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Assessment of Human Pharmaceuticals in Drinking Water Catchments, Tap and Drinking Fountain Waters

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Abstract: The occurrence of pharmaceuticals in water catchments and drinking waters raises potential risks to public health. Therefore, after addressing the major aquatic contamination pathway, the wastewater treatment plants (WWTPs), and, subsequently, surface waters, 18 human pharmaceuticals from 6 therapeutic groups (antibiotics, lipid regulators, selective serotonin reuptake inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs) and hormones) were analyzed in drinking water catchments, tap and drinking fountain waters. This was performed by solid phase extraction (SPE) and liquid chromatography coupled with tandem mass detection (LC-MS/MS). The 97 samples analyzed were collected from 31 different sites in the center of Portugal. All samples presented concentrations below the method detection limits (MDLs) that ranged between 1.13 to 5.45 ng L^{-1}. The achieved results contributed to a better knowledge on the Portuguese and European context of drinking water, since there is a knowledge gap regarding this matrix. Comparing our data with other studies, published worldwide, we can observe that median concentrations of pharmaceuticals were reported in the low ng L^{-1} levels, values close to our MDLs. Consequently, it is unlikely that, in light of the current knowledge, the presence of pharmaceuticals in drinking water presents a threat to human health.

Keywords: environmental contaminants; pharmaceuticals; chemicals; Portugal; occurrence and fate

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1. Introduction

The increasing use of pharmaceuticals worldwide, classified as a group of emerging contaminants, presenting different characteristics and, consequently, producing different environmental exposure profiles, represents an environmental issue which has been raising increasing concerns in recent years [1,2]. The continued introduction of these chemicals into the environment is of concern, since albeit in trace amounts, they are designed to exert specific biological effects [3,4].

Its occurrence in the water compartment has been continuously documented throughout the world, mainly through the use of solid phase extraction (SPE) followed by liquid chromatography coupled with tandem mass detection (LC-MS/MS), with human excretion being the main source of pharmaceuticals in the aquatic environment [3]. Consequently, the widespread presence of pharmaceuticals in environmental samples is more likely to occur from wastewater treatment plants (WWTPs) that incompletely remove these compounds [5], an issue that was first studied in 1976 [6]. Pharmaceuticals are then released into the environment, through the effluents and sludges, as parent compounds, metabolites and as transformation products, formed during water treatments promoting contamination of surface water, groundwater and even drinking water [7]. Groundwater systems globally around 50% of the world's drinking water, with the remaining being mainly originated

in surface waters [8]. Therefore, it is important to evaluate not only drinking waters but also its catchments in order to evaluate the removal of pharmaceuticals in water treatment plants (WTPs) [9].

Due to cumulative and multigenerational exposure, the environmental impact of pharmaceuticals and the potential for negative ecotoxicological effects on the aquatic environment, even at sublethal concentrations, has been recognized worldwide. Once released into the environment, they can remain bioactive and pose a toxicological risk to non-target organisms as well as for human health [10,11]. The main risks associated with their presence in the aquatic environment is not only acute toxicity but also their genotoxicity, emergence of bacterial resistant genes, and endocrine disruption [12,13]. Examples of ecotoxicological effects are the Gyps vulture population decline in India after consumption of contaminated carcasses with diclofenac and the feminization in male fish in rivers due to the presence of estrogens [14]. Additionally, as referred, antibiotic presence in the aquatic environment increases the selective pressure promoting the emergence and/or dissemination of bacteria resistant genes, one of the major public health concerns [13]. Additionally, the occurrence of several pharmaceuticals, together with the array of other contaminants present in the water compartment increases the complexity of these matrices' toxicity, promoting non-additive interactions that can lead to unexpected toxic effects [12].

Despite the risk evidence, legal limits have not yet been established in drinking water and therefore, routine monitoring programs have not yet been implemented, as is the case for the evaluation of regulated chemical and microbial parameters [15].

The pharmaceuticals in study, key representatives of the major therapeutic groups, were selected based on their high consumption in Portugal, legislation, previous data on their occurrence in water bodies, their attenuation/persistence in surface waters as well as considering the relative concern about their potential toxicological impact [16–20].

