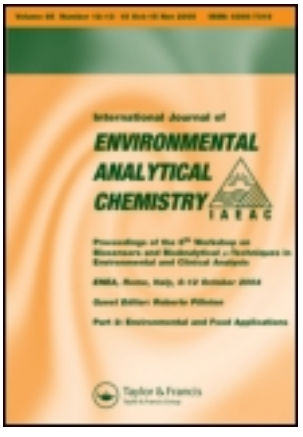

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Occurrence of environmentally relevant pharmaceuticals in Italian drinking water treatment plants

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Occurrence of environmentally relevant pharmaceuticals in Italian drinking water treatment plants

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The occurrence and seasonal distribution of 14 pharmaceutical substances of different classes were investigated in two drinking water treatment plants (DWTPs) supplied by the two main Italian rivers, the Po and the Adige (Northern Italy). The therapeutic categories of the selected pharmaceuticals included anti-inflammatory drugs, β-blockers, lipid regulators, diuretics, anti-epileptics, antibiotics and a steroidal hormone. The named compounds were assessed in samples collected from the river water inlet and after each purification stage (sand filtration, ozone treatment, granular active carbon (GAC)). Six of the 14 selected pharmaceuticals were found in all analysed samples, with concentration levels ranging from 1 ng l^{-1} for atorvastatin to 69 ng l^{-1} for atenolol in the drinking water produced. The granular active carbon stage resulted the most efficient in eliminating the examined chemicals from the water (removal range: 12–95%, average: 68%), while the sand filtration stage resulted the least effective treatment (removal range: 4–37%, average: 13%). The observed differences between winter and summer conditions, in terms of residual concentrations and number of detected analytes, seemed to depend mainly on the quality of the river water supplies. To the best of our knowledge, these are the first reported data on the presence of pharmaceuticals in drinking water obtained from the water treatment of these two rivers.

Keywords: pharmaceuticals; drinking water treatment plant; LC-MS

1. Introduction

The presence and distribution of pharmaceuticals in surface natural waters are of increasing scientific and regulatory concern owing to their high biological activity and the huge number of active principles approved for use (approximately 3,000 substances only in the EU for humans). Pharmaceuticals enter the aquatic environment directly (e.g. from applications in aquaculture) or indirectly via wastewater treatment plants. Wastewater is considered the most important source of pharmaceuticals for surface and coastal waters [1–3]. Many of these compounds are classified as non-biodegradable [4]. Moreover, conventional treatments cannot efficiently remove such chemicals in sewage treatment plants [5–9]. Hospital wastewater effluents have also been recognised as relevant sources of pharmaceuticals [10,11].

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Many analytical methods have been already proposed for the determination of pharmaceuticals in water samples [12–15]. The applied analytical methods are preferably based on solid-phase extraction (SPE) followed by liquid chromatography coupled with mass spectrometry, usually via electrospray interface (SPE-LC-ESI-MS), which allows developing multi residual methods for a wide assortment of aqueous matrices with satisfactory sensitivity, in the low to sub $ng\,l^{-1}$ range, and selectivity [11,14,16,17]. Methods based on GC-MS have also been proposed, but they are usually limited to individual substances or to a narrow class of compounds, since these analytes generally exhibit medium to high polarity, so often require a derivatisation procedure prior to chromatographic separation and detection [18].

The widespread environmental occurrence of pharmaceuticals has been already demonstrated for both wastewater, surface and ground waters, with concentration levels in the $ng\,l^{-1}$ range, with occasional maximum concentrations of several $\mu g\,l^{-1}$ [4,6,12,19–21]. Less information is, however, available on the occurrence and distribution of pharmaceuticals in drinking waters, especially when produced by purification of surface water supplies, such as rivers and lakes. A list of selected literature references on some identified pharmaceuticals in drinking waters is summarised in Table 1, where the number of searched and found analytes is also reported. A few papers have already highlighted that conventional technologies in drinking water treatment plants (DWTPs) cannot completely eliminate pharmaceuticals and their possible metabolites from the drinking water produced, posing an additional risk for human health [12,20,21].

The present study focused on two DWTPs located approximately 30 kilometres from the deltas of the two longest Italian rivers, the Po and Adige, which cumulatively account for a drainage basin of approximately 17,000,000 inhabitants in the Padana Valley (Northern Italy). An analytical method was developed for the determination of 14 pharmaceuticals that were selected according to sales data in Italy and previous occurrence from literature data. All purification stages (sand filtration, ozone treatment, active carbon) of the examined DWTPs were investigated to ascertain their capabilities to prevent contamination by such contaminants in the drinking water produced.

Table 1. Literature references on the number of identified pharmaceuticals and on the concentration range in drinking water.

State	Identified pharmaceuticals (total examined)	Concentration range ($ng\,l^{-1}$)	Ref.
Canada	2 (18)	0.1–8.0 (1)	[21]
Canada	5 (10)	3.4–4.2 (2)	[22]
Finland	2 (5)	8.0 (1)	[23]
France	8 (17)	0.2–210 (2)	[29]
Germany	2 (3)	120–400 (1)	[34]
Germany	3 (2)	50–250 (1)	[35]
Italy	3 (16)	0.6–24 (2)	[28]
Spain	11 (12)	0.8–330 (1)	[30]
USA	2 (5)	510–5000 (1)	[36]
USA	6 (24)	1.4–4.9 (1)	[33]

(1)DWTP outlet.
(2)Tap water.

