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Review

The behavior of pharmaceutically active compounds and contrast agents during wastewater treatment – Combining sampling strategies and analytical techniques: A critical review

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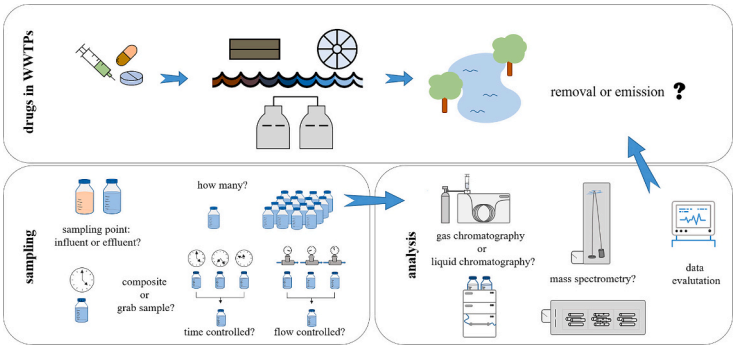
HIGHLIGHTS

- Overview of most relevant techniques for analysis of wastewater samples
- Discussion of suitability of sampling and analysis strategies
- Review on the research of nine PhACs and CAs in wastewater
- Comparison of analytical methods and sampling strategies over several decades
- Evaluation of the results with regard to sampling and analysis approach

GRAPHICAL ABSTRACT



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ABSTRACT

Increasing consumption of pharmaceuticals and the respective consequences for the aquatic environment have been the focus of many studies over the last thirty years. Various aspects in this field were investigated, considering diverse pharmaceutical groups and employing a wide range of research methodologies. Various questions from the perspectives of different research areas were devised and answered, resulting in a large mix of individual findings and conclusions. Collectively, the results of the studies offer a comprehensive overview. The large variety of methods and strategies, however, demands close attention when comparing and combining information from heterogeneous projects.

This review critically examines the application of diverse sampling techniques as well as analytical methods in investigations concerning the behavior of pharmaceutically active compounds (PhACs) and contrast agents (CAs) in wastewater treatment plants (WWTPs). The combination of sampling and analysis is discussed with regard to its suitability for specific scientific problems. Different research focuses need different methods and answer different questions.

An overview of studies dealing with the fate and degradation of PhACs and CAs in WWTPs is presented, discussing their strategic approaches and findings. This review includes surveys of anticancer drugs, antibiotics,

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analgesics and anti-inflammatory drugs, antidiabetics, beta blockers, hormonal contraceptives, lipid lowering agents, antidepressants as well as contrast agents for X-ray and magnetic resonance imaging.

1. Introduction

Analytical chemistry has advanced tremendously in the last decades. Instruments are optimized and offer more sensitivity, more selectivity, faster data evaluation and easier handling. Analytical techniques are becoming more refined and improved. Several years ago, samples had to be prepared elaborately prior to analysis in order to detect low concentrated analytes. Now, detection limits are becoming lower and lower (Richardson and Ternes, 2022; Radjenović et al., 2007a, 2007b). These enhancements are especially important in environmental studies. Matrix loaded samples and low analyte concentration do not constitute insurmountable hurdles anymore. Concentrations of rarely prescribed pharmaceuticals can now be determined in sewage sludge or seawater (Horstmann et al., 2021; Park et al., 2020).

The advancements in analysis did not diminish the relevance of correct data interpretation: what does an especially high or an extraordinary low analyte concentration mean for the research query? We need to know details about the origin of the sample to be able to bring the results in a bigger context (Lopez et al., 2022; Ort et al., 2010).

This review focuses on the combination of sampling strategy and analytical technique to approach the behavior of pharmaceutically active compounds (PhACs) and contrast agents (CAs) during wastewater treatment, especially concerning the question if they are removed in the process.

How were PhACs and CAs analysed in wastewater? How and where were the samples taken? What conclusions were drawn from the results? Were the PhACs and CAs removed during wastewater treatment?

The use of pharmaceuticals increased immensely in recent years (Peña et al., 2021). Drugs are administered in great quantities for human and veterinary treatment. Subsequently, they are eliminated from the body, either in their original form or following metabolic biotransformation. Water-soluble compounds are excreted primarily by the kidneys, while more hydrophobic compounds are eliminated by the liver (Barreto and Koubek, 2021). After excretion, PhACs, CAs and their residues enter the sewage system (Daughton and Ternes, 1999).

The removal of pollutants from domestic and industrial waste water is initially the job of wastewater treatment plants (WWTPs). Wastewater, emerging from hospitals, homes, official buildings and industrial sites, containing pharmaceuticals and their residues, is transported to local WWTPs. There, it is treated and subsequently returned to streams, rivers, lakes and finally into the oceans. In the 1990s, the first reports compiled information about the potential of drugs and respective transformation products to reach the aquatic environment (Halling-Sørensen et al., 1998; Raloff, 1998; Hirsch et al., 1999). Clofibrate, a lipid-lowering agent, was one of the first drugs found in ground and tab water in Berlin (Raloff, 1998; Richardson and Bowron, 1985; Heberer and Stan, 1997). Since then, a steadily increasing number of studies and projects worldwide were designed and conducted in order to assess the environmental risk by exposing medical substances and metabolites to the environment. Multiple studies in the last decades have shown that the conventional wastewater treatment processes have strong limitations on the removal of PhACs. A wide range of medicinal substances is not efficiently eliminated during wastewater treatment (Petrović et al., 2003; Pérez and Barceló, 2007; Batt et al., 2008; Künnemeyer et al., 2009; Deblonde et al., 2011; Telgmann et al., 2012; Afonso-Olivares et al., 2016).

It became clear that the continuous emission of medical substances that cannot be held back in WWTPs can lead to a bioaccumulation of these compounds with unknown impact. More importantly: drugs are developed with the intention of performing biological effects – they might also have the necessary properties to provoke effects in the aquatic environment (Halling-Sørensen et al., 1998).

The possibility that a high input of PhACs, CAs and/or their metabolites or transformation products has a negative impact on the aquatic environment has been noted in the literature for decades. However, until the 1990s, chemical analysis tools were mainly inadequate for the detection of drugs in the environment due to insufficient detection limits and specificity (Daughton and Ternes, 1999).

Strategies and approaches to explore the occurrence and fate of PhACs and CAs during wastewater treatment are numerous (Richardson and Ternes, 2022). A wastewater sampling and an adequate analysis technique have to be chosen. The first investigation on a specific PhAC in a WWTP often involves simple one-shot analysis of grab samples of water from one or several stages of the treatment. The presence or absence of the analyte then sets the next steps when refining the research question. The sampling strategy and analysis technique are defined by the research focus. For example: the analysis of a grab sample can only reveal if the examined drug is present in that one sample, taken at one specific time point and at one sampling location, whereas the analysis of a multitude of composite samples gives insight into the whereabouts of the drug over a period of time. As per the choice of an analytical technique: the direct analysis of a wastewater sample by means of inductively coupled plasma mass spectrometry (ICP-MS) can reveal the content of an element in very low concentrations but omits information of the species. The alternative electrospray ionization mass spectrometry (ESI-MS) offers great options for compound characterization but is usually only reliable when hyphenated to high pressure liquid chromatography (HPLC). And the choice of a chromatographic method is a whole new chapter. In the past, a number of reviews covering several PhACs or CAs have been published. Hernandez et al. critically reviewed LC-MS methods for the determination of antibiotics in environmental waters (Hernández et al., 2007). Methods used to measure estrogens in environmental waters were reviewed by Gabet in 2007 (Gabet et al., 2007). In 2012, the development and progress of analytical methods for the determination of MRI contrast agents was evaluated (Telgmann et al., 2012). An extensive listing of studies regarding antidepressants in WWTPs has been recently published by Laimou-Geraniou (Laimou-Geraniou et al., 2023). Other articles covered a wider range of PhACs: Petrovic presented a broad overview of the occurrence and behavior of several trace contaminants including analgesics and lipid lowering agents in wastewater (Petrović et al., 2003). Richardson and Ternes outlined emerging contaminants, including many PhACs, and analytical approaches in a very detailed summary of topical projects (Richardson and Ternes, 2022). Andreu et al. summarized approaches for sample preparation and analytical techniques while Radjenovic et al. presented an overview of analytical methodologies applied in environmental monitoring of PhACs and their degradation products (Andreu et al., 2007; Radjenović et al., 2007a, 2007b). Lambropoulou et al. reviewed the occurrence and removal of transformation products of pharmaceuticals in detail. The authors discussed several PhAC groups and a wide range of the respective transformation products and their analysis in wastewater (Evgenidou et al., 2015). A recent publication by Anand et al. reviews several aspects of pharmaceuticals in WWTPs, including transformation, bioaccumulation and analysis methods (Anand et al., 2022). Ort et al. critically reviewed the sampling of pharmaceuticals and personal care products according to tradition or standard laboratory protocols. Appropriate sampling modes and frequencies were discussed in detail and recommendations were offered to the reader (Ort et al., 2010).

These reviews give a detailed and comprehensive overview of essential parts in this research area. Nevertheless, some of the articles are becoming outdated. This together with the fact, the most of the

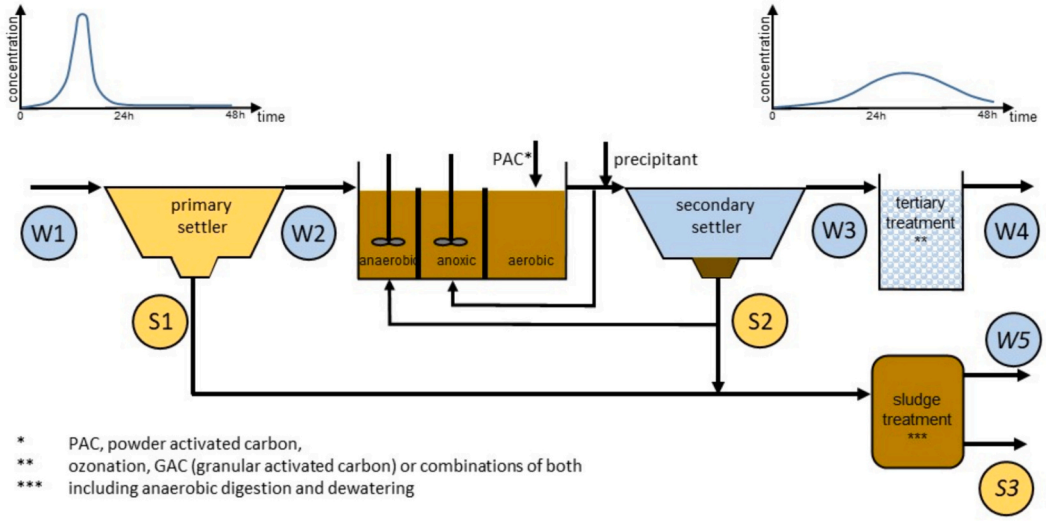

Fig. 1. Outline of a WWTP with an activated sludge system and sludge treatment. The residence time distribution for main stream is shown for a conservative compound which is not degraded or removed. W1 through W4 are sampling points for the water phase. S1 and S2 are sampling points for sludge samples. Additional samples from sludge treatment (*S3*) and reject water (*W5*) are also possible.

Table 1
Sampling points and types in a WWTP as well as respective potentially gained information from comparison.

Sampling points	Type of sampling	Gained information	Necessary data from WWTP
W1 and W2	24-h composite	Amount of analytes linked to solid fraction	Hydraulic loading rate of primary settler, HRT, TSS
W2 and W3	24-h composite	Biodegradation, volatilization, adsorption (together with S2), formation of metabolites and transformation products	HRT, SRT, COD loading rate of biological treatment step, with or without nitrogen removal
W3 and W4	24-h composite	Removal efficiency of the chosen technology, formation transformation products	HRT, ozone and/or activated carbon dosage
W5	24-h composite	Amount of analytes released from the solid phase after anaerobic digestions	HRT, SRT, TSS and temperature of the anaerobic digester
S1	24-h composite	Amount of analytes linked to solid fraction	Hydraulic loading rate of primary settler, HRT, TSS
S2	grab sample	Removal of analytes adsorped to biomass	SRT, mass balance for excess sludge
S3	grab sample	Removal and fate of analytes during anaerobic digestion	HRT, SRT, TSS and temperature of the anaerobic digester

HRT: hydraulic retention time, SRT: solid retention time, TSS: total suspended solids, COD: Chemical oxygen demand.

publications either focus on PhACs and/or CAs or on sampling or on analytical strategies, we are missing a combined overview of these related topics together.

In this review the view from environmental engineering on the one side and analytical chemistry from the other side come together to discuss the suitability of sampling and analysis strategies for the examination of PhACs and CAs in the WWTP process. Both aspects belong together very closely and the expertise from analytical and environmental chemistry to water technology and environmental engineering is required. Analytical techniques cannot be developed without knowledge on expected matrix load or analyte concentration. Sample preparation only makes sense when it is known what analytical technique will be used. Analysis results can only be interpreted when appropriate sampling was carried out. A research question can only be answered when sampling and analysis was carried out in accordance to the research question.

Many medicinal compounds have been investigated in the last decades. The studies differ from substance to substance, focusing on different aspects, ranging from general questions to more specific and detailed issues. After a discussion on techniques and strategies, this review gives an overview of the research of the last decades. The studies on the behavior of anticancer drugs, antibiotics, analgesics, antidiabetics, betablockers, hormonal contraceptives, lipid lowering agents, antidepressants and contrast agents during wastewater treatment will be reviewed.

2. Fundamentals: drug sources, wastewater treatment, sampling, preparation and analysis techniques

In the following, we will give a short overview of the essential background of the relevant topics. We will focus on the information needed to review the investigations on PhACs and CAs presented in part 3.

2.1. Layout of WWTPs and suitable sampling strategies

Fig. 1 shows the flow scheme of the wastewater treatment technology used most often worldwide. A WWTP works in three consecutive stages: a primary stage that includes grit removal and sedimentation, a secondary stage consisting of an aeration tank for further purification and a third stage for the removal of nitrogen. A tertiary treatment for micropollutant removal is optional. The activated sludge system featured in Fig. 1 includes carbon and nitrogen removal and an anaerobic tank, which allows for biological phosphorous removal (Henze et al., 2002). Chemical phosphorous removal is indicated by the addition of precipitant (iron (III) or aluminium (III) salts) into the influent of the secondary settler. As last step a tertiary treatment is integrated, which can be conducted by ozonation, activated carbon or combinations of

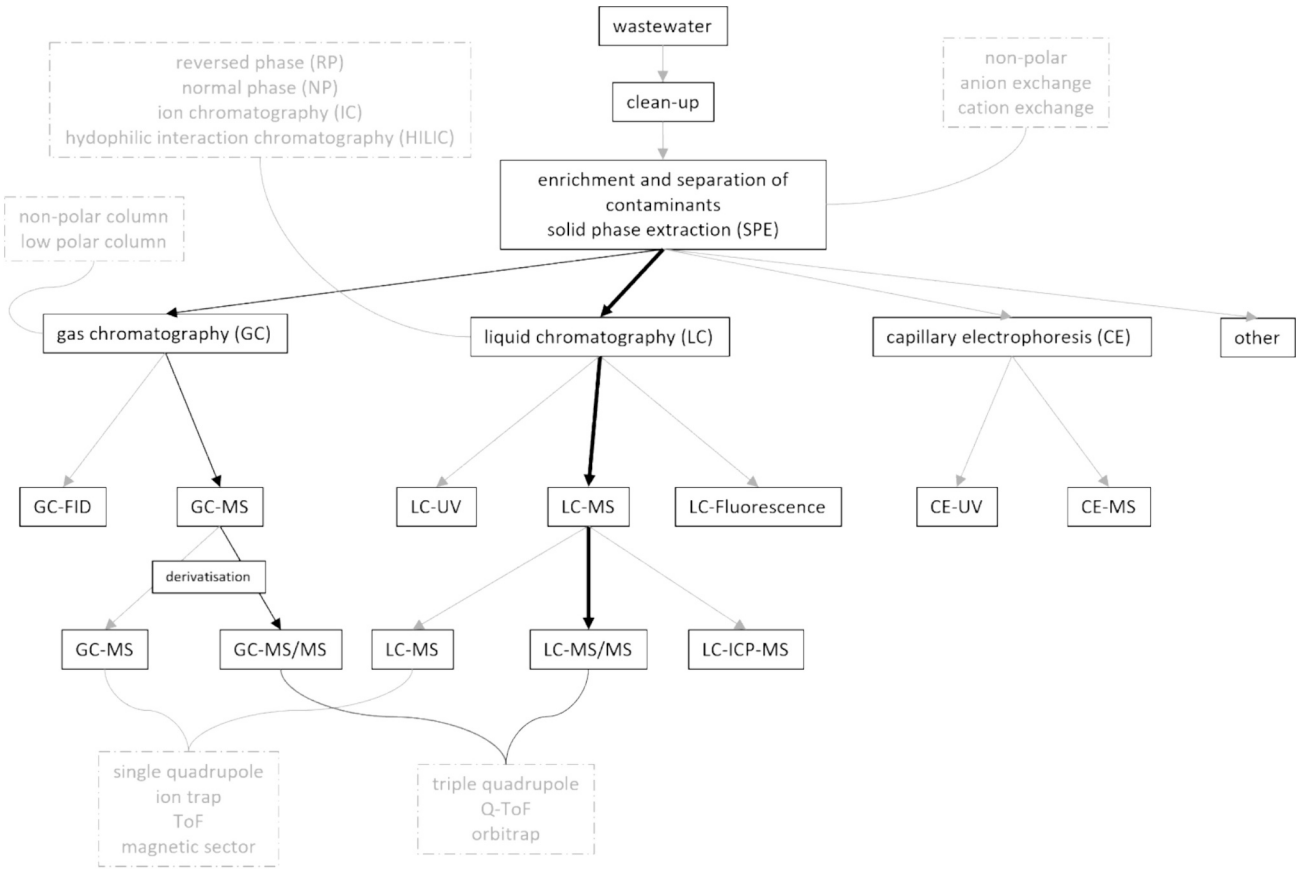

Fig. 2. Work flow for determination of PhACs and CAs in wastewater samples. Black arrows indicate analysis strategies used predominantly in the last decades. Thick arrows indicate the most often used analysis method employed nowadays.

both. Powder activated carbon can directly be added into the activated sludge tank.

The statement of which processes are integrated within the WWTP under investigation is strongly recommended for all publications dealing with the removal of micropollutants. Additional advisable parameters are shown in Table 1.

In this setup for aerobic biological treatment (samples W1 through W3), the removal mechanisms for pharmaceutically active compounds (PhACs) and contrast agents (CAs) can be

- Biochemical degradation
- Adsorption to biomass
- Volatilization due to aeration or strong mixing
- Photolysis in the secondary settler due to sunlight

The latter will be of interest during dry weather (high hydraulic retention time) and long time periods with sunlight in summer. In addition, anaerobic biochemical removal and resolution can take place during anaerobic sludge treatment and subsequent dewatering.

Tertiary treatment is not integrated in all WWTPs. It can be both chemical (oxidizing processes with ozone or hydrogen peroxide) and physical (adsorption with both powder or granular activated carbon). Another option can be membrane filtration. Currently, ozone and activated carbon or a combination of both are the most often used treatment steps in municipal WWTP (Hollender et al., 2009; Mailler et al., 2016). PhACs and CAs behave very differently during the wastewater treatment process. Their molecular characteristics can differ strongly even within a structurally similar group of compounds. To study the behavior of pharmaceutical residues – whether they are removed, transformed or not affected at all – diverse locations in the WWTP can be sampled. The sampling strategy has a big impact on the interpretation of the results.

Biological treatment systems are designed in different ways. However, independent of whether the wastewater treatment is done with pre- or post denitrification, with and without biological phosphorous removal, the sampling spots for studies on PhACs and CAs removal during the biological step have to be placed at W2 (influent biological treatment train) and W3 (effluent secondary clarifier). The residence time distribution of a conservative component in Fig. 1 shows that grab samples will not be very helpful to identify removal rates. 24-h composite sampling over one week and under dry weather conditions is the best way for identification of micropollutant removal within the biological treatment step. Ort et al. very nicely described and explained the different ways for 24-h composite sampling and differentiate between continuous and time discrete systems, which can sample time-, flow- and volume-dependent. Moreover, they pointed to the connected sewer system, which also will have an impact on the distribution of PhACs and Cas in the influent of WWTP. The latter will then influence the liquid phase sample in front of the primary settler (W1). As most analyses on PhACs and CAs are done from the liquid phase (after removal of solids) there should not be a huge difference between W1 and W2. Finally, the optional tertiary treatment step would require a fourth sample (W4) from the liquid phase (Ort et al., 2010).

