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ORIGINAL ARTICLE

Trace Analysis of Pharmaceutical Residues in Wastewater Treatment Plants in Rio de Janeiro, Brazil

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KEYWORDS

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ABSTRACT: The occurrence and fate of trace-level contamination of pharmaceuticals in the aquatic environment has been recognized as one of the emerging issues in environmental chemistry. The effluents of wastewater treatment plants, usually directly emitted to the environment, often contain the anti-inflammatory drug diclofenac. Diclofenac was chosen because it is of high consumption; by background literature indicate toxic effects on biota and the lack of profile in sewage removal provided by the city. For this purpose, a survey on the presence of diclofenac in urban wastewater of Rio de Janeiro was carried out. It were evaluated diclofenac concentration in the affluent and effluent from wastewater treatment plant (WWTP) Penha and Ilha do Governador, Rio de Janeiro, Brazil. Samples were collected along the line of treatment of each WWTP, and for clean up the samples were solid phase extraction (SPE), analysed by high performance liquid chromatography (HPLC), assisted by diode array detector (DAD) techniques. The removal efficiency of pharmaceuticals in the wastewater treatment plants was roughly evaluated. Diclofenac was detected in all samples analysed wastewater (treated and raw), which confirms the low removal efficiency of conventional treatment systems, aerobes and anaerobes.

INTRODUCTION

The constant exponential growth in human population has created a corresponding intensification in the demand for the earth's limited supply of freshwater. Consequently, protecting the integrity of our water resources has become in the most essential environmental issues to be done. Numerous recent pollution problems are a result of the incessant release of chemical substances into the environment. Their presence is one of the central emerging issues to public and environmental health concern [1]. Although the occurrence of pharmaceuticals in the environment are nowadays considered emerging pollutants as being

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biologically active molecules they are considered potentially hazardous for aquatic organisms and health of the ecosystem, since they are continuously entering the aquatic environment *via* sewage systems, and can be found in a number of water bodies [2, 3]. Thus, pharmaceuticals are becoming ubiquitous aqueous pollutants and as a result, they are detected in remote aquatic environments [4]. Even if the effects of pharmaceuticals on living organisms are useful, designed to be biologically active, but some of them are not readily biodegraded [5, 6].Even if the effects of pharmaceuticals on living organisms are useful, designed to be biologically active, but some of them are not readily biodegraded [5, 6]. Sewage and sewage treatment plants are believed to be the main source of human pharmaceutical contamination [7], in the sense that WWTP's compromise the point of release into receiving waters [6]. Thousands of tons of pharmaceuticals are used every year, in both human and veterinary medicine, and are released to the environment through metabolic excretion and improper disposal techniques [8]. Human-use pharmaceuticals enter sewage effluents via urine and faeces and by improper disposal. These compounds are not completely degraded at the wastewater treatment plants, and many of them are discharged into the environment through many sources and pathways [8, 9]. Despite this general finding, the question arises what risks these traces of pharmaceuticals pose for aquatic ecosystems. Figure 1 presents a schematic picture showing the sources and pathways of pharmaceuticals into various water bodies.

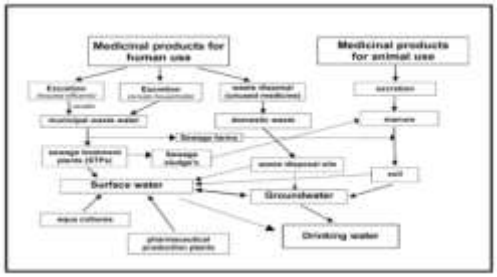


Figure 1. Sources and pathways for the occurrence of pharmaceutical residues in the aquatic environment. Modified from Heberer [10].

Diclofenac (2-[(2,6-dichlorophenyl)-amino]benzene acetic acid) (Figure 2), is a popular pharmaceutical drug, mostly used as the sodium salt (diclofenac-Na), for human medical care as an analgesic, antiarthritic, antirheumatic compound belonging to the group of the nonsteroidal anti-inflammatory drugs [11]. It is available in a number of formulations with the generic names e.g. Diclometin, Diclomex and Voltaren [12]. It is used worldwide and has a production volume estimated to be in the hundreds of tons annually, in the form of tablets, capsules, suppositories, intravenous solutions, and in ointments and gels for dermal application. It is readily metabolized after oral use, but assimilation is lower after dermal application [13].

