Coupled chromatographic and mass-spectrometric techniques for the analysis of emerging pollutants in the aquatic environment

Klaas Wille, Hubert F. De Brabander, Eric De Wulf, Peter Van Caeter, Colin R. Janssen, Lynn Vanhaecke

We present a comprehensive overview of recent developments in analytical chemistry for the most environmentally important groups of chemicals of emerging concern (CECs), including pharmaceuticals and personal-care products, pesticides, steroid hormones, perfluorinated compounds, alkylphenolethoxylates, bisphenol A and phthalates. Due to both the typically very low concentrations at which CECs occur and environmental samples being complex matrices demanding extensive extraction and clean-up procedures, very specific, sensitive analytical procedures are needed.

In this context, we discuss state-of-the-art instrumentation for sample preconcentration, analyte separation and detection. We could observe several prominent trends: the common use of liquid chromatography (LC) to allow separation of CECs (instead of gas chromatography); the development and the application of multi-class methods; and, the increasing popularity of high-resolution, full-scan analysis, combined with a trend towards the use of sub-2-μm-particle sizes and high flow rates (ultra-high-performance LC).

Overall, due to the recent advances in instrumentation, we could see significant progress in the analytical chemistry of CECs in environmental matrices.

Keywords: Aquatic environment; Chemical of emerging concern (CEC); Chromatography; Coupled technique; Emerging pollutant; Environmental matrix; Extraction procedure; Mass spectrometry; Personal-care product (PCP); Pharmaceutical and personal-care product (PPCP)

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1. Introduction

The introduction of new, more sensitive analytical equipment for the detection of chemicals in complex sample matrices on the one hand and growing knowledge about their ecotoxicological effects on the other hand have drawn the attention to new compounds, which have been largely outside the scope of monitoring and regulation. These so-called "chemicals of emerging concern" (CECs) or "emerging contaminants" were previously undetected or had not been considered as a risk [1]. The term emerging contaminants is somewhat ambiguous, since these contaminants are not necessarily new substances [2]. CECs encompass a diverse group of compounds, including algal and cyanobacterial toxins, brominated and organophosphate flame

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retardants, plasticizers, hormones and other endocrine-disrupting compounds (EDCs), pharmaceuticals and personal-care products (PPCPs), drugs of abuse and their metabolites, disinfection by-products, organo-metallics, nanomaterials, polar pesticides and their degradation/transformation products, perfluorinated compounds (PFCs), and surfactants and their metabolites [3]. Both the extensive environmental distribution of CECs and their potential ecotoxicological effects at very low concentrations have attracted increasing interest among researchers, regulatory authorities and the public [1,2].

In addition, the introduction of the European Union legislation on Registration, Evaluation, Authorization and Restriction of Chemical substances (REACH) has drawn attention to emerging, more polar anthropogenic pollutants. According to Hogenboom et al. [4], REACH will drive producers to develop newly-designed chemicals that will be less persistent, bioaccumulative or toxic. Generally, these newly-designed chemicals could be characterized as hydrophilic compounds, which may result in higher mobilities in the aquatic environment [4]. This shift in focus from persistent organic pollutants (POPs) and heavy metals towards more polar CECs has already become apparent in the scientific literature of the past decade on environmental chemistry [3].

Concentrations of CECs in aquatic systems are very low, typically in the ng/L up to the low µg/L concentration range. Relatively low concentrations of CECs may also be expected to occur in biotic matrices, so very sensitive analytical procedures are needed to obtain sufficiently low limits of detection (LODs) to enable measurement of environmental concentrations. In addition, biotic samples are complex matrices containing high amounts of possible interfering compounds that demand extensive extraction and clean-up procedures to obtain extracts amenable to analysis. As a result, the reliable quantification of CECs in both aqueous and biological samples has appeared as a huge challenge to environmental analytical chemists.

We review the possible solutions to encounter these challenges within this work. In this context, we discuss state-of-the-art instrumentation for sample preconcentration, analyte separation and detection. PPCPs (Fig. 1), pesticides (Fig. 2), estrogenic compounds, alkylphenolethoxylates (APEOs), bisphenol A (BPA) and phthalates (Fig. 3) are contaminants of particular concern, as many of them exhibit endocrine-disrupting properties. We therefore review the analytical chemistry of these groups of CECs. We also consider PFCs (Fig. 4), since these bioaccumulative chemicals are known to be abundant in the aquatic environment, where they could exert possible adverse effects on humans and wildlife.

2. Environmental chemistry of emerging micropollutants

In recent years, advances in instrumentation have resulted in significant progress in the detection of CECs in environmental matrices. Within this review, it is not our objective to give a complete compilation of papers dealing with analysis of CECs. Instead, we aim to discuss the current performance in quantifying CECs in the aquatic environment and to highlight some recent advances.

2.1. Pharmaceuticals

2.1.1. Water and passive-sampler analysis. Overall, hundreds of papers have been published on pharmaceutical analysis of untreated and treated waters [5]. Still, it took until 2007 for the US Environmental Protection Agency (EPA) to publish the EPA Method 1694, a standardized methodology for the analysis of more than 70 pharmaceuticals in environmental matrices. Recently, this standard protocol was improved by Ferrer et al. [5]. Within both methodologies, the target pharmaceuticals are divided into several sub-groups of compounds, each with their specific optimized analytical procedure. This division into smaller subgroups is required because of their typical physicochemical properties and chemical structures. Consequently, the development of multi-class methods, which has been the general trend in recent years, demands a compromise in the selection of experimental conditions (e.g., sample preparation, separation and detection) [3]. Nevertheless, a typical procedure for the analysis of a broad group of pharmaceuticals in aqueous matrices has been reported in the literature (Fig. 5) [6]. This procedure included filtration and acidification for acidic pharmaceuticals, extraction, an additional clean-up step (if necessary), derivatization in the case of detection with gas chromatography (GC), and, finally, detection with GC or liquid chromatography (LC) in combination with mass spectrometry (MS).

All studies reporting on procedures for pharmaceutical analysis up to 2007 were thoroughly reviewed by Fatta et al. [6] and Kot-Wasik et al. [7]. In recent years, there has been a tremendous progress in analytical techniques for trace analysis in environmental samples, so we discuss below the most recent studies on pharmaceutical analysis, from sample preparation to analyte separation and detection.

At first, to remove particulate matter and to avoid clogging of the sorbent used for solid-phase extraction (SPE), filtration of water samples was suggested prior to the concentration procedure [8]. However, together with suspended solids, filtration also removes the fraction of target compounds sorbed to particulates. It was therefore recommended to wash the glass-fiber filters with

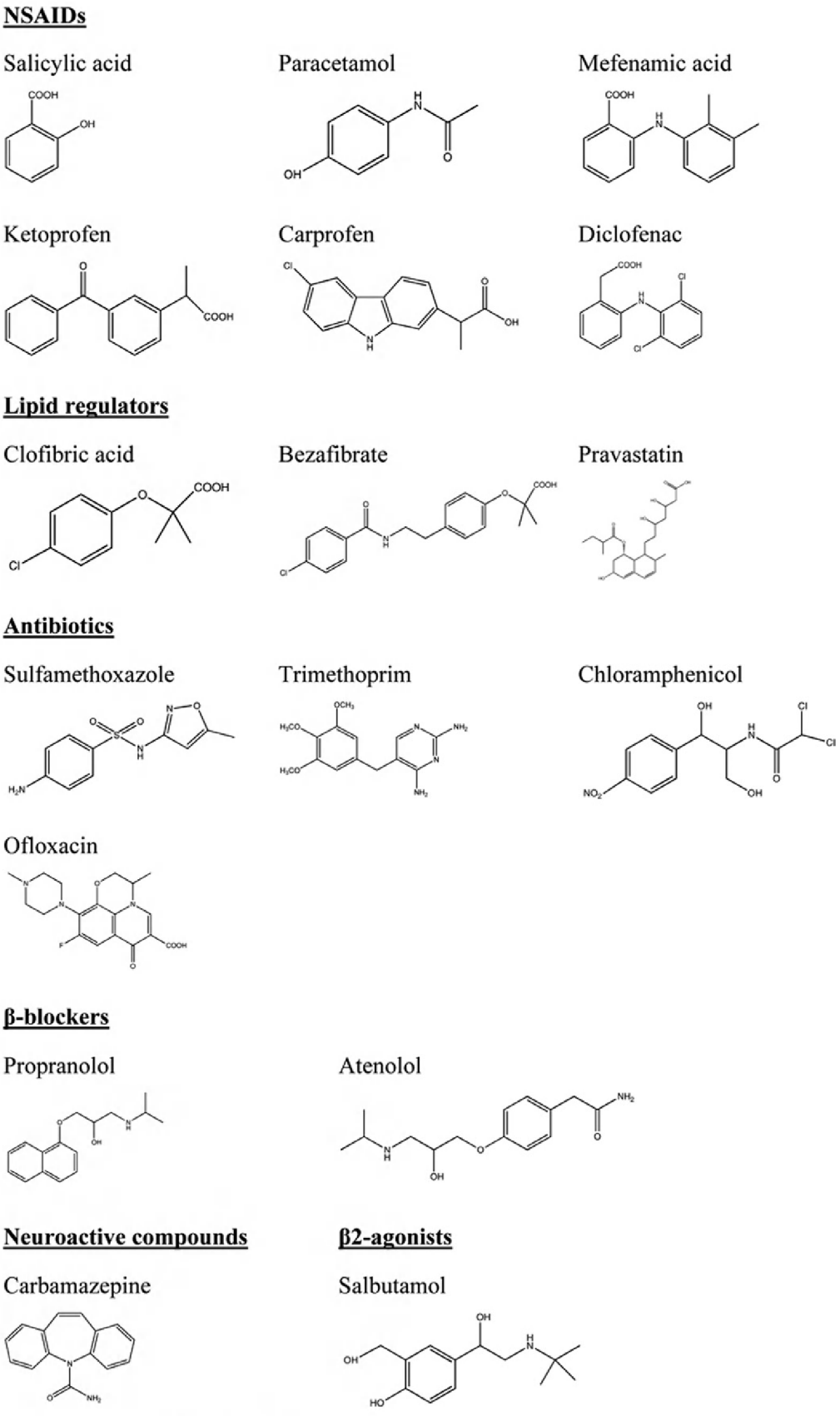


Figure 1. Chemical structures of some environmentally important pharmaceuticals.

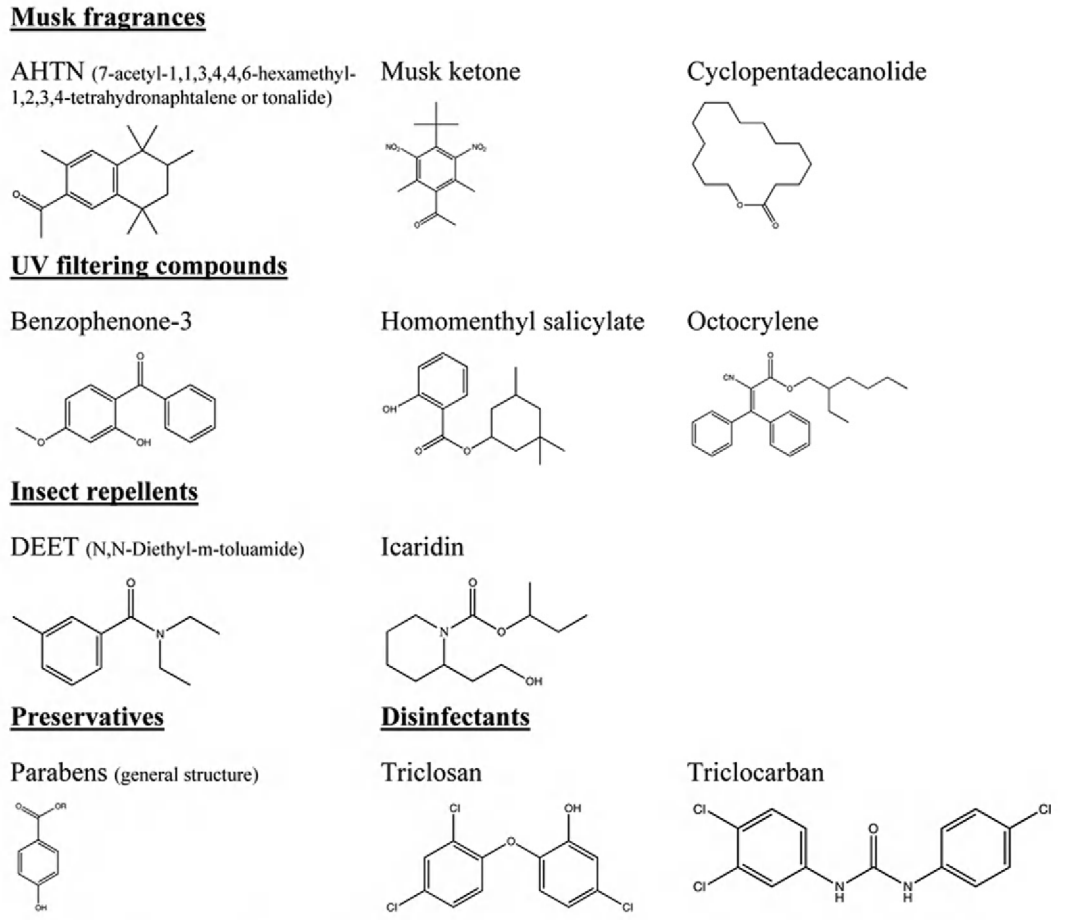


Figure 2. Chemical structures of some environmentally important personal-care products (PCPs).

