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Miniaturized liquid chromatography in environmental analysis. A review

S. Cortés-Bautista, C. Molins-Legua *, P. Campíns-Falcó *

Department Analytical Chemistry, University of Valencia, 46100, Burjassot, Valencia, Spain

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ABSTRACT

The greater and more widespread use of chemicals, either from industry or daily use, is leading to an increase in the discharge of these substances into the environment. Some of these are known to be hazardous to humans and the environment and are regulated, but there is a large and increasing number of substances which pose a potential risk even at low concentration and are not controlled. In this context, new techniques and methodologies are being developed to deal with this concern. Miniaturized liquid chromatography (LC) emerges as a greener and more sensitive alternative to conventional LC. Furthermore, advances in instrument miniaturization have made possible the development of portable LC instrumentation which may become a promising tool for in-situ monitoring. This work reviews the environmental applications of miniaturized LC over the last 15 years and discusses the different instrumentation, including off- and on-line pretreatment techniques, chromatographic conditions, and contributions to the environmental knowledge.

1. Introduction

In recent decades, numerous efforts from both academic and industry have been made for the development of miniaturized LC, which is considered an important topic in the field of LC. The first reported use of a column with an internal diameter (i.d.) of 1 mm was by Horváth et al. for the separation of ribonucleotides in the late 1960s [1]. Ten years later, Tsuda and Novotny established the required equipment for working with capillary LC, implementing modifications on conventional injectors and detector systems aiming to reduce the band-broadening effects [2]. Table 1 shows the main reviews concerning this topic during the last 15 years. As can be seen, up to 2019, there are revisions about its applications in food [3] and drug analysis [4], the fundamentals of the instrumentation [5–8], and its coupling with mass spectrometry [7,9,10]. Advances in automation and on-line pretreatment configuration have been reviewed for the period 2020 – 2022 [11–16]. However, these contributions have not focused much on miniaturized LC for the analysis of environmental samples. Only one review was published [14], as shown in Table 1. The scope here is the revision of strategies in LC miniaturization based on the publications related to the environmental field over the last 15 years.

The determination of organic pollutants is a very important research topic in the field of environmental analytical chemistry [17,18]. The production and consumption of chemicals has resulted in the discharge of thousands of compounds into the environment, leading to the contamination of water bodies in particular [19]. In addition, these compounds may be present in other environmental matrices such as soil, air, or biota [20]. The high persistence and mobility of some of these substances causes to migrate away from the site of application, reaching surface and ground water [21] and consequently, bioaccumulating in the food chain [22]. The EU Water Framework Directive [23] establishes the control of a list of persistent chemicals in the environment, which can be bioaccumulated and have the risk of causing adverse effects to human health and the environment. These regulated compounds are worldwide controlled and monitored by analytical methods. Many of the substances classified as priority pollutants are pesticides (organochlorides, organophosphates, triazines, sulfonylureas, and other). There are several regulation around the pesticide application and concentration limits in the environment and drinking waters [24–27]. The legal limit established by the Directive (EU) 2020/2184 in drinking water is generally 0.1 $\mu g \cdot L^{-1}$ for individual and 0.5 $\mu g \cdot L^{-1}$ for the total pesticides while, for surface waters, the environmental quality standard is variable for each pesticide, ranging from $ng \cdot L^{-1}$ to $\mu g \cdot L^{-1}$ [28]. Other compounds like volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), alkylphenols, etc. are also included. Another group of unregulated substances known as contaminants of emerging concern (CECs) are receiving high interest, and might be considered in future regulations due to their potential risk to human health and the environment. This group include pharmaceuticals, personal care products, licit and illicit drugs, endocrine disruptors (EDs), newly registered

* Corresponding authors.
E-mail addresses: cmolins@uv.es (C. Molins-Legua), pilar.campins@uv.es (P. Campíns-Falcó).

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Table 1
Main reviews concerning miniaturized liquid chromatography instrumentation and applications over the last 15 years.

Title	Topic	Year	REF
Capillary-liquid chromatography (CLC) and nano-LC in food analysis	Applications	2013	[3]
Current applications of miniaturized chromatographic and electrophoretic techniques in drug analysis	Applications	2014	[4]
From conventional to miniaturized analytical systems.	Instrumentation	2014	[5]
Instrument platforms for nano liquid chromatography	Instrumentation	2015	[6]
Evolution in miniaturized column liquid chromatography instrumentation and applications: An overview	Instrumentation/ applications	2015	[7]
Advances in microscale separations towards nanoproteomics applications	Applications	2017	[8]
Nanoscale separations based on LC and CE for food analysis: A review	Applications	2019	[9]
Nano liquid chromatography columns	Instrumentation	2019	[10]
Nano-liquid chromatography-mass spectrometry and recent applications in omics investigation	Instrumentation/ applications	2020	[11]
A review of portable high-performance liquid chromatography: the future of the field?	Instrumentation	2020	[12]
Miniaturized liquid chromatography focusing on analytical columns and mass spectrometry	Instrumentation	2020	[13]
Miniaturized analytical methods for determination of environmental contaminants of emerging concern- A review	Instrumentation/ application	2021	[14]
Recent advances in on-line upfront devices for sensitive bioanalytical nano LC methods	Instrumentation	2021	[15]
Current advances and applications of online sample preparation techniques for miniaturized liquid chromatography systems	Instrumentation/ applications	2022	[16]

pesticides, surfactants, high-technology rare earth elements, natural and engineered nanomaterials, among others [29]. Hence the development of analytical methods for the sensitive and selective determination of organic pollutants is of great interest [30,31]. Recently, Puri et al. [32] conducted a comprehensive review providing a global overview of policies and regulations concerning emerging contaminants in the environment and biota, emphasizing the need to investigate the presence and potential adverse effects of these substances for their regulation.

Traditionally, gas chromatography (GC) and LC have been used for the determination of environmental pollutants, with GC coupled to mass spectrometry (MS) being the preferred technique for the determination of hydrophobic pollutants and LC coupled to MS [7] for the determination of hydrophilic micropollutants. Stricter regulations, the need to control emerging pollutants, and the search for environmentally friendly methodologies have driven the demand for faster, more cost-effective, and low waste generation analytical methods. Also, the need to detect some substances at ultra-trace levels requires highly sensitive methods. In this context, the miniaturized LC techniques emerge as a suitable tool to achieve these goals.

Miniaturization and automatization are two of the main areas of research in chromatography technology [16]. Miniaturized LC arises as a result of decreasing the analytical column internal diameter, which is directly related to the system flow rate and all others system components by the next scaling factor $(d_1/d_2)^2$, where d_1 and d_2 are the internal diameter of conventional and miniaturized analytical columns, respectively [33]. Table 2 shows the nomenclature initially proposed by Chervet et al. [34] where the column i.d. is correlated with the flow rate.

The reduction on the scale has as main drawback the increase in extra-column volume which results in band-broadening and the consequent loss of efficiency [7]. Nevertheless, much research has been done

Table 2
Denominations of the different chromatographic systems attending to its column internal diameter and flow rate [34].

Denomination	Column i.d.	Flow rate
HPLC	4.6 – 3.2 mm	2.0 – 5.0 mL·min^{-1}
Microbore LC	3.2 – 1.5 mm	0.5 – 0.1 mL· min^{-1}
Micro LC	1.5 – 0.5 mm	100 – 10 μL· min^{-1}
Capillary LC	500 – 150 μm	10 – 1 μL· min^{-1}
Nano LC	150 – 10 μm	1 – 0.1 μL· min^{-1}
Open Tubular LC	50 – 5 μm	< 0.1 μL· min^{-1}

to minimize this effect [35–37] increasing the performance of the different parts of the chromatographic system, particularly in connection tubing and end fittings. Working with lower flow rates supposes a decrease in solvent usage and therefore in waste production. Another consequence of the miniaturization is the low sample volumes needed to carry out the analysis, which is of special importance when the sample is scarce, as in bioanalysis. The reduction in the column i.d. also reduces radial diffusion and hence the analyte concentration increases in inverse proportion with r^2, however this enhancement in sensitivity is countered by the lower injectable volume which is proportional to the amount of stationary phase in the column [38]. Nano liquid chromatography (nLC) makes the coupling to MS easier increasing the sensitivity as nano-electrospray ion sources are much more effective transferring ions to the detector than conventional electrospray [39]. Despite this, nLC is still not very popular for rutinary applications. The difficulties in locating and stopping leaks, along with the problems of clogging due to the small internal diameter (10–50 μm) tubing, have contributed to its reduced use.

On the other hand, the miniaturization of LC has made possible the emergence of portable LC instrumentation. Portable LC is still under development since 1986, however, latest models are managing to solve the challenges derived from portable systems (robustness, portability and, autonomy, among others). Rahimi et al. comprehensively reviewed the different portable chromatographic systems highlighting the trends in the field [12]. Currently, a lot of research has been done to develop new portable LC systems, however, not many applications in environmental field have been reported. It is expected that more applications will be developed in the future due to the possibilities that these instruments offer in place [40].

