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Stereoisomeric profiling of drugs of abuse and pharmaceuticals in wastewaters of Valencia (Spain)



Pablo Vazquez-Roig^a, Barbara Kasprzyk-Hordern^{b,*}, Cristina Blasco^a, Yolanda Picó^a

^a Laboratory of Nutrition and Bromatology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés s/n, 46100 Burjassot, Valencia, Spain

^b University of Bath, Department of Chemistry, Faculty of Science, Bath BA2 7AY, UK

HIGHLIGHTS

- Enantiomeric profiling of chiral drugs was undertaken at three WWTPs.
- Degradation efficiency of WWTPs was compound and enantiomer dependent.
- Atenolol was enriched with either *S*(–)– or *R*(+)–enantiomer in different WWTPs.
- Amphetamine and MDMA were enriched with *R*(–)–enantiomers
- 1*S*,2*S*(+)–pseudoephedrine was more readily degradable than its diastereomer

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ABSTRACT

The enantiomeric and diastereomeric profiling of chiral pharmaceuticals (ephedrine, norephedrine, atenolol and venlafaxine) and illicit drugs (amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) and 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA)) was undertaken over a period of fourteen consecutive days in three wastewater treatment plants (WWTPs) in the city of Valencia, Spain. Degradation efficiency of WWTPs was found to be compound and enantiomer dependent. Selective enantiomer enrichment was observed for several target analytes. Amphetamine and MDMA were enriched with *R*(–)–enantiomers. 1*S*,2*S*(+)–pseudoephedrine was found to be more readily degradable during activated sludge treatment than its diastereomer 1*R*,2*S*(–)–ephedrine. Atenolol underwent enrichment with either *S*(–)– or *R*(+)–enantiomer in different WWTPs. This unexpected enantiomeric variation in the stereoselective degradation of atenolol could be attributed to different processes utilized during activated sludge treatment. The application of (enantiomeric) profiling of wastewater revealed usage patterns of chiral drugs in the Valencia region.

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1. Introduction

More than 50% of pharmaceuticals and illicit drugs currently in use are chiral (Lien et al., 2006). Although they are usually manufactured as racemic compounds, they can be stereoselectively degraded in humans (for example as a result of stereoselective metabolism) and/or during wastewater treatment as a result of microbial processes. Since activity and toxicity of these compounds are often isomer-dependent (Kasprzyk-Hordern, 2010), it is of importance to understand the influence of wastewater treatment processes in the selective degradation of chiral drugs in order to improve the performance of wastewater treatment plants (WWTPs) and to protect the receiving aquatic environment. Fluoxetine, for example, is one of the most toxic human

pharmaceuticals reported so far. Its ecotoxicity is currently assessed for the racemate (Kasprzyk-Hordern, 2010). However, recent research indicates that toxic effects of fluoxetine are enantiomer dependent: *S*(+)–fluoxetine is 9.4 times more toxic to *Pimephales promelas* than *R*(–)–fluoxetine (Stanley et al., 2007). Specific inter-species toxic effects also exist among enantiomers; for example, *S*(–)–propranolol has a higher chronic toxicity to *Fathead Minnows* than its enantiomer, but the opposite is true in *Daphnia magna* (Nikolai et al., 2006).

There is a lack of information on the stereoselective and/or stereospecific fate and effects of chiral pharmaceuticals and illicit drugs in the environment. Although chiral HPLC methods have been extensively used for stereoisomer separation of drugs in pharmaceutical preparations they are usually not directly amenable to the analysis of chiral drugs in complex environmental matrices and at trace concentrations (Evans and Kasprzyk-Hordern, 2014). Recently, new HPLC methods using chiral columns packed with antibiotics or proteins coupled with

* Corresponding author. Tel.: +44 1225 385013; fax: +44 1225 386231.
E-mail address: B.Kasprzyk-Hordern@bath.ac.uk (B. Kasprzyk-Hordern).

tandem mass spectrometry have been successfully applied in the analysis of several chiral drugs in WWTPs and/or surface waters (Bagnall et al., 2012; Kasprzyk-Hordern and Baker, 2012b; MacLeod and Wong, 2010; Nikolai et al., 2006). Among these chiral drugs are: atenolol, metoprolol, fluoxetine, venlafaxine, ibuprofen, ketoprofen, naproxen, amphetamine, methamphetamine and ephedrine (MacLeod and Wong, 2010; Nikolai et al., 2006; Barclay et al., 2012; Hashim and Khan, 2011; Fono and Sedlak, 2005; Buser et al., 1999; López-Serna et al., 2013; Kasprzyk-Hordern and Baker., 2012b).

This paper presents for the first time the results of a two week study of three WWTPs in Valencia City and surroundings aiming at estimating the occurrence and stereoselective fate of five chiral pharmaceuticals: (ephedrine, pseudoephedrine, norephedrine, atenolol and venlafaxine) and five illicit drugs (amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyethamphetamine (MDEA)).

2. Materials and methods

2.1. Sampling

In a sampling campaign of two weeks (April 17–May 1 of 2012), influent and effluent 24 h-composite samples (time-proportional mode) were collected in three WWTPs serving the city of Valencia (Spain) and the surrounding towns: Pinedo-I, Pinedo-II and Quart-Benager (Fig. 1). These WWTPs have tertiary treatments but they differ in the type of technology utilized for secondary treatment: Pinedo-I utilizes only activated sludge, Pinedo-II utilizes activated sludge and phosphorus removal, and Quart-Benager utilizes activated sludge and nitrogen removal (see operational details of studied WWTPs in Table 1). Pinedo-I treats only wastewaters of the city of Valencia. Pinedo-II is the biggest and serves Valencia and surrounding towns. It has a very complete primary treatment and a secondary treatment, digestion and dehydration of sludge (200,000 m³/day). This installation is complemented by a treatment with Densadeg, filters, sand and UV disinfection with a capacity of 350,000 m³/day (100,000 m³/day for

Table 1
Operational parameters of studied WWTPs.

