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Occurrence of non-steroidal anti-inflammatory drugs in Tehran source water, municipal and hospital wastewaters, and their ecotoxicological risk assessment

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Abstract Pharmaceuticals are becoming widely distributed in waters and wastewaters and pose a serious threat to public health. The present study aimed to analyze non-steroidal anti-inflammatory drugs (NSAIDs) in surface waters, drinking water, and wastewater in Tehran, Iran. Thirty-six samples were collected from surface waters, tap water, and influent and effluent of municipal and hospital wastewater treatment plants (WWTP). A solid-phase extraction (SPE) followed by liquid chromatography–tandem mass spectrometry method was used for the determination of pharmaceuticals, namely ibuprofen (IBP), naproxen (NPX), diclofenac (DIC), and indomethacin (IDM). IBP was found in most of the samples and had the highest concentration. The highest concentrations of NSAIDs were found in the municipal WWTP influents and hospital WWTP effluents. In the municipal WWTP influent samples, the concentrations of IBP, NPX, DIC, and IDM were 1.05, 0.43, 0.23, and 0.11 μg/L, respectively. DIC was found only in one river sample. All NSAIDs were detected in tap water samples. However, their concentration was very low and the maximum values for IBP, NPX, DIC, and IDM were 47, 39, 24, and 37 ng/L, respectively, in tap water samples. Results showed that the measured pharmaceuticals were detected in all rivers with low concentrations in nanograms per liter range, except DIC which was found only in one river. Furthermore, this study showed that the aforementioned pharmaceuticals are not completely removed during their passage through WWTPs. A potential environmental risk of selected NSAIDs for the urban wastewater has been discussed. However, given their low measured concentrations, no ecotoxicological effect is suspected to occur.

Keywords Non-steroidal anti-inflammatory drugs · Monitoring · Surface waters · Wastewater · Tehran · Iran · Liquid chromatography–tandem mass spectrometry · Risk assessment

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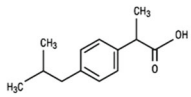
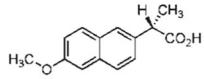
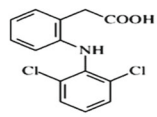
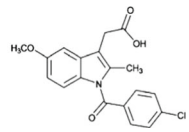
Introduction

In the last two decades, the occurrence of pharmaceuticals in aquatic environments has become an issue of international attention which attracted the scientific community (Daneshvar et al. 2010). Pharmaceuticals are a large class of chemical contaminants which may originate from human usage and excretions, such as over-the-counter and prescription medications. These materials after disposal to municipal sewage systems find their way to the aquatic environment and groundwater aquifers (Ziylan and Ince 2011; Glen R. Boyd et al. 2003). Although human and animal excretions are among the most important associated source of pharmaceutically active compounds (PhACs), other sources such as emission from production sites, manufacture spill accidents, septic tanks, aquaculture, direct disposal of surplus drugs in households, underground leakage from sewage infrastructures, therapeutic treatment of livestock on fields, and effluents from farms are of significance, as well (Zwiener and Frimmel 2000; Clara et al. 2005; Kosjek et al. 2007; Ziylan and Ince 2011). The removal of many pharmaceuticals in sewage treatment plants (STP) is often incomplete, and these are directly discharged to water resources (Andreozzi et al. 2003). As a consequence, the presence of pharmaceuticals has become ubiquitous in natural waters, even to the extent of entering drinking water facilities which can affect water quality and public health (Santiago-Morales et al. 2013).

Among pharmaceuticals, non-steroidal anti-inflammatory drugs (NSAIDs) are the most frequently detected medicines, and their environmental distribution is widespread (Weigel et al. 2004; Gentili 2007). The annual production of NSAIDs is estimated to be several kilotons (Cleuvers 2004). They produce their therapeutic activities through inhibition of cyclooxygenase (COX), the enzyme that makes prostaglandins (PGs) which causes swelling and pain and are used commonly in the treatment of symptoms like inflammation, pain, and fever (Vane and Botting 1998; Hernando et al. 2006). The molecular structures, physical and chemical properties of selected NSAIDs are presented in Table 1. Based on selected characteristic properties these drugs have ability to be persistent in the aqueous environment. The bioconcentration factor (BCF) of all drugs is low and ranges between 1 and 1.31. This is means that the bioaccumulation potential of the chemicals is low. Log K_{ow} (octanol–water partition coefficient) describes the drug lipophilicity and represents the ability of drug to pass into lipid-rich zones from aqueous environment. Log K_{ow} is related to water solubility, soil/sediment adsorption coefficients, and bioconcentration factors for aquatic life. All selected drug has narrow Log K_{ow} ranges located between 3 and 4.5 (Singh et al. 2014). The PSA (polar surface area) affects on molecular transportation through membranes and, thus, allows an estimation of the apparent volume of distribution in the body (Fatemi and Ghorbannezhad 2011).

Ibuprofen (IBP; (rac)-2-(4-isobutylphenyl)propionic acid) is among the most widely used medicines (third most popular drug) in the world, with an annual global production of several kilotons (Ali et al. 2009). A significant degree of ingested IBP (70–80 % of the therapeutic dose) is excreted as the parent compound (free or conjugated) or in the form of metabolites (Buser et al. 1999). The physiochemical properties of IBP led to high mobility in aquatic environments, which was identified up to 2370 μg/L in surface water in the UK (Ali et al. 2009). Naproxen (NPX; 6-methoxy-α-methyl-2-naphthalene acetic acid) is a non-steroidal anti-inflammatory drug used to treat mild-to-moderate pain, inflammation, rheumatoid arthritis, psoriaticarthritis, and gout (Suzuki et al. 2014). NPX has a dissociation constant of 4.2 to 4.9 that led to high mobility in the natural aquatic environment (Suzuki et al. 2014). NPX has been detected in the range of 0.1–2.6 μg/L in wastewater treatment plant effluents and 0.01–0.1 μg/L in surface waters (Glen Raul Boyd et al. 2005; Tixier et al. 2003). Diclofenac (2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid) is a common NSAIDs which is used in the form of oral tablets or topical gel. During the revision of the Water Framework Directive (2000/60/EC) in the European Union, it was proposed to classify diclofenac (DIC), 17-α-ethynilestradiol and 17-β-estradiol as priority substances (Vieno and Sillanpää 2014; EU 2013). Indomethacin (IDM) is another polar compound which is poorly removed in the activated sludge conventional treatment and has been reported at concentration of 42 and 17 ng/L in the influent and effluent of the STP, respectively, in Alcalá de Henares (Madrid) (Rosal et al. 2010). According to Tran et al. (2014), more than 80 PhACs from different prescription classes were found at concentrations up to the milligrams per liter level in sewage, surface, and groundwater at various locations in Europe and the USA. However, a little information is available on pharmaceuticals occurrence in developing countries like Iran where pharmaceuticals are rapidly