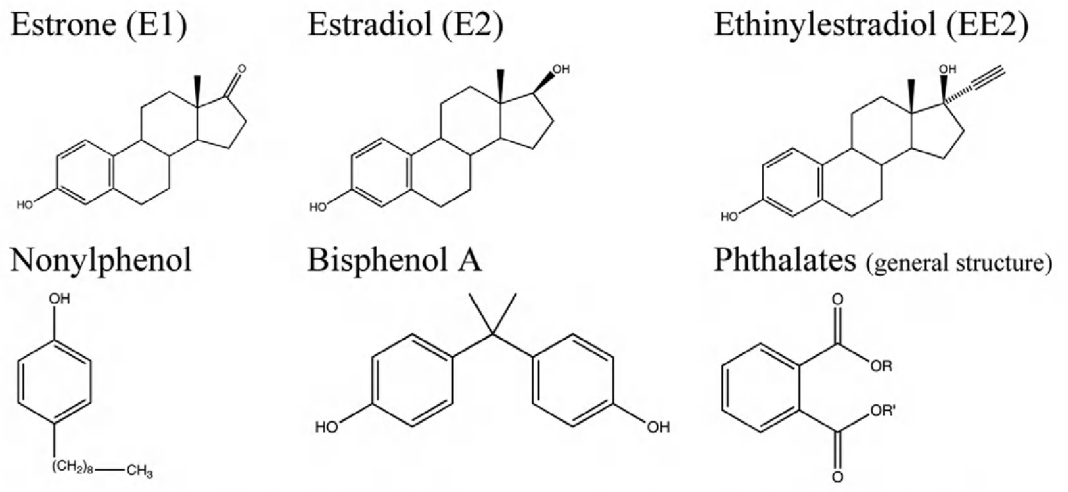


Figure 3. Chemical structures of environmentally important endocrine-disrupting compounds (EDCs).

methanol after filtration [8]. Centrifugation of wastewater samples may also be performed in the case of observable suspended particulate matter.

Before sample extraction, the pH of the samples is adjusted or reagents may be added to optimize the extraction efficiency. Studies suggested adjusting the pH

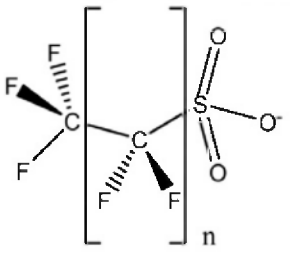
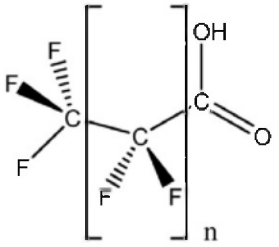
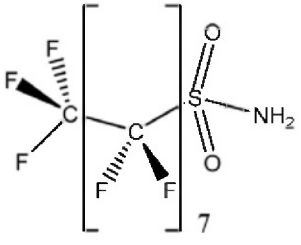
Compound	Abbreviation	Structural formula	Structure
Perfluoroalkyl sulfonates	PFASs		
Perfluorobutane sulfonate	PFBS	$C_4F_9SO^-_3$	n = 3
Perfluorohexane sulfonate	PFHxS	$C_6F_{13}SO^-_3$	n = 5
Perfluorooctane sulfonate	PFOS	$C_8F_{17}SO^-_3$	n = 7
Perfluorodecane sulfonate	PFDS	$C_{10}F_{21}SO^-_3$	n = 9
Perfluoroalkyl carboxylic acids	PFCAs		
Perfluoropentanoic acid	PFPA	$C_5HF_9O_2$	n = 3
Perfluorohexanoic acid	PFHxA	$C_6HF_{11}O_2$	n = 4
Perfluoroheptanoic acid	PFHpA	$C_7HF_{13}O_2$	n = 5
Perfluorooctanoic acid	PFOA	$C_8HF_{15}O_2$	n = 6
Perfluorononanoic acid	PFNA	$C_9HF_{17}O_2$	n = 7
Perfluorodecanoic acid	PFDA	$C_{10}HF_{19}O_2$	n = 8
Perfluoroundecanoic acid	PFUnA	$C_{11}HF_{21}O_2$	n = 9
Perfluorododecanoic acid	PFDoA	$C_{12}HF_{23}O_2$	n = 10
Perfluorotetradecanoic acid	PFTeA	$C_{14}HF_{25}O_2$	n = 12
Perfluorooctane sulfonamide	PFOSA	$C_8H_2F_{17}SO_2N$	

Figure 4. Chemical structures of the most environmentally important PFCAs, PFSAs and PFOSA.

to acidic, basic or neutral conditions, depending on the analyte [8]. In addition, chelating agents (e.g., di- or tetrasodium ethylenediaminetetraacetate: Na_2EDTA or Na_4EDTA), quenching agents (e.g., ascorbic acid) and other preservatives could be added to samples prior to extraction [5].

Further sample preparation and clean-up is necessary for three main reasons:

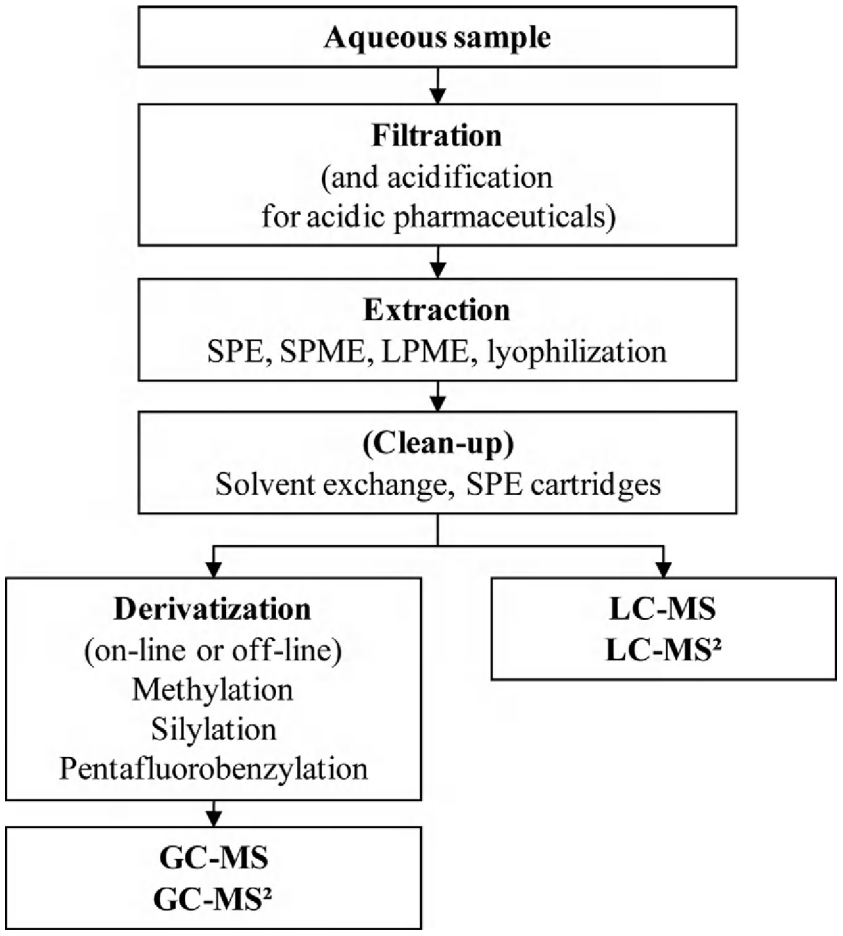


Figure 5. Typical procedure for the analysis of pharmaceuticals in aqueous matrices, as reported by Fatta et al. [28].

(1) to remove interferences that would otherwise affect the determination of analytes;
(2) to concentrate analytes to detectable concentrations; and,
(3) to perform solvent switching to the desired solvent conditions used for detection.

To date, SPE is still the most frequently applied sample-preparation technique. Using SPE, retention of pharmaceuticals was improved by developing new polymeric sorbents, mostly hydrophilic-hydrophobic balanced material. The copolymer of divinylbenzene and vinylpyrrolidone, better known as Oasis HLB, is currently the most commonly used SPE sorbent for extracting multi-class pharmaceuticals, next to the copolymer sorbents of Isolute ENV+, Strata-X and Chromabond HR-X [8,9]. Elution of these cartridges is performed with polar solvents (mixtures of methanol and water), often containing traces of acids [9]. Sorbents of sol-gels and carbon nanotubes are used less frequently [7]. SPE has generally been performed off-line (i.e. prior to separation and detection of pharmaceuticals). Currently, on-line SPE is emerging as an effective technique, coupled online with an LC system or as a fully-automated system [9].

Next to SPE, promising extraction and clean-up techniques for pharmaceuticals in environmental matrices involve the use of molecularly-imprinted polymers (MIPs) [9]. Due to the specific cavities designed for template molecules, MIP sorbents provide increased selectivity and specificity for target analytes. Consequently, the level of co-extracted matrix compounds is reduced, which leads to fewer matrix effects and better sensitivity. The use of MIPs as a selective sorbent in a SPE procedure (MISPE) has been successfully applied for several therapeutic classes (e.g., MIP4SPE β-blockers, SupelMIP non-steroidal anti-inflammatory drugs (NSAIDs) and SupelMIP antidepressant) [9]. However, the sorbent material must be custom-made, and multi-class analysis is not achievable using this technique [10].

Two other approaches, enabling extraction, clean-up and concentration of pharmaceuticals in aqueous samples simultaneously, are solid-phase microextraction (SPME) and liquid-phase microextraction (LPME). A reduction in processing time, labor, costs and matrix effects is achieved using SPME and LPME, but the sensitivity and the precision tend not to be as good as the commonly used SPE techniques [10]. Stir-bar sorptive

extraction (SBSE), a technique related to SPME, which is usually followed by liquid or thermal desorption (LD or TD) in combination with GC, was recently applied to detect ibuprofen, naproxen and ketoprofen [11].

The conventional techniques of sampling, based on the collection of discrete grab or spot samples of water, are used in most aquatic monitoring programs. Although these conventional sampling techniques are very useful, generally, the determination of time-weighted average (TWA) concentrations over extended sampling periods of pollutants in the aquatic environment is impossible. As a result, the use of passive samplers, which are designed to obtain TWA concentrations, has gained in popularity. In addition, these techniques mimic biological uptake in a more straightforward manner by determining the pollution level of contaminants in relation to their freely dissolved concentration. With respect to pharmaceuticals, the use of passive sampling devices [e.g., polar organic chemical integrative samplers (POCISs) and Chemcatcher passive samplers] was recently reported [20]. Also, poly(ethylene-co-vinyl acetate-co-carbon monoxide) (PEVAC) and polydimethylsiloxane (PDMS) have been used as passive sampling materials for pharmaceuticals in the aquatic environment [12]. However, an important negative aspect of the passive-sampler approach involves the need for laboratory-calibration studies to enable correct calculations of concentration. After exposure, the passive sampler devices are usually extracted using polar organic solvents (e.g., methanol, acetone, acetonitrile, ethyl acetate and mixtures) [20].

