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Review

On-line sample extraction and purification for the LC–MS determination of emerging contaminants in environmental samples

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ABSTRACT

In the present paper the application of on-line preparation techniques applied to LC–MS for the determination of emerging contaminants in environmental samples are critically reviewed highlighting the advantages but also the limits and the way to overcome them. Recent methods are collected in a detailed table and discussed in the text according to the different classes of compounds. The implementation of on-line SPE made possible the effective development of faster methods by reducing the analysis time and thus increasing the analytical productivity. The on-line SPE method are particularly suitable in studies where only limited sample material is available, as the sample volume can be as low as 1 mL. Most of the multiresidual methods have been developed using wettable and polar embedded reverse phases, sometimes sequentially packed with weak ionic exchangers in order to widen the polarity range of the adsorbents. On-line techniques have been also applied in cleanup of extracts of solid environmental samples, such as sediment, soil and biota, in order to minimize the ionization suppression effects from the matrix. Many applications employed RAM, often coupled to monolithic pre-concentration columns, but there is an increasing number of application of TFC for the determination of emerging pollutants in environmental samples.

Contents

Abbreviations: BPA, bisphenol A; EC, emerging contaminants; EDC, endocrine disrupter compounds; FSS, fractionized sampling and stacking strategy; HRMS, high resolution mass spectrometry; MIP, molecular imprinted polymers; MSPD, matrix solid phase dispersion; MW, molecular weight; PFAA, perfluoroalkylacids; PFAS, perfluorinated compounds; PFCA, perfluoroalkylcarboxylic acids; PFOA, perfluorooctanoic acid; PFOS, perflorooctanesulfonic acid; PFPA, perfluoroalkylphosphonic acids; PFSA, perfluoroalkylsulfonic acids; PLE, pressurized liquid extraction; RAM, restricted access media; SPE, solid phase extraction; TFC, turbulent flow chromatography; TP, transformation products; UHPLC, ultra high performance liquid chromatography; WAX, weak anionic exchangers; WCX, weak cationic exchangers; WWTP, wastewater treatment plant.

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1. Introduction

Until the beginning of the ‘90, non-polar hazardous compounds, i.e. persistent organic pollutants and heavy metals, were in the focus of interest and awareness as priority pollutants, and consequently were part of intensive monitoring programs. Nowadays these compounds are less relevant for the industrialized countries since a drastic reduction of emission has been achieved due to the adoption of appropriate measures and elimination of the dominant pollution sources.

However, the emission of so-called “emerging” or “new” unregulated contaminants has emerged as an environmental problem and there is a widespread consensus that this kind of contamination may require legislative intervention. This group is mainly composed of products used in large quantities in everyday life, such as human and veterinary pharmaceuticals, personal care products, surfactants and surfactants’ residues, plasticizers and different industrial additives. Main sources of emerging contaminants are untreated urban wastewaters and wastewater treatment plant (WWTP) effluents. Most current WWTP are not designed to treat this type of substances and the high portion of emerging compounds and their metabolites can escape elimination in the WWTP and enter the aquatic environment via sewage effluents. These contaminants do not need to be persistent in the environment to cause negative effect since their high transformation/removal rates can be compensated by their continuous introduction into environment. Physico-chemical characteristics of the various emerging contaminants (ECs) are very different, they can vary even in the same class of compounds, such as the case of the homologues series of perfluoroalkylacids (PFAA), and this fact represents an analytical challenge when a multicomponent method has to be developed. Nevertheless most of the investigated ECs are polar and biological active molecules (e.g. veterinary and human pharmaceuticals, illicit drugs and drugs of abuse) and their metabolites and transformation products (TP) are generally even more polar. Thereby the strategies and materials, originally developed for the extraction of hydrophobic compounds from environmental samples, need to be adjusted or designed for highly polar compounds. A part from some hydrophobic or very small and volatile emerging compounds, the chromatographic technique of election was the liquid chromatography coupled to the mass spectrometry.

Growing interest on ECs from both research and regulatory perspectives makes necessary to collect more data on their occurrence in the water bodies. The need for productivity in environmental monitoring pulls the research towards automated methods, with a special regards to the on-line coupling of preparation techniques, both for extraction and clean-up, to chromatographic separation. But a complete risk assessment of the substances requires also the identification and quantitation of their metabolites and transformation products, which was made possible by the introduction of high resolution mass spectrometry (HRMS). The inclusion of improved clean up steps before the chromatographic analysis is also an effective way to minimize the matrix load and their effects on ionization when HRMS is used for the identification of unknown compounds.

In the present paper we critically review the recent application of on-line preparation techniques applied to LC–MS for the determination of ECs in environmental samples highlighting the advantages but also the limits and the way to overcome them.

This review does not aim to describe all the applications of on-line SPE techniques which have been described and discussed in recent excellent reviews [1–3], but to survey only the techniques that have been actually used for environmental analysis of the main classes of ECs. Finally we collected and discussed all the recent applications of on-line techniques for ECs in environmental samples (Table 1).

2. On-line techniques: general considerations

Since the late eighties the automation of sample preparation in liquid chromatography has been explored for the determination of pesticides and their metabolites in environmental waters [4,5], which allowed reducing sample manipulation and total analytical time. Currently automation in SPE extraction and injection in a LC system, called on-line SPE, can be achieved by two main configurations: (*a*) a SPE extractor, serially connected with the LC system by switching valves, which uses disposable SPE cartridges, such as e.g. Symbiosis^{TM} from Spark Holland (Emmen, The Netherlands). These systems provide a constant SPE flow rate and allow to run sample preparation and LC analysis in parallel. High pressure SPE cartridges with 8 μm particles are available to increase the selectivity and reduce the elution volumes; (*b*) a dual-column switching set-up which serially connects the extraction or purification column with the separation one by using one or two multichannel switching valves [6]. This configuration, which employs a dual LC column systems, one for pre-concentration and the other for analytical separation, can be implemented in every LC system, but it is also commercialized as stand-alone prep station, such as e.g. EQuan^{TM} system developed by Thermo Fisher Scientific (Waltham, MY, USA).

The different configurations and solutions of on-line SPE systems were recently reviewed and exhaustively discussed from the technical point of view [2]. Instrumental set ups can vary from the simplest, where the same column acts as concentration and separation phases [7], to the most complex with a robotic autosampler which controls up to three multi-way valves [2].

On-line techniques have some advantages, such as reduction of solvent consumption, reuse of SPE columns, time savings and less exposure to hazardous solvents, but also some disadvantages because the system is less flexible in the choice and combination of extraction solid phases and it is not possible to add internal standards after the extraction step [8,9]. Sample manipulation is reduced to centrifugation or filtration and thereby the contamination from laboratory materials and atmosphere, which could be very significant for plastic additives, perfluorinated compounds (PFAS) and metabolites of caffeine and nicotine, is avoided. But minimal sample pretreatment can lead to inject environmental samples with a heavy load of particles and matrix components which can be accumulated and eluted from the front of the extraction column when the elution is carried out in the common backflush mode. If more complex environmental samples, such as wastewater or extracts of solid samples, shall be managed, a cleanup phase must be added to the enrichment step, which is based on restricted access media (RAM) or turbulent flow chromatography (TFC) described in Section 3.

