

## QUALITATIVE DETERMINATION OF KEY EMERGING XENOBIOTICS IN MIXED WASTEWATER DISCHARGE

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### Abstract

Nowadays, it is unattainable to follow, detect or identify all the chemicals that are excreted into the environmental aquatic bodies. The number of chemicals introduced into the environment in modified structures, as metabolites or as mixtures of chemical cocktails, is growing progressively. Illicit drugs and endocrine disruptive substances (EDCs) (emerging xenobiotics) are not excluded from this fate. Illicit drugs are xenobiotics with psychoactive effects and influences onto the human body and mind, and are excreted into the environment mostly in forms of metabolites. In sense of these facts, a high level of importance is appointed on an optimal and effective analytical practices and high performing equipment, but far more important is a good detection model for emerging substances, especially for detection of emerging xenobiotics in a highly polluted sample, as wastewater. This paper will show the results of qualitative analyses for complex sample matrix of mixed wastewater and results of detection for selected xenobiotics with endocrine disruptive and psychoactive effects.

### Introduction

The safety and quality of surface water that is used as a source for drinking water production and for the significant number of human activities have to be an important topic for society, since the pollution can cause serious effects on human health [1, 2]. Newly recognized environmental contaminants identified as emerging substances are perceived as globally unregulated substances [3]. The emerging substances include organic and inorganic pollutants, such as pharmaceuticals, personal care products, flame retardants, endocrine disruptive compounds, industrial chemicals, biological metabolites, hormones, toxins and other chemicals [4, 5].

The sewerage system in Novi Sad is designed and constructed as a mixed wastewater channelling system that collects domestic and industrial wastewater mixed with the urban, sub-urban and rural runoff, making maintenance of regular water quality very difficult. The system does not incorporate treatment of wastewater before discharge.

Xenobiotic is a chemical compound foreign to a given biological system. With respect to animals and humans, xenobiotics include drugs, drug metabolites, and environmental compounds such as pollutants that are not produced by the body. In the environment, xenobiotics include synthetic pesticides, herbicides, hormones, illicit and licit drugs and medication, agricultural and industrial pollutants that would not be found in nature [6].

The illicit use of substance makes it more difficult to research and observe, especially when excreted levels are in nano levels or less. Illicit drugs are the latest group of emerging compounds

identified in the aquatic environment [7]. Mixed wastewater from urban areas are the dominant source of illicit drugs and their metabolites in surface water. The results represent an introduction to a new approach to organic contamination monitoring of waste and surface water. 24h composite samples of mixed urban wastewater were collected over a period of 7 days from selected location in the city of Novi Sad in order to determine concentration levels of cocaine, benzoylecgonine, amphetamines, ecstasy, methamphetamine and cannabis metabolite THC – COOH, caffeine, antibiotics, pesticides and other. For the target determination 41 analytes in total were selected - 16 pesticides, 9 hormones, 11 sterols and 5 illicit drugs.

### Exerimental

In order to identify the list of compounds that could be expected in aquatic environment in Novi Sad and it's vicinity, screening analyses of waste (four samples per cycle) and surface water (six samples per cycle) were performed during the three cycles of sampling in 2011 [8]. Detailed description of the sampling, sample preparation and analysis is available in Milić et al., 2013.

The samples were collected from collectors GC1 and GC2 in glass bottles and stored at 4 °C. For screening analyses a 1000 ml aliquot of water sample were spiked with internal standard to achieve final concentration of 1µg/L and extracted with two 50 ml portions of DCM for 30 minutes. Extract was dried with anhydrous sodium sulfate. Kuderna-Danish apparatus was used to evaporate to final volume of 1 ml. A 50 µl of extract was injected into Agilent 6890 GC Agilent 5973 MSD system equipped with PTV injector that was programmed from 60 °C to 260 °C. Capillary GC analysis was performed on a 30 m x 250 mm I.D., 0.25 mm df DB-XXLB and HP-5MS column. Helium was used as carrier gas. The MSD was used in SIM mode for all samples.

Each target compound during target analyses was qualified by two qualifier ions. The group of pesticides were analyzed using GC-MS according to modified ISO 6468 procedure, VOCs by GC-MS according to ISO 10301 procedure. Pesticides and hormones were analyzed using SPE-HPLC-DAD according to modified ISO 11369 procedure [9]. During the screening analyses a high number of substances were identified, from which 41 were selected as key substances for the target analyses according to lists of priority and hazardous priority and emerging substances. For quantification of selected analytes validated target methods - liquid chromatography tandem mass spectrometry (HPLC-MS<sup>2</sup>) or high-resolution mass spectrometry (HPLC-HRMS) are used.

