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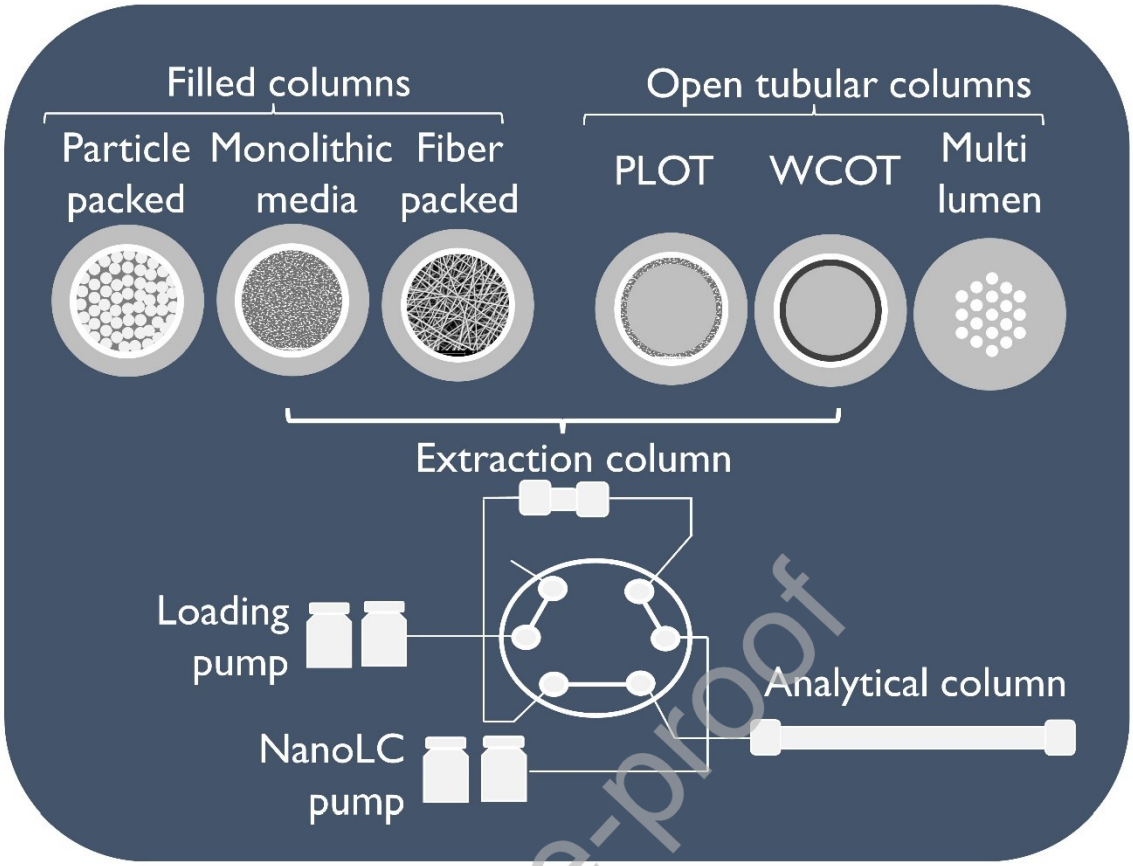
Graphical abstract



Highlights

- Novelties in sorptive phases for online microextraction columns are reviewed.
- Advances in particulate, monolithic, open-tubular, and fibrous extraction media.
- The development of fully miniaturized column switching approaches is highlighted.
- Focus on modern bioanalytical applications of fully miniaturized column-switching.

Abstract

Column switching (CS) emerged as a strategy for direct injection of raw biological samples and currently is the more versatile technique for the fully automated integration of the sample preparation and the chromatography analysis. At the miniaturized scale, CS addresses the matrix complexity and allows the injection of sample volumes larger than those supported by the capillary/nano analytical columns. Detectability can be significantly improved while preserving the analytical capillary/nano column and the proper functioning of the instrument. In the last two decades, fully miniaturized CS systems have been under continuous development. Innovative polymeric, inorganic, and nanomaterials-based extraction phases have been introduced and exploited in diverse column formats, including particle-packed, monolithic, fiber-packed, and open tubular, extraction media. This paper reviews the more recent advances in microextraction column technology for fully miniaturized column-switching systems. Modern sorptive phases and extraction will be described, discussing their potentialities, advantages, and limitations. The overview will emphasize the significance of those developments in bioanalytical applications.

Keywords: Microextraction; automated sample preparation; online SPE; in-tube SPME; column; switching; bioanalysis

Abbreviations: **γ-MAPS**, 3-(triethoxysilyl) propyl methacrylate; **ADS**, alkyl-diol-silica; **APTES**, 3-Aminopropyl)triethoxysilan; **BAMC**, boronate affinity capillary column; **BSA**, bovine serum albumin; **CE,** capillary electrophoresis; **CNTs**, carbon nanotubes; **-MWNTs**, carboxylic-multi walled carbon nanotubes; **c-SWNTs**, carboxylic-single walled carbon nanotubes; **DMF**, N,N-dimetilformamida; **EDMA-co-VDM**, ethylene glycol dimethacrylate-co-vinyl azlactone; **ESI**, electrospray ionization; **FIT-SPE**, fiber-in-tube solid-phase microextraction; **FLD**, fluorescence detector; **GC**, gas chromatography; **GMA**, glycidyl methacrylate; **GO**, graphene oxide; **HILIC**,

hydrophilic interaction chromatography; **HPLC,** high performance liquid chromatography; **IT-SPME**, in-tube solid pahse microextraction; **LC,** liquid chromatography; **LDHS**, layered double hydroxides; **LLLE**, liquid-liquid extraction; **LOD**, limits of detection; **MS**, mass spectrometry; **MIP**, molecularly imprinted polymers; **MOF,** metal-organic framework; **MTEOS**, triethoxymethylsilane; **NPs**, nanoparticles; **OT,** open-tubular column; **PDMS,** polydimethylsiloxane; **PAHs**, polynuclear aromatic hydrocarbons; **PCFs**, photonic crystal fibers; **PD**, polydopamine; **PEEK**, polyether ether ketone; **PEGs**, polyethylene glycols; **PEDOT**, poly(3, 4-ethylenedioxythiophene); **PLIs**, polymeric ionic liquids; **PLOT,** porous-layer open tubular; **PS-DVB,** poly(styrene-co-divinylbenzene); **PS-OD-DVB; PTFE**, politetrafluoroetileno; **RAM**, restricted access media; **RP**, reversed-phase; **SAX**. strong anion-exchange; **SPE**, solid-phase extraction; **SPME**, solid-phase microextraction; **UHPLC**, ultra-high performance liquid chromatography; **VPBA**, 4-vinylphenylboronic acid; **WCOT**, wall coated open tubular columns; **ZIFs**, zeolitic imidazolate frameworks

1 Introduction

Since the last century, analytical chemistry has made significant technological and theoretical advancements, resulting in a "new era" of high-performative methods. New research fields have been springing up, demanding more sensitive and greener approaches for target and non-targeted analysis of many compounds. Significant preponderance has received analytical techniques such as liquid and gas chromatography (LC and GC), mass spectrometry (MS), and capillary electrophoresis (CE).

Liquid chromatography can be considered one of the most studied techniques evolved in the last years, as discussed in a series of reviews published last year [1–3].

Such interest emerged from its versatility in analyzing a broad spectrum of compounds covering 75% of the analytes monitored in routine analyses [4]. LC instrumentation has remarkably progressed during this period. New developments include connections with zero-dead volumes or developing instrumental parts capable of withstanding high-pressure levels (up to 1000 bar) while working with flow rates (μL. and nL. min^{-1}) [5]. Miniaturized LC modes have widespread, and capillary- and nanoLC columns (0.5 – 0.075 mm i.d.) have become to replace the conventional ones (4.6 – 2.1 mm i.d.). Miniaturized LC brings significant analytical advantages as (i) gains in chromatographic efficiency and sensitivity; (ii) reduction on chemicals and sample consumption, and then, less toxic waste generation; as well as (iii) the development of portable-LC instruments for in-field analysis (Maciel et al., 2020).