In the environmental area, usually, independently to the separation technique used, a sample preparation is mandatory. Thus, in addition to the reduction of conventional analytical systems, the downscale of the sample preparation though microextraction techniques can help to overcome the limitations of the conventional procedures. The first miniaturization approach was the solid phase microextraction (SPME) in 1990 [41] and liquid phase microextraction in 1996 [42] and since then other miniaturized extraction techniques have emerged such as sorptive extraction (SBSE) [43] or dispersive liquid-liquid microextraction (DLLME) [44,45] among others, increasing the enrichment factor and reducing the extraction time and solvents usage. Nevertheless, some of these techniques can involve multiple steps, which increase the analysis time, complexity, and the potential risk of sample contamination and loss of analytes. In this context, on-line sample treatment arises as faster, simpler, and greener alternative over off-line procedures. As most sample preparation techniques used in miniaturized LC are not miniaturized yet, the discussion here is focussed on the applied sample preparation techniques, regardless of classical or miniaturized types. Usually, fully miniaturized systems do not allow to process large amount of sample limiting the sensitivity. This problem can be reduced by using trap columns in an on-line column switching system which allows to inject larger sample volumes. As column switching systems, some studies employed commercial trap-packed columns for analyte preconcentration [15]. Another alternative is the use of in-tube (IT)-SPME, in which a tube containing the extractive phase is used in replacement of the injection loop or in the circuit of an automatic injection system [46].

Miniaturized LC has been applied in fields such as food science [47–49], pharmaceutical analysis [50,51], environmental sciences [52, 53], biochemistry (proteomics and lipidomics) [54,55], and others (see Fig. 1). Biochemistry stands out as the predominant application area, representing approximately 65 % of publications, a percentage significantly higher than that of conventional LC, which accounts for approximately 40 % (Web of Science database) although the number of publications are very different, 340,000 and 24,000 for conventional and miniaturized LC (capillary and nano), respectively. nLC coupled to MS/MS is of special interest in proteomics since a high number of proteins at low concentrations can be identified in a single chromatographic run [56]. On the other hand, for areas as pharmacology, food science, toxicology and in particular environmental analysis, the technique has not gained as much attention as shown in Fig. 1. Given the advantages of miniaturized LC and the easier and more accessible the technique has become over time, it opens for potential opportunities in environmental applications.

The aim of this work is to review the strategies proposed in the last 15 years in miniaturized LC (cLC, nLC and portable LC) applied to the analysis of different matrices in the field of environmental analysis. This paper reviews fundamental aspects and advances, such is the case of portable LC instrumentation. Relevant points concerning the sample treatment have been considered in order to implement automated procedures. The article has been focused on the applications described for the determination of organic pollutants. In addition, the strengths, weaknesses, and opportunities of the technique have been discussed.

2. Pumps, injectors, columns, and detectors

The essential instrumentation for any LC consists of a solvent delivery system to carry the mobile phase through the system, an injector to introduce the sample, an analytical column where the separation takes place, and a detector to record the analytical signal. For each of these components, different variants are available depending on the analytical application to be addressed.

Solvent delivery units comprise the mobile phase reservoirs and the pumping system. For modern LC instruments, dual-piston (either in-series or in-parallel) binary or quaternary pumps are commonly used. In-parallel dual-piston pumps provide accurate mobile phase flow rates and compositions with minimal pulsations. Other pumping systems, such as syringe pumps, are reserved for portable LC equipment due to their easier miniaturization [53,57]. The reduction in the column size

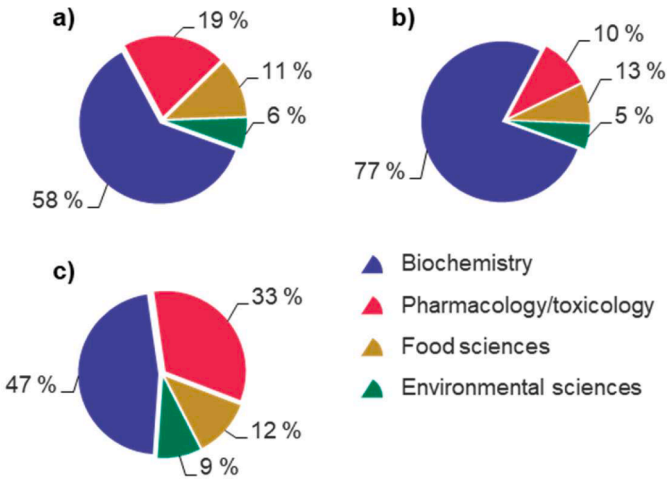

Fig. 1. Percentage of publications reported in the Web of Science database according to the field of application for the topics a) capillary LC (8200 publications), b) nano LC (16,000 publications), and c) conventional LC (340,000 publications) as a topic search in the Web of Science (WoS) (consulted in March 2024).

produces a consequent reduction in the working flow rate which, for the applications reviewed in this paper ranges from 5 to 20 μL min^{-1} for cLC and from 0.01 to 1.7 μL min^{-1} for nLC (Table 3). To achieve nano and capillary flow rates, a mobile phase split valve was coupled to the pump outlet. Nowadays, many pumps are capable to operate at flow rates ranging from μL to nL per minute. Chromatographic run times of 8 to 39 min have been reported, which, at the mentioned flow rates, entails a minimal mobile phase consumption (from 80 to 480 μL per run in the case of cLC and from 2.4 to 160 μL for nLC) compared to conventional LC (various mL per run), as Table 3 shows.

Regarding the injectors, these are typically based on switching injection valves. In these, the sample is first loaded (manually or by means of an automatic injector) into a sample loop of a determined volume and then, by switching the valve position, the mobile phase flows through the loop, transporting the sample to the analytical column. The reduction in the i.d. of the analytical column must be accompanied by a reduction in the injection volume to ensure suitable efficiency. The maximum sample volume that can be injected without efficiency loss can be calculated using the following expression:

$$V_{s,max} = 0.36 \cdot \sqrt{L d_p} \cdot d_c^2 \cdot (k+1)$$

Where L, d_p, d_c, and k are the length, particle diameter, and internal diameter of the analytical column and the retention factor respectively. Following this equation, for a nano column with dimensions 100 mm x 0.1 mm, 3 μm, injection volumes down to 4 nL should be injected [91]. Nowadays, there are commercial injection valves with internal loops in the form of a groove in the valve rotor able to load volumes down to 4 nL. In the literature injection volumes varying from 0.04 to 75 μL and 0.004 to 20 μL have been used for capillary and nano LC, respectively (Table 3) .

Unlike injectors and pumps, analytical columns exhibit greater diversity, with specific phases available for different analytes. As can be seen in Table 3, filled columns were used in all works. Concerning the packed columns, these were mostly commercial C18 with particle sizes of 1.7 to 5 μm for cLC [52,53,57–74]. Smaller particles ranging from 1.8 to 3.5 μm were used in nLC [57,75–84]. The cLC columns used have diameters between 200 μm and 500 μm and lengths between 15 and 35 cm, while nLC columns have diameters between 75 μm and 100 μm and lengths between 5 cm and 20 cm. Compared to conventional LC, miniaturized LC has a limited variety of commercial phases available, and thus the development of new sorbent materials remains a challenge. In this context, some monolithic columns have been used as alternative to silica-based supports [85–90]. A commercial monolithic column, Onyx C_{18} (150 × 0.2 mm i.d.), has been used for the determination of triazines and their degradation products [87] and phthalates [86]. The monolithic columns show porous structure and offer low resistance to mobile phase flow allowing to work with higher flow rates. Lin et al. prepared fused-silica capillaries (250-μm i.d.) in which an alkyl methacrylate-based (AIMA) monolith with 1,6-hexanediol ethoxylate diacrylate (HEDA) as crosslinker was used [85]. The capillary was used to separate polar small molecules such as sulfonylurea herbicides by cLC. The separation was carried out in 5 min applying a steep gradient at a flow rate of 16 μL·min^{-1} with good efficiency and a R_s value (from 1.45 to 6.76). In a later study [89], they used AIMA-based capillary columns with divinylbenzene (DVB) as crosslinker for the separation of different phenol derivates. The separation was carried in approximately 10 min at a flow rate of 200 μL·min^{-1} with good repeatability (RSD < 2 % in retention time) and stability.

The chiral separation using nLC presents a great potential, however, not many applications have been described compared with conventional LC. The use of nLC reduces the amount of stationary phase as well as avoiding the use of large amount of solvent, which are usually very expensive in enantiomeric separations [92]. Rocchi et al. used a novel sub-2 μm vancomycin silica hydride stationary phase for enantiomers separation of basic and acidic racemic compound including multiclass

Table 3
Chromatographic separation information for the reported applications.

