Detection of Pharmaceuticals in the Environment

Keila Isaac-Olivé and Amado Enrique Navarro-Frómeta

Contents

Abstract In this chapter, for articles addressing drugs as pollutants of natural and wastewater, we discuss the temporal distribution of studies, as well as the main countries of origin for such studies. Focus is given to those articles with more than 200 citations. The main sampling techniques, sample extraction, and detection methods accepted by the USEPA and USGS (based on LC-MS/MS and LC-MS) are reviewed, as well as those methods not based on MS, between 2014 and 2017. From this analysis, we propose directions for future research. Finally, a case study is presented that addresses the analysis of the surface waters of the Izúcar de Matamoros region and the Atoyac River in Puebla, as well as the Zahuapan River in Tlaxcala, Mexico. By GC/MS, quantitative determination of naproxen, diclofenac, and triclosan, at stations on the Zahuapan (Tlaxcala), Atoyac, and Nexapa (Puebla) rivers, was performed during the period of 2012–2013. We found that 30% of the publications related with drugs as pollutants of natural and wastewater were published between 2014 and 2017. Spain has published the most of such articles (accounting for 25% of all publications). Three of the articles have more than 1,000

K. Isaac-Olivé
Facultad de Medicina, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico

A.E. Navarro-Frómeta (✉)
Universidad Tecnológica de Izúcar de Matamoros, Puebla, Mexico
e-mail: navarro4899@gmail.com

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citations. HPLC/MS/MS was the most commonly used method, followed by GC/MS. However, less sophisticated detection alternatives were also used. GC/MS was used to demonstrate the presence of naproxen, diclofenac, triclosan, and carbamazepine in the Mexican Central Plateau and the inability of the wastewater treatment plants (WWTPs) to remove completely these contaminants.

Keywords Analytical methods, Contaminant, Pharmaceuticals, Surface waters, Wastewaters

1 Introduction

On August 10 1897, the German chemist Felix Hoffmann (under Arthur Eichengrün's direction) discovered acetylsalicylic acid, a substance that has analgesic, anti-inflammatory, and antipyretic properties. Two years later, in 1899, the pharmaceutical company Bayer began its commercialization under the name Aspirin [1]. Thirty-one years later, in September 1928, Sir Alexander Fleming discovered penicillin, likely the most widely used antibiotic in the world [2]. These two discoveries, which occurred 118 and 89 years ago, respectively, could be considered as the beginning of an era of environmental contamination by drugs. This is because the treated organism does not absorb 100% of the medicine, and the waste water treatment plants (WWTPs) do not remove them completely. Therefore, the drug returns to the water. The rapid rise of the pharmaceutical industry and the overuse of drugs have increased the antibiotic resistance of bacteria, as well as the levels of these drugs in the surface waters, often at concentrations detrimental to the ecosystem and human health.

However, the first works on the determination of pharmaceuticals in natural waters did not appear until around 80 years after the onset of environmental contamination by drugs. In 1977, Hignite and Azarnoff quantified salicylic acid levels in sewage water effluent [3]. In 1990, Aherne, Hardcastle, and Nield quantified the levels of the anticancer drug bleomycin in sewage treatment works effluent, river, and drinking water samples [4]. However, it was not until the late 1990s that this topic began to be of interest to the scientific community. Figure 1 shows the temporal distribution of published articles addressing the amounts of drugs in natural waters (e.g., rivers, lakes, drinking water, tap water, wastewater, and treatment plant effluents), excluding seawater, which appear in the Scopus database.

We detected a rapid increase in the number of such publications beginning around the year 2000 (Fig. 1). It is worth mentioning that prior to this, there had been several studies investigating drug levels (mainly antibiotics) in other matrices, such as foods (milk and fruits), working areas, and in the soil. Such studies are still being done now. However, here we focus only on those studies addressing natural and wastewaters.

Commonly detected concentrations of drugs in natural and wastewater are in the range of ng L^{-1} to μg mL^{-1}, depending on the drug. These values are sufficient to have a toxic effect on the environment but low enough to require analytical methods

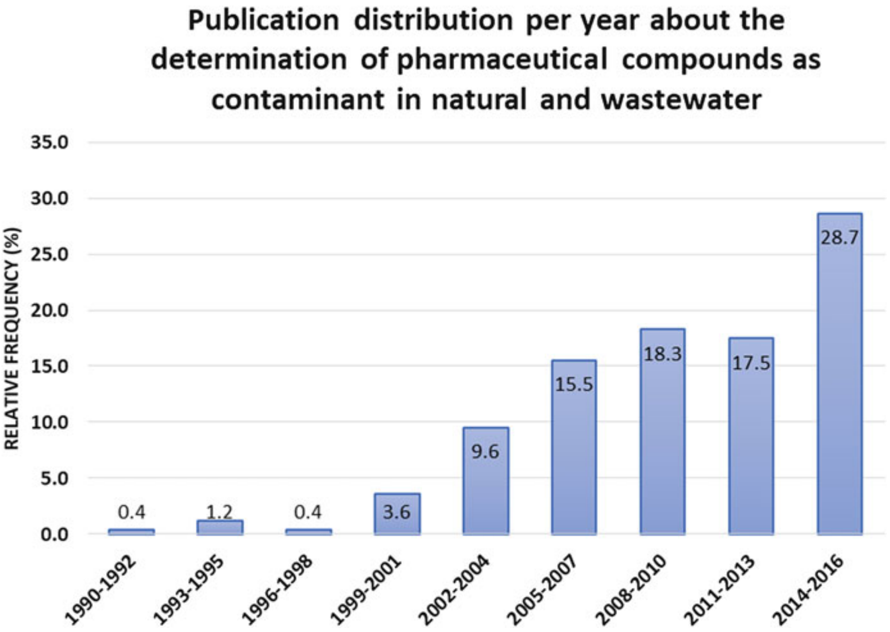

Fig. 1 Number of articles per year (until 2016) related to the determination of pharmaceutical contaminants in waters (excluding seawater) reported in Scopus

of high sensitivity and low detection limit. This might account for the almost 100-year lag between the widespread use of aspirin and penicillin and serious scientific interest in drug contamination of natural and wastewaters.