A partly blind spot in the treatment process often is the solid fraction and its contribution to distribution and removal of PhACs and CAs. The sampling of primary sludge and excess sludge is necessary for a complete investigation concerning compounds that bind to the solid fraction during the WWTP process. As the primary sludge is going along with daily changes depending on wastewater composition (similar to W1) the excess sludge does have a certain retention time, which ranges between 3 and >20 days. As the primary sludge is constantly removed from the primary settler it will represent the hourly/daily changes depending on wastewater composition (similar to W1). However, the activated sludge

originates from the biological processes in the biological treatment train and needs recirculation into the process constantly. It thus has a certain retention time which ranges between 3 and >20 days, depending on whether the treatment plant is optimized for high sludge yield (biogas production) or low sludge production (high removal of chemical oxygen demand (COD), decreased costs for sludge management). Therefore, the results obtained from analysis of S1 (composite sample) and S2 (grab sample) can be different.

Additional sampling of reject water from sludge dewatering (not included in Fig. 1) can be necessary since reintroduction of PhACs and CAs after anaerobic digestions into the main train is possible. Table 1 gives as overview of the discussed sampling points and the information that can be gained from the investigation of the respective samples.

PhACs and CAs behave very differently during the wastewater treatment process. The molecular characteristics are important parameters when assessing the impact of different removal processes. The molecular characteristics can differ strongly even within a structurally similar group of compounds. The physico-chemical properties play a particularly crucial role. Therefore, studies on behavior of PhACs and CAs within the entire wastewater process should be supported with parameters like polarity, solubility in water and susceptibility to photolysis by sunlight.

2.2. Sample clean-up and enrichment

Water samples can often be injected into analysis systems without further preparation. Sample preparation can be necessary for samples with high matrix loads or for samples with very low analyte concentration. Suspended material can be removed by filtration or centrifugation. Solid phase extraction (SPE) is nowadays the most common choice for subsequent treatment whereas liquid-liquid extraction (LLE) is only rarely applied. The sorbent material of the SPE stationary phase can be specifically selected for the removal of interfering compounds and enrichment of the respective analytes. In a general procedure, an aqueous sample containing analyte and interfering compounds is applied onto an SPE cartridge. Subsequently, there are two basic options: the analyte molecules are enriched on the sorbent material whereas other compounds do not show interaction and can be washed from the adsorbent in the next step. In a final step, the analyte is removed from the sorbent by elution with an appropriate solvent. Or the analyte molecules do not show interaction with the adsorbent, but the interfering compounds are retained. In this case, the analyte can be washed from the adsorbent in the second step. Depending on the volume of eluent, the analyte can also be enriched in the final step.

The sorbent material is chosen depending on the properties of the substances: a wide range of polar and unpolar sorbents are available, as well as anion and cation exchange cartridges or hydrophilic polymer SPE that contains both non-polar and polar functional groups (Mompelat et al., 2013).

In part 3, you will find that a wide range of sorbents were used for the preparation of environmental samples with PhACs and CAs.

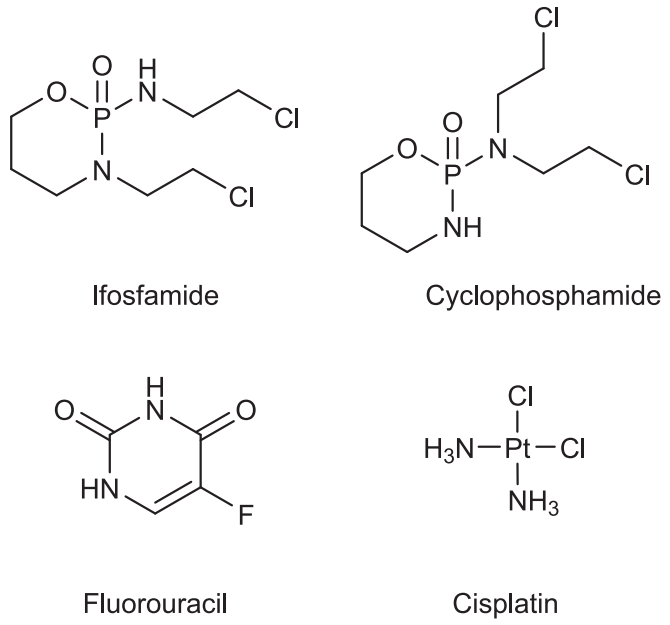

Fig. 3. Structures of various anticancer drugs: ifosfamide, cyclophosphamide, fluorouracil and cisplatin.

2.3. Portfolio of analytical techniques

The choice of analytical method must be made on the basis of the analyte's characteristics. The polarity plays an important role during method development: Is it a small ion or a big polar molecule? Or is it a rather inert compound?

Fig. 2 shows an overview of potential analysis routes. The common approach for wastewater samples is the hyphenation of a separation method and a detection method. Separation can be achieved with gas chromatography (GC) or high performance liquid chromatography (HPLC). The choice of appropriate GC or HPLC stationary phase is strongly dependent on the analyte's properties, especially its polarity. GC columns are available in a wide range of polarity. HPLC is mainly carried out on reversed phase (RP) columns, for example octadecyl (C_{18}) or phenyl. More polar substances can be separated with hydrophilic interaction liquid chromatography (HILIC) stationary phases.

For most PhACs and CAs, GC and HPLC were employed in the past decades with divers columns.

From the beginning of environmental analysis, mass spectrometry (MS) was usually the detection technique employed after separation. You will find exceptions in the literature review; they are, however, rare. The choice of MS type is dependent on availability (early studies were carried out with single quadrupole MS, because it was the only type commercially available are the time), but also on the research question: is quantitation the focus of the study, then analysis in MS/MS mode with a triple quadrupole MS are selected. Tandem MS not only allows for quantitation, but also for the performance in multiple-reaction-monitoring (MRM) which improves analyte identification. During HPLC-MS/MS analysis in MRM mode, the ratio of two so-called MRM transitions can, together with the retention time, be used to confirm the presence of an analyte in the samples (Radjenović et al., 2007a, 2007b).

When the identification of unknown compounds is required, MS instruments with a higher mass accuracy are necessary. High resolution (HR) mass spectrometers (resolution >15,000, $\Delta m < 10$ ppm) can differentiate better between two different analytes than instruments with lower resolutions, allowing for the determination of exact masses. Orbitrap and quadrupole time-of-flight instruments are examples for HRMS.

Elemental analysis does not play a big role for PhACs and CAs: inductively coupled plasma (ICP-)MS is highly sensitive, but requires a heteroatom. You will therefore find it only being discussed in the sections about platinum based anticancer drugs and gadolinium based contrast agents.

Due to the commonly low concentrations of PhACs and CAs in wastewater, the sensitivity of the analytical method is very important. Optimization of the limit of quantitation (LOQ) and limit of detection (LOD) is in general one of the most essential exercise during method development.

3. Studies of the occurrence, behavior and fate of PhACs and CAs during wastewater treatment

In the following, studies on the behavior of PhACs and CAs during wastewater treatment will be reviewed. We will discuss the choice of

Table 2
Details and results of wastewater analysis regarding anticancer drugs in the past decades.

Sampling		Literature
Grab samples		Azuma et al., 2015; Negreira et al., 2013; Ferrando-Climent et al., 2014; Česen et al., 2015; Llewellyn et al., 2011; Kümmerer et al., 1997; Camacho-Muñoz and Kasprzyk-Hordern, 2015; Gómez-Canela et al., 2014; Cristóvão et al., 2021; Busetti et al., 2009
	Flow proportional	Buerge et al., 2006
Composite samples	Time proportional	Česen et al., 2015; Yin et al., 2010
	No details	Ulvi et al., 2022; Busetti et al., 2009; Martín et al., 2011

Preparation		Literature
SPE	Non-polar (reversed phase)	Ulvi et al., 2022; Busetti et al., 2009; Česen et al., 2015; Martín et al., 2011; Kümmerer et al., 1997; Llewellyn et al., 2011; Castiglioni et al., 2005; Rabii et al., 2014; Yin et al., 2010; Steger-Hartmann et al., 1996; Kiffmeyer et al., 1998; Negreira et al., 2013; Ferrando-Climent et al., 2014; Gómez-Canela et al., 2014; Cristóvão et al., 2021; Camacho-Muñoz and Kasprzyk-Hordern, 2015
	Cation exchange	Gómez-Canela et al., 2014; Castiglioni et al., 2005
	Anion exchange	Azuma et al., 2015; Camacho-Muñoz and Kasprzyk-Hordern, 2015

Analysis					Literature
Method	Derivatization	Column	MS	LOD (ng/L)* of cyclophosphamide**	
GC–MS	Acetylation	Non-polar	Single quadrupole	0.55	Steger-Hartmann et al., 1996; Česen et al., 2015; Kümmerer et al., 1997
HPLC-DAD	–	Reversed phase	–	80,000,000	Kiffmeyer et al., 1998
HPLC-FL	–	Reversed phase	–	*na*	Kiffmeyer et al., 1998
HPLC-MS	–	Reversed phase	Triple quadrupole	0.5	Buerge et al., 2006; Azuma et al., 2015; Negreira et al., 2013; Negreira et al., 2014; Cristóvão et al., 2021; Llewellyn et al., 2011; Castiglioni et al., 2005; Yin et al., 2010
			Orbitrap-HRMS	4.4	Gómez-Canela et al., 2014
		Chiral	Triple quadrupole	*na*	Camacho-Muñoz and Kasprzyk-Hordern, 2015

Results		Literature
Concentration in influent	Up to 27 ng/L (cyclophosphamide)	Buerge et al., 2006; Česen et al., 2015; Rabii et al., 2014; Yin et al., 2010
Concentration in effluent	Up to 21 ng/L (cyclophosphamide)	Buerge et al., 2006; Česen et al., 2015; Llewellyn et al., 2011; Castiglioni et al., 2005; Rabii et al., 2014; Yin et al., 2010
Load influent	0.97 g/day (cyclophosphamide)	Buerge et al., 2006
Load effluent	0.72 g/day (cyclophosphamide)	Buerge et al., 2006
Removal efficiencies	Between 3 % and 82 % (cyclophosphamide)	Ulvi et al., 2022; Česen et al., 2015

na: LOD not determined in effluent in the respective study.
* Lowest LOD of listed studies (determined for analysis in effluent).
** Cyclophosphamide is one of the most frequently analysed compounds and therefore used for comparison between studies.

sampling strategy and analytical technique with regard to the compound characteristics and research question. The interpretation of the results based on the respective approach will be reviewed.

A generally chronological order gives us the opportunity to assess changes in technological availabilities and advances as well as resulting changes of practice.

In general, we focus on the behavior of drugs in conventional WWTPs. Mostly studies with real samples from the treatment process are discussed, sporadic examples from accompanying batch experiments or laboratory-scale WWTPs are included at times. Advanced wastewater treatment is a very important research field and has direct relevance to the question on the behavior of PhACs and CAs in WWTPs. However, the development of novel techniques for wastewater treatment is a big challenge on its own and will therefore only be mentioned marginally in this review.

Metabolites and transformation products will occur in this review, but also only when part of a study focusing on the original compound. The field of metabolites and transformation products is very important and affecting the fate of PhACs and CAs during wastewater treatment. It is, however, also too big to be covered in the same work.

In this review we will cover research on anticancer drugs, antibiotics, analgesics, antidiabetics, betablockers, hormonal contraceptives, lipid lowering agents, antidepressants and contrast agents. This selection covers a range of compounds that belong to the most often used pharmaceuticals. The PhACs and CAs differ in application, drug dosage, molecular structure, characteristics and behavior. Thus, the selection offers a great overview of the behavior of PhACs and CAs during wastewater treatment.

3.1. Anticancer drugs

Anticancer drugs are effective in the treatment of cancerous diseases. They inhibit the growth and multiplying of cancer cells. There are several major classes of anticancer drugs: alkylating agents, antimetabolites, monoclonal antibodies, microtubule inhibitors, steroid hormones and others. These agents work very differently; some keep cells

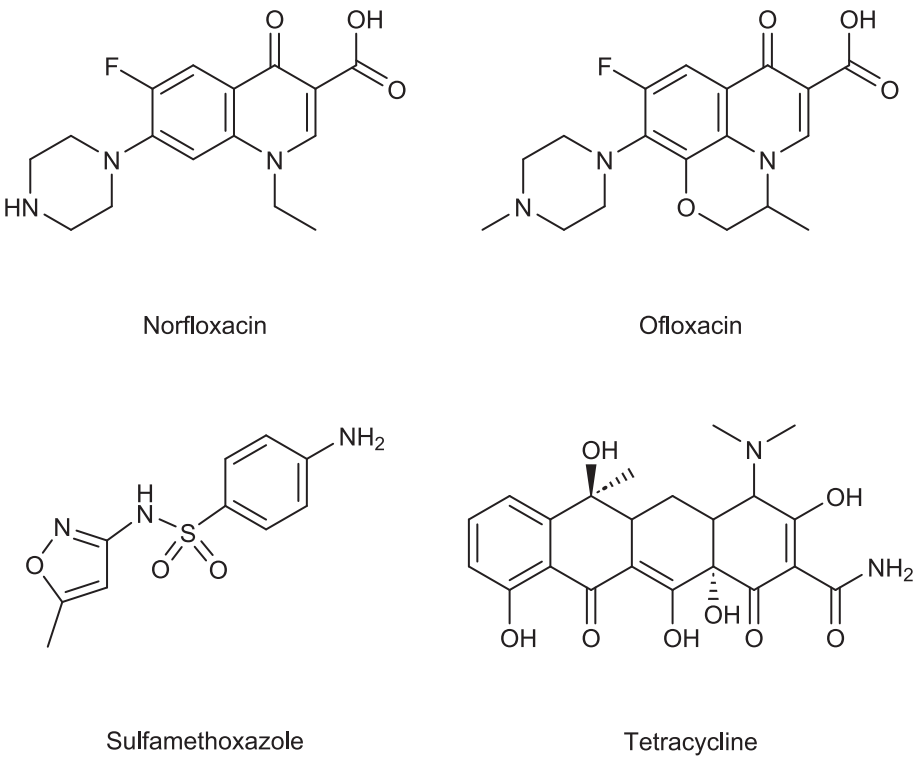

Fig. 4. Structures of various antibiotics. The two fluoroquinilones (norfloxacin, ofloxacin), one sulfonamide (sulfamethoxazole) and one tetracycline (tetracycline).

from reproducing by damaging its DNA, others interfere with DNA and RNA by acting as a substitute for the normal building blocks, and others work by stopping cells from dividing to form new cells. The anticancer drugs are consumed worldwide on a regular basis. Consumption was reported to exceed 20 tons in Germany in 2012, including 102 different drugs (Kümmerer et al., 2016). As very active compounds, anticancer drugs and their metabolites are excreted into wastewater. The highly toxic nature of the majority of the agents, including cytotoxic, fetotoxic, mutagenic and teratogenic properties, calls for a comprehensive risk assessment, including observations on their behavior during wastewater treatment. As the structures shown in Fig. 3 indicate, anticancer drugs come in very different polarities: cisplatin is a highly polar molecule whereas ifosfamide is not water soluble.

The first analytical method, to the author's knowledge, was developed in 1995 by Steger-Hartmann et al. Two anticancer agents, ifosfamide and cyclophosphamide, were determined by GC–MS, preceded by a two-step SPE procedure. The two drugs were determined in samples of a laboratory-scale WWTP and in hospital effluent (Steger-Hartmann et al., 1996). In 1997, the authors then determined anticancer drugs in wastewater. They measured concentrations up to 43 ng/L (ifosfamide) in effluent samples (Kümmerer et al., 1997).

In 1998, Kiffmeyer et al. developed a different method. The analysis of ten cytostatic drugs was performed using SPE with subsequent HPLC separation and quantitative determination with DAD and fluorescence detection. Detection limits as low as 0.002 mg/L were achieved. A simulation of the degradation processes occurring in a WWTP showed that cisplatin and cyclophosphamide are not biodegradable, but cytarabine and 5-fluorouracil are biodegradable in different magnitudes (Kiffmeyer et al., 1998).

Another analytical method was developed by Azuma et al. for a selection of anticancer agents. Based on strong anion based SPE sample preparation followed by a HPLC-MS/MS analysis, high concentrations of up to 1032 ng/L bicalutamide were detected in WWTP effluent, showing that wastewater treatment is not efficient for their removal (Azuma et al., 2015). Buerge et al. performed wastewater sampling to obtain flow-proportional composite samples of influent and effluent. After SPE treatment, the samples were analysed by means of HPLC-MS/MS. The sampling procedure allowed the determination of the cyclophosphamide mass flow in influent (0.97 g/day) and effluent (0.72 g/day). The direct comparison of these values suggest incomplete removal. However, a removal efficiency was not calculated. Presumably because the samples were taken simultaneously and thus disregarding the hydraulic retention time in the WWTP (Buerge et al., 2006). In 2011, Llewellyn et al. also detected cyclophosphamide in WWTP effluent by means of HPLC-MS/MS (Llewellyn et al., 2011).

In 2013, Negreira et al. applied isotope dilution for the quantification of 13 cytostatics and 4 metabolites in wastewater to improve the correctness of the data by avoiding matrix effects. WWTP influent samples were analysed with a fully automated on-line SPE HPLC-MS/MS method. Detection limits as low as 0.5 ng/L were achieved with this set-up and several compounds could be detected in influent wastewater (Negreira et al., 2013).

Several studies were carried out by means of SPE and HPLC-MS, obtaining similar LODs and similar values for anticancer agent concentration in influent and effluent (Castiglioni et al., 2005; Martín et al., 2011; Rabii et al., 2014; Yin et al., 2010, Cristóvão et al., 2021).

A sampling campaign by Ferrando-Climent et al. in 2014 took the hydraulic retention time of the WWTP into account: samples in the effluent were taken 27 h after sampling the influent. However, the samples were taken as grab samples and therefore only represent a snapshot of the drug concentration. Nevertheless, with a SPE procedure followed by HPLC-MS/MS analysis, six anticancer agents were detected in WWTP influent samples and most of them also in the effluent. Although not directly comparable to the influent concentrations, the high content of anticancer drugs in the effluent samples confirmed a poor removal efficiency. Detection limits as low as 1.1 ng/L were achieved (Ferrando-Climent et al., 2014).

Česen et al. compared cyclophosphamide concentrations in influent and effluent of a WWTP. Additionally, they determined the removal efficiency of two anticancer drugs in lab scale flow-through bioreactors. The results (42 % and 18 % for cyclophosphamide and ifosfamide, respectively) give some insight into the behavior of the two compounds

during wastewater treatment, suggesting that a large proportion of the drugs is findings its way into the aqueous environment (Česen et al., 2015). In the same year, Gómez-Canela tested automated SPE followed by HPLC-Orbitrap-MS as analysis method and achieved detection limits as low as 4.4 ng/L for cyclophosphamide (Gómez-Canela et al., 2014). The use of enantionselective HPLC-MS/MS was employed in 2015 by Camacho-Muñoz for the analysis of several PhACs. An excellent method detection limit of 0.08 ng/L was achieved for ifosfamide (E1) in water. It could, however, not be detected in wastewater samples (Camacho-Muñoz and Kasprzyk-Hordern, 2015).

The concentrations of several PhACs, including anticancer drugs, were determined by Ulvi et al. at two sampling dates, in the attempt to compare WWTP removal efficiencies in winter and summer. Composite samples were taken to allow for results as precise as possible. The removal efficiency of cyclophosphamide was calculated to be 82 % in summer and 3 % in winter. The authors explained the extreme difference with the biodegradation kinetic, which is slower in winter on account of the low temperatures (Ulvi et al., 2022). Such a temperature effect is still very unexpected. However, the results also differ compared to the results of Česen et al. (2015). As no details regarding the sampling of the composite samples are provided from Ulvi et al., it is difficult to determine the reason for the discrepancy.

