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Pharmaceutical contamination in wastewater treatment plants: occurrence, challenges in detection and insights on high-performance liquid chromatography as an effective analytical tool in environmental matrices — a review

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ABSTRACT

Environmental pollution caused by pharmaceuticals is a significant issue among various other challenges currently faced. The main and secondary source of these pollutants is wastewater and sewage sludge. It is extremely important to effectively determine the content of pharmaceuticals in wastewater and sewage sludge and develop methods for neutralizing and removing these substances. Pharmaceutical contamination in wastewater treatment plants has emerged as a growing concern due to its potential environmental and human health impacts. This review paper presents an in-depth analysis of the occurrence, challenges in detection, and the promising role of high-performance liquid chromatography (HPLC) as an analytical tool for assessing pharmaceutical pollutants in diverse environmental matrices, including wastewater, treated wastewater, and sewage sludge. The manuscript provides a comprehensive overview of the efficiency, advantages, and limitations of HPLC in pharmaceutical analysis, including detection limits, error analysis, chemical requirements, precision, and analysis time. The study focuses on the application of HPLC for the determination of pharmaceuticals in wastewater matrices, highlighting its utility in profiling target compounds across different concentration ranges. The paper also discusses the significance of HPLC in overcoming the analytical challenges associated with pharmaceutical detection in wastewater treatment processes. The review offers insights into the capabilities of HPLC for analysing pharmaceuticals, comparing its performance with other analytical methods, and discussing its potential for routine monitoring and environmental risk assessment. Overall, this manuscript aims to provide a comprehensive understanding of the analytical methods for pharmaceutical determination in wastewater and sewage sludge matrices, emphasizing the benefits and limitations of HPLC. The findings of this review contribute to advancing knowledge in the field of pharmaceutical pollution, aiding researchers, environmental practitioners, and policymakers in developing effective strategies for monitoring and mitigating the environmental impacts of pharmaceutical contamination in wastewater treatment plants.

Keywords: Pharmaceuticals; Multi-analysis; Wastewater; Sewage sludge; Emerging contaminants; Environmental matrices; High-performance liquid chromatography (HPLC); Detection challenges; Occurrence; Detection limits

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1. Introduction

Currently, environmental pollution is constantly increasing. A lot of waste ends up in the natural environment, along with contaminants that are difficult to remove, such as micro- and nanoplastics, pharmaceuticals, heavy metals, and chemical pollutants. Pharmaceutical substances are a large group of chemical pollutants and are one of the emerging contaminants [1]. Pollution of the natural environment with pharmaceuticals can be divided into soil pollution and water pollution. However, pharmaceuticals are constantly migrating between these ecosystems affecting the living organisms in these ecosystems. Pharmaceuticals are a very diverse group of environmental pollutants.

Testing the presence of pharmaceuticals in the environment and determining the impact of these substances is very difficult. The first place where pharmaceuticals should be tested are wastewater treatment plants (WWTPs), which are the main source of environmental pollution by pharmaceuticals and their transformation products [2–4]. WWTPs produce sewage sludge and treated wastewater, which are released into the environment. Both treated wastewater and sewage sludge carry high levels of micropollutants, including pharmaceuticals and their transformation products. To be able to minimize the content of these substances in sewage and sewage sludge, the first necessary step is to determine the content of pharmaceuticals. However, due to the variety of pharmaceuticals and the complexity of the matrix, which is treated wastewater and sewage sludge, carrying out the determination can be problematic. The use of high-performance liquid chromatography (HPLC) has become the gold standard in these analyses, however, there is still no universal method for detecting pharmaceuticals in these matrices.

In this paper, we would like to present the problem of environmental pollution by pharmaceuticals and the possibilities offered by using HPLC for the analysis of these substances. One of the objectives is to investigate the occurrence and extent of pharmaceutical contamination in wastewater treatment plants, including wastewater, treated wastewater, and sewage sludge matrices. The paper presents the applicability and evaluation of the HPLC method for the determination of pharmaceuticals in wastewater, treated wastewater, and sewage sludge, and the parameters that may affect the effectiveness of these analyses are considered. The main purpose of this work is to collect and systematize information on the possibility of modification and application of the HPLC method in the determination of pharmaceuticals in these three main matrices derived from WWTPs. The collection of this information can be used in designing and optimizing methods using HPLC to determine pharmaceuticals in wastewater and sewage sludge. The objective of this research is to provide insights into the efficiency, precision, and detection limits of different pharmaceutical compounds using HPLC and to discuss the potential implications of pharmaceutical contamination in wastewater treatment plants on the environment and human health.

Overall, the main objective of this review is to examine the problem of pharmaceutical contamination in wastewater treatment plants, present the role of HPLC as a valuable tool for pharmaceutical analysis, and provide comprehensive insights into the challenges, advantages, and limitations associated with using HPLC for determining pharmaceuticals in different environmental matrices.

2. Sources of pharmaceuticals in the environment and the role of wastewater treatment plants in the distribution of pharmaceuticals

Pharmaceuticals and their transformation products are found worldwide in soil, surface water, drinking water, and all other environmental matrices [2,5,6]. Their presence has been demonstrated in marine waters and bottom sediments, as well as in inland waters and soils [7]. In addition, new drugs are constantly being developed, the fate of which in the environment is not yet known. In 2022 U.S., The Food and Drug Administration (FDA) has approved 37 new drug molecules. Due to the large number of pharmaceuticals and transformation products, it is difficult to determine the specific environmental pollution caused by a particular pharmaceutical. All ecosystems interpenetrate and micropollutants migrate between them. Each pharmaceutical has a different affinity to the water and soil environment, which is why pollution with these substances is uneven. On the example of diclofenac, a popular substance from the group of non-steroidal anti-inflammatory drugs (NSAIDs), it can be observed that its presence has been confirmed in various matrices: surface water (maximum level worldwide: 57.1 μg/L), groundwater (13.4 μg/L), wastewater (836 ug/L), seawater (10.2 μg/L), drinking water (56 ng/L), soil (257 μg/kg), sediment (309 ng/g), suspended soil (1.3 μg/g), sludge (4,968 μg/kg), leachate (108 μg/L) and even in organisms tissue: plasma of fish (11.9 μg/L), mussel (4.5 μg/kg), and plant (11.6 μg/kg) [8]. Pharmaceuticals contamination is not limited to a specific region but occurs globally. What's more, pharmaceuticals, which are primarily used for human and veterinary purposes, can enter the environment through various pathways such as wastewater discharge, improper disposal, or agricultural practices. Once released, these compounds can transform and persist in the environment, leading to their detection in different compartments. The main sources of environmental pollution with pharmaceuticals include the pharmaceutical industry – production process of drugs, wastewater treatment plants (WWTPs), agriculture (animals breeding, livestock including fish farming), septic systems, and landfills (leachate) (Fig. 1 major pathways of the release of human and veterinary pharmaceuticals into the environment [2,9–13]).

Sources of pharmaceuticals in the environment can be divided into two groups. The first group is point sources, which include sources of pollution that are easy to determine, with a known location (Fig. 1). This group includes WWTPs, industrial and hospital sewage, and household septic systems in one of the studies, in which it was confirmed that less than 120 different pharmaceuticals end up in the environment from hospital effluents [14]. The second group – diffuse sources – are sources of pollution with a difficult-to-specify place of release into the environment. These sources include runoff from agricultural fields fertilized with manure, runoff from livestock farming, as well as leakages from WWTPs and sewage systems. Dispersed

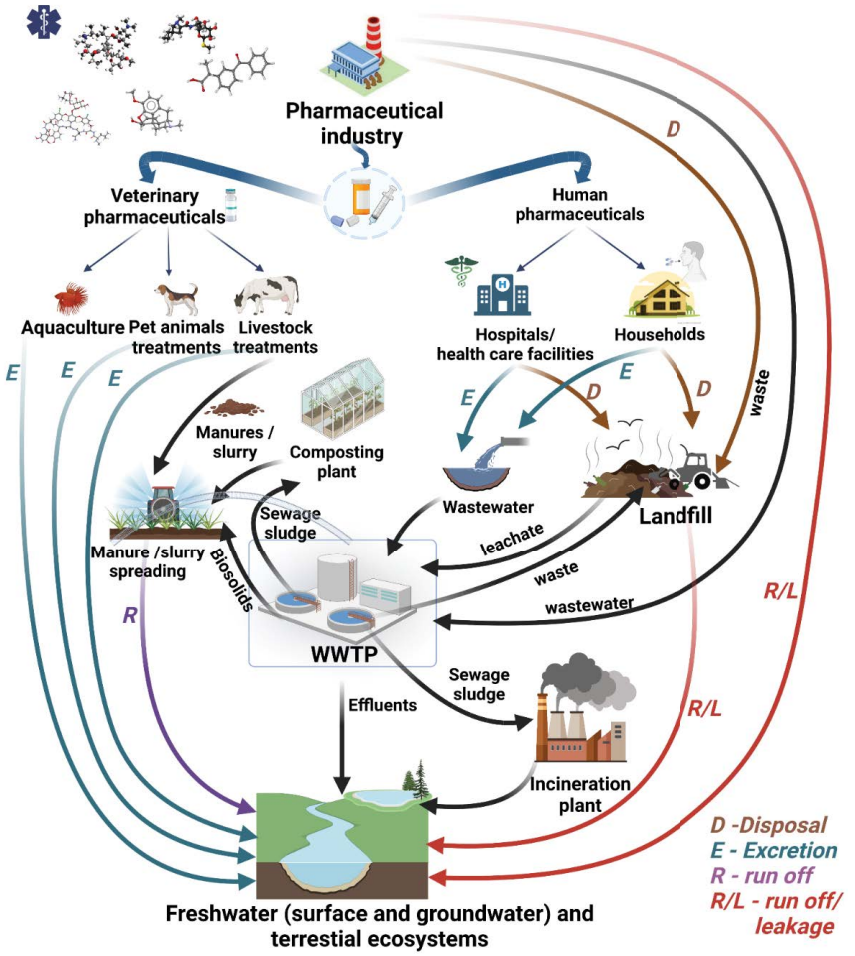


Fig. 1. Major pathways of the release of human and veterinary pharmaceuticals into the environment [2,9–13].

sources of pollution by pharmaceutical substances are less of a problem for the natural environment in a short time manner because, due to the penetration into the environment through a larger, dispersed area, they do not reach high concentrations and are more easily attenuated naturally. It should be noted that the largest percentage of pharmaceuticals and their transformation products enter the environment through municipal wastewater treatment plants, which treat municipal wastewater, as well as hospital and industrial wastewater with high content of pharmaceuticals [3]. Understanding the widespread occurrence of pharmaceuticals in environmental matrices is crucial for assessing their potential ecological impacts, as well as evaluating the risks associated with human exposure to these substances. It underscores the need for effective monitoring, management, and remediation strategies to mitigate the environmental and health risks posed by pharmaceutical contamination.