Analytical column	Injection volume	Flow rate ($\mu L \cdot min^{-1}$)	Run time	Mobile phase volume	Detection technique	LODs ($\mu g\ L^{-1}$)	REF
Capillary packed C18 (5 cm x 150 μm, 1.7 μm)	1 μL	3	5.5 min	16.5 μL	MS/MS	n.a.	[52]
Capillary packed C18 (10 cm x 150 μm, 1.7 μm)	40 nL	2	5.2 min	10.μL	On-column LED 255 nm	15	[53]
Capillary packed C18 (15 cm x 500 μm, 5 μm)	15 μL	10	15 min	150 μL	DAD	0.5 – 10	[57]
Nano packed C18 (15 cm x 100 μm, 3.5 μm)	440 nL	0.5	20 min	10 μL	DAD	0.5 – 12	
Capillary packed C18 (10 cm x 150 μm, 1.7 μm)	40 nL	2	8 min	16 μL	On-column LED 255	100 – 10,000	
Capillary packed C18 (15 cm x 500 μm, 3.5 μm)	32 μL	n.a.	35 min	n.a.	DAD	10	[58]
Capillary packed C18 (25 cm x 500 μm, 5 μm)	5 μL	10	25 min	250 μL	DAD	0.02 – 0.03	[59]
Capillary packed C18 (25 cm x 500 μm, 5 μm)	5 μL	10	30 min	300 μL	DAD	0.002 – 0.009	[60]
Capillary packed C18 (15 cm x 300 μm, 5 μm)	7 μL	20	20 min	400 μL	DAD	1.4 – 7.6	[61]
Capillary packed C18 (15 cm x 300 μm, 5 μm)	3 μL	15	32 min	480 μL	DAD	0.7 – 1.3	[62]
Capillary packed C18 (15 cm x 500 μm, 3.5 μm)	5 μL	10	29 min	290 μL	DAD in series with MS	0.01 – 0.2	[63]
Capillary packed C18 (15 cm x 300 μm, 5 μm)	3 μL	10	34 min	340 μL	DAD	0.1 – 1.7	[64]
Capillary packed C18 (35 cm x 500 μm, 5 μm)	75 μL	20	11 min	220 μL	DAD	0.015 – 0.05	[65]
Capillary packed C18 (15 cm x 300 μm, 2 μm)	n.a.	4	22 min	88 μL	MS	0.00005 – 0.0125	[66]
Capillary packed C18 (5 cm x 530 μm, 3.5 μm)	800 nL	12	8 min	50 μL	MS	0.1 – 0.6[a]	[67]
Capillary packed C18 (15 cm x 500 μm, 5 μm)	2 μL	10	10 min	100 μL	EC	1.0 – 2.0	[68]
Capillary packed C18 (15 cm x 500 μm, 5 μm)	1 μL	18	13 min	234 μL	MS/MS	0.0051 – 0.0068	[69]
Capillary packed C18 (15 cm x 500 μm, 5 μm)	0.5 μL	10	18 min	180 μL	DAD	0.01 – 0.03	[70]
Capillary packed C18 (15 cm x 500 μm, 5 μm)	2 μL	10	17 min	170 μL	DAD in series with MS	0.04 – 0.15	[71]
Capillary packed C18 (15 cm x 500 μm, 3.5 μm)	5 μL	15	21 min	315 μL	MS/MS	0.000003 – 0.005	[72]
Capillary packed C18 (25 cm x 500 μm, 5 μm)	8 μL	10	39 min	390 μL	DAD	1.13 – 2.95	[73]
Capillary packed C18 (15 cm x 500 μm, 3.5 μm)	3 μL	6	20 min	120 μL	DAD	0.0017 – 0.005	[74]
Nano packed phenyl (25 cm x 100 μm, 3 μm)	20 μL	0.5	25 min	12.5 μL	UV	0.70 – 29.52	[75]
Nano packed silica amylose tris(3-chloro-5-methylphenylcarbamate) immobilized (25 cm x 100 μm,	100 nL	0.75	20 min	15 μL	On-capillary UV	n.a.	[76]
Nano packed C18 (75 μm, 3 μm)	1 μL	0.3	40 min	12 μL	MS/MS	1.28 – 128[a]	[77]
Nano packed diol hydride-based vancomycin modified (11 cm x 75 μm, 1.8 μm)	40 – 90 nL	0.01 – 1.7	Variable, depending on each analyte and conditions	–	On-column UV–vis	n.a.	[78]
Nano packed C18 (15 cm x 100 μm, 3 μm)	0.17 μL	0.9	9.5 min	8.6 μL	MS	0.0001 – 0.0278	[79]
Nano packed C18 (5 cm x 75 μm, 3.5 μm)	0.94 μL	0.2	12 min	2.4 μL	DAD	1.0	[80]
Nano packed C18 (5 cm x 75 μm, 3.5 μm)	1.2 μL	0.7	8.4 min	6 μL	DAD	0.025 – 0.5	[81]
Nano packed C18 (5 cm x 75 μm, 3.5 μm)	2.5 μL	0.5	20 min	10 μL	DAD	0.025	[82]
Nano packed C18 (15 cm x 75 μm, 3.5 μm)	20 μL	0.4	10 min	4 μL	LEI-MS/MS	0.10 – 0.45	[83]
Nano packed C18 (15 cm x 75 μm, 3 μm)	1 μL	0.5	66 min	33 μL	FT-ICR-MS	n.a.	[84]
Capillary monolithic alkyl methacrylate (15 cm x 250 μm)	n.a.	16	5 min	80 μL	DAD	n.a.	[85]
Capillary monolithic C18 (15 cm x 200 μm)	32 μL	5	25 min	125 μL	DAD	0.005 – 1.5	[86]
Capillary monolithic C18 (15 cm x 200 μm)	30 μL	5	18 min	90 μL	DAD	0.02 – 0.1	[87]

(*continued on next page*)

Table 3 (*continued*)

Analytical column	Injection volume	Flow rate ($\mu L \cdot min^{-1}$)	Run time	Mobile phase volume	Detection technique	LODs ($\mu g\ L^{-1}$)	REF
Nano monolithic poly (MQD-co-HEMA-EDMA) (20 cm x 100 μm)	20 nL	20	15 min	300 μL	DAD	6600 - 20,000	[88]
Capillary monolithic AIMA-DVB (15 cm x 250 μm)	0.2 μL	200	10 min	2000 μL	DAD	n.a.	[89]
Nano monolithic CNT-based benzyl polymethacrylate (20 cm x 100 μm)	4 nL	0.5	13 min	6.5 μL	UV–vis	0.02 – 0.22	[90]

[a] LOD expressed in $ng \cdot g^{-1}$ instead of $\mu g \cdot L^{-1}$.

phenoxi herbicidies and NSAIDs [78]. Different flow rates ranging from 0.01 to 1.7 $\mu L \cdot min^{-1}$ were employed with injection volumes from 40 to 90 nL. The use of sub-2 μm particles offers some advantages over the 3 – 5 μm particles, such as increased resolution and the possibility to reduce the analysis time. This chiral stationary phase was effective in the resolution of chiral acidic compounds, showing better chromatographic performance than that obtained by employing 5 μm silica diol particles modified with vancomycin.

The different detectors reported are listed in Table 3. DAD is one of the preferred choices in environmental applications [61,74,80] due to its low-cost, simplicity, and range of applications. LODs from ng to $\mu g \cdot L^{-1}$ were obtained in the reported applications. Compared to conventional LC, the DAD flow-cells for miniaturized LC typically have a shorter path length to minimize the dead volumes related to the detector. Although this reduction diminishes the absorption of the analytes, the LODs achieved were low enough to reach the limits established by the legislation, especially when on-line sample treatment based on IT-SPME was used [63,65,81,82,86,87], which will be reviewed in the next section. As an example, the LOQs obtained for the analysis of cephalosporins by ion-pairing liquid-liquid extraction (IP-LLE) coupled to cLC-DAD ranges from 4.3 to 22.7 $\mu g \cdot L^{-1}$ [61] while the LOD for the analysis of diclofenac by IT-SPME-cLC [80] was 1 $\mu g \cdot L^{-1}$ which is suitable for its monitoring in environmental water samples. Moliner et al. [74] achieved LODs ranging from 1.7 to 5 $ng \cdot L^{-1}$ by using a magnetic IT-SPME-cLC-DAD. The lower LODs obtained, in comparison to other studies using DAD, are due to the high recoveries obtained by the magnetic IT-SPME. Nevertheless, these values are not comparable with those obtained by MS detectors, which are in the order of $pg \cdot L^{-1}$ to $ng \cdot L^{-1}$ as Table 3 indicates.

MS detectors are widely used in both, conventional and miniaturized LC due to their high sensitivity, accuracy, selectivity, and the possibility to identify and obtain structural information of a compound. Moreover, when working with ESI interfaces, the reduction in the flow rates increases ion transfer efficiency, which translates to an increase in the sensitivity [93]. Unlike in the analysis of regulated contaminants, MS/MS is the preferred detection method for emerging pollutants for both cLC and nLC [52,66,67,69,71,72].