Very good results for the analysis of platinum-based cytostatic drugs were in the past achieved by means of HPLC coupled to ICP-MS (Arenas et al., 2022). Especially with regard to generally low concentrations of the analyte, detection and quantification with ICP-MS appears to be a promising approach. However, to the author's knowledge, platinum-based anticancer drugs have not yet been analysed in wastewater with ICP-MS.

Table 2 gives an overview of the reviewed studies. The behavior of anticancer drugs in WWTPs was investigated in multiple surveys. GC–MS and HPLC-MS were applied for analysis, with HPLC-MS becoming the more frequently selected technique over the years. MS/MS is the most used detection technique, providing an LOD of 0.5 ng/L for cyclophosphamide in effluent samples. In the beginning the focus on most published studies is on the development of adequate analytical methods. The general question of occurrence in WWTP influent and, more importantly, effluent was contemplated. Sampling methods were very different and no standardised sampling strategy was adopted. The majority of samples were collected as 24-h composite samples or grab samples. The observation of many different anticancer drugs in WWTP effluent suggests that wastewater treatment is inefficient to remove all cytostatic drugs from wastewater. The removal efficiency in detail has, however, only been investigated by few studies and only for a few compounds. Since the findings for removal efficiencies vary strongly (3 to 82 %) for cyclophosphamide, concrete numbers remain to be determined in order to assess the impact on anticancer drugs on the aqueous environment.

3.2. Antibiotics

Antibiotics are one of the most important groups of pharmaceuticals in today's human and veterinary medicine. The antibacterial agents are used to treat or prevent bacterial infections, either by killing or inhibiting the growth of bacteria. Various antibiotics exist and each type only works against certain types of bacteria or parasite. Often used groups of antibiotics include penicillins (e.g. phenoxymethylpenicillin), macrolides (e.g. erythromycin), quinolones (e.g. ciprofloxacin) and many more (Fig. 4). Despite a range of potential side effects, they are in general considered safe and well tolerated. The usage of antibiotics is immense: the average total consumption for antibacterial agents was calculated as 16.4 defined daily doses per 1000 inhabitants per day in the EU in 2021 (ESAC, 2022). They are only partially metabolized in the human body and are excreted up to 90 % via urine and feces, ending up in the wastewater (Harrower et al., 2021; Kumar et al., 2005). Due to the frequent use of antimicrobial agents in recent years, several strains of bacteria have developed resistance to many types of antibiotics. The release of these compounds from WWTPs and subsequent accumulation of both antibiotic resistant bacteria and antibiotics could have severe consequences. The question concerning the behavior of the drugs during wastewater treatment and the subsequent behavior in water bodies is therefore critical and despite this not fully understood (Kümmerer et al., 1997).

One of the first studies that investigated the fate of antibiotics after entering the sewage system was conducted in 1998 by Hirsch et al. A method for the examination of antibiotics in WWTP effluent, surface and ground waters by means of HPLC-MS/MS was developed. Due to expected low concentrations, the method included SPE procedures for analyte enrichment. 18 antibiotics could be quantified down to the lower ng/L range in different water matrices (Hirsch et al., 1998). A year later, the authors published a study employing this method for the analysis of various water samples. WWTP effluent from five plants and surface water samples from 14 locations were taken as grab samples. The investigated WWTP effluents and surface water samples showed the presence of one degradation product, as well as two antibiotic agents (roxithromycin and sulfamethoxazole) with concentrations up to 6 μg/L. Other antibiotics could not be detected (Hirsch et al., 1999).

Many studies followed, developing analytical methods to investigate antibiotics and their residues: in 2001, Zhu et al. reached LODs as low as 3.1 μg/L for oxytetracycline, tetracycline, and chlortetracycline in lagoon water by extraction using polymeric and non-polar cartridges before analysis with HPLC-MS (Zhu et al., 2001). Reverté et al. determined Ciprofloxacin in WWTP influent and effluent samples in 2003 (Reverté et al., 2003). In 2004, Yang et al. published the determination of seven tetracycline and six sulfonamide compounds in pristine and wastewater-influenced surface water (Yang et al., 2004). Göbel et al. emphasizes in 2004 that the analysis of the metabolites of sulfonamide along with their active parent compounds is important because they transform back to the parent compounds in wastewater environments. This study also assessed that during sample preparation, erythromecin is transformed to erythromecin-H_2O at pH 4, which needed to be taken into account during analysis (Göbel et al., 2004).

Antibiotic polyether ionophores in river water were studied by Cha et al. in 2005 with a similar method based on SPE and HPLC-MS/MS, providing detection limits as low as 0.03 μg/L (Cha et al., 2005). Over the years, HPLC-MS/MS methods could be optimized to reach LODs as low as 1.8 ng/L for norfloxacin and 24 ng/L for sulfamethoxazole (Golovko et al., 2014; Camacho-Muñoz and Kasprzyk-Hordern, 2015; Gao et al., 2012).

Ferdig et al. compared MS detection to fluorescence detection for the analysis of (fluoro)quinolones. LOQs were better by a factor of at least an order of a magnitude for MS detection (Ferdig et al., 2005). Another approach was the determination of sulfamethoxalole, trimethoprim, and lincomyrin by means of CE-UV. Sulfamethoxazole was detected in concentrations up to 106.1 ppb in wastewater samples (Gibbons et al., 2011).

In these studies, samples were commonly taken as grab samples. The focus of the investigation was on the method development. Sample preparation by SPE and subsequent separation with HPLC followed by determination with tandem MS became the method of choice (Díaz-Cruz and Barceló, 2006; Seifrtová et al., 2009). The improving sensitivity and selectivity of tandem MS made HPLC-MS/MS the ideal technique for the determination of a broader range of substances at trace levels in environmental studies (Hernández et al., 2007; Andreu et al., 2007). Detection and quantification limits were additionally improved by the optimization of SPE procedures: the careful optimization of SPE strategies allowed for a detection limit as low as 0.01 ng/L for sulfonamides (Díaz-Cruz et al., 2008).

As antibiotics comprise a wide spectrum of substances, methods were usually developed for a specific group of antibiotics. Tetracylines as well as sulfonamides being the most studied antibiotic families (Petrovic et al., 2006).

Table 3
Details and results of wastewater analysis regarding antibiotics in the past decades.

Sampling		Literature
Grab samples		Reverté et al., 2003; Petrovic et al., 2006; Miao et al., 2004; Periša and Babić, 2014; Tran et al., 2016; Le-Minh et al., 2012; Chen et al., 2022; Gros et al., 2013; Camacho-Muñoz and Kasprzyk-Hordern, 2015; Gao et al., 2012; Gibbons et al., 2011; Ferdig et al., 2005
	Flow proportional	Gurke et al., 2015; Lindberg et al., 2005; García-Galán et al., 2010; Göbel et al., 2004
Composite samples	Time proportional	Golovko et al., 2014
	No details	Gao et al., 2012; Seifrtová et al., 2009; Xiao et al., 2008; Pérez-Parada et al., 2011; Lopez et al., 2022

Preparation		Literature
SPE	Non-polar (reversed phase)	Zhu et al., 2001; Reverté et al., 2003; Yang et al., 2004; Cha et al., 2005; Golovko et al., 2014; García-Galán et al., 2010; Hirsch et al., 1998; Hirsch et al., 1999; Camacho-Muñoz and Kasprzyk-Hordern, 2015; Gao et al., 2012; Ferdig et al., 2005; Seifrtová et al., 2009; Petrovic et al., 2006; Periša and Babić, 2014; Le-Minh et al., 2012; Miao et al., 2004; Xiao et al., 2008; Pérez-Parada et al., 2011; Gros et al., 2013; Chen et al., 2022; Gurke et al., 2015; Gibbons et al., 2011; Göbel et al., 2004; Papageorgiou et al., 2016
	Cation exchange	Gros et al., 2013
	Anion exchange	Camacho-Muñoz and Kasprzyk-Hordern, 2015

Analysis				Literature
Method	Column	MS	LOD (ng/L)* of norfloxacin and/or sulfamethoxazole**	
CE-UV	–	–	*Na*	Gibbons et al., 2011
HPLC-FL	Reversed phase	–	6.9 (norfloxacin)	Ferdig et al., 2005; Seifrtová et al., 2009
HPLC-MS	Reversed phase	Single quadrupole	*Na*	Reverté et al., 2003
		Ion trap	1.8 (norfloxacin)*** 24 (sulfamethoxazole)***	Zhu et al., 2001; Yang et al., 2004; Cha et al., 2005; Lindberg et al., 2005
		Triple quadrupole	1 (norfloxacin) 1.7 (sulfamethoxazole)	Golovko et al., 2014; Camacho-Muñoz and Kasprzyk-Hordern, 2015; Gao et al., 2012; Seifrtová et al., 2009; Tran et al., 2016; Perisa et al., 2014; Le-Minh et al., 2012; Miao et al., 2004; Xiao et al., 2008; Gros et al., 2013; Gurke et al., 2015; Lopez et al., 2022; Hirsch et al., 1998; Hirsch et al., 1999; Göbel et al., 2004; Papageorgiou et al., 2016
		Q-ToF	*Na*	Chen et al., 2022; Pérez-Parada et al., 2011; Lopez et al., 2022; García-Galán et al., 2010;
	chiral	Triple quadrupole	*Na*	Gao et al., 2012

Results		Literature
Concentration in influent	Up to 3130 ng/L (azithromycin) and up to 1208 ng/L (ofloxacin)	Lopez et al., 2022; Xiao et al., 2008
Concentration in effluent	Up to 2704 ng/L (azithromycin) and up to 503 ng/L (ofloxacin)	Lopez et al., 2022; Xiao et al., 2008; Göbel et al., 2004
Load influent	Up to 0.124 g/(d 1000 inhabitants) sulfamethoxazole	Papageorgiou et al., 2016
Load effluent	Up to 0.019 g/(d 1000 inhabitants) sulfamethoxazole	Papageorgiou et al., 2016
Removal efficiencies	Between 42 % and 82 % (sulfamethoxazole)	Golovko et al., 2014; 210, Gurke et al., 2015; Lopez et al., 2022
	Between 86 % and 98 % (ciprofloxacin)	Lopez et al., 2022; Seifrtová et al., 2009; Golovko et al., 2014

na: LOD not determined in effluent in the respective study.
* Lowest LOD of listed studies (determined for analysis in effluent).
** Norfloxacin and sulfamethoxazole are frequently analysed compounds and therefore used for comparison between studies.
*** Determined from LOQ.

Although the beginning of the research regarding antibiotics in the environment prioritised analytical techniques, water samples were often analysed as proof of concept. Very early on, the presence of individual antibiotic substances or respective degradation products could be proven in wastewater effluent and surface water. These findings shed the first light on the behavior of antibiotics during wastewater treatment: removal efficiency is poor (Tran et al., 2016; Peixoto et al., 2016; Periša and Babić, 2014; Cernoch et al., 2012; Le-Minh et al., 2012; Szymańska et al., 2019; Mehata et al., 2022; Nannou et al., 2020).

However, analytical strategies were commonly developed for certain substance groups and often a complete quantification was neglected.

In 2004, Miao et al. started a thorough investigation by quantifying 31 antimicrobials from the macrolide, quinolone, quinoxaline dioxide, sulfonamide, and tetracycline classes in the treated effluent of eight WWTPs in Canada. The concentrations of the substances did not exceed 1 μg/L (Miao et al., 2004). Flow-proportional sampling of raw sewage, sludge and WWTP effluent was conducted by Lindberg et al. Results obtained by HPLC-MS/MS analysis indicated that fluoroquinolones have a tendency for sorption to sludge, whereas sulfamethoxazole and trimethoprim remain primarily in the liquid phase (Lindberg et al., 2005).

One of the first studies that also included composite samples for influent and effluent of a WWTP in the investigation was published in 2008: 24-h samples were collected. MS/MS analysis allowed for quantification. Comparing influent and effluent samples of eight quinolone and fluoroquinolone antibiotics, the authors found that the reduction of analyte concentration varied from 50 % to 82 %. However, as only one influent and one effluent sample of each WWTP are compared, leaving out the influence of hydraulic and sludge retention time, a total balancing of substance input and output is not reliable (Xiao et al., 2008).

Decreasing detection and quantification limits allowed for the determination of a wide presence of e.g. sulfonamides in WWTP influent and effluent, as well as ground water and surface water. García-Galán

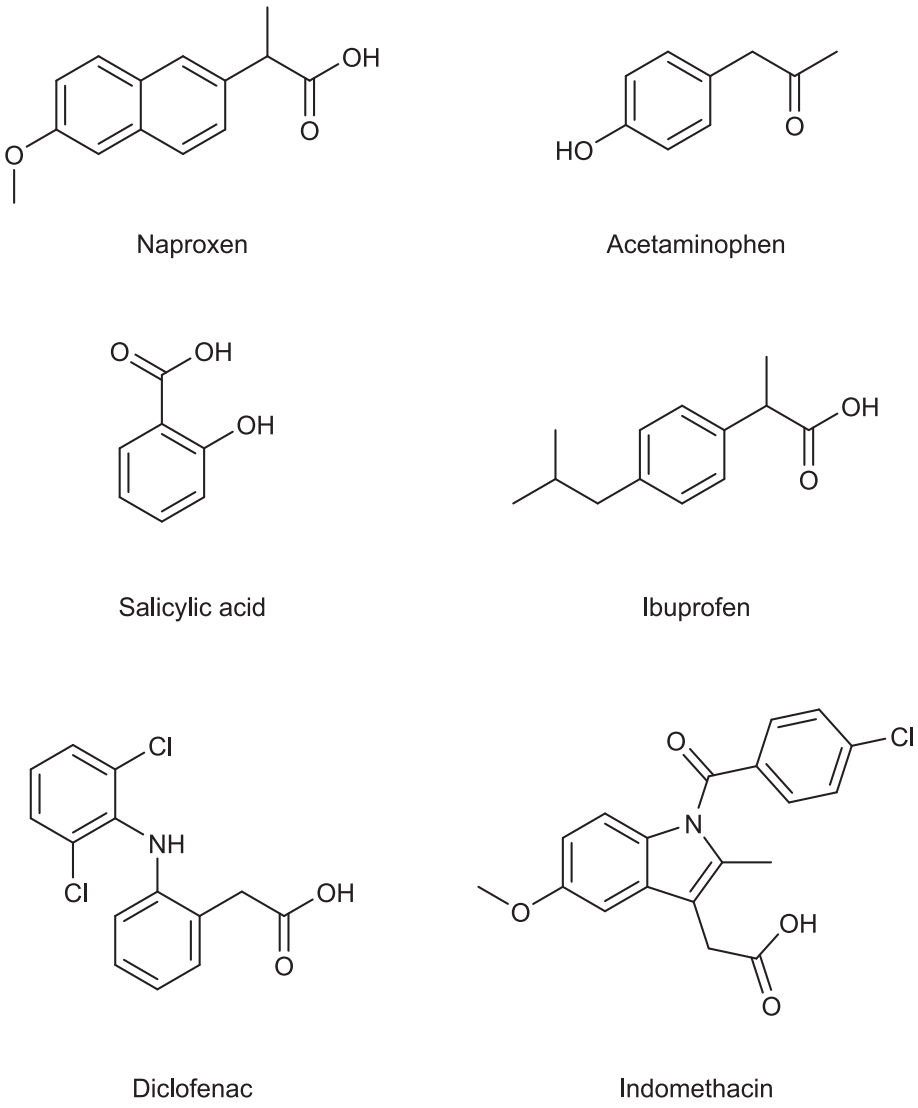

Fig. 5. Structures of various analgesics: naproxen, acetaminophen, salicylic acid, ibuprofen, diclofenac and indomethacin.

et al. optimized the LOD to be as low as 0.12 ng/L (for sulfamerazine) in WWTP effluent water (García-Galán et al., 2010). The implementation of HPLC-QTOF-MS/MS, combining the highly accurate mass measurement with the structural information, enabled identification and structural elucidation of amoxicillin and its main transformation products (Pérez-Parada et al., 2011). Le-Minh et al. successfully incorporated the analysis of the metabolites of sulfonamide in wastewater as Göbel et al. had recommended in 2004 (Le-Minh et al., 2012; Göbel et al., 2004). Representative metabolites were also included in the method development of Gros et al. who developed a multi-residue analytical method based on SPE and HPLC-MS/MS. Eleven out of 53 investigated antibiotic compounds were detected in WWTP influent and effluent, metabolites could not be detected (Gros et al., 2013).

A study in 2014, combining the analysis of 2-h mixed samples of influent and effluent WWTP samples and the analysis of sludge samples indicated that the WWTP process removed odor 90 % of triclosan from wastewater, while only 25 % of ofloxacin was eliminated (Pasquini et al., 2014). Another study by Chen et al. investigated the presence of antibiotics in different treatment stages. A mass balance indicates that biodegradation was the major route of removal. The calculations compared the antibiotics concentration between liquid and solid phase. The determined removal efficiency varied strongly (e.g. 5.4 % to 99.9 % erythromycin), which might be due to the fact that samples were neither collected as composite samples nor flow-controlled (Chen et al., 2022).

The determination of removal efficiencies based on a mass balancing with composite samples from WWTP influent and effluent was performed by Gurke et al. in 2015. For ten consecutive days, 24-h composite samples were collected flow-proportionally in the influent and effluent of a WWTP. The length and manner of sampling guarantees a high reliability of the results. The removal efficiencies of several pharmaceutical compounds were calculated. An efficiency of 42.4 % was determined for sulfamethoxazole (Gurke et al., 2015). In another study, influent and effluent load of antibiotics were compared and removal efficiencies determined in a range between 20 % (trimethoprim), 58 % (sulfamethoxazole) and 86 % (norfloxacin) determined (Golovko et al., 2014). The removal efficiency of several antibiotics were also determined by comparison of analyte concentrations in 24-h composite samples collected over seven consecutive days in an advanced treatment plant. Removal rates between 10 % (clindamycin), 82 % (sulfamethoxazole) and 100 % (tetracycline) after secondary treatment and >65 % for all compounds after tertiary treatment were determined (Lopez et al., 2022). Obviously, the removal efficiency is strongly dependent on the substance and its structural properties. The differences between studies, which can be clearly shown when looking at the three calculated removal efficiencies for sulfamethoxazole, may result from parameters of the sampled WWTP, such as dilution rates, very high temperature

differences, or different solid retention times (STP). However, we assume that huge impact comes with the chosen sampling strategies. Composite samples were taken flow- or time proportional, which has a certain potential for variations within the results.

The behavior of antibiotics during wastewater treatment has been investigated intensively over the last decades (Table 3). This might be due to the growing challenge of bacterial resistance. Compared to other PhACs, whose impact on the aqueous environment can only be estimated, the impact on bacterial resistance against antibiotics is a rather imminent threat, resulting in a significant interest. Due to the large number of compounds, it is still difficult to paint a complete picture of antibiotic behavior in WWTPs. The calculated removal efficiencies, however, vary strongly, especially between compounds (10–100 %) (Lopez et al., 2022). One reason can be the different polarities of the different compounds employed as antibiotics, as the polarity has a major impact on the water solubility and the behavior during biochemical degradation and adsorption to biomass. Sulfonamides are more polar than quinolones and much more polar than tetracyclines (Díaz-Cruz and Barceló, 2006). However, the majority of the studies show that removal of most compounds is incomplete. This does suggest that the total amount of antibacterial agents reaching surface water is high. The determination of effluent loads of all compounds should be the next step in order to estimate the input of antibiotics into the aquatic environment.

3.3. Analgesics and anti-inflammatory drugs

Analgesics and anti-inflammatory drugs are a broad category of compounds that are used to treat acute or chronic pain and reduce inflammation. Acetaminophen, for example, is the most widely used medicine worldwide and is, unlike other compounds discussed is this article, readily available without prescription in most countries (Brune et al., 2015). Analgesics and anti-inflammatory drugs can be classified by their therapeutic effects, mechanism of action, and structure. The group of non-opinoid analgesics is the most common form of analgesics and includes acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs). This chapter focuses on these compounds. Studies on the behavior of painkillers commonly comprise a range of several analgesics (Fig. 5).

