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Article

Guideline values and human risk assessment for the presence of anti-inflammatory drugs remaining in drinking water after lab scale treatment

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This study aimed to determine whether the anti-inflammatory drugs that are most commonly consumed in Brazil, including diclofenac, ketoprofen, naproxen, indomethacin, ibuprofen and acetaminophen, are present in drinking water and to derive guideline values to characterize the human risk. These pharmaceuticals were quantified in surface waters by LC-MS/MS with solid phase extraction, both before and after conventional treatment on a laboratory scale, using a jar test assay. The methods used to quantify these drugs showed good results: the chromatographic analysis obtained correlation coefficients between 0.9952 and 0.9991, with limits of quantification of 0.5 $ng.mL^{-1}$ - 50 $ng.mL^{-1}$ and precision standard deviations (0.08 - 2.08). Only ketoprofen and ibuprofen were not completely removed through the jar test. Environmental samples were collected and handled by the same method; the values obtained for ketoprofen and ibuprofen after treatment were 18.67 – 19.65 $ng.L^{-1}$ (±17%) and 166.70 – 244.73 $ng.L^{-1}$ (±14%), respectively. Human risk was assessed by comparing the guideline values for each compound to the concentrations obtained in the environmental samples, considering the toxicological backgrounds, following WHO (2011) method. The results suggest that the concentrations of ketoprofen and ibuprofen found in drinking water do not pose a risk to human health, even with chronic consumption.

Keywords: Emerging contaminant. LC-MS/MS. Human risk assessment. Pharmaceuticals in drinking water.

INTRODUCTION

Until the 1970s, the worldwide concern with water quality was mainly limited to its microbiological aspects. The interest of the scientific community and researchers in evaluating the presence of xenobiotics or emerging contaminants in different environments, such as the aquatic environment, led to the creation of environmental protection agencies such as the USEPA (United States Environmental Protection Agency) in 1970. Since then, many studies have been published about the presence and possible toxicity of drugs and their transformation products in the environment (Ternes, 2001; Raimundo, 2007; Kumar, Chang, Xagoraraki, 2010; Richardson, Ternes, 2011; Richardson, 2012; Stuart *et al.,* 2012; Leung *et al.,* 2013; Cai *et al.,* 2015**)**.

In 2008, Brazil was ranked first among Latin American countries and 50th in the world in terms of the excessive use of medications (Souza, Silva, Neto, 2008). Such consumption and production may lead to the shedding of pharmaceuticals in wastewater, either as a result of human metabolism or by industrial spill. Pharmaceuticals and personal care products are the groups that are most frequently found in surface and drinking waters (Stumpf *et al.,* 1999; Richardon, 2012; Cai *et al* 2015). The detection of these pharmaceuticals in drinking water raises considerable public concern, especially when human-based guideline values are not available (Schriks *et al.,* 2010).

Such contaminations are not recent events; the first findings on the occurrence of drug residues in an aquatic environment date to 1965 and were related to drugs and

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substances that could interfere with the endocrine system. In 1976, Garrison, Pope and Allen used extractive methods and chromatographic analysis to report on the presence of other contaminants in waste water treatment plants in the United States.

New technologies allowed for the development of more sensitive, accurate and precise methods that can identify and quantify substances at concentrations as low as ng/L.

Even at low concentrations, the residue of biologically active drugs can have serious effects on aquatic biota, which, when they are not removed in sewage treatment, may result in health problems for most susceptible species because they can reach drinking water used by humans or animals (Melo *et al*., 2009; Cai *et al.,* 2015; Villanueva *et al.,* 2014) Anti-inflammatory drugs are one of drugs most frequently found in the environment. Such contamination can cause several toxic effects ranging from gastrointestinal problems to kidney damage, particularly within sensitive populations.

Issues that are pertinent to this type of contamination should be investigated. Guideline values for contaminants must be established, and long-term studies must be performed to analyze their possible chronic effects (Ghiselli, 2006; Richardson, Ternes, 2011).

Therefore, this paper aims to contribute to the evaluation of the conventional process of water treatment in Brazil by determining the degree of retention of anti-inflammatory painkiller drugs and deriving guideline values to characterize the human risk.

MATERIAL AND METHODS

Material

The drugs acetaminophen, ketoprofen, diclofenac, ibuprofen, indomethacin and naproxen, were purchased from Sigma Aldrich Brazil (batch numbers: 099K0127V; BCBD6676V; BCBD6672V; 122K0676V; BCBC9386V; 078K1629) with purities ranging from 98.5 to 100%.

