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REVIEW

Transformation products of emerging contaminants in the environment and high-resolution mass spectrometry: a new horizon

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Abstract It is crucial to study the presence of transformation products (TPs) of emerging contaminants that can be potentially found in the environment after biological or chemical degradation. This review focuses on the potential and shortcomings of high-resolution mass spectrometry (HRMS) to identify these TPs, with emphasis on recent developments in mass analyzers, data evaluation, and compound identification workflows and applications. Advances in HRMS technologies, including direct introduction or in-line chromatographic separation modes, ionization techniques, mass analyzers, and detection methods, have led to powerful tools to assess the molecular changes and the opening of new horizons to identify unknown molecules. Advances in HRMS pertaining to the generation of analytical data for the main methods to identify TPs, including nontargeted and targeted approaches as they are applied to elucidate the structure of TPs, are also discussed.

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Introduction

In recent years, the growing presence of emerging contaminants, such as pharmaceuticals (both human and veterinary), personal-care products, illicit drugs, pesticides, and perfluorinated compounds detected in the water cycle, has been referred to as one of the most imperative environmental concerns [1–3]. After human and/or animal use, these compounds are excreted or release unchanged and as free and/or conjugate metabolites. Transformation products (TPs) of emerging contaminants can be found in wastewater treatment plants (WWTPs) or in the environment as a result of a multitude of abiotic and biotic processes (such as hydrolysis, photolysis, oxidation, and microbial metabolism) acting on the parent compounds or the metabolites [4–7]. TPs are of environmental concern particularly if they are biologically active or resistant to biodegradation [8–10]. However, there is only limited information in the literature on the fate of these TPs, and many of them remain undiscovered [11]. The importance of further investigation of the subject is highlighted by the fact that some TPs are equally active as or even more active than the precursor compounds on aquatic ecosystems or, in the last instance, on humans [10, 12–15].

There are already some reviews that address the experience of several research groups in the analysis of emerging contaminants, including their TPs, with particular emphasis on liquid chromatography (LC)–mass spectrometry (MS)-based techniques, describing the state of the art and highlighting gaps and future needs [9, 16–19]. Recently, some reviews have been published that included current information regarding

the background of (biotic and abiotic) transformations of emerging contaminants [7, 9, 14, 15, 20–22], including their fate, occurrence, and distribution. Different reviews [1, 23–26] have also presented and discussed the main analytical techniques used for the determination of the emerging contaminants and their TPs.

Historically, identification of TPs has been conducted using radiolabeled compounds, thin-layer chromatography, and gas chromatography or LC with unspecific detectors [4]. However, a serious weakness of all these methods is that they lack sufficient specificity for identification of the TPs. Thus, MS is a key element. Because of the polar nature of TPs, the most commonly used separation technique is high-performance LC (HPLC) or simply LC [17, 27]. TPs can be identified using nominal mass. However, quadrupoles are not sensitive in full-scan mode (required to identify unknowns), and much of this work has been done with standards or pure water solutions at relatively high concentrations [24, 28]. Conventional LC–MS interfaces involve soft ionization techniques that produce little fragmentation but provide information on the molecule. The fragmentation of the analyte required for the structural elucidation is most commonly produced by collision-induced dissociation, which can be done in a specialized collision cell or in the intermediate-pressure part of the mass spectrometer (so-called in-source collision-induced dissociation). The fragmentation pattern of the molecule helps, after the application of long and complicated processes and the use of a number of additional confirmatory analyses, to establish the structure of unknown TPs [21, 22]. An additional obstacle to identify these TPs is that there are no extensive standard libraries that would help to identify the chemical composition of the molecule and its fragments (unlike in gas chromatography). Another limitation of the nominal-mass LC–MS methods to determine traces of these TPs is the need to use target methods, i.e., they detect only what they are designed to look for. Ultratrace target methods, based on low-resolution MS, are designed to preserve only a small amount of information about the composition of the sample; the rest of this analytical information is lost [29–31].

High-resolution MS (HRMS) transcends the major limitations of nontarget LC–MS and targeted MS/MS analysis. HRMS instruments [e.g., time-of-flight (TOF) and Orbitrap instruments] provide high-quality information by combining sensitive full-spectrum data with high mass resolution and mass accuracy [32, 33]. In theory, the presence of an unlimited number of compounds can be investigated at the proper sensitivity, without requiring the preselection of analytes or even without having reference standards available. In HRMS, ions are measured at a high resolving power, i.e., ions with slightly different *m*/*z* ratios can be distinguished from one another [34]. The most important feature of HRMS is the capacity to determine the molecular formulas of analytes from accurate-mass measurements. An HRMS instrument can be coupled to quadrupoles and ion traps to add MS/MS and/or MS^n capabilities that allow to obtain mass spectra of multiple product ions with accurate-mass measurements [1, 35]. For this reason, HRMS is a promising technique that has opened new horizons in screening and rapid identification of a wide range of compounds and unknowns. The trends and emerging applications of HRMS in the analysis of micropollutants, but without pinpointing the problem of TPs, have also been reviewed by several authors [23, 28, 36, 37].

This review compiles information from publications that have appeared in the last 5 years (2011–2015) related to the study of TPs in the environment to clearly demonstrate the potential of HRMS for the identification of the myriad of TPs that are still unknown. The advantages and disadvantages of this technique are also examined, and strategies are proposed in order to improve current analytical performance and streamline data analysis.

Approaches to mimic degradation processes and to identify TPs

There are two different approaches to identify TPs: laboratory studies and environmental screening, as schematized in Fig. 1. These approaches are complementary and mutually enriching rather than the opposite of each other. Commonly, laboratory studies offer the advantage of simulating transformation processes under well-defined conditions with appropriate control that facilitates the establishment of differences in the samples that contain the compounds. However, the identification in the environment of at least a few of these compounds is the next necessary step. Tables 1 and 2 show the most recent applications in laboratory and environmental monitoring studies, respectively.

TPs are mostly generated during water treatment processes, including oxidation and biotransformation by activated

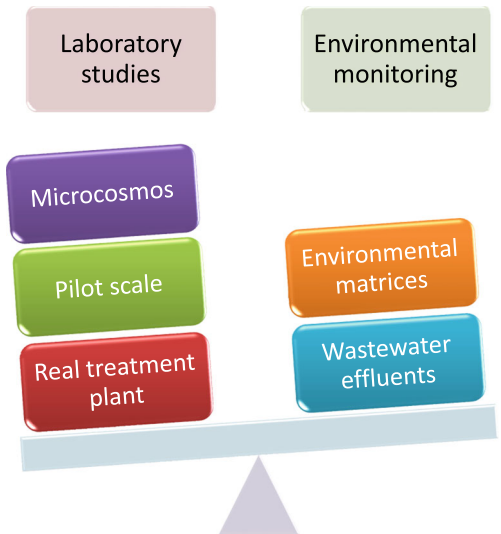

Fig. 1 Different types of degradation experiments

Table 1 Identification of emerging contaminant transformation products (*TPs*) in the environment based on laboratory studies