Characteristics	Pinedo-I	Pinedo-II	Quart-Benager
Coordinates UTM (ETRS 89 zone 30N)	X:728552 Y: 4368031 Z: 5	X:728371 Y: 4368153 Z: 5	X:722456 Y: 4370419 Z: 22
Population served (thousands)	351,198	942,774	166,942
Flows (m ³ /day)	100,602	242,580	35,903
Wastewater (% industrial/ domestic)	0/100	0/100	60/40
Treatment	AS	AS/N removal	AS/P removal
Average daily sewage flow (m ³ /d)	106,537	236,396	37,998
Designed treatment capacity (m ³ /d)	124,800	200,000	60,000
Influent BOD (mg/L) ^a	248.2	263.7	318.1

AS: Activated Sludge; N: Nitrogen; P: phosphorus; BOD: Biological Oxygen Demand.

^a Annual average.

regeneration of the ecological flow of Natural Park) and 250,000 m³/day for irrigation. Quart-Benager serves the towns of the industrial belt of Valencia.

24 h-composite samples were taken in 2 L plastic bottles (polyethylene) with Teflon protected caps and were transported to the laboratory for their immediate analysis. If this was not possible, the samples were frozen at -20°C until analysis to prevent degradation of the target residues.

2.2. Chemicals

All reference standards (\pm)-amphetamine (AMP), (\pm)-methamphetamine (MAMP), (\pm)-MDA, (\pm)-MDMA, (\pm)-MDEA, (–)-ephedrine (EPH), (+)-pseudoephedrine (PEPH), (\pm)-norephedrine (NOR), (\pm)-atenolol (ATE), and (\pm)-venlafaxine (VEN) were purchased from LGC Standards (Teddington, UK) and Sigma-Aldrich (Gillingham, UK). The surrogate standard (SS) (\pm)-MDA-d5 was added to the samples before solid-phase extraction (SPE). Internal standards (IS): (\pm)-methamphetamine-d5, (\pm)-MDMA-d5, (\pm)-MDEA-d5 and (\pm)-atenolol-d7

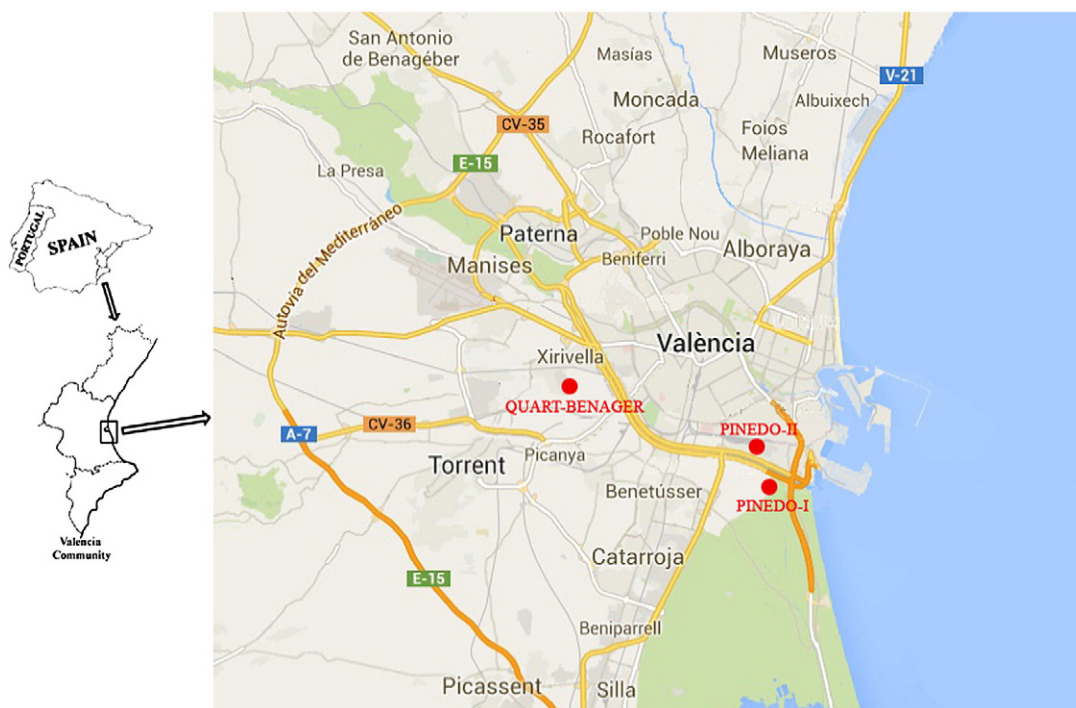


Fig. 1. Sampling locations in Valencia.

were added to SPE extracts before their analysis with chiral LC–MS. All glassware was silanized with dimethylchlorosilane (5% DMDCS in toluene) to minimize sample loss through adsorption of basic analytes onto OH-sites present on glass surface.

2.3. Analytical methodology

Once in the laboratory, and after the addition of SS, all samples were filtered through a 0.45 μm glass microfiber filter and subsequently extracted following a protocol described elsewhere (Kasprzyk-Hordern et al., 2010). Briefly, 50 mL of water was extracted using an HLB cartridge. The cartridge was then rinsed with Milli-Q water, dried under vacuum for 15 min, frozen and then eluted prior to the analysis with 6 mL of methanol. The eluent was evaporated to dryness and reconstituted in 500 μL of methanol/water (25:75, v/v). 5 μL of IS at 10 ng/ μL was added to each sample. Vials were stored at $-18\text{ }^\circ\text{C}$ until analysis. For each sample, three extractions were carried out and subsequently analyzed.

Waters ACQUITY UPLC™ system (Waters, Manchester, UK) consisting of ACQUITY UPLC™ binary solvent manager and ACQUITY UPLC™ sample manager was used for the separation of analytes. Chiral-CB column, 100 \times 2 mm, 5 μm (Chromtech, Congleton, UK) was used for the separation of enantiomers of chiral pharmaceuticals and illicit drugs. The separation of chiral drugs was carried out in isocratic conditions with a mobile phase (pH, 5.0) composed of 90% H_2O , 10% 2-propanol and 1 mM ammonium acetate. Injection volume was 20 μL . The column was kept at 25 $^\circ\text{C}$ and the temperature in the sample manager was kept at 4 $^\circ\text{C}$. The flow rate of mobile phase was 0.075 mL/min, which allowed for the introduction of mobile phase from LC into MS without splitting.

