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Wastewater-based epidemiology to assess pharmaceutical consumption. Spanish perspective

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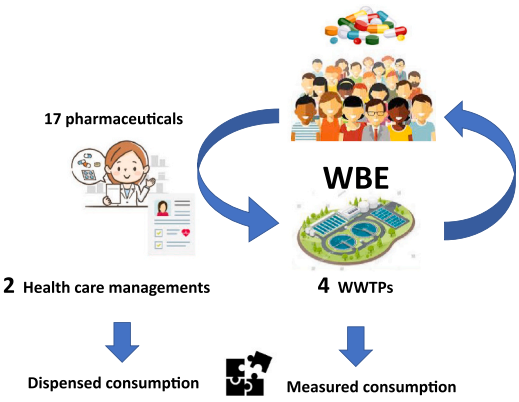
HIGHLIGHTS

- Monitoring pharmaceuticals in four Spanish wastewater treatment plants (WWTPs)
- Similar population normalized daily loads of pharmaceuticals in the studied WWTPs.
- Wastewater-based epidemiology suitable approach to provide pharmaceutical measured consumption
- Comparison with dispensed pharmaceuticals with good match (ratio within 0.8 and 1.2)
- First Spanish study comparing dispended pharmaceuticals with WBE approach

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewater-based epidemiology (WBE) is a well-established approach that can provide objective and real-time data on the consumption of substances such as pharmaceuticals. However, most of the studies reported so far compares consumption data obtained using WBE with those derived from prescription data from public health systems, which is often incomplete and might represent a source of uncertainty.

This study aims to compare the measured pharmaceutical consumption back calculated with the WBE approach with consumption derived from dispensed pharmaceuticals in two regions of Spain, managed by two different Health Systems. To do so, a group of 17 pharmaceuticals, including the most representative ones of every therapeutic family, were monitored in influent wastewater (IWW) samples collected over a week campaign

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in spring 2022 at four different wastewater treatment plants (WWTPs) in Spain: two WWTPs in Madrid city (center of Spain) and two WWTPs in Catalonia (Northeast of Spain).

Population-normalized daily loads (PNDL) revealed that the patterns of pharmaceutical occurrence in the different WWTPs are very similar, being acetaminophen, 4-acetamidoantipyrine and valsartan the pharmaceuticals with the highest PNDL values: 17162 ± 1457 mg day^{-1} 1000 inh^{-1} for acetaminophen, 2365 ± 696 and 2429 ± 263 mg day^{-1} 1000 inh^{-1} for 4-acetamidoantipyrine, 2006 ± 541 and 2041 ± 352 mg day^{-1} 1000 inh^{-1} for valsartan.

Pharmaceutical PNLDs were then transformed into measured pharmaceutical consumption (MC) and compared with dispensed consumption (DC) data obtained from the pharmacies in the catchment area where the WWTPs are located. A ratio MC/DC within 0.8 to 1.2 was obtained for 11 out of the 17 studied pharmaceuticals. Highlighting a match in all the cardiovascular system pharmaceuticals, with the exception of losartan (1.29–1.39 ratio) and valsartan (1.35–1.43) in all WWTPs. In summary, the degree of correlation between MC/DC is higher than those previously reported comparing with the prescribed pharmaceutical consumption.

1. Introduction

Pharmaceutical consumption has been increasing for many years, driven by a growing need for medicines to treat age-related and chronic diseases, and by changes in clinical practice. For instance, consumption of medicines for chronic diseases, including lipid-modifying agents, antidiabetic agents and antidepressants, increased by around 10 % on average in OECD countries between 2019 and 2021. Moreover, this trend is expected to rise in the next years (OECD, 2023). Intake of 5–10 pills/patient/day in residents of senior residences, which is translated in a total consumption of pharmaceuticals of hundreds of milligrams per day/person, is a typical datum to figure out pharmaceutical consumption in elder population (Lacorte et al., 2018).

Pharmaceutical consumption is highly dependent on socio-demographic and economic status, life-style and environmental factors and thus its monitoring helps to understand society, economy and health systems in a country or worldwide. Nevertheless, pharmaceutical data collection is not an easy task. Conventionally, prescription data, medical records, pharmaceutical sales, and personal questionnaires (which are then extrapolated to the whole community) have been the only data sources (WHO, 2003). However, none of them are considered optimal since all are subjected to data bias (Boogaerts et al., 2021a; Escolà Casas et al., 2021). For instance, not all the quantity of pharmaceuticals sold is eventually consumed, or the individual behavior might not be generalized to the whole community, and at the end these indicators may led to misleading information. Furthermore, another limitation is the lack of spatial details and the low frequency of data reporting (often in yearly bases) (Escolà Casas et al., 2021).

Wastewater-based epidemiology (WBE) is a consolidated approach to assess the exposure to or the consumption of different chemical substances through the analysis of influent wastewater (IWW) samples. It is based on the measurement of appropriate biomarkers (i.e. pharmaceuticals and metabolites) that are excreted by the population into the sewers. Concentration of these biomarkers in IWW are converted to population-normalized daily loads (PNDL) by multiplying them by the daily wastewater flow and dividing them by the catchment population served by the wastewater treatment plant (WWTP) (Boogaerts et al., 2021a; Senta et al., 2020). Among the main advantages of WBE are the possibility to obtain qualitative and quantitative up-dated information in an economic and almost in real-time manner to readily identify patterns of use/consumption. However, there are some disadvantages such as the eventual instability of biomarkers in sewer systems, the influence of the sampling mode and the uncertainty in the estimation of the population served by the WWTP (Boogaerts et al., 2021a; Choi et al., 2018; Senta et al., 2020).

WBE was first applied to estimate illicit drug consumption (Zuccato et al., 2005) and has since then become a widely used tool to monitor drug consumption (Asicioglu et al., 2021; Bijlsma et al., 2021a; Campo et al., 2023; Gracia-Lor et al., 2024; Rodríguez-Álvarez et al., 2015; Salgueiro-Gonzalez et al., 2024), but also other lifestyle indicators such as tobacco (Asicioglu et al., 2021; Rousis et al., 2023; Thanh et al., 2022), alcohol (Asicioglu et al., 2021; Rodríguez-Álvarez et al., 2015; Rousis et al., 2023), and pharmaceutical consumption (Adhikari et al., 2023; Ahmed et al., 2021; Boogaerts et al., 2021b; Carnevale Miino et al., 2024; Escolà Casas et al., 2021; Laimou-Geraniou et al., 2023; Rice et al., 2020; Riva et al., 2020; van Nuijs et al., 2015).

