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A SIMPLE SPE-UPLC-MS/MS METHOD FOR DETERMINATION OF 9 ANTIBIOTICS IN SURFACE WATER

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Abstract. The overuse of antibiotics is losing their effectiveness due to increased antibiotic resistance in bacteria. Over the past two decades, the evaluation of antibiotic residues in the environment has received considerable attention. In this work, a combined SPE-UPLC-MS/MS method was developed and validated for the simultaneous determination of 9 antibiotic compounds belonging to 3 different groups which are tetracyclines (tetracycline, chlortetracycline, oxytetracycline), fluoroquinolones (ciprofloxacin, norfloxacin, ofloxacin), and macrolides (azithromycin, erythromycin, clarithromycin) in surface water. All target analytes were separated on a reversed-phase column (Water BEH C18 column, 1.7 µm particle size, 100 mm in length × 2.1 mm in diameter) coupled with a tandem mass spectrometer employing positive electrospray ionization (+ESI). All target analytes were well separated with an overall run time of 16 minutes. The limit of detection of antibiotics ranged from 0.2 to 10 µg L^{-1}. The extraction process was successfully optimized using an HLB column (Oasis, 6 mL, 200 mg, Waters) with recoveries from 71 to 125 %. The optimized method was finally applied to analyze ten surface water samples (from lake and river). Ofloxacin, clarithromycin and erythromycin were the most frequently detected compounds in lake water samples at concentrations ranging from 35 to 570.3 ng L^{-1}, while only CIP was found in river samples. The other compounds were also detected in both types of samples, but in concentrations below the limit of quantification.

Keywords: macrolides, fluoroquinolones, tetracyclines, UPLC-MS/MS, surface water.

Classification numbers: 2.4.2, 2.4.4, 5.2.1.

1. INTRODUCTION

Over the past twenty years, pharmaceutical residues have been found in water samples as important emerging contaminants resulting from human activities. In the late 1990s, reports began to appear about the detection of pharmaceuticals and cosmetics in water sources, including groundwater and surface water [1, 2]. Initially, pharmaceutical residues were of little concern because the concentrations of these substances were lower than those detected by laboratory analytical equipment or because the contaminants were believed to have been diluted by natural water and removed from water treatment plants. However, the interest gradually increased as three classes of drugs including endocrine, antibiotic, and antidepressant were found and reported [3 - 5].

Pharmaceuticals have been widely used to save millions of lives through the prevention and treatment of diseases. Many studies have shown that these substances can cause harmful effects on organisms living in the environment, and more importantly, can lead to an increase in antibiotic resistance [6]. Antibiotic resistance was recently a global problem and was particularly serious in Viet Nam where the use of antibiotics is often uncontrolled [7]. This issue is a growing public health concern because not only are first generation antibiotics ineffective, but also more expensive pharmaceuticals for newer generations including other antibiotics are facing significant resistance from bacteria [8].

In this research, an analytical method was developed for analyzing nine pharmaceutical residues including ciprofloxacin (CIP), ofloxacin (OFL), norfloxacin (NOR), azithromycin (AZI), erythromycin (ERY), clarithromycin (CLA), tetracycline (TET), oxytetracycline (OXY), and chlortetracycline (CHL) in surface water by ultra-high performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS/MS) using electrospray ionization. These compounds are reported to be the most consumed antibiotics in Viet Nam [9]. Surface water samples were extracted and purified using hydrophilic-lipophilic balance solid phase extraction column (HLB - SPE) cartridges. The purpose of this study was to optimize the conditions on the UPLC-MS/MS instrument, the different sample extraction conditions, and validate the analytical method. Finally, the developed method was applied to analyze ten surface water samples that were collected from West Lake and Duong River in Gia Binh district, Bac Ninh province.

