

Postprint of: Dogan A., Płotka-Wasyłka J., Kempieńska-Kupczyk D., Namieśnik J., Kot-Wasik A. Detection, Identification and Determination of Chiral Pharmaceutical Residues in Wastewater Problems and Challenges Trends in Analytical Chemistry 122 (2020), 115710 DOI: [10.1016/j.trac.2019.115710](https://doi.org/10.1016/j.trac.2019.115710)

© 2019. This manuscript version is made available under the CC-BY-NC-ND 4.0 license

<http://creativecommons.org/licenses/by-nc-nd/4.0>

Detection, Identification and Determination of Chiral Pharmaceutical Residues in Wastewater: Problems and Challenges

Aysegul Dogan^{1,2}, Justyna Płotka-Wasyłka ^{2,a}, Dagmara Kempieńska-Kupczyk², Jacek Namieśnik², Agata Kot-Wasik²

¹ Department of Analytical Chemistry, Faculty of Pharmacy, Hacettepe University, 06100, Sıhhiye, Ankara, Turkey.

² Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, 11/12 G. Narutowicza Street, 80-233 Gdańsk, Poland

^aCorresponding author email: juswasyl@pg.edu.pl

Highlights

- Chiral drugs as wastewater-based epidemiology biomarkers
- Environmental fate of chiral pharmaceuticals
- Problems and challenges connected with determination of chiral drugs in wastewater
- Future trends in chiral pharmaceutical analysis

Abstract

Chiral pharmaceuticals (CPs) are widely used in different areas of human life, thus they are frequently detected in different ecosystems. However, before CPs reach the environment, wastewater is subjected to different treatment processes in order to remove them. Nevertheless, such processes may affect the chirality of CPs, thus it is very important to monitor CP levels during the wastewater treatment. This review addresses the present state of knowledge concerning the input, occurrence, fate and effects of CPs in the environment. It focuses primarily on wastewater analysis, problems and challenges connected with trace levels of CP enantiomers and highly complex matrices of samples. Analytical approaches used in detection, identification and determination of enantiomers are presented. The application of the results of wastewater analysis to obtain information on the population's health and behaviour has been included and discussed. Moreover, the prospects of the future trends in green enantiomeric analysis are described.

Keywords

Chiral pharmaceuticals, enantiomers, wastewater, wastewater-based epidemiology, chiral analysis, environmental fate, enantiomeric fraction

Abbreviations

ADME, Absorption, distribution, metabolism and excretion; CBH, Cellobiohydrolase; CPs, Chiral pharmaceuticals; EF, Enantiomeric fraction; FDA, the American Food and Drug Administration; GC, Gas chromatography; GAC, Green Analytical Chemistry; GAPI, Green Analytical Procedure Index; K_{ow} , water/octanol partition coefficient; K_{sw} , sediment/water partition coefficient; K_{ws} , water/sediment partition coefficient; LC, Liquid chromatography; MS, Mass spectrometry; MS/MS, tandem mass spectrometry; NSAIDs, Nonsteroidal antiinflammatory drugs; SPE, Solid phase extraction; U.S. EPA, United States Environmental Protection Agency; WBE, wastewater-based epidemiology; WHO, World Health Organization; WTP, Water treatment plant; WWTP, Wastewater treatment plant

Content:

Introduction	2
Sources and Environmental fate of chiral pharmaceuticals	6
Problems and challenges connected with determination of pharmaceuticals in the wastewater	12
Analytical performance used for the determination of chiral pharmaceuticals in wastewat	13
Conclusions and future trends	31
Acknowledgement	34
References	34

1. Introduction

Pharmaceuticals are compounds that have been used in increasing amounts for many years either to prevent or to treat diseases. Drug usage is constantly increasing due to changes and innovations in medical treatment methods, as well as the increase in diseases caused by environmental conditions, consumption, stress and increasing elderly population.

Drugs are subjected to several processes after intake entering the body, such as absorption, distribution, metabolism and excretion, as stated in ADME parameters in pharmacokinetics of drugs [1]. After drugs are metabolised, the remaining amount is excreted either unchanged, as a parent compound, or changed, as its metabolites, into the wastewater [2]. Considering billions of people living in the world, the number of drugs they consume, and the waste from the pharmaceutical industry which continues to develop at a rapid pace, it is not surprising that the amounts of drugs reach levels significant for the environment, as well as for human and animal health. Although the effects caused by drug residues in wastewater are not known exactly, there are increasing difficulties in the treatment of diseases that develop in humans and animals, especially due to microorganisms that cause resistance to antibiotics, cancer drugs and many other drug molecules. Antibiotics, an emerging environmental pollutant group, are widely used as human and veterinary drugs to prevent or treat infectious diseases and to promote animal husbandry, aquaculture and

agriculture growth [3]. Municipal wastewater systems are an important route for the disposal of pharmaceuticals and the concentrations measured in the wastewater treatment plant (WWTP) reflect the antibiotic levels in the region. In the composition of the sewage system, the determination of the amount of drug molecules used, paying attention to rational and reasonable use of medicines, is of increasing importance in today's environmental and health policies in the world. With these studies, not only should the drug levels in the wastewater be monitored, but also the frequency and dosage regime of regional drug usage should be determined. In addition, it is thought that significant conclusions about substance addiction will be drawn with the methods that will be developed for the determination of banned drug molecules. Biopharmaceuticals which contain molecules based on proteins and synthesised using biotechnological techniques, such as recombinant human insulin, are thought to be biodegraded or denatured in the environment, because they are almost identical to the natural products [4].

A large percentage of commercial and research phase pharmaceutical compositions are enantiomers, and many of these exhibit significant enantioselective differences in pharmacokinetics and pharmacodynamics. The importance of the chirality of drugs is increasing, and their use as racemates or enantiomers has been discussed in the pharmaceutical literature in recent years. The evidence that stereoselectivity problems have increased in the drugs or drug residues in the environment has made enantioselective analysis with chromatographic methods the focus of research.

Chiral substances have the same structure of the molecule, but are different in the formation of their atoms in space. The word chiral comes from the Greek word $\chi\epsilon\iota\rho$ (hand), thus the chiral molecules are known for being right- and left-handed. The optical isomer pairs in relation to the chiral centre are called enantiomers [5]. The most general definition of enantiomers is that they are substances with identical chemical structures that do not overlap with the mirror image of itself [6]. An atom containing a stereocentre (asymmetric centre) can be a stereoisomer with the change of any two groups around it. The idea of separation of chiral isomers was realised in 1848 (Fig. 1). Chirality plays an important role in life activities since amino acids, enzymes, nucleic acids, fats, carbohydrates, metabolic intermediates, and many other biomolecules are chiral. In addition to the biological systems, due to the different biological properties of enantiomers, chirality has an important place in many areas, such as the pharmaceutical industry, chemical industry, petrochemical industry, agrochemicals and food industry. It is especially important in medical practice. Approximately 56% of the pharmaceuticals currently in use are chiral and 88% of these are administered in racemic proportions [7], while single enantiomer formulations of some marketed medicines exhibited increased potency of one stereoisomer over the other [5]. Although it has the same chemical structure, most enantiomers in racemic drugs have different pharmacokinetic, pharmacodynamic, biological and toxic effects. Experts of the American Food and Drug Administration (FDA) emphasize that the effect of each enantiomer on the body of the racemic drugs should be explained one by one and it is



also underlined that the new chiral compounds should be developed as single enantiomers [8, 9]. Some of the enantiomers forming the active pharmaceutical ingredients (api) may be more effective or different than their isomers. For instance, the S-enantiomer of thalidomide had a teratogenic effect and the R-enantiomer had a sedative effect [10]. In another example, the R-enantiomer of verapamil is used as a multidrug resistance regulator in cancer chemotherapy, while the S-enantiomer is used as a calcium channel blocker. It is also known that the R-enantiomer of verapamil has a cardiotoxic effect [7]. The awareness in such cases led the scientific world to evaluate the drug molecules in terms of chiral molecules and, if possible, to deliver single enantiomer-based products.

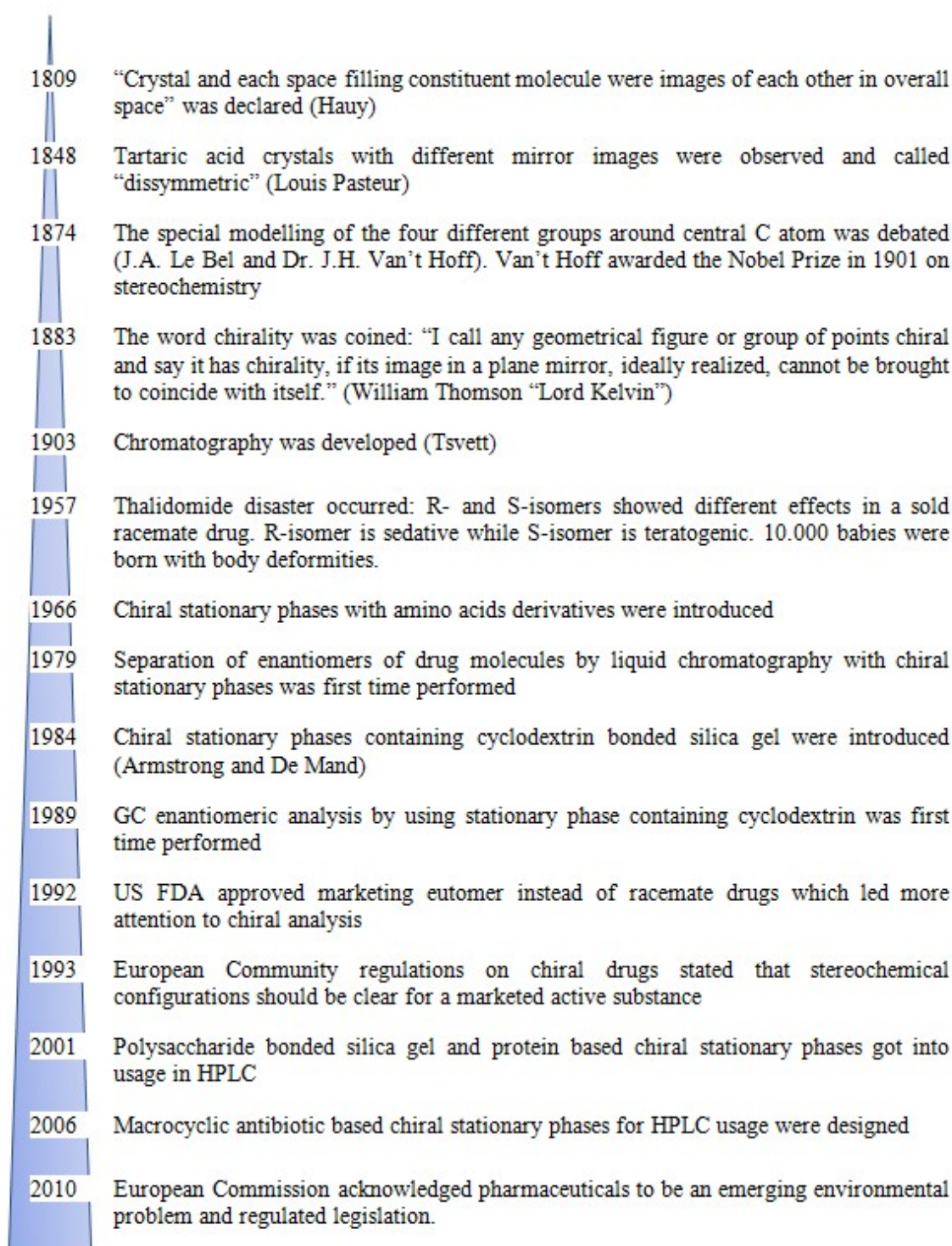


Fig. 1. Chiral analysis milestones [11-16]

The pharmaceuticals can enter wastewater systems either directly from humans or indirectly by rain from animals' excretion of veterinary pharmaceuticals [17]. The natural conditions and seasons are directly

related with the amount discharged [18]. The previous studies have demonstrated that only a part of the pharmaceutical contaminants can be eliminated from water systems worldwide [19-22]. The rest of the drugs stays in the sewage or is transported into the aquatic environment, from where it can get to human beings once more. Even if the studies [23] show that the amounts in drinking water samples did not exceed the relevant limits, their accumulation in humans due to the circulation in water has not been fully explained [24-26]. To indicate the type and the fate of the pharmaceutical pollution, more data should be obtained. Not only the parent compound, but also the metabolites and degradation products should be kept in consideration for screening [27-31]. The observations performed only for the parent molecule would not be enough to define the total impact on the environment [32-34].