Several studies have applied WBE to estimate pharmaceutical consumption, and correlate the derived figures with the prescription data obtained from the health entities in the specific country where the study took place, such as United Kingdom (Ceolotto et al., 2024; Rice et al., 2020), Belgium (Boogaerts et al., 2021b, 2023; van Nuijs et al., 2015), Italy (Riva et al., 2020), Czech Republic (Carnevale Miino et al., 2024), Slovenia (Laimou-Geraniou et al., 2023), Spain (Escolà Casas et al., 2021), United States and Mexico (Adhikari et al., 2023) or Australia (Ahmed et al., 2021). Some of these studies lied in one single WWTP (Boogaerts et al., 2023; Ceolotto et al., 2024; Rice et al., 2020) that was monitored during different periods of time to provide data on the periodicity (Boogaerts et al., 2023; Rice et al., 2020) and seasonal patterns (differences among summer and winter periods) (Carnevale Miino et al., 2024; Laimou-Geraniou et al., 2023), as well as to evaluate the effect of Covid-19 pandemic and post-pandemic periods on pharmaceutical consumption (Laimou-Geraniou et al., 2023; Petromelidou et al., 2024a). Other studies showed spatial trends by monitoring different WWTPs allocated in different areas of the same country (Ahmed et al., 2021; Escolà Casas et al., 2021; Laimou-Geraniou et al., 2023; Riva et al., 2020; van Nuijs et al., 2015), or even combined different areas and different time periods (Laimou-Geraniou et al., 2023). For instance, IWW from six different WWTPs across Slovenia, covering approximately 30 % of the Slovenian population, were monitored to back-calculate antidepressant consumption, obtaining spatiotemporal figures that improved the data derived from other classical sources of pharmaceutical consumption, such as medical records or personal questionnaires (Laimou-Geraniou et al., 2023).

Despite the high number of studies, all of them (except one (Boogaerts et al., 2023)) rely on prescription data for comparison purposes. These data are highly influenced by the country and its medical health care system but, in general, they are incomplete since some pharmaceuticals are sold over-the-counter. Additionally, in most of the countries that benefit from public health care systems, part of the population holds an additional private insurance, which sometimes does not record their prescriptions into the public servers. All these issues have arisen uncertainties in the comparison between estimated pharmaceutical consumption data derived from WBE and official consumption data. This uncertainty can be decreased by taking into account the dispensed pharmaceuticals in pharmacies, as no distinction between public or private prescriptions is given. However, only in one study in Belgium the estimated consumption data from WBE was compared to the data from pharmacies (Boogaerts et al., 2023). Nonetheless, the drugs selected were antidepressants and opioids, which, apart from being medically prescribed are known to be illicitly consumed; which makes the data correlation highly uncertain.

In Spain only one study has applied WBE to estimate pharmaceutical

consumption (Escolà Casas et al., 2021) although the data of the two WWTPs monitored were compared with the reference database of the regional Health System (Catalonia, NE Spain). In a previous study from our group (Fontanals et al., 2023b), PNDL for a group of pharmaceuticals were calculated in six WWTPs across Spain and a comparative dataset for pharmaceutical consumption patterns across the different cities in Spain was provided. In the present study, we selected four out of these WWTPs belonging to two different areas of Spain (Madrid, center of Spain, and Tarragona and Reus, medium cities in Catalonia, Northeast Spain) to obtain measured pharmaceutical consumption through WBE approach. Additionally, these two areas also hold two different health care management systems that provided with dispensed pharmaceuticals in each corresponding area. With all the data, the objective is to triangulate pharmaceutical consumption through WBE and to compare it, for the first time in Spain to pharmaceuticals dispensed in pharmacies. The study aims to help consolidating the WBE approach as a tool to estimate pharmaceutical consumption Moreover, from the national point of view, authorities may evaluate the potential of this methodology to monitor the consumption of the pharmaceuticals, beyond the dispensing data in pharmacies, as well as the consumption of illicit substances which are less regulated than pharmaceuticals.

2. Experimental

2.1. Reagents and standards

Analytes (listed in Table S1) were purchased from Merck KGAa (Darmstadt, Germany) except codeine that was from Cerilliant (Round Rock, TX, USA). These commercial standards are provided as methanol (MeOH) or acetonitrile (ACN) solutions or as solids. Individual standard solutions were prepared at a concentration of 1000 mg L^{-1} in HPLC grade MeOH. Mix standard solutions at lower concentration were freshly prepared in H_2O/MeOH (90/10, v/v). All standard solutions were stored in the dark at −20 °C. Ultrapure water was supplied by a Synergy UV water purification system (Merck Millipore, Burlington, MA, USA) and HPLC grade MeOH, MS grade ACN and MS grade water were all supplied from Carlo Erba (Val de Reuil, France). Formic acid (HCOOH), used as mobile phase additive, was acquired from Merck KGAa. SPE cartridges (Oasis HLB, 150 mg, 6 mL) were purchased from Waters (Milford, MA, USA).

2.2. Sampling

IWW samples were collected at four WWTPs located in two different regions in Spain. Two WWTPs were in the center of Spain: Madrid 1 (352,188 inhabitants served) and Madrid 2 (727,176 inhabitants served), which together serve 30 % of the total population of the metropolitan area of Madrid; the other two WWTPs were located in Catalonia, Northeast Spain: one in Tarragona (146,498 inhabitants served) and another in Reus (105,563 inhabitants served) that comprises Camp de Tarragona region.

Table S2 overviews the location and the main characteristics of the WWTPs included in this study. Samples were collected for seven consecutive days in a conventional week (avoiding festivities or special events) of spring from 15 to 21 March 2022 in Madrid and from 29 March to 04 April 2022 in Tarragona and Reus. In total, twenty-eight 24 h-composite samples were directly collected in 0.5 L polypropylene bottles using automatic sampling devices working in flow- (Reus) or time- (remaining WWTPs) proportional mode. After collection, samples were transported to the laboratory and stored in the dark at −20 °C. Daily wastewater flow rates (m^3 day^{-1}) (detailed in Table S2 and used to calculate daily excretion loads) and other details concerning the sampling or the quality of the wastewater samples were compiled from the WWTPs facilities using a standardized questionnaire.