2. MATERIALS AND METHODS

2.1. Chemicals, reagents, and materials

All target compounds (CIP, OFL, NOR, AZI, ERY, CLA, TET, OXY, CHL) and organic solvents (acetonitrile (ACN), methanol (MeOH)) were purchased from Sigma-Aldrich (Singapore). An internal standard, ofloxacin-D3 (OFL - D3) was supplied by Toronto Research Chemicals (TRC, Toronto, Canada). 100 % formic acid (FA) (Optima MS grade) and 25 % ammonium hydroxide solution (reagent grade) were provided by Merck (Germany). Oasis mix-mode cation exchangers (MCX, 3 mL, 60 mg) and hydrophilic-lipophilic balance (HLB, 6 mL, 200 mg) solid phase extraction cartridges were purchased from Waters (USA), and the bond elute ENV (200 mg, 6 mL) was purchased from Agilent (USA). The ultrapure water (18.3 MΩ.cm) was produced from the Smart2pure 12 UV water purification system (Thermo, England) and was used throughout this study.

2.2. Sample collection

Ten surface water samples were collected in West Lake, Ha Noi, and Duong River at Gia Binh district, Bac Ninh province according to the protocol from Vietnamese standard TCVN No.6663 - 6:2018 [16]. These sampling points were chosen because West Lake was considered the discharge point of domestic wastewater from Tay Ho district where the population is densely concentrated, while Duong River was affected by major industrial activities of Bac Ninh province. The sampling location was mapped as in Figure 1. All samples were collected and kept in plastic bottles pre-rinsed with ultrapure water in the laboratory and rinsed several times with field samples. At the laboratory, the samples were filtered using a GF/F filter (Whatman, Ø = 47 mm, pore size < 0.7 µm) with the help of a vacuum pump. The filtered samples were stored at 4 ℃ and analyzed within 48 hours or stored at -80 ℃ for further analysis.

Figure 1. Surface water sampling points at Duong River (left) and West Lake (right).

2.3. Analytical methods

2.3.1. Chromatographic conditions

An ultra-high performance liquid chromatograph (ACQUITY UPLC, H-class, Waters, USA) combined with a mass spectrometer in tandem (Xevo - TQD, Waters, USA) was used for the analysis. The target analytes were separated on a reserved phase C18 column (BEH, C18, 100 × 2.1 mm, 1.7 µm particle size) operating in a multiple reaction monitoring (MRM) mode followed by positive electrospray ionization (+ESI). Each compound was monitored by 2 MRM transitions. The higher signal was used for quantification and the lower was used for confirmation. The identification of target analytes was based on their retention time, two transitions, and the relative ratio of two transitions. The MS parameters of each analyte were optimized by direct injection of individual standard solution at 1 mg L^{-1} in methanol. The source parameters were also optimized to achieve the optimal conditions such as desolvation temperature at 500 °C, source temperature at 150 °C, desolvation gas flow at 1000 L h^{-1}, cone gas flow at 10 L h^{-1}, and capillary voltage at 3.0 kV. All cone voltages (CV) and collision energies (EV) applied to individual analytes are detailed in Table 1.

Table 1. Operating conditions for all target analytes on MSMS detector.

Analytes	RT (min)	Precursor ion (m/z)	Product ions (m/z)	CV (V)	CE (eV)	Ion Ratio
NOR	3.50	320.2	276.1	40	22	21.3
			302.0	40	22	
OFL	3.50	362.3	261.3	25	20	2.1
			318.3	25	20	
OXY	3.50	461.1	426.1	30	20	2.6
			443.2	30	13	
CIP	3.70	332.1	288.1	42	18	7.8
			314.1	42	22	
TET	4.10	445.1	98.00	36	42	2.0
			154.1	36	28	
CHL	5.00	479.0	444.0	30	30	7.3
			462.0	20	18	
AZI	7.00	375.0	116.3	35	25	1.2
			158.1	35	25	
ERY	9.90	734.5	158.1	30	25	2.4
			576.3	30	20	
CLA	11.90	748.5	158.0	30	30	4.5
			590.4	30	20	

2.3.2 Sample preparation for LC-MS/MS

Samples were thawed and left at room temperature before experimenting. An isotope-labeled internal standard (OFL-D3) was added to the sample to obtain a final concentration of 50 µg L^{-1} via analysis. The HLB SPE cartridges were conditioned with 5 mL of ACN and then with 5 mL of ultrapure water at pH 5. After the conditioning step, 200 mL of water samples to which 8.32 mL of 0.25 M EDTA was added at pH 5 was percolated through the cartridge at a flow rate of 10 - 12 mL min^{-1}. Afterward, HLB SPE cartridges were washed with 5 mL of ultrapure water at pH 5 to remove interferences and dried for 60 min under vacuum to remove excess water. The elution step was performed with 5 mL of the H_2O/ACN mixture (40/60, v/v. The eluents were evaporated under a gentle stream of nitrogen until dryness and then reconstituted to 1 mL of H_2O/ACN (90/10, v/v). Finally, the solutions were filtered using a syringe with a 0.2 µm pore size and injected into the UPLC-MS/MS system under optimal operating conditions. Other solid phase extraction materials such as MCX (Waters, USA) and ENV+ (Sigma, Singapore) were also tested to investigate drug enrichment in both surface and wastewater samples.