Even with advanced detectors [e.g., high-resolution (HR) or triple-quadrupole mass spectrometers (QqQ-MS)], good chromatographic separation is needed for the quantification of pharmaceuticals in environmental matrices down to ng/L-concentration levels [10]. GC is the preferred technique for separation of non-polar and volatile pharmaceuticals, while, for GC analysis of the more polar pharmaceuticals, a derivatization step is required using typical derivatizing agents [e.g., acid anhydrides, benzyl halides, alkylchloroformates, diazomethane and silylating reagents including N-methyl-N-(trimethylsilyl) trifloroacetamide (MSTFA) or N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (MTBST FA)] [9].

Despite the benefits of GC with respect to selectivity and sensitivity, the loss of analytes during the time-consuming derivatization process and the background noise are matters of concern [10]. In recent years, LC has therefore become the preferred technique for multi-class pharmaceutical analysis [9,10]. Current LC analyses for pharmaceuticals use reversed-phase columns, often end-capped with polar groups and mobile phases comprising acetonitrile, methanol and water. Besides, special columns allowing enantioselective separation of chiral pharmaceuticals have also been developed [9]. Generally, solvent modifiers in the form of proton acceptors (e.g., ammonium acetate) and/or proton donors (e.g., formic acid) are added to the mobile phase to enhance ionization efficiencies of basic and acidic pharmaceuticals, respectively [3]. The main negative aspect for LC analysis of pharmaceuticals in environmental matrices, is the occurrence of matrix effects. Due to co-extracted matrix constituents, the MS analysis may suffer from signal suppression or enhancement, thereby disturbing adequate quantification. Nowadays, the development of ultra-high-performance LC (U-HPLC) enables faster separation of compounds than conventional LC, due to the use of columns packed with sub-2-μm particles. U-HPLC provides improved speed of analysis, better resolution, increased sensitivity and a reduction of matrix effects [10]. In general, considerably improved separation of pharmaceuticals in complex matrices could be obtained using U-HPLC [12].

Identification and quantification of pharmaceuticals in environmental matrices is usually performed by MS techniques. The most common interface for pharmaceutical analysis in environmental matrices is the electrospray ionization (ESI) source [9,10]. In particular, in complex environmental samples, ESI efficiency can be affected by co-extracted sample components, resulting in ionization-suppression or enhancement effects and subsequent poor analytical accuracy and reproducibility. These effects can be reduced by extensive clean-up procedures prior to LC-MS analysis, improved chromatographic separation and dilution of the final extract. However, the most common technique is compensation for matrix effects by using isotope-labeled internal standards, to enable reliable quantification. Nevertheless, evaluation of matrix effects is usually included in the validation study of new analytical approaches for pharmaceutical analysis using ESI [5].

A second "soft" ionization technique, atmospheric pressure chemical ionization (APCI), has been used less often for pharmaceutical analysis. APCI has been reported to be less susceptible to matrix effects, but, generally, sensitivity is less than that obtained with ESI [10].

With respect to MS, the most commonly applied mass analyzers for pharmaceutical analysis are the QqQ mass detectors, which provide precise quantification, and high selectivity and sensitivity [10]. Using QqQ technology, typical LODs are in the low-ng/L range of concentration [10]. Concerning the performance and the confirmation criteria for residues in complex matrices, European Union Commission Decision 2002/657/EC includes a system of identification points (IPs) [13]. An ion (or precursor ion) contributes one IP, and each multiple-reaction monitoring (MRM) product ion yields 1.5 IPs [13]. Using QqQ, the minimum of four IPs is accomplished easily, by comparing two MRM precursor-to-product-ion transitions [10]. Also, ion-trap mass analyzers have been reported in the literature as excellent detection apparatus for pharmaceutical analysis [8].

Using ion-trap instruments, a minimum of four IPs is obtained by detection of the precursor ion, as well as at least two product ions.

Recent advances in instrumentation resulted in a gain in popularity of HR, full-scan analysis. Time-of-flight (ToF) and Orbitrap-based MS have proved to be very suitable alternatives to QqQ instruments, enabling accurate-mass screening of a virtually unlimited number of analytes, targeted and untargeted compounds [4]. Thanks to the high mass resolution, the advantages of these instruments include increased selectivity and reduction in false positives. Besides, the high scan rates of these instruments, compared to the older QqQ systems, are suitable for the narrow peak widths that are encountered with U-HPLC analysis. In addition, the full-scan data permit retrospective analysis of an unlimited number of pharmaceuticals [10].

So far, the use of single ToF and Orbitrap instruments for analysis of pharmaceuticals in environmental matrices is rather limited [4,12]. For many applications with respect to pharmaceuticals, providing specific fragments upon QqQ or ion-trap analysis, these HR, full-scan analyses are less sensitive than the classical QqQ analyzers. Therefore, more commonly, the combination of two MS techniques with complementary features has been used. These highly-sophisticated MS analyzers, or so-called hybrid tandem mass spectrometers, constitute the latest trend in environmental chemistry, increasing instrument versatility and the scope of the method. In addition, their sensitivities approach that of QqQ systems [10]. Moreover, they allow identification of unknowns, since HR measurements of fragmentation products of analytes are also possible. The hybrid systems that have been used for pharmaceutical analysis include triple quadrupole/linear ion-trap MS (QTRAP or QLIT), quadrupole/time-of-flight MS (QToF), and ion-trap/Orbitrap MS [4,10,14]. However, criteria within CD 2002/657/EC [13] related to the reliable identification and confirmation of pharmaceuticals using HRMS systems are incomplete, so additional criteria to be implemented have been suggested by Nielen et al. [15]. In general, very high mass accuracy (<5 ppm) is offered by modern ToF and Orbitrap systems, providing reliable identification and quantification of pharmaceuticals in environmental matrices [12,14].

MS techniques are also applied upon GC separation of pharmaceuticals. GC-MS is typically performed using electron-impact (EI) ionization, in full-scan mode for identification and selected-ion-monitoring (SIM) mode for quantification. Limits of quantification (LOQs) in the low-ng/L range of concentration have been obtained, and no obvious matrix effects have been observed using GC-MS.

Less expensive detection techniques than MS include fluorescence detection (FLD), ultraviolet (UV) detection, diode-array detection (DAD) and immunoanalytical techniques. Generally, rather low sensitivities are obtained using these techniques, limiting their use to aqueous matrices containing large amounts of pharmaceuticals (e.g., wastewaters).

2.1.2. Sediment analysis. Despite the rather low lipophilicity of pharmaceuticals, interaction of the polar functional groups of pharmaceuticals with organic matter and/or minerals may result in adsorption to solids. Furthermore, the application of sewage sludge as a fertilizer to agricultural land and the reuse of manure containing veterinary medicines may also introduce pharmaceuticals into the soil. In accordance with aqueous samples, the presence of pharmaceuticals in sediment, soil and sewage sludge has been studied extensively. Analytical methods for the determination of specific groups of pharmaceuticals, including NSAIDs, anti-depressants, antibiotics and β-blockers [16,17], and multi-class methods [16] were reported in recent years.

The main difference with water analysis relates to sample preparation and extraction. Generally, pharmaceuticals are extracted from dried solid samples by conventional Soxhlet extraction, microwave-assisted (micellar) extraction [MA(M)E], ultrasound-assisted extraction (UAE), supercritical-fluid extraction (SFE), or pressurized liquid extraction (PLE) [16,17]. Nowadays, Soxhlet extraction has become less attractive due to the time required and its solvent consumption [9]. All five techniques were compared for the determination of four NSAIDs in river sediment [17]. Based on extraction efficiencies, average solvent consumption and extraction time, MAE was found to be the most effective extraction method. However, the most commonly used approaches seem to be UAE and PLE, which provide good extraction efficiencies and demand less extraction time and solvents [9]. Typically, mixtures of water and rather polar solvents (e.g., methanol and acetonitrile) are used for achieving good recoveries during extraction. An additional clean-up step using SPE is usually required. Finally, analysis of extracts is performed using the techniques described in the previous section.

2.1.3. Biota analysis. The presence of pharmaceuticals in aquatic organisms has rarely been studied, due to both the complexity of the matrix and the levels at which pharmaceuticals occur. Ramirez et al. [18] reported a screening method for the detection of 23 pharmaceuticals in fish tissue, based on extraction using a 1:1 mixture of 0.1 M aqueous acetic acid and methanol before analysis using LC-MS/MS. More recently, PLE and SPE followed by U-HPLC coupled to QqQ-MS enabled the quantification of 11 pharmaceuticals in tissue of marine organisms [19]. To the best of our knowledge, no more attention has been paid to the analysis of pharmaceuticals in aquatic organisms. To obtain more information regarding their presence in marine organisms, an increasing demand exists for reliable analytical methods

allowing the quantification of these micropollutants in biotic matrices.

2.2. Personal-care products

In general, the methodologies for PCP analysis in environmental samples are quite similar to those described for pharmaceuticals [9], so we provide only a shortened discussion here of the typical applications for each group of PCPs.

2.2.1. Water analysis

2.2.1.1. UV-filtering compounds. UV-absorbing compounds are increasingly applied as a result of a growing concern about UV radiation and skin cancer. This group includes benzophenones, salicylates, cinnamates, camphor derivates, triazines, benzotriazoles, benzimidazole derivates, and dybenzoyl methane derivates. Sorptive extraction in combination with GC analysis seems to be the proper analytical technology for determination of hydrophobic and volatile UV-filtering compounds in water samples [9].

Recent studies have reported the use of SBSE for the extraction of PCPs from aqueous samples followed by TD-GC-MS or LD-(U-HPLC)-MS [21]. SBSE followed by direct analysis in real-time MS (DART-MS) provided a shorter analysis time, but LODs are poorer and only semi-quantitative results can be obtained.

Other analytical approaches for PCP determination include microextraction by packed sorbent (MEPS) coupled directly to large-volume injection-GC-MS, SPME with on-fiber derivatization by silylation followed by GC–MS/MS analysis, and SPE with GC-MS. With respect to the benzophenones, derivatization of phenolic groups was needed to enable their detection in the low-ng/L range. All these GC methods are limited to those compounds that are volatile or can be derivatized for GC determination.

If multi-class determination of UV-filtering compounds in combination with other emerging pollutants, including pharmaceuticals and pesticides, is intended, (U-)HPLC coupled to QqQ-MS after SPE extraction with Oasis HLB cartridges [22], is generally the approach applied more for pharmaceutical residues.

2.2.1.2. Insect repellents. The group of insect repellents comprises chemically diverse substances. N,N-diethyl-m-toluamide (DEET) and 1-piperidinecarboxylic acid 2-(2-hydroxyethyl) 1-methylpropyl ester (called Icaridin) have been reported as the most important analytes within this group [9]. These compounds have generally been analyzed with GC-MS. Rodil et al. [22] enabled the detection of eight insect repellents by SBSE followed by TD-GC-MS. Recently, a multi-class method using Oasis HLB cartridges followed by LC–MS/MS allowed the determination of four insect repellents, obtaining LODs in the low-ng/L range of concentration [22].

2.2.1.3. Fragrances. The group of fragrances can be divided into nitro-aromatic musks, polycyclic musks and macrocyclic musks. Compounds of the nitro-aromatic and polycyclic musk classes are considered ubiquitous, persistent and bioaccumulative pollutants, with the potential to generate toxicologically active compounds.

Recently, Bester [23] reviewed the analytical approaches that have been used for the determination of fragrances. The use of SPE as a sample-preparation technique has been reported only occasionally. Due to the hydrophobic, volatile and lipophilic properties of fragrances, other extraction techniques clearly are more useful than SPE [23]. Liquid-liquid extraction (LLE) has been applied for extraction of fragrances as well as SBSE with LD and microwave-assisted headspace SPME. Typically, good chromatographic separation is obtained by GC, commonly followed by MS detection [23].

2.2.1.4. Preservatives. The most common preservatives are the P-hydroxybenzoic esters or parabens, which are used in PCPs but are also preservatives in pharmaceuticals and food products. Typically, the high usage and low degradability of these compounds may lead to their prevalence in the environment, there exhibiting estrogenic activities. Parabens have therefore typically been analyzed together with other EDCs using SPE followed by LC-MS or GC-MS (see below). So far, little effort has been made to determine this group of compounds separately in environmental matrices.

