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Review

Recent Advances and Uses of Monolithic Columns for the Analysis of Residues and Contaminants in Food

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Abstract: Monolithic columns are gaining interest as excellent substitutes to conventional particle-packed columns. These columns show higher permeability and lower flow resistance than conventional liquid chromatography columns, providing high-throughput performance, resolution and separation in short run times. Monoliths possess also great potential for the clean-up and preparation of complex mixtures. *In situ* polymerization inside appropriate supports allows the development of several microextraction formats, such as in-tube solid-phase and pipette tip-based extractions. These techniques using porous monoliths offer several advantages, including miniaturization and on-line coupling with analytical instruments. Additionally, monoliths are ideal support media for imprinting template-specific sites, resulting in the so-called molecularly-imprinted monoliths, with ultra-high selectivity. In this review, time-saving LC columns and preparative applications applied to the analysis of residues and contaminants in food in 2010–2014 are described, focusing on recent improvements in design and with emphasis in automated on-line systems and innovative materials and formats.

Keywords: monolith; solid-phase; MIP; chromatography; food; residues; contaminants

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1. Introduction

Nowadays, there is a growing demand for high-yield separation processes. Laboratories belonging to different areas are interested in cost-effective methodologies, with reduced analysis time. In the last 30 years, high performance liquid chromatography (HPLC) has become one of the most frequently-used methods for the analysis of mixtures of compounds in a wide variety of fields, from the quality control of drugs to the determination of pollutants or food additives [1]. This is due to its universal applicability and remarkable assay precision. The heart of each chromatography method is the column, which enables the resolution of compounds based on selectivity and column performance. This technique allows the separation of low and high molecular weight compounds, as well as different polarities and acid-base properties in various matrices. However, to solve all of the existing analytical problems in complex matrices, fast or ultra-fast chromatography methods are necessary to achieve sensibility, robustness and high resolution within an acceptable analysis time. Nowadays, there are several new approaches in HPLC that enable the reduction of the analysis time without compromising resolution and separation efficiency. Among them, high temperature liquid chromatography (HTLC), ultra-high performance liquid chromatography (UHPLC), fused core columns, hydrophilic interaction liquid chromatography (HILIC) and the use of monolithic columns have been reviewed in the bio-analytical area [2]. It is a little known fact that the very first monolithic columns were initially used in gas chromatography (GC) more than 30 years ago. Inorganic particles and porous polymer beads were the most commonly-used stationary phases in gas-solid chromatography (GSC) [3].

In addition to the use of analytical methods that allow the separation and detection of different compounds in a mixture, it is necessary to develop and optimize an extraction method. Modern trends in sample preparation for food applications include the use of on-line solid phase extraction (SPE) methods or the use of more SPE-based selective approaches, such as molecularly-imprinted polymers (MIPs) [4–6].

2. Synthesis and Characterization of Monoliths

2.1. Monoliths: General Features

Two types of columns have been used as stationary phases for routine HPLC: packed columns and monolithic columns. The particle size, the distribution and the quality of the packing of the particles within the column determine the column quality. In the case of packed columns, silica microspheres are the most used and consist of a tube packed with 3–5-μm porous silica microparticles. In contrast to HPLC packed columns, monolithic columns are made of a single piece of porous material, which is also called a "silica rod". This kind of material entirely fills the column volume without any of the interparticle voids typical of packed columns [7]. One of the main advantages of monolithic columns is that they can work at high flow rates (up to 10 mL min^{-1}) in conventional column lengths (4.6 mm I.D.) without generating high back-pressures [8]. According to the size and function, there are two main types of pores in monolithic columns: the flow pores and the mesopores filled with the "stagnant" mobile phase, in which the solute molecules migrate to access the active adsorption sites. Large flow pores are responsible for the permeability of the monolith, and they allow LC separations at low pressures. The inner pores of the particles in the packed columns correspond to the mesopores in the