A XevoTQD (triple quadrupole) mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization source was used for enantiomeric profiling. The analyses were analyzed in positive ion mode with a capillary voltage of 3 kV, a source temperature of 150 $^\circ\text{C}$ and a desolvation temperature of 200 $^\circ\text{C}$. A cone gas flow of 0 L/h and desolvation gas flow of 450 L/h were used. Nitrogen, used as a nebulizing and desolvation gas, was provided by a high purity nitrogen generator (Peak Scientific Instruments Ltd, UK). Argon (99.999%) was used as a collision gas. MassLynx 4.1 (Waters, UK) software was used to collect and analyze the obtained data. Mass spectrometry analyses were performed in the selected reaction monitoring (SRM) mode, measuring the fragmentation of the protonated pseudo-molecular ions of each chiral drug (Table S1, Supplementary material). A dwell time of 200 ms per ion pair was used to maintain high sensitivity of the analysis and required a number of data points across the chromatographic peak. All instrumental and method validation parameters such as: linearity and range, accuracy, precision, detection and quantification limits and calibration curve were determined (Table S2, Supplementary material). A detailed discussion of this method is presented elsewhere (Kasprzyk-Hordern and Baker, 2012a,b; Kasprzyk-Hordern et al., 2010; Bagnall et al., 2012).

The relative concentration of enantiomers of chiral drugs was expressed as the enantiomeric fraction (EF) and was calculated using the following equation:

$$EF = \frac{(+)}{(+)+(-)}$$

where (+) and (–) are concentrations for (+) and (–)-enantiomers. In the case of amphetamine, methamphetamine, MDMA, MDA and MDEA, enantiomers were identified as R(–)- and S(+)-enantiomers. In the case of atenolol and venlafaxine, they were identified as R(+)- and S(–)-enantiomers.

Ephedrine/pseudoephedrine has two chiral centers and as a result two pairs of enantiomers. The ephedrine enantiomers have a diastereomeric relationship with pseudoephedrine enantiomers. Therefore, for

1R,2S(–)-ephedrine and 1S,2S(+)-pseudoephedrine diastereomeric fraction (DF) was also calculated:

$$DF = \frac{1S,2S(+)\text{-pseudoephedrine}}{1R,2S(-)\text{ephedrine} + 1S,2S(+)\text{pseudoephedrine}}$$

Removal efficiency for each compound and WWTP were calculated from mean concentrations of analytes measured in WWTP effluent and influent samples using the following equation:

$$\% \text{Removal Efficiency} = 1 - \left(\frac{[\text{Effluent}]}{[\text{Influent}]} \right) * 100.$$

100% elimination was assumed when the analyte was detected in the influent water but not in the effluent.

2.4. Estimation of drug usage at the community level in main urban areas of Valencia

Drug usage in the city of Valencia and adjacent residential areas was calculated from drug concentrations measured at the inlets of the investigated WWTPs. The stability of compounds in raw wastewater and sorption to suspended particle matter was taken into account as suggested by Baker et al. (2012, 2014). The consumption was calculated with the usage of the following formula:

$$Q \left(\text{mg} \cdot \text{day}^{-1} \cdot 1000 \text{ people}^{-1} \right) = \frac{\left(Q_{\text{day}} * 1000 \right) * \left(\frac{100}{U_{\text{ex}}} \right) * M_{\text{ratio}}}{\frac{N_{\text{inh}}}{1000}}$$

where Q_{day} is the load of the DTR, U_{ex} is the percentage of the DTR urinary excretion, M_{ratio} is the parent drug/DTR molar ratio and N_{inh} is the number of inhabitants linked to the WWTPs estimated assuming an organic per-capita load of 60 g $\text{BOD}_5 \text{ Hab}^{-1} \text{ day}^{-1}$ (Andreottola et al., 1994). DTR percentages of excretion were obtained from Postigo et al. (2011) for AMP, MAMP, MDMA and ephedrine, and from Lai et al. (2011) for atenolol and venlafaxine.

Q_{day} was calculated using the following equation:

$$Q_{\text{day}} \left(\text{g day}^{-1} \right) = \frac{\left(\left(\text{Conc} * \left(\frac{-\text{Sta}}{100 - \text{Sta}} + 1 \right) \right) + \text{Sorp} \right) * \text{Flow}}{1 * 10^9}$$

where Conc corresponds to the drug target residue (DTR) concentration in wastewater (ng L^{-1}), Sorp is the DTR concentration sorbed to suspended particulate matter (ng L^{-1}), Flow is the wastewater influent volume over a 24 hour period ($\text{m}^3 \text{ day}^{-1}$) and Sta is the stability change of each compound (%) after 12 h (see Baker et al., 2012 for stability data). In the case of atenolol sorption to SPM and stability factors were not available. Therefore, these parameters were not used in load calculations. For the list of parameters used in above calculations see Table S3.

3. Results and discussion

Daily loads of the investigated pharmaceuticals and drugs of abuse in analyzed influent wastewater samples are presented in Figs. 2, 3 and 4 for Pinedo-I, Pinedo-II and Quart-Benager WWTPs respectively.