Analytical chemistry is involved in the fundamentals of wastewater treatment and several environmental explorations. The findings of wastewater analysis enhanced as the modern hyphenated techniques and chiral column choices developed. The analyses of wastewater in terms of pharmaceuticals, illicit drugs, industrial chemicals, nutrients, and biological markers are gaining attention among the scientific and regulatory societies. In fact, several review papers are focused on CPs and its determination in the environment as well as wastewater [4, 5, 16, 17].

However, they usually refer to a specific and narrow issue. Therefore, in this review, chiral active pharmaceuticals used for wastewater-based epidemiology purposes, methods of their determination and challenges connected with it will be discussed briefly. In addition, future trends in the use of enantioselective analyses in environmental science are presented.

2. Sources and environmental fate of chiral pharmaceuticals

Experts of the World Health Organization (WHO) declare that analytical studies can be used to carry out epidemiological investigations to study determinants [35]. Waste can give a rough idea of the general health of the whole population. Therefore, the newly emerging wastewater-based epidemiology (WBE) approach were introduced. WBE is a non-invasive methodology and can be an effective way to improve public health [36]. WBE provides valuable, objective evidence of the amount and type of foreign chemical (xenobiotic) compounds to which a population is exposed, such as protein biomarkers, toxic substances, metabolites and digested foods [37]. To estimate public health status, WBE requires human- and disease-specific biomarkers. The specified knowledge obtained from the analysis of wastewater will make it possible to define population-based biomarkers [38].

WBE is the fastest way to express the behaviour of a population, because it is not necessary to wait for samples to be gathered for an analysis project. The objective data obtained from wastewater analysis is anonymous; it allows to have information about the general population [39, 40].

The sensitive analysis methods and advanced detector technologies should be used because the amount of the drug or its single enantiomer in the wastewater may be even 1000 times lower than in human body fluids. These trace amounts (ng/l or parts per trillion) could be detected with hyphenated techniques and expert analysis [41, 42]. The application of enantioselective analysis for the purpose of WBE has only been presented in several studies, generally concerning the presence of illicit drugs and their consumption [43]. The researchers focused on fluoxetine, but they concluded that its high levels in wastewater were associated with disposal of unused drugs, not their regular consumption [44].

CPs reach the aquatic environment as a result of human and animal activity. However, before they end up in water ecosystems, wastewater is subjected to various abiotic and biotic treatment/purification processes that are supposed to remove CPs from waste (Fig. 2). Nevertheless, the conventional WWTP is not particularly designed to remove CPs and the efficiency of their removal depends on the type of treatment process [45, 46]. In the beginning, CPs are exposed to abiotic processes, such as adsorption, sedimentation, thermal and photodegradation, which are not expected to be enantioselective, so the enantiomers should be equally removed. During secondary treatment, they are also exposed to microbial degradation that may be enantioselective and/or enantiospecific and therefore may lead to changes in the enantiomeric composition due to enrichment or depletion of one particular enantiomer [46-48]. The parameter that provides information on the composition of enantiomers in a sample is called enantiomeric fraction (EF), and can be defined by the following formula:

$$EF = \frac{S}{S+R} \quad (1)$$

where [S] and [R] are the fractions of S and R-enantiomers respectively. In case of the racemate, the EF value is 0.5, whereas in case of single enantiomers the value of EF is 0 (R-enantiomer) or 1.0 (S-enantiomer). Evaluation of the EF value can be also used as indicator of the effectiveness of the removal processes [46, 48-50]. For instance, it was shown that during wastewater treatment S-ibuprofen was preferentially degraded. The EF value decreased from the range of 0.79- 0.86 in influent to 0.63-0.68 in effluent. Furthermore, due to the similarity of its EF values in surface waters and effluents, EFs of ibuprofen were proposed as markers of discharge of wastewater [51].

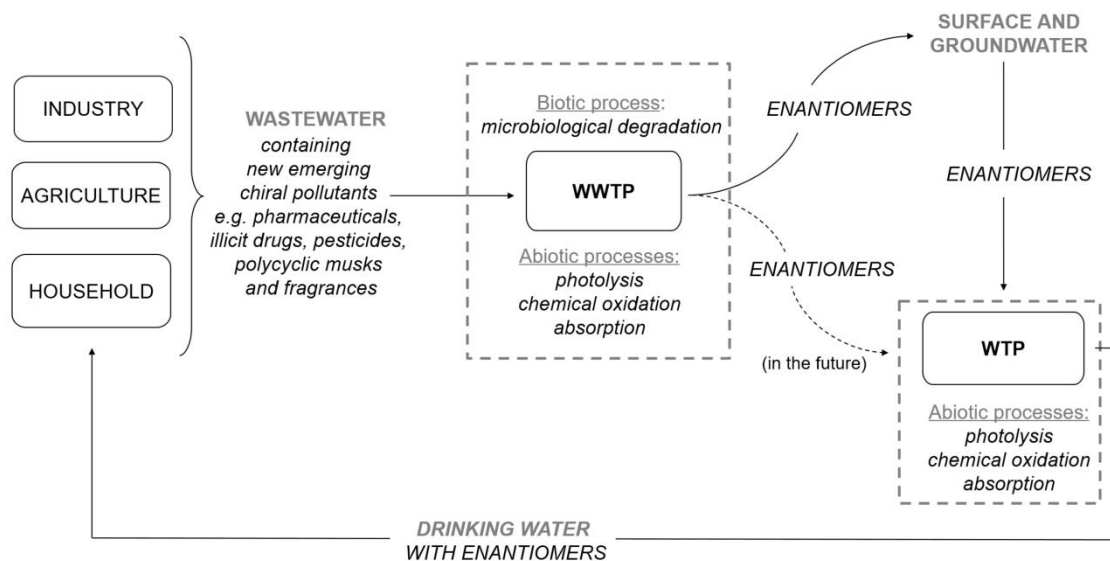


Fig. 2. The pathway of chiral pharmaceuticals from the sources to the environment with indication of purification/treatment processes which may affect their chirality.

Currently, some alternative solutions of elimination of pharmaceuticals are being proposed. They are generally used as tertiary treatment and include wetlands, bioremediation using biomolecules or microorganisms (e.g. fungi, bacteria) or remediation capitalizing on the uptake potential of plants, such as *Echinodorus horemanii* and *Eichornia crassipes* [45]. The obtained results look promising, but there is still little information on enantioselectivity of these treatment processes. However, most of them used living organisms, so there is a big possibility of occurrence of selective removal [52]. Such alternative solutions in treatment processes are also needed due to the search for alternative water resources. The ever-growing water demand leads to changing the idea of wastewater management from ‘purification and discharge’ to ‘recycle, reuse and recovery of resources’. According to the *United Nations World Water Assessment Programme Report 2017: Wastewater. The Untapped Resource* published by UNESCO [53], the use of effluents for ecosystem services may reduce freshwater abstractions and allows fisheries to thrive by recharging depleted aquifers. In addition, effluent can also be considered as a source of

drinking water that could solve the problem of water shortage in some countries. So far, recycling sewage into drinking water has been used in several cities, such as San Diego (USA), Singapore, and most notably Windhoek (Namibia), [53, 54]. CPs may also undergo transformation in water treatment plants (WTPs) [55]. However, there are no data on the enantiomeric profiling of CPs in tap water. Furthermore, the presence of pharmaceuticals in drinking water generates concern about the effectiveness of water treatment techniques. Although their concentration in tap water is low, the effects of long-term exposure at low levels are still unknown [56].

CPs introduced to environmental waters are subjected to different processes, as it is presented in Fig 3. However, only a few studies focus on stereoselective and/or stereospecific fate and effects of CPs on the environment [50, 57]. Stereoselectivity in degradation processes has been reported in several papers [48, 57]. It was noticed that the changes of river flow influence the enrichment of some enantiomers. This variation in degradation was explained by changes in microbial diversity, resulting from modification of water parameters [58]. Furthermore, it was found that S-ibuprofen degraded faster in lake ecosystems, whereas R-ibuprofen undergoes biodegradation more rapidly in rivers. Nevertheless, this observation cannot be taken as a rule because of the complex nature of stereoselectivity, diversity of microorganisms in different ecosystems and different chemical structures of CPs [59].

Most studies on the environmental fate of CPs focused on the degradation of their enantiomers and overlooked the possibility of the change of EF values being caused by chiral inversion. This process is induced by enzymes and leads to the conversion of one enantiomer to its antipode [57, 59]. It was reported that bacteria, such as *Nocardia corallina* or *Nocardia diaphanozonaria*, produce enzymes that invert R-ibuprofen to its S-enantiomer [59]. The role of chiral inversion in the environmental fate of CPs was also confirmed in another study [60]. Some researchers suggest that chiral chemicals can be also stereoselectively adsorbed to soil or 231 sediment, but the mechanism and factors that influence this process are rather unknown and 232 poorly understood [57]. Due to the fact that solid matrices (e.g. organic matter, minerals) are 233 mainly chiral structures, there is a belief that they may serve as a chiral environment in which 234 enantiomers may behave differently. Stereoselectivity in adsorption may also lead to unavailability of one enantiomer for microbial degradation, transport or uptake by non-target organisms [61]. Stereoselective sorption to soil was firstly reported in the case of diastereomers



of synthetic growth hormone called trenbolone [62]. Several years later, it was observed that polar interactions are important in sorption of CPs [57, 63].

Pharmaceuticals also have the potential for bioaccumulation in organisms along the food chain, which causes threat to flora, fauna and human beings [45]. However, there is still a need to investigate individual enantiomers, as they may display differences in bioaccumulation, transport, persistence and toxicity in the environment [57].