2.3.3 Method validation

The method validation was evaluated for various parameters, including linear range, method detection limit (MDL), instrument detection limit (IDL), and correlation. The linear range was investigated over a six-point calibration ranging from 5 to 500 µg L^{-1}. The MDL and MQL were determined using a signal-to-noise ratio of 3 and 10, respectively, and on a real sample matrix. If any compound was not detected in the real sample, the MDL and MQL were then calculated based on the spiked sample. The sample volume for analysis was 200 mL of surface water.

3. RESULTS AND DISCUSSION

3.1. Chromatographic separation

Various experiments with different mobile phase compositions were investigated to optimize chromatographic separation for all target analytes. Formic acid (FA) was added to the mobile phase for providing protons in the ESI+ process and was also used to improve compound separation. Different gradients were performed to optimize chromatographic separation. Briefly, the mobile phases were composed of H_2O (phase A) and ACN (phase B), both of which were acidified with 0.5 % FA. The gradient started with 90 % A, followed by a linear decrease of phase A to 65 % for 0 - 10 minutes. Then, it was linearly dropped down to 5 % from 10 to 11 minutes and kept at this state for 2 minutes. Finally, the proportion of phase A was re-increased to 90% to reach the initial condition for the next injection. The total running time was 16 minutes per injection. The flow rate of the mobile phase was continuously kept at 0.3 mL min^{-1} and the column chamber temperature was kept constant at 35 ^{o}C. The sample injection volume was set at 10 µL using an auto-sampler.

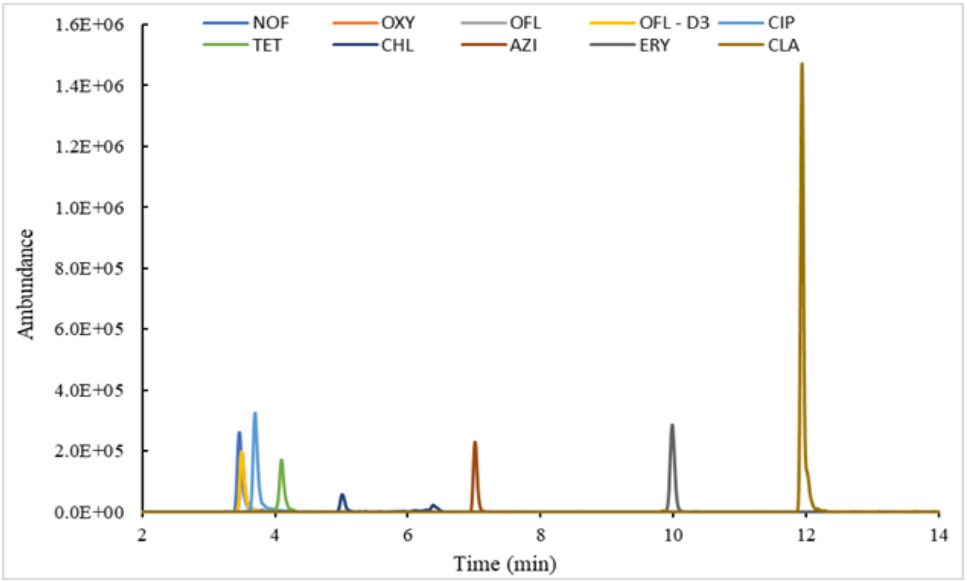

Figure 2. Extracted ion chromatogram (EIC) of all target analytes at 100 µg L^{-1}.

Figure 2 shows the extracted chromatogram of all analytes that were well separated. The sharp peaks were not split, peaks were smooth, and tailing was reduced in all target analytes.