Compound	Process	TPs	Determination	Working mode	Information	Comments	Reference
Biodegradation							
Acyclovir, penicyclovir	Batch with activated sludge	Acyclovir: carboxyacyclovir. Penicyclovir: 8 TPs (oxidation)	UHPLC–LTQ Orbitrap Velos	MS/dd MS^2	Identification: empirical formula and MS/MS	Additionally 1D (^{1}H NMR, ^{13}C NMR) and 2D (^{1}H,^{1}H COSY, ^{1}H-^{13}C HSQC) NMR spectroscopy	[38]
Codeine	Aerobic batch with activated sludge	8 TPs: double bond shifts, introduction of hydroxyl groups, amine demethylation	UHPLC–LTQ Orbitrap Velos	MS/dd MS^2 and dd MS^3	Identification: empirical formula and MS/MS	Additionally 1D and 2D NMR	[39]
5:3 polyfluorinated acid	Aerobic batch with activated sludge	4:3 acid, 3:3 acid, PFBA, PFPA via "1-carbon removal pathways"	LC–LTQ Orbitrap (NI)	MS (100–1,000 *m/z*)/ CID MS^2	Mass defect data filter. Identification: empirical formula and MS/MS	This is the first report to identify key biotransformation intermediates	[40]
Metoprolol	Batch with activated sludges	Known metabolites (*O*-DMTP, MTPA and α-HMTP) and new TPs	TFC–UHPLC–LTQ Orbitrap Velos	MS/dd MS^2	Postulation of fragmentation pathways of MS/MS spectra	MTP and its TPs were monitored in a full-scale MBR and in a full-scale conventional urban WWTP	[41]
Methotrexate	Batch with activated sludge	9 TPs, 8 of them described for the first time	UHPLC–Q-Orbitrap (PI and NI)	MS/dd MS^2	Automatic software to identify the TPs	Among the TPs identified was 2,4-diamino-N^{10}-methylpteroic acid	[42]
UV-filter sulisobenzone	Batch with activated sludge	Structures of 9 TPs are proposed	LC–DAD–LTQ Orbitrap MS	MS/MS^6	Automatic software to identify the TPs	Additional methods such as NMR spectroscopy to confirm the TPs	[43]
Irgarol and terbutryn	Batch with activated sludge	2 TPs: Iorgarol sulfoxide and terbutryn sulfoxide as toxic as parent	UHPLC–LTQ Orbitrap MS (PI)	MS and MS/MS	Identification: empirical formula and MS/MS	Additionally, NMR was performed. Quantification on environmental samples	[44]
Triclosan	Aerobic batch with activated sludge	Triclosan *O*-sulfate and other TPs	UHPLC–LTQ Orbitrap MS (NI)	MS and MS/MS	Identification: empirical formula and MS/MS	Additionally, NMR was performed	[45]
MDPV	Batch with activated sludge	12 TPs, some of them only reported previously in rats	UHPLC–Q-Orbitrap MS	MS and MS/MS	Identified via their HR MS^2 spectra and LC properties	Based on OECD guideline 314A	[46]
Benzotriazole	Batch with activated sludge	Up to 42 TPs	UHPLC–LTQ Orbitrap MS	MS and MS/MS	Accurate masses and external database, confirmation of MS/MS ions	TPs found in WWTPs. Initial reactions studied also using CSIA	[47]
Pharmaceuticals	pH-dependent batch experiments with activated sludge	Different TPs	UHPLC–Q-Orbitrap MS	MS (50−750 *m/z*) /dd MS^2	Identified via their HR MS^2 spectra and LC properties	Influence of pH on the biotransformation of pharmaceuticals with cationic–neutral speciation	[48]
6:2 fluorotelomer alcohol	Batch experiments with *Phanerochaete chrysosporium*	PFCAs, ≥8 carbons	UHPLC–LTQ Orbitrap MS	MS and MS/MS	Accurate masses and external database, confirmation of MS/MS ions	Bacteria and fungi appear to contribute differently toward the environmental loading of FTOH-derived PFCAs	[49]
Tetracyclines, erythromycin	Batch with enzymes from *Trametes versicolor*	3 TPs for tetracycline and 5 TPs for erythromycin	UHPLC–LTQ Orbitrap	MS (100–1,000 *m/z*)/dd MS^2 of the 5 most intense ions	Xcalibur 2.2 (Thermo) used for data interpretation	TPs were mostly dehydration and oxidation products	[50]
N-Ethyl perfluoroctane sulfonamide	Batch with soil	PFOS	UHPLC–QqTOF	MS and MS/MS	Identified via their HR MS^2 spectra and LC properties	OECD guideline 304A	[51]
9 pharmaceuticals	Batch with sediments	16 TPs	UHPLC–QqTOF-MS	MS^E mode, at LE (2 eV) and HE (20 eV)	Software helps to automatically identify the compounds	Data processing based on peak detection, time-trend filtration, and structure assignment was established	[52]
Fluoroquinolones and macrolides	Pilot-scale. MBR	6 novel TPs, mostly conjugated compounds	UHPLC–QqTOF-MS (NI and PI)	MS and MS/MS	Elemental composition of precursor and product ions	The dynamics of the TPs identified in MBR effluents was followed over a period of 65 days	[53]
Abiotic degradation							
Amoxycillin	Laboratory experiment in alkaline and acid media	4 main TPs. Amoxicilloic acid methyl ester was reported for the first time	LC–QqTOF-MS/MS	MS and MS/MS (obtained using a CE of 5–10 eV)	Identification: empirical formula and MS/MS	Screening of TPs in wastewaters and river water. Transformation pathway is through β-lactam ring cleavage	[54]

Table 1 (continued)

Compound	Process	TPs	Determination	Working mode	Information	Comments	Reference
4 analogues of sildenafil	Photodegradation	6 common TPs and 9 unique TPs	UHPLC–Orbitrap (QExactive) MS	MS/dd MS^2	Elemental composition	Detection of TP488 and TP448 in effluent samples	[55]
Ibupofen, paracetamol, bisphenol A	Photodegradation ($1O_2$) and solar energy	Identification of the main TPs	UHPLC–QqTOF-MS	MS scan	Elemental composition	Use of "blank experiments" to establish differences with the treated samples	[56]
Sildenafil (Viagra) and its N-demethylated human metabolite	Photodegradation: simulated sunlight	Identification of the main TPs	UHPLC–ESI-QqTOF-MS	MS/dd MS^2	Elemental composition	Hydrogen/deuterium-exchange experiments	[57]
Carbamazepine	Photodegradation: UV and simulated solar irradiation	Several recalcitrant hydroxy-TPs and keto-TPs identified	UHPLC–QqTOF-MS (PI)	MS and MS/MS	Identification: empirical formula and MS/MS	*Daphnia magna* bioassay showed that the mixtures were highly toxic	[58]
Chloroacetamide herbicides	Photodegradation: UV treatment of water	15 TPs	LC–QqTOF-MS	MS and MS/MS	Identification: empirical formula and MS/MS	The main reactions were dechlorination, hydroxylation, and cyclizations	[59]
Ciprofloxacin	Photodegradation	11 TPs	UHPLC–LTQ Orbitrap	MS (100–400 *m/z*) and MS/MS	Identification: empirical formula and MS/MS	Formation of stable TPs in wastewater treatment and in surface waters	[60]
Diclofenac and ibuprofen	Photodegradation (sonophotocatalysis)	7 TPs of ibuprofen. 10 TPs of diclofenac	UHPLC–QqTOF (PI diclofenac and NI ibuprofen)	MS and MS/MS	Identification: empirical formula and MS/MS	No differences were observed in the TPs for each substrate among the experiments	[61]
Zanamivir	Photodegradation: solar irradiation	Several TPs	UHPLC–LTQ Orbitrap (PI)	MS and MS/MS	Identification: empirical formula and MS/MS	Sun test experiments suggest that humic acid constituents influence the zanamivir degradation profile in surface water	[62]
Estrone	Oxidation: ozonation (O_3)	593 peaks were associated with ozonation TPs (16 candidates)	UHPLC–Orbitrap (LTQ Orbitrap XL) MS	MS^n, CID, HCD	Elemental composition	Use of a control to identify the possible TPs	[63]
Metoprolol	Oxidation: ozonation (O_3)	7 OPs were identified, and 4 of them were reported for first time	LC–QqTOF-MS (PI)	MS and MS/MS	Identification: empirical formula and MS/MS	Metoprolol degraded via aromatic ring opening and the degradation of aliphatic chain and aromatic ring	[64]
Diazepam	Chemical oxidation: Fe^0/EDTA/O_2 degradation)	Several TPs depending on the degradation conditions	LC–QqTOF-MS (PI)	MS and MS/MS	Identification: empirical formula and MS/MS	OH was the main oxidizing species formed in this process	[65]
Thiabendazole	Oxidation: Fenton degradation (low pH+H_2O_2+$FeSO_4$ $7H_2O$)	12 TPs	UHPLC–QqTOF-MS	MS and MS/MS	Identification: empirical formula and MS/MS	Two main pathways we identified that lead to the transformation of this pesticide in water	[66]
Ciprofloxacin and sulfamethoxazole	Chemical oxidation: decomposition of $S_2O_8^{2-}$ by Fe(II)	Several TPs: piperazine > benzene ring>pyridine ring	LC–Q-Orbitrap-MS	MS (50–500 *m/z*)/dd MS^2 (10 ions)	Identification: empirical formula and MS/MS	Application to groundwater remediation. Similar degradation in river	[67]
Ofloxacin	Fenton oxidation: low pH+H_2O_2 +$FeSO_4$ $7H_2O$	7 TPs in different types of water samples	LC–TOF-MS (PI)	MS including fragment ions	Identification: empirical formula	The degradation is different among deionized, surface, simulated effluent and real effluent waters	[68]
Iopromide	Reverse osmosis	8 new TPs identified	UHPLC–LTQ Orbitrap-MS	MS/CID MS^2 and MS^3	Elemental composition and MS^n	TPs also detected during electrolysis in real reverse osmosis concentrates	[69]
UV filters	Chlorination	3 chlorinated TPs	LC–QqTOF-MS	MS and MS/MS	Identification: empirical formula and MS/MS	Chlorinated atoms are attached to the phenolic ring	[70]
Cannabis	Hydrolysis, photo-degradation, chlorination	1 TP from hydrolysis,8 TPs from chlorination, several TPs frm photodegradation	LC-QqTOF MS (PI and NI)	MS^E (LE 4 eV and HE 15–40 eV)	Use of software to automatically identify TPs	Real WWTPs and in river water (sunlight photodegradation TPs detected)	[71]
Mixed degradation modes							
o-Phenylphenol, bisphenol A, and dextrorphan	Abiotic nitration. Biotransformation in activated sludges	4 TPs of *o*-phenylphenol , 4 TPs of bisphenol A , 1 TP of dextrorphan	UHPLC–QqTOF–UHPLC–LTQ Orbitrap	MS and MS/MS	Identification: empirical formula and MS/MS	Reaction leading to nitrophenols is due to the formation of radicals from nitrous acid	[72]

