Transformation products of antibacterial drugs in environmental water: Identification approaches based on liquid chromatography-high resolution mass spectrometry

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ABSTRACT

In recent years, the presence of antibiotics in the aquatic environment has caused increasing concern for the possible consequences on human health and ecosystems, including the development of antibiotic-resistant bacteria. However, once antibiotics enter the environment, mainly through hospital and municipal discharges and the effluents of wastewater treatment plants, they can be subject to transformation reactions, driven by both biotic (e.g. microorganism and mammalian metabolisms) and abiotic factors (e.g. oxidation, photodegradation, and hydrolysis). The resulting transformation products (TPs) can be less or more active than their parent compounds, therefore the inclusion of TPs in monitoring programs should be mandatory. However, only the reference standards of a few known TPs are available, whereas many other TPs are still unknown, due to the high diversity of possible transformation reactions in the environment. Modern high-resolution mass spectrometry (HRMS) instrumentation is now ready to tackle this problem through suspect and untargeted screening approaches. However, for handling the large amount of data typically encountered in the analysis of environmental samples, these approaches also require suitable processing workflows and accurate tandem mass spectra interpretation. The compilation of a suspect list containing the possible monoisotopic masses of TPs retrieved from the literature and/or from laboratory simulated degradation experiments showed unique advantages. However, the employment of *in silico* prediction tools could improve the identification reliability. In this review, the most recent strategies relying on liquid chromatography-HRMS for the analysis of environmental TPs of the main antibiotic classes were examined, whereas TPs formed during water treatments or disinfection were not included.

1. Introduction

In the last decades, there is a growing concern about the presence of anthropogenic organic contaminants in water sources for their potential impact on both the environment and public health. Currently, among the various commercial categories of contaminants of emerging concern (CECs), there is specific attention on pharmaceuticals [1], as there are over 3000–4000 active ingredients on the market [2].

Pharmaceuticals can enter water bodies through various pathways, including improper hospital and industrial discharges, and municipal wastewater effluents. Indeed, wastewater treatment plants (WWTPs) may not be able to completely remove these substances, leading to their persistence in the environment [3–5]. Furthermore, the application of manure on agricultural soils could be a significant source of veterinary antibiotics [6], which is a major issue for their contribution to the antimicrobial resistance emergency [7–9].

Once in the environment, pharmaceuticals, and their human/animal metabolites can undergo transformation processes occurring through various mechanisms, influenced by both biotic and abiotic factors, including microbial activity, UV radiation, and temperature [10,11]. While biochemical transformations of pharmaceuticals are regulated by specific enzyme-mediated pathways, making them reasonably predictable and limited, on the other hand, abiotic processes are extremely diverse and non-specific (e.g., catalyzed by radicals). This results in a multitude of transformation products (TPs) with different levels of occurrence [12], and different chemical properties and biological activity compared to the parent compounds. TPs with distinct environmental behavior and ecotoxicological profiles can be generated based on

the prevailing chemical or biochemical processes involved (such as biodegradation, photolysis, or hydrolysis). The environmental matrices where these processes occur (surface water, groundwater, soil, sediment, wastewater effluents, etc.) and specific site conditions also play a crucial role in determining the resulting TPs [12]. Generally, TPs are more polar [1] and, in some cases, more persistent than the original molecules [13].

Studies have reported the presence of a wide range of pharmaceutical compounds in rivers, lakes, groundwater, and even drinking water sources. Commonly detected pharmaceuticals include painkillers, antibiotics, antidepressants, antihypertensives, contraceptives, and antiepileptic drugs. Nonetheless, for an exhaustive contamination assessment, it is important to include pharmaceutical TPs in environmental monitoring programs [1].

The most investigated pharmaceutical TPs are those belonging to antibacterials, for the increased global usage of these drugs in both human and animal treatments, and their possible contribution to the development of antibiotic-resistant bacteria [9]. Nonetheless, only a little information is available for antibacterial TPs on their formation mechanisms and occurrence in the environment.

The term 'antibiotic' should be reserved exclusively for natural and semi-synthetic compounds with antibacterial properties. Nevertheless, in the current usage of the term, it also encompasses completely synthetic drugs [14]. Consequently, this review will employ the term "antibiotic" without distinguishing based on origin.

This review aims to provide an overview of the most recent analytical strategies based on liquid chromatography-mass spectrometry for the tentative identification of environmental TPs, i.e., those formed by natural processes and human/animal metabolism, in water samples; therefore, TPs formed during WWTP or disinfection processes such as oxidation, chlorination, ozonation, etc. will not be included. The main antibiotic classes and their TPs reported in the literature will be discussed, focusing attention on the suspect screening and untargeted approaches offered by high-resolution mass spectrometry (HRMS) for the tentative identification of compounds whose reference standards are not available or are even still unknown. The issue of TP determination is attracting the interest of the research community in the last years, driven by improved HRMS instrumental performance and the development of more sophisticated computational tools. However, the literature about TPs is still fragmented and incomplete, and a general lack of a common operative strategy can be observed. Nevertheless, the characterization of TPs is important, especially in the case the TPs can still have some activity. Compared to the reviews previously published on this topic, the present work objective is to provide a more comprehensive overview of the last developments, and to summarize the results on the study of TPs of the main antibiotic classes. Lack of information or drawbacks are also provided, to prompt future research in the field.

2. Antibiotics and their transformation products

Antibiotics are widely used in humans for infection treatments, and in food-producing animals for growth promotion and infection prevention. The main antibacterial classes include β-lactam antibiotics (penicillins, cephalosporins, carbapenems, and monobactams), tetracyclines, sulfonamides, macrolides, and (fluoro)quinolones (see Supplementary Figs. S1-S4). According to the sale data provided by the European Medicines Agency, penicillins (31.2%), tetracyclines (25.8%), sulfonamides, (9.9%), and macrolides (8.5%) were the most used antibacterials for food-producing animals in Europe (31 countries) in 2021. Together, these four classes accounted for 75.4% of the total sales [15]. On the other hand, new-generation cephalosporins and quinolones are more often used in humans than in animals; however, there are specific differences from country to country and depending on the type of considered antibiotics [16].

Despite this, data on the usage of antibiotics do not always reflect their occurrence in the environment, which depends on several factors.

The most frequently detected antibacterials are sulfonamides, followed by quinolones, macrolides, and tetracyclines [17], at concentration levels ranging from ng L^{-1} to μg L^{-1} in different countries [18,19].

For both humans and animals, after administration, a significant proportion of antibiotics, ranging from 50% to 90%, is excreted through urine and feces as a combination of parent compounds and metabolites [18,20]. Most metabolites are produced in a two-phase process involving enzyme-catalyzed reactions. In phase I, these reactions result in the formation of new or modified functional groups, or cleavage through oxidation, reduction, and hydrolysis, whereas in phase II, conjugation with hydrophilic moieties takes place primarily through glucuronidation, sulfation, acetylation, and methylation.

Moreover, the concentrations of antibacterial drugs commonly detected in surface water and WWTP influents and effluents also depend on their half-life in the environment. This is influenced by factors such as hydrolysis, photolysis, adsorption, bioaccumulation, and biodegradation, among others [21]. In aqueous media, for example, photolysis of antibiotics can be directly induced by the absorbed solar light or indirectly by the action of photosensitive compounds reacting with them [11,22,23].

The potentially harmful effects of TPs on humans and ecosystems have not been fully elucidated yet [22]. However, it seems that some TPs could be back-transformed into the parent compound [24] (e.g., the acylated forms of sulfonamides) and other TPs could possess an antibacterial activity similar to or greater than that of the parent compound [25]. For example, Löffler *et al.* [9] reported in their study that tetracycline and sulfonamide TPs often showed a higher mutagenicity and carcinogenicity level, respectively, than their parent compounds.

Some researchers suppose that TP concentration in environmental matrices could be much higher than those of parent compounds [1,26], considering that a single molecule can generate several TPs.