Automation with extraction of high volume samples [10] is the preferable way to achieve high concentration factors and thereby low detection limits of emerging contaminants, but the use of on-line techniques drastically reduces sample volume, which is a factor that weights upon sampling time, transport and storage, and increases productivity, allowing to increase sampling frequency or extend the sampling area for monitoring purpose [11].

The challenge of the on-line SPE methods is still to optimize pre-concentration and elution procedures to achieve a satisfactory accuracy in a single run for a wide numbers of EC which include classes of compounds with different physico-chemical variables. As noted by [3], it is very uncommon to find papers which detail the optimization procedure and highlight the difficulties and the failures of the techniques. For example the optimization of an on-line SPE–UHPLC method for the determination of 12 PFAAs in

Table 1
On-line SPE methods for the analysis of emerging contaminants in environmental samples.

Substances	Matrices	Analytical technique; injection volume	SPE column elution mode	Analytical column	Limit of detection	Recovery (%)	Ref.
Perfluorinated compounds							
12 PFAA	Drinking and surface waters	UHPLC–HESI–MS/MS; 5 mL	Hypersil Gold aQ (20 mm × 2.1 mm, 12 μm); Back-flush	Hypersil Gold PFP (50 mm × 2.1 mm; 1.9 μm)	0.2–5 ng L^{-1}	76–134 for PFCA; 87–115 for PFSA	[11]
PFOA and PFOS	Tap, river and pond waters, water eluate from the frying pan by heating	In tube-SPME–LC–MS; 40 μL	GC capillary column (CP-Pora PLOT amine, 60 cm × 0.32 mm I.D.)	Inertsil ODS-3 (50 mm × 2.1 mm, 5 μm)	1.5–3.2 ng L^{-1}	(81.1 ± 4.1)–(85.4 ± 2.1)	[14]
PFOS	River water	TFC–LC/APPI-MS; 1 mL	TFC C18 (50 mm × 1 mm I.D., 50 μm porous particles); Back-flush	Zorbax Eclipse XDB C18 (150 mm × 4.6 mm I.D., 5 μm)	5.35 ng L^{-1}	94.3–97.2	[27]
21 PFAS	Fish (after digestion and solvent extraction)	TFC–LC/MS–MS	C18 XL and Cyclone (50 mm × 0.5 mm, 60 μm)	Hypersil Gold PFP (50 mm × 3 mm, 3 μm)	0.007–0.2 ng L^{-1}	50–120	[30]
21 PFAS (PFAA, PFOSA, PFPA)	Ultrapure and tap water, treated wastewater	LC–MS/MS; 5 mL	Hypersil GOLD aQ (20 mm × 2.1 mm, 12 μm); Straight-flush	Hypersil Gold PFP (50 mm × 3 mm, 3 μm)	0.27–11 ng L^{-1}	34-–126	[44]
6 PFAA	Groundwater and aqueous soil extracts	LC–ESI–MS/MS; 1 mL	Hypersil Gold Silica based C18 (20 mm × 2.1 mm, 12 μm)	Hypersil Gold Silica based C18 (50 mm × 2.1 mm, 3 μm)	2.4–8.3 ng L^{-1}	–	[45]
8 PFAA and FOSA	River water	UHPLC–MS/MS; 350 μL	Poros HQ (30 mm × 2.1 mm, 10 μm); Back-flush	Zorbax Eclipse XDB-C18 (50 mm × 4.6 mm, 1.8 μm)	3–15 ng L^{-1} (LOD) 9–49 ng L^{-1} (MDL in river water matrix)	91.2–101.9	[46]
PFOA and PFOS	River water	nano-LC–nanospray-MS; 1 mL	Kromasil C18 (5 mm × 1.0 mm I.D.; 5 μm, 100 Å)	Two columns (150 mm × 0.1 mm I.D. and 150 mm × 0.3 mm I.D) packed with 3.5 μm 100 Å Kromasil C18 particles	0.5–1 ng L^{-1}	95	[47]
Pharmaceutical products							
Macrolide antibiotics	River water; primary and final sewage effluent	LC–ESI–MS/MS; 1 mL	RAM-Capcell PakMF Ph-1 (20 mm × 4.0 mm, 5 μm); Back-flush	Symmetry C18 (150 mm × 4.6 mm, 5 μm)	2–6 ng L^{-1}	86.5–98.3	[19]
Omeprazole	River and estuarine water and wastewater	LC–UV–vis; LC-IT-MS/MS; 0.5 mL; LC–MS/MS; 1 mL	RAM-BSA C8 (50 mm × 4.6 mm, Luna, 10 μm, 100 Å); Straight-flush	Tris-(3,5-dimethylphenylcarbamate) of amylose coated onto APS-Nucleosil (150 mm × 4.6 mm, 500 Å, 7 μm, 20%, w/w)	5000 ng L^{-1} (UV–vis), 25 ng L^{-1} (IT-MS/MS)	96.9–110 (LC–UV–vis) 91–113 (LC–ITMS/MS)	[20]
Antiepileptics	River water	LC–MS; LC–MS/MS; 50 mL	RAM-MIP for cyclobarbital (10 mm × 4.0 mm); Back-flush	Cosmosil 5C18-MS-II (150 mm × 2.0 mm) and Guard column Cosmosil 5C18-MS-II (10 mm × 4.6 mm)	0.5–5 ng L^{-1}	–	[23]
Nonsteroidal anti-inflammatory drugs (NSAIDs)	River water	LC–MS/MS; 50 mL	RAM-MIP for flufenamic acid (10 mm × 4.0 mm); Back-flush	Cosmosil 5C18-MS-II (150 mm × 2.0 mm) and Guard column Cosmosil 5C18-MS-II (10 mm × 4.6 mm)	0.1–0.15 ng L^{-1}	90–100	[24]
58 Pharmaceuticals and 19 metabolites (antibiotics and metabolites, cardiovascular drugs and metabolites, β-Agonists, barbiturates, antidiabetic and metabolite, antineoplastic)	Ground-, river and wastewaters	TFC–LC–ESI–MS/MS; 7.5 mL in total for ESI pos and ESI neg runs	TurboFlow^{TM} Cyclone P, C18-P XL and Cyclone MAX (all 50 mm × 1.0 mm, porous particle 30 μm) connected in line	Betasil phenyl-hexyl (50 mm × 3 mm, 3 μm)	0.01–49.3 ng L^{-1}	16–184	[28]
25 Antibiotics: tetracyclines, macrolides, fluoroquinolones, quinolones, cephalosporines, lincosamides, sulfonamides, dihydrofolate reductase inhibitor	Tap, surface and wastewater	TFC-HRMS LTQ-Orbitrap; 10 μL	TurboFlow^{TM} Cyclone (50 mm × 0.5 mm, porous particle 60 μm, 60 Å)	Hypersil Gold (50 mm × 2.1 mm, 3 μm)	10–53 ng L^{-1}	(35 ± 15)–(128 ± 4)	[31]
Sulfonamides (SAs) and metabolites	Groundwater	LC–ESI–MS/MS; 5 mL	Oasis HLB	Atlantis C18 (150 mm 2.1 mm, 3 μm) + guard column	0.09–11 ng L^{-1}	34.3–134.4	[32]
Illicit drugs (nicotine, cocaine, benzoylecgonine, morphine,	Environmental waters	LC–ESI–MS; 10 mL	Stainless steel precolumn (20 mm × 2 mm I.D.) packed		0.5–2 ng L^{-1}	63–105 (river water)	[33]