monolithic columns. The presence of mesopores increases the total pore surface area and sample capacity of monolithic beds. The structure of monolithic media can be represented as a network of small mesopores, which are responsible for the retention and separation selectivity, interconnected by large flow-through pores [9], and this should lead to higher permeability. Thus, you can get fast separations at the high flow of the mobile phase and moderate back-pressures in comparison to particle-packed columns with similar efficiency [10]. The first generation of commercial monolithic columns has been marketed as "Chromolith Performance" columns by Merck since 2000. These columns are made of a C_{18} chemically-bonded silica monolith. These kinds of materials have some important limitations in their morphology: the broad size distribution, variable geometry and random spatial distribution of the through-pores. The large domain size is in contrast with decreasing diameters of packed bed particles. Furthermore, a radially heterogeneous morphology introduces a mobile phase velocity bias between the local regions of the column cross-section. Additionally, the drawbacks of these columns include the lower column-to-column and batch-to-batch reproducibility. However, the investigations of Kele *et al.* with these HPLC monolithic columns proved that the reproducibilities achieved are similar to those obtained with commercial columns packed with silica-based reversed-phase packing materials [11]. Second generation monolithic columns were also commercialized by Merck, as a welcome addition to the Merck chromatography family of products. The monolithic columns of this second generation can perform fast, high-resolution separations, while keeping the pressure drop to a minimum [12,13].

Rods are prepared by a polymerization process either *in situ* in a column tube, such as in glass tubes or fused silica capillaries, or in column molds, in which the monolith can later be replaced [1]. The main classification of monolithic materials used in chromatography is according to the nature of their construction materials, organic polymer or silica-based columns. Organic polymers were used for the first monolithic columns, and they were prepared for gas chromatography [3]. Monoliths prepared from organic polymers used as supports for enzyme immobilization and for the preparation of bioreactors are most often formed from either acrylamide derivatives or acrylate/methacrylate-based monomers [14]. In general, to prepare all kinds of monolithic columns, a polymerization mixture with monomers, initiator and porogenic solvent is necessary, and then, this leads to macroporous materials with large through-pores. The polymerization conditions, especially temperature, affect the monolithic structure. Two decades after their introduction, polyacrylamides were used to prepare columns. With the use of these materials, Frechet *et al.*, 2000, obtained a permanent macroporous structure [15]. However, the use of polymeric materials in HPLC is accompanied by several disadvantages, such as their lower efficiency compared with silica-based columns. The preparation of organic polymer capillary monolithic columns is simple: a fused silica capillary is filled with a polymerization mixture, sealed at both ends, and by heating or by UV, polymerization is initiated [16]. When the polymerization is completed, the seals at the ends of the capillary are removed; the capillary is cut to the required length and washed with an appropriate solvent to remove the porogen and other soluble compounds from the pores of the monolithic column. Generally, organic polymers offer wider variability in chemistries and better biocompatibility than silica [14]. Most of the methods controlling the surface chemistry of porous polymer monoliths described so far rely on copolymerization of functional monomers, chemical modification of reactive groups of the monolith or grafted chains originating from functional monomers [17]. However, it is of great interest to explore new approaches enabling the modification of