2. Experimental

2.1 *Materials and methods*

Atenolol (ATE, ≥98%), bezafibrate (BEZ, ≥98%), flumequine (FLU, ≥98%), furosemide (FUR, ≥98%), gemfibrozil (GEM, ≥99%), hydrochlorothiazide (HCT, ≥99%), trimethoprim (TRI, >98%), meclofenamic acid (MEC, ≥98%, used as recovery standard) were from Sigma (Sigma-Aldrich, St. Louis, MO, USA; Sigma-Aldrich Chemie GmbH, Stenheim, Germany); carbamazepine (CAR, >99%), clofibric acid (CLO, >97%), ibuprofen (IBU, >99%), mestranol (MES; >99%), dihydrocarbamazepine (DHC, >99%, used as recovery standard), were from Aldrich (Sigma-Aldrich, St. Louis, MO, USA; Sigma-Aldrich Chemie GmbH, Stenheim, Germany); propanolol (PRO, >98%), bisphenol A (BPA, >99%, used as internal standard), benzophenone (BP, >99%, used as internal standard) were from Fluka (Buchs, Switzerland); naproxen (NAP, >99.9%) was from Riedel-de Haën (Buchs, Switzerland); atorvastatin (ATO, >99%) was from Kemprotec Limited (Middlesbrough, UK). Solvents (acetonitrile, methanol, 2-propanol, acetic acid) were HPLC ultra-gradient purity from Romil (Dublin, Ireland). Formaldehyde solution (~36% in water) and ammonium acetate buffer (HPLC-MS grade) were from Fluka (Buchs, Switzerland). Water for chromatographic purposes was purified using a Milli-Q system (Millipore, Bedford, MS, USA). The employed SPE sorbent phase was Strata-X from Phenomenex (Torrance, CA, USA). Individual stock solutions were prepared in 2-propanol and stored at 2°C in the dark. The working standard solutions were weekly prepared by diluting the analytical standards in 2-propanol in order to avoid solvent evaporation during sample storage. Both working solutions and sample extracts were stored in brown glass vials (Agilent) at 2°C. Laboratory materials for analytical purposes were accurately cleaned with ammonium persulfate solution and then rinsed two times with 2-propanol before their use. GF/F glass fibre filters (Whatman, Landspert, NJ, USA) were pre-cleaned by sonication with 2-propanol (2 h) and then gently dried overnight (12 h at 80°C). Owing to the potential hazard, all standards and application samples were handled with appropriate safety precautions.

2.2 *Sampling and sample pre-treatment*

Po and Adige are the first and second longest rivers in Italy, respectively. Approximately 15,800,000 and 1,400,000 inhabitants live in their catchment areas, respectively. Average 6-h water samples were manually collected on February and June 2006 at four sampling points: river inlet (1); after sand filtration (2); after ozone treatment (3); and after granular active carbon treatment (GAC) (4) from the drinking water treatment plants (DWTPs) of Corbola (Rovigo, Italy) and Cavarzere (Venice, Italy), which catch water supply from the Po and Adige rivers, respectively. Acetonitrile (1%, vol : vol) and formaldehyde (4%, vol : vol) were added to the water samples just after collection in order to prevent bacterial degradation and as organic modifier, and were stored in the dark at 2°C in dark glass bottles prior to extraction. The suspended particulate matter (SPM) in the water samples was eliminated by filtration on 0.7 μm glass fibre filters (GF/F). All filtration and SPE procedures were performed within 6 h after sampling.

2.3 *Extraction from water samples*

The selected analytes were extracted (500 ml, triplicate determination) from water samples (river water, various DWTP stages, drinking water) by SPE on Strata-X sorbent phase

(500 mg, 6 ml) using an automated Aspec XL extractor by Gilson (Middleton, WI, USA). Two selected recovery standards (dihydrocarbamazepine, meclofenamic acid, 100 ng l^{-1} each) were added to the aqueous samples prior to extraction. In addition to real samples, 500 ml of Milli-Q water was concurrently extracted as a procedural 'blank'. The SPE sorbent phase was conditioned at 3 ml min^{-1} with a sequential elution of acetonitrile (8 ml), methanol (8 ml) and Milli-Q water (5 ml). The aqueous sample was then passed through the cartridge at 7 ml min^{-1}. Potential interfering compounds were removed from the sorbing material by eluting them with 20 ml of Milli-Q water. The cartridge was then dried under vacuum in a SPE Manifold System by Supelco (Bellefonte, CA, USA) for 60 minutes. Analytes were subsequently eluted at 2 ml min^{-1} with methanol (4 × 3 ml aliquots); a 30 seconds waiting time between each extraction step was included in the extraction procedure since it was shown to improve some recovery yields and their standard deviations. The combined aliquots were then concentrated to 100 µl under a gentle stream of nitrogen in the automated evaporator set at 25°C. The final extract was then diluted to 200 µl with Milli-Q water and then stored in 2 ml Teflon®-lined screw-capped brown glass vials stored at 2°C until their injection (20 µl) in the HPLC system.

