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DETERMINATION OF SELECTED ACIDIC PHARMACEUTICALS AND CAFFEINE IN ERGENE BASIN, IN TURKEY

DÖKMECİ A.H. [1,*]
SEZER K.[2]
DÖKMECİ I.[3]
İBAR H.[2]

[1] *Department of Emergency and Disaster Management*
School of Health, Namik Kemal University
Tekirdağ, Turkey
[2]*Department of Chemistry, Faculty of Sciences*
Trakya University, Edirne, Turkey

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*to whom all correspondence should be addressed:
e-mail: hdokmeci@nku.edu.tr

ABSTRACT
In this study, surface and wastewater in Çorlu, Tekirdağ has been monitored for ibuprofen, naproxen and diclofenac as non-steroidal anti-inflammatory drugs (NSAIDs), salicylic acid as an analgesic and caffeine. For this goal, samples were collected from 5 areas during winter and summer times (W1, W2, W3, W4 and W5) working in the field of a wastewater treatment plant site, only Çerkezköy industrial district W4. Different solid-phase extractions, pH and derivatization conditions were tested with some anti-inflammatory drugs and caffeine of Gas-Chromatography-Mass Spectrometry in environment samples and their identification and quantification at trace levels were made (ng L^{-1}). Diclofenac (LOQ = 4.3 ng L^{-1}) and ibuprofen (LOQ = 134.1 ng L^{-1}) could not be determined. Other concentration levels of arranged drugs range between 2.12 -13.58 ng L^{-1} naproxen, 15.74-18.74 ng L^{-1} salicylic acid and 5.8-121.2 ng L^{-1} caffeine.

KEYWORDS: GC-MS; SPE; Derivatization; Acidic Pharmaceuticals; Caffeine.

1. INTRODUCTION

Nowadays, with the influence of the rapidly increasing importance of environmental pollution, many xenobiotics with which we were unfamiliar before have been identified in the aqueous environment with the help of recently developed analytical methods (Daughton, 2008). In environmental analyses, besides traditional chemicals, pharmaceuticals and personal care products have also been classified under the polluting category by the studies which have been done. The medical and veterinary drugs used by humans and animals, houses, factories or hospitals to be discharged into treatment plants or directly into untreated surface water, groundwater and soil have been the focus of recent studies which have tried to explain their outcomes (Kümerer *et al*., 2009; Fatta-Kassinos *et al*., 2011). In many countries there are studies about these products. However in Turkey, scarcely a few studies have been done on this issue. This is because quite expensive technologies are required for the identification and measurement of pharmaceuticals and personal care products (PPCP). In this study; some anti-inflammatory drugs which are widely used in many countries including Turkey and some of the most common in domestic wastewater, industrial wastewater and sewage in surface water, diclofenac, naproxen, ibuprofen and salicylic acid (acetyl salicylic acid metabolite) as well as caffeine, a stimulant in use, have been detected with the help of the SPE GC-MS analytical method due to its high sensitivity of detection and low cost. Solid-phase extraction (SPE) is popularly used for sample extraction and analyte enrichment. The Oasis HLB sorbent (divinylbenzene/ N-vinylpyrrolidone copolymer), exhibiting both hydrophilic and lipophilic retention features, has recently emerged. It has got superb wetting properties which create no negative “running dry” impacts on analyte recovery (Rodriguez *et al*., 2003; Ollers *et al*., 2001). The Oasis HLB cartridge was used due to the usability and high sensitivity of acidic, basic and neutral drugs in the solid phase

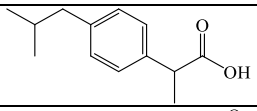
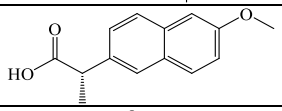
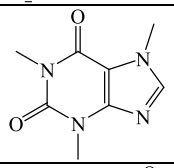
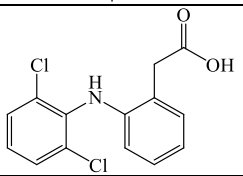
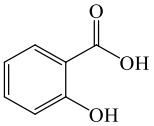
extraction of the surface waters (Tixier *et al*., 2003; Tixier *et al*., 2002; Dévier *et al.,* 2013). During this study, solely silylation reagents were used since the selected compounds consisted of various practical groups. The goal of this paper was to present a GC–MS dependent analytical technique for the simultaneous determination of selected neutral and acidic pharmaceutically active compounds (PhACs) (Table I). A particular focus point was the optimization of enrichment and derivatization processes which were carried out through a multi-factorial experimental design. Consequently, the developed analytical method was used to find out the incidence of pharmaceutical compounds in surface and wastewater, which might be considered as a contribution to further drinking water treatment studies in terms of selecting relevant compounds (Yu *et al*., 2007).