This study aims to provide a clearer view of pharmaceutical contamination in drinking water catchments (surface and groundwaters), in tap and drinking fountains in the center of Portugal. The selected pharmaceuticals include not only the parent compounds (17), but also one metabolite, belonging to different therapeutic groups, such as antibiotics (Ciprofloxacin (CIP), Erythromycin (ERY), Azithromycin (AZI) and Clarithromycin (CLA)), lipid regulators (Gemfibrozil (GEM) and Bezafibrate (BEZ)), antiepileptics (Carbamazepine (CAR)), selective serotonin reuptake inhibitors (SSRIs) (Citalopram (CIT), Fluoxetine (FLU), Sertraline (SER)), non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics, still referred to only as NSAIDs, (Diclofenac (DIC), 4-hidroxy-diclofenac (4-OH-DIC), Naproxen (NAP), Ibuprofen (IBU), Paracetamol (PARA)) and hormones (Estrone (E1), 17β-estradiol (E2) and 17α-ethinylestradiol (EE2)).

Previously, the determination of pharmaceuticals in several water bodies, such as WWTPs' influents (WWIs) and effluents (WWEs) as well as surface waters enabled the tracking of the major pathway of pharmaceutical contamination in the Portuguese aquatic environment, namely in the selected region. This study allowed to improve the analytical methodology sensibility and characterize the pharmaceutical contamination in the remaining aquatic matrices until it reaches human consumption, namely the quality of the drinking water catchments, tap and fountain water [3,19,21,22]. This will allow to present a global picture of the pharmaceutical's contamination in the aquatic environment, an important input for setting prioritizing measures and sustainable strategies, to minimize their impact in the aquatic environment.

2. Materials and Methods

2.1. Sampling Site and Collection

The 97 water samples were collected from 31 different sites, in the center of Portugal, by national authorities, to ensure correct sampling procedures (Figure S1 and Table S1, Supplementary Materials). Samples (2 L) were collected throughout seven sampling campaigns between March and April of 2017 in the water catchment points and in the treated waters of five WTPs. Four WTPs had one water catchment point and one WTP

presented two. This sampling included surface waters (7), groundwaters (35) and treated waters (35). Sampling (2 L) was also performed in September of 2017 in eighteen drinking fountains and two tap waters of the same region.

After collection into high-density polyethylene containers, previously rinsed with bi-distilled water, samples were refrigerated for transportation. On arrival at the laboratory they were acidified to pH 3 with formic acid and stored at −20 °C until analysis.

2.2. Standards and Chemicals

All pharmaceutical standards CIP, ERY, AZI, CLA, GEM, BEZ, CAR, CIT, FLU, SER, DIC, 4-OH-DIC, NAP, IBU, PARA, E1, E2 and EE2, with purity degree ≥ 98% or certified reference material, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock and intermediate solutions were prepared in acetonitrile at 500 μg mL^{-1} and 100 μg mL^{-1}, respectively, and stored at −20 °C for a maximum of 6 months. Mixed standard working solutions, renewed before each analytical run, and prepared at concentrations ranging between 20 and 250 ng mL^{-1}, in a mixture of water-methanol (90:10 v/v), were used for linearity, accuracy and repeatability assays. For the labelled surrogates, a concentration of 250 ng mL^{-1}, also in water-methanol (90:10 v/v), was used.

Sigma-Aldrich (St. Louis, MO, USA) supplied methanol and acetonitrile and Ultrapure Milli-Q water was obtained from a Millipore Milli Q system (Bedford, MA, USA). Formic acid (98%) was obtained from Merck (Darmstadt, Germany).

2.3. Experimental Procedure

The analytical procedure was based on a previously reported and revalidated method for the identification and quantification of these pharmaceuticals in surface water [21]. Since low concentrations were expected a high volume of sample was used for SPE, mainly to preconcentrate the samples, being the main difference from the previous validated analytical methodology.

Briefly, after sample collection acidification to pH3 was performed with formic acid and the samples frozen. After defrosting and reaching room temperature, 1.1 L of each sample was filtered through glass microfiber filters (1.0 μm, 934-AH) and 0.45 and 0.2 μm polyamide membrane filters from Whatman Schleicher and Schuell (Piscataway, NJ, United States of America) and from Whatman (Dassel, Germany). One liter of sample was spiked with surrogate standards (0.5 mL) and loaded into SPE cartridges Oasis HLB (200 mg, 6 mL), from Waters Corporation (Milford, MA, USA), previously conditioned with 2 mL methanol and 2 mL Milli-Q water. After rinsing with 5 mL of methanol/Milli-Q water (10:90 v/v) and left to dry for 15 min, elution was performed with 6 mL methanol. Finally, the eluate was evaporated to dryness under a gentle stream of nitrogen, at 40 °C, and the dried extracts were stored at −20 °C until analysis, that took place in a maximum of 48 h.