3.1. Occurrence of chiral drugs in wastewaters of Valencia

MDA, MDEA and norephedrine were not detected in any analyzed wastewater samples. Amphetamine, methamphetamine and MDMA were present at trace ppt levels. On the other hand, atenolol and venlafaxine were quantified at the highest concentrations reaching 1322 ng/L for R(+)-atenolol and 390 ng/L for R/S(\pm)-venlafaxine, both in Pinedo-II WWTP. A detailed list of concentrations of chiral

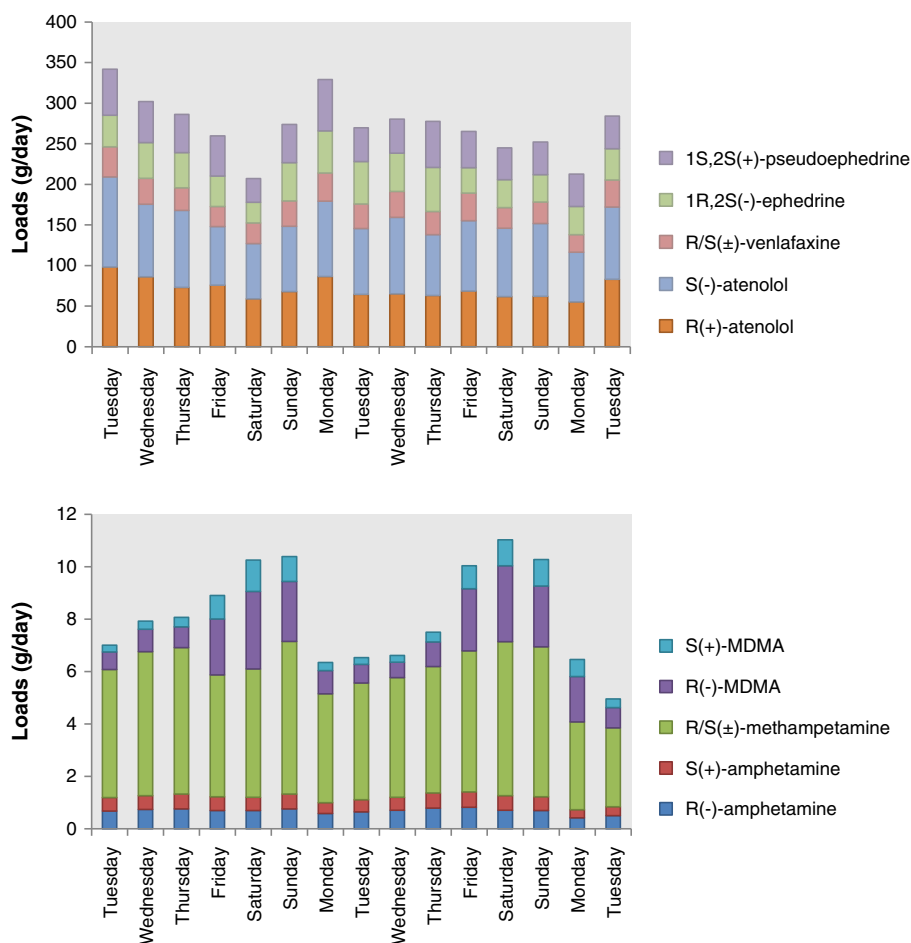


Fig. 2. Loads (g/day) of chiral drugs in influent waters from Pinedo-I WWTP.

drugs can be found in Tables S4, S5 and S6 (Supplementary material). As expected, Pinedo-II with 100% communal inputs was characterized by the highest concentrations of chiral drugs and Quart-Benager WWTP with 60% industrial contribution to wastewater by the lowest. Illegal drugs were not detected in any of the samples collected in this WWTP probably due to high dilution of domestic wastewater with industrial effluent.

Furthermore, as can be seen from Figs. 2 and 3 (representing data from Pinedo-I and Pinedo-II WWTPs) loads of illicit drugs increased during weekend days. Such an effect was not observed in the case of prescription medications: atenolol and venlafaxine. These temporal changes in daily illicit drug loads are attributed to their increased use during weekends (e.g. MDMA) and are a phenomenon which has recently been widely reported (Huerta-Fontela et al., 2007; Van Nuijs Alexander et al., 2009; Pedrouzo et al., 2011).

3.2. Enantiomeric profiling of wastewaters

Enantiomers of venlafaxine and methamphetamine were not fully separated in the chromatographic column probably because of matrix components interfering with the separation process. Thus, their enantiomeric fraction could not be calculated. Enantiomeric fractions and diastereomeric fractions of other chiral drugs studied are shown in Fig. 5.

With the exception of a few cases, there was nearly no inter-day variation observed in the value of *EF* for the target drugs quantified in wastewater. In contrast, significant temporal (months and seasons) variations in enantiomeric fractions were reported by other authors (Kasprzyk-Hordern and Baker, 2012b; MacLeod and Wong, 2010;

Nikolai et al., 2006). Physicochemical properties of water including temperature, pH, oxygen, nitrogen and phosphorus concentration, etc. have direct influence on the behavior of microorganisms during wastewater treatment and may have a decisive influence regarding the *EF* values (Gasser et al., 2012). Since these parameters are practically constant during the two week sampling campaign, no high inter-day variations in *EF*s were expected.

MDMA is illegally synthesized as racemate. However, humans metabolize mainly *S*(+)-MDMA, which leads to the enrichment of MDMA with *R*(-)-enantiomer (*EF* < 0.5). Therefore MDMA, if consumed, is likely to be present in wastewater enriched with *R*(-)-enantiomer. Such a situation was observed in both Pinedo-I and Pinedo-II WWTPs. Amphetamine is usually produced as racemate and similarly to MDMA, *S*(+)-amphetamine metabolizes faster than *R*(-)-enantiomer if administered in a racemic form (Kasprzyk-Hordern and Baker, 2012b). Indeed, *EF*s of amphetamine were quantified to be 0.42 and 0.38 in Pinedo-I and Pinedo-II WWTPs respectively. Unfortunately, MDMA and amphetamine were practically not detected in effluents, so the enantioselective fate of these compounds in the WWTPs studied could not be verified.