Onghena et al. [72] compared the results obtained using a cLC (C18 packed, 3.5 μm, 500 μm x 15 cm) analytical column coupled to a MS detector for the simultaneous determination of 18 PFAS in river waters with those obtained by a conventional LC-MS/MS and UHPLC-MS/MS (C18 packed, 1.8 μm, 2.1 mm x 5 cm analytical column). The LODs provided by the cLC range from 0.03 to 15 $ng \cdot L^{-1}$ which were higher than those obtained with the HPLC-MS/MS. Compared to UHPLC, the sensitivity and linearity were similar; however precision and analysis time were higher. This study demonstrates that the use of cLC is a cheaper and greener alternative for the determination of PFAS in environmental samples.

More recently, Celma, et al. [52] determined 35 illicit drugs and NPSs by means of cLC-MS/MS using a C18 capillary column (1.7 μm, 150 μm x 5 cm). The results were compared with those obtained by UHPLC-MS/MS using a C18 (5 cm x 2.1 mm, 1.7 μm) analytical column. Sensitivity obtained for the cLC was overall higher, increasing the sensitivity up to 50 times for the more polar compounds and up to 6 times for the less polar ones. However, it should be considered that these results were obtained for 1 μL and 3 μL injection volumes for cLC and UHPLC, respectively. Also, analysis time and solvent usage were lower for cLC (Table 3).

Finally, even without a preconcentration step, the usage of an electrochemical (EC) detector allowed to achieve suitable LODs (between 1 and 2 $\mu g \cdot L^{-1}$), which could be useful for large-scale environmental monitoring. Thus, this procedure simplified the determination of various phenolic compounds in treated wastewater, reducing not only the analysis time but also the amount of solvents [68].

3. Sample treatment and miniaturized LC

3.1. Off-line mode

The off-line procedure involves the treatment of the sample separately from the chromatographic system, followed by the injection into the liquid chromatograph. As it is shown in Table 4, sample volumes varying from 2 to 500 mL were processed, with sample preparation times varying between 3 and 100 min depending on the extraction technique and application. Solid phase extraction (SPE) is the most popular choice, with different commercial extractive phases attending to the nature of the analytes. SPE cartridges packed with octadecyl-bonded silica (C18) phase were chosen for the extraction of methylxanthines [53] and estrogens [69] in surface waters achieving recoveries close to 100%. Hydrophilic-lipophilic balanced (HLB) packed cartridges, which use a N-vinylpyrrolidone-divinylbenzene copolymer enhancing the retention of polar compounds, have been used for the extraction of illicit drugs [52] and carbamate pesticides [70] with recoveries of 90% and 75 – 95%, respectively. A weak anionic exchange (WAX) cartridge was used for the extraction of perfluoroalkyl acids from 250 mL river water sample with an average recovery of 75% [72]. Finally, a commercial styrene-divinylbenzene copolymer SPE cartridge was used to extract dissolved organic matter (DOM) and disinfectant by-products (DBPs) from drinking water samples in a treatment plant with an average recovery of 50 ± 3% [84].

Other less common phases for SPE synthesized in the laboratory have also been utilized [59,60,71,73,75]. Magnetic sorbents prepared from magnetic metallic nanoparticles (NPs) (typically Fe_3O_4 NPs) have gained interest in recent years due to their unique features, including high surface/area, low toxicity, and ease of surface modification. These have been reported in various works, for example, a magnetic material based on Fe_3O_4 magnetic NPs incorporated in a silica matrix by using a sol-gel procedure was proposed by Moliner-Martinez et al. [71] to extract and preconcentrate some pharmaceutical compounds from environmental water samples prior to cLC-MS. The magnetic adsorbent has been developed by a polarity-controlled procedure by means of CTAB addition to the medium. The extraction was completed in less than 10 min with recoveries ranging from 87 to 93%. Similarly, an ionic liquid-functionalized magnetic silica NPs (IL-MNPs) sorbent has been proposed by Bouri et al. as a preconcentration material [73]. Imparting magnetism to the IL-functionalized silica and then performing magnetic separation represents a promising alternative. Good recoveries were obtained (90 – 97%), and no influence of the extraction time was

Table 4
Characteristics of the reported pretreatment techniques in the last 15 years for the determination of different contaminants by cLC and nLC in environmental samples.

Sample	Analyte	Sample amount	Sample treatment	Time (min)	ER (%)	Coupling mode	REF
Wastewater	NPS and illicit drugs	5 mL	SPE (HLB)	90	n.a.	Off-line	[52]
Wastewater	Trimethylxanthines	25 mL	SPE (C18)	n.a.	n.a.	Off-line	[53]
Wastewater	Biocides	100 μL	None (portable LC) IT-SPME (TEOS-MTEOS-SiO_2NPs) (cap/nanoLC)	0	n.a.	On-line	[57]
Bivalves	DEHP	0.1 g	MSPD and IT-SPME (TRB-5 PDMS)	30	n.a.	On-line	[58]
Wastewater	Sulfonylurea pesticides	500 mL	MMISPE	10	94 – 102	Off-line	[59]
River water	Sulfonylurea pesticides	2 mL	Au-NP coated sorbent SPE	20	84 – 105	Off-line	[60]
Spring and river water	Cephalosporines	2 mL	Ion-paired LLE	10	74 – 108	Off-line	[61]
River and ground water	Sulfonylurea pesticides	12 mL	HF-LPME	60	70 – 119	Off-line	[62]
Surface waters	Multiclass pesticides	4 mL	IT-SPME TRB-5 PDMS)	0	3 – 65	On-line	[63]
Ground and river water	Sulfonylurea pesticides	4 mL	SALLE (acetonitrile)	3	83 – 96	Off-line	[64]
Marine water	Irgarol-1051 and diuron	4 mL	IT-SPME (TRB-35% PDMS)	0	10	On-line	[65]
Creek water	β-blockers and antibiotics	100 μL	C18 SPE trap column	1	n.a.	On-line	[66]
Soil	PFAS	1 g	Puae (methanol)	15	70 – 110	Off-line	[67]
Treated wastewater	Phenolic compounds	–	–	–	–	Off-line	[68]
River water	Estrogens	100 mL	SPE (C18)	100	95 – 100	Off-line	[69]
River and ground water	Carbamate pesticides	500 mL	SPE (HLB)	75	75 – 95	Off-line	[70]
Treatment plant and river water	NSAIDs	20 mL	Silica supported Fe3O4 magnetic NPs extraction	10	87 – 93	Off-line	[71]
River water	PFAS	250 mL	SPE (WAX)	40	72 – 82	Off-line	[72]
River water	Sulfonylurea pesticides	50 mL	IL-MNPs	10	90 – 97	Off-line	[73]
Standard solution	NSAIDs and β-blockers	200 μL	Magnetic IT-SPME	0	70 – 100	On-line	[74]
Standard solution	Multiclass pesticides	100 mL	MWCNTs DSPE /C18 SPE trap column	15/ 4.5	36 – 102	Off-line/ on-line	[75]
Natural waters	Racemic pharmaceuticals	3 mL	DLLME (isoamyl acetate)	30 min	70 – 116	Off-line	[76]
Benthic invertebrates	Psychiatric drugs	n.a.	Micro-QuEChERS/switch column (C18)	7		On-line	[77]
Standard solution	Phenoxies and enantiomeric NSAIDs	–	–	–	–	–	[78]
Lake water	Pharmaceuticals, pesticides, and its metabolites	88 μL	SPE (HLB)	10	7 – 124	On-line	[79]
River water	Diclofenac	8 μL	IT-SPME (TRB-5 PDMS, TRB-5 c-SWNTs, TRB-5 c-MWNTs, SiO_2/PEG spherical Fe_3O_4 NPs, SiO_2/PEG c-SWNTs, and SiO_2/PEG rod-shaped Fe_3O_4 NPs)	< 1	5 (TRB-5) 75 (TRB-5 c-SWNTs)	On-line	[80]
Transition waters	Triazine pesticides	0.5 mL	IT-SPME (TEOS-MTEOS-SiO_2NPs)	0	n.a.	On-line	[81]
River, irrigation, transition, and sea water	Tribenuron-methyl	200 μL	Two capillaries IT-SPME (TEOS-MTEOS reinforced with TiO_2 and CuO respectively)	0	n.a.	On-line	[82]
Soil	Herbicides	1 g	LLE and M-WATF	120	n.a.	On-line	[83]
Drinking water treatment plant	DOM and DBP	150 mL	SPE (SDVB polymer)	n.a.	50 ± 3	Off-line	[84]
Standard solution	Phenylurea pesticides	–	–	–	–	–	[85]
Sea water	Phthalates	4 mL	IT-SPME (TRB-5 PDMS)	0	n.a.	On-line	[86]
Commercial herbicide sample	Enantiomeric phenoxies	–	–	–	–	–	[88]
Standard solution	Phenol derivates	n.a.	–	–	–	Off-line	[89]
Tap and seawater	PAHs	100 mL	CNT-benzyl methacrylate-co-ethylene dimethacrylate monolith SPE	70	81 – 95	Off-line	[90]

observed between 5 and 30 min of extraction time. The use of this sorbent combines the high surface area of nanomaterials with the magnetic properties, allowing them to be isolated from the solution by applying a magnetic field. The optimum conditions permitted the omission of a clean-up step, with the material being utilized up to 20 times without a loss in extraction efficiency, reaching a preconcentration factor of 100. The utilization of magnetic SPE was also reported by Lerma et al. who employed a magnetic molecular imprint (MMIP) as sorbent [59]. The processing of up to 500 mL of sample enabled the attainment preconcentration factor of 1000 (recoveries from 94 to 102%) within 10 min.