For LC-MS/MS analysis, the dried eluate was reconstituted into 0.5 mL of water-methanol (90:10 v/v), and microfiltered. A 20 μL injection volume was used and a gradient of (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid at 200 μL min^{-1} were used (Table S2, Supplementary Materials).

Chromatographic separation was achieved with a column Waters Spherisorb ODS2 (150 × 2.1 mm, 3 μm) (Waters Corporation, Milford, MA, USA) preceded by a guard cartridge of the same packing material (10 × 4.6 mm, 5 μm) (Waters Corporation, Milford, MA, USA). The LC-MS/MS analyses were performed using a Liquid Chromatograph of High Performance (Thermo Finnigan, San Jose, CA, USA) coupled to a Linear Ion Trap (LIT-MS) (LTQ XL, Thermo Scientific, San Jose, CA, USA).

The MS was operated in the two electrospray ionization mode (negative and positive) using multiple reaction monitoring (MRM) acquisition (Table S3, Supplementary Materials). Nitrogen was used as nebulizing gas, with a sheath gas flow of 70 (arbitrary unit) and the auxiliary sweep gas flow of 10 (arbitrary unit). Source and capillary temperatures were set at 0 °C and 235 °C and voltages at 4.0 and 15 V, respectively. Collision gas was helium with a normalized collision energy range from 15.0% to 37.0% depending on the

target compound. Retention time, product ions, and collision energy are also presented in (Table S3, Supplementary Materials).

3. Results and Discussion

3.1. Analytical Quality Control

Analytical quality control was performed encompassing different performance criteria such as sensitivity, linear range, matrix effects (ME), accuracy and precision (Table S4, Supplementary Materials). Linearity was studied analyzing in triplicate six concentration levels, using standard solutions between 20 and 250 ng mL^{-1}, that correspond, according to the analytical methodology, to the range of 10 to 125 ng L^{-1}, and also in matrix-matched calibrations, at the same concentrations. Linearity, achieved for every compound in the working standard solutions, was reliable as shown by the fact that the correlation coefficients (r^2) ranged from 0.9989, for E1 and 1, for CIT. In matrix-matched solutions, r^2 values ranged between 0.9987, for E1 and 1, for CIT.

MEs equaled the percentage of the matrix-matched calibration slope (B) divided by the slope of the standard calibration in solvent (A). Thus, the ratio (B/A × 100) was defined as the absolute matrix effect (ME%). The obtained value was interpreted as follows: a value of 100% denoted an absence of MEs, above 100% signal enhancement and below 100% signal suppression. MEs were considered negligible, since the values varied from 93.55 to 102.68%, for IBU and E2, respectively.

The method detection (MDLs) and quantification limits (MQLs) were estimated through the matrix-matched calibration curve as |3.3Sy/x|/b and |10Sy/x|/b, respectively, where b is the slope and Sy/x the residual standard deviation of the linear function. MDL and MQL values ranged from 1.13 to 5.45 ng L^{-1}, and from 3.41 to 16.53 ng L^{-1}, for CIT and E1, respectively (Table 1).

Table 1. Matrix-matched linearity, matrix effect, method detection limits (MDLs), method quantification limits (MQLs) of the selected pharmaceuticals.

