Screening thousands of emerging contaminants in Asopos river basin by UPLC-QToF-MS

Nikolopoulou V.I.[1], Nika M.C.[1], Aalizadeh R.[1], Alygizakis N.[1,2], Thomaidis N.S.[1,*]

[1] National and Kapodistrian University of Athens, Department of Chemistry, Laboratory of Analytical Chemistry, Greece, Panepistimiopolis Zografou, 15771 Athens

[2] Environmental Institute, Okružná 784/42, 97241 Koš, Slovak Republic

*corresponding author: Thomaidis N.S.: e-mail: ntho@chem.uoa.gr

Abstract

Monitoring activities over several decades have revealed the ubiquitous presence of organic contaminants in environmental compartments. Asopos river basin, located in Sterea Ellada, has been the focus of many environmental studies over the last decades, mainly due to its geomorphology and the extensive industrial activities that take place in this territory. The detection of high Cr(VI) concentrations, causing potential carcinogenic effects, have attracted the attention of media and raised the consciousness of the citizens. Although there are many studies in recent literature focusing on the occurrence of heavy metals in Asopos river basin, there is a lack of information concerning the presence of emerging contaminants. The aim of this study is the determination of thousands of emerging contaminants in the environmental samples (river water and sediments) form Asopos river basin, following wide-scope target screening methodologies. Solid-Phase Extraction (SPE) by mixed-mode sorbent was used during the sample preparation of river water samples to ensure the extraction of various classes of compounds with a wide range of physicochemical properties. The extracts were analyzed by complimentary chromatographic techniques, including Reversed Phase (RP) and Hydrophilic Interaction Liquid Chromatography (HILIC) coupled to Quadrupole-Time-of-Flight Mass Spectrometry (QToF-MS). Moreover, the application of suspect and non-target screening, using advanced chemometric techniques, will provide a holistic view of the pollution in the area under investigation.

Keywords: Emerging Contaminants, River Water, Sediments, wide-scope Target and Suspect Screening, Non-target Screening, HRMS

1. Introduction

Recent environmental studies have shown the ubiquitous presence of organic contaminants in environmental compartments including river water and sediments. The site of interest of this study is the river basin of Asopos, which is located in Sterea Ellada, north of Athens, due to its geomorphology and the industrial activities taking place in this territory. Asopos river has been the focus of several research studies,

mainly because of the high media coverage surrounding the presence and carcinogenic effects of Cr(VI). The major focus of previous efforts was the monitoring of metals, arsenic and physicochemical properties using existing regulatory standards. However, nowadays, the development of high-resolution mass spectrometric techniques has enabled the detection of thousands of organic contaminants such as industrial chemicals, pharmaceuticals, personal care products and other chemicals from domestic activities and the plant protection products used in agricultural activities in the area of Oinofyta. The aim of the study was the development of an advanced methodology for the extended monitoring of emerging contaminants in environmental samples from Asopos river basin.

2. Methods

2.1. Sampling

The selection of two sampling sites (one sampling site close to industrial and agricultural activities in the Area of Oinofyta, whereas the other one was located to the estuaries of the river) provided information for the levels of emerging contaminants and potential spills. The sampling campaign provided trend information for daily (sampling for 30 consecutive days) and seasonal trends (7 consecutive days in the beginning of every season). Two portable autosamplers were used for collecting 24-h composite river samples.

2.2. Sample Preparation

2.2.1. River water

The river water samples were stored in the dark at 4 °C until the extraction after the adjustment of the pH to 6.5 (±0.2). 1L of each water sample was filtered through regenerated cellulose filters to separate total suspended matter from the water sample. Internal Standards mix solution was spiked in each sample and sample clean-up and pre-concentration was realized by Solid-Phase Extraction (SPE) with mixed-mode sorbents. This approach was used to ensure the extraction of compounds with a wide range of physicochemical properties and low limits of detection. The elution of the analytes from the sorbent materials was performed

by a basic solution, followed by an acidic solution. The extract was evaporated to dryness under a gentle nitrogen stream and reconstituted to a final volume of 250 µL (MeOH-Milli-Q water, 50/50 v/v). The extract was filtered directly into a vial using a syringe fitted with RC membrane filter and was ready for being analyzed in the Liquid Chromatography High Resolution Mass Spectrometry [Gago-Ferrero P. et al, 2015].