Table 1 (*Continued*)

Substances	Matrices	Analytical technique; injection volume	SPE column elution mode	Analytical column	Limit of detection	Recovery (%)	Ref.
6-acetylmorphine, codeine, dihydrocodeine, EDDP and methadone			manually with Oasis WCX (particle size of 30 μm); Back-flush	Fused-Core Ascentis Express C18 (50 mm 4.6 mm, 2.7 μm)		51–115 (WWTP)	
40 Pharmaceuticals (analgesic, NSAIDs, antibiotics, antineoplasic, anti-epileptic agent, antifungal, histamine H2 receptor, andepressants, beta-blockers, diuretic, hormones, lipid regulators)	Tap and surface water	LC–ESI–MS/MS; 1 mL	Strata-X (20 mm × 2.0 mm, 25 μm) positive mode, Hypersil Gold C18 (20 mm × 2.1 mm, 12 μm) negative mode	Hypersil Gold C18 (150 mm × 2.1 mm, 3 μm for ESI+ and 100 mm × 2.1 mm, 3 μm for ESI−)	0.7–15 ng L^{-1} (drinking water), 2–15 ng L^{-1} (surface water)	78.6–130.5 (surface water)	[34]
Veterinary antibiotics	Swine wastewater	LC/MS/MS; 100 μL	Oasis HLB (20 mm × 2.1 mm I.D., 25 μm)	Zorbax SB-Aq (100 mm × 2.1 mm I.D., 1.8 μm)	10–100 ng L^{-1}	87–100	[35]
Illecit drugs: benzodiazepines, cannabinoids, hallucinogens, stimulants, opioids	Influent wastewater	LC–ESI–MS/MS; 1 mL	Oasis HLB (20 mm × 2.1 mm, 15 μm); Back-flush	Hypersil Gold aQ (50 mm × 2.1 mm, 5 μm) + guard column	1–50 ng L^{-1} (LOQ)		[36]
25 Human and veterinary pharmaceuticals drugs of abuse	Surface and waste-waters	UHPLC–MS/MS	Strata-X (20 mm × 2 mm, 25 μm)	Zorbax Eclipse XDB-C18 (50 mm × 4.6 mm, 1.8 μm)	2–400 ng L^{-1}	>85 ± 13	[37]
Antibiotics, antiviral, nasal decongestants	Treated sewage effluent and surface water	LC–MS/MS; 1.1 mL	Hypersil Gold C18 (20 mm × 2.1 mm, 12 μm); Back-flush	Hypersil Gold C18 (50 mm × 2.1 mm, 3 μm) and Guard column C18 (2 mm × 2 mm, 3 μm)	0.2–3.1 ng L^{-1}	30–157 (effluent) 21–152 (surface water)	[55]
Nerve agent degradation products	River water and water from a rain pool	LC–ESI–MS; 0.5 mL	Hypercarb (10 mm × 2.1 mm, 5 μm); Back-flush	ZIC-HILIC (150 mm × 1 mm, 3.5 μm, 200 Å)	10–70 ng L^{-1}	80–106	[56]
Sulpiride	River water	LC–MS; 2 μL	I-MIP packed into a stainless steel column (100 mm × 4.6 mm i.d.)	(100 mm × 4.6 mm D.I.)	3900 ng L^{-1}	97–102	[57]
Sulfonamides (SAs)	Wastewater	LC–MS/MS; 2.5 (influent) −5 mL (effluent);	Oasis HLB	Atlantis C18 (150 mm 2.1 mm, 3 μm) + guard column	0.01–2.35 ng L^{-1}	–	[58]
12 Licit and illicit drugs (benzodiazepines, fluoxetine, carbamazepine, codeine, morphine, amphetamine)	Water from influent and effluent of WWTP	HPLC–MS/MS; 5 mL	Pursuit C18 (20 mm × 2.0 mm, 10 μm)	Varian Pursuit C18 (150 mm × 2.0 mm, 4.6 μm)	5–150 ng L^{-1}	–	[59]
18 Drugs of abuse: Amphetamine-like compounds; Cocainics; Cannabinoids; Opioids; Lysergic acid	Sewage water	LC–HESI–HRMS; 5 mL	HyperSep Retain PEP SPE (20 mm × 2.1 mm, 12 μm)	Hypersil Gold aQ (150 × 2.1 mm,3 μm); guard column Hypersil Gold^{TM} aQ (10 × 2.1 mm, 3 μm)	0.6–1.7 ng L^{-1}	83–163	[60]
Caffeine and carbamazepine	Water samples from streams, brooks, and storm sewer outfall pipes	LC–ESI–MS/MS; 1 mL	Hypersil Gold C18 (20 mm × 2.1 mm, 12 μm); Back-flush	Hypersil Gold C18 (50 mm × 2.1 mm, 3 μm) and Guard column Hypersil Gold C18 (2 mm × 2 mm, 5 μm)	0.2–9 ng L^{-1}	87–110	[61]
Cytostatic anticancer drugs and metabolites	Groundwater, river water and WWTP effluent and influent	LC–MS/MS; 5 mL	PLRP-s (10 mm × 2 mm, 15-25 μm)	Purospher STAR RP-18 ec (125 mm × 2.0 mm, 5 μm)	0.1–54 ng L^{-1}	(60 ± 3)–(120 ± 11)	[62]
Chemotherapeutic agents	Municipal wastewater	LC–ESI–MS/MS; 2 mL in 1 mL loop	Hypersil Gold PFP (20 mm × 2 mm, 12 μm)	Hypersil Gold PFP (100 mm × 2.1 mm, 3 μm) + guard column (10 mm × 2.1 mm, 3 μm)	4–20 ng L^{-1}	(47 ± 22)–(90 ± 10)	[63]
Endocrine disruptors compounds (EDCs) and compounds suspected to be EDCs							
Bisphenol A	Tap and lake waters; effluent from WWTP	LC–ESI–MS/MS; 100 mL	Immunoaffinity monolithic column (50 mm × 4.6 mm) with BPA antibodies covalently immobilized; Back-flush	Diamonsil C18 (150 mm × 4.6 mm, 5 μm)	0.3 ng L^{-1}	87–113	[15]
Alkylphenolic compounds and steroid sex hormones	River sediment (after PLE)	LC–MS; 25 μL	RAM-LiChrospher ADS C4 (25 mm × 4 mm, 25 μm); Back-flush	LiChrospher 100 RP18 (250 mm × 4 mm, 5 μm) and Guard column LiChrospher 100 RP18 (4 mm × 4 mm, 5 μm)	0.5–5 ng g^{-1}	62–104	[21]
EDCs (natural and synthetic estrogens and their conjugates, antimicrobials, parabens, bisphenol A, alkylphenolic compounds, benzotriazoles and organophosphorus flameretardants)	Sediment and sewage sludge (after PLE)	TFC–LC–MS/MS; 20 μL	NI: Cyclone (50 mm × 0.5 mm), PI: Cyclone-MCX (50 mm × 0.5)	NI: Hypersil GOLD (75 mm × 2.1 mm, 3 μm), PI: Purospher STAR RP-18 (125 mm × 2 mm I.D., 5 μm)	0.0025–321 ng L^{-1}	53–115	[29]

