Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com

Sample treatment for the determination of emerging organic contaminants in aquatic organisms

Mireia Núñez, Francesc Borrull, Eva Pocurull*, Núria Fontanals

Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira i Virgili, Sescelades Campus, Marcel·lí Domingo s/n, 43007 Tarragona, Spain

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Emerging organic contaminants
Aquatic organisms
Sample treatment
Extraction
Clean-up

ABSTRACT

Chromatography coupled to mass spectrometry has become an important tool for determining emerging organic contaminants (EOCs) in environmental samples such as aquatic organisms. Sample treatment, which includes extraction and clean-up, continues to play an important role in the analysis of complex matrices. Indeed it often becomes a bottleneck in the compromise between time and efficiency when obtaining suitable extracts for analysis. This article focuses on the state of the art in the treatment of aquatic organism samples for determining EOCs. A review is carried out of the most recent relevant publications from 2011 up to the present, in which new methods for determining EOCs in aquatic organisms were developed. The most common extraction techniques employed in these studies, like pressurised liquid extraction, solid–liquid extraction, QuEChERS, microwave-assisted extraction and matrix solid-phase extraction along with the subsequent clean-up steps, are also examined. The most important parameters involving extraction and clean-up are discussed.

1. Introduction

In recent years the use of various chemical substances in everyday consumer products as well as in industrial processes has continued to be widespread, which means that some can be considered emerging organic contaminants (EOCs) due to their continuous release into the environment. Consequently EOCs include a huge and increasing number of chemicals found in items such as personal care products (PCPs), water disinfection by-products, nanomaterials and pharmaceuticals, among others. To date EOCs have been characterised mostly in different aquatic environments which they reach via different routes, for instance in effluents from waste water treatment plants, livestock activities and so on [1,2]. The continuous discharge of these EOCs into the environment may lead to a degradation of ecosystems, and one of the main concerns related to their presence is that they could then bioaccumulate in non-target species and produce side effects in them [3]. Another potential concern involves possible biomagnification through the food chain, whereby they could eventually cause risks to humans. To address this issue, analytical methods need to be developed in order to obtain information about their presence in organisms (mainly in species present in the human diet) [4] and ecotoxicological studies need to be carried out to establish their potential effects in non-target species.

The analytical procedures to determine EOCs in aquatic organisms involve sample treatment, separation and detection. Separation and detection are performed predominantly by liquid chromatography (LC) or gas chromatography (GC), usually coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS), due to the selectivity, specificity and sensitivity achieved [5–10]. Meanwhile sample treatment, which includes extraction and clean-up, is still a critical step. As the objective here is to obtain extracts suitable for quantitative analyses, extracting the target analytes and removing potential interferences are the main aims of sample treatment. This type of matrix is rich in undesired components that may not only co-extract with the analytes but also affect their response. In addition, most EOCs are commonly present in environmental samples at low concentration levels and should therefore be expected at trace levels in aquatic organisms. To overcome these problems, tedious sample treatment is usually required, which makes these studies more challenging. As for the complex matrices, fish samples and particularly muscle tissue are the most common matrices analysed for most EOCs [5,8]. However, other aquatic organisms are also studied, for example bivalves such as mussels. It should be mentioned that some studies where fish is analysed are intended for food safety, which would reduce the number of studies for environmental proposes.

This paper reviews the most common extraction and clean-up procedures to determine EOCs in aquatic organisms published over the last five years. Pharmaceuticals, PCPs (UV filters, insect repellents, parabens, antimicrobials and synthetic musk fragrances), brominated flame retardants such as polybrominated diphenyl ethers (PBDEs), perfluorinated compounds (PFCs) and oestrogens were selected from the range of EOCs because of their widespread use. Detailed information on studies carried out before 2011 can be found in other reviews [5–10].

* Corresponding author. Fax: +34 977558446.
Email address: eva.pocurull@urv.cat (E. Pocurull)

https://doi.org/10.1016/j.trac.2017.09.007
0165-9936/

2. Extraction

As mentioned earlier, solid tissues are the most common matrix analysed. They are usually freeze-dried and then ground and homogenised to obtain similar-sized particles before extraction [3,11–13]. Sometimes sodium sulphate (Na_2SO_4) is used to dry the sample instead of freeze-drying [14,15]. Because of this pre-treatment, concentrations are usually expressed in dry weight, although the wet weight or percentage of lipid content respectively are also used when wet tissues or lipid content are measured [8].

Some studies have described enzymatic digestion [16–18] or alkaline digestion in the case of PFCs [19,20] as being successful pre-treatments to release binging analytes, eliminate organic matter interferences and achieve accurate measurements respectively.

Once the sample has been pre-treated, most of the techniques currently applied to extract EOCs from aquatic organisms are based on partitioning analytes between the sample matrix (solid) and a liquid phase, which is usually an organic solvent. On comparison with other studies covered by previous reviews it can be seen that the traditional Soxhlet extraction technique, which used to be widely used to extract different EOCs from aquatic organisms [7–9], has gradually been replaced by techniques requiring less time and less solvent, such as pressurised liquid extraction (PLE), solid–liquid extraction (SLE) and, to a lesser extent, microwave-assisted extraction (MAE), matrix solid-phase dispersion (MSPD) and QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). This latter technique is now gaining in popularity and its use has spread to more EOC groups than shown in previous studies. All these techniques will therefore be reviewed along with their recent applications.

2.1. Solid–liquid extraction

SLE is still used today to extract EOCs from aquatic organisms due to its simplicity and the fact it requires no expensive equipment. Table 1 shows the most relevant publications from 2011 onwards. The classic technique of shaking by hand usually ensures the partitioning of the analytes between the solid matrix and the organic solvent [19] and an Ultra-Turrax device [21–23] has been used to favour homogenisation, as in the case of PFCs in mussels [21]. However, ultrasounds are generally preferred to promote contact between the matrix and the solvent [14,16,24,25], becoming the extraction technique known as ultrasound-assisted solvent extraction (USE).

The efficiency of SLE depends mainly on the nature of the organic solvent used. Methanol (MeOH) is the most common solvent employed despite the different chemical nature of EOCs. It has been successful in extracting, among other compounds, UV filters [24], hormones [26], pharmaceuticals [25] and PFCs [19,23,27,28] from various fish species and mussels. Acetonitrile (ACN) has also been used to extract a group of pharmaceuticals and personal care products (PPCPs) [16] and PFCs [21] from fish and mussels respectively. In some cases solvent mixtures have been necessary to extract analytes with a wider range of polarity. For instance, a mixture of hexane:dichloromethane (DCM) [14] was used to extract 89 EOCs including polychlorinated biphenyls (PCBs), pesticides, chlorobenzenes, brominated and chlorinated flame retardants, musk fragrances and antimicrobials from fish, clams and polychaete worms. Unfortunately one of the main drawbacks of SLE is the volume of organic solvent needed, which can be as high as in classic Soxhlet extraction, up to 150 mL even with USE, when the amount of sample extracted is about 0.1–10 g.

Extraction time is another key factor in assuring quantitative extractions. This varies from 10 to 30 min for most applications [14,16,19–21,23–25]. In the case of USE, although ultrasound frequency can be modified to enhance extraction, this does not cause any significant decrease in extraction time. However, the centrifugation step usually needed after SLE to separate the extract causes a large increase in extraction time [16,21,23–25]. On average, centrifugation takes 10–30 min and the whole procedure (extraction and centrifugation) usually had to be repeated two or three times to achieve suitable results [14,16,20,24,25]. The complete extraction can therefore take more than 1 h per sample. An example of this is the extraction of UV filters from different fish species [24] by USE. Peng et al. [24] applied 3 extractions of 15 min and 20 mL of MeOH (solvent) each, alternating with centrifugations of 10 min at 4000 rpm to achieve recoveries of between 42% and 120%.