2.1. β-lactams

The β-lactams are the largest group of antibiotics, which include penicillins, cephalosporins, monobactams, and carbapenems. The main compounds of this class are penicillins, first discovered by Fleming in 1928, characterized by a thiazolidin ring linked to the common structure of the β-lactam ring [27] (see Fig. S1). The compounds of natural origin are produced by different species of *Penicillium spp,* whereas the semi-synthetic drugs are derivatives of 6-aminopenicillanic acid. The substitution at position 6 of the ring distinguishes the various compounds and determines their antibacterial activity and pharmacokinetics.

Penicillins act by interfering with the synthesis of bacterial cell walls, and they are widely used for human and veterinary medicine, especially in Europe [15,28]. The main representatives of this antibiotic class are amoxicillin (AMX), penicillin G (PEN G), ampicillin (AMP), oxacillin (OXA), and cloxacillin (CLX).

Cephalosporins are produced from *Cephalosporium acremonium* fermentation. Cephalosporins are characterized by a core β-lactam ring attached to a dihydrothiazine ring [27] (see Fig. S1), also known as 7-aminocephalosporanic acid or cephem ring, with different possible substituents on C3- and C7-positions (R2 and R1 moieties, respectively, Fig. S1) [29]. Currently, there are 53 cephalosporins classified by the World Health Organization. Five (cefalonium, cefonicid, ceftiofur, cefovecin, cefquinome) are restricted to veterinary use, while the others are used in humans as a second- or third-line therapy. As such, the consumption of cephalosporins is limited compared to other important antimicrobial groups, especially fluoroquinolones and macrolides. Nonetheless, tons of cephalosporins are consumed each year and they contribute significantly to the development of antibiotic-resistant bacteria in hospitals and WWTPs. Cephalosporins have variable structures according to the different substituents, which determine the environmental behavior and fate of these compounds. Heterogeneous properties result for the compounds of this antibiotic class (in terms of solubility, acid-base characteristics and the octanol-water partition coefficient

(K_{ow})) due to the effect of substituents. For instance, most cephalosporin structures have two or more ionization centers, therefore they can be positively or negatively charged, or zwitterionic, depending on the pH value. Nonetheless, in water cephalosporins present fast hydrolysis rates (days < $t_{1/2}$ < weeks) at environmentally relevant conditions (i.e., neutral pH and 20 °C) [29]. The hydrolysis can occur on the β-lactam ring or on suitable substituents, which in the latter case does not change the bioactive part of the β-lactam structure. In water environments, the most commonly reported cephalosporins include cefalexin, cefradine, and cefotaxime.

Carbapenems are synthetic compounds with a β-lactam ring fused with a dihydropyrrole ring. Side chains include hydroxyethyl and sulfur-containing moieties (Fig. S1). They have the largest spectrum against bacteria; therefore, they are employed as last-line therapy in infections by resistant bacteria. Imipenem, meropenem, doripenem and ertapenem are some main compounds of this class [30].

All β-lactams have a carboxylic group partly ionized and are very polar. The β-lactam ring common to all this family of natural or semi-synthetic antibiotics is highly unstable and subject to various reactions encompassing hydrolysis [31], molecular rearrangement, and polymerization [27]. Therefore, components of this antibiotic class are rarely detected in environmental waters [32,33]. For example, when the β-lactam ring undergoes hydrolysis, AMX is transformed into AMX penicilloic acid, which can further degrade via decarboxylation at low pH values to form amoxicillin penilloic acid [28,32].

2.2. Sulfonamides

Sulfonamides, whose activity was discovered in the 1930 s, are a class of synthetic antibacterial drugs commonly used in both human and veterinary medicine to treat infections. They are particularly prevalent in veterinary medicine due to their effectiveness and low cost; they are the third most-sold veterinary antibacterial class in Europe [6,15].

Sulfonamides are N-substituted derivatives of sulfanilamide (Fig. S2) and, depending on the heterocyclic *N*-containing ring (*R* substituent), they can be differentiated into five-membered and six-membered sulfonamides [34]. They are amphoteric compounds, and weak acids, with pK_{a1} and pK_{a2} values approximately ranging from 1.5 to 3.0 and 5.0–10.5, respectively [34–37], which make sulfonamides positively charged up to pH 2, neutral for pH values between 2 and 5, and negatively charged at pH> 5 [38]. Sulfonamides are quite soluble in water, with Log K_{ow} values generally < 1 [37,39], and this scarce adsorption capacity leads to their high mobility into the environment [34].

Sulfonamide parent compounds and TPs have been detected in surface water, and in WWTP effluents [6] at concentration levels up to μg L^{-1}. Sulfamethoxazole (SMX) and sulfapyridine (SPY) are the most detected sulfonamides in water samples, followed by sulfadiazine (SDZ) and sulfamethazine (SMZ) [40]. Consequently, the investigation of environmental sulfonamide TPs has mainly focused on these compounds along with a few others. In the literature, there are reported more than 600 sulfonamide TPs, mainly belonging to SMX, SDZ, and SMZ [6]; nonetheless, the information about TP occurrence in the environment is scarce, and data on their degradation and toxicity are quite limited. Although the structural homogeneity of this antibiotic class leads to the hypothesis that similar transformation reactions occur for all of them [25], there is still an incomplete understanding of possible sulfonamide transformation pathways. The R substituent is retained to strongly affect the mechanisms and kinetics of sulfonamide transformation reactions [34]. However, the theoretical approaches for predicting the occurrence of biological TPs are not exhaustive, given the complexity stemming from multiple enzyme-induced reactions and microbial mechanisms [25].

The literature mainly reports the transformation reactions occurring to SMX, and these involve acetylation [25,41], various types of single or multiple hydroxylation (N-hydroxylation, hydroxylation at aromatic ring or R-moiety, ipso-hydroxylation), nitrosation, nitration, deamination, desulfonation, formylation, glucuronidation, glucosidation [25]; also, multiple biotransformations can occur [25,41]. Nonetheless, the same TPs can likely be formed from the other structural analog sulfonamide drugs.

2.3. Tetracyclines

Tetracyclines, first discovered in the 1940 s, are broad-spectrum antibiotics that are active against both Gram-positive and Gram-negative bacteria (Fig. S3). In veterinary medicine, they are the second most used antibiotic class in Europe [15], and the first one in the United States [42] and in the world [43]. The most common tetracyclines are tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), and doxycycline (DOX) [43].

Tetracyclines are very polar and amphoteric molecules, with multi-ionic groups. They have a strong ability to form chelate complexes with certain cations, in particular Ca^{2+} and Mg^{2+}, which affect their pharmacological behavior, absorption, and efficacy.

They are not completely absorbed by humans and animals and are excreted as the original molecule (about 50–80%) and/or free or conjugated metabolites [26]. Tetracyclines have been detected in environmental matrices, including wastewater, natural and drinking water, as well as sediment, and activated sludge [43] up to μg L^{-1} level [26]. Nonetheless, their concentration levels in the environment do not reflect the large amounts used for food-producing animals, very likely because tetracyclines undergo transformation reactions quickly [44]. As for other antibacterial classes, both abiotic (e.g., hydrolysis, photolysis, oxidation, etc.) and biotic pathways can lead to the formation of TPs. For example, photolysis and hydrolysis reactions seem to generate epi-tetracyclines, anhydro-tetracyclines, 4-epi-anhydrotetracyclines, and demethyl-tetracyclines, whereas demethylation of tetracyclines occurs via different processes, including photodegradation [26]. For tetracyclines, photolysis in surface water is considered as one of the main degradation processes depending on the specific water conditions [44].

2.4. Quinolones

Quinolones are synthetic broad-spectrum antibiotics effective against a wide range of bacteria, including Gram-negative and Gram-positive ones. They are used in livestock and aquaculture treatments. Their base structure is constituted by 2 condensed rings, both containing an N-residue in position 1 [27,45] (Fig. S4).

Nalidixic acid was the first quinolone discovered and introduced in the 1960 s. Since then, to improve its antimicrobial properties, various modifications have been introduced in its structure, including fluorine atoms, to give a new generation of quinolones known as fluoroquinolones. Fluoroquinolones are classified into generations, and the most used drugs are the second-generation ciprofloxacin (CIP), ofloxacin (OFX), and norfloxacin (NOR), the third-generation levofloxacin (LEV), and the fourth-generation moxifloxacin (MOX).