Table 1 (*Continued*)

Substances	Matrices	Analytical technique; injection volume	SPE column elution mode	Analytical column	Limit of detection	Recovery (%)	Ref.
Bisphenol A and its chlorinated derivatives	River, WWTP, and drinking waters	LC–MS/MS; 1 mL	Hypersil Gold C18 (20 mm × 2.1 mm, 12 µm, 175 Å)	Ascentis Express C18 (fused-core)	–	–	[38]
Bisphenol A, bisphenol AF, tetrachlorobisphenol A, and tetrabromobisphenol A	Tap, source and river waters, effluent from WWTP	UHPLC–ESI–MS/MS; 2.5 mL	Direct Connect HP XBridge^{TM} C18 column (30 mm × 2.1 mm, 10 µm); Back-flush	Acquity Shield RP18 (100 mm × 2.1 mm, 1.7 µm)	0.5–18 ng L^{-1}	85.1–110.8	[39]
Natural and synthetic estrogenic endocrine disruptors	Raw sewage and treated wastewater effluent and drinking water intakes	LC–MS/MS; 1–3 mL	Hypersil Gold C18 (20 mm × 2.1 mm, 12 µm); Back-flush	Hypersil Gold (50 mm × 2.1 mm, 3 µm) and Guard column Hypersil Gold (2 mm × 2 mm, 5 µm)	2–50 ng L^{-1} (1 mL) 0.5–25 ng L^{-1} (3 mL)	85–110	[40]
Endocrine disruptors compounds (EDCs) and compounds suspected to be EDCs (natural and synthetic estrogens, antimicrobials, disinfectants, preservatives, BPA, alkylphenolic, anticorrosives, organophosphorus flame retardants compounds, and caffeine)	Surface river and waste-waters	LC–MS/MS; 5 mL (surface river water), 2 mL (waste water)	Hypersil Gold Aqua (20 mm × 2.1 mm, 12 µm), Back-flush	LiChrospher 100 RP18 (250 mm × 4 mm)	0.0024–62 ng L^{-1} (surface water), 0.053–187 ng L^{-1} (wastewater)	54–128 (surface water), 51–137 (wastewater)	[42]
Emerging contaminants (7 estrogens, 3 progestogens and 5 acidic pesticides)	Sewage, river and drinking waters	LC–ESI–MS/MS; 20 mL	PLRP-s (10 mm × 2 mm I.D., 15–25 µm particle size) and Hysphere Resin GP (10 mm × 2 mm I.D., 10–12 µm)	Purospher Star RP18 (125 mm × 2 mm I.D., 5 µm)	0.03–3.94 ng L^{-1}	69–108	[43]
Endocrine-disrupting compounds	Sewage samples from WWTP	UHPLC–MS/MS; 5 mL	Oasis HLB (2.1 mm × 30 mm, 20 µm)	Acquity BEH C18 (2.1 mm × 50 mm, 1.7 µm)	0.3–2.1 ng L^{-1}	–	[64]
8 Estrogenic and progestagenic steroid hormones	Urban wastewater	LC–APCI–MS/MS; 1–10 mL	Two Hypersil Gold aQ (20 mm × 2 mm, 12 µm) in tandem	Hypersil Gold (100 mm × 2.1 mm, 1.9 µm, 55 °C)	8–60 ng L^{-1}	71–95	[65]
Estrogens and androgens	River water; influent and effluent from WWTP	HPLC–ESI–MS/MS; 50 mL	IonPac NG1 (4 mm × 35 mm); Back-flush	Acclaim PA2 (3 mm × 150 mm, 3 µm)	0.5–5 ng L^{-1}	31.8–120.0	[66]
20 Natural and synthetic steroid hormones	Surface and waste-waters	LC–APPI–MS/MS; 30 mL (liquid sample)	Either Oasis HLB and HySphere-C18(EC) (10 × 2 mm)	HyPURITY C18 (250 mm × 2.1 mm, 5 µm)	1.3–6.9 ng L^{-1} (liquid sample)	28–195 (liquid sample)	[67]
Alkylphenolic compounds and steroid sex hormones	Liquid and solid samples from WWTPs	UHPLC–ESI–MS/MS	two Oasis HLB in parallel (50 mm × 2.1 mm, 20 µm)	Acquity BEH C18 (50 mm × 2.1 mm, 1.7 µm)	0.3–2.1 ng L^{-1} (liquid sample), 0.1–1.9 ng g^{-1} (solid samples)	72–104	[68]
Personal care products and other emerging contaminants							
Benzotriazole UV stabilizers (BUVs)	River water, sewage influent and effluent	HPLC–MS/MS; 20 mL	Acclaims Polar Advantage II (PA II) (33 mm × 3.3 mm, 3 µm, 120 Å); Back-flush	SymmetryShield C18 (150 mm × 4.6 mm, 5 µm)	0.21–2.17 ng L^{-1}	76–114	[49]
Benzotriazole UV stabilizers (BUVSs)	Coastal marine and waste-waters	UPLC–MS/MS; 5 mL	Oasis HLB Direct Connect HP (30 mm × 2.1 mm, 20 µm)	Acquity UPLC BEH C18 (50 mm × 2.1 mm, 1.7 µm)	0.9–4.5 ng L^{-1}	(58 ± 9.1)–(94 ± 7.1)	[50]
1H-Benzotriazole, 5-methyl-1H-benzotriazole and 2 (2H-benzotriazol-2yl)-4-methyl-6-(2-propenyl) phenol	Wastewater	HPLC–MS/MS; 5 mL	Bond Elut, PLRP-s (15–25 µm)	LiChroCART Purospher STAR RP-18 ec (125 mm × 2.0 mm, 5 µm); guard column LiChroCART 4-4 Purospher STAR RP-18 ec (5 µm)	0.3–1.1 ng L^{-1}	(84 ± 7)–(109 ± 7)	[69]
Sucralose	Seawater	HPLC–HESI–HRMS; 10 mL	Hypersep Retain PEP (20 mm × 3 mm, 12 µm)	Hypersil Gold (50 mm × 2.1 mm, 3 µm) + guard column (10 mm × 2.1 mm, 3 µm)	1.4 ng L^{-1}	98.06 ± 0.04	[51]
Phthalates (DEHP, DEP, DBP, MEHP)	Sea and transition waters	In Tube-SPME–Cap-LC–DAD, 4 mL	GC TRB-5 capillary column (40 cm × 0.32 mm I.D.,coated with 5% diphenyl –95% polydimethylsiloxane, 3 µm coating thickness)	Onyx Monolithic C18 (150 mm × 0.2 mm I.D.)	5–1500 ng L^{-1}	95 ± 4–103 ± 7	[13]
						91 ± 5	[52]

Table 1 (*Continued*)

