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The occurrence of Diclofenac in the particulate phase of wastewaters

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ABSTRACT

Abstract:*Diclofenac is one of the most consumed drugs in the world and in Algeria. It is classified in the therapeutic group of non-steroidal anti-inflammatory drugs (NSAIDs). The presence of pharmaceuticals in the environment is known in the recent decades as "emerging contaminants", the most part of these substances can enter in the aquatic systems from the excretion of these molecules after human consumption or veterinary use. Our work focuses on the presence of these molecules in the wastewater's particulate phase of two wastewaters treatment plants (WWTPs) in Algiers. The particulate phase was isolated by filtration of wastewater on glass fibre filters (GFF) with diameter pores of 1.6 and 0.7 µm, and then the filters were extracted using an ultrasound bath followed by a centrifugation step. The filtrate was diluted in Ultra-pure water and extracted with solid phase extraction (SPE) cartridges. The samples were analysed by gas chromatography coupled with mass spectrometry (GC-MS) after a derivatization step with N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) to reduce the polarity of our molecule and increase its volatility, which make the analysis more suitable.*

I. Introduction

The pollution of aquatic systems by classic contaminants as polycyclic aromatic hydrocarbons (HAPs), pesticides and polychlorinated biphenyls (PCBs), has attracted the world's attention for their consequences on the environments, the organisms and human health [1, 2].
In the last decades, a new category of pollutants has been occurred in the environment under the name of "emerging contaminants", as the presence of the pharmaceutical products in the aquatic systems. Depending on their great consumption, pharmaceuticals have been detected in different water's types, in wastewaters (influents and effluents) [3-6], in surface waters [7-9], and even in drinking waters [3, 9-11], rising concerns about their implications on the ecosystems and human health.
The wastewater treatment plants are not designed to eliminate this kind of pollutants, many reports indicated their removal efficiencies by WWTPs, ranging from 50 to 90% [12]. The presence of pharmaceuticals in surface water and drinking water is due to the continual discharge of these molecules via multiple pathways, engendering a so-called "Pseudo-persistence" [6].
Solid Phase Extraction (SPE) are the most used method for the extraction and the concentration of water samples searching the presence of pharmaceutical compounds in the environment [13]. The instrumentation mainly used in the analysis of pharmaceuticals in water are Gas Chromatography coupled with Mass Spectrometry or in tandem (GC/MS GC/MS/MS) and Liquid Chromatography coupled with Mass Spectrometry or in tandem (LC/MS or LC/MS/MS) [14]. Analysis of acidic pharmaceuticals bay GC/MS need a derivatization step to reduce their polarity and rise their volatility [15].
The present study was focused on the occurrence of a pharmaceutical product "Diclofenac", belonging to the therapeutic group of non-steroidal anti-inflammatory drugs (NSAIDs) in the particulate

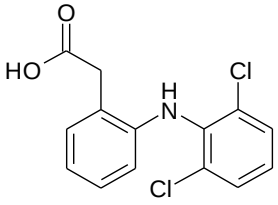
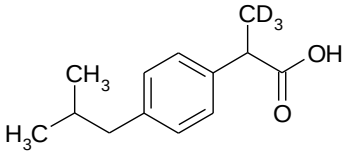
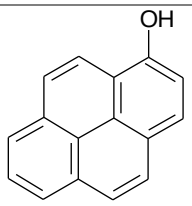
phase of wastewaters in Algiers, which their aquatic systems have a direct impact on the Mediterranean Sea. The samples are concentrated using solid phase concentration (SPE) method. The analysis ware performed by GC/MS after a silylation step withN-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA).

II. Materials and methods

II.1. Chemicals and reagents

Isotope-labeled standard Ibuprofen-d3 (IBU-d3) and 1-Hydroxypyrene (HPY) both with purity higher than 98%, Na2EDTA (99%), Ortho-phosphoric acid (85%), HPLC-grade methanol and ethyl acetate were purchased from Sigma-Aldrich. Oasis® hydrophilic–lipophilic balanced (HLB, 3 cc, 60 mg) cartridges were purchased from Waters (USA). GFF glass fibre filters pore size 1.6 µm and Nylon filters pore size 0.45 µm were from Filtres-Fioroni. MSTFA were purchased from Sigma–Aldrich. Ultrapure water with resistivity 18.2 MΩ x cm was obtained from milli-Q system (Millipore). The chemical structures and proprieties of the target pharmaceutical product and the surrogate standards are given in Table 1.

Table 1.*Chemical structures and proprieties of pharmaceuticals.*

Compounds	**CAS number**	**Molecular formula**	**MW (g.mol^{-1})**	**Chemical stricture**
Diclofenac (DIC)	15307-79-6	$C_{14}H_{11}Cl_2NO_2$	296	
Ibuprofen-d3 (IBU-d3)	121662-14-4	$C_{13}D_3H_{15}O_2$	209	
1-hydroxypyren (HPY)	5315-79-7	$C_{16}H_{10}O$	218	

II.2. Sample collection

Water samples were collected during November 2014 at two WWTPs of BeniMessous and Reghaia as 24-h composite samples .Reghaia WWTP can serves a Population equivalent of 400000 inhabitants with Average daily flow of 80000 m3/day. This WWTP treats Mixed Wastewaters (domestic and industrial). BeniMessous WWTP serves 250000 inhabitants with an average daily flow of 50400 m3/day and treats domestic sewage. The two WWTPs Discharges the treated water to the Mediterranean Sea (Figure1).