In line with the current trend in analytical chemistry to develop environmentally-friendly methods, some authors use a closed extractor fitted with a sonic probe to perform the extraction, this being known as focused ultrasound solid–liquid extraction (FUSLE) [29]. FUSLE not only reduces the amount of organic solvent needed (5–20 mL) but also the amount of sample (0.01–1.0 g) and extraction time (from seconds to a few minutes). For instance, hormones [26] were extracted using FUSLE from 0.5 g of mussel using 2 extractions of 1 min and 10 mL of MeOH, alternating with centrifugations of 5 min at 2800 × g each, and the recoveries obtained were over 88%. FUSLE has also successfully been applied to extract PFCs [30,31] and pharmaceuticals [29] from fish and mussels.

Thus, although the main disadvantage of USE is the large volumes of solvent required, however, it allows the extraction of large amounts of sample at relatively low cost.

2.2. QuEChERS

Although originally developed to determine pesticides in fruits and vegetables [32], in recent years the QuEChERS technique has been extended to extract EOCs from different matrices such as aquatic organisms because of its simplicity and the fact it requires no expensive equipment. Table 2 shows details from recent publications that employ this extraction technique. As mentioned before, the samples are usually freeze-dried and therefore water is added (between 2 and 10 mL) to enable phase separation. The most common extracting solvent used is ACN (between 1 and 10 mL), but a mixture of ACN:MeOH (75:25) has also been used by Pereira et al. [33] because MeOH increased the extraction of quinolones and tetracyclines from fish samples. In Jakimska et al. [34], the ratio V_{ACN}:V_{water} (4:1, 2:1 and 4:3) was evaluated in order to optimise the extraction efficiency of endocrine disrupting compounds (EDCs) such as preservatives, hormones and antibacterials, among others, from fish samples. The ratio 2:1 was chosen as a compromise between recoveries.

The addition of salts is required to favour phase separation between water and the organic solvent, and depending on the salts there are different QuEChERS methods: the Original Method, which uses the anhydrous $MgSO_4$ and NaCl salt composition; the European Standard Method EN 15662 (EN method), which uses citrate buffer as salts; and the AOAC Official 2007.1 Method (AOAC method), in which the acetate buffer is used. All three methods have been applied to extract EOCs from aquatic organisms.

Among other compounds, PBDEs and PCPs were extracted from different fish tissues employing the original method described by Anastassiades et al. [32], obtaining recoveries higher than 60% for most of the compounds [35]. In contrast, in the work by Jakimska et al. [34] different salt compositions were evaluated, of which the ac-

Table 1
Analytical methods based on SLE technique.

Matrix	Analytes	Extraction	Clean-up	Determination	Recoveries	Ref.
Amphipod	29 Pharmaceuticals	Agitation (0.1 g sample) ACN/5 min/2500 rpm	SPE Oasis HLB W: H_2O E: 1:1 ethyl acetate:acetone	LC–(ESI)MS/MS(QqQ)	41–89%	[22]
Fish	17 PFCs	Mechanical agitation (1 g sample) 0.01 M KOH in MeOH/15 min	SPE Oasis WAX W: 20 mM $NH_4CH_3CO_2$; MeOH E: 99.5:0.5 MeOH:NH_4OH SPE Envicarb E: 80:1 MeOH:CH_3COOH	LC–(ESI)MS/MS(QqQ)	65–125%	[19]
Fish	PFCs	Agitation (2.5 g sample) 200 mM NaOH + MeOH/30 min + 4 M HCl/10 min/4000 rpm	SPE Oasis WAX W: 25 mM acetate buffer; MeOH E: MeOH (2% NH_4OH)	LC–(ESI)MS/MS(QqQ)	95–109%	[23]
Mussel	10 PFCs	Agitation (1 g sample) ACN + centrifugation 20 min/5000 rpm	SPE Oasis HLB W: H_2O E: MeOH	LC–(ESI)HRMS(TOF)	90–106%	[21]
Fish	21 PFCs	Agitation (2 g sample) MeOH + NaOH/4 h/125 rpm 4000 rpm	TFC C_{18} column	LC–(ESI)MS/MS(QqQ)	55–94%	[27]
Fish Crustacea Cephalopoda	UV filters and UV stabilizers	Ultrasonication (2–4 g sample) MeOH 3× (15 min + 10 min/4000 rpm)	GPC Biobeads S-X3 E: 1:1 ethyl acetate:cyclohexane Silica gel column E: DCM:ethyl acetate (1:1)	UHPLC-(APCI)MS/MS(QqQ)	42–120%	[24]
Fish	2 Pharmaceuticals	USE (1 g sample) MeOH + 0.05 M HCl 2× (20 min/30°C + 15 min/3000 × g)	SPE Strata X W: H_2O E: MeOH	LC–(ESI)HRMS(Q-TOF)	40%	[25]
Fish, Clam and Polychaete worms	89 Compounds: (PCBs, pesticides, chlorobenzenes, BFR, CFR, musks fragrances, antimicrobials)	USE (5–10 g sample) 1:1 hexane:DCM 3 × 15 min	GPC Bio-Beads E: hexane:DCM (1:1) 1st fraction rejected 2nd fraction divided for GC and LC: 25% LC & 75% GC passed through deactivated florisil column E: hexane:DCM	GC–(EI)MS/MS(QqQ) UHPLC-(ESI)MS/MS (QqQ)	40–119%	[14]
Fish tissues (Plasma, liver, brain)	17 PPCPs	Enzymatic digestion (acetate buffer and β-glucuronidase/sulfatase) USE (0.5 g sample; 1 mL plasma) ACN 2× (10 min + 10 min/5000 × g) LLE 2× (5% NaCl + 1 M CH_3COOH + MTBE + 20 min shake + 10 min centrifugation 720 × g) 2× ($NaCO_3$ + 20 min shake + 10 min 720 × g)	Silica gel column (3 g) 1rst fraction: DCM 2nd fraction: 7:3 DCM:acetone; liver & brain pass through GPC (3:1 cyclohexane:ethyl acetate) 3rd fraction: acetone 4th fraction: 6:4 acetone:MeOH 2nd + 3rd + 4th fractions combined through SPE Oasis HLB W: H_2O (20% MeOH) E: MeOH:MTBE (7:3)	LC–(ESI)MS/MS(QqLIT)	Liver: 92–109% Brain: 88–118% Plasma: 90–110%	[16]
Fish	15 PFCs	USE (1 g sample) NaOH 0.2 M in MeOH ACN 2× (15 min sonication and 15 min shake + HCl neutralisation + 30 min/ 9000 × g)	Hexane dSPE ENVI-Carb CH_3COOH	UHPLC-(ESI)MS/MS(QqQ)	23–149%	[20]
Fish	14 PFCs	FUSLE (0.5 g sample) 9:1 ACN: H_2O 2.5 min/10% irradiation power/0°C	SPE Oasis WAX W: HCOOH (2%); H_2O:MeOH (95:5) E: acetone (2.5% NH_4OH)	UHPLC-(ESI)MS/MS(QqQ)	29–117%	[30]
Mussel	Hormones	FUSLE (0.5 g sample) MeOH 2× (1 min + 5 min centrifugation/ 2800 × g)	Hexane SPE Oasis HLB W: H_2O (5% MeOH) E: MeOH	LC–(ESI)MS/MS (QqLIT)	88–100%	[26]

Table 1 (*Continued*)

Matrix	Analytes	Extraction	Clean-up	Determination	Recoveries	Ref.
Mussel and fish tissues (muscle and liver)	Tricyclic antidepressants	FUSLE (0.5 g sample) 95:5 ACN:H_2O/30 s/10% power/0°C ice-water bath	SPE Evolute-CX W: H_2O; MeOH E: acetone (2.5% NH_4OH)	LC–(ESI)MS/MS(QqQ)	Liver: 89–109% Muscle: 94–114% Mussel: 86–104%	[29]
Mussel and fish tissues (muscle and liver)	14 PFCs and 10 potential precursors	FUSLE (0.5 g sample) 9:1 ACN:H_2O/2.5 min/10% irradiation power/0°C ice-water bath	SPE Evolute-WAX W: HCOOH (2%); H_2O:MeOH (95:5) Envi-Carb E: acetone (2.5% NH_4OH)	UHPLC-(ESI)MS/MS(QqQ)	Liver: 66–111% Muscle: 83–146% Mussel: 77–119%	[31]

BFR: Brominated flame retardants; CFR: chlorinated flame retardants; DCM: dichloromethane; dSPE: dispersive solid-phase extraction; E: elution; EI: electron impact; ESI: electrospray ionisation; FUSLE: focused ultrasound solid–liquid extraction; GC: Gas chromatography; GPC: gel permeation chromatography; HRMS: high resolution mass spectrometry; LC: liquid chromatography; MS/MS: tandem mass spectrometry; PFC: perfluorinated compounds; QqLIT: hybrid triple quadrupole linear ion trap; QqQ: triple quadrupole; SPE: solid-phase extraction; TFC: Turbulent flow chromatography; TOF: time of flight; UHPLC: ultra-high performance liquid chromatography; W: wash.