2.5. Macrolides

Macrolides are a group of antibiotics exhibiting strong effectiveness against both Gram-positive and Gram-negative cocci. They are frequently prescribed for individuals with penicillin allergies or to combat penicillin-resistant infections, particularly in food-producing animals.

The main macrolides share a common chemical structure, featuring a macrocyclic lactone ring with a typical size of 12–16 atoms; this ring is linked to one or more deoxy, neutral, and/or amino-sugars through glycosidic bonds [33] (Fig. S4). The number of carbon atoms is used to classify macrolides, and the most widely used are erythromycin (ERY) and clarithromycin (CLA) (14 carbons), azithromycin (AZI) (15 carbons), and spiramycin (16 carbons) [27].

Their log K_{ow} values are in the range of 2.3–5.1, therefore macrolides can establish hydrophobic interactions and possess a strong adsorption capacity onto sediment [46]. Photodegradation products are the most investigated TPs of this class.

2.6. Aminoglycosides

Aminoglycosides antibiotics inhibit protein synthesis and are used in both human and veterinary medicine, as well as in plant agriculture. Streptomycin, first isolated from Streptomyces griseus, was discovered in 1943. Together with streptomycin, kasugamycin, and gentamycin are used in plant agriculture; neomycin, dihydrostreptomycin, apramycin, gentamicin, kanamycin, paromomycin, and neomycin are used in veterinary medicine, and enter the environment without transformation because the absorption by the gastrointestinal tract of animals is limited and they are rapidly excreted in the urine if injected. However, the aminoglycosides of highest concern are those used for human therapy and include gentamicin, tobramycin, and mikacin [47]. Effluents from hospitals and wastewater from factories producing these drugs are the main sources of introduction into the environment. Indeed, these antibiotics were detected in raw and treated wastewater, and also in seawater. Streptomycin was the highest in marine waters, and gentamicin was detected in groundwater. No aminoglycoside has been identified in drinking water [33].

2.7. Other antibacterial classes

2.7.1. Amphenicols
Amphenicols are broad-spectrum antibiotics especially effective against anaerobic microorganisms. Chloramphenicol was the first compound available in the market of this class and was originally isolated from *Streptomyces venezuelae*, a soil bacterium. This compound was extensively used for both human and veterinary infections. Today chloramphenicol antibiotics include chloramphenicol and the two analogs thiamphenicol and florfenicol. These compounds enter the environment intact or as glucuronides, especially from the hospital sewage [48]. Degradation products are then obtained via natural attenuation, including photolysis, hydrolysis, and biodegradation, and constitute a health risk as some of them were found far more toxic than their parent compounds for both people and aquatic organisms. However, it should also be noted that chloramphenicols are not effectively degraded by natural degradation, therefore most of the attention was focused on characterizing the TPs obtained by other processes.

2.7.2. Lincosamides
Lincosamides are molecules made up of a pyrrolidine ring with attached sugar. The main compounds in the lincosamides class include lincomycin, which is the first molecule of the class and was originally discovered from *Streptomyces lincolnensis*, clindamycin, and pirlimycin. Information on the biotransformation of these compounds is limited [49]. The clindamycin sulfoxide TP was detected and quantified by hydrophilic interaction chromatography (HILIC)-MS in aqueous environmental samples [50] suggesting it to be persistent in river water but with no further investigation of other potential TPs.

2.7.3. Glycopeptides
Glycopeptide antibiotics are made up of a cyclic heptapeptide with two sugar moieties. They are produced by *Streptomyces* species and are used when other antibiotics fail. The most representative compound in the class is vancomycin, which is used in veterinary medicine [51]. This molecule is relatively stable to degradation in WWTPs and its presence was reported in the aquatic environment. Other compounds of the class include polymyxins, teicoplanin, and bleomycin. No transformation products were reported for vancomycin after abiotic degradation [49].

3. Analytical approaches for the determination of antibacterials and their transformation products in water samples

3.1. Sample preparation

The environmental samples, including influents and effluents from WWTPs, and surface waters, contain several thousands of organic substances of natural and anthropogenic origin. Therefore, to analyze antibacterials and their TPs, likely present at ng L^{-1} level, it is necessary to carry out some sample enrichment and/or clean-up steps. For aqueous environmental samples, after pre-treatment often consisting of a filtration step [38], solid-phase extraction (SPE) is generally employed [1], and among the several available sorbents, hydrophilic-hydrophobic polymers are the preferred materials for CECs, including pharmaceuticals [38,52–56] and their TPs. Indeed, OASIS HLB was the most used sorbent for extracting parent compounds and TPs of tetracyclines [26], sulfonamides [38,40,57], and multiclass pharmaceuticals [32,56,58, 59], even if in some works graphitized carbon black (GCB) gave better recoveries for sulfonamides [41]; also, Strata X was used for penicillins [60] and macrolides [61]. A multilayered SPE constituted by Oasis HLB, Oasis WAX, Oasis WCX, and Bond Elut PPL was used for multiclass halogenated pharmaceuticals, including 1 fluoroquinolone [62]. Finally, the QuEChERS (quick, easy, cheap, effective, rugged, and safe) protocol was used instead of SPE to extract multiclass pharmaceuticals from freeze-dried WWTP influent and effluent samples before Fourier transform ion cyclotron resonance (FT-ICR) MS analysis [63].

3.2. Liquid chromatography-mass spectrometry detection

3.2.1. Chromatographic stationary phases
For the identification of antibacterials and their TPs in environmental water, (ultra)high-performance liquid chromatography coupled to mass spectrometry (LC-MS) via electrospray source (ESI) is the technique of choice [22,64,65]. Generally, the chromatographic stationary phase is a reversed-phase (RP), mostly C18, which is widely used for sulfonamides [25,38,57], tetracyclines [26,44], fluoroquinolones [62,66], penicillins [60], cephalosporins [27] and multiclass antibiotics [22,32,58], but alternative phases such as pentafluorophenyl (PFP) for sulfonamides [40,41], phenyl for penicillins [60], and C3 for multiclass antibacterials [56] were proposed, too.

3.2.2. Mass spectrometry analysis: targeted vs. untargeted and suspect screening strategies
Targeted methods are typically performed by low-resolution (LR)MS using triple-quadrupole (QqQ) [58,61,67–70] or quadrupole-linear ion trap (QTRAP) [56,57] mass analyzers in multiple reaction monitoring (MRM) acquisition mode [38]. Nonetheless, for wide-scope target screening (> 2000 contaminants, including some antibiotics and available TPs), also hybrid quadrupole-time of flight (Q-TOF) instrumentation was used [71].

For suspect screening and untargeted analysis, the employment of HRMS is mandatory, and Q-TOF [22,25,44,59,60,72], and Q-Orbitrap [32,41,62] hybrid mass analyzers are generally used. Only one group used the FT-ICR mass spectrometer for suspect screening analysis in direct injection mode [63].

When operating in LRMS, the unambiguous identification and the quantitation of the compounds of interest can be obtained by comparing retention time and relative MRM transition intensities if authentic standards are available. This approach was used for investigating both parent compounds and TPs of tetracyclines [26], sulfonamides [57], and multiclass pharmaceuticals including some fluoroquinolones (LEV and CIP), macrolides (ERY, AZI, and CLA), and penicillins (AMX) [67]. Nonetheless, many TPs are still unknown [73], and only a few standards are commercially available for those that are known.