3. Pharmaceuticals in wastewater, treated water and sewage sludge: occurrence, fate, and implications

The occurrence of pharmaceuticals in treated wastewater can be attributed to two primary factors. Firstly, it is a consequence of the elevated concentration of pharmaceutical substances present in municipal wastewater, which subsequently enters the wastewater treatment plant. This phenomenon is closely linked to the extensive consumption of pharmaceutical drugs by the human population. In both highly developed nations and rapidly developing countries, there is a constant surge in the availability and utilization of pharmaceutical medications. Therefore, the inflow of pharmaceutical substances to wastewater treatment plants will also increase. In addition, new pharmaceutical substances will be delivered to the wastewater treatment plant along with wastewater, as the pharmaceutical market is also constantly growing [15]. Under the theoretical and ideal conditions in a WWTP, with 100% removal efficiency, the process manages to remove all these substances from the wastewater. However, the lack of adaptation of the technological processes used in WWTPs to remove drug particles and the lack of optimized methods of wastewater treatment is the second reason why treated wastewater is the largest source of environmental pollution with these substances. Moreover, the 3rd degree of wastewater treatment is not obligatory in many countries. Only in a few of them is this process a legal obligation, or an obligation coming from the necessity of water reuse. An example of a country

where the modernization of the WWTPs and the use of 3rd degree wastewater treatment reduced the release micropollutants into the environment is Switzerland [16]. China also introduced a new environmental protection act under which all wastewater treatment plants in Beijing were to be reconstructed. Wastewater treatment plants have been extended with tertiary wastewater treatment, and the solutions that have been used are a biological filter, ultrafiltration, ozonation, and NaClO disinfection. The main purpose of this act was to limit the content of the main pollutants, such as carbon, nitrogen, phosphorus, and microbial organisms in the treated effluent. However, the upgrades also affected the content of micropollutants such as pharmaceuticals and reduced their concentration in wastewater by 45%–74% [17]. Unfortunately, in many countries and areas, even the 2nd degree of wastewater treatment is still not an obligation. Some drug molecules are not retained, neutralized, or removed in any of the currently applied wastewater treatment processes. Therefore, has become extremely important to properly detect pharmaceutical substances contained in treated wastewater, which are the main product during wastewater treatment directly affecting the water and environment due to direct and constant discharge. Together with municipal and industrial wastewater, significant amounts of biological, mechanical, and chemical pollutants are delivered to WWTPs. Chemical pollutants include pharmaceuticals, their metabolites, and transformation products. The currently used wastewater treatment methods are not effective in removing these pollutants, and the lack of appropriate legal regulations means that drug concentrations in wastewater treatment products are not determined [18]. Along with treated effluent and sewage sludge, pharmaceuticals end up in the natural environment [19]. Treated wastewater is most often discharged into rivers close to the treatment plant. However, due to the need for a circular economy system, more and more attention is being focused on the use of wastewater treatment products in various areas of life may occur the risk of the direct release of pharmaceuticals into the environment. Treated wastewater, also known as reclaimed water or recycled water, can be utilized for various purposes, including agricultural and urban areas irrigation, industrial uses, and groundwater recharge, and can be discharged into surface water bodies, such as rivers or lakes, to enhance flow regimes during periods of low flow or drought conditions, toilet flushing [20–23].

Conventional wastewater treatment methods currently are not suitable for the complete removal of pharmaceuticals. Fig. 2 shows the basic technological diagram of the wastewater treatment plant, considering the processes that take place at individual stages. Primary wastewater treatment takes place in a bar screen, grift chamber, and primary clarifier. The basic processes during primary wastewater treatment are sorption, desorption, biotransformation, biodegradation, and complexation. These processes show low efficiency in removing pharmaceuticals [28]. After this stage, the wastewater is subjected to secondary treatment, in which biological treatment is used. Depending on the construction of the sewage treatment plant, treatment can take place in several chambers with variable oxygen conditions or in sequential biological reactors, where the parameters change periodically. Pharmaceuticals with high removal efficiency (>80%) are removed in anaerobic processes (removal efficiency 26%–156%) [28]. The main processes that occur during secondary wastewater treatment are sorption, desorption, biodegradation, biotransformation, complexation, flotation, oxidation, and photodegradation. These processes remove pharmaceuticals from wastewater, but they are not 100% effective. Therefore, the sewage sludge contains pharmaceuticals in high amounts, for example, diclofenac – up to

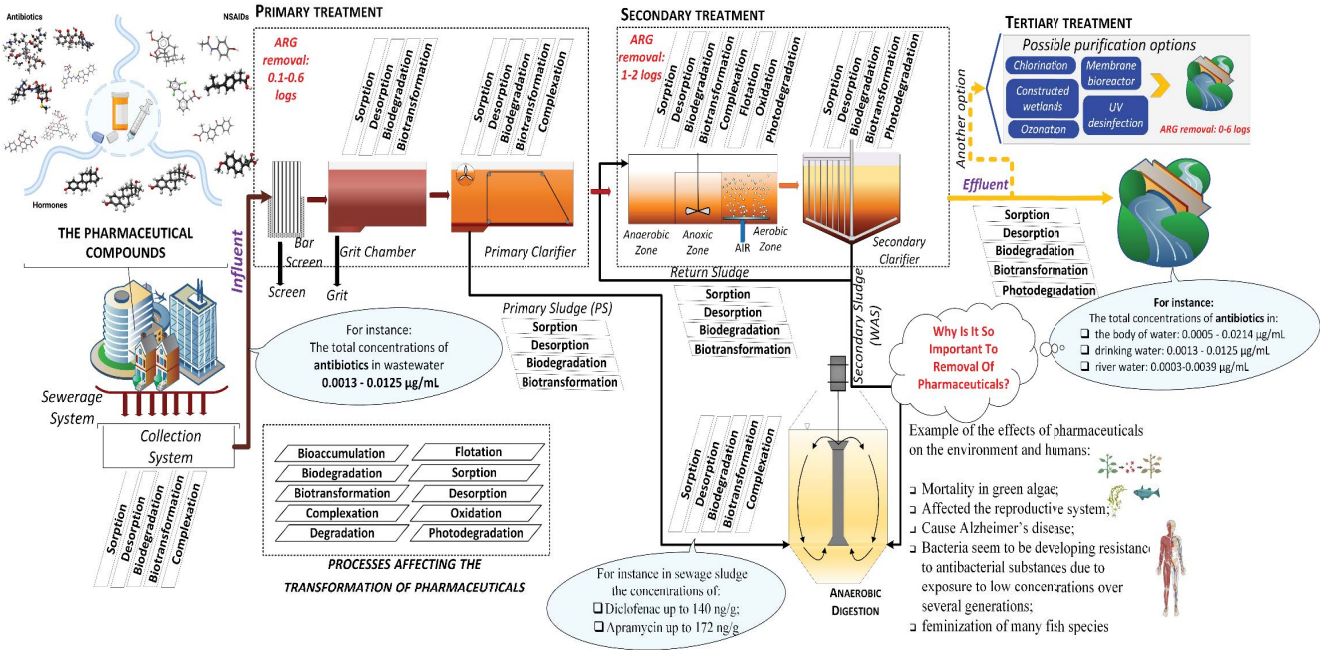


Fig. 2. Pathways and the fate of pharmaceuticals resistance in the WWTP [12,24–27].

140 ng/g or apramycin up to 172 ng/g. In the example of antibiotics, the degree of their removal in anaerobic processes is between 60%–97%, depending on the analysed drug [11]. On the other hand, the use of tertiary wastewater treatment allows for increasing removal efficiency. Among others, advanced oxidation processes (AOPs), photocatalysts, or adsorbents are used for antibiotic removal. The AOPs include ozone (O_3)/UV – 87%–100% removal, O_3/hydrogen peroxide (H_2O_2) – 92%–100% removal, O_3/H_2O_2/UV – 80%–100% removal, Fenton process – 74%–76% removal, electrochemical oxidation – 99% removal, sonochemical oxidation – 100% removal [11].

Table 1 shows the different wastewater treatment processes that researchers use to find the most effective method. The problem is that these studies often focus on a single pharmaceutical, and the concentrations used in the analysis are much higher than those found in the natural environment, so the processes do not reflect the actual removal rates. Table 1 shows the concentrations that were used by the investigators in the analyses. It is therefore necessary to expand research in this area because the large-scale introduction of tertiary wastewater treatment could significantly reduce the release of pharmaceuticals into the environment.

Figs. 3 and 4 show the concentration of these pharmaceuticals in the influent and treated effluent, for which no tertiary wastewater treatment was conducted. This problem is present all over the world, both in European, Asian, and North American countries, and the presence of drugs in treated effluent is documented. Figs. 3 and 4 show how high concentrations of pharmaceuticals have been recorded around the world. For pharmaceuticals in influent wastewater, the scale reaches 620,000 ng/L (and the highest concentration is achieved by naproxen labeled in Europe – Fig. 4a). Fig. 3 refers to the large group of pharmaceuticals in the environment: antibiotics. Fig. 3a collects data on antibiotics present in influent wastewater on different continents. It is worth paying attention to the dominant antibiotics on a given continent – the variability of the dominant drug results from diverse health care systems and the availability of modern drugs. In Europe, the dominant antibiotics reaching WWTPs are ciprofloxacin, erythromycin, sulfamethazine, and trimethoprim. In Asia, the main antibiotics in influent wastewater are vancomycin and azithromycin. In North America, the dominant antibiotics in influent wastewater are ciprofloxacin, as in Europe, and additionally representatives of tetracyclines: tetracycline and oxytetracycline. In treated wastewater, the presence of antibiotics is distributed differently than in wastewater influent (Fig. 3b). In treated wastewater, antibiotics with the highest concentrations were for Europe – vancomycin, ofloxacin and ciprofloxacin, for Asia – lincomycin and erythromycin, and for North America – trimethoprim and clarithromycin. Fig. 4 refers to hormones, NSAIDs, and other pharmaceuticals. Fig. 4a summarizes the data on influent wastewater. Attention is drawn to the high concentration of NSAIDs and antihypertensive. The first group of drugs are popular over-the-counter drugs, hence their large

Table 1
Brief summary of research studies in which different methods of wastewater treatment were used for removing pharmaceuticals from wastewater

Method	Pharmaceutical	Matrix	Initial concentration	Removal efficiency (%)	References
Advanced oxidation process					
Bio-electro-Fenton system (under the continuous flow mode)	Metoprolol	Synthetic wastewater	10 μg/L	95	[29]
Photo-Fenton	Paracetamol	Synthetic wastewater	100 mg/L	100	[30]
Ozonation	Amoxicillin	Industrial wastewater	125 mg/L	80–98	[31]
Photocatalytic ozonation	Carbamazepine	Treated domestic wastewaters	5 mg/L	91 (total organic carbon removal)	[32]
TiO_2 photocatalysis	Diclofenac	Deionized water	0.5 mg/L	80	[33]
Sorption					
Sorption on pomelo peel derived biochar	Sulfamethoxazole	Swine wastewater	100 μg/L	82.44–88.15	[34]
Sorption on methanol-modified biochar	Tetracycline	Aqueous solution	100 mg/L	95%	[35]
Wetlands					
Rural wastewater treatment wetlands	Diclofenac	Domestic wastewater	1,000 ng/L	52–73	[36]
Wetland mesocosms planted with *Scirpus validus*	Carbamazepine	Nutrient solution	100 μg/L	79–99	[37]