Table 2
Analytical method that employ QuEChERS as extraction technique.

Matrix	Analytes	Extraction	Clean-up	Determination	Recoveries	Ref.
Mussel	2 anticonvulsants and 6 TPs	2 g sample; 10 mL H_2O + 10 mL ACN + QuEChERS EN method	dSPE Na_2SO_4/PSA/C_{18} HCOOH	UHPLC-(HESI)HRMS(Orbitrap)	67–100%	[38]
Molluscs	2 pharmaceuticals	1 organism; 250 µL ACN + 100 µL H_2O + QuEChERS citrate buffer	Hexane	Nano LC-(ESI) MS/MS(QqLIT)	>85%	[41]
Benthic invertebrates	35 EOCs (pharmaceuticals and metabolites, hormones, PFCs, alkylphenols, pesticides, plasticiser)	1–4 organisms; 500 µL ACN + 500 µL H_2O + QuEChERS citrate buffer	Hexane	Nano LC-(ESI)MS/MS(QqLIT)	*C. riparius* 38–121% *G. fossarum* 50–120% *P. antipodarum* 47–102%	[40]
Fish	19 EDCs (triazoles, stimulants, hormones, flame retardants, plasticisers, antibacterials, preservatives)	0.5 g sample; ACN:H_2O (2:1) + QuEChERS AOAC method	dSPE $MgSO_4$/PSA/C_{18}	UHPLC-(ESI)MS/MS(QqLIT)	*C. carpio* 46–125% *S. glanis* 13–109% *L. graellsii* 32–121%	[34]
Fish	32 veterinary drugs (macrolides, penicillins, quinolones, sulphonamides and tetracyclines)	5 g sample; 2 mL H_2O + 10 mL ACN:MeOH (75:25) QuEChERS AOAC method	Not reported	UHPLC-(ESI)MS/MS (QqQ)	69–125%	[33]
Bivalves and fish (tissues: liver, muscle and gonad)	9 synthetic musk	0.2 g of sample; 1 mL ACN + QuEChERS EN method	dSPE PSA/C_{18}/$MgSO_4$	GC–(EI)MS(Q)	46–120%	[37]
Bivalves	7 pharmaceuticals	1 g of sample; 10 mL H_2O + 10 mL ACN + QuEChERS EN method	dSPE silica gel	LC–(ESI)MS/MS(QqQ)	61–95%	[39]
Mussel	1 pharmaceutical and TPs	100 mg sample; 5 mL H_2O + 10 mL ACN + QuEChERS acetate buffer	Heptane	LC–(ESI)MS/MS(QqQ)	73–117%	[36]
Fish tissues (liver and gonad)	33 EOCs (PBDEs, PCBs, musk fragrances, PAHs, pesticides, plasticiser, UV filter, alkylphenol)	5 g of sample; 5 mL ACN + Original method	dSPE 2× ($MgSO_4$/PSA/C_{18})	GC–(EI)MS(Q)	Liver 28–108% Gonad 61–113%	[35]

dSPE: dispersive solid-phase extraction; EI: electron impact; ESI: electrospray ionisation; GC: Gas chromatography; HRMS: high resolution mass spectrometry; LC: liquid chromatography; MS: mass spectrometry; MS/MS: tandem mass spectrometry; PBDE: polybrominated diphenyl ethers; PCB: Polychlorinated biphenyl; PSA: primary secondary amine; QqLIT: hybrid triple quadrupole linear ion trap; Q: quadrupole; QqQ: triple quadrupole; SPE: solid-phase extraction; TP: transformation product; UHPLC: ultra-high performance liquid chromatography.

C. riparius: *Chironomus riparius*; *G. fossarum*: *Gammarus fossarum*; *P. antipodarum*: *Potamopyrgus antipodarum*; *C. carpio*: *Cyprinus carpio*; *S. glanis*: *Silurus glanis*; *L. graellsii*: *Luciobarbus graellsii*.

etate buffer was the best option for extracting 19 EDCs belonging to different classes. The same buffer was employed by Daniele et al. [36] to extract diclofenac and some of their transformation products from mussel. Saraiva et al. [37] applied the EN method to extract musk from seafood, obtaining recoveries between 46% and 120%. The citrate buffer was also used by Martínez-Bueno [38] to extract two anticonvulsants and six of their transformation products from mussel samples. However, in this study the $MgSO_4$ contained in the buffer was substituted for Na_2SO_4, which, according to the authors, efficiently absorbs the water. The final method provided recoveries of between 67% and 100%. The EN and the AOAC QuEChERS methods were both tested in our previous study [39] to extract seven pharmaceuticals from different bivalve species, with the EN method being the one that achieved the highest extraction recoveries.

As in the case of SLE, a miniaturisation of this technique (micro-QuEChERS) has also recently been applied [40,41], in which the volumes employed were at μL level and the amount of salts was also reduced. In Berlioz-Barbier [41], for instance, fluoxetine and carbamazepine were extracted from two species of gastropod employing 100 μL of water, 250 μL of ACN and 100 mg of citrate buffer, obtaining recoveries higher than 85%.

Therefore, viewing the sorted examples using QuEChERS it seems that this technique is going to continue being used in the future, mainly because of the results achieved and the simplicity of the technique.

2.3. Matrix solid-phase dispersion

Despite its advantages (simplicity, small sample size, short extraction time, less solvent than conventional techniques and no equipment required), MSPD has been less widely used than the previous techniques because the samples have to be ground up with a dispersing agent (also known as solid support) and packed into a column, which makes this technique laborious. Nevertheless, there are some recent publications that employ this extraction technique can be found in Table 3.

Florisil [42], diatomaceous earth [43] and primary secondary amine (PSA) [44] are solid supports that have been used in the extraction of different flame retardants, musk fragrances and PFCs from bivalve samples. The analytes are eluted using a suitable organic solvent while interfering matrix compounds are selectively retained in the column. It should be noted that MSPD can simultaneously perform extraction and clean-up by placing a layer of co-sorbent at the bottom of the MSPD column [45]. In recent studies silica gel [43], a combination of deactivated and activated silica [42] and a combination of silica, acidified silica with 10% H_2SO_4 and deactivated Florisil with 5% water [44] have been employed for clean-up purposes. In Ziarrusta et al. [42], for example, the use of deactivated and activated silica obtained cleaner chromatograms, repetitive retention times and low %RSD (values up to 5%) in repeatability.

Table 3
Analytical methods that employ MSPD or MAE extraction techniques.