Substances	Matrices	Analytical technique; injection volume	SPE column elution mode	Analytical column	Limit of detection	Recovery (%)	Ref.
Di(2-ethylhexyl) phthalate (DEHP)	Bivalves (after extraction by MSPD)	In Tube-SPME–Cap-LC–DAD; 2 mL	GC TRB-5 capillary column (40 cm × 0.32 mm I.D.,coated with 5% diphenyl –95% polydimethylsiloxane, 3 μm coating thickness)	Zorbax SB C18 (105 mm × 0.5 mm I.D., 3.5 μm)	10,000 ng L^{-1} in extract; 170 ng g^{-1} for 0.1 g of lyophilized mussel		
Di(2-ethylhexyl) phthalate (DEHP)	Coastal sediment (after extraction by MSPD)	In Tube-SPME–Cap-LC–DAD; 3 mL	GC TRB-5 capillary column (40 cm × 0.32 mm I.D.,coated with 5% diphenyl –95% polydimethylsiloxane, 3 μm coating thickness)	Zorbax SB C18 (35 mm × 0.5 mm I.D., 5 μm)	8000 ng L^{-1} in extract; 90 ng g^{-1} for 0.3 g sediment	80 ± 20	[53]
Microcystins	Drinking water	Flow-Injection-SPE–ESI–MS	RP-SDVB Disk Monolithic column (12 mm diam., 3 mm thicknesss)	–	1.3 ng injected		[7]
Microcystins and nodularin	Surface and drinking waters	UHPLC–MS/MS; 1 mL	HLB (20 mm × 2.1 mm, 25 μm); Back-flush	Acquity UPLC BEH C18 (50 mm × 2.1 mm, 1.7 μm)	0.5–40 ng L^{-1}	71–116	[54]
Multiresidual methods							
88 Polar organic micropollutants (pesticides, pesticide TPs, pharmaceuticals, pharmaceuticals TPs, biocides, biocide TPs, corrosion inhibitors, artificial sweetener)	Ground-, surface and waste-waters	HPLC–MS/MS; 20 mL	SPE cartridge prepared in-house with 10 mg Oasis HLB (15 μm) + 10 mg of a mixture of Strata X-AW (33 μm), Strata X-CW (25 μm) and Isolute ENV+ (70 μm) in a ratio of 1/1/1.5 (X-AW/X-CW/ENV+); Back-flush	Atlantis T3 (150 mm × 3.0 mm I.D., 3 μm), in-line filter (0.5 μm pore size)	0.1–206 ng L^{-1} (LOQ)	22–297	[17]
37 Substances: pesticides, pharmaceuticals, steroids, alkylphenols, chlorophenols, plasticisers	Surface water	LC–MS/MS; 2.5 mL	Oasis HLB (20 mm × 2.1 mm, 5 μm); Back-flush	Kinetex XB C18 (100 mm × 2.1 mm, 1.7 μm)	0.1–10 ng L^{-1} (LOQ)	(86 ± 1)–(114 ± 2)	[70]
Micropollutants	Drinking, surface, and waste-waters	UHPLC–HRMS; 1 mL	C18Hypersil Gold (20 mm × 2.1 mm, 12 μm); Back-flush	C18 core–shell Accucore RP-MS (50 mm × 2.1 mm, 2.6 μm)	–	–	[71]
Trace organic compounds: pharmaceuticals, personal care products, industrial compounds, hormones, pesticides	Wastewater, surface water, groundwater, septic tank	LC–MS/MS; 1.7 mL	PLRP-s (12.5 mm × 2.1 mm, 15-20 μm); Back-flush	Poroshell 120 EC-C18 (50 mm × 2.1 mm, 2.7 μm)	0.1–13.1 ng L^{-1}	(69.2 ± 8.3)–(145.1 ± 1.8)	[72]

water samples was described in detail in Ref. [11]. The optimization process of an on-line SPE method can be long and troublesome procedure because the effects of the elution gradient, sample volume as well as matrix modifications should be investigated. Understanding how these factors impact on an on-line SPE methodology helps to develop a rapid and reliable method for the simultaneous determination of ECs in water. Generally, the coupling of on-line SPE with UHPLC is not straightforward because of the high back pressure generated by the combination of a high flow rate with low particle size (<2 μm). The use of on-line SPE coupled with UHPLC has made possible the development of faster methodology, by reducing the analysis time and thus increasing the sample throughput. However, if a multi-residual analysis of a series of analytes with a broad polarity range as the emerging contaminants is carried out, it may be difficult to achieve a satisfying analysis for all target compounds because of the variability in SPE recovery and the loss of chromatographic efficiency. Ideally by the time the extraction column is switched into the analytical flow path, the trapped analytes should be eluted and re-focused onto the analytical column by the analytical elution gradient. However in multi-residual analysis, the gradient elution for reversed-phase separations usually starts with high percentage of water in the mobile phase, and the slow elution from the SPE pre-concentration column results in peak broadening, which may cause a decrease in sensitivity [12].

In the on-line SPE method, during the sample loading step, analytes are trapped by the stationary phase of the pre-concentration column. Then elution of analytes is achieved in back-flush mode by putting in-line the pre-concentration column with the eluting mobile phase.

The first approach is to transfer the separation gradient elution program, optimized by direct injection of analytes, to the on-line procedure. In this case the gradient program started soon after the end of the loading of the sample into the pre-concentration column. By using these settings, peak broadening and distortion has been observed for the more polar compounds, which show lower affinity for the pre-concentration column stationary phase, are poorly focused on the pre-concentration column and are transferred as a broaden band into the separation column. To overcome this problem it is possible to implement the “solvent plug injection technique” [12]. This technique provides elution bands of only a few seconds width with high percentage solvent in order to obtain a rapid transfer of the analytes from the pre-concentration column to the chromatographic column as well as to keep them focused. The high-elution strength solvent plugs can be generated by the same UHPLC pump, inserting a high percentage solvent step in the elution gradient at the beginning of the elution, in order to provide narrow high-elution strength eluent band with all the analytes [11,12]. A simple alternative way is to anticipate the start of the gradient program on separation column when the loading on pre-concentration column is still on going, and by this way, when the mobile phase is switched on the pre-concentration column, mobile phase has already an higher solvent percentage, which makes possible to quickly transfer analytes on the separation column with a focusing effect [11].

In a field different from environmental chemistry, a novel fractionized sampling and stacking strategy (FSS) for online SPE–UHPLC has been proposed [2]. FSS was a peak compression strategy developed to prevent band broadening and distortion caused by excessive solvents with high elution strength, which has been a main obstacle to conjunction of sample preparation techniques with UHPLC. Such a strategy was based on online dividing a sample solution into fractions by plugs of weak mobile phase, followed by head-column stacking process, aiming to obtain a quite sharp sample zone. FSS enables UHPLC to tolerate much larger injection volume of solvents with high elution strength.

3. Stationary phases for on-line solid phase extraction

If a simultaneous analysis of EC with different physico-chemical characteristics shall be developed, the choice of sorbent type is one of the most critical step. Few specific applications of in-tube-SPME–LC, which used a short piece of GC capillary column (40–60 cm) as adsorption phase, for the extraction of phthalates [13] PFOA and PFOS [14] were described. A part from these experimental works, the most of the on-line extraction of ECs in environmental samples used solid sorbents with particles of larger diameters (15–70 μm particles) for high-throughput analysis. Higher flow rates during the extraction can be achieved by using short monolithic columns or disks [7] which seem to be a promising SPE material for fast on-line extraction. Monolithic phases, derivatized with antibodies for bisphenol A (BPA), allowed the highly selective on-line extraction of BPA from surface and waste waters [15]. Synthetic organic-inorganic hybrid monolithic column extracted β-lactam antibiotics from river waters [16]. In these examples monolithic phases were in-house synthetized and derivatized for the target analytes, but the employ of the monolithic phases in current monitoring has been restrained by the limitation in the commercially available adsorption chemistries.