Atenolol belongs to the group of beta-blockers and is marketed as racemate. Pharmacological action of beta-blockers in humans is stereoselective: *S*(-)-enantiomers usually reveal much higher cardiac beta-blocking potency than *R*(+)-enantiomers. On the other hand *R*(+)-enantiomers have higher activity in blocking β_2 receptors in ciliary processes (Kasprzyk-Hordern, 2010). Modest stereoselectivity in renal clearance of atenolol in humans in favor of *S*(-)-enantiomer results in the enrichment of excreted atenolol with this enantiomer (Mehvar and Brooks, 2001). Indeed, in this study, atenolol was found

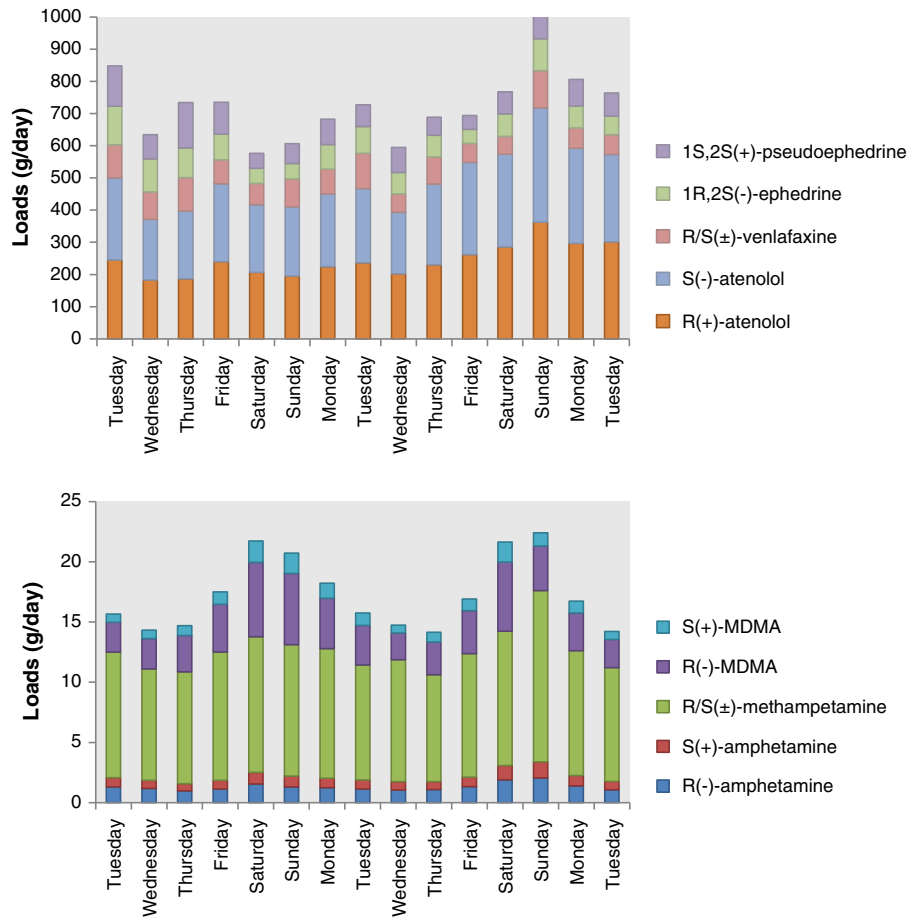


Fig. 3. Loads (g/day) of chiral drugs in influent waters from Pinedo-II WWTP.

to be enriched with the *S*(−)-enantiomer in raw wastewater. Additionally, further enrichment of atenolol with *R*(+) or *S*(−)-enantiomer was observed during wastewater treatment and depended on the WWTP technology utilized. In Pinedo-II WWTP, atenolol was enriched with *S*(−)-enantiomer, while in Pinedo-I and Quart-Benager WWTPs atenolol was enriched with *R*(+) enantiomer. Enrichment of atenolol with *R*(+) enantiomer has been previously reported by Kasprzyk-Hordern and Baker (2012a,b). On the other hand, Nikolai et al. (2006), MacLeod and Wong (2010) and López-Serna et al. (2013) reported the enrichment of atenolol with *S*(−)-enantiomer. Although, in this work,

all studied WWTPs utilize activated sludge treatments, Pinedo-II also has biological nitrogen removal. During this process, influent waters undergo nitrification with different bacteria in aerobic conditions. These bacteria might favor degradation of *R*(+) -atenolol, leading to the enrichment of atenolol with *S*(−)-enantiomer. It is worth mentioning that nitrification process is highly favored at higher temperatures of wastewaters (Antoniu et al., 1990). Such a situation was observed in Pinedo-II where recorded temperatures varied from 18.4 to 20.3 °C. More pronounced enrichment of atenolol with *S*(−)-enantiomer has also been observed in summer season in two Canadian WWTPs utilizing

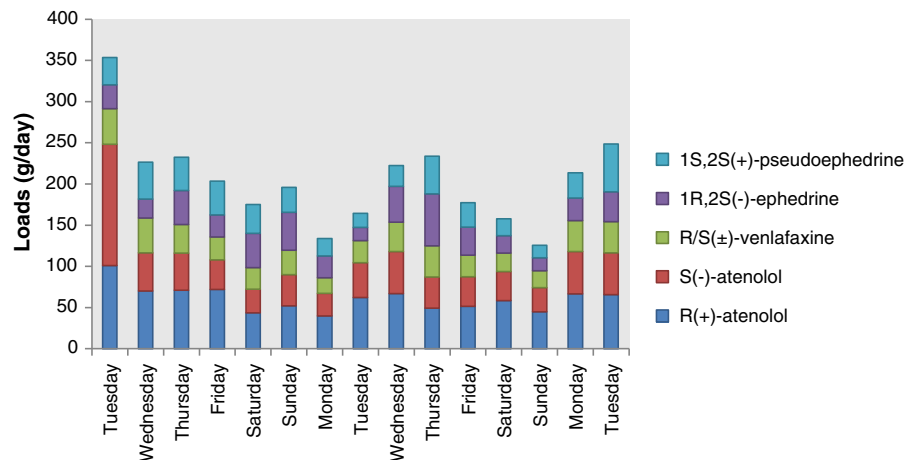


Fig. 4. Loads (g/day) of chiral drugs in influent waters from Quart-Benager WWTP.