Most of the published papers are based on the combination of solid-phase extraction (as the separation and concentration method) and MS/MS detection (mass spectrometry). Both of these methods were first developed in the 1990s and were widely introduced in analytical methods by the early 2000s, coinciding with the first serious studies investigating drug levels in natural and residual waters. Also around this time, concern about antibiotics resistance and environmental contamination began to grow. Thus, prior to the year 2000, there was neither the technical ability nor societal interest to determine the concentration of drugs (e.g., antibiotics, NSAIDs, beta-blockers, antidepressants, anticancer drugs, and hormones) and their metabolites in natural and wastewater.

Research groups from 12 countries account for 72% of publications investigating drug levels in natural and wastewater (Fig. 2). European countries (Spain, Germany, France, Portugal, Poland, Italy, and the Czech Republic) account for 47% of these publications. Spanish groups are most productive, accounting for almost 25% of these publications. Several of the identified articles (including reviews) have received more than 200 citations (Table 1).

Article numbers 3, 5, and 7 from Table 1 have more than 1,000 citations each and can be considered the classic articles in this field. From Fig. 1, it can be seen that the

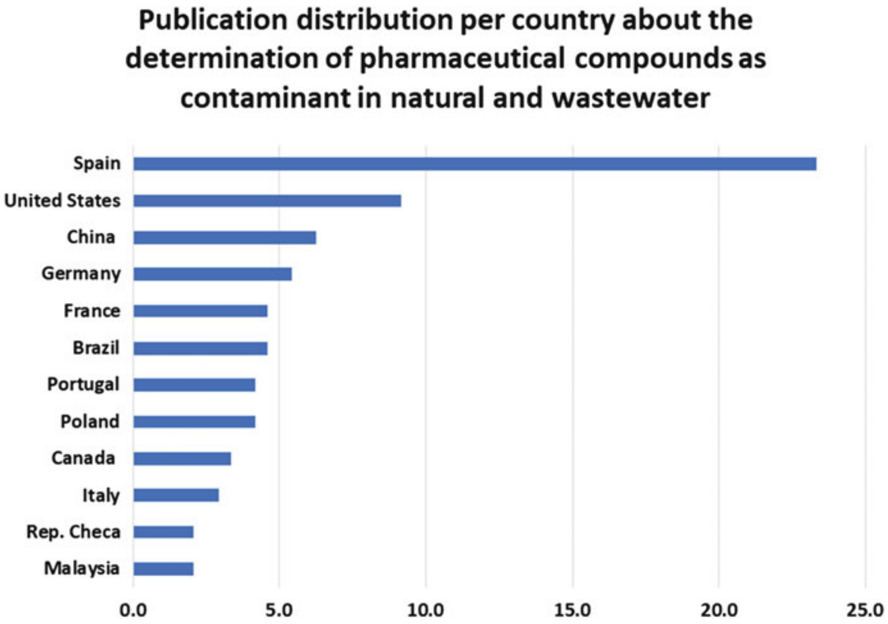

Fig. 2 Countries with the highest percent of publications related to the determination of drugs in natural and wastewaters. Source: Scopus

number of published articles plateaued between 2002 and 2013, before again increasing rapidly. Almost 30% of the identified articles were published between 2014 and 2017.

Here, we analyze the methods (including sampling, concentration, separation, and detection methods) used in studies investigating the pharmaceutical contaminants in surface and wastewater published since 2014. We also describe the findings of a case study entitled "The determination of some pharmaceuticals in the central Plateau of Mexico using GC-MS."

2 Sampling Methods

Without a good sampling protocol, it is impossible to obtain reliable analytical results. For this reason, numerous sampling recommendations and standards have been developed by various agencies and countries. Among the international methods, the ISO 5667 standards are recognized by the World Health Organization [25]. As an example of a regional standard, in Mexico, the National Program of INECC-Sampling is recommended by the Institute of Ecology of Mexico for the sampling of priority substances in priority matrices [26].

The objective of sampling is to obtain a sample representing, as accurately as possible, the environment from which it is taken. At the same time, it must be

Table 1 Scientific articles with more than 200 citations each about the determination of pharmaceuticals as contaminants in natural and wastewaters

No.	Title of the article	Year	Reference
1	Drugs and drug metabolites as environmental contaminants: Chlorophenoxyisobutyrate and salicylic acid in sewage water effluent	1977	[3]
2	Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil	1999	[5]
3	Occurrence of antibiotics in the aquatic environment	1999	[6]
4	Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands	1999	[7]
5	Behavior and occurrence of estrogens in municipal sewage treatment plants – I. Investigations in Germany, Canada and Brazil	1999	[8]
6	Determination of drug residues in water by the combination of liquid chromatography or capillary electrophoresis with electrospray mass spectrometry	2001	[9]
7	Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: A review of recent research data	2002	[10]
8	Pharmaceuticals, personal care products, and endocrine disruptors in water: Implications for the water industry	2003	[11]
9	Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, USA and Ontario, Canada	2003	[12]
10	Determination of antibiotic residues in manure, soil, and surface waters	2003	[13]
11	Strategic survey of therapeutic drugs in the rivers Po and Lambro in Northern Italy	2003	[14]
12	Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction-liquid chromatography-mass spectrometry	2004	[15]
13	Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromsø/Norway with emphasis on ibuprofen and its metabolites	2004	[16]
14	Determination of pharmaceutical compounds in surface- and groundwater samples by solid-phase extraction and high-performance liquid chromatography-electrospray ionization mass spectrometry	2004	[17]
15	Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant	2004	[18]
16	Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters	2005	[19]
17	Liquid chromatography-tandem mass spectrometry for the analysis of pharmaceutical residues in environmental samples: A review	2005	[20]
18	Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges	2006	[21]
19	Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography-positive electrospray ionisation tandem mass spectrometry	2007	[22]
20	Occurrence and fate of antibiotics in the Seine River in various hydrological conditions	2008	[23]
21	Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation	2010	[24]

Source: Scopus

sufficiently small that it is safe to handle in the laboratory yet sufficiently large to ensure the necessary amount of analytics for determination. Thus, the following considerations are used when planning sampling protocols: objective of the sampling; characteristics of the target (e.g., water body, soil); location of the sampling points and their accessibility; frequency and type of sampling; material to be used in the sampling and preparation; requirements for the preparation, transport, and conservation of samples; the logistical aspects, including occupational safety and health measures to be observed by the staff.