One of the first investigation of the behavior of anti-inflammatory drugs was carried out in 2003 by Lee et al. with GC–MS. Salicylic acid, ibuprofen, acetaminophen, naproxen, ketoprofen, diclofenac, and indomethacin were determined in influent and effluent WWTP samples by means of GC–MS after sample preparation with SPE and derivatization with TMS. LODs of 0.01 μg/L were estimated for all compounds but indomethacin. Concentrations as high as 1.03 μg/L salicylic acid were determined in effluent (Lee et al., 2003). Similar method parameters could be achieved by Metcalfe et al. who also determined similar concentrations in WWTP samples (Metcalfe et al., 2003). In the same year, Koutsouba et al. achieved a much improved LOD of 0.6 ng/L for ibuprofen and 1 ng/L diclofenac and confirmed the presence of analgesics in influent and effluent of a WWTP (Koutsouba et al., 2003).

A number of studies employed GC–MS for the determination of analgesics and anti-inflammatories (Han et al., 2006; Verenitch et al., 2006; Togola and Budzinski, 2007, Antoniou et al., 2009). Method optimization was the focus of Lin et al.: different SPE cartridges were tested. Additionally, injection-port derivatization resulted in a rapid and quantitative method for the trace determination of PhACs in aqueous samples, including ibuprofen, naproxen, ketoprofen, diclofenac (Lin et al., 2009). Ibuprofen and naproxen were analysed by means of enantiomeric GC. This led to additional information and to the characterization of distinct differences in treated wastewater effluent compared to untreated untreated sewage. As the chiral inversion might occur during wastewater treatment, a distinction of treated and untreated sources of the compounds is possible with this strategy (Khan et al., 2014). An unusual technique for the determination of ibuprofen and acetaminophen in wastewater samples was applied by Gibbons et al. in 2011. A fast, simple and low-cost CE-UV method was developed (Gibbons et al., 2011).

The first analysis with HPLC separation was carried out by Santos et al. in 2005. Acetaminophen, diclofenac, ibuprofen, ketoprofen and naproxen were determined in influent and effluent samples that were collected as flow proportional 24-h samples. After preconcentration with SPE, HPLC-UV analysis was carried out. Detection of ibuprofen and naproxen was also executed with fluorescence. The method was not suitable for acetaminophen determination which could not be extracted from the samples. Diclofenac could not be detected in the WWTP samples with this method. Ibuprofen was found high concentrated (up to 143 μg/L) in influent. The presence of ibuprofen, ketoprofen and naproxen in effluent samples confirmed the hypothesis, that analgesics and anti-inflammatory drugs cannot be removed completely by conventional wastewater treatment (Santos et al., 2005).

The majority of following studies based were hyphenations of HPLC-MS/MS analysis following sample preparation with SPE (McEneff et al., 2014; Al-Tarawneh et al., 2015; Ghoshdastidar et al., 2015; Camacho-Muñoz and Kasprzyk-Hordern, 2015; Dasenaki and Thomaidis, 2015; Morosini et al., 2017; Oliveira et al., 2015). Gros et al. performed an elaborate method development for a range of pharmaceuticals including eight analgesics and anti-inflammatories. LODs between 1 ng/L (mefenamic acid) and 21 ng/L (ketoprofen) were achieved. In WWTP effluent, acetaminophen was determined in concentrations up to 5990 ng/L (Gros et al., 2006). Similar results were determined by Al-Odaini et al. and Yan et al. (Yan et al., 2014; Al-Odaini et al., 2010). In another study, ibuprofen, diclofenac, ketoprofen, and naproxen were determined in several 24-h composite WWTP samples with HPLC-MS/MS after SPE. In the multi-residue study, diclofenac and ibuprofen were the PhACs most resistant to the wastewater treatment, showing the highest concentrations in all effluents (Patrolecco et al., 2015).

Although tandem MS proved to be the method of choice for detection, other mass filters were tested as well. In 2006, Petrovic et al. determined a wide range of PhACs in wastewater, including analgesics. Following a SPE procedure, analysis was carried out with UPLC-Q-TOF. LODs ranged between 20 ng/L (diclofenac) and 150 ng/L (ketoprofen, ibuprofen). As expected, Q-ToF showed some disadvantages in terms of sensitivity compared to triple quadrupole instruments. However, Q-ToF offered the advantage of unequivocal identification of target PhACs (Petrovic et al., 2006). Similar observations were made by Robles-Molina et al. in 2014 (Robles-Molina et al., 2014). The advantage of identification capabilities of high mass accuracy data was also emphasized by Cahill et al. who evaluated the application of an orbitrap. Excellent LODs were achieved: 1.9 ng/L were determined for ibuprofen (Cahill et al., 2012). With double focusing magnetic sector high resolution MS, several antiinflammatoriy drugs were determined in influent and effluent WWTP samples in another study. The analgesic found in the highest concentrations was paracetamol (up to 67,107 ng/L) (Vergeynst et al., 2015).

As important part of the analytical strategy for environmental samples, the sample preparation including clean-up and preconcentration was optimized for analgesics in several studies. Sample preparation was commonly executed with SPE. Several alternatives were tested over the years. Nödler et al. and Sousa et al. compared several sorbents to optimize the procedure (Nödler et al., 2010; Sousa et al., 2011). In another project, molecular imprinted SPE (MISPE) was considered to be straightforward and fast (Zorita et al., 2008). Dual SPE (dSPE) enabled the simultaneous extraction of PhACs with acidic and basic characteristics, including naproxen and diclofenac (Unceta et al., 2010). A rare study looking into the metabolites of diclofenac was carried out in 2008. Analysis of effluent samples with HPLC-MS/MS showed the wide occurrence of diclofenac and its metabolites. The results indicate that not only diclofenac but also its metabolites are globally entering the aqueous environment (Stülten et al., 2008).

Early on, the removal of analgesics and anti-inflammatory drugs

Table 4
Details and results of wastewater analysis regarding analgesics in the past decades.

Sampling		Literature
Grab samples		Koutsouba et al., 2003; Han et al., 2006; Verenitch et al., 2006; Togola and Budzinski, 2007; Lin et al., 2005; Khan et al., 2014; Gibbons et al., 2011; Ghoshdastidar et al., 2015; Camacho-Muñoz and Kasprzyk-Hordern, 2015; Yan et al., 2014; Al-Odaini et al., 2010; Petrovic et al., 2006; Robles-Molina et al., 2014; Cahill et al., 2012; Zorita et al., 2008; Nödler et al., 2010; Sousa et al., 2011; Antoniou et al., 2009; Gao et al., 2012; Gros et al., 2006; Lin et al., 2009
Composite samples	Flow proportional	Dasenaki and Thomaidis, 2015; Bueno et al., 2009; Pasquini et al., 2014
	Time proportional	Metcalfe et al., 2003; Morosini et al., 2017; Oliveira et al., 2015; Lee et al., 2003; Santos et al., 2005
	No details	McEneff et al., 2014; Al-Tarawneh et al., 2015; Patrolecco et al., 2015; Vergeynst et al., 2015; Gros et al., 2007; Kleywegt et al., 2016; Huang et al., 2011; Lacina et al., 2013; Aydin et al., 2019; Lopez et al., 2022; Gao et al., 2012

Preparation		Literature
SPE	Non-polar (reversed phase)	Koutsouba et al., 2003; Han et al., 2006; Verenitch et al., 2006; Togola and Budzinski, 2007; Lin et al., 2005; Khan et al., 2014; Gibbons et al., 2011; Ghoshdastidar et al., 2015; Yan et al., 2014; Petrovic et al., 2006; Robles-Molina et al., 2014; Cahill et al., 2012; Nödler et al., 2010; Gao et al., 2012; Gros et al., 2006; Lin et al., 2009; Bueno et al., 2009; Pasquini et al., 2014; Metcalfe et al., 2003; Lee et al., 2003; Santos et al., 2005; McEneff et al., 2014; Al-Tarawneh et al., 2015; Patrolecco et al., 2015; Vergeynst et al., 2015; Kleywegt et al., 2016; Huang et al., 2011; Lacina et al., 2013; Aydin et al., 2019; Camacho-Muñoz and Kasprzyk-Hordern, 2015; Dasenaki and Thomaidis, 2015; Becerra-Herrera et al., 2015; Stülten et al., 2008; Papageorgiou et al., 2016
	Cation exchange	Khan et al., 2014; Al-Odaini et al., 2010
	Anion exchange	Camacho-Muñoz and Kasprzyk-Hordern, 2015; Sousa et al., 2011

Analysis					Literature
Method	Derivatization	Column	MS	LOD (ng/L)* of ibuprofen and/or diclofenac**	
GC–MS	Alkylation	Non-polar	Single quadrupole	250 (diclofenac) 50 (ibuprofen)	Togola and Budzinski, 2007; Metcalfe et al., 2003
			Ion trap	1.0 (diclofenac) 0.6 (ibuprofen)	Koutsouba et al., 2003; Verenitch et al., 2006; Lin et al., 2005; Lin et al., 2009
	Silylation	Low polarity x mid polar	ToF	2.88 (diclofenac) 1.33 (ibuprofen)	Lacina et al., 2013
	Silylation	Non-polar	Single quadrupole	22 (diclofenac) 12 (ibuprofen)	Han et al., 2006; Lee et al., 2003
	Chiral derivatization	Non-polar	Triple quadrupole	*Na*	Khan et al., 2014
CE-UV	–	–	–	*Na*	Gibbons et al., 2011
HPLC-UV	–	Reversed phase	–	*Na*	Santos et al., 2005
HPLC-FL	–	Reversed phase	–	*Na*	Santos et al., 2005
HPLC-MS	–	Reversed phase	Ion trap	17 (diclofenac) 62 (ibuprofen)	Sousa et al., 2011; Bueno et al., 2009; Morosini et al., 2017; Patrolecco et al., 2015; McEneff et al., 2014
			Triple quadrupole	5.4 (diclofenac)*** 12 (ibuprofen)	Ghoshdastidar et al., 2015; Yan et al., 2014; Al-Odaini et al., 2010; Zorita et al., 2008; Nödler et al., 2010; Dasenaki and Thomaidis, 2015; Oliveira et al., 2015; Al-Tarawneh et al., 2015; Gros et al., 2007; Huang et al., 2011; Aydin et al., 2019; Lopez et al., 2022; Stülten et al., 2008; Lee et al., 2003; Březinova et al., 2018; Papageorgiou et al., 2016
			ToF	20 (diclofenac) 55 (ibuprofen)	Petrovic et al., 2006; Robles-Molina et al., 2014; Vergeynst et al., 2015; Lopez et al., 2022; Becerra-Herrera et al., 2015
			Orbitrap	1.9 (ibuprofen)	Cahill et al., 2012
			Double focusing	35 (diclofenac) 312 (ibuprofen)	Vergeynst et al., 2015
		chiral	Triple quadrupole	0.7 (ibuprofen)	Camacho-Muñoz and Kasprzyk-Hordern, 2015

Results		Literature
Concentration in influent	Up to 98,700 ng/L diclofenac and up to 758,000 ng/L ibuprofen	Koutsouba et al., 2003; Han et al., 2006; Gibbons et al., 2011; Petrovic et al., 2006; Cahill et al., 2012; Gros et al., 2006; Metcalfe et al., 2003; Morosini et al., 2017; Santos et al., 2005; Al-Tarawneh et al., 2015; Patrolecco et al., 2015; Vergeynst et al., 2015; Lacina et al., 2013; Aydin et al., 2019; Lopez et al., 2022; Dasenaki and Thomaidis, 2015; Becerra-Herrera et al., 2015; Sousa et al., 2011; Sultana et al., 2017
Concentration in effluent	Up to 10,960 ng/L diclofenac and up to 24,600 ng/L ibuprofen	Koutsouba et al., 2003; Han et al., 2006; Verenitch et al., 2006; Togola and Budzinski, 2007; Lin et al., 2005; Al-Odaini et al., 2010; Zorita et al., 2008; Nödler et al., 2010; Sousa et al., 2011; Metcalfe et al., 2003; Morosini et al., 2017; Santos et al., 2005; McEneff et al., 2014; Al-Tarawneh et al., 2015; Patrolecco et al., 2015; Vergeynst et al., 2015; Lopez et al., 2022; Dasenaki and Thomaidis, 2015; Becerra-Herrera et al., 2015; Stülten et al., 2008; Sultana et al., 2017; Camacho-Muñoz and Kasprzyk-Hordern, 2015; Bueno et al., 2009; Petrovic et al., 2006; Lacina et al., 2013; Aydin et al., 2019; Gros et al., 2006

(*continued on next page*)

Table 4 (*continued*)

Results		Literature
Load influent	Up to 1.190 mg/(d 1000 inhabitants) diclofenac	Papageorgiou et al., 2016
Load effluent	Up to 0.541 mg/(d 1000 inhabitants) diclofenac	Papageorgiou et al., 2016
Removal efficiencies	Between 22 % and 100 % (diclofenac) and between 22 % and 100 % (ibuprofen)	Han et al., 2006; Metcalfe et al., 2003; Patrolecco et al., 2015; Vergeynst et al., 2015; Huang et al., 2011; Gros et al., 2007; Kleywegt et al., 2016; Lacina et al., 2013; Aydin et al., 2019; Lopez et al., 2022; Sultana et al., 2017; Lin et al., 2009

na: LOD not determined in effluent in the respective study.
* Lowest LOD of listed studies (determined for analysis in effluent).
** Diclofenac and ibuprofen are frequently analysed compounds and therefore used for comparison between studies.
*** Determined from LOQ.

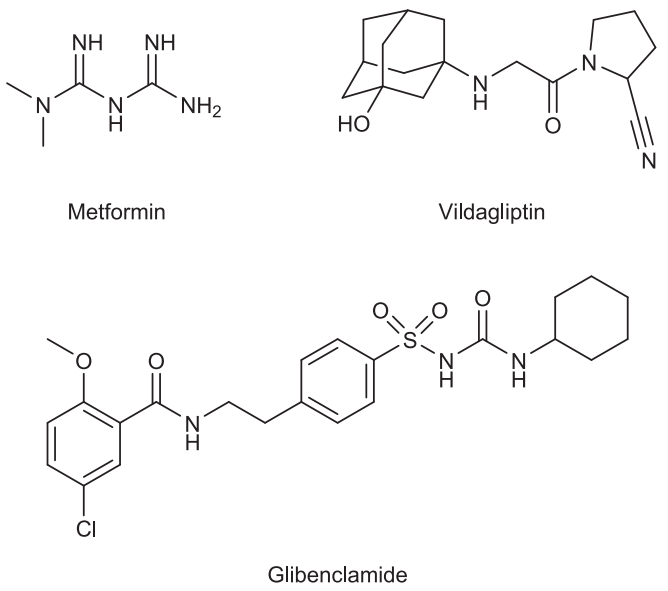

Fig. 6. Structures of various antidiabetics: metformin, vildagliptin and glibenclamide.

during wastewater treatment was investigated. Although high concentrations of the compounds were detected in the effluent samples of WWTPs from the beginning, surprisingly high removal rates of several pain killers were determined. With a simple plain comparison of concentrations in influent and effluent in 24-h composite samples, Gros et al. determined the removal efficiencies of different WWTPs for a wide range of PhACs. The results showed a high variation between different sites. Conventional biological wastewater treatment was found to be unable to efficiently remove a wide range of PhACs studied (Gros et al., 2007). However, more elaborate sampling for detailed results were necessary. A determination of removal efficiencies based on the loads in grab samples taken according to the hydraulic retention time of a WWTP confirmed the results of Gros et al.: 72 % - 97 % for six analgesics and anti-inflammatories (Lin et al., 2009). A similar strategy led to similar results a few years later: 76 % - 100 % for acetaminophen and ibuprofen, 51 % - 75 % for naproxen (Kleywegt et al., 2016). As did a study by Huang et al. in 2011: elimination rates between 72 % (diclofenac) and 100 % (indomethacin) were calculated in composite samples (Huang et al., 2011).

An elaborate sampling was carried out by Lacina et al.: for one month, 24-h influent and effluent mixed samples were collected time-dependent in 2-h intervals. Samples were analysed with two dimensional GC with ToF-MS (GCxGC-ToF-MS) after SPE and derivatization. Removal efficiencies of 72.4 % (ketoprofen) and 99.8 % (salicylic acid) were determined (Lacina et al., 2013). More recent studies determined removal efficiencies in the same range for several analgesics and anti-inflammatories (Aydin et al., 2019; Lopez et al., 2022).

Another approach with the aim of a total overview of input and output of so called indicator compounds in WWTPS was carried out in 2017 by Sultana et al. Ibuprofen removals were determined in the range of 85–97 % in five of six sampled WWTPs. Naproxen could not be determined in all samples and showed a strong variation in calculated removal efficiencies (Sultana et al., 2017).

Besides the technology of the sampled WWTP, the removal efficiency is strongly dependent on the substances and their structural properties. Special attention towards ibuprofen was paid in a study by Březinova et al.: the metabolites carboxyibuprofen and hydroxyibuprofen enter the wastewater system along with the unaltered ibuprofen, but can also be formed during anoxic and/or aerobic conditions. By employing UPLC-MS/MS and sampling of composite samples of a full-scale constructed wetland, the authors determined different removal efficiencies for the three compounds: 44.7 %, 29.3 % and 47.5 % for IBU, OH-IBU and CA-IBU, respectively (Březinova et al., 2018).

As seen in the results of the analgesics, differences between studies can very high. Table 4 shows how different sampling strategies can be. We assume that the differences in calculated removal efficiencies can partly be linked to sampling.

The number of references cited in this chapter alone shows that the behavior of analgesics and anti-inflammatory drugs is investigated rather intensively compared to other PhACs. Despite the high number of available compounds. A wide range of sample preparation techniques, including SPE but also several other approaches, was explored. Separation has been carried out with GC, HPLC and even CE. Detection was commonly performed by MS, but even here other techniques besides tandem MS were evaluated. Low LODs allowed for the analysis of the majority of the substances in wastewater samples. The wastewater treatment procedure is not capable of removing all analgesics and anti-inflammatories, this was shown early on. But removal efficiencies have in most studies been determined to be >70 % - which is a relief, as the concentrations (and loads) of the drugs were found to be among the highest of the PhACs in wastewater.

3.4. Antidiabetics

Type-2 diabetes mellitus is a chronic metabolic disorder caused by the body becoming resistant to the effects of insulin. The number of people affected by diabetes accounts for >200 million on a worldwide scale (Kosma et al., 2015). Pharmaceuticals used to treat diabetes alter the glucose level in the blood. There are different classes of antidiabetic drugs, working in different ways (Fig. 6). Insulin is given intravenously to patients suffering from diabetes mellitus type 1. Type 2 diabetes can be treated with biguanides (usually metformin), sulfonylureas, meglitinide, dipeptidyl peptidase 4 (DPP-4) inhibitors, sodium-glucose cotransporter (SGLT2) inhibitors, α-glucosidase inhibitors and thiazolidinediones. Due to the large number of patients and the corresponding frequent usage of antidiabetic drugs, the input into the sewage system is very high.

The first data on the occurrence of metformin in sewage and surface waters was presented in 2009 by Scheurer et al. The analytical method, including pre-concentration by SPE and determination with HPLC-MS/MS achieved a detection limit of 10 ng/L. Influent and effluent of three WWTPs was sampled as 24-h composite sample. During sampling,

Table 5
Details and results of wastewater analysis regarding antidiabetics in the past decades.

