DETERMINATION OF FLUOROQUINOLONE ANTIBIOTICS IN WASTEWATER USING HPLC-FLD

Florinela Pirvu[1,2], Vasile-Ion Iancu[1], Iuliana Paun[1], Marcela Antoaneta Niculescu[1], Gabriel Valentin Serban[1], Nicoleta Vasilache[1], Florentina Laura Chiriac[1]

[1]National Research and Development Institute for Industrial Ecology-ECOIND, 57-73 Drumul Podu Dambovitei, district 6, 060652, Bucharest, florinela_pirvu@yahoo.com, Romania
[2] University Politehnica of Bucharest, Faculty of Biotechnological Systems Engineering, 313 Splaiul Independentei, 060642. Bucharest, Romania

INTRODUCTION

The proliferation of antibiotics in the environment as well as their persistence is a problem of global concern. They can induce antibiotic resistance which can threaten both human health and the ecosystem. Fluoroquinolone (FQ) antibiotics represent an emerging class of contaminants that are widely used in human and veterinary medicine, resulting in their presence in wastewater, effluents and water bodies. Even if they are found at a low concentration, FQs can stimulate antibacterial resistance. The threat to human health regarding antibiotic resistance may be associated with ingested antibiotics thus altering the microbiome of humans, creating pressure on the microbiome of the environment. Health risk assessment is based on the number of infectious diseases due to exposure to antibiotic-resistant bacteria. The present work presents a developed and validated method for the determination of ciprofloxacin (CIP) and norfloxacin (NOR) in wastewater.

MATERIALS AND METHODS

The experiments to establish the optimal conditions for chromatographic separation and detection were carried out on an Agilent 1200 HPLC system.

The optimal HPLC separation parameters of fluoroquinolones established experimentally are as follows:
- Chromatographic column: Zorbax Eclipse Plus C18 (15 x 3 mm, 5 µm);
- Column temperature: 30°C;
- Injection volume: 20 µL;
- Mobile phase: H3PO4 25mM (brought to pH=3): ACN; 88/12, v/v;
- Mobile phase flow rate: 1 mL/min;
- Elution: isocratic;
- FLD detection: λex (excitation wavelength), 280 nm and λem (emission wavelength), 440 nm;
- Separation duration: 7 minutes.

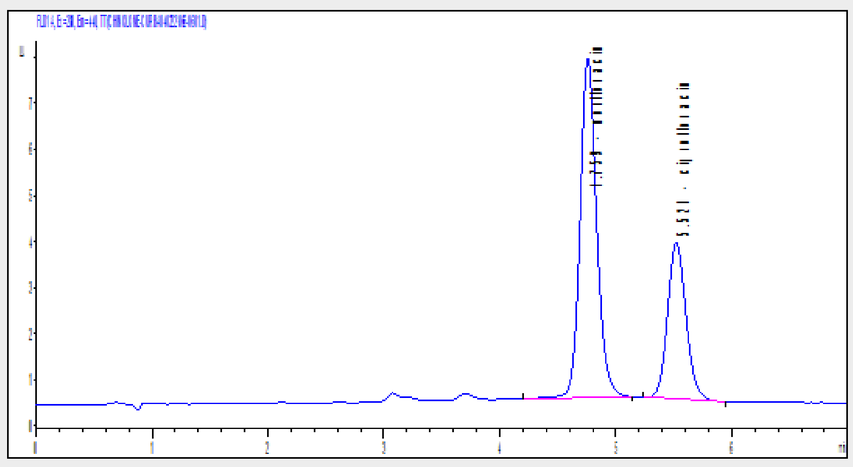

Fig. 1. Chromatogram of a mixed solution of NOR and CIP concentration 100 µg/L

Using the Agilent ChemStation software, the calibration functions for each compound were drawn, passing the concentration values, in µg/L, on the ordinate, and the average values of the areas of the chromatographic peaks, expressed in area units, corresponding to the injections, on the abscissa performed for each calibration solution.