2.2.1.5. Disinfectants. The main compounds within the group of disinfectants are triclosan and triclocarban, which are often used in soap, toothpaste, and other consumer products. Triclosan especially has been frequently analyzed as a marker compound in many studies. Obviously, the combination of LLE or SPE with GC-MS has been routinely applied for detection of triclosan in aqueous matrices, while some studies also report the use of SPE followed by LC-MS. The use of various derivatization procedures and LC methods has been suggested as a way to improve the analytical performance in analyzing triclosan [9].

2.2.1.6. Multi-class methods. As stated above, there was a trend towards multi-residue methods [3]. Modern analytical equipment enables the determination of large numbers of analytes within one analytical run. With respect to PCPs, several analytical methods have been reported on the simultaneous determination of several groups of PCPs. Recently, Gonzalez-Marino [24] reported the simultaneous determination of preservatives and disinfectants in water Using Oasis HLB SPE and elution with methanol at neutral pH followed by LC-MS/MS in negative ion mode, while Cuderman and Heath [25] described the determination of UV filters and

antimicrobial agents or disinfectants in environmental water samples using SPE with GC-MS. With SPE using two polymeric cartridges (Oasis HLB and Bond Elut Plexa), a U-HPLC–(ESI)-MS/MS run of 9 min was developed for the simultaneous determination of four preservatives, two antimicrobial agents and five UV filters by Predrouzo et al. [21], combining positive-ion and negative-ion modes and using methanol as organic solvent. Furthermore, analyses of PCPs have been regularly performed in combination with pharmaceuticals and EDCs. Nine important pharmaceuticals and PCPs were simultaneously extracted by SPE using Oasis HLB cartridges with subsequent GC-MS detection [26]. Another comprehensive approach was reported by Guitart and Readman [27], who described a method for the determination of some PPCPs, phenolic EDCs and steroids by SPE using Oasis HLB cartridges followed by GC-MS. They obtained LODs in the low-ng/L range of concentration for all compounds.

2.2.2. Sediment analysis. Several PCPs (e.g., triclosan, triclocarban, and most UV-filtering compounds) show affinity to solid matrices [e.g., sediment, suspended particulate matter (SPM) and sewage sludge]. As a consequence, to allow correct evaluation of the ecological impact of these substances, evaluation of their prevalence in solid matrices is important. Several analytical approaches were therefore reported recently.

Rodil et al. [28] developed a methodology for the determination of UV-filtering compounds based on the use of non-porous polymeric membranes in combination with PLE, followed by detection with LC–atmospheric pressure photoionization (APPI)–MS/MS. Fast determination of synthetic polycyclic musks in sewage sludge and sediment was enabled by microwave-assisted headspace SPME followed by GC-MS [29].

As for the analysis of aqueous samples, multi-residue methods are also gaining in popularity for sediment analysis. A new analytical strategy for the determination of UV-filtering compounds, four preservatives and two antimicrobials in sewage sludge was reported by Nieto et al. [30]. The combination of PLE and U-HPLC-MS/MS resulted in LODs <10 ng/g. More recently, a sensitive method was also developed and validated for the determination of diverse groups of hormone-like PPCPs and steroid hormones in sewage sludge [31]. Sample extraction was performed by UAE with a mixture of acetonitrile and water, followed by Oasis HLB SPE and analysis with U-HPLC-MS/MS in ESI positive-ion mode.

2.2.3. Biota analysis. It has been demonstrated that environmental exposure to pseudopersistent PCPs resulted in accumulation of the parent compounds, their metabolites, or both in tissues of aquatic organisms.

Balmer et al. [32] demonstrated low, but detectable, concentrations of UV-filtering compounds in fish, obtained upon extraction of 20-g samples with dichloromethane/cyclohexane (1:1) followed by gel-permeation chromatography (GPC) and GC-MS analysis.

More recently, Zenker et al. [33] reported an analytical strategy for the determination of UV-filtering compounds in fish. One gram of fish tissue was suspended in 5 mL of methanol and 5 mL of acetonitrile. Next, the mixture was homogenized, centrifuged, sonicated and filtrated before LC–ESI-MS/MS analysis.

Nakata et al. [34] reported an analytical method for the analysis of synthetic musks in fish. Briefly, sample tissues were extracted with dichloromethane/hexane (8:1) using a Soxhlet apparatus, before GPC and GC-MS analysis. In general, analytical methods for the determination of PCPs in biotic tissue are limited in scope. However, two analytical methods have been developed for the determination of a group of 10 extensively used PCPs in fish [29]. The methods comprised extraction with acetone, clean up with silica gel, GPC and derivatization before analysis by GC–SIM–MS or GC–MS/MS techniques.

2.3. Pesticides

Within this study, we consider only those analytical techniques developed for the environmentally prevalent, more polar pesticides. The chemical classification of these modern pesticides is quite complex, since they are characterized by a variety of chemical structures and functional groups. The most relevant groups of polar pesticides include the organonitrogen pesticides (ONPs) and the organophosphorus pesticides (OPPs), both covering a wide group of compounds. These pesticides are universally applied and have replaced the withdrawn group of the organochlorine pesticides (e.g., chlordane, dieldrin, and DDT) [3].

In literature, there are numerous studies on the analysis of pesticide residues in environmental matrices. Still, the determination of modern pesticides and their degradation products at very low concentration levels in real samples is complicated because of their polarity and thermolability. In addition, there is a need to improve significantly the sensitivity and the selectivity of the analytical methodologies to meet the requirements established in Directive 98/83/EC regarding water for human consumption, which has to comply with the 100-ng/L level of concentration in legislation for individual pesticides. We therefore give below an overview of the various approaches and recent trends used for determination of the more polar pesticides in environmental samples. Some environmentally important pesticides are shown in Fig. 6.

2.3.1. Water and passive-sampler analysis. To remove suspended particulate matter and sediment, it was suggested first to filtrate or to centrifuge water samples. Jansson and Kreuger [35] evaluated nine different filter

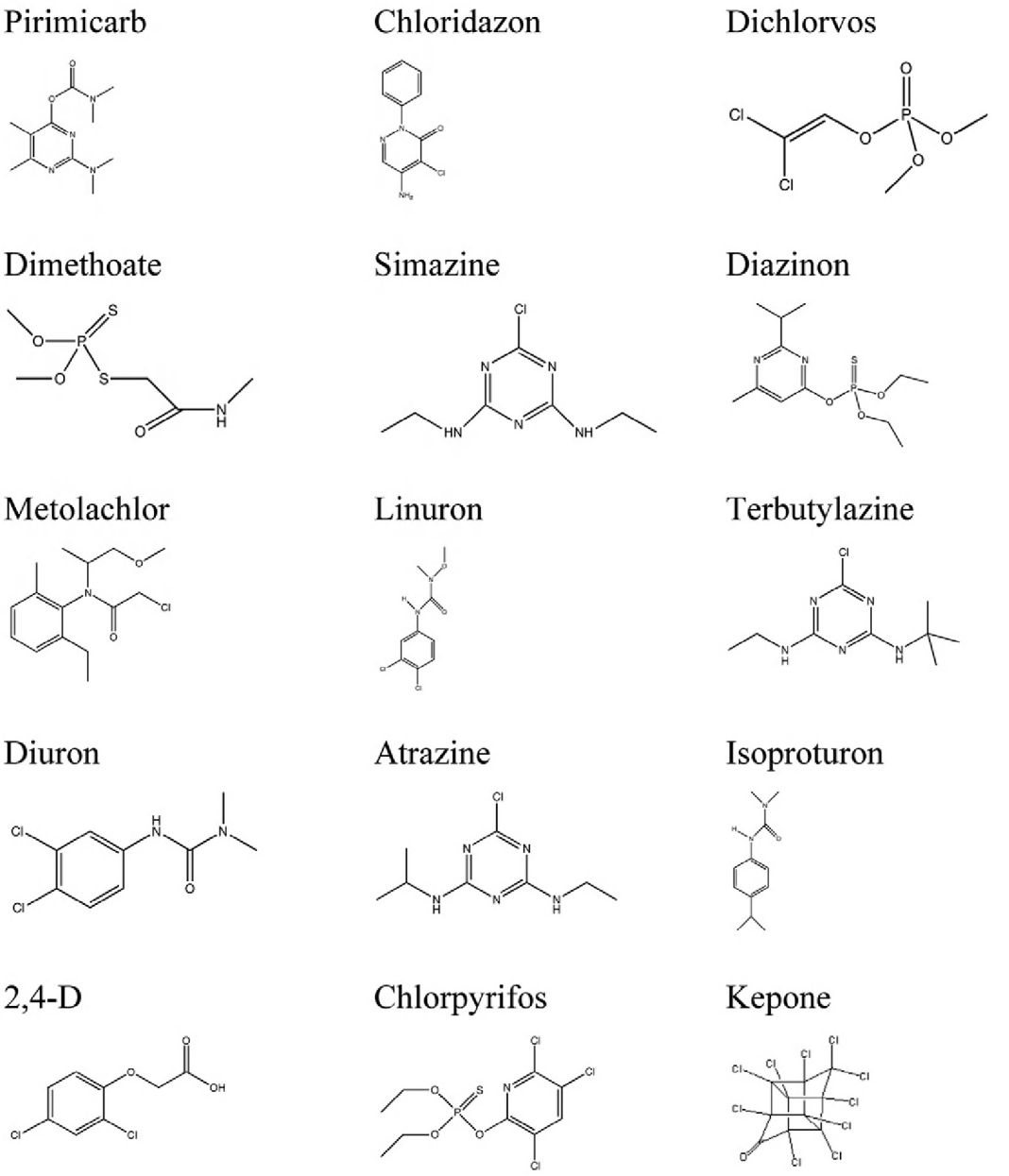


Figure 6. Chemical structures of some environmentally important pesticides.

materials prior to analysis of 95 pesticides in surface water. Some pesticides were completely adsorbed upon filtration, while the highest recoveries were obtained using PTFE and regenerated cellulose (RC) filters. However, as many studies reported analytical approaches without this previous pretreatment step.

In contrast to the practice in PPCP analyses, the pH of the water samples is only rarely adjusted for pesticide analysis. Regarding the extraction and the enrichment of pesticides, several strategies have been reported. The use of conventional LLE has been recommended by the US EPA for pesticide analysis because of the high degree of enrichment, as opposed to the general use of SPE in pharmaceutical and PCP analyses. The main drawbacks of LLE include low extraction efficiencies for polar compounds, the time taken, the needs for large quantities of solvents and further clean up, and the risk emulsion forming during agitation. To eliminate some of these inconveniences, LLE has been replaced by miniaturized liquid extraction procedures [e.g., LPME, single-drop microextraction (SDME) and membrane-assisted solvent extraction (MASE)]. However, the most popular extraction technique is, as with other polar micropollutants, SPE, which combines both isolation and enrichment of the target compounds. A wide range of commercially-available SPE sorbents has been applied, with the polymer-based OASIS HLB as the most distributed adsorbent [14].

However, optimal elution conditions for pesticides slightly differ from those for pharmaceuticals, with a lower percentage of water and a higher percentage of organic solvents. The automated approach, namely on-line SPE, was recently also applied to extraction of pesticides [35]. Typical on-line enrichment cartridges are PLRP-S columns, comprising stable polymeric reversed-phase material. SBSE, SPME and MIPs have been employed only sporadically for pesticide extraction. Finally, the use of multi-walled carbon nanotubes as solid-phase extractant has been reported once, obtaining LODs in the μg/L range [36].

As in pharmaceutical monitoring, the introduction of passive samplers has enabled the determination of the TWA pesticide concentration in aqueous matrices. Still, there are only few studies available on the use of passive sampling devices for the more polar pesticides. POCISs and Chemcatcher passive sampling devices have been designed for the sampling of polar contaminants. Their use for polar pesticide sampling was illustrated recently [40]. In addition, PEVAC, PDMS and membrane-assisted passive samplers (MAPSs) have also been used for pesticide sampling in the aquatic environment [12].

Also, as in pharmaceutical analysis, both LC and GC have been applied for the separation of pesticides. Nowadays, the tendency towards the use of more polar, less volatile and thermostable pesticides has stimulated the application of LC in pesticide-residue analysis [14]. However, high-usage pesticides remain volatile and thermostable, so GC is suitable for them and is still reported in literature.