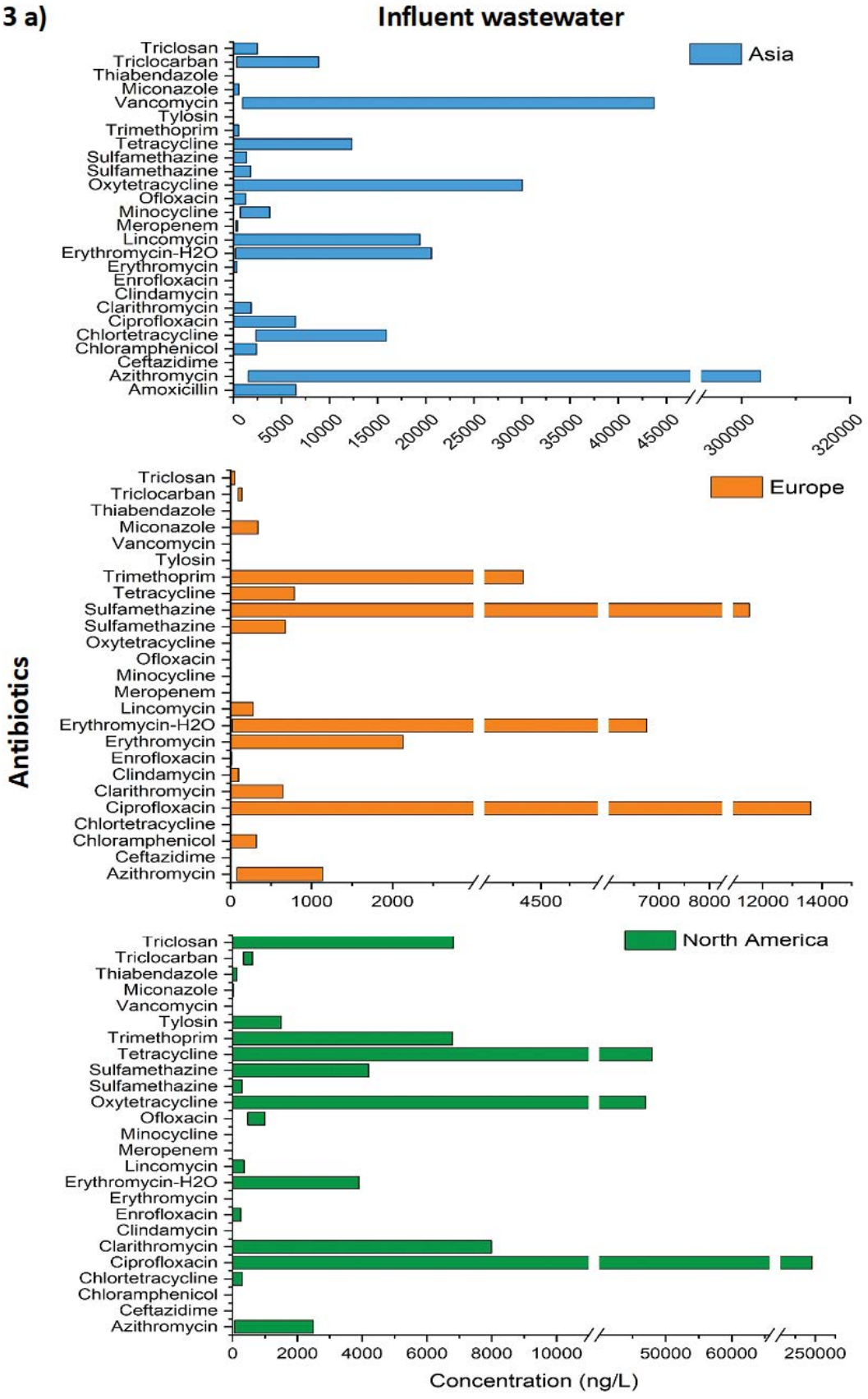


Fig. 3 (Continued)

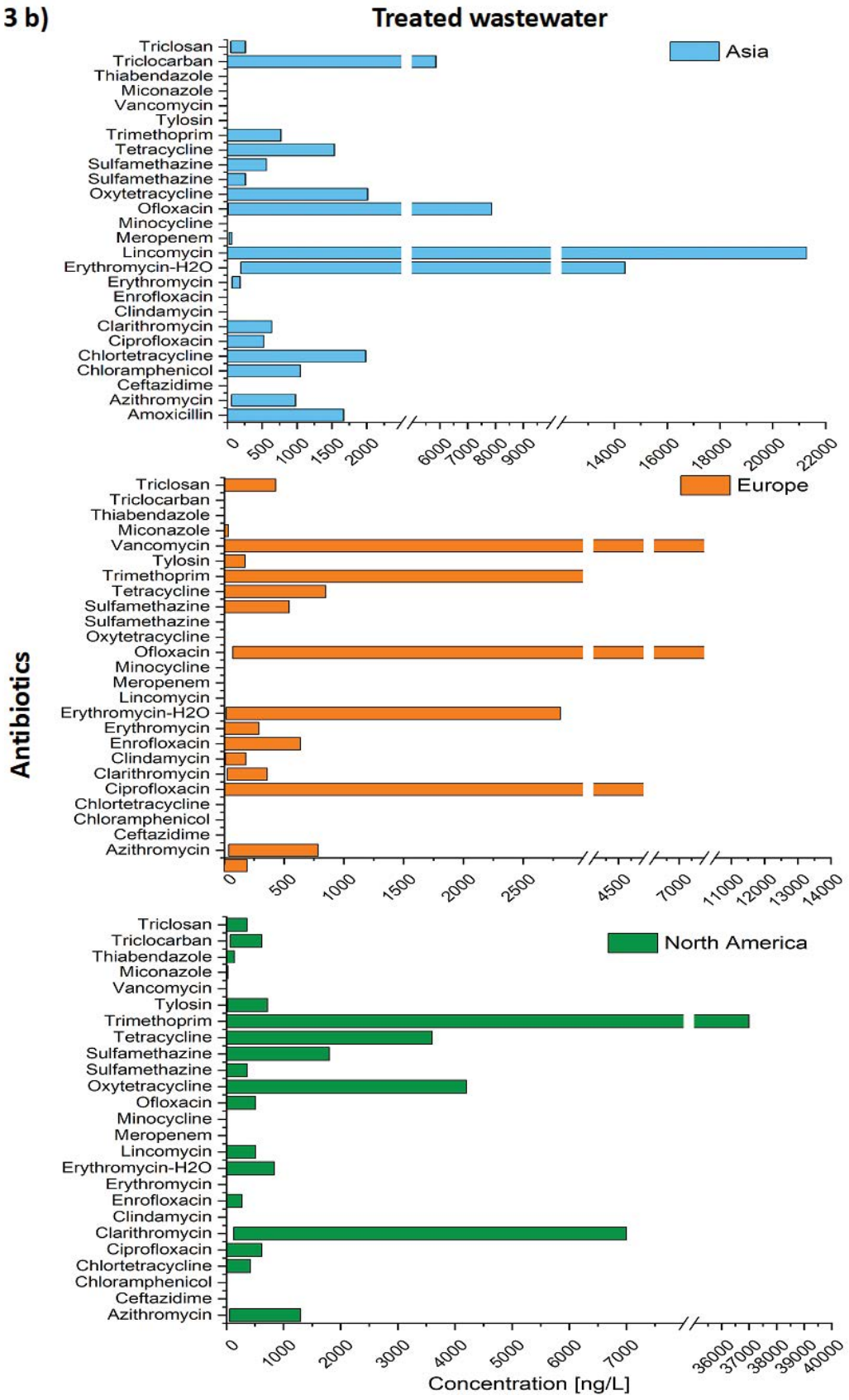
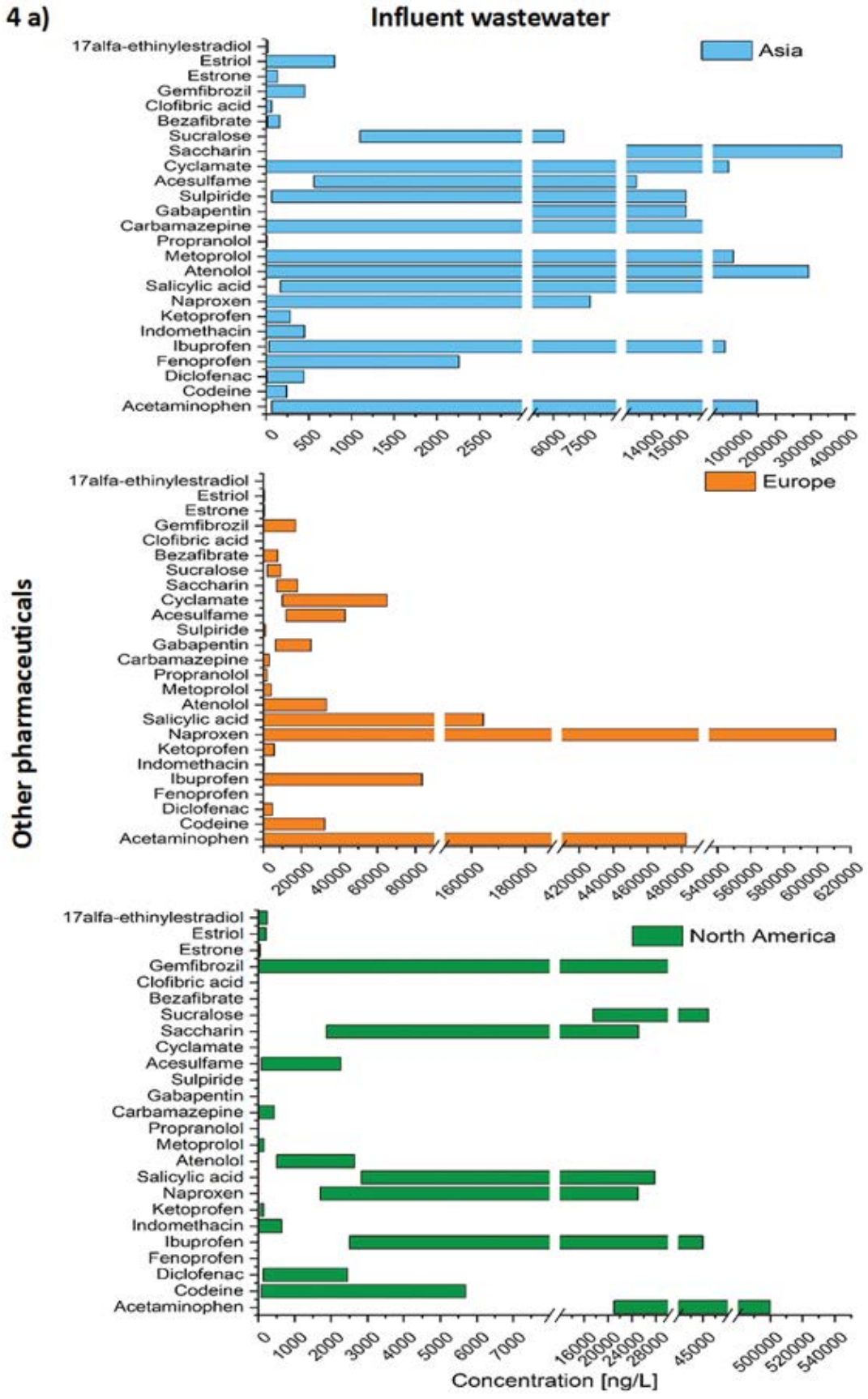


Fig. 3. Concentration of selected antibiotics in raw influent and treated effluent (ng/L) collected from full-scale WWTPs [24,38].