Table 1 Physical and chemical properties of the selected NSAIDs (Ziylan and Ince 2011; Scheytt et al. 2005; Limnell et al. 2011; Tran et al. 2014; Yamamoto et al. 2007; Singh et al. 2014)

Compound	IBP	NPX	DIC	IDM
Chemical structure				
CAS RN	15687-27-1	2204-53-1	15307-86-5	53-86-1
Molecular formula	$C_{13}H_{18}O_2$	$C_{14}H_{14}O_3$	$C_{14}H_{11}Cl_2NO_2$	$C_{19}H_{16}ClNO_4$
Molec. weight (g/m)	206.3	230.3	296.2	357.79
Vapor pressure (mmHg)	1.86×10^{-4}	1.27×10^{-6}	6.14×10^{-8}	5.12×10^{-10}
Solubility (in water) (mg/L)	21	15.9	2.37	9
pK_a(20 °C)	3.5−4.9	4.2−4.9	4.1−4.5	4.5
logK_{ow}	3.84	3	4.27	4.51
Henry's constant (at m^3/m)	1.5×10^{-7}	3.4×10^{-10}	3.13×10^{-14}	4.7×10^{-12}
$t_{1/2}$[a]	360	360	900	900
BCF[a]	1	1	1.31	1.31
Log *D* (pH = 5.5)[b]	2.60	2.14	3.21	2.23
PSA[b]	37.30	46.53	49.33	68.53

Log D distribution coefficient, *BCF* bioconcentration factor, *PSA* polar surface area, $t_{1/2}$ half-life in water

[a]www.chemspider.com

[b]www.chemicalize.org

growing, and environmental regulations are not very well established.

To the best of our knowledge, there is no report on environmental pharmaceutical contamination investigations in Iran, including water samples, hospital effluents, WWTPs influents and effluents, river waters and tap waters. In this context, pharmaceuticals monitoring studies in Iran aquatic environments are required, entailing different type of environmental samples. From the perspective of a geographic area, it is the first study to report on the occurrence of these compounds in Iran. This study aimed to use a developed analytical method to evaluate the presence of NSAIDs (IBP, NPX, DIC, and IDM) in the aquatic environment in order to provide an overview of the occurrence of NSAIDs (IBP, NPX, DIC, and IDM) in Tehran, Iran. An environmental risk assessment for wastewater samples based on measured environmental concentration (MEC) and predicted no-effect concentration (PNEC) has been also carried out.

Materials and methods

Chemicals and materials

Pharmaceuticals (IBP, NPX, DIC, and IDM) were purchased from Hakim Pharmaceutical Co. (Tehran, Iran) and were of analytical grade, with purity of 98 % or higher. Stock standard solutions of pharmaceuticals were prepared in methanol (HPLC grade, Merck) at 100 mg/L and were stored at −20 °C. Working standard solutions were daily prepared from the stock standard solution using methanol as solvent and kept at 4 °C prior to analysis. Amber glassware was used to prevent light degradation of pharmaceuticals.

Geographical characterization and sample collection

A total of 36 water samples were analyzed in order to evaluate the occurrence of NSAIDs in Tehran, Iran. Tehran is the capital of Iran and has a population of approximately 8 million people. Eight rivers, 4

influents, and 4 effluents of WWTPs, 3 inlet water treatment plants (WTPs), 2 hospital effluents, and 21 tap waters in Tehran (Fig. 1) were studied. Table 2 presents a summary of the characteristics of the studied locations. Composite samples corresponding to 24 h were collected from influents and effluents of WWTPs and hospital effluents; however, grab samples were taken from rivers and tap waters.

For tap water samples, Tehran City was divided into five districts (north, south, west, east, and central) and three grab samples were collected from each district (one more sample from the central districts). The populations served by WWTP1, WWTP2, WWTP3, and WWTP4 were 100,000, 85,000, 2000, and 30,000, respectively. All rivers (except Karaj River) pass through different regions of Tehran City including the densely populated areas. The Jalalie water treatment plant uses the Karaj River water as one of the raw water sources. Effluent samples of two general hospitals at the central part of Tehran were also collected. Tehran has five water treatment plants with three sources of water. Therefore, samples were collected from three sources of water at the plant intake, prior to any water treatment process.

All samples were collected in 0.5-L amber glass bottles with screw cap. For tap water samples, excess quenching agent (sodium thiosulfate) was added to dechlorinate the sample. Sampling campaigns were performed in July 2014. Sample collection, preservation, and storage were done according to the US EPA Method Guideline (USEPA 2007). The samples were immediately transported to the laboratory and pH of the samples was adjusted to 2 with 6 M HCl and kept at 4 °C. They were filtered through 1.2-μm glass microfiber filters (GF/C, Whatman, UK), followed by 0.20-μm nylon membrane filters (Millipore Co., Durapore, Ireland) in order to remove particulate matter and colloids. The samples were then extracted by using SPE during 24–72 h after sampling.