Table 1 (continued)

Compound	Process	TPs	Determination	Working mode	Information	Comments	Reference
Benzodiacepines, diazepam, oxazepam	Pilot WWTP. Activated sludges, photochemical treatment and absorption to carbon	8 TPs of diazepam and 4 TPs ofoxazepam	UHPLC–QqTOF-MS (PI)	MS and MS/MS	Use of software to automatically identify TPs	Confirmation in real wastewater and river water	[27]
Furosemide	Electro-Fenton process and screening of active microorganisms	3 TPs fully identified plus 5 TPs not fully identified	UHPLC–QqTOF-MS	(1) MS in V-mode; (2) MS/MS; (3) MS in W-mode	Identification: empirical formula and MS/MS	Multidisciplinary approach	[73]

CE collision energy, *CID* collision-induced dissociation, *COSY* correlation spectroscopy, *CSIA* compound-specific isotope analysis, *DAD* diode array detection, *dd* data dependent, *ESI* electrospray ionization, *FTOH* fluorotelomer alcohol, *HCD* high-energy C-trap dissociation, *HE* high energy, *α-HMTP* α-hydroxymetoprolol, *HR* high resolution, *HSQC* heteronuclear single quantum coherence, *LC* liquid chromatography, *LE* low energy, *MBR* membrane bioreactor, *MDPV* methylenedioxypyrovalerone, *MS* mass spectrometry, *MTP* metropolol, *MTPA* metoprolol acid, *NI* negative ionization, *O-DMTP* O-desmethylmetoprolol, *PFBA* perfluorobutanoic acid, *PFCAs* perfluorocarboxylic acids, *PFOS* perfluorooctanesulfonic acid , *PI* positive ionization, *Q* quadrupole, *QqTOF* quadrupole time-of-flight, *TFC* turbulent flow chromatography, *TOF* time of flight, *UHPLC* ultra-high-performance liquid chromatography, *WWTP* wastewater treatment plant

sludges [87]. A large number of chemicals used in industry and households, including pharmaceuticals, biocides, and personal-care products, are released into aquatic environments because of their incomplete removal in WWTPs [3, 88]. These plants are optimized for nutrient removal and biological degradation of natural organic compounds, but more recalcitrant chemicals are only partially degraded during the treatment process [16, 22, 32, 34, 36]. As a consequence, their TPs are formed and emitted via WWTP effluents into the aquatic environment. Once released into the environment, emerging contaminants are also subject to processes (e.g., biodegradation in soil, sediment, and biota, as well as hydrolysis and photochemical degradation in surface water and groundwater) that contribute to their elimination.

The laboratory studies are focused on the different types of degradation mechanisms—biotic and abiotic. They can be at different scales. For batch processes, bench-scale testing is typically conducted on samples of 1–20 L or less, whereas pilot-scale testing is performed with samples of 20–100 L. Demonstration scale essentially involves operating the equipment at full commercial feed rates over extended time periods to prove operational stability.

Biodegradability is mostly evaluated under different treatment conditions in activated sludge batch experiments [38–43, 50]. The sludge is aerated to suspend the microorganisms and is used as the biodegradation medium or autoclaved for use as a sterile control to eliminate the influence of abiotic processes. Batches spiked and not spiked with the analyte are also run in parallel to avoid interferences. These experiments can be performed under different operational conditions: aerobic, anaerobic, and anoxic. The pH, dissolved oxygen content, temperature, and content of total suspended solids are monitored and adjusted to allow direct comparison with environmental conditions [39, 44, 51, 52]. The sorption behaviors of compounds in soils or sewage sludges can be determined by adjusting experimentally these parameters according to several OECD guidelines, among them 304A and 314A [46, 51]. Several recent studies have identified micropollutant TPs in laboratory studies with activated sludge and field investigations in WWTPs, pointing to oxidative and hydrolytic reaction pathways [38–52]. Evaluation of the importance of different reaction pathways, however, is very difficult owing to problems related to the identification of TPs (e.g., the lack of protocols and standardized methods, spectral libraries, or analytical standards to confirm the identity) and their quantification (e.g., again lack of analytical standards). Batch experiments have also been performed to establish biodegradation in soil and sediments, where microorganisms play an important role in degradation [51, 52]. Li et al. [52] used a test system with artificial river water and sediment collected from two rivers to identify microbial TPs from nine pharmaceuticals in a water/sediment test, with special focus on TPs that are

Table 2 Identification of emerging contaminant TPs in environmental matrices

Compound	Matrix	Extraction	Determination	Working mode	Software	Comments	Reference
Water							
Dipyrone	Effluent wastewater	SPE (Oasis HLB)	UHPLC–QqTOF (ESI)	MS^E (LE 4 eV and HE (15–40 eV)	Automatic search for metabolites or TPs	Nontarget screening: metabolites can share fragment ions	[74]
Around 160 metabolites	Effluent wastewater	SPE (Oasis HLB)	UHPLC–QqTOF	MS^E (LE 4 eV and HE (15–40 eV)	MassFragment from Water to automatically search for TPs	Suspect screening: the retrospective search for TPs in parent-positive effluent	[75]
Systematic identification of TPs	Effluent wastewater	SPE (Oasis HLB)	UHPLC–QqTOF (ESI+)	MS and MS/MS	Construction of accurate-mass database of compounds and fragments	Suspect screening: (1) automatic screening; (2) identification of possible TPs; (3) confirmation by MS/MS analysis	[76]
Systematic identification of TPs	Effluent wastewater	SPE (Chromabond HR-X)	LC–LTQ Orbitrap (ESI+/ESI-)	MS/dd MS^2	Filtering of the list, stepwise identification. Selection of peaks for identification	Suspect and nontarget screening	[77]
Systematic identification of TPs	Wastewaters and surface waters	SPE (Oasis HLB)	LC–QqTOF (ESI+)	MS^E (LE 4 eV and HE (15–40 eV)	Automatic search of a homemade database	Suspect screening	[78]
Systematic identification of TPs	Wastewaters and surface waters	SPE (Oasis HLB)	LC–QqTOF (ESI+)	MS/IDA MS^2 (peaks>100 cps)	Automatic search of a homemade database with an MS/MS library	Suspect and nontarget screening	[79]
Organic trace substances and TPs	Water analysis	SPE (Isolute ENV)	LC–QqTOF (ESI+/ESI-)	MS and MS/MS	Automatic search for metabolites or TPs. DAIOS database	Nontarget screening	[80]
Systematic identification of TPs	River water	Direct injection analysis	UHPLC–QqTOF. GC×GC–TOF	MS/IDA MS^2	Automatic extraction of *m/z*	Comprehensive strategy to detect TPs	[81]
Soil and sediment							
Several pesticide TPs	Surface waters and soils from rice production	SPE (Bond Elut C18) for water. Ethyl acetate for soil and sediments	UHPLC–QqTOF	MS^E (LE 4 eV and HE (15-40 eV)	Searching against a homemade database	Post-target search of suspected compounds	[82]
Triclocarban and its TPs	River sediment	PLE (2×5 min; flushing for 60 min at 80 °C and 1,500 psi) using methanol	UHPLC–Q-Orbitrap	MS and MS/MS	Identification: empirical formula and MS/MS	TCC, DCC, and 3-Cl-TCC were ubiquitously detected. Target screening	[83]
>180 emerging contaminants and TPs	Lake sediments	PLE: ethyl acetate and acetone at 80 °C	UHPLC–APPI–Q-Orbitrap	MS/ddMS^2	Identification: empirical formula and MS/MS	Retrospective analysis of the full-scan data. Target screening. Suspect searching	[84]
Biota							
Velafaxin and its metabolites	Marine mussels (*Mytilus galloprovincialis*)	Modified QuEChERS method	UHPLC–Orbitrap (QExactive) MS (PI)	AIF. Nontarget searching	Database searching using ToxID 2.1.2	Target screening. Identification of other nontarget compounds	[85]

