Detecting emerging pollutants in environmental compartment by Ultra High Performance Liquid Chromatography - Time of Flight Mass Spectrometry

Carmona Eric[1,*], Pico Yolanda.[1], Thomaidis Nikolaos.[2]

[1] Environmental and Food Safety Research Group (SAMA-UV), Desertification Research Centre CIDE (CSIC-UV- GV), Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, Burjassot, 46100 Valencia, Spain.

[2] Trace Analysis and Mass Spectrometry group (TrAMS), Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, 157 71 Athens, Greece.

*corresponding author:

e-mail: eric.carmona@uv.es

Abstract.

Pharmaceuticals, illicit drugs, personal care products and pesticides, among others, are emerging pollutants widely distributed in water. In this work, the occurrence of these pollutants in waste and surface waters as well as in fish (*Anguila anguila*) has been study. A screening of these kind of compounds, in order to detect all the compounds present in these matrices, was carried out with a Ultra High Performance Liquid Chromatograph coupled to a Time of Flight Mass Spectrometry (UHPLC-QToF MS/MS) and compared with a library of more than 1200 compounds. More than 200 compounds in the effluents were identified at low concentrations. Less compounds were detected in water of the Albufera Lake, 90 emerging pollutants Two extraction methods, for biota samples were compared, the QuEChERS (one of the most used methods) and other developed in the laboratory based in a McIlvaine-EDTA buffer extraction. Resulting better peak areas in the McIlvaine-EDTA buffer method than QuEChERS and detecting some compounds that do not appear with the last method (55 compounds were detected with the McIlvaine extraction and 35 with QuEChERS).

Keywords: Emerging pollutants, WWTP, effluents, lake, water, fish, UHPLC, qToF

1. Introduction

A large number of compounds, named emerging pollutants, are continuously entering the aquatic environment due mostly to the human or animals, agriculture and industrial activity (Murray *et al*., 2010). In case of human activity, these substances are intake as nourishment or for the health care and excreted by the urine or feces (Khetan *et al*. 2007) (Carmona *et al*. 2014). Some of these emerging pollutants, as pharmaceuticals or cosmetics, are characteristics of human activity (Hardon *et al*. 2004). Agriculture residues (pesticides, fertilizers, etc..) arrive to the water depending on conditions like the crop closeness to the surface water, climate or soil properties that conditions the leaching of the pesticide ingredients (Masiá *et al*. 2014) (Székács *et al*. 2014). Industrial contaminants depend and vary widely on the industrial activity. These contaminants are release to water due to a poor residues treatment or illegal dumping to the natural waters. The occurrence of these substances have been and are studied in environmental water (Carmona *et al*. 2014) (Alygizakis *et al*. 2016) (Petrovic *et al*. 2014) (Masiá *et al*. 2015) (Andrés-Costa *et al*. 2014) being crucial the methods that have been developed to detect this kind of pollution (Nannou *et al*. 2015) (Cortéjade *et al*. 2016) (Petrie *et al*. 2016). Most of these analytical methods uses liquid-chromatographic analysis in tandem with mass spectrometry techniques (LC-MS/MS) (Campo *et al*. 2014). In this study will be used the extraction method developed for a triple quadrupole HPLC-MS/MS for water by Carmona *et al*. 2014 [2_ENREF_2, 3]. A new method for the extraction of pharmaceuticals from fish, which will be compared with QuEChERS method. This method will be apply to a UHPLC-QToF MS/MS in order to screen different compounds of emerging concern in effluents, lake water and fish with a library of more than 1200 compounds that includes pharmaceuticals, personal care products, illicit drugs, pesticides or perfluoroalkyl substances among others.

2. Materials and methods

2.1. Site description and sampling collection

Lake Water samples (AW) were taken from 10 different points in the Albufera Lagoon, during the summer of 2016. Sampling points were taken in both, the lake and the channels that feed it, tourist ports or flooded rice crops in the Natural Park as shown Figure 1. *Anguila anguila* was used as Fish sample (FA) because are aquatic animals, which are in critical, endangered due to habitat degradation and the reduction in water quality (European Commission DG Environment News Alert Service) and these lives in Albufera Lake. Five samples of this fish were taken from aquaculture and 10 fish-samples from the lake. WWTP effluent samples were collected in three different plants, Pinedo I (PI) and Pinedo II (PII), which receives residual water from Valencia City (Spain), and Quart-Benager (QB), which receives that of the Valencian metropolitan