Reagents, glassware and consumables: Glass fiber filters (GF/F 07 μm); C18 chromatographic column (Phenomenex – Endcapping 125 X 3 mm); automatic pipettes (10-40 μL; 40-200 μL); flasks (10, 25, 100 and 1000 ml); Beakers; Isolute C18 cartridge (Sep-pak® Vac 6cc – 500 mg- Waters® batch: 027536242A); Methanol (HPLC - Carlo Erba); ammonium acetate (mass spectrometric grade - Carlo Erba); Acetonitrile (HPLC - Carlo Erba); Acetone (p.a. - Merck), acetic acid (p.a. - Merck), formic acid (Merck - HPLC), sulfuric acid (p.a. - Carlo Erba); hydrochloric acid (p.a. - Carlo Erba); C18 cartridge (Sep-pak ® Vac 3cc - 500 mg, Waters ®batch: 027 536 242 nd); Sodium sulfate (p.a - Merck), sodium formate (High-Performance Liquid Chromatography (HPLC) or mass spectrometric (MS)); septum vial cap and septum compatible with HPLC Agilent®; filter unit (0.22) PTFE Liquid Chromatography – Tendem mass spectrometry (LC/MS-MS); disposable Falcon tube (5 mL), test tube (10, 100 and 1000 mL), Pasteur pipettes; purification system Milli Q - Plus ® Millipore, Millipore filter ®, regular detergent, commercial humic acid (Sigma-Aldrich).

Equipments: high-performance liquid chromatograph coupled with a mass spectrometer (Applied Biosystems® - API2000/MS/MS System); analytical balance (Denver Instrument® APX60); DR/2010 Spectrophotometer - Hach Company (Serial No.: 000 500 018 060) and 2100N turbidimeter - Hach company.

Methods

Pharmaceuticals in the environmental samples were quantified by LC/MS-MS after solid phase extraction (SPE). After quantification, the samples were subjected to jar testing, which mimics the steps of water treatment, to evaluate the amount of contaminants removed from the treated water.

Chromatographic method validation

To validate the chromatographic method, solutions with increasing concentrations of drugs (0.5 - 500 ng mL^{-1}) were prepared to construct the calibration curve and determine the linearity, precision and accuracy of the method. The mobile phase consisted of Phase A (methanol) and Phase B (ammonium acetate 1 mM) in a concentration gradient ranging from 45-80% of A and 20-55% of B, an injection volume of 20 μL, a column temperature of 122 °F, and a pressure of 150 bar. Electrospray ionization (ESI) in positive mode was used for all drugs except for ibuprofen, which showed better results in negative mode.

Solid Phase Extraction (SPE)

The SPE concentration of the drugs was based on EPA method 1694, using a C18 cartridge. This method enhanced the samples concentrations by approximately 300 times (EPA, 2007).

Jar Test method

Standardization of the jar test was performed using samples that had been prepared in the laboratory. Known concentrations of the analytes of interest (ranging from

35 to 150 ng/L) were added to six jars (five jars with known concentrations and one control, in triplicate) with a capacity of 2 liters each, and their removal was assessed. Each jar was subjected to all steps of conventional water treatment: oxidation, coagulation, flocculation and disinfection with the possibility of pH, temperature, coagulant and chlorine adjustments throughout the process. All the jar tests were carried out with humic acid aqueous solution (20 mg L^{-1}), which is the main component of organic matter that is present in natural fresh water (Ishiwatari, 1969). The following operating parameters were optimized: oxidant concentration (1-10 ppm Cl), pre-oxidation time (5-30 min), coagulant concentration (10-100 mg L^{-1} of $FeSO_4$) and optimum coagulation pH (3-8). The maximum allowable concentration of residual chlorine in drinking water (2 mg. L^{-1}) was also considered.

Analysis of the samples

The samples were collected at Guarapiranga dam and at the University of São Paulo Olympic streak (São Paulo, Brazil) in December 2012. The first one is located at Alto Tietê in the southwest of São Paulo metropolitan region and is used as source for drinking water after treatment. The second one is located in the University of São Paulo, in the west region of the São Paulo city and is only used for recreation.