Lerma et al. also reported the use of silica-supported AuNPs functionalized with N-methylimidazolium as extracting material [60]. The AuNP–ion liquid (IL)–silica sorbent demonstrated superior recovery values for all the tested analytes, with values ranging from 85 to 105%, compared with the Au–tetraethyl orthosilicate (TEOS) (70 to 96%) and C18 sorbents (75 to 107%). The results of this study, demonstrate that pH is a critical variable in the adsorption process of sulfonylureas onto the two proposed sorbents. The sorbents exhibited stability up to at least 350 retention–elution cycles, with no significant loss of their uptake capacity upon repeated use. A composite of carbon nanotubes and (CNT)-benzyl polymethacrylate monolith was employed as a SPE adsorbent and stationary phase material to separate a range of PAHs including naphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, pyrene, benz[a]anthracene, chrysene, benz[k]fluoranthene and dibenz[a,h]anthracene in sea and tap water by nLC [90]. The incorporation of CNTs to the monoliths improved the extraction performance up to 95%. Asensio et al. have proposed an off-line dispersive solid phase extraction (DSPE) method with multi-walled carbon nanotubes (MWCNTs) for environmental applications (12 pesticides) combined with nLC [75]. In comparison with conventional SPE procedures, where the loading step requires a long period of time depending on the amount of sample, dispersive SPE significantly reduces the extraction time while maintaining the recoveries (36 to 102% for DSPE and 31 to 95% for SPE). Furthermore, DSPE enables the processing of large sample volumes to achieve higher preconcentration factors.

In addition to solid-phase extraction techniques, liquid-phase extraction (LPE) has also been employed in various applications, as detailed in Table 4. A ion-paired salting-out assisted LLE (IP-SALLE) prior to the analysis by cLC coupled to diode array detector (DAD) was proposed by Quesada-Molina et al. [61] for the determination of cephalosporin antibiotics (cefazolin, cephalexin, cephadroxil, cephoperazone, cefquinome, cephapirin, cephamandole, and cephalonium) in spring and river water samples. The extraction procedure involved the use of cetrimonium bromide (CTAB) as ion-pairing agent, ammonium sulphate for salting-out, and acetone/water mixtures (1 mL acetone in 2 mL of water) as extractive solution. This approach yielded satisfactory recoveries (74 – 108%) in 10 min. Gure et al. tested three phases of hollow fiber liquid phase microextraction (HF-LPME) combined with cLC for the determination of eight sulfonylurea herbicides (chlorosulfuron, foramsulfuron, nicosulfuron, oxasulfuron, primisulfuron-methyl, prosulfuron, triasulfuron, and triasulfuron-methyl) [62]. Enrichment factors were obtained in the range of 71 to 548. However, the procedure has two significant drawbacks: the lengthy extraction time (60 min) and the lack of reusability of the fibers. The same authors employed 1 mL of ACN as organic phase and $(NH_4)_2SO_4$ as salting-out agent for the extraction of residues of nine sulfonylurea pesticides in ground and river water [64]. In comparison to their previous work, this method drastically reduced the extraction time (3 min) and lowered the sample and solvent volumes while maintaining good recoveries. In a separate study, Hemida et al. employed a portable ultrasound-assisted extraction (pUAE) using ammonium hydroxide as solvent for the extraction of various per- and polyfluoroalkyl substances (PFAS) obtaining recoveries between 70 and 110% for the majority of analytes [67]. One of the main disadvantages of LLE over SPE is the larger volumes of solvents required, which often prove to be expensive and hazardous, and deviate from the principles of green chemistry. As a result, the miniaturization of LPE has emerged as an excellent alternative due to the lower volumes of sample and solvent required, more efficient sample enrichment, faster sample preparation, and easier automation. As an example, Salido et al. [76] extracted a number of drugs from natural waters by means of DLLME utilizing 300 μL of isoamyl acetate as a natural extractant in less than 20 min obtaining recoveries over 70%.

3.2. On-line mode

On-line coupling to minLC implies that the entire sample is introduced into the system, and the extraction and preconcentration processes occur within the system itself. On-line sample preparation represents an important step in order to miniaturize the whole analytical procedure. This option minimizes the volume of solvent and sample required while significantly reducing the sample processing time. The operational principle is similar in most cases and is based on column switching configurations where the analytical column and an extractive column are connected to a switching valve. The sample is first loaded manually or by means of an auxiliary pump into the extractive column/capillary, where the analytes are retained. Then, by switching the valve position, the mobile phase elutes the analytes to the analytical column.

In general terms, on-line sample treatment techniques for miniaturized LC can be classified depending on weather a trap column or IT-SPME is employed. In the former case, and in its simplest configuration, a single valve is used to load the sample into the trap column via an external pump. The retained analytes are then eluted to the analytical column (Fig. 2, a). This set-up was employed by Asensio et al. [75] for the preconcentration of twelve multiclass pesticides. Compared to direct injection, the on-line preconcentration resulted in an increase in sensitivity of up to 100-folds. Similarly, Berlioz et al. [77] employed a C18 trap column for the on-line preconcentration of the psychiatric drugs carbamazepine and fluoxetine in benthic invertebrates. In this case, the loading time is critical to maximize the sensitivity. If the loading time is too low, the analytes may not be eluted to a sufficient extent, while if it is too high, they may be completely eluted to the waste.

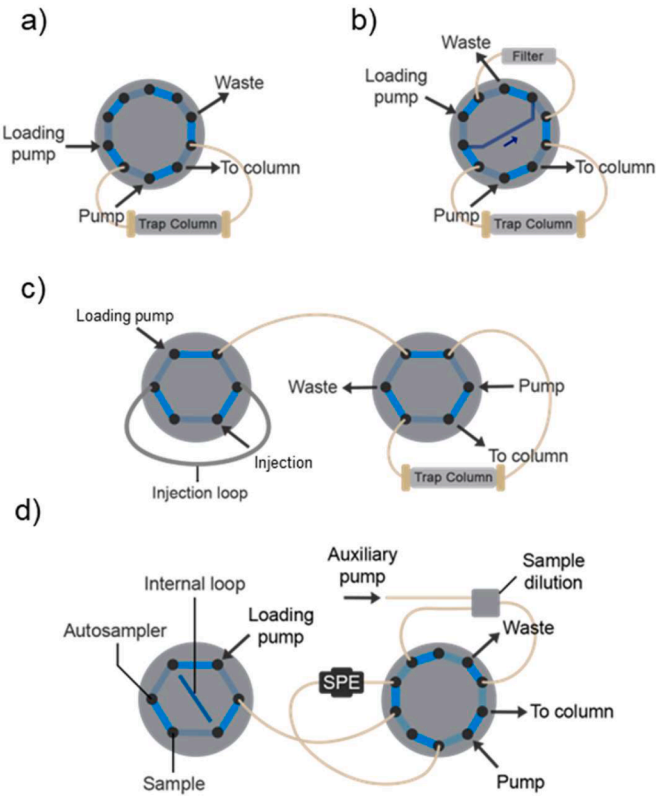

Fig. 2. Schematic of the different column switching configuration reported in the literature.

In a similar vein, Roberg-Larsen et al. [66] proposed a column switching configuration (Fig. 2, b) coupled to cLC, which consisted of an on-line automatic filtration and filter back flush (AFFL) SPE using large sample volumes. This method was used for the determination of pharmaceutical products (sulfamethoxazole, trimethoprim, atenolol, azithromycin, clarithromycin, propranolol, diclofenac, and sulfapyridine) in environmental water samples using 10-port switching valve. The injection of 100 μL of non-filtered water did not result in a pressure rise or clogging of the SPE/capillary columns, which is particularly noteworthy given the susceptibility of miniaturized LC systems to such issues. Termopoli et al. employed a μ-water-assisted trap focussing (μWATF) for the preconcentration of five pesticides (Fig. 2, c) [83]. In this configuration, two 6-port/2-position Rheodyne injection valves were used. In the first valve, the sample is injected and loaded into a sample loop of a fixed volume. Subsequently, water is pumped using a μ-pump at 20 $\mu L \cdot min^{-1}$ going through a T-union which divides the flow in two streams. One part of the flow is directed to the sample loop, while the remainder is directed to a second T-union, where both flows converge. The resulting flow is directed to the trap column in the second valve where the analyte is retained. Finally switching the valve position causes the mobile phase to elute the analytes into the nano analytical column. This method allows for the injection of 20 μL (or higher) of sample volume (organic solvent), speeding up the analysis time, while the water-assisted dilution increases the focusing on the trap column achieving a 400-fold increase of the limits of quantitation (LOQ) of selected pesticides (clomazone, paclobutrazol, fludioxonil, metolachlor and pirimiphos methyl) in comparison to direct injection.