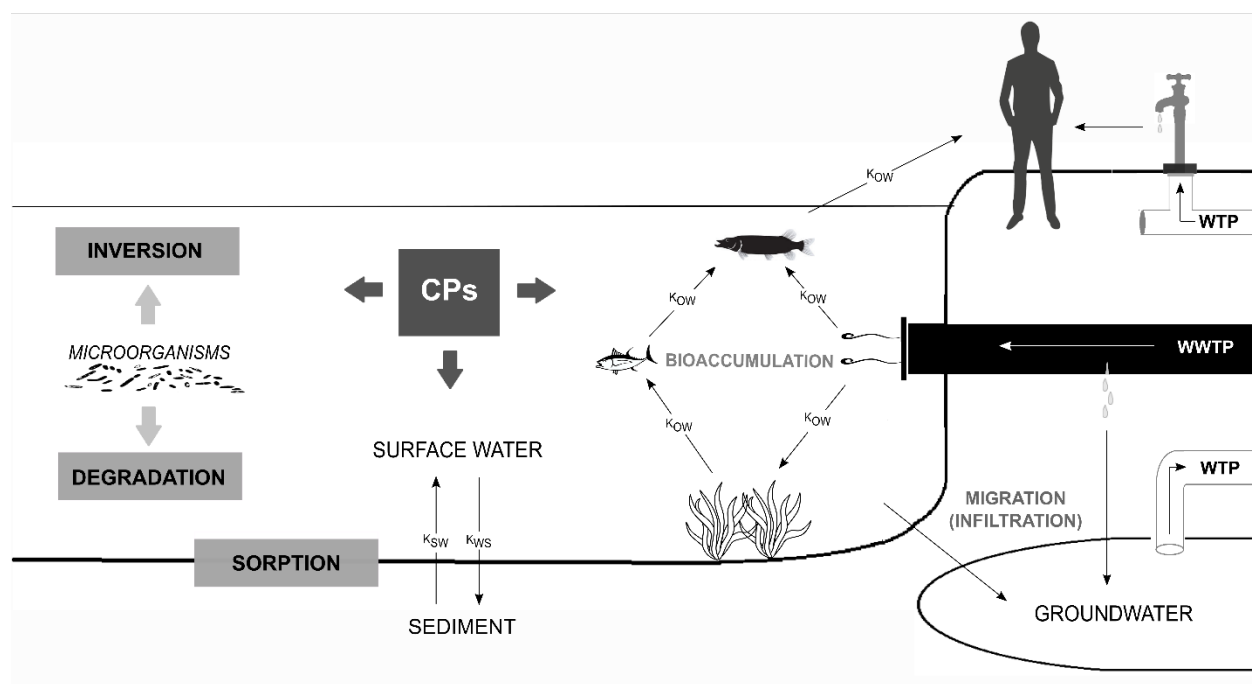


Fig. 3. Environmental fate of chiral pharmaceuticals (CPs). Partition coefficients, such as K_{ow} (octanol/water partition coefficient), K_{sw} (sediment/water partition coefficient) and K_{ws} (water/sediment partition coefficient) represent these contaminants' movement through some environmental compartments.

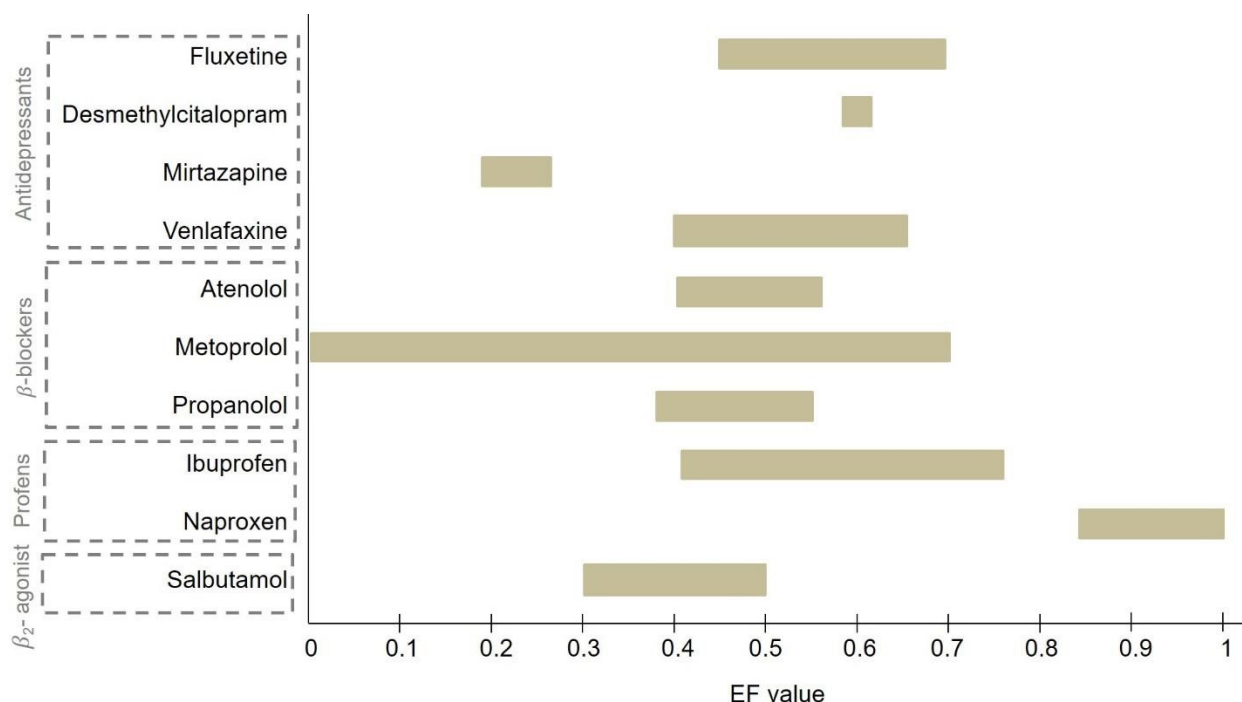


Fig. 4. Range of EF values of chiral pharmaceuticals recorded in the samples of surface water [33, 48, 57, 64].

Due to the processes that are described above, different enrichment of enantiomeric forms of CPs occurs in the environment (Fig. 4). Antidepressants, such as fluoxetine, venlafaxine and desmethylocitalopram (citalopram metabolite) were found to be generally enriched with S-enantiomers, whereas mirtazapine was enriched with R-enantiomer. The enrichment of β blockers and salbutamol with R-enantiomer was also often described. As it was mentioned before, ibuprofen in the environment was mainly enriched with S-enantiomer and naproxen was found to be enriched with R-enantiomer [33, 48, 57, 64]. The main concern is that the different enantiomeric forms of the same pharmaceutical can differently interact with organisms [64]. In order to assess ecotoxic effects of enantiomers, several studies using environmentally relevant species were performed [48, 54]. It was shown that S-fluoxetine and S-atenolol inhibited the growth of *Tetrahymena thermophila* (a freshwater protozoan), whereas R-atenolol increased mortality of one type of algae (*Pseudokirchneriella subcapitata*). Furthermore, S- propranolol and S- fluoxetine revealed higher ecotoxicity towards the fathead minnow (*Pimephales promelas*) [34]. However, it is worth noting that aquatic organisms in natural ecosystems are exposed to mixtures of enantiomeric forms of CPs, therefore the ecotoxicity of such compounds may increase due to synergistical effects [45].

3. Problems and challenges connected with determination of pharmaceuticals in the wastewater

Despite the fact that analytical chemists have been focused on the determination of pharmaceuticals in wastewater for many years, there are still several problems and challenges connected with this issue. The schematic representation of such problems is presented in Fig. 5, while their description is posted further.

- Complex matrix
- Lack of/ low detection and quantification limit
- Fluctuations in the findings, depending on season or population
- Drugs metabolism and degradation products need to be known
- Problems for total concentration
- The source of the suspected drug must be away from free disposal of unused drugs not to give false results
- The increasing use of pharmaceuticals with nano-drug delivery systems in medicine
- Too many analyses need to be performed to generalize the drug usage for a particular season, region and population
- False negative identification because of too low concentrations Challenges
- Low concentrations need to be analysed with good sensitivity detectors by high cost instruments
- False positive results due to improper identification
- Interpretation should be done by a highly qualified person

Fig. 5. Schematic representation of problems and challenges connected with determination of pharmaceuticals in the wastewater

One of the most fundamental problems in the analysis of wastewater samples is the low detection and quantification limit. The pharmaceuticals, either in the form of a parent molecule or its metabolites, are excreted at a much lower level than the intake amount. Mass spectrometers, which usually have better sensitivity than conventional detection systems, and which allow analysis of lower levels of analytes, have a large contribution to this field. Many methodologies have been developed for the analysis of drug molecules in wastewater samples. Most of them were conducted with liquid chromatography coupled with tandem mass spectrometry [19, 23, 28, 65-71] and few of them were performed using GC-MS [20, 28, 72] instruments for drug molecules in wastewater. Trace level drugs, such as illicit drugs and drugs for rare diseases, require more selective and sensitive methods of analysis. Furthermore, the content of pharmaceuticals in the water layer and the residuals in the sludge layer must be taken into consideration to generalize the results for one provenance. Thus, the hydrophilicity and lipophilicity of CPs are of special importance. Analytical results obtained from the analysis of wastewater from the

treatment plants or apparatus that mimic wastewater plants are mainly related with the sample collection step. The better the sample represents the whole, the more reliable the results [73].

4. Analytical performance used for the determination of chiral pharmaceuticals in wastewater

The separation of the enantiomers from each other is very difficult, because they have identical chemical properties. For such a separation, chromatographic methods (GC and LC) based on specially designed columns for chiral analysis are available in the literature, as it is presented in Table 1. HPLC analysis of CPs has been gaining much attention starting from 1970's. Polyacrylamide chiral stationary phases were first used for the separation of thalidomide, chlortalidone, glutemide, hexobarbital, and mandelic acid by Blaschke in 1980. Of the pharmaceuticals studied, thalidomide was found to be the best chromatographically resolved. Different kinds of adsorbents were investigated to obtain the best resolution of the racemates. Not only the type of adsorbent, but also the small changes in synthesis of the same adsorbent affected the resolution. Polyacrylamides, microcrystalline cellulose triacetate, aminoacidic chelates, cellulose and starch, chiral crown ethers, and some other optically active adsorbents were examined for resolution of enantiomers. This paper also focuses on the pharmaceutical toxicology of the enantiomers investigated. The attempt of replacing the toxic solvent, benzene, with toluene, which practically gave the same results, is also emphasised in this review. During chromatographic separation, the separation time between enantiomers was shortened by the raise in the temperature after the complete elution of the first enantiomer [74].

In 1980 Pirkle worked on developing a broad spectrum of ionically bonded chiral stationary phases used for separation of enantiomers by application of HPLC. The need of an adsorbent having three different binding sites for an analyte was met in this new type

of global adsorbent with acidity, hydrogen bond receptor, hydrogen bond donor and steric interactions [75]. The adsorbent was to be the first commercially available chiral column.

After this, plenty of chiral column developments were achieved successfully and they were used on preparative and analytical scale. Proteins [76], polysaccharides [77], cyclodextrins [78], macrocyclic antibiotics [79] and chiral ion exchangers [80, 81] got into use after the synthetic polymer-based chiral stationary phases of Blaschke [74]. The presence of any chiral compound interacting non-covalently with other chiral compounds is sufficient for considering it as a potential chiral selector in liquid chromatography. The chiral columns were mainly developed with a chiral selector and inert carrier adsorbent materials like silica. The most frequently faced problem of the chiral stationary phases was their very low stability across solvents, since the chiral selectors were non-covalently bonded [77].

The commercially available columns put forward the topic of reversible enantiomeric retention mechanisms. Mobile phase type and composition, modifier, and acidic/basic additives play an important role in the retention of enantiomers. It has been difficult to predict or reverse the order of enantiomers with cyclodextrins, macrocyclic antibiotics, peptides, many alkaloids and polysaccharide derivatised columns [82]. The studies demonstrate that the addition of modifier to mobile phases generally reverses the enantiomer retention order. Also, temperature and pH of the mobile phase has an effect on the order [83-85]. The addition of different acidic/basic additives to the mobile phases can reverse the order, especially when analysing acidic analytes. The typical acidic/basic additives can cause a decrease in the sensitivity, which can be especially important in low concentration analytes [86]. All of these attempts to predict and/or change the order of the enantiomers are important for industrial pharmacy, as they “can” lead to the reduction in solvent and time consumption, as well as to achieving an easier way of enantiomeric purification.

Nowadays the modified macrocyclic chiral stationary phases via Edmond degradation of vancomycin was evaluated by researchers. The possibility of using these more stable chiral columns in normal phase, reversed phase, polar organic and polar ionic modes allowed these chiral phases to serve as broad spectrum stationary phases for different kinds of analytes. Ionizable enantiomers can be separated in polar ionic mode using 100% methanol containing trace amounts of acid and base or a nonvolatile salt. A methanol and acetonitrile mixture with the addition of acids and bases can be classified as a polar organic mode which would also give rise to hydrogen bonding. A methanol mixture with an ammonium salt with pH adjustment can be used as a reversed phase for developing ionic interactions. Normal phase is usually usually not needed for enantiomeric separations on macrocyclic glycopeptides [87].