Sampling		Literature
Grab samples		Kafeenah et al., 2018; Al-Odaini et al., 2010; Ghoshdastidar et al., 2015
Composite samples	Flow proportional	Dasenaki and Thomaidis, 2015
	Time proportional	Oliveira et al., 2015
	No details	Scheurer et al., 2009; Scheurer et al., 2012; Kosma et al., 2015; Zheng et al., 2022

Preparation		Literature
SPE	Non-polar (reversed phase)	Ghoshdastidar et al., 2015; Dasenaki and Thomaidis, 2015; Kosma et al., 2015; Bones et al., 2006; López-Serna et al., 2010; Martín et al., 2012; Kafeenah et al., 2018; Oertel et al., 2018
	Cation exchange	Scheurer et al., 2009; Scheurer et al., 2012; Al-Odaini et al., 2010

Analysis				Literature
Method	Column	MS	LOD (ng/L)* of metformin**	
HPLC-MS	Reversed phase	Triple quadrupole	9	Kafeenah et al., 2018; Al-Odaini et al., 2010; Ghoshdastidar et al., 2015; Dasenaki and Thomaidis, 2015; Oliveira et al., 2015; Zheng et al., 2023; López-Serna et al., 2010; Oertel et al., 2018
		Ion trap	*Na*	Bones et al., 2006
		Q-ToF	2.6***	Martín et al., 2012
		Orbitrap	5.1	Kosma et al., 2015
	HILIC	Triple quadrupole	10	Scheurer et al., 2009; Scheurer et al., 2012; Zheng et al., 2022

Results		Literature
Concentration in influent	Up to 215,000 ng/L (metformin)	Kafeenah et al., 2018; Dasenaki and Thomaidis, 2015; Scheurer et al., 2009; Scheurer et al., 2012; Kosma et al., 2015; Zheng et al., 2022; Dasenaki and Thomaidis, 2015
Concentration in effluent	Up to 53,000 ng/L (metformin)	Kafeenah et al., 2018; Al-Odaini et al., 2010; Ghoshdastidar et al., 2015; Dasenaki and Thomaidis, 2015; Scheurer et al., 2009; Scheurer et al., 2012; Kosma et al., 2015; Zheng et al., 2022
Removal efficiencies	Between 68 % and 98 % (metformin)	Kafeenah et al., 2018; Scheurer et al., 2009; Scheurer et al., 2012

na: LOD not determined in effluent in the respective study.
* Lowest LOD of listed studies (determined for analysis in effluent).
** Metformin is the most frequently analysed compound and therefore used for comparison between studies.
*** Determined from LOQ.

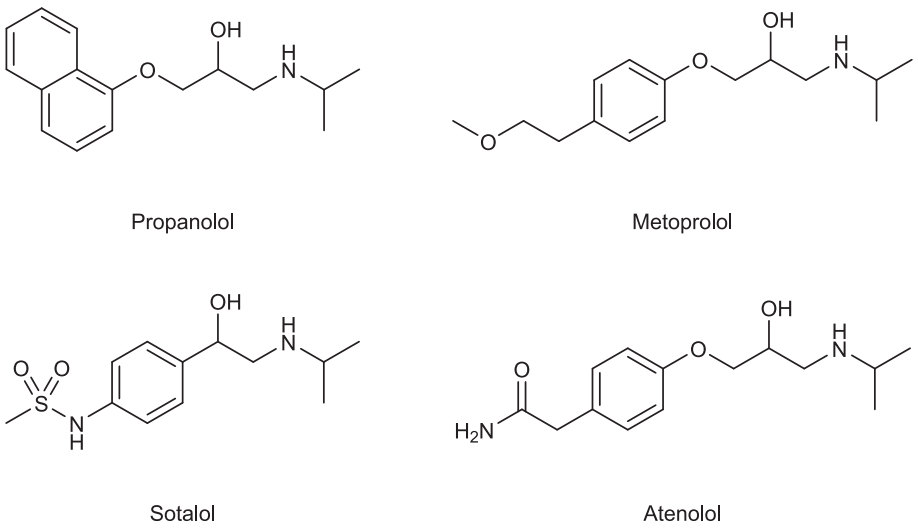

Fig. 7. Structures of lipophilic (propanolol, metoprolol) and hydrophilic (sotalol, atenolol) betablockers.

the residence time of the plants was taken into account, allowing an estimation of the removal rates. These removal efficiencies in the three sampled WWTPs were between 79 and 98 % (Scheurer et al., 2009). In 2010, Al-Odaini et al. optimized the technique with SPE and HPLC-MS/MS. In effluent, LODs between 0.4 ng/L (glibencalmid) and 9 ng/L (metformin) could be achieved. With this method, concentrations between 5 ng/L (glibenclamid) and 65 ng/L (glicazide) were detected in WWTP effluent (Al-Odaini et al., 2010). In the same year, glibenclamide was part of an extensive study determining 74 contaminants in environmental waters, including wastewater. With a fully automated method including on-line SPE and HPLC-MS/MS, a detection limit of 13.54 ng/L was achieved for glibenclamide in WWTP effluent (López-

Serna et al., 2010).

Analytical strategies for the determination of antidiabetics in water, in the majority of the cases focusing on metformin, were optimized in the following years, always consisting of a SPE procedure and HPLC-ESI-MS analysis (Bones et al., 2006; Oertel et al., 2018). Martín et al. conducted a comprehensive survey of a wider range of antidiabetic drugs. Glibenclamide, metformin, pioglitazone, rosiglitazone, sitagliptin and vildagliptin were determined with a method based on SPE and determination with HPLC-MS (Q-ToF-MS). A LOQ of 8.2 ng/L (metformin) was reached. All but two substances could be determined in WWTP effluent grab samples (Martín et al., 2012).

Metformin was part of several multianalyte studies analysing WWTP influent and/or effluent, achieving method detection limits as low as 0.3 ng/L. All studies employed a SPE procedure and HPLC-MS/MS (Dasenaki and Thomaidis, 2015; Kafeenah et al., 2018; Oertel et al., 2018). A different approach was made by Zheng et al.: the application of direct injection HPLC-MS/MS led to an LOD of 0.04 μg/L for metformin. The method utilised a hydrophilic interaction HPLC together with simple filtration through 0.2 μm regenerated cellulose filter. The concentration of metformin in WWTP influent and effluent samples could easily be determined (Zheng et al., 2022; Zheng et al., 2023). In the same year, Oliveira et al. developed a direct injection HPLC-MS/MS method for even more analytes: 185 pharmaceutical and personal care products were determined in 24-h composite samples from WWTPs. Metformin was the compound measured in the highest concentration among all analytes: up to 720 μg/L in WWTP effluent (Oliveira et al., 2015). With a LOD of 12 ng/L for metformin, very high concentrations of this antidiabetic in WWTP effluent could be confirmed in another study: up to 10,608 ng/L (Ghoshdastidar et al., 2015).

The assessment of elimination mechanisms was examined when Scheurer et al. investigated guanylurea in WWTP effluent. Corresponding 24-h composite influent and effluent wastewater samples were analysed by SPE and HPLC-MS/MS. Due to the analyte's polar nature, a HILIC column was successfully employed. Concentrations of metformin

Table 6
Details and results of wastewater analysis regarding beta blockers in the past decades.

Sampling		Literature
Grab samples		Huggett et al., 2003; Al-Odaini et al., 2010; Dahane et al., 2013; Rice et al., 2020; Hernando et al., 2004; Salem et al., 2012; Ghoshdastidar et al., 2015
Composite samples	Flow proportional	Ternes, 1998; Gurke et al., 2015
	No details	Lee et al., 2007; van Nuijs et al., 2010; Asimakopoulos et al., 2017; Lopez et al., 2022

Preparation		Literature
SPE	Non-polar	Huggett et al., 2003; Rice et al., 2020; Gurke et al., 2015; van Nuijs et al., 2010; Asimakopoulos et al., 2017; Lopez et al., 2022; Hernando et al., 2004; Ghoshdastidar et al., 2015; Papageorgiou et al., 2016
	Cation exchange	Al-Odaini et al., 2010; Lee et al., 2007; van Nuijs et al., 2010; Asimakopoulos et al., 2017; Salem et al., 2012
	Anion exchange	van Nuijs et al., 2010

Analysis					Literature
Method	Derivatization	Column	MS	LOD (ng/L) * of metoprolol**	
GC–MS	Silylation, acetylation	Non-polar	Single quadrupole	25	Huggett et al., 2003; Ternes, 1998
HPLC-MS	–	Reversed phase	Triple quadrupole	3.3***	Al-Odaini et al., 2010; Lee et al., 2007; Gurke et al., 2015; Lopez et al., 2022; Salem et al., 2012; Silva et al., 2021; Ghoshdastidar et al., 2015; Papageorgiou et al., 2016
			Ion trap	*Na*	Dahane et al., 2013; Asimakopoulos et al., 2017
		Supercritical LC	Single quadrupole	10	Rice et al., 2020
		HILIC	Triple quadrupole	*Na*	van Nuijs et al., 2010
		Enantioselective	Triple quadrupole	0.3	MacLeod et al., 2007

Results		Literature
Concentration in influent	Up to 1190 ng/L metoprolol and up to 6211.7 ng/L atenolol	Lee et al., 2007; Dahane et al., 2013; Rice et al., 2020; van Nuijs et al., 2010; Asimakopoulos et al., 2017; Lopez et al., 2022; Salem et al., 2012
Concentration in effluent	Up to 995 ng/L metoprolol and up to 1680 ng/L atenolol	Al-Odaini et al., 2010; Lee et al., 2007; Dahane et al., 2013; Rice et al., 2020; Asimakopoulos et al., 2017; Lopez et al., 2022; Salem et al., 2012; Ghoshdastidar et al., 2015
Load influent	Between 0.005 g/(d 1000 inhabitants) and 1.218 g/(d 1000 inhabitants) metoprolol****	Ternes, 1998; Lopez et al., 2022; Papageorgiou et al., 2016
Load effluent	Between 0.003 g/(d 1000 inhabitants) and 0.385 g/(d 1000 inhabitants) metoprolol****	Ternes, 1998; Lopez et al., 2022; Papageorgiou et al., 2016
Removal efficiencies	Between 9 % and 83 % (metoprolol)	Ternes, 1998; Lee et al., 2007; Lopez et al., 2022

na: LOD not determined in effluent in the respective study.
* Lowest LOD of listed studies (determined for analysis in effluent).
** Metoprolol is the most frequently analysed compound and therefore used for comparison between studies.
*** Determined from LOQ.
**** Calculated for better comparison based on available information in reference.

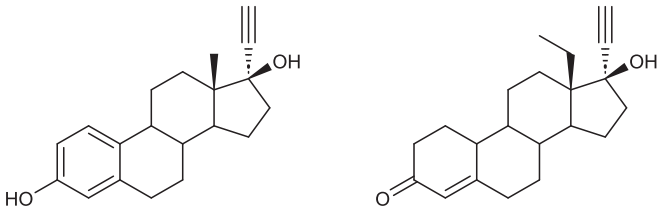

Fig. 8. Structures of two steroidal hormonal contraceptives: 17α-ethinylestradiol and levornogestrel.

and its degradation product guanylurea were determined. Metformin elimination was calculated to be >88 % in all five sampled WWTPs. In the WWTP where the concentration of metformin decreased from 105 to 2.7 μg/L, the guanylurea concentration increased from 3 to 99 μg/L. This indicates clearly that biodegradation is the reason of metformin elimination (Scheurer et al., 2012). Similar studies by Kosma et al. and Yao et al. confirmed these observations (Kosma et al., 2015; Yao et al., 2018; Tisler and Zwiener, 2018).

The behavior of the highly polar metformin during wastewater treatment has been investigated elaborately (Table 5). High concentrations were detected in WWTP effluent samples in various studies. This would indicate inefficient removal during wastewater treatment. However, the removal efficiency calculated in a few studies is throughout >79 %, rather high compared to many other PhACs. Presumably, metformin is efficiently removed in WWTP, but introduced in such high levels that its concentration in WWTP effluent can still be very high. This example shows how important it is to compare influent and effluent samples, best as composite samples. Besides that, the main boundary conditions such as biomass concentration and HRT should be provided. The latter then would provide a more detailed picture of removal.

Only little is known about other antidiabetics; they were detected in WWTP effluent also, but information on removal efficiency has yet to be determined.

3.5. Beta blockers

Beta blockers are predominantly prescribed for hypertension, migraine and cardiovascular disease. They work by blocking the receptor cites for adrenaline and noradrenaline. Their primary effect is the reduction of the heart rate, which lowers the blood pressure (Gottschau et al., 2022). Cardiovascular drugs belong to the most frequently prescribed pharmaceuticals. Owing to the fact that the lipophilic beta blockers (e.g. propranolol, alprenolol) are extensively metabolized and eliminated predominantly, the investigation of their behavior during wastewater treatment is not straightforward. The more hydrophilic beta blockers (e.g. atenolol, nadolol, sotalol) are almost exclusively excreted unchanged (Lee et al., 2007). Due to their relatively high hydrophilic properties, they are expected to be eliminated insufficiently during wastewater treatment (Stankiewicz et al., 2015) (Fig. 7).

One of the first studies investigating a beta blocker in wastewater was carried out in 1998 by Ternes. Seven beta blockers were determined in influent and effluent WWTP samples. Several methods were developed and tested: GC–MS with a two-step derivatization with silylation and acetylation achieved a LOD of 0.025 μg/L. Composite influent and effluent WWTP samples were analysed. All seven substances were detected. Metoprolol, being the most often prescribed beta blocker, was found in the highest concentrations. Nevertheless, the authors indicate that the removal efficiencies of propranolol and metaprolol are high (96 % and 83 %, respectively). The principal excreted metabolites were not examined (Ternes, 1998).

A lot of ground was covered by this study although it is already 26 years old. However, analytical techniques advanced over the years and optimized methods were developed and applied. Beta blockers were often part of multiresidue studies that included several environmental waters like wastewater, river and lake water.

Huggett et al. employed GC–MS preceded by SPE and determined concentrations up to 1.9 μg/L of propranolol in WWTP grab effluent samples (Huggett et al., 2003). In 2004, beta blockers were determined in wastewater with SPE followed by HPLC-MS/MS. LOD ranged between 0.017 μg/L (solatol) and 0.75 μg/L (betaxolol) (Hernando et al., 2004). Similar LODs in a similar range were reached by Lee et al. in 2007 with the same method (Lee et al., 2007). Improved LODs as low as low as 3.3 ng/L could be achieved for a range of beta blockers in the in following years (Al-Odaini et al., 2010; Silva et al., 2021; Dahane et al., 2013; Ghoshdastidar et al., 2015; Rice et al., 2020). Common SPE cartridges were employed as a standard procedure in these studies (Hernando et al., 2007). Dahane et al. introduced successfully self-made cartridges packed with multi-walled carbon nanotubes as an alternative SPE material (Dahane et al., 2013). As an alternative for HPLC separation based on C18 columns, HILIC was introduced in 2010 (van Nuijs et al., 2010). Rice et al. successfully employed supercritical fluid chromatography coupled with MS/MS. LODs between 0.6 ng/L (bisoprolol) and 10 ng/L (metoprolol) could be achieved (Rice et al., 2020).

Beta blockers could be found in most WWTP influent and also in the effluent samples. In studies by Salem et al. and van Nuijs et al., atenolol was the compound most prevalent with concentrations up to 2118 ng/L (van Nuijs et al., 2010; Salem et al., 2012). In a study by Ghoshdastidar, metoprolol could be detected in concentrations up to 995 ng/L in effluent samples (Ghoshdastidar et al., 2015).

Already in 2007, the removal efficiency was determined for several beta blockers by comparison of influent and effluent composite samples by means of SPE HPLC-MS/MS. It ranged between 9 % (metoprolol) and 37 % (bisoprolol) (Lee et al., 2007). These values differ strongly from the results of Ternes from the year 1998 (Ternes, 1998). Lopez et al., however, determined a removal efficiency for metoprolol closer to the value of Ternes: 75 % (Lopez et al., 2022). Gurke et al. determined the removal efficiencies of further compounds. The removal efficiencies of three beta blockers were determined by mass load comparisons of flow-proportional 24-h influent and effluent composite samples over a period of ten days. The removal was compound specific: atenolol 22.6 %, bisoprolol 20.3 %, sotalol 11.9 % (Gurke et al., 2015).

It can be assumed that differences in calculated removal efficiencies of metoprolol determined by Lee et al. (2007), Ternes (1998), and Lopez et al. (2022), may stem from different WWTP parameters. As for other groups of PhACs discussed above the length of sampling, as well as the intervals of time or flow proportional sampling can lead to different findings.

For more insight into the removal process itself, enantiomeric fractions in WWTP influent and effluent were compared; analysed with as enantioselective HPLC column hyphenated to MS/MS. Significant differences were observed for several drugs including atenolol and propranolol. This indicated that the compounds are being degraded by a biological process, such as enzyme-mediated bacterial biodegradation (MacLeod et al., 2007). The analysis of sludge samples revealed that propanolol belonged to the most prevalent of the measured compounds; it was found in >95 % of the samples in concentrations up to 70.3 ng/g. Atenolol and metoprolol were found in ca. 30 % and ca. 50 % of the samples, respectively. Concentrations were between 0 and 57.2 ng/g. The results indicate that beta blockers compounds end up in sewage sludge after wastewater treatment. This must be taken into account when considering its potential use in agriculture (Silva et al., 2021).

A wide range of studies investigated the behavior of beta blockers during wastewater treatment (Table 6). Analytical methods were successfully designed and applied for WWTP sample analysis. HPLC-MS/MS analysis with LOD as low as 3.3 ng/L (for metoprolol, Lopez et al., 2022) allowed for the analysis of the substances in influent and effluent samples. The observation of high beta blocker concentrations in the effluent suggested that wastewater treatment was not sufficient to remove the

Table 7
Details and results of wastewater analysis regarding hormonal contraceptives in the past decades.

Sampling		Literature
Grab samples		Ternes et al., 1999; Snyder et al., 1999; Belfroid et al., 1999; Kelly, 2000; Kuch and Ballschmiter, 2001; Kolodziej et al., 2003; Chimchirian et al., 2007; Ingrand et al., 2003; Ferguson et al., 2001; Petrovic et al., 2002; Cargouët et al., 2004; Johnson et al., 2000; Kuster et al., 2008; Stavrakakis et al., 2008; Viglino et al., 2008; Sun et al., 2009; Liu et al., 2014; Belhaj et al., 2015; Gunatilake et al., 2014; Čelić et al., 2017; Aborkhees et al., 2020; Merlo et al., 2020; Huang and Sedlak, 2001; Al-Odaini et al., 2010
Composite samples	Flow proportional	Laganà et al., 2004; Chang et al., 2011
	No details	Desbrow et al., 1998; Larsson et al., 1999; Ternes et al., 1999; Baronti et al., 2000; Lopez et al., 2000; Petrovic et al., 2002; Vulliet et al., 2007

Preparation		Literature
SPE	Non-polar (reversed phase)	Desbrow et al., 1998; Larsson et al., 1999; Ternes et al., 1999; Kelly, 2000; Kuch and Ballschmiter, 2001; Kolodziej et al., 2003; Chimchirian et al., 2007; Ingrand et al., 2003; Lopez et al., 2000; Petrovic et al., 2002; Cargouët et al., 2004; Laganà et al., 2004; Vulliet et al., 2007; Kuster et al., 2008; Stavrakakis et al., 2008; Viglino et al., 2008; Sun et al., 2009; Liu et al., 2014; Chang et al., 2011; Caban et al., 2013; Belhaj et al., 2015; Gunatilake et al., 2014; Aborkhees et al., 2020; Huang and Sedlak, 2001
	Cation exchange	Al-Odaini et al., 2010

Analysis					Literature
Method	Derivatization	Column	MS	LOD (ng/L)* of 17α-ethinylestradiol**	
GC–MS	Silylation	Non-polar	Single quadrupole	6.7	Caban et al., 2013; Gunatilake et al., 2014
			Ion trap	0.3***	Ternes et al., 1999; Belfroid et al., 1999; Kelly, 2000; Belhaj et al., 2015
		High polarity	Single quadrupole	*Na*	Chimchirian et al., 2007
	Acylation	Non-polar	Single quadrupole	0.2	Cargouët et al., 2004
			Triple quadrupole	0.1	Kolodziej et al., 2003; Huang and Sedlak, 2001
	Alkylation	Non-polar	Single quadrupole	0.1	Kuch and Ballschmiter, 2001
HPLC-FL	–	Normal phase	–	*Na*	Snyder et al., 1999
HPLC-MS	–	Reversed phase	Single quadrupole	0.18	Lopez et al., 2000; Ferguson et al., 2001; Petrovic et al., 2002; Vulliet et al., 2007
			Ion trap	*Na*	Ingrand et al., 2003
			Triple quadrupole	0.078	Baronti et al., 2000; Johnson et al., 2000; Laganà et al., 2004; Kuster et al., 2008; Stavrakakis et al., 2008; Viglino et al., 2008; Sun et al., 2009; Liu et al., 2014; Chang et al., 2011; Čelić et al., 2017; Aborkhees et al., 2020; Merlo et al., 2020; Al-Odaini et al., 2010

Results		Literature
Concentration in influent	Up to 46.7 ng/L 17α-ethinylestradiol	Sun et al., 2009; Liu et al., 2014; Chang et al., 2011
Concentration in effluent	Up to 90 ng/L 17α-ethinylestradiol	Ternes et al., 1999; Baronti et al., 2000; Vulliet et al., 2007; Viglino et al., 2008; Sun et al., 2009; Chang et al., 2011; Merlo et al., 2020; Belfroid et al., 1999
Removal efficiencies	64 %	Ternes et al., 1999

na: LOD not determined in effluent in the respective study.
* Lowest LOD of listed studies (determined for analysis in effluent).
** 17α-Ethinylestradiol is the most frequently analysed compound and therefore used for comparison between studies.
*** Determined from LOQ.

compounds before entering the aqueous environment. The determination of respective removal efficiencies of common WWTPs varied strongly. Results between 9 and 83 % indicate a high uncertainty. More elaborate sampling strategies and a larger number of WWTPs will increase the knowledge on beta blocker behavior during wastewater treatment.