Water samples were collected in white and opaque plastic bottles (4-10 L) that had previously been cleaned with regular detergent and running water; the bottles had screw caps and were stored in a place with temperature control (approximately 20 °C), for 24 hours until solid phase extraction and jar tests were performed. The parameters of color, turbidity and pH were evaluated before extraction and chromatographic analysis. After the extraction process by SPE using 1 liter of each sample, the samples were re-suspended in 1 ml of methanol; 300 µL of this solution was aliquoted and increased to a volume of 1 mL with water and formic acid (pH 4). Drug removal from water was evaluated in two spiked samples, before and after treatment by chromatographic analysis.

Quantitative pharmaceuticals risk assessment

Once the analytical phase was concluded, the quantitative pharmaceuticals risk assessment (QPhRA) was calculated as follows: (1) Hazard identification, (2) Exposure assessment, (3) Dose-response relationship, and (4) Risk characterization (Kumar, Chang, Xagoraraki, 2010).

Hazard identification was supported by literature review; exposure assessment was based on an analysis of the surface water after the conventional treatment. The dose-response relationship was based on the most restrictive and relevant NOAEL (No-observed adverse effect level) found in the literature. The risk characterization was given by Hazard Quotient calculation (formula 2) that is the division product of Exposure Dose by Guideline value, according to the US EPA, 2011.

FORMULA 1 - *The guideline value was found using formula 1,* (WHO, 2011):

$$GV = \frac{(TDI \times bw \times P)}{C}$$

where: GV = Guideline Value (mg L^{-1} per day); bw = body weight (70 kg); P = fraction of the TDI allocated to drinking water (10-20%, usually); C = daily drinking-water consumption (2 L day^{-1}); TDI = Tolerable daily intake (mg kg^{-1} per day)

The guideline value is based on the multiplication of the Tolerable Daily Intake (mg per kg d^{-1}) (TDI) by the Body Weight (BW) and the fraction of the TDI allocated to drinking water (P), divided by the chronic consumption (C). The TDI was calculated based on the most restrictive NOAEL, as found in literature, allocated to the factors of uncertainties that are applicable to each study.

FORMULA 2 - *Hazard Quotient (HQ)* EPA, 2011.

$$HQ = \frac{ED}{GV}$$

where: HQ = Hazard Quotient; ED = Exposure Dose (mg L^{-1}); GV = Guideline Value (mg L^{-1})

According to US EPA, 2011: If HQ > 1.0, then harmful effects cannot be ruled out; if HQ = 1.0, contaminant alone is not likely to cause risk; and if HQ < 1.0, harmful effects are not likely.

RESULTS AND DISCUSSION

Chromatographic method

The chromatographic method showed adequate linearity, precision, accuracy and limits of detection and quantification that are adequate to quantify the analytes in question. In all of the analyses, a weighting factor of 1/x for homoscedastic results, with smaller variance, was used. The limit of detection and quantification of the compounds ranged from 0.50 µg L^{-1} to 50 µg L^{-1}, based on injections of decreasing amounts to evaluate the height of the peak and baseline, as presented in Table I. For all drugs a negative control (blank) was evaluated to guarantee the reliability of the method.

The chromatogram obtained for all drugs, using the selected method, is shown in Figure 1.

Jar test standardization and removal evaluation

The evaluation of turbidity, color, and absorption at λ 254 nm showed that significant reductions and/or reductions below the values set out in Brazilian legislation were obtained only after all processing steps were done, using the following conditions: pre-oxidation with 1 ppm Cl (as NaClO) for 10 min, 23 mg.L^{-1} of coagulant, and coagulation pH optimum between 5-6. In these conditions, the jar test process produced a water sample with a pH 6.0, with a color beneath 1 uT and a chlorine residual of 0.65 mg L^{-1}, thus demonstrating that the concentrations of the added analytes were sufficient to ensure the reliability of the process, according to Brazilian decree 2914/2011. (Brasil, 2011).

The process of compound removal was shown to be highly capable of removing most of the analyzed compounds. Acetaminophen had high removal rates but still remained in some samples of treated water. The ketoprofen showed very low or no removal in alignment with what was found in the literature; ibuprofen showed great removal variation, as shown in Table I (Stumpf *et al.,* 1999; Vieno *et al.,* 2007).

