Detection, ecological risk assessment and removal efficiency of diclofenac and caffeine in wastewater treatment plant

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Abstract. Pharmaceutical residues that are considered as emerging contaminants have received increased interest in the last years due to their adverse effects on the environment. This is the first study on the assessment of diclofenac and caffeine in municipal waste water of Egypt. The study aims also to report the removal efficiency of waste water treatment plant and the ecological risk assessment of diclofenac and caffeine. Diclofenac and caffeine were detected in both influent and effluent of Tezmant waste water treatment plant (WWWP) in Beni-Suef governorate, Egypt with detection frequency of 100% by using UPLC-MS/MS method. The maximum detected concentrations were 27.85 µg/L for caffeine in February and 1.07µg/L for diclofenac in January, respectively. Caffeine showed higher removal efficiency (95.2%) than that of diclofenac (69.6%). Based on Predicted No Effect Concentration (PNEC) obtained from the literature, it is observed that diclofenac has high risk (RQ>1) on fish and low risk on algae and Daphnia. Meanwhile, caffeine showed low risk on all of the studied species.

Keywords:Diclofenac; Caffeine; Wastewater Treatment Plants; Removal efficiency; Solid phase extraction; Ecological risk assessment

1. Introduction

Pharmaceuticals are biological active compounds that are very important in our life because of their effects on living organisms even at low concentration for treatment of illness and the reduction of pain. However, they may impose harmful effects on different organs through their side effects. Discharging of Pharmaceutical residues to the environment may induce acute and chronic effects on living organisms [1–5]. Recently, there has been a great concern about pharmaceutical residues in the environment due to their persistency and continuous input [6,7]. They find their way to the environment through many routs such as effluents of municipal wastewater treatment plants (MWWTPs) and landfill effluents.

Among the largest consumed pharmaceutical drugs in Egypt and many countries around the world are diclofenac and caffeine. Diclofenac is non-steroidal anti-inflammatory drug that is used for treatment of several diseases including arthritis and rheumatoid diseases. Caffeine is a natural alkaloid...
that is extracted from some plants. Caffeine is used increasingly as a stimulant to the central nervous system and it is found in coffee, tea and many pharmaceutical products [8]. Caffeine and diclofenac are detected in waste water and other water masses around the world as well as drinking water [9–11]. Although the detected concentrations are very low (from ng/L to µg/L), their continuous input to the environment induced undesirable ecological effects such as decreases of vulture population in Pakistan [12,13] and human health effects. The presence of diclofenac in drinking water even at low concentration is a very serious problem to humans as it causes renal lesions as well as cytotoxicity to liver and kidney [14–17].

Although there are several studies worldwide evaluating the residual concentrations of diclofenac and caffeine, the scarcity of available information on their quantities in different compartments of the aquatic ecosystems in Egypt as well as their reported drastic ecological and human health effects necessitated the present study. This study aims to detect the residual concentration, removal efficiency and ecological risk assessment of both caffeine and diclofenac in a municipal wastewater treatment plant that serves a large population in Beni-Suef governorate, Egypt. The massive use of Egyptian community of the two investigated drugs also added to the value of the present study.

2. Materials and method

2.1 Chemicals and materials

Diclofenac sodium standard (DCF) (98.74%) and caffeine (CAF) (99.45%) obtained from sigma Aldrich. All reagents used were of HPLC grade obtained from Fisher scientific company (methanol, Acetonitrile and water). Ethylenediamine tetraacetic acid disodium salt dihydrates (EDTA) (99.0–101.0%) and sodium phosphate monobasic dehydrate are obtained from Sigma-Aldrich. Formic acid (analytical reagent grade, 98%), Hydrochloric acid, 36.5-38% (ACS BASIC Scharlau), Phosphoric acid (Laboratory reagent group) were supplied by Fischer Scientific. Oasis HLB cartridge (200 mg, 6 cm3) Waters (Milford, MA, USA) is used. Glass microfiber filter (1 µm GF/B, 0.45 µm) Whatman is used to remove suspended solids and syringe filter (PVDF) 0.45 µm pore size used in filtration of sample before injection to UPLC.

2.2 Sampling

Twenty-four 24-h composite samples were collected from the influent and effluent of Tezmant municipal waste water treatment plant in Beni-Suef governorate, Egypt. Sampling was carried out in winter season (from December 2015 to February 2016). Samples were collected in dark amber glass bottles, then transported to the lab in ice bag and extracted in the same day of collection to reduce the degradation of the target compounds.