On the contrary, with the advent of new-generation HR mass spectrometers, mainly based on TOF or Orbitrap mass analyzers, suspect

screening, and untargeted approaches are becoming prevalent in environmental applications [64,65]. They rely on HRMS for the determination of elemental composition by accurate mass measurements (with mass accuracy <5 ppm and often <2 ppm), and the tentative structural identification of compounds by their tandem mass spectra. The untargeted approach consists of a full scan acquisition in the selected m/z range followed by fragmentation spectra acquisition either in data-dependent acquisition (DDA) or data-independent acquisition (DIA) mode [74], the latter being the preferred one with TOF mass analyzers. In DDA mode, after a survey full-scan MS acquisition, the ions that meet predefined criteria (mainly the intensity threshold) are selected within a 1–2 m/z window and fragmented; moreover, dynamic exclusion setting can avoid redundant tandem mass spectra acquisition. Instead, the DIA mode is a sequence of full-scan MS acquisitions performed at low and high collision energy to obtain information on precursor and fragment ions, respectively. The advantage of operating in DIA over DDA mode is the larger amount of MS and MS/MS data that can be collected in a single acquisition, without the limit of the instrumental cycle time [62,65] typically encountered in DDA mode. On the other hand, the interpretation of tandem mass spectra obtained through DIA mode is more laborious than those obtained through DDA. Indeed, all the fragments of the precursors having the m/z value within the set m/z isolation window are shown at the same time, complicating the coupling between precursor and fragment ions [62]. The HRMS data acquired both in DDA and DIA modes enable a retrospective analysis of TPs that were not initially considered [32,75].

The first step for the identification of TPs following the suspect screening approach is retrieving from the literature the information about antibiotic transformation reactions [25,40,41]. Furthermore, TPs of small molecules can be predicted in-silico through various freely or commercially available computational tools, which generally provide the possible products of human metabolism and/or microbial and abiotic degradation [1,65,76–78].

All these data are used to compile a list of molecular formulas and relative monoisotopic masses of possible TPs. Then, the list can be implemented either in the mass spectrometric DDA method or in the data processing workflow [1]. In the first case, the m/z of interest will be selected and fragmented despite their intensity, allowing the determination of TPs present at very low concentrations also through the interpretation of their tandem mass spectra. Instead, during data processing, the match between the accurate masses of precursor ions acquired in HRMS and the exact masses reported in the list can help to easily extrapolate the putative TPs, whose tentative identification still requires the acquisition of their tandem mass spectra.

In some cases, simulated laboratory experiments are carried out by incubating one or more compounds under certain conditions to allow the occurrence of transformation reactions [25,41,60,62]. At the end of the experiment, TPs generally present also at low concentrations can be tentatively identified through their accurate mass and tandem mass spectrum interpretation. Various simulated experiments were performed to study the kinetics and pathways of antibiotic photodegradation and the toxicity of their TPs. Baena-Nogueras et al. [79] subjected 9 sulfonamides and 6 fluoroquinolones to simulated natural sunlight, identifying more than 200 phototransformation products, most of which were described for the first time. The sequential formation and disappearance of TPs observed during the experiments led the authors to hypothesize several photodegradation pathways. Nonetheless, it also confirms that simulation experiments of a single degradation factor can provide insight into reaction mechanisms, but it cannot reflect the presence of TPs in the environment where several biotic and abiotic factors, as well as water characteristics and organic matter content [23], contribute to TP generation and concentration level.

Relative chromatographic retention times can support the hypothesized transformation and, in some cases, also the localization of the modification. The retention times can be predicted from the physicochemical characteristics of the compounds, including their log K_{ow}

values, or using simple algorithms. However, more sophisticated *in silico* approaches have been developed, based on quantitative structure–retention relationship (QSRR) modeling, which predicts retention times from the molecular structure and physicochemical characteristics. One very accurate and attractive QSRR method increasingly used in HRMS applications is the artificial neural networks (ANNs) [80–82].

3.3. Processing workflows

Once the data are acquired, either in DDA or DIA mode, a processing workflow to reduce the large amount of data and complexity is required [83,84]. The workflows may involve tasks such as peak detection, peak annotation, blank subtraction, data compression, feature grouping or componentization (i.e., grouping isotopes, adducts, and all signals that likely belong to a single molecular structure), and finally feature identification [83,84]. Statistical analysis can be performed to extrapolate significant differences in feature intensities between samples.

Besides manufacturer's software tools, such as Compound Discoverer (Thermo Fisher Scientific), and Mass Profiler Professional (Agilent Technologies), open-source platforms, e.g. XCMS online [85], and MZmine 2 [86], are available for data processing [65,87,88]. However, since these tools operate with different algorithms, and some processing parameters must be set by the user without an easily predictable impact on the results, considerable differences can be obtained among research groups [63,87].

Operating by a suspect screening approach, as described above, a mass list with the possible TP exact masses and formulas can be implemented in the processing workflow for rapid recognition of the m/z of interest. Compound tentative identification could be obtained by matching with mass spectral libraries. Although these databases are continuously implemented with new validated HR tandem mass spectra by international collaborative studies, they are far from being complete, especially for TPs, whose presence is only suspected (and often the authentic standards are not commercially available) or even unknown [41].

Indeed, various levels of identification confidence can be assigned based on the standard availability and the potential match between experimental tandem mass spectra and mass spectral databases, e.g. mzCloud (https://www.mzcloud.org/, HighChem LLC, Slovakia) and MassBank (http://www.massbank.eu/, MassBank consortium) [89].

When metabolites and TPs maintain the characteristic structure of the parent compounds (i.e., the transformation reaction occurred on the R moiety), they can be recognized by the common fragments [1,72]. This strategy is generally applied to tandem mass spectra obtained by DIA methods, and it consists in extracting the ion chromatogram with a narrow window at the m/z value of a diagnostic fragment from the parent compound. The resulting chromatographic peaks, occurring at different retention times than the parent compound, are expected to represent individual TPs [1].

Despite its potential to find out TPs of environmental organic contaminants, the Kendrick mass defect filtering is still rarely applied, except for perfluoroalkyl substances [74,90].

4. Antibacterial transformation products

4.1. β-lactams

4.1.1. Penicillins

The 2 penicillins AMX and AMP were subject to simulated solar irradiation for photodegradation experiments in HPLC and river water samples [60]. Then, analysis was performed by Q-Orbitrap MS retrieving from the literature data on photolysis, hydrolysis, methanolysis, and metabolism TPs of the 2 antibiotics. The resulting list containing 52 and 15 possible TPs for AMX and AMP, respectively, was used for compound identification. AMP showed higher stability than AMX to photodegradation, as confirmed also by the presence of their

TPs. The wastewater samples were analyzed by Q-TOF MS, and 15 (9 and 6 for AMX and AMP, respectively) out of the 32 TPs evaluated were detected in influent samples, whereas only 6 AMP TPs were detected in effluent samples.

Angeles et al. [32] retrospectively performed a suspect screening on surface water samples. A large suspect list containing more than 1100 compounds was extracted from 2 database lists retrievable from the US Environmental Protection Agency (EPA) website. These lists contain the commercial antibiotics and their TPs, and emerging substances in environmental matrices with potential negative effects. As expected [28, 60], AMX was not detected in any samples, whereas there were 2 of its TPs, namely penicilloic acid and AMX penilloic acid, confirmed through the respective reference standards.

4.1.2. Cephalosporins

Parent compounds are usually investigated in water environment, although there is evidence that TPs can be more toxic and more persistent than parent drugs, and some of them may retain antimicrobial activity. RP-LC-MS analysis is the technique of choice for structural elucidation of TPs in these studies. Photodegradation is considered a main degradation pathway for cephalosporins. In a study on cefazolin, cephapirin, cephalexin, and cephradine photodegradation by direct and indirect processes, the results indicated that decarboxylation, opening of the β-lactam ring, and loss of the R1 substituent are the main reactions involved [91]. In addition, cephalosporin side chains appear to play a pivotal role in both affecting the reactivity of the specific cephalosporin and the production of toxic TPs, as discovered for cefazolin, whose TP is more toxic than the parent compound due to reaction of the R2

substituent. Cephalosporin C, chosen as the model compound of the entire class, was studied for degradation by gamma radiation to assess the type and nature of product compounds [92]. The gamma radiation process can be implemented in WWTPs. The study indicated that higher irradiation and acidic pH favored the degradation of cephalosporin C. Ten products were tentatively identified as a result of four degradation pathways involving the thioether sulfur oxidation, β-lactam ring opening, acetyl dissociation from dihydrothiazine ring and D-α-aminohexylamide group abscission (Fig. 1). All but three products were less or as toxic as the parent compound.