Recently, there are two types of columns used in LC analysis of CPs, Chiral CBH and Chirobiotic columns, which differ in the chiral selectors they have.



(CBH) enzyme which is immobilised on 5µm spherical silica particles. CBH columns are mainly used for enantioselective analysis of basic pharmaceuticals. Ion exchange, hydrophobic and hydrogen bonding are types of the mechanisms for the retention of enantiomers in this column type. Chirobiotic phases are macrocyclic glycoproteins silica-packed in such a way as to analyse components for chiral recognition. They can be applied either in normal phase or reverse phase operations. Chirobiotic V (vancomycin) and Chirobiotic T (Teicoplanin) are two types of chiral columns containing macrocyclic glycopeptides as chiral selector. Chirobiotic V is specialised for neutral molecules, amides, acids, esters and amines, while Chirobiotic T is used for acidic compounds, including carboxylic acids and phenols, small peptides, neutral aromatic analytes, and cyclic aromatic and aliphatic amines, β -blockers and dihydrocoumarins [88]. Based on the comparison of application of chiral columns (Chirobiotic V and Chiral CBH) which has been published by Bagnall et al. [89], it can be concluded that the CBH column is more selective towards certain β -blockers than the Chirobiotic V column. On the other hand, very long retention times (>90 min) for propranolol separated using the CBH column led to the use of the Chirobiotic V column at the enantiomeric level. Moreover, in case of antidepressants, the Chirobiotic V column was observed to be more selective than the CBH column. It was reported by Evans et al. [34] that the solid matrix should be taken into account to calculate the mass balance of CPs in wastewater. CPs were found to be often non-racemic in wastewater matrices. Enantiomeric composition of pharmaceuticals differed in liquid and solid wastewater matrices and it was observed that the biological wastewater treatments were capable of changing the enantiomeric fraction. In 2016, Ma et al.[64] presented in their work the use of the Chirobiotic V column for metoprolol, propranolol, atenolol, fluoxetine, venlafaxine and Chiralpak AD-RH chiral column for NSAIDs, such as ibuprofen, flurbiprofen and naproxen. Naproxen was found, as well as its S-enantiomer, whereas atenolol and flurbiprofen were not detected in the matrix. The stereoselective metabolism enrichment trend in the wastewater samples was also realised by E. Castrignanò et al. [90]. They compared three types of columns specified for enantiomeric separation: CBH, Chirobiotic V and Chirobiotic T. It was reported by H.R. Buser et al. [91] that ibuprofen was present in influents of WWTPs at concentrations of up to 3 µg/L with a high enantiomeric excess of the pharmacologically active S-enantiomer. They found out that the S-enantiomer of ibuprofen was excreted in a much greater concentration than the R-enantiomer, and the S-enantiomer degraded faster in the sewage system and apparently in surface water. They also observed that enantioselective degradation was not changed due to pharmaceutical concentrations in raw sewage, as the two enantiomers recoveries were the same at each stage of the analytical procedure. Similar results were presented by Fono and Sedlak [92], which also agreed that the S enantiomer exhibits higher biodegradability than the R-enantiomer. Matamoros et al. [93] found out that the EF values in raw sewage and effluents of various wastewater treatments depended on the compounds. The low correlation found for ibuprofen was



explained in terms of enantioselective degradation kinetics under prevailing aerobic and anaerobic conditions. They proved that the degradation type, whether it was aerobic or anaerobic, marked the difference in removal capacity. S-ibuprofen degraded faster than R-ibuprofen and the degradation was not enantioselective. The results for naproxen were different thus it was degraded enantioselectively under prevailing aerobic and anaerobic conditions. Moreover, it was found out that some of the illicit drugs were detected after treatment as only one type of enantiomer and traced the chiral change in the composition [94]. Kasprzyk-Hordern et al. stated that stereoselectivity was found to be related to the type of chiral drug, the treatment technology used and the season. Furthermore, each enantiomer in the aquatic environment should be evaluated separately because of different ecotoxicity. In 2013, Li et al. explained that EF depends on the biological processes the molecules underwent [58]. The relationship between sampling time and concentration showed high variability in these processes, depending on environmental conditions. Vazquez-Roig et al. [50] provided a profile of the used chiral drugs from wastewater in one region. Wastewater treatment technology was determined to make a difference in the type of enantiomer found in the analysis. In 2014, the green mobile phase composition of ethanol/10mM aqueous ammonium acetate buffer (92.5/7.5, v/v), pH 6.8, with isocratic gradient and a flow rate of 0.32 mL/min was investigated by Ribeiro et al. [49]. This eco-friendly composition of the mobile phase and a lower flow rate led the analysis to a greener area. The method proved the capability of green analysis to track enantiomeric separation. In 2017, Evans et al. [33] focused on the microbial degradation of chiral drugs, which was discovered to be stereoselective as they had hypothesised. The enantiomers were found to enrich the parent drug amounts in the matrices, which could lead to the enrichment of stereoselective metabolic pathways with the other enantiomer.

In LC, CPs can be also separated on conventional achiral column. However, this is an indirect method, as it involves the derivatization step. Derivatization is often used when the location of functional groups prevents the chiral centre from interacting with the stationary phase. Due to the use of chiral reagents (e.g. 2,3,4,6-tetra-O-pivaloyl- β -D-glucopyranosyl isothiocyanate or R-1-phenylethylamine), diastereomers are formed during this process. There are four derivatization methods used for the formation of these derivatives: derivatization with solidphase reagents, solid-support-assisted derivatization methods, homogeneous-solution derivatization and on-column derivatization methods. However, these methods have some disadvantages. Firstly, the sample preparation step is usually time-consuming and laborious. It is often caused by many liquid-liquid extraction operations, used to enrich and isolate analytes from the matrix. Secondly, the conversion of analytes is also generally time-absorbing. Despite several attempts to simplify the derivatization procedure, this process is not often performed in a “green” way, because toxic and hazardous reagents or solvents are still being used [57, 94].



In the experiments conducted with LC-MS and LC-MS/MS, the lack of standardization or validation studies and data poses problems with the reliability of the experiments. Most of the papers in the literature are based on in-house methods. Although frequently detected pharmaceuticals, such as caffeine and paracetamol, are present in high concentrations, methods such as sample enrichment are needed for most other drug molecules. Besides, even with the sensitive LC-MS/MS technique it should be kept in mind that false positive results and blank contamination can be observed at low concentration analyte levels.

In 2007 U.S. EPA (United States Environmental Protection Agency) declared a method (1694) for analysing water samples that was advised to be used only for screening rather than quantifying [95]. The method allows to evaluate the samples for the absence or presence of drugs. The bias factor becomes more important when the levels of analytes go down to ppt or sub-ppt level; therefore, the statistical evaluations, system suitability tests and validation should be performed carefully [96]. Nanomolecules (drug size 1-100 nm) and other newly applied drug delivery systems for targeted active pharmaceutical compounds are ultra-low in concentration compared to the direct intake amounts.

Even if the amounts of CPs observed in wastewater are rather small, the amounts delivered as the uncleaned portion from -WWTPs to the drinking water systems are still growing, and thus more research should be performed to investigate it more thoroughly.

443 Table 1. Main information on analytical procedures used for the determination of chiral pharmaceuticals in wastewater

Analytes	Extraction type	Determination technique	Selected conditions and comments	LOD/LOQ (ng/L)	Recovery	Ref.
(S)-(+)- and (R)-(-)- Ibuprofen and metabolites as methyl esters Clofibric acid	SPE Macroporous polystyrene divinylbenzene copolymer adsorbent Elution with methanol/methylene chloride	GC-MS	16-m non-commercial enantioselective column (0.25 mm i.d) SRM. Temperature programmed: 70 °C, 2 min isothermal, 20 °C/min to 120 °C, then at 2.5 °C/min to 152 °C, then at 20 °C/min to 230 °C, followed by an isothermal hold at this temperature	Influent: 990-3300 Effluent: 2-81	Ibuprofen in surface water at concentrations of 0.1-1 ng/L, with acceptable recoveries (50- 90%). The WWTP samples with much higher concentrations of IB	[91]

Propranolol and its enantiomers	SPE: C18 cartridge, elution with methanol	GC-MS/MS	MDN-5S column (30 m, 0.25 mm, 0.25 mm film thickness) 1.0-min hold at 100 °C, 3 °C/min ramp to 300 °C with a 1.0-min hold at the end of the program.	LOD: 0.1 to 1.0 ng/L	15% to 90%	[92]
---------------------------------	---	----------	---	----------------------	------------	------

Ibuprofen Naproxen	SPE: Oasis HLB cartridges, elution with hexane/ethyl acetate (1:1)	GC-MS	Chiraldex column 20 m 0.25 mm 0.12 µm film thickness 100 °C for 2 min and then the temperature was programmed at 2 °C /min to 200 °C, with the final temperature being held at 200 °C for 5 min.	Removal efficiency was studied.		[93]
-----------------------	--	-------	---	---------------------------------	--	------

Atenolol	SPE: Oasis HLB cartridges, elution with MeOH	LC-MS/MS	Chirobiotic V (250 mm×4.6 mm, 5 μm)	LOD:	Influent: 86%	[97]
Metoprolol				Influent: 0.3-3.7	Effluent: 78%	
Nadolol				Effluent: 0.1-0.7		
Pindolol						
Propranolol				LOQ:		
Sotalol				Influent: 1-13	Effluent: 0.2-2.5	
Atenolol	SPE: Oasis HLB cartridges, elution with MeOH	LC-MSMS	Chirobiotic V (250 mm×4.6 mm, 5 μm)	LOD:	67%-106%	[98]
Metoprolol				Influent: 17-110		
propranolol				Effluent: 4.4-17		
			90:10 MeOH:water with 20 mM NH ₄ OAc, 0.1% formic acid (pH 4) run isocratic for 45 min at 0.5 mL/min			
			90:10 methanol/water with 0.1% TEAA adjusted to pH 4.0 with acetic acid, run isocratically at 1.0 mL/min			

Amphetamines	SPE: Oasis HLB (60 mg, 3 mL) elution with methanol	LC-MS/MS	Chiral CBH (100 mm × 2 mm, 5 μm)	IDL: 0.1-0.4	[94]
Ephedrines				IQL: 0.4-1.25	
Venlafaxine				MDL:0.5-3.5	
			90% H ₂ O, 10% 2- propanol and 1 mM ammonium acetate (pH:5) Column: 25 ° C sample manager :4°C. The flow rate 0.075 mL/min	MQL:2.2-11.75	

Amphetamine Methamphetamine	SPE: HLB cartridges elution with methanol	LC-MS/MS	Chiral CBH (100 × 2 mm, 5 μm): 90% H ₂ O, 10% 2-propanol and 1 mM ammonium acetate (pH:7) flow rate: 0.075 mL/min with run time of 65 min.	Chirobiotic column: IDL: 0.2-4.0 IQL: 0.5-15.0	>75% in both column	[89]
MDMA						
Atenolol						
Metoprolol Propranolol Venlafaxine			Chirobiotic V (250 × 4.6 mm, 5 μm): 4 mM ammonium acetate and 0.005% formic acid, flow rate: 0.1 mL/min with a run time of 40 min.	River water: MDL:0.2- 10.4 MQL:0.3-39.0		
Fluoxetine				Sewage effluent: MDL:0.6- 22.8 MQL:1.3-85.7		
				CBH Column: IDL: 1.3-5.0 IQL: 5.0-25.0		
				River water: MDL:2.1- 10.7 MQL:9.1-51.7		