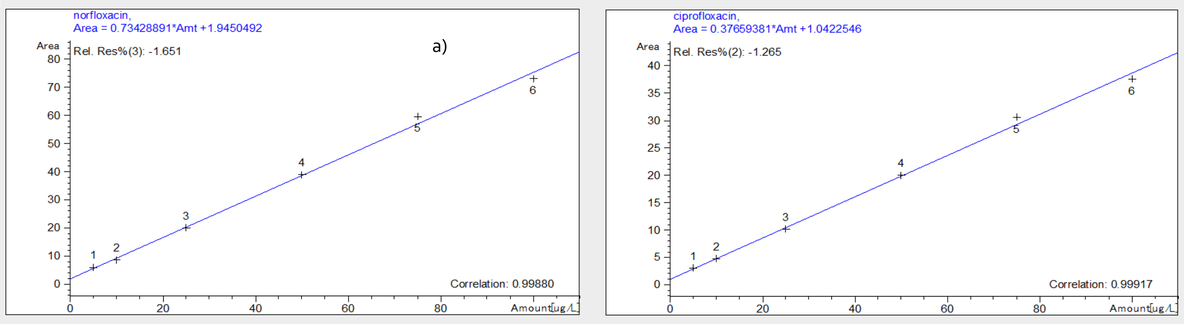

Fig. 2. Calibration curves for a) NOR, b) CIP

The laboratory experiments regarding the separation of the two fluoroquinolone drugs from aqueous matrices were performed quantitation as well as for the elimination of interfering potentials. For the extraction and concentration of the target analytes from aqueous matrices, the extractor Dionex SPE AutoTrace 280 (Thermo Scientific) and Strata X-WA Polymeric Cartridges with 33 µm anion exchangers with the size of 500mg/6ml from Phenomenex were used.

RESULTS

In this study, an HPLC-DAD method was developed and validated for the determination of Norfloxacin and Ciprofloxacin in wastewater samples, in a chromatographic run-time of only 7 minutes. All liquid-chromatographic conditions (nature and composition of the mobile phase, injection volume, detection wavelength, column temperature, etc.) were optimized for the fast separation of the two analytes with high sensitivity (µg/L) from complex m wastewater and surface water matrices. The recovery yields of the HPLC-DAD method were 87.2% for norfloxacin and 90.3% for ciprofloxacin.

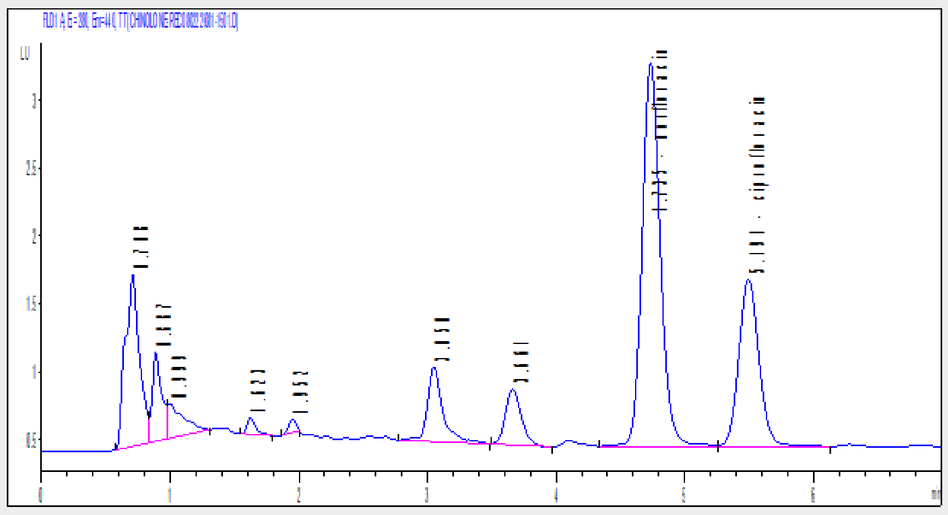

Fig. 3. Chromatogram of the two pharmaceutical compounds in real wastewater sample after using the cartridges Strata XAW Polymeric Anion Cartridges

From the chromatographic analysis of the wastewater samples taken from the WWTP (Effluent), it can be observed that the concentrations values of norfloxacin are situated in the range between 54.3 and 89.9 ng/L, and for ciprofloxacin the concentrations obtained was in the range of 56.5 and 289.8 ng/L), table 1 and figure 4.