Analytical procedure

Extraction was conducted using SPE C18 classic cartridges (1000 mg) from Waters. The SPE procedure was adapted from Hernando et al. (2006). Briefly, conditioning of cartridges was performed with 12 mL of methanol followed by 10 mL of deionized water (HPLC grade). The volume load for all samples was 240 mL. Before SPE, internal standard was added to all samples at a concentration of 1 μg/L. The flow rate in the loading step of the samples was 30 mL/min. The cartridges were washed with 10 mL deionized water, while drying (30 min) was performed by vacuum prior to elution with 1 × 15 mL of MeOH. The obtained extract was evaporated to dryness under a gentle stream of nitrogen and reconstituted in 1 mL of acetonitrile for further analysis. The sample extracts were preserved at −20 °C prior to the analysis by LC-MS/MS. Finally, 10 μL of each sample was injected into LC-MS/MS.

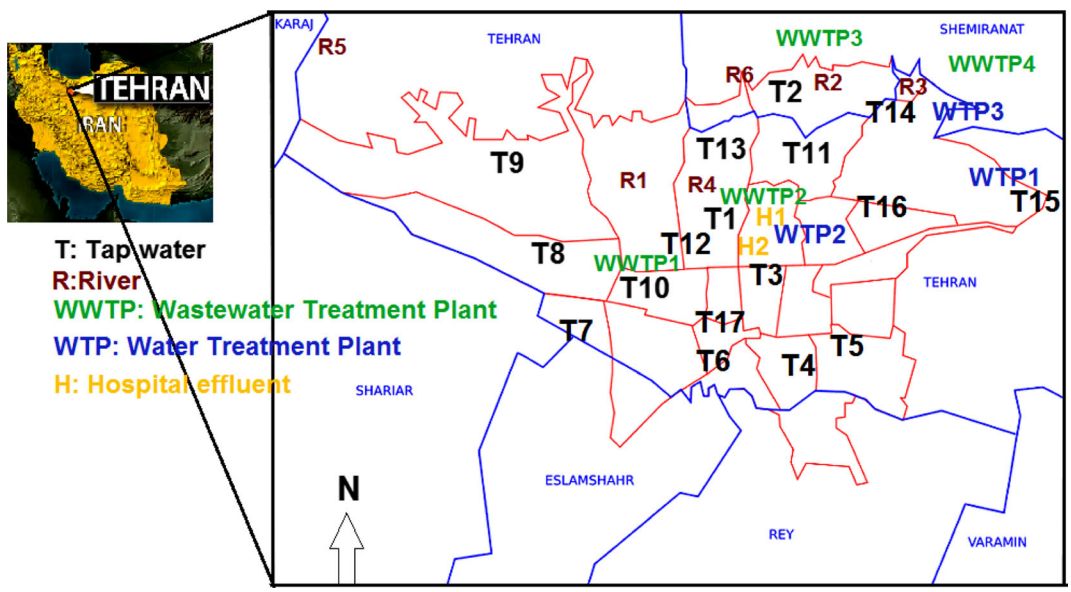


Fig. 1 Location and numbering of sampling points in the Tehran, Iran

Chromatographic separation was performed on a "Simmetry" C-18 reversed phase column 150 × 2.1 mm i.d. with 3 μm packing (Waters). The flow rate of the mobile phase was 0.2 mL/min. Mobile phase were MeOH and HPLC-grade water containing 2 % (*v*/*v*) ammonium format (pH = 8) and the linear gradient was from 20 to 100 % of solvent A (MeOH) within 40 min, at a flow rate of 0.2 mL/min. MS analysis was performed using a Micromass Quattro triple quadrupole mass spectrometer equipped with a Z-spray electrospray interface (Manchester, UK) in negative-ion mode. Instrument control, data acquisition, and evaluation were carried out with Masslynx NT software (version 3.4). The operating parameters were as follows: electrospray source block and desolvation temperature (100 and 300 °C), capillary and cone voltages (3.5 kV and 20 V), argon collision gas (2.5×10^{-3} mbar), cone nitrogen gas flow and desolvation gas (109 and 508 L/h). A total of 200 μL/min of the LC column effluent was diverted to the ESI source.

Validation studies of the analytical method

Validation procedures were performed as per an in-house standard operating procedure (SOP), a document based on the FDA Guidance for Bioanalytical Method Validation (Chan et al. 2004). The following assessments were carried out: sensitivity and lower limit of quantification (LLOQ), selectivity, matrix effects, calibration curve suitability, intra- and inter-assay precision and accuracy, carryover effect, extraction yield, dilution integrity, reproducibility of response, and interference from degradation of target compound metabolites. Furthermore, the following stability studies were performed: stock, working and spiking solution stability, freeze–thaw stability, post preparative stability, short- and long-term storage stability. The stability of stock, working, and spiking solutions was evaluated for 2 weeks. To do this, the stock, spiking, and working solutions of the analytes and I.S. were analyzed at the time of preparation. Next, the solutions were stored at −20 °C for 14 days and injected on 10 occasions during the observation period. After exposure to different conditions of temperature and time, the stabilities of all the analytes in real matrices were investigated by analyzing spiked samples at three concentration levels in triplicate. The results were compared with those for freshly prepared QC samples, and the percentage concentration deviation was calculated. For short-term stability, the spiked samples were kept at room temperature (25 °C) for 4 and 12 h before sample preparation. The stability was also evaluated after storage of the spiked samples at −70 °C for 60 days. The freeze–thaw stability test was performed over three freeze–thaw cycles for 4 days. In each freeze–thaw cycle, the spiked samples were frozen for 24 h at −70 °C and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for the next 24 h at −70 °C. During each cycle, a triplicate of 1 mL aliquots were analyzed and the results were averaged.

Potential environmental risk

Environmental risk of each pharmaceutical compound was evaluated by calculating the risk quotient values (RQ) obtained from the measured environmental concentrations (MEC) and the predicted no effect concentrations (PNEC). PNEC values were estimated from the lowest toxicity data of several aquatic organisms: *Vibrio fischeri* (bacteria), algae, and *Daphenia magna* species reported in the literature. PNEC values were estimated to be 1000 times lower than the toxic concentration reported for the most sensitive species assayed to consider the toxicity of the other aquatic species which are more sensitive than those used in toxicity studies and allows accounting for extrapolation from intra- and inter-species variability in sensitivity (Paíga et al. 2013; Camacho-Muñoz et al. 2010). RQ values equal or higher than 1 imply significant ecotoxicological risk to aquatic organisms, whereas RQ lower than 1 indicates no risk (Camacho-Muñoz et al. 2010; Paíga et al. 2013; Ginebreda et al. 2010).