3.2. Optimization of sample preparation

3.2.1. Solid Phase Extraction (SPE) optimization

Three types of solid phase extraction cartridges were used to evaluate the extraction of analytes from the water matrices: HLB cartridges (200 mg, 6 mL, OASIS, Waters, USA), MCX cartridges (60 mg, 3 mL, OASIS, Waters, USA), and ENV+ cartridges (200 mg, 6 mL, ISOLUTE®). All experiments were performed in triplicate. Blank samples were prepared using tap water instead of real water samples. All these columns (MCX, ENV+, and HLB) were made from reversed-phase and could operate over a wide range of pH. In addition, these columns were linked with different functional groups including sulfonic, secondary amine (MCX), and hydroxylated polystyrene-divinylbenzene copolymer, respectively. In acidic solution, most analytes were protonated and favored to interact with the stationary phases. In contrast, the HLB column that was made from N-vinylpyrrolidone, divinylbenzene preferentially operated at neutral pH. For MCX and ENV+ cartridges, the water sample was adjusted to pH 3 with FA, the cartridge was activated with 3 mL of MeOH and 3 mL of H_2O, respectively. For the HLB cartridge, the water sample was adjusted to neutral pH, the cartridge was conditioned with 5 mL of ACN and then with 5 mL H_2O. The sample was loaded into the cartridge at a flow rate of 10 - 12 mL min^{-1}. The SPE cartridge was dried under vacuum for 30 min. Finally, the analytes were eluted with 5 mL of 2 M MeOH/NH_4OH solution (90/10, v/v) for MCX and ENV+ cartridges. The elutes were converted to alkaline media by adding NH_4OH. The analytes in alkaline media preferentially changed their form to anions which were easier to be eluted with 5 mL of ACN/H_2O (60/40, v/v) for the HLB cartridge. The eluents were then collected and dried under a gentle nitrogen stream at 50 °C until dryness and reconstituted with 1 mL of H_2O/ACN (90/10, v/v). The solution was filtered using a 0.2 µm cellulose acetate membrane filter and analyzed by UPLC-MS/MS.

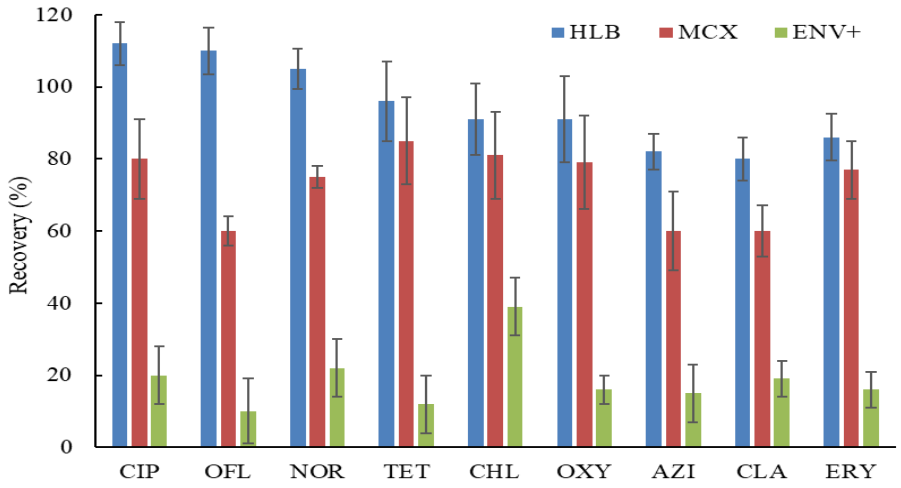

Figure 3. Recoveries of all analytes in surface water on different solid phase extraction materials.

Figure 3 shows the recovery of the analytes through three types of SPE cartridges. The recovery of target analytes was highest for the HLB cartridge, ranging from 80 to 112 %. The recovery was good for quinolones and tetracyclines, while the macrolide group had a worse yield (83 %). The results were similar to a previous work in which the range of 85 - 115 % was reported [10]. Among various types, HLB cartridges have high recovery and good reproducibility due to their chemical composition containing lipophilic divinylbenzene groups

and hydrophilic N-vinylpyrrolidone groups which allow their working over a wider range of pH (from pH 1 to 14) [11]. The ENV+ cartridge is only effective for some compounds as it is recommended for very polar organic compounds that are not retained on C8 or C18 [11]. However, it can also retain neutral compounds at neutral pH (including macrolides) through hydrophobic interactions. Therefore, the HLB column was selected as the extraction column for the subsequent optimization processes.