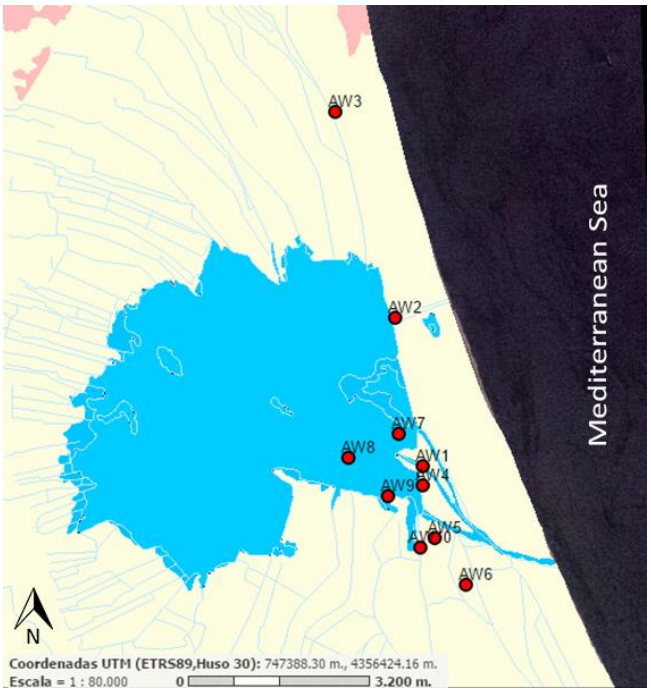

Figure 1. Map of the Albufera water sampling area.

area. PI receives a flow around 101 674 m3/day, PII 219 774 m3/day and QB 60 000 m3/day.

2.2. Sample preparation

Water samples were prepared according the method established in Carmona *et al*. 2014. Samples previously filtered, were extracted with Strata X cartridges, eluted with methanol reconstituted with 1ml of 30:70 (% v/v) Methanol/Water. Fish samples were prepared with two different methods. The first one is the traditional method, QuEChERS, developed by Anastassiades *et al*. 2003, using $MgSO_4$, NaCl, $Na_3Cit{\cdot}2H_2O$ and $Na_2HCit{\cdot}1.5H_2O$ for the extraction. The clean-up of the supernatant uses C18, PSA and $MgSO_4$. On the other hand, a method using a McIlvaine-EDTA buffer was tested where 5ml of distilled water, 5ml of methanol and 5ml of McIlvaine-EDTA buffer was added to 5g of fish. After sonicate and centrifuge to 3000rpm during 6min, supernatant was added to 200ml of distilled water and concentrating with a Solid Phase Extraction (SPE) with the same procedures than waters in the method of Carmona *et al*. 2014.

2.3. Chromatographic analysis

Chromatographic analysis was performed using an Ultimate 3000 UHPLC from Thermo Fischer Scientific (Dreieich, Germany) coupled with a MaXis Impact Time of Flight Mass Spectrometry from Bruker (Billerica, MA, USA) in non-target screening. For the compounds separation, an Acclaim RSLC C18 column (2.1 x 100 mm, 2.2 µm) from Thermo was used preceded by a guard column of the same packaging material, thermostated at 30^0C. For positive ionization mode (PI), the mobile phases are water/methanol 90/10 (solvent A) and methanol (solvent B) both amended with 5mM ammonium formate and 0.01% formic acid. For negative ionization mode (NI), the mobile phases consisted of water/methanol (solvent A) and methanol (solvent B) both acidified with 5mM ammonium acetate.

3. Results

More than 150 compounds in positive mode (highlighting some compounds as LSD or fluconazole detected in a high frequency) and more than 50 in negative (detecting in all samples carbamazepine, dinoterb or flufenamic acid) both in low concentrations (from 1 ng L^{-1} up to 400 ng L^{-1}) were detected in wastewater effluents. Less compounds were found in water of L'Albufera lake where wide screening search against the database tentatively identify 60 compounds in positive and 30 in negative ionization mode mode at concentrations between 0.5 ng/L to 200 ng/L) detecting in every sample PFBuS, acetamiprid or salicylic acid. QuEChERS method and the one developed in our laboratory using McIlvaine-EDTA buffer for the extraction of *Anguila anguila* were compared with the same analysis procedure than waters. With the QuEChERS extraction were detected 35 different compounds and with the McIlvaine-EDTA procedure 55 compounds were detected including Allopurinol, DEET or MDA. Furthermore, recoveries were better in the McIlvaine-EDTA extraction. Concentrations of emerging pollutants were determined in fish from 0.5 ng/g to 150 ng/g. This procedure provides acceptable recoveries (60% - 120% for water and 40% - 104% for fish) and relative standard deviation (RSDs < 20%) at the limits of detection from 2.5 ng/L for water and 5 ng/g for fish.

4. Conclusions

According the results, the Waste Water Treatment Plants are not completely ready for the treatment of the emerging pollutants since do not remove all the compounds and these are going to the environment, in this case, to the Albufera lake and their biota. Although the low concentrations do not appear to have harmful effects on human health, but some studies indicate that, these concentrations could have an effect on the environment (Ginebreda *et al*. 2010). The developed method provides reliable results with the screening of emerging pollutants in environmental matrices detecting a big amount of different compounds. Furthermore, the method developed in the laboratory for the extraction of these compounds of emerging concern in biota, using the McIlvaine-EDTA buffer improves the results of the classical methods for the extraction of these matrices.

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