Results and discussion

Analytical method validation

In order to construct the calibration curves and make quality control samples (QCs), distilled water was used as a blank matrix. To check for any possible matrix effect due to the differences between the real and blank samples, a real water sample (mixture of surface and tap water 50:50) was selected and for each analyte the calibration curves were constructed in real and blank samples. Thereafter, the slope sand intercepts of the acquired calibration equations were compared (*t* test, $\alpha = 0.05$). In all cases, no

Table 2 Characterization and geographical localization of the surface waters, WWTPs, hospitals, and tap waters

No.	Sample	Location	GPS coordinates	
1	T1	Fatemi Square	35° 43′ 08″ N	51° 21′ 25″ E
2	T2	SBM University	35° 47′ 60″ N	51° 23′ 38″ E
3	T3	Hor Square	35° 41′ 18″ N	51° 23′ 35″ E
4	T4	Bokharayi Street	35° 38′ 48″ N	51° 25′ 20″ E
5	T5	Azadegan Park	35° 37′ 53″ N	51° 25′ 42″ E
6	T6	Cheraghi–Kazemi–Saeedi	35° 38′ 20″ N	51° 21′ 16″ E
7	T7	Khalij e fars–Azadegan	35° 38′ 22″ N	51° 15′ 43″ E
8	T8	Azadegan–Karaj Road	35° 41′ 40″ N	51° 14′ 43″ E
9	T9	Shahidbaqeri Town	35° 45′ 17″ N	51° 13′ 09″ E
10	T10	Shahran Second Square	35° 45′ 45″ N	51° 17′ 23″ E
11	T11	Africa Boulevard	35° 46′ 11″ N	51° 25′ 13″ E
12	T12	Sadeqyeh	35° 43′ 42″ N	51° 20′ 05″ E
13	T13	Pounak Square	35° 45′ 43″ N	51° 20′ 09″ E
14	T14	Aqdasyeh	35° 48′ 02″ N	51° 28′ 59″ E
15	T15	Hakimyeh	35° 43′ 21″ N	51° 35′ 54″ E
16	T16	Resalat (Narmak)	35° 44′ 10″ N	51° 29′ 34″ E
17	T17	Yaftabad crossroads	35° 39′ 39″ N	51° 20′ 45″ E
18	WTP1	Tehranpars water treatment plant	35° 44′ 40″ N	51° 35′ 12″ E
19	WTP2	Jalalyeh water treatment plant inlet	35° 42′ 44″ N	51° 23′ 47″ E
20	WTP3	Sohanak water treatment plant inlet	35° 47′ 36″ N	51° 31′ 51″ E
21	R1	Kan River	35° 44′ 46″ N	51° 15′ 54″ E
22	R2	Darband River	35° 48′ 24″ N	51° 25′ 43″ E
23	R3	Darabad River	35° 49′ 0.4″ N	51° 28′ 55″ E
24	R4	Farahzadi River	35° 45′ 09 ″ N	51° 20′ 30″ E
25	R5	Karaj River	35° 49′ 47″ N	51° 02′ 22″ E
26	R6	Darake River	35° 42′ 24″ N	51° 23′ 25″ E
27	$WWTP1_{in}$	Ekbatan wastewater treatment plant inlet	35° 42′ 2.5″ N	51° 18′ 33″ E
28	$WWTP1_{ef}$	Ekbatan wastewater treatment plant outlet	35° 42′ 3.3″ N	51° 18′ 32″ E
29	$WWTP2_{in}$	Shahrake Gharb wastewater treatment plant inlet	35° 44′ 54″ N	51° 22′ 3.3″E
30	$WWTP2_{ef}$	Shahrake Gharb wastewater treatment plant outlet	35° 44′ 46″ N	51° 22′ 06″ E
31	$WWTP3_{in}$	Sahebqeranyeh wastewater treatment plant inlet	35° 48′ 22″ N	51° 28′ 23″ E
32	$WWTP3_{ef}$	Sahebqeranyeh wastewater treatment plant outlet	35° 48′ 22″ N	51° 28′ 23″ E
33	$WWTP4_{in}$	Mahallati wastewater treatment plant inlet	35° 48′ 3.7″ N	51° 30′ 23″ E
34	$WWTP4_{ef}$	Mahallati wastewater treatment plant outlet	35° 48′ 2.6″ N	51° 30′ 21″ E
35	H1	EmamKhomayni Hospital wastewater treatment plant	35° 42′ 29″ N	51° 22′ 58″ E
36	H2	Shariati Hospital wastewater treatment plant	35° 43′ 15″ N	51° 23′ 09″ E

T tap water, *R* river, *H* hospital WWTPs

significant differences were observed, which indicates similar analytical responses for real and blank matrices. The calibration curve parameters were obtained under the optimized condition. Linearity of the calibration curves was determined to be in the range of 0.01–2 μg/L for all the analytes. Coefficients of estimation ranged from 0.958 to 0.975. The limit of detection (LOD) was calculated as three times the baseline noise (S/N = 3), after 5 successive extractions of blank samples. According to the International Conference on Harmonization of Technical Requirements for Analytical Methods

(ICH) criteria for analytical method validation, the limit of quantification (LOQ) for each analyte was determined as the lowest concentration on the calibration curve with a precision of less than 20 % coefficient of variation (CV%) and an accuracy of 80 to 120 % (Chan et al. 2004). The corresponding LOQs for IBP, NPX, DIC, and IDM were 0.02, 0.03, 0.02, and 0.02 μg/L, respectively, which indicates sensitivity of the method. As mentioned above, the LOD and LOQ for each analyte were also determined in a real blank matrix and no significant differences were found (t test, $\alpha = 0.05$). The precision of the method was evaluated in terms of repeatability (or interday precision) by calculating the analyte concentrations inequality control samples, prepared at three levels (each six replicates) on three consecutive days. Interday precision values for all the analytes were always <12 %. Expression of the intraday precision is based on the coefficient of variation (CV%) of determined responses of six replicates of quality control (QC) samples, which were prepared at three levels and reported in Table 3. The estimated recoveries at three different concentration levels are also shown in Table 3. To determine the recoveries, mean peak area of each analyte at each concentration level was determined for final extracts of blank water samples spiked with the analytes and compared with that of standard solutions at the same concentrations after correction for volume changes. All these results indicate the feasibility and reliability of the developed method for determining target analytes in water samples.