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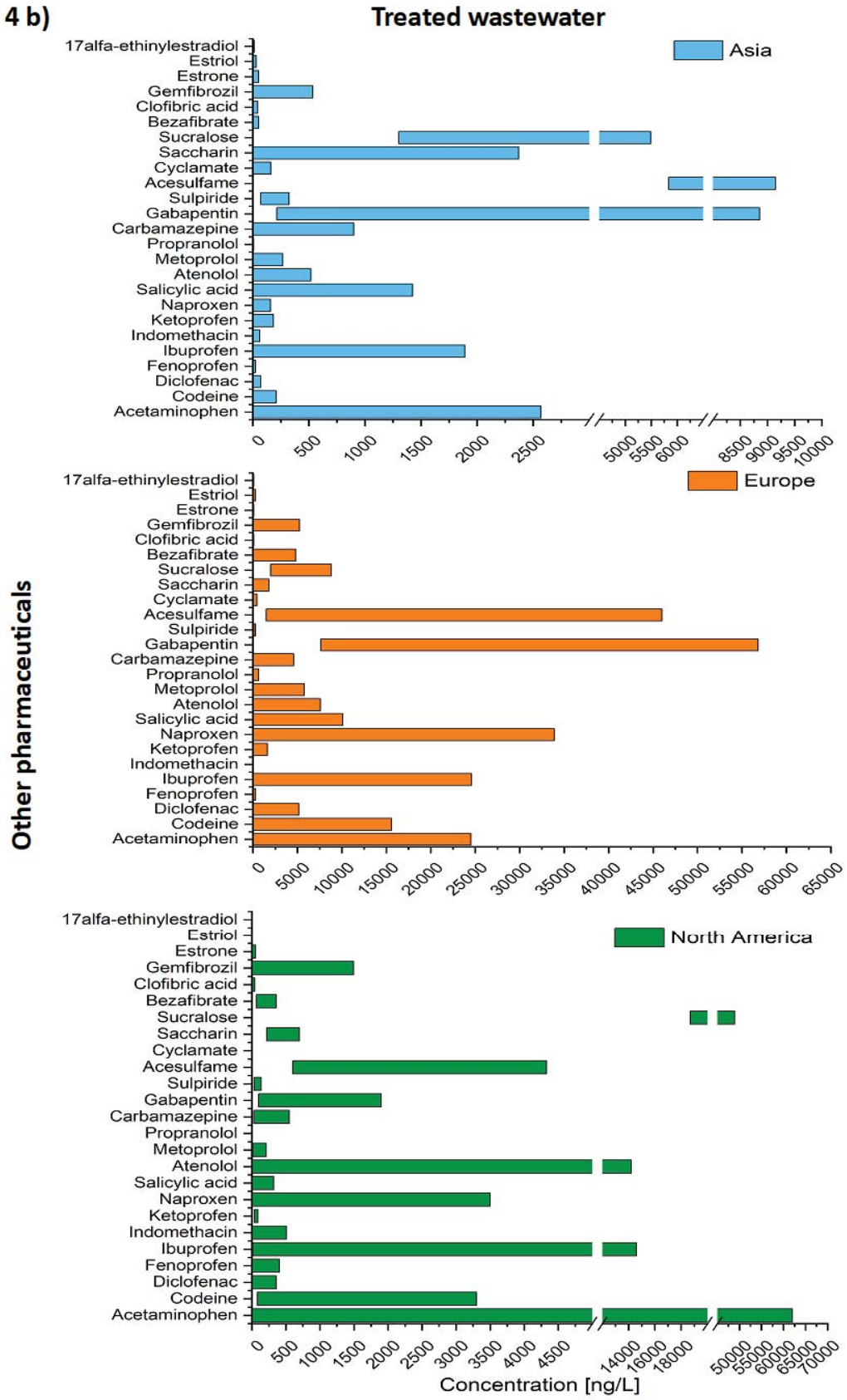

Fig. 4. Concentration of selected pharmaceuticals from diverse groups in raw influent and treated effluent (ng/L) collected from full-scale WWTPs [24,38].

amount in wastewater. The second group consists of drugs permanently taken by patients with cardiovascular diseases. On each of the continents, high concentrations of acetaminophen, that is, paracetamol, were detected. Unfortunately, more disturbing data are presented in Fig. 4b, as they indicate high concentrations of pharmaceuticals in treated wastewater. Such a high concentration of pharmaceuticals in treated wastewater indicates a big problem related to the lack of adjustment of the process of removing micropollutants from wastewater.

Additional processes allow to increase the efficiency of removing pharmaceuticals and other micropollutants from wastewater, however, it should be remembered that during these processes, for example, AOPs, many transformation/degradation products are generated. For example, Fig. 5 shows various alternative pathways for the degradation of diclofenac, a pharmaceutical that is commonly found in the environment. As previously mentioned, various transformation/degradation products are formed depending on the processes to which the substance is subjected.

Various processes are used in the research to find the most effective solution in removing pharmaceuticals, while ensuring that the resulting products do not show a stronger toxic effect than the starting substance. These processes are introduced as wastewater pre-treatment [42] or as tertiary wastewater treatment [43]. To obtain the highest efficiency of removing pharmaceuticals, the biological method is most often coupled in research, including the dominant modern systems using a membrane biological reactor (MBR), with AOPs [43–46]. The use of AOPs brings the greatest removal efficiency. It is a very large group of different processes. The mechanisms of AOPs are shown in Fig. 6.

4. Impact on the environment, animals, and human health of pharmaceuticals after discharge from wastewater treatment plants

The United States Environmental Protection Agency (EPA) and the European Environment Agency (EEA) recognize pharmaceuticals as contaminants of emerging

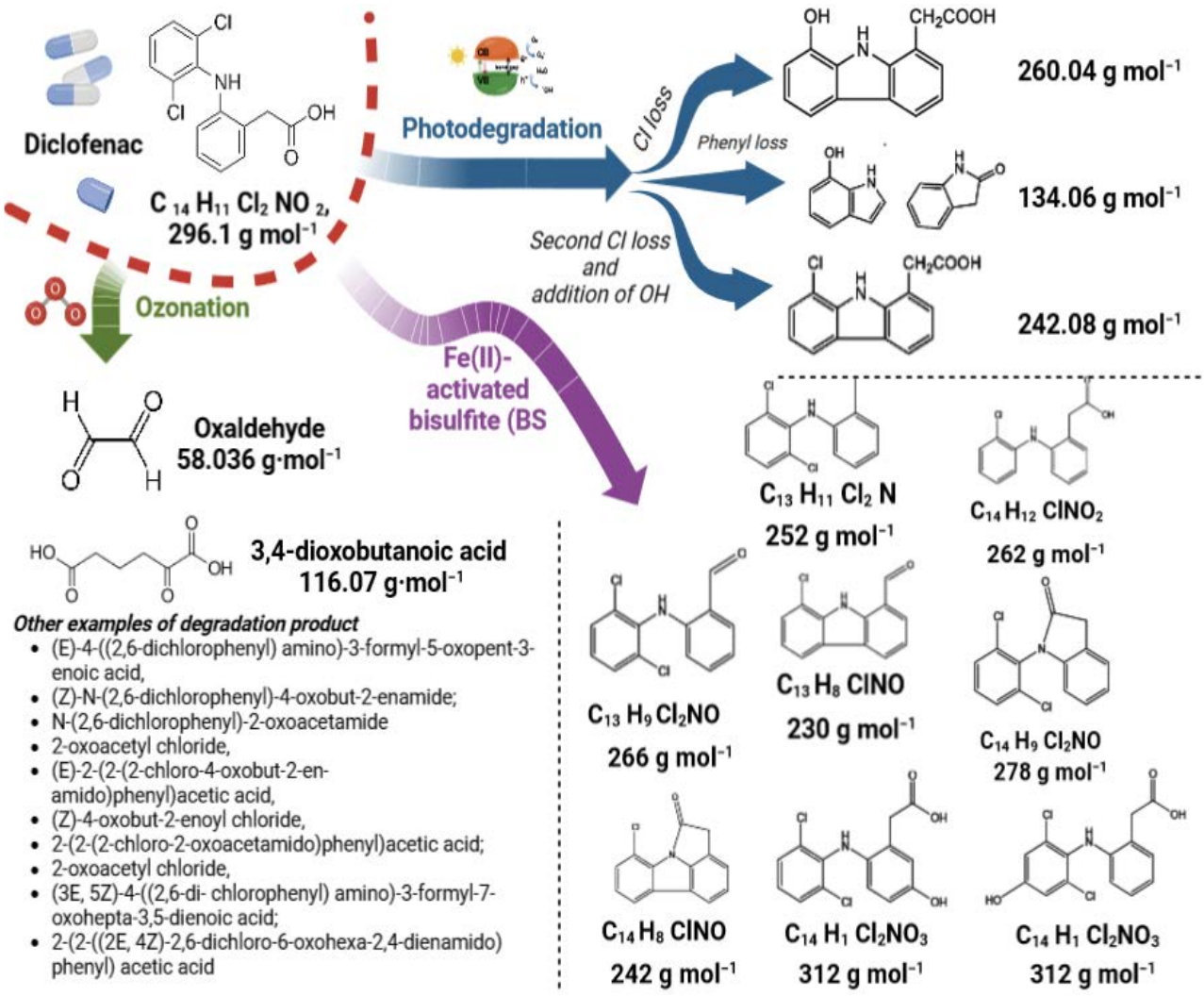

Fig. 5. Examples of products produced during the degradation of diclofenac using selected advanced oxidation processes methods [39–41].

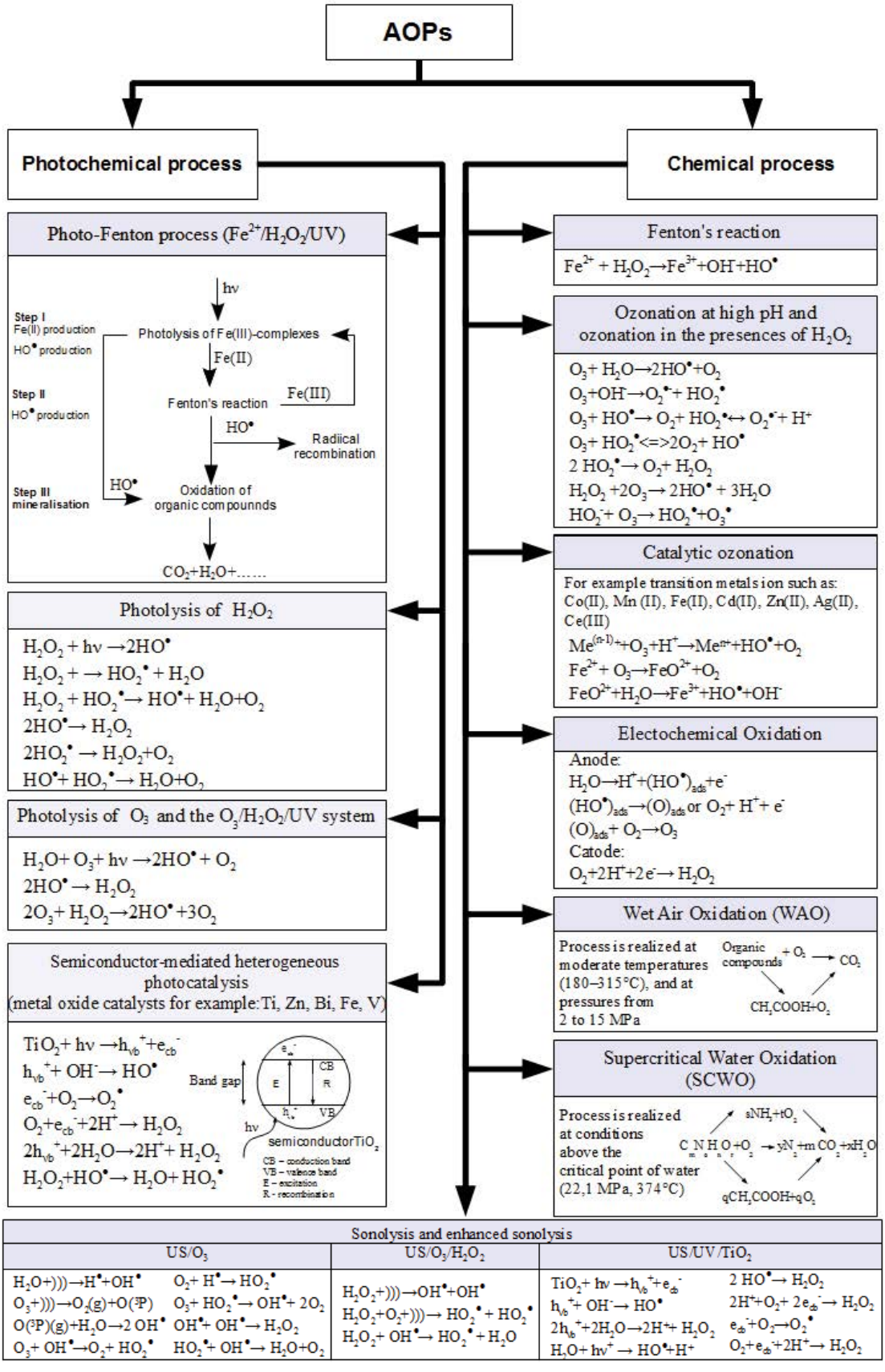

Fig. 6. Different mechanisms of advanced oxidation processes [47,48].