On the other hand, more high-throughput and sensitive methods are growing in demand in bioanalytical chemistry due to their crucial role in discovering and surveillance drugs, biomarkers, and metabolites [7]. Despite LC's current level of excellence in performing such analyses, most current methods still demand a preliminary sample preparation step [7]. biological samples are complex mixtures, which contain many interferents (e.g., carbohydrates, lipids, salts, etc.) while most of the target compounds are present at trace levels, usually between μg L^{-1} – ng L^{-1} [8,9]

Suitable sample preparations for bioanalysis should: (i) increase detectability by pre-concentrating the target analytes while eliminating most interferents; (ii) reduce the matrix effect helping to enhance even more the analytes' signal and reproducibility; and (iii) clean-up the extract to improve equipment lifetime and method robustness [9]. In modern times, the increasing number of biological samples has encouraged new developments on modern high-throughput methods while maintaining an ecological consciousness. The two most efficient and recognized strategies to cope with are

miniaturizing and automating the sample preparation step [9]. Despite the excellent performance reported by conventional techniques (e.g., liquid-liquid extraction – LLLE – and solid-phase extraction – SPE), they often require multi-steps and substantial amounts of chemicals and samples to be performed adequately [10]. On the contrary, miniaturized techniques demand lower amounts of them being considered a greener approach. However, even with these good characteristics, such approaches still involve multi-steps, possibly conducting to analytical errors and reducing productivity [10].

The combination of miniaturization and automation allows the most lingered drawbacks in sample preparation: labor-intensiveness and longstanding procedures. Automated approaches enhance laboratory productivity by reducing the steps and accelerating time-per-analysis while diminishing sample handling by operators [11]. Nowadays, there are several miniaturized, automated sample preparation techniques, being most of them emerged as an adaptation from its non-automated version [12]. Despite the good reportings of such approaches, the existence of different microextraction devices (e.g., MEPS syringe, SPME fiber, SPE cartridge), per se, does not favor an effective coupling with LC without the existence of a suitable interface [12]. As shown in **Figure 1**, geometrically distinct microextraction devices often require specifically-designed platforms or interfaces to execute an automated sample preparation properly.

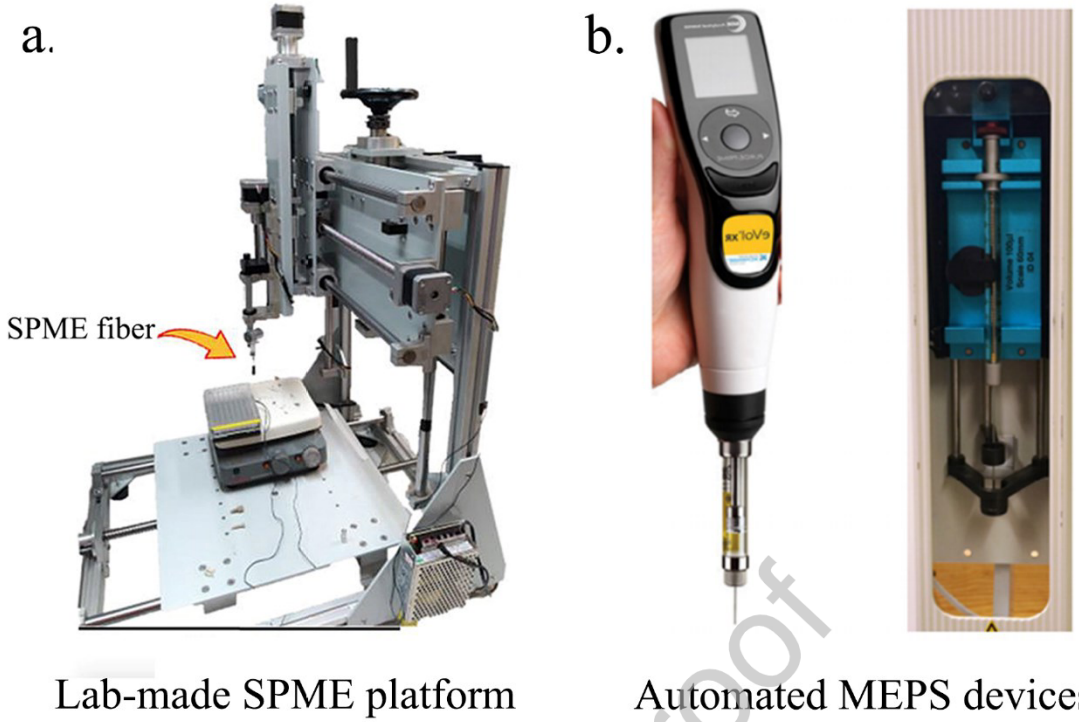


Figure 1. Representation of specifically-designed interface to automate microextraction techniques: (a) lab-made SPME platform; and (b) Automated MEPS devices. It was adapted from [13,14] with the permission of Elsevier, 2021.

The better alternative to overcome such inconveniences is the column switching technique, where extraction column online connected with the chromatographic analytical column. Both sample preparation and chromatographic separation happen using the same type of hardware connected via a six or ten-port rotating valve [15]. The sample is injected into the first column (extractive), where target compounds are trapped, while most of the matrix interferents pass directly through the waste. The switching valve rotates after a pre-defined loading time, allowing the chromatographic mobile phase flow within the microextraction column, eluting the target compounds,

and transferring them towards the second column (analytical) without further sample manipulations [15]. Column switching gathers techniques as in-tube SPME, online SPE, and similar approaches [15,16].

Figure 2 shows the two most used arrangements in these systems: flow-through and draw-eject procedures. In the flow-through mode **(Figure 2a)**, the sample passes only one time through the extraction column. In contrast, placing the extraction column between the injection loop and the injection needle, the draw-eject mode **(Figure 2b)** allows multiple cycles of analytes-extractor interactions [17,18]. While the draw-eject mode can be performed in some instruments, the flow-through mode requires a more straightforward setup [17]. However, when the amount of sample available is reduced (e.g., biomedicine), the draw/eject process allows a more efficient preconcentration of the target compounds [18].