2.3 Extraction

The sample was filtered using 1 µm then 0.45 µm glass microfiber filters and acidified with 0.1 M HCL to pH=2, then 0.5 g EDTA was added. Solid phase extraction cartridge was conditioned three times by 5 mL methanol and then three times by 5 mL deionized water for activation. The sample was then passed through the cartridge. After that the cartridge was washed by deionized water and dried for about 20 minutes under a vacuum. Elution process was made by 5 mL methanol twice. The extract was evaporated by using rotary evaporator at 50 °C then re-dissolved in 2 mL methanol and 1 mL 0.1% formic acid solution (0.1% formic in methanol). The final extract was cleaned by filtration with 0.45 µm PVDF syringe filter before UPLC-MS-MS analysis.

2.4 Analysis

Diclofenac and caffeine were analyzed by using Ultra-performance Liquid Chromatography tandem mass spectrometry System UPLC-MS/MS (waters) with positive ion mode electrospray. The used detector is Triplet Quadrapole. First, the separation done using C18 Column, 3.5 µm particle size (4.6 ×50 mm) which was maintained at 40°C using the mobile phase consisting of water containing 0.3% formic acid and 0.1 ammonium formate (solvent A) and acetonitrile- methanol (in ratio 1:1, v/v) (solvent B) with Positive Ion Electrospray mode at a flow rate of 0.15- 0.30 ml/min at a gradient
elution. The elution program started with 5% eluent B for 4 min then increased to 88%, 100% at time 22.5 and 23 min consequently. The injection volume was 15µL. Mass spectrometry conditions are source temperature at 140°C and desolvation temperature 350°C. Quantification was done based on peak areas of target compounds. Standard stock solutions of diclofenac and caffeine (1 mg/mL) were prepared in methanol, and then proper dilution was performed to prepare working solution of both drugs in concentration of (1 µg/mL) in methanol. The calibration curve was done in the range of 0.001-1 μg/L (9 points) for caffeine and 0.01-1 μg/L (10 points) for diclofenac by dissolving them in methanol. The correlation coefficients (R2) of the calibration curves are shown in table 2. The limit of detection (LOD) was determined as the lowest concentration gives a signal to noise (S/N) ratio of 3 and the Limit of Quantification (LOQ) was measured with S/N ratio of 10. The recoveries of the target compounds were carried out by spiking three water samples with standard drugs at concentration of 100, 200 and 300 ng/L (Table 1).

Table 1. Main analytical parameters of the target pharmaceuticals.

| Compound     | LOD (ng/L) | LOQ(ng/L) | R²   | Recovery % |
|--------------|------------|-----------|------|------------|
| Diclofenac   | 2.85       | 8.55      | 0.982| 58.79      |
| Caffeine     | 0.31       | 0.95      | 0.985| 80.97      |

* LOD = Limits of detection,  
* LOQ = limit of quantitation  
* Data are expressed as average of 3 determinations.

2.5. Removal efficiency estimation

The efficiency of wastewater treatment plant for the removal of caffeine and diclofenac was calculated by the concentration of them in the aqueous phase in the influent and effluent [18,19] as presented in equation (1).

$$\% \text{ Removal efficiency} = \frac{C_{\text{influent}} - C_{\text{effluent}}}{C_{\text{influent}}} \times 100$$  \hspace{1cm} (1)

2.6. Ecotoxicological risk assessment

The expected risk resulted from the discharge of the effluent waste water containing caffeine and diclofenac to the environment was calculated by using Risk Quotient (RQ) which is the ratio of the measured environmental concentration (MEC) to its Predicted No Effect Concentration (PNEC).

$$\text{RQ} = \frac{\text{MEC}}{\text{PNEC}}$$  \hspace{1cm} (2)

Where MEC corresponds to the highest detected effluent concentration

PNEC is the safe dose of compound concentration and it is measured using equation (3).

$$\text{PNEC} = \frac{(\text{EC}_{50} \text{ or LC}_{50})}{\text{AF}}$$  \hspace{1cm} (3)

Where (EC_{50}) is the median effective concentration, AF (Assessment factor) is the safety factor or uncertainty factor which set 1000 as recommended by European Commission [20]. According to Hernando et al. 2006, RQ values will determine the resulted risk as following: RQ is ≥ 1 indicates high ecological risk, 0.1 ≤ RQ < 1 a median risk is suspected and 0.01 ≤ RQ < 0.1 low risk may appear.