3.2.2. pH optimization

pH has a significant effect on the chemical form of the analytes and their interaction with the stationary phase of the SPE cartridge [11]. Figure 4 shows the recoveries of the analytes at different pHs. All the MLs, FQs, and TCs structures have at least one or more acid or base groups, the adjustment of pH was needed to reach a better sorption capacity of the analytes in the HLB phase [11]. The results indicated that the quinolone group (CIP, OFL, NOR) had a good extraction over a pH range of 3 to 5, while for the tetracycline group (TET, CHL, OXY), the best performance was achieved at pH > 3. The group of macrolides (AZI, CLA, ERY) showed a maximum extraction efficiency in the neutral pH range. Therefore, water samples were adjusted to pH 5 with formic acid for the optimal recovery of all analytes. Similar results were also reported in recent publications [12, 10].

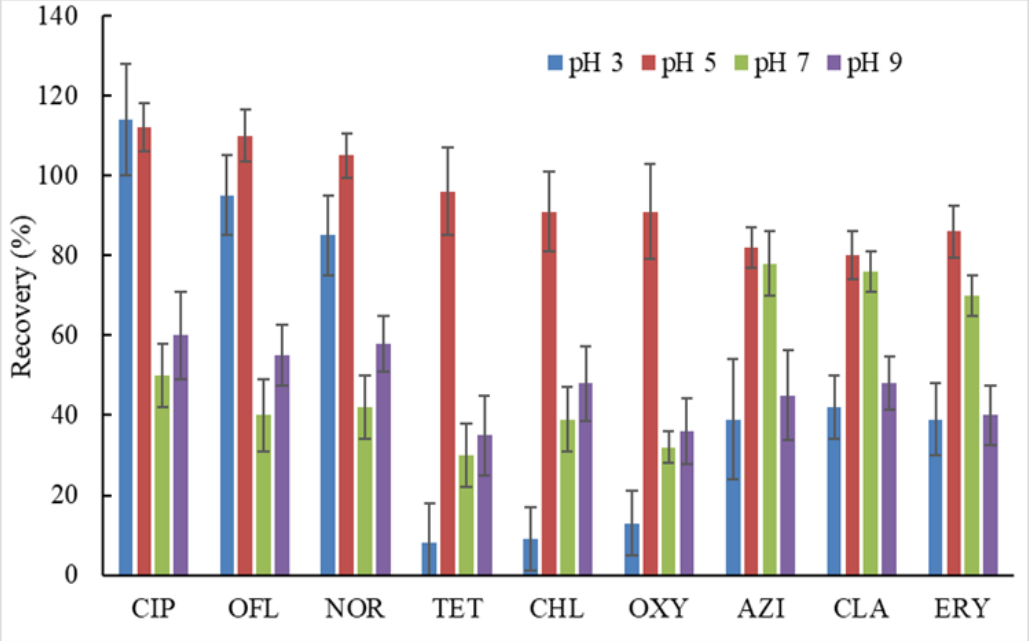

Figure 4. Recoveries of all analytes in surface water at different pH values.

3.3. Method validation

3.3.1. Calibration curves and linearity

The linear range was investigated by establishing a calibration curve with six concentration levels from 5 to 500 µg L^{-1}. The analyte concentrations were evaluated by internal standard OFL

- D3 with a concentration of 50 µg L^{-1} for each calibration point. Six-point calibrations were daily prepared in ultrapure water and injected three times into the UPLC-MS/MS system under optimal conditions. The standard curve showed the relationship of the peak area ratio to the concentration of analytes as a linear function. The ratio of peak area was calculated by dividing the peak area of compounds by the peak area of the appropriate isotopic labeled internal standard. The results of Table 2 show that the correlation coefficient R^2 of the standard curves is relatively good and is in the range of $0.99 \leq R^2 \leq 1$.