Table 2 (continued)

Compound	Matrix	Extraction	Determination	Working mode	Software	Comments	Reference
(Oxo) carbamazepine	Marine mussels (*Mytilus galloprovinciatis*)	Modified QuEChERS method	UHPLC–Orbitrap (QExactive) MS (PI)	AIF. Nontarget searching	Database searching using ToxID 2.1.2	Target searching. Identification of other nontarget compounds	[86]

AIF all-ion fragmentation, *APPI* atmospheric pressure photoionization, *3-Cl-TCC* 3,3′,4,4′-tetrachlorocarbanilide, *DCC* 4,4′-dichlorocarbanilide, *GC* gas chromatography, *HLB* hydrophilic–lipophilic balance, *IDA* information-dependent acquisition, *PLE* pressurized liquid extraction, *QuEChERS* quick, easy, cheap, effective, rugged, and safe, *SPE* solid-phase extraction, *TCC* triclocarban

not easily degraded and thus accumulate during the incubation period. The biotransformation in soil of two representative perfluorooctanesulfonic acid precursors—*N*-ethyl perfluorooctane sulfonamide and *N*-ethyl perfluorooctane sulfonamide ethanol—has also been studied using a similar approach [51].

Most of the pilot-scale experiments are performed using alternative water treatment technologies such as membrane bioreactors that better simulate the conditions in the full-scale system. These membrane bioreactors are commonly fed with synthetic wastewater and inoculated with activated sludge from the full-scale WWTP. The TPs are commonly searched for in the membrane bioreactor effluent [53]. Batch and pilot-scale experiments need to be correlated with the detection of the TPs identified in the real WWTPs or in the environment. Those studies that report searching for the identified TPs in real matrices demonstrated that at least some of them are present in wastewaters and surface waters [41, 44, 47], pointing out the interest in small-scale simulation to increase knowledge of TPs [46, 51].

The physicochemical degradation occurs concurrently with engineered processes such as oxidation reactions with chlorine, chlorine dioxide, or ozone and transformations by ultraviolet light. There have also been a number of batch and pilot-scale studies [55–71]. Among the various advanced oxidation processes, heterogeneous photocatalysis using titanium dioxide as a catalyst is a technology that appears to be a promising tool for water and wastewater treatment as it has been successfully applied for the removal of pharmaceuticals and other micropollutants from water in laboratory-scale reactors [58]. Another rapidly developing field in advanced oxidation processes for applications in environmental remediation is the use of ultrasound irradiation to destroy or accelerate the destruction of liquid-phase contaminants. In the case of reverse osmosis concentrates, a subsequent electrochemical treatment using boron-doped diamond electrodes offers potential removal of contaminants, since electrolysis itself profits from enhanced electrical conductivity owing to the high salinity and the capability of boron-doped diamond to generate hydroxyl radicals [69]. In situ chemical oxidation is an emerging technology for groundwater remediation because of its applicability to a wide range of contaminants, relatively fast treatment, potentially enhanced postoxidation microbial activity, and cost-effectiveness [67]. Chlorination is the most commonly used chemical process for disinfecting swimming pools and drinking water. Formation of halogenated disinfection by-products in chlorinated water is unavoidable, particularly for substances containing phenolic and/or amino groups [70]. Biotic and abiotic degradations occur together to transform emerging contaminants during the biological wastewater treatment. An abiotic transformation process of recent interest is the nitration of phenol moieties and the formation of

nitrophenolic TPs during biological wastewater treatment [72].

Environmental studies have been performed in WWTP effluents and in surface water matrices. Nonetheless, in the field of environmental chemistry, the detection of trace levels creates an extra difficulty in the analytical development required as well as in its performance [81]. These studies as outlined in Table 2 are much scarcer. A number of studies have focused on effluent wastewaters because the concentrations of TPs are higher than in surface waters [74–76]. Extraction procedures able to isolate and preconcentrate the analytes are extremely important, and conventional solid-phase extraction (SPE) is commonly used in the case of water. These procedures are sometimes also used in laboratory studies. The extraction procedures are not sophisticated, but are generic and robust since the physicochemical characteristics of the TPs are sometimes unknown. Hydrophilic-lipophilic balance reversed-phase sorbents are the most popular [74–76] owing to their ability to retain quite polar compounds.

The long-term contamination of sediments by emerging contaminants such as pharmaceuticals, personal-care products, household chemicals, or pesticides as well as their possible degradation products has not been well explored [82–84]. Pressurized liquid extraction followed by quick, easy, cheap, effective, rugged, and safe (QuEChERS)-like cleanup has been used to reduce solvent consumption and to avoid the use of time-consuming cleanup techniques that can increase the loss of polar compounds.

The use of marine organisms as a tool for the monitoring of a pharmaceutical and its TPs in marine environments was evaluated in some studies [85, 86]. The extraction method applied to these matrices is the QuEChERS extraction that involves miniaturized extraction with acetonitrile by salting-out with sodium chloride and magnesium sulfate and a clean-up step, which is done by mixing the acetonitrile extract with several sorbents (dispersive SPE). Successful determination of TPs of venlafaxine and carbamazepine has been reported.

The features of LC separation prior to HRMS

A question that arises when one is collecting information about how to determine the TPs of different emerging contaminants is whether it is necessary to use a separation technique