There are three basic types of samples. Grab samples are those collected at a specific time and place and thus provide only a snapshot of what exists. Therefore, composite samples are preferable, which are obtained by mixing proportions according to the quantities and mass flows at the time and sampling point (e.g., by mixing samples taken every 24 h in a discharge of treated wastewater according to the measured flow at each sampling time). These samples are more useful for determining the concentrations of the target analyte [27]. In some cases, it might be possible to use autosamplers [28].

In recent years, passive sampling, which is representative of the actual concentrations of the analytes and their variability over time, has received increasing attention. Passive sampling involves the use of devices that do not have moving or mechanical parts, that do not require energy or supervision during use, and that are able to retain the target analyte(s). Two of the most commonly used passive samplers for organic contaminants are the semipermeable membrane device (SPMD) and the polar organic chemical integrative sampler (POCIS). For example, the POCIS is adequate for sampling water-soluble organic contaminants, thus providing time-weighted average concentrations of the analytes over deployment periods that range from 1 week to several months [29, 30].

3 Methods of Sample Extraction (Clean Up)

A major problem in the analysis of pharmaceuticals in environmental samples is their low concentrations. Therefore, extraction and concentration techniques are of great importance. As with other parts of the analytical procedure, the implementation of the proper extraction and concentration technique of the target analytes depends not only on the analytes and the environmental matrix but also on the practical limitations of the laboratory.

Solid-phase extraction (SPE) with cartridges of selective sorbents with different Hydrophilic-Lipophilic Balance (HLB) is the most used procedure in the analysis of pharmaceuticals. In this approach, the sample is passed through the sorbent, and the analytes of interest are retained in the cartridge. Subsequently, the target analytes are eluted with a suitable organic solvent. The simultaneous analysis of many different substances that differ in their physical and chemical properties implies that their optimal conditions of extraction could not be achieved. Therefore, new phases, combinations of solvents, and experimental conditions for the removal of specific groups of analytes are continuously reported in the literature. In addition to the SPE cartridges, SPE disks, which enable the handling of larger sample flows, thereby shortening the time of analysis, are also used. Although SPE is typically performed off-line, online SPE, in which the separation device is automated and attached to the detection devices, has been described [31, 32].

Lyophilization, ultrasound-assisted extraction, and liquid-liquid extraction continue to be used but less frequently. The miniaturization of extraction techniques has also improved efficiency and effectiveness. Examples include dispersive liquid-liquid microextraction in which some microliters of the extraction solvent are mixed with a few milliliters of a dispersive organic solvent and then injected into the aqueous sample, forming a cloud of organic droplets, thus producing a very large area of contact and enabling efficient extraction of the analytes. Remarkable advances have also been made in the use of solid-phase microextraction, which is based on a similar principle as SPE. Stir bar sorptive extraction is another effective procedure, in which a bar magnet coated with a suitable polymer is introduced into the sample and, after a time, when equilibrium is reached, the analyte is desorbed with an appropriate solvent [31, 32].

Between 2014 and 2017, the cleanup methods have changed little. During this period, about 64% of cleanup methods were based on extraction by SPE cartridges (e.g., OASIS HLB, Strata-X, ODS, Hypersil GOLD), and 26% have used similar methods (e.g., stir bar sorptive extraction [SBSE] and liquid desorption [LD] [33, 34], extraction based on ionic liquids [35, 36], styrene-divinylbenzene-based extraction [SDB] covered by a polyethersulfone [PES] membrane [37], extraction based on SPE disk [38–40], and other sorbent materials such as graphene [41], fabric phase sorptive extraction [42], molecularly imprinted polymers [43], and polydimethylsiloxane [PDMS] tubing fashioned into a loop) [44]. The remaining 10% of studies have used methods that do not require a cleanup procedure since the detection is performed directly in the untreated sample.

By analyzing the recent literature, we detected a tendency to develop new adsorbent materials for use in the cleanup process. These materials have been designed either to detect a compound or group of similar compounds in a sample or as materials of high adsorption capacity for many different compounds. It is preferred that these materials are eco-friendly (i.e., that they do not require solvents for the desorption of the analyte [e.g., thermal desorption] or that they require solvents of low toxicity and very low volume). It is worth mentioning that the development of adsorbent materials based on nanostructures has the potential to revolutionize the cleanup process because it is expected that the adsorption capacity, and hence the concentration of the analyte, will be much greater for such materials than for conventional materials based on microstructures.

4 Separation, Detection, and Identification

The gas and liquid chromatography separation techniques are the most commonly used in the analysis of pharmaceuticals, especially the latter. The separation of organic substances has been advanced by the development of reversed-phase liquid chromatography, which allows the use of polar substances as solvents for the chromatographic separation. The most used phase is C18. In recent years, the development of UHPLC, which enables a higher resolution and greater peak capacity, has expanded the possibilities for pharmaceutical analyses in environmental matrices. The most relevant aspects of the development of both chromatographic techniques can be found in classical texts [45–47].

The development of atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) interfaces has enabled the successful coupling of liquid chromatography with mass spectrometry (MS), high-resolution mass spectrometry (HRMS), and tandem mass spectrometry (MS/MS), using triple quadrupoles, hybrid quadrupole/time-of-flight (Q-TOF), and ion-trap mass spectrometers, among others. As mentioned above, the choice between methods mostly depends on the economic resources of the laboratories. For this reason, gas chromatography coupled with mass spectrometry is used for the detection of the most volatile compounds, being necessary the derivatization that makes the GC/MS a more laborious and time-consuming method. Given that the most important aspect of detection and identification by mass spectrometry is the ionization of the compounds present in the sample mixture, which then reach the MS, it is sometimes necessary to use additives to improve the efficiency of the ionization. This also explains the advantage of ESI in the analysis of pharmaceuticals due to the possibility of change between negative (in the analysis of acidic compounds [e.g., antibiotics]) and positive mode (in the analysis of neutral and basic analytes). It is necessary to emphasize that, for the same compound, the observed fragments might differ depending on experimental conditions and the instrument used. It is also important to consider the interference and the matrix. The APCI and the use of labeled standards can reduce these interferences, but these standards are not always available for all of the compounds of interest [31, 32, 48].

Finally, it should be noted that very powerful and sophisticated instrumentation alone is insufficient. Expert technical knowledge is also required for their proper operation, as well as for proper selection of the column and the ability for troubleshooting [45].