Matrix	Analytes	Extraction	Clean-up	Determination	Recoveries	REF.
Bivalves	8 PFCs	MSPD sample mixed with diatomaceous earth E: ACN	Na_2SO_4 + silica gel	LC–(ESI)MS/MS(QqQ)	Clam: 64–114% Mussel: 97–115% Cockle: 92–126%	[43]
Bivalves	15 BFR (PBDEs among others)	MSPD 0.5 g sample mixed with PSA E: DCM	silica + acidified silica (10% H_2SO_4) + deactivated Florisil	GC–(CI)MS(Q)	Mussel: 99–120% Clam: 46–110% Cockle: 70–101%	[44]
Bivalves	40 analytes (PAHS, PCBs, PBDEs, musk fragrances, pesticides)	MSPD 0.3 g sample mixed with Florisil E: DCM	deactivated silica + activated silica	GC–(EI)MS/MS(QqQ)	Mussel: 64–109%	[42]
Mussel	2 UV filters, 2 pharmaceuticals	MAE (3 g of sample) 1:1 acetone:heptane; T[a] increased to 110°C within 15 min and maintained for 5 min	RPLC Sperisorb ODS2 E: 70:30 MeOH:H_2O with gradient	GC–(EI)MS/MS(IT)	89–122%	[53]
Fish and mussel	11 antibiotics and metabolites	MAE (2 g of sample) (Proteinase-K) H_2O/5 min/50 W + 5 min/centrifugation 8000 × g HCOOH + 2× (5 mL DCM + manual agitation)	Not reported	LC–(ESI)MS/MS(QqLIT)	Anchovy: 64–99% Wedge sole: 63–96% Hake: 63–99% Mussel: 61–97%	[17]
Fish	15 hormones	MAE (100 mg sample) 4 mL MeOH; 450 W; 60°C; 7 min	SPE Phree cartridge	UHPLC-(ESI) MS/MS(QqQ)	Muscle: >56%	[55]
Mussel	3 UV filters	MAE (3 g of sample) 1:1 acetone:heptane; 110°C within 15 min.	RPLC Sperisorb ODS2 E: 70:30 MeOH:H_2O with gradient	GC–(EI)MS/MS(IT)	89–116%	[54]

BFR: brominated flame retardants; CI: chemical ionisation; DCM: dichloromethane; E: elution; EI: electron impact; ESI: electrospray ionisation; GC: Gas chromatography; GPC: gel permeation chromatography; IT: ion trap; LC: liquid chromatography; MAE: Microwave extraction; MSPD: matrix solid-phase dispersion; MS: mass spectrometry; MS/MS: tandem mass spectrometry; PAH: Polycyclic aromatic hydrocarbon; PCB: polychlorinated biphenyl; PBDE: polybrominated diphenyl ether; PFC: perfluorinated compounds; PSA: primary secondary amine; QqLIT: hybrid triple quadrupole linear ion trap; Q: quadrupole; QqQ: triple quadrupole; RPLC: reversed-phase liquid chromatography; SPE: solid-phase extraction.

MSPD has been employed to extract different groups of EOCs such as musk fragrances and PBDEs [42], PFCs [43] and brominated flame retardants [44] in bivalve samples, with the recoveries obtained for most of the compounds ranging from 64% up to 126%.

2.4. Pressurised liquid extraction

In recent years PLE has expanded its field of application and, compared with previous reviews, has established itself as one of the most extensively used techniques for extracting different EOCs from aquatic organisms. Table 4 shows some examples in which PLE has been used. The most important parameters to be optimised are the extraction solvent followed by temperature, extraction time and number of cycles [46]. As regards extraction solvents, MeOH has been successfully applied to extract pharmaceuticals from a wide variety of aquatic organisms [11,47,48]. However, a high matrix effect (ME) was encountered when mussels were analysed [49] and consequently ultrapure water was used as a compromise between recoveries and ME. Poorly cleaned extracts were also encountered when MeOH was tested to extract UV filters from fish [12]. In addition, Vallecillos et al. [13] described that fatty precipitates appeared in the PLE extracts when MeOH was tested to extract musk fragrances from fish and mussel samples. Solvent mixtures have also been employed [3,4,12,19,21,50–52] to enhance the extraction of analytes with a

Table 4
Analytical methods that employ PLE techniques.

Matrix	Analytes	Extraction	Clean-up	Determination	Recoveries	Ref.
Fish tissues (homogenate, liver and muscle)	20 pharmaceuticals	0.5–1 g sample; MeOH/3 cycles/5 min/50°C	In-cell neutral alumina GPC (LC-DAD) Acquity HSS T3 column	UHPLC-(ESI)MS/MS(QqLIT)	*L. graellsii*: 31–108% *C. carpio*: 19–79% *S. glanis*: 26–75%	[11]
Mussel	5 pharmaceuticals	1 g sample; 3:1 ACN:H_2O/ 3 cycles/5 min/60°C	In-cell activated neutral alumina SPE Strata-X cartridge W: H_2O E: 1:1 ethyl acetate:acetone	LC–(ESI)MS/MS(IT)	83–104%	[3]
Mussel and other bivalves	11 pharmaceuticals	1 g sample; 3:1 ACN:H_2O 1% HCOOH/3 cycles/ 10 min/100°C	In-cell neutral alumina SPE Strata-X W: H_2O E: MeOH	UHPLC-(ESI)MS/MS(QqQ)	95–103%	[21]
Bivalves	23 pharmaceuticals and metabolites	0.5 g sample; 1:2 MeOH:H_2O/3 cycles/ 5 min/50°C	In-cell neutral alumina SPE Oasis HLB W: H_2O E: MeOH	UHPLC-(ESI)MS/MS(QqLIT)	Oyster: 33–101% Clam: 30–74% Mussel: 30–116	[4]
Fish	8 UV filters	1 g sample; 1:1 ethyl acetate:DCM/2 cycles/ 5 min/100°C	In-cell Florisil SPE Isolute C_{18} E: 1:1 ethyl acetate/DCM; DCM	LC–(ESI)MS/MS(QqLIT)	36–112%	[12]
Mussel	7 pharmaceuticals	1 g sample; H_2O/1 cycle/ 10 min/100°C	SPE Oasis MAX W: H_2O (5% NH_4OH); MeOH E: MeOH (5% HCOOH)	LC–(ESI)MS/MS(QqQ)	61–90%	[49]
Fish and mussel	10 musk fragrances	0.5 g sample; DCM/1 cycle/5 min/60°C	In-cell Florisil	GC–(EI)-MS/MS(IT)	Fish: 61–109% Mussel: 45–91%	[13]
Dolphin (Liver)	UV filter	1 g liver; 1:1 DCM:hexane/2 cycles/ 10 min/100°C	Acid attack 4× (H_2SO_4) SPE alumina E: hexane:DCM (1:2)	UHPLC-(ESI)MS/MS(QqQ)	Not reported	[51]
Fish Crustacean	4 UV filters and 4 stabilizers	1:1 hexane:DCM/3 cycles/ 5 min/100°C	In-cell PSA GPC 2 Envirogel GPC columns (19 × 300 mm/19 × 150 mm) dSPE PSA	GC(EI)HRMS(TOF) LC(ESI)HRMS(Q-TOF)	46–85%	[50]
Fish	11 pharmaceuticals	1 g sample; DCM/1 cycle/ 10 min/80°C	SPE Oasis MCX W: ACN E: MeOH(5% NH_4OH)	LC–(HESI)MS/MS(QqQ)	19–85%	[15]
Fish	18 PCBs, 7 PBDEs	1 g sample; 70:30 toluene:acetone/3 cycles/ 5 min/120°C	Silica gel column (H_2SO_4) activated Florisil column Florisil/Carbopack C/Celite 545 column	GC–(EI)HRMS(Double sector)	60–120%	[19]
Clam	Tetracycline antibiotics	3 g sample; MeOH/1 cycle/15 min/70°C	In cell copper (II) isonicotinate	LC–(ESI)MS/MS(QqQ)	85–94%	[47]
Molluscs	22 antibiotics	0.1 sample; MeOH/2 cycles/10 min/70°C	SPE Oasis HLB W: H_2O E: MeOH (5% NH_3)	LC–(ESI)MS/MS(QqQ)	Not reported	[48]
Biofilm	13 EDCs (parabens, plasticiser, hormones, flame retardants, antibacterial) 44 pharmaceuticals	200 mg sample; Citric buffer (pH 4):ACN/3 cycles/5 min/60°C	SPE Oasis HLB W: H_2O E: MeOH	UHPLC-(ESI)MS/MS(QqLIT)	24–137%	[52]

DAD: diode array detector; DCM: dichloromethane; E:elution; EI: electron impact; ESI: electrospray ionisation; GC: Gas chromatography; GPC: gel permeation chromatography; HRMS: high resolution mass spectrometry; IT: ion trap; LC: liquid chromatography; MS/MS: tandem mass spectrometry; QqLIT: hybrid triple quadrupole linear ion trap; QqQ: triple quadrupole; Q-TOF: quadrupole time of flight; SPE: solid-phase extraction; TOF: time of flight; UHPLC: ultra-high performance liquid chromatography; W: wash. *L. graellsii*: *Luciobarbus graellsii*; *C. carpio*: *Cyprinus carpio*; *S. glanis*: *Silurus glanis*.

wide range of polarity. For instance, 23 pharmaceuticals and some of their metabolites were extracted from different bivalve species with a mixture of MeOH:water (1:2). According to the authors, the addition of water favours the recovery of antibiotics [4]. In the work by McEneff et al. [3] another mixture, ACN:water; (3:1; v:v), was employed to extract different pharmaceuticals in mussels, and Couderc et al. [19] used a mixture of toluene:acetone (70:30) to extract PBDEs from fish samples.