2.3. Sample preparation and LC-MS/MS analysis

The analytical method was previously optimized for a similar group of compounds (Fontanals et al., 2023b). In short, IWW samples without pH adjustment were filtrated through 1.2 μm glass fiber followed by 0.45 μm Nylon filters (Whatman, Maidstone, UK). Then, 100 mL of the filtrated sample were loaded at a flow-rate of 4–6 mL min^{-1} into Oasis HLB (150 mg) cartridges previously conditioned with 6 mL of MeOH and 6 mL of ultrapure water. Then, the cartridges were rinsed with 5 mL of ultrapure water, air-dried for 2 min, and the analytes were eluted with 5 mL of MeOH. Extracts were evaporated to dryness using a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) and reconstituted with 1.0 mL of H_2O/MeOH (90/10, v/v), which were then diluted to 1/10 (due to the high concentration of some of the pharmaceuticals) using the same solvent, and filtered through a 0.22 μm PTFE syringe filter (Scharlab, Barcelona, Spain).

The diluted extracts were analyzed using a 1200 series liquid chromatography (LC) system coupled to a mass spectrometry (MS) detector with a triple quadrupole (QQQ) analyzer and an electrospray ionization (ESI) source (Agilent Technologies, Santa Clara, CA, USA). The LC system was equipped with a degasser, a quaternary pump, an autosampler and a column oven compartment. A Luna Omega Polar C18 (150 mm × 3 mm, 5 μm) column with a precolumn (4 mm × 3 mm, 5 μm) from Phenomenex (Torrence, CA, USA) heated at 30 °C was used. The mobile phase consisted of (A) 0.1 % HCOOH in water and (B) 0.1 % HCOOH in ACN. Gradient elution started at 5 % B, which was maintained for 3 min, increased to 75 % B in 25 min and then to 100 % B in 2 min, held for 1 min and returned to the initial conditions in 2 min, which were maintained during 5 min for column equilibration. The flow-rate was set at 0.4 mL min^{-1} and the injection volume was 10 μL.

ESI parameters set in the positive ionization mode (except for hydrochlorothiazide) were optimized and the compromised values are: gas temperature, 320 °C; gas flow, 12 L min^{-1}; nebulizer pressure, 45 psi; and capillary voltage, 3000 V. Cone voltages (100 to 150 V) and collision energy (CE) (5 to 40 eV) were optimized for each compound to obtain precursor ions and two product ions (except for tramadol) with the optimum values listed in Table S1. The acquisition was performed under dynamic multiple reaction monitoring (dMRM) mode using the most abundant precursor/product ion transition as quantifier (Q) and the second most abundant transition as qualifier (q). Data were collected using MassHunter software from Agilent Technologies. For confirmation purposes, both the ratio of these transitions (q/Q) considering a tolerance level of ±40 % of relative standard deviation (%RSD) and the retention time (considering ±0.1 min) were assessed following SANTE guidelines (EU, 2021).

Quantification was performed using linear regression curves by injecting in triplicate eight standard solutions at concentrations ranging from the individual instrumental limit of quantification (IQL) to 1000 μg L^{-1}. IQL was set as the lowest concentration in the calibration curve and that accomplishes a signal-to-noise (S/N) ratio of 10. They ranged from 0.1 to 1 μg L^{-1}. Instrumental limit of detection (IDL) accomplishes the S/N ratio of 3 and were between 0.05 and 0.2 μg L^{-1}. The recovery rates of the method when samples were spiked at 10 μg L^{-1} ranged from 41 to 105 % and matrix effect from −23 to +26 %, except for bezafibrate (−64 %) and omeprazole (47 %). Precision of the method was assayed in terms of repeatability and reproducibility as the %RSD of five replicated samples spiked at 10 μg L^{-1} that were analyzed the same day or in different days, respectively. Table S3 compiles the data on the validation parameters.

2.4. Wastewater-based epidemiology back calculation

Daily mass loads (μg day^{-1}) (Eq. (1)) were calculated by multiplying pharmaceutical concentrations (ng L^{-1}) measured in the 24 h composite IWW samples by their corresponding wastewater flow rates (m^3 day^{-1}) (Table S2). Daily mass loads were then normalized by dividing them by

the estimated population (Table S2) of the area served by the WWTP that sourced the samples (Eq. (2)) to end with population-normalized daily loads (PNDL) (mg day^{-1} 1000 inh^{-1}). Population served by each WWTP was estimated based on the criteria considered more realistic by WWTP managers, i.e. chemical oxygen demand, biochemical oxygen demand, or census (Table 2S), which is also adopted in other studies related to WBE.

Pharmaceutical MC was estimated by multiplying PNDL values by a specific correction factor (CF) that considers the fraction of drug excreted as biomarker after human metabolism. The CF applied for each pharmaceutical was obtained from literature (see the details in Table S4).

$$\text{Daily load}\left(\mu g\,\text{day}^{-1}\right) = \text{concentration}\left(\mu g\,\text{L}^{-1}\right) \times \text{wastewater daily flow rate}\left(\text{L day}^{-1}\right) \quad (1)$$

$$\text{PNDL}\left(\text{mg day}^{-1}\ 1000\ \text{inh}^{-1}\right) = \text{daily load} \times 10^{-3} \times \left(1000\ \text{inh}^{-1}\right) \quad (2)$$

$$\text{Measured consumption (MC)}\left(\text{mg day}^{-1}\ 1000\ \text{inh}^{-1}\right) = \text{PNDL} \times \text{CF} \quad (3)$$

2.5. Dispensed consumption data

Dispensed pharmaceutical data for each pharmaceutical identified with a specific Anatomical Therapeutic Classification (ATC) code (Table S5) were obtained from the corresponding public organisms serving the geographical area of each WWTP. Data were provided in form of defined daily doses (DDD) along the whole year 2022. DDD figures were then multiplied by the dose (in mg) (Table S5), which is the assumed average maintenance dose per day for a drug used for its main indication in adults, and it is recommended by the WHO as a measuring unit for drug monitoring (WHO, 2023). Moreover, only dispensations for oral administration were considered (excluding parenteral, dermal and rectal administration) to better encompass with the excretion of pharmaceuticals.