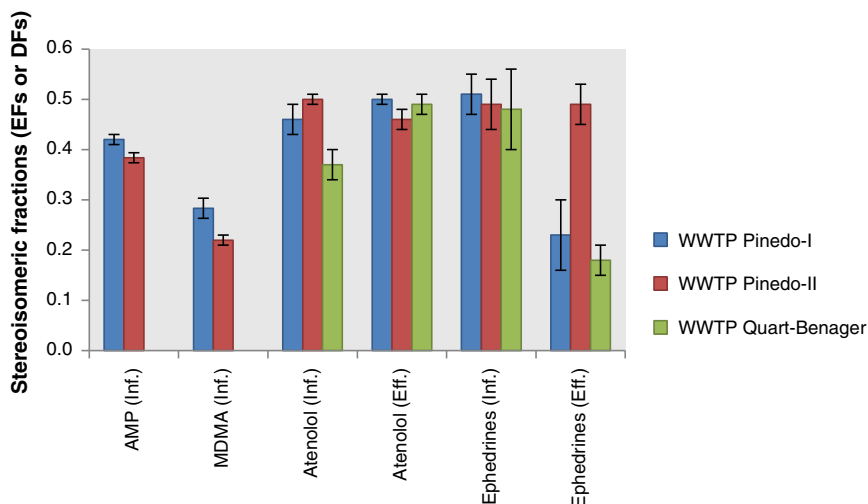


Fig. 5. Enantiomeric or diastereomeric fractions of chiral drugs in influent and effluent samples of the three studied WWTPs.

activated sludge and nitrogen removal: Capital Region (MacLeod and Wong, 2010) and Edmonton Gold Bar (Nikolai et al., 2006). Further studies have to be carried out to verify mechanisms underlying differential degradation patterns of enantiomers of atenolol.

Ephedrine and pseudoephedrine have two chiral centers and as a result exist in the form of two pairs of enantiomers. Only two isomers are natural: 1*R*,2*S*(−)-ephedrine and 1*S*,2*S*(+)-pseudoephedrine. Both isomers are marketed in pure isomeric forms. It is interesting to note that both 1*R*,2*S*(−)-ephedrine and 1*S*,2*S*(+)-pseudoephedrine were present in raw wastewater at similar concentration levels. Furthermore, the enrichment of ephedrines with 1*R*,2*S*(−)-ephedrine was observed in WWTP Pinedo I and Quart-Benager. This is of significant ecotoxicological importance as 1*R*,2*S*(−)-ephedrine is known to be more potent than 1*S*,2*S*(+)-pseudoephedrine. Further work is being undertaken to verify ecotoxicity of ephedrine isomers. It is important to note that no stereoselectivity was observed in WWTP Pinedo II. Such an outcome concurs with results presented by Kasprzyk-Hordern and Baker (2012a,b).

3.3. Removal of chiral drugs during wastewater treatment

The average elimination rates of chiral drugs in the studied WWTPs ranged from −29 to 100% (see Fig. 6). Degradation efficiency of WWTPs

was compound and enantiomer dependent. Amphetamines and ephedrines were highly degraded in all three studied WWTPs. Due to both low concentration levels in wastewater influent and high removal during treatment, amphetamine and methamphetamine were not detected in effluent samples. Stereoselective degradation was observed in the case of MDMA as *S*(+)-MDMA was more readily degraded than *R*(−)-MDMA. Similarly, 1*S*,2*S*(+)-pseudoephedrine was more readily degraded than its diastereomer 1*R*,2*S*(−)-ephedrine in Pinedo-I and Quart-Benager WWTPs. However, in Pinedo II similar degradation of two studied diastereomers was observed.

Atenolol was found to be poorly removed with efficiencies differing between studied WWTPs (see Fig. 6). The removal efficiency of *S*(−)-atenolol was higher than *R*(+)-enantiomer, with the exception of Pinedo-II WWTP. A similar situation was observed in the case of MDMA. Additional research is, however, needed to fully understand and explain the differences observed. Venlafaxine was poorly degraded in Pinedo-I WWTP (18%), but in Pinedo-II and Quart-Benager its concentration increased after treatment. This low degradation has been also reported by other authors (Kasprzyk-Hordern and Baker, 2012a, b). Probable explanation could be the cleavage of free venlafaxine from their glucuronide conjugated forms due to fecal bacterial enzymes, the back-reversion of its main metabolite *O*-desmethylvenlafaxine into its original form, hydrolysis, and desorption from particulate matter

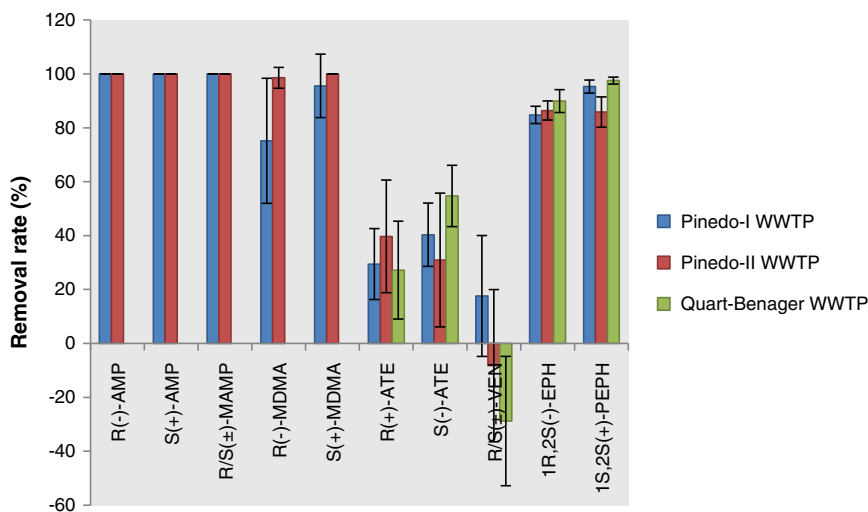


Fig. 6. Median removal rates (%) of studied chiral drugs during wastewater treatment.

during wastewater treatment (Ternes et al., 1999; Gasser et al., 2012). It is suggested that different strains of bacteria present during N and P removal in Pinedo-II and Quart-Benager WWTPs could favor degradation of venlafaxine conjugates and transformation of its metabolite.