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Ibuprofen, diclofenac, naproxen, salicylic acid, caffeine, hexamethyldisilizane (HMDS), trifluoroacetic acid (TFA), N-methyl-N-trimethylsilytrifluoroacetamide (MSTFA), N,O-bis[trimethylsilyl]trifluoroacetamide (BSTFA), hexane, methanol (MeOH), ethyl acetate (EtOAc) and hexane were all purchased from Sigma–Aldrich (Merck, Darmstadt, Germany, purity>98%). Ultrapure water was obtained with a Milli-Q System (Millipore, Anamed, Turkey), Oasis HLB 3 cc/60 mg, Oasis MCX Cartridges 3cc/60 mg, were purchased from Waters (Milford, MA, USA). Supelco C18 3cc/60 mg was purchased from Supelco (Torrance, CA, USA). Glass microfiber filters (pore size 0.7 µm) were from Whatman (Maidstone, UK).

Table 1. Compounds, Therapeutic group, Molecular Structures, Molecular Weight, Retention time, linearity and m/z ratio after derivatization of detected compounds

Compound	Therapeutic Group	Molecular Structures	MW (g mol^{-1})	R_T (min)	m/z $[M\text{-}Si(CH3)_3]^+$ -(CH3)n (n:1, 2, 3)
İbuprofen	NSAID		206	7.06	73, 160, 234, 263, 278
Naproxen	NSAID		230	9.13	185, 243, 287, 302
Caffeine	Stimulant		194	8.32	109, 194 (molecular ion)
Diclofenac	NSAID		295	10.35	214, 242, 277, 352, 367
Salicylic Acid	Acetyl salicylic acid metabolite		138.12	6.56	73, 267

2.2. Study Area, Sampling and sample preparation

The study area selected as Çorlu is the most populous district (approximately 350,000 people) of Tekirdağ with textile, glass, paper, metal, oil, food, pharmaceutical, cosmetic, chemical and leather industries in and around. These industries discharge their wastewater treatment or without treatment into the Ergene River and its tributaries. In addition, as there are no domestic wastewater treatment plants in Çorlu, this discharged wastewater mingling with surface water directly reaches up to the Marmara Sea. 5 stations numbered between W1-W5 have been determined for the study area.

Representing Çorlu municipal and hospital wastewater in the center, 3 points (W1, W2, W3) have been selected. The W1 station represents the wastewater from Vatan Hospital and the surrounding residential areas; W2 represents the wastewater from public and military hospitals and surrounding residential areas whereas W3 represents the wastewater from Şifa Hospital and residential areas in the neighborhood. About 180 m^3 day^{-1} of domestic and hospital effluents reach the Çorlu River and beyond to the Ergene River. The Velimeşe station (W4), the closest point to the output of the treatment plants of the Çerkezköy Industrial Zone, is located on the Çorlu River which is one of the major branches of the Ergene River. It collects collect effluent and/influent wastewater from Çerkezköy, Veliköy, Velimeşe, Çorlu and Muratlı municipalities and factories in the region. According to data from 2010, 80,000 m^3 of domestic and industrial wastewater is discharged per day from the Wastewater Treatment Plant of Çerkezköy Industrial Zone into the Çorlu River. There are lots of pharmaceutical and cosmetics companies like Atak Pharma, Cosmetics and Cosmetics Chemistry Incorporation; Bilim Pharma, Industry and Commercial Incorporation; Hunca Cosmetics, Industry, Incorporation; Koçak Pharma, Drug and Chemistry Industry and Commercial Incorporation; Zentiva Chemical Products, Industry and Commercial Incorporation. Besides those, there are approximately 190 chemical, textile, leather, paint, electronics, iron and steel industrial organizations operating in the region. Çerkezköy Industrial Zone consists of physical treatment (coarse and fine gratings, and the grease sand pool, balancing ponds and pumping Pool), chemical treatment (fast and slow mixer, chemical sedimentation ponds), biological treatment (biophosphorus tanks, air tanks, last settling tanks) and sludge dewatering (sludge thickening tanks, sludge holding tanks, decanters, filter presses) units. The Ergene River is the most important river by which approximately 300,000 square meters of 1st, 2nd and 3rd class farm fields in the Thrace Region are fed. Moreover, the Ergene River is the most important branch of the international waterway the Meriç River while also being the most significant source of surface water in the Ergene Basin. The Ergene River drainage area is 10,730 km^2 and has an average annual rate of about 28 m^3 sec^{-1}. (TBMM, 2002). Therefore, the W5 station has been selected as the Seyitler locale due to its representation of the domestic and industrial wastewater discharge into Ergene River.