In another study, the hydrolysis and photolysis of ceftriaxone were investigated in ultrapure and natural water. Stability to hydrolysis was higher in the latter. UHPLC-HRMS analysis with up to MS^3 fragmentation was used to assess the nature of the nine products tentatively identified in the study [93]. More products were obtained by hydrolysis in ultrapure water than in Danube River water, indicating that the 4 products detected in natural water result from a common pathway. The compounds originated by decarboxylation of the parent cephalosporin, followed by dihydrothiazine ring opening and rearrangement, side chain loss, and final loss of central core structure, to result in the main products. Photolysis provided 8 products, involving additional reactions with ring opening of the side chains and demethylation. As previously reported, some of the intermediate products still bore the untouched cephalosporin core structure (Fig. 2).

In another study, degradation by sunlight was simulated to investigate the TPs of cefuroxime axetil [94]. Also in this case, the ecotoxicological analyses and in vitro cytotoxicity studies indicated greater toxicity of the irradiated mixture. Interestingly, the *in silico* toxicity
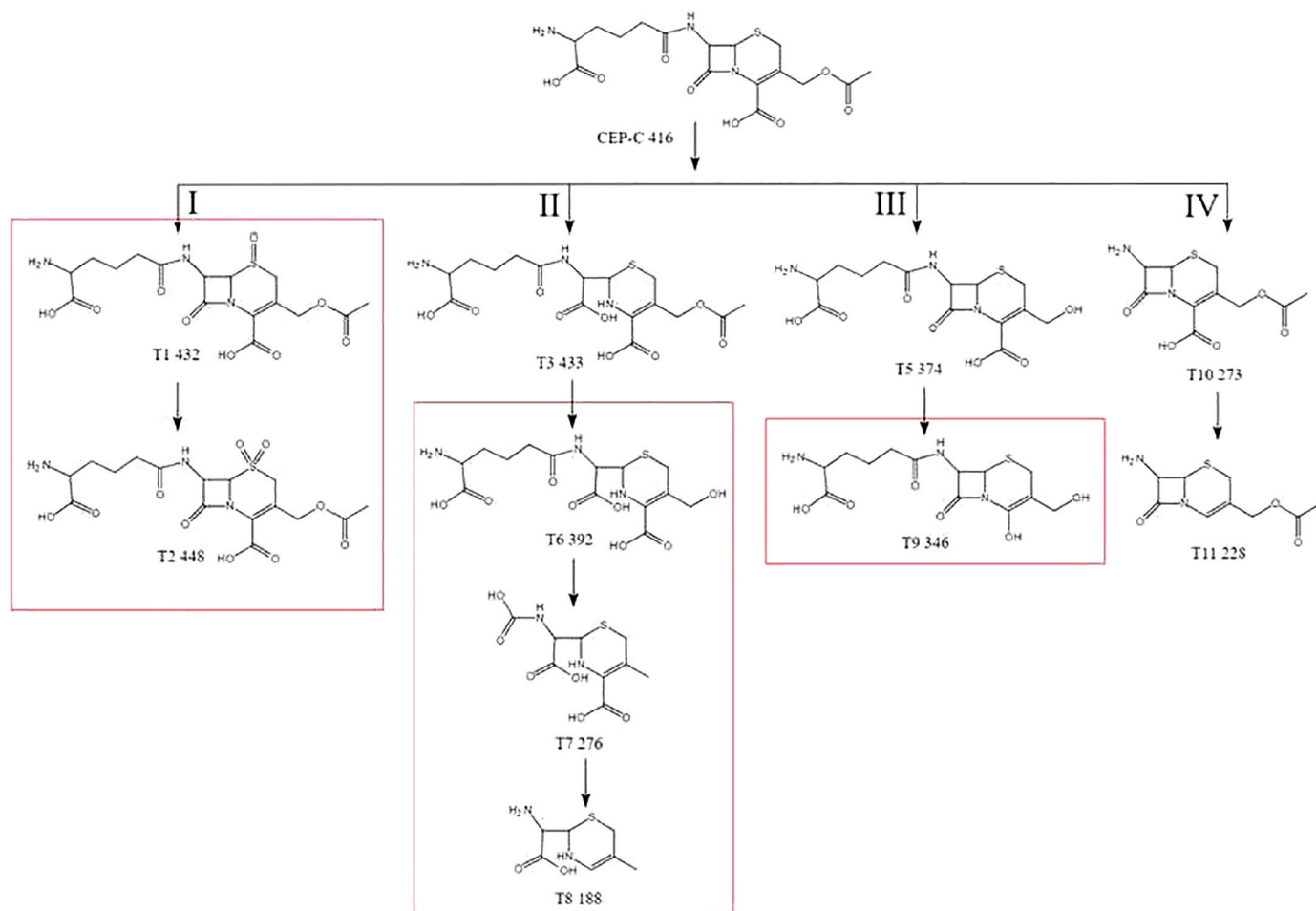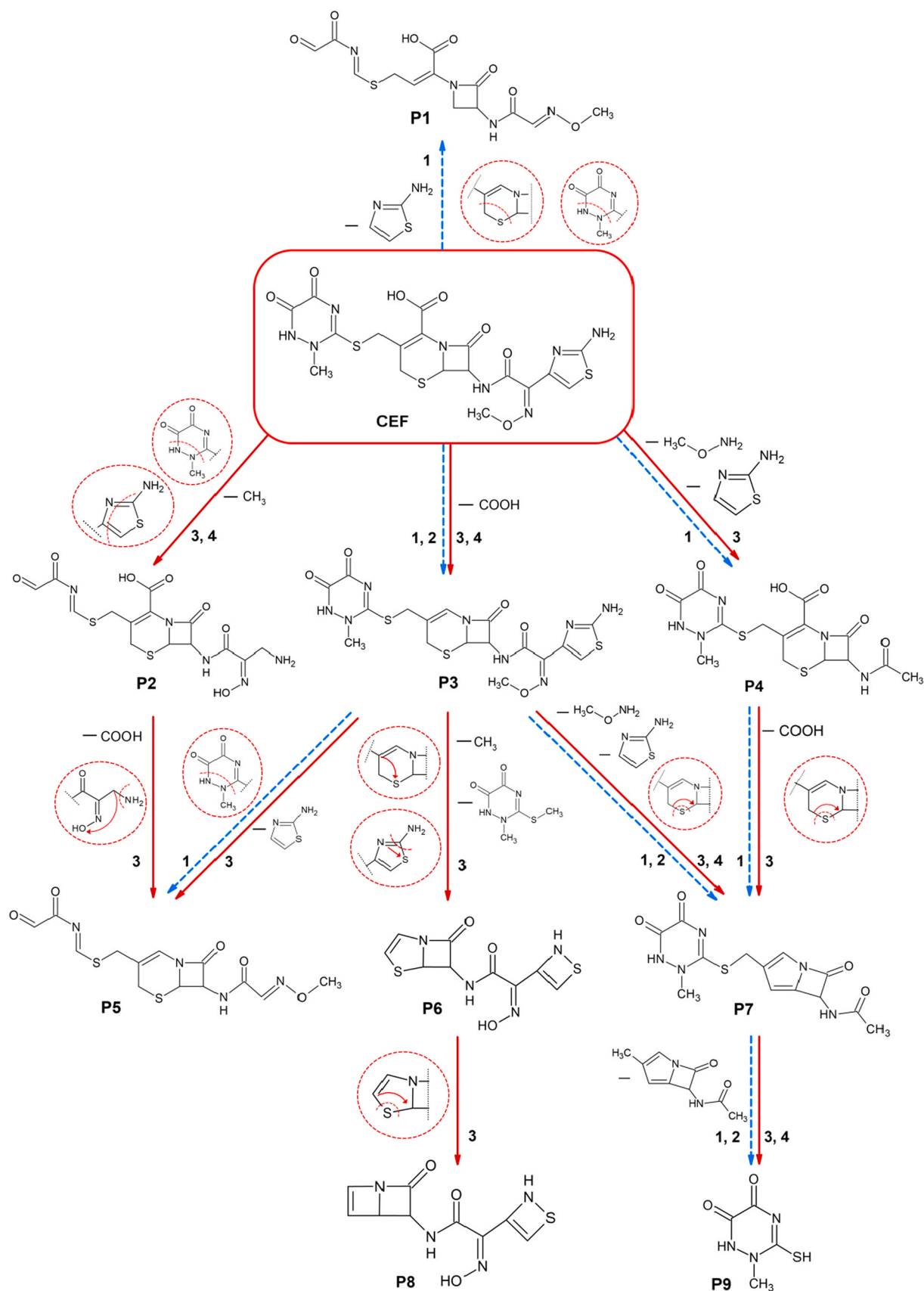

Fig. 1. Possible pathways of Cephalosporin C degradation by gamma radiation (the intermediate products identified in the study are labeled with the red box). Reprinted from Ref. [92]. Copyright (2021), with permission from Elsevier.