The most recently published analytical approaches for the determination of pesticides in environmental matrices rely on detection with MS. Both APCI and ESI have been applied as ionization sources, with generally much wider applications using ESI. Upon ionization, QqQ technology is the most frequently applied MS tool for quantitative pesticide analysis in aqueous matrices. In general, the lowest LODs, ranging from pg/L to ng/L concentration levels, were achieved by on-line SPE coupled to LC–MS/MS using a QqQ instrument [35]. The combination of quadrupole with linear ion-trap technology (QTRAP) has also been used, providing excellent sensitivity and selectivity for pesticide analysis.

Since QqQ and ion-trap systems operate at unit resolution, their capability for analysis of non-target screening of pesticides and retrospective analysis is limited. To obtain more information on the pesticide composition in water samples, including targeted as well as untargeted substances, use of full-scan HRMS instruments for screening purposes has gained widespread acceptance.

The use of ToF and QToF instruments for both pesticide screening and quantification was frequently reported in recent literature, providing reliable accurate mass measurements and high sensitivity in full-scan mode [14].

Accurate mass measurements using Orbitrap MS, whether or not in combination with linear ion-trap MS (LIT/Orbitrap), have hardly been used for pesticide analysis so far [12]. In addition, some less important detection techniques have been reported in the literature (i.e. UV, DAD and FLD).

The use of GC analysis with flame-ionization detection (GC-FID), surface-assisted laser desorption/ionization MS (SALDI-MS) and enzyme-linked immunosorbent assay (ELISA) have also been reported.

2.3.2. Sediment analysis. Much less effort has been addressed to the detection of polar pesticides in sediment, soil and sewage-sludge samples. In recent years, the conventional methods for pesticide extraction from solid matrices, including Soxhlet or mechanical shaking, were replaced by advanced extraction techniques (e.g., PLE and sonication). Typically, rather polar extraction solvents are used (e.g., acetonitrile, acetone and methanol and mixtures). Additional clean-up steps include the use of (dispersive) SPE or HPLC fractionation, thereby obtaining extracts ready for analysis. Finally, analysis of the extracts is performed using the techniques described in the previous section.

2.3.3. Biota analysis. There are also very few reports on the detection of polar pesticides in aquatic organisms.

Lehotay et al. [37] monitored more than 60 pesticides in oysters originating from Chesapeake Bay (Maryland, USA). Oyster tissue was blended with acetonitrile and a series of SPE cartridges were used for clean-up. Analysis was performed using GC-MS on a quadrupole instrument in SIM mode. The LODs obtained for oyster samples were <5 ng/g wet weight.

More recently, Buisson et al. [38] also described an analytical procedure for detection of pesticides in oysters. Oyster samples were first homogenized with an Ultra Thurax unit, then extracted by accelerated solvent extraction using acetonitrile, and finally purified on a Florisil column. Analysis was performed using LC-ESI (+)-MS.

Another study reported an analytical procedure for the determination of 29 herbicides and related metabolites in freeze-dried clams [39]. Using a mixture of 4:1 methanol/water, the analytes were extracted with UAE and manual agitation. After centrifugation, removal of methanol and reconstitution in water, the extracts were cleaned and concentrated using Oasis HLB cartridges. Analysis was performed using LC-QqQ-MS, obtaining LODs for simazine, atrazine and terbuthylazine of 0.21 ng/g, 0.042 ng/g and 0.012 ng/g, respectively. Wille et al. [19] recently reported an analytical approach based on PLE and SPE followed by U-HPLC coupled to

Table 1. Recent analytical approaches to quantify estrogens in aqueous matrices

Matrix	EDCs studied	Sample pre-treatment	Extraction method	Derivatization	Analytical method	Ref.
Seawater	E1, E2, E3, EE2, daidzein, genistein, BPA, NP, OP	Filtration	SPE HLB	–	LC-QqQ-MS	[41]
Wastewater	E1, E2, EE2	Filtration	Online SPE HLB	Dansyl chloride	LC-QqQ-MS	[49]
Wastewater	E1, E2, E3, EE2, PPCPs, other steroids	pH adjustment (pH 2) and filtration	SPE HLB	–	LC-QqQ-MS	[52]
Wastewater	E1, αE2, βE2, E3, EE3, other steroids	Filtration and pH adjustment (pH 3.4)	SPE Strata-X and silica clean-up	–	LC-MS	[50]
Surface waters and groundwaters	E1, αE2, βE2, E3, EE3, other steroids	Filtration and pH adjustment (pH 3)	SPE Strata C18-E	–	LC-QqQ-MS	[41]
Surface water and wastewater	E1, βE2, EE2	Filtration (influent)	SPE C18 and silica gel (influent)	–	LC-MS/MS	[53]
Wastewater, and surface and drinking waters	E1, E2, E3, EE2, DES, other steroids, PPCPs, pesticides	Filtration	Online SPE PLRP-s (cross linked styrenedivinylbenzene)	–	LC-QqQ-MS	[41]
Surface water and wastewater	E1, βE2, EE2, other steroids, BPA, OP, NP	Filtration	SPE C18 + silica gel (steroids) and HLB (phenols)	–	LC-MS/MS	[47]
Surface water	βE2	–	MIPs	–	LC-MS	[46]
Surface water	E1, βE2, E3, EE2, DES	–	In-tube SPME	–	LC-QqQ-MS	[45]
Surface water and wastewater	E1, αE2, βE2, E3, EE2, conjugated forms	Filtration	SPE HLB and Florisil clean-up	–	LC-QqQ-MS	[51]
Surface water	E1, αE2, βE2, E3, EE2	Filtration	Discovery DSC-18Lt column	–	cLC-QqQ-MS	[48]
Wastewater	E1, βE2, EE2, progesterone	Filtration	SPE HLB/HPLC fractionation	–	UPLC-QqQ-MS	[41]
Wastewater, and surface and well waters	E1,βE2, E3, EE2	Filtration	SPE C18 and NH_2	–	LC-QqQ-MS/ELISA	[41]
Wastewater, and surface and well waters	E1, E2, E3, EE2, daidzein, genistein	Filtration	SPE C18 and NH_2	–	ELISA/LC-QqQ/UPLC–QTOF	[41]
Well water	E1, αE2, βE2, EE2, DES, MeEE2, other steroids	–	SBSE-LD	–	LC-DAD	[43]
Wastewater	E1, βE2, E3, EE2	Addition of formaldehyde, filtration and pH adjustment (pH 3–5)	SPE HLB + LC-NH_2	MSTFA + mercaptoeth. + NH_4I (for GC)	GC-MS/LC-QqQ-MS	[41]
Effluent and surface water	E1, βE2, E3, EE2, MeEE2, other steroids	Addition of formaldehyde, filtration and pH adjustment (pH 3–5)	SPE HLB	MSTFA + mercaptoeth. + NH_4I	GC-EI-MS	[41]
Surface water	E1, αE2, βE2, EE2	pH adjustment (pH 7)	SPE C18XF Speedisk + Si and NH_2	MSTFA	GC – ion trap MS	[41]
Water	E1, βE2, other steroids, OP, NP, BPA, phthalates	Addition of sodium carbonate and acetic anhydride	SBSE-TD	–	GC-MS	[44]

Table 1. continued

Matrix	EDCs studied	Sample pre-treatment	Extraction method	Derivatization	Analytical method	Ref.
Surface water and effluent	E1, E2, EE2, BPA, OP, NP	Filtration	SPE HLB	BSTFA + 1% TMCS + pyridine	GC-MS	[41]
Surface, wastewater and seawater	E1, αE2, βE2, E3, EE2, MeEE2, NP, OP, BPA	Filtration	SPE HLB	BSTFA	GC-ion trap-MS	[54]
Wastewater	E1, αE2, βE2, EE2, MeEE2, other steroids, NP, BPA, phthalates, phytoestrogens	–	LLE with dichloromethane and clean-up with Florisil	BSTFA:trimethyl-chlorosilane (99:1)	GC-HRMS	[42]
Surface and tap waters	E1, EE2, NP, PPCPs	Filtration in case of high turbid samples	SPE HLB	MTBSTFA	GC-MS	[55]
Wastewater	E1, αE2, βE2, other steroids, OP, NP, BPA, phthalates, one pharmaceutical	Filtration and pH adjustment (pH 2–3)	SPE HLB	BSTFA	GC-MS/E-screen assay	[56]
Wastewater	E1, αE2, βE2, E3, EE2, MeEE2, other steroids, OP, NP, BPA, phthalates, phytoestrogens	Filtration in case of Yeast Assay	LLE with dichloromethane	BSTFA	GC-HRMS/Recombinant Yeast Assay	[41]
Surface water	EE2	Filtration and pH adjustment (pH 7)	–	–	Immunoassay method	[57]
Rain, surface water and wastewater	E1, αE2, βE2, EE2, OP, NP, BPA, phthalates	Filtration	–	–	ER-CALUX	[41]

QqQ-MS for the quantification of 14 pesticides in tissue of marine organisms, obtaining LOQs of 1–10 ng/g.

2.4. Endocrine-disrupting compounds

Many environmental pollutants acting as EDCs are regulated pollutants [e.g., dioxins, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), and new emerging, more polar pollutants (e.g., estrogenic compounds, APEOs, BPA and phthalates)]. Since the present review deals with the various approaches used for determination of emerging pollutants, we review below the analytical chemistry of the emerging EDCs. We discussed above the analytical approaches for PPCPs (sub-section 2.2.) and pesticides (sub-section 2.3), which may also induce endocrine disruption.

2.4.1. Estrogenic compounds

The group of natural and synthetic estrogens, of which E1, βE2, αE2, E3 and EE2 are important representatives, has been identified as the class with the highest EDC potential. Consequently, a lot of research has been devoted to the analysis of these estrogenic compounds [41]. Table 1 (for aqueous matrices) and Table 2 (for solid matrices) summarize the recently published (beginning from 2005) analytical approaches to quantify estrogens in environmental matrices. The analytical approaches for estrogenic compounds regularly include the simultaneous detection of other classes of EDCs (e.g., PPCPs, APEOs, phthalates, BPA or phyto-estrogens).

2.4.1.1. Water analysis. As can be seen from Table 1, chemical analysis of aqueous samples generally required pre-treatment as well as an extraction step. Sample pre-treatment typically included filtration, while pH adjustment and addition of chemical preservatives occurred only sporadically. However, filtration was absent in LLE [42], SBSE [43,44], SPME [45] or extraction using MIPs [46], which have been applied rarely, since SPE is the preferred technique for isolation and concentration of estrogens.

Typical SPE sorbents used for estrogens are Oasis HLB (hydrophilic-lipophilic balanced copolymer) [41] and octadecyl silica bonded phases [47,48]. Comparison of Strata C18-E with styrenedivinylbenzene Strata-X for extraction of a wide group of steroids led to comparable retention capacities for all analytes. Extraction disks, which provide a larger contact area between the sorbent and the matrix, have also been used for steroid extraction from aqueous samples [41]. Also, on-line SPE using Oasis HLB [49] or PLRP-s [41] sorbents have been applied for estrogen extraction.

Upon extraction, further clean-up steps using silica gel [50], Florisil [42,51] or NH_2 cartridges [41] were often applied.