monolithic supports to obtain materials adapted for specific applications; thus, more dedicated and less common applications of monoliths have recently been found [18]. Moreover, the incorporation of nanostructures in the polymeric scaffold, as well as the preparation of hybrid structures had been described by Arrua *et al.*, 2012 [19]. There is a variety of nanoparticles that have been successfully used to modify porous polymer monoliths, and this highlights that the field of micro-/nano-material-functionalized polymer monolithic columns is still in its infancy and needs more attention from analysts [20]. Affinity monolith chromatography (AMC) is a type of liquid chromatography that uses a monolithic support and a biologically-related binding agent as a stationary phase. Formats have ranged from traditional columns, to disks, microcolumns and capillaries. Many types of binding agents have been used with monolithic supports in AMC, including antibodies, enzymes, proteins, peptides, lectins, immobilized metal ions and dyes. Thus, a variety of applications have been reported that are based on methods, such as bioaffinity chromatography, immunoaffinity chromatography or immunoextraction, immobilized-metal-ion affinity chromatography (IMAC), dye-ligand affinity chromatography and chiral separations [21]. Nanoparticle-based monoliths are a less popular member of the monolith family. They have emerged as a new class of substrates in sample preparation and separation science, as it has been summarized and highlighted in a recent mini-review of the major advances developed in the last three years [22]. The modification of monolithic supports can also be achieved using "templating" approaches, generating new families of porous materials. To synthetized nanoparticle-templated monoliths, nanoparticles are added as a suspension to the polymerization mixture. After polymerization, the nanoparticles are removed by washing the monolith with a strong base [23]. This procedure has been used to increase the ion-exchange capacity of monoliths. Monoliths can also be modified using high internal phase emulsions (HIPEs), achieving the so-called polyHIPEs, reviewed by Silverstein in 2014 [24].

2.2. Molecularly-Imprinted Monoliths

The molecularly-imprinted technique (MIT) is one of the most promising techniques for preparing polymers with the desired and predetermined selectivity and provides specific binding sites or catalytic sites in the molecularly-imprinted polymer (MIP). Molecularly-imprinted polymers (MIPs) are synthetic materials with recognition sites that specifically bind target molecules in mixtures with other compounds. In contrast to classical SPE sorbents used for clean-up procedures, MIPs are more selective and allow the elution of analytes from the cartridges, nearly free from co-extracted compounds. Several polymerization methods can be used to obtain MIPs for SPE. Traditionally, MIPs have been prepared by bulk polymerization, because it does not require sophisticated instrumentation and because the reaction conditions can be easily controlled. Although this procedure is tedious and time consuming, it is the most widely-used method for the preparation of MIPs [25]. MIT has appeared as an interesting solution to solve the problem of the recognition ability using conventional SPE materials. In recent years, a number of analytical methods utilizing MIT have been applied for the analysis of residues in food, and existing methodologies have been improved [26,27]. Nevertheless, there is a growing interest in alternative routes for preparing MIPs to better control morphology and, thus, to explore new applications [27]. Recent advances in MIT include the development of monolithic columns for chromatographic separations and the development of new options for the extraction and

microextraction of analytes [1,15]. Monolithic imprinting, as one of the methods for preparing MIP, combines the advantage of monolithic column and molecular imprinting technology [28]. MIP monoliths can not only concentrate, but also selectively separate the target analytes from real samples, which is crucial for the quantitative determination of analytes in complex samples.

3. Applications in Food Safety

Nowadays, there is a growing demand for efficient separation techniques and reduced analysis time to cope with the large number of residues (and contaminants) that may be present in food samples. Modern approaches include the use of monolith columns for chromatography, molecularly-imprinted polymers and novel stationary phases [7,8,16,29].

Not many analytical methods for the determination of residues and contaminants in food using monoliths can be found in the literature. Table 1 summarizes the implementation of commercial and/or custom-made monolithic sorbents in food analysis (chromatography and sample preparation) in the last five years (2010–2014).

Table 1. Summary of the existing methods using monolith-based technology for the analysis of residues and contaminants in food (period 2010–2014) *.