The samples were filtered with 1.6 µm and 0.45 µm; the pH was adjusted at 2.5~3 with Ortho-phosphoric acid 5% [7, 11]. The storage of the samples was in darkness at 4 °C and the extraction was elaborated within 24 h.

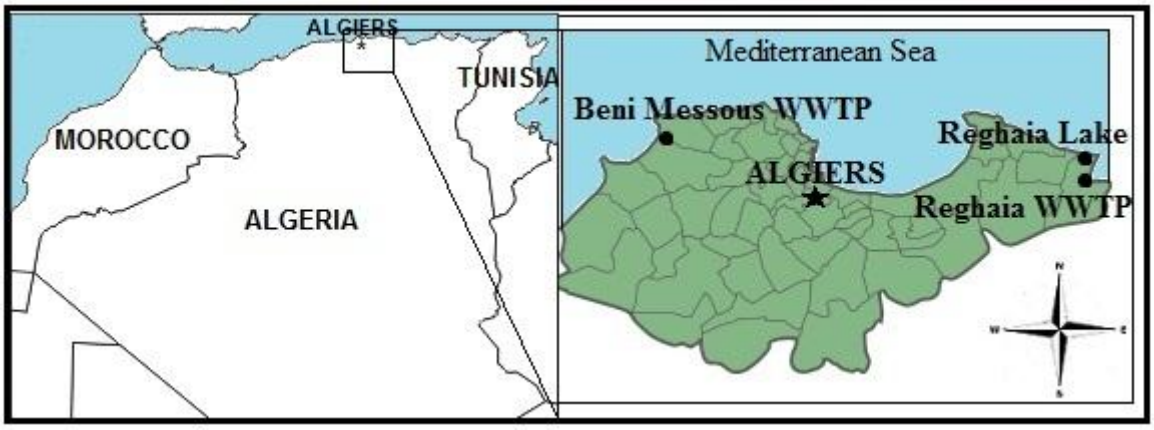


Figure 1.*Map of the sampling sites* [16]

II.3. Extraction procedures

The Filters were placed in centrifuge tubes of 15 mL, with 10 mL of extraction solvent (Phosphoric acid 1M / Methanol (20/80 v / v)). After the agitation, the samples were placed in an ultrasound bath for 15 min, and then they were centrifuged (2500 rev / min for 5 min). The supernatants were recovered in a glass tube and then diluted in 100 mL of ultra-pure water and the pH was adjusted to 3 with orthophosphoric acid 5% [17, 18]. The diluted extracts were extracted on Oasis HLB cartridges (60mg, 3ml), the extraction procedure are illustrated in Figure (2).

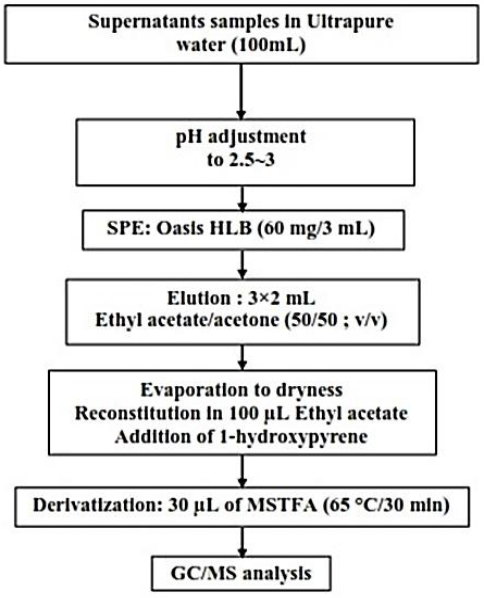


Figure 2. *The organogram of the extraction procedure.*

II.4. GC-MS analysis

The samples were analysed by GC-MS (Agilent 7890A GC system and 5975C Series MSD) with High Sensitivity Triple-Axis Detector. The column of separation was HP-5 MS capillary column (5% diphenyl/95% dimethylsiloxane; 30 m × 0.25 mm × 0.25 µm film thickness). The carrier gas was ultrapure helium (purity > 99.999%, Air Liquide) set at a constant flow mode (1.3 ml/min).1 µL of extract were injected into the GC in splitless mode at 250 °C using an Agilent 7693 Autosampler/G4513A series injector. The GC oven was programed at 70 °C, hold 2 min, then increased to 280 °C with a rate of 10 °C/min, then hold for 5 min at 280 °C.