Not strictly using a trap column, Stravs et al. employed an on-line SPE prior to the analysis by nLC-MS [79]. The configuration is depicted in Fig. 2d. In a first step, the sample is loaded into an internal sample loop of a 6-port/2-position injection valve. Then, a loading pump transports the sample at a fixed flow rate and loading time to a second

10-port/2-position valve. At this stage, the sample passes through a C18 SPE cartridge, where the analyte is retained while the matrix is discarded. Finally, by switching the second valve position, the mobile phase flows through the SPE cartridge eluting the analyte into the analytical column. This configuration also incorporates an auxiliary pump that enables sample dilution prior to its entry into the analytical column. Only 88 μL of sample is required, and the extraction is performed using 200 μL of solvent in less than 10 min.

Concerning the IT-SPME, it uses a capillary packed or coated with the extractive phase instead of the injection loop or within the sampling circuit of an automatic injector. The IT-SPME capillaries are segments of open tubular columns, generally GC columns, which are filled with the extractive phase. Campíns-Falcó research group explored the possibilities of IT-SPME coupled to cLC and nLC for the determination of pollutants in environmental samples [57,58,63,65,74,80–82,86,87,94]. Different configurations, from the simplest which involves the usage of a single injection valve and one pump (Fig. 3a and b) [57,58,80,81,86,87,94] to more complex such as the usage of two valves with one SPME capillary and one pump (Fig. 3c and f) [57,63], two pumps (Fig. 3d) [80], or two pumps and two SPME capillaries (Fig. 3e) [82] have been used.

Muñoz et al. coupled the IT-SPME (Fig. 3a) to cLC for the analysis of di(2-ethylhexyl) phthalate (DEHP) in bivalves to further help to establish an environmental quality standard for biota and estimate its bioconcentration factors. Before injection, a matrix SPE was performed to extract the DEHP from the samples [58]. For the IT-SPME, a 40 cm length and 0.32 mm i.d. 95% dimethyl 5% diphenylpolysiloxane (TRB-5) capillary was employed. The method exhibited favourable analytical characteristics and represented a cost-effective approach for the determination of DEHP in biota samples, processing a sample in less than 30 min (Table 4).

In a study by Pla-Tolós et al. [65], an IT-SPME-cLC (Fig. 3a) procedure was optimized for the determination of irgarol-1051 and diuron, two antifouling biocides, in marine waters. The peak areas obtained for different% of diphenyl groups (TRB-5, TRB-20, TRB-35, and TRB-50) in the capillary loop and injection volumes were compared. Despite the low extraction efficiencies (but in accordance with the technique), the relative high sample volume processed (up to 4 mL) allows for a significant increase sensitivity, reaching LODs of 0.015 $\mu g \cdot L^{-1}$ which are adequate for regulatory purposes. The advantages of this miniaturized system are the short analysis time, the small amount of sample, and satisfactory precision (RSD < 3.5 %).

One of the main limitations is the low extraction efficiency achieved with these capillaries. Therefore, many efforts have been done to develop a new extractive materials to improve selectivity and extractive capacity. Depending on the application and the analyte characteristics different sorbents and packaging can be used. A comprehensive review of the different options is provided by Ponce et al. [95]. A capillary column with a polydimethylsiloxane (PDMS) coating and the same coating modified with carboxylated single-wall carbon nanotubes (c-SWCNTs) and carboxylated multiwall carbon nanotubes (c-MWCNTs) was used to extract on-line triazines and their degradation products in transition water [87]. The immobilization of c-SWCNTs and c-MWCNTs on PDMS columns did not result in a significant enhancement of the

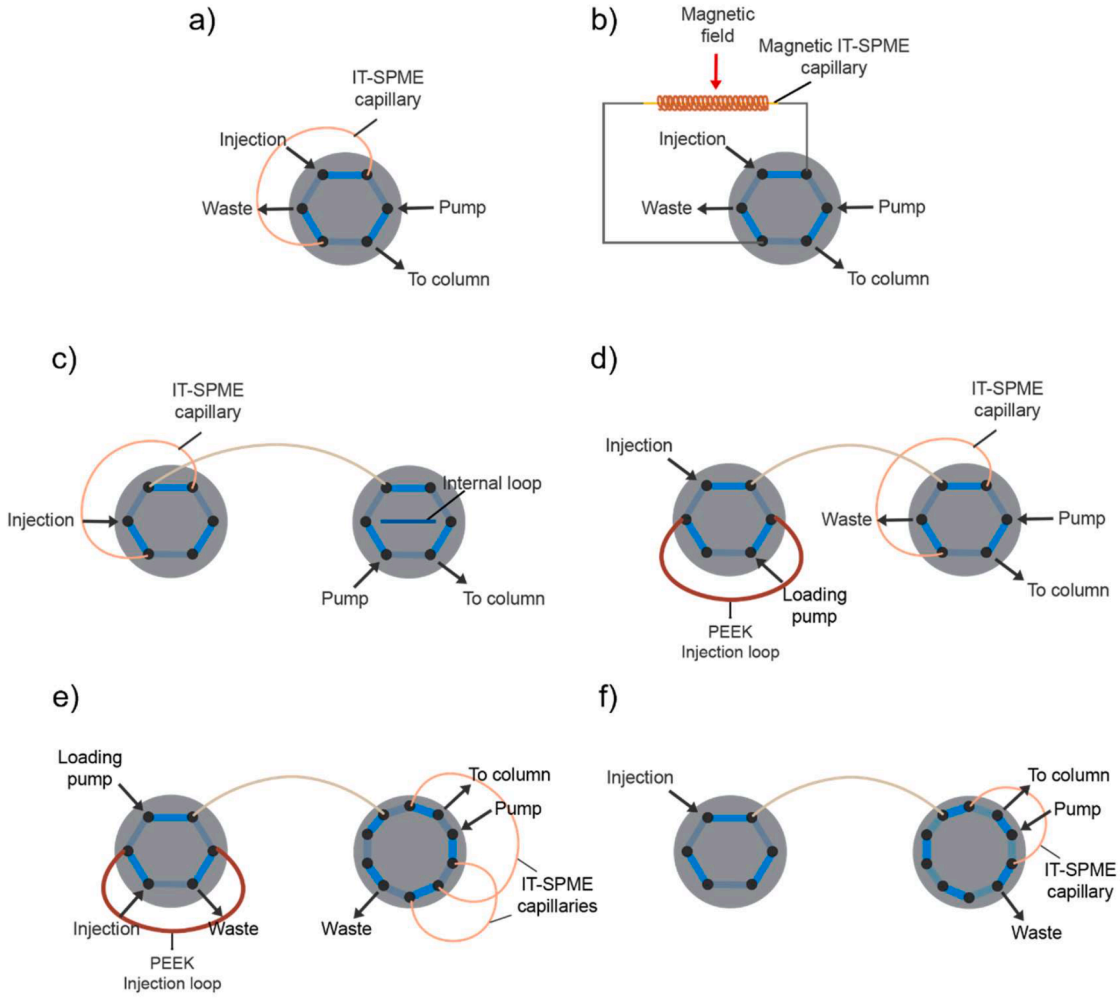

Fig. 3. Schematic of the different IT-SPME configurations reported in the literature.

extraction and preconcentration of triazines in the IT-SPME. The slight improvement found for some of the tested compounds did not compensate the effort required to prepare the CNTs coatings, and so a commercial TBR-5 capillary column was selected as the best option for the extraction of triazines. Another IT-SPME extractive phase for the extraction of intact and degraded triazines (terbutylazine-2-hydroxy, terbuthylazine-desethyl, atrazine, propazine, terbutylazine and irgarol-1051) in transition water was proposed by Serra-Mora et al. The new extractive phase reported consisted of a tetraethyl orthosilicate-trimethoxymethylsilane (TEOS-MTEOS) based polymer enhanced with SiO_2 NPs synthesized in an empty fused silica capillary (75 μm i.d.) [81]. The results were compared with those obtained using TRB-5, c-SWCNTs-TRB-5, c-MWCNTs-TRB-5, and TEOS-MTEOS (without the SiO_2 NPs) extractive phases (Fig. 4). The TEOS-MTEOS--SiO_2 NPs phase provided a signal increase factor between 4.5 to 5.8 compared to the above-mentioned extractive phases. This signal increase was attributed, on the one hand, to the interaction between the hydroxyl groups of the TEOS-MTEOS polymer and the analytes (hydrogen bonding and ion exchange interactions) and, on the other hand, to the presence of the SiO_2 NPs that alters the surface morphology and roughness increasing the superficial area. They also compared the performance of IT-SPME-cLC and IT-SPME-nLC demonstrating that the sensitivity in nLC improved between 10 and 25 % when using the same extractive phase. The proposed method has shown high analytical performance in terms of linearity, sensitivity, system precision and accuracy values for practical applications.