On the contrary in the market there is a wider choice in particle sorbents which includes traditional silica-bonded phase, polymeric materials, ion-exchange, mixed mode sorbents.

The need to extract a significant volume of water without the risk of loss of performance or poor stability and retain hydrophilic compounds makes preferable to employ polymeric phases with increased wettability. The wettability of reversed phase media can be increased by the introduction of polar functional groups introduced as silanol end-capping (e.g. Thermo Gold AQ and analogous phases) or included in the polymeric backbone (e.g. *N*-vinylpyrrolidone in Waters Oasis HLB or derivatized piperidones in Phenomenex Strata X). The selectivity of polymeric phases can be modulated by introducing functional groups in the polymers which modify also the principal partition or adsorption mechanism and transform reverse phases even in anion and cation exchangers.

Two divergent approaches were followed for selectivity tuning in the on-line extraction of emerging contaminants. The first is to tailor mixed mode cartridges to the wide polarity range of ECs in multicomponent analysis, by filling each extraction cartridge with hydrophobic, WAX and WCX phases [17]. The opposite approach is to maximize selectivity for specific classes of compounds by using molecular imprinted polymers (MIP) which is a technology where recognition sites are created by copolymerization of a target molecule in a macromolecular matrix [9]. MIPs are effective in extraction of polar compounds because the polymer-molecules interaction is not based on hydrophobic mechanisms and yield cleaner extracts than classic hydrophobic phases.

MIPs have been demonstrated to be able to reduce the matrix background in the on-line extraction of pesticides and pharmaceuticals [9], but they were often employed in combination with RAM which excludes biopolymers and biological molecules on the basis of size exclusion principles (Table 1). RAMs are porous sorbent which have a bimodal mechanism: adsorption of low weight molecules on the biocompatible surface and elimination of larger molecules, such as humic acids or biopolymers, by size exclusion on a certain pore size (common cut-off is about 15 kDa).

RAMs, used in the automation of extraction of environmental samples, consist of silica porous spherical particles, with diol groups bonded to the external surface and alkyl chains (C4, C8, or C18) bonded to the internal surface of the pores, are currently used [18]. Other RAMs possess long hydrophilic polyoxyethylene chains and hydrophobic phenyl groups on the surface of 80 Å silica which limit the access of large molecules and retain small molecules longer [19]. The capacity of a hydrophobic (C8 or C18) RAM coated by bovine serum albumin (RAM BSA) to exclude humic and fulvic acids in river and waste waters has been discussed for the determination of selected pharmaceuticals [20]. RAM worked also as an on-line purification system of solvent extracts coming from the pressurized liquid extraction (PLE) of sediment for the determination of estrogenic compounds [21].

First attempts of coupling RAM and MIP phases were based on a two steps procedure: the sample was firstly fractionated on RAM column and then transferred by an organic solvent to MIP phase [22]. Currently an integrated RAM–MIP phase, where MIP adds selectivity features to the clean-up potency of RAM, was prepared by a multi-step swelling and polymerization method followed by a hydrophilic surface modification technique [23]. RAM–MIP could exclude water-soluble oligomers of humic materials and selectively recognize target compounds, such as antiepileptics [23] and non steroidal anti-inflammatory drugs [24], in river waters.

The alternative for the on-line purification from biomolecules is TFC, which is a technique introduced in the late 1990s for the analysis of biological fluids that combines high-throughput, high reproducibility and reduced time-consuming samples cleanup [25]. The sample is injected at high flow rate, higher than 1 mL min^{-1}, into 0.5–1.0 mm internal diameter columns packed with large particles (30–60 μm) whose pores are functionalized with different chemistries depending on the type of column. The high linear velocity is possible only because of the low back pressure into the column. Under the turbulent flow conditions the improved mass transfer across the bulk mobile phase allows all molecules to improve their radial distribution, though a laminar zone around the stationary phase particles still exists, where diffusional forces still dominate the mass transfer process [26]. The small molecules, which diffuse faster than large molecules, have time to interact with stationary phase and to bind to pores, while the large molecules are quickly flushed to waste. The resolution capability of this column is low, so the analytical separation is achieved by conventional analytical column using laminar flow.

In recent years this approach was applied to dirty environmental waters, such as e.g. wastewaters, and extracts of sediment samples for the determination of PFAS [27], pharmaceuticals [28] and endocrine disrupters [29] (Table 1). It shows some advantages like minimum sample manipulation, low error introduction, very efficient extraction and good reliability, but exhibits also some limitations. While larger biological molecules are efficiently removed, physical mechanism of exclusion probably is not very efficient for environmental matrix molecules with lower molecular weight (MW), as shown by the UV spectra of wasted fractions of extracts of different origin collected after the TFC treatment (Fig. 1). In fact, target compounds and matrix molecules have similar dimensions and, consequently, same diffusion capability in the stationary phase and would not be washed out by flow. Besides, if the spectrum of polarities and acidities of analytes molecules is wide, it is necessary to use different TFC columns in series to cover the whole range of properties, but, for the same reason, also a large number of matrix molecules may establish bond within the functionalized pores [28].

Notwithstanding these general considerations, TFC found applications in the purification of digested food, included fish, for the determination of 21 PFAS [30]. An interesting application is the coupling of TFC with high resolution mass spectrometer for the detection of unknown compounds, such as the transformation products of tetracycline and erythromycin during enzymatic treatment [31]. On-line TFC as sample clean-up was used to remove matrix, get high robustness and reduce sample manipulation. In addition, this process helps to maintain clean the ultra-high sensitive LTQ-Orbitrap and thus increases its lifetime.

4. Application to environmental samples

On-line extraction and purification techniques have been applied for the determination of various classes of emerging contaminants in environmental samples, both liquid, such as river, ground waste waters, and solid, such as aquatic biota and sediment (Table 1).