Once the extraction solvent is selected, the temperature is usually optimised to increase the recoveries, bearing in mind ME [12] or avoiding analyte (e.g. pharmaceuticals) degradation [11]. For example, 100°C was chosen for extracting UV filters from fish using a mixture of ethyl acetate:DCM (1:1; v:v) [12].

As Table 4 shows, between 1 and 3 cycles with extraction times of between 5 and 15 min for each cycle are common values. The sample amount required is generally between 0.1 and 3 g, the most common values being 0.5 and 1 g.

When comparing PLE with other extraction techniques such as QuEChERS that do not require any equipment, Vallecillos et al. [13] reported that both extraction techniques were suitable for extracting musk fragrances from fish and mussel. However, PLE with DCM as an extracting solvent achieved lower ME than QuEChERS employing the EN method and slightly better validation parameters. In contrast, Martínez-Bueno et al. [38] studied the influence of the extraction methodology when determining two anticonvulsants and some of their transformation products. QuEChERS and PLE were again compared, with higher average recoveries being obtained for most of the analytes when QuEChERS was used. Moreover, QuEChERS required less solvent and sample and also a shorter extraction time. Jakimska et al. [34] also compared the same extraction techniques to determine different EDCs in fish samples, with QuEChERS being selected as the best option again, since PLE led to high ME due to the co-extraction of other matrix components that could not be removed in the clean-up step. Nevertheless, QuEChERS, PLE and USE were compared in extracting 20 pharmaceuticals in different fish species and tissues. Although the QuEChERS recoveries were higher than 40%, PLE was selected due to the higher recoveries of relevant compounds (such as diclofenac and propanolol), lower %RSD and fewer matrix interferences (compared with QuEChERS) [11]. In another work by the same group [52], a comparison was made between PLE and USE using the same solvent (citric buffer (pH4):ACN; 1:1) when extracting 13 EDCs and 44 pharmaceuticals in biofilm. Despite the fact that similar recoveries were obtained, PLE was said to prevail over USE in terms of reproducibility (%RSD < 20%), indicating greater robustness. The main disadvantages of PLE are its high cost due to the equipment needed and a limited selectivity. For this reason further clean-up of the extract is required. However, it is an attractive technique since if offers several advantages such as being fast, using less solvent volumes. Moreover, the equipment allows a semi-automated process where different samples can be processed sequentially and different sample sizes can be accommodated (different cell and vessel volumes are available).

2.5. Microwave-assisted extraction

Although MAE has been used to extract different EOCs from different environmental matrices, it has been less widely used than PLE in the case of aquatic organisms even though both techniques require equipment. Current applications are shown in Table 3. Only a few studies have recently used this extraction technique [17,53–55]. It has been used to extract UV filters and pharmaceuticals from mussels [53,54] and hormones from fish samples [55], for instance, and also to extract pharmaceuticals from fish and mussels, in which the extraction was combined with enzymatic digestion [17]. The nature of the solvent employed by the extraction is very important. It is common practice to use a binary mixture (heptane:acetone) in which only one of the solvents absorbs microwaves [53,54]. Other important parameters affecting the extraction process are the power applied, temperature and extraction time [46]. In the work by Fernández-Torres et al. [17], for example, MAE was carried out using 2 g of mussel/fish sample with an extraction time of 5 min with 5 mL of water at 50 W to extract veterinary antibiotics from different fish species and mussels, obtaining recoveries of between 61% and 99%.

However, as state above, this technique is not widely used, mainly because it becomes expensive and that a subsequent clean-up step is usually necessary due to the efficiency of MAE.

3. Clean-up

Most of the extraction techniques for aquatic organism samples are not very selective. Endogenous components present in the matrix, which can include ionic species, highly polar compounds, various organic molecules and analogous compounds or metabolites with a chemical structure close to that of the target analytes, are usually also extracted [5–8]. Therefore the removal of co-extracted matrix components is critical, and different clean-up procedures have been described for use during or after extraction to minimise the negative effects. The different clean-up strategies are detailed in Tables 1–4 along with the extraction techniques already described. Due to the complexity of the matrix in several studies, more than one clean-up step is usually required and different clean-up strategies are combined. Solid-phase extraction (SPE) and gel permeation chromatography (GPC) are the most common strategies.

Hexane is sometimes added to the extract prior to clean-up in order to eliminate co-extracted nonpolar and fatty compounds. This strategy has been used before SPE [26] and dispersive solid-phase extraction (dSPE) [20], and thus an already cleaner extract is contacted with the SPE sorbent. Another strategy reported in the literature is to perform an acid attack using H_2SO_4 [51]. However, sulphuric acid cannot then be employed for the analysis of certain compounds since they may be degraded [7]. Other acids (HCOOH or CH_3COOH) have also been added to the clean-up step [20,38]; according to Martínez-Bueno [38], the addition of HCOOH favours the disruption of the compound-protein binding, which affects the recovery and ME.

3.1. Solid-phase extraction

The most commonly used clean-up strategy is SPE, which is applied after different extraction techniques for several EOCs. The main sorbents chosen for SPE are high capacity ones such as Oasis HLB [4,16,21,22,26,48,52] and, to a lesser extent, Strata–X [3,21,25]. As mentioned in previous sections, the extraction solvents employed are usually organic. This means that the extracts must be completely or partly evaporated before being loaded into the cartridge, and are then usually diluted to a certain mL up to 100 mL or 200 mL with ultrapure water and, if necessary, adjusted to a certain pH. In some studies, chelating agents are added in order to bind the residual metals present in the matrix [4,52]. Once the extract has been loaded into the cartridges, ultrapure water is usually employed to clean the matrix [3,4,21,22,25,48,52], although water containing a percentage (5%–20%) of MeOH [16,26] has also been used. MeOH is the most common solvent employed for eluting the compounds from the sorbent. Nevertheless, the addition of 5% NH_4OH in MeOH has been successfully used to elute antibiotics [48]. Other eluting solvents such

as a mixture of ethyl acetate:acetone (1:1) have also been employed to elute pharmaceuticals from Oasis HLB sorbent, when amphipod *Gammarus* sp. extract was percolated [22], and Strata-X sorbent from mussel extract [3]. A mixture of MeOH:Methyl *tert*-butyl ether (MTBE) (7:3) has been used to elute PCPs from different fish tissues from the Oasis HLB sorbent [16]. Isolute C_{18} is another cartridge described in the literature and supplied better results than the Oasis HLB sorbent for most lipophilic UV filters [12].

More selective mixed-mode ion-exchange sorbents like Oasis MCX [15], Evolute-CX [29], Oasis MAX [49] and Oasis WAX [23,30] have also been employed. The elution from the Oasis WAX cartridge of the target analytes was performed with 2.5% NH_4OH in MeOH or acetone. In the case of strong cation-exchangers (Oasis MCX sorbent and Evolute-CX), organic solvents (ACN and MeOH) were used to clean the matrix without losing the selectively retained analytes, and the elution took place with 5% NH_4OH in MeOH [15] or 2.5% NH_4OH in acetone [29].

Polar sorbents in the normal phase, such as alumina [51], silica gel [16,19,24] and Florisil [14,18,19] columns or cartridges with different levels of activity, are employed separately or in combination for the removal of nonpolar lipids and other nonpolar molecules [5]. However, larger volumes (up to 160 mL) of solvent are usually used in these cases in order to elute the compounds.

Apart from clean-up, SPE [18,56] and liquid–liquid extraction (LLE) [57] have also been used to extract EOCs from non-solid matrices such as blood and bile from aquatic organisms.