3.4. Drug usage at the community level in the main urban areas of Valencia

Fig. 7 shows drug consumption estimates for each drug and two WWTPs investigated: Pinedo I and II. None of the illicit drugs was detected in Quart-Benager WWTP. This is probably due to high contribution of industrial wastewater (up to 60%) in analyzed wastewater influent. Such high contribution from industry to wastewater collected in Quart-Benager made back-calculation of drug usage estimates in this particular WWTP impossible. Therefore, below discussion concerns Pinedo I and II only.

As expected, usage of prescription drugs was found to be much higher than usage of illicit drugs. Venlafaxine consumption was estimated in the range of 10–33 g/day/1000 inh, which is much higher than estimates reported by Baker et al. (2014) and Lai et al. (2011). The excretion of venlafaxine in the urine accounts for only 4.7% of the intake dose as venlafaxine is excreted mainly as O-demethyl-venlafaxine (Howell et al., 1993). Low excretion rates of venlafaxine and using parent compound rather than its metabolite (and therefore also accounting for direct disposal of unused drug) could result in overestimation of the population consumption (Boleda et al., 2009). Therefore, it is inappropriate to monitor concentrations of venlafaxine for consumption estimation. Indeed, Baker et al. (2014) reported significant overestimation of consumption estimates for venlafaxine using wastewater analysis (1276 mg/day/1000 inh) when compared to the UK National Health Service prescription cost analysis data (417 mg/day/1000 inh). Results for venlafaxine presented in Fig. 7 should be therefore considered on a semi-quantitative basis. A more appropriate DTR is likely to be the main metabolite O-desmethylvenlafaxine (Baker et al., 2014).

Daily estimates of atenolol use revealed a constant pattern of atenolol usage throughout the two week sampling campaign (0.7–2.2 g/day/1000 inh). Two week average usage of atenolol was estimated to be 1 g/day/1000 inh in Pinedo I WWTP. On the other hand, atenolol use based on analysis of Pinedo II wastewater was higher and denoted 1.3 g/day/1000 inh. This indicates that population served by Pinedo II either consumed higher quantities of atenolol (which is unlikely) or disposed unused atenolol to the sewerage system. Indeed, slight enrichment of atenolol with *S*(–)-enantiomer in Pinedo I influent indicated that large proportion of atenolol residue present in analyzed wastewater could be associated with consumption of atenolol rather than direct disposal of unused drug. This is because, as discussed in Section 3.2, atenolol after consumption by humans is excreted in the urine enriched with *S*(–)-enantiomer. In contrast, atenolol quantified in Pinedo II was racemic. This indicates that large proportion of atenolol might have originated from direct disposal of unused atenolol or other sources. However, future work will have to be undertaken to investigate this phenomenon and to fully understand sources of atenolol loads in wastewater. However, it is worth emphasizing that according to Spanish Agency of Medicines and Sanitary Products, consumption of atenolol has been steadily increasing over recent years and was estimated at 7.63 doses/1000 inh/day in 2006 (Spanish Agency of Medicines and Sanitary Products, 2006). Assuming 100 mg of atenolol per one daily dose, 7.63 doses equal 0.763 g/day/1000 inh, which agrees with our estimates. This suggests that wastewater analysis could be used to estimate drug use in studied communities. Chiral analysis provides yet another dimension to wastewater based estimates of drug use as it allows for tracking of origin of drug residue in wastewater and as a result, it helps with making a distinction between direct disposal of drugs (those with enantioselective metabolism) and their consumption.

Data presented in Fig. 7 revealed an irregular pattern of amphetamine use. The highest levels corresponded to Friday (8.7 mg/day/1000 inh) and Saturday (9.6 mg/day/1000 inh) during the second

weekend of sampling in Pinedo-II, while in Pinedo-I the highest levels were recorded for Monday (13.0 mg/day/1000 inh) and Tuesday (13.3 mg/day/1000 inh). Zuccato et al. (2008) also reported similar patterns. Amphetamine residues in wastewater may be also a result of metabolism of other amphetamine-type drugs, such as methamphetamine, phenproporex and phenetylline (Postigo et al., 2011), and thus being their higher consumption not limited to weekend periods. This however seems not to be the case in this study as enantiomeric fractions are constant at 0.42 ± 0.01 and 0.38 ± 0.01 (amphetamine enriched with *R*(–)-enantiomer) in the case of Pinedo-I and -II respectively, indicating consumption of racemic amphetamine (Kasprzyk-Hordern and Baker, 2012a). Consumption of this drug in Valencia is similar to other European cities (Thomas et al., 2012; Ort et al., 2014) and clearly below amphetamine estimates reported in Antwerp (Belgium) and the main cities of The Netherlands.

Methamphetamine data showed increased (although irregular) use during weekend in Pinedo-II, while in Pinedo-I a random distribution was observed (Fig. 7). Usage of this drug in the Valencia region is slightly above the European average, with the exception of some cities of Finland, Norway and Czech Republic (Thomas et al., 2012), which presented loads of methamphetamine reaching 350 mg/day/1000 inhabitants.

MDMA data, similarly to methamphetamine data, revealed a clear weekly pattern of increased use during weekend in Pinedo-I and -II WWTPs (Fig. 7). Furthermore, its use was found to be high in the Valencia region when compared to many European cities (Thomas et al., 2012). Usage of MDMA ranged from 7 mg/day/1000 inhabitants to 49 mg/day/1000 inhabitants throughout the two week sampling campaign. It was only exceeded by Antwerp (Belgium), London (UK), Amsterdam, Eindhoven and Utrecht (The Netherlands). Furthermore, enantiomeric fractions of MDMA were found to be consistent throughout the two week sampling campaign and denoted 0.22 ± 0.01 and 0.28 ± 0.02 (MDMA enriched with *R*(–)-enantiomer) in the case of Pinedo-I and -II respectively. This indicates that MDMA residue quantified in wastewater was due to consumption and not direct disposal of unused drug. Kasprzyk-Hordern and Baker (2012a) drew similar conclusions. Interestingly, Emke et al. (2013) reported racemic MDMA in a study of a WWTP in Utrecht, which indicated the direct disposal of unused MDMA possibly as a result of a police raid at a nearby illegal production facility.