Therapeutic Group/Compound	Matrix-Matched Linearity (r^2)	Matrix Effect (%)	MDL (ng L^{-1})	MQL (ng L^{-1})
Antibiotics				
Azithromycin (AZI)	0.9995	100	3.29	9.97
Ciprofloxacin (CIP)	0.9988	100.96	5.26	15.93
Clarithromycin (CLA)	0.9999	98.77	1.51	4.58
Erythromycin (ERY)	0.9991	98.84	4.49	13.60
Lipid regulators				
Bezafibrate (BEZ)	0.9998	100.00	2.26	6.84
Gemfibrozil (GEM)	0.9994	94.02	3.78	11.46
Antiepileptic				
Carbamazepine (CAR)	0.9992	101.27	4.22	12.80
Selective serotonin reuptake inhibitors (SSRIs)				
Citalopram (CIT)	1.0000	100.00	1.13	3.41
Fluoxetine (FLU)	0.9999	100.61	1.84	5.59
Sertraline (SER)	0.9996	99.44	3.04	9.22
Non-steroidal anti-inflammatory drugs (**NSAIDs**)				
Diclofenac (DIC)	0.9991	101.96	4.61	13.97
4-hydroxydiclofenac (4-OH-DIC) (metabolite)	0.9990	99.33	4.92	14.92
Ibuprofen (IBU)	0.9994	93.55	3.66	11.09
Naproxen (NAP)	0.9992	101.27	4.38	13.27
Paracetamol (PARA)	0.9995	99.20	3.57	10.82
Hormones				
Estrone (E1) (natural hormone/metabolite)	0.9987	100.68	5.45	16.53
17β-estradiol (E2)	0.9991	102.68	4.56	13.82
17α-ethinylestradiol (EE2)	0.9990	99.44	4.94	14.98

Recovery tests were performed to determine the accuracy and precision of the method by spiking a surface water at three levels, 20, 60 and 125 ng L^{-1} (n = 3), in three different days, and each sample was analyzed in triplicate. Accuracy, determined using the matrix-matched calibration curve, varied between 94.02 and 99.88%; as for precision, evaluated through the relative standard deviation (RSD) of intra-day and inter-day repeatability, was below 8.70 and 6.85%, respectively. These values are considered reliable and similar to other methods developed for the same purpose [4,23,24].

3.2. Occurrence and Comparison with Other Studies

In the 97 water samples analyzed none of the drinking water catchments (surface and groundwater), drinking fountains and tap water samples presented concentrations above the MDLs. The drinking water fountains' sampling was performed in a low population density region and with low contamination pressure, which can justify the observed results. As for the surface and groundwaters' catchments for drinking waters, with the exception of Boavista catchment, they were also located far from high population density and contaminated areas. This seems to be a good option for the location of drinking water catchments. Naturally, that if the catchments presented concentrations below the MDLs, as expected, the drinking waters obtained from these catchments presented similar results.

When observing the concentration of pharmaceuticals in other water matrices provided by other studies, it is possible to identify decreasing levels of contamination in WWIs, WWEs and surface waters [3,21]. Generally, observing previous Portuguese results, compounds are found in WWI with much higher concentration values than in WWE [3,19,25]. In both matrices, WWIs and WWEs, 100% detection frequency was observed. The highest average and concentration found in WWIs were 41 and 150 μg L^{-1}, respectively; as in WWEs, the values were of 1.8 and 33 μg L^{-1}, respectively, both for PARA [19]. Performing the environmental risk assessment, we can observe that the WWEs still pose a threat to aquatic ecosystems [3,19,26].

The range of pharmaceutical concentrations observed in Portuguese rivers was considerably lower than the ones recorded in the WWEs, with detection frequencies of 27.8% and average (7.78–39.21 ng L^{-1}) and maximum (69.15 ng L^{-1}) concentration in the low ng L^{-1} levels [21]. In surface waters, several compounds were not detected, namely the hormones E1, E2 and EE2, NSAIDs IBU and NAP and the antibiotic CIP. These concentrations in surface waters appear to be primarily related to the river flow rates, suggesting that, the dilution factor is the main accountable for pharmaceutical river concentrations [21].

The European Medicines Agency (EMA) Guideline on Environmental Risk Assessment (ERA) suggest the use of a factor (0.25) to derive the concentrations in groundwaters from surface waters [22,23]. Notably, the obtained results showed that the concentrations in groundwater, below the MDLs (<5.45 ng L^{-1}), were considerably lower than those obtained by the use of the EMA factor in surface water concentrations (averages of 1.95–9.80 ng L^{-1}), questioning the accuracy of this factor. Our results show that the concentrations in groundwaters must be more than 7 times lower (factor of 0.14) than the ones in surface waters.

Regarding the pharmaceutical with the highest concentrations in several water matrices, namely PARA, one can compare the observed levels: 150 μg L^{-1} in WWI, 32 μg L^{-1} in WWE, 69.15 ng L^{-1} in surface waters and <3.57 ng L^{-1} in the present study. These values imply a reduction of 78.7% from WWIs to WWEs and of 99.8% from WWEs to surface water. Moreover, the concentration decreases from WWI to this study more than 99.99% [3,19,21,24].