3. Results and discussion

3.1. Occurrence of diclofenac and caffeine

Twenty-four samples were collected from influent and effluent of wastewater treatment plant during winter season (2016) in December, January and February. As shown in Figure 1, the lowest detected concentration was in December for both diclofenac and caffeine. Caffeine concentrations were higher than diclofenac in all months.
Diclofenac was detected in all of the samples with maximum concentration of 1.07 µg/L and minimum concentration below limit of quantification (Table 2). The detected concentrations were lower than that reported by Papageorgiou et al., 2016 (4.869 µg/L) and Kosma et al. 2014 (5.16 µg/L) in Greece but also Lower concentrations were detected in Thailand (0.382 µg/L) [21].

Caffeine detection frequency was 100% for all influent and effluent samples and It was detected with relatively high maximum concentration (27.85 µg/L) (Table 2) that was similar to the majority of previous studies on occurrence of caffeine for example, 192 µg/L in Mediterranean Coast [22], 96.65 µg/L in Greece [10], 89.5 µg/L in China [23]. Otherwise, there are relatively low concentrations of caffeine such as that reported by the following research articles: 5.559 µg/L in Greece [3] and 4.550 µg/L in Thailand [21].

![Figure 1. Occurrence of diclofenac and caffeine in Tezmant wastewater treatment plant, Egypt.](image)

The high concentration of caffeine may be due to the high rate of drinking coffee and tea in the Egyptian community.

### Table 2. Concentration of diclofenac and caffeine in both influent and effluent of municipal waste water treatment plant.

| compound  | Influent | Effluent |
|-----------|----------|----------|
|           | Min (µg/L) | Max (µg/L) | Frequency | Min (µg/L) | Max (µg/L) | Frequency |
| Caffeine  | 6.67     | 27.85   | 100%       | <LOQ      | 2.32     | 100%       |
| Diclofenac| 0.03     | 1.07    | 100%       | <LOQ      | 0.65     | 100%       |

#### 3.2. Removal efficiency

There are many parameters that affect the removal efficiency of the waste water treatment plant for the pollutant such as its physicochemical properties that affect whether a compound will remain in the aqueous phase or adsorbed to sludge and wastewater treatment plant operating conditions. Adsorption onto suspended solids, aerobic, and anaerobic biodegradation, and chemical degradation (via processes such as hydrolysis) and volatilization are the primary removal mechanisms for pharmaceutical residues in waste water. As shown in Figure 2, Diclofenac removal rate was 69.6% that can be rationalized by high adsorption to sewage sludge and suspended solids [24,25]. However, hydrolysis is not expected to contribute to their removal as its structure lack functional groups that may trigger hydrolysis under environmental condition, also volatilization has negligible effect (low Kc henry law constant). High removal efficiency (74 %) was reported by Fernández-lópez et al. (2016). However, there are other studies that reported low removal efficiency of 40 % in Japan [26] and 17% in Germany [27]. Low removal efficiency of diclofenac could be due to the presence of chlorine group in the molecule decreases biodegradation kinetic constants, kbi. The biodegradation process proceeded through the following series: migration of the organic pollutant from the bulk solution to the microorganism, penetrating (to) microorganism membrane and then enzymatic oxidation for the
pollutant. In the case of diclofenac, enzymatic oxidation step is slow as chlorine is electron-withdrawing group and the oxidation process needs oxygen which is electrophilic [26,28–30].

Figure 2. Removal efficiency of WWTP for diclofenac and caffeine.

Caffeine removal from the WWTP was very high that are with large commitment with other results 94.8% [3], 99% [22], 96% [31], >99% [32]. Low removal recorded by Santos et al., 2007 (38 to 86%). Caffeine main removal mechanism is biodegradation because of its slow photo catalysis and its low sorption [8,33].

3.3. Risk assessment
The environmental risk assessment gives us the priorities for the compound that showed a risk for further studying and how to treat it. As shown in table 3, the acute toxicity of diclofenac and caffeine has been calculated for fish, algae and invertebrate and based on the maximum concentration in the effluent of the Waste water treatment plant to present the worthiest case scenario. It was observed that there is only a high risk from diclofenac on fish (RQ>1) and low risk on the other species. Diclofenac high risk was also observed in other studies that reported high diclofenac concentration than our study[3,10]. On the other hand, there is a low risk from caffeine on all the studied species (0.01 ≤ RQ < 0.1) although caffeine concentration is higher than diclofenac. This observation reflects that the toxicity of the pollutants depends on many factors other than concentration.