In general most studies claimed that aquatic organisms are complex matrices and require clean-up. However, after this clean-up is carried out, in some studies it is difficult to see whether it was worth it or at what level it improves recoveries or decreases ME.

3.2. Chromatographic approaches

GPC is a clean-up strategy widely used for separating large molecules (e.g. lipids) on the basis of size exclusion [46]. The Bio-Beads S-X3 is a commonly employed column [24,58]. A disadvantage of this technique is that after GPC additional steps are usually needed because of the difficulty involved in removing all lipids by GPC alone [14,16,24,58]. In other words, after GPC the samples were passed through a silica gel column or cartridge [24,58], a Florisil column [14], an Oasis HLB cartridge [16] or cleaned by dSPE with PSA [50]. Another disadvantage is the large volumes obtained, which makes the clean-up step tedious and increases analysis time.

In Peng et al. [24], for example, GPC was employed to clean the extract obtained from USE, which was evaporated to dryness and redissolved in ethyl acetate:cyclohexane (1:1) before being subjected to a GPC column. The analytes were eluted with the same solvent, in which the first 15 mL were rejected and the following 16 mL collected. However, some analytes still suffered from ME and for this reason the collected eluate was concentrated again for solvent exchange before further clean-up with a silica gel column, which, according to the authors, reduced the ME. In Huerta et al. [11], GPC was chosen between different strategies (SPE using Florisil cartridges and SPE using Oasis HLB followed by GPC) since lower recoveries were obtained for the other strategies, and single GPC clean-up provided satisfactory results for most of the compounds. The extract was passed through an EnviroPrep column (300 × 21.2 mm; 10 μm) coupled to a PLgel guard column using DCM:MeOH (9:1) as mobile phase. The fraction between 13.5 and 26.5 min was collected and a diode array detector was used to monitor the sample. In this case no further clean-up was required.

Another type of chromatography, reversed-phase liquid chromatography (RPLC), has been widely used as clean-up for the determination of UV filters [53,54]. According to Zenker et al. [59], RPLC used as clean-up for UV filters with different properties enabled more efficient separation compared to SPE or GPC. However, for the UV filters that had similar physicochemical properties, the clean-up with GPC or SPE was very useful.

Another type of chromatography is turbulent flow chromatography (TFC) which has recently employed as clean-up in the case of PFCs [27,28]. Although not many studies employed this clean-up technique TFC is a promising technique for rapid sample preparation [60]. In Campo et al. [27], for example, TFC was employed to clean the extract obtained after shaking and alkaline digestion to determine 21 PFCs obtaining recoveries between 55% and 94%. The main advantages of this technique are that saves considerably in sample preparation time and allows analysis to be performed when limited sample quantities are available [60]. However, considerable amount of work is required for optimization of different parameters (elution mode, suitable column, injection volumes, mobile phase composition, etc.). Moreover, high solvent consumption is usually necessary [60].

3.3. Strategies related to PLE

PLE makes it possible to perform an in-cell clean-up in which a sorbent is placed at the bottom of the extraction cell or mixed with the sample instead of the inert material in order to retain interfering substances. Alumina [3,4,11,21] was the most common sorbent for the clean-up when pharmaceuticals were extracted from fish [11] and bivalves [3,4,21]. Other less widely used sorbents include silica gel [61], Florisil [12,13] and PSA [50]. The amount of sorbent is variable and depends on the volume of the extraction cell and ranges from 1 g to 20 g [3]. Looking at the examples detailed in Table 4, it can be seen that this strategy is usually combined with further clean-up steps such as SPE [3,4,12,21] or GPC [11,50]. This was the case in the work by Gago-Ferrero et al. [12], in which 1 g of Florisil was selected as in-cell clean-up when UV filters were determined from fish samples, since it was observed that it improved extraction efficiency and supplied a cleaner extract and a better chromatographic peak shape, although the extract required one further clean-up step with SPE. Other examples are shown in Table 4. However, in some recent studies the in-cell clean-up was the only purification step taken. Jiao et al. [47] used 3 g of copper (II) isonicotinate as in-cell sorbent for fatty samples such as clams and discovered that no further clean-up step was necessary. This was also the case in the work by Vallecillos et al. [13], in which in-cell clean-up with Forisil was the only purification step carried out for musk fragrances in fish and mussel samples. As can be seen in Table 4, in-cell clean-up is almost always employed with PLE, since it offers several advantages as compared to the previous clean-up strategies, it does not involve a lengthening of the analysis time, requires no additional parameters of the extraction to be modified and does not entail higher costs.

Another cleaning strategy made possible by PLE is on-cell clean-up, which is when a solvent with complementary properties to the one used in the extraction is passed through the sample. Although this strategy was tested [11], it was not included in any final method in recent publications.

3.4. Dispersive solid-phase extraction

This clean-up step is commonly carried out after QuEChERS extraction (see Table 2), and usually it does because it is simple and fast, but can also be performed after other techniques such as SLE

[20]. With QuEChERS, dSPE using PSA, C_{18} and graphitised carbon black (GCB), among other sorbents, has been tested [34,38]. However, in recent publications the most common mixture is that containing PSA and C_{18} because it removes nonpolar compounds such as lipids from fish [34,35,37] and bivalve [37,38] samples. $MgSO_4$ is also added to remove excess water and improve analyte partitioning [40]. In the study by Jakimska et al. [34], after extraction different dSPEs such as $MgSO_4$/PSA, $MgSO_4$/PSA/C_{18}, PSA/C_{18}/GCB/$MgSO_4$ and PSA/GCB/$MgSO_4$ were tested, with the $MgSO_4$/PSA/C_{18} mixture being selected since according to the authors it can be used with samples with a high lipid content and provides suitable results (recoveries higher than 50% and %RSD lower than 18%). Other studies did not carry out a clean-up step after QuEChERS [33], while others used hexane [40,41] to promote separation of the lipidic fraction. Nevertheless, it is always advisable to include a clean-up step after the extraction of such complex samples as aquatic organisms.

4. Conclusions

Sample treatment is still the most time-consuming step in the analytical method in order to achieve extracts compatible with the detection techniques. As regards extraction techniques, although the classic Soxhlet extraction is still employed, alternative less time-consuming techniques have been widely used. SLE (another traditional technique) continues to be used due to its simplicity. PLE and QuEChERS offer the advantage of significantly reducing the amount of organic solvent consumed. Moreover, PLE offers a semi-automated extraction process and the possibility of efficient in-cell clean-up using selective sorbents. Meanwhile QuEChERS has also been noted as a powerful extraction technique and has recently been gaining in popularity.

As for clean-up techniques, despite the long purification procedures that can be involved, GPC and adsorption chromatography, for example, are still widely used. In-cell clean-up in the case of PLE and dSPE in the case of QuEChERS have been described as saving time and enabling cheaper clean-ups.

Some miniaturisation methods have also appeared to make sample treatment shorter and reduce solvent consumption with the aim of being more environmentally friendly while obtaining promising results.

In view of improvements that the extraction and clean-up techniques have undergone during these last years, it is expected that further progress will emerge in the future to address the challenging analysis of aquatic organisms.

Acknowledgements

The authors would like to thank the Spanish Ministry of the Economy and Competitiveness (Project CTQ-2014-52617-P) for the financial support received.

References

[1] A. Pal, K.Y.H. Gin, A.Y.C. Lin, M. Reinhard, Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects, Sci. Total Environ. 408 (2010) 6062–6069.

[2] D.J. Lapworth, N. Baran, M.E. Stuart, R.S. Ward, Emerging organic contaminants in groundwater: a review of sources, fate and occurrence, Environ. Pollut. 163 (2012) 287–303.

[3] G. McEneff, L. Barron, B. Kelleher, B. Paull, B. Quinn, The determination of pharmaceutical residues in cooked and uncooked marine bivalves using pressurised liquid extraction, solid-phase extraction and liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 405 (2013) 9509–9521.

[4] D. Álvarez-Muñoz, B. Huerta, M. Fernández-Tejedor, S. Rodríguez-Mozaz, D. Barceló, Multi-residue method for the analysis of pharmaceuticals and some of their metabolites in bivalves, Talanta 136 (2015) 174–182.