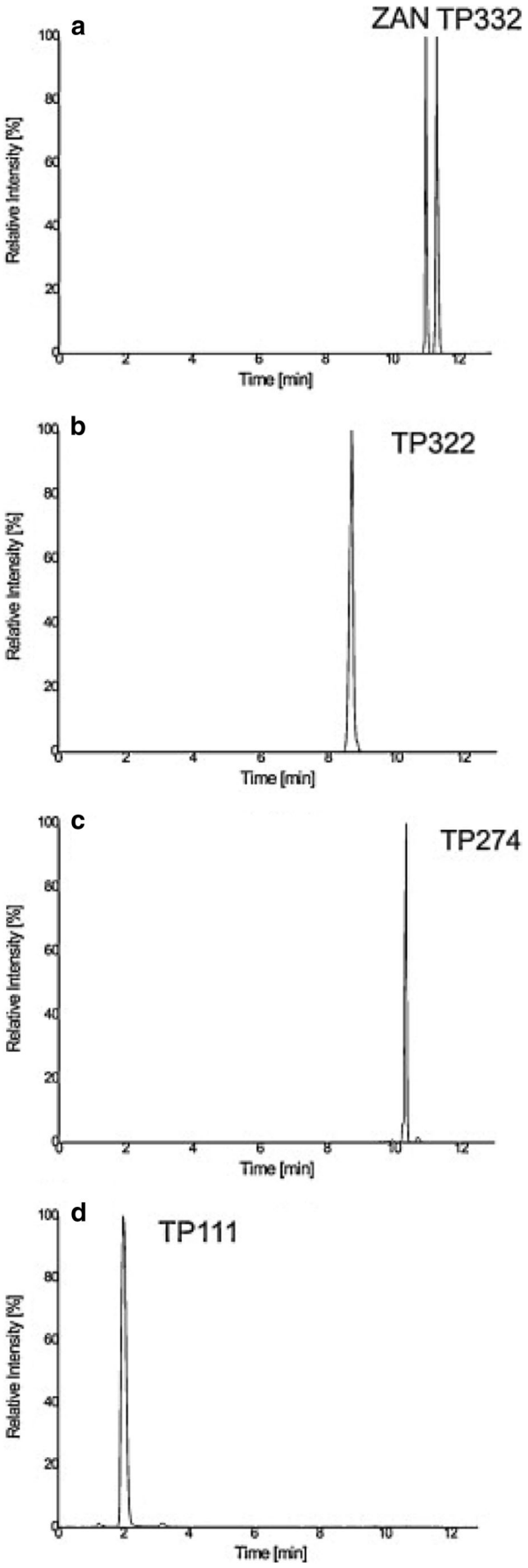

Fig. 2 Extracted ion chromatograms corresponding to separation by ultra-high-performance liquid chromatography (UHPLC) of zanamivir (*ZAN*) and its photoproducts on a hydrophobic interaction liquid chromatography column (acquired with an LTQ Orbitrap mass spectrometer) present in a irradiated high-performance liquid chromatography water sample (initial zanamivir concentration of 40 mg L^{-1}). The figure depicts the ion traces of the molecular ions of **a** zanamivir (*m*/*z* 333) and TP332 (*m*/*z* 333), **b** TP322 (*m*/*z* 323), **c** TP274 (*m*/*z* 275), and **d** TP111 (*m*/*z* 112). (Reproduced from [62] with permission of Elsevier)

prior to the determination by HRMS, taking into account its specificity.

Analysis of the literature summarized in Tables 1 and 2 shows that HRMS is always used in combination with LC, which has been used indistinctly as either classical HPLC or the recently developed ultra-high-performance LC [38–41]. The major advantages of ultra-high-performance LC (1.7-μm particle size in the stationary phase) over classical HPLC (particle size between 3 and 5 μm) include improved resolution within a shorter retention time and higher analytical sensitivity.

The columns used are mostly reversed-phase LC columns and, in a few particular cases, hydrophilic interaction LC (HILIC) columns [62]. The attractiveness of the latter variant arises from the fact that the mobile phase composition is fully compatible with electrospray ionization, with an elevated percentage of organic solvent in the mobile phase enhancing evaporation of the solvent and altering ion suppression and thus improving detection sensitivity [62]. HILIC columns play an interesting role in achieving chromatographic separation of very polar parent compounds or even their more polar TPs. Figure 2 illustrates the chromatographic separation on a HILIC column achieved for zanamivir and its TPs. The reported advantages of the HILIC column in comparison with a C_{18} column were that it substantially improves the retention of zanamivir (an antiviral) and that it separates an isobaric TP, TP332, that was co-eluted with the parent compound.

Chromatographic separation is very important for the identification of the TPs because firstly it helps to properly identify the mass spectra corresponding to the TPs. The parent compound and the TPs normally have part of the molecule in common, and the mass spectra have ions in common; therefore, the separation is important. In addition, this separation, which is usually based on the polarity, gives additional information about whether the assigned structure matches the expected polarity.

The role of HRMS in identification and structural elucidation of compounds

The high-resolution mass analyzers commonly employed are TOF, Orbitrap, and hybrid systems (e.g., quadrupole TOF [27, 51–54, 56, 57, 59, 61, 64–66, 70–76, 78–82], linear ion trap Orbitrap [38–41, 43–45, 47, 49, 50, 60, 62, 63, 69, 77], or quadrupole Orbitrap [42, 46, 48, 55, 67, 83–86]). As can be seen in Tables 1 and 2, despite the fact that HRMS can provide accurate mass, with which the empirical formula of the molecule can be calculated, and thus make possible elucidation of the molecular structure, it is hardly used alone, and all applications confirm the identification by an MS/MS study. The occurrence and variations in patterns of multi-isotopic elements often make the identification of TPs easy. Many emerging contaminants contain chlorine or bromine; these produce a distinctive mass spectral isotopic pattern corresponding to

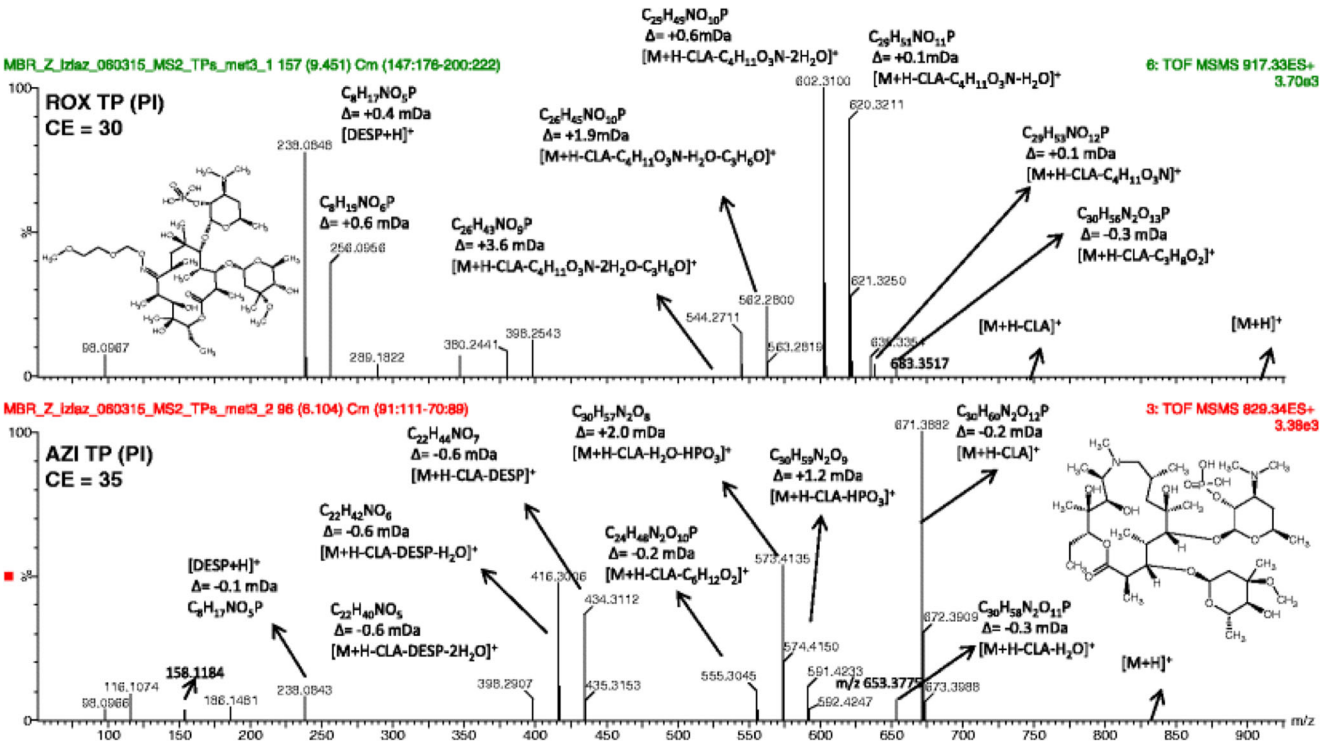

Fig. 3 Time-of-flight (*TOF*) tandem mass spectrometry (*MSMS*) spectra of the transformation products of azithromycin (*AZI*) and roxithromycin (*ROX*) identified in membrane bioreactor effluent obtained in positive polarity mode. *CE* collision energy, *CLA* cladinose, *ES* electrospray, *DESP* phosphorylated desosamine, *PI* positive ionization. (Reproduced from [53] with permission of Springer)

their natural isotopic abundance. By searching for compounds that show the same isotopic pattern as the parent compound, one can quickly associate TPs with the parent drug. The isotopic pattern capabilities have been demonstrated for several compounds, such as diazepam [27, 65].