Stability studies

The stability of target analytes was assessed under different conditions. All the analytes showed similar behaviors. Therefore, to avoid presenting a long list of similar quantities, the result for IBP is summarized in Table 4. Upon storage of samples kept frozen at −70 °C for 2 months and during three freeze–thaw cycles, reliable stability behaviors of all the analytes were observed (all within ±12 %). The stability studies indicated acceptable variations in the analytes concentrations over a span of 4 and 12 h, at room temperature. Therefore, the final samples can be handled under normal laboratory conditions without incurring any significant loss of detection.

Occurrence of pharmaceuticals in Tehran water sources

The NSAIDs pharmaceuticals group is one of the most studied in view of occurrence and removal rates in WWTPs, due to high consumption rates. An example of a chromatogram obtained by LC-MS/MS for the target analytes is presented in Fig. 2. The chromatograms include (I) total ion

Table 3 Estimated recoveries and method precision for target analytes at different concentrations ($n = 6$) in QC samples

Compound	Sample	Nominal conc. (μg/L)	Mean of calc. conc.[a] (μg/L)	CV% of calc. conc.	RE% of calc. conc.[b]	Recoveries (%)	CV% recovery
IBP	QC1	0.050	0.044	11.3 %	12 %	81 %	13.2 %
	QC2	0.250	0.222	9.4 %	11.2 %	85 %	10.3 %
	QC3	1.500	1.416	7.5 %	5.6 %	88 %	8.6 %
NPX	QC1	0.050	0.046	12.2 %	8 %	82 %	12.4 %
	QC2	0.250	0.231	9.2 %	7.6 %	87 %	10.5 %
	QC3	1.500	1.421	8.3 %	5.3 %	91 %	8.7 %
DIC	QC1	0.050	0.046	12.7 %	8 %	78 %	13.1 %
	QC2	0.250	0.228	10.2 %	8.8 %	84 %	11.2 %
	QC3	1.500	1.451	7.3 %	3.3 %	88 %	9.3 %
IDM	QC1	0.050	0.045	11.6 %	10 %	82 %	11.4 %
	QC2	0.250	0.236	8.4 %	5.6 %	86 %	9.2 %
	QC3	1.500	1.438	6.7 %	4.1 %	92 %	8.4 %

[a] Calculated concentration

[b] Relative error = [1 − (calculated conc./nominal conc.)] × 100

Table 4 Stability of target analytes in spiked real samples

Nominal concentration (μg/mL)	Determined concentration (μg/mL)	Precision (CV%)	Accuracy (%)
Short-term stability (4 h)			
0.10	0.081 ± 0.009	11.25	80.67
0.50	0.452 ± 0.023	5.15	90.39
1.00	0.896 ± 0.037	4.10	89.59
Short-term stability (12 h)			
0.10	0.081 ± 0.008	9.29	80.67
0.50	0.419 ± 0.016	3.87	83.72
1.00	0.090 ± 0.024	2.69	90.02
Freeze–thaw stability			
0.10	0.093 ± 0.011	11.84	92.67
0.50	0.462 ± 0.023	4.98	92.39
1.00	0.930 ± 0.030	3.22	93.00
Long-term stability			
0.10	0.092 ± 0.10	11.13	91.69
0.50	0.464 ± 0.020	4.33	92.79
1.00	0.964 ± 0.058	5.96	96.39

chromatogram, (II) NPX, (III) DIC, (IV) IBP, (V) IBP-d3 (IS), and (VI) IDM, all the analytes were dissolved in acetonitrile. The concentration of all chromatograms was 10 μg/L. The target pharmaceuticals showed the same behavior in terms of occurrence and removal rates as reported in previous literatures (Quintana and Reemtsma 2004; Miao et al. 2002; Hernando et al. 2006).

The occurrence of non-steroidal anti-inflammatory drugs (IBP, NPX, DIC, and IDM) was investigated in Tehran source waters, including several rivers, wastewater treatment plants, hospital effluents, and tap waters (Table 5). The range of concentrations was between non-detection and several nanograms per liter. In general, Tehran has five water treatment plants with three inlet water sources, and sampling was carried out in each one. Analysis of the samples collected from the three inlet water treatment plants showed that the target pharmaceuticals only existed in one of them with the exception of NPX. The raw sources for drinking water production mainly include surface water from the Latial, Amirkabir, and Lar dams. Since, inlet of treatment plants is from dams, most of the entering pharmaceuticals become diluted to very low concentration, and they are eliminated in the lake by hydrolysis, sorption, biodegradation, and photolytic degradation. The concentration of NSAIDs in lake or dams was low at the time of sampling (summer), probably because of high temperature and low water flow rate which are the two most important factors. Our results are in agreement with the values reported for the anti-inflammatory drugs in the lake of Haapajärvi, Southeastern Finland (Togunde et al. 2012).