Pharmaceutical dispensation data on pharmacies were obtained for the two regions where WWTPs are allocated. On the one hand, dispensation data in Madrid city in 2022 (for comparison with Madrid 1 and Madrid 2 WBE estimations) were obtained from the *General Directorate of Pharmacy and Health Products* from the *Madrid Health Service* in Madrid Autonomous Community. These data include prescriptions from the public and private medical sectors (both primary health care centers and hospitals) as well as those pharmaceuticals bought in pharmacies without medical prescription. On the other hand, concerning to Catalonia region (for comparison with Tarragona and Reus WBE estimations), data on pharmacy dispensation were obtained from the *Data Analytics Program for Research and Innovation in Health* in *Health Quality and Evaluation Agency of Catalonia* from the *Government of Catalonia* in the Catalonia Autonomous Community. These data in form of DDDs and organized by cities, towns and villages, includes prescription from the public and private primary health care centers (excluding hospitals) as well as those pharmaceuticals acquired in pharmacies without medical prescription, and completely covers the regions where the WWTPs are allocated.

The population size (census) of Madrid city was sourced from Instituto de Estadística (Spanish Stadistical Institute, 2022) in Madrid Autonomous Community; while the census for each city and town served by Tarragona and Reus WWTPs were obtained from Idescat (Stadistical Institute of Catalonia, 2022). Dispensation data in each region were normalized as treatment per 1000 inhabitants per day, according to Eq. (4).

$$\text{Dispensed consumption (DC)}\left(\text{mg day}^{-1}\ 1000\ \text{inh}^{-1}\right) = \sum \text{DDD}_{2022} \times \text{dose}_{\text{pharmaceutical}}\text{inmg} \Big/ 365\ \text{days} 1000 \Big/ \text{inhabitants} \quad (4)$$

3. Results and discussion

3.1. Pharmaceutical selection and quantification

Pharmaceuticals and one metabolite (i.e. 4-acetamidoantipyrine as metabolite of metamizole) included in the study were selected based on preliminary data on their occurrence in the environment (Escolà Casas et al., 2021; Fontanals et al., 2023b; Gómez-Canela et al., 2019; Munné et al., 2023; Paíga et al., 2019; Petromelidou et al., 2024b). In addition, data on their prescription in Spain in health care centers were considered (www.observatorisalut.gencat.cat; www.observatorioresultados.sanidadmadrid.org). Moreover, most of the pharmaceuticals selected were among those mainly used for chronic treatments, in order to reduce daily and seasonal variability. Dispensation data on hospitals were not considered, since these figures were not available in the region of Catalonia. In this way, pharmaceuticals typically administrated in hospitals, such as iopromide (X-ray contrast agent) or morphine, were removed from the study. Another pharmaceutical excluded was trimethoprim since, in some of the medicines dispensed, it is combined with sulfamethoxazole due to its synergetic effect and this might cause some confusion in the reported DDD and the following calculation of consumption. Similar correlation problems were already found by Carnevale Miino et al. (2024) in the determination of the consumption of trimethoprim and sulfamethoxazole.

In addition, some of them were also removed due to the bad performance in the analytical method. This is the case of ibuprofen, whose dispensation data revealed that it is a highly administrated anti-inflammatory drug; however, its performance in the developed method is not suitable since only one product ion and at low response could be acquired. In addition, ibuprofen is dispensed for oral administration (the one used for the consumption calculation) but also in form of creams and ointment for dermal administration, and this might be also a source of error in data calculation.

Finally, biomarker stability in IWW and/or sorption onto particulate matter or biofilms should also be considered. To overcome this, stability data was taken from previous studies (Ceolotto et al., 2024; Fontanals et al., 2023a) and pharmaceuticals and biomarkers were selected based on their (mostly high) stability in IWW as well as under storage conditions.

The analytical method had been already developed in a previous study (Fontanals et al., 2023b), except for the extract dilution step. This dilution was included to allow the quantification of those pharmaceuticals whose concentrations in IWW were higher than the upper limit of the calibration curve. This is the case of acetaminophen, whose concentrations in IWW were up to 105,000 ng L^{-1}. Another case is 4-acetamidoantipyrine (metabolite of metamizole), whose concentrations in IWW were up to 19,000 ng L^{-1}.

The analytical performance of the developed method in terms of MQLs, MDLs, percentage of apparent recoveries and matrix effect is presented in Table S3. All compounds presented suitable matrix effects, which is attributed to the extract dilution, except for omeprazole (47 % of signal enhancement) and bezafibrate (64 % of ion suppression). These matrix effects have a direct impact on the apparent recoveries obtained (140 % and 35 %, respectively). The extract dilution also affected the limits of the method, which per se are 10 times higher than those found when the extract was not diluted (Fontanals et al., 2023b). However, this did not affect the quantification of pharmaceuticals; on the contrary, it improved the quantification of those compounds occurring at high levels. Table 1 collects the data on the occurrence of pharmaceuticals in IWW.

Table 1
Concentration range and the mean concentration in ng L^{-1} of the studied pharmaceuticals in the IWW in the studied WWTP.