EPA-1694 is the most widely used HPLC/MS/MS method [49]. This method uses the SPE and HPLC/MS/MS for the analysis of many pharmaceuticals in water. The analytes are divided into four groups according to their physical and chemical properties. Water samples are treated using an acid pH (2) first, and an alkaline pH (10) is employed later for separation by SPE. The analysis includes four different conditions for the chromatographic analysis (column, mobile phase, and gradient) and the detection and analysis by MS/MS (ESI+ and ESI−), depending on the polarity and extraction conditions of the compounds. Although this is not a regulatory method, it is a great starting point for the development of a method adjusted to the needs and analytes-of-interest, which explains the development of many variations of the standard method [50].

Another useful method is the USGS method for the analysis of 14 pharmaceuticals in water by HPLC/MS [51]. In this method, the sample filtrate is separated by SPE with a cartridge of styrene-divinylbenzene. The extracts are reduced under nitrogen and then reconstituted with the primary eluent used in HPLC. The chromatography is carried out using a reverse-phase octadecylsilane HPLC column, which is coupled to an electrospray ionization interface and quadrupole mass spectrometer for detection, identification, and quantitation. Regarding the GC/MS methods, their successful application depends on the column selection and analysis conditions, including the derivatization that will permit the identification and quantification of the desired analyte.

As stated above, LC-MS/MS remains the most used analytical technique for the analysis of pharmaceutical contaminants in waters, followed by gas GC/MS. This tendency can be seen in the recently published work. For example, of the seven articles addressing this subject that were published in 2017, four of them employed LC-MS/MS as a detection technique [33, 52–54] and two used GC/MS [44, 55]. The choice of technique depends on the volatility and polarity of the analyte(s) of interest, as well as the number of analytes in the sample. For instance, using LC-MS/MS, Robles-Molina et al. [56] and Moschet et al. [37] quantified 400 and 322 analytes, respectively, whereas Sghaier et al. [55] determined 13 using GC/MS. Both methods reach detection limits ranging from ng L^{-1} to μg L^{-1}. However, these are not the only methods available. Table 2 shows methods used for the same purpose in the period 2014–2017, which are not based on MS and are sometimes more accessible and reach equally low detection limits.

About 60% of the scientific works included in Table 2 correspond to research groups from countries that are not listed in Fig. 2 such as South Africa, Viet Nam, Argentina, Mexico, and Iran. Similarly, 70% of works in Table 2 were done in developing countries (South Africa, Argentina, Viet Nam, Mexico, Brazil, and Iran), where sophisticated MS/MS-based techniques are not widely available. This, however, drives the development of analytical methods based on more conventional techniques. Although these methods are less versatile, since they do not allow the detection of many analytes, they are efficient in the detection of those for which they are designed. For example, conventional analytical methods such as fluorescence have great potential when they are coupled to multivariate calibration algorithms, as it is shown in Table 2 [39, 64].

5 A Case Study: Some Pharmaceuticals in the Central Plateau of Mexico Detected by GC/MS

The results presented here include the determination of naproxen (NAP), diclofenac (DCFN), carbamazepine (CMP), and triclosan (TCS) in the Nexapa river and the discharges of wastewater from the cities of Atlixco and Izúcar de Matamoros

Table 2 Analytical methods employed from 2014 to 2017 for analyzing pharmaceutical compounds in waters which are not based on mass spectrometry (MS)

Analytes	Separation and detection methods	Limit of detection or quantification	Year/country/ reference
Benzodiazepines	GC-FID: FID, flame ionization detector	LOD (μg L^{-1}), 0.02–0.05	2014/Iran/[57]
17α-Ethinylestradiol (EE2)	HPLC-FD: FD, fluorescence detection	LOQ (ng L^{-1}), 2.1	2014/Brazil/[58]
Estrogenic compounds	HPLC-DAD-FD: DAD, diode array detector; FD, fluorescence detection	LOD (μg L^{-1}), FD: 0.30–0.57, DAD: 13.8–37.1	2014/Spain/[35]
Diclofenac	No separation method. Detection: fluorescence of the dye released in solution after diclofenac binds cyclodextrin-based polymer	LOD (μmol L^{-1}), 1	2015/Switzerland/ [59]
Alprazolam	No separation method. Detection: differential pulse adsorptive cathodic stripping voltammetry (PD-AdsCSV)	LOQ (μg L^{-1}), 0.4	2015/Brazil/[60]
Carbamazepine 17-α-ethinylestradiol (EE2), endocrine disruptor (bisphenol A)	LC-PAD: LC, liquid chromatography on Onyx Monolithic C18 column; PAD, photodiode array detector	LOD (ng L^{-1}), 0.01–209	2015/Mexico/[61]
Carbamazepine, glyburide, and three endocrine disruptors	SFC-DAD: SFC, supercritical fluid chromatography; DAD, diode array detection	LOD (μg L^{-1}), 0.10–1.59	2015/Mexico/[62]
Carbamazepine, ofloxacin, piroxicam	No separation method. Detection: excitation-emission photoinduced fluorescence	LOD (ng mL^{-1}), 0.04–0.3	2015/Argentina/ [39]
Fluoroquinolones	HPLC-FD: FD, fluorescence detection	LOD (ng L^{-1}), 2	2015/Italy/[41]
Estrogens and metabolites	UPLC-FD: FD, fluorescence detection	LOD (ng mL^{-1}), 0.18–0.45	2015/Spain/[43]
Various (8)	Capillary electrophoresis (CE) with capacitively coupled contactless conductivity detection (C4D)	LOD (mg L^{-1}), 0.2–0.8	2016/Viet Nam/[63]
Estrogens, progestogens, and androgens	HPLC-PDA-FD-MCR-ALS: PDA, photodiode array; FD, fluorescence detector; MCR-ALS, multivariate curve resolution/ alternating least squares	LOD (ng L^{-1}), 6–24	2016/Argentina/ [64]
Steroid hormones, 17β-estradiol (E2), and estriol (E3)	HPLC-DAD: DAD, diode array detector	LOD (μg L^{-1}), 1.14–2.510	2017/South Africa/ [65]

(between 2003 and 2005 as part of a project funded by the CONACyT, SIZA-20020803007), as well as stations on the Zahuapan (Tlaxcala) and Atoyac (Puebla) and Nexapa rivers (Puebla) (between 2012 and 2013 as part of project 11-CAP2-1756, funded by the Spanish Agency for International Development Cooperation). Although it is not a pharmaceutical, TCS is associated with the use of drugs and hospital facilities and, as such, is a useful indicator of the intensity of pharmaceutical discharge into water bodies.