Compound	Matrix	Monolith	Application	Reference	Observations
Benzimidazole anthelmintics and metabolites: albendazole, albendazole sulfoxide, albendazole sulfone, 2-aminoalbendazole sulfone, fenbendazole, oxfendazole, fenbendazole sulfone, mebendazole, thiabendazole, 5-hydroxythiabendazole	Egg, milk, chicken, pork	Poly(MAA-co-EGDMA)	PMME	[30]	Capillary column
Quinolones: arbofloxacin, norfloxacin, ciprofloxacin, danofloxacin, difloxacin, oxolinic acid, flumequine, enrofloxacin	Pork	MIP (MAA, EGDMA)	MISPE columns	[31]	Room temperature ionic liquid-mediated polymerization
Fluoroquinolones: ciprofloxacin, difloxacin, danofloxacin, enrofloxacin	Milk	MIP (MAA, EGDMA)	MIPMME	[32]	Fused-silica capillary
Pyrethroids: fenpropathrin, permethrin	Water	Poly(GMA-co-EDMA)	PMME	[33]	Fused-silica capillary
Melamine	Milk products and eggs	Poly(AMPS-co-EDMA)	PMME	[34]	On-line extraction (precolumn)
Azo-dyes: Para-Red, Sudan (I, II, III, IV)	Spices	Endcapped C18	LC column	[35]	Commercial column, narrow-bore

Table 1. *Cont.*

Compound	Matrix	Monolith	Application	Reference	Observations
Cyromazine and melamine	Bovine milk	Silica C18	Spin-column (centrifugal device)	[36]	Commercial MonoSpin® columns
Thiamphenicol	Milk, honey	MIP (4-VP, EGDMA)	PT-MIPMME	[37]	Synthesized in micropipette tip
Thiamphenicol	Milk	MIP (4-VP, EGDMA)	PT-MIPMME	[6]	Synthesized in micropipette tip
Organonitrogen pesticides: alachlor, dichloran, etaconazole, hexaconazole, imazalil, linuron, prochloraz, propiconazole, tebuconazole	Honey	Silica	Purge-and-trap extraction	[38]	Capillary filled with monolith
Triazines: cyanazine, simazine, atrazine, prometon, ametryn, prometryn	Cereal	Poly(MAA-co-EGDMA)	PMME	[39]	Fused-silica capillary
Sulfamethoxazole	Milk	MIP (AM, 4-VP)	PT-MIPMME	[40]	Synthesized in micropipette tip
PHAs: acenaphthylene, fluoranthene, pyrene, benzo[k]fluoranthen, benzo[b]fluoranthene, benzo[ghi]perylene, fluorine, phenanthrene, anthracene, chrysene, acenaphthene, benzo[a]anthracene, dibenzo[a,h]anthracene, naphthalene, Benzo[a]pyrene Indeno[1,2,3 cd]pyrene	Seafood	Poly(SMA-co-DVB)	CEC	[41]	On-line preconcentration coupled to APCI-MS
Penicillin antibiotics: amoxicillin, ampicillin, penicillin G, oxacillin, cloxacillin, dicloxacillin	Milk, honey	Poly(VI-co-DVB)	SBSE	[42]	Monolithic coating of stir bars
Fluoroquinolones: ofloxacin, lomefloxacin, ciprofloxacin, enrofloxacin	Milk	MIP (organic-inorganic hybrid composite)	MISPE monoliths	[43]	Stainless steel column for on-line MISPE; only sample centrifugation

Table 1. *Cont.*

Compound	Matrix	Monolith	Application	Reference	Observations
Fluoroquinolones: ofloxacin, ciprofloxacin, enrofloxacin	Honey	MIP (MAA, EGDMA)	MISPE monoliths	[28]	Stainless steel LC column for on-line MISPE; only sample centrifugation
Malachite green	Fish feed	Endcapped C18	LC column	[44]	Commercial column
Nitrosamine	Sausage	Silica	superheated water extraction and on-line trap extraction	[45]	Capillary filled with monolith
Difenoconazole	Water, grape juice	MIP (MAA, EGDMA)	PT-MIPMME	[46]	Synthesized in micropipette tip
Parabens: methyl paraben, ethyl paraben, propyl paraben, butyl paraben	Wine	Functionalized polymer	SPME	[47]	Pillarene functionalized
Tylosin, josamycin	Muscle, liver, milk, eggs, baby food, formulae	Endcapped C18	LC column	[48]	Commercial column
4 Pesticides: fludioxonil, cyprodinil, flusilazole, triflumizole	Fruit, vegetable	Poly(BMA-co-EDMA)	SPME	[49]	Graphene-modified
Aflatoxins: B1, B2, G1, G2	Water	MIP (MAA, EGDMA)	On-line capillary MISPE	[50]	Core monolith (polyTRIM) grafted with MIP layer
Isoprocarb	Rice	MIP (MAA, MTMS, EGDMA)	PT-MIPMME	[51]	Hybrid monolith, in micropipette tip
Trichlorfon	Vegetables	MIP (MAA, MAPS)	MICEC	[52]	
Benzimidazole anthelmintics: fenbendazole, thiabendazole, mebendazole, albendazole, oxfendazole	Milk, honey	Poly(MAA-co-EDMA)	SPME	[53]	Fiber-based SPE