concern (CEC). This means that pharmaceuticals are often found at low levels in surface waters and can pose a threat to aquatic life [49,50]. Therefore, a regulatory framework for the environmental risk assessment of medicinal products of human use (ERA) was created, which is intended to minimize the toxicity of drugs on the environment [51,52]. Despite the introduction of the ERA, pharmaceuticals entering the environment affect aquatic and soil life [6,53]. Moreover, not only are pharmaceuticals a problem, but their transformation products (TPs) are as well. In many cases, they are more ecotoxic than the parent compound [6]. Pharmaceuticals and TPs are present in the environment in many processes, for example, adsorption, and bioaccumulation, which can introduce these compounds into the food chain and lead to potential human exposure.

One of the most recognized issues associated with the presence of antibiotics in the environment is the emergency of antibiotic resistance among environmental bacteria. This phenomenon is mainly modulated by the influx of antibiotic molecules into the environment. Bacteria produce resistance systems to a significant number of currently used antibiotics, moreover, due to the ongoing processes of gene exchange between bacterial strains, and horizontal gene transfer, resistance mechanisms spread very quickly in the environment [54–56]. Therefore, infection with environmental bacteria can cause severe infections that are difficult to treat and may even lead to the death of the patient [57,58]. Various ways of affecting the human body should be considered, including percutaneous absorption or accidental drinking of contaminated water [53]. Antibiotics present in the soil and water can affect not only the microorganisms living in it but also some of its physicochemical properties, that is, acidity or concentration of organic matter [59]. Drugs such as antibiotics, in addition to affecting microorganisms, also affect higher living organisms. They can be toxic to the fish population and accumulate in their tissues. They also accumulate in plant tissues [53].

NSAIDs are a very big problem in the environment. This group of pharmaceuticals includes, for example, ibuprofen, naproxen, diclofenac, and acetylsalicylic acid. They are substances with analgesic, anti-inflammatory, and antipyretic properties. They are present in the environment in high concentrations because they are available over the counter (OTC drugs) and often abused by patients [28,60]. A representative of this group, widely discussed in the literature, is diclofenac. It shows several toxic effects on aquatic organisms as well as the ability to accumulate in their tissues [61,62]. Other representatives of this group, that is, ibuprofen [63] or naproxen [64] also show toxic effects on environmental organisms. In the case of this group of drugs, mixtures of these substances, even in low concentration, are characterized by the highest toxicity [65,66].

Other groups of pharmaceuticals whose presence is considered in terms of toxicity to environmental organisms are cytostatics. These substances are used in cancer therapy, and their task is to inhibit the growth of cancer cells and even kill them. Unfortunately, these drugs often show cytotoxic activity to healthy cells as well [40]. Despite the special attention paid to therapeutic regimens in the treatment of cancer, waste containing residues of these drugs ends up in wastewater treatment plants, and then into the aquatic environment [67]. Due to the specific activity of cytotoxic drugs, even in small concentrations, they can have a toxic effect on the cells of organisms. The current state of knowledge about their presence in the environment and their impact on human health indicates some risk groups like children and lactating women [67,68]. Therefore, it is necessary to determine the impact of human exposure to cytostatics present in the environment. The most frequently monitored cytostatics in the environment include fluorouracil, methotrexate, tamoxifen, ifosfamide, and cyclophosphamide [69]. For the drugs, 5-fluorouracil and methotrexate toxicity studies have been performed on freshwater mussels of the species *Elliptio complanata*. It was found that these drugs can affect the processes occurring in mussel tissues [70].

Another group of pharmaceuticals that has an environmental effect is synthetic steroid hormones. Synthetic steroid hormones found in the environment may interfere with the reproductive processes of organisms such as fish. They cause the feminization of many fish species and thus reduce the population of these organisms in natural water reservoirs [71]. Both natural and synthetic estrogens that occur in the aquatic environment exhibit biological activity at low concentrations. Constant exposure of aquatic vertebrates and invertebrates to low estrogen concentrations can significantly affect their reproduction [72]. Estrogen that is often monitored in environmental studies is synthetic ethinylestradiol. It is the most common hormone found in two-component hormonal drugs, hence its high presence in the environment. It is characterized by high biological activity, and thus, has a large impact on aquatic organisms. One of the effects of exposure of organisms to estrogens is an increase in the concentration of the precursor protein – vitellogenin (Vtg). With the increase in the Vtg concentration, an increased number of intersex individuals, reduced production of eggs and sperm, reduced quality of gametes, and even complete feminization of male fish are observed. In addition, ethinylestradiol affects metabolic processes, modifying the activity of enzymes, and affecting genetic processes [73]. Also, in the case of soil, 17α-ethinylestradiol, and 17β-estradiol are critical contaminants from sewage sludge [19,74,75]. Hormones of anthropogenic origin are the main cause of hormonal imbalance in water and soil because they are endocrine disruptors [71,76]. Those substances can mimic the natural hormones produced by living organisms and interfere with various functions [1].

Neuroactive drugs, drugs for metabolic diseases, and many others are also a problem [77]. In a global study of pharmaceuticals found in rivers around the world, the most frequently detected substances were carbamazepine, metformin, and caffeine [78]. Drug molecules entering the human body in this way can disrupt the homeostasis of the body and affect various processes. In vitro studies have shown that psychoactive substances present in small amounts in the environment may interact with genetic factors, and thus cause a different impact as neurological disorders [79]. In addition, human exposure to chiral pharmaceuticals in the environment, through drinking, eating, or skin contact may have carcinogenic potential [4].

There are many more negative effects of environmental pollution caused by pharmaceuticals, but they are difficult to determine. This is due to limited research in this

area and a large diversification of pharmaceutical contamination in different parts of the world. This problem results from differences in the development of a given society, availability of drugs, methods used in water treatment as well as different physicochemical conditions prevailing in each place. Therefore, concentrations of drugs in the environment should be controlled. Moreover, the impact on living organisms and danger for public health should be monitored. The difficulty in determining the concentration of pharmaceuticals is also due to their constant migration between the water and soil environment. In addition, with the migration of these substances, a series of transformations occur that result in the formation of many different transformation products. The transformation pathways are different, and the resulting products have different environmental toxicity. Fig. 7 shows the migration routes of pharmaceuticals in the environment, and in the example of diclofenac, possible ways of its degradation are presented. Depending on the process taking place, different products are formed. This is extremely challenging both in terms of introducing new solutions in wastewater treatment plants and in terms of monitoring the natural environment and the impact of pharmaceuticals on the ecosystem.

Determination of the content of pharmaceuticals in both treated wastewater and sewage sludge is difficult due to the complex composition of such a matrix and the large number of different pharmaceutical substances that need to be determined. HPLC coupled with a detector has found wide applications in the detection of pharmaceuticals. It is used to test pharmaceutical substances in various matrices, including environmental ones. Thanks to the changes in the conditions of analysis, it is possible to detect pharmaceuticals from different groups, characterized by different chemical structures. These tests allow for a qualitative and quantitative assessment of the sample.

5. Methods of determination of pharmaceuticals in environmental matrices

The natural environment is composed of many ecosystems that constantly interpenetrate. Also, pollution from pharmaceuticals migrates in the environment between water and soil ecosystems. Pharmaceutical residues are found in both water and soil. Due to the diversity of environmental samples, it is difficult to find a universal tool to perform an analysis of pharmaceuticals. For solid samples, also for sewage sludge, each analysis usually consists of 4 stages: extraction of contaminants, cleaning-up the sample, proper analysis, and appropriate detection and processing of results. Each of these stages is important for the analysis and the quality of the results obtained. The first stage, that is, the extraction of a solid sample to the liquid phase, can be carried out in many ways. These include the Soxhlet apparatus, and its automatic version, ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, and pressurized hot water extraction novel methodologies include matrix solid-phase dispersion (MSPD) or QuEChERS (quick, easy, cheap, effective, rugged and safe) [82–85]. Sample clean-up is the first common step for soil and water analysis. Solid-phase extraction (SPE) is the most popular technique for the clean-up after extraction from sewage sludge and wastewater. Many factors affect the efficiency of this process. The first is the type of sorbent used in the SPE. Examples of sorbents are hydrophilic–lipophilic balance (HLB), strong mixed-mode anion exchanger (MAX), and the mixed-mode polymeric sorbent (MCX). Hydrophilic–lipophilic balanced sorbents are the most widely used [27,82,86,87]. Factors that also affect the SPE are the pH of the sample, the selection of the appropriate eluent, the load volume of the sample solution, or the addition of a buffer [86]. The prepared samples are subjected to instrumental analysis. In environmental samples, including samples of sewage sludge and wastewater, liquid chromatography (LC) found the greatest application [88,89]. In addition to liquid chromatography, gas chromatography (GC) or capillary electrophoresis (CE) is also used [89,90]. Modern devices used to detect pharmaceuticals include an aptamer-based graphene field effect transistor (AptG-FET) platform [91].

6. HPLC for the detection of pharmaceuticals in wastewater and sewage sludge

Among these methods, HPLC is a method that is routinely used to determine pharmaceuticals in the environment [92]. Both the parameters of the HPLC and the type of detector with which the HPLC is coupled affect the detection limit of pharmaceuticals. Currently, it is most often coupled with a tandem mass spectrometer. In addition to tandem mass spectrometry (MS/MS), the detector can be an absorption UV-Vis detector, for example, DAD (diode array detector) also known as PDA (photodiode array), fluorescence detector (FLD), electrochemical detector (El-D) or conductometric detector (CD).

Table 2 presents an example of HPLC application in the determination of selected groups of pharmaceuticals in wastewater and sewage sludge samples. Table 2 also presents the detected concentrations of individual pharmaceuticals in wastewater and sewage sludge. High concentrations were found in the case of representatives of NSAIDs and antibiotics. Table 3 presents the use of the HPLC method for the multi-analysis of pharmaceuticals in wastewater and sewage sludge samples. In multi-analyses, the most frequently used detector was MS/MS. The use of this detector in conjunction with HPLC allows for the precise separation of the analyte and the simultaneous determination of many different substances.

