Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. XII, 2023
Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064

SPATIAL DISTRIBUTION OF PHARMACEUTICALS IN THE LOWER DANUBE RIVER WATER

Valentina Andreea CALMUC, Madalina CALMUC, Maxim ARSENI, Ira-Adeline SIMIONOV, Alina ANTACHE, Stefania-Adelina MILEA, Catalina ITICESCU, Puiu Lucian GEORGESCU

REXDAN Research Infrastructure, "Dunarea de Jos" University of Galati, 98 George Cosbuc Street, Galati, Romania

Corresponding author email: valentina.calmuc@ugal.ro

Abstract

Pharmaceuticals are part of the emerging pollutants class found in aquatic ecosystems. The presence of these contaminants in the aquatic ecosystem can have harmful effects on living organisms due to their toxicity and ability to accumulate in tissues. In this study, water samples taken from stations located in the Lower Danube River Basin were analysed in order to identify and quantify some classes of pharmaceuticals. To confirm the presence of pharmaceutical traces in surface water samples, a High-resolution UHPLC-MS/MS was used. The obtained results demonstrated that the most frequently identified pharmaceutical residues in the water samples were: caffeine > carbamazepine > metformin > sulfamethoxazole > trimethoprim > clindamycin > ketoprofen > diclofenac > clarithromycin. The highest recorded concentration was 118.52 ng/L for caffeine possibly due to the presence of this compound especially in drinks and food and to a lesser extent also in medicines and the lowest value was 0.36 ng/L for trimethoprim.

***Key words**: surface water, pharmaceuticals, emerging pollutants, Danube river.*

INTRODUCTION

The pollution of the Danube River Basin is a topic of interest especially for scientists, governments and residents of the riverside cities, because it is the second longest river in Europe, has a wide variety of fauna and flora and is an important source of drinking water (Popescu et al., 2022; Woitke et al., 2003). Pharmaceutical substances (PhACs) are considered emerging pollutants that are continuously discharged into the aquatic environment (Hejna et al., 2022). Until now, this class of pollutants is not regulated by law, but some of these compounds are on the Watch List adopted by the European Commission (EU) 2020/1161. The latest version of the "Watch List" was revised in 2020 and contains the following pharmaceutical compounds for human use: amoxicillin, ciprofloxacin, sulfamethoxazole, trimethoprim, venlafaxine and o-desmethylvenlafaxine (*Commission Implementing Decision (EU) 2020/1161*).

The main sources of surface water pollution with this class of contaminants are: the pharmaceutical industry, wastewater from hospitals and homes, surface runoff from agricultural land and animal farms (Gaw et al., 2014; Vatovec et al., 2021).

The presence of pharmaceutical residues in aquatic ecosystems has toxic effects on aquatic biota. Depending on the class to which they belong and the concentration in which they are found, pharmaceutical substances can cause the physiological and behavioural disorders in certain fish species. For example, contraceptives can cause feminisation of fish (Gross-Sorokin et al., 2006), antidiabetics have potential effects of disrupting the endocrine system in fish, antibiotics determine the development of antibiotic resistance and analgesics cause damage to some organs in fish (Khan et al., 2020; Patel et al., 2019).

The main aim of this study is to determine the occurrence of some classes of pharmaceuticals in the Danube River Lower Basin water.

MATERIALS AND METHODS

In order to determine the presence of pharmaceutical residues in the water of the Danube River Lower Basin, 500 mL of water were collected from 6 sampling stations located near the Galati city in 2021 (Figure 1).