The results found in this study were consistent with those previously reported in Portuguese drinking and groundwaters by other authors with concentrations ranging from 0.005 to 23.8 ng L^{-1} and where hormones were also not detected [25–27]. Overall, the levels of pharmaceuticals detected in drinking water catchments and drinking water were generally below 50 ng L^{-1} [15,28–30]. In fact, the concentrations reported by other authors confirm effectively low concentrations values in these water types. Therefore, it is unlikely

that pharmaceuticals pose significant threats to human health at the concentrations that may occur in drinking waters [31,32].

Moreover, a limited amount of publications are available with regard to the occurrence of pharmaceuticals in mineral, drinking and groundwaters worldwide [33]. Besides the aspects previously referred, it is also important to compare our study with the distribution of pharmaceuticals in these water compartments in the different continents, as identified in Figure 1 and Figure S2 (Supplementary Materials), regarding their detection frequencies and median concentrations. The main factors that could impact this data are the pharmaceuticals consumption, the existence and efficacy of wastewater and WTPs, the dilution from wastewater to surface water, soil characteristics and the climate (sunlight and temperature) that can influence pharmaceuticals degradation [7,19,21,23].

Observing the data on the occurrence of pharmaceuticals in these water matrices, as expected, groundwaters presented higher detection frequencies and medians followed by drinking water and mineral water. We can also observe that the European continent is clearly the most studied, whereas for Africa and Asia, there is limited information. Although there are fewer studies regarding the African continent, the detection frequencies were higher (100 and 67%), and their median concentrations (20 and 23 ng L^{-1} for CAR and PARA, respectively) similar to those on the European and American continents. This data reflects the lower pharmaceutical consumption but also the lower development of wastewater and WTPs and lower dilution factor of wastewaters due to low precipitation.

It is in Europe that higher detection frequencies and median concentrations were observed. Although the development in the wastewater and WTPs and the dilution factor observed in this continent, the observed results are probably related to the higher pharmaceuticals consumption. Antibiotics were the therapeutic group with the higher median concentration (33.8 ng L^{-1}), with AZI being the main contributor (median of 89.7 ng L^{-1}), followed by NSAIDs, antiepileptics and lipid regulators that presented similar patterns. NSAIDs were found with a detection frequency of 35% and median concentration of 15.1 ng L^{-1}. It is well known that antiepileptics are among the most frequently found pharmaceutical compounds in drinking and groundwaters (45.3%) and presented median concentration of 13.7 ng L^{-1}. Concerning the remaining therapeutic groups, the medians were, in decreasing order: SSRIs (1.4 ng L^{-1}) and hormones (0.7 ng L^{-1}).

In America, the median concentrations of the therapeutic groups varied from not detected, for lipid regulators, to 22.7 ng L^{-1}, for NSAIDs. On the other hand, in Asia, the antibiotics, SSRIs and lipid regulators were evaluated and were not detected. Concerning the median concentrations, antiepileptics (13.6 ng L^{-1}) were followed by NSAIDs (6.9 ng L^{-1}).

In general, pharmaceutical compounds were detected in drinking and groundwaters around the world in trace concentrations, which are in agreement with the results obtained in this study.