The MS/MS spectrum can be recorder dependent or independent of the precursor ion data. In the data-dependent acquisition (DDA) mode, a precursor ion is selected for MS/MS, which is inherently biased owing to the existence of many compounds in the sample. For the precursor, a non-overlapping *m/z* window is selected, and all the precursor ions within the window are co-fragmented, yielding more fragment ions per sample, which increases the analyte identification score.

The commonest way to work with hybrid mass spectrometers has been with DDA or information-dependent acquisition. Both refer to the same way of working, but the nomenclature depends on the instrument. In this mode, the high-resolution MS/MS instrument first performs a survey scan, from which

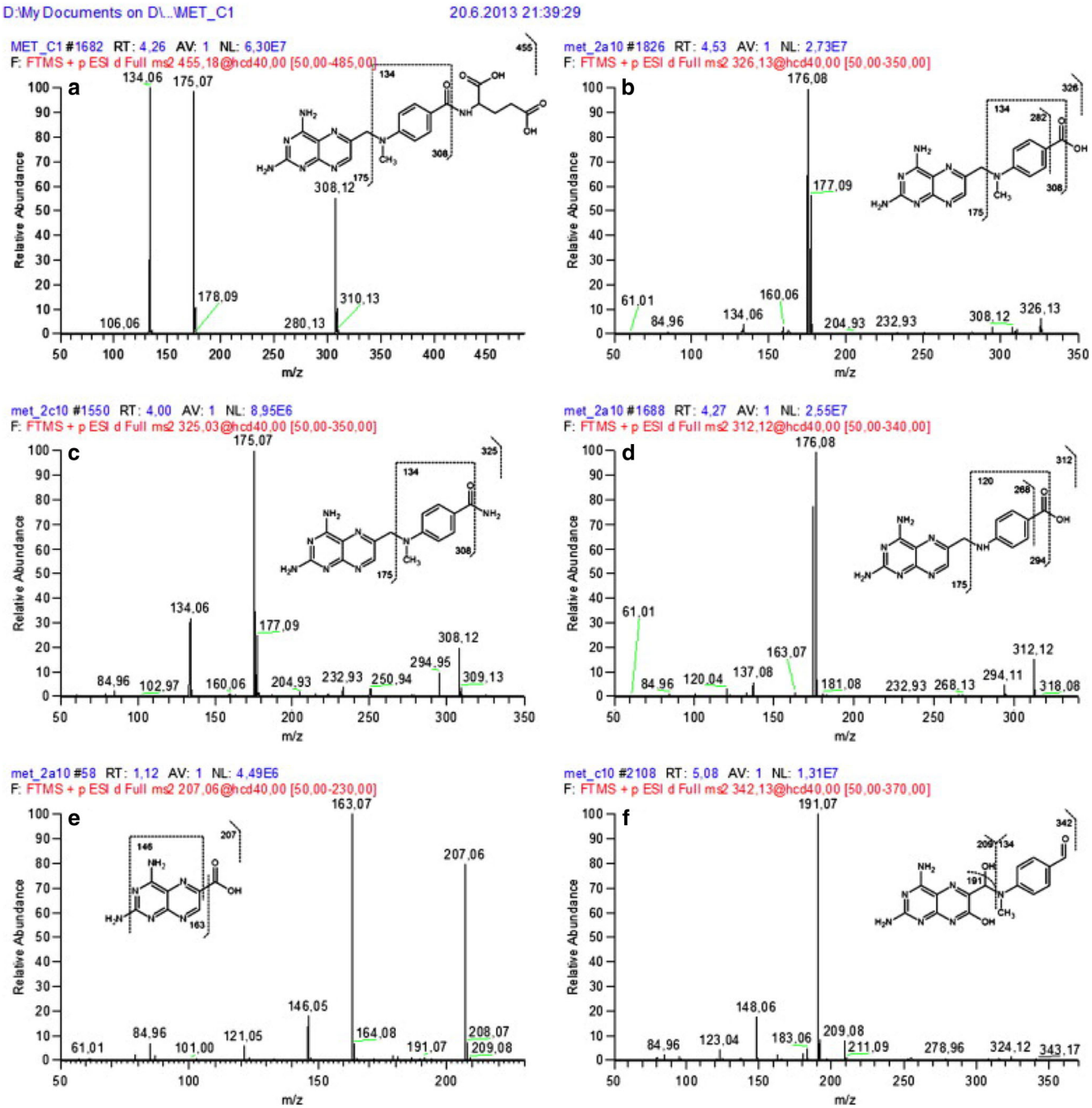

Fig. 4 The positive electrospray ionization (*ESI*) Orbitrap data-dependent tandem mass spectrometry spectra of methotrexate (**a**) and its biotransformation products: DAMPA (**b**), TP325 (**c**), TP312 (**d**), TP207 (**e**), and TP342 (**f**). (Reproduced from [53] with permission of Elsevier)

certain ions chosen "a priori" by the analyst or "not chosen" but meeting some pre-defined criteria (e.g. the intensity above a predefined threshold value) are selected, isolated, fragmented, and sequenced by product ion scanning in the instruments. Additional selection criteria, such as dynamic exclusion, background subtraction, and charge state selection, are also used to prevent redundant acquisition of the most abundant ions, or to avoid acquiring product ion spectra of the interferences. With use of this strategy, co-eluted matrix compounds or noisy peaks can be easily excluded, facilitating the identification and quantification of known or novel analytes in a single run. Figure 3 shows the fragmentation pattern of macrolide TPs obtained in a QqTOF instrument after analyst selection of the precursor ions. Figure 4 illustrates data-dependent MS/MS acquisition of the five most intense ions detected in a full-scan spectrum with dynamic exclusion enabled. The intensity threshold for triggering the MS/MS events was set to 80,000 counts in the full-scan event. Parent ions were fragmented by high-energy C-trap dissociation (HCD). With use of DDA, MS/MS data were obtained in a single run for all compounds investigated.

Data-independent acquisition (DIA) experiments are conducted using a hybrid system, with full-scan data being recorded in a TOF, Orbitrap, or other high-resolution mass analyzer. A major difference in these methods lies in the width (or the absence) of the precursor windows in the first stage of ion isolation. This, in turn, determines the range of ions being transmitted, fragmented, and sequenced. A consequence of having a wide precursor isolation window in DIA approaches is that it produces a very complex data structure and requires coherent and intricate data processing.

MS^E is the commonest DIA approach. It acquires two full-scan mass spectra in an unbiased and parallel manner. As such, it increases both the number of compounds detected and the reproducibility. During data acquisition, the energy of the gas-filled traveling-wave collision cell is dynamically switched between a low-energy and a high-energy state. This produces alternating composite mass spectra of all intact molecular ions, followed by hypothetical fragmented mass spectra of all precursors. MS^E has been a popular choice, with a number of reported applications to identify TPs [52, 74, 75, 78, 82]. Figure 5 shows the detection and identification of clopidogrel carboxylic acid in an effluent wastewater sample [75]. The accurate mass of the protonated molecule of the suspected clopidogrel carboxylic acid (retention time 5.64 min) was *m/z* 308.0524 from the low-energy MS spectrum (Fig. 5, panel B, bottom). The combined spectrum for this chromatographic peak showed a typical monochlorinated isotopic pattern, in accordance with the elemental composition of the protonated molecule. The high-energy TOF-MS spectrum was also investigated (Fig. 5, panel B, top). Up to seven