Solid phase extraction

All analytes, except ibuprofen, showed the same recovery profile in the two homogeneous matrices: distilled water and water with humic acid. Acetaminophen had a recovery of approximately 20±2,5%, but diclofenac showed a high recovery of 100±7,8%. Ketoprofen also showed high recovery (approximately 99%), as did naproxen (103.4%) and indomethacin (90.3%), with deviations below 10%. Ibuprofen showed a wide variation in the first evaluated controls in both the distilled water matrix and the distilled water with humic acid. This analysis was repeated three times more with strict control of pH and luminosity, which resulted in an average recovery of 48±9.3% (58%, 47.5%, 39.5%).

The solid phase extraction efficiency is considering at the end of the experiments (removal evaluation) in order to obtain the final real concentration.

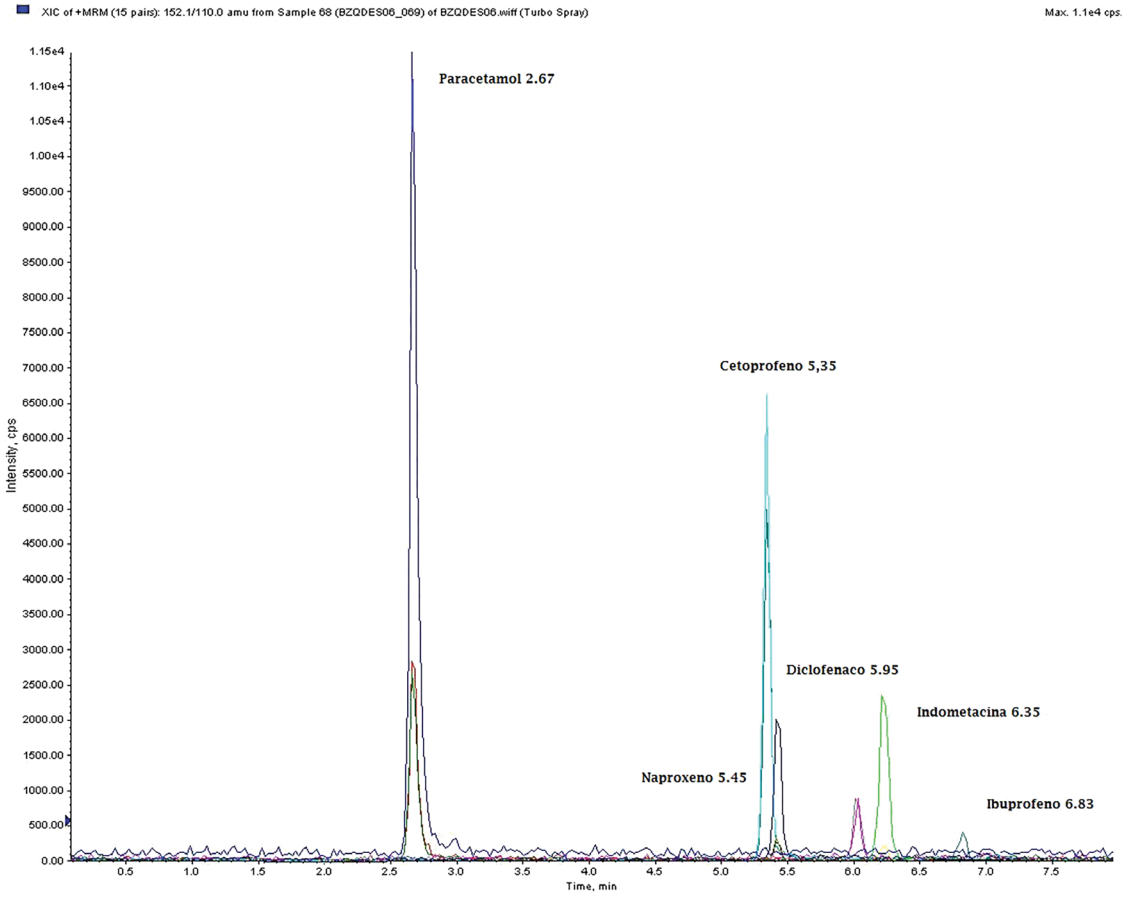

FIGURE 1 - Validation of the method: Chromatogram of drugs: acetaminophen, diclofenac, naproxen, ketoprofen, indomethacin and ibuprofen.