3.6. Hormonal contraceptives

Hormonal contraception is a highly effective birth control method that acts on the endocrine system. The original hormonal method was introduced in 1960, meanwhile there are two types of hormonal contraceptive formulations: progesterone-only methods which contain only progesterone or one of its synthetic analogues, and combined methods which contain an estrogen and a progestin. Their effect is the reduction of ovulation frequency and thickening cervical mucus or the suppression of ovulation, respectively (Fig. 8).

The impact of estrogenic compounds on the environment was discussed as early as 1994, when Purdom et al. hypothesized that WWTP effluent was estrogenic to fish (Purdom et al., 1994). Based on this presumption, the first study on contraceptives in wastewater was then reported in 1998. Desbrow et al. carried out a thought-out investigation on estrogenic chemicals in WWTP effluent. 24-h composite samples were taken from seven WWTPs and concentrated with SPE. The pretreated sample was then chromatographed twice with a semipreparative C_{18} column. After the extraction of the estrogenic fractions and

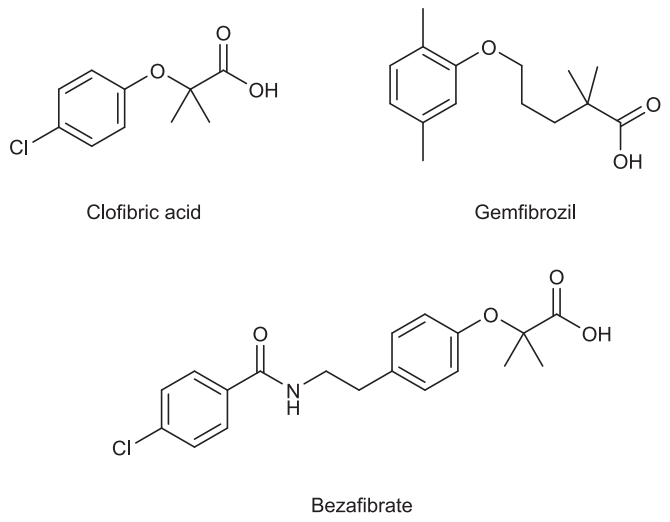

Fig. 9. Structures of clofibric acid (metabolite of clofibrate), gemfibrocil and bezafibrate (lipid lowering agents).

derivatization, the analytes were determined by GC–MS. The non-polar 17α-ethinylestradiol, the main compound of the combined oral contraceptive pill, was detected in some of the effluent samples in concentrations from 0.2 to 7.0 ng/L (Desbrow et al., 1998). Larsson et al. also detected 17α-ethinylestradiol in similar concentrations in continuously sampled WWTP effluent. Extraction pretreatment and extractive acetylation of the substances was followed by GC–MS analysis (Larsson et al., 1999). Ternes et al. determined 17α-ethinylestradiol among natural estrogens in WWTP effluents. Several WWTPs in different countries were sampled with different sampling strategies: composite samples from influent and effluent as well as grab samples of influent and effluent. With a combination of SPE extraction, clean-up, derivatization and GC–MS/MS analysis, a LOQ of 1 ng/L was achieved. Concentrations similar to prior studies were determined. In Brazilian WWTPs, a removal efficiency of 64 % was estimated. This could not be confirmed in samples from German WWTPs, where 17α-ethinylestradiol was not appreciably removed (Ternes et al., 1999). Similar results could be achieved by Belfroid in 1999 (Belfroid et al., 1999). Snyder et al. analysed several estrogenic compounds, including 17α-ethinylestradiol, in wastewater samples by means of HPLC separation and fluorescence detection (Snyder et al., 1999).

Many studies followed with an analogue approach (Kuch and Ballschmiter, 2001; Kolodziej et al., 2003). Researchers employed similar analytical strategies, in the beginning mainly with GC–MS or GC–MS/MS. Studies focused mainly on the determination of 17α-ethinylestradiol which was usually analysed together with other natural estrogenic compounds and other endocrine disruptors. Concentrations of 17α-ethinylestradiol were therefore determined very early in the discussion about the impact of synthetic hormones on fish population.

After the early achievements of well performing methods, researchers continued the optimization of analytical strategies (Chimchirian et al., 2007). In 2000, Kelly tackled the issue of matrix difficulties during wastewater pretreatment and introduced SPE disks for sample preparation (Kelly, 2000). At the same time, Kuch et al. employed GC–MS to obtain a higher sensitivity. After common SPE and extractive derivatization pretreatment, grab wastewater samples were analysed by high resolution GC–MS. For 17α-ethinylestradiol a LOD of 0.1 ng/L in effluent samples could be achieved (Kuch and Ballschmiter, 2001). In 2003, GC–MS/MS was also employed to determine medroxyprogesterone, a progesterone which is often added to 17α-ethinylestradiol in combination pills, for the first time. In grab wastewater samples, it could be determined with a LOD as low as 0.2 ng/L (Kolodziej et al., 2003).

Determination by GC–MS generally requires that the steroids be derivatized to more volatile molecules. The LODs of the presented studies were adequate for the successful proof of the presence of synthetic estrogens in WWTP effluent (Cargouët et al., 2004). LODs, however, ranged between >1 and 0.1 ng/L. They often bordered on the actual concentrations of the analytes, making it impossible to determine the removal efficiency of wastewater treatment. GC–MS/MS allowed for more sensitive (0.1 ng/L – 1 ng/L for treated effluent) analysis, yet still requires derivatization procedures (Caban et al., 2013). In the following years, HPLC coupled to MS was more and more introduced as suitable method for environmental analysis. The advantage that derivatization is not required and sample preparation became more straightforward made it attractive for high throughput analysis.

Baronti et al. developed procedures with SPE preconcentration followed by HPLC-MS in 2000. 24-h composite samples of raw and treated sewages were analysed. A removal rate of 17α-ethinylestradiol could not be determined. A LOQ of 0.3 ng/L in effluent was achieved (Baronti et al., 2000). Many studies included the testing of extraction sorbents. Immunosorbent extraction worked well for levonorgestrel, but led to low recoveries for 17α-ethinylestradiol. Non-polar cartridges generally led to good result (Ingrand et al., 2003; Ferguson et al., 2001).

Studies of contraceptives in environmental waters were over time also extended to progestogens. SPE followed by HPLC-MS became the analytical method of choice. In 2002, Petrovic et al. determined a range of natural and synthetic steroids, including 17α-ethinylestradiol, norethindrone, levonorgestrel, progesterone in 24-h WWTP composite samples (Petrovic et al., 2002). A series of studies employing a similar strategy based on SPE and HPLC-MS/MS followed (Al-Odaini et al., 2010). Grab samples or 24-h composite samples were taken in influent and/or effluent. LODs for 17α-ethinylestradiol and other synthetic hormones employed as contraceptives were usually in the ng/L range, which made it impossible to determine a removal efficiency, since concentrations were in the same range (Laganà et al., 2004; Vulliet et al., 2007; Kuster et al., 2008; Caban et al., 2013; Liu et al., 2014; Belhaj et al., 2015; Aborkhees et al., 2020; Merlo et al., 2020; Huang and Sedlak, 2001; Johnson et al., 2000).

In 2008, Stavrakakis et al. presented an optimized method with additional purification, leading to a LOD below 0.3 ng/L for 17α-ethinylestradiol and a smaller sample volume (Stavrakakis et al., 2008). In the same year, the runtime of HPLC-MS/MS was optimized, but detection limits in this study were too high for most contraceptives (Viglino et al., 2008). Another approach for optimization was the improvement of the SPE procedure; Sun et al. were able to reduce the preparation time to 15 min (Sun et al., 2009). A major leap in LOD was achieved by Celic et al. in 2017; a detailed sample preparation procedure led to a LOD of 0.078 ng/L for 17α-ethinylestradiol in WWTP effluent. Applicability of the method was confirmed by analysis of diverse environmental waters (Čelić et al., 2017).

Other analytical tests were tried for the determination for steroid hormones in wastewater: ELISA (Manickum and John, 2015; Pu et al., 2008), voltammetric determination (Monteiro et al., 2022), carbon isotope measurements (Griffith et al., 2012) or two dimensional GC (Gunatilake et al., 2014). Major leaps in parameters such as quantitation limit, run time or samples preparation effort were not achieved.

As quantitation limits were often too high for an accurate determination of the removal efficiency of WWTPs for hormonal contraceptives, Chang et al. carried out aerobic degradation tests. In these simulations, high removal efficiency (91–100 %) was found for androgens and progestogens compared with estrogens (67–80 %), with biodegradation being the major removal route in WWTPs (Chang et al., 2011).

The presumption of Purdom et al. (1994) that WWTP effluent might contain a substance that is estrogenic to fish could be proven quite quickly: 17α-ethinylestradiol was found in measurable concentrations in a study four years after the hypothesis and in many studies since. A general agreement on the concentration range in WWTP effluent samples could be observed – a rare observation when browsing through

studies regarding PhACs in environmental waters. The choice of analytical method changed between GC–MS and HPLC-MS, resulting in a wide range of optimized methods with low LODs (Table 7). Early studies on the removal efficiency of several synthetic hormones came to contradictory results. More insights were given by batch experiments which supported the hypothesis of high removal rates. The next step should be the improvement of LODs and LOQs for the determination of a removal efficiency in real WWTP samples in order to be able to assess the impact on hormonal contraceptives on the aqueous environment.

Table 8
Details and results of wastewater analysis regarding lipid lowering agents in the past decades.

Sampling		Literature
Grab samples		Antoniou et al., 2009; Becerra-Herrera et al., 2015; Boix et al., 2016; Cahill et al., 2012; Garcia-Ac et al., 2009; Gros et al., 2006; Hernando et al., 2004; Kuster et al., 2008; Lin et al., 2005; Lin et al., 2009; Miao and Metcalfe, 2003a, 2003b; Koutsouba et al., 2003; Miao and Metcalfe, 2003a, 2003b; Nödler et al., 2010; Petrovic et al., 2006; Robles-Molina et al., 2014; Sousa et al., 2011; Tete et al., 2020; Togola and Budzinski, 2007; Unceta et al., 2010; Verenitch et al., 2006; Zhou et al., 2010; Zorita et al., 2008; Al-Odaini et al., 2010; Han et al., 2006
	Flow proportional	Bueno et al., 2009; Yuan et al., 2015; Gurke et al., 2015
Composite samples	Time proportional	Morosini et al., 2017; Golovko et al., 2014; Oliveira et al., 2015
	No details	Gros et al., 2007; Huang et al., 2011; Lacina et al., 2013; Lee et al., 2003; Metcalfe et al., 2003; McEneff et al., 2014; Patrolecco et al., 2015; Unceta et al., 2010

Preparation		Literature
SPE	Non-polar (reversed phase)	Becerra-Herrera et al., 2015; Bueno et al., 2009; Cahill et al., 2012; Garcia-Ac et al., 2009; Gros et al., 2006; Hernando et al., 2004; Gros et al., 2007; Huang et al., 2011; Koutsouba et al., 2003; Kuster et al., 2008; Lacina et al., 2013; Lin et al., 2005; Lin et al., 2009; Miao and Metcalfe, 2003a, 2003b; Koutsouba et al., 2003; Miao and Metcalfe, 2003a, 2003b; Nödler et al., 2010; Metcalfe et al., 2003; Petrovic et al., 2006; Patrolecco et al., 2015; Robles-Molina et al., 2014; Tete et al., 2020; Togola and Budzinski, 2007; Verenitch et al., 2006; Zhou et al., 2010; Yuan et al., 2015; Yan et al., 2014; Gurke et al., 2015; Golovko et al., 2014; Han et al., 2006
	Cation exchange	Morosini et al., 2017; Al-Odaini et al., 2010
	Anion exchange	Sousa et al., 2011

Analysis					Literature
Method	Derivatization	Column	MS	LOD (ng/L)* of gemfibrozil and/or clofibric acid**	
GC–MS	Alkylation	Non-polar	Single quadrupole	1 (gemfibrozil)***	Togola and Budzinski, 2007
			Ion trap	0.3 (gemfibrozil)	Lin et al., 2005; Verenitch et al., 2006; Koutsouba et al., 2003; Metcalfe et al., 2003
		Low polarity	ToF	1.33 (clofibric acid)	Lacina et al., 2013
	Silylation	Low polarity	Single quadrupole	*Na*	Lee et al., 2003
HPLC-MS	–	Reversed phase	Triple quadrupole	1 (gemfibrozil) 2 (clofibric acid)	Garcia-Ac et al., 2009; Gros et al., 2006; Hernando et al., 2004; Gros et al., 2007; Huang et al., 2011; Lin et al., 2009; Miao and Metcalfe, 2003a; Miao and Metcalfe, 2003b; Nödler et al., 2010; Zhou et al., 2010; Yuan et al., 2015; Zorita et al., 2008; Gurke et al., 2015; Golovko et al., 2014; Al-Odaini et al., 2010; Oliveira et al., 2015
			Ion trap	10 (gemfibrozil)	Bueno et al., 2009; Morosini et al., 2017; Patrolecco et al., 2015; McEneff et al., 2014; Sousa et al., 2011; Unceta et al., 2010
			ToF	25 (clofibric acid)	Becerra-Herrera et al., 2015; Boix et al., 2016; Petrovic et al., 2006; Robles-Molina et al., 2014; Tete et al., 2020
			Orbitrap	0.7 (gemfibrozil)	Cahill et al., 2012
		- (direct injection)	Triple quadrupole	*Na*	Oliveira et al., 2015

Results		Literature
Concentration in influent	Up to 19,760 ng/L gemfibrozil and up to 12,955 ng/L clofibric acid	Becerra-Herrera et al., 2015; Garcia-Ac et al., 2009; Gros et al., 2006; Patrolecco et al., 2015; Sousa et al., 2011; Zorita et al., 2008; Tete et al., 2020
Concentration in effluent	Up to 8320 ng/L gemfibrozil and up to 9820 ng/L clofibric acid	Becerra-Herrera et al., 2015; Bueno et al., 2009; Cahill et al., 2012; Garcia-Ac et al., 2009; Gros et al., 2006; Kuster et al., 2008; Lee et al., 2003; Miao and Metcalfe, 2003a, 2003b; Metcalfe et al., 2003; McEneff et al., 2014; Nödler et al., 2010; Patrolecco et al., 2015; Sousa et al., 2011; Tete et al., 2020; Togola and Budzinski, 2007; Verenitch et al., 2006; Zhou et al., 2010; Zorita et al., 2008
Load influent	Up to 0.38 g/(d 1000) habitants (clofibric acid, gemfibrozil, bezafibrate, pravastatin)	Lin et al., 2009
Load effluent	Up to 0.07 g/(d 1000) habitants (clofibric acid, gemfibrozil, bezafibrate, pravastatin)	Lin et al., 2009
Removal efficiencies	Between 5 % and 100 % (clofibric acid, gemfibrozil, bezafibrate, pravastatin)	Lin et al., 2009; Lee et al., 2003; Patrolecco et al., 2015; Yuan et al., 2015; Gurke et al., 2015

na: LOD not determined in effluent in the respective study.
* Lowest LOD of listed studies (determined for analysis in effluent).
** Gemfibrozil and clofibric acid are frequently analysed compounds and therefore used for comparison between studies.
*** Determined from LOQ.

3.7. Lipid lowering agents

Cardiovascular disease, in particular coronary heart disease, is the principal cause of morbidity and mortality (WHO Statistics 2023). Elevated plasma total cholesterol and low-density lipoprotein cholesterol levels have been shown to be predictive of coronary heart disease (Barter, 2000). Lipid lowering drugs are employed for the decrease of low density lipoprotein or the increase of high density lipoprotein. Statins are one of the most frequently prescribed types of cholesterol-lowering drugs and can be lipophilic or hydrophilic. Statins decrease cholesterol output by blocking the rate-controlling enzyme that the liver uses to make cholesterol. Other agents include fibrates, niacin and lecitin (Fig. 9).

Clofibric acid, the metabolite of clofibrate, was one of the first pharmaceutical products detected in groundwater, bank filtrates and even drinking water (Richardson and Bowron, 1985; Heberer and Stan, 1997). Heberer et al. determined clofibric acid by means of SPE, followed by derivatization and GC–MS. High concentration of up to 270 ng/L clofibric acid were determined in drinking water, confirming that these polar contaminants are not sufficiently eliminated during drinking water treatment.

In 2003, Metcalfe et al. determined bezafibrate, clofibric acid (the metabolite of clofibrate) and gemfibrozil in influent and effluent WWTP samples. Grab samples were prepared with SPE before derivatization with methylation. GC–MS was applied for analysis. The LOD for all three analytes was 50 ng/L. Concentrations as high as 1.3 μg/L (gemfibrozil) were determined in effluent. This was one of the first studies that showed that lipid lowering agents are not fully removed during wastewater treatment (Metcalfe et al., 2003).

The statin drugs atorvastatin, lovastatin, pravastatin and simvastatin were determined in the same year. Miao et al. employed SPE and HPLC-MS/MS in the mobile phase for the analysis of grab samples (influent and effluent of WWTP). The LOD was lower than for the above mentioned GC–MS method: between 0.2 ng/L (simvastatin) to 9.8 ng/L (pravastatin). Up to 59 ng/L of pravastatin could be determined in the effluent samples, confirming that the WWTP were not able to remove the statins efficiently (Miao and Metcalfe, 2003a). Miao et al. followed up with a study examining atorvastatin, novobiocin, roxithromycin by means of SPE and microbore HPLC-MS/MS in positive and negative switching mode. All analytes could be determined in one injection in a 4 min run. Atorvastatin was found in WWTP effluent with an average concentration of 22.4 ng/L (Miao and Metcalfe, 2003b).

In the following years, several other studies employed GC–MS for the determination of clofibric acid, gemfibrozil and fenofibrate (Lin et al., 2005; Togola and Budzinski, 2007; Lee et al., 2003; Antoniou et al., 2009). Koutsouba et al. achieved an LOD of 1.8 ng/L for clofibric acid, but did not detect the drug in influent and effluent of a WWTP (Koutsouba et al., 2003). Verenitch et al. could determine as low as 0.3 ng/L of gemfibrozil in effluent samples (Verenitch et al., 2006).

In the following years, the prevalence of HPLC-MS/MS as analytical method of choice increased. SPE was commonly applied for sample pretreatment, as, especially in the first years, a sample concentration was needed as well as sample clean-up (Nödler et al., 2010). Hernando et al. showed an enrichment factor of 100-fold with an elaborate optimization of the procedure (Hernando et al., 2004).