Vanlafaxine	SPE: Oasis HLB, 200 mg	LC-MS/MS	Chirobiotic V column (250 mm × 2.1 mm, 5 μm)	LOD: 4-15	>72%	[58]
			Tetrahydrofuran:ammonium acetate (10 mM) pH 6.0 (10:90; v/v) with a flow rate of 0.2 ml/min.			
Venlafaxine	SPE: Oasis MCX cartridges, methanol for elution	LC-MS/MS	Chirobiotic V 5 m (150 × 2.1 mm, 5 μm.) Ethanol/10mM aqueous ammonium acetate buffer (92.5/7.5, v/v), pH 6.8, performed in isocratic mode with a flow rate of 0.32 mL/min. Column oven and autosampler were set to 20 ° C and 4 ° C	IDL: 163-2868	>72%	[49]
Fluoxetine				IQL: 495-4935		
Norfluoxetine (Metabolite)				MDL:0.65- 11.5		
Alprenolol				MQL:1.98-19.7		
Bisoprolol						
Metoprolol						
Propranolol						
Salbutamol						

Ephedrine Norephedrine	SPE: Oasis HLB (60 mg, 3 mL), elution with methanol	LC-MS/MS	Chiral-CBH column (100 × 2 mm, 5 μm): 90% H ₂ O, 10% 2-propanol and 1 mM ammonium acetate (pH: 5.0) at a flow rate of 0.075 mL/min	MDL Influent: 0.4-3.3	Influent: 21%-125%	[50]
Atenolol				Effluent: 0.3-2.5	Effluent: 54%-122	
Venlafaxine						
Illicit Drugs				MQL		
				Influent: 1.3-11.1		
				Effluent: 1.1-8.4		

Amphetamine Methamphetamine MDMA	SPE: Oasis HLB, elution with methanol	LC-MS/MS	Chirobiotic V (250 x 2.1 mm, 5 µm): methanol, 4 mM ammonium acetate and 0.005% formic acid (rest of the analytes)	Chirobiotic V: IDL: 0.01-1.85	Influent relative recovery:>20%	[34]
MDA				IQL: 0.03-6.16		
Venlafaxine			CBH (100 × 2 mm, 5 µm) (pH, 5.0) 90% H ₂ O, 10% 2- propanol and 1 mM ammonium acetate. flow rate: 0.075 mL/min, (amphetamine-ephedrine derivatives)	CBH: IDL: 0.05- 1.83	Effluent relative recovery:>46%	
Desmethylvenlafaxine Citalopram				IQL: 0.17-6.11		
Metoprolol						
Propranolol						
Sotalol				Influent: MDL: 0.03-28.74		
Mirtazapine Salbutamol				MQL: 0.01-95.81		
Fluoxetine Desmethylcitalopram Atenolol					Effluent: MDL: 0.02-32.73	
Ephedrine Pseudoephedrine Alprenolol				MQL: 0.09-109.08		



Metoprolol	SPE: Oasis HLB, elution with MeOH (0.1% HCOOH)	LC-MS/MS	Chirobiotic V (250 × 4.6 mm, 5 µm) column for metoprolol, propranolol, atenolol, fluoxetine, venlafaxine	Chiral drugs on	>%72 (except indomethacin: 46%)	[64]	
Propranolol				Chirobiotic V:			
Atenolol				IDL:100-500			
Fluoxetine				90% MeOH, 10% H ₂ O (0.1% HCOOH, pH=4) at a flow rate of 0.65 mL/min			IQL: 300-1700
Venlafaxine				MDL: 100-600			
Ibuprofen				MQL: 400-2100			
Flurbiprofen				Chiralpak AD-RHchiral column (150 × 4.6 mm, 5 µm) for NSAIDs as ibuprofen, flurbiprofen, naproxen			NSAI on Chiralpak:
Naproxen	Acetonitrile:10 mM ammonium acetate buffer (pH 5.0, formic acid adjusted) (35:65 v:v), at flow rate of 0.4 mL/min	IDL: 300-9900					
			IQL: 1100-33000				
			MDL: 400-11000				
			MQL: 1200-37000				
			LOD:0.02-2.3				
			LOQ:0.07-23				



56 drug biomarkers
(opioid analgesics,
amphetamines, cocaine,
heroin, stimulants,
anaesthetics, sedatives,
anxiolytics, designer
drugs,
phosphodiesterase-5
(PDE5) inhibitors,
amphetamine and
methamphetamine drug
precursors)

SPE: Oasis HLB,
elution with MeOH

LC-MS/MS

Chiralpack CBH: 1 mM
ammonium acetate/methanol
85:15 v/v at 0.1 mL/min

IDL: 10-10000

IQL: 20-50000

Influent:

MDL: 0.1-61.2

MQL: 0.1-306.1

SPE relative
recovery: 75%
- 127%

[90]

Fluoxetine	SPE: Oasis HLB, elution with MeOH	LC-MSMS	CBH: 90:10 water:IPA, 1 mM NH ₄ OAc isocratically at 0.075 mL/min	Influent: MDL: 0.06- 28.74	Influent: SPE relative recovery: 45% - 148%	[33]
Venlafaxine				MQL: 0.11-95.81		
Desmethylvenlafaxine						
Citalopram				Effluent:	Effluent:	
Desmethylcitalopram				MDL: 0.02- 32.73	SPE relative recovery: 46% - 132%	
Mirtazapine				MQL: 0.07-109.08		
Atenolol						
Metoprolol						
Sotalol						
Propranolol						
Alprenolol						
Salbutamol						
Amphetamine						
Methamphetamine						
MDMA						
MDA						

90 chiral and achiral micropollutants (Propranolol	SPE: Oasis HLB, elution with MeOH	LC-MS/MS	Chirobiotic V (100 × 2 mm, 5 μm) (beta-blockers and anti-depressants) with 4 mM NH ₄ OAc in MeOH containing 0.005% HCOOH	Chirobiotic V	Chirobiotic V	[99]
Atenolol				Influent: MDL: 0.07- 28.7	Influent: > 72% (except: atenolol: 21)	
Metoprolol			CBH (100 × 2 mm, 5 μm) (amphetamine-like compounds) and a mobile phase consisting of 1 mM NH ₄ OAc in 85:15 H ₂ O: MeOH	MQL: 0.18-95.8	Effluent: > 73 (except atenolol: 55)	
Mirtazapine				Effluent:		
Citalopram				MDL: 0.05- 32.7	CBH Influent: > 76%	
Desmethylcitalopram, Fluoxetine				MQL: 0.17-109.1	Effluent: -	
MDMA				CBH Influent: MDL: 0.3- 0.8		
Amphetamine as chiral drugs)				MQL: 1.3-2.9		
				Effluent: -		



444 5. Conclusions and future trends

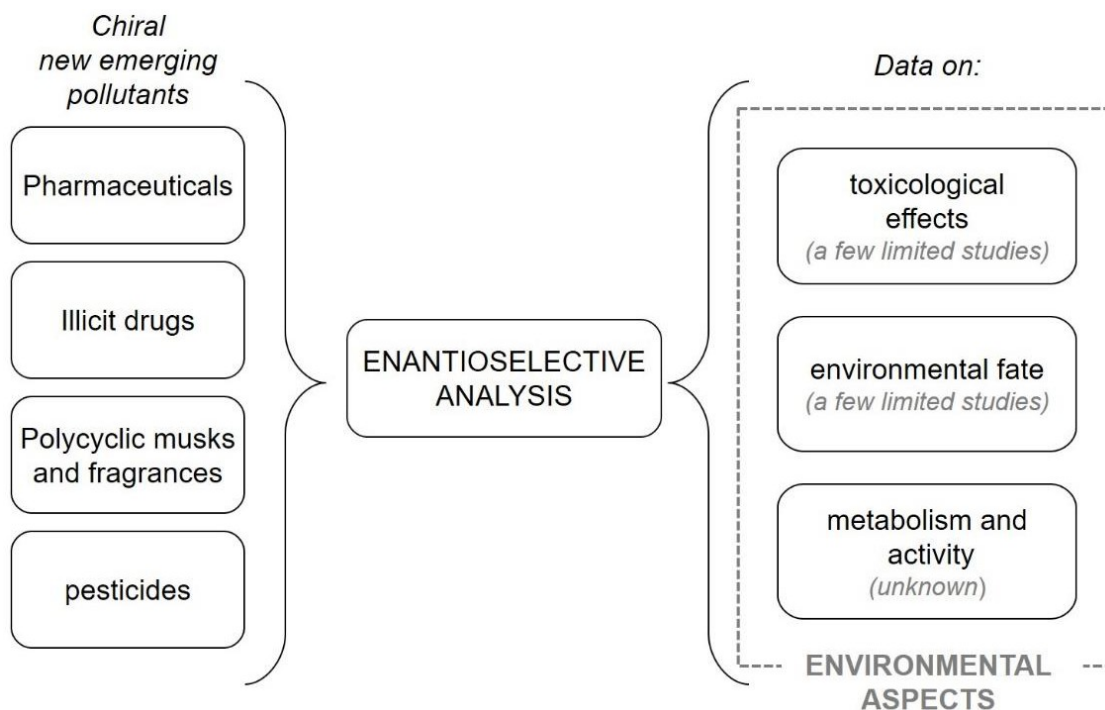
445
446 CPs play an important role in the life of animals, including humans. However, nowadays
447 they represent a great threat to the environment. Their enantiomers may exhibit toxic effects in
448 different environmental media due to the different pharmacokinetic characteristics. Non-chiral
449 compounds may be formed when chiral drug substances are degraded or vice versa. In addition,
450 many of the metabolites of non-chiral pollutants may also show chiral properties and some
451 metabolites may be more toxic than chiral compounds. However, there are ~~still~~ many unknowns
452 about the processes that CPs are subjected to in the environment.

453 Wastewater is the main source of CPs in the environment. They, in turn, are the sources
454 of information on human health and lifestyle. The use of chiral compounds as WBE biomarkers
455 would enable to estimate the public health status. However, their detection in a matrix as complex
456 as wastewater may cause problems and result in some challenges. Since CPs and their chiral
457 metabolites are at lower concentrations than other chiral pollutants, their analysis requires
458 hyphenated techniques and has been the subject of less research. Lack of standardization of
459 sample collection, storage, sample preparation procedure, analysis and identification steps are
460 some of the problems that today's analysts face. Even though there are some procedures applied
461 in the analysis of pharmaceuticals in environmental samples that are also used for the
462 determination of chiral compounds, they do not consider the possibility of occurrence of chiral
463 processes during the analytical procedure. Detection, identification and determination of CPs in
464 wastewater is challenging and of the highest demand. Furthermore, greener solutions in the
465 analysis of CPs should be the topic of scientific concern in the next years.

466 Despite the fact that the knowledge concerning CPs in wastewater is at quite a good level,
467 there are still many issues that should be considered in the future. There is still a lot to do when it
468 comes to analytical chemistry and environmental science. First of all, the new procedures for the
469 online sample treatment and analysis of the wastewater should be developed and introduced in
470 routine analysis. Proceeding in such a way will make it easier to follow the fate of
471 pharmaceuticals in terms of environmental and human health aspects. Therefore, it is crucial that
472 these new approaches can be applied to simultaneous detection of new CPs that occur in the
473 environment, their metabolites and products of transformation, as well as for evaluation of the



474 enantiomer-specific toxicity to aquatic organisms. Moreover, it would contribute to further
475 development of researches in “unknown-known” and “unknown-unknown” fields/area. There is
476 still little information on environmental processes and the impact of CPs and other pollutants in
477 ecosystems (Fig 6.).



478

479 *Fig. 6. Future trends in the use of enantioselective analyses in environmental science.*

480

481 The priority of the most frequently detected pharmaceuticals can be evaluated statistically
482 in terms of the season, population and its characteristics, and other key influencing factors. The
483 information obtained from wastewater analysis about drugs can be summarised as national data
484 of drug usage trend as well.

485 Human impact on the aquatic environment should be kept in mind when following the
486 toxicological pathway analytically. It should be remembered that the amount of drug residues in
487 drinking water is related to the success in wastewater treatment.