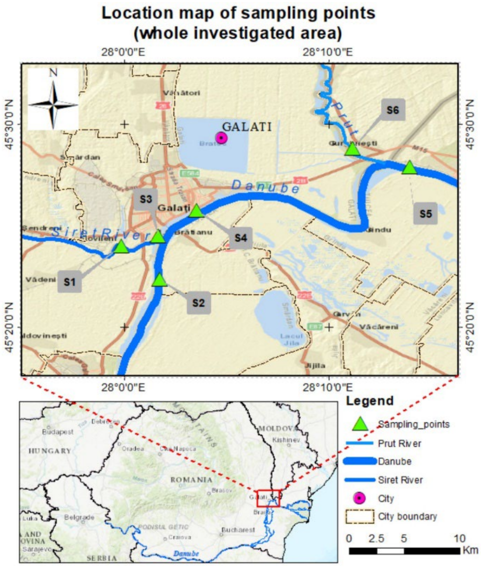

Figure 1. Map of sampling stations

Extraction of target compounds was performed using the Dionex AutoTrace 280 Thermo Scientific automated solid phase extraction instrument (Figure 2). After conditioning the Branchia C18 cartridges, 500 mg/ 6 mL with 5 mL methanol followed by 5 mL water, loading of 100 mL water sample adjusted to pH 3. Elution of the analytes was performed with 6 mL methanol. The eluate was concentrated by evaporation in a nitrogen atmosphere of purity 5.0, at a temperature of 42°C using the Reacti-Therm Heating Thermo Scientific equipment (Figure 3). The eluate was redissolved with 25 µl of methanol and 225 µl of water (Chitescu et al., 2015).

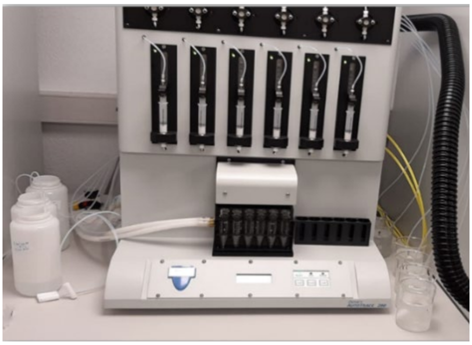

Figure 2. Extraction of pharmaceuticals from water samples

Figure 3. Concentration of eluate under a N_2 flow

Analytical standards purchased from Sigma-Aldrich were used for the identification and quantification of pharmaceutical residues in the analysed water samples for the following compounds: caffeine, ketoprofen, diclofenac, carbamazepine, metformin, clindamycin, sulfamethoxazole, trimethoprim and clarithromycin.

The identification and quantification of the compounds was carried out with the Vanquish Flex UHPLC Systems equipment coupled to the Orbitrap Exploris 120 mass spectrometer - Thermo Fisher Scientific (Figure 4).

Figure 4. Identification and quantification of analytes

The column used to separate the compounds was Accucore C18 (100 x 2.1 mm, 2.6 µm).

The mobile phase consisted of 2 solvents A - ultrapure water with 0.1% formic acid (98% - 100% LC-MS grade) and B - methanol (LC-MS grade) with 0.1% formic acid (98-100% LC-MS grade).

The detection of the compounds was carried out with the Orbitrap Exploris 120 mass spectrometer at a high resolution of 120,000 (FWHM) at m/z 200. To confirm the presence of the compounds in the water samples, MS/MS analysis were performed.

RESULTS AND DISCUSSIONS

In order to identify and quantify the pharmaceutical compounds present in the water samples, the standards of the compounds tracked in the samples were analysed. Table 1 and Figure 5 show the retention times and chromatograms obtained for each analysed compound.

Table 1. Retention time of analyzed pharmaceuticals

Compound	Formula	Adduct	m/z	RT Time (min)
Metformin	$C_4H_{11}N_5$	+ H	130,1087	0.44
Trimethoprim	$C_{14}H_{18}N_4O_3$	+ H	291,1452	3.21
Caffeine	$C_8H_{10}N_4O_2$	+ H	195,0877	3.38
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	+ H	254,0594	3.69
Clindamycin	$C_{18}H_{33}ClN_2O_5S$	+ H	425,1871	4.91
Carbamazepine	$C_{15}H_{12}N_2O$	+ H	237,1022	5.72
Clarithromycin	$C_{38}H_{69}NO_{13}$	+ H	748,4842	6.50
Ketoprofen	$C_{16}H_{14}O_3$	+ H	255,1016	6.65
Diclofenac	$C_{14}H_{11}Cl_2$NO2	+ H	296,024	8.03

