Detection of Emerging Pollutants Oxytetracycline and Paracetamol and the Potential Aquatic Ecological Risk Associated with their Presence in Surface Waters of the Arges-Vedea, Buzau-Ialomita, Dobrogea-Litoral River Basins in Romania

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Abstract. Pharmaceuticals are a major group of emerging pollutants frequently identified in surface waters. The occurrence of pharmaceuticals as environmental pollutants is a problem involving multiple aspects and continue to cause new and serious challenges to ecosystems and human health. For example, antibiotics exert a selective pressure on bacterial communities in aquatic environments, leading to the selection and increase in the prevalence of antibiotic-resistant bacteria, which is a major problem in the effective treatment of bacterial infections, while for analgesics, a number of adverse effects such as accumulation in tissues and behavioral changes were observed in aquatic organisms. In this paper, the detection by UHPLC-MS/MS of two widely used pharmaceuticals was performed, oxytetracycline (antibiotic) and paracetamol (analgesic) respectively, in surface water matrix from the Arges-Vedea, Buzau-Ialomita and Dobrogea-Litoral river basins in Romania. The potential aquatic ecological risk (RQ) was also estimated by the ratio between Measured Environmental Concentration (MEC) and Predicted No-Effect Concentration (PNEC).
1. Introduction

Water pollution with emerging pollutants such as pharmaceuticals is a major global problem involving multiple aspects, generating growing concerns about potential adverse effects on environment and human health. Detection of organic micropollutants, such as compounds that affect the endocrine system, pharmaceuticals and personal care products, in wastewater and the aquatic environment has led to growing concerns about potential adverse effects on environment. However, knowledge about the nature and extent of the impact of pharmaceuticals on the environment is still limited [1]. Despite the insufficient knowledge of this emerging environmental problem, the scientific community tends to agree that ways of limiting the impact of pharmaceuticals on environment should be studied, thus anticipating the necessary actions in the near future.

Risk assessment studies for aquatic species and humans are in progress. Nevertheless, the risk of exposure to pharmaceuticals has proven higher for aquatic species than for humans, and some substances such as oxytetracycline (OXY) and paracetamol (PAR) are thought to be present in water at a risk level that is not negligible for water biota [2, 3]. Due to the extremely high dispersion of paracetamol (PAR) in nature, its environmental fate and ecotoxicological assessment should be a priority [4]. Even though the detected concentrations of paracetamol at a low level, a variety of potential negative effects have been described including, e.g., reproductive or DNA damage, accumulation in tissues, oxidative stress, lipid peroxidation, and behavioral changes observed in algae, microcrustaceans, mollusks, or teleost fish [5].

Oxytetracycline (OXY) is one of the most used antibiotics in human medicine and for the treatment of animals reared in terrestrial environment and in aquaculture [6]. This raises concerns due to its effects in human and animal health, the environmental contamination and the consequences arising therefrom [7]. Antimicrobial resistance (AMR) is one of the ten threats identified by the World Health Organization (WHO) in 2019, since it affects modern healthcare and the effective prevention and treatment of an ever-increasing range of infections. A few studies have provided evidence of the presence of oxytetracycline affecting nontarget organisms such as fish, oysters, clams, and crustaceans following discharge in the vicinity of aquaculture operations [8].

The identification and quantification of pharmaceutical compounds in water or wastewater has become a major scientific task requiring highly sophisticated analytical methodologies which are able to detect in levels of nanograms per liter (ng/L) [9, 10]. Although pharmaceuticals have been detected in the aquatic environment as reported in numerous studies and analytical measuring techniques for pharmaceuticals have been improved, there is still very little data available on their concentration levels in environment. In this context, this paper presents a method developed for the detection of two pharmaceutical compounds in water (oxytetracycline and paracetamol), as well as an assessment of the potential aquatic ecological risk associated with their presence in river basins in Romania.