The frequency of detection in tap water samples were 41, 23, 6, and 35 % for IBP, NPX, DIC, and IDM, respectively. Ibuprofen showed higher concentrations and frequency among other compounds. Although, the target pharmaceuticals were not detected in the two inlets of water treatment plants, several detections have been made in the distribution systems. It is because besides surface water, there are several groundwater wells from which water is disinfected and introduced to water storage reservoirs and transferred to a distribution system. Several pharmaceuticals and organic pollutants were found at maximum concentration levels in groundwater wells that were used as source water for drinking (Heberer et al. 1998; Barnes et al. 2008). The infiltration of contaminants from surface water, leaks from landfills and sewer drains may probably contribute to the presence of NSAIDs in groundwaters and water

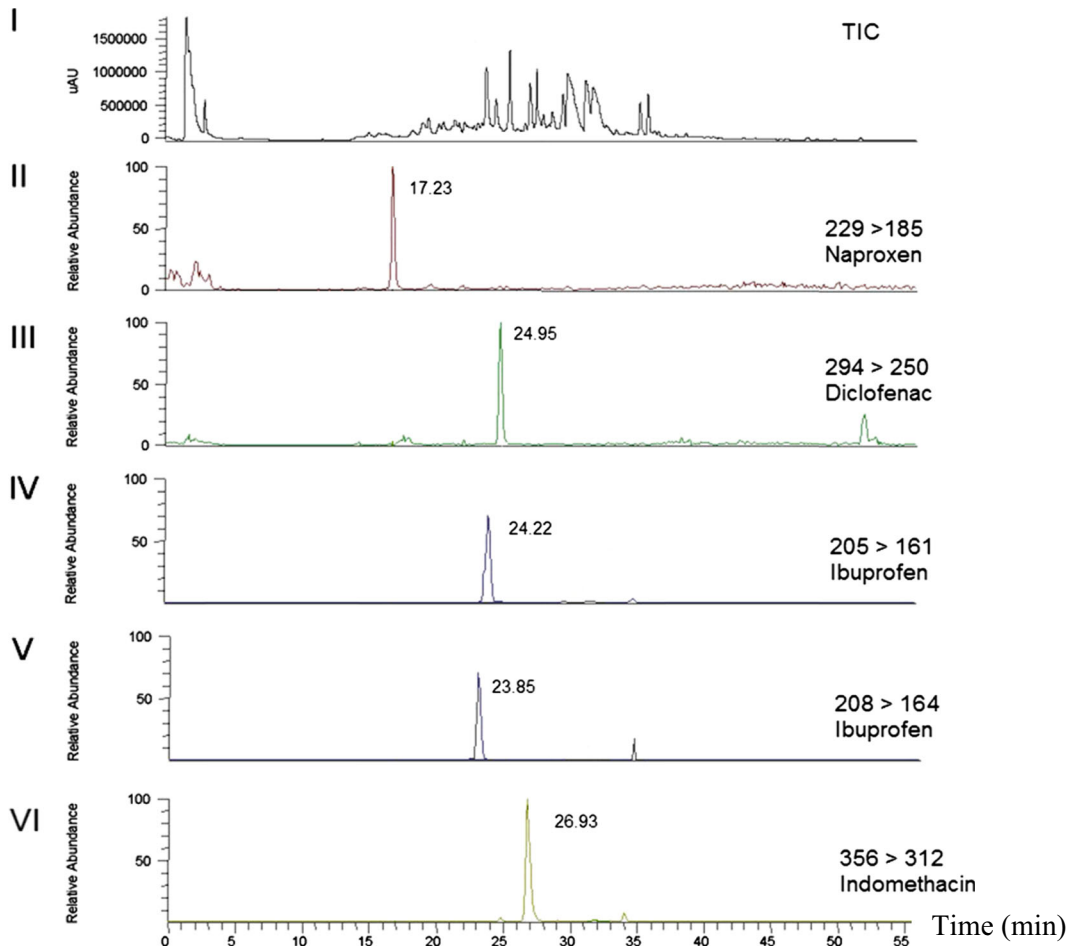


Fig. 2 Extracted multiple reaction monitoring (MRM) chromatograms for target pharmaceuticals in a real sample

from wells (Huerta-Fontela et al. 2011). It is worth mentioning that DIC, IPB, and NPX were detected in concentration up to 96 ng/g in soil and sludge samples in Poland (Kumirska et al. 2015). These pharmaceuticals in soil matrices can further penetrate to the groundwater source. However, drinking water may also be polluted directly by pharmaceuticals disposed into wastewater through infiltration in distribution systems. The obtained concentrations are similar to those of Kosjek et al. (2007), who studied these compounds in Slovene rivers and potable water. They did not detect traces of NSAIDs in any potable water samples. Lin et al. (2009) reported the concentration of IBP in tap water in the range of 1–8 ng/L. However, in the present study, NSAIDs were found in some samples at low-range contamination (ng/L).

Results of the occurrence of pharmaceuticals through the four WWTPs are shown in Table 5. All pharmaceuticals were detected in the WWTPs influents and effluents studied. The unit operations for all municipal WWTPs were extended aeration with the exception of Ekbatan (WWTP1) that was A_2O processes. In general, the pharmaceuticals (IBP, DIC, NPX, and IDM) were detected at low-level concentrations in influents and effluents. However, IBP and DIC were detected at higher concentrations. According to Iranian Administration of Health, the consumptions of IBP, NPX, DIC, and IDM were 389, 65, 37.7, and 5.8 t, respectively, in 2013 for the entire country. IBP was found at a high concentration (up to 1000.05 ng/L) which is probably due to their high consumption and high excretion as the parent compound (70–80 % of the therapeutic dose in the case of ibuprofen) (Buser et al. 1999). The high concentration of DIC is probably associated with its use in human and veterinary medicine and in every possible route of administration from oral