	Concentration (ng L^{-1})							
	Madrid 1		Madrid 2		Tarragona		Reus	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
4-Acetamidoantipyrine	7856–15,056	9858 ± 3313	5405–19,049	13,275 ± 4776	9648–12,372	11,535 ± 1824	12,057–15,921	15,128 ± 1824
Acetaminophen	37,276–78,510	55,402 ± 21,734	29,073–91,232	67,472 ± 22,329	87,444–105,379	100,821 ± 8049	81,069–107,732	98,267 ±,115
Atenolol	310–1650	716 ± 463	344–1453	1019 ± 353	1444–2471	1723 ± 362	714–1081	890 ± 119
Bezafibrate	<MDL–4824	157 ± 168	46–422	253 ± 121	<MDL–223	39 ± 104	1037–12,654	1127 ± 104
Carbamazepine	17–93	47 ± 27	49–173	86 ± 47	73–86	80 ± 5	79–142	108 ± 25
Codeine	105–287	191 ± 77	87–514	359 ± 134	301–435	353 ± 55	282–336	326 ± 40
Diazepam	27–166	55 ± 55	98–502	224 ± 136	19–92	36 ± 25	28–49	35 ± 8
Diclofenac	421–1957	1009 ± 566	533–4852	3159 ± 1909	711–2842	1403 ± 757	1141–4818	2041 ± 1256
Hydrochlorothiazide	1264–4497	2682 ± 1198	1312–4786	3528 ± 1161	3539–4930	4145 ± 516	4185–5251	4753 ± 409
Losartan	691–1728	1135 ± 393	699–2584	1864 ± 655	1079–1708	1470 ± 258	1529–1936	1742 ± 158
Omeprazole	129–560	386 ± 168	131–1745	1037 ± 591	501–750	592 ± 107	510–770	594 ± 130
Pentoxifylline	32–95	63 ± 25	34–119	87 ± 28	41–110	76 ± 28	147–394	289 ± 89
Quetiapine	151–258	211 ± 48	354–795	592 ± 149	102–146	121 ± 17	372–467	415 ± 41
Tramadol	436–1642	1006 ± 454	550–3530	2297 ± 930	1206–1866	1573 ± 260	2167–2691	2449 ± 213
Trazodone	193–360	275 ± 66	355–865	621 ± 189	163–195	182 ± 17	199–242	217 ± 17
Valsartan	4662–12,383	8403 ± 2823	4756–17,291	11,043 ± 4242	3381–10,312	7034 ± 2549	9837–15,546	12,678 ± 2003
Venlafaxine	230–1032	590 ± 278	348–3286	1853 ± 954	2596–3001	2816 ± 202	1387–2061	1609 ± 233

3.2. Population-normalized daily loads of pharmaceuticals

Population-normalized daily loads (PNDL) for the studied pharmaceuticals in all the WWTPs are presented in Fig. 1 and Table S6. On average, the highest PNDLs were found by far for acetaminophen (17,162 ± 1457 mg day^{-1} 1000 inh^{-1} in Tarragona WWTP), followed by 4-acetamidoantipyrine (2365 ± 696 and 2429 ± 263 mg day^{-1} 1000 inh^{-1} in Madrid1 and Reus WWTPs, respectively) and valsartan (2006 ± 541 and 2041 ± 352 mg day^{-1} 1000 inh^{-1} in Madrid 1 and Reus WWTPs, respectively). Except for the slightly lower values of acetaminophen and 4-acetamidoantipyrine in Madrid 2, the patterns of pharmaceutical PNDLs were very similar across the four WWTPs. This is consistent with the similarities in pharmaceutical prescription across Spain (Bijlsma et al., 2021b; Escolà Casas et al., 2021; Gómez-Canela et al., 2019; Munné et al., 2023). Acetaminophen presence in IWW samples has been widely reported in different WWTPs across Spain (Bijlsma et al., 2021b; Escolà Casas et al., 2021; Gómez-Canela et al., 2019). For instance, Bijlsma et al. (2021b) and Gómez-Canela et al. (2019) found this pharmaceutical at the highest level among other pharmaceuticals in IWW samples from a Northern town and from the North-East region of Spain, respectively. In fact, some NSAID/analgesic pharmaceuticals including acetaminophen can be sold over-the-counter in many countries, which may imply increased adverse effects both in humans and the environment (Paíga et al., 2019).

Other pharmaceuticals showing high PNDLs are atenolol, tramadol, venlafaxine, losartan, and diclofenac. This data agrees with a study (Fontanals et al., 2023b) including these four WWTPs, among other Spanish regions monitored in 2021, which reinforces the hypothesis that no temporal trends can be found in the PNLD data, at least with the data in these two consecutive years. As an example, the mean PNLD for atenolol in Tarragona WWTP in 2021 was 340 mg day^{-1} 1000 inh^{-1}, whereas in 2022 it is 294 mg day^{-1} 1000 inh^{-1}. Losartan values in all WWTP monitored, however, were higher in 2021 (392 to 1464 mg day^{-1} 1000 inh^{-1}) than in 2022 (85 to 338 mg day^{-1} 1000 inh^{-1}). In fact, these low values are more in agreement with a study conducted in different regions of Italy with losartan PNDL values between 56 and 86 mg day^{-1} 1000 inh^{-1} (Riva et al., 2020).

Venlafaxine PNDL values agree with the values reported in other studies conducted in Slovenia (Laimou-Geraniou et al., 2023), Belgium (Boogaerts et al., 2023) and UK (Rice et al., 2020); in some of these studies (Boogaerts et al., 2023; Laimou-Geraniou et al., 2023), however, its main metabolite (*O*-desmethylvenlafaxine) was also monitored, and the authors found that both (parent compound and metabolite) are present in the analyzed IWW. In fact, venlafaxine is one of the most frequently prescribed antidepressants, and studies conducted worldwide have observed that both parent compound and metabolite occurred in IWW (Boogaerts et al., 2019; Yavuz-Guzel et al., 2022).

Trazodone is another antidepressant described as one of the most frequently prescribed (Laimou-Geraniou et al., 2023); nevertheless, the PNDL values are in the range of 19–94 mg day^{-1} 1000 inh^{-1}, which are lower than those presented by venlafaxine (58–530 mg day^{-1} 1000 inh^{-1}). In any case, trazodone values are in agreement with those reported in the other countries (Laimou-Geraniou et al., 2023).

As said, the comparison of the PNDL values among the four WWTPs shows very similar patterns, with the exception of: venlafaxine, whose values in Tarragona WWTP are higher than the rest of the studied WWTPs; quetiapine, with lower values in Tarragona WWTP; and bezafibrate and pentoxifylline, with much higher values in Reus WWTP. In general, PNLD values in Madrid (and more specifically in Madrid 1) are more heterogeneous. This could be attributed to the uncertainty in the catchment population estimation.