2. Experimental

2.1. Sampling locations

Testing the detection methods of the emerging micropollutants paracetamol and oxytetracycline were performed on surface water matrices collected during May - October 2019, from the Arges-Vedea river basin (figure 1. (a)), Dambovita River - Budesti area respectively, Gîlina Wastewater Treatment Plant area, upstream and downstream of Gîlina WWTP, Arges R. - Hotarele and Clătăști area, Sabar R., Ciorogârla R. - upstream and downstream of Domnîștii locality; Buzau-Lâlomita river basin (figure 1. (b)), Ialomița R. respectively - upstream and downstream of Slobozia WWTP, Prahova R. - upstream and downstream of WWTP Campina, Dambo R. - upstream and downstream of Ploiești WWTP and the Dobrogea-Litoral river basin (figure 1. (c)), respectively, Danube River - Fetesti (km 43), Bala (km 9.5), Izvoarele, upstream and downstream of Tulcea, Sulina and Sf. Gheorghe.
2.2. Sample collection
Water samples were collected as 1 L in glass bottles by means of water sampler (figure 2.). After collection all samples were stored in coolboxes and delivered on the sample collection day to the laboratory where water samples were stored at 4°C until analysis. All water samples were filtered through 0.2 μm polyethersulfone (PES) filters (Sartorius Stedim Biotech GmbH, Germany) before direct injection into UHPLC-MS/MS system.

2.3. Methods and material

2.3.1. Equipment. Identification of paracetamol and oxytetracycline was performed using the SPE-online-UHPLC-MS/MS Thermo Fisher Scientific™ EQuan MAX Plus™UltiMate 3000 system coupled with TSQ Quantiva triple-stage quadrupole mass spectrometer (figure 3.). The system delivers exceptional quantitative performance with sensitivity attogram level (10⁻¹⁸g). The technique used is the most sensitive and selective compared to other frequently used analytical techniques, allowing the detection and quantification of emerging pollutants in the category of pharmaceuticals at ng / L level.
2.3.2. Methods. Method developed in this study for identifying oxytetracycline and paracetamol by UHPLC-MS/MS method meets the requirements at the EU level regarding the detection limit, i.e. it should be at least the same as the predicted no-effect concentration values in the corresponding matrix.

2.3.3. Chemicals and Standards. The reference standards used for this analysis were paracetamol (PAR) (≥99.9% purity) and oxytetracycline (OXY) (≥92.9% purity), produced by Chiron AS (Norway). Ultra-pure water was obtained from a Millipore Milli-Q water purification system used in preparing all standard solutions. HPLC grades acetonitrile and formic acid for LC/MS were provided by Scharlau Chemie SA and VWR Chemicals.

2.4. Ecological risk assessment
The ecological risk quotient (RQ) associated with the presence of oxytetracycline and paracetamol in aquatic ecosystems was also calculated for potential ecological risk assessment, using the ratio between the measured environmental concentration (MEC) of pharmaceutical micropollutants detected in surface water and predicted no-effect concentration (PNEC) values, the environmental concentration at which no adverse effect on aquatic ecosystem function is to be expected [11, 12, 13]. The PNEC values are estimated on the basis of available acute or chronic ecotoxicity data from the scientific literature for several aquatic organisms: bacteria, algae, invertebrates, and fish, where the ecotoxicity data is adjusted with an appropriate assessment factor. The measured environmental concentrations (MECs) used for the risk assessment corresponded to the maximum concentrations found in surface water. The calculated RQ value is then used to prioritize pharmaceuticals that are likely to pose a high risk (RQ ≥ 1.0); medium risk (1.0 > RQ ≥ 0.1); compounds with 0.01 < RQ < 0.1 have low risk and an RQ < 0.01 indicates that the respective compound displays no ecological risk to the aquatic ecosystem [14, 15, 16].

3. Results and Discussions
The results of the pharmaceutical products’ detection were achieved by selected-reaction monitoring (SRM) experiments on a TSQ Quantiva triple quadrupole mass spectrometer (table 1). Method development for the detection of paracetamol and oxytetracycline was carried out in accordance with the quantitative confirmation criteria described in Decision 2002/657/EC, which implements Directive 96/23/EC on the analytical methods and the interpretation of results.

| Table 1. Detection results of the analyzed pharmaceutical products. |
|---------------------------------------------------------------|
| Compound | CAS number | Retention Time (min) | Polarity | Precursor (m/z) | Product (m/z) | Collision Energy (V) | Chemical formula |
|----------|------------|----------------------|----------|----------------|--------------|---------------------|-----------------|
| Oxytetracycline | 79-57-2 | 4.7 | Positive | 283 | 283 | 39 | C_{22}H_{24}N_{2}O_{9} |
| | | | | 426 | 426 | 20 | Molecular weight: 460,148 g/mol |
| | | | | 443 | 443 | 14 | |
| Paracetamol (acetaminophen) | 103-90-2 | 4.15 | Positive | 65 | 65 | 29 | C_{8}H_{9}NO_{2} |
| | | | | 93 | 93 | 23 | Molecular weight: 151.163 g/mol |
| | | | | 110 | 110 | 16 | |
Screening of pharmaceutical micropollutants revealed the presence of oxytetracycline and paracetamol in concentrations of the ng/L order, in the analyzed surface water sample (figures 4.).