2.4 *Chromatographic separation and MS detection*

The sample extracts were injected into an Agilent 1100 HPLC system (Palo Alto, CA, USA) using an Agilent G1313A autosampler. The chromatographic separation of the selected analytes was performed using a Phenomenex Fusion Sinergy column (150 × 2 mm) filled with 2.5 µm C18 reversed-phase packing (Phenomenex Srl, Castel Maggiore, BO, Italy) protected by two 4 × 2 mm guard columns containing the same stationary phase. The LC column temperature was set at 25°C by an Agilent G1316A thermostatted column compartment. Operation and settings of the HPLC system were controlled by Agilent Chemstation rev. 9.01 software. The mobile phase (flow: 0.2 ml min^{-1}) was a mixture of methanol (A) and water (B), both containing ammonium acetate (2 mmol l^{-1}) buffer acidified at pH 3.5 with acetic acid. Two elution gradients were used to separate and detect all selected analytes, due to the different ionisation conditions in the MS detector: (A) which starts at 10% A, hold for two minutes and then linearly increased to 99% over 30 minutes for atorvastatin, bezafibrate, clofibric acid, furosemide, gemfibrozil, hydrochlorothiazide, ibuprofen, naproxen; (B) which starts at 2% A, hold for two minutes and then increased to 99% after 30 minutes, for atenolol, carbamazepine, flumequine, mestranol, propanolol, trimethoprim. The developed separation/detection method can be easily run in a fully automated way (only two injections needed) for all selected chemicals, since mobile and stationary phases remain the same, and it can be applied to all MS detectors, as the employed one, that cannot shift from positive to negative mode during the same chromatographic run under MS-MS mode.

The detection and quantification were performed by using an Agilent 1100 Ion Trap SL detector, via Electro Spray Interface (ESI) operating in MS^2-MRM mode under both negative (NI, gradient A) and positive ionisation (PI, gradient B) conditions. Nebulising and drying gases were nitrogen kept at 50 psi and 350°C, 10 l min^{-1}, respectively. Mass spectrometer was controlled by Agilent 1100 series LC/MSD Trap Control Ver. 4.1 software. Capillary, cone and capillary exit voltages for each examined compound, as well as monitored ions, are reported in Table 2. The End Plate Offset was at 500 V for all analytes.

Table 2. Selected parent, product and confirmatory ions monitored under MS-MS mode for the investigated analytes, and optimised parameters for MS-MS detection under Positive (a) and Negative (b) ionisation mode.

Compound	Retention time (min)	Precursor ion, $[M+H]^+$	Product ion (m/z)	Confirmatory ions	Capillary (V)	Skimmer (V)	Cap. Exit (V)	Oct. 1 DC (V)	Oct. 2 DC (V)	Trap drive	Oct. RF (V)	Lens 1 (V)	Lens 2 (V)	Frag. ampl.	Cut off (m/z)
ATE	10.2	267.3	225.2	190.2/208.2	−2200	46	145	13.5	0.25	60.0	160	−8.3	−100	1.5	144
CAR	34.2	237.3	194.2	179.2/220.2	−5000	15	175	9.0	0.40	59.0	160	−8.5	−100	2.8	155
FLU	36.4	262.3	238.1	244.1/220.1	−4000	32	135	9.0	0.00	75.0	175	−8.9	−100	5.8	160
MES	52.1	311.4	293.3	121.4	−5000	15	130	9.3	1.50	72.0	200	−9.2	−97	1.8	230
PRO	23.0	260.3	183.2	218.2	−5000	32	140	6.0	1.50	45.0	150	−7.5	−100	1.9	130
TMP	14.4	291.3	230.2	261.2/275.2	−3800	50	160	9.0	2.80	77.0	160	−3.0	−71	2.0	140
Compound	Retention time (min)	Precursor ion, $[M-H]^-$	Product ion (m/z)	Confirmatory ions	Capillary (V)	Skimmer (V)	Cap. exit (V)	Oct. 1 DC (V)	Oct. 2 DC (V)	Trap drive	Oct RF (V)	Lens 1 (V)	Lens 2 (V)	Frag. ampl.	Cut off (m/z)
ATO	26.1	557.7	397.1	453.1/521.1	+5000	−15	−185	−14.4	−1.60	86.0	300	4.4	100	2.3	240
BEZ	30.5	360.8	273.9	316.0	+5000	−15	−175	−14.0	−0.40	63.0	250	6.4	100	2.2	185
CLO	28.3	213.7	127.0	167	+4500	−15	−55	−9.0	−2.80	41.0	137	4.3	100	1.2	86
FUR	20.2	329.8	284.9	269.9	+4400	−24	−105	−15.0	0.00	64.0	250	6.9	100	2.2	153
GEM	29.2	249.3	121.0	127.1	+5000	−15	−100	−9.3	0.00	56.0	150	6.4	100	1.4	86
HCT	11.6	296.7	268.8	204.8	+2800	−15	−160	−15.8	0.00	91.2	230	8.1	100	1.8	215
IBU	32.5	205.3	161.0	175.0	+5000	−15	−55	−7.0	0.00	35.0	120	5.2	78	1.2	78
NAP	27.9	229.3	185	170.0	+5000	−15	−55	−8.8	−2.80	41.5	152	5.1	100	1.3	92

Notes: Abbreviations: Cap.: Capillary; Oct.: Octupole; RF: Radio Frequency; Frag. ampl.: Fragmentation Amplitude.