Table 1. *Cont.*

Compound	Matrix	Monolith	Application	Reference	Observations
Quinoxaline and sulfonamide antimicrobials: sulfaquinoxaline, sulfamethoxazole, sulfametoxydiazine, mequindox, quinocetone	Chicken, pork, egg	MIP (MAA, EGDMA)	MIMCC	[54]	Fused-silica capillaries
PCBs: 28,52,101,118,138,153,180	Red wine	Poly(BMA-co-EGDMA)	PMME	[55]	Functionalized with allylamine-β-cyclodextrin and nano-cuprous oxide
Azo-dyes: Sudan I, II, III, IV	Tomato sauce, egg yolk	Poly(OM/VI-DVB)	SPME	[56]	Fibers

* Abbreviations: AM, acrylamide; AMPS, 2-acrylamido-2-methyl-1-propanesulfonic acid; APCI, atmospheric pressure chemical ionization; BMA, butyl methacrylate; DVB, divinylbenzene; EDMA, ethylene dimethacrylate; LC, liquid chromatography; MAA, methacrylic acid; MAPS, γ-methacryloxypropyltrimethoxysilane; MICEC, molecularly-imprinted capillary electrochromatography; MIMCC, molecularly-imprinted monolithic capillary columns; MIP, molecularly-imprinted polymer; MIPMME, molecularly-imprinted polymer monolith microextraction; MS, mass spectrometry; OM, octadecyl methacrylate; PHAs, polycyclic aromatic hydrocarbons; PCBs, polychlorinated biphenyls; PT-MIPMME, pipette tip-based molecularly-imprinted polymer monolith microextraction; PMME, polymer monolith microextraction; SMA, stearyl methacrylate; SPME, solid-phase microextraction; SBSE, Stir bar sorptive extraction; VI, vinylimidazole; 4-VP, 4-vinylpyridine.

3.1. LC and GC Separations

Monolithic columns are gaining popularity, as they have been demonstrated to be a good alternative to particle-packed columns in HPLC. These columns possess some unique characteristics that make them an excellent tool in the analytical laboratory. When compared to conventional particle-packed columns, they provide a lower pressure drop and higher total porosity and separation efficiency. Monoliths are frequently designed to be used for liquid chromatography (LC) and capillary electrochromatography (CEC). The major chromatographic features of monolithic columns arise from their mesopore/macropore structure, with low back-pressure, even at high flow rates. The drawbacks of these columns include the existence of only a few stationary phases and lower column-to-column and batch-to-batch reproducibility. However, some studies have already demonstrated the high degree of reproducibility of some last-generation monolithic columns [11]. Fast separations in the second dimension of two-dimensional LC × LC are achieved using short and efficient columns, including monolithic columns, which do not require ultrahigh pressures to provide high efficiency at high flow rates [57].