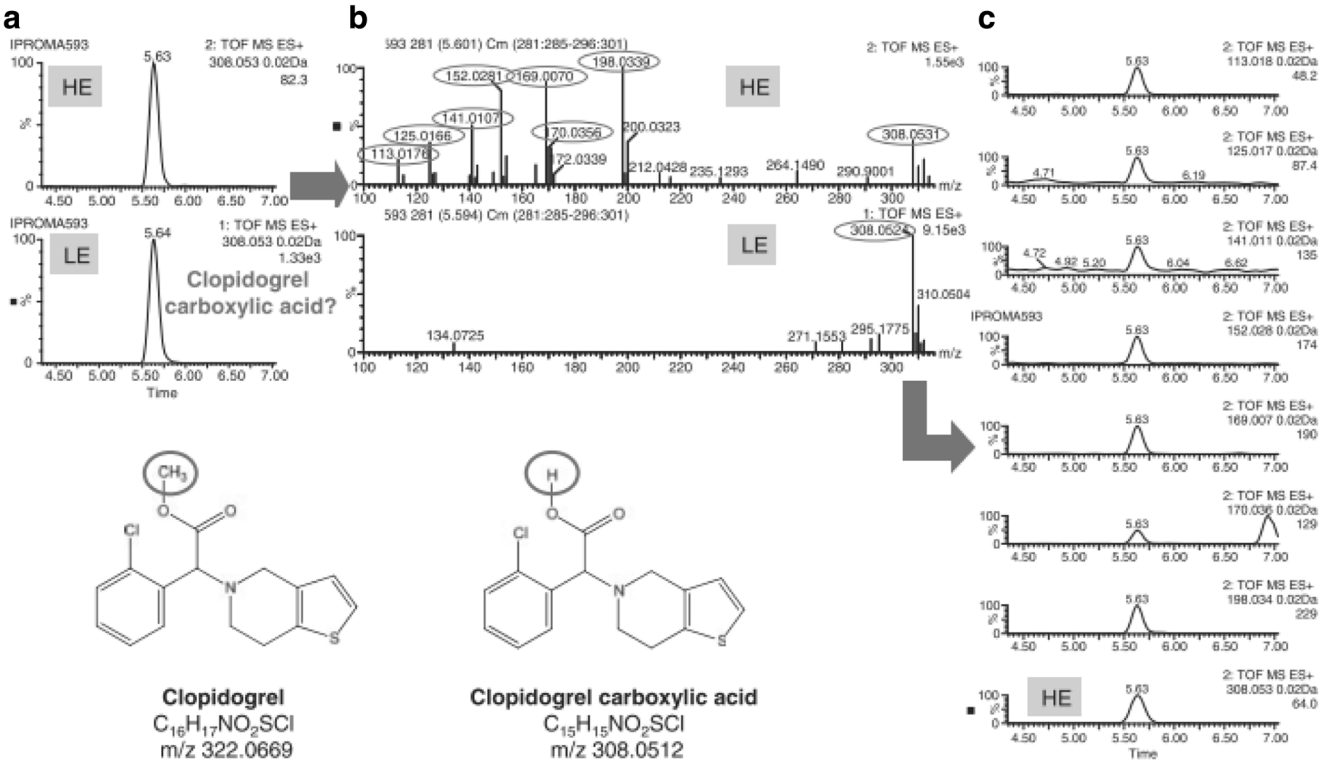

Fig. 5 Detection and identification of clopidogrel carboxylic acid in effluent wastewater by UHPLC–quadrupole time-of-flight (*QqTOF*) mass spectrometry (*MS*) (MS^E approach). Chromatograms (*A*) and spectra (*B*) of the sample. *C* extracted ion chromatograms with a 20-mDa mass window for different ions observed in high-energy (*HE*) mode. *ES* electrospray, *LE* low energy. (Reproduced from [75] with permission of Elsevier)

fragment ions, all showing the chlorine isotopic pattern, were obtained. A possible concern of the MS^E approach is that co-eluted compounds might "contaminate" the high-energy spectrum, which might also contain ions unrelated to the analyte, complicating the interpretation of the spectrum. As Fig. 5, panel C shows, all extracted ion chromatograms, for the protonated molecule and for the seven main fragment ions, led to a chromatographic peak at the same retention time (5.63 min).

All-ion fragmentation (AIF) is the DIA system developed for the Orbitrap. AIF, despite being named differently, is an acquisition mode similar to MS^E in which all precursor ions are fragmented without a preselection by the quadrupole. Fragmentation, however, is obtained with the HCD cell, located at the far side of the C-trap. During filling of the HCD collision cells, the energy can be set to step between values at specified percent values around the chosen middle energy regardless of the ion's characteristics. Figure 6 illustrates the AIF MS/MS spectrum of carbamazepine TPs, which was obtained in an additional experiment using a 10-eV. In HCD operation mode, ions were passed from the C-trap into a multipole collision cell, where they were fragmented and stored. The HCD cell voltages were then applied, and ions were transferred back into the C-trap and injected into the Orbitrap mass analyzer for detection.

LC–HRMS enables one to determine many target polar contaminants and detect their TPs because of its sensitivity and selectivity across a wide range of *m*/*z* values. In addition to target compounds [77], LC–HRMS detects other contaminants not considered a priori in the analysis but present in the samples (screening of suspects or unexpected compounds against a library or database), even after measurement (post-target screening), and identifies the chemical nature of unknown or unidentifiable signals (Fig. 7).

Most of the wide-target and suspect screening is done using an algorithm that extracts *m*/*z* values from a database, either homemade or commercially provided by the instrument manufacturer. These databases automatically extract peaks of an accurate mass by comparing them with those compiled in a database of known compounds. In addition to the theoretical accurate mass (calculated according to the empirical formula), if available, other characteristics of the molecule, such as the isotopic pattern, double bounds (double bond equivalents), and MS/MS data, help to better match the possible chemicals [22, 45, 77]. The database can be automatically extended in

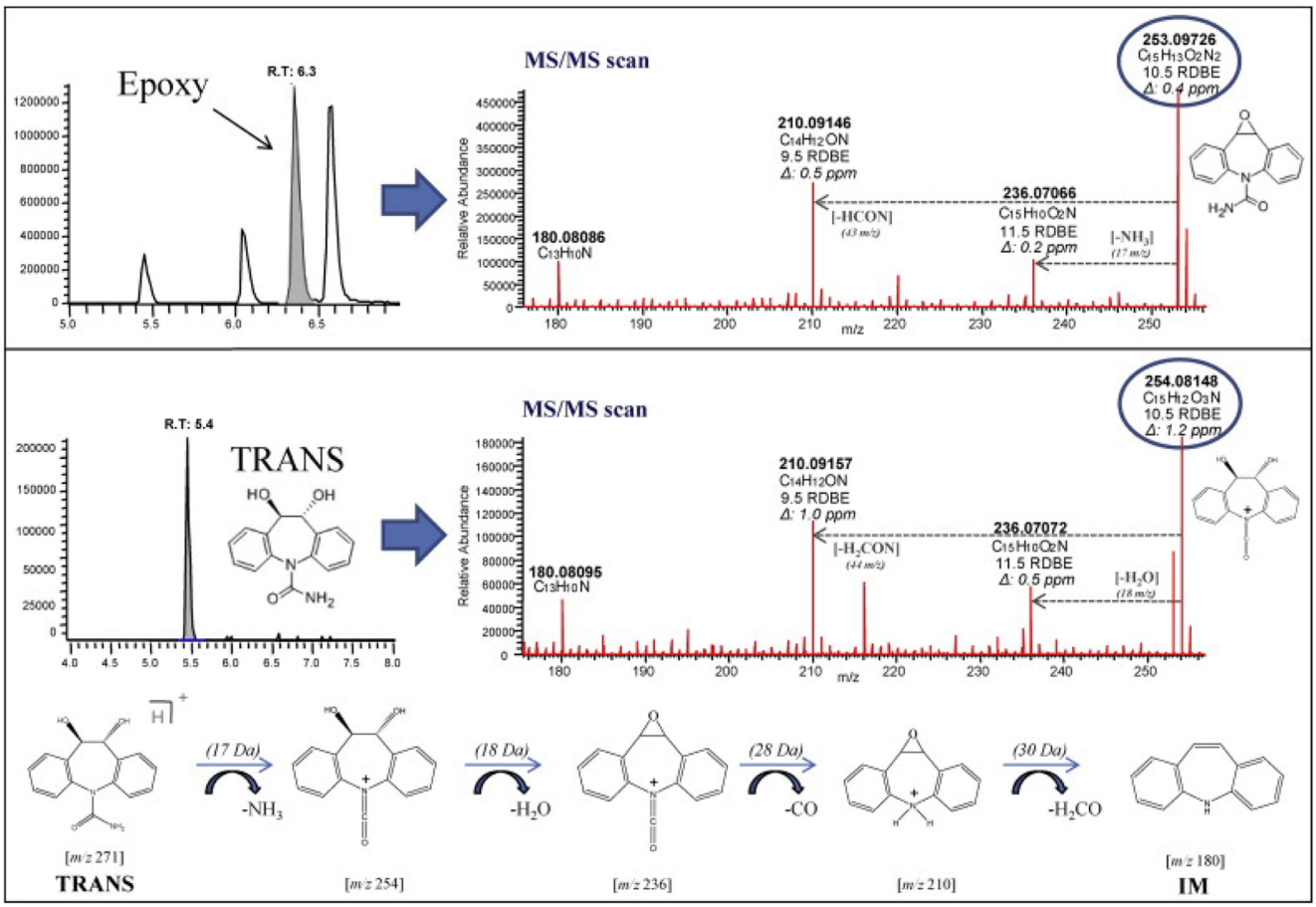

Fig. 6 Product ion mass spectra obtained using a quadrupole Orbitrap instrument by all-ion fragmentation for epoxycarbamazepine (*epoxy*) and *trans*-carbamazepine (*TRANS*) at 10 eV, and proposed fragmentation pathway for *trans*-carbamazepine. *MS/MS* tandem mass spectrometry, *RDBE* ring and double bond equivalents, *R.T.* retention time. (Reproduced from [86] with permission of Elsevier)