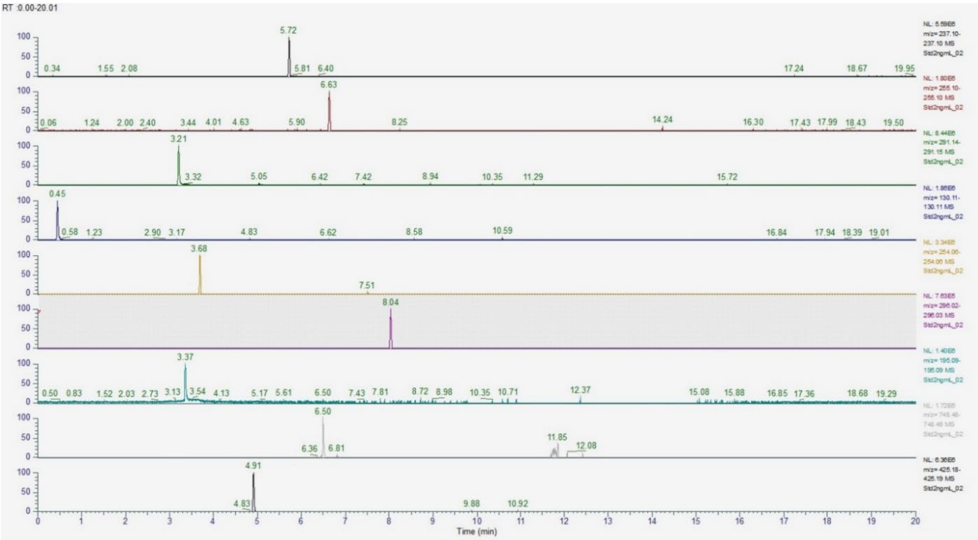

Figure 5. Chromatograms of analyzed pharmaceutical residues

Metformin is an anti-diabetic drug used by people with type 2 diabetes (Oosterhuis et al., 2013). This compound was registered in 5 sampling stations. The lowest value was 0.74 ng/L (S2) and the highest value was recorded for station 1.83 ng/L (S3) (Figure 6).

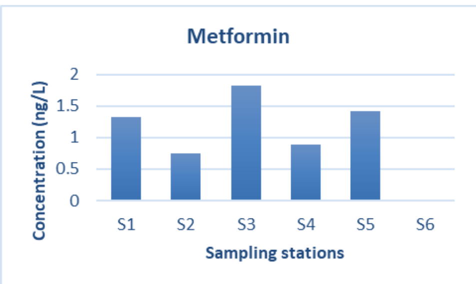

Figure 6. Spatial distribution of metformin concentration concentration

Trimethoprim is an antibiotic that belongs to the diaminopyrimidine class (Straub, 2013). The values of trimethoprim concentrations are in the range of 0.36 ng/L - 9.17 ng/L (Figure 7).

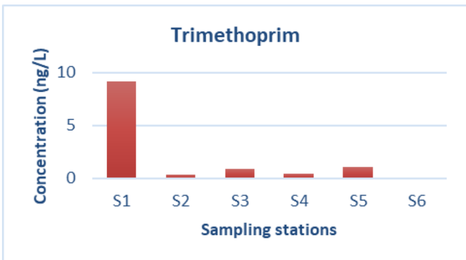

Figure 7. Spatial distribution of trimethoprim

For the caffeine, values were recorded in all 6 sampling stations. This alkaloid is one of the most consumed psychoactive substances worldwide. It is found in food, drinks and medications (Edwards et al., 2015). The highest concentration was 118.52 ng/L, in station S3 (Figure 8).

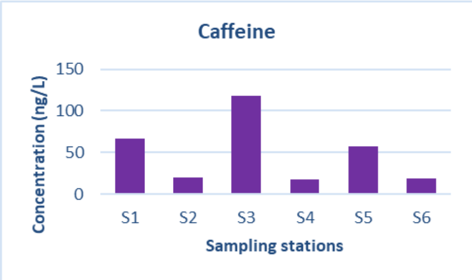

Figure 8. Spatial distribution of caffeine concentration

Sulfamethoxazole is part of the sulfonamides class and is one of the most prescribed antibiotics (Patrolecco et al., 2018). The concentrations of sulfamethoxazole in surface waters were detected in the range 3.57 ng/L - 4.36 ng/L (Figure 9).

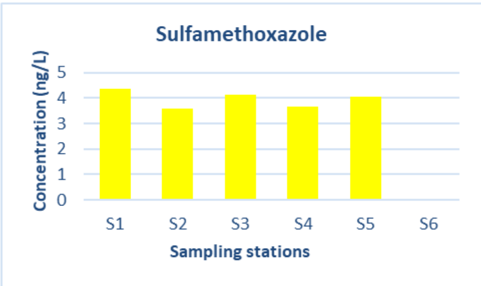

Figure 9. Spatial distribution of sulfamethoxazole concentration

Clindamycin is an anti-bacterial drug used to treat various acute and chronic infections (Koba et al., 2017). This compound was detected in 4 of the 6 sampling stations, the lowest value was 3.32 ng/L (S4), and the highest concentration was 3.54 ng/L (S3) (Figure 10).