Figure 4. UHPLC-MS/MS selected-reaction monitoring (SRM) transitions screening for pharmaceutical compounds - surface water sample taken from the Danube River, downstream of Tulcea town.

Diagrams 5 and 6 show the distribution of the analyzed pharmaceutical micropolllutants (oxytetracycline, paracetamol) in the area of the river basins under study: Arges-Vedea, Buzau-Ialomita and Dobrogea-Litoral.

Figure 5. Distribution of oxytetracycline (OXY) in aquatic environments from the areas under study.

Figure 6. Distribution of paracetamol (PAR) in aquatic environments from the areas under study.
The results of the pharmaceutical micropollutants detection in the aqueous matrix and calculated ecological risk quotient (RQ) are presented in Table no. 2. Paracetamol was occasionally detected at the level of Romania, in the Danube basin (the Danube River) and the three main tributaries (Arges, Olt and Siret) [17], and as far as the detection of oxytetracycline concerns, no investigations were performed for the surface waters in Romania.

Table 2. Concentrations of paracetamol and oxytetracycline detected in surface water (MECsw), their predicted no-effect concentration (PNEC), and calculated ecological risk quotient (RQ).

| Pharmaceutical micropollutants | PNEC [18] (ng/L) | Location                                      | MECsw (ng/L) | RQ (MECsw/PNEC) |
|--------------------------------|------------------|-----------------------------------------------|--------------|-----------------|
| Paracetamol (acetaminophen)    | 4.8 × 10^5       | Arges River - Clatesti                         | 74           | <0.01           |
|                                |                  | Dambovita River – downstream of Glina WWTP    | 64.8         | <0.01           |
|                                |                  | Sabar River                                   | 65.5         | <0.01           |
|                                |                  | Ciorogarla River                              | 9.6          | <0.01           |
|                                |                  | Ialomita River – downstream of Slobozia WWTP  | 117.4        | <0.01           |
|                                |                  | Prahova River                                 | NF*          | -               |
|                                |                  | Dambovita River - area Ploiesti WWTP          | 1414         | <0.01           |
|                                |                  | Danube River – area Sf. Gheorghe               | 183.7        | <0.01           |
| Oxytetracycline                | 4.0 × 10^7       | Arges River - Hotarce                          | 328          | <0.01           |
|                                |                  | Dambovita River – area Glina WWTP             | 935.5        | <0.01           |
|                                |                  | Sabar River                                   | 34.1         | <0.01           |
|                                |                  | Ciorogarla River                              | 313.1        | <0.01           |
|                                |                  | Ialomita River - downstream of Slobozia WWTP  | 1032         | <0.01           |
|                                |                  | Prahova River                                 | NF*          | -               |
|                                |                  | Dambovita River - area Ploiesti WWTP          | 2450         | <0.01           |
|                                |                  | Danube River – area Sulina                     | 399.5        | <0.01           |

NF* - not found

The PNEC values were estimated by dividing the pharmaceuticals chronic toxicity values towards fish with an assessment factor of 100, which is required to extend the chronic toxicity values for fish to other aquatic organisms [18]. The studied antibiotic and analgesic (oxytetracycline and paracetamol) were detected in the sample analyzed at μg to ng/L concentrations that are unlikely to pose a risk to aquatic ecosystems. Considering the potential risks due to the presence of pharmaceutical products in the aquatic environment, it is obvious the need to deepen and spread knowledge on this type of contamination, especially regarding the monitoring of the ecological behavior [19, 20].

4. Conclusions

The assessment of the possible pharmaceutical micropollutants risk on aquatic ecosystems is of particular importance both for setting up regulations at the European level within the Water Framework Directive (WFD 60/CE/2000) [21] and for the possibility of developing new methods and technologies for removing antibiotics from water in order to protect the environment and human health. In this context, a method was presented for the identification and quantification of two emerging micropollutants oxytetracycline and paracetamol and the possible risk posed by them to the aquatic ecosystems based on PNEC was evaluated. Taking into account potential risks due to the presence of pharmaceuticals in water, more work is needed to expand knowledge on this kind of contamination and of treatment technologies designed to limit the release of antibiotics and analgesics into the aquatic environments.
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