2.2.2. Sediment

Sample preparation for sediments was carried out using Ultrasound Assisted Extraction [Gago-Ferrero P. et al, 2015]. 0.2 g freeze-dried sediment was spiked with an Internal Standards mix and kept in contact for 30 min. The sample was extracted with 2 mL MeOH-Milli-Q water (FA 0.5%, 0.1% EDTA), 50:50 (v/v), by vortex, followed by ultrasound extraction. The extract was centrifuged, and the supernatant was collected in a glass tube. This procedure was repeated two more times with a total of 6 ml supernatant. The total extract was evaporated under a gentle nitrogen stream to dryness and then reconstituted to 0.2 mL with a final proportion of MeOH-Milli-Q water, 50/50 v/v. Finally, the extract was filtered through RC filters, transferred to a glass vial and it was ready for injection in the chromatographic system.

2.3. Instrumental Analysis

The extracts were analyzed by the complimentary chromatographic techniques, achieving chemical characterization of the samples, facilitating the detection and separation of compounds with wide range of polarity in the samples, including Reversed Phase Liquid Chromatography (RPLC) and Hydrophilic Interaction Liquid Chromatography (HILIC) coupled to Quadrupole-Time-of-Flight Mass Spectrometry (QToF-MS) due to its increased selectivity. For RP analysis an Acclaim^{TM} C18 column was used with a gradient elution program of the mobile phase (ratio and flow rate) for both ionization modes. For HILIC analysis, the samples were analyzed after reconstitution of the extract with a final proportion of ACN-Milli-Q water, 95/5 v/v, using an Acquity UPLC$^®$ BEH Amide column. The MS data were acquired through Data Dependent and Independent Acquisition modes (DDA and DIA). The same extract was used for wide-scope target, suspect and non-target screening.

2.4. Data Treatment

River water samples and sediments were analyzed not only for the priority pollutants according to the Directive 2013/39/EU, but also for emerging contaminants from various classes of compounds. In-house databases consisting of 3,000 compounds combined (emerging contaminants, naturally occurring compounds and endogenous metabolites) was used for wide-scope target screening and the detection criteria was based on retention time, mass accuracy, isotopic pattern of the precursor ions and MS/MS fragmentation pattern [Gago-Ferrero et al, submitted]. Concerning the suspect and non-target analysis, the NORMAN Digital Sample Freezing Platform was used for archiving, processing, analysing, data mining and retrieving information from the environmental samples [Alygizakis N.A. et al, 2019].

3. Results

3.1. Target Screening

The preliminary screening results indicate the presence of numerous industrial chemicals such as benzothiazoles, benzotriazoles, surfactants from the homologue series of Linear Alkylbenzene Sulfonates (LAS) and Perfluorinated Alkylated Substances (PFASs) in both sampling sites. Plant protection productswere present such as the herbicides MCPA and Dicampa. Additionally Metolachlor, Azoxystrobin and Fluometuron potentially originating from the nearby agricultural activities, were also detected. Compounds from different classes of compounds including stimulants such as Nicotine and Caffeine and pharmaceuticals such as Metformin and Isoconazole were also among the identification.

3.2. Suspect and Non-target Screening

The wide-scope screening strategies supplemented the overview of the level of pollution, indicating the occurence and spatial distribution of emerging contaminants and prioritizing unidentified features for future identification efforts [Alygizakis N.A. et al, 2019]. Moreover, chemometric tools for time versus concentration analysis (TrendTrAMS) developed in the Laboratory of Analytical Chemistry will be applied for the first time for the analysis of environmental samples of Greece [Nika M.-C. et al, 2017]. The results of this research will provide an overview for the state of organic pollution in the Asopos river basin and highlight potential sources of concern.

References

Alygizakis N.A., Oswald P., Thomaidis N.S., Schymanski E.L., Aalizadeh R., Schulze T., Oswaldova M., Slobodnik J. (2019), *Trends in Analytical Chemistry,* **115,** 129-137.

Gago-Ferrero P., Schymanski E.L., Bletsou A.A., Aalizadeh R., Hollender J. and Thomaidis N.S. (2015), *Environmental Science and Technology*, **49**, 12333–12341.

Gago-Ferrero P., Borova V., Dasenaki M.E. and Thomaidis N.S. (2015), *Analytical and Bioanalytical Chemistry*, **407**, 4287–4297

Gago-Ferrero P., Bletsou A.A., Damalas D.E., Aalizadeh R., Singer H.P., Hollender J. and Thomaidis N.S. (2019), , *Analytical and Bioanalytical Chemistry* (Submitted).

Nika M.-C., Aalizadeh R. and Thomaidis N.S. (2017), 15th International Conference on Environmental Science and Technology, (31 August to 2 September 2017, Rhodes, Greece,https://cest2017.gnest.org/sites/default/files/presentation_file_list/cest2017_01272_oral_paper.pdf)