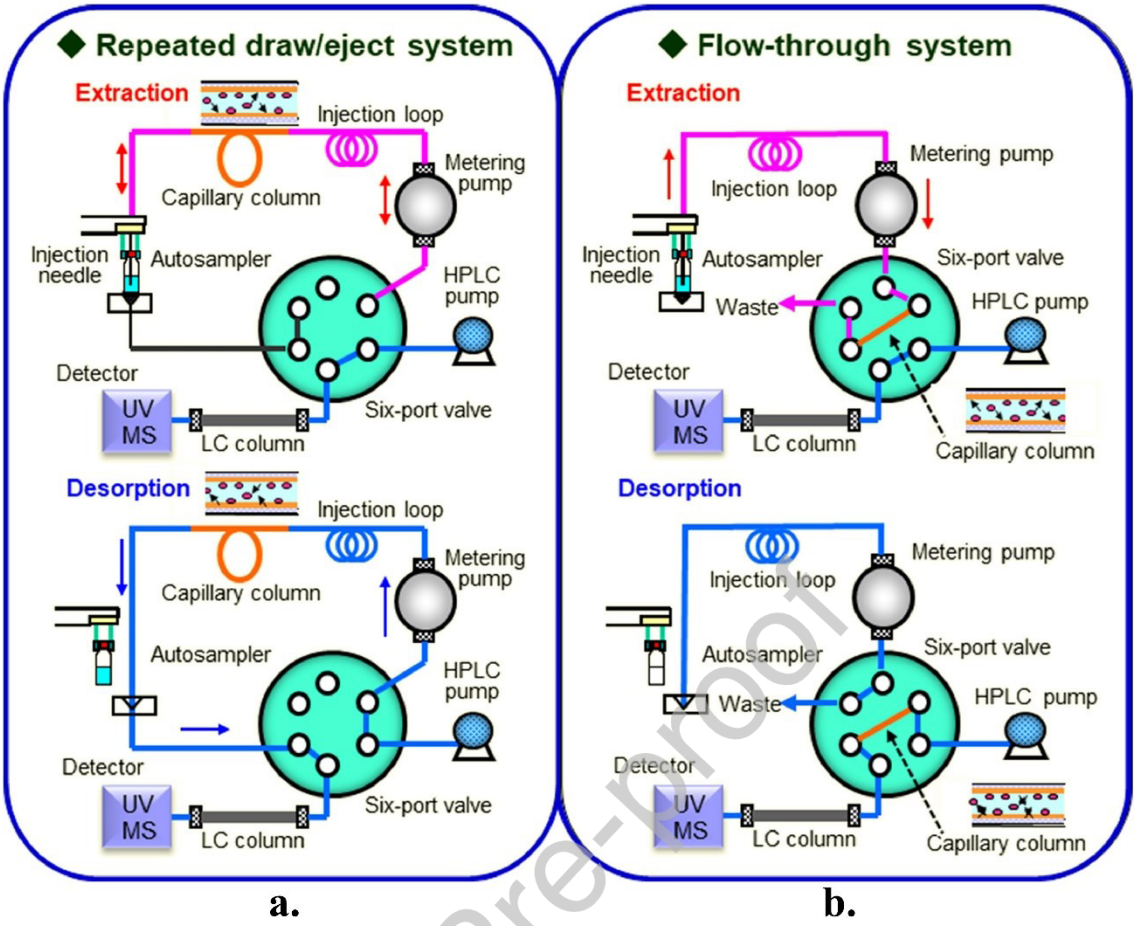


Figure 2. Representative scheme of an IT-SPME approach coupled with liquid chromatography highlighting the most used operation modes: (a) Draw/Eject, and (b) Flow-through. It was adapted from [12] with the permission of Elsevier, 2021.

Bearing in mind the advantages that emerged from miniaturization and automated sample preparation, this review summarizes the essential characteristics of column switching systems focusing on modern extractive phases and fully miniaturized approaches (e.g., using capillary and nanoLC).

The following sections will review the four main types of extractive columns used for column switching approaches: i) particle-packed, ii) monolithic-filled, iii) fiber-packed, iv) and open tubular.

As this topic is a still-in-development field, there are few applications reported in the last five years – our timeframe here – and due to this fact, the authors pursued to include a discussion on the recent advances and future trends which might conduct this field in the coming years. In this context, attention was given to recent applications and manuscripts discussing relevant characteristics on the covered subject that could be important for fully miniaturized column switching systems.

2. Particle-packed columns

2.1 Columns packed with silica-based particles

2.1.1 Classical reverse-phase particles

Despite their significant advantages (e.g., better chromatographic resolution and efficiency), cap and nanoLCa still bear the drawback of the low injection volumes that typically compromise the sensitivity of the methods. One of the best strategies to overcome large-volume injection at the cap/nanoscale is using a reversed-phase (RP) extraction column before chromatographic analysis. RP extraction columns can eliminate salts and low-retained interfering substances while pre-concentrate target compounds. RP particle-packed extraction columns have larger dimensions than the cap/nano analytical ones (i.e., d_p and i.d.) to support larger samples at µL min-1 loading flow rates.

Analytical gains can be derived from the complementary character of diverse particulate RP phases. For example, Gu et al. [19] combined offline and online sample preparation to extract only polyunsaturated fatty acids from high-complex human serum before the nanoLC-MS analysis. The method exploited the complementary properties of the OASIS HLB SPE cartridge and the online C18 extraction column (75 µm i.d. × 2 cm, 3 µm d_p) to clean up the sample as much as possible. Another recent research [20]

introduced an in-lab-made "T" dispositive to perform water dilution of a high-organic percentage sample trapping into a C18 extraction column (300 µm i.d. x 0.5 cm, 3.5 µm d_p). Such an approach supports 20 µL of injection volume, focalizing and pre-concentrating the analytes for further separation into a C18 nanocolumn (75 µm i.d. x 15 cm x 3.5 µm d_p). Compared to the conventional scale, LODs 400 times higher were obtained. Although this system has not been applied yet for bioanalytical-interesting compounds, such results suggest a promising way to improve even more trace-level analysis sensitivity than those in bioanalysis.

The use of particle-packed RP extraction columns in miniaturized LC is predominantly in omics analysis [19,21–30]. This tendency might be related to the considerable injection volume supported by them and their adequate extraction capacity. [31] **Table 1** gathers the last five years of selected bioanalytical applications (not fully inclusive) using miniaturized extraction columns packed with classical reversed-phase particles for fully automated analysis of different matrices.

Table 1. Bioanalytical applications of miniaturized extraction columns packed with RP particles coupled to miniaturized LC.

Analytes	**Matrix**	**Analytical technique**	**Extraction column (1D)**	**Injection volume**	**REF**
Quecetin and derivatives	Human epithelial cells (Caco-2)	nanoLC-MS/MS	C18 (20 mm x 300 µm i.d., 5 µm of particle size)	5 µL	[16]
Steroids	Starfish *Lethasterias*	nanoLC-	C18 (5 mm x	0.2 µL	[18]

	fusca	MS/MS	300 µm i.d., 5 µm of particle size)		
Several xenobiotics	Benthic invertebrates (*Chironomus riparius*)	nanoLC-MS/MS	C18 (5 mm x 300 µm i.d., 5 µm of particle size)	1 µL	[19]
Metabolites	Human breast cells	nanoLC-MS/MS	C18 (20 mm x 75µm i.d.)	--	[20]
Bioactive compounds	Snake venoms	nanoLC-MS	C18 (5 mm x 300 µm i.d., 3 µm of particle size)	1 µL	[21]
Proteins	Ovarian tissues	nanoLC-MS	C18 (20 mm x 75µm i.d.)	--	[22]
Somatostatin	Rat plasma	nanoLC-MS/MS	C18 (20 mm x 100 µm i.d., 5 µm of particle size)	1 µL	[23]
Lipids	Human serum/ pig serum and cerebrospinal fluid	nanoLC-MS/MS	C18 (20 mm x 75µm i.d., 3 µm of particle size)	4 µL	[24]
Fatty acids	Human serum	nanoLC-MS	C18 (20 mm x 75µm i.d. 3 µm	1 µL	[14]

			of particle size)		
Glycoproteins	Human serum and plasma	nanoLC-MS/MS	C18 (5 mm x 300 µm i.d., 5 µm of particle size)	5 µL	[25]
Peptides	Dried human blood	nanoLC-MS	C12 (5 mm x 150 µm i.d., 4 µm of particle size)	1 µL	[17]

2.1.2 Silica-based particles with restricted access material (RAM)

The term restricted access material (RAM) was introduced in the early '90s and represented classes of materials that allow direct injection of biosamples. The direct injection of biological fluids is only possible because of RAMs' particular surface structure, specially designed to prevent the adsorption of macromolecules from the matrix while retaining the analytes in its inner structure [32]. The alkyl-diol-silica (ADS) particles are a typical commercially available RAM, while the use of bovine serum albumin (BSA), glycidyl methacrylate (GMA), and sugars are common to produce lab-made RAM sorbents [33].

Between 2006 and 2008, Santos-Neto and collaborators present three interesting applications of fully miniaturized CS systems with RAMs. For direct injection of fluoxetine in human plasma, Chromobond C18 particles (45 µm dp) were employed to produce lab-made RAM-BSA-C18 microextraction columns (50 mm x 520 µm i.d.). Separations were carried out in a lab-made Phase Sep C18 “Stable pH” (100 mm x 520 µm, 3.0 µm dp) analytical column followed by UV detection. The authors highlighted

the system's potential for further downscaling and coupling to ESI-MS [34]. In another work, for the direct analysis of human plasma and urine, the authors compared ADS commercial particles with lab-made RAM (modified with BSA) to produce microextraction columns with i.d.s between 200 - 320 µm. Separations were carried out in lab-made YMC ODS-AQ analytical columns (50 x 0.2 – 0.25 mm, 5.0 µm dp). In this study, the authors determined the RAM-ADS particles as the most appropriate sorbent, as it presented adequate exclusion of macromolecules and eliminated the matrix effect using ESI-MS/MS [35]. Finally, for analysis of 5 antidepressants from direct injection of human plasma, commercial RAM materials were employed to produce 200 µm i.d extractive columns. The separations were carried out in a lab-made YMC ODS-AQ (85 mm x 250 µm, 5.0 µm dp) analytical column coupled to capLC-MS/MS. The use of RAM-ADS particles resulted in insignificant matrix effects; also, with a fully miniaturized LC system, better ESI-MS conditions were achieved [36].