4.1. Pharmaceuticals

Nowadays, a large number of analytical methodologies, using different modalities of on-line sample preparation followed by LC–MS/MS, are available for pharmaceutical determination in aqueous and solid environmental samples, being majority of them multi-residual methods targeting compounds from different therapeutical classes (see Table 1). The simultaneous analysis of multi-class pharmaceutical compounds, with quite different physico-chemical characteristics, represents a great challenge and often requires very meticulous optimization of operational parameters. Various authors indicated that optimization of the type of online SPE cartridge or preconcentration column, washing mobile phase composition, washing volume and flowrate are critical to obtain best sensitivity for analysis [28,32,33]. Using completely automatized EQuan^{TM} system Idder et al. [34] developed and validated a multiresidual method for the analysis of 40 pharmaceutical residues in drinking and surface waters. The method was evaluated as very convenient and efficient compared to classical off-line SPE method because of its shorter total run time including sample preparation and smaller sample volume (1 mL vs up to 1 L). The limits of detection are between 0.7 and 15 ng L^{-1} for drinking waters and 2–15 ng L^{-1} for surface waters. Similar configurations using Strata X or Oasis HLB in the enrichment step were proposed for the analysis sulfonamide antibiotics [32], veterinary antibiotics [35], illicit drugs and stimulants [36] and mixtures of human and veterinary drugs and illicit drugs [37]. The instrumental configuration involving turbo-flow chromatography (TFC) for the sample pretreatment followed by LC–ESI–MS/MS was successful applied by López-Serna et al. [28] for the multi-residue analysis of 58 pharmaceuticals and 19 metabolites and transformation products in environmental waters such as groundwater and river water. However, the TFC technology was found to be unsuitable for clean-up and preconcentration of more complex samples such as raw and treated wastewater. As already mentioned in section 3, the physical mechanism of TFC, based on diffusion phenomenon, is highly effective in case when matrix molecules are bigger than 8 kDa (i.e. proteins, carbohydrates, lipids and others biological macromolecules in biological matrices). However, environmental and wastewaters contain impurities and matrix components with MW lower than 2 kDa, thus resulting in poor discrimination of matrix components using TFC.

4.2. Endocrine disruptors compounds

There are several on-line analytical methodologies described in the literature for the determination of EDCs in surface river water and wastewater (see Table 1). Most of these methods cover different groups of EDCs simultaneously, i.e. natural and synthetic estrogens, alkylphenolic compounds and other suspect EDC such as parabens, benzotriazoles, flame retardants. Several dedicated methods have been developed for the analysis of one specific group of compounds with similar polarities, structures or similar activities (for example for the analysis of bisphenol A and its chlorinated derivatives [38], BPA, bisphenol AF, tetrachlorobisphenol A, and tetrabromobisphenol A [39], natural and synthetic estrogens [40]. The most challenging group of EDCs is natural and synthetic hormones, because of extremely low LODs required for their analysis. The European Commission included 17β-estradiol (E2), estrone (E1) and 17α-ethinylestradiol (EE2) in the watch list of substances for Union-wide monitoring and stated in the Decision 2015/495/EU of 20 March 2015 that the maximum acceptable method detection limits are 0.4 ng L^{-1} for 17β-estradiol and estrone, and 0.035 ng L^{-1} for 17α-ethinylestradiol.

However, achieving such low LODs in real environmental samples (i.e. surface water) is extremely difficult task that requires

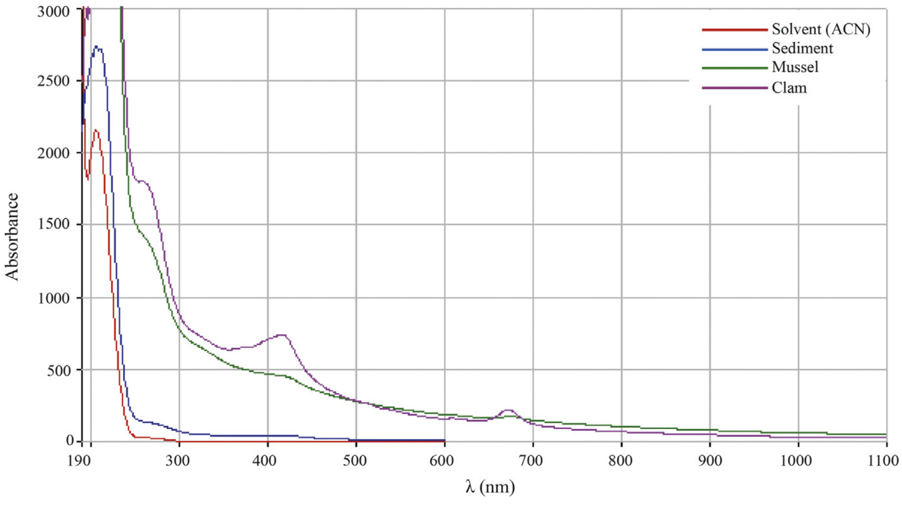


Fig. 1. UV spectra of the wasted fractions of different matrices after the TFC treatment. Blank solvent (red line); extracts of river sediment (blue), mussel (green) and clam (violet). (Valsecchi et al., unpublished results) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

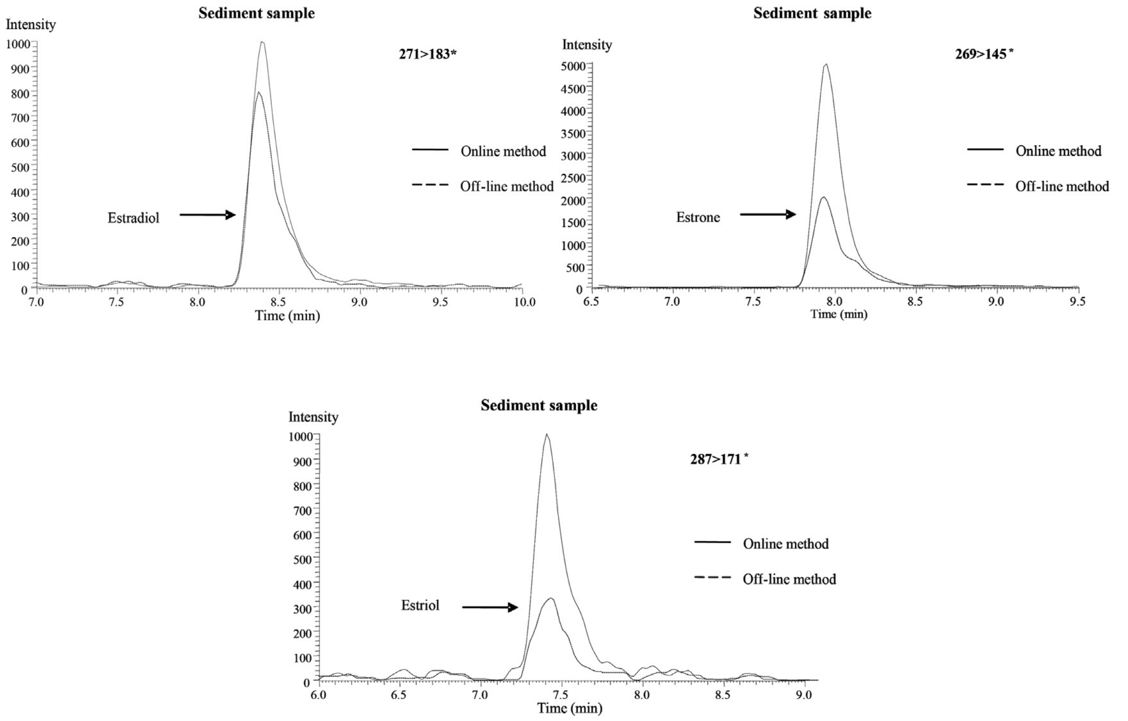
either large volume off-line SPE or specifically optimized on-line approach combined with the high sensitivity detection using the last generation MS/MS instruments [41]. While, several authors reported LODs for E1 and E2 in the range of required 0.4 $\mathrm{ng\,L^{-1}}$ [42,43], none of the methods described in the literature achieves detection of EE2 at required 0.035 $\mathrm{ng\,L^{-1}}$ making the on-line approach particularly suitable and necessary for such demanding task. The application of fully automated method based on dual column switching using TFC–LC–MS/MS for the purification of extracts obtained from sediment and sewage sludge, allowed an improved efficiency of selective extraction and minimization of the matrix effects, as compared to the offline technique [29]. The comparison of reconstructed ion chromatograms of MS/MS detection corresponding to $[\mathrm{M-H}]^-$ of estradiol (E2), estrone (E1) and estriol in off-line SPE–LC–MS/MS and on-line TFC–LC–MS/MS conditions are shown in the Fig. 2, showing significantly lower background noise, and consequently lower LODs in case of on-line TFC purification.