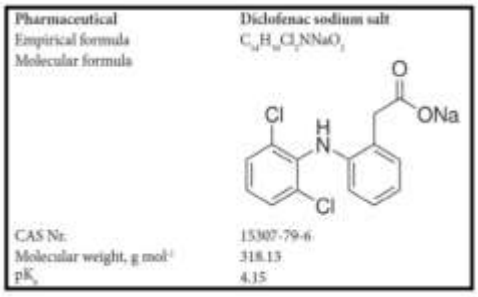


Figure 2. The structure and main properties of Diclofenac (2-[(2, 6-dichlorophenyl)-amino] benzene acetic acid)

Many pharmaceuticals in use have been proven to cause adverse effects in organisms, e.g., the decline of vulture population due to the consumption of livestock treated with diclofenac causing a 95% decrease of

oriental white-backed vulture in India subcontinent [14]. Even if it were found in the bile of wild fish caught downstream of a wastewater treatment plant [15]. Some studies have reported physiological and behavioral effects on fish exposed to environmental or near environmental concentrations of pharmaceuticals [16]. Reported to induce cytological changes in rainbow and brown trout tissues (kidney and gills) at environmental concentrations [17, 18, and 19]. More recently found that exposure of rainbow trout to diclofenac can interfere with the biochemical functions of the fish and lead to tissue damage [20]. In addition, diclofenac has been found to affect the gene expression in fish. The compound has also been connected with the serious decline of the Gyps vultures in Asia due to renal failure and visceral gout, and ultimately death [21].With growing interest on environmental issues, several intriguing questions related to the removal performance in the WWTPs Penha and Ilha do Governador, to assess the diclofenac removal efficiency in the processes. This study addresses the basic concepts, sources, mode of action, levels, analytical measurement, bioavailability, and biological role in the environment. An attempt has been made to answer the queries presented by the environmentalists working on various aspects of pharmacs pollution in the environment.

MATERIALS AND METHODS

Characterizing the WWTP

Sewage treatment plants can be equipped with primary, secondary and tertiary treatment steps. Primary treatment, often called physical treatment, involves the removal of big objects, floating solids and suspended solids (both fine and coarse) from raw sewage. This step often removes grease as well. Secondary treatment involves biological processes and results in decanted effluents and separated sludge containing microbial mass together with pollutants. The tertiary process removes pollutants not adequately removed by the secondary treatment, particularly nitrogen and phosphorus, often accomplished by some means of chemical treatment, sand filters, or other methods. During the tertiary treatment, microorganisms such as pathogens and viruses should also be removed by disinfection [22]. The WWTPs Penha and Ilha do Governador are submitted to the following operational parameters: pH, total suspended solids, fixed suspended solids, volatile suspended solids, chemical oxygen demand, biochemical oxygen demand, total Kjeldahl nitrogen, ammonia nitrogen, nitrate, nitrite and phosphorus.

Activated sludge

The activated sludge process make use of biological sludge full of microorganisms often combined with bubbling air or oxygen to reduce the organic content from the sewage [23]. Under ideal conditions, a nitrification process takes place in which ammonia is converted to nitrite and nitrate and ultimately to nitrogen gas. This usually takes place in the aeration tank. The microorganisms grow and reproduce by using the organic material as food, and at the same time, they are mixed with air, which results in their aggregation [22, 23]. These biological solids or sludges are more readily sedimented in the secondary clarifiers where they are separated from the treated wastewater. Some fraction of the sludge is returned to the head of the aeration system (40 to 60% of the wastewater flow) while the rest goes to waste. This wasted activated sludge is removed from the treatment process to keep the ratio of biomass to food supplied (sewage or wastewater) in balance [24].

WWTP Penha

The WWTP Penha operates with biofilters and activated sludge, treating a flow of around 1,600 L^{s-1}. The pre-treatment starts by medium and fine screening, with removal of solids, followed by removal of sands, oils,

and greaset. Greases are incinerated. The primary treatment consists of an accelerated lamellar settling in four tanks, with optional physical and chemical treatment, and of three tanks of equalization/homogenization. The secondary biological treatment is performed by means of a continuous-flow activated sludge system with conventional aeration in six aerated tanks by surface aeration, followed by a secondary lamellar settling, in 12 settlers of rectangular plant, with biological sludge recirculation.