2.2 Columns packed with other particulate materials

2.2.1 Molecularly imprinted polymers (MIP)

MIPs are synthetic materials with interaction sites designed to bind a class of substances exclusively. That selectivity derives from the polymerization in the presence of a template substance, a functional monomer, a cross-linker agent, and a polymerization initiator. The monomer-template interaction forms a pre-polymerization complex via reversible interactions (ionic or hydrogen-bond, hydrophobic interaction, etc.) [37]. This pre-polymerization complex is cross-linked, forming the three-dimensional MIP polymeric structure. After the polymerization, the template molecules are removed from the polymeric surface, washing with a proper solvent to leave three-dimensional cavities with molecular recognition capabilities [38].

Besides a high selectivity, the MIPs are also resistant and stable in a wide range of pH, bearing a high concentration of organic solvents and relatively high temperatures. [39]MIPs can be easily combined with column-switching approaches, making them helpful extraction phases for column production [38,40]. The production of restricted access media-MIP (RAM-MIP) materials has also been reported using the in-tube solid-phase microextraction (IT-SPME) format [41]. Regarding the use of MIPs in fully miniaturized column-switching systems, there were not found recent reports. However, MIPs have great potential for column [42]

switching bioanalysis. For example, in a recent bioanalytical application of MIP in a column-switching system, three commercially available sorbents were evaluated to produce efficient stainless-steel extraction columns. Authors packed between 70–80 mg of the MIP sorbent into a 25 mm × 3 mm i.d. guard-column hardware [43]. Even though the three commercial sorbents presented very similar and satisfactory results, and according to the authors, are substitutable, they aim to produce lab-made MIPs specifically designed to reach better compatibility between extraction and separation.

2.2.2 Miscellaneous particulate sorbents

Several other materials have been used as sorbents in packed extraction columns, including carbon allotropes, enzyme-based materials, and metal-organic frameworks, among many others. [44–49] This variety enhances the chances of matching an ideal material to a specific target compound, leading to better selectivity and extraction performance.

Miniaturized extraction columns packed with carbon-based materials are acquiring growing applications [47,48,50–53]. Carbon allotropes exhibit excellent physicochemical properties for extraction purposes, such as high surface area, delocalized π-π electron system, structure-axial oxygen-based active sites, simple

manufacturing procedure, and easy functionalization [54]. These properties result in several interaction mechanisms between target compounds and the sorbent (e.g., hydrogen bonding and hydrophobicity), which allows the extraction of different molecules [46]. For example, Toffoli et al. [51] demonstrated the suitability of packed graphene-based extraction columns for the online extraction and pre-concentration of several target compounds, including antibiotics, veterinary drugs, preservatives, cannabinoids, and mycotoxins.

Enzyme-based packed columns are a standard in proteomic analysis. For example, considering the difficulties in performing high-sensitivity proteomic analysis of volume-limited samples, Hata et al. [49] proposed a trapping step using an extraction column packed with an immobilized enzyme (trypsin) responsible for lysing and digesting mammalian cells in a single workflow. The sample losses were reduced as much as possible in the sample preparation step, which is a positive characteristic once these volume-limited samples could be used more efficiently from an analytical and economic viewpoint. Sun and Jiang [45] presented another exciting approach focusing on reducing sample losses[45]. Instead of employing a conventional RP extraction column for trapping phosphopeptides, the authors changed to a strong anion-exchange (SAX) phase. By these means, they reported gains in extraction efficiency once most of the polar phosphopeptides lost in an RP extractive phase were efficiently trapped in the SAX phase.

Metal-organic frameworks are also excellent candidates for producing high-efficient extraction columns due to their high surface area, porous tunability, and easy chemical functionalization. Despite the few bioanalytical applications acting as an extraction column so far, they still can represent a promising alternative to achieve high selective methods for column-switching systems [44,55].

Finally, other alternatives such as ionic liquids (ILs), deep eutectic solvents, or natural compounds (e.g., chitosan) have already been tested as sorbents for online extraction columns [56,57]. They possess unique properties highlighting their environmentally-friendliness [58]. New applications employing alternative materials in miniaturized extraction columns for column switching-based could be expected in the future.

2.3 Columns packed with fibrous materials

Fibrous materials offer competitive extraction capabilities and are an excellent alternative media for sample preparation via modern sorbent-based techniques [59,60]. Diverse polymeric, inorganic, or mixed materials produce fibers liable of diversified interaction mechanisms for suitable retention for a wifely variety of molecular structures [61,62]. Moreover, the addition of polymeric crown ethers [63] or molecularly imprinted particles [64] in the fibrous structure can lead to the obtention of very selective fibrous extracting materials. Molecularly imprinted fibers are prepared by adding the template with the polymer/precursor during the fiber fabrication process [65,66]. Likewise, some nanofibrous polymers, such as Poly-ε-caprolactone, also have recently been demonstrated to be a simple alternative to restricted access media for online treatment of protein-containing biological samples [67].

In column switching approaches, fiber-packed extraction columns provide almost the same column permeability and low backpressures of the open tubular columns but with the packed columns' sample capacity. Two types of fiber-packed extraction have been described in the last years: i) fiber-in-tube columns [40] and ii) nanofibrous packed columns [62].

2.3.1 Fiber-in-tube columns

The use of fiber-packed microextraction columns in online column-switching approaches has its more remote antecedent in the so-called fiber-in-tube solid-phase microextraction (FIT-SPE) introduced by Jinno's research group [68]. FIT-SPE used a short PEEK tube (0.25 mm i.d.) capillary packed with hundred heterocyclic polymeric fibers (Zylon®) of 11.5 µm of diameter, organized in a similar format. The extraction media then compassed a series of narrow coaxial channels, facilitating the sample percolation, offering reduced back pressure and lower clogging risk while demonstrating excellent preconcentration/enrichment capabilities.

In the first demonstration of fully miniaturized online extraction [69], a FIT-SPE cartridge — prepared with a PEEK tube of 0.50-mm i.d. x· 5.0-mm length, packed with Zylon® filaments — was installed in the rotor of the injection valve of a Micro-LC-UV-Vis system (**Figure 3a**). This FIT-SPE setup provided limits of quantification less than 1 ng mL^{-1} in the analysis of phthalates and tricyclic antidepressant drugs. Another fully miniaturized FIT-SPE approach, featuring a polymer-coated fibrous material as the extraction medium, demonstrated suitable performance in the online sample preparation of tricyclic antidepressants (TCAs) [70]. In this case, the microextraction column was prepared by packing ca. 330 Zylon® fibers (11.5 µm o.d.) in a 0.32 mm i.d. capillary and sequentially coated with 5% solution of HR-52 (5%-phenyl-polydimethylsiloxane, Shinwa Chemical) polymer in n-hexane/acetone (90/10). This FIT-SPE device allowed the direct analysis of biologic fluids by coupling with a Capcell Pak C18 MG (1.0 mm i.d., 150 mm length, 5 µm d.p) analytical column through a six-port valve. Limits of quantification were suitable for the analysis of TCAs in clinical and forensic situations.

Although, to the best of our knowledge, no recent reports are describing the online coupling with capillary/nano-LC, since its introduction FIT-SPE-LC demonstrated excellent performance in many research areas, including the treatment of biological samples [40]. For example, Hu and coworkers developed a molecularly imprinted coating based on a FIT-SPE-LC method to analyze antibiotics in pork liver and chicken samples [71]. Selective antibiotics extraction was achieved employing a PEEK tube (0.50 mm i.d. x 6 cm) packed with a longitudinal arrangement of silica fibers (125 μm o.d.), coated with ofloxacin MIP (**Figure 3b**). Feng et al. introduced a FIT-SPE device packed with copper wires chemically functionalized with ionic liquids (ILs) for estrogens' microextraction [72]. Metal support not only increases the stability of in-tube SPME devices but also improves the sensitivity by increasing sample volume with a high sampling rate, which is associated with the excellent extraction ability of ILs coating yields high enrichment factors (611–1661) and low LODs (0.02–0.05 g L−1). Ling and coworkers developed a FIT-SPME column packed with carbon bundles electrochemically modified with the conducting polymer poly(3, 4-ethylene dioxythiophene) (PEDOT) as a suitable sorbent for online microextraction of the sulfonamides in aqueous and plasma samples [73]. The coated fibers were stable under harsh conditions. The electrochemical modification demonstrated feasibility for immobilizing conducting polymer for highly selective extraction of target molecules, with excellent extraction efficiency.