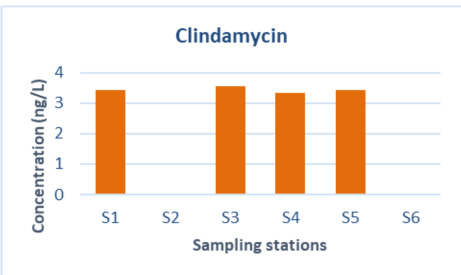

Figure 10. Spatial distribution of clindamycin concentration

Carbamazepine is an anticonvulsant used especially as a treatment for epilepsy (Cunningham et al., 2010). Based on the results obtained, it was observed that this compound was detected in all 6 sampling stations. Carbamazepine concentrations varied from 0.16 ng/L in station S2 to 9.07 ng/L in station S1 (Figure 11).

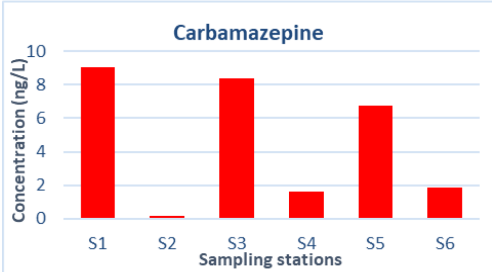

Figure 11. Spatial distribution of carbamazepine concentration

Ketoprofen is a non-steroidal anti-inflammatory drug (NSAIDs) used especially to relieve inflammation and pain (Alkimin et al., 2020). Figure 12 illustrates that only 2 values were obtained for this pharmaceutical residue, respectively 5.55 (S3) ng/L and 5.37 ng/L (S5).

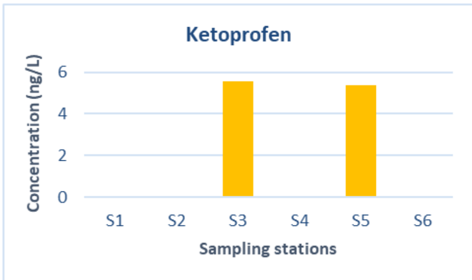

Figure 12. Spatial distribution of ketoprofen concentration

Diclofenac belongs to the category of non-steroidal anti-inflammatory drugs (Peters et al., 2022). For this pharmaceutical compound, only one value was recorded, in the water sample taken from station S3 (Figure 13).

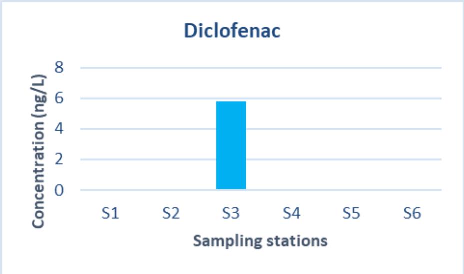

Figure 13. Spatial distribution of diclofenac concentration

The macrolide antibiotic clarithromycin (Baumann et al., 2015) was also analyzed in the water samples, but was not detected at any sampling station.

CONCLUSIONS

The method applied to determine the pharmaceutical compounds tracked in the analyzed water samples proved to be suitable for their identification and quantification.

The most frequently detected compounds in the water samples were caffeine and carbamazepine, while the antibiotic clarithromycin was not detected in any of the 6 sampling stations.

Also, the obtained results highlight the fact that the fewest pharmaceutical compounds were identified in sampling station S6. Considering the fact that one of the major sources of pollution with pharmaceutical substances is municipal waste water, the most pharmaceutical compounds were identified in the stations that are located near the urban agglomeration of the Galati city (S1, S3, S5).

To confirm the identity of each analysed compound, at least two fragment ions with the appropriate ion-ratio were detected.

ACKNOWLEDGEMENTS

The technical support was provided by the Rexdan Research Infrastructure, created through the project An Integrated System for the Complex Environmental Research and Monitoring in the Danube River Area, REXDAN, SMIS code 127065, project co-financed by the European Regional Development Fund through the Competitiveness Operational Programme 2014-2020, contract no. 309/10.07.2020.

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