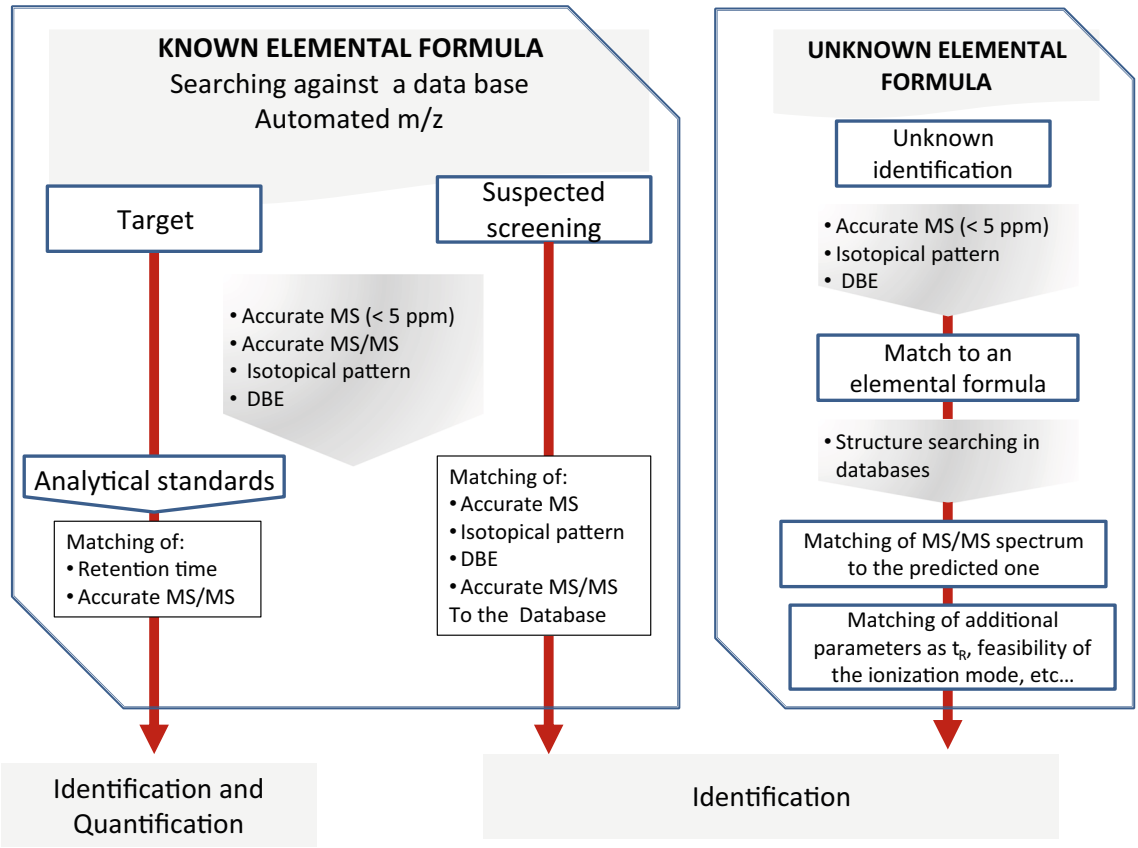

Fig. 7 Different workflows to identify transformation products by high-resolution mass spectrometry (*MS*). *DBE* double bond equivalents

the laboratory by injecting analytical standards, or if there are no analytical standards available, the empirical formula found in the literature can be added [44, 77].

The prediction of possible TPs to add their empirical formula to the library can be done using in silico prediction tools [20]. Commercially available or freely accessible programs have been applied in the prediction step, but more so in biochemical studies than in environmental ones.

The identification of unknown signals is commonly very difficult because there is no information available on the empirical formula corresponding to the analytes. Then, peaks are detected using automated peak detection and spectral deconvolution algorithms able to find thousands of peaks in an individual water sample [11, 15, 77]. Subsequently, the most probable empirical formula can be established according to the accurate mass and isotopic pattern of the mass spectrum corresponding to the peak. However, each molecular formula can be related to a quite large number of candidate structures, which have to be ranked or filtered to obtain a useful list of possible compounds for confirmation against the reference standards. In these cases, high-resolution instruments generate huge quantities of data that are difficult to export to the appropriate software and that are complex to evaluate. There is a range of free or commercial software able to predict MS properties of candidates, such as MS^n fragmentation energies, product ion spectra, retention times, and ion mobility drift times, to facilitate the task by comparing predicted behavior with experimental data [28, 11, 77].

For this reason, postacquisition data-processing tools are necessary; computer-aided techniques provide rapid, accurate, and efficient data mining. There are quite a large number of software options for nontarget screening depending on the instrument vendor. The main problem with such software is the high price and the lack of compatibility between instruments. There are some free options, but they are often not user-friendly, as is required for analysts not specialized in bioinformatics [1, 74].

The use of mass spectral libraries for the confirmation of compounds is still limited for LC–(HR)MS data, as the libraries are small and the comparability of spectra is limited among different instruments. The compounds are identified by comparison with literature data, from retention times and MS and MS/MS analysis. The identity is confirmed on the basis of the mass accuracy of the molecular ion (5 ppm or less), the appropriate number of rings and double bonds, and the isotopic profile confirmed by simulation and the coincidence of the characteristic MS/MS data. The elucidation of the possible structure is done using chemical databases such as ChemSpider, and a search in the scientific literature related to the compound.

The ultimate confirmation of the identity always implies the use of analytical standards and the comparison of the behavior of the suspected substance identified with the behavior of the standards. This is time-consuming and very expensive.

Conclusions and future trends

The ability to perform identification of unknowns on the basis of accurate-mass measurements is one of the most powerful applications of HRMS to identify TPs of emerging contaminants. The adoption of "omics" techniques such as in the environmental sciences has opened up new horizons in the study of transformation processes. Unknown identification is further enhanced with MS/MS or multistage MS (MS^n) since fragmentation of precursor ions reveals additional structural information that helps eliminate potential matches. In HRMS methods, the detection is performed over a wide *m/z* range (e.g., 50–1,000 *m/z*). Therefore, the possibility to reanalyze acquisition files to screen samples for the presence of "new" TPs is an attractive feature that is not possible using target analysis with quadrupole instruments.

It is important to stress that now that technological advancements have put such powerful instruments in the hands of scientists, emphasis should be given to the correct method. Key steps before HRMS analysis such as sample preconcentration and purification, ionization, and separation are thus essential to this technique. Sample preconcentration can be improved by using SPE phases with different chemistries such as reversed-phase columns and ion exchangers in order to maximize analyte retention. Finally, LC remains one of the most important aspects of method development since proper separation facilitates identification and can reduce matrix interferences. Recent techniques such as HILIC offer an interesting alternative to established techniques such as reversed-phase LC for the separation of polar compounds.

The applications outlined in this review for HRMS in environmental analysis of emerging contaminant TPs show its capacity to help solve some of the problems faced by scientists in the field because of the complexity of environmental samples. HRMS, coupled wit the use of proper sample preparation techniques, databases, and data-processing algorithms, has shown potential both for the screening of thousands of compounds simultaneously in a given sample and to identify new substances generated during wastewater treatment or in the environment. HRMS has the potential to become one of the most important analytical techniques for elucidation of TPs.

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