In February and June 2009 seasonal (winter and summer) surface water samples were collected from 5 locations. Water samples of 3 liters obtained from the stations were taken to laboratories in amber glass bottles. All samples were filtered with the GFF fiber filters on the same day. Analyses were stored until the next day at 4 ^{0}C. Prior to the extraction, water samples were pH adjusted using HCl to pH 2 (1 mol L^{-1}).

All glassware was meticulously purified by washing with detergent first, rinsing by pure water afterwards and by heating in the end at 450 °C overnight.

2.3.Surface and wastewater characteristics

The measurement of pH, dissolved oxygen, conductivity and temperature was performed by a Hach-Lange Brand HQ 40D dual-channel multimeter while blurring by the Hach-Lange Brand 2100 P portable turbidity meter at sampling points was in a land-scale manner. The measurements of Total Organic carbon (TOC), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solid (TSS) and Adsorbable Organo-xenobiotics (AOX) were made by standard methods at TUBITAK Marmara Research Center Environmental Laboratory.

2.4.Instrumentation

The derivatized pharmaceutical compounds were analyzed on a GC–MS system consisting of a Thermo Trace GC- DSQII MS and an auto-sampler AI-3000 (Thermo Electron Corporation, Austin, TX, USA). A fused-silica column (Thermo TR-5MS, 60m×0.25 mm, 0.25µm) was connected to a glass liner. The injector temperature was set at 280 ^{0}C operated at splitless mode and injector volume was set at 1 µL. The temperature of the column was set to 150 ^{0}C at the injection point, held 1 min. and elevated to 270 ^{0}C (15 ^{0}C min^{-1}), then held for 10 minutes. The temperature of the transfer line was set to 270 ^{0}C. The carrier gas was helium (purity 99.999 %) at a flow rate of 1 mL min^{-1}. Mass spectra were obtained in full scan mode in preliminary experiments, and later on in selected ion mode (SIM) using electron impact ionization (70 eV). In order to obtain lower detection limits, the background subtraction method was applied to the chromatograms obtained from the injection of the standard solutions, real samples and blank solutions using Xcalibur software. SPE extractions were applied by use of the Visiprep DL Vacuum Manifold (12 port) from Supelco (Bellefonte, PA, USA). Obtained extracts were dried on an IKA RV10 Rotevaporator (IKA® Werke

GmbH&Co.Kg, Germany) connected in-line with the Vacuum pump V-700 from Buchi (Flawil, Switzerland).