Table 3 shows the location of the sampling stations on the studied rivers. The location of the sample sites in the subbasin of the Nexapa river can be found elsewhere (Supplemental Fig. S2 of [66]). Sampling was conducted in accordance with the procedures set out in the ISO 5667 series concerning the sampling of surface water and the NMX-003-1980 that regulates the sampling of wastewater.

Relevant details about the sampling points include that Point 2 is located at the site of impact of a transfer to the Nexapa river of 4 $m^3 s^{-1}$ of heavily polluted waters of the Atoyac River, after its passage through the City of Puebla. Point 7 is in the Zahuapan River at the entrance of the Tlaxcala State, while Point 8 is located after the passage of the river through the city of Tlaxcala. Point 9 is located before the exit of the Atoyac River from the city of Puebla. The details of the analytical procedures can be found in [66, 67].

Figure 3 shows the profiles of the concentrations of the studied compounds during the period of 2003–2005, adjusted by distance-weighted least squares. The least contaminated point, located near the source of the Nexapa river, shows the lowest concentrations. The impact of the Atoyac contaminated water transferred to the Nexapa river is reflected in the increased concentrations of the compounds at Point 2, with an attenuation downstream until Point 6, which is located after the town of Izúcar de Matamoros, where the contaminants increase because of the contributions of wastewater from the city, which at that time was without a WWTP.

The concentrations in the discharges of the cities of Atlixco and Izúcar de Matamoros were about ten times greater than those found in the Nexapa river (data not shown), which confirms that it is the use, excretion, and inadequate disposal of pharmaceuticals that account for the presence of these contaminants in the surface waters.

Table 3 Coordinates of the sampling points in the highlands, 2012–2013

Point	North latitude	West longitude	Height (m)
Los Molinos (1)	57,021° 18’	23,393° 98’	1,927
Santo Domingo Atoyatempan (2)	54,235° 18’	22,616° 98’	1,821
Las Fajanas (3)	48,662° 18’	25,984° 98’	1,636
Alchichica (4)	37,617° 18’	28,091° 98’	1,353
Puctla 1 (5)	34,055° 18’	28,359° 98’	1,240
Puctla 2 (6)	18° 33. 624’	98° 28. 777’	1,250
Zahuapan (Tlaxco) (7)	37,284° 19’	5,694° 98’	2,654
Zahuapan (City of Tlaxcala) (8)	19,248° 19’	15,204° 98’	2,229
Atoyac (City of Puebla) (9)	19° 01. 051’	98° 14. 275’	2,104

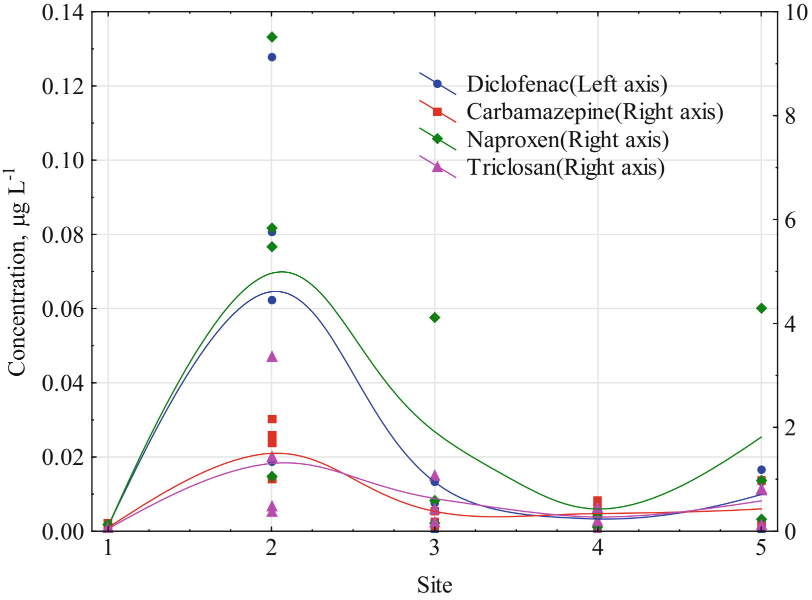

Fig. 3 Concentrations of the drugs analyzed during 2003–2005 in the Nexapa river stations from Los Molinos to Puctla

Table 4 shows the concentrations of some of the compounds studied during 2012–2013. From these data, it can be concluded that in their passage through the cities, the rivers receive high inputs of the pharmaceuticals. The data in Table 5, obtained from samples taken at the WWTP before the first settler (Point 51), before the biofilter (Point 52), before the second settler (Point 53), and prior to the chlorination for discharge to the river (Point 54), indicate the insufficient removal of the drugs in a conventional WWTP.

This case study demonstrates that it is possible to study the impact of pollution caused by the anthropic use and disposal of pharmaceuticals to the environment using GC/MS as a tool, which is more affordable than LC-MS or LC-MS/MS approaches that have high acquisition, operation, and maintenance costs.

6 Future Perspectives

The determination of drugs in the environment is likely to be of ongoing interest to the scientific community. Such monitoring of water, soil, plants, and food is likely to become routine. There is now a need to regulate the maximum permissible and tolerable concentration, and, once established, these will need to be revised periodically. Therefore, it is necessary to have effective, affordable, and inexpensive methods for this task.

Table 4 Descriptive statistics of some of the target compounds studied in the Zahuapan, Nexapa, and Atoyac rivers (μg L^{-1})

	Mean ± SD ‖ median(min; max), annual	Mean(min;max), annual		
	Nexapa	Atoyac	Zahuapan	Tlaxco
TCS	1.89 ± 2.06 ‖ 0.94(0.00;6.27)	1.42(0.29;2.97)	7.45(0.31;15.32)	0.03(0.00;0.05)
NAP	1.43 ± 1.61 ‖ 1.04(0.03;8.69)	0.91(0.73;1.22)	0.95(0.36;1.56)	0.04(0.01;0.06)
DCFN	0.12 ± 0.13 ‖ 0.08(0.00;0.55)	0.15(0.09;0.24)	0.28(0.12;0.37)	0.00(0.00;0.01)

Table 5 Removal of drugs in the WWTP of Izúcar de Matamoros (concentrations, μg L^{-1}, mean ± SD)

Point	51	52	53	54
TCS	19.34 ± 6.87	14.26 ± 5.30	5.76 ± 1.68	5.80 ± 1.32
NAP	1.89 ± 0.89	1.61 ± 0.89	2.20 ± 0.41	2.06 ± 0.12
DCFN	0.60 ± 0.13	0.58 ± 0.03	0.46 ± 0.63	0.57 ± 0.02

Little is known about pharmaceutical contamination of water in Africa. There is a recent review work highlighting the scarcity of this type of research in this continent [68]. Most parts of Latin America, Asia, and Australia have the same situation [69]. Also, little is known about the coefficients of transfer of water to land, crops, animals, and humans. It is necessary to develop mathematical models to identify the main sources of contamination and generalize the toxicity study models.