Fig. 2. Tentative intermediates and pathways of ceftriaxone degradation: hydrolysis in ultrapure water (1), hydrolysis in Danube River water (2), UVA-B photolysis (3), and solar/H$_2$O$_2$ (4) suggested by ultra-high-performance liquid chromatography-linear ion trap-Orbitrap mass spectrometry. ⤍ shows hydrolysis, ➤ shows direct photolysis and/or solar/H$_2$O$_2$ treatment. Reprinted from [93]. Copyright (2021), with permission from Elsevier.

evaluation provided different results, which indicate the need to obtain substantial experimental data on this topic. The characterization of TPs provided 9 putative structures, originating from oxidation of the thioether to sulfoxide, hydroxylation of the dihydrothiazine ring, ring openings, and hydrolysis of the 1-acetoxyethyl carboxylate group. The main product was, however, the simple isomerization of the oxime moiety in the R1 side chain. Similar degradation pathways were reported for UV irradiation of cephalexin [95].

Although these studies are very interesting and supported by computational analysis, still TPs are not confirmed nor quantified to improve the confidence of suggested molecular structures. In this sense, the use of HRMS to elucidate the structure of TPs is essential and should be preferred to LRMS, which is not applicable for the purpose due to lack of pure standard reference compounds. In addition, although some pathways are similar between the studies, product compounds can have very different structures. Availability of pure standard compounds and investigation in water real samples would improve knowledge on cephalosporin TPs in the environment, which is currently overlooked in the aquatic environment, where the attention is mostly focused on other compound classes.

4.1.3. Other β-lactams

The TPs of other β-lactams other than the one discussed previously are far less investigated or completely overlooked in the current literature, probably due to the limited use of these drugs. Studies on the degradation of carbapenems follow the precursors but do not investigate the nature and bioactivity of products [30]. This knowledge gap is not limited to the environmental fate in water and represents a possible investigation topic for future research [96] focused also on antibiotics that are less employed.

4.2. Sulfonamides

Mass spectrometric acquisition of sulfonamides is generally carried out in positive ion mode, and their characteristic fragment ions are at m/z 92.0495, 108.0444, and 156.0114.

N-acetylation, which can originate from both human and animal metabolisms and aerobic bacteria, is the most reported sulfonamide transformation in the literature. Furthermore, the commercial availability of the standards of the acetylated forms of the main sulfonamides allows their targeted analysis [57,58] (see Supplementary Table S1). In wastewater samples collected in Spain, Gómez-Canela et al. [58] detected Ac-SMX at concentration levels up to 324 ng L^{-1} (mean value 105 ng L^{-1}). Much lower concentration levels were found in two Chinese river water samples, where acetylated forms of SMX, SDZ, SMZ, SPY, and sulfamerazine were in the range 0.1–60 ng L^{-1} except for Ac-SPY, detected up to 133 ng L^{-1} [57]. Seasonal variation was reported for Ac-SMX, Ac-SDZ, and Ac-SMZ in wastewater effluent and receiving water samples collected in China, from few to 500 ng L^{-1} [68]. The acetyl-sulfonamides can be easily recognized in the HR tandem mass spectra by the two diagnostic fragments at m/z 134.0605 and 198.0224 [38,40,41,57]. Other frequently detected sulfonamide transformations include deamination, with characteristic product ions at m/z 77.0384, 93.0699, and 141.0006, and formylation, with characteristic product ions at m/z 120.0443, 136.0394, and 184.0063 in positive ion mode [25,41]. Moreover, hydroxylation and glucuronide conjugation have been reported, even if only limited information is available on their occurrence in the environment. In Chinese river water samples, hydroxy-SPY, SMX-β-D-Glucuronide, and 4-nitro-SMX, were detected below 10, 10, 3.2 ng L^{-1}, respectively, whereas 5-[4-(acetylamino)benzenesulf-ony-loxy]-SPY acetate was detected at trace levels only in a few samples [57].

When the transformation occurs on the R moiety, i.e., keeping the characteristic sulfonamide structure, the typical diagnostic ions at m/z 92.0495, 108.0444, and 156.0114 are generally the most intense in the spectrum.

Even in the absence of authentic standards, differences in chromatographic retention times can support the identification. For example, N4-methylated forms of sulfonamides, recognizable by 3 characteristic product ions at m/z 106.0651, 122.0601, and 170.0271, are expected to have a retention time longer than the parent compound due to the reduction of the polarity by the methylation reaction.

Nonetheless, when multiple interactions with the chromatographic stationary phase are possible, the relative retention times of isomeric forms are difficult to predict. For example, using a PFP column, Montone et al. [41] were unable to discriminate between N4-hydroxy-acetyl-SMX and N4-acetyl-hydroxy-SMX, N4-hydroxy-acetyl-SDZ and N4-acetyl-N4-hydroxy-SDZ, methyl-N4-acetyl-hydroxy-SPY, and methyl-N4-hydroxy-acetyl-SPY, and the two hydroxylated forms of SMX and SPY occurring on the homolog structure moieties.

In environmental samples, SMX TPs are the most frequently detected by all the authors [25,40,41], whereas some differences were observed for TPs of SPY [41] and SDZ [40].

For determining environmental sulfonamide TPs, Majewsky et al. [25] studied SMX TPs from the literature data and the tandem mass spectra of the available TP standards acquired by LC-HRMS with a Q-TOF mass analyzer. For each sulfonamide, 29 potential TPs were predicted. By neglecting the breakdown reactions and calculating the mass shifts from the parent compound for each of the 15 transformation reactions, it was possible to predict 5 characteristic product ions for the tentative identification of TPs of all the considered sulfonamides (Figs. 3 and 4). To verify the strategy's reliability, a suspect screening was applied to samples obtained by incubating SPY and SDZ in activated sludge. In this way, 13 TPs were tentatively identified.

Montone et al. [41] carried out an analog simulated experiment by incubating SMX, SPY, and SDZ in a batch reactor with activated sludge for 20 days to allow TP formation. The samples were analyzed by LC-HRMS using a quadrupole-orbitrap (Q Exactive) mass analyzer in DDA mode. Data were processed with the software Compound Discoverer, including a mass list of hypothetic TPs compiled by the literature data on possible sulfonamide transformation pathways. A total of 44 TPs for the 3 sulfonamides (18, 13, and 13 TPs for SMX, SPY, and SDZ, respectively) were manually validated, including multiple TPs (up to 3 different transformations). Finally, the list of tentatively identified TPs was included in the mass spectrometric acquisition method to analyze WWTP samples. By this suspect screening strategy, besides parent compounds, 4 SMX TPs and 5 SPY TPs were tentatively identified; 5 of 9 TPs were acetylated forms, confirming that these are the main sulfonamide transformations. The numerical difference between TPs detected in the batch reactor simulation experiment and WWTP samples could be ascribed to the low concentration levels in the real samples and possible back-transformation reactions in the environment.