2.5. Determination of Optimum Derivatization Conditions

The samples in reaction vials which had undergone the drying process undertook 3 types of silylation so as to determine the optimum conditions for derivatization. Three different derivatization reagents were selected for this purpose. 125 µl of standard solutions which would have analyte concentrations at pre-determined levels in the injector (Ibuprofen:259pg/µL, Naproxen: 301 pg μL^{-1}, Diclofenac: 385 pg μL^{-1}, Salicylic acid: 323 pg μL^{-1}) were taken into 3 separate vials for each analyte, were dried as explained above and they went into reaction with selected sylilation reagents: 1) 125 µl pyridine, 250 µl HMDS (Hexamethyldisilazane) + 25 µl TFA (Trifluoro acetic acid), 2) 150 µl pyridine + 250 µl BSTFA (N,O-bis-trimethylsilyl-trifluoroacetamide) and, 3) 150 µl pyridine + 250 µl MSTFA (N-methyl-N-trimethylsilyl-trifluoroacetamide). These samples were inserted into a microwave reaction system (Matthews, MARS) at 70 ^{0}C, and had incubation for 60, 90 and 120 minutes respectively (Sebok *et al*., 2009). Looking at the results, the best results for naproxen and diclofenac were given by the BSTFA reagent at 70 ^{0}C for 60 minutes whereas the best results for ibuprofen and salicylic acid were provided by the HMDS reagent at 70 ^{0}C for 90 minutes (Table 2). Being less expensive, the HMDS reagent was selected for the further study with the reaction condition of 70 ^{0}C for 90 minutes. Table 2 represents the reaction yield of each silylation reagent by integration unit (integrated area/pg analyte).

Table 2. Values of Integration Unit of chromatographic peaks obtained from different derivatization conditions

Injector conc.	**Integration Unit (pg μL^{-1}) ▼**			
Derivatization conditions▼	**İbuprofen (259 pg μL^{-1})**	**Naproxen (301 pg μL^{-1})**	**Diclofenac (385 pg μL^{-1})**	**Salicylic Acid (323 pg μL^{-1})**
I-1(HMDS:70^{o}C-60 min)	2.25E+03	1.09E+03	3.52E+03	3.85E+03
I-2(BSTFA: 70^{o}C-60 min)	7.76E+03	1.31E+04	4.28E+03	5.23E+03
I-3(MSTFA:70^{o}C-60 min)	1.78E+03	4.63E+03	1.94E+03	7.12E+03
II-1(HMDS:70^{o}C-90 min)	3.08E+04	5.27E+03	1.81E+03	2.25E+03
II-2(BSTFA: 70^{o}C-90 min)	3.28E+03	5.43E+03	1.07E+03	3.34E+03
II-3(MSTFA:70^{o}C-90 min)	3.12E+03	5.34E+03	1.66E+03	5.23E+03
III-1(HMDS:70^{o}C-120 min)	3.23E+03	5.18E+03	1.05E+03	3.13E+03
III-2(BSTFA: 70^{o}C-120 min)	5.61E+03	5.94E+03	2.14E+03	4.72E+03
III-3(MSTFA:70^{o}C-120 min)	3.28E+03	5.28E+03	2.05E+03	4.98E+03

2.6. Optimization of Solid Phase Extraction

In order to ascertain the recovery of analytes at different pH values, ultra pure water samples with 500 mL volume were spiked with analytes at different concentrations (Ibuprofen: 2074 pg, Naproxen: 2410 pg, Caffeine: 2410 pg, Dicolfenac: 3081 pg, Salicylic acid: 1600 pg) and adjusted to the desired pH values (2, 4, 7) using HCl (1 mol L^{-1}) and NaOH (1 mol L^{-1}). With the use of 3 different extraction cartridges (Supelco C-18, Oasis HLB, Oasis MCX) the recovery rates of the analytes in interest were identified. Prior to the extraction, pre-cleaning and conditioning procedures were implemented on the selected cartridges.

The target compounds and the SPE material are the most influential factors determining the choice of elution solvent and volume. The most common elution solvents of Oasis HLB are regarded as methanol, ethyl acetate and acetone (Rodriguez *et al*., 2003; Ollers *et al*., 2001; Koutsouba *et al*., 2003; Sacher *et al*., 2001). Determination of the solvent, solvent elution rates and volumes are based on experimental trials. Prior to the loading of samples to the cartridges, 5 ml of n-hexane, 5 mL of ethyl acetate, 5 mL of methanol and 10 mL of Mili Q Water (adjusted to pH 2 with HCl) were strained from the cartridge under a vacuum of 12-15 mL min^{-1} flow rate respectively. Spiked solutions with a volume of 500 ml were passed through the cartridges at flow rate 5 mL min^{-1}. After extracting all the samples, the cartridge was dried after being exposed to vacuum forced air flow for 1-2 hours. The samples were ready for the sililation reaction after being eluted by 5 mL of n-hexane,

5 ml of EtOAc and 10 ml of MeOH, the volume of the eluents was reduced to 2 mL by rotevapoartor and then transferred to the reaction vials (4 mL) and evaporated to dryness using a gentle stream of nitrogen.