Based on their previous work [71], Moliner-Martínez et al. proposed a silica supported Fe_3O_4 NPs capillary to perform a magnetic IT-SPME coupled to cLC with the purpose of improving the extraction efficiency of various non-steroidal anti-inflammatory drugs (NSAIDs) and β-blockers [74]. In this configuration the IT-SPME capillary was encircled by a magnetic coil and connected to a single conventional 6-port/2-position injection valve (Fig. 3b). In this way, the current applied to the magnetic coil can be modified by means of a power supply, thereby adjusting the external magnetic field. The application of a variable magnetic field resulted in a quantitative extraction of the target analytes between 70 and 100 %, which are higher than those achieved by other IT-SMPE methods (between 10 and 30 %).

Apart from single-valve configurations, configurations with two injection valves have also been reported. Masiá et al. employed a two-valve configuration where the sample was first injected into the valve containing the IT-SPME extraction capillary (Fig. 3c) [63]. After sample injection, a few μL of methanol was injected to desorb the analytes into a second valve incorporating a sample-loop. Finally, the second valve position is switched so that the analytes are directed through the capillary analytical column by the mobile phase. In comparison one valve IT-SPME configuration where the entire extractive capillary volume is injected into the system, the desorption of the analytes into a low volume sample loop allows preconcentration without performance loss. However, as the injected volume is lower, sensitivity is reduced. The device was equipped with a GC TRB-5 capillary column used as preconcentration loop, and two conventional six-port injection valves. This methodology was applied to the determination of pesticides (chlorfenvinphos, chlorpyriphos, trifluralin, diuron, terbuthylazine, atrazine, isoproturon and simazine) in surface waters. Enrichment factors from 2.5 to 10 were achieved (Table 4). In this case, the extractive capillary and sample loop are placed in the first and second valves, respectively, and then injection and desorption are carried out manually. Alternatively, the sample can be manually loaded into a sample loop in the first valve and then transferred to the IT-SPME capillary in the second valve by means of a loading pump (Fig. 3d). This configuration was used by González-Fuenzalida et al. coupled to nLC for the determination of diclofenac in river water samples [80]. Different extractive phases with nanomaterials (TRB-5 cSWNTs, SiO_2/PEG spherical Fe_3O_4 NPs, SiO_2/-PEG cSWNTs, SiO_2/PEG rod-shaped Fe_3O_4 NPs, and PDMS Fe_3O_4 NPs gel) were tested. In all cases, the analysis was carried out in less than 1 min with recoveries ranging from 5 for the TRB-5 phase to 75% for the TRB-5 cSWNTs phase. The same configuration, but with two extractive capillaries in the second valve, was also reported by Serra-Mora et al. (Fig. 3e) [82]. In this case, the extractive capillaries were connected to a 10-port/2-position injection. TEOS-MTEOS-CuO and TiO_2 capillaries were employed which exhibited high extraction efficiency for polar and less polar herbicides respectively. Thus, the coupling of both capillaries is relevant when analytes of different polarities are present [96]. Cortés-Bautista et al. employed a two-valve configuration where the sample was first injected into a 6-port/2-position valve which was used to transfer the sample to a second 10-port/2-position injection valve where a TEOS-METOS-SiO_2NPs extraction capillary was placed (Fig. 3f). This configuration was coupled to both cLC and nLC with DAD for the determination of biocides in wastewater. The method characteristics in terms of figures of merit, greenness, and sustainability were evaluated and quantified by means of the HEXAGON tool, a method evaluation

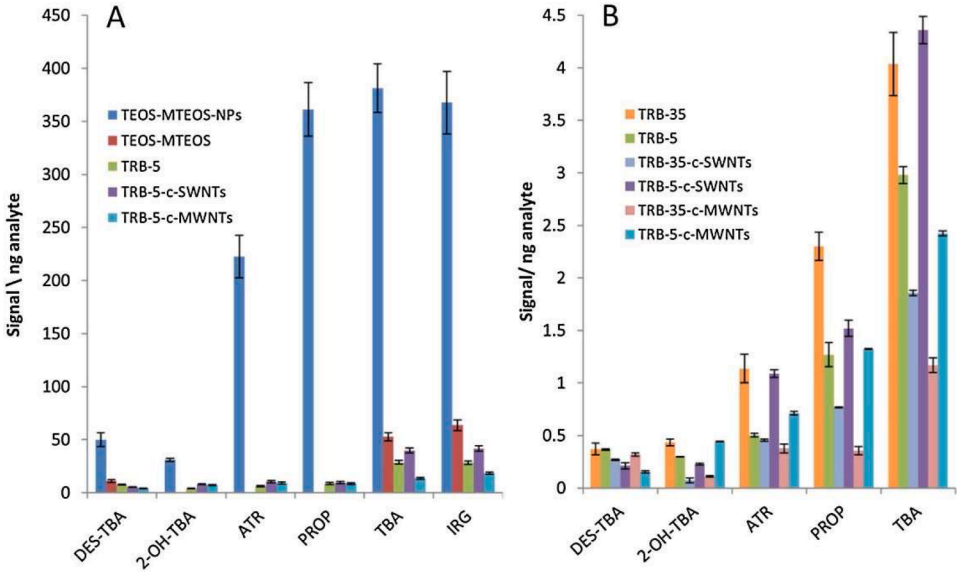

Fig. 4. Signal for 1 ng of intact or degraded triazines loaded in IT-SPME-nanoLC (A) and IT-SPME-CapLC (B), using TEOS-MTEOS-NPs, TEOS-MTEOS, TRB-5, TRB-5-c-SWNTs,TRB-5-MWNTs, TRB-35, TRB-35-c-SWNTs and TRB-35-MWNTs extractive phases. From Serra-Mora et al. (2017) [79].(With authorization to be published).

tool proposed by Ballester-Caudet et al. [97], and the results were compared with those obtained by means of a portable miniaturized LC and a UHPLC-MS (see Fig. 5). Superior results in terms of sustainability, greenness, and economic cost were obtained for the portable LC, followed by the miniaturized benchtop LC, while lower LODs were provided by IT-SPME capillary/nanoLC-DAD and UHPLC-MS.

4. Contributions to the environmental field

Based on the reviewed articles, the pollutants were categorized into analytes with an established concentration limit, including pesticides and other pollutants such as phenols, PAHs, and phthalates, and compounds without legislated limits which covers contaminants such as estrogens, pharmaceutical compounds and, PFAS among others. As Fig. 6 shows, the total amount of emerging and regulated contaminants determined by miniaturized LC is comparable (44 and 56% respectively). As for regulated contaminants, pesticides predominate (34% of the total analyzed pollutants) over the PAHs (4%), phthalates (2%) and phenolic compounds (4%) while for emerging contaminants, new psychoactive substances (NPSs) and illicit drugs, pharmaceuticals and PFAS share a similar interest (19, 21 and 13% respectively). Table 4 indicates the different matrices analyzed and the analytes tested.

Besides the development of analytical methods, other points to be considered are their applicability to real samples and the concentration of contaminants found in environmental samples. In only a few works positive samples are identified; some examples are discussed below.

In a study, 12 sea water samples from different ports or marinas in the region of Valencia were analyzed for irgarol-1051 and diuron [65]. All samples presented concentrations of diuron and irgarol-1051 below the LODs except for two samples in which irgarol-1051 was found at 0.02 $\mu g \cdot L^{-1}$, still below the environmental quality standards (EQS). Other regulated contaminants such as phenol and 2-chlorophenol were also found in effluent wastewater at concentrations of 3.7 and 14.9 $\mu g \cdot L^{-1}$ [68]. Diethyl phthalate and DEHP were detected at concentrations of 1.8 and 0.4 $\mu g \cdot L^{-1}$ in sea water, respectively [86]. The analysis of PAHs in sea water by Al-Rifai et al. [90] showed the presence of fluorene at 0.86 $\mu g \cdot L^{-1}$, anthracene at 0.64 $\mu g \cdot L^{-1}$, pyrene at 0.14 $\mu g \cdot L^{-1}$ and benz[a]anthracene at 0.19 $\mu g \cdot L^{-1}$. Naphthalene and chrysene were also detected but not quantified. For these samples, the concentration levels of the PAHs were higher than the Environmental Protection Agency's minimum concentration levels in drinking water. Fruit washing residual which may contain high concentrations of the biocides potassium sorbate, imazalil, and pyrimethanil, were determined by IT-SPME-nLC-DAD, IT-SPME-cLC-DAD and portable minLC-LED/UV [57]. Concentrations in the range of 1800 – 1700 $mg \cdot L^{-1}$, 150 – 480 $mg \cdot L^{-1}$, and 3.2 – 120 $mg \cdot L^{-1}$ for potassium sorbate, imazalil, and pyrimethanil were found respectively.