On the other hand, weekly patterns of the PNDL data (Fig. S1) only show a slight difference between weekdays and weekends in the case of psychoactive pharmaceuticals, such as codeine, trazodone, tramadol, and venlafaxine. This difference is observed in all the WWTPs but in the ones located in Madrid region. This might be explained by the typical

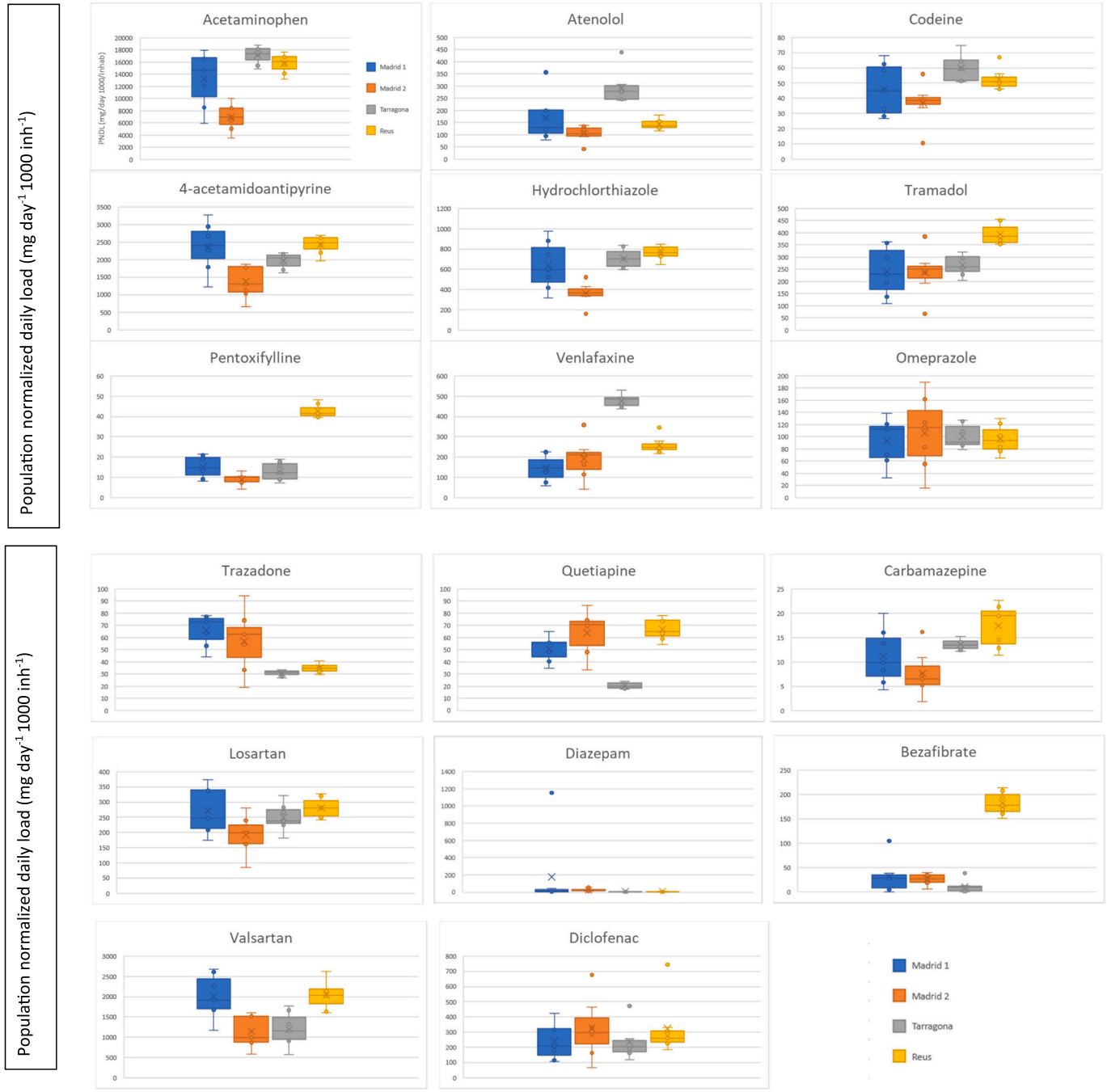

Fig. 1. Population normalized daily loads (PNDLs) in mg day^{-1} 1000 inh^{-1} for each pharmaceutical in the four WWTPs monitored.

floating population movement to big cities for recreational purposes during the weekend. In fact, this scenario is widely observed in the monitoring of illicit drugs (Bijlsma et al., 2021a; Campo et al., 2023; Gracia-Lor et al., 2024), or of prescribed drugs that are consumed illicitly (Laimou-Geraniou et al., 2023). Nevertheless, it should be born in mind that the weekly patterns might be unnoticeable, since, on the one hand, many students leave the city (catchment area) during the weekend; and, on the other hand, during working days are more people entering to the city, which at the end, balance the catchment population. This observation was already reported in pharmaceutical PNLD in Belgium (Boogaerts et al., 2023).

3.3. *Measured consumption vs. dispensed consumption*

Measured consumption (MC) was back-calculated by applying the corresponding CFs which were contrasted from literature (see information in Table S4) and compared to dispensed consumption (DC) obtained from dispensation data within the same region to test correlation of the datasets. In Fig. 2, data were classified in plots according to the main therapeutic classes, i.e. pharmaceuticals for the cardiovascular system, medicines for the nervous system, and analgesics and others (which contain one anti-inflammatory, one pharmaceutical for the respiratory system, and another one for the food track). This distribution was maintained for each WWTP in a separate plot.

A general overview of the plots in Fig. 2 shows a close relationship between MC and DC values, with MC/DC ratios within the 0.8 to 1.2