2.7. pH Optimization

In order to determine the optimum pH for the recovery of acidic and neutral compounds prior to extraction of the real environmental samples, stock solutions were spiked with pure water and adjusted to pH 2, pH 4 and pH 7. With the help of OASIS HLB absorbent, pharmaceutical compounds in the prepared water samples were analyzed by GC-MS. The best recoveries for acidic drugs (recoveries between 56 and 97%) ranged at pH 2 whereas the recovery for caffeine at pH 2 was very close to the recovery at pH 7 (recoveries between 35-40%). As a result of these experiments, the optimum pH for all the selected compounds was chosen as pH 2 (Table 3).

Table 3. Recovery Rates for Different pHs (n=3)

Compounds (pg 500 mL^{-1})	**pH2**	**pH4**	**pH7**
Salicylic acid (1600)	75.92%	30.60%	3.00%
İbuprofen (2074)	71.42%	41.80%	21.00%
Naproxen (2410)	97.00%	97.40%	36.00%
Diclofenac (3081)	56.00%	20.00%	13.70%
Caffeine (2410)	35.00%	35.50%	40.00%

2.8. Cartridge Selection

In order to specify the optimum recovery rates in the extractions of pharmaceutical drugs, 3 different cartridges were used as adsorbents; Oasis HLB (1 g), Oasis MCX (60 mg) and Supelco C 18 (200 mg). Prior to the loading of analyte solutions, cartridges were preconditioned with 5 mL of n-hexane, 5 mL of EtOAc, 5 mL of MeOH and 10 mL of ultrapure water (pH 2). All compounds were eluted with 5 mL of n-hexane, 5 mL of EtOAc and 10 mL of MeOH. The best recovery rates for acidic compounds ibuprofen, naproxen and diclofenac were achieved by MCX cartridge (respectively; 100.2, 115.30, 100.2%). The best recovery rates for caffeine and salicylic acid were performed by Oasis HLB (respectively; 74.50, 83.08%). Compared with Oasis HLB and Oasis MCX, the recovery rates of C18 were rather low (Table 4). Despite the straining of the collected water and waste water samples prior to the analysis, they caused obstruction in C18 and MCX cartridges. Therefore, Oasis HLB cartridge (at pH 2) was selected for all compounds to be used.

*Table 4***.** Recovery rates of different SPE cartridges at pH 2

Compounds (pg 500 mL^{-1})	**Supelco C-18**	**Oasis HBL**	**MCX**
Ibuprofen (2074 pg 500 mL^{-1})	%16.50±6	%72.84±10	%121.30±2
Naproxen (2410 pg 500 mL^{-1})	%6.50±4	%84.90±13	%115.00±3
Caffeine (2410 pg 500 mL^{-1})	%65.30±5	%74.50±6	%69.10±12
Diclofenac (3081 pg 500 mL^{-1})	%62.45±6	%89.17±11	%100.20±7
Salicylic acid (1600 pg 500 mL^{-1})	%25.65±12	%83.05±14	%74.56±10

2.9.Validation of GC-MS analysis

2.9.1. Linear correlation coefficients, Limits of Detections and Limits of Quantifications

The limit of detection (LOD) of the method was defined at the three times magnitude of the peak signal/background signal ratio (signal to noise ratio of 3) and the limit of quantification LOQ was defined as S/N=10. A series of different concentration of analytes (4 µg L^{-1}, 8 µg L^{-1}, 16 µg L^{-1}, 32 µg L^{-1}, 64 µg L^{-1}) were prepared as external standards in 400 µL volume of derivatization solution (125 µL Pyridine + 250 µL HMDS + 25 µL Trifluoroacetic acid) according to the optimized silylation with HMDS. Each one of the prepared solutions (3 vials containing the same concentrations) was

injected three times at injector volume 1 µL in splitless mode. Mass spectra were obtained in SIM (Selected Ion Monitoring) mode. Chromatographic peaks of selected compounds were integrated using a Merlin (Xcalibur Software) signal to noise calculator. Detection parameters of the analyte compounds are given below in Table 5, where ILD represents the detection limit of the analyte concentrations in the injector that gives S/N=3, ILQ represents the quantification limit of analyte concentration in the injector (S/N=10). MLD (Method Limit of Detection) and MLQ (Method Limit of Quantification) represents the detection and quantification limits of analytes in wastewaters. Correlation coefficients (R^2) in the concentration range 4-64 µg/L show that caffeine gives a better correlation compared with other compounds, probably due to not being included in the derivatization reaction.