It is clear that at present there is a great concern for the presence of drugs (among other emerging contaminants) in the environment. International agencies such as the WHO and other national agencies of great relevance, such as the EPA and even civil society organizations begin to pay increasing attention to this problem. However, the focus of attention has been directed to inland waters, and it is necessary to pay greater attention to the presence of the drugs into the marine environment [70, 71].

All this requires the development of methods with low detection limits (this does not necessarily mean that the method must be sophisticated or expensive). Development is required in new adsorbent materials, which have a greater capacity of adsorption, thereby obtaining higher coefficients of concentration. The development of multivariate methods along with specific sensors for ELISA type of assays would contribute to in situ determinations that allow real-time monitoring campaigns.

It is necessary to identify effective methods for degrading or eliminating these compounds from natural and residual waters. The use of natural treatment systems such as constructed wetlands shows high potential over other more expensive technologies [72, 73]. Associated with this, it is also necessary to improve the quality control of the determinations that are made. This will require certified reference materials that will allow methods to be validated and inter-comparative studies to be made. Analytical chemistry must develop eco-friendly and sensitive methods that require small sample volumes that respond in a short time with precision and accuracy, reach low detection limits, and are accessible to most scientists. Only in this way will it be possible to monitor the health of the planet and, thus, to preserve life.

References

1. Sneader W (2000) The discovery of aspirin: a reappraisal. BMJ 321(7276):1591–1594
2. Diggins FWE (1999) The true history of the discovery of penicillin, with refutation of the misinformation in the literature. Br J Biomed Sci 56(2):83–93
3. Hignite C, Azarnoff DL (1977) Drugs and drug metabolites as environmental contaminants: chlorophenoxyisobutyrate and salicylic acid in sewage water effluent. Life Sci 20(2):337–341
4. Aherne GW, Hardcastle A, Nield AH (1990) Cytotoxic drugs and the aquatic environment: estimation of bleomycin in river and water samples. J Pharm Pharmacol 42(10):741–742
5. Stumpf M, Ternes TA, Wilken RD, Rodrigues SV, Baumann W (1999) Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. Sci Total Environ 225(1): 135–141
6. Hirsch R, Ternes T, Haberer K, Kratz KL (1999) Occurrence of antibiotics in the aquatic environment. Sci Total Environ 225(1):109–118
7. Belfroid AC, Van der Horst A, Vethaak AD, Schäfer AJ, Rijs GBJ, Wegener J, Cofino WP (1999) Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. Sci Total Environ 225(1):101–108
8. Ternes TA, Stumpf M, Mueller J, Haberer K, Wilken RD, Servos M (1999) Behavior and occurrence of estrogens in municipal sewage treatment plants – I. Investigations in Germany, Canada and Brazil. Sci Total Environ 225(1):81–90
9. Ahrer W, Scherwenk E, Buchberger W (2001) Determination of drug residues in water by the combination of liquid chromatography or capillary electrophoresis with electrospray mass spectrometry. J Chromatogr A 910(1):69–78
10. Heberer T (2002) Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol Lett 131(1):5–17
11. Snyder SA, Westerhoff P, Yoon Y, Sedlak DL (2003) Pharmaceuticals, personal care products, and endocrine disruptors in water: implications for the water industry. Environ Eng Sci 20(5): 449–469
12. Boyd GR, Reemtsma H, Grimm DA, Mitra S (2003) Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, USA and Ontario, Canada. Sci Total Environ 311(1):135–149
13. Christian T, Schneider RJ, Färber HA, Skutlarek D, Meyer MT, Goldbach HE (2003) Determination of antibiotic residues in manure, soil, and surface waters. Acta Hydrochim Hydrobiol 31(1):36–44
14. Calamari D, Zuccato E, Castiglioni S, Bagnati R, Fanelli R (2003) Strategic survey of therapeutic drugs in the rivers Po and Lambro in northern Italy. Environ Sci Technol 37(7): 1241–1248
15. Rodriguez-Mozaz S, de Alda MJL, Barceló D (2004) Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction–liquid chromatography–mass spectrometry. J Chromatogr A 1045(1):85–92
16. Weigel S, Berger U, Jensen E, Kallenborn R, Thoresen H, Hühnerfuss H (2004) Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromsø/Norway with emphasis on ibuprofen and its metabolites. Chemosphere 56(6):583–592