488 The recent reports will function as a starting point for the future contamination problems.
489 The alteration of some pharmaceuticals can lead the national health institutes to take action on the
490 type and dosage of prescription drugs.

491 Both environmental and analytical methodologies currently applied should be performed
492 within the “green” frame. Green Analytical Chemistry (GAC) aims to reduce the impact of
493 analytical procedures on the environment. The most common mode of application is the
494 miniaturization of the devices and the reduction of the extractions in the sample preparation steps.
495 The other, less frequently observed approach is to skip the sample preparation steps and apply
496 direct analysis methods. In the multiple benchmark analysis, GAC uses many experimental tests,
497 calculation models and tools to assess the ecological risks associated with human health and
498 environmental stressors and the effects of curative and mitigation strategies on risk reduction.
499 With this modelling, it is thought that the analytical separation mechanism and sample
500 preparation steps will be the most appropriate approach.

501 The new optimization procedure will enable the inclusion of GAC concept in the selection
502 of the optimum parameters of the liquid chromatographic separation. Multi-criteria decision
503 analysis, which enables decision making by converting many variables of the process into a
504 single score, is one of the parameters to be tested within the project.

505 In terms of GAC, the proposed highlights, which suggest the usage of eco-friendly mobile
506 phases, should be given more attention, as the chiral analysis is mainly performed with high
507 amounts of toxic solvents. On-line sample collection and elimination of the derivatization step
508 with direct screening methods will also qualify as green approaches.

509 All of the existing tools used for the evaluation of “greenness” of the developed
510 procedures have their own advantages and disadvantages; however, the most ideal solution would
511 be to apply them all, so as to obtain as much information as possible. Nevertheless, in reality,
512 such an approach is overly time-consuming. Therefore, GAPI (Green Analytical Procedure
513 Index) [100], which is a new tool that can evaluate the green character of the whole analytical
514 methodology – from the sample collection step to the analysis – can be proposed.

515 In addition to what is mentioned above, one other topic should be greatly considered: to
516 work in accordance with the idea of sustainable environment. Green Chemistry and GAC evolved
517 from the academic sphere into the real world; therefore, there is enormous research activity on
518 "greening" all aspects associated with the analysis of any kind of sample, including wastewater.
519 Considering this fact, it can be stated with absolute conviction that GAC could be really useful in
520 the analysis of CPs in wastewater. The application of fast, cheap and environmentally friendly
521 and safe procedures in such an analysis may change the quality of life in developing countries.



522

523 **Acknowledgement**

524 The research is funded by the Scientific and Technological Research Council of Turkey
525 (TUBITAK) within the TUBITAK-BIDEB 2219-International Postdoctoral Research Scholarship
526 Program.

527 Aysegül Dogan would like to express her deep gratitude to her postdoctoral advisor, Prof.
528 Jacek Namieśnik, for his help and support throughout her stay at Gdańsk University of
529 Technology, Gdańsk, Poland in 2018-2019.

530

531

532 **References**

533

534

535 1. L. Di, E.H. Kerns, ADME properties of drugs, Wiley Encyclopedia of Chemical Biology.
536 (2007) 1-8. <https://doi.org/10.1002/9780470048672.wecb003>.

537 2. K. Kümmerer, Drugs in the environment: emission of drugs, diagnostic aids and
538 disinfectants into wastewater by hospitals in relation to other sources—a review, Chemosphere. 45
539 (2001) 957-969. [https://doi.org/10.1016/S0045-6535\(01\)00144-8](https://doi.org/10.1016/S0045-6535(01)00144-8).

540 3. H. Rho, B. Shin, O. Lee, Y.-H. Choi, J. Rho, J. Lee, Antibiotic resistance profile of
541 bacterial isolates from animal farming aquatic environments and meats in a peri-urban
542 community in Daejeon, Korea, J. Environ. Monit. 14 (2012) 1616-1621.
543 <https://doi.org/10.1039/C2EM30168G>.

544 4. K. Kümmerer, Antibiotics in the aquatic environment—a review—part I, Chemosphere. 75
545 (2009) 417-434. <https://doi.org/10.1016/j.chemosphere.2008.11.086>.

546 5. N.M. Maier, P. Franco, W. Lindner, Separation of enantiomers: needs, challenges,
547 perspectives, J. Chromatogr., A. 906 (2001) 3-33. [https://doi.org/10.1016/S0021-9673\(00\)00532-](https://doi.org/10.1016/S0021-9673(00)00532-X)
548 [X](https://doi.org/10.1016/S0021-9673(00)00532-X).

549 6. S.W. Smith, Chiral toxicology: it's the same thing... only different, Toxicol. Sci. 110
550 (2009) 4-30. <https://doi.org/10.1093/toxsci/kfp097>.

551 7. L.A. Nguyen, H. He, C. Pham-Huy, Chiral drugs: an overview, Int. J. Biomed. Sc. 2
552 (2006) 85.

553 8. U.S.F.a.D. Administration. Development of New Stereoisomeric Drugs. 1992.

554 9. W.H. De Camp, The FDA perspective on the development of stereoisomers, Chirality. 1
555 (1989) 2-6. <https://doi.org/10.1002/chir.530010103>.

556 10. T. Eriksson, S. Björkman, B. Roth, Å. Fyge, P. Höglund, Stereospecific determination,
557 chiral inversion in vitro and pharmacokinetics in humans of the enantiomers of thalidomide,
558 Chirality. 7 (1995) 44-52. <https://doi.org/10.1002/chir.530070109>.

559 11. I. Ali, V. Gupta, H.Y. Aboul-Enein, Chirality: a challenge for the environmental
560 scientists, Curr. Sci. 84 (2003) 152-156.

561 12. G. Blaschke, H. Kraft, K. Fickentscher, F. Köhler, Chromatographic separation of



- 562 racemic thalidomide and teratogenic activity of its enantiomers (author's transl), *Arzneim.-*
563 *Forsch.* 29 (1979) 1640.
- 564 13. H. Gerlach, Chirality: A Relational Geometric-Physical Property, *Chirality*. 25 (2013)
565 684-685. <https://doi.org/10.1002/chir.22216>.
- 566 14. E. Gil-Av, B. Feibush, R. Charles-Sigler, Separation of enantiomers by gas liquid
567 chromatography with an optically active stationary phase, *Tetrahedron Lett.* 7 (1966) 1009-1015.
568 [https://doi.org/10.1016/S0040-4039\(00\)70231-0](https://doi.org/10.1016/S0040-4039(00)70231-0).
- 569 15. T. Kościelski, D. Sybilska, J. Jurczak, Separation of α - and β -pinene into enantiomers in
570 gas-liquid chromatography systems via α -cyclodextrin inclusion complexes, *J. Chromatogr., A*.
571 280 (1983) 131-134. [https://doi.org/10.1016/S0021-9673\(00\)91547-4](https://doi.org/10.1016/S0021-9673(00)91547-4).
- 572 16. Y. Zhang, S. Yao, H. Zeng, H. Song, Chiral separation of pharmaceuticals by high
573 performance liquid chromatography, *Curr. Pharm. Anal.* 6 (2010) 114-130.
574 <https://doi.org/10.2174/157341210791202636>.
- 575 17. A. Nikolaou, S. Meric, D. Fatta, Occurrence patterns of pharmaceuticals in water and
576 wastewater environments, *Anal. Bioanal. Chem.* 387 (2007) 1225-1234.
577 <https://doi.org/10.1007/s00216-006-1035-8>.
- 578 18. J. Santos, I. Aparicio, E. Alonso, M. Callejón, Simultaneous determination of
579 pharmaceutically active compounds in wastewater samples by solid phase extraction and high-
580 performance liquid chromatography with diode array and fluorescence detectors, *Anal. Chim.*
581 *Acta.* 550 (2005) 116-122. <https://doi.org/10.1016/j.aca.2005.06.064>.
- 582 19. K.K. Barnes, D.W. Kolpin, E.T. Furlong, S.D. Zaugg, M.T. Meyer, L.B. Barber, A
583 national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the
584 United States—I) Groundwater, *Sci. Total Environ.* 402 (2008) 192-200.
585 <https://doi.org/10.1016/j.scitotenv.2008.04.028>.
- 586 20. K.K. Barnes, D.W. Kolpin, M.T. Meyer, E.M. Thurman, E.T. Furlong, S.D. Zaugg, L.B.
587 Barber, Water-quality data for pharmaceuticals, hormones, and other organic wastewater
588 contaminants in US streams, 1999-2000, US Geological Survey Open-File Report. 94 (2002).
- 589 21. T.A. Ternes, A. Joss, H. Siegrist. Peer reviewed: scrutinizing pharmaceuticals and
590 personal care products in wastewater treatment. ACS Publications; 2004.
- 591 22. T.A. Ternes, M. Meisenheimer, D. McDowell, F. Sacher, H.-J. Brauch, B. Haist-Gulde,
592 G. Preuss, U. Wilme, N. Zulei-Seibert, Removal of pharmaceuticals during drinking water
593 treatment, *Environ. Sci. Technol.* 36 (2002) 3855-3863. <https://doi.org/10.1021/es015757k>.
- 594 23. D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T.
595 Buxton, Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams,
596 1999– 2000: A national reconnaissance, *Environ. Sci. Technol.* 36 (2002) 1202-1211.
597 <https://doi.org/10.1021/es011055j>.
- 598 24. N. Bolong, A. Ismail, M.R. Salim, T. Matsuura, A review of the effects of emerging
599 contaminants in wastewater and options for their removal, *Desalination*. 239 (2009) 229-246.
600 <https://doi.org/10.1016/j.desal.2008.03.020>.
- 601 25. T. Heberer, Occurrence, fate, and removal of pharmaceutical residues in the aquatic
602 environment: a review of recent research data, *Toxicol. Lett.* 131 (2002) 5-17.
603 [https://doi.org/10.1016/S0378-4274\(02\)00041-3](https://doi.org/10.1016/S0378-4274(02)00041-3).
- 604 26. H.-Y. Kim, I.-S. Lee, J.-E. Oh, Human and veterinary pharmaceuticals in the marine
605 environment including fish farms in Korea, *Sci. Total Environ.* 579 (2017) 940-949.
606 <https://doi.org/10.1016/j.scitotenv.2016.10.039>.
- 607 27. M.D. Celiz, J. Tso, D.S. Aga, Pharmaceutical metabolites in the environment: analytical
608 challenges and ecological risks, *Environ. Toxicol. Chem.* 28 (2009) 2473-2484.

609 <https://doi.org/10.1897/09-173.1>.

610 28. D. Fatta, A. Achilleos, A. Nikolaou, S. Meric, Analytical methods for tracing
611 pharmaceutical residues in water and wastewater, *TrAC, Trends Anal. Chem.* 26 (2007) 515-533.
612 <https://doi.org/10.1016/j.trac.2007.02.001>.

613 29. A. Göbel, C.S. McArdell, M.J.-F. Suter, W. Giger, Trace determination of macrolide and
614 sulfonamide antimicrobials, a human sulfonamide metabolite, and trimethoprim in wastewater
615 using liquid chromatography coupled to electrospray tandem mass spectrometry, *Anal. Chem.* 76
616 (2004) 4756-4764. <https://doi.org/10.1021/ac0496603>.

617 30. O. H. Jones, N. Voulvoulis, J. Lester, Human pharmaceuticals in wastewater treatment
618 processes, *Critical Reviews in Environmental Science and Technology.* 35 (2005) 401-427.
619 <https://doi.org/10.1080/10643380590956966>.

620 31. J.B. Quintana, S. Weiss, T. Reemtsma, Pathways and metabolites of microbial
621 degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater
622 treated by a membrane bioreactor, *Water Res.* 39 (2005) 2654-2664.
623 <https://doi.org/10.1016/j.watres.2005.04.068>.