Fast LC in the minimum possible time is a major trend in modern food analytical chemistry. So far, only UHPLC methods using sub-2-μm particulate columns fulfil this demand, offering analysis times in the 5-min range. Alternative solutions employ a conventional HPLC instrumentation with

monolithic columns, allowing faster analysis than the particle-packed columns of the same length at the same operating pressure [9,57]. Recently, a simple and sensitive method for the quantification of malachite green in fish feed was developed using a commercial LC C18 monolithic column in combination with mass spectrometry [44]. Nasr *et al.*, 2014, measured two macrolide antibiotics in various animal tissues (muscle, liver), eggs, milk, baby food and formulae using the same monolithic column, producing well-resolved peaks with very high sensitivity within a reasonable analytical time (10 min) [48]. Samples were injected directly into the chromatographic system with no previous treatment other than homogenization, dilution and filtration. In 2011, Zacharis *et al.* developed and validated an analytical method for the determination of banned colorants in spices, using a narrow-bore commercial monolithic column [35]. The analysis time, achieved using conventional HPLC instrumentation, is comparable and, in some cases, even lower than the previously reported UHPLC approaches. This new narrow-bore monolithic column provides high performance separations at very low operating pressures. This unique feature makes the column compatible not only with UHPLC instruments, but also with conventional HPLC, offering an interesting hybrid solution. Additionally, the typical working flow rates are ideal for mass spectrometric detection.

CEC is a separation technique that combines the features of HPLC and CE. It can be coupled to a wide variety of detection systems, such as UV, conductivity, laser-induced fluorescence and mass spectrometry (MS). These detectors are available to couple on-line with CEC. Capillary monolithic columns are also used as stationary phases for CEC. In a recent work, on-line preconcentration CEC separation of 16 polycyclic aromatic hydrocarbons present in seafood was performed using a polymeric monolith as the separation column [41]. The analytes were successfully determined at very low levels using atmospheric pressure chemical ionization mass spectrometry (APCI-MS). On some occasions, the monolith is prepared with a molecular imprinting technique, allowing the so-called molecularly-imprinted capillary electrochromatography (MICEC). Recently, Zhao *et al.* applied a MICEC method for the rapid determination of organophosphorus pesticide trichlorfon residues in vegetable samples with good accuracy [52]. Coupling the molecular imprinting technique to CEC can take advantage of the high specific and good adsorption abilities of MIPs, helping to overcome the low sensitivity of CEC. The current trends in the development of molecularly-imprinted capillary columns as stationary phases in CEC have been presented in a very recent revision by Mu *et al.* [58].

The use of monoliths in gas chromatography is one of the least common applications. The early monolithic GC columns were displaced by the overwhelmingly popular open capillary columns. These monolithic stationary phases have re-emerged in the last few decades, including both polymer- and silica-based options [3,59]. Studies using monoliths for GC separation of residues and contaminants that are monitored in food matrices could not be found in the literature of the last five years. However, a few applications on other fields have been reported. For example, the potential of silica under its monolithic form as a stationary phase in GC for the separation of very volatile compounds has been demonstrated [60]. Furthermore, monolithic columns have been suggested as interesting formats for GC × GC [61]. Monoliths have been used also in supercritical fluid chromatography (SFC) in food analysis, more dedicated to food constituents, such as lipids and fat-soluble vitamins [62]. These monolithic columns allow working with complicated samples at high flow rates, the result being separations in very short run times.

3.2. Preparative Solutions

Food is usually considered a rather complicated matrix in the development of analytical methods. New methods and techniques should be developed to monitor the presence of residues and contaminants in food. Generally, the analysis of this kind of sample requires a pretreatment step to reduce matrix content and interfering compounds that would compromise the accuracy of the results. With this regard, monoliths possess a great potential for the clean-up and preparation of complex mixtures. Several porous monolith microextraction formats, including for example in-tube solid-phase microextractions, stir bar sorptive extraction (SBSE) and spin columns, have been published in the last decade, as may be appreciated in the existing reviews on the topic [16,63,64]. Microextraction techniques using porous monoliths offer several advantages, including miniaturization, high-throughput performance and on-line coupling with analytical instruments. Additionally, they may be considered as solvent-free and portable. This part of the review is focused on the methods of sample preparation for the determination of residues and contaminants in food matrices by monolith-based techniques.