A combination of targeted and non-targeted analysis was proposed by Kokoszka et al. [40] using a QTRAP mass spectrometer. The DDA-based non-targeted analysis allowed the identification of 12 SMX and SDZ TPs in environmental samples. These TPs are derived from both biotic (e.g., acetylation, methylation, and oxidation) and abiotic pathways (e.g., chemical reactions).

In a recent work, molecular networking nontarget strategy allowed to explore the transformation pathways of 5 sulfonamides with similar structures (namely SMX, SMZ, sulfadimidine, sulfamonomethoxine and sulfathiazole) in wastewater biological treatment batch experiments. Fourteen of the 45 TPs identified were previously unknown. The main transformation patterns were pterin-chelation and formylation and acetylation, methylation and deamination reactions [97].

4.3. Tetracyclines

In the case of tetracyclines, most studies follow a targeted approach for the 4 main compounds, namely TC, OTC, CTC, and DOX, and their known TPs, i.e., their 4-epi forms, anhydroTC, 4-epi-anhydroTC, isoCTC, demethyl-CTC, and demethyl-DOX [26], whose reference
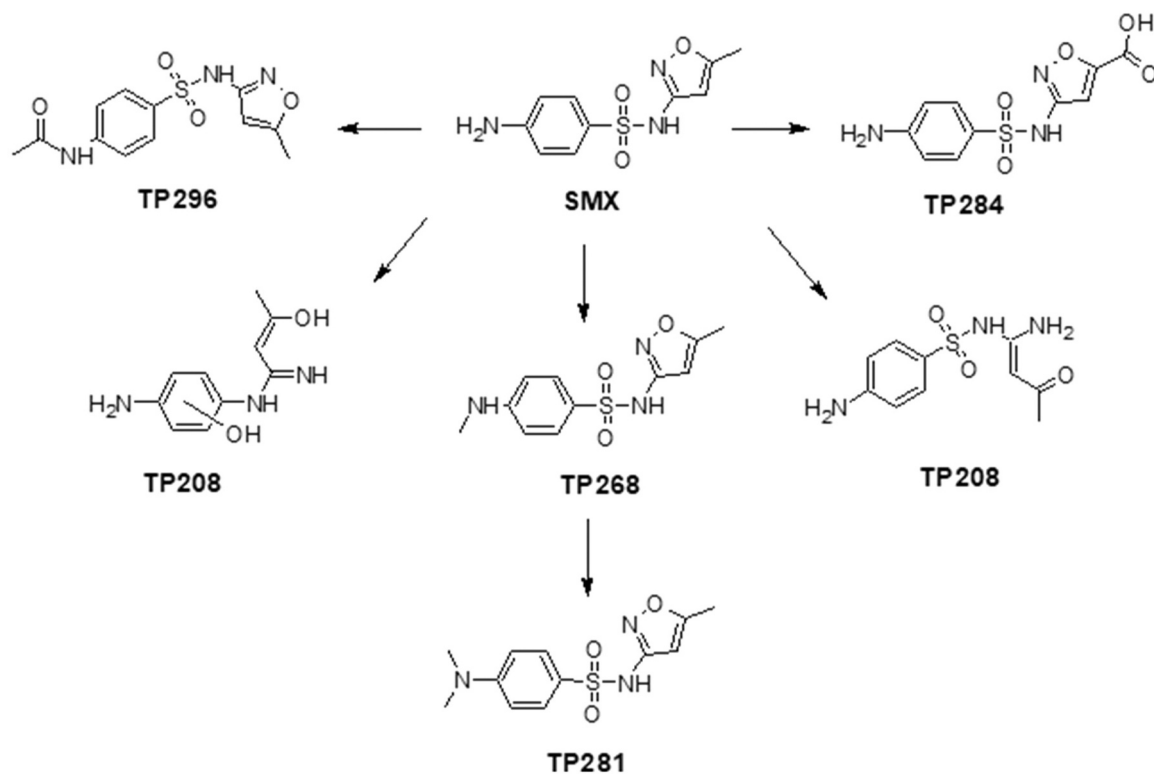
Fig. 3. The degradation pathway of sulfamethoxazole (SMX) in the environment.
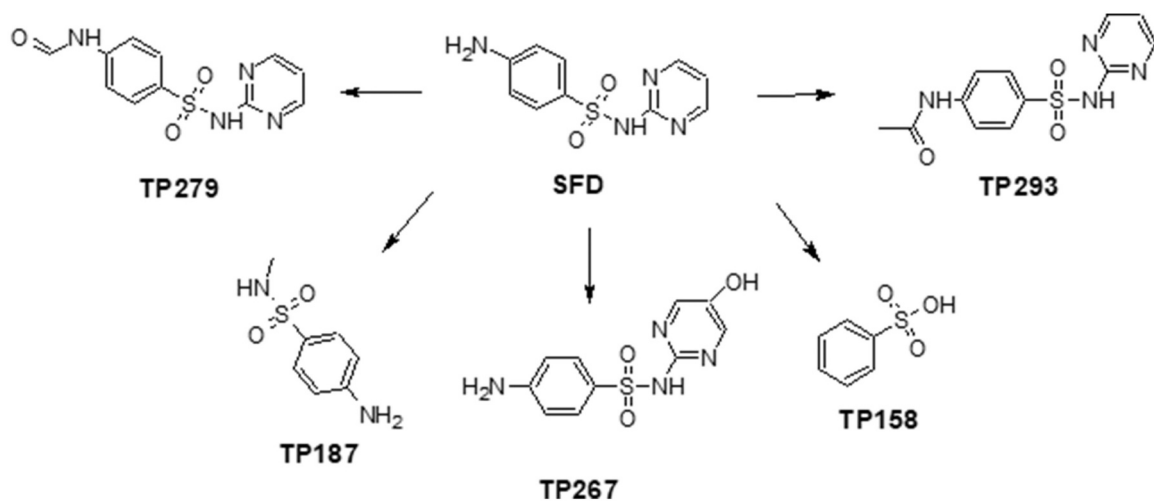Reprinted from Ref. [40]. Copyright (2021), with permission from Elsevier.

Fig. 4. The degradation pathway of sulfadiazone (SFD) in the environment.
Reprinted from Ref. [40]. Copyright (2021), with permission from Elsevier.

standards are commercially available (see Supplementary Table S2). For these tetracycline TPs, maximum concentration ranged between 21 and 47 and 5.4–10.7 ng L^{-1} in some influent and effluent samples, respectively, whereas in sludge up to 1190 ng g^{-1} was found for epiTC [26].

TC (m/z 445.1605) was subjected, together with other 2 drugs, to a simulated photodegradation experiment, and 7 TPs were identified by LC-Q-TOF MS [59] based on accurate mass measurement and tandem mass spectra. These 7 TPs were 2 dehydro-TC (m/z 443.1449), 2 anhydro-TC (m/z 427.1500), 1 monohydroxylated at the aromatic ring form (m/z 461.1555; exact position of –OH group was not determined), 1 derived from the previous one with a subsequent hydroxylation (m/z 477.1504), 1 N-desmethyl-TC (m/z 431.1449), 1 hydroxylated form

(m/z 459.1398) and epi-TC (m/z 445.1605). Some of these TPs were never reported before or they were reported with a different structure. The method was finally applied in a target fashion to influent and effluent wastewater samples and untreated and treated water samples; however, neither the TC parent compound nor their TPs were detected.

4.4. Fluoroquinolones

Boix et al. [66] studied the biotransformation of 5 pharmaceuticals, including OFX, under laboratory-controlled conditions in surface water and by active sludge. After drug TP identification by LC–QTOF MS, also applying the common fragment strategy, a retrospective analysis was

performed in effluent wastewater and surface water samples. However, differently from the other non-antibiotic pharmaceuticals, only 1 transformation, namely a hydroxylation *(m/z* 378.1465), was found for ofloxacin exclusively in the activated sludge experiment and not in the surface water experiment.