III. Results and discussion

III.1. GC/MS method

The compounds were identified in full-scan mode (m/z 40–550) by injection of individual standard solutions firstly and then a mixture of standards solution. The Retention times and m/z ratios (trimethylsilyl (TMS) derivatives).are given in Table 2. The linearity were calculated and calibrated to the ratio of peak areas of analyte and the internal standard with five-point calibration curves. The range of linearity are from 10 to 2000 ng/L and the curves have a correlation coefficient (r2) higher than 0.99, which means a satisfactory linearity (Table 2).
The concentrations were determined following equation 1:

$$CA = \frac{Analyte\ area}{I.S.\ area} X \frac{CI.S}{RRF} \textbf{(1)}$$

Were CA: concentration of analyte (ng/L), I.S: Internal Standard and CI.S: concentration of Internal Standard (ng/L) [19].

The relative response factor (RRF) was determined from the slope of the line.

III.2. Occurrence of Diclofenac in the particulate phase of wastewaters

The concentrations of diclofenac in particulate phase of wastewater influents and effluents are presented in Table 2. The highest concentration were detected in Reghaia influent (383 ng/L) and the lowest concentration in BeniMessous effluent (188 ng/L). A diminution of the concentration was observed in comparing the concentrations between influents and effluents for the both plants as illustrated in Figure 3. The presence of diclofenac in the final influents prove the incapability of the classical WWTPs for the total elimination of pharmaceuticals. These molecules can present a high risk for the aquatic systems living organisms and for human health by the infiltration into surface water and even in drinking water. The continual dishrag of pharmaceuticals (excretion) increase their presence and the so-called pseudo-persistence.

Table 2.*Retention times, m/z ratios and the concentrations detected of Diclofenac in wastewater particulate phase.*

Compounds		**Retention time (min)**	**m/z ratio**	**Reghaia WWTP**		**Beni Messous WWTP**		**r^2**
				Influent ($ng.L^{-1}$)	**Effluent ($ng.L^{-1}$)**	**Influent ($ng.L^{-1}$)**	**Effluent ($ng.L^{-1}$)**	
Pharmaceutical	DIC	19.923	214	383	267	345	188	0.9982
Deuterated standard for quantification	IBU-d3	13.188	163					
Standard for recovery validation	HPY	21.395	290					

The concentration of diclofenac in the surface water is depending on their elimination in the treatment plants. In addition, the dilution phenomena play an important factor for the presence of pharmaceuticals in aquatic systems, which means high presence in closed aquatic systems, a moderate presence in semi-open aquatic systems and low in the open systems [3].

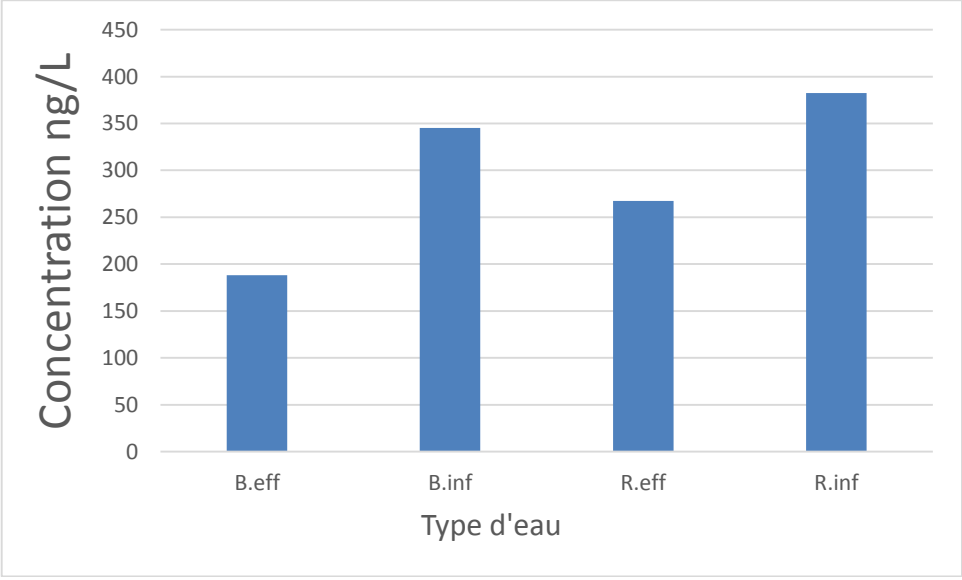


Figure 3.*Comparison of the concentrations influents and effluents.*

IV. Conclusion

In this work, an SPE extraction method assisted with GC/MS detection has been used for studying the presence of one NSAIDs "Diclofenac", in wastewater particulate phase of influents and effluents in two WWTPs situated in Algiers. The analytical method has been verified and the derivatization reaction has improved the volatility and thermal stability of the molecule, allowing good resolution.

Our study shows the presence of diclofenac in the two treatment plants and especially in the wastewater before treatment (influents). The presence of Diclofenac in effluents indicate the persistence of this pharmaceutical product during WWTPs elimination processes. Indeed, the introduction of effluents containing pharmaceutical compounds into aquatic systems engender high risks for the ecosystem, aquatic living organisms and human health.

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