Similarly, the Yamini research group reported a novel electrochemically controlled fiber-in-tube solid-phase microextraction of antipsychotic drugs from biological samples [74]. Eight stainless-steel wires were packed into the stainless-steel column. A nanostructured Cu-Cr-Al ternary layered double hydroxide/polythiophene (LDH) coating was prepared on the inner surface of the stainless-steel tube on the

surfaces of the stainless-steel wires by a facile in situ electrodeposition method. LDHs such as conducting polymers were appropriate for extracting acidic and basic analytes, and Cu-Cr-Al-LDH-PTh nanostructure coating showed an excellent extraction efficiency for cationic analytes. This method's superior extraction efficiency may be attributed to combining the eight steel fibers and the steel tube.

2.3.2 Nanofibers-packed columns

More recently, the development of fiber-packed microextraction columns has focused on using electrospun polymer nanofibres as extraction media [61,62]. Diverse polymers with suitable chemical stability for column switching applications can be provided nanofibres via electrospinning. However, due to their very reduced diameter, electrospun nanofibers have low mechanical stability, and their three-dimensional structures are prone to collapse. Therefore, typical nanofibrous extraction phases manufactured via electrospinning are obtained in two-dimensional fabrics and then cut and packed into the microextraction column hardware. Obtaining repeatable and uniform packings is a challenging task.

The potential of the nanofibrous materials as extraction media has been exploited in diverse formats in packed sorbent-based microextraction, including micro solid-phase extraction (μ-SPE) [75], microextraction by packed sorbent (MEPS) [76], planar disks, and pipette tips [77]. Some specialists have considered that nanofibers could become preferred materials for efficient online extractions in chromatographic systems (Háková et al., 2020). However, few reports describe the online integration of nanofibers-packed columns in column switching liquid chromatography systems, and to the best of our knowledge, studies dealing with systems at a fully miniaturized scale have not been reported yet.

Most of the reported applications of the nanofibers-packed columns correspond to the development of LC methods in a conventional scale, using online [62]SPE hardware of 4.6 µ.m i.d. —probably due to the challenges of pack nanofibers in very narrow columns —. The first demonstration of nanofibers' use in online extractions coupled to liquid chromatography was reported in 2014 by Bagheri et al. [78]. They developed an online automated method for determining clodinafop propargyl from water, soil, and wheat samples, employing a µ-SPE cartridge packed with 11.5 mg of electrospun Polyamide nanofibers (**Figure 3c**). The nanofibers-packed column demonstrates competitive performance face conventional µ-SPE devices. Since then, nanofibers-packed columns' suitability in the development of column switching methods for the analysis of biological samples has been demonstrated in many recent reports [62]. Háková et al. introduced a microextraction column packed with polycaprolactone nanofibers functionalized with a dopamine coating for online extraction of bisphenols, beta-blockers, nonsteroidal drugs, and phenolic acids in water, plasma, and urine [79]. The polydopamine coating shows an improved ability to extract polar compounds, especially in determining hydrophilic compounds, such as phenolic acids, in liquid samples with a high content of strongly retained lipophilic interferences. The same research group recently developed a column switching method for determining parabens in bovine milk and human serum [67]. The authors demonstrated in this case that a microextraction column packed with Poly-ε-caprolactone nanofibrous polymer provides excellent macromolecule exclusion capabilities, becoming a feasible substitute for the RAM media. The parabens were selectively and efficiently retained, while the ballast protein macromolecules were quantitatively eluted from the nanofibrous sorbent.

From another approach, Mahdi Moein and coworkers manufactured an innovative sol-gel-based molecularly imprinted polymer (MIP) nanofiber via electrospinning on the surface of a stainless-steel wire [65]. After being prepared, the fiber was packed into a metallic needle and used for online extraction of acesulfame sweetener from beverage samples (**Figure 3d**). This extraction device demonstrated excellent reusability and allowed the development of an automated, straightforward, and fast HPLC method.

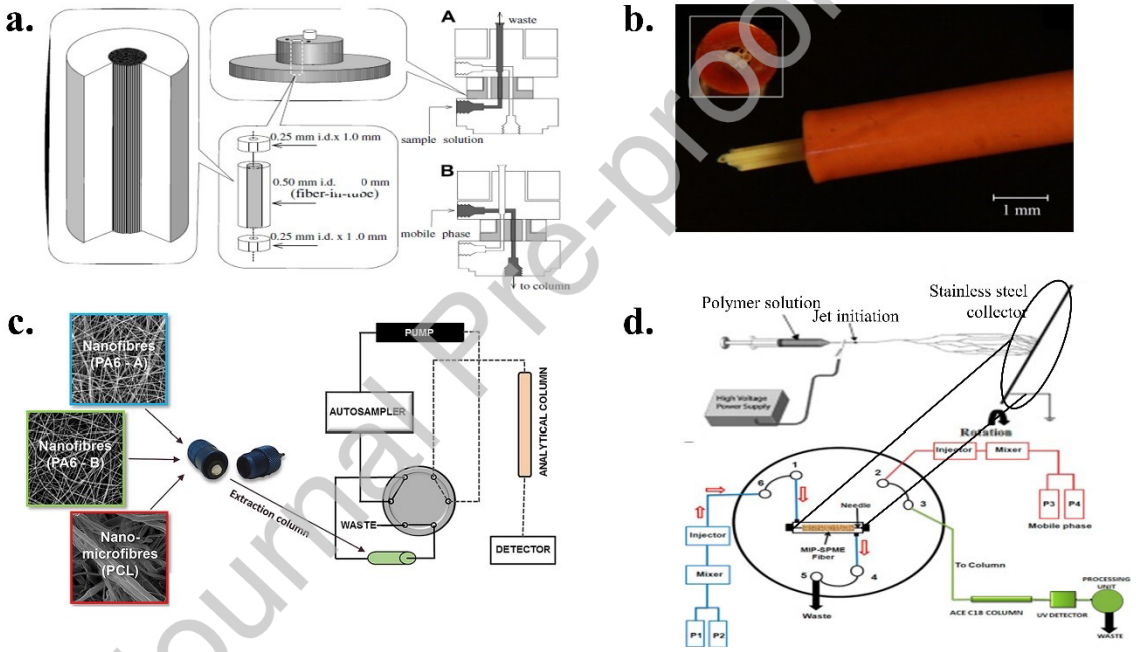


Figure 3. Examples of different fiber-packed microextraction columns. **a)** fully Miniaturized fiber-in-tube (FIT) system with the extraction cartridge installed in the microinjector valve [69]; **b)** Micrograph of the multiple fibers with molecularly imprinted coating packed into a peek tube [71]; **c)** on-line µ-SPE-HPLC set up featuring electrospun polyamide nanofibers sorbent [78]; **d)** Molecularly imprinted sol-gel nanofibers based solid phase microextraction coupled on-line to LC [65]. It was adapted the cited references with permission of Elsevier, 2021.

3. Monolithic columns

Monolithic stationary phases arose in the early '90s as an alternative to particle-packed columns [80]. A single piece of porous materials forms the monolith, which can perform separations at high flow rates with low backpressures. Monoliths can come from organic or inorganic materials or even be combined into hybrid organic-inorganic phases [81]. For monolith use in fully miniaturized CS systems, the inner surface of a silica capillary is modified with 3-(triethoxysilyl) propyl methacrylate (γ-MAPS) to allow the chemical anchoring of phase to the capillary wall, avoiding problems such as monolithic detachment [82].