4. Conclusions

Two weeks monitoring of three WWTPs in the region of Valencia revealed that, as expected, atenolol, (pseudo)ephedrine and venlafaxine were present in wastewater at the highest concentrations. On the other hand, MDA, MDEA and norephedrine were not detected in any of the analyzed samples. Except for ephedrine, drugs of abuse were quantified in influent wastewater at very low levels, being practically not detected in effluent samples. The elimination patterns of the target compounds depended on WWTP and the studied substance. Drugs of abuse were highly degraded in all three WWTPs, while atenolol was poorly removed. Venlafaxine, depending on technology utilized, was either poorly removed or formed because of cleavage of venlafaxine conjugates, back-reversion of metabolites or unexpected processed taking place during treatment.

Throughout the two week sampling campaign, there was nearly no inter-day variation in the value of *EF* for any particular drug in all three WWTPs studied. However, enantiomeric enrichment was compound and wastewater treatment plant dependent. Remarkably, depending on wastewater treatment technology utilized, the enrichment of atenolol with either *R*(+)- or *S*(–)-enantiomer was observed. This unexpected enantiomeric variation in the stereoselective degradation of atenolol could be attributed to different processes utilized during activated sludge treatment. Additional studies on the effect of WWTP

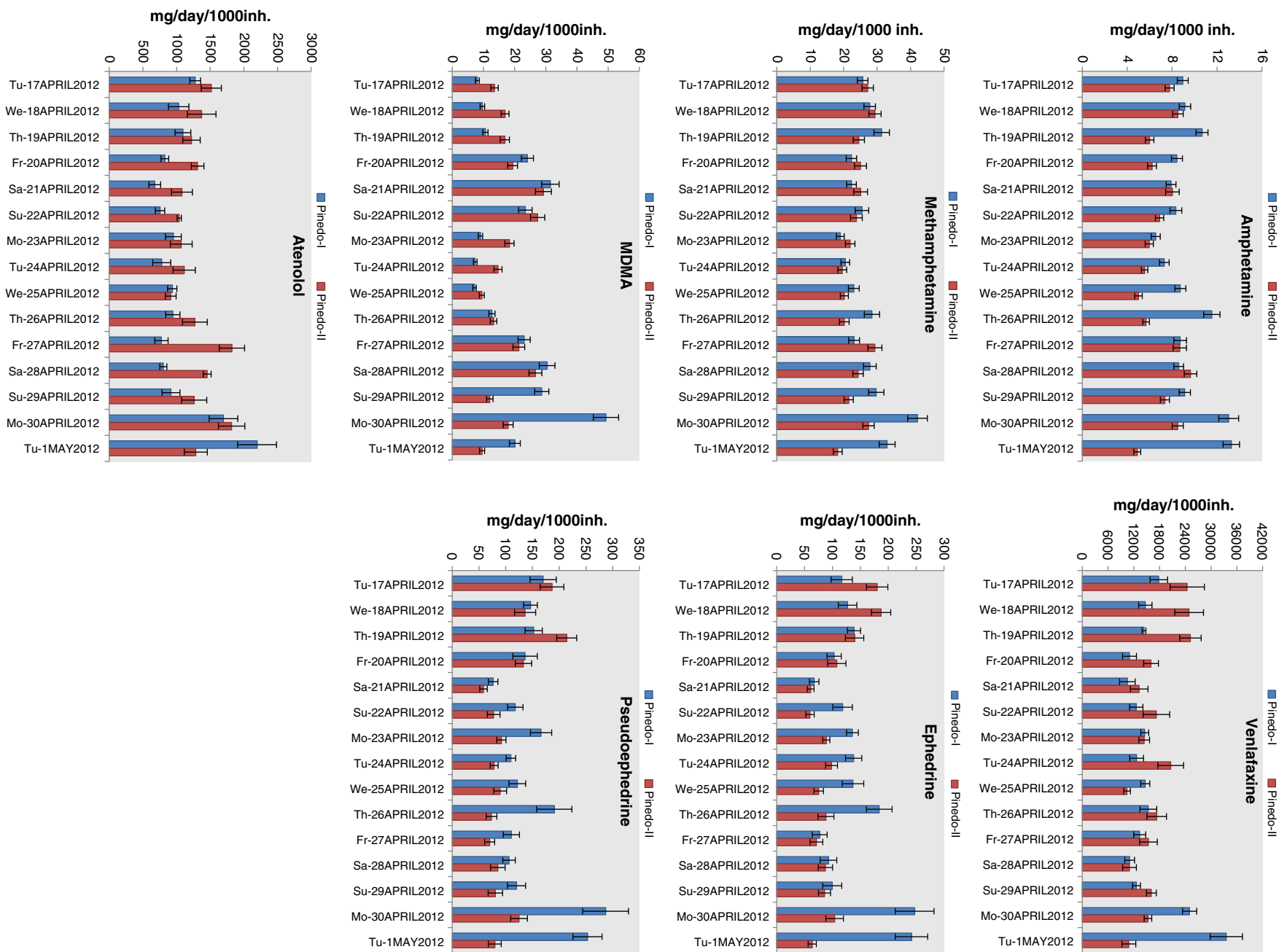


Fig. 7. Daily usage of chiral drugs expressed as mg/day/1000 inhabitants.

technologies in the stereoselective degradation of chiral drugs will be undertaken to explain these phenomena.

The application of wastewater enantiomeric profiling revealed usage patterns of chiral drugs in the Valencia region. Usage of amphetamine showed an irregular pattern throughout the two week sampling campaign, while methamphetamine in Pinedo-II WWTP showed a slight increase (although still irregular) in daily loads throughout the weekend. However, such an effect was not observed in Pinedo-I WWTP. MDMA showed a clear weekly pattern of increased daily loads during weekend, in both Pinedo-I and -II WWTPs. In summary, Valencia showed a level of consumption above many European cities, but below levels observed in many large cities of The Netherlands, Finland and Belgium.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2014.06.098>.

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