Table 1. Pharmaceutical residue concentration values determined in WWTPs

Effluents	Conc, ng/L	Conc, ng/L
	Norfloxacin	**Ciprofloxacin**
EF-1	62.2	131.4
EF-2	54.3	142.1
EF-3	67.5	56.7
EF-4	73.2	203.8
EF-5	71.8	65.5
EF-6	90.5	284.5
EF-7	78.1	130.4
EF-8	98.9	109.2
EF-9	87.8	289.8
EF-10	62.4	31.8

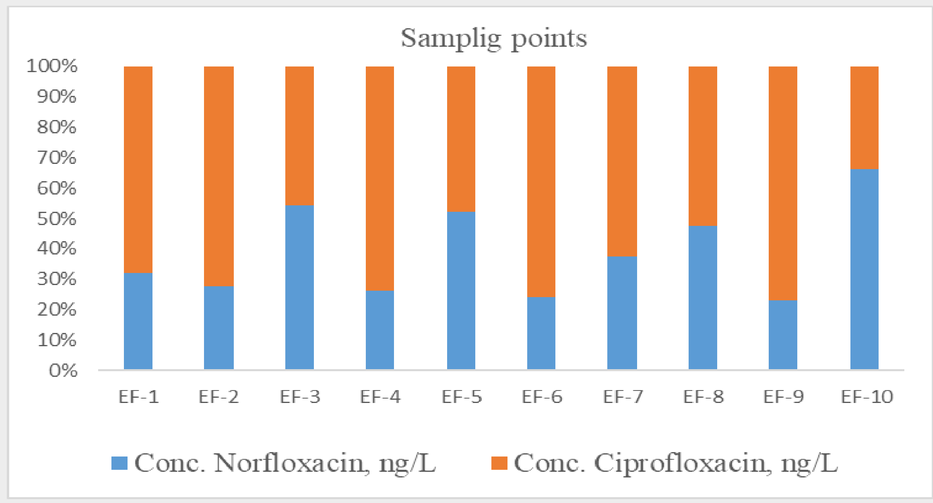

Fig. 4. Percentage evaluation of the two analytes in sewage treatment plants

CONCLUSIONS

From the chromatographic analysis of the wastewater samples taken from the area of the treatment plants, a decrease in the CIP concentration values is observed in the order: EF-9 (289.8 ng/L) > EF-6 (284.5 ng/L) >EF-4 (203.8 ng/L) > EF-2 (142.1 ng/L) > EF-1 (131.4ng/L) >EF-7(130.4 ng/L) > EF-8 (109.2 ng/L) >EF-5 (65.5 ng/L) > EF-3 (56.7 ng/L), and an increase in the value of NOR concentrations in the order EF-2 (54.3 ng /L) <EF-1 (62.2 ng/L) <EF-3 (67.5 ng/L)< EF-5 (71.8 ng/L) < EF-4 (73.2 ng/L) < EF-7(78.1 ng/L) < EF-9(87.8 ng/L) < EF-6 (90.5 ng/L) < EF-8 (98.9 ng/L).

ACKNOWLEDGEMENTS

The authors acknowledge the financial support offered by the Ministry of Research, Innovation, and Digitization of Romania through the Romanian National Research Program “Nucleu”, contract no. 3N/2022, A.A.1/2023, Project code PN 23 22 01 01.