TABLE I - Percentage of removal of the evaluated compounds analyzed (in triplicates with SD <5% between them). Comparison of the peak areas before and after conventional treatment

Pharmaceuticals	Jar Sample	Concentration before treatment (ng ml^{-1})	Concentration after treatment (ng mL^{-1})	Removal %
Acetaminophen	Sample 1	1650	14.1	**99.14**
	Sample 2	2920	242.3	**91.72**
	Sample 3	1500	<LOD	**100.00**
	Sample 4	2210	8.7	**99.60**
	Sample 5	1510	<LOD	**100.00**
Diclofenac	Sample 1	2140	<LOD	**100.00**
	Sample 2	2990	<LOD	**100.00**
	Sample 3	2460	<LOD	**100.00**
	Sample 4	3290	<LOD	**100.00**
	Sample 5	3290	<LOD	**100.00**
Ketoprofen	Sample 1	2110	2150	**0**
	Sample 2	4320	4730	**0**
	Sample 3	3360	2890	**13.88**
	Sample 4	1460	1510	**0**
	Sample 5	1900	1890	**3.63**
Naproxen	Sample 1	3220	<LOD	**100.00**
	Sample 2	1980	<LOD	**100.00**
	Sample 3	1700	<LOD	**100.00**
	Sample 4	2850	3.74	**99.88**
	Sample 5	1470	<LOD	**100.00**
Indomethacin	Sample 1	1910	2.82	**99.87**
	Sample 2	2240	3.32	**99.87**
	Sample 3	1320	2.37	**99.85**
	Sample 4	815	3.92	**99.59**
	Sample 5	3220	5.78	**99.84**
Ibuprofen	Sample 1	5940	3560	**40.14**
	Sample 2	2830	1660	**41.20**
	Sample 3	2370	884	**62.76**
	Sample 4	1700	1500	**11.74**
	Sample 5	7070	1610	**77.20**

< LOD: Value below the limit of detection of the analytical method

Removal evaluation of the compounds in the environmental samples

Water from the Olympic streak showed pH 9.0, 4.38 uT of turbidity and apparent color 52 PtCo L^{-1}. Water from Guarapiranga showed pH approximately 6.0, 10.7 uT of turbidity and apparent color of 70PtCo L^{-1}. Both pH values are in accordance with the regular pH value for superficial water, according to Brazilian legislation CONAMA n^0 357 (Brasil, 2005).

Part of the samples (1 liter) was subjected to SPE to assess the actual concentration of each drug in the water in a triplicate analysis. This extraction method was validated in a similar manner as that previously performed, and samples were taken for chromatographic analysis. Table II shows the results of the SPE for the actual samples.

The water collected from the USP Olympic streak shows only 8 ng / L diclofenac, but in the water collected from Guarapiranga, all evaluated compounds were found, except acetaminophen, which was not detected in any of

TABLE II - The concentrations of analytes obtained after SPE (300x concentrated) and analytes concentration in environmental samples (n=3), considering the SPE concentration and recovery factor

Drugs	Mean concentration after SPE * ($ng.mL^{-1}$)				Efficiency of SPE %	Final (real) Concentration ($ng.L^{-1}$)	
	USP	(SD)	Guarapiranga	(SD)		USP	Guarapiranga
Acetaminophen	<LOD		<LOD		16-20	<LOD	<LOD
Diclofenac	2.27	(11%)	8.62	(19%)	95-100	7.57-7.96	28.73 - 30.25
Ketoprofen	<LOD		5.60	(17%)	90-100	<LOD	18.67 - 19.65
Naproxen	<LOD		3.30	(7.5%)	95-100	<LOD	11.00 - 11.58
Indomethacin	<LOD		10.70	(2.4%)	75-97	<LOD	36.77 - 47.56
Ibuprofen	<LOD		29.00	(14.4%)	39.5 -58	<LOD	166.70 - 244.73

<LOD: Value below the detection limit of the method; SD: Standard Deviation. All samples were analyzed in triplicate with standard deviations < 20%.

the samples (Table III). According to Xagoraraki *et al.* (2008), acetaminophen may be degraded and transformed by free chlorine. The rate of this degradation is affected by pH and chlorine/acetaminophen molar ratio. The highest degradation rates were observed at pH 9.0, and the lowest degradation rates were observed at pH 6.0. In both cases, acetaminophen may be converted into the toxic byproduct, 1,4- benzoquinone.

In addition to the SPE procedures, the jar test was performed using an actual sample spiked with the evaluated drugs. Analytes were added at concentrations ranging from 35-150 ng/mL. The samples were analyzed in triplicate, with standard deviations that ranged up to 20%.