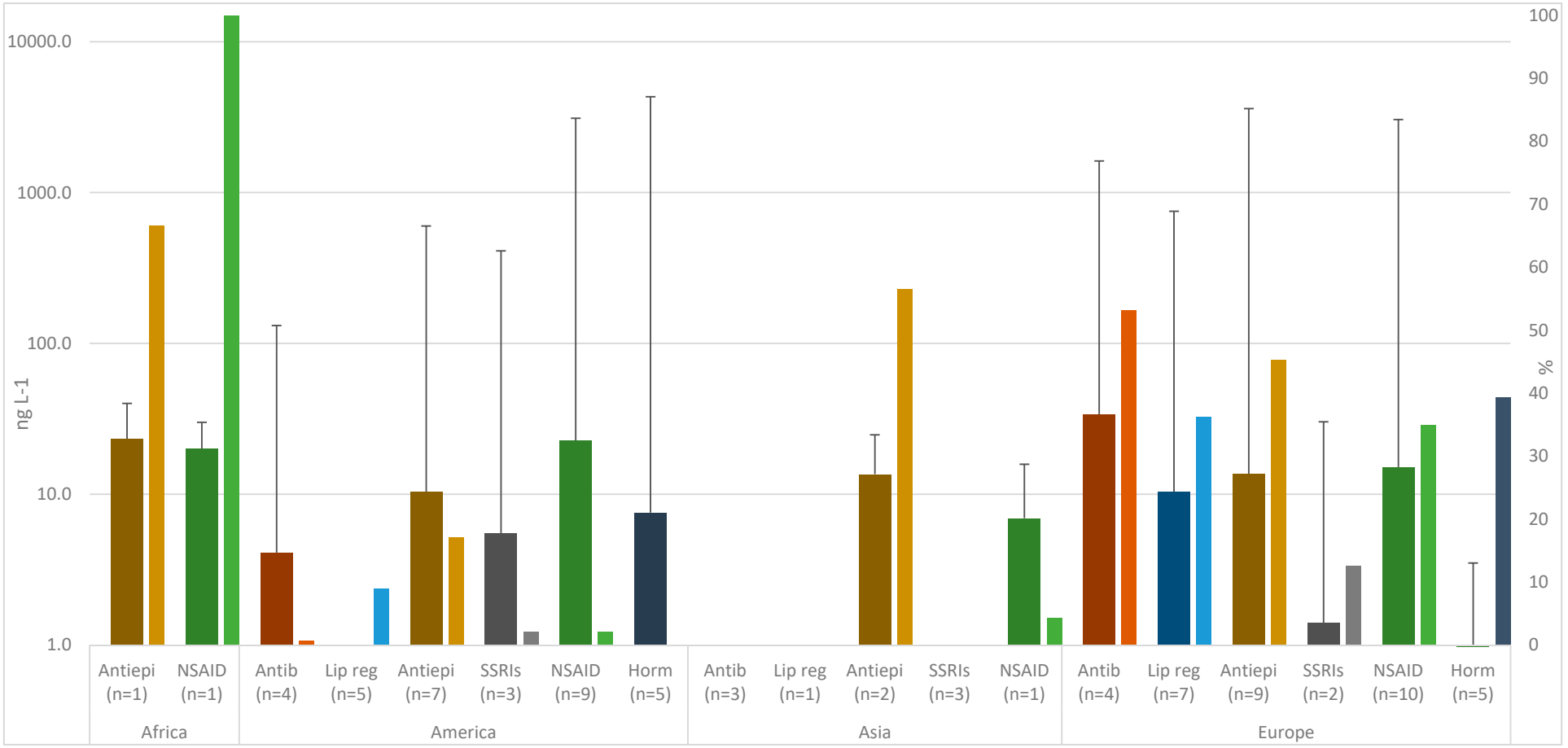

Figure 1. Median (**left** column), maximum (**top** wiskers) concentrations and detection frequencies (**right** column) in drinking and groundwaters by continent. Antib—Antibiotics; Lip reg—Lipid regulators; Antiepi—Antiepileptics; SSRIs—Selective serotonin reuptake inhibitors; NSAIDs—Non-steroidal anti-inflammatory drugs; Horm—hormones [4,25,31–59].

4. Conclusions

The 97 samples of drinking water catchments, tap and drinking fountain waters presented pharmaceutical concentrations below the MDLs. Our findings, with all values below the MDLs (1.13 to 5.45 ng L^{-1}), are in agreement with previous studies found in the scientific literature, where the reported concentrations ranged from 0.005 to 23.8 ng L^{-1} in Portuguese drinking and groundwaters. In addition, around the world, similar concentrations were also detected in these water matrices with the different continents presenting median concentration by decreasing order: Europe, Africa, America and Asia.

In this context, limited data on the occurrence of pharmaceuticals in drinking water, impacted by other contaminated environmental matrices, are a challenge in assessing potential human health risks from exposure to trace concentrations of pharmaceuticals. Overall, more systematic studies will help to further understand the fate and occurrence of pharmaceuticals in the environment, especially in drinking water.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11157062/s1, Figure S1: Sampling location, Figure S2: Median concentrations for each pharmaceutical in mineral, drinking and groundwaters by continent, Table S1: Sampling site and type of water, Table S2: Gradient elution scheme, Table S3: Target compounds organized in their therapeutical groups and their internal standards, Table S4: Analytical quality control for the quantification of each pharmaceutical in water.

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