Recent developments in monolithic phases for bioanalysis include surface modification and functionalization for microextraction selectivity enhancement [83]. **Table 2** summarizes the most recent monolithic microextraction columns coupled with capLC or nanoLC for bioanalytical applications. Details of the presented works will be discussed in the following sections.

Table 2. Recent applications of monolithic extraction columns coupled to miniaturized LC for analyzing biological samples.

Analytes	**Matrix**	**Analytical technique**	**Extraction column**	**Injection volume**	**LOD.**	**Ref.**
Dopamine	Urine	capLC-UV	Online SPE-LC: fused silica capillary	60 nL (calibration curve	0.12 mg L-1	[79]

			VPBA monolithic column (250 mm x 320 µm i.d.) Comprehensive SPE-LC: 2 fused silica capillary VPBA monolithic columns (90 mm x 320 µm i.d.)	method) or 20 µL (standard addition method)		
Cis-diol nucleosides (uridine, cytidine, adenosine, and guanosine)	Phosphate buffer	nanoLC-UV	Fused silica boronate affinity capillary column (µBAMC) (10 mm x 75 µm i.d.)	1 µL	50 ng mL-1 (uridine)	[80]
Protein and peptides	Standard E. Coli tryptic	capLC-MS/MS.	Fused silica capillary γ-poly-(LMA-	4 µL	--	[81]

	digest		co-HDDMA) monolithic column (50 mm x 250 µm i.d.) and Acclaim PepMap100 C18 trapping column (5 mm x 300 µm i.d., 5 µm particle size)			
Lung cancer biomarker ProGRP	Human serum	nanoLC-MS	Fused silica capillary antibody-immobilized (EDMA-co-VDM) monolithic column (150 mm x 180 µm i.d.) and Acclaim PepMap C18 RP trapping	20 µL	520 pg mL-1	[82]

			column (5mm x 1 mm i.d., 3 μm particle size)			
Cocaine and benzoylecgonine	Plasma and saliva	nanoLC-UV	Fused silica cappilary monolithic MIP (MAA-co-TRIM) (50 mm x 100 μm i.d.)	50 nL	6.1-14.5 ng mL-1	[83]
Cl−, Br−, NO3− and HPO42-	Weak organic and inorganic acids and hydrogen peroxide	capLC-CD	Anion exchange monolithic column IonSwift MAC-200 (80 mm × 0.75 mm i.d.)	50 μL	2.1–32.6 ng L-1	[84]
Caffeine and metabolites (theobromine, paraxanthine, and theophylline)	Serum, saliva, and urine	capLC-DAD	Fused silica capillary monolithic column (TEOS-MTEOS) with	25 or 100 μL of diluted samples	0.1-0.5 μg mL-1	[85]

			TiO2 and SiO2 NPs (30 cm x 320 µm i.d.)			

3.1 Monolithic phases with boronate functionalization

The recent use of boronate functionalized monolithic columns focused on cis-diol compounds' selective isolation, such as dopamine neurotransmitters and some nucleosides. Janků and coworkers developed a capillary monolithic trapping column containing 4-vinylphenylboronic acid (VPBA) for microextraction and another monolithic capillary column with zwitterionic functionality chromatographic separations [84]. The monolithic columns were in-situ synthesized in polyimide-coated fused silica capillaries with 320 µm i.d. The researchers developed two different instrumental arrangements: i) one for online microextraction (one extractive VPBA column followed by a zwitterionic separation column) and ii) another for comprehensive SPE-LC (two extractive VPBA columns, followed by the zwitterionic column), resulting in very selective methods with potential application for focusing and separation of other cis-diol neurotransmitters. Lik Espina-Benitez and collaborators employed monolithic columns for a novel in-line coupling between a boronate affinity capillary column (µBAMC) and a reversed-phase separation column [85]. Monoliths were synthesized into PTFE-coated fused-silica capillaries of 75 µm i.d., functionalized with 3-(mercaptopropyl)trimethoxysilane and 3-(acrylamido)phenylboronic acid or with n-Octadecyl dimethyl(dimethylamino)silane. By the boronate functionalization, the authors observed a remarkable increase in the active sites' of the monoliths, with remarkable gains on the preconcentration capacity and LODs of the method.

3.2 Methacrylate based monolithic columns

Polymethacrylate monoliths are versatile materials employed for different bioanalytical approaches, such as proteomics [86], immunosorbent production [87], and molecularly imprinted monoliths [88]. Capriotti and collaborators developed a multidimensional nanoLC system for shotgun proteomics, featuring two sequential capillary extractive columns. A polymethacrylate monolith, a C18 packed column, and a fused silica analytical nanocolumn — (25 cm × 75 μm i.d., 2.2 μm dp) packed with Acclaim-C18 particles—, were online coupled [86]. With this setup, peptides from complex samples were selectively trapped, divided into two different fractions, and independently analyzed. The authors highlighted the setup potential for protein identification in bottom-up proteomics without a comprehensive approach. More than 1476 proteins were identified from the standard E. Coli tryptic digest.

In a similar configuration, Levernæs and coworkers developed a multidimensional method, integrating a proteotypic epitope peptide immunoextraction with nanoLC-MS/MS. The authors prepared ethylene glycol dimethacrylate-co-vinyl azlactone (EDMA-co-VDM) monoliths, immobilized with anti-protein antibodies [87]. Samples were injected into the antibody immobilized column for the analytes' immunocapture, with other peptides discrimination. Then, the retained peptides were sequentially trapped into the C18 extractive column and eluted to the Acclaim PepMap C18 RP column (150 mm x 75 μm i.d., 3 μm dp), for separation. The authors demonstrated the monolithic EDMA-co-VDM polymer's applicability for immobilizing antibodies and selective peptide extraction with insignificant secondary interactions and low backpressure.

Bouvarel and collaborators (2020) produced a molecularly imprinted monolith, using cocaine as a template molecule for online microextraction nanoLC-UV analysis [88]. The extractive columns used methacrylic acid as monomer and were *in-situ* synthesized in fused silica PTFE-coated capillaries (100 μm i.d), separations were carried out in a nanoLC column Acclaim PepMap100 C18 (150 mm x 75 μm, 3 mm dp). After successfully coupling the imprinted monolithic with nanoLC, authors foresee developing a lab-on-chip microfluidic device with possible electrochemical, fluorescent, or UV detection.

3.3 Ion-exchange monolithic columns

Ye et al. combined several columns of different diameters, including an ion-exclusion column, a monolithic anion-exchange concentrator column, and capillary anion-exchange columns [89]. loading samples into the ion-exclusion column, inorganic anions were retained and separated from the weak acid matrix. In the next step, the retained anions were eluted from the ion-exclusion column and concentrated in the monolithic column. Then, with a switching valve's help, the concentrated anions were eluted from the monolithic column to the in-series capillary columns utilizing a capillary pump. It is relevant to highlight that the ion-exclusion column, an IonPac ICE-AS1 (250 mm × 9 mm i.d., 7.5 μm dp), had a much larger internal diameter than the IonSwift MAC-200 monolithic column used for preconcentration of anions, or the IonPac AG18-Fast (35 mm × 0.4 mm i.d., 13 μm dp) and IonPac AS18-Fast (150 × 0.4 mm i.d., 7.5 μm dp) capillary columns. Thus, the monolithic column demonstrated some advantages, including a high permeability and the capacity to provide efficient separations in a broad range of linear velocities. Monolith received a relatively high flow rate of 0.55 mL min-1 from the ion-exclusion column for preconcentration

analytes. Also, it employed a much lower flow rate (0.01 mL min-1) for efficient separation of preconcentrated anions, as authors observed that high separation efficiency of inorganic anions is achieved by the three columns (MAC-200, IonPac AG18-Fast, and IonPac AS18-Fast) connected in series.

3.4 Monolithic columns modified with NPs

Aiming to quickly analyze small amounts of biological samples in a preterm newborn's hospital, Ponce-Rodríguez et al. (2019) [90] tested different capillary CS arrangements. The authors employed 320 μm i.d. OT and monolithic extractive columns (modified with TiO_2 and SiO_2 nanoparticles) in the first dimension, followed by commercial analytical packed or monolithic columns in the second dimension. The setup demonstrates suitability for fast analysis of caffeine in paired serum and saliva samples from preterm newborns in treatment for apnea. Moreover, it presented the excellent potential for implementation in routine analysis.