WWTP Ilha does Governador

The WWTP Ilha do Governador operates with activated sludge, treating a flow of around 525 L^{s-1}. The treatment process works with the steps: the elevated influent pass through a screening formed by grids – a coarse and a medium, responsible for removing coarse solids, followed by the removal of oils, greases and sands, in two grit chambers/degreasers. The resulting by-products of this pre-treatment are later deposited in controlled landfill. The influent is then fed to a homogenization tank e with 4,000 m^3 working volume, whose function is to regularize peaks of pollutant load. The subsequent primary treatment consists of a preliminary step of physical and chemical treatment, with application of aluminium sulphate, hydrated lime and a polyelectrolyte on two lines of coagulation/flocculation tanks, with primary settling in two tanks of circular plant with bottom and surface scraper. The primary effluent is then fed to the secondary treatment, which is performed by means of activated sludge system, in six aeration tanks provided of two surface aerators each and sludge recirculation tank. The next step is the secondary settling in two settlers of circular plant, with bottom and surface scraper.

Sampling

Samplings were carried out along the line of treatment of the WWTPs Penha and Ilha do Governador. The sites sampled were: (1) influent, (2) post preliminary treatment, (3) post-primary treatment and (4) final effluent. Samples were taken in triplicate and stored in amber vials of 250 ml. During the 12h scheduled for sample collection, which occurred between 8 and 20 h, it was gathered eight samples per site during the collection period. The collections in the WWTPs were performed in February-March 2013, and the results express the consolidated obtained from each analysed site.

Reagents

Diclofenac, triethylamine, toluene, clofibric acid, mefenamic acid and 2, 3, 4, 5, 6-pentafluorobenzyl bromide was all purchased from Sigma-Aldrich (Rio de Janeiro, Brazil). Stock solutions of all compounds were prepared by dissolving the compounds in methanol, and the solutions were stored in glass-stoppered bottles at 4°C prior to use. Working aqueous standard solution was prepared daily. Ultrapure water was provided by a Milli-Q system (Millipore, Rio de Janeiro, Brazil).

Filtration and solid-phase extraction (SPE)

With Millipore hazardous waste filtration system 250 ml portions of each sample was filtered (0.45 µm). The pH was adjusted to 2 with HCl. Subsequent extraction of solid matter retained by the 0.45 µm filter with diethyl ether did not show any presence of analytes of interest [25]. Extraction was performed by percolation through an ENVI-18 reverse phase packed tube at a flow rate of approximately 3ml/min by applying a low vacuum. The solid phase was previously conditioned by flushing with 3 ml acetone, followed by 3 ml methanol and 3 ml of water adjusted to pH <2. At the end of percolation, Erlenmeyer flasks were washed with 3 x 15 ml of acidified water, which are also passed through the cartridge. After drying the solid phase for 1h under vacuum, the analytes were eluted with 6 ml of methanol. The methanol extract was evaporated until dryness under a gentle stream of nitrogen.

Derivatization and clean-up

Derivatization was performed at 90°C for 1 h using 400 ml of 2, 3, 4, 5, 6-pentafluorobenzyl bromide (2% in toluene) and 4 ml of triethylamine [10]. The derivatized extract was passed through a SiOH cartridge conditioned with toluene. The analytes were eluted with 15ml of toluene. The eluate volume was reduced under a gentle stream of nitrogen between 100 and 1500ml; to be inside the range of concentration tested in the calibration curve. If higher/smaller concentration was found, the samples were diluted/concentrated and analysed a second time.

Instrumentation

The HPLC analysis was carried out in a Varian system (HPLC-DAD) with a Varian 920-LC model liquid chromatograph equipped with a 900-LC model autosampler, gradient pump, 330 models DAD, and the Galaxie software for data acquisition and processing. The analyses were carried out in the gradient mode using a Pursuit C_{18} (microsorb-MV100-5, 250 x 4.6 mm). The injection volume of samples was 50 µl.

Method validation

The limit of detection (LOD) and limit of quantification (LOQ) of the chromatographic analysis were estimated as 3 and 10 times the baseline noise, respectively. The overall method detection limit (MDL) was then determined by division of the LOD with E_e for each specific matrix.

Reproducibility, determination of recoveries and detection limits

To determine the initial concentration and to quantify the reproducibility of the whole method, an unspiked sample was analysed four times. To determine the recoveries, samples of wastewater were spiked with the pharmaceutical substance at four concentrations: 50%, 100%, 150% and 200% of the initial concentration or about 5, 10, 15 and 20 times the limit of detection for compounds not found in the wastewater tested (clofibric acid). Samples were taken through the analytical procedure. The experimental quantities expressed as a function of the theoretical quantities enabled to determine a regression line. The recovery rate was then derived from the slope.