Table 3. Acute toxicity of the studied pharmaceuticals.

| Compound  | Species  | EC₅₀,LC₅₀(mg/L) | References | PNEC (mg/L) | MEC (mg/L) | RQ   |
|-----------|----------|-----------------|------------|-------------|-------------|------|
| Diclofenac| Algae    | 16              | [34]       | 0.016       | 0.00065     | 0.04 |
|           | Fish     | 0.09            | [35]       | 9 × 10⁻³    | 0.026       | 7.22 |
|           | Invertebrates | 23           | [34]       | 0.023       |             | 0.028|
| Caffeine  | Algae    | 46              | [36]       | 0.046       | 0.00232     | 0.05 |
|           | Fish     | 87.5            | [37]       | 0.0875      |             | 0.026|
|           | Invertebrates | 182           |            | 0.182       |             | 0.013|
4. Conclusion

Both of caffeine and diclofenac were detected in all of the collected samples. However, caffeine was detected in high concentration (27.85 µg/L – maximum concentration in the influent) and showed high removal efficiency (95.2%), its ecological risk is low for algae, Daphnia and fish. Moreover, diclofenac was detected in relatively low concentration in comparison with caffeine, and was found to have a high risk effect on fish. Therefore, further studies on detection of diclofenac and caffeine in surface and drinking water is essential, also the acute and chronic environmental risk on different organisms is required.

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References

[1] K’oreje K O, Vergeynst L, Ombaka D, De Wispelaere P, Okoth M, Van Langenhove H and Demeestere K 2016 Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya Chemosphere 149 238–44
[2] Crane M, Watts C and Boucard T 2006 Chronic aquatic environmental risks from exposure to human pharmaceuticals Sci. Total Environ. 367 23–41
[3] Papageorgiou M, Kosma C and Lambropoulou D 2016 Seasonal occurrence, removal, mass loading and environmental risk assessment of 55 pharmaceuticals and personal care products in a municipal wastewater treatment plant in Central Greece Sci. Total Environ. 543 547–69
[4] Richardson B J, Lam P K S and Martin M 2005 Emerging chemicals of concern: pharmaceuticals and personal care products (PPCPs) in Asia, with particular reference to Southern China Mar. Pollut. Bull. 50 913–20
[5] Verlicchi P, Aukidy M Al and Zambello E 2012 Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment — A review Sci. Total Environ. 429 123–55
[6] Kümmmerer K 2009 Antibiotics in the aquatic environment—a review—part I. Chemosphere 75 417–34
[7] Ternes T A 1998 Occurrence of drugs in German sewage treatment plants and rivers Water Res. 32 3245–60
[8] Buerge I J, Poiger T, Müller M D and Buser H-R 2003 Caffeine, an anthropogenic marker for wastewater contamination of surface waters Environ. Sci. Technol. 37 691
[9] Carmona E, Andreu V and Picó Y 2014 Occurrence of acidic pharmaceuticals and personal care products in Tuna River Basin: From waste to drinking water Sci. Total Environ. 484 53–63
[10] Kosma C I, Lambropoulou D A and Albanis T A 2014 Investigation of PPCPs in wastewater treatment plants in Greece: occurrence, removal and environmental risk assessment Sci. Total Environ. 466 421–38
[11] Vieno N, Tuhkanen T and Kronberg L 2007 Elimination of pharmaceuticals in sewage treatment plants in Finland Water Res. 41 1001–12
[12] Collier A C 2007 Pharmaceutical contaminants in potable water: potential concerns for pregnant women and children Ecohealth 4 164–71
[13] Oaks J L, Gilbert M, Virani M Z, Watson R T, Meteyer C U, Rideout B A, Shivaprasad H L, Ahmed S, Chaudhry M J I and Arshad M 2004 Diclofenac residues as the cause of vulture population decline in Pakistan Nature 427 630
[14] Hartmann J, Bartels P, Mau U, Witter M, Tümpling W v., Hofmann J and Nietzschmann E 2008 Degradation of the drug diclofenac in water by sonolysis in presence of catalysts Chemosphere 70 453–61
Martínez C, Canle M L, Fernández M I, Santaballa J A and Faria J 2011 Applied Catalysis B: Environmental Aqueous degradation of diclofenac by heterogeneous photocatalysis using nanostructured materials Appl. Catal. B Environ. 107 110–8

R. T. H. C, A. H, R. E and H.-R. K 2004 Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part II. Cytological effects in liver, kidney, gills and intestine of rainbow trout (Oncorhynchus mykiss) Aquat. Toxicol. 68 151–66

Schwaiger J, Ferling H, Mallow U, Wintermayr H and Negelea R D 2004 Toxic effects of the non-steroidal antiinflammatory drug diclofenac: part I: histopathological alterations and bioaccumulation in rainbow trout Aquat Toxicol 68 141

Santos L H M L M, Gros M, Rodríguez-mozaz S, Delerue-matos C, Pena A, Barceló D and Montenegro M C B S M 2013 Science of the Total Environment Contribution of hospital effl uents to the load of pharmaceuticals in urban wastewaters: Identi fi cation of ecologically relevant pharmaceuticals Sci. Total Environ. 461–462 302–16