In general, studies either focused on statins or around clofibric acid, gemfibrozil and bezafibrate. Often, the lipid lowering agents were part of a multi-residue study. SPE was applied in most cases prior to HPLC-MS/MS analysis. LODs ranged between 1 ng/L to 60 ng/L. In grab samples of WWTP influent and effluent the PhAcs were determined in high concentrations up to 9820 ng/L (clofibric acid) (Becerra-Herrera et al., 2015; Bueno et al., 2009; Cahill et al., 2012; Garcia-Ac et al., 2009; Gros et al., 2006; Kuster et al., 2008; Lee et al., 2003; Metcalfe et al., 2003; McEneff et al., 2014; Miao and Metcalfe, 2003a, 2003b; Nödler et al., 2010; Patrolecco et al., 2015; Sousa et al., 2011; Tete et al., 2020; Togola and Budzinski, 2007; Verenitch et al., 2006; Zhou et al., 2010;

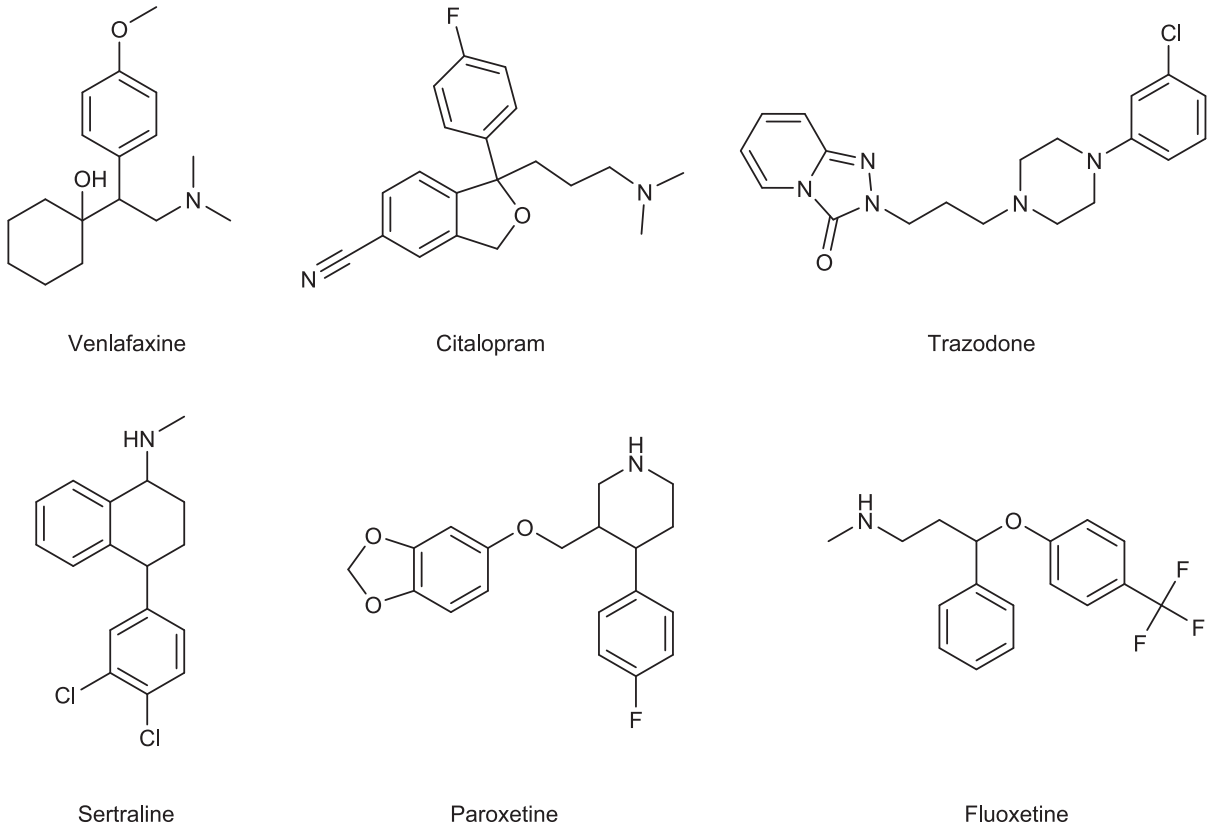

Fig. 10. Structures of various antidepressants: venlafaxine, citalopram, trazodone, sertraline, paroxetine and fluoxetine.

Zorita et al., 2008).

As important part of the analytical strategy for environmental samples, the sample preparation including clean-up and preconcentration was optimized in several studies: Zorita et al. achieved a LOQ of 3.5 ng/L for clofibric acid when using molecular imprinted SPE (MISPE). The method was straightforward and the application of wastewater samples was successful (Zorita et al., 2008). The method development of rotating-disk sorptive extraction by Becerra-Herrara showed a significant challenge due to its novelty and micellar extraction with SDS surfactants (Becerra-Herrera et al., 2015).

Although tandem MS proved to be the method of choice for detection, other mass filters were tested as well. In 2006, Petrovic et al. determined a wide range of PhACs in wastewater, including clofibric acid, gemfibrozil, bezafibrate, pravastatin and mevastatin. Following a SPE procedure, analysis was carried out with HPLC-Q-TOF. LODs ranged between 25 ng/L (clofibric acid) and 150 ng/L (mevastatin). As expected, Q-ToF showed some disadvantages in terms of sensitivity compared to triple quadrupole instruments. However, Q-ToF offered the advantage of unequivocal identification of target PhACs (Petrovic et al., 2006). Most studies sticked to MS/MS. However, in between 2014 and 2020, (Q)-ToF was employed for lipid lowering agents in wastewater three more times (Boix et al., 2016; Robles-Molina et al., 2014; Tete et al., 2020). The sensitive full-scan acquisition allowed Robles-Molina the determination of 400 analytes (Robles-Molina et al., 2014). Tete et al. employed HPLC-Q-ToF, but could not achieve sensible LODs (Tete et al., 2020). A critical evaluation of the use of an orbitrap as mass filter showed comparable data from the more commonly employed triple quadrupole MS. For gemfibrozil, a LOD of 0.7 ng/L could be achieved. Additionally, high mass accuracy was obtained (Cahill et al., 2012).

Following analytical method development and the first analysis of grab samples from WWTP influent and effluent that showed traces of lipid lowering agents in the treated water, sampling procedures were adapted for the better interpretation of concentration data.

In 2003, Lee et al. collected the first composite WWTP samples of lipid lowering agents. Grab samples were collected for 24 h to make up composite samples. After SPE and derivatization, GC–MS analysis was carried out. The mixing of composite samples allowed for the determination of removal efficiencies: 5 % of clofibric acid was removed during wastewater treatment (Lee et al., 2003). Gros et al. also collected 24-h composite samples and calculated a removal efficiency for the lipid regulators. However, a general trend on removal capacities could not be shown as different sites were sampled and different types of treatment applied (Gros et al., 2007). Lin et al. took grab samples in different WWTPs according to the hydraulic retention time and calculated removal efficiencies for clofibric acid, gemfibrozil, bezafibrate and pravastatin accordingly. They determined efficiencies between 14 and 81 %, strongly depending on the WWTP (Lin et al., 2009). In other studies, the concentration of clofibric acid in 24-h composite sample was below the LOQ thus removal efficiencies were difficult to determine (Lacina et al., 2013; Patrolecco et al., 2015; Morosini et al., 2017). Bezafibrate was determined by Yuan et al. in composite samples of two WWTPs with two different set-ups. The removal efficiencies displayed a large difference between a WWTP with standard set-up: 35.1 %, and a WWTP with modified activated sludge biological treatment (C-orbal oxidation ditch): 69.3 % (Yuan et al., 2015). Similar observations were made by Patrolecco et al. In four different WWTPs, removal efficiencies ranged from 46 to 100 % for clofibric acid and from 21 to 78 % for gemfibrozil. A large sampling campaign showed a seasonal impact: removal was higher in winter than in spring (Patrolecco et al., 2015). Gurke et al. determined a removal efficiency of 48.8 % for bezafibrate in an elaborate flow-controlled sampling over 10 days (Gurke et al., 2015). In derogation thereof, a significant removal of gemfibrozil could not be determined by Sultana et al. (Sultana et al., 2017).

Unfortunately, no details can be found for the sampling strategy of Patrolecco et al., as the study shows high variations in calculated removal efficiencies in between different WWTPs. In general, the removal efficiency can depend on the operation of the WWTPs. Dilution, which goes along with storm water and HRT, as well as the solid retention time (STP) can lead to different results between different WWTPs. Very high temperature differences, which have an impact on the biodegradation process can also influence the results. However, we assume that different sampling strategies will have an impact on the calculated removal efficiencies.

In conclusion, it can be said that lipid lowering agents have been in the focus of environmental studies for around 20 years. The analytical methods involved sample preparation and/or preconcentration with SPE and analysis with GC–MS and, in growing prevalence, HPLC-MS/MS. The LODs are higher compared to other PhAC groups, but appropriate for most studies. From the beginning, the drugs were found in WWTP effluent, strongly indicating that the treatment is not sufficient for their removal thus proving their input into environmental waters. More extensive sampling considering the hydraulic retention time and mixing during the treatment process allowed for the determination of removal efficiencies. These, however, very strongly between the studies and within the studies when samples were taken during different seasons or in different WWTPs (see Table 8). A concrete removal efficiency of conventional wastewater treatment cannot yet be stated.

3.8. Antidepressants

Antidepressants are a class of medications prescribed to treat clinical depression, anxiety disorders, chronic pain and some addictions. Antidepressants are often used in combination with each other. There are different types, including selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, serotonin modulators and stimulators, tricyclic antidepressants and monoamine oxidase inhibitors (Fig. 10).

One of the first studies of antidepressants in WWTPs was carried out with capillary electrophoresis (CE). Himmelsbach et al. carried out sample enrichment with SPE and subsequent evaporation to dryness before analysing effluent samples with CE-ESI-MS. Rather high LODs between 13 μg/L (trazodone) and 53 μg/L (sertraline) could be achieved in riverwater. In several effluent samples, venlafaxine, citalopram and trazodone could be detected. Four other antidepressants could not be detected (Himmelsbach et al., 2006).

Following studies were carried out with HPLC-MS, usually with tandem MS. Gros et al. developed a multi-residue method and achieved LODs between 7 ng/L (paroxetine) and 20 ng/L (fluoxetine). The researchers determined up to 630 ng/L carbamazepine in WWTP samples (Gros et al., 2006). In 2008, Schultz et al. investigated WWTP effluent and stream grab samples. Venlafaxine was the predominant antidepressant observed in wastewater and river water samples, found in the wastewater effluent in high concentrations up to 2190 ng/L (Schultz and Furlong, 2008). 24-h composite samples collected in a volume proportional manner were analysed with HILIC-MS/MS after SPE in 2010. HILIC provided a good separation of all compounds. In the composite samples, venlafaxine was also determined in the highest concentrations of all analytes (van Nuijs et al., 2010).

Several studies were carried out by means of SPE and HPLC-MS, obtaining similar LODs. The concentrations of antidepressants and other psychiatric drugs varied very much (Busetti et al., 2009; Metcalfe et al., 2010). In general, several compounds were investigated per study. The first study with up to 30 psychoactive compounds in one analysis was carried out in 2014 by Sheng et al. with HPLC-MS/MS. Concentrations between 10.6 ng/L (imipramine) and 163.9 ng/L clozapine were determined in wastewater from three WWTPs in Beijing (Sheng et al., 2014). Borova et al. achieved excellent LODs as low as 0.04 ng/L (in water) for the analysis of tricyclic and tetracyclic antidepressants with SPE and HPLC-MS/MS. In influent and effluent grab samples up to 669.9 ng/L doxepin was determined (Borova et al., 2014). In another study, venlafaxine could be detected in high concentrations in WWTP effluent from 16 WWTPs (Ghoshdastidar et al., 2015). Commonly, the

Table 9
Details and results of wastewater analysis regarding antidepressants in the past decades.

Sampling		Literature
Grab samples		Himmelsbach et al., 2006; Gros et al., 2006; Busetti et al., 2009; Borova et al., 2014; Sheng et al., 2014; Ghoshdastidar et al., 2015; Boogaerts et al., 2023; Schultz and Furlong, 2008
Composite samples	Flow proportional	Gurke et al., 2015; van Nuijs et al., 2010
	Time proportional	Vergeynst et al., 2015; Lajeunesse et al., 2012; Golovko et al., 2014
	No details	Busetti et al., 2009; Metcalfe et al., 2010; Asimakopoulos et al., 2017; Kleywegt et al., 2016; Lopez et al., 2022

Preparation		Literature
SPE	Non-polar	Himmelsbach et al., 2006; Gros et al., 2006; Sheng et al., 2014; Ghoshdastidar et al., 2015; Schultz and Furlong, 2008; Gurke et al., 2015; van Nuijs et al., 2010; Vergeynst et al., 2015; Golovko et al., 2014; Busetti et al., 2009; Kleywegt et al., 2016; Lopez et al., 2022; Silva et al., 2014; Asimakopoulos et al., 2017; Papageorgiou et al., 2016
	Cation exchange	Borova et al., 2014; van Nuijs et al., 2010; Lajeunesse et al., 2012; Metcalfe et al., 2010; Asimakopoulos et al., 2017
	Anion exchange	Boogaerts et al., 2023; van Nuijs et al., 2010
	Molecularly imprinted polymers	Demeestere et al., 2010

Analysis				Literature
Method	Column	MS	LOD (ng/L)* of citalopram and venlafaxine**	
CE-MS	Non-polar	ToF	*na*	Himmelsbach et al., 2006
HPLC-MS	Reversed phase	Ion trap	22.5 (citalopram)	Silva et al., 2014; Asimakopoulos et al., 2017
		Triple quadrupole	0.1 (citalopram)	Himmelsbach et al., 2006; Gros et al., 2006; Busetti et al., 2009; Sheng et al., 2014; Gurke et al.,
			2.1 (venlafaxine)***	2015;, Ghoshdastidar et al., 2015; Boogaerts et al., 2023; Lajeunesse et al., 2012; Golovko et al., 2014; Metcalfe et al., 2010; Kleywegt et al., 2016; Lopez et al., 2022; Demeestere et al., 2010; Schultz and Furlong, 2008; Papageorgiou et al., 2016
		magnetic sector	*na*	Vergeynst et al., 2015
		Q-ToF	*na*	Lopez et al., 2022;
	HILIC	Triple quadrupole	*na*	van Nuijs et al., 2010

Results		Literature
Concentration in influent	Up to 504.6 ng/L citalopram and up to 2982 ng/L venlafaxine	Borova et al., 2014; Schultz and Furlong, 2008; van Nuijs et al., 2010; Vergeynst et al., 2015; Lajeunesse et al., 2012; Golovko et al., 2014; Metcalfe et al., 2010; Asimakopoulos et al., 2017; Kleywegt et al., 2016; Lopez et al., 2022
Concentration in effluent	up to 766.2 ng/L citalopram and up to 2563 ng/L venlafaxine	Sheng et al., 2014; Ghoshdastidar et al., 2015; Schultz and Furlong, 2008; Lajeunesse et al., 2012; Metcalfe et al., 2010; Asimakopoulos et al., 2017; Kleywegt et al., 2016; Lopez et al., 2022; Himmelsbach et al., 2006; Borova et al., 2014; Vergeynst et al., 2015; Golovko et al., 2014
Load influent	Up to 0.093 g/(d 1000 inhabitants) venlafaxine****	Lopez et al., 2022; Papageorgiou et al., 2016
Load effluent	Up to 0.030 g/(d 1000 inhabitants) venlafaxine	Lopez et al., 2022; Papageorgiou et al., 2016
Removal efficiencies	Between 0 % and 40 % (citalopram) and between 0 % and 25 % (venlafaxine)	Gurke et al., 2015; Vergeynst et al., 2015; Lopez et al., 2022; Silva et al., 2014; Golovko et al., 2014

na: LOD not determined in effluent in the respective study.
* Lowest LOD of listed studies (determined for analysis in effluent).
** Citalopram and venlafaxine are frequently analysed compounds and therefore used for comparison between studies.
*** Determined from LOQ.
**** Calculated based on information in reference.

analysis of antidepressants and other psychiatric drugs was carried out with HPLC coupled to tandem MS. Vergeynst et al. introduced double focusing magnetic sector HRMS in 2015 in a multi-residue analysis. Venlafaxine was successfully determined in WWTP 24-h time integrated samples with concentrations up to 403 ng/L in influent and 365 ng/L in effluent (Vergeynst et al., 2015). A wide range of tricyclic and tetracyclic antidepressants as well as selective serotonin reuptake inhibitors and serotonin and norepinephrine reuptake inhibitors in a study of Asimakopoulos. With SPE followed with HPLC-MS/MS, citalopram, venlafaxine and bupropion could be determined in 24-h composite WWTP samples (Asimakopoulos et al., 2017).

Several SPE material was tested for method development. Due to the wide range of compounds that are being employed as antidepressants, different SPE cartridges, including non-polar as well as anion or cation exchange material, can be found in literature. Some other studies went beyond ordinary SPE and tested other approaches. Demeestere et al. were the first to evaluate the application potential of molecularly imprinted SPE (MISPE) for environmental analysis of venlafaxine, trazodone, citalopram, paroxetine, lorazepam, fluoxetine and diazepam. Already in 2010, they achieved LODs in the low ng/L range with this technique (Demeestere et al., 2010). Recently, Boogaerts et al. developed a high-throughput method based on 96-well anion exchange SPE requiring only 2 mL sample. They observed that preconcentration with this approach is less than with offline-SPE (20-fold vs. 250 fold), but much faster while requiring less solvent and less sample (Boogaerts et al., 2023).

As shown by the findings of antidepressants in the studies presented above, conventional wastewater treatment is unable to efficiently

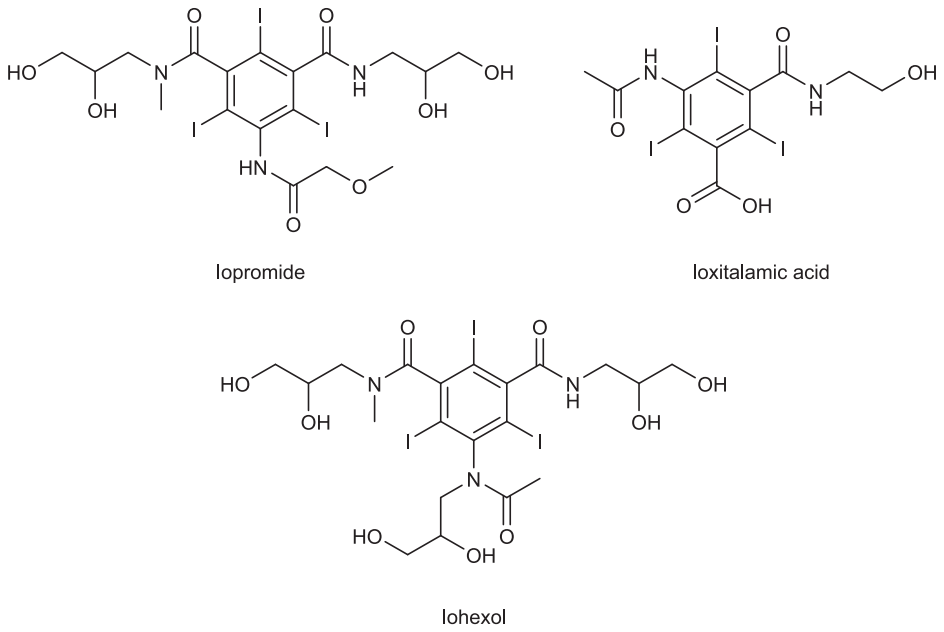

Fig. 11. Structures of several X-ray contrast agents: iopromide, iohexol and ioxitalamic acid.

remove the drugs. However, more elaborate studies have to be contemplated for a better insight into the removal capacities of wastewater treatment. An adequate sampling strategy is inevitable for this enterprise. In 2012, Lajeunesse et al. determined several antidepressants in 24-h equal volume composite samples of WWTP influent and effluent taken over a period of six months. Venlafaxine was the compound detected with the highest concentrations. Up to 2982 ng/L were reported in influent samples. Removal efficiencies were calculated based on the comparison of influent and effluent samples. For the six sampling dates, the results showed a strong variation between 7.8 % and 39 % for venlafaxine. For citalopram, the removal efficiency was determined to be between 3.5 % and 41 % (Lajeunesse et al., 2012). In the same year, Gurke et al. developed an elaborate sampling strategy based on the flow-proportional sampling of 24-h composite samples of WWTP influent and effluent over a period of ten consecutive days. A removal efficiency of 7.7 % was calculated for venlafaxine and 15.3 % for amitriptyline. The chosen approach including the flow proportional sampling ensures a high reliability of the results and allows for the precise determination of the removal efficiency values (Gurke et al., 2015). Silva et al. decided to collect time proportional 24-h composite influent and effluent samples and calculated mass loading by multiplying the analyte concentration by the mean daily flow rate. They reason that although discharges of PhACs can fluctuate daily, monthly or even seasonally, since antidepressants are used chronically, a more comprehensive monitoring using more periodical sampling was not necessary. Citalopram, fluoxetine, paroxetine and sertraline were analysed by means of SPE and HPLC-MS/MS. Removal efficiencies around 40 % were calculated for citalopram and around 19 % for paroxetine (Silva et al., 2014). In 2014, Golovko et al. determined removal efficiencies between 1 % (venlafaxine) and 81 % (sertraline) with time proportional sampling. These values differ rather strongly from earlier studies (Golovko et al., 2014). The removal efficiency calculation of Kleywegt et al. took into account the hydraulic retention time of the sampled WWTP. The effluent collection time was staggered 16 h relative to that of the influent. In this study, reduction of citalopram, venlafaxine, amitriptyline and sertraline was determined to be between 0 and 25 %. Fluoxetine showed 26–50 % reduction and paroxetine 51–75 % reduction (Kleywegt et al., 2016). Two campaigns in which samples were collected over seven consecutive days were published recently. The removal efficiency of several PhACs was estimated for each analyte from the average weekly concentration. For venlafaxine a removal efficiency of ca. 25 % was calculated in effluent after secondary treatment (Lopez et al., 2022).