To overcome the disadvantages of bulk polymers (crushing, grounding, sieving, irregular particle size and shape, *etc*.), monolithic polymers prepared by *in situ* polymerization directly inside appropriate supports are becoming more frequent. The most common polymer monolith microextraction (PMME) procedure is performed with an extraction device composed of a regular plastic syringe, a monolithic capillary tube and a plastic pinhead [39]. Furthermore, elastic monolithic fiber can be synthetized inside glass capillaries (eliminated after polymerization) to obtain monolithic fibers for solid phase microextraction, as demonstrated by Zhang *et al.* in their recent method for the determination (SPME) of benzimidazole residues in milk and honey [53]. Another example is the very recent method developed by Wang *et al.* for solid-phase microextraction of Sudan dyes in tomato sauce and egg yolk [56]. The SPME was performed with monolithic fibers based on dual functional monomers, enabling low limits of detection in both matrices with high precision and satisfactory recoveries. A graphene-modified monolithic column was successfully utilized for purification and enrichment of four pesticides in fruit and vegetable samples. Compared with direct HPLC analysis and preconcentration with unmodified monolith, the incorporation of graphene into the monolith increased the enrichment capacity for the analytes [49]. Spin-columns offer the possibility of developing miniaturized versions of SPE extractions. The columns can be packed with conventional SPE particles, but also with monolithic materials. In these columns, a monolithic disk is packed into a spin column, and the operations (sample loading, washing and elution of the target compounds) are only carried out by centrifugation. In addition, many samples can be processed simultaneously. This simple method requires a low elution volume and does not require solvent evaporation. The spin-column technique has been used to extract various analytes in different matrices, mainly for toxicological analysis of human specimens (urine and serum) [65–67]. Very few examples of the application of these devices in the analysis of food can be found in the literature of the last five years, basically due to the complex characteristics of edible matrices. Furusawa used a commercial silica spin mini-column (MonoSpin®) to extract cyromazine, an insecticide, and its metabolite, melamine, in bovine milk [36]. The author described the method as "ultra-safe, idiot-proof and inexpensive", as well as solvent-free, all of them clear positive aspects of this spin-columns. Pipette tip extraction is another miniaturization option for sample preparation, and some commercially manufactured pipette tip-assembled monoliths are available on the market [65].

This type of extraction is introduced for high throughput as a miniaturized version of the SPE cartridge. Furthermore, the monolith plug eliminated the need for frits, a potential source of secondary adsorption. Finally, monolithic material sorptive extraction, the so-called MonoTrap, appears as a modern commercially available option in both disk and rod formats. Theses monoliths may be applied in the headspace of a vial (volatile compounds) or under agitation immersed in a solution, the extraction times usually being relatively long [47]. On the other hand, the PMME procedure can be performed on-line, as well, using stainless steel and/or capillary monolithic columns. On-line fully automated polymer monolith microextraction was performed to separate melamine residues present in milk products and eggs and successfully coupled to HPLC determination [34]. The monoliths were synthesized inside capillary columns. A different approach is the on-line purge-and-trap, and trap extraction only, using capillaries filled with monolith and coupled to GC separations, has also been reported [38,45]. In the GC oven, one end of the absorbed monolith capillary was connected to a bare fused silica tubing that was inserted into the injection port of GC, and the other end was connected similarly to a GC column.