Table 5. LOD and LOQ values of compounds in interest

Compound	ILD (pg µL^{-1})	ILQ (pg µL^{-1})	MLD (ng L^{-1})	MLQ (ng L^{-1})	R^2
Salicylic acid	1.05	3.5	1.7	5.7	0.9998
Ibuprofen	24.8	82.8	39.7	134.1	0.9897
Caffeine	3.5	11.7	5.6	19.0	0.9999
Naproxen	0.4	1.3	0.6	2.1	0.9984
Diclofenac	0.8	2.7	1.3	4.3	0.9984

3. RESULTS AND DISCUSSION

3.1. Characteristics of surface water and wastewater quality parameters

The pH, dissolved oxygen (DO), conductivity, turbidity and temperature measurements of the samples collected from W1, W2, W3, W4 and W5 stations were performed instantaneously by land-scale devices in the field. Furthermore, BOD, COD, TSS, AOX and TOC were measured to determine the characterization of measures of water (Table 6).

Table 6. Water quality parameters of samples

Stations of samples	BOD mg L^{-1}	COD mg L^{-1}	TSS mg L^{-1}	AOX mg L^{-1}	TOC mg L^{-1}	Turbidity	pH	EC (µS cm^{-1}) 25^{0}C	DO mg L^{-1}	T ^{0}C
W1	168	427	603	0.077	121.8	322	8.02	1130	1.38	15.8
W2	239	743	262	0.082	203.7	286	8.41	1242	5.44	16.3
W3	162	469	98	0.14	139.7	126	7.51	1198	1	16.2
W4	58	163	69	0.121	35	111	8.84	5240	0.2	16.1
W5	90	255	176	0.114	52.2	120	7.83	4851	1.2	15.7

3.2. Environmental samples

Pharmaceutical compounds, derivatized in surface water and wastewater collected from five different points in February 2009 and June 2009 were analyzed at GC-MS (for results see Table 7).

4. DISCUSSION

Ibuprofen; Annual consumption is estimated to be hundreds of tons of ibuprofen (Koutsouba *et al*.,2003). Between 200-800 mg is used per tablet. In many studies, it has been identified either in wastewater treatment plant output or in surface and underground waters. This is because of its very frequent use in prescriptions and its widespread use (Andreozzi *et al*.,2003; Ternes *et al*.,1998; Winkler *et al*.,2001). Although ibuprofen's half-life ($t_{1/2}$) is less than a day (Tauxe-Wuersch *et al*., 2005), it has been defined as $t_{1/2}$ = 50 days in water systems (Buser *et al*., 1998; Singer *et al*., 2002). Ibuprofen was found in neither periods. Its non-detection might be an indicator of ibuprofen being resolved in the wastewater treatment plant or its turning into metabolites.

Table 7. Determined concentrations of the selected NSAIDs (Standard Deviations in % are given in parentheses)

	W1 (ng L^{-1})	W2 (ng L^{-1})	W3 (ng L^{-1})	W4 (ng L^{-1})	W5 (ng L^{-1})
Winter					
İbuprofen	nd	nd	nd	nd	nd
Naproxen	nd	nd	nd	13.58(±1.5)	nd
Caffeine	nd	5.8(±3.8)	6.4(±5.5)	121.2(±8.1)	22.4(±3.5)
Diclofenac	nd	nd	nd	nd	nd
Salicylic acid	nd	nd	nd	15.74(±8.39)	nd
Summer					
İbuprofen	nd	nd	nd	nd	nd
Naproxen	nd	nd	2.12(±5.3)	10.48(±2.3)	nd
Caffein	12.3(±5.8)	6.6(±3.2)	nd	95.13(±7.4)	nd
Diclofenac	nd	nd	nd	nd	nd
Salicylic acid	nd	nd	nd	18.74 (±3.3)	nd