Table III shows that except for ibuprofen and ketoprofen, most of the compounds were degraded with the traditional method of treating water, following the same trend of the samples produced in the laboratory with humic acid and as reported by Cai *et al.* (2015). Diclofenac, indomethacin and naproxen are present in the sample from Guarapiranga; they are almost entirely removed in the water treatment station after the chlorination step.

Ketoprofen and ibuprofen, which were present in Guarapiranga water, were still present in the treated water at $ng.mL^{-1}$ concentrations. A Quantitative Pharmaceutical Risk Assessment (QPhRA) was carried out for these two compounds.

Quantitative Pharmaceuticals Risk Assessment (QPhRA)

Human risk assessment of the compounds that were present in the aquatic environment and those that were not removed by water treatment (ibuprofen and ketoprofen) are discussed.

The concentrations of ibuprofen (166.70 to 244.73 ng L^{-1}) and ketoprofen (18.67 to 19.65 ng L^{-1}) that remained in the treated water were used to assess the risk, though these data are not adequate to characterize the entire water treated by conventional methods.

Although these drugs were present in low concentrations, it is important to consider the duration of the exposure and the sensitive population. With chronic exposure, anti-inflammatory drugs can induce kidney damage and gastrointestinal disturbances, as well as dyspnea, nausea, vomiting, irritation and ulcer, as describe in some toxicological reviews: HSDB, 2014; ECHA, 2008 and EMA, 1995. Based on these toxicological review studies of ketoprofen and ibuprofen and considering the most restrictive toxicological values found in the literature (Tables IV and V), it was possible to determine the tolerable daily intake to find a threshold value (guideline value) to evaluate the exposure risk, according to Formula 1, previously presented in this article.

The guideline value for ibuprofen exposure (Table IV), is calculated from Formula 1, where the TDI is 0.40 mg kg^{-1} per day, BW is 70 kg, the P is 20% and C is 2 liters. per day. The result was a guideline value of 2.8 mg L^{-1} per day.

The risk for ibuprofen was estimated by dividing 244.73 x 10^{-6} mg L^{-1}, the maximum concentration found in treated water, by the established guideline value of 2.8 mg L^{-1};according to formula 2. The obtained HQ value was < 1.0.

The guideline value of 0.14 mg L^{-1} /d for ketoprofen exposure (table V) is calculated from Formula 1, assuming a TDI of 0.02 mg kg^{-1}/d, a BW of 70 kg, P of 20% and C 2 liters per day (WHO, 2011).

The risk for ketoprofen was estimated by dividing the maximum concentration found in treated water

TABLE III - Mean values of concentration of analytes added before and after the jar test procedure (two samples of each compartment with known concentration) and removal percentage of each analyte in samples of USP Olympic Streak and Guarapiranga Dam

Olympic streak (ng.mL^{-1})				Guarapiranga Dam (ng.mL^{-1})			
		Sample 1	**Sample 2**			**Sample 1**	**Sample 2**
	Before	41.5	17.5		Before	34.9	53.9
Acetaminophen	After	<LOD	<LOD	**Acetaminophen**	After	<LOD	<LOD
	Removal	100%	100%		**Removal**	100%	100%
	Before	93	48.8		Before	81.1	157
Diclofenac	After	<LOD	<LOD	**Diclofenac**	After	<LOD	<LOD
	Removal	100%	100%		**Removal**	100%	100%
	Before	63.7	36.9		Before	65.23	91.07
Ketoprofen	After	54.1	43.2	**Ketoprofen**	After	90	118
	Removal	15%	0%		**Removal**	0%	0%
	Before	65.4	34.7		Before	59.3	98.1
Naproxen	After	<LOD	<LOD	**Naproxen**	After	<LOD	<LOD
	Removal	100%	100%		**Removal**	100%	100%
	Before	54.9	25.6		Before	51.47	88.67
Indomethacin	After	<LOD	<LOD	**Indomethacin**	After	<LOD	<LOD
	Removal	100%	100%		**Removal**	100%	100%
	Before	57.66	50.17		Antes	82.7	85.5
Ibuprofen	After	37.3	50.9	**Ibuprofen**	Depois	93.8	90.23
	Removal	35%	0%		**Removal**	0%	0%

<LOD: below the detection limit of the method

(19.65 x 10^{-6} mg L^{-1}), by the established guideline value of 0.14 mg L^{-1} (Formula 2). The HQ value obtained was <1.0.