6.1. Factors affecting HPLC analysis efficiency

Because there is an extensive range of parameters that can be modified during HPLC, the method of determining pharmaceuticals by chromatography is constantly developing. Moreover, new analysis models allow for more accurate and extensive testing of environmental samples, including wastewater and sewage sludge samples. The SPE, parameters such as the pH of the sample, the addition of buffer, the selection of the appropriate eluent (or several eluents), and the load volume are important during the HPLC process. In addition, the type of chromatographic column used, the flow rate of the analyte through the column, and the injection volume, temperature, and pressure are also important.

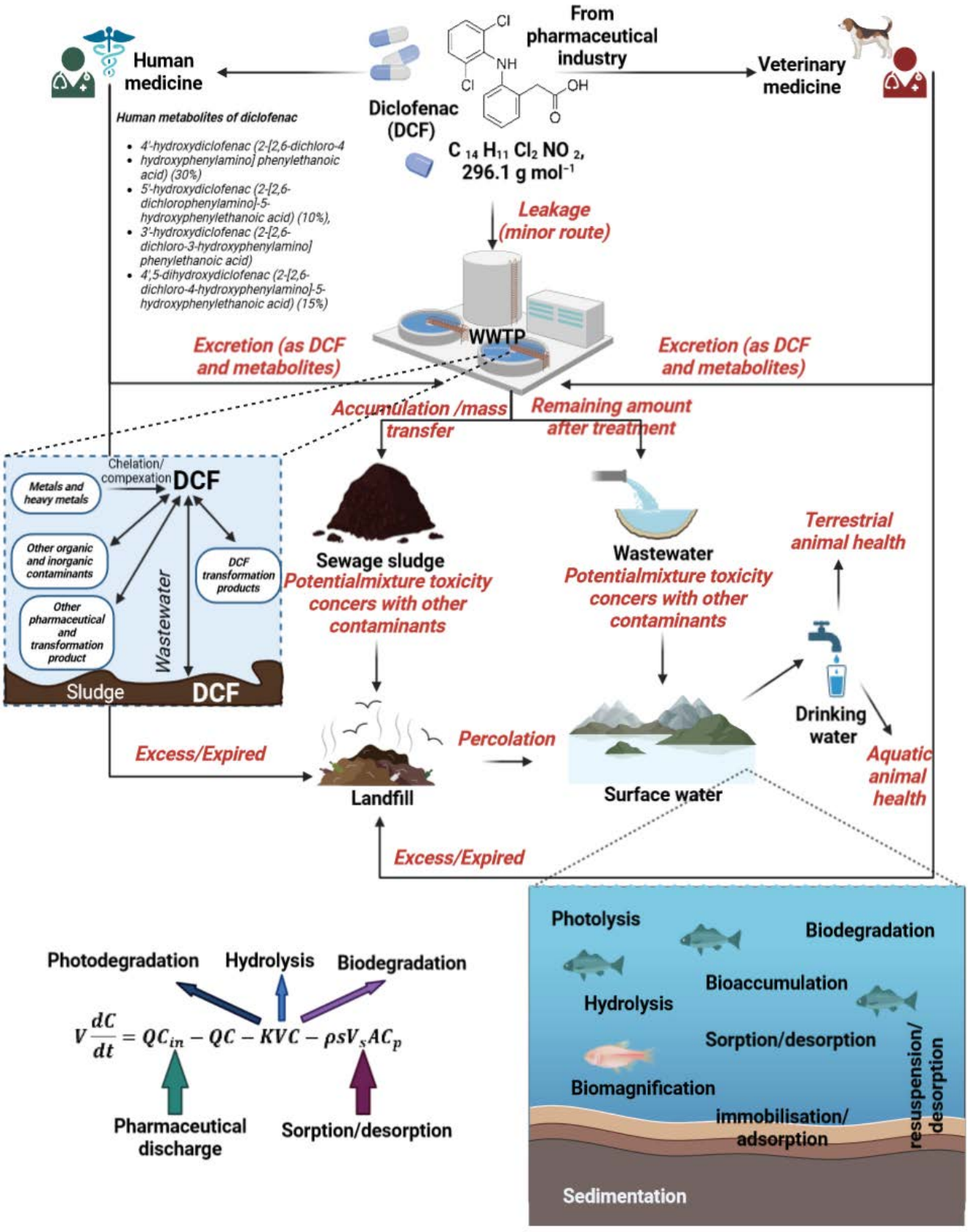

Fig. 7. Fate of diclofenac in the environment [61,80,81].

Table 2
Use of HPLC to determine selected groups of pharmaceuticals

Sample type	Analysis	Analyte	Detected concentration (ng/L or ng/g for solid samples)	References
		Steroid hormones		
Treated wastewater	SPE-HPLC-ESI-MS/MS	Estrone, 17β-estradiol, 17α-ethinylestradiol, testosterone, progesterone, 17α-hydroxyprogesterone, medroxyprogesterone acetate	39, <LOQ, 15, 2, 10, <LOD, <LOD	[93]
Influent and treated wastewater	SPE-HPLC-MS/MS	Androstenedione, androsterone, testosterone, estrone, 17β-estradiol, 17α-hydroxyprogesterone, megestrol acetate, progesterone, hydroxypregnenolone, pregnanediol	38–2.2 × 10^2, 1.5 × 10^2–1.1 × 10^3, 11–26, 2.3–37, N.D.-9, N.D.-66, N.D.-5, 2–22, 51–2.6 × 10^2, N.D.-4.0 × 10^2	[94]
Influent and treated wastewater	Dispersive liquid–liquid microextraction-solid floating organic drop (DLLME-SFO)-HPLC/PDA	Progesterone	N.D.-16.687 ± 6.233	[95]
Wastewater influent	SPE-LC-ESI-MS/MS	10 anabolic-androgenic steroids and 14 endogenous hormones include: 5α-dihydrotestosterone, androstenedione, dehydroepiandrosterone, epitestosterone, estriol, estrone, progesterone, testosterone, β-estradiol	59–180, <LOQ–204, <LOQ-139, 15–39, <LOQ-611, <LOQ-190, 2–63, <LOQ-41, <LOQ-37	[96]
Treated wastewater	Immunoaffinity extraction-HPLC-ESI-MS	17α-Estradiol, estrone, 17β-ethinylestradiol	0.77–6.44, 1.61–17.6, N.D.	[97]
Sewage sludge	Microwave-assisted extraction (MAE)-UHPLC-MS/MS	17α-Ethinylestradiol, 17β-estradiol, cortisone, norgestrel	31.5–1.44 × 10^3, N.D., N.D.-17.3 ± 4.27, 430–1.35 × 10^3	[98]
Sewage sludge	Ultrasonic liquid extraction (ULE)-HPLC-FLD	Estrone, 17α-ethinylestradiol	8–4,800, <LOD	[99]
		Non-steroidal anti-inflammatory drugs (NSAIDs)		
Wastewater	SPE-HPLC-PDA	Paracetamol, ketoprofen, naproxen, diclofenac, ibuprofen	N.D.-224.11, N.D.-2,705.1, N.D.-1,743.4, N.D.-1,6967.8, N.D.-2,436.7	[100]
Treated wastewater	SPE-HPLC-UV-Vis	Paracetamol, diclofenac sodium, ibuprofen, indomethacin	2.8 × 10^6–3.9 × 10^6, 3.1 × 10^6–3.4 × 10^6, 1.25 × 10^6–2.9 × 10^6, 1.4 × 10^6–1.5 × 10^6	[101]
Treated wastewater	SPE-HPLC-CCD	Diclofenac, naproxen, ibuprofen	N.D., 9.67 × 10^3–1.243 × 10^5, N.D.	[102]
Sewage sludge	Matrix solid-phase dispersion (MSPD)-LC-QTOF-MS	Valdecoxib, etoricoxib, parecoxib, celecoxib, 2,5-dimethylcelecoxib	N.D., 1.9–14.7, N.D., 6.5–21.6, N.D.	[103]
		Antibiotics		
Wastewater	HPLC-DAD	Amoxicillin, doxycycline	6.24 × 10^5–7.11 × 10^5, 9.07 × 10^5–1.064 × 10^6	[104]
Treated wastewater	SPE-HPLC-UV	Amoxicillin, azithromycin	6.9 × 10^4–8.47 × 10^5, 1.17 × 10^5–4 × 10^5	[105]
Influent and treated wastewater	SPE-HPLC-FLD	Ciprofloxacin, difloxacin, enrofloxacin, fleroxacin, gatifloxacin, lomefloxacin, moxifloxacin, norfloxacin, ofloxacin, orbifloxacin, sarafloxacin	12.2–1,292, <MDL-20.6, <MDL-15.6, 8.5–506, <MDL, <MDL, <MQL-81.5, <MDL-86.8, 19.4–569, <MDL, <MDL	[106]

Table 2 (Continued)

Table 2

Sample type	Analysis	Analyte	Detected concentration (ng/L or ng/g for solid samples)	References
		Antibiotics		
Treated influent and sewage sludge	Online SPE-LC-MS/MS	Ethionamide, metronidazole, trimethoprim, norfloxacin, ofloxacin, ciprofloxacin, albendazole, sulfamethoxazole, clindamycin, erythromycin, azithromycin, clarithromycin, roxithromycin	Max. = 194.7, Max. = 164,264.4, Max. = 8,815.2, Max. = 261.6, Max. = 9,662.412, Max. = 501,575.5, Max. = 763,610.9, Max. = 9,054.4, Max. = 87.1, Max. = 201.9, Max. = 527.7, Max. = 1,822.4, Max. = 6,012.8	[107]
		Cytostatics		
Influent and treated wastewater	SPE-UHPLC-MS/MS	Etoposide, cyclophosphamide, vinblastine, vincristine	375.8–5,141, 55.94–1,218, 1,835, 1,851	[108]
Influent and treated wastewater	SPE-HPLC-QqQ-MS (triple quadrupole-mass spectrometry)	Cytarabine, cyclophosphamide, docetaxel, doxorubicin, epirubicin, etoposide, 5-fluorouracil, gemcitabine, ifosfamide, irinotecan, methotrexate, mitomycin C, paclitaxel, vinorelbine	9.2–14, <MDL, <MDL, 4.5, <MDL, 3.4–15, <MDL, 7–9.3, 1.2–3.5, <MDL, <MDL, <MDL, <MDL, 9.1	[109]

LOQ: limit of quantification; LOD: limit of detection; MDL: method detection limit; N.D.: non-detect.

Moreover, depending on the selected detector, relevant parameters should be considered, for example, wavelength for UV detectors. Fig. 8 presents a diagram of the determination of pharmaceuticals in wastewater and sewage sludge along with parameters that may change during the analysis.

6.1.1. pH of the sample

In the case of analysing samples from WWTPs containing many compounds, there is no ideal pH for all compounds, therefore this parameter should be selected experimentally, considering what pharmaceuticals are analysed. Choosing the right pH will allow you to get symmetrical and sharp peaks during the analysis. Sharp and symmetrical peaks are essential for quantitative analysis. The presence of such peaks allows to obtain low limit of detection (LOD) and repeatable retention times (RT). During SPE optimization, the best results were obtained for samples with pH 5, for which the highest recovery was obtained for most of the analytes [86,129,130]. In the case of NSAIDs, which are acidic in nature, it was confirmed that the extraction value increases with decreasing pH. In the case of steroid hormones, the change in the pH parameter had no effect on the extraction efficiency [112]. The pH range of the mobile phases, that is, the eluent, which can be used in chromatography is strictly defined by the bed matrix of a given column. For silica gel columns, this range is in the range of 2–8 pH units, while for columns with a polymer base it extends to pH 10 or more. This parameter is crucial for separation efficiency. The pH of the eluent affects not only the form of the analyte itself but also the ionization of the stationary phases.