Both the advantages of molecularly-imprinted polymers (MIPs) and solid-phase extraction (SPE) are frequently combined for sample clean-up, exhibiting good extraction efficiency, reusability and selectivity. Conventional sorbents usually develop non-specific interaction sites that lead to the co-extraction of interfering compounds. To overcome this problem, MIPs have also become increasingly attractive options for monolithic materials [32,68]. Novel formats include MIP monoliths prepared in a stainless steel column, in a tip of a micropipette or in fused-silica capillaries by *in situ* polymerization. Thus, the monolith may be used directly as the SPE sorbent with no need for crushing or sieving. MIP monoliths prepared inside stainless steel chromatographic columns appear as interesting pre-columns for on-line clean-up and the preconcentration of analytes. As an example, Lv *et al.* developed a method to extract fluoroquinolones (FQs) present in milk and honey using an on-line MIP-based monolithic precolumn [28,43]. By using this MIP monolith, FQs were selectively isolated from biological samples, and the impurities were eliminated simultaneously, showing high selectivity and sufficient accuracy to be used on trace levels of FQs analysis in biological samples. On-line extraction can be performed also using monolithic capillary columns, as has been demonstrated by Szumski *et al.* 2014 [50]. In this study, a monolith was grafted with MIP material and applied to the on-line isolation of aflatoxins. MIPs can also be synthesized as fibers. MIP monoliths of the desired length are obtained, the thickness depending on the inner diameter of the capillary used. These fibers have been used for SPME of food samples, as in a recent method developed to determine the insecticide, chlorpyrifos, in fruits [69]. A common limit for most of these fibers is their fragility, but in this case, the MIP fiber proved to be firm and stable. A relatively new approach is the preparation of MIP monoliths inside a pipette tip and matched to a syringe for performing the polymer monolith microextraction [6,37,40,46,51]. Pipette tip-based molecularly-imprinted polymer monolith microextraction (PT-MIPMME) has been used for the selective extraction of sulfamethoxazole in milk, achieving high recoveries and low standard deviations [40]. This type of extraction was used also for the selective extraction of difenoconazole in tap water and grape juice using PT-MIPMME [46]. In a similar approach, the metallic needle of the pinhead of a syringe can be replaced by a polymer monolith and coupled to a syringe infusion pump. Following this methodology, molecularly-imprinted monolithic capillary columns (MIMCC) were used to selectively extract FQs from phosphate buffer

diluted milk samples [32]. A novel porous, chemically stable and long-lifetime MIMCC for on-line extraction of antimicrobials was *in situ* fabricated with sulfaquinoxaline as the template and used for trace analysis of five antimicrobials in food samples [54].

Over the past few years, there has been growing interest in the use of monolithic materials as SBSE coatings [64]. In this approach, the commercial stir bars are coated with monolithic material and applied as sorptive extraction media. Recently, an SBSE method based on monolithic material as the coating was successfully applied to extract penicillins in milk and honey samples [42]. Samples were diluted with water and were then directly used for sorptive extraction, with no additional step to eliminate fats and proteins in milk samples and/or sugar in honey samples prior to extraction.

4. Conclusions

This review confirms that monoliths are finding their way into separation science and into the solid-phase extraction field. Several articles have been published in the last five years, reporting the superb performance of monoliths in a wide range of applications for food analysis, using novel designs and preparation options. There has been a remarkable evolution in column technology for liquid chromatography. Although columns packed with silica particles have predominated, monolithic columns have also emerged as a viable strategy. Commercial and custom-made columns appear as excellent options for the development of methods to determine residues and contaminants in foodstuff. With the increasing number of samples, miniaturization has been the key to achieve the high-throughput capacity of extraction methods. Small-sized devices, fast chromatography and on-line extractions are among the major advantages of monoliths. With the availability of monoliths, the drawbacks from the conventional particle-packed bed options can be eliminated. Furthermore, molecularly-imprinted monolithic polymers have for improving the selectivity of analytical methods. In the case of MIP monoliths, the most demonstrated their suitability widely-used supports to extract residues and contaminants in food have been capillaries and micropipette tips, following a clear miniaturization trend.

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Author Contributions

M. Díaz-Bao: bibliography compilation, manuscript writing; R. Barreiro and J.M. Miranda: guidance; A. Cepeda: supervision and manuscript revision; P. Regal: initial design, manuscript writing. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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