Table 5 Occurrence and levels (μg/L) of NSAIDs in samples

No.	Sample	Concentration (μg/L)			
		IBP	NPX	DIC	IDM
1	T1	nd	nd	nd	nd
2	T2	nd	nd	nd	nd
3	T3	0.035	0.037	nd	0.027
4	T4	nd	nd	nd	nd
5	T5	0.047	0.039	nd	0.034
6	T6	nd	nd	nd	nd
7	T7	nd	0.039	nd	0.035
8	T8	0.022	nd	nd	nd
9	T9	nd	nd	nd	nd
10	T10	0.045	0.037	0.024	0.037
11	T11	nd	nd	nd	nd
12	T12	nd	nd	nd	0.022
13	T13	0.037	nd	nd	nd
14	T14	0.023	nd	nd	0.021
15	T15	nd	nd	nd	nd
16	T16	nd	nd	nd	nd
17	T17	0.025	nd	nd	nd
18	T18	nd	nd	nd	nd
19	WTP1	nd	nd	nd	nd
20	WTP2	0.021	nd	nd	nd
21	WTP3	0.035	nd	0.022	0.025
22	R1	0.029	0.041	0.025	0.041
23	R2	0.029	0.033	nd	0.025
24	R3	0.031	nd	nd	0.025
25	R4	0.022	nd	nd	0.028
26	R5	0.037	0.029	nd	0.026
27	$WWTP1_{in}$	1.051	0.43	0.23	0.11
28	$WWTP1_{ef}$	0.043	0.042	0.033	0.057
29	$WWTP2_{in}$	0.849	0.34	0.20	0.096
30	$WWTP2_{ef}$	0.045	0.054	0.033	0.039
31	$WWTP3_{in}$	0.233	0.088	0.044	0.039
32	$WWTP3_{ef}$	0.031	0.037	0.024	0.028
33	$WWTP4_{in}$	0.437	0214	0.12	0.087
34	$WWTP4_{ef}$	0.035	0.033	0.022	0.029
35	H1	0.141	0.092	0.027	0.025
36	H2	0.29	0.084	0.077	0.039

nd not detected

to intramuscular (Buser et al. 1998). The lower concentration of NPX may contribute to its high biodegradability which can be considered as the possible elimination mechanism for this drug (Tixier et al. 2003). Our results are comparable to the results from other studies (Buser et al. 1999; Metcalfe et al. 2003; Rosal et al. 2010; Kim et al. 2007) which showed the concentration of selected drugs in WWTPs influents and effluents. A significant portion of PhACs (30 to 90 %) are excreted unchanged or with their metabolites in urine and feces, which enter into the sewage network and then the municipal wastewater treatment plants (WWTPs) (Metcalfe et al. 2003). With passage of these compounds through the WWTPs, their concentration becomes significantly reduced. The values are shown in Fig. 3. In this study, the average removal rates for IBP, NPX, DIC, and IDM were 92, 79, 73, and 50 %, respectively, which are in the range of most previous reports. The WWTP3 showed a lower removal rate of pharmaceuticals which may be due to old facility (more than 20 years operation) and the location which is in a relatively cold climate zone. The removal rates for IBP, NPX, DIC, and IDM vary between 75 and 98 % (Jones et al. 2007; Castiglioni et al. 2006), 50–98 % (Kosjek et al. 2007; Fent et al. 2006), 0–90 % (Gómez et al. 2007; Lindqvist et al. 2005; Zorita et al. 2009), and 11–33 % (Jelic et al. 2011; Rosal et al. 2010), respectively. In general, a removal efficiency between 48 and 98 % was reported for NSAIDs in WWTPs (Lin et al. 2009; Gros et al. 2007). The highest reduction in IBP and NPX concentration during the biological treatment processes is in agreement with previous literature (Jones et al. 2007). However, the performance of activated sludge treatment for breakdown of pharmaceuticals varies from complete to very poor degradation (Kulik et al. 2008). There are two elimination processes that generally contribute to wastewater treatment and contain acidic pharmaceuticals such as

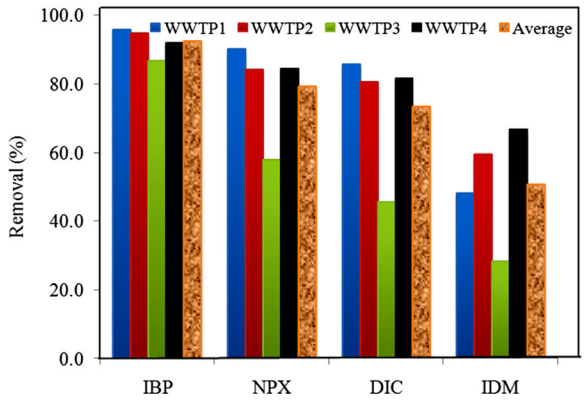


Fig. 3 Percentage removal of pharmaceuticals through WWTP (Calculated using (influent concentration − effluent concentration)/influent concentration × 100)

Table 6 PNEC values calculated from ecotoxicological studies reported in the literature and RQ values for WWTP1

Pharmaceutical compound	Species	EC_{50} (mg/L)	PNEC (μg/L)	Reference	Risk quotients (RQ) for WWTP1
IBP	*Vibrio fischeri* (bacteria)	37.5	37.5	Camacho-Muñoz et al. 2010	0.028
	Daphnia magna	9.06	9.06	Jones et al. 2002	0.116
	Algae	5.7	5.7	Paíga et al. 2013	0.184
NPX	*Vibrio fischeri* (bacteria)	21.2	21.2	Camacho-Muñoz et al. 2010	0.02
	Daphnia magna	25	25	Boström and Berglund 2015	0.017
	Algae	626	626	Camacho-Muñoz et al. 2010	0.001
DIC	*Vibrio fischeri* (bacteria)	11.4	11.4	Camacho-Muñoz et al. 2010	0.020
	Daphnia magna	20	20	Ferrari et al. 2004	0.012
	Algae	14.5	14.5	Ferrari et al. 2004	0.015
IDM	*Vibrio fischeri* (bacteria)	29	29	Yamamoto et al. 2007	0.004
	Daphnia magna	22	22	Yamamoto et al. 2007	0.005
	Algae	39	39	Yamamoto et al. 2007	0.003

NSAIDs: adsorption of all acidic compounds (with low to moderate log K_{ow}) to fatty-greasy settled particles that cause a reduction of between 17 and 57 % and biodegradation (Zorita et al. 2009; Fent et al. 2006). Acidic pharmaceuticals ($4.2 < pK_a < 4.9$) such as IBP, INM, NPX, and DIC are available as ions at neutral pH and are highly hydrophilic. Hence, they remain in the aqueous phase and are not readily adsorbed by sludge (Nikolaou et al. 2007). For these pharmaceuticals, biodegradation is assumed as a significant removal route in the aerobic and anaerobic parts of the activated sludge. For example, diclofenac was shown to have a significant biodegradation in WWTP with SRT for at least 8 days (Kreuzinger et al. 2004). However, typical WWTPs are observed to be inadequate for complete or effective removal of these compounds from wastewater (Nikolaou et al. 2007; Ziylan and Ince 2011). Furthermore, photodegradation could be a main factor in Tehran, at the time of sample collection (high sun radiation at the middle of summer).