### Results and discussion

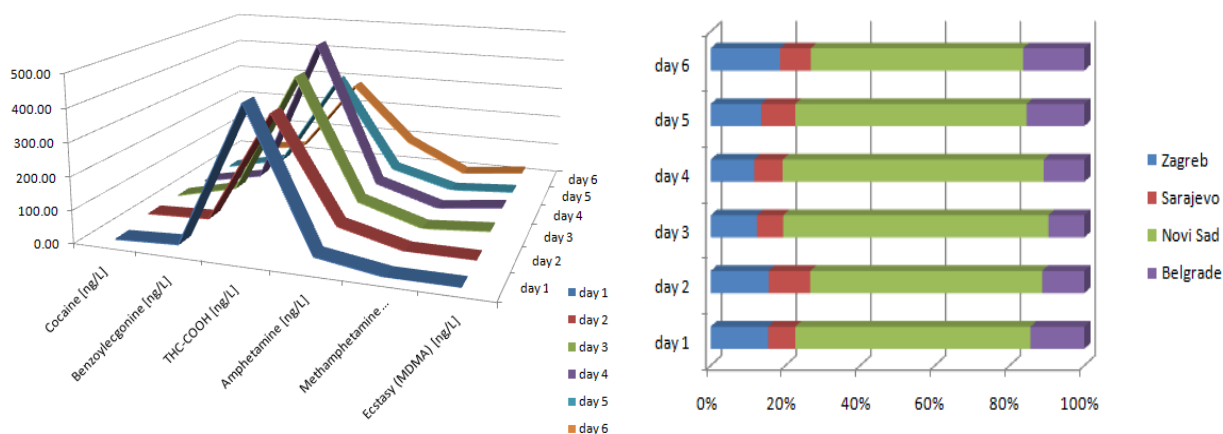
In the samples of wastewater collected from two main discharges of wastewater (GC1 and GC2), the presence of all monitored analytes was confirmed in concentration levels of ng/L. [10, 11] Pesticides were detected in the highest concentrations, especially in the wastewater. According to the concentration ratio of p,p'-DDD and p,p'-DDT at sampling point with the highest concentrations, GC1, the value of 1.29 indicated historical contamination with these chemicals. p,p'-DDD, p,p'-DDE and p,p'-DDT at sampling site GC1 were determined in concentrations more than eight times higher than annual average values in EU countries. Hexachlorobenzene (Lindane) was found in concentrations three to five times higher than AA EQS at GC1 and GC2. The significant quantity of the hormones androstane-17-one, -cholestan-3-ol, cholest-5-en-3-ol, cholestan-3-one, cholestane, 3- hydroxy-, (3.beta,5.alpha)-, stigmast-5-en-3-ol and coprostanol were detected in urban effluent at the sampling points of GC1 and GC2. The presence of hormones is mainly highlighting the impact of faecal pollution, due to the lack of the WWTP. The hormones were only detected above the LOD, but it was not possible to quantify them. In sampling point GC2, estriol has been detected in concentration of 4.1±0.5 ng/mL.

During the determination of the 5 selected analytes from the group of illicit drugs, THC-COOH singled out as very interesting for the site of Novi Sad. THC-COOH was recorded in significantly higher concentration in all samples from the city of Novi Sad, compared to the region, but also to all selected sites in Europe that were also involved in this research. The concentration levels of all 5 analytes from the group of illicit drugs are presented in Table 1.

**Table 1.** Measured concentration levels of selected illicit drugs in Novi Sad

Illicit drug	Measured concentrations in [ng/L] for Novi Sad						LOQ
	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	
Cocaine	4.40	17.00	14.00	5.30	n.d.	16.00	12.00
Benzoyllecgonine	14.00	25.00	60.00	43.00	43.00	39.00	10.00
Amphetamine	31.00	50.00	61.00	60.00	52.00	95.00	25.00
MDMA	n.d.	n.d	16.00	24.00	14.00	22.00	20.00
THC-COOH	442.00	368.00	435.00	500.00	345.00	284.00	10.00

Up to 4 times higher concentrations of all selected analytes were detected during the weekend (from Friday till Sunday). According to the results shown in Table 1. and Figure 1, THC-COOH has a significantly higher concentrations in regard to other illicit drugs, as well as to other cities in the region.



**Figure 1.** Concentration levels of selected compounds and contribution of Novi Sad in THC-COOH excretions to surface water in Balkan region

### Conclusion

In recent years a new problem has emerged in our water environment, namely, the presence of endocrine disruptive compounds (EDCs) that may affect the reproductive functions of human beings and wild life. Most potent EDCs are pesticides, hormones and illicit drugs. In our research, it is shown that all three groups of emerging xenobiotics from the group of EDCs are present in the mixed wastewater of city of Novi Sad, as a result of discharging of wastewater into the Danube without any treatment. Out of 41 selected endocrine disruptive compounds, 20 were detected, with pesticides in the highest concentrations. From the group of illicit drugs, THC-COOH was detected in the highest concentrations. Republic of Serbia is in the midst of planning efforts that will lead to selection of a water plan and wastewater treatment facilities that should serve for decades. Wastewater reclamation and reuse will be a major part of both water supply and wastewater treatment planning. City of Novi Sad is in the planning and design stage of

WWTP which means that is of essential importance to have and obtain up-to-date specific data on wastewater quality and quantity.

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