According to the guidelines and hazard quotients discussed above, it is clear that the concentrations of ibuprofen and ketoprofen found in treated water are below the exposure limit set for both.

CONCLUSION

The validation parameters of the chromatographic method used showed good linearity, accuracy and precision as well as an acceptable limit of detection and quantification for analyzing samples that contain diclofenac, ketoprofen, naproxen, indomethacin, ibuprofen and acetaminophen. The extraction method in solid phase showed linear values, low standard deviations and high precision for all samples, which made it a reliable extraction technique to focus on the analytes of interest. The removal test using the jar test values show the linear removal of the compounds in samples produced in the laboratory and in environmental samples. It is important to state that the experiment was done in a lab scale and the removal of the compounds from water may be overestimated once in a real scale there are a lot of other compounds and it is not possible to control chemical reaction for example.

Acetaminophen was not detected in the analyzed environmental samples, and spiked samples showed complete removal after the jar test. According to Xagoraraki *et al*. 2008, acetaminophen may be degraded by free chlorine, depending on the water pH and acetaminophen molar ratio, and it can be converted into the toxic byproduct 1,4-benzoquinone. Indomethacin and naproxen were detected only in water from Guarapiranga, whereas diclofenac was detected in samples from Guarapiranga and the Olympic streak. However, diclofenac showed high percentage of removal by the jar test after chlorination; therefore, it is unlikely to reach treated water.

Ketoprofen and ibuprofen peaked in water from Guarapiranga and had low or no percentage removal after treatment via jar test; thus, these two compounds are present in the treated water, as also reported by Cai *et al.* (2015). The removal methods of these compounds suggested by Melo *et al.* (2009) – ozonation and photo-Fenton - are expensive and is not part of the conventional

TABLE IV - Determination of the reference dose of ibuprofen (base value for no carcinogenic substances)

Determination of the reference dose of ibuprofen	
NOAEL	Based on the most restrictive NOAEL found in toxicity studies available, of 40 mg/kg/bw/day for one year (ECHA, 2008).
Point of departure (PD)	40 mg/kg-d
Uncertainty factors	10 to animal-human extrapolation; 10 intraspecies variability;
Uncertainty factor final (UF):	100
Reference dose TDI (based on PD/UF)	0.40 mg/kg/d
Critical effects (endpoint)	Statistical analysis revealed higher kidney weights for the animals receiving 100 mg/kg/day, which was considered to be related to dosing with the test substance. Examination of the kidneys revealed areas of cortical pitting in 9/10 receiving 100 mg/kg/day.

TABLE V - Determination of the Reference Dose of ketoprofen (base value for not carcinogenic substances)

Determination of the reference dose of ketoprofen	
NOAEL	Based on the most restrictive NOAEL found in literature: dose of 2 mg/kg/day derived from the results of the teratogenicity toxicity study in rabbits (EMA, 1995).
Point of departure (PD)	2 mg/kg-d
Uncertainty factors	10 animal to human; 10 sensitive population
Uncertainty factor final (UF):	100
Reference dose (based on PD/UF)	0.02 mg/kg/d
Critical effects (endpoint)	In rabbits, ketoprofen was maternotoxic for doses higher than 2 mg/kg b.w./day after oral administration.

method of treatment performed in the state of São Paulo.

Based on the guideline value and hazard quotient, the results suggest human risk only in concentrations higher than 2.8 mg L^{-1} and 0.14 mg L^{-1} for ibuprofen and ketoprofen, respectively; therefore, no significant risk to humans is indicated at the concentrations found. Other authors, such as Schwab *et al.* (2005) and Webb *et al.* (2003), also reported no risk or a low possibility of health risk due to exposure to these pharmaceuticals. In this study, risk assessment based on the mixture of chemicals was not reported.

Although indomethacin, diclofenac and naproxen do not show peaks in the chromatograms after water treatment, it does not necessarily mean that they are completely eliminated. Reaction with the oxidation products may be added to the water generated by-products, which are known as "disinfection byproducts" and are often more toxic than their precursors.

Because some of these compounds, such as acetaminophen and diclofenac, react with the oxidizing agent used in water treatment plants, disinfection products may be produced, which are reportedly more toxic than their precursors (Bedner, Maccrehan, 2006; Xangoraraki *et al.,* 2008). Therefore, it is recommended to qualitatively and quantitatively evaluate the formation of these products after the chlorination process to assess human risk from consuming this water.

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