An unambiguous statement on the behavior of antidepressants during wastewater treatment cannot be made. The large choice of approaches for sampling results in many studies whose results are difficult to compare due to different strategies (see Table 9). HPLC-MS/MS was the most common analytical technique, providing appropriate LODs, that prevailed from the beginning of antidepressant analysis in WWTP samples. Several strategies for sample preparation were successfully tested and many studies carried out in order to shed light on the behavior of these compounds during wastewater treatment. The number of compounds, however, is high and the amount of information is very different for the different drugs. Removal efficiencies that were determined in the last two decades for diverse antidepressants show discrepancies for some compounds and yet need to be confirmed.

3.9. Contrast agents

3.9.1. X-ray

Iodinated contrast media are the most commonly used agents for intravascular X-ray examinations. They are designed to exhibit extremely high chemical and biological stability. This maintains their efficiency during X-ray examinations and prevents undesired toxicological effects caused by degradation products (Fig. 11).

In the 1990s, Kümmerer et al. measured adsorbable organic halogens (AOX) in the effluent of six different hospitals. Day time composite samples and night time composite samples were prepared from 2-h samples. Concentrations up to 1.17 mg/L AOX were determined. Since a speciation of the different analytes was not performed, the origin of the high concentrations remained unclear, but it was assumed that iodine containing X-ray contrast media contributed significantly to the AOX concentrations which would later reach the sewage system (Kümmerer et al., 1998).

In 2000, Hirsch et al. presented an elaborate method development for the determination of six iodinated X-ray contrast agents in wastewater. With an optimized SPE procedure, followed by a HPLC-MS/MS analysis, a LOQ of 50 ng/L could be achieved for X-ray diagnostics in WWTP grab samples. Iopromide was determined in concentrations up to 3.08 μg/L in effluent samples (Hirsch et al., 2000). The same author presented an elaborate study on the behavior of iodinated X-ray contrast

Table 10
Details and results of wastewater analysis regarding contrast agents for X-ray in the past decades.

Sampling		Literature
Grab samples		Hirsch et al., 2000; Busetti et al., 2008; Seitz and Winzenbacher, 2017
Composite samples	Flow proportional	Ternes and Hirsch, 2000
	Time proportional	Carballa et al., 2004
	No details	Busetti et al., 2008; Seitz and Winzenbacher, 2017; Putschew et al., 2001; Busetti et al., 2010; Lopez et al., 2022; 244
Sludge		Ternes et al., 2005

Preparation		Literature
SPE	Non-polar	Hirsch et al., 2000; Ternes and Hirsch, 2000; Carballa et al., 2004; Putschew et al., 2001; Lopez et al., 2022
None	Direct injection	Busetti et al., 2008; Seitz and Winzenbacher, 2017

Analysis				Literature
Method	Column	MS	LOD (ng/L)* of iopromide**	
HPLC-MS	Reversed phase	Triple quadrupole	16.7	Hirsch et al., 2000; Busetti et al., 2008; Seitz and Winzenbacher, 2017; Ternes and Hirsch, 2000; Carballa et al., 2004; Putschew et al., 2001; Busetti et al., 2010; Lopez et al., 2022
		Q-ToF	*na*	Lopez et al., 2022
	Ion chromatography	ICP-MS	*na*	Sacher et al., 2005

Results		Literature
Concentration in effluent	Up to 21.000 ng/L iopromid	Hirsch et al., 2000; Busetti et al., 2008; Seitz and Winzenbacher, 2017; Ternes and Hirsch, 2000; Carballa et al., 2004; Putschew et al., 2001; Busetti et al., 2010
Load influent	1.205 g/(d 1000 inhabitants) iopromide***	Ternes and Hirsch, 2000
Load effluent	1.256 g/(d 1000 inhabitants) iopromide***	
Removal efficiencies	0 % to 75 % (iopromide)	Ternes and Hirsch, 2000; Lopez et al., 2022

na: LOD not determined in effluent in the respective study.
* Lowest LOD of listed studies (determined for analysis in effluent).
** Iopromide is one of the most frequently analysed compounds and therefore used for comparison between studies.
*** Calculated for better comparison based on available information in reference.

agents the same year. Daily composite samples, taken flow proportionally, of influent and effluent of a WWTP were analysed to determine the concentrations, loads and the removal efficiency of iodinated X-ray contrast media. Concentrations between 0.16 μg/L (ioxithalamic acid) and 8.1 μg/L (iopromide) were found in effluent samples of a municipal WWTP. A comparison between the loads in influent and effluent showed no removal during wastewater treatment. Potential metabolites were not found. This study covered a lot of ground regarding the behavior of contrast agents during wastewater treatment. It took many aspects into account, answering the questions of removal efficiency and potential transformation (Ternes and Hirsch, 2000). Putschew et al. reached a similar LOQ in a method for the analysis of iodinated contrast media. Enrichment with SPE was employed with consecutive HPLC-MS/MS analysis. Iodinated X-ray contrast media was detected and quantified in a WWTP effluent with iopromide being the prevalent compound (21 μg/L). The contrast media could also be detected in a receiving channel and lake. In the receiving lake, the concentrations are still high with values between 0.5 and 4 mg/L, confirming that the low removal capacity of WWTPs leads to a high input of X-ray contrast agents into environmental waters (Putschew et al., 2001). Iopromide was part of a multi-residue study in wastewater by Carballa et al. in 2004. Five sampling points were chosen in a WWTP. A different sampling approach was tested: integrated 24-h samples were mixed from 1-h samples while taking the operating hydraulic retention time into account. Samples were enriched by SPE and analysed with HPLC-MS/MS. Significant concentrations of iopromide were found in the effluent. Removal efficiency was calculated taking into account the measured concentration at the inlet of the plant, the biological reactor and the final effluent. Comparison with influent concentration confirmed prior observations that there is no significant elimination of this compound during the treatment (Carballa et al., 2004). An additional study was carried out by Ternes et al. in 2005. Activated sludge was sampled as grab samples in two wastewater treatment plants. After SPE, the analytes were determined by HPLC-MS/MS. The iodinated compounds could not be detected. Hence, sorption onto sludge was assumed to be negligible which corresponds to their polar properties. This observation also corroborates the proposed low removal efficiencies (Ternes et al., 2005).

Analysis of X-ray contrast media was generally carried out with SPE followed by HPLC-MS/MS. A new approach was adopted by Sacher et al. in 2005. The method is based on an IC separation and a subsequent detection by ICP-MS. Without prior sample enrichment, LOQs below 40 ng/L (in water) could be achieved. As the ICP-MS does not differ between iodine species, specificity is low compared to the simultaneous run SPE-HPLC-MS/MS method (Sacher et al., 2005). Busetti et al. introduced direct injection HPLC-MS/MS in order to circumvent laborious sample preparation and the risk of bad recoveries. The method proved to be faster and considerably cheaper than standard methods and could successfully be validated. All eight X-ray contrast agents measured where detected in wastewater samples in concentrations around 9.5 μg/L (Busetti et al., 2008).

In a survey on 84 anthropogenic compounds, Seitz and Winzenbacher analysed 24-h composite samples of WWTP influent and effluent with direct injection HPLC-MS/MS. Iomeprole was the contrast agent found with the highest concentration in untreated and treated wastewater (44 μg/L). Followed closely by iopromide, iohexol, iopamidol and diatrizoic acid. Elimination efficiencies were calculated with regard to compound concentrations in influent and effluent sample. Surprisingly, with prior studies in mind, the elimination of iohexol was calculated to be around 70 %. However, elimination of iomeprole and iopamidol showed high variations between WWTPs and were difficult to evaluate. Details of the sampling are not given and the authors attributed high variations to specific behavior in the different plants (Seitz and Winzenbacher, 2017). Recently, Lopez et al. also estimated a high removal efficiency of >75 % for iopromide. 24-h composite samples were collected in this study over seven consecutive days (Lopez et al., 2022). As also observed for beta blockers, the calculated removal efficiencies differ strongly. In conclusion, it can be said that the behavior of iodinated X-ray contrast media has been evaluated with HPLC-MS/MS analysis from the start. High concentrations of the compounds were determined. In multi-residue studies, iodinated contrast agents often belonged to the most prevalent compounds – in influent and also in effluent samples. Early on, sampling strategies were developed to determine removal efficiencies. In the majority of cases, very low

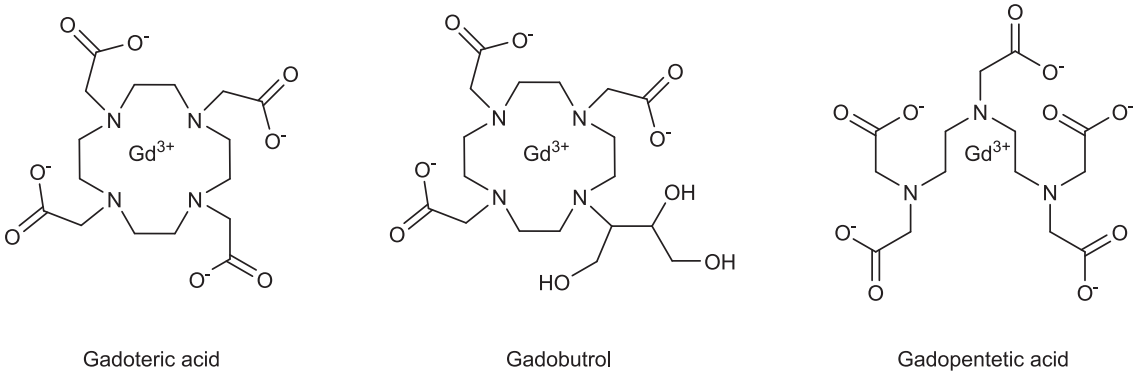

Fig. 12. Structures of several MRI contrast agents: gadoteric acid, gadobutrol and gadopentetic acid.

Table 11
Details and results of wastewater analysis regarding contrast agents for MRI in the past decades.

Sampling		Literature
Grab samples		Möller et al., 2002; Künnemeyer et al., 2009; Verplanck et al., 2010
Composite samples	Flow proportional	Telgmann et al., 2012; Verplanck et al., 2010
Sludge		Telgmann et al., 2012; Verplanck et al., 2010

Preparation		Literature
SPE	Non-polar	Möller et al., 2002; Knappe et al., 2005

Analysis				Literature
Method	Column	Detection	LOD (ng/L)* of Gd or Gd-complex**	
Direct injection analysis	–	ICP-OES	31.45 (Gd)	Künnemeyer et al., 2009
		ICP-MS	*na*	Möller et al., 2002; Telgmann et al., 2012; Knappe et al., 2005; Verplanck et al., 2010
HPLC-MS	HILIC	ICP-MS	130 (Gd-complex)	Künnemeyer et al., 2009; Telgmann et al., 2012
	Ion chromatography	ICP-MS	*na*	Telgmann et al., 2012

Results		Literature
Concentration in influent	Up to 874 ng/L gadolinium	Künnemeyer et al., 2009; Telgmann et al., 2012; Verplanck et al., 2010
Concentration in effluent	Up to 330 ng/L gadolinium	Möller et al., 2002; Künnemeyer et al., 2009; Telgmann et al., 2012; Verplanck et al., 2010
Load influent	0.193 g/(d 1000 inhabitants) gadolinium***	Telgmann et al., 2012
Load effluent	Up to 116 g/day (gadolinium)	Telgmann et al., 2012; Knappe et al., 2005
Removal efficiencies	1 % to 10 % (gadolinium)	Telgmann et al., 2012; Verplanck et al., 2010

na: LOD not determined in the respective study.
* Lowest LOD of listed studies (determined for analysis in water).
** Total Gd is determined in studies employing direct injection analysis, the LOD of complexed Gd is determined in studies employing a separation technique prior to detection.
*** Calculated for better comparison based on available information in reference.

removal capacities were determined for several of the iodinated compounds. However, recently rather high removal rates were observed. The discrepancy for iopromide between the two studies (Ternes and Hirsch, 2000; Lopez et al., 2022) cannot be explained. However, the sampling strategies differ, the studies involved different compounds and the WWTPs are diverse (Table 10).

3.9.2. Magnetic resonance imaging (MRI)

Contrast agents for magnetic resonance imaging (MRI) are based on paramagnetic metal ions because of their magnetic moment. Gadolinium (Gd) have been employed in contrast agents for MRI since the 1980s. The highly toxic Gd ions are complexed with polyaminocarboxylic acid chelating agents (Fig. 12). They are in general considered safe and well-tolerated (Telgmann et al., 2013).

A challenge arose in 1996 when high concentrations of anthropogenic Gd were discovered in surface waters. This suggested that the Gd complexes are not eliminated during wastewater treatment (Bau and Dulski, 1996). It was assumed that the complexes pass the WWTP unhindered due to their high polarity. Because it was not known if Gd was still complexed, which is highly relevant regarding its toxicity, and because its effect on surface water was not foreseeable, a detailed study of the behavior and fate of the contrast agents after entering the sewage system was required (Bau et al., 2006). Möller et al. determined anthropogenic Gd in surface water and WWTP effluents by means of ICP-MS. The choice of instrumentation, which in contrast to most other studies in this review leaves out analyte identification, is based on the valid presumption that almost all Gd present originates from Gd contrast media. The authors chose different locations in the plant for grab

sampling. Elevated Gd concentrations could be detected in all wastewater samples. The finding of high Gd concentrations in the effluent (up to 278 μmol/L) indicated strongly that neither chemical reactions nor bacteria in the biological treatment of the plant eliminate the Gd effectively from the liquid phase (Möller et al., 2002). In 2005, Knappe et al. determined the Gd concentration in the effluent of seven wastewater treatment plants in Berlin with ICP-MS. A total load of Gd in WWTP effluent between a few g/day to 116 g/day were determined – values varying heavily between different WWTPs on different locations (Knappe et al., 2005). The first time-dependent monitoring of Gd was achieved, giving first quantitative statements about its behavior during the passing through the WWTPs. In both studies an element-selective detector was chosen, quantifying total Gd concentrations without focus on the species. At this point it could not be determined if the detected Gd was still complexed or underwent species transformation.

The first speciation of the Gd complexes in environmental samples was introduced by Künnemeyer et al. in 2009. A hyphenation of HPLC with ICP-MS lead to the identification and differentiation of three Gd-based contrast agents in grab samples of wastewater. A LOQ of 3.3 nmol/L (in water) was achieved. A comparison between the concentrations of complexed and total Gd was made in order to find out if all Gd is present in its complexed form. Slightly less complexed Gd content was observed in the samples, indicating species transformation (Künnemeyer et al., 2009). A detailed study was carried out by Verplanck et al. in 2010. An elaborate sampling strategy was designed to survey a wide range in WWTP operations. Solid phase samples of sludges were collected as a series of grab samples from four different WWTPs. In addition, influent and effluent aqueous samples were collected as 24-h flow proportional composites. The start of the 24-h sampling was lagged for the primary and secondary effluent samples to try to match the hydraulic residence times of the various WWTP operations, trying to follow a 24-h slug of water through the plant. Gd concentrations were determined with ICP-MS. In sludge, no Gd was found. High Gd content could by measured in influent samples (1300 to 2090 pmol/L) and less Gd content in effluent samples (1010 to 1520 pmol/L). However, the idea of following a 24-h parcel of water through the plant was diminished due to different residence times in secondary treatment operations (Verplanck et al., 2010).

In 2012, the behavior of Gd complexes during wastewater treatment was studied in composite samples with a range of analytical techniques. An elaborate sampling strategy, covering 2-h samples over one week in influent and effluent allowed the balancing of Gd input and output of the plant. Samples from sludge and the dewatering station gave insight into details of potential Gd elimination and transformation. The mixture of speciation analysis with HPLC-ICP-SFMS as well as IC-ICP-MS and total content determination with isotope dilution analysis (ICP-MS) guaranteed an extensive overview of Gd complex behavior. For HPLC-ICP-SFMS, an LOD of 0.13 μg/L was achieved. The total input of Gd during the sampled week was 237.0 g and the total load of Gd in the effluent was 213.0 g. The balancing suggested that 10 % of Gd is removed from the liquid phase during wastewater treatment. Detailed analysis of samples from the dewatering process indicated that species transformation took place during anaerobic sludge treatment (Telgmann et al., 2012). Over the last decade, studies have been reported with improving quantification limits of Gd-based contrast agents. Samples from a nature reserve attached to the effluent of a WWTP have been analysed with HPLC-ICP-SFMS enhanced with dry aerosol by Birka et al. The mass balancing of complexed and total Gd content indicated the presence of further Gd species. This result also suggests species transformation in WWTPs where the samples of this study originated (Birka et al., 2013). Recently, Horstmann et al. improved the LOD of Gd contrast agents even further by means of a novel automated solid phase extraction method and by improving the ion transmission of the employed ICP-MS. LODs between 18 ng/L (Magnevist) and 24 ng/L (Gadovist) were achieved. Two contrast agents could even be determined in Australian seawater (Horstmann et al., 2021).

The analysis of Gd contrast agents differs a little from the analysis of many of the PhACs we read about in this review: the fact that it contains a hetero element allows for the determination with element specific techniques and additionally, Gd is almost exclusively present as anthropogenic complex. LODs for the Gd complexes in wastewater effluent are rather high compared to other presented substances, but were appropriate for Gd quantification in WWTP influent and effluent. Many studies were carried out to confirm its presence in WWTP effluent and attached environmental waters. Early on, it was shown that almost no removal occurs during wastewater treatment. A look on the molecular structure shows clearly a high polarity which is the reason for high water solubility and the resistance towards removal during wastewater treatment. Although the question of the potential transformation has still to be addressed in order to evaluate the impact on Gd complex on the environment, the presented analytical methods and employed sample strategies constitute a perfect base for future research (Table 11).

4. Conclusion

This review extensively assessed a large number of environmental studies researching the behavior of PhACs and CAs in WWTPs. The range of applied analytical techniques is wide. However, two techniques were clearly prevalent: LC-MS and GC–MS. Advancement in instrumentation led to better analysis results: a general trend for all PhACs and CAs showed optimized detection and quantification limits.

The assessment of the projects highlighted the importance of the choice of sampling strategy: grab samples of WWTP effluent are optimal for a fast determination if the analyte is removed during wastewater treatment. The investigation of elaborate composite samples gives more detail into the process and allows for the determination of removal efficiencies.

In environmental studies, the choice of analytical method and the choice of sampling strategy is important. The analytical method has to be suited for the analyte characteristics as well as the expected matrix and analyte concentration. The sampling procedure must be taken into account in order to correctly interpret the analysis results.

CRediT authorship contribution statement

Lena Telgmann: Writing – original draft, Visualization, Conceptualization. **Harald Horn:** Writing – review & editing, Conceptualization.

Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

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