6.1.2. Selection of appropriate eluent

It is extremely important, both for the SPE stage and for the proper HPLC analysis, to choose the right mobile phase. The most used phases are a mixture of acetonitrile (ACN) and methanol (MeOH), ultrapure water, and formic acid, acetic acid, ammonium hydroxide, ammonium formate and/or ammonium acetate to adjust the pH of the aqueous phase. In the case of a mixture of organic phases, a buffer is also added to adjust the pH. It is also important to determine whether the elution will be isocratic or gradient during analysis. In the isocratic elution, the composition of the eluents is constant, while in the gradient, one composition of the mixtures changes during the analysis. For example, during the optimization of the HPLC process for pharmaceuticals, the best recovery effects were obtained using a mixture of the organic phase – MeOH with the addition of 2% formic acid in water (80/20, v/v) [14,86,130,131].

6.1.3. Load volume of the sample solution

Appropriate selection of the volume of the sample solution transferred to the SPE column allows for the best recovery results. The volume used must be appropriate so as not to overload the SPE column. Too much sample solution volume can also cause the sorbent to exceed the breakthrough volume [86,106,132].

Table 3
Examples of determination of pharmaceuticals from samples from WWTPs

Sample type	Analyte	Analysis	Detector	References
Wastewater effluent	43 compounds, including human prescription pharmaceuticals, veterinary drugs, and hormones	UHPLC	MS/MS	[54]
Influent and effluent wastewater	Amoxicillin, acetylsalicylic acid, cloxacillin, metronidazole, doxycycline hyclate, norfloxacin, trimethoprim, albendazole, ciprofloxacin, caffeine, theophylline, metformin, chloroquine	HPLC	DAD	[86]
Influent and effluent wastewater	Carbamazepine, gemfibrozil, clofibric acid, fenofibrate, fenoprofen, ibuprofen, naproxen, ketoprofen, and diclofenac	HPLC	UV/FLD	[110]
Tap water and drinking water	Naproxen, ketoprofen, diclofenac, ibuprofen, antipyrine, isopropylantipyrine, carbamazepine, clofibric acid, paracetamol, aminopyrine, sulfamethoxazole, erythromycin, bezafibrate, 17β-estradiol	HPLC	MS/MS	[111]
Influent wastewater	NSAIDs (ketoprofen, naproxen, ibuprofen, and diclofenac) and three estrogens (17ß-estradiol, 17α-ethynylestradiol, and estriol)	HPLC	UV	[112]
Wastewater and surface water	Diclofenac, sulfamethoxazole, acetaminophen, carbamazepine, and gemfibrozil	HPLC	PDA	[113]
Influent and effluent wastewater	Acetaminophen, azithromycin, carbamazepine, ciprofloxacin, clarithromycin, clindamycin, cloxacillin, diclofenac, doxycycline, erythromycin, irbesartan, losartan, metronidazole, naproxen, norfloxacin, sulfamethoxazole, tetracycline, trimethoprim, venlafaxine	LC	MS/MS	[114]
Wastewater	Carbamazepine, ciprofloxacin, ceftiofur, diclofenac, erythromycin, lincomycin, ofloxacin, pyrimethamine, spiramycin, sulfamethoxazole, sulfapyridine, testosterone, trimethoprim, and thiamphenicol	HPLC	MS/MS	[115]
Wastewater	NSAIDs, hormones, and triclosan	HPLC	DAD/FLD	[116]
Wastewater	39 drugs and metabolites, including eight glucuronide conjugates	HPLC	MS/MS	[117]
Influent and effluent wastewater	142 anthropogenic compounds of emerging concern (CECs) include steroid hormones, antibiotics, NSAIDs, diabetes	UHPLC	MS/MS	[118]
Influent and treated wastewater	Steroid hormones, hormone conjugates, oral contraceptives, and macrolide antibiotics	HPLC	MS/MS	[119]
Sewage sludge	Clofibric acid, diclofenac, oxytetracycline	HPLC	UV	[120]
Sewage sludge	Sixty pharmaceuticals and personal care products	UHPLC	MS/MS	[27]
Sewage sludge and sediment from marine outfalls	5-fluorouracil, gemcitabine, methotrexate, vincristine, and vinblastine	UHPLC	MS/MS	[121]
Sewage sludge	β-blockers, carbamazepine, and its metabolite, natural and synthetic estrogens, a progestogen, parabens, bisphenol A, three antiseptics triclosan, triclocarban, 2-phenylphenol	UHPLC	MS/MS	[122]
Sewage sludge and biochar	Diclofenac, phenazone, ibuprofen, carbamazepine, sulfamethoxazole, clarithromycin, roxithromycin, erythromycin, bezafibrate, fenofibrate acid, metoprolol, and propranolol	HPLC	MS/MS	[123]

6.1.4. Addition of the buffer

Adding a buffer to prepare the sample solution can have a positive effect on recovery. An example of this would be the addition of ethylene diamine tetraacetic acid (EDTA). In the presence of EDTA, no significant differences were found for most of the pharmaceuticals analyzed in the study. However, for the antibiotics: doxycycline hyclate and metronidazole a statistically significant better recovery was obtained. Adding EDTA to the solution prevents metal ions from interfering with the extraction of these compounds [86,106]. In the study of fluoxetine and norfluoxetine, it was shown that the addition of buffer does not affect the result, but its addition allows to reduce the run time [133].

6.1.5. Type of chromatographic columns

Reverse-phase HPLC (RP-HPLC) is the most used mode of chromatographic analysis – it is used in more than 90% of all analyses. When selecting the appropriate analytical column, which is the "heart" of HPLC analysis, attention should be paid to many factors. These include:

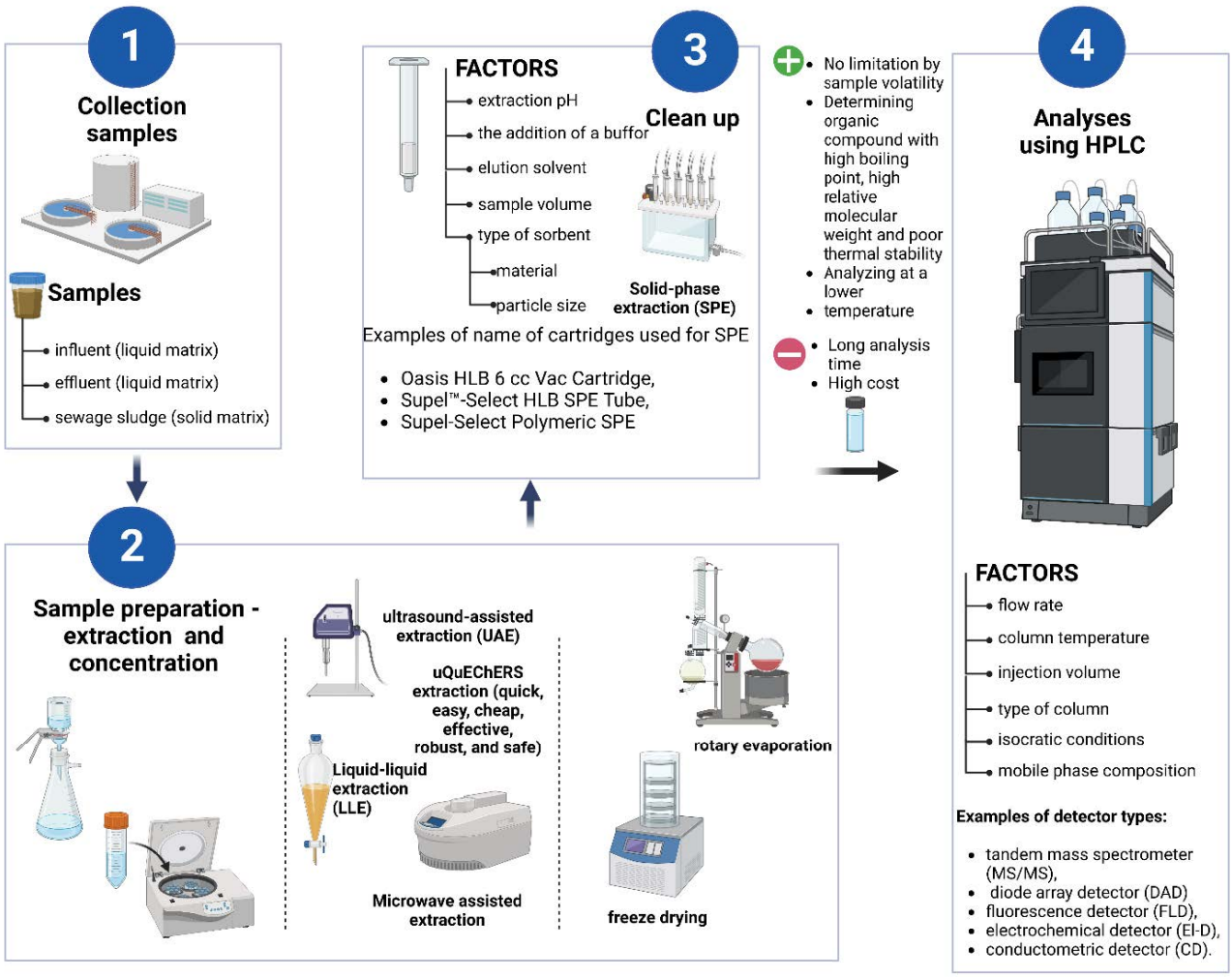

Fig. 8. Scheme of determination of pharmaceuticals form samples collected from WWTPs using high-performance liquid chromatography [10,124–128].

- chemical structure,
- physicochemical properties, for example, type of medium (monolithic, porous, or non-porous), geometry (surface area, pore diameter, volume, particle size, and shape),
- chemical properties of the bed (type of ligands attached and their density),
- composition stationary phase carrier (silica, polymers, or carbon),
- column length.

For the determination of pharmaceuticals in environmental samples, including WWTPs, C18 columns are most often used – these are octadecyl-bonded silica columns [13,134].

6.1.6. Injection volume

The injection volume of the sample on the chromatographic column is an essential element in performing the analysis. Firstly, changing the injection volume taken from the same sample creates opportunities to prepare a calibration curve. On the other hand, the injection volume should be adjusted to find the correct balance between chromatographic efficiency and analytical sensitivity. Usually, the injection volume does not exceed 10% of the effective volume of the column. An interesting voice in the discussion on the role of the described factor in the chromatographic analysis is the work by Werres et al. [135]. Based on calculated minimum plate heights about the retention factor (in isocratic elution) or peak capacity (in gradient elution), these authors determined the influence of injection volume on the efficiency of the analysis. The study showed that for metronidazole and acetylsalicylic acid at k = 2.2, the loss in efficiency due to an increase in injection volume is most pronounced. Furthermore, the authors suggest that for isocratic elution only at retention times more significant than eight, the injection bandwidth is sufficient to increase the injection volume. However, too significant an increase in retention time is disadvantageous due to an increase in peak variance.

The authors also showed that, to a lesser extent, the injection volume influences the analyses conducted in

gradient elution. For carbamazepine, for example, only a slight decrease in peak capacity was observed, even with an injection volume equal to 160% of the effective column volume. In contrast, sotalol and metronidazole showed a constant drop in peak capacity if the injection volume was higher than 1,000 nL.