624 32. E. Eljarrat, P. Guerra, D. Barceló, Enantiomeric determination of chiral persistent organic
625 pollutants and their metabolites, *TrAC, Trends Anal. Chem.* 27 (2008) 847-861.
626 <https://doi.org/10.1016/j.trac.2008.08.010>.

627 33. S. Evans, J. Bagnall, B. Kasprzyk-Hordern, Enantiomeric profiling of a chemically
628 diverse mixture of chiral pharmaceuticals in urban water, *Environ. Pollut.* 230 (2017) 368-377.
629 <https://doi.org/10.1016/j.envpol.2017.06.070>.

630 34. S.E. Evans, P. Davies, A. Lubben, B. Kasprzyk-Hordern, Determination of chiral
631 pharmaceuticals and illicit drugs in wastewater and sludge using microwave assisted extraction,
632 solid-phase extraction and chiral liquid chromatography coupled with tandem mass spectrometry,
633 *Anal. Chim. Acta.* 882 (2015) 112-126. <https://doi.org/10.1016/j.aca.2015.03.039>.

634 35. WHO <https://www.who.int/topics/epidemiology/en/> (accessed). [cited date Cited].

635 36. P.M. Choi, B.J. Tschärke, E. Donner, J.W. O'Brien, S.C. Grant, S.L. Kaserzon, R.
636 Mackie, E. O'Malley, N.D. Crosbie, K.V. Thomas, Wastewater-based epidemiology biomarkers:
637 Past, present and future, *TrAC, Trends Anal. Chem.* 105 (2018) 453-469.
638 <https://doi.org/10.1016/j.trac.2018.06.004>.

639 37. B. Kasprzyk-Hordern, L. Bijlsma, S. Castiglioni, A. Covaci, P. de Voogt, E. Emke, F.
640 Hernandez, C. Ort, M. Reid, A. van Nuijs, Wastewater-based epidemiology for public health
641 monitoring, *Water and Sewerage Journal.* 4 (2014) 25-26.

642 38. Z. Yang, G. Xu, J. Reboud, B. Kasprzyk-Hordern, J.M. Cooper, Monitoring Genetic
643 Population Biomarkers for Wastewater-Based Epidemiology, *Anal. Chem.* 89 (2017) 9941-9945.
644 <https://doi.org/10.1021/acs.analchem.7b02257>.

645 39. C.G. Daughton, T.A. Ternes, Pharmaceuticals and personal care products in the
646 environment: agents of subtle change?, *Environ. Health Persp.* 107 (1999) 907-938.
647 <https://doi.org/10.1289/ehp.99107s6907>.

648 40. W. Hall, J. Prichard, P. Kirkbride, R. Bruno, P.K. Thai, C. Gartner, F.Y. Lai, C. Ort, J.F.
649 Mueller, An analysis of ethical issues in using wastewater analysis to monitor illicit drug use,
650 *Addiction.* 107 (2012) 1767-1773. <https://doi.org/10.1111/j.1360-0443.2012.03887.x>.

651 41. S. Castiglioni, E. Zuccato, C. Chiabrando, R. Fanelli, R. Bagnati, Mass spectrometric
652 analysis of illicit drugs in wastewater and surface water, *Mass Spectrom. Rev.* 27 (2008) 378-
653 394. <https://doi.org/10.1002/mas.20168>.

654 42. C. Postigo, M.J. Lopez de Alda, D. Barceló, Fully Automated Determination in the Low
655 Nanogram per Liter Level of Different Classes of Drugs of Abuse in Sewage Water by On-Line

656 Solid-Phase Extraction-Liquid Chromatography– Electro spray-Tandem Mass Spectrometry,
657 *Anal. Chem.* 80 (2008) 3123-3134. <https://doi.org/10.1021/ac702060j>.

658 43. E. Castrignanò, Z. Yang, R. Bade, J.A. Baz-Lomba, S. Castiglioni, A. Causanilles, A.
659 Covaci, E. Gracia-Lor, F. Hernandez, J. Kinyua, Enantiomeric profiling of chiral illicit drugs in a
660 pan-European study, *Water Res.* 130 (2018) 151-160.
661 <https://doi.org/10.1016/j.watres.2017.11.051>.

662 44. B. Petrie, J. Youdan, R. Barden, B. Kasprzyk-Hordern, New framework to diagnose the
663 direct disposal of prescribed drugs in wastewater—a case study of the antidepressant fluoxetine,
664 *Environ. Sci. Technol.* 50 (2016) 3781-3789. <https://doi.org/10.1021/acs.est.6b00291>.

665 45. A. Courtier, A. Cadiere, B. Roig, Human Pharmaceuticals: Why and how to reduce their
666 presence in the environment, *Curr. Opin. Green Sustain. Chem.* 15 (2019) 77-82.
667 <https://doi.org/10.1016/j.cogsc.2018.11.001>.

668 46. L. Duan, Y. Zhang, B. Wang, S. Deng, J. Huang, Y. Wang, G. Yu, Occurrence,
669 elimination, enantiomeric distribution and intra-day variations of chiral pharmaceuticals in major
670 wastewater treatment plants in Beijing, China, *Environ. Pollut.* 239 (2018) 473-482.
671 <https://doi.org/10.1016/j.envpol.2018.04.014>.

672 47. C.L. Amorim, I.S. Moreira, A.R. Ribeiro, L.H. Santos, C. Delerue-Matos, M.E. Tiritan,
673 P.M. Castro, Treatment of a simulated wastewater amended with a chiral pharmaceuticals
674 mixture by an aerobic granular sludge sequencing batch reactor, *Internat. Biodeter. Biodegr.* 115
675 (2016) 277-285. <https://doi.org/10.1016/j.ibiod.2016.09.009>.

676 48. S.E. Evans, B. Kasprzyk-Hordern, Applications of chiral chromatography coupled with
677 mass spectrometry in the analysis of chiral pharmaceuticals in the environment, *Trends Environ.*
678 *Anal. Chem.* 1 (2014) e34-e51. <https://doi.org/10.1016/j.teac.2013.11.005>.

679 49. A.R. Ribeiro, L.H. Santos, A.S. Maia, C. Delerue-Matos, P.M. Castro, M.E. Tiritan,
680 Enantiomeric fraction evaluation of pharmaceuticals in environmental matrices by liquid
681 chromatography-tandem mass spectrometry, *J. Chromatogr., A.* 1363 (2014) 226-235.
682 <https://doi.org/10.1016/j.chroma.2014.06.099>.

683 50. P. Vazquez-Roig, B. Kasprzyk-Hordern, C. Blasco, Y. Picó, Stereoisomeric profiling of
684 drugs of abuse and pharmaceuticals in wastewaters of Valencia (Spain), *Sci. Total Environ.* 494
685 (2014) 49-57. <https://doi.org/10.1016/j.scitotenv.2014.06.098>.

686 51. C. Caballo, M. Sicilia, S. Rubio, Enantioselective determination of representative profens
687 in wastewater by a single-step sample treatment and chiral liquid chromatography–tandem mass
688 spectrometry, *Talanta.* 134 (2015) 325-332. <https://doi.org/10.1016/j.talanta.2014.11.016>.

689 52. P. Verlicchi, E. Zambello, How efficient are constructed wetlands in removing
690 pharmaceuticals from untreated and treated urban wastewaters? A review, *Sci. Total Environ.*
691 470 (2014) 1281-1306. <https://doi.org/10.1016/j.scitotenv.2013.10.085>.

692 53. U. WWAP. (United Nations World Water Assessment Programme). Paris: Unesco; 2017.

693 54. [https://www.pri.org/stories/2016-12-15/recycling-sewage-drinking-water-no-big-deal-](https://www.pri.org/stories/2016-12-15/recycling-sewage-drinking-water-no-big-deal-theyve-been-doing-it-namibia-50-years(accessed))
694 [theyve-been-doing-it-namibia-50-years\(accessed\)](https://www.pri.org/stories/2016-12-15/recycling-sewage-drinking-water-no-big-deal-theyve-been-doing-it-namibia-50-years(accessed)). [cited date Cited].

695 55. Y. Zhou, S. Wu, H. Zhou, H. Huang, J. Zhao, Y. Deng, H. Wang, Y. Yang, J. Yang, L.
696 Luo, Chiral pharmaceuticals: Environment sources, potential human health impacts, remediation
697 technologies and future perspective, *Environ. Int.* 121 (2018) 523-537.
698 <https://doi.org/10.1016/j.envint.2018.09.041>.

699 56. Y. Valcárcel, S.G. Alonso, J. Rodríguez-Gil, A. Gil, M. Catalá, Detection of
700 pharmaceutically active compounds in the rivers and tap water of the Madrid Region (Spain) and
701 potential ecotoxicological risk, *Chemosphere.* 84 (2011) 1336-1348.
702 <https://doi.org/10.1016/j.chemosphere.2011.05.014>.



- 703 57. E. Sanganyado, Z. Lu, Q. Fu, D. Schlenk, J. Gan, Chiral pharmaceuticals: A review on
704 their environmental occurrence and fate processes, *Water Res.* 124 (2017) 527-542.
705 <https://doi.org/10.1016/j.watres.2017.08.003>.
- 706 58. Z. Li, E. Gomez, H. Fenet, S. Chiron, Chiral signature of venlafaxine as a marker of
707 biological attenuation processes, *Chemosphere.* 90 (2013) 1933-1938.
708 <https://doi.org/10.1016/j.chemosphere.2012.10.033>.
- 709 59. S.J. Khan, Biologically mediated chiral inversion of emerging contaminants,
710 Transformation Products of Emerging Contaminants in the Environment. (2014) 261-280.
711 <https://doi.org/10.1002/9781118339558.ch08>.
- 712 60. T. Suzuki, Y. Kosugi, M. Hosaka, T. Nishimura, D. Nakae, Occurrence and behavior of
713 the chiral anti-inflammatory drug naproxen in an aquatic environment, *Environ. Toxicol. Chem.* 714
33 (2014) 2671-2678. <https://doi.org/10.1002/etc.2741>.
- 715 61. R. Celis, B. Gámiz, M. Adelino, M. Herмосín, J. Cornejo, Environmental behavior of the
716 enantiomers of the chiral fungicide metalaxyl in Mediterranean agricultural soils, *Sci. Total*
717 *Environ.* 444 (2013) 288-297. <https://doi.org/10.1016/j.scitotenv.2012.11.105>.
- 718 62. B. Khan, X. Qiao, L.S. Lee, Stereoselective sorption by agricultural soils and liquid-
719 liquid partitioning of trenbolone (17 α and 17 β) and trendione, *Environ. Sci. Technol.* 43 (2009)
720 8827-8833. <https://doi.org/10.1021/es902112v>.
- 721 63. E. Sanganyado, Q. Fu, J. Gan, Enantiomeric selectivity in adsorption of chiral β -blockers
722 on sludge, *Environ. Pollut.* 214 (2016) 787-794. <https://doi.org/10.1016/j.envpol.2016.04.091>.
- 723 64. R. Ma, B. Wang, S. Lu, Y. Zhang, L. Yin, J. Huang, S. Deng, Y. Wang, G. Yu,
724 Characterization of pharmaceutically active compounds in Dongting Lake, China: Occurrence,
725 chiral profiling and environmental risk, *Sci. Total Environ.* 557 (2016) 268-275.
726 <https://doi.org/10.1016/j.scitotenv.2016.03.053>.
- 727 65. A.L. Batt, M.S. Kostich, J.M. Lazorchak, Analysis of ecologically relevant
728 pharmaceuticals in wastewater and surface water using selective solid-phase extraction and
729 UPLC-MS/MS, *Anal. Chem.* 80 (2008) 5021-5030. <https://doi.org/10.1021/ac800066n>.
- 730 66. M. Clara, B. Strenn, O. Gans, E. Martinez, N. Kreuzinger, H. Kroiss, Removal of selected
731 pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and
732 conventional wastewater treatment plants, *Water Res.* 39 (2005) 4797-4807.
733 <https://doi.org/10.1016/j.watres.2005.09.015>.
- 734 67. A. Joss, S. Zabczynski, A. Göbel, B. Hoffmann, D. Löffler, C.S. McArdell, T.A. Ternes,
735 A. Thomsen, H. Siegrist, Biological degradation of pharmaceuticals in municipal wastewater
736 treatment: proposing a classification scheme, *Water Res.* 40 (2006) 1686-1696.
737 <https://doi.org/10.1016/j.watres.2006.02.014>.
- 738 68. B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Multiresidue methods for the analysis
739 of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by
740 solid-phase extraction and ultra performance liquid chromatography-electrospray tandem mass
741 spectrometry, *Anal. Bioanal. Chem.* 391 (2008) 1293-1308. <https://doi.org/10.1007/s00216-008-1854-x>.
- 742 1854-x.
- 743 69. B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, The removal of pharmaceuticals,
744 personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its
745 impact on the quality of receiving waters, *Water Res.* 43 (2009) 363-380.
746 <https://doi.org/10.1016/j.watres.2008.10.047>.
- 747 70. S.D. Kim, J. Cho, I.S. Kim, B.J. Vanderford, S.A. Snyder, Occurrence and removal of
748 pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters,
749 *Water Res.* 41 (2007) 1013-1021. <https://doi.org/10.1016/j.watres.2006.06.034>.