Table 2. Recent analytical approaches to quantify estrogens in solid matrices, including sediment, biota and passive samplers

Matrix	EDCs studied	Sample pre-treatment	Extraction method	Derivatization	Analytical method	Ref.
Sediment	E1, αE2, βE2, E3, EE2, other steroids	Freeze-dried	Sonication with ACN/ H2O, SPE EDS-1, GPC and Florisil clean-up		LC-MS/MS	[41]
Sediment	E1, βE2, EE2	–	MASE, SPE Strata X-AW and silica gel		LC-ToF-MS/LC-QqQ-MS	[41]
Sediment	E1, βE2, E3, NP, OP, BPA	Freeze-dried	Ultrasonic extraction with methanol, SPE HLB + HPLC fractionation		LC-QqQ-MS/YES assay and ELISA	[41]
Biosolids and sludge	E1, βE2, other steroids, OP, NP, BPA, phthalates	Addition of water, Na_2CO_3,acetic anhydride; filtration and oven-dried	SBSE-TD	–	GC-MS	[43]
Soil	E1, αE2, βE2, E3	Air dried and sieved	PLE (acetone or ethyl acetate) + SPE C18	MSTFA:hexane (1:5)	GC-MS	[41]
Sediment	E1, βE2, E3, EE2, DES	Drying	Sonication (hexane–acetone) + SPE HLB		GC-MS/(GC x) GC-MS-ToF	[41]
Sludge samples	E1, βE2, E3, EE2	Freeze-dried and sieved	PLE with methanol:acetone (1:1) and SPE HLB + LC-NH_2	MSTFA + mercaptoeth. + NH_4I	GC-MS/LC-QqQ-MS	[41]
Suspended solids and sediment	E1, αE2, βE2, E3, EE2, MeEE2, NP, OP, BPA	Freeze-dried	Ultrasonic assisted extraction with methanol:acetone (1:1) and SPE Florisil	BSTFA	GC-ion trap-MS	[54]
Sediment and suspended solids	E1, αE2, βE2, EE2	Sieved and freeze-dried	PLE (acetone/ methanol) + HPLC fraction.	MSTFA	GC-ion trap-MS	[41]
Fish plasma and bile	E1, βE2, other steroids	Centrifugation	SPE C18 and NH_2	MSTFA + mercaptoeth. + NH_4I	GC-MS	[41]
Sediment and biota	E1, αE2, βE2, EE2, OP, NP, BPA, phthalates	Filtration			ER-CALUX	[41]
Passive samplers	E1, βE2, EE2, BPA, PPCPs	–	POCIS	–	GC-MS	[41]
Passive samplers	E1, αE2, βE2, E3, EE2, MeEE2, BPA, OP, NP	–	POCIS	BSTFA	GC-ion trap-MS	[58]
Wastewater	E1, αE2, βE2, other steroids, OP, NP, BPA, phthalates, one pharmaceutical	–	Empore SDB-RPS	BSTFA	GC-MS/E-screen assay	[56]

Analysis of estrogens has been performed using LC-MS as well as GC-MS (Table 1). Prior to GC-MS analysis, derivatization of the hydroxyl or carbonyl groups is carried out to enhance the thermal stability and the volatility of the compounds and to reduce the polarity due to decreasing dipole-dipole interactions [41]. The most common derivatization technique for estrogens involves silylation using derivatization mixtures of MSTFA or N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) [41]. Derivatization prior to LC-MS analysis is rare, since estrogens can be analyzed directly using LC techniques [49], so LC-MS-based methods for detection and quantification of steroids have been used increasingly [41].

So far, only a few U-HPLC-MS methods have been reported in literature [41].

Recently, Kozlik et al. [48] reported the use of capillary LC (cLC) for estrogen analysis, which is a promising approach towards green chemistry that preserves the separation advantages of classical LC. Combined with MS, sensitivity was sufficiently high using this technique.

As can be deduced from Table 1, QqQ-MS has been frequently applied after LC separation [41,47,48]. Comparison of LC-QqQ-MS, U-HPLC-ToF-MS and ELISA for estrogen analysis in water samples led to the conclusion that the highest sensitivity was obtained after appropriate sample pre-treatment followed by LC-QqQ-MS [41]. However, the UPLC–QTOF-MS/MS method provided a shorter analysis time and improved selectivity for confirmation and screening purposes, while the ELISA technique could be directly applied, without previous sample extraction or clean-up, to obtain relatively low LODs.

Besides ELISA, other bioanalytical techniques [e.g., RYA (Recombinant Yeast Assay), E-screen assay and ER-CALUX (estrogen-responsive chemically-activated luciferase expression) have also been applied [44]. Bioanalytical techniques are generally intended to determine total estrogenic potency and are frequently complemented with chromatographic (LC or GC) analyses. Finally, DAD and FLD have rarely been used, mainly due to their lesser sensitivity and selectivity [41].

2.4.1.2. Solid matrices and passive samplers. Estrogenic compounds are medium-polar to relatively non-polar substances, with log K_{ow} values in the range 2.5–5.3. Consequently, we can expect sorption of estrogens to suspended matter and a tendency for them to accumulate in sediments.

Estrogen extraction is usually performed by using polar or medium-polar organic solvents or their mixtures. UAE [54], PLE [41] and MASE [41] have been applied to enhance the extraction efficiency and to shorten the extraction time (Table 2). Further purification is usually needed and carried out by SPE, HPLC fractionation or GPC. Few studies have reported on the analysis of estrogens in biota. An analytical approach for estrogen analysis of fish plasma and bile using GC-MS and a bioanalytical approach using ER-CALUX has been reported [41]. Recently, several studies also described the use of passive samplers for estrogenic compounds [41,58].

As shown in Table 2, GC-MS analysis with preceding derivatization has been used more frequently than LC-MS for estrogen analysis in solid samples. Also, different GC-MS procedures for underivatized analytes have been described [41]. It was found that GC×GC coupled with ToF-MS provided unequivocal identification of target analytes due to better resolution. An HR-LC-ToF-MS method has also been reported [41].

2.4.2. Alkylphenolethoxylates and bisphenol A

Alkylphenolethoxylates (APEOs) belong to the class of the non-ionic surfactants, which are molecules comprising a hydrophobic part, usually an alkyl or alkylaryl chain, and a hydrophilic part, which can vary greatly. Both the surfactants and the metabolites [octylphenol (OP) and nonylphenol (NP)] are relatively persistent and have been shown to cause endocrine disruption. Since bisphenol A (BPA) is routinely analyzed together with the APEOs (and metabolites), we also consider this well-known EDC in this sub-section.

2.4.2.1. Water analysis. With respect to the analysis of water samples, we expect APEOs to degrade. The most common procedure for conserving APEOs is by acidifying the sample to a pH below 3 [59]. In addition, it is essential to process water samples as quickly as possible. As in the extraction of many emerging pollutants, SPE has replaced LLE and may be considered as the extraction technique applied most for APEOs and BPA.

Polymer-based sorbents have been preferred to C18 cartridges, since the long-chain APEOs and the metabolites OP and NP were retained. Using Oasis HLB cartridges, lower recoveries were frequently obtained for NP, a very environmentally important compound within this group because of its endocrine-disrupting potency. Sequential SPE or elution using solvents with different desorption potential and polarity has therefore been successfully applied to cover a broad range of APEOs [59].

Besides, the use of more specific extraction techniques for APEOs and BPA [e.g., SBSE, SPME, LPME and dispersive liquid-liquid microextraction (DLLME)] has also been reported [43].

Wang and Schnute [60] reported a U-HPLC-QTRAP method without previous sample preparation for the simultaneous quantification of NP and BPA, obtaining LODs in the range 0.04–0.057 μg/L.

Both GC-MS, with preceding derivatization, and LC-MS have been employed for analysis of aqueous samples

[60]. Anyhow, LC analysis is preferred for the longer chain APEOs, since these are not volatile enough for GC analysis. However, quantification of APEOs can be a matter of concern when using LC separation with C18 columns, since co-elution can be expected for the APEOs that differ in only ethoxylate chain length [59]. In LC-MS, QqQ-MS has often been used, while accurate mass measurements of APEOs using QToF-MS have also been reported [61].

In addition, UV detection and FLD have been applied for the determination of BPA and NP in water [43].

2.4.2.2. Solid samples, biota and passive samplers. Their physicochemical profiles suggest that some APEOs and degradation products have a strong affinity to SPM and organic matter. They also tend to bind to sediments and to accumulate in aquatic organisms, due to their high lipophilicity and lower water solubilities [59].

Extraction from sediment can be performed by Soxhlet extraction, UAE, SFE, MAE and PLE [59]. PLE offers several advantages over the other methods, and was therefore found to be the main extraction technique for these compounds in sediment matrices. Further purification of the extract obtained is usually performed by SPE [59].

With respect to biota, only a few studies have been conducted. Tavazzi et al. [62] described a PLE method followed by LC-MS analysis for the determination of OP, NP and BPA in fish liver. The use of passive samplers (POCISs) for the determination of BPA, NP and three steroids has also been reported [61].

2.5. Phthalates

Phthalates are considered ubiquitous environmental pollutants. As a result, considerable attention must be paid to the possible occurrence of sample contamination, which is a major problem throughout the analytical process. Phthalates present in laboratory equipment could contribute to sample contamination and result in systematic errors and false positives. Consequently, very specific analytical approaches, as we discuss below, are required to ensure the reliability of analytical results.

2.5.1. Water and passive-sampler analysis. Prior to analysis, phthalates need to be extracted from aqueous samples. To this end, traditional techniques, including LLE [42] and SPE [56], have rarely been reported. More common, extraction based on (in-tube) SPME using polymer-coated fibers has been applied successfully [63].

Penalver et al. [63] compared different fibers to optimize an SPME method for the most common phthalates, obtaining the best results using the polydimethylsiloxane–divinylbenzene fiber. An important advantage of the use of SPME for phthalate extraction includes the reduced risk for secondary contamination during sample handling. Other techniques (e.g., SBSE and LPME) have also been used to extract phthalates, as has DLLME [43]. Finally, a novel method, termed ionic liquid cold-induced aggregation dispersive liquid–liquid microextraction (IL-CIA-DLLME), was recently used for analysis of phthalates [64].

The use of passive samplers comprising styrene-divinylbenzene Empore SDB-RPS disks for the determination of 15 EDCs, including several phthalates, in wastewaters was reported by Tan et al. [56]. Also, semi-permeable membranes charged with Tenax TA have been applied as passive sampling devices for monitoring phthalates in the aquatic environment. Prior to GC-MS analysis, the targeted phthalates were thermally desorbed from the sampler with a stream of helium.

Upon sample pre-treatment, phthalates have been determined with GC-MS and LC-MS. LC methods coupled to DAD or UV detectors have also been applied to quantify phthalates in water samples.

2.5.2. Solid matrices. Several different methods for the extraction of phthalates from sediment or sludge samples have been reported (e.g., Soxhlet extraction, UAE and PLE). Due to the complexity of the sample matrix, sample preparation frequently included an additional clean-up step based on SPE with C18 sorbent or GPC with further fractionation using silica gel.

With respect to phthalate extraction from biota samples, recent literature is very limited. Chaler et al. [65] reported an analytical approach based on alkaline digestion with subsequent fractionation on an alumina/silica column. No other recent studies have examined the analysis of phthalates in aquatic organisms.

Both GC and LC have been employed for phthalate analysis in environmental matrices [65]. Recently, successful application of a GC-APCI-ToF-MS method was also reported [66].

2.6. Perfluorinated compounds

The occurrence of PFCs in the aquatic system has been extensively studied. Still, the quality of data obtained from analysis of PFCs in environmental matrices is a major issue of concern [67]. Very recently, the final report of the third interlaboratory study on PFCs in environmental matrices was published [67]. We could see a relatively wide variance in PFC data of aqueous samples, probably caused by a combination of problems that are characteristic for PFC analysis, namely the occurrence of cross contamination, matrix interferences and branched isomers. These inconveniences render quantitative analysis of PFCs in environmental matrices a challenging task, as reviewed below.

2.6.1. Water analysis. Several aspects of sampling and sample pre-treatment have been assessed as essential for straightforward analysis of PFCs. First, the

sampling depth may affect the results for PFC concentration. Decreasing PFC concentration with increasing water depth has been reported by Yamashita et al. [68]. This can be associated with their surface-active character and, in ocean waters, with the global ocean-circulation system [68]. Second, the sample equipment should be carefully selected to avoid contamination with and adsorption of PFCs [68]. Prior to sampling, it was suggested to rinse sample bottles using (semi-)polar solvents [3]. Acidification of samples to conserve them was discouraged to prevent volatilization and adsorption to the sample container [3].

Filtration of water samples is also a matter of concern for PFC analysis. The surface-active nature of these substances may result in sorption to the filter material. Furthermore, several filters are sources of contamination for PFOA and perfluorononanoate (PFNA), so, except in water samples visibly containing particulate matter, it was suggested to avoid filtration as a sample-preparation step. Schultz et al. [69] recommended centrifugation as an alternative sample-clean-up step.