Among emerging contaminants, pharmaceuticals have attracted considerable attention and numerous studies have focused on their determination in environmental waters. The study by Moliner et al. was the only one to report the presence of pharmaceuticals in the analyzed samples [71]. Of the five river samples, acetylsalicylic acid was found in 3 of them in a concentration range of 0.60 – 0. 90 $\mu g \cdot L^{-1}$, acetaminophen was found in 2 samples at 1.7 – 3.1 $\mu g \cdot L^{-1}$, diclofenac and ibuprofen in only one sample at 0.17 $\mu g \cdot L^{-1}$ and 0.12 $\mu g \cdot L^{-1}$ respectively. Another major group of emerging contaminants are PFAS. In two studies, these were determined by miniaturized LC in environmental samples. In a first study, Onghena et al. [72] analyzed six river water samples by cLC-MS. Of the 18 PFAS studied, only 7 were found in concentrations ranging from 0.8 $ng \cdot L^{-1}$ for perfluorooctanoic acid to 148 $ng \cdot L^{-1}$ for sodium perfluorocantesulfonate. Perfluoroheptanoic acid, perfluorooctanoic acid were more frequent in the samples (found in 3 samples), followed by perfluorononanoic acid (found in 2 samples). Long-chain PFAS were not detected in these samples, probably due to their lower solubility and less frequent production and consumption compared to short-chain PFAS. In a second study, Hemida et al. [67] determined 12 PFAS in soil samples by at-site ultrasound-assisted extraction combined with portable cLC-MS. The samples were analyzed in the field (8 samples) and in the laboratory (17 samples) and the results were compared. Perfluorohexanesulfonic acid was the only analyte found in all samples with a mean concentration of 2.4 $ng \cdot g^{-1}$. The other PFAS were found in concentrations up to 30.2 $ng \cdot g^{-1}$ for perfluorohexanoic acid, 1.7 $ng \cdot g^{-1}$ for perfluorononanoic acid, 7.5 $ng \cdot g^{-1}$ for perfluoroundecanoic acid, 7.9 $ng \cdot g^{-1}$ for perfluorododecanoic acid and 394.7 $ng \cdot g^{-1}$ for perfluorooctanesulfonic acid.

5. Remarks and future trends

Miniaturized LC has been established as a common tool for analysis in proteomics and pharmacology, but despite its potential benefits, it has not received much attention in environmental analysis. In the present work, we have reviewed the applications of miniaturized LC (cLC and nLC) to the analysis of contaminants in environmental samples over the last 15 years, focusing on the sample treatment techniques and instrumentation. This review aims to highlight the advantages of the technique in this field.

The technique has been applied to the determination of a wide range of analyte classes and matrices, providing suitable results in all cases.

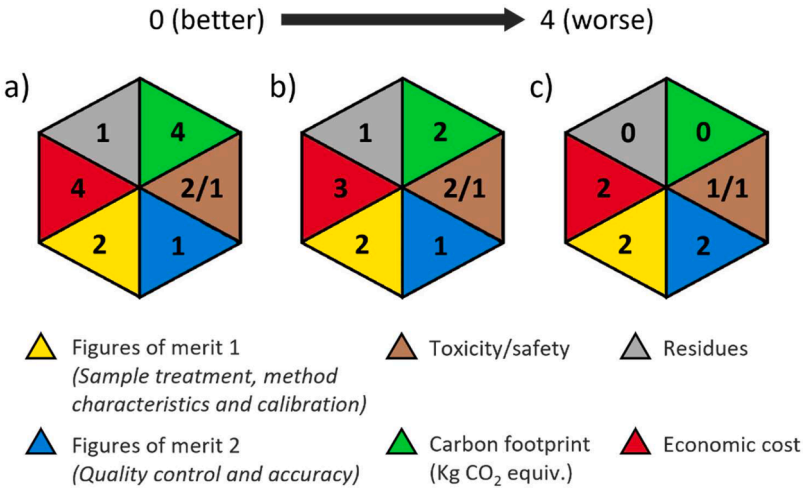

Fig. 5. HEXAGON tool for a) UHPLC-MS, b) capillary and nano LC-DAD, and c) portable miniaturized LC. (Adapted from Cortés-Bautista et al. (2021) [81] with permission from Elsevier). (With authorization to be published).

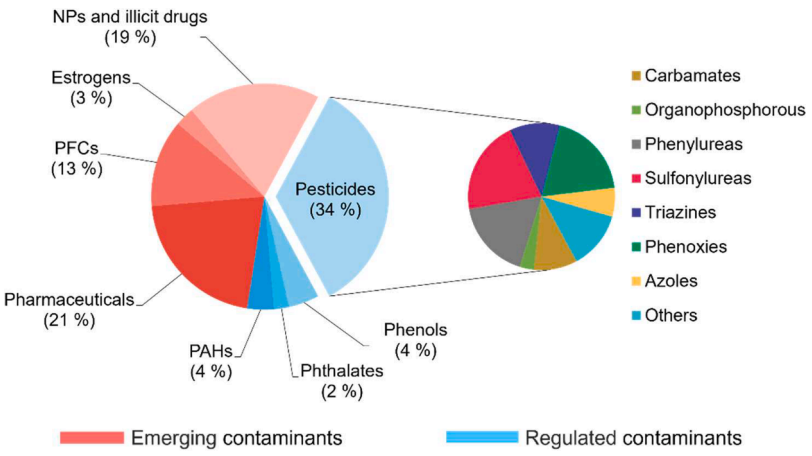

Fig. 6. Comparison of the total amount of pollutants analysed by miniaturized LC in environmental samples.

Although some articles reported difficulties with retention time reproducibility, most applications provided chromatographic separations with high efficiency and improved sensitivity compared to conventional LC. Moreover, the lower flow rates greatly reduced the amount of mobile phase required per run, not only making the methodology greener but also lowering the costs. In combination with miniaturized LC, on-line sample treatment is often performed. This simplifies the sample preparation step and improves the environmental analysis performance by carrying out the injection, extraction, preconcentration, separation, and detection in a single run. In this sense, column switching configurations based on trap columns and IT-SPME were employed with good results, meeting the required LODs set by legislation. In contrast to trap columns which are more tied to the C18 extractive phase, IT-SPME has explored a more diverse number of phases (including TRB-5, TRB-35, TEOS-MTEOS-NPs, and magnetic sorbents, among others) allowing it to be more selective in retaining the contaminants of interest.

Regarding the instrumentation, valve-based injectors and dual-piston reciprocating pumps are mainly used in benchtop miniaturized liquid chromatographs. A wider variety of injectors and pumps are used in portable systems to minimize weight while maintaining compactness. When moving from conventional to miniaturized analytical columns, the method applicability might be restricted due to the lower diversity of commercially available miniaturized columns and their higher price. Thus, some studies have proposed home-made monolithic columns instead of the commonly used packed C18 columns. Although the use of these kind of columns is not frequent, it is an interesting trend to explore as it expands the analysis possibilities while reducing the costs. With respect to detectors, DAD and MS are chosen for most applications. While DAD is preferred for its accessibility, MS detectors are opted for their higher sensitivity, selectivity, and compound identification. Also, miniaturized spectrophotometric UV/vis detectors are light enough to be used in portable systems, whereas MS is still rather limited to laboratory applications.

Finally, the occurrence of contaminants in environmental samples was discussed. Although the data are limited, it can be observed that most of the regulated pollutants were not found and the quantified ones were within the environmental quality standard concentration. Compared to the regulated ones, the percentage of found analytes for emerging contaminants was higher for all matrices in concentrations varying from $\mu g \cdot L^{-1}$ for some pharmaceuticals to $ng \cdot L^{-1}$ for estrogens and PFAS.

As a last remark, the miniaturization of LC instruments has introduced a new trend in environmental analysis — the development of portable systems. The growing demand for instruments capable of in-situ analysis makes portable LC a powerful tool for real-time monitoring of environmental samples. Despite their great potential, these systems are still in the development stage. It is noteworthy that the recent introduction of the first commercially available miniaturized portable LC which marks a potential turning point that could lead to an increase in applications in the coming years. In view of the reviewed applications, a promising outcome is expected for minLC in the environmental analysis, marked by a trajectory towards enhanced analytical capabilities, sustainability, greenness and portability.

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CRediT authorship contribution statement

S. Cortés-Bautista: Writing – original draft, Methodology, Investigation, Formal analysis. **C. Molins-Legua:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **P. Campíns-Falcó:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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