2.5 *Quantitative analysis*

Identification of analytes in real samples was performed by both retention time (RT) and compound mass/charge ratio. The structural confirmation of analytes in real samples was performed by matching the exhibited mass spectrum with reference mass spectrum recorded with pure standards. Identification was considered positive when RT was ±1 min from the standard reference RT, and the ratio by the monitored quantification fragment generated by MS^2 MRM mode and a second 'confirmatory' fragment was ±20% of the ratio exhibited by a reference standard. Quantification of all analytes was performed by internal standard method by using benzophenone (BP) and bisphenol-A (BPA) as MS internal standards for the PI and NI mode, respectively. The presence of both recovery and internal standards residues in the collected samples has been preliminary investigated. A correction was also made in order to include possible matrix effects (see 'Results and discussion' section below). As all analysed procedural blanks gave <MDL values for recovery standards and negligible (<0.5%, with respect to added internal standard) concentration values, no correction for blank was applied for them. Recovery experiments (4 replicates) were conducted by spiking selected analytes to real samples. The recovery efficiency obtained from spiked samples was corrected by subtracting the signal contributions attributed to compounds, when detectable, in the corresponding samples.

The method detection limits (MDLs) were determined in real sample extracts as the minimum analyte concentrations which could generate a signal-to-noise (S/N) ratio of 3, taking into account the concentration factor and the injection volume (Table 3). Six calibration levels in the 3 × MDL-100 ng range (as injected amount), spaced by a 2 × criterium, were applied. The obtained linear calibration plots showed $R^2 > 0.9958$ linearity for all investigated compounds. The intra-day precision, represented as relative

Table 3. Method validation parameters (method detection limits-MDLs, Recovery efficiencies, Relative standard deviations) for the selected pharmaceuticals determined in the examined water matrices.

	River water			Drinking water		
Compound	MDL ($ng\,l^{-1}$)	Mean recovery (6 replicates) (% RSD) at 1000 $ng\,l^{-1}$	Mean recovery (6 replicates) (% RSD) at 100 $ng\,l^{-1}$	MDL ($ng\,l^{-1}$)	Mean recovery (6 replicates) (% RSD) at 100 $ng\,l^{-1}$	Mean recovery (6 replicates) (% RSD) at 20 $ng\,l^{-1}$
ATE	6.9	58 (11)	34 (13)	3.5	66 (6)	48 (8)
ATO	0.6	33 (12)	18 (6)	0.2	72 (2)	45 (6)
BEZ	2.8	98 (2)	100 (8)	1.1	105 (5)	50 (10)
CAR	2.3	20 (7)	22 (9)	2.1	40 (3)	24 (4)
CLO	1.5	38 (10)	49 (15)	0.9	106 (5)	98 (9)
FLU	1.2	12 (3)	11 (12)	0.1	23 (7)	17 (7)
FUR	8.0	104 (7)	102 (9)	1.4	103 (2)	68 (5)
GEM	0.4	31 (13)	22 (15)	0.2	50 (3)	32 (4)
HCT	1.5	46 (6)	55 (12)	0.8	58 (1)	33 (8)
IBU	7.1	32 (13)	25 (14)	1.2	78 (2)	56 (9)
MES	1.4	43 (11)	31 (13)	1.0	85 (9)	75 (8)
NAP	1.7	38 (10)	28 (12)	1.1	61 (4)	74 (11)
PRO	2.6	35 (4)	22 (7)	1.6	75 (2)	67 (6)
TRI	1.2	25 (5)	32 (11)	1.1	71 (3)	36 (8)

standard deviation (RSD) from 10 replicate injections (20 µl of a real sample extract spiked with 50 ng of each analyte, in order to mimic the matrix effect) was in the 1–11% range for drinking water and 3–15% range for river water, respectively.

3. Results and discussion

3.1 *Selection of chemicals*

The selection of pharmaceuticals was based on national consumption figures, human rate of excretion as parent compound and environmental occurrence in Italian and other Southern European surface waters [19,23,24]. The therapeutic categories of the selected pharmaceuticals included anti-inflammatory drugs (ibuprofen, naproxen), β-blockers (propanolol, atenolol), lipid regulators (bezafibrate, gemfibrozil, atorvastatin), a metabolite of a lipid regulator (clofibric acid), diuretics (furosemide, hydrochlorothiazide), anti-epileptic drugs (carbamazepine), antibiotics (trimethoprim, flumequine) and a steroidal hormone (mestranol). In Table 4, the available data about consumption of selected substances in Italy, as well as their average elimination rates in sewage treatment plants, are reported. The simultaneous determination of all selected pharmaceuticals was

Table 4. Available data about consumption, removal by STP and excretion as parent compound by human body of the selected analytes.