3.3.2 Instrument detection limit and Method detection limit

The results of determining the instrument detection limit (IDL) and method detection limit (MDL) are shown in Table 2. According to the US - EPA standard (American Environmental Protection Agency EPA 1694), IDL is calculated as the signal-to-noise ratio at the lowest concentration detected in a set of 3 calibration solutions. In our study, the IDLs were expressed as the absolute amount of the analyte injected into the UPLC-MS/MS system, while the MDLs were calculated from the real or spiked samples and expressed as concentrations. The analytes had good sensitivity to the MDL in the range of 0.2 - 10 µg L^{-1}. The sensitivity of the developed method was comparable to those in recent reports and good enough for the analysis of these antibiotics in surface water [13, 9, 10].

Table 2. Regression equation, MDL, IDL of all target analytes.

Analytes	Linear range µg L^{-1}	R^2	IDLs pg injected	MDLs µg L^{-1}
NOF	5 - 500	0.9959	0.69	2.0
OFL	5 - 500	0.9998	0.97	1.0
OXY	10 - 1000	0.9982	3.32	5.0
CIP	5 - 500	0.9981	0.52	2.0
TET	10 - 1000	0.9945	3.32	5.0
CHL	10 - 1000	0.9926	5.46	10.0
AZI	5 - 500	0.9913	0.10	0.3
ERY	5 - 500	0.9962	0.08	0.2
CLA	5 - 500	0.9975	0.01	0.2

3.4. Environmental application

Ten surface water samples were prepared using the solid phase extraction procedure and analyzed by UPLC-MS/MS under optimal operating conditions. The obtained results are shown in Table 3. Out of the 9 target antibiotics, OFL, ERY, and CLA were detected in the lake samples and only CIP was detected in river samples.

In the West Lake water sample, ERY was detected at 60 % of the sites with concentrations ranging from 175.5 ng L^{-1} to 747.3 ng L^{-1}. The level of OFL was slightly lower than ERY, it was observed in the concentration range from 126 ng L^{-1} to 570.3 ng L^{-1} at all sites. In contrast, CLA concentrations were the lowest and ranged from 35.5 ng L^{-1} to 75 ng L^{-1}. Besides, in river water samples, only CIP was detected at all points in the river with a concentration of CIP from 35.5

ng L^{-1} to 75 ng L^{-1}. The other analytes were almost not detected or only detected at concentrations between MDL and MQL. Through the above results, quinolone antibiotics were detected with the highest concentration and frequency. These were also antibiotics that have been detected in high concentrations in domestic and hospital wastewater in countries such as Vietnam, China, and Sweden [9, 14, 15].

Table 3. Concentration level of pharmaceutical residues in surface water (ng L^{-1}).

Analytes	HT1	HT2	HT3	HT4	HT5	GB1	GB2	GB3	GB4	GB5
OFL	570.3	543.0	216.8	187.3	126.0	ND	ND	ND	ND	ND
CIP	ND	ND	ND	ND	ND	110.5	112.0	19.5	110.0	111.5
NOF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TET	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
OXY	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CHL	ND	ND	ND	ND	ND	ND	<MQL	<MQL	<MQL	ND
AZI	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CLA	75.0	39.3	52.0	35.5	ND	<MQL	<MQL	<MQL	<MQL	<MQL
ERY	747.3	ND	504.0	175.3	ND	ND	ND	ND	ND	ND

