, G.P., Elsworth, J.D., Blau, K., Sandler, M., Lees, A.J., Stern, G.M., 1978. Deprenyl is metabolized to methamphetamine and amphetamine in man. Br. J. Clin. Pharmacol. 6 (6), 542–544.
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.
Rodríguez-Álvarez, T., Rodil, R., Cela, R., Quintana, J.B., 2014a. 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.
Rodríguez-Álvarez, T., Rodil, R., Rico, M., Cela, R., Quintana, J.B., 2014b. Assessment of local tobacco consumption by liquid chromatography–tandem mass spectrometry sewage analysis of nicotine and its metabolites, cotinine and trans-3′-hydroxycotinine, after enzymatic deconjugation. Anal. Chem. 86 (20), 10274–10281.
Rousis, N.I., Zuccato, E., Castiglioni, S., 2016. Monitoring population exposure to pesticides based on liquid chromatography-tandem mass spectrometry measurement of their urinary metabolites in urban wastewater: a novel biomonitoring approach. Sci. Total Environ. 571, 1349–1357.
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.
Sanganyado, E., Lu, Z., Fu, Q., Schlenk, D., Gan, J., 2017. Chiral pharmaceuticals: a review on their environmental occurrence and fate processes. Water Res. 124, 527–542.
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 (10), 3255–3268.
Senta, I., Rodríguez-Mozaz, S., Corominas, L., Petrovic, M., 2020. Wastewater-based epidemiology to assess human exposure to personal care and household products – a review of biomarkers, analytical methods, and applications. Trends Environ. Anal. Chem. 28, e00103.
Siffel, C., Page, M., Maxwell, T., Thun, B., Kolb, N., Rosenlund, M., von Bredow, D., Keja, J., 2020. Patterns of lisdexamfetamine dimesylate use in children, adolescents, and adults with attention-deficit/hyperactivity disorder in Europe. J. Child. Adolesc. Psychopharmacol. 30 (7), 439–447.
Thomas, K.V., Bijlsma, L., Castiglioni, S., Covaci, A., Emke, E., Grabic, R., Hernandez, F., Karolak, S., Kasprzyk-Hordern, B., Lindberg, R.H., de Alda, M.L., Meierjohann, A., Ort, C., Pico, Y., Quintana, J.B., Reid, M., Rieckermann, J., Terzic, S., van Nuijs, A.L. N., de Voogt, P., 2012. Comparing illicit drug use in 19 European cities through sewage analysis. Sci. Total Environ. 432, 432–439.
Tscharke, B.J., White, J.M., Gerber, J.P., 2016. Estimates of tobacco use by wastewater analysis of anabasine and anatabine. Drug Test. Anal. 8 (7), 702–707.
Vazquez-Roig, P., Kasprzyk-Hordern, B., Blasco, C., Picó, Y., 2014. Stereoisomeric profiling of drugs of abuse and pharmaceuticals in wastewaters of Valencia (Spain). Sci. Total Environ. 494-495, 49–57.
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.
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. Clin. Pract. Epidemiol. Ment. Health 9, 1–9.