Compound	Main uses	Consumption (ton), Italy 2001 [19]	Removal in SWTP	% Excretion as parent compound
ATE	β-blocker	22.1	0–76%[9]	90%[19]
ATO	lipid regulator	n.a.*	n.a.*	5%[37]
BEZ	lipid regulator	7.6	0-98%[9], 15–100%[38], 50%[39], 83%[40]	45%[37], 50%[19]
CAR	anticonvulsant	n.a.*	0%[9], 7%[40], 8–81%[41], 30%[42]	1–2%[37]
CLO	lipid regulator	n.a.*	0–30%[9], 34%[39], 51%[40]	6%[40]
FLU	antibiotic	n.a.*	n.a.*	n.a.
FUR	diuretic	6.4	0–62%[9]	40%[37], 90%[19]
GEM	lipid regulator	n.a.*	46%[39], 55%[43], 69%[40], 75%[42]	6%[45]
HCT	diuretic	14.7	0–77%[9]	24%[37], 95%[19]
IBU	anti-inflamatory	1.9	0–100%[9], 30–99%[41], ~60%[45], 75%[39], 79–100%[38], 90%[38], >90%[43], 96%[42]	1–8%[37], 10%[19]
MES	hormone therapy	n.a.*	n.a.*	n.a.*
NAP	anti-inflamatory	n.a.*	15–99.7%[41], ~50%[45], 55–98%[38], 66%[40], 78%[39], 80%[43], 93%[42]	10%[44]
PRO	β-blocker	n.a.*	32%[42], 96%[40]	<1%[40]
TMP	antibiotic	n.a.*	49%[42], 66–96%[41]	60%[37]

*n.a.: not available.

the first goal of the work, followed by the environmental investigation in the two drinking water treatment plants.

3.2 *Optimisation of the LC conditions and MS detection*

Because of the wide structural differences, electrospray ionisation interface (ESI) under both negative (NI) and positive ionisation (PI) modes was tested for the optimisation of ionisation parameters leading to the formation of $[M–H]^-$ and $[M+H]^+$ pseudomolecular ions, and optimised parameters are reported in Table 2. Atenolol, carbamazepine, flumequine, mestranol, propanolol and trimethoprim exhibited the best ionisation efficiency under positive mode, while atorvastatin, bezafibrate, clofibric acid, furosemide, gemfibrozil, hydrochlorothiazide, ibuprofen and naproxen showed the best ionisation efficiency under NI mode, as expected on the basis of their functional groups and on the possible stability of their pseudomolecular ions. The MS-MS mode was also investigated for both confirmation and quantification purposes. The most intense product and confirmatory ions monitored under MS-MS mode are reported in Table 2.

The chromatographic separation was performed on Phenomenex Fusion Synergi RP stationary phase thermostatted at 25°C, a Reversed-Phase stationary phase with an embedded polar group, which offered the best performance in terms of selectivity and peak symmetry, in comparison with other tested stationary phases. The opportunity of a fully resolved simultaneous chromatographic separation for all analytes was first investigated in order to improve the overall throughput of the proposed analytical method. Unfortunately, the partial co-elution of some analytes moved the strategy to a splitting sample analysis, simply based on their ionisation mode, in order to increase their response factors, that would have affected their S/N values applying only the detector selectivity. Preliminary experiments were then carried out in order to apply the same mobile phase to both separations. Two different gradients were then developed (see 'Experimental' section for details). Different mobile phases, composed by water/acetonitrile and water/methanol, at different pH values and at different buffer concentrations, were also tested. A mobile phase based on methanol and water mobile, both containing ammonium acetate at $2\,mmol\,l^{-1}$, with pH set at 3.5 resulted in the best compromise between chromatographic separation and MS response factors, as previously reported [25]. The slightly acidic mobile phase selected resulted, moreover, in improved peak shapes for analytes detected under negative ion mode, so balancing the partial signal suppression due to the acidic mobile phase. The low buffer concentration allowed for the minimisation of the formation of $[M+NH_4]^+$ and $[M+CH_3COO]^-$ adducts during the ionisation process. In Figure 1 the chromatographic separations, under both NI and PI detection modes, respectively, of the selected analytes in a drinking water extract are presented.

3.3 *Sample extraction*

On the basis of preliminary experiments (data not reported), Strata-X was selected for the extraction step, as it offered higher recoveries and/or lower standard deviation for some analytes, in comparison with other tested sorbents. The extraction procedure was optimised in order to achieve satisfactory recoveries for all selected chemicals. The best efficiencies were obtained adjusting the samples pH at 7, while both higher (10) and lower (2.5) values gave lower recoveries for some compounds. The recoveries exhibited by the