4.3. Perfluorinated compounds (PFAS)

Commercial stations for automated SPE have been used in several monitoring campaigns in order to process dozens of samples [10]. Although these systems allowed to reach higher concentration factors (1:1000) in order to measure PFAS in low contaminated samples such as sea water at $\mathrm{pg\,L^{-1}}$ level [10] and to achieve reduction in sample loading time, the preparation steps are labor intensive and the risk of contamination from manipulation, which is a very important source of errors for PFAS, is not avoided. The implementation of on-line SPE made possible the effective development of faster methods by reducing sample manipulation and analysis time and thus increasing the analytical productivity in the LC–MS/MS determination of PFAS in natural and wastewater samples (Table 1). On-line SPE methods are particularly suitable in studies where only limited sample material is available, as the sample volume can be as low as 1 mL.

The challenge of the on-line SPE methods is still to optimize pre-concentration and elution procedures to achieve a satisfactory accuracy in a single run for a wide numbers of PFAS which include classes of compounds with different physico-chemical variables. The factors that influence the recoveries of the different PFAAs in on-line SPE have been studied and optimized in a study carried out by using a UHPLC–MS/MS system [11]. Extraction has been carried out by using a polar embedded reverse phase column, which has been demonstrated to be able to retain a wide range of PFAS, including perfluoroalkylcarboxylic acids (PFCA, from 4 to 18 carbon atoms), perfluoroalkylsulfonic acids (PFSA, from 4 to 10), perfluoroalkylphosphonic acids (PFPA, 6, 8 and 10) with good LODs and LOQs ranging from 0.8–10 $\mathrm{ng\,L^{-1}}$ to 3–50 $\mathrm{ng\,L^{-1}}$ [11,44]. This method were validated for different types of matrices (ultrapure, tap and waste-water). As an alternative an endcapped, ultrapure silica-based C18 column were also used to preconcentrate a narrower range of PFAA [45]. Furthermore polymeric packings have been tested as pre-concentrators in on-line SPE–UHPLC–MS/MS analysis. While the Dionex HRGP gave a poor sorption, the Poros HQ column, working in perfusion mode, permitted good performances in terms of both sorption and chromatographic separation [46]. Poros HQ columns are polymeric packings, surface-coated with fully quaternary polyethyleneimine, designed for strong anion exchange chromatography of biomolecules in the perfusion mode because they consist of crosslinked poly(styrene-divinylbenzene) flow-through particles with a bimodal pore size distribution for very rapid mass transport.

C18 microbore columns were also used as enrichment column in an on-line SPE with nano-LC/nano-ESI–MS method for rapid and sensitive determination of PFOA and PFOS in river water [14,47]



***Precursor ion > SRM1 (m/z)**
Spiked samples at a final concentration of 25 $\mu\mathrm{gL^{-1}}$, injected volume 20 μL

Fig. 2. Comparison between online and off-line cleanup in estrogen analysis. Reconstructed ion chromatograms of MS/MS corresponding to $[\mathrm{M-H}]^-$ of estradiol, estrone and estriol obtained under NI conditions of sediment spiked samples applying the online and off-line methodologies (Elsevier® 2014, Reprinted with permission from [29]).

developed an automated in-tube SPME method to determine PFOS and PFOA by using an open tubular fused-silica capillary with an inner surface coating as the SPME device. A GC capillary column (60 cm × 0.32 mm i.d.) was used as the in-tube SPME device, and placed between the injection loop and injection needle of the autosampler. CP-Pora PLOT amine (basic modified styrene divinylbenzene polymer) showed the best recovery results compared to other GC phases. The extracted compounds could be desorbed easily from the capillary by the mobile phase, and no carryover was observed. However, in-tube SPME method has some limitations in the extraction of "dirty" environmental samples because the capillary used for the extraction is prone to be clogged.

A rapid on-line SPE–HPLC–MS/MS method was developed for the identification and quantitation of nine PFAS in environmental, biological and food samples [46]. Fish filet was extracted with methanol and the clean supernatant, after dilution and acidification, was subjected to on-line SPE HPLC–MS/MS analysis on a Poros HQ preconcentration phase. Aquatic biota and sediment samples were on-line purified by TFC coupled to LC–MS/MS which is a very promising technique tested for a great variety of food, including aquatic biota, after alkaline digestion and solvent extraction [30,48]. TFC was also applied to determine PFOS at trace level in river water coupling an LC/APPI-MS [27].

4.4. Other emerging compounds

Benzotriazoles were determined in river, marine and wastewaters by using polar embedded reverse phases as enrichment sorbents [49,50], getting LODs of ng L^{-1} level with injection volumes of 5–20 mL. Sucralose in seawater was on-line concentrated by using a highly porous polystyrene divinylbenzene modified with urea functional groups [51].

Phthalates were measured in salted waters by using in-tube-SPME coupled to monolithic capillary LC with Diode Array Detection [13]. The same purification method was used for extracts of lyophilized mussels and sediments [52,53].

Cyanotoxins in drinking waters were measured by on-line SPE–UHPLC–MS/MS using HLB as the concentration phase [54], while a selective screening method for microcystins was developed by directly interfacing the Flow Injection-SPE, with polymeric RP monolithic disks to an electrospray ionization-Ion Trap MS [7].

5. Conclusions

Only the implementation of on-line SPE made possible the effective development of faster methods by reducing the analysis time and thus increasing the analytical productivity. The method is labor-saving and cost effective, especially in the dual column configuration where the SPE column can be reused up to 300 times (depending on the characteristics of the samples). The on-line SPE method are particularly suitable in studies where only limited sample material is available, as the sample volume can be as low as 1 mL. Also, compared to more conventional methods, the on-line SPE method uses very small amounts of solvent for sample cleanup and reduces the risk of sample contamination because often the only sample preparation necessary for water samples is centrifugation.

Most of the multiresidual methods have been developed using wettable and polar embedded reverse phases, sometimes sequentially packed with weak ionic exchangers in order to widen the polarity range of the adsorbents. Interesting alternatives are monolithic columns, also derivatized for immunoaffinity chromatography, or GC column for in-line capillary SPE, which allow to use higher flow rates in the sample extraction step and reduce the system backpressures.

On-line techniques have been also applied in clean-up of extracts of solid environmental samples, such as sediment, soil and biota, in order to minimize the ionization suppression effects from the matrix. Many applications employed RAM, often coupled to monolithic pre-concentration columns, but there is an increasing number of application of TFC for the determination of emerging pollutants in environmental samples. The physical principle of TFC makes this technique highly effective for eliminating large biomolecules, such as in the cleanup of biota extracts, while it is rather ineffective for environmental and wastewaters containing impurities and matrix components with MW lower than 2 kDa.

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