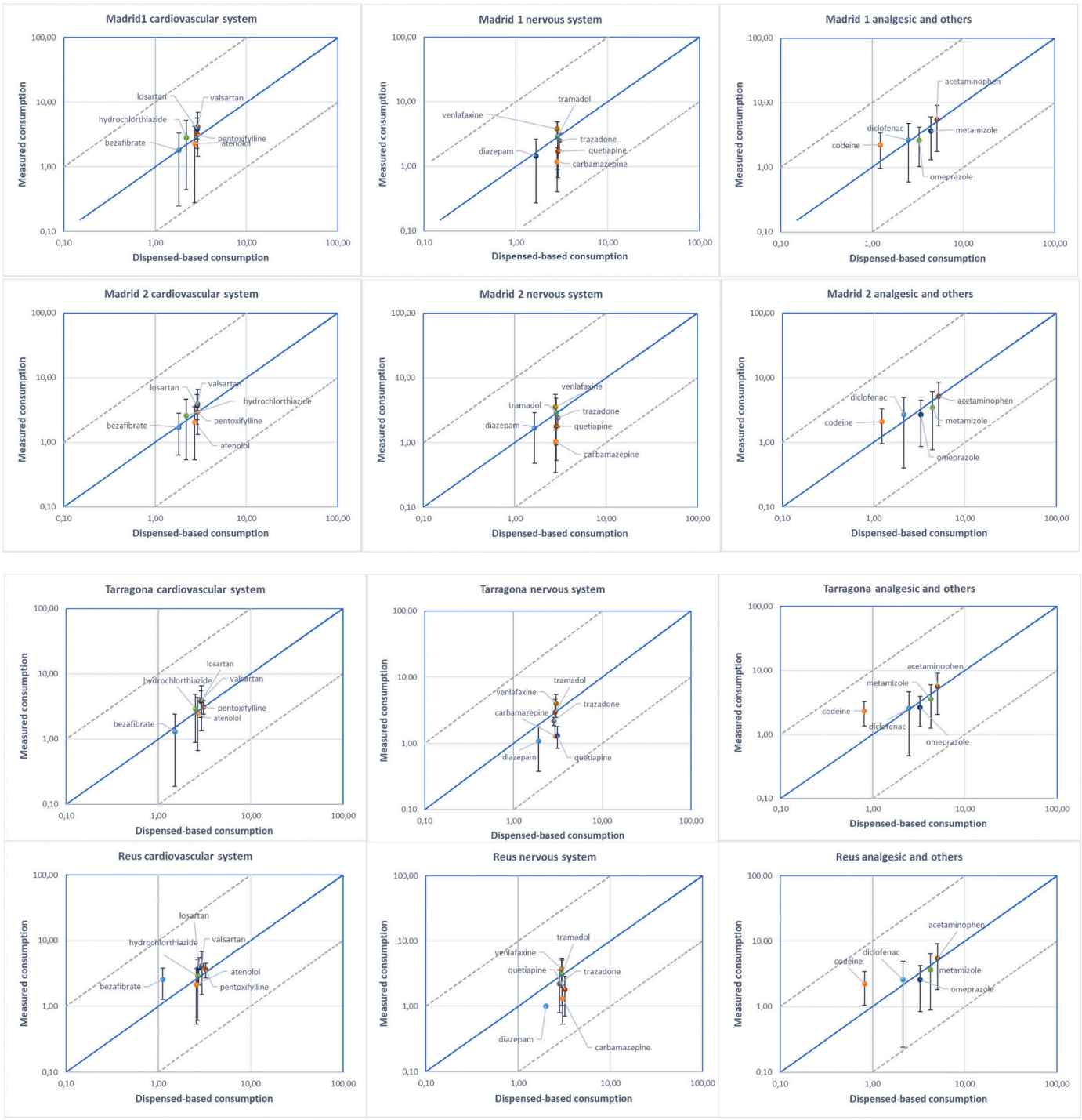

Fig. 2. Comparison of measured consumption (MC) (Eq. (3)) and dispensed consumption (DC) (Eq. (4)), both in mg day^{-1} 1000 inh^{-1} for the studied pharmaceuticals grouped by therapeutic class. Solid line indicates a match between measured and dispensed consumption. The dashed lines indicate one order of magnitude difference.

order for 11 out of 17 pharmaceuticals. By therapeutic class, all cardiovascular system pharmaceuticals MD/DC ratio fitted within the abovementioned range except losartan (1.29–1.39 ratio) and valsartan (1.35–1.43 ratio) in all WWTPs, hydrochlorothiazide (1.30 ratio) in Madrid 1, and bezafibrate in Reus (2.29 ratio). Bezafibrate in Reus presented a 0.5 order of magnitude higher MC than DC, which was already discussed in the previous section where the bezafibrate PNDL in Reus was abnormally high. The overestimation of losartan and valsartan might be due to the omission of the fecal excretion rates in this study, as losartan and valsartan are pointed to be pharmaceuticals more excreted via feces rather than urine (Escolà Casas et al., 2021).

Within the "nervous system" group, the lower correlation was found for carbamazepine in all WWTPs, and for diazepam in Tarragona and Reus (MC/DC ratios close to 0.5). In the case of carbamazepine, this might be attributed to the metabolization of this compound (Ceolotto et al., 2024; Massano et al., 2023). In fact, Ceolotto et al. (2024) monitored 10,11-dihydro-10-hydroxycarbamazepine and carbamazepine-10,11-epoxide metabolites together with the parent compound and found a good agreement between prescribed and estimated consumption. But when they estimated carbamazepine consumption only from

carbamazepine residues in IWW, they found a poor correlation (0.2) between measured and prescribed consumptions. These results corroborate that carbamazepine itself is not a valid biomarker to estimate its consumption by WBE (Carnevale Miino et al., 2024). Therefore, a careful revision of the monitoring pharmaceuticals and its metabolites should be taken into account for this type of studies. Diazepam underestimation in Tarragona and Reus might be attributed to the omission of hospital dispensation data in this region since, as it has been reported elsewhere, diazepam is commonly found in effluents from hospital WWTPs (Adhikari et al., 2023). In fact, correlation of the diazepam MC/DC in Madrid area (where hospital dispensation was included) matched almost perfectly (0.88 to 1.02 ratio).

Within the "analgesics and others" group, (Fig. 2), a good correlation between MC and DC values was observed for all pharmaceuticals in all locations but for codeine. In this case, the MC was always higher than the DC, which might be attributed to its presence in other co-formulations, such as cough and cold medicines.