[5] B. Huerta, S. Rodríguez-Mozaz, D. Barceló, Pharmaceuticals in biota in the aquatic environment: analytical methods and environmental implications, Anal. Bioanal. Chem. 404 (2012) 2611–2624.

[6] S. Valsecchi, M. Rusconi, S. Polesello, Determination of perfluorinated compounds in aquatic organisms: a review, Anal. Bioanal. Chem. 405 (2013) 143–157.

[7] A.M. Peck, Analytical methods for the determination of persistent ingredients of personal care products in environmental matrices, Anal. Bioanal. Chem. 386 (2006) 907–939.

[8] P. Gago-Ferrero, M.S. Díaz-Cruz, D. Barceló, An overview of UV-absorbing compounds (organic UV filters) in aquatic biota, Anal. Bioanal. Chem. 404 (2012) 2597–2610.

[9] K. Bester, Analysis of musk fragrances in environmental samples, J. Chromatogr. A 1216 (2009) 470–480.

[10] A. Papachlimitzou, J.L. Barber, S. Losada, P. Bersuder, R.J. Law, A review of the analysis of novel brominated flame retardants, J. Chromatogr. A 1219 (2012) 15–28.

[11] B. Huerta, A. Jakimska, M. Gros, S. Rodríguez-Mozaz, D. Barceló, Analysis of multi-class pharmaceuticals in fish tissues by ultra-high-performance liquid chromatography tandem mass spectrometry, J. Chromatogr. A 1288 (2013) 63–72.

[12] P. Gago-Ferrero, M.S. Díaz-Cruz, D. Barceló, Multi-residue method for trace level determination of UV filters in fish based on pressurized liquid extraction and liquid chromatography–quadrupole-linear ion trap-mass spectrometry, J. Chromatogr. A 1286 (2013) 93–101.

[13] L. Vallecillos, E. Pocurull, F. Borrull, Influence of pre-treatment process on matrix effect for the determination of musk fragrances in fish and mussel, Talanta 134 (2015) 690–698.

[14] H. Zhang, S. Bayen, B.C. Kelly, Co-extraction and simultaneous determination of multi-class hydrophobic organic contaminants in marine sediments and biota using GC-EI-MS/MS and LC-ESI-MS/MS, Talanta 143 (2015) 7–18.

[15] J. Wang, P.R. Gardinali, Analysis of selected pharmaceuticals in fish and the fresh water bodies directly affected by reclaimed water using liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 404 (2012) 2711–2720.

[16] R. Tanoue, K. Nomiyama, H. Nakamura, T. Hayashi, J.-W. Kim, T. Isobe, R. Shinohara, S. Tanabe, Simultaneous determination of polar pharmaceuticals and personal care products in biological organs and tissues, J. Chromatogr. A 1355 (2014) 193–205.

[17] R. Fernández-Torres, M.A. Bello López, M. Olias Consentino, M. Callejon Mochon, M. Ramos Payan, Enzymatic-microwave assisted extraction and high-performance liquid chromatography-mass spectrometry for the determination of selected veterinary antibiotics in fish and mussel samples, J. Pharm. Biomed. Anal. 54 (2011) 1146–1156.

[18] O. Ros, J.K. Izaguirre, M. Olivares, C. Bizarro, M. Ortiz-Zarragoitia, M.P. Cajaraville, N. Etxebarria, A. Prieto, A. Vallejo, Determination of endocrine disrupting compounds and their metabolites in fish bile, Sci. Total Environ. 536 (2015) 261–267.

[19] M. Couderc, L. Poirier, A. Zalouk-Vergnoux, A. Kamari, I. Blanchet-Letrouve, P. Marchand, A. Venisseau, B. Veyrand, C. Mouneyrac, B. Le Bizec, Occurrence of POPs and other persistent organic contaminants in the European eel (Anguilla anguilla) from the Loire estuary, France, Sci. Total Environ. 505 (2015) 199–215.

[20] U. Eriksson, A. Karrman, A. Rotander, B. Mikkelsen, M. Dam, Perfluoroalkyl substances (PFASs) in food and water from Faroe Islands, Environ. Sci. Pollut. Res. 20 (2013) 7940–7948.

[21] K. Wille, J.L. Kiebooms, M. Claessens, K. Rappé, J. Vanden Bussche, H. Noppe, N. Van Praet, E. De Wulf, P. Van Caeter, C.R. Janssen, H. De Brabander, L. Vanhaecke, Development of analytical strategies using U-HPLC-MS/MS and LC-ToF-MS for the quantification of micropollutants in marine organisms, Anal. Bioanal. Chem. 400 (2011) 1459–1472.

[22] T.H. Miller, G.L. McEneff, R.J. Brown, S.F. Owen, N.R. Bury, L.P. Barron, Pharmaceuticals in the freshwater invertebrate, Gammarus pulex, determined using pulverised liquid extraction, solid phase extraction and liquid chromatography-tandem mass spectrometry, Sci. Total Environ. 511 (2015) 153–160.

[23] V. Ciccotelli, M.C. Abete, S. Squadrone, PFOS and PFOA in cereals and fish: development and validation of a high performance liquid chromatography-tandem mass spectrometry method, Food Control 59 (2016) 46–52.

[24] X. Peng, J. Jin, C. Wang, W. Ou, C. Tang, Multi-target determination of organic ultraviolet absorbents in organism tissues by ultrasonic assisted extraction and ultra-high performance liquid chromatography tandem mass spectrometry, J. Chromatogr. A 1384 (2015) 97–106.

[25] M.E. Valdés, M.V. Ame, M.A. Bistoni, D.A. Wunderlin, Occurrence and bioaccumulation of pharmaceuticals in a fish species inhabiting the Suquia River basin (Cordoba, Argentina), Sci. Total Environ. 472 (2014) 389–396.

[26] J.M. Ronan, B. McHugh, A sensitive liquid chromatography/tandem mass spectrometry method for the determination of natural and synthetic steroid estrogens in seawater and marine biota, with a focus on proposed water frame-