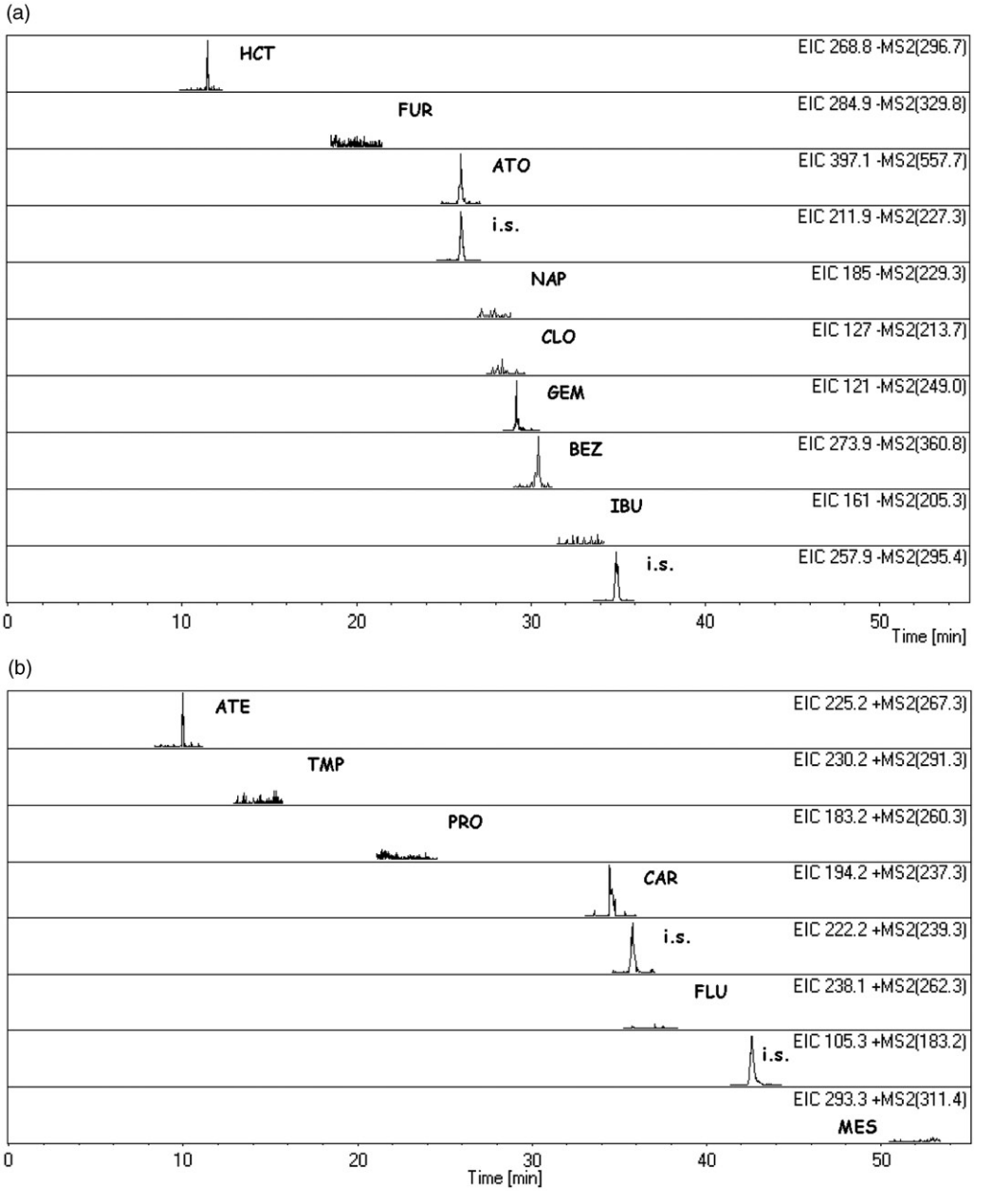

Figure 1. HPLC-ESI-MS chromatograms (a, NI mode; b, PI mode) of a water sample extract (drinking water from the Po river DWTP, winter session). Concentration range 2–69 $ng\,l^{-1}$.

proposed procedure in the various investigated matrices (river water, sand filtration, drinking water) are presented in Table 3. As expected on the basis of the different chemical structures and properties, the observed recoveries were quite different for each individual compound, and a decrease of efficiency was also observed, for the river water, the most critical matrix among those investigated. Recoveries at 100 $ng\,l^{-1}$ spiking level in river

water and 20 ng l^{-1} in Milli-Q water were 11–102% (average: 39%) and 17–98% (average: 52%) respectively. The last values resulted very similar also in drinking water spiked samples. Clofibric acid, atorvastatin, propanolol, ibuprofen and mestranol were observed to be more affected by matrix effects, with an average recovery decrease higher than 60% moving from drinking water to river water matrix. Vice versa, furosemide, bezafibrate and hydrochlorothiazide were instead recovered with similar efficiencies in all examined matrices (recovery decrease: 1–5%).

3.4 *Matrix-induced suppression*

In addition to recovery experiments in Milli-Q water at various concentration levels, potential effects of the matrix in inducing MS signal suppression were also investigated [11,25].

A relatively strong matrix-induced suppression of ion signals was recorded for all examined compounds when spiked river water extracts were analysed, with a decrease of response factor by 8–66% (average: 47%) of their original values, with respect to those obtained with standard solutions. A decrease of observed linearity (R^2: 0.9884–0.9952, average: 0.9917) and reproducibility (RSD: 3–15%) was also observed (Table 3). No significant differences were observed when water extracts from sand filtration sampling, ozone and GAC stage were analysed. Even if the matrix effect was significant only for river sample extracts, the use of internal standard method was applied to the quantification of analytes in all extracts.

3.5 *Environmental applications*

The two investigated DWTPs are located at the end of the respective river drainage basins of the Po and Adige rivers (Padana Valley, Northern Italy), which collect both treated and untreated sewage (currently, only approximately 50% of sewage in the Padana Valley is actually treated) from urban, industrial and agricultural settlements, with an overall input of approximately 114,000,000 and 4,800,000 equivalent inhabitants, respectively.