In general, the degree of correlation between MC and DC (this study) is much higher than the degree of correlation between MC and prescribed consumption (PC) reported in previous studies, both in Spain (Escolà Casas et al., 2021) and also other countries (Adhikari et al., 2023; Carnevale Miino et al., 2024; Laimou-Geraniou et al., 2023; Riva et al., 2020). PC is mostly derived from prescriptions in public health centers, excluding prescriptions in private centers and over-the-counter consumption. In the particular case of Catalonia region and according to statistical data, 28.5 % of the population has dual (public and private) health coverage (Departament de Salut, Generalitat de Catalunya, 2022), a percentage that might be similar across Spain. Therefore, the use of dispensation data is much more accurate than the use of only prescription data from public health centers. Despite that in Tarragona and Reus the hospital dispensation was not taken into account, this might be compensated by the fact that some of the pharmaceuticals, although dispensed, are not (totally) consumed at the end; or the pharmaceuticals prescribed for chronic diseases might be dispensed in a period of time longer than those consumed. Additionally, it should consider the fluctuation of inhabitants caused by the influx and efflux of visitors in the cities. As a result, some dispensed pharmaceuticals may not be consumed in the same area, and vice versa.

It should be mentioned that dispensation figures in the two regions (Madrid and Tarragona and Reus through Catalonia database) were in line for all the studied pharmaceuticals but for bezafibrate, which was more dispensed in Madrid than in Catalonia, and diazepam, half dispensed in Madrid as compared to Catalonia. These differences might be attributed to prescription or consumption of other drugs from the same family to treat the same disease.

3.4. Limitations of the study

The results of this study may not be extrapolated to national level, since the population served by the four studied WWTPs only covers 2.8 % of the Spanish population. Nevertheless, they show a good correlation between MC (WBE-derived) values and DC values in two different regions of Spain and in two types of cities: on the one hand, Madrid, which is a large-sized city in the center of Spain; on the other hand, Tarragona and Reus, which are medium-sized cities located in the Northeast region of Spain.

Another concern lies in the population estimates, population served by Tarragona WWTP was obtained from the census, whereas in the remaining WWTPs it was estimated through markers such as the chemical oxygen demand or the biological oxygen demand, which fitted more accurately to consider variation in the socio-demographics of the catchment population. These WWTP markers have been reported to diminish the variability source and have been successfully applied in previous studies (Di Marcantonio et al., 2022; Laimou-Geraniou et al., 2023). However, DC was always calculated using the census. Despite these limitations, the results in the different WWTPs matched quite suitably. Nevertheless, other authors suggested the use of dynamic population markers such as mobile phones to track population variations (Boogaerts et al., 2023); which the authors proved to be a valuable tool to evaluate relative changes in population, but more research is necessary to evaluate its capability to estimate absolute population numbers (Boogaerts et al., 2023).

One of the main sources of uncertainty in WBE is the excretion factors used to calculate CFs and, finally, consumption values. In this study, and for most of the pharmaceuticals considered, we sourced them from excretion rates reported in literature with contrasted studies on pharmacokinetics (Ceolotto et al., 2024; Escolà Casas et al., 2021); however, for some substances (i.e. diazepam and metamizole) the information (although available) was limited and not up dated. It should be also born in mind that most WBE studies and excretion rate literature sources rely on urine excretion; and, as pointed by Escolà Casas et al. (2021), fecal excretion should be also considered since some of pharmaceuticals are excreted more through feces than through urine. Actually, valsartan and losartan showed underestimated MC values because of this. Another weak point of this study is the omission of already known metabolites of some of the pharmaceuticals, as it was already discussed for venlafaxine and carbamazepine in previous sections. Thus, these issues should be considered in future WBE studies.

One strong point of this study is that the consumption estimated by WBE (MC) is compared to the consumption derived from dispensed pharmaceuticals (DC) instead from prescribed pharmaceuticals (PC), which normally does not include prescriptions from private centers and over-the-counter medicines. In any case, this can reduce the uncertainty, but not eliminate it, since some pharmaceuticals are dispensed but not (totally) consumed (Carnevale Miino et al., 2024). Direct disposal is another factor that might become an important source of uncertainty if the parent compound is used as biomarker (Boogaerts et al., 2023). However, no abnormal PNDL values were observed in any case.

Additionally, WBE-derived consumption (MC) comes from only one week of sampling and, consequently, it may not be representative of a whole year. However, the selected week (seven consecutive days, representing "normal" conditions at WWTP) was in the same period of spring (period with less influence from pharmaceuticals related to flu and cold) for all four sampling points, and most of the pharmaceuticals are administrated for chronic diseases. This week might reflect a baseline consumption. In fact, most of the WBE studies rely on data taken in one week (Bijlsma et al., 2021a; Laimou-Geraniou et al., 2023; Rice et al., 2020) or 3–5 days (Escolà Casas et al., 2021; Kasprzyk-Hordern et al., 2023), while others have used one sample per month during a longer period (Carnevale Miino et al., 2024; Petromelidou et al., 2024b). Boogaerts et al. (2023) demonstrated that the weekly mean of the PNDLs of different biomarkers was even similar to 3-day average as long as it contains one weekend day.

4. Conclusions

This study confirms the potential of WBE as a valuable approach to monitor pharmaceutical consumption, since a good correlation was found between WBE-derived consumption and consumption obtained from dispensed pharmaceuticals in pharmacies.

Moreover, WBE-derived consumption has shown a higher degree of correlation with dispensed pharmaceutical data than it has shown with prescribed pharmaceutical figures in previous studies. Consumption derived from pharmaceutical prescription does not normally include prescriptions from private centers and over-the-counter medicines. Thus, and for the first time, we propose the comparison between WBE and dispensation data to validate WBE as a tool to estimate pharmaceutical consumption. Some variables that should be carefully considered are the selection of biomarkers, the calculation of correction factors, and the estimation of the population that is consuming pharmaceuticals in the catchment area and/or is served by the studied WWTP.

The present study should also serve to consolidate WBE for future monitoring of biomarkers, whose data using other indicators are scarce.

CRediT authorship contribution statement

Núria Fontanals: Writing – original draft, Validation, Investigation. **Rosa Maria Marcé:** Investigation, Funding acquisition. **Rosa Montes:** Writing – original draft, Methodology. **Rosario Rodil:** Methodology, Conceptualization. **Iria González-Mariño:** Writing – original draft, Validation, Data curation. **Yolanda Valcárcel:** Validation, Methodology. **Sara Rodríguez-Mozaz:** Writing – review & editing, Methodology, Conceptualization. **Francesc Borrull:** Supervision, Funding acquisition. **José Benito Quintana:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Eva Pocurull:** Writing – review & editing, Supervision, Methodology, Conceptualization.

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.176108.

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