Diclofenac; Less than 1% of diclofenac compound is excreted unchanged from the body. Its half-life is $t_{1/2}$ = 4 hours. When it enters the aquatic environment, it dissociates by photodegradation within less than a day (Buser *et al*., 1998; Ayscoughet al., 2000). Diclofenac is variable around the compound. It has been detected in low concentration in wastewater treatment plants although it has not been detected in surface water samples in which wastewater is discharged (Ashton *et al*., 2004). Biotransformation occurs in the surface waters due to biological degradation; however it is less important than abiotic transformation reactions. Hydrolysis of important drugs for the environment is usually neglected, although photodegradation sometimes play an important role on water surfaces. Photolysis of diclofenac in surface waters showed that it has been the main removal process (Buser *et al*.,1998). During the study, diclofenac was found in neither period.

Caffeine; Caffeine as a stimulant is available in medicine for influenza, cold and in anti-inflammatory drugs. Being found in nature as well as in bodily disposals due to its intake via food, it is possible to detect it in very high concentrations in surface and waste water samples. A recent study (Pollack *et al*., 2009) suggested that exposure to caffeine may exacerbate the effects of other environmental stressors on corals, making them more likely to undergo bleaching. In our study, for both periods, the highest rate was detected to be 121.2 ng L^{-1}. The highest concentration was found at the W4 station. In many studies, caffeine stands among the most commonly existing compounds in the domestic wastewater treatment plant output, surface waters and underground waters (Tixier *et al.*,2002; Buser *et al.*,1998; Ashton *et al.*, 2004; Wiegel *et al.*, 2004; Gross *et al.*, 2004). This also shows that caffeine is permanent over long distances in the direction of surface water flow.

Salicylic acid; Acetyl salicylic acid as an analgesic and anti-inflammatory pharmaceutical is among the most common. It is used both in the paint industry and in medicine and veterinary sciences as well (Stellman *et al*., 1998). In 2000, close to 1000 tons per year in European countries, and 18 tons in Britain were available for use (Jones *et al*., 2002). Salicylic acid is a metabolite of acetyl salicylic acid. We detected the highest concentrations of salicylic acid at the W4 station for each period, respectively at 15.74-18.74 ng L^{-1}. The unstable status of salicylic acid in the waters of the wastewater treatment plant output is not well known (Brun *et al*., 2006). Like caffeine, the detection of salicylic acid in high concentrations W4 station might indicate its permanence over long distances in surface water.

Naproxen; Naproxen, with its wide use in both medicine and veterinary sciences, is among the non-prescription anti-inflammatory drugs group (Metcalfe *et al*., 2003). It is excreted out of the body, with around 60 % as non-metabolized (Kosjek *et al*., 2005). Some studies of water systems have reported the half-life of naproxen as being 50 days (Kosjek et al., 2004), while another study has reported it as being less than a day (Kosjek *et al*., 1985). The concentration of NSAIDs residues in surface water bodies receiving WWTP effluents may range between 0.4 and 0.5 µg L^{-1} (e.g. naproxen, diclofenac, Ibuprofen) , a range which may cause chronic toxicity in some aquatic

organisms or undesired health effects in humans upon prolonged exposure (Ziylan and Ince, 2011). As with caffeine and salicylic acid, the highest concentrations of naproxen were found at the W4 station both during winter and summer periods, respectively at 13.58-10.48 ng L^{-1}.

5. CONCLUSIONS

Regarding the results; despite having a wastewater treatment plant, the high rate of drug components (except for ibuprofen and diclofenac) at the W4 station might be due to its representation of all domestic and industrial wastewater of Çerkezköy region as well as the low removal rates of the drugs in the Wastewater Treatment Plant (WWTP). Since there is no Domestic Wastewater Plant in Çorlu, unremoved drugs reach up to surface waters through the wastewater. We know that these components do not exist solely in the wastewater while many organic and inorganic chemicals are also included in this water. Therefore, the monitoring of drug components would be recommended at wastewater discharge standards in Turkey while the optimization of existing WWTP is also required. This study stands as one of the first studies in Turkey which refers to the detection and reporting of the concentrations of pharmaceutical products in wastewater and surface waters. From this point of view, it will shed light to develop further studies on this issue or in other regions of Turkey.

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