4 Open tubular extraction columns

Although mainly used in the analytical dimension, open tubular columns (OT) also exhibit some advantages as extraction media, particularly for column switching in miniaturized liquid chromatography (capillary and nano-LC). OT columns work at flow rates that are more compatible with cap/nanoLC, and their extraction phases — in the format of a thin film coating the inner wall of the tube — provide high permeability and reduced back pressures [91]. Samples can be drawn and ejected through the preconcentration OT column in a sequence of repetitive cycles, enhancing analytes uptakes and methods LODs [92–94]. Moreover, thanks to the vast diversity of coating

materials, OT columns can provide diversified chemical interactions with adequate retention, even under up to 100 % of organic solvent [91].

The use of OT columns as an extraction media in fully miniaturized column switching goes back to the introduction of the in-tube SPME by Gou and Pawliszyn, who for the first time reported the use of a segment of polyethylene glycol (PEG)-coated GC column as extraction media, online coupled to capLC [95]. Carbamates were efficiently extracted/preconcentrated, passing the water samples in repetitive draw/eject cycles, demonstrating sensitivities 24-62 times greater than the obtained with the equivalent in-tube SPME setup at a conventional scale.

In the last two decades, the OT columns' attributes as extraction media in fully miniaturized column switching systems have been explored and exploited to analyze diverse samples in many scientific areas. Although most reported applications correspond to environmental samples, OT columns successfully address biological samples. [57]Table 3 summarizes recent reports describing OT extraction columns' hyphenation with capLC and nanoLC to analyze biological and forensic samples.

Table 3. Recent applications of OT extraction columns online coupled with miniaturized LC for the analysis of biological and forensic samples

Analytes	**Matrix**	**Analytical technique**	**Extraction column (1D)**	**Injection volume**	**LOD**	**REF**
DEHP and degradation	Bivalves and water	CapLC-DAD	TRB-5 400 x	2.0 mL	170 µg kg^{-1}	[92]

products			0.320 mm, 3 µm f.t			
Phthalates	Urine	CapLC-DAD	TRB-5 300 x 0.320 mm, 3.0 µm f.t	4.0 mL	0.05-1.5 µg L^{-1}	[93]
Methamphetamines	Oral fluid	CapLC-FLD	TRB-5/MWCNTs 300 x 0.320 mm, 3 µm f.t	20-50 µL	0.5-0.8 ug mL^{-1}	[94]
Quaternary ammonia compounds (C_{12}-BAK)	Biocide formulations	CapLC-DAD	TRB-35 430 x 0.320 mm, 3 µm f.t	100 µL	0.006-0.03 µg mL^{-1}	[95]
Meropenem	endotracheal tubes	CapLC-DAD	TBR-35 90cm	500 µL	3.0 µg/L	[96]
Caffeine, theobromine, paraxanthine, and theophylline	f serum, saliva, and urine	CapLC-DAD	ZB FFAP 900 x 0.32 mm, 1 µm f.t.	25 µL	0.1-0.5 µg/L	[85]
Diphenylamine	Hand Gunshot	CapLC-DAD	TRB-35 900 x 0.32	300 µL	0.15 µg/L	[97]

	Residues		mm, 3 µm f.t.			
Cannabinoids (THC, CBD, and CBN)	Extracts of Cannabis Plants, Surfaces: Plastic bags, aluminum, paper, skin	nanoLC-DAD	TRB-5 150 x 0.320 mm, 3 µm f.t	10 µL	2-15 ng mL^{-1}	[98]
carbonyl compounds	hand scent	nanoLC-DAD	Immobilized SiO_2-Fe_3O NPs	8 µL	0.03-0.08 ng / cm2 min)	[99]
Diclofenac	pharmaceutical and water samples	nano-LC-UV-Vis	PDMS TRB-5 PDMS TRB-5 c-SWNTs PDMS TRB-5 c-SWNTs PDMS TRB-5 c-MWNTs	--	1.0 ng mL−1for	[100]

			SiO2/PEG spherical Fe3O4 SiO2/PEG spherical Fe3O4 SiO2/PEG spherical Fe3O4 SiO2/PEG c-SWNTs SiO2/PEG spherical Fe3O4 10 SiO2/PEG spherical Fe3O4 SiO2/PEG rod-shaped Fe3O4 SiO2/PEG. PDMS-Fe3O4 gel SiO2/PEG spherical			

			Fe3O4 Between: 170-310 mm and 75 -100 µm i.d.			

Most of the reported applications of open tubular columns in fully miniaturized column-switching approaches use segments of commercial GC columns. Those are wall-coated open tubular columns (WCOT) with non-porous stationary phases, synthesized from modified polydimethylsiloxane polymers (PDMS) [105], such as TBR-5 (5% diphenyl-95% PDMS) and TRB-35 (35% diphenyl-65% PDMS). Selectivity and polarity of the PDMS can also be tunable by substitution of the methyl groups with: i) functional moieties such as phenyl, cyano, and vinyl [106,107], ii) polyethylene glycols (PEGs), Carbowax-20M, superox, innovax, and iii) various groups as GeO2-PDMS, TiO2-PDMS, polypyrrole, and 3-mercaptopropyltrimethoxylanosilica, among many others [93]. However, the application in liquid chromatography of commercial GC columns is somewhat limited. GC-Like stationary phases barely retain polar molecules and exhibit limited stability under organic solvents and very acidic or alkaline conditions.

In the last years, necessary research has been performed to develop LC amenable OT extractant coatings capable of enhanced extraction selectivity and improved sensitivity when coupled online with capLC and nanoLC. Falcó's research group has proposed and introduced a plethora of lab-made OT extraction columns with diversified chemistry, developed explicitly for online coupling with capLC and nanoLC. Recently

those researchers reviewed the development of OT extraction media, distinguishing four types of modified PDMS coatings: i) reinforced with Carbon allotropes, ii) reinforced with SiO2 NPs, iii) reinforced with Metal and Metal Oxide NPs, and iv) reinforced with magnetic extraction phases [18].

4.1 Coatings based on allotropic forms of carbon

Due to their unique characteristic for extraction purposes, such as the large surface area and the honeycomb delocalized π electrons systems, allotropic forms of carbon, such as carbon nanotubes, graphene, and fullerene, have been used in recent years as an alternative source of hydrophobic interactions in the preparation of both extraction and separation phases [54]. In OT columns, the hydrophobicity increases compensate to some extent the low sample capacity.

PDMS coatings reinforced with Carbon allotropes have proved suitable for enhancing the PDMS extraction of apolar and aromatic compounds. For example, Falcó's research group evaluated the possibility of improving the extraction efficiency of PDMS phases by immobilization of carboxylic-single walled carbon nanotubes (c-SWNTs) and carboxylic-multi walled carbon nanotubes (c-MWNTs) on the activated surface of TRB-5- and TRB-35 capillary columns [108]. Activated capillaries were prepared by treating with APTES, glutaraldehyde, and dispersion of c-SWCNTs and c-MWCNTs in DMF: 1,3-dicyclohexylcarbodiimide. CNTs-functionalized coatings did not provide any improvement for the extraction of the polar analytes. However, a significant enhancement in the extraction of nonpolar compounds, such as pyriproxyfen, PAHs [108], amphetamines [109], or diclofenac [104], was observed. The incorporation of CNTs to the PDMS film favored the coating's affinity for the analytes due to the

additional π-π interactions established between the aromatic rings of the analytes and the CNTs.

Similarly, but online coupled with LC at a conventional scale, Zhang and coworkers developed a graphene oxide (GO) OT microextraction column [110]. In this case, the inner surface of a poly(tetrafluoroethylene) (PTFE) tube functionalized — using mussel-inspired chemistry — with multiple layers of polydopamine (PD) and GO via covalent and non-covalent anchoring. This OT column was employed in the online extraction of PAHs, followed by their analysis by HPLC-FLD. The multilayer graphene-modified columns exhibited good stability, high sampling speed, and excellent extraction efficiency, with enrichment factors from 1082- to 2331 and limits of detection in the sub-pg/mL order.