4. CONCLUSIONS

In this study, a simple method for analyzing 9 antibiotics belonging to 3 groups on surface water by ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) was successfully developed and validated. Ten surface water samples were applied and the procedure for sample cleaning was investigated. The recoveries in the range of 71 - 125 % were accepted in environmental analysis. Relatively low MDLs (at ng L^{-1} level) were achieved, allowing the application of the developed method for monitoring antibiotic residues. Three compounds (OFL, CLA, ERY) were detected in West Lake with concentrations of 35.5 - 747.3 ng L^{-1}, while only CIP was found in Duong River with concentrations of 19.5 - 112 ng L^{-1}.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Jones O. A. H., Voulvoulis N., and Lester J. N. - Human pharmaceuticals in wastewater treatment processes, Crit. Rev. Environ. Sci. Technol. **35** (4) (2005) 401-427. doi:10.1080/ 10643380590956966.
2. Cardoso O., Porcher J. M., and Sanchez W. - Factory-discharged pharmaceuticals could be a relevant source of aquatic environment contamination: Review of evidence and need for knowledge, Chemosphere **115 (**1) (2014) 20-30. doi:10.1016/j.chemosphere. 2014.02.004.
3. Sumpter J. P. and Jobling S. - Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment, Environ. Health Perspect. **103 (7)** (1995) 173-178. doi:10.1289/ehp.95103s7173.
4. Jobling S., Nolan M., Tyler C. R., Brighty G., and Sumpter J. P. - Widespread sexual disruption in wild fish, Environ. Sci. Technol. **32** (17) (1998) 2498-2506. doi: 10.1021/ es9710870.
5. Kolpin D. W., Furlong E. T., Meyer M. T., Thurman E. M., Zaugg S. D., Barber L. B. and Buxton H. T. - Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance, Environ. Sci. Technol. **36** (6) (2002) 1202-1211. doi: 10.1021/es011055j.
6. Baquero F., J. Martínez L., and Cantón R. - Antibiotics and antibiotic resistance in water environments, Curr. Opin. Biotechnol. **19** (3) (2008) 260-265. doi: 10.1016/j.copbio. 2008.05.006.
7. Mao W., Vu H., Xie Z., Chen W., and Tang S. - Systematic review on irrational use of medicines in China and Viet Nam, PLoS One **10** (3) (2015). 1-16, doi: 10.1371/ journal.pone.0117710.
8. Nguyen K. V., Do N. T. T., Chandna A., Nguyen T. V., Pham C. V., Doan P. M., A. Q., Nguyen C. K. T., Larsson M. - Antibiotic use and resistance in emerging economies: A situation analysis for Viet Nam, BMC Public Health **13** (1), (2013), doi: 10.1186/1471-2458-13-1158.
9. Tran N. H., Hoang L., Nghiem L. D., Nguyen N. M. H., Ngo H. H., Guo W., Trinh Q. T., Mai N. H., Chen H., Nguyen D. D. - Occurrence and risk assessment of multiple classes of antibiotics in urban canals and lakes in Ha Noi, Viet Nam, Sci. Total Environ. **692** (2019) 157-174, doi: 10.1016/j.scitotenv.2019.07.092.
10. Ye Z., Weinberg H. S., and Meyer M. T. - Trace analysis of trimethoprim and sulfonamide, macrolide, quinolone, and tetracycline antibiotics in chlorinated drinking water using liquid chromatography electrospray tandem mass spectrometry, Anal. Chem. **79** (3) (2007) 1135-1144. doi: 10.1021/ac060972a.
11. Seifrtová M., Nováková L., Lino C., Pena A., and Solich P. - An overview of analytical methodologies for the determination of antibiotics in environmental waters, Anal. Chim. Acta **649** (2) (2009) 158-179. doi: 10.1016/j.aca.2009.07.031.
12. Sturini M., Speltini A., Maraschi F., Rivagli E., and Profumo A. - Solvent-free microwave-assisted extraction of fluoroquinolones from soil and liquid chromatography-

fluorescence determination, J. Chromatogr A **1217** (47) (2010) 7316-7322. doi: 10.1016/ j.chroma.2010.09.053.

13. Mookantsa S. O. S., Dube S., and Nindi M. M. - Development and application of a dispersive liquid-liquid microextraction method for the determination of tetracyclines in beef by liquid chromatography mass spectrometry, Talanta **148** (2016) 321-328. doi:10.1016/j.talanta. 2015.11.006.
14. Duong H. A., Phung T. V., Nguyen T. N., Phan T. L. A., and Pham H. V. - Occurrence, Distribution, and Ecological Risk Assessment of Antibiotics in Selected Urban Lakes of Ha Noi, Viet Nam, J. Anal. Methods Chem. **2021** (2021). doi: 10.1155/2021/6631797.
15. Paíga P., L. Santos H. M., Ramos S., Jorge S., Silva J. G., and Delerue-Matos C. - Presence of pharmaceuticals in the Lis river (Portugal): Sources, fate and seasonal variation, Sci. Total Environ. **573** (2016) 164-177. doi: 10.1016/j.scitotenv.2016.08.089.
16. TCVN (2018) https://tieuchuan.vsqi.gov.vn/tieuchuan/view?sohieu=TCVN+6663-6%3A2018