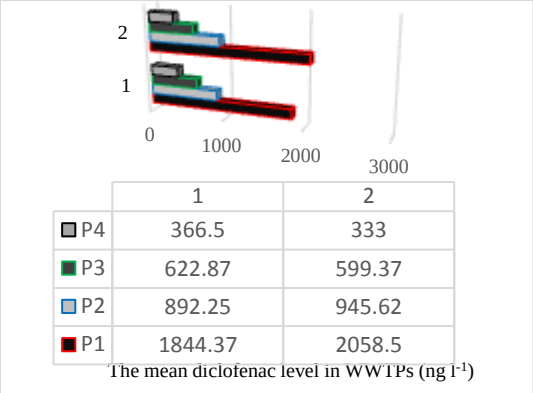
Deviation standards of slopes were calculated with the method of least squares. Recoveries after filtration, SPE, derivatization and clean-up generally exceeded 70%. Seeing that relative standard deviations on the reproducibility and standard deviation on recoveries varied from 2% to 16%, the precision is sufficient. Limits of detection (signal/noise ratio of 3) and limits of quantification (s/n ratio of 10) of the entire analytical procedure were calculated with a spiked sample and were corrected for recovery. Limits of detection and quantification were in a range, which allows the detection and the quantification of diclofenac in wastewaters.

STATISTICAL ANALYSIS

Statistical analyses were run using the software Origin 7.5 (OriginLab Corporation).

RESULTS

The determination of diclofenac levels in wastewater samples showed extremely complex, due to interferences that are not eliminated in clean-up process and which absorb UV radiation, which causes severe signals appear at retention times similar to those of the species of interest. However, diclofenac was detected in the influent and effluent of sewage treatment plants monitored, which confirms the low capacity removal submitted by conventional activated sludge techniques. The concentration values found for diclofenac in sewage effluent are consistent with reported results of a similar nature [26, 29]. Diclofenac has been found in the sewers of all WWTPs evaluated, and occurrence pattern was of the same order of magnitude for all samples (Figure 3).



The mean diclofenac level in WWTPs (ng l^{-1})

Figure 3. The mean diclofenac concentration (ng l^{-1}) at sites of samples collection (P1, P2, P3, P4) in WWTP Penha (1) and WWTP Ilha do Governador (2)

The results obtained from the effluents collected along the line of treatment of the WWTPs Penha and Ilha do Governador are presented in Tables 1 and2.

Table 1. The diclofenac concentration (ng l^{-1}) in effluent samples of the WWTP Penha

WWTP Penha								
Sites	**Statistical analyses**							
	X	**Sd(yEr±)**	**Se(yEr±)**	**Min**	**Q_1**	**Median**	**Q_3**	**Max**
1	1844.37	267.51	94.58	1316	1732	1922.5	2008.5	2113
2	892.25	82.87	29.30	744	847.5	914.5	945	980
3	622.87	103.88	36.72	469	535.5	629.5	715.5	753
4	366.5	64.16	22.68	245	330	383	408	445

X-arithmetic mean; Sd- standard deviation; Se- standard error; Q_1 – Percentile 25; Q_3 – Percentile 95, Max – Maximum; Min – Minimum

Table 2. The diclofenac concentration (ng l^{-1}) in effluent samples of the WWTP Ilha does Governador

WWTP Ilha do Governador								
Sites	**Statistical analyses**							
	X	**Sd(yEr±)**	**Se(yEr±)**	**Min**	**Q_1**	**Median**	**Q_3**	**Max**
1	2058.5	416.35	147.20	1437	1815	2002.5	2368	2660
2	945.62	138.12	48.83	679	861	990	1036	1112
3	599.37	71.00	25.10	458	561.5	623.5	644.5	678
4	333	28.42	10.04	305	312.5	322.5	350	389

X-arithmetic mean; Sd- standard deviation; Se- standard error; Q_1 – Percentile 25; Q_3 – Percentile 95, Max – Maximum; Min – Minimum

In some samples from WWTP Ilha do Governador were observed the diclofenac loads increased in the effluent in relation to the influent. According Zorita et al. [30] this fact is probably due to deconjugation of conjugated glucuronides or sulphates and / or desorption of diclofenac solid particles.

Contradictory to the results obtained for diclofenac research at WWTP Penha and Ilha do Governador, some studies demonstrate a high removal of diclofenac with

biological treatment [31, 32]. One possible explanation for the different efficiencies of found for this drug are the conditions on which the cause sewage treatment, as well as the WWTP design [31], since the removal of diclofenac is higher anoxic conditions, acid pH and high solar radiation [33]. In turn, the reduction diclofenac photolytic degradation will also depend on some additional parameters, such as eutrophic conditions, degree of particulate material and still depth of the watercourse [10].