The concentrations of NSAIDs in the various rivers are listed in Table 3. The concentrations in the rivers varied from below detection limits to 41 ng/L. It can be seen that IBP and NPX were ubiquitous in river water samples. The high concentration of IBP may be due to the high consumption of this pharmaceutical in the form of prescript and over-the-counter. All rivers with the exception of Karj, pass through Tehran city and in the Darband and Darake rivers, there are several restaurants in the catchment area of the river. The rivers may receive wastewater discharges from these restaurants. However, along these rivers, no discharges from industrial activities were found. Moreover, in Tehran, urban and hospital wastewater effluents are discharged into the surface water after treatment processes and thus could contaminate the receiving water. The high concentration of IDM in spite of low consumption as compared to other drugs may be related to low biotransformation and photodegradation. To the best of our knowledge, there is no information regarding the fate of IDM in previous literature. DIC was found in the Kan River but at very low concentrations, 25 ng/L. Laboratory assays performed with surface water revealed that DIC could be eliminated efficiently by direct phototransformation (Tixier et al. 2003). Similar concentrations for IBP (0.07 μg/L), NPX (0.07 μg/L), and IDM (0.04 μg/L) were reported for German rivers and streams, while DIC was reported in higher concentration (Fatta et al. 2007). IBP (0.022 μg/L), NPX (0.007 μg/L), DIC (0.013 μg/L), and IDM (0.036 μg/L) were reported to have similar concentration with that observed in river Tagus, Portugal, although these pharmaceuticals were not detected in river Zezere (de Jesus Gaffney et al. 2015). However, DIC and NPX were not observed in Vico Lake and Blosena Lake in Italy (Mainero Rocca et al. 2015). Shanmugam et al. (2014) reported NPX, DIC, and IBP concentrations ranging from not detectable (ND) to 28, ND to 103, and ND to 200 (ng/L), respectively, in the three studied rivers in Southern India.

Table 5 also shows the ranges of concentration of selected pharmaceuticals in the effluents of hospital wastewater samples collected from two general

hospitals in Tehran. As shown, all investigated drugs were observed at least once in the effluents of hospital wastewater. IBP showed the highest concentration followed by NPX. However, high initial chlorine concentrations (about 30 mg/L) are used in chlorination and most of the target compounds are degraded practically. Recently, Noutsopoulos et al. (2015) showed that DIC and NPX are degraded more than 90 and 60 %, respectively, through chlorination with chlorine initial concentration of 30 μg/L. The concentration ranges of NSAIDs shown in this survey are almost similar in magnitude to that detected by other studies reported in earlier literature (Tran et al. 2014).

Potential environmental risk

Ecotoxicological risk assessment was carried out in surface and drinking waters and WWTP effluents, in order to investigate the potential risk of the target pharmaceutical to aquatic organisms present in the environment. Acute EC_{50} values of pharmaceutical compounds used in this investigation were obtained from different literatures and are presented in Table 6. According to the EU-Directive 93/67/EEC, the compounds were classified based on their EC_{50} values: <0.1 mg/L = extremely toxic to aquatic organisms; 0.1–1 mg/L = very toxic to aquatic organisms, 1–10 mg/L = toxic to aquatic organisms, 10–100 mg/L = harmful to aquatic organisms, <100 mg/L = non-toxic to aquatic organisms (Sanderson et al. 2003). Table 6 shows the risk quotients calculated from mean concentrations for *V. fischeri*, *Daphnia magna*, and algae in the WWTP influents and effluents (due to very low RQ values, only WWTP1 with highest RQ was shown). With regard to this, none of the selected NSAIDs exceeded the RQ limit value that is why no toxicological effect is expected to occur (RQ higher than 1). Nevertheless, RQ values are very low, and IBP has the highest RQ value of 0.028, 0.116, and 0.184 for *V. fischeri*, *D. magna*, and algae, respectively. The results of this study are in agreement with that of Lolić et al. (2015), who observed that daphnids and algae had similar sensitivity to the detected pharmaceuticals and never exceeded the threshold value of one. Although, the RQ values are low, more attention should be paid to the environmental risk associated with the anti-inflammatory drug IBP. However, mixtures of various therapeutic classes of pharmaceuticals are present in the aquatic environment, which allows synergic or additive effects, resulting in higher toxicities than single classes (Paíga et al. 2013).

Conclusions

To the best of our knowledge, this study provides the first quantitative data on the occurrence of the selected NSAIDs pharmaceuticals in Tehran, Iran's water environment. In conclusion, based on the results, all studied pharmaceuticals were frequently detected in Tehran waters sources. Overall, IBP was found in the highest concentration among NSAIDs. The detection of NSAIDs in the effluents of hospital and WWTPs indicates that these effluents are the two important sources of pharmaceuticals that contribute to the environmental pollution. The WWTPs showed insufficient removal of all these compounds. The contamination of all river water samples shows that the target compounds are ubiquitously present and persistent in the water environment. Phototransformation was demonstrated as the main removal route of DIC in the surface water, and consequently, DIC was detected in only one river. Environmental risk assessment based on MEC/PNEC approach was evaluated, and this shows that there is no expected ecotoxicological risk for aquatic organisms due to the presence of selected pharmaceuticals in surface waters and WWTP effluents. More studies need to be conducted to evaluate the risk assessment of other pharmaceuticals, for a better understanding of their impact on public health, as well as their degradation metabolites to the aquatic environment.

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