Table 4 contains a list of exemplary factors influencing the effectiveness of the assay. It includes the type of sample, the type of detector, the extraction method used, as well as the types of columns and mobile phase in HPLC. Parameters such as the LOD and the limit of quantification (LOQ) are important in HPLC analyses and indicate the possible application of the method. Another important parameter during HPLC is the recovery (Rec.), which refers to the extraction efficiency of a given analytical method. It is expressed as a percentage of the known amount of analyte that passes through the column. As shown in Table 4, most HPLC analyses use a gradient of eluents. The mobile phase consists mainly of organic solvents, the most commonly used is ACN.

6.2. Advantages and disadvantages of HPLC in pharmaceuticals determination

When performing HPLC using environmental samples, as well as wastewater and sewage sludge samples, is a matrix effect. Extracts from such matrices are rich in various components isolated together with analytes and may interfere with the course of quantification. The variability of the composition of the samples often causes differences in the results of the determinations. Matrix effects, affecting the results of quantitative analyses, are commonly associated with the liquid chromatography-mass spectrometry (LC-MS) technique. The mechanism of influence of the matrix composition on the obtained results, in this case, is the enhancement or suppression of the ionization of the analytes, caused by the presence of other sample components in the ion source of the mass spectrometer. Research shows that the size of the matrix effect depends, among others, on the method of preparing the sample for analysis, the type and concentration of the analyte, as well as the parameters of the detector. The effects of matrix components can be reduced by improving sample purification or optimizing chromatographic conditions. When developing a method for the determination of pharmaceuticals in wastewater and sewage sludge samples, the matrix effect must be considered. To minimize it, external calibration, internal standards, matrix-matched extract calibration, standard addition before HPLC-MS/MS, and standard addition over the whole procedure can be used [123,131,140]. In one of the studies dealing with experimental modeling of HPLC-FLD, to consider the matrix effect, standard curves were made on river water. In addition, a river water chromatogram was used as an internal control [141]. Another problem with HPLC for the determination of pharmaceuticals in wastewater and sewage sludge is the lack of a universal assay method. The development of new methods of HPLC analysis for environmental matrices is difficult due to the wide variety of these matrices. In addition, the "ideal method" should allow us to detect many pharmaceuticals and their transformation products during one analysis. when creating a new HPLC method, factors related to the determined pharmaceuticals should also be considered: the chemical structure of the drug and its physicochemical properties, that is, solubility, polarity, and pH of the drug molecule. In multi-analyses, where many pharmaceuticals are determined simultaneously, the process conditions should be adjusted to all analyzed compounds. It is also necessary to determine the LOD and LOQ for each of the analyzed compounds.

Despite some limitations, HPLC is the most widely used method for the determination of pharmaceuticals in environmental samples, including wastewater and sewage sludge. This is because HPLC has a lot of advantages. HPLC is economical and uses a minimal amount of organic solvent. The analyses are characterized by high precision and accuracy [142]. HPLC analyses are short and, due to high automation, easy to perform. In addition, the advantage of HPLC is the wide possibility of modifying the process by changing one of the parameters. Currently, there are many modern chromatography columns on the market, characterized by various properties. The most important advantage of HPLC is the high efficiency of the process. Moreover, many methods are used to optimize and increase HPLC efficiency. Chemometrics can be used in modelling the HPLC process which allows for the preparation of the appropriate experimental design and maximizes the detection, estimation, and control of all sources of variation [143]. An example is the use of Box–Behnken design to develop a UV/Vis spectrophotometry and HPLC method for febuxostat quantification, also in wastewater [142].

7. Summary and conclusions

Pharmaceuticals from many different groups are found in the environment. The constantly expanding pharmaceutical market poses a threat to the environment. WWTPs are the largest source of environmental pollution with pharmaceutical substances. The inflow of pharmaceutical substances, as well as new pharmaceutical substances to wastewater treatment plants, is increasing. Currently, the adaptation of the technological processes used in WWTPs to remove drug particles is quite low. One of the future challenges to WWTPs is the treatment method optimization in the removal of many different pharmaceuticals in one process. Thanks to the effective analysis of this problem, it is possible to apply new solutions to remove pharmaceuticals from wastewater. HPLC is the basic tool for pharmaceutical presence in environmental samples determination and is widely used. Such a wide application of HPLC in the analysis of pharmaceuticals in wastewater and sewage sludge is due to the many advantages of this method. The first is that many different pharmaceuticals can be measured in a single HPLC analysis, and the analysis is very efficient. Thanks to this, despite the costs associated with the purchase of appropriate reagents or chromatographic columns, the determination of pharmaceuticals using HPLC is economical. The second advantage of HPLC is the high sensitivity of the analysis, accuracy, and repeatability. In addition, small amounts of sample are required for HPLC analysis, which is extremely helpful when designing the experiment. Modification of parameters, that is, type of column, eluent, flow rate, allows for optimization of the process and accurate determinations. Although pharmaceuticals are the

Table 4
Examples of selected factors affecting HPLC analysis efficiency

Matrix	Pharmaceutics	Extraction, analysis	Sorbent type	Method parameters	Recovery concentration	References
Wastewater	Three penicillin's (amoxicillin, ampicillin, penicillin G), two cephalosporins (ceftazidime, ceftriaxone), and two tetracyclines (tetracycline, doxycycline)	SPE-HPLC-DAD-ES-I(+)-MS	Oasis HLB	Column: C18 (3 μm, 100 × 3 mm), MP: gradient elution: formic acid and MeCN	LOD = 0.07–0.92 μg/mL, LOQ = 0.21–2.77 μg/mL	[136]
Wastewater	Macrolides, quinolones, quinoxaline-dioxides, sulfonamides, tetracyclines	SPE-HPLC-DAD	Oasis HLB	Column: (5 μm, 125 × 4 mm), MP: gradient (0.01 M oxalic acid and ACN), detection: 280 nm	Rec = 68.3% to 97.9%	[137]
WWTP influents and effluents	Fluoroquinolones, macrolides, sulphonamides and DHFR inhibitors, steroid hormones, hormone conjugates	SPE-HPLC-MS (APCI SRM)	DVB-phobic speed disk cartridges	Column: RP18 (150 × 2 mm, 3 mm), MP: gradient (aqueous ammonium acetate, ACN)	Rec = 76% to 103%, LOQ = 2–6 ng/L	[98]
Wastewater	Diclofenac, ibuprofen, paracetamol, indomethacin	SPE-HPLC-UV	Oasis HLB	Column: BDS Hypersil Cyano column (250 × 4.6 mm, 5 μm), MP: gradient (phosphate buffer pH 3.0, ACN), detection: 220 nm	LOD = 6.38 ng/mL – paracetamol, 1.51 ng/mL – diclofenac, 1.18 ng/mL – ibuprofen, 2.29 ng/mL – indomethacin LOQ = 19.3 ng/mL – paracetamol, 4.57 ng/mL – diclofenac, 3.58 ng/mL – ibuprofen, 6.94 ng/mL – indomethacin	[80]
Wastewater	Paracetamol, metamizole, salicylic acid, aspirin, ibuprofen, ketoprofen, diclofenac, naproxen, dexamethasone, prednisolone, carvedilol, metoprolol, propranolol, sotalol, carbamazepine	SPE-HPLC-DAD	Nexux, bond elute ENV, oasis HLB	Columns: LiChroCart Purospher®Star C18 (250 × 3 mm, 5 μm particle size), TSK-GEL ODS (150 × 4.6 mm, 5 μm), Chromolith®RP-18e (100 × 4.6 mm, monolithic), Develosil®RPAQUEOUS-AR-5 C30 (250 × 4.6 mm, 5.8 μm), LiChrosorb RP-8 (250 × 4 mm, 7 μm); MP: gradient (0.1% formic acid in water, MeOH, ACN), detection: 200–450 nm	LOD = 0.007–0.183 μg/L, LOQ = 0.013–0.548 μg/L	[138]

Wastewater influent and treated effluent	Carbamazepine, clofibric acid, diclofenac, fenofibrate, fenoprofen, gemfibrozil, ibuprofen, ketoprofen, naproxen, (17β-estradiol, 17α-ethinylestradiol and estrone	SPE-HPLC-UV-FVD	Si-C18 and Strata X	Column: Alltima C18 (250 × 4.6 mm, 5 μm), MP: gradient (ACN, water acidified at pH 3.6 with glacial acetic acid), detection: 230 nm	LOQ = 30–1,100 ng/L (influent), 10–850 ng/L (effluent)	[110]
Wastewater influent and effluent	Metronidazole, doxycycline hyclate, norfloxacin, trimethoprim, albendazole, ciprofloxacin, caffeine, theophylline, metformin, chloroquine	SPE-HPLC-DAD	HLB, MAX and MCX	Column: Kromasil C18, (4.6 × 150 mm, 5 μm), MP: gradient (water containing 0.1% formic acid, v/v, MeOH, ACN), detection: 250 nm	LOD = 0.1–0.8 μg/L, LOQ = 0.3–2.6 μg/L	[86]
Wastewater influent and effluent	Trimethoprim, clarithromycin, ciprofloxacin, triclosan, paracetamol, ibuprofen, diclofenac, fluoxetine, carbamazepine, c-10–11-epoxide, propranolol, atorvastatin, metformin, estrone, 17α-ethynylestradiol, estriol	SPE-HPLC-MS/MS	Oasis HLB prime	PI modes: Column: Waters XBridge BEH C18 column (2.1 × 100 mm, 2.5 μm), MP: gradient (0.1% formic acid and ACN) NI modes: Column: Phenomenex Kinetex EVO C18 (3.0 mm, 100 mm, 2.6 μm), MP: gradient: (0.025% ammonium hydroxide and ACN)	PI modes: LOD = 0.039–0.213 μg/L NI modes: LOD = 0.272–2.177 μg/L	[139]
Primary sludge, secondary sludge, anaerobic digested sludge, and dehydrated sludge samples	estrone and 17α-ethinylestradiol	ULE-HPLC-FD	–	Column: ACE®C18 (150 mm × 4.6 mm × 5 μm), MP: isocratic (ultrapure water, ACN)	LOD = 0.305 ± 0.003 μg/g – estrone and 0.0516 ± 0.0006 μg/g 17α-ethinylestradiol	[99]
Sewage sludge	Diclofenac, phenazone, ibuprofen, carbamazepine, sulfamethoxazole, clarithromycin, roxithromycin, erythromycin, bezafibrate, fenofibric acid, metoprolol, and propranolol	PLE-HPLC-MS/MS	–	Column: Atlantis T3 (100 × 2.1 mm, 3 μm), MP: gradient (ACN, MeOH containing 0.1% formic acid)	LOD = 0.4–20 ng/g, LOQ = 1.2–68 ng/g	[123]

SPE: Solid-phase extraction; LOD: limit of detection, LOQ: limit of quantification; ACN: acetonitrile, MCX: mixed mode cation exchange; MS: mass spectrometry; MeOH: methanol; MP: mobile phase; MDL: method detection limit; FD: fluorescence detection; MPC: mixed-phase cation exchange cartridges; PI: positive ionization, NI: negative ionization.

subject of ongoing research, and the techniques for their analysis are constantly being improved, further research is needed due to their nature and the dynamics of change. One of the most used solutions is the SPE with the Oasis HLB sorbent because these filling the columns can extract a wide range of polar analytes over a wide pH range.

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