Generally, low concentrations of PFCs (pg/L–ng/L) are found in water samples, requiring their pre-concentration and isolation. To this end, LLE and SPE, both followed by solvent evaporation, have frequently been reported. The use of these techniques is common, as also shown in the third interlaboratory study on PFCs [67]. Within this final report, it was found that SPE was predominantly used for nearly 80% of the water samples [67].

LLE and SPE were compared by Gonzalez-Barreiro et al. [70]. Using LLE, the overall PFC concentration (aqueous and particulate fraction) can be determined, since filtration is avoided, but this technique is limited to long-chain PFCs ($C \geqslant 8$). Apparently, SPE was best suited to PFCs with less than 10 carbon atoms, including the most important contaminants, PFOS and PFOA [70].

An SPE approach enabling the determination of short-chain and long-chain PFCs has also been described. Based on its publication, the ISO 25101/2006 method was established using weak anion-exchange (Oasis WAX) SPE cartridges. The use of C18 and certainly Oasis HLB cartridges for PFC enrichment has also been frequently reported [70,71].

As an alternative, direct determination of PFCs from aqueous samples has been reported [69]. Without sample pre-treatment, PFCs were quantified using large-volume injection, thereby obtaining LOQs in the 1 ng/L range.

LC coupled to quadrupole MS in negative-ionization mode is the preferred instrumental method for the determination of ionic PFCs in environmental matrices, including PFCAs and PFSAs. Upon LC separation, QqQ-MS provides excellent quantification of PFCs at very low concentration levels.

Controversially, it has been reported that the use of MS^n (using QqQ or IT-MS) for the detection of PFSAs, including the major contaminant PFOS, is complex and less efficient [72]. Since these substances exhibit very high stability even at extreme conditions (e.g., high collision energies), the use of QqQ-MS or IT-MS typically results in PFOS fragments of $[FSO_3]^-$ and $[SO_3]^-$, with m/z ions of 99 and 80, respectively. Unfortunately, interferences that co-elute with PFOS could be observed with the same retention time and one similar transition.

Another powerful analytical approach for PFC determination involves the use of HR full-scan accurate-mass measurements. (Q)ToF-MS has been reported to be the optimal detector for quantification of PFCs, combining high selectivity with high sensitivity.

Recently, LC-ToF-MS was applied for the determination of 14 PFCs in surface water, sewage water and seawater with LOQs in the range 2–200 ng/L [71]. In addition, HRMS using an Orbitrap instrument has proved to have excellent applicability for PFC determination as well, however, this has not been published in the literature earlier. Alternatively, the ionic PFCAs and PFSAs may be analyzed with GC as well. This however needs to be preceded by a derivatization with a mixture of 2,4-difluoroaniline and N,N-dicyclohexylcarbodiimide or with iso-propanol [73]. The non-ionic PFCs, such as the fluorotelomer alcohols and fluoroalkyl sulfonamides, are, due to their higher volatility, directly amenable to GC. Langlois et al. [73] reported an HRGC method for both ionic and non-ionic PFCs. In this context, the separation of PFOS isomers was performed using the HR of a capillary GC-column coated with 5%-phenyl-95%-methylpolysiloxane.

Specific measures have been described to overcome some characteristic difficulties during PFC analysis.

First, background contamination in the analytical blanks is a major problem in the analysis of PFCs, especially when the low-ng/L concentration range is targeted [74]. It was therefore suggested to avoid the use of Teflon materials throughout the extraction procedure, to clean and to dry all glass material, to replace the internal parts of the HPLC system made of PTFE with stainless steel and polyether ether ketone (PEEK), and to place an additional HPLC column between the pump and the injector to separate sample PFCs and PFCs originating from the system [71].

Second, the limited use of mass-labeled analogues to compensate for ionization effects can contribute to the occurrence of deviations in analytical results. For this reason, it is highly recommended to use an isotopically-labeled internal standard for every single PFC that requires quantification [67].

Finally, the occurrence of branched isomers should also be taken into consideration. For example, technical PFOS contains up to 30% differently branched isomers [73]. These branched isomers may have varying ioni-

zation efficiencies, resulting in a systematic quantification bias of PFCs. Recently, an HPLC-MS/MS method for isomer-specific quantification of PFCs in water samples was reported. Examination of PFC-isomer profiles may also be interesting for the study of the environmental fate of PFCs and to distinguish historical from recent contamination [75].

2.6.2. Solid matrices. Since the first report on the global distribution of PFOS was published in 2001, a large number of studies have been dedicated to the detection of PFCs in biota. The most important techniques to extract PFCs from solid matrices include ion-pair extraction (IPE) and solid-liquid extraction (SLE). Several recent studies used IPE methods, which comprise ion-pairing of PFCs with tetrabutylammonium hydrogen sulfate (TBA) followed by extraction with methyl-*tert*-buthyl ether (MTBE) [76]. However, according to the recent interlaboratory study on PFCs in environmental matrices, SLE using a medium polar solvent (e.g., methanol or acetonitrile) is considered the foremost applied approach for PFC extraction from biotic tissue [67].

The SLE sediment method of Powley et al. [77] and the altered version for biotic samples [78] have been regularly adopted or modified by environmental chemists [71]. Further clean-up of the extract obtained is usually performed by SPE or treatment with activated carbon (ENVIcarb) to reduce possible interferences [71]. This was also apparent from the interlaboratory study on PFCs in environmental matrices [67].

Another popular extraction method in this context is the approach of Berger and Hauskas [79], comprising extraction with methanol/water (50/50; 2 mM ammonium acetate) followed by filtration. Malinsky et al. [80] included a freezer-incubation step of the acetonitrile/tissue extracts to facilitate protein precipitation for improved analyte recoveries.

The use of PLE for PFC extraction was reported once by Llorca et al. [81], who compared three different sample-preparation techniques for PFC analysis in fish. It was found that PLE using water as extraction solvent with subsequent SPE was chosen over IPE and alkaline digestion followed by SPE.

Finally, microextraction with tetrahydrofuran was also successfully applied for analysis of PFCs in biota [82].

In general, extraction methods, similar to those for biota, have been used for sediment and sewage-sludge samples. Bao et al. [83] performed sediment extraction with TBA and MTBE, while a SLE method using 9 mL of methanol and 10 mL of a 1% glacial acetic-acid solution was applied by Gomez et al. [84]. Also, Ahrens et al. [85] used methanol as extraction solvent for PFC extraction from sediments.

So far, the use of PLE for sediment samples has not been reported.

In recent years, PFC analysis has typically been performed using LC coupled to different MS techniques (e.g., ion-trap MS, tandem MS, QqQ-MS, ToF-MS or QTRAP-MS) [71,80,83].

The use of U-HPLC for PFC analysis in solid matrices has been reported once [84].

Also the separation and the quantification of the PFC isomers have been demonstrated [80].

As for aqueous matrices, it has been reported that correct quantification of PFCs in solid matrices is challenging [67], so the measures mentioned above to enable accurate quantification in aqueous matrices should also be taken into consideration for solid samples.

With respect to quantification, it has been demonstrated that analytical results obtained with extracted matrix-matched calibration differ only slightly from solvent (unextracted) calibration [80]. This definitely facilitates quantification, especially when only a limited number of clean control matrix samples are available.

3. Conclusions and further research recommendations

We thoroughly reviewed current literature on the determination of CECs in the aquatic environment, thereby considering aqueous as well as solid matrices derived from the aquatic environment. Obviously, we paid considerable attention to the analysis of CECs in environmental matrices in the past decade.

Within this field of environmental chemistry, we could observe some prominent trends. First, the common use of GC to allow separation of the analytes has to a large extent been replaced by LC, due to the rather hydrophilic character of the CECs. However, GC has not been completely ruled out, since it is still the method of choice for separating some typical, more hydrophobic CECs (e.g., steroids, alkylphenols, fragrances and phthalates) displaying ionization issues in LC-MS analysis. Nevertheless, alternative LC applications have recently been reported for most of these groups as well.

Second, we could see a clear trend towards multi-residue and multi-class methods [3]. Recent advances in instrumentation have enabled the simultaneous detection of a large number of compounds within one analytical run. Also, the recent emergence of higher resolution LC equipment enabling the use of sub-2 μm particle sizes and high flow rates (U-HPLC) allows CECs to be resolved more easily, and that results in shorter analytical run times. Also, we propose the use of on-line SPE to shorten the analysis time [3].

Finally, there is another remarkable trend in MS. We could perceive increased popularity of HR full-scan analysis. ToF and Orbitrap instruments proved to be very suitable alternatives to QqQ instruments, thereby allowing accurate-mass screening of a virtually

unlimited number of analytes, targeted as well as untargeted, although, for some applications, their sensitivity is insufficient [4]. In this perspective, hybrid mass spectrometers [e.g., triple-quadrupole/linear ion trap MS (QTRAP or QLIT), quadrupole/time-of-flight MS (QToF), and ion-trap/Orbitrap MS] offer even greater potential by combining the sensitivity of QqQ systems with the versatility and identification potential of HRMS.

Despite these remarkable evolutions and achievements in analytical approaches, we can list some typical problems within this domain. First, pitfalls in identification and quantification still arise due to the lack of standardized criteria for identification and confirmation of CECs in environmental matrices. Criteria comparable to the European criteria laid down under Commission Decision 2002/657/EC [13], concerning the determination of analytes in products of animal origin, or to SANCO/10684/2009, on pesticide-residue analysis in food and feed, are missing for environmental matrices [e.g., (sea)water and biota]. For example, the evaluation of matrix effects is not always taken into consideration within validation procedures of newly developed analytical methods, while the occurrence of matrix interferences is a well-known source of false positives or false negatives and erroneous quantification using LC-MS methods. There exists therefore a great need for a standardized validation procedure for analytical methods for environmental applications.

A second issue relates to the use of modern instruments based on accurate HRMS, which in recent years have proved to be powerful screening and confirmation tools. Still, appropriate identification criteria using these systems are currently incomplete in the commonly used procedure prescribed by Commission Decision 2002/657/EC [4,13]. Both the criteria concerning mass resolution and mass accuracy, and the system of IPs have not yet been fully specified for these MS systems. As was suggested by several authors [15], additional criteria for the use of these accurate-mass LC-MS technologies should therefore be implemented in the standardized validation procedures.

Furthermore, several critical comments and useful recommendations concerning quantitative data obtained with new analytical methods could be enumerated. Recently, this was demonstrated once again by Van Leeuwen et al. [67], who reported the results of an interlaboratory study on the analysis of PFCs in environmental matrices. Some typical sources that could contribute to the variance of analytical data included the occurrence of matrix effects, the limited use of mass-labeled internal standards, the need for blank and recovery correction, and the perceived failure in separation of isomers (especially for PFCs). However, the most important aspect was related to the CECs usually being present at concentration levels close to the LODs of analytical methods, which results in less precise quantification and a higher variance. For newly developed methods, we therefore definitely recommend utilizing sufficient mass-labeled internal standards and performing extensive validation at environmentally relevant concentrations, thereby including the evaluation of matrix effects, accuracy and the natural background levels in so-called control samples. In addition, we also suggest participation in international interlaboratory studies or other data-quality tests, which may be good verification tools for the reliability of analytical methods.

Another important shortcoming within the domain of environmental chemistry is the current lack of comparability of analytical methods and results. In the literature, widely differing analytical methodologies have been used for obtaining concentrations of CECs in environmental matrices. There exists therefore a great need for standardized "state-of-the-art" methods for the different groups of CECs.

EPA Method 1694 and ISO 25101/2006 method were mentioned as standardized methodologies for analysis of pharmaceuticals and PFCs in water samples, respectively. EPA methods for analysis of different groups of pesticides are also available. However, these standardized methods were rarely applied in recent monitoring studies. We believe that the development of advanced up-to-date standardized methods and the widespread application thereof would result in reliable, comparable monitoring data.