Fagnani et al. [62] carried out an interesting comparison between DDA and DIA performance in the identification of TPs of various halogenated pharmaceuticals, including OFX, obtained through a photolysis simulation experiment. An in-house library containing information on both precursors and possible TPs was built for suspect screening analysis of freshwater samples. However, as observed also by other researchers [41], even in the presence of the precursor molecules, the number of TPs detected in water samples was quite smaller than in the lab simulation experiment. In the case OFX, none of the 19 TPs identified in the photolytic experiment was detected in freshwater samples. Unlike DDA mode, DIA mode was able to detect OFX in all freshwater samples, reaching lower detection limits.

4.5. Macrolides

Several TP standards are available for macrolides (see Supplementary Table S3). By a targeted analysis of macrolides and their TPs in wastewater and river water samples from Croatia, Senta et al. [61] found very high concentration levels (up to mg L^{-1}) only in industrial effluents for ERY-oxime, anhydro N-demethyl-ERY, descladinosyl-AZI, and N-desmethyl-AZI; in municipal wastewater and river water samples, TP presence was sporadic and always below 1 μg L^{-1}.

Ibáñez et al. [72] carried out a collaborative study for the screening of drugs and their TPs in treated wastewater samples using 2 LC-Q-TOF MS systems. Among pharmaceuticals, there were also AZI and CLA, and some of their TPs were (tentatively) identified as well. The first laboratory built a database containing pharmaceuticals that were used for targeted analysis in the samples. The second laboratory applied a general screening for investigating pharmaceutical parent compounds and TPs, using two homemade databases depending on whether the reference standards were available at the lab (target screening) or not (suspect screening). A retention time (t_R) prediction approach, based on a previously developed ANN method [80] was implemented to aid in the tentative identification of metabolites. Supported also by tandem mass spectra interpretation, *N*-desmethyl-CLA was identified in target screening, 14-hydroxy-CLA was tentatively identified in suspect screening, and Met 590 CLA was tentatively identified by the common fragmentation pathway.

4.6. Aminoglycosides

There is limited data on the TPs of aminoglycosides in water. The degradation of aminoglycosides in the environment was found facilitated by high temperature and low pH. Gentamicin and kanamycin decompose to produce 2-deoxystreotamine in 6 N HCl. The product does not have antibacterial activity anymore. Photolysis could not efficiently degrade aminoglycosides, as light is not readily absorbed, but photodegradation was observed in nature due to the participation of organic matter that makes aminoglycosides more susceptible to radical attacks [98]. The degradation of parent molecules was investigated under environmental significant conditions, including the photodegradation in simulated natural waters [99], with few exceptions. The degradation of streptomycin was studied in water to assess the influence of conditions, including light exposure, solution pH, temperature, ionic strength, and dissolved organic matter [100]. In addition, the main TPs were identified through HILIC LRMS. Four products were detected that probably originated from the hydrolysis of the glycosidic linkage between sugar moieties and structure rearrangement. The antibacterial activity of the streptomycin solution was also determined during degradation and found weaker than that of the parent compound.

4.7. Amphenicols

TPs for this class were mainly investigated for chloramphenicol and its two analogs, considering processes of photolysis and biodegradation. RP-LC separation with MS detection is the technique of choice in these studies; both low- [101–103] and high-resolution [104] instrumentation was employed. Chloramphenicol, thiamphenicol, and florfenicol have 11, 5, and 15 TPs, respectively, that originate from reactions including defluorination, oxidation by ●OH, photoinduced hydrolysis, and cleavage of the side chain. The main reaction pathways involve hydroxylation and ring-opening, with ●OH playing an important role in the photolysis process [48]. To improve the degradation of chloramphenicols, engineered water treatments are needed, which in turn also produce TPs, recently reviewed elsewhere [48]. Although interesting and pioneering, these studies suffer from the limitation that HRMS is not always the available detector, so further studies, employing more recent instrumentation, are needed to confirm the suggested structures of TPs, also with synthetic standard compounds. Further research in the field is important because computational studies indicated how the toxicity of TPs generated from chloramphenicols in water may possess multi-endpoint toxicity, containing developmental toxicity, carcinogenicity, mutagenicity, and genotoxicity, and contribute to antimicrobial resistance in case the original structure to the parent compound is retained [105].

4.8. Multiclass antibacterials

A combination of targeted analysis and suspect screening was used for the determination of 16 selected antimicrobials (sulfonamides, tetracyclines, fluoroquinolones, macrolides, lincosamides, glycopeptides, and synthetic antimicrobials) and their TPs, respectively, in river water and municipal wastewater samples [56]. Using a QTRAP mass spectrometer, suspect screening analysis was first performed in (pseudo) MRM mode, compiling a list of 300 potential TPs from the literature data, then by DDA using a combination of enhanced full scan and product ion modes, i.e., in the linear ion trap (LIT). This allowed the tentative identification of 14 fluoroquinolone, 6 tetracycline, 5 macrolide, 5 sulfonamide, and 3 synthetic antimicrobial TPs, for a total of 33 TPs for 10 of the 16 selected drugs. The highest number of TPs detected in untreated wastewater confirms that biotic transformation reactions can occur before WWTP processes.

Segalin et al. [22] studied the behavior of 3 antibiotics, namely the fluoroquinolones NOR and CIP, and the sulfonamide SMX, under photolysis simulated condition in distilled water. The aim was to identify the resulting TPs and their toxicity, and no application to real samples was carried out. Analyses were performed by LC-Q-TOF MS in DDA mode and provided the identification of 10, 15, and 15 TPs for SMX, CIP, and NOR, respectively. Among them, 6 TPs have not been reported in the literature before. The study of the degradation pathway was more addressed to antimicrobial removal than their fate in the environment.

Even if no antibiotic TPs were detected in wastewater samples, an interesting strategy was proposed by Perkons et al. [63]. By employing FT-ICR MS, the authors applied the direct infusion-HRMS for the rapid and comprehensive detection of active pharmaceutical ingredients and their TPs. Both a wide-scope suspect screening and a semi-quantitative determination of target analytes were carried out. The compound tentative identification was supported by an in-house database containing hundreds of pharmaceuticals and TPs, tandem mass spectra matching with a mass spectral library, and an *in-silico* fragmentation tool. The advantages of the method are its rapidity, and less laborious data processing (e.g., peak picking, retention time alignment, etc.); moreover, the absence of chromatographic separation allows simultaneous analysis of hydrophobic and hydrophilic compounds. Nonetheless, the authors reported a poorer sensitivity compared to the conventional LC-HRMS applications, and only 16–18 out of 71 TPs

present in the suspect list were identified in the samples.

5. Conclusions

Although the discovery of antibiotics revolutionized medicine in the 20th century [5], recently the worldwide presence of these compounds and their TPs in environmental water has become a major concern for the possible consequences on human health and ecosystems, and the development of antibiotic-resistant bacteria.

Quantification of antibiotic TPs in environmental water is possible only by targeted analysis of compounds whose reference standards are available or can be easily synthesized; therefore, it is limited to a few compounds already known. Furthermore, not only quantification data are scarce, but they strictly depend on sample typology and geographical location of sample collection.

Several identification strategies based on HRMS have been proposed for antibiotic TPs, following either a suspect or an untargeted screening. Various suspect lists have been compiled; however, it should be necessary to reorganize and validate these lists through international collaborative studies. The continuous discovery of new TPs demonstrates that further elucidation of the degradation pathways of the different antibacterial classes is of utmost importance for a comprehensive environmental assessment. In particular, TPs characterized by residual activity or toxicity should be continuously monitored and regulated. Nonetheless, the lack of reference standards hinders the unambiguous identification of TPs and their routinely analysis in monitoring programs.

Besides the development of more effective technologies for antibiotic removal in hospital discharge and WWTPs, an alternative approach to tackling the persistence of parent compounds and their TPs in the environment could be the design of active pharmaceutical ingredients that could be sufficiently active and stable during storage and usage but rapidly degradable after excretion into the environment [106].

CRediT authorship contribution statement

Carmela Maria Montone: Investigation. **Benedetta Giannelli Moneta:** Investigation. **Aldo Laganà:** Supervision. **Susy Piovesana:** Writing - Review & Editing. **Enrico Taglioni:** Investigation. **Chiara Cavaliere:** Conceptualization, Writing - Original Draft.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpba.2023.115818.

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