The two DWTPs, each one with a daily production of approximately 28,000 m^3 day^{-1}, have the same configuration, composed by the following 'standard' purification stages: sand filtration, ozone treatment, and Granular Active Carbon (GAC) treatment, and are run by the same company. Under these conditions, any detectable difference in the quality of their drinking water produced could be attributed only to the different water source. In fact, Po and Adige river waters have significantly different physico-chemical properties, such as temperature, salt content and pH [26]. To the best of our knowledge, these are the first reported data on the presence of pharmaceuticals in drinking water obtained from the treatment of these two river waters.

The resulting average concentrations found in the final purified water are reported in Table 5, while a typical HPLC-ESI-MS chromatogram of a drinking water sample extract from river Po DWTP is presented in Figure 1.

Six of the 14 selected chemicals were recorded in the entering river waters. The detected pharmaceuticals were found also in each intermediate purification stage and in the final drinking waters, indicating that they had not been completely eliminated by the examined DWTPs. All purification stages were shown to contribute to the reduction of the investigated contaminants, with an overall elimination efficiency (for chemicals found at

Table 5. Residual concentrations of the selected pharmaceuticals in the four sampling points of the investigated DWTPs (a: Adige river DWTP; b: Po river DWTP).

Compound	Winter session				Summer session			
	Adige river inlet	After sand filtration	After ozone treatment	After GAC[1] treatment	Adige river inlet	After sand filtration	After ozone treatment	After GAC[1] treatment
	Concentration ($ng\,l^{-1}$)							
ATE	831 ± 62	746 ± 33	481 ± 18	25 ± 1	197 ± 25	39 ± 2	<MDL	<MDL
ATO	4 ± 0.3	4 ± 0.3	3 ± 0.1	1 ± 0.1	<MDL	<MDL	<MDL	<MDL
BEZ	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
CAR	105 ± 9	88 ± 5	36 ± 3	4 ± 1	60 ± 4	65 ± 3	39 ± 2	17 ± 1
CLO	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
FLU	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
FUR	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
GEM	26 ± 2	25 ± 1	12 ± 0.4	3 ± 0.1	<MDL	<MDL	<MDL	<MDL
HCT	16 ± 1	10 ± 0.5	7 ± 0.5	8 ± 0.3	9 ± 1	3 ± 0.2	2 ± 0.5	2 ± 0.5
IBU	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
MES	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
NAP	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
PRO	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
TRI	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
	Po river inlet	After sand filtration	After ozone treatment	After GAC[1] treatment	Po river inlet	After sand filtration	After ozone treatment	After GAC[1] treatment
	Concentration ($ng\,l^{-1}$)							
ATE	1824 ± 152	756 ± 38	[2]	69 ± 2	51 ± 4	<MDL	[2]	<MDL
ATO	35 ± 3	25 ± 2	[2]	4 ± 0.2	<MDL	<MDL	[2]	<MDL
BEZ	26 ± 2	10 ± 0.3	[2]	2 ± 0.2	<MDL	<MDL	[2]	<MDL
CAR	182 ± 15	71 ± 5	[2]	11 ± 0.8	126 ± 11	152 ± 12	[2]	<MDL
CLO	<MDL	<MDL	[2]	<MDL	<MDL	<MDL	[2]	<MDL
FLU	<MDL	<MDL	[2]	<MDL	<MDL	<MDL	[2]	<MDL

(*Continued*)

Table 5. Continued.

	Winter session				Summer session			
	Po river inlet	After sand filtration	After ozone treatment	After GAC[1] treatment	Po river inlet	After sand filtration	After ozone treatment	After GAC[1] treatment
				Concentration ($ng\,l^{-1}$)				
FUR	<MDL	<MDL	[2]	<MDL	<MDL	<MDL	[2]	<MDL
GEM	10 ± 0.8	5 ± 0.3	[2]	2 ± 0.1	<MDL	<MDL	[2]	<MDL
HTC	52 ± 6	8 ± 0.7	[2]	4 ± 0.2	61 ± 7	<MDL	[2]	2 ± 0.2
IBU	<MDL	<MDL	[2]	<MDL	<MDL	<MDL	[2]	<MDL
MES	<MDL	<MDL	[2]	<MDL	<MDL	<MDL	[2]	<MDL
NAP	<MDL	<MDL	[2]	<MDL	<MDL	<MDL	[2]	<MDL
PRO	<MDL	<MDL	[2]	<MDL	<MDL	<MDL	[2]	<MDL
TRI	<MDL	<MDL	[2]	<MDL	<MDL	<MDL	[2]	<MDL

[1]GAC: granular activated carbon.
[2]This stage was not operating during the sampling session.