work directive environmental quality standards, Rapid Commun. Mass Spectrom. 27 (2013) 738–746.
[27] J. Campo, M. Lorenzo, F. Pérez, Y. Picó, M. Farré, D. Barceló, Analysis of the presence of perfluoroalkyl substances in water, sediment and biota of the Jucar River (E Spain). Sources, partitioning and relationships with water physical characteristics, Environ. Res. 147 (2016) 503–512.
[28] J. Campo, F. Pérez, A. Masia, Y. Picó, M. Farré, D. Barceló, Perfluoroalkyl substance contamination of the Llobregat River ecosystem (Mediterranean area, NE Spain), Sci. Total Environ. 503 (2015) 48–57.
[29] H. Ziarrusta, L. Mijangos, A. Prieto, N. Etxebarria, O. Zuloaga, M. Olivares, Determination of tricyclic antidepressants in biota tissue and environmental waters by liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 408 (2016) 1205–1216.
[30] I. Zabaleta, E. Bizkarguenaga, A. Iparragirre, P. Navarro, A. Prieto, L.A. Fernández, O. Zuloaga, Focused ultrasound solid-liquid extraction for the determination of perfluorinated compounds in fish, vegetables and amended soil, J. Chromatogr. A 1331 (2014) 27–37.
[31] I. Zabaleta, E. Bizkarguenaga, A. Prieto, M. Ortiz-Zarragoitia, L.A. Fernández, O. Zuloaga, Simultaneous determination of perfluorinated compounds and their potential precursors in mussel tissue and fish muscle tissue and liver samples by liquid chromatography-electrospray-tandem mass spectrometry, J. Chromatogr. A 1387 (2015) 13–23.
[32] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, J. AOAC Int. 86 (2003) 412–431.
[33] R. Pereira Lopes, R. Cazoria Reyes, R. Romero-González, J.L. Martínez Vidal, A. Garrido Frenich, Multiresidue determination of veterinary drugs in aquaculture fish samples by ultra high performance liquid chromatography coupled to tandem mass spectrometry, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 895 (2012) 39–47.
[34] A. Jakimska, B. Huerta, Z. Barganska, A. Kot-Wasik, S. Rodríguez-Mozaz, D. Barceló, Development of a liquid chromatography-tandem mass spectrometry procedure for determination of endocrine disrupting compounds in fish from Mediterranean rivers, J. Chromatogr. A 1306 (2013) 44–58.
[35] S.A. Morrison, K.K. Sieve, R.E. Ratajczak, R.B. Bringolf, J.B. Belden, Simultaneous extraction and cleanup of high-lipid organs from white sturgeon (Acipenser transmontanus) for multiple legacy and emerging organic contaminants using QuEChERS sample preparation, Talanta 146 (2016) 16–22.
[36] G. Daniele, M. Fieu, S. Joachim, A. James-Casas, S. Andres, P. Baudoin, M. Bonnard, I. Bonnard, A. Geffard, E. Vulliet, Development of a multi-residue analysis of diclofenac and some transformation products in bivalves using QuEChERS extraction and liquid chromatography-tandem mass spectrometry. Application to samples from mesocosm studies, Talanta 155 (2016) 1–7.
[37] M. Saraiva, J. Cavalheiro, L. Lanceleur, M. Monperrus, Synthetic musk in seafood products from south Europe using a quick, easy, cheap, effective, rugged and safe extraction method, Food Chem. 200 (2016) 330–335.
[38] M.J. Martínez Bueno, C. Boillot, H. Fenet, S. Chiron, C. Casellas, E. Gómez, Fast and easy extraction combined with high resolution-mass spectrometry for residue analysis of two anticonvulsants and their transformation products in marine mussels, J. Chromatogr. A 1305 (2013) 27–34.
[39] M. Núñez, F. Borrull, N. Fontanals, E. Pocurull, Determination of pharmaceuticals in bivalves using QuEChERS extraction and liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 407 (2015) 3841–3849.
[40] A. Berlioz-Barbier, A. Bulete, J. Fabure, J. Garric, C. Cren-Olive, E. Vulliet, Multi-residue analysis of emerging pollutants in benthic invertebrates by modified micro-quick-easy-cheap-efficient-rugged-safe extraction and nanoliquid chromatography-nanospray-tandem mass spectrometry analysis, J. Chromatogr. A 1367 (2014) 16–32.
[41] A. Berlioz-Barbier, R. Baudot, L. Wiest, M. Gust, J. Garric, C. Cren-Olive, A. Bulete, MicroQuEChERS-nanoliquid chromatography-nanospray-tandem mass spectrometry for the detection and quantification of trace pharmaceuticals in benthic invertebrates, Talanta 132 (2015) 796–802.
[42] H. Ziarrusta, M. Olivares, A. Delgado, O. Posada-Ureta, O. Zuloaga, N. Etxebarria, Multiscreening determination of organic pollutants in molluscs using matrix solid phase dispersion, J. Chromatogr. A 1391 (2015) 18–30.
[43] E. Villaverde-de-Saa, J.B. Quintana, R. Rodil, R. Ferrero-Refojos, E. Rubi, R. Cela, Determination of perfluorinated compounds in mollusks by matrix solid-phase dispersion and liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 402 (2012) 509–518.
[44] E. Villaverde-de-Saa, C. Valls-Cantenys, J.B. Quintana, R. Rodil, R. Cela, Matrix solid-phase dispersion combined with gas chromatography-mass spectrometry for the determination of fifteen halogenated flame retardants in mollusks, J. Chromatogr. A 1300 (2013) 85–94.
[45] M. García-López, P. Canosa, I. Rodríguez, Trends and recent applications of matrix solid-phase dispersion, Anal. Bioanal. Chem. 391 (2008) 963–974.
[46] N. Fidalgo-Used, E. Blanco-González, A. Sanz-Medel, Sample handling strategies for the determination of persistent trace organic contaminants from biota samples, Anal. Chim. Acta 590 (2007) 1–16.
[47] Z. Jiao, S. Zhang, H. Chen, Determination of tetracycline antibiotics in fatty food samples by selective pressurized liquid extraction coupled with high-performance liquid chromatography and tandem mass spectrometry, J. Sep. Sci. 38 (2015) 115–120.
[48] W. Li, Y. Shi, L. Gao, J. Liu, Y. Cai, Investigation of antibiotics in mollusks from coastal waters in the Bohai Sea of China, Environ. Pollut. 162 (2012) 56–62.
[49] M. Núñez, F. Borrull, E. Pocurull, N. Fontanals, Pressurized liquid extraction followed by liquid chromatography with tandem mass spectrometry to determine pharmaceuticals in mussels, J. Sep. Sci. 39 (2016) 741–747.
[50] K.H. Langford, M.J. Reid, E. Fjeld, S. Oxnevad, K.V. Thomas, Environmental occurrence and risk of organic UV filters and stabilizers in multiple matrices in Norway, Environ. Int. 80 (2015) 1–7.
[51] P. Gago-Ferrero, M.B. Alonso, C.P. Bertozzi, J. Marigo, L. Barbosa, M. Cremer, E.R. Secchi, A. Azevedo, J. Lailson-Brito Jr., J.P.M. Torres, O. Malm, E. Eljarrat, M.S. Díaz-Cruz, D. Barceló, First determination of UV filters in marine mammals. Octocrylene levels in Franciscana dolphins, Environ. Sci. Technol. 47 (2013) 5619–5625.
[52] B. Huerta, S. Rodríguez-Mozaz, C. Nannou, L. Nakis, A. Ruhi, V. Acuña, S. Sabater, D. Barceló, Determination of a broad spectrum of pharmaceuticals and endocrine disruptors in biofilm from a waste water treatment plant-impacted river, Sci. Total Environ. 540 (2016) 241–249.
[53] E. Gómez, M. Bachelot, C. Boillot, D. Munaron, S. Chiron, C. Casellas, H. Fenet, Bioconcentration of two pharmaceuticals (benzodiazepines) and two personal care products (UV filters) in marine mussels (Mytilus galloprovincialis) under controlled laboratory conditions, Environ. Sci. Pollut. Res. 19 (2012) 2561–2569.
[54] M. Bachelot, Z. Li, D. Munaron, P. Le Gall, C. Casellas, H. Fenet, E. Gómez, Organic UV filter concentrations in marine mussels from French coastal regions, Sci. Total Environ. 420 (2012) 273–279.
[55] R. Guedes-Alonso, Z. Sosa-Ferrera, J.J. Santana-Rodríguez, Determination of steroid hormones in fish tissues by microwave-assisted extraction coupled to ultra-high performance liquid chromatography tandem mass spectrometry, Food Chem. 237 (2017) 1012–1020.
[56] M. Lahti, J.-M. Brozinski, A. Jylha, L. Kronberg, A. Oikari, Uptake from water, biotransformation, and biliary excretion of pharmaceuticals by rainbow trout, Environ. Toxicol. Chem. 30 (2011) 1403–1411.
[57] F. Chen, Z. Gong, B.C. Kelly, Rapid analysis of pharmaceuticals and personal care products in fish plasma micro-aliquots using liquid chromatography tandem mass spectrometry, J. Chromatogr. A 1383 (2015) 104–111.
[58] I.-S. Lee, U.-J. Kim, J.-E. Oh, M. Choi, D.-W. Hwang, Comprehensive monitoring of synthetic musk compounds from freshwater to coastal environments in Korea: with consideration of ecological concerns and bioaccumulation, Sci. Total Environ. 470 (2014) 1502–1508.
[59] A. Zenker, H. Schmutz, K. Fent, Simultaneous trace determination of nine organic UV-absorbing compounds (UV filters) in environmental samples, J. Chromatogr. A 1202 (2008) 64–74.
[60] M. Farré, L. Kantiani, M. Petrovic, S. Pérez, D. Barceló, Achievements and future trends in the analysis of emerging organic contaminants in environmental samples by mass spectrometry and bioanalytical techniques, J. Chromatogr. A 1259 (2012) 86–99.
[61] B. Subedi, M.A. Mottaleb, C.K. Chambliss, S. Usenko, Simultaneous analysis of select pharmaceuticals and personal care products in fish tissue using pressurized liquid extraction combined with silica gel cleanup, J. Chromatogr. A 1218 (2011) 6278–6284.