- 750 71. H. Matsuo, H. Sakamoto, K. Arizono, R. Shinohara, Behavior of pharmaceuticals in
751 waste water treatment plant in Japan, *Bulletin of environmental contamination and toxicology*. 87
752 (2011) 31-35. <https://doi.org/10.1007/s00128-011-0299-7>.
- 753 72. G.K. Brown, S.D. Zaugg, L.B. Barber, editors. *Wastewater analysis by gas*
754 *chromatography/mass spectrometry*. Proceedings of the US Geological Survey Toxic Substances
755 Hydrology Program Technical Meeting, Contamination of Hydrologic Systems and Related
756 Ecosystems, March; 1999: Citeseer.
- 757 73. G.E. Cordy, N.L. Duran, H. Bouwer, R.C. Rice, E.T. Furlong, S.D. Zaugg, M.T. Meyer,
758 L.B. Barber, D.W. Kolpin, Do pharmaceuticals, pathogens, and other organic waste water
759 compounds persist when waste water is used for recharge?, *Groundwater Monitoring &*
760 *Remediation*. 24 (2004) 58-69. <https://doi.org/10.1111/j.1745-6592.2004.tb00713.x>.
- 761 74. G. Blaschke, *Chromatographic Resolution of Racemates*. *New analytical methods* (17),
762 *Angewandte Chemie International Edition in English*. 19 (1980) 13-24.
763 <https://doi.org/10.1002/anie.198000131>.
- 764 75. W.H. Pirkle, J.M. Finn, J.L. Schreiner, B.C. Hamper, A widely useful chiral stationary
765 phase for the high-performance liquid chromatography separation of enantiomers, *Journal of the*
766 *American Chemical Society*. 103 (1981) 3964-3966. <https://doi.org/10.1021/ja00403a076>.
- 767 76. S. Allenmark, B. Bomgren, H. Borén, Direct liquid chromatographic separation of
768 enantiomers on immobilized protein stationary phases: III. Optical resolution of a series of N-
769 aroyl d, l-amino acids by high-performance liquid chromatography on bovine serum albumin
770 covalently bound to silica, *J. Chromatog., A*. 264 (1983) 63-68. [https://doi.org/10.1016/S0021-](https://doi.org/10.1016/S0021-9673(01)95006-X)
771 [9673\(01\)95006-X](https://doi.org/10.1016/S0021-9673(01)95006-X).
- 772 77. Y. Okamoto, M. Kawashima, K. Hatada, *Chromatographic resolution*. 7. Useful chiral
773 packing materials for high-performance liquid chromatographic resolution of enantiomers:
774 phenylcarbamates of polysaccharides coated on silica gel, *J. Am. Chem. Soc.* 106 (1984) 5357-
775 5359. <https://doi.org/10.1021/ja00330a057>.
- 776 78. W.L. Hinze, T.E. Riehl, D.W. Armstrong, W. DeMond, A. Alak, T. Ward, Liquid
777 chromatographic separation of enantiomers using a chiral. beta-cyclodextrin-bonded stationary
778 phase and conventional aqueous-organic mobile phases, *Anal. Chem.* 57 (1985) 237-242.
779 <https://doi.org/10.1021/ac00279a055>.
- 780 79. D.W. Armstrong, Y. Tang, S. Chen, Y. Zhou, C. Bagwill, J.-R. Chen, Macrocyclic
781 antibiotics as a new class of chiral selectors for liquid chromatography, *Anal. Chem.* 66 (1994)
782 1473-1484. <https://doi.org/10.1021/ac00081a019>.
- 783 80. C.V. Hoffmann, R. Pell, M. Lämmerhofer, W. Lindner, Synergistic effects on
784 enantioselectivity of zwitterionic chiral stationary phases for separations of chiral acids, bases,
785 and amino acids by HPLC, *Anal. Chem.* 80 (2008) 8780-8789.
786 <https://doi.org/10.1021/ac801384f>.
- 787 81. N.M. Maier, W. Lindner, Stereoselective chromatographic methods for drug analysis,
788 *Chirality in drug research*. 33 (2006) 189-260.
- 789 82. B. Chankvetadze, C. Yamamoto, Y. Okamoto, Enantioseparation of selected chiral
790 sulfoxides using polysaccharide-type chiral stationary phases and polar organic, polar aqueous-
791 organic and normal-phase eluents, *J. Chromatogr. A*. 922 (2001) 127-137.
792 [https://doi.org/10.1016/S0021-9673\(01\)00958-X](https://doi.org/10.1016/S0021-9673(01)00958-X).
- 793 83. A. Karlsson, A. Aspegren, The use of mobile phase pH and column temperature to
794 reverse the retention order of enantiomers on chiral-AGP®, *Chromatographia*. 47 (1998) 189-
795 196. <https://doi.org/10.1007/BF02466580>.
- 796 84. M. Okamoto, Reversal of elution order during the chiral separation in high performance

797 liquid chromatography, *J. Pharmaceut. Biomed.* . 27 (2002) 401-407.
798 [https://doi.org/10.1016/S0731-7085\(01\)00646-X](https://doi.org/10.1016/S0731-7085(01)00646-X).

799 85. R.W. Stringham, J.A. Blackwell, Factors that control successful entropically driven chiral
800 separations in SFC and HPLC, *Anal. Chem.* 69 (1997) 1414-1420.
801 <https://doi.org/10.1021/ac9609283>.

802 86. K. Pihlainen, R. Kostianen, Effect of the eluent on enantiomer separation of controlled
803 drugs by liquid chromatography–ultraviolet absorbance detection–electrospray ionisation tandem
804 mass spectrometry using vancomycin and native β -cyclodextrin chiral stationary phases, *J.*
805 *Chromatog. A.* 1033 (2004) 91-99. <https://doi.org/10.1016/j.chroma.2003.12.073>.

806 87. G. Hellinghausen, D.A. Lopez, J.T. Lee, Y. Wang, C.A. Weatherly, A.E. Portillo, A.
807 Berthod, D.W. Armstrong, Evaluation of the Edman degradation product of vancomycin bonded
808 to core-shell particles as a new HPLC chiral stationary phase, *Chirality.* 30 (2018) 1067-1078. 809
<https://doi.org/10.1002/chir.22985>.

810 88. C. Handbook, A guide to using macrocyclic glycopeptide bonded phases for chiral LC
811 separations, Advanced Separation Technologies Inc. (2004) 1.

812 89. J. Bagnall, S. Evans, M. Wort, A. Lubben, B. Kasprzyk-Hordern, Using chiral liquid
813 chromatography quadrupole time-of-flight mass spectrometry for the analysis of pharmaceuticals
814 and illicit drugs in surface and wastewater at the enantiomeric level, *J. Chromatogr., A.* 1249
815 (2012) 115-129. <https://doi.org/10.1016/j.chroma.2012.06.012>.

816 90. E. Castrignanò, A. Lubben, B. Kasprzyk-Hordern, Enantiomeric profiling of chiral drug
817 biomarkers in wastewater with the usage of chiral liquid chromatography coupled with tandem
818 mass spectrometry, *J. Chromatogr., A.* 1438 (2016) 84-99.
819 <https://doi.org/10.1016/j.chroma.2016.02.015>.

820 91. H.-R. Buser, T. Poiger, M.D. Müller, Occurrence and environmental behavior of the
821 chiral pharmaceutical drug ibuprofen in surface waters and in wastewater, *Environ. Sci. Technol.*
822 33 (1999) 2529-2535. <https://doi.org/10.1021/es981014w>.

823 92. L.J. Fono, D.L. Sedlak, Use of the chiral pharmaceutical propranolol to identify sewage
824 discharges into surface waters, *Environ. Sci. Technol.* 39 (2005) 9244-9252.
825 <https://doi.org/10.1021/es047965t>.

826 93. V. Matamoros, M. Hijosa, J.M. Bayona, Assessment of the pharmaceutical active
827 compounds removal in wastewater treatment systems at enantiomeric level. Ibuprofen and
828 naproxen, *Chemosphere.* 75 (2009) 200-205. <https://doi.org/10.1016/j.chemosphere.2008.12.008>.

829 94. B. Kasprzyk-Hordern, V.V. Kondakal, D.R. Baker, Enantiomeric analysis of drugs of
830 abuse in wastewater by chiral liquid chromatography coupled with tandem mass spectrometry, *J.*
831 *Chromatogr., A.* 1217 (2010) 4575-4586. <https://doi.org/10.1016/j.chroma.2010.04.073>.

832 95. US. Environmental Protection Agency Method 1694: Pharmaceuticals and Personal Care
833 Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS. Office of Water, Office of
834 Science and Technology Engineering and Analysis ...; 2007.

835 96. K. Kümmerer, Pharmaceuticals in the environment, *Annu. Rev. Env. Resour.* 35 (2010)
836 57-75. <https://doi.org/10.1146/annurev-environ-052809-161223>.

837 97. S.L. MacLeod, P. Sudhir, C.S. Wong, Stereoisomer analysis of wastewater-derived β -
838 blockers, selective serotonin re-uptake inhibitors, and salbutamol by high-performance liquid
839 chromatography–tandem mass spectrometry, *J. Chromatogr., A.* 1170 (2007) 23-33.
840 <https://doi.org/10.1016/j.chroma.2007.09.010>.

841 98. L.N. Nikolai, E.L. McClure, S.L. MacLeod, C.S. Wong, Stereoisomer quantification of
842 the β -blocker drugs atenolol, metoprolol, and propranolol in wastewaters by chiral high-
843 performance liquid chromatography–tandem mass spectrometry, *J. Chromatogr., A.* 1131 (2006)

844 103-109. <https://doi.org/10.1016/j.chroma.2006.07.033>.

845 99. B. Petrie, K. Proctor, J. Youdan, R. Barden, B. Kasprzyk-Hordern, Critical evaluation of
846 monitoring strategy for the multi-residue determination of 90 chiral and achiral micropollutants
847 in effluent wastewater, *Sci. Total Environ.* 579 (2017) 569-578.

848 <https://doi.org/10.1016/j.scitotenv.2016.11.059>.

849 100. J. Płotka-Wasyłka, A new tool for the evaluation of the analytical procedure: Green
850 Analytical Procedure Index, *Talanta.* 181 (2018) 204-209.

851 <https://doi.org/10.1016/j.talanta.2018.01.013>.

852