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References

[1] C. Zwiener, F.H. Frimmel, Anal. Bioanal. Chem. 378 (2004) 862.
[2] H. Söderström, R.H. Lindberg, J. Fick, J. Chromatogr., A 1216 (2009) 623.
[3] M. Petrovic, M. Farré, M. Lopez de Alda, S. Perez, C. Postigo, M. Köck, J. Radjenovic, M. Gros, D. Barcelo, J. Chromatogr., A 1217 (2010) 4004.
[4] A.C. Hogenboom, J.A. van Leerdam, P. de Voogt, J. Chromatogr., A 1216 (2009) 510.
[5] I. Ferrer, J.A. Zweigenbaum, E.M. Thurman, J. Chromatogr., A 1217 (2010) 5674.
[6] D. Fatta, A. Nikolaou, A. Achilleos, S. Meric, Trends Anal. Chem. 26 (2007) 515.
[7] A. Kot-Wasik, J. Debska, J. Namiesnik, Trends Anal. Chem. 26 (2007) 557.
[8] K. Wille, H. Noppe, K. Verheyden, J. Vanden Bussche, E. De Wulf, P. Van Caeter, C.R. Janssen, H.F. De Brabander, L. Vanhaecke, Anal. Bioanal. Chem. 397 (2010) 1797.
[9] W.W. Buchberger, J. Chromatogr., A 1218 (2011) 603.
[10] C.S. Wong, S.L. MacLeod, J. Environ. Monit. 11 (2009) 923.
[11] E. Van Hoeck, F. Canale, C. Cordero, S. Compernolle, C. Bicchi, P. Sandra, Anal. Bioanal. Chem. 393 (2009) 907.
[12] K. Wille, M. Claessens, K. Rappé, E. Monteyne, C.R. Janssen, H.F. De Brabander, L. Vanhaecke, J. Chromatogr., A 1218 (2011) 9162.

[13] European Commission, Decision 2002/657/EC, Off. J. Eur. Commun. L221 (2002) 8.
[14] M. Ibanez, J.V. Sancho, D. McMillan, R. Rao, F. Hernandez, Trends Anal. Chem. 27 (2008) 481.
[15] M.W.F. Nielen, M.C. van Engelen, R. Zuiderent, R. Ramaker, Anal. Chim. Acta 586 (2007) 122.
[16] E. Pérez-Carrera, M. Hansen, V.M. León, E. Björklund, K.A. Krogh, B. Halling-Sørensen, E. González-Mazo, Anal. Bioanal. Chem. 398 (2010) 1173.
[17] J. Antonić, E. Heath, Anal. Bioanal. Chem. 387 (2007) 1337.
[18] A.J. Ramirez, M.A. Mottaleb, B.W. Brooks, C.K. Chambliss, Anal. Chem. 79 (2007) 3155.
[19] K. Wille, J.A.L. Kiebooms, M. Claessens, K. Rappé, J. Vanden Bussche, H. Noppe, N. Van Praet, E. De Wulf, P. Van Caeter, C.R. Janssen, H.F. De Brabander, L. Vanhaecke, Anal. Bioanal. Chem. 400 (2011) 1459.
[20] Z. Zhang, A. Hibberd, J.L. Zhou, Anal. Chim. Acta 607 (2008) 37.
[21] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, Anal. Bioanal. Chem. 397 (2010) 2833.
[22] R. Rodil, J.B. Quintana, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, J. Chromatogr., A 1216 (2009) 2958.
[23] K. Bester, J. Chromatogr., A 1216 (2009) 470.
[24] I. Gonzalez-Marino, J.B. Quintana, I. Rodriguez, R. Cela, Rapid Commun. Mass Spectrom. 23 (2009) 1756.
[25] P. Cuderman, E. Heath, Anal. Bioanal. Chem. 387 (2007) 1343.
[26] C.-P. Yu, K.-H. Chu, Chemosphere 75 (2009) 1281.
[27] C. Guitart, J.W. Readman, Anal. Chim. Acta 658 (2010) 32.
[28] R. Rodil, S. Schrader, M. Moeder, J. Chromatogr., A 1216 (2009) 8851.
[29] S.-F. Wu, W.-H. Ding, J. Chromatogr., A 1217 (2010) 2776.
[30] A. Nieto, F. Borrull, R.M. Marcé, E. Pocurull, J. Chromatogr., A 1216 (2009) 5619.
[31] Y. Yu, Q. Huang, J. Cui, K. Zhang, C. Tang, X. Peng, Anal. Bioanal. Chem. 399 (2011) 891.
[32] M.E. Balmer, H.-R. Buser, M.D. Müller, T. Poiger, Environ. Sci. Technol. 39 (2005) 953.
[33] A. Zenker, H. Schmutz, K. Fent, J. Chromatogr., A 1202 (2008) 64.
[34] H. Nakata, H. Sasaki, A. Takemura, M. Yoshioka, S. Tanabe, K. Kannan, Environ. Sci. Technol. 41 (2007) 2216.
[35] C. Jansson, J. Kreuger, J. AOAC Int. 93 (2010) 1732.
[36] Y.S. Al-Degs, M.A. Al-Ghouti, A.H. El-Sheikh, J. Hazard. Mater. 169 (2009) 128.
[37] S.J. Lehotay, J.A. Harman-Fetcho, L.L. McConnell, Mar. Pollut. Bull. 37 (1998) 32.
[38] S. Buisson, V. Bouchart, E. Guerlet, J.P. Malas, K. Costil, J. Environ. Sci. Health, Part B 43 (2008) 655.
[39] R. Carafa, J. Wollgast, E. Canuti, J. Ligthart, S. Dueri, G. Hanke, S.J. Eisenreich, P. Viaroli, J.M. Zaldívar, Chemosphere 69 (2007) 1625.
[40] H. Nyoni, L. Chimuka, B. Vrana, E. Cukrowsk, Anal. Chim. Acta 694 (2011) 75.
[41] G. Streck, Trends Anal. Chem. 28 (2009) 635.
[42] M.G. Ikonomou, S.-S. Cai, M.P. Fernandez, J.D. Blair, M. Fischer, Environ. Toxicol. Chem. 27 (2008) 243.
[43] C. Almeida, J.M.F. Nogueira, J. Pharm. Biomed. Anal. 41 (2006) 1303.
[44] B.L.L. Tan, D.W. Hawker, J.F. Muller, L.A. Tremblay, H.F. Chapman, Water Res. 42 (2008) 404.
[45] K. Mitani, M. Fujioka, H. Kataoka, J. Chromatogr., A 1081 (2005) 218.
[46] Y. Watabe, T. Kubo, T. Nishikawa, T. Fujita, K. Kaya, K. Hosoya, J. Chromatogr., A 1120 (2006) 252.
[47] C. Stavrakakis, R. Colin, V. Hequet, C. Faur, P. Le Cloirec, Environ. Technol. 29 (2008) 279.
[48] P. Kozlík, Z. Bosáková, E. Tesarová, P. Coufal, R. Cabala, J. Chromatogr., A 1218 (2011) 2127.
[49] A. Salvador, C. Moretton, A. Piram, R. Faure, J. Chromatogr., A 1145 (2007) 102.
[50] E. Vulliet, J.-B. Baugros, M.-M. Flament-Waton, M.-F. Grenier-Loustalot, Anal. Bioanal. Chem. 387 (2007) 2143.
[51] C. Miege, P. Bados, C. Brosse, M. Coquery, Trends Anal. Chem. 28 (2009) 237.
[52] R.A. Trenholm, B.J. Vanderford, J.C. Holady, D.J. Rexing, S.A. Snyder, Chemosphere 65 (2006) 1990.
[53] S. Zuehlke, U. Duennbier, T. Heberer, J. Sep. Sci. 28 (2005) 52.
[54] A. Arditsoglou, D. Voutsa, Environ. Sci. Pollut. Res. 15 (2008) 228.
[55] Z. Yu, S. Peldszus, P.M. Huck, J. Chromatogr., A 1148 (2007) 65.
[56] B.L.L. Tan, D.W. Hawker, J.F. Muller, F.D.L. Leusch, L.A. Tremblay, H.F. Chapman, Environ. Int. 33 (2007) 654.
[57] N.A. Martínez, R.J. Schneider, G.A. Messina, J. Raba, Biosens. Bioelectron. 25 (2010) 1376.
[58] A. Arditsoglou, D. Voutsa, Environ. Pollut. 156 (2008) 316.
[59] T. Vega Morales, M.E. Torres Padron, Z. Sosa Ferrera, J.J. Santana Rodriguez, Trends Anal. Chem. 28 (2009) 1186.
[60] J. Wang, W.C. Schnute, Rapid. Commun. Mass Spectrom. 24 (2010) 2605.
[61] E. Magi, C. Scapolla, M. Di Carro, C. Liscio, J. Mass Spectrom. 45 (2010) 1003.
[62] S. Tavazzi, E. Benfenati, D. Barcelo, Chromatographia 56 (2002) 463.
[63] A. Penalver, E. Pocurull, F. Borrull, R.M. Marce, J. Chromatogr., A 922 (2001) 377.
[64] H. Zhang, X. Chen, X. Jiang, Anal. Chim. Acta 689 (2011) 137.
[65] R. Chaler, L. Canton, M. Vaquero, J.O. Grimalt, J. Chromatogr., A 1046 (2004) 203.
[66] F. David, P. Sandra, P. Hancock, LC-GC Europe 24 (2011) 16.
[67] S.P.J. Van Leeuwen, M.-P. Strub, W. Cofino, G. Lindström, B. van Bavel, Third interlaboratory study on perfluorinated compounds in environmental and human matrices, Report R-11/04, IVM Institute for Environmental Studies, VU University, Amsterdam, The Netherlands, 2011.
[68] N. Yamashita, S. Taniyasu, G. Petrick, S. Wei, T. Gamo, P.K.S. Lam, K. Kannan, Chemosphere 70 (2008) 1247.
[69] M.M. Schultz, D.F. Barofsky, J.A. Field, Environ. Sci. Technol. 40 (2006) 289.
[70] C. González-Barreiro, E. Martínez-Carballo, A. Sitka, S. Scharf, O. Gans, Anal. Bioanal. Chem. 386 (2006) 2123.
[71] K. Wille, J. Vanden Bussche, H. Noppe, E. De Wulf, P. Van Caeter, C.R. Janssen, H.F. De Brabander, L. Vanhaecke, J. Chromatogr., A 1217 (2010) 6616.
[72] B. Veyrand, H. Kadar, A. Barbarossa, S. Durand, P. Marchand, J.P. Antignac, G. Pagliuca, B. Le Bizec, Proc. 29th Int. Symp. Halogenated Environmental Organic Pollutants POPs, San Antonio, Texas, USA, 2010.
[73] I. Langlois, U. Berger, Z. Zencak, M. Oehme, Rapid Commun. Mass Spectrom. 21 (2007) 3547.
[74] S. Taniyasu, K. Kannan, M.K. So, A. Gulkowska, E. Sinclair, T. Okazawa, N. Yamashita, J. Chromatogr., A 1093 (2005) 89.
[75] J.P. Benskin, L.W.Y. Yeung, N. Yamashita, S. Taniyasu, P.K.S. Lam, J.W. Martin, Environ. Sci. Technol. 44 (2010) 9049.
[76] H. Yoo, N. Yamashita, S. Taniyasu, K.T. Lee, P.D. Jones, J.L. Newsted, J.S. Khim, J.P. Giesy, Arch. Environ. Contam. Toxicol. 57 (2009) 552.
[77] C.R. Powley, S.W. George, T.W. Ryan, R.C. Buck, Anal. Chem. 77 (2005) 6353.
[78] C.R. Powley, S.W. George, M.H. Russell, R.A. Hoke, R.C. Buck, Chemosphere 70 (2008) 664.
[79] U. Berger, M. Haukas, J. Chromatogr., A 1081 (2005) 210.
[80] M.D. Malinsky, C.B. Jacoby, W.K. Reagen, Anal. Chim. Acta 683 (2011) 248.
[81] M. Llorca, M. Farré, Y. Picó, D. Barceló, J. Chromatogr., A 1216 (2009) 7195.

[82] N. Luque, A. Ballesteros-Gomez, S. van Leeuwen, S. Rubio, J. Chromatogr., A 1217 (2010) 3774.
[83] J. Bao, W. Liu, L. Liu, Y. Jin, X. Ran, Z. Zhang, Chemosphere 80 (2010) 123.
[84] C. Gómez, J. Vicente, B. Echavarri-Erasun, C. Porte, S. Lacorte, Mar. Pollut. Bull. 62 (2011) 948.
[85] L. Ahrens, N. Yamashita, L.W.Y. Yeung, S. Taniyasu, Y. Horii, P.K.S. Lam, R. Ebinghaus, Environ. Sci. Technol. 43 (2009) 6969.