17. Cahill JD, Furlong ET, Burkhardt MR, Kolpin D, Anderson LG (2004) Determination of pharmaceutical compounds in surface-and ground-water samples by solid-phase extraction and high-performance liquid chromatography–electrospray ionization mass spectrometry. J Chromatogr A 1041(1):171–180
18. Carballa M, Omil F, Lema JM, Llompart M, García-Jares CK, Rodríguez I, Gómez M, Ternes T (2004) Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. Water Res 38(12):2918–2926
19. Lindqvist N, Tuhkanen T, Kronberg L (2005) Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. Water Res 39(11):2219–2228
20. Petrović M, Hernando MD, Díaz-Cruz MS, Barceló D (2005) Liquid chromatography–tandem mass spectrometry for the analysis of pharmaceutical residues in environmental samples: a review. J Chromatogr A 1067(1):1–14
21. Batt AL, Bruce IB, Aga DS (2006) Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges. Environ Pollut 142(2): 295–302
22. Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2007) Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry. J Chromatogr A 1161(1):132–145
23. Tamtam F, Mercier F, Le Bot B, Eurin J, Dinh QT, Clément M, Chevreuil M (2008) Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. Sci Total Environ 393(1):84–95
24. Rosal R, Rodríguez A, Perdigón-Melón JA, Petre A, García-Calvo E, Gómez MJ, Agüera A, Fernández-Alba AR (2010) Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. Water Res 44(2):578–588
25. WHO (World Health Organization) (2011) Guidelines for drinking-water quality, 4th edn. WHO, Geneva, p 541
26. INECC-CCA (2010) Manual de métodos de muestreo y preservación de muestras de las sustancias prioritarias para las matrices prioritarias del PRONAME. México, p 55
27. Migliaccio KW, Harmel D, Smiley PC (2010) Surface water quality sampling in streams and canals. In: Li Y, Migliaccio K (eds) Water quality concepts, sampling, and analyses. CRC Press, Boca Ratón
28. Anderson CW, Rounds SA (2010) Use of continuous monitors and autosamplers to predict unmeasured waterquality constituents in tributaries of the Tualatin River, Oregon. U.S. Geological Survey scientific investigations report 2010–5008, p 76
29. Alvarez DA (2010) Guidelines for the use of the semipermeable membrane device (SPMD) and the polar organic chemical integrative sampler (POCIS) in environmental monitoring studies. U.S. Geological Survey, techniques and methods 1–D4, p 28
30. Bayen S, Segovia E, Loh LL, Burger DF, Eikaas HS, Kelly BC (2014) Application of polar organic chemical integrative sampler (POCIS) to monitor emerging contaminants in tropical waters. Sci Total Environ 482-483:15–22
31. Primel E, Caldas S, Escarrone A (2012) Multi-residue analytical methods for the determination of pesticides and PPCPs in water by LC-MS/MS: a review. Special issue: recent advances in separation science. Open Chem 10(3):876–899
32. Dasgupta D, Sengupta TK (2015) Techniques and methods: detection of antibiotics in environmental samples. In: Mendez-Vilas A (ed) The battle against microbial pathogens: basic science, technological advances and educational programs. Formatex Research Center, Badajoz
33. Aparicio I, Martín J, Santos JL, Malvar JL, Alonso E (2017) Stir bar sorptive extraction and liquid chromatography–tandem mass spectrometry determination of polar and non-polar emerging and priority pollutants in environmental waters. J Chromatogr A 1500:43–52
34. Pintado-Herrera MG, González-Mazo E, Lara-Martín PA (2014) Atmospheric pressure gas chromatography–time-of-flight-mass spectrometry (APGC–ToF-MS) for the determination of

regulated and emerging contaminants in aqueous samples after stir bar sorptive extraction (SBSE). Anal Chim Acta 851:1–13

35. Socas-Rodríguez B, Hernández-Borges J, Asensio-Ramos M, Herrera-Herrera AV, Palenzuela JA, Rodríguez-Delgado MÁ (2014) Determination of estrogens in environmental water samples using 1, 3-dipentylimidazolium hexafluorophosphate ionic liquid as extraction solvent in dispersive liquid–liquid microextraction. Electrophoresis 35(17):2479–2487
36. Aftafa C, Pelit FO, Yalçinkaya EE, Turkmen H, Kapdan İ, Ertaş FN (2014) Ionic liquid intercalated clay sorbents for micro solid phase extraction of steroid hormones from water samples with analysis by liquid chromatography–tandem mass spectrometry. J Chromatogr A 1361:43–52
37. Moschet C, Vermeirssen EL, Singer H, Stamm C, Hollender J (2015) Evaluation of in-situ calibration of Chemcatcher passive samplers for 322 micropollutants in agricultural and urban affected rivers. Water Res 71:306–317
38. Caban M, Lis E, Kumirska J, Stepnowski P (2015) Determination of pharmaceutical residues in drinking water in Poland using a new SPE-GC-MS (SIM) method based on Speedisk extraction disks and DIMETRIS derivatization. Sci Total Environ 538:402–411
39. del Carmen Hurtado-Sánchez M, Lozano VA, Rodríguez-Cáceres MI, Durán-Merás I, Escandar GM (2015) Green analytical determination of emerging pollutants in environmental waters using excitation–emission photoinduced fluorescence data and multivariate calibration. Talanta 134:215–223
40. Křesinová Z, Linhartová L, Petrů K, Krejčová L, Šrédlová K, Lhotský O, Kameník Z, Cajthaml T (2016) Method for analysis of psychopharmaceuticals in real industrial wastewater and groundwater with suspended organic particulate matter using solid phase extraction disks extraction and ultra-high performance liquid chromatography/time-of-flight mass spectrometry. J Chromatogr A 1440:15–22
41. Speltini A, Sturini M, Maraschi F, Consoli L, Zeffiro A, Profumo A (2015) Graphene-derivatized silica as an efficient solid-phase extraction sorbent for pre-concentration of fluoroquinolones from water followed by liquid-chromatography fluorescence detection. J Chromatogr A 1379:9–15
42. Racamonde I, Rodil R, Quintana JB, Sieira BJ, Kabir A, Furton KG, Cela R (2015) Fabric phase sorptive extraction: a new sorptive microextraction technique for the determination of non-steroidal anti-inflammatory drugs from environmental water samples. Anal Chim Acta 865:22–30
43. Guedes-Alonso R, Santana-Viera S, Sosa-Ferrera Z, Santana-Rodríguez JJ (2015) Molecularly imprinted solid-phase extraction coupled with ultra high performance liquid chromatography and fluorescence detection for the determination of estrogens and their metabolites in wastewater. J Sep Sci 38(22):3961–3968
44. Wooding M, Rohwer ER, Naudé Y (2017) Determination of endocrine disrupting chemicals and antiretroviral compounds in surface water: a disposable sorptive sampler with comprehensive gas chromatography–time-of-flight mass spectrometry and large volume injection with ultra-high performance liquid chromatography–tandem mass spectrometry. J Chromatogr A 1496:122–132
45. Jennings WG, Poole CF (2012) Milestones in the development of gas chromatography, in gas chromatography. Elsevier, Amsterdam, pp 1–17
46. Poole CF (2012) In: Poole CF (ed) Gas chromatography. Elsevier, Amsterdam
47. Fanali S, Haddad PR, Poole CF, Schoenmakers P, Lloyd D (2013) In: Fanali S, Haddad PR, Poole CF, Schoenmakers P, Lloyd D (eds) Liquid chromatography. Elsevier, Amsterdam
48. Scognamiglio V, Antonacci A, Patrolecco L, Lambreva M, Litescu SC, Ghuge S, Rea G (2016) Analytical tools monitoring endocrine disrupting chemicals. TrAC Trends Anal Chem 80:555–567
49. USEPA (United States Environmental Protection Agency) (2007) Method 1694: pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS. Washington