4.2 Coatings based on NPs

On the other hand, for the extraction of more polar compounds, porous TEOS-MTEOS coatings doped with SiO2, CuO, or TiO2 NPs have been demonstrated to be a better option. For example, Serra-Mora and coworkers prepared OT extraction columns from PEG, TEOS, MTEOS, and variable amounts of SiO2 NPs [111] for online extraction of polar herbicides, coupled to capLC. Compared to commercial capillary columns with polydimethylsiloxane (PDMS) and polyethylene glycol (PEG)-based coatings, the TEOS-MTEOS/ SiO_2 NPs phase provided particular extractions enhancements for the most polar compounds (log Kow < 1). That improvement was mainly attributed to the combination of different extraction mechanisms, such as hydrophobic, ion-dipole, dipole-dipole, and hydrogen bonding interactions. T

he same research group synthesized and tested TEOS-MTEOS coatings modified with SiO_2 and TiO_2 NPs to extract various pollutants (saccharine, naphthalene

fluoranthene, and some phenylurea organophosphorus herbicides) in both CapLC and NanoLC systems [112]. Compared with TRB-35, FFAP, and PS-DVB commercial OT columns, the TiO_2 doped coating provided better extraction efficiency for most assessed analytes. The SiO_2 NPs functionalized phase showed stronger interaction with compounds nitro groups or moieties through dipole-dipole or hydrogen bonding interactions [113]. Given the diverse range of NPs (TiO_2, ZrO_2, Al_2O_3, Fe_2O_3, ZnO, and CuO NPs) used, the preparation of phases doped with NPs with complementary selectivities, such as CuO and TiO_2 have allowed the preparation of OT extraction columns providing suitable extraction efficiency for analytes in a wide range of polarity [18].

4.3 Coatings based on magnetic NPs

Magnetic hybrid extraction phases are obtained by immobilizing Fe_3O_4 NPs supported on silica onto the internal surface of fused silica capillaries [114]. Applying an external magnetic field around the OT column, NPs create regions with field gradients. Diamagnetic analytes are strongly retained in the regions where the field is minimal. Hence, the analytes are released and transferred to the analytical column after extraction by inversion of the magnetic field's polarity. Compared with the extraction in the absence of a magnetic field, magnetic IT-SPME significantly improved the extraction recoveries of pharmaceutical compounds, such as acetylsalicylic acid, acetaminophen, atenolol diclofenac, and ibuprofen [114].

4.4 Miscellaneous coatings

Although, to the best of our knowledge, there are not yet reports on the coupling at a fully miniaturized scale, other modern materials have received considerable

attention as novel sources of unique interaction mechanisms for the preparation of OT extraction columns. Some examples of those are conductive polymers, layered double hydroxides (LDHS), polymeric ionic liquids (PLIs), metal-organic frameworks (MOFs), and chitosan-derived phases, among many others. Queiroz's research group recently summarized a synopsis on applying all those materials for in-tube SPME [115].

For example, Souza et al. recently described the preparation of capillary OT columns coated with polymeric ionic liquid (PILs) for in-tube solid-phase microextraction of endocannabinoids in plasma samples [116]. PILs based WCOTs extraction media were prepared into 1.0 cm × 0.53 mm-id fused silica capillaries by in-situ thermal-initiated polymerization of the IL monomer ([VC6IM][Cl], [VC16IM][Br], and [(VIM)2C10]2 [Br] ionic liquids) and the cross-linker agent. The obtained thickness coating (1.7 μm.) exhibits good stability so that one extraction column was used more than ninety times without significant changes in extraction efficiency. Although coupled to UHPLC-MS/MS at the conventional scale, this study shows that the careful structural tuning of polymeric ionic liquids can produce selective sorbent phases, with great potential application in fully miniaturized column switching systems.

Evenly, MOFs also have allowed the preparation of alternative coatings with suitable retention for diverse analytes [117]. Zhang and coworkers prepared OT extraction columns innerly coated with zeolitic imidazolate frameworks (ZIFs) [44]. Although online coupled at conventional HPLC, a ZIF-8-PDA-OT column demonstrated excellent extraction capabilities in analyzing PAHs, with high enrichment factors and very suitable sensitivity.

Finally, with OT-LC resurgence, a series of new coatings involving diversified sorbents, such as micelles [118], cyclodextrins [119], and imprinted polymers (MIP) [120], among others, also have been explored. Diverse analytical OT columns for

reverse-phase (RP), hydrophilic interaction chromatography (HILIC) [118], chiral chromatography [119], and micellar chromatography also have been introduced and employed in the separation of diverse organic compound classes. Some of those coatings have demonstrated outstanding potential as extraction phases, and reports describing their use on OT-based column switching approaches at a fully miniaturized scale should appear in the coming years.

4.5 Multi-lumen Capillary OT columns

From another point of view, to improve the OT columns' loading capacity, alternative tube formats such as photonic crystal fibers (PCFs) have been explored. Rodriguez et al. functionalized a PCF (126 channels of 4.2 μm i.d) with C18 to produce multi-channel OT columns [121]. When coupled to mass spectrometry, those multi-lumen capillary columns demonstrated excellent performance as online extraction column, analytical column, and even nanoESI emitter. Also, the Lundanes research group developed an in-tube SPME setup involving an OT extraction column in a multidimensional miniaturized approach. The columns were prepared into a PCF of 126 channels of 8 μm i.d., coated with poly(styrene-co-octadecene-co-divinylbenzene) (PS-OD-DVB) [122]. This PS-OD-DVB-PCF column was assessed in an online extraction of sulfonamides followed by their analysis via open tubular liquid chromatography-tandem mass spectrometry (OT-LC-MS/MS). The relatively large surface area of the 12 cm long tube allowed sample loading up to 80 times larger than the injectable in a single column of 10 μm i.d and the same length. Besides, the system demonstrated faster sample loading and excellent analyte refocusing compared to monolithic and particle-packed extractant columns.

5 Concluding remarks

Despite the level of excellence that modern analytical techniques have been showing, sample preparation is still crucial and often the most time-consuming and laborious part of the whole process. In our opinion, this characteristic negatively contributes to three aspects: (i) increasing time per analysis and decreasing analytical throughput; (ii) the multiple-step impact on the volume of reagents and sample required which are not environmentally friendly; and (iii) the handle-dependency of sample preparation techniques is still one of the most error-producing stages of the analytical workflow. For these reasons, many researchers are looking for automated and greener sample preparation approaches nowadays. As observed, one of the most widely used strategies is by using the column switching-based liquid chromatography methods such as In-Tube SPME-LC and online SPE-LC. For these reasons, such automated sample preparation approaches have been attracting increasing attention from the scientific community. In such a context, column switching approaches currently represent greener and higher-throughput alternatives to the conventional non-automated techniques. Using them hyphenated with miniaturized LC can enhance the analytical productivity/efficiency and sustainability even more. Although all advancements reached in modern analytical instrumentation, the present literature also spotlights the synthesis of new sorbent materials as an important core of studies to improve sample preparation That is related to the increasing necessity for more selectivity, extraction performative, and robust materials to cope with high-complex bioanalytical samples. This review shows that the dominant classes of extractive phases for such techniques are the traditional OT and the monoliths. However, other promising classes have been reported including greener ILs and DES, chitosan-based compounds, immunosorbents, and high-selective hybrid materials composed of RAM, MIP, ILs, MNs, NPs. Likewise,

incorporating naturally-occurring compounds to work as sorbents is being investigated worldwide.

From the authors' point of view, we can expect more researchers focused on improving the coupling between these automated sample preparation approaches with capLC and nanoLC to decrease ionization suppression and enhance efficiency, being much more sustainable than in conventional scale – using HPLC, for example. The direct coupling between miniaturized extraction columns upfront to mass spectrometry could be already underscored as another trend for the years to come. To conclude, the excellent levels of performance already achieved by these automated miniaturized systems deserve an honorable mention. However, the challenges and problems still springing up in bioanalysis should not be overlooked, and more researchers aiming for even better results must be encouraged worldwide.

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Declaration of competing interest

The authors declare that they have no conflict of interest related to this work.

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