DISCUSSION

The presence of drugs in WWTP influent is expected, since these compounds are not completely absorbed by the human body and thus are eliminated through excretion. However in this study for this xenobiotic high concentration in raw sewage, which can be explained by the indiscriminate use by this population, were registered. The observed concentrations in the effluent were also high, which implies a reduced efficiency on WWTPs in removing it and how current systems of sewage treatment can not totally eliminate this pollutant. Some improvements or modifications should be studied for a complete removal of this compound in order to mitigate risks to public health [34]. The continuous increase in the presence of this drug substance in the drinking water supply is one of the world's problems compromising the quality of water intended for human consumption and the inherent losses on aquatic environments impacted by this compound. Due to this continuous and increasing consumption and their incomplete elimination in WWTPs, pharmaceuticals in general can be detected in rivers, lakes, and coastal waters [35, 38]. The removal efficiency of WWTPs varies depending on the pharmaceutical, and the treatment applied [39]. In general, the WWTP systems are characterised by a high degree of dilution. Dilution is one of the reasons why pharmaceutical compounds are not sufficiently removed. When discharged to surface water they may form a threat to aquatic life and in the worst case may re-enter the water cycle. Source control, i.e. sanitation approaches based on separation at source are based on separation and separation of wastewater streams of different origin. Specific treatments, targeting different flows, may enable elimination of pharmaceuticals and minimisation of the emission of human pharmaceuticals to the environment. However, there are no regulations dealing with the actual levels of pharmaceuticals in various environmental compartments, e.g. minimum removal rates in WWTPs or maximum drug concentration in surface water. In the European Union there are only a scarce guidelines recommending more environmental risk assessment when predicted environmental concentrations of pharmaceuticals are equal to or higher than 10ng l [40]. In recent years, a number of new technologies have been developed in order to guard against contamination of aquatic environments by xenobiotic compounds. Although this study holds up in a timely reviewed for the presence and concentration of diclofenac in various stages of sewage treatment, it serves as a guide for future studies to consider a more detailed temporal analysis of WWTP, the compounds and the physical, chemical and biological parameters that act to sewage disposal the drug in the effluent. None of the studied sewage treatment plants were able to remove diclofenac from wastewater. In addition, the removal of this pharmaceutical was not dependent on the sampling period. Heberer et al. [10] pointed out a slightly higher elimination rate of 17% in different WWTPs in Berlin. Diclofenac was detected at concentrations ranging from 50–500 ng/L and 40–650ng l, respectively, in WWTP influents from Croatia and Spain [41]. Diclofenac was observed in WWTP influents at concentrations of 1,900ng l in Germany [42] and 251ng l in Japan [9]. Vieno et al. [28] observed the occurrence of this anti-

inflammatory in raw sewage in Finland at concentration of 0.46μg l^{-1}. In WWTPs located at different urban areas in Greece, diclofenac concentrations in the influent ranged between 0.012 and 0.56μg l^{-1}, and in effluent between 0.010 and 0.365μg l^{-1} [27]. On the other hand, in the WWTPs studied by Ternes [26] and Zhang et al. [43] with biological treatment, up to 75% of Diclofenac was removed. Thereby, the removal of this substance was very variable between different WWTPs studied by various authors.

CONCLUSION

This study demonstrates the need for sensitive and reliable analytical methods for investigating the occurrence and fate of pharmaceuticals in wastewater treatment systems. The diclofenac levels were relatively high in the effluents, and thus, the risk for surface water contamination was important. This requires a good and extended knowledge about sources, occurrence, fate, toxicity *etc*. Apart from regulating the waste disposal from the pharmaceutical industry, a deeper understanding of the fate and removal of human drugs and their degradation products in WWTPs is essential. This would help reduce or minimise the introduction of pharmaceuticals into the environment and thereby protect our water bodies. The disposal of unused medication via the toilet seems to be of minor importance but many of the pharmaceuticals applied in human medical care are not completely eliminated in the human body. Frequently they are excreted only slightly transformed or even unchanged mostly conjugated to polar molecules. These conjugates can without difficulty be cleaved during sewage treatment and will then be released into the aquatic environment mostly by effluents from WWTPs. To address the problem of unwanted occurrence of pharmaceuticals in the environment, a more integrated approach is needed to evaluate the real risks of pharmaceuticals and to regulate them.

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