50. Cimetiere N, Soutrel I, Lemasle M, Laplanche A, Crocq A (2013) Standard addition method for the determination of pharmaceutical residues in drinking water by SPE–LC–MS/MS. Environ Technol 34(22):3031–3041
51. Furlong ET, Werner SL, Anderson BD, Cahill JD (2008) Determination of human-health pharmaceuticals in filtered water by chemically modified styrene-divinylbenzene resin-based solid-phase extraction and high-performance liquid chromatography/mass spectrometry. U.S. Geological Survey Techniques and Methods, book 5, sec. B, Chap. B5, p 56
52. Mokh S, El Khatib M, Koubar M, Daher Z, Al Iskandarani M (2017) Innovative SPE-LC-MS/MS technique for the assessment of 63 pharmaceuticals and the detection of antibiotic-resistant-bacteria: a case study natural water sources in Lebanon. Sci Total Environ 609:830–841
53. Meng T, Cheng W, Wang M, Wan T, Cheng M, Zhang CC, Jia ZY (2017) The distribution of antibiotics in water of a river basin in South China. IOP Conf Ser Earth Environ Sci 82:012078
54. Kay P, Hughes SR, Ault JR, Ashcroft AE, Brown LE (2017) Widespread, routine occurrence of pharmaceuticals in sewage effluent, combined sewer overflows and receiving waters. Environ Pollut 220:1447–1455
55. Sghaier RB, Net S, Ghorbel-Abid I, Bessadok S, Le Coz M, Hassan-Chehimi DB, Trabelsi-Ayadi M, Tackx M, Ouddane B (2017) Simultaneous detection of 13 endocrine disrupting chemicals in water by a combination of SPE-BSTFA derivatization and GC-MS in transboundary rivers (France-Belgium). Water Air Soil Pollut 228(1):2
56. Robles-Molina J, Lara-Ortega FJ, Gilbert-López B, García-Reyes JF, Molina-Díaz A (2014) Multi-residue method for the determination of over 400 priority and emerging pollutants in water and wastewater by solid-phase extraction and liquid chromatography-time-of-flight mass spectrometry. J Chromatogr A 1350:30–43
57. Ghobadi M, Yamini Y, Ebrahimpour B (2014) SPE coupled with dispersive liquid–liquid microextraction followed by GC with flame ionization detection for the determination of ultra-trace amounts of benzodiazepines. J Sep Sci 37(3):287–294
58. Melo SM, Brito NM (2014) Analysis and occurrence of endocrine disruptors in Brazilian water by HPLC-fluorescence detection. Water Air Soil Pollut 225(1):1783
59. Xiao P, Weibel N, Dudal Y, Corvini PFX, Shahgaldian P (2015) A cyclodextrin-based polymer for sensing diclofenac in water. J Hazard Mater 299:412–416
60. Nunes CN, Pauluk LE, Dos Anjos VE, Lopes MC, Quináia SP (2015) New approach to the determination of contaminants of emerging concern in natural water: study of alprazolam employing adsorptive cathodic stripping voltammetry. Anal Bioanal Chem 407(20):6171–6179
61. Salvatierra-Stamp VDC, Ceballos-Magaña SG, Gonzalez J, Jurado JM, Muñiz-Valencia R (2015) Emerging contaminant determination in water samples by liquid chromatography using a monolithic column coupled with a photodiode array detector. Anal Bioanal Chem 407(16):4661–4670
62. del Carmen Salvatierra-Stamp V, Ceballos-Magaña SG, Gonzalez J, Ibarra-Galván V, Muñiz-Valencia R (2015) Analytical method development for the determination of emerging contaminants in water using supercritical-fluid chromatography coupled with diode-array detection. Anal Bioanal Chem 407(14):4219–4226
63. Le MD, Duong HA, Nguyen MH, Sáiz J, Pham HV, Mai TD (2016) Screening determination of pharmaceutical pollutants in different water matrices using dual-channel capillary electrophoresis coupled with contactless conductivity detection. Talanta 160:512–520
64. Pérez RL, Escandar GM (2016) Multivariate calibration-assisted high-performance liquid chromatography with dual UV and fluorimetric detection for the analysis of natural and synthetic sex hormones in environmental waters and sediments. Environ Pollut 209:114–122
65. Olatunji OS, Fatoki OS, Opeolu BO, Ximba BJ, Chitongo R (2017) Determination of selected steroid hormones in some surface water around animal farms in Cape Town using HPLC-DAD. Environ Monit Assess 189(7):363

66. Biache C, Navarro A, Czechowski F, Lu Y, Philip RP (2015) Thiosteranes in samples impacted by fecal materials and their potential use as marker of sewage input. Environ Pollut 196:268–275
67. Navarro A, Herrera J, Marrugo J, Bayonne J, Moral L (2014) Organic micro-pollutants in rivers of Mexico: the case of the river Nexapa Rivers. In: Ramos M, Aguilera V (eds) The engineering sciences and technology, handbook T-IV. ECORFAN, Valle de Santiago, pp 49–63
68. Madikizela LM, Tavengwa NT, Chimuka L (2017) Status of pharmaceuticals in African water bodies: occurrence, removal and analytical methods. J Environ Manag 15(193):211–220
69. Hughes SR, Kay P, Brown LE (2013) Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ Sci Technol 47(2):661–677
70. McEneff G, Schmidt W, Quinn B (2015) EPA research programme 2014–2020. Pharmaceuticals in the aquatic environment: a short summary of current knowledge and the potential impacts on aquatic biota and humans. EPA research report 142
71. Lyons G (2014) Pharmaceuticals in the environment: a growing threat to our tap water and wildlife. A CHEM trust report
72. Li Y, Zhu G, Ng WJ, Tan SK (2014) A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: design, performance and mechanism. Sci Total Environ 468–469:908–932
73. Auvinen H, Havran I, Hubau L, Vanseveren L, Gebhardt W, Linnemann V, Van Oirschot D, Du Laing G, Rousseau DP (2017) Removal of pharmaceuticals by a pilot aerated subsurface flow constructed wetland treating municipal and hospital wastewater. Ecol Eng 100:157–164