Kosma C I, Lambropoulou D A and Albanis T A 2010 Occurrence and removal of pharmaceutically active compounds in wastewaters in Greece HazWasManagement2010 179 1–4

European Commission - Joint Research Centre - Institute for Health and Consumer Protection 2003 Technical Guidance Document on Risk Assessment Inst. Heal. Consum. Prot. Eur. Chem. Bur. Part II. Available online http://echa. Eur. eu/documents/10162/16960216/tgdpart2_2ed_en.pdf 398

Tewari S, Jindal R, Kho Y L, Eo S and Choi K 2013 Major pharmaceutical residues in wastewater treatment plants and receiving waters in Bangkok, Thailand, and associated ecological Chemosphere 91 697–704

Gómez M J, Martínez Bueno M J, Lacorte S, Fernández-Alba A R and Agiéria A 2007 Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast Chemosphere 66 993–1002

Zhou, Haidong, Chunying Wu, Xia Huang, Mijun Gao, Xianghua Wen, Hiroshi Tsuno and H T 2010 Occurrence of Selected Pharmaceuticals and Caffeine in Sewage Treatment Plants and Receiving Rive ... Science (80-). 82 2239–48

Sipma J, Osuna B, Collado N, Monclús H, Ferrero G, Comas J and Rodriguez-Roda I 2009 Author’s personal copy Comparison of removal of pharmaceuticals in MBR and activated sludge systems Desalination 250 653–9

Ternes T A, Herrmann N, Bonerz M, Knacker T, Siegrist H and Joss A 2004 A rapid method to measure the solid-water distribution coefficient (K-d) for pharmaceuticals and musk fragrances in sewage sludge Water Res. 38 4075–84

Fernández-López C, Guilén-Navarro J M, Padilla J J and Parsons J R 2016 Comparison of the removal efficiencies of selected pharmaceuticals in wastewater treatment plants in the region of Murcia, Spain Ecol. Eng. 95 811–6

Kimura K, Hara H and Watanabe Y 2007 Elimination of selected acidic pharmaceuticals from municipal wastewaters by activated sludge systems and membrane bioreactors Environ. Sci. Technol. 41 3708–14

Heberer T, Reddersen K and Mechlis[K]inski A 2002 From municipal sewage to drinking water: fate and removal of pharmaceutical residues in the aquatic environment in urban areas. Water Sci. Technol. 46 81–8

Jelic A, Gros M, Ginebreda A, Cespedes-Sánchez R, Ventura F, Petrovic M and Barceló D 2010 Title: Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment Water Res. 45 1165–76

Cirja M and Ivasechkin Ø P 2007 Factors affecting the removal of organic micropollutants from wastewater in conventional treatment plants ( CTP ) and membrane bioreactors ( MBR ) Factors affecting the removal of organic micropollutants from wastewater in conventional treatment plants ( Rev. Environ. Sci. Bio/Technology 7 61–78

Andreozi R, Cesaro R, Marotta R and Pirozzi F 2006 Evaluation of biodegradation kinetic
constants for aromatic compounds by means of aerobic batch experiments *Chemosphere* **62** 1431–6

[32]  Heberer T 2016 Tracking persistent pharmaceutical residues from municipal sewage to drinking water. *J. Hydrol.* **266** 175–89

[33]  Ternes T A, Bonerz M and Schimdt T 2001 Determination of neutral pharmaceuticals in waste waters and rivers by liquid chromatography electrospray tandem mass spectrometry *J. Chromatogr. A* **938** 175–85

[34]  Grung M, Källqvist T, Sakshaug S, Skurtveit S and Thomas K V. 2008 Environmental assessment of Norwegian priority pharmaceuticals based on the EMEA guideline *Ecotoxicol. Environ. Saf.* **71** 328–40

[35]  Dietrich D R and Prietz A 1999 Fish embryotoxicity and teratogenicity of pharmaceuticals, detergents and pesticides regularly detected in sewage treatment plant effluents and surface waters *Toxicologist* **48** 151

[36]  Sanderson H, Johnson D ., Wilson C ., Brain R ., and Solomon K . 2003 Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids, and algae by ECOSAR screening *Toxicol. Lett.* **144** 383–95

[37]  Fernández C, González-Doncel M, Pro J, Carbonell G and Tarazona J V. 2010 Occurrence of pharmaceutically active compounds in surface waters of the henares-jarama-tajo river system (madrid, spain) and a potential risk characterization *Sci Total Env.* **408** 543–51