inlet, but not in the produced drinking water, a precautionary concentration value of MDL/2 was assumed in order to calculate the removal efficiency) ranging between 56% and 99% (average elimination efficiency: 88%) for both DWTPs. The Granular Active Carbon (GAC) stage was the most efficient in eliminating the examined chemicals from the water (removal range: 12–95%, average: 68%), even if not to an exhaustive extent, while the sand filtration stage resulted in the least effective treatment (removal range: 4–37%, average: 13%). A difference was observed between the winter and the summer sessions: 6 and 5 pharmaceuticals were found in the drinking water produced at Po and Adige DWTPs, respectively, during the winter session, while none and 2, respectively, were detected during the summer session. The occurrence of pharmaceutical residues in the final drinking water was shown to depend strongly on the river water quality: not only were more (6 vs. 3) substances found in the river water supply during the winter session, but also their measured concentrations were much higher, approximately 1.8–4 and 1–36 times for the Adige and Po rivers, respectively, considering only pharmaceuticals detected in both sessions. The recorded substances are not expected to have a seasonal consumption, due to their therapeutical applications, so the overall quality of the analysed drinking waters seems to be affected by the seasonality of the river water supplies. Available data from literature about removal efficiencies by STP, as well as excretion percentages as parent compounds (Table 4), could not help in clarifying this hypothesis, since most removal efficiencies are highly divergent, and excreted fractions are generally low (i.e. <10%) or contrasting (such as for furosemide and hydrochlorothyazide). On the basis of these literature data, removal during mechanical/biological sewage treatment (which is known to be more efficient during the summer due to higher temperature) and natural degradation, both biochemical and photochemical, in the river waters, are expected to decrease the quality of the produced drinking water during winter conditions. In addition, a possible enhanced re-forming of the parent chemicals from conjugate due to higher temperatures, with an increase of their concentrations in water, should also be considered for some pharmaceuticals [27]. Both DWTPs exhibited anyway high removal efficiency during the winter sessions: 56–97% (average: 82%) at Adige DWTP, and 80–96% (average: 90%) at the Po DWTP. The removal value found in the river Po DWTP during the winter session is even more interesting since the ozonation stage was unfortunately not operating during the planned sampling session, indicating that physico-chemical properties of river water supplies could probably mainly affect the removal efficiencies, since the plants are actually identical.

A comparison with previous available reports on drinking waters allows one to summarise some similarities and differences in the recorded panel of substances. Atenolol showed the highest concentration levels (25–69 $ng\,l^{-1}$) (see Table 5). This compound has been previously searched for, but not found, in Italian drinking waters and, unfortunately, no analytical detection limits were reported [28]. A possible increased consumption over recent years could also explain this finding. Carbamazepine was the second score, with a 4–17 $ng\,l^{-1}$ range in the final drinking water. This pharmaceutical had already previously been detected in other countries, such as Canada and France, in the 0.4–2.3 and 43 $ng\,l^{-1}$ range, respectively [21,29]. In a recent study on rejection of pharmaceuticals in nanofiltration and reverse osmosis membrane drinking water treatment [30], carbamazepine was detected in the 0.5–5.7 $ng\,l^{-1}$ range after the two cited purification stages. However, in the studied plant, nanofiltration and reverse osmosis are not the final purification steps. Gemfibrozil and bezafibrate were indeed identified at very low concentration levels (2–3 $ng\,l^{-1}$). These two pharmaceuticals were previously looked for

also in Italian, European and Canadian drinking waters, but they were not detected [28,29,31,32]. High concentrations of gemfibrozil (288–298 ng l^{-1}) were instead detected after nanofiltration and reverse osmosis [30]. Hydrochlorothiazide was found in 2–9 ng l^{-1} concentration level, while 0.8–330 ng l^{-1} range was detected after nanofiltration and reverse osmosis steps [30]. Atorvastatin, which was investigated, to the best of our knowledge, for the first time in drinking waters, was found at 1–4 ng l^{-1}. Clofibric acid and ibuprofen, which were already identified in EU and Canadian drinking waters at low concentration levels (3.2–5.3 and 0.6–8.5 ng l^{-1}), were not detected in the present study [28,29,31,32], probably because the residual concentration levels were below the available limit of detection during the sampling period. Flumequine was found in the USA in 2.0–2.5 ng l^{-1} range [33] and not identified in the Po and Adige DWTPs, although the developed MDL values were one order lower in the present work.

4. Conclusions

Residual concentrations of pharmaceuticals were investigated for the first time in Northern Italy drinking water treatment plants (DWTPs) treating river waters. Six of the 14 selected pharmaceuticals were recorded in the collected river water samples, as well as in all intermediate purification stages and in the drinking water produced. Although the calculated removal efficiency demonstrated that a good management of the examined DWTPs can actually strongly reduce the concentrations of such contaminants along all purification stages, the residual values found in the drinking water produced demonstrated that current purification procedures are not adequate to completely eliminate such polar contaminants, especially during winter conditions, when residual concentration in the river water inlet are much higher than during the summer season. Although the individual residual concentrations were systematically below their no observed effect concentrations (NOEC), it should be emphasised that the selected chemicals exhibit very high biochemical activity, the long-term effects of which are currently largely unknown in humans. An additional potential concern could arise from their possible 'mixture' effects: only a very limited number of pharmaceuticals were actually searched for, while many more, in addition to their related chemicals (such as metabolites and degradation products), could be also present in the drinking waters produced. It has to be emphasised that additional, preliminary water samples collected in other Italian rivers and in the Venice lagoon (data not shown) showed in the same period a completely different pattern of detected pharmaceuticals, among the selected ones, indicating that this class of contaminants is highly site-specific, at least in the investigated geographic area.

Current work is focusing on the determination of these and other pharmaceuticals and related chemicals, such as possible metabolites and degradation products, in other water bodies, as well as on the evaluation of the potential toxicological effects of pharmaceuticals mixtures at realistic concentration levels on humans and environmental health.

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