REVIEW PAPER

A review of micro-solid-phase extraction techniques and devices applied in sample pretreatment coupled with chromatographic analysis

STEFANO DUGHERI^{1*}, GIORGIO MARRUBINI²,
NICOLA MUCCI³, GIOVANNI CAPPELLI³,
ALESSANDRO BONARI⁴, ILENIA POMPILIO⁴,
LUCIA TREVISANI³ and GIULIO ARCANGELI³

¹ Industrial Hygiene and Toxicology Laboratory, Careggi University Hospital, Florence, Italy

² Department of Drug Sciences, University of Pavia, Pavia, Italy

³ Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

⁴ General Laboratory, Careggi University Hospital, Florence, Italy

Received: April 20, 2020 • Accepted: May 04, 2020

Published online: August 4, 2020

ABSTRACT

Sample pretreatment is one of the most crucial and error-prone steps of an analytical procedure; it consents to improve selectivity and sensitivity by sample clean-up and pre-concentration. Nowadays, the arousing interest in greener and sustainable analytical chemistry has increased the development of microextraction techniques as alternative sample preparation procedures. In this review, we aimed to show two different categorizations of the most used micro-solid-phase extraction (μSPE) techniques. In essence, the first one concerns the solid-phase extraction (SPE) sorbent selection and structure: normal-phase, reversed-phase, ion-exchange, mixed-mode, molecular imprinted polymer, and special techniques (e.g., doped cartridges for specific analytes). The second is a grouping of the commercially available μSPE products in categories and sub-categories. We present every device and technology into the classifications paying attention to their historical development and the actual state of the art. So, this study aims to provide the state-of-the-art of μSPE techniques, highlighting their advantages, disadvantages, and possible future developments in sample pretreatment.

KEYWORDS

micro-solid-phase extraction, solid-phase extraction, liquid chromatography, gas chromatography, sample pretreatment, sample purification

INTRODUCTION

Correct sample preparation is a fundamental step for the success of analytical processes. Therefore, it is essential to know the different techniques to select the one that best meets the needs of the analysis. It is also necessary to understand the working principles of the various techniques, to maximize the process, and obtain optimum results [1, 2]. Solid-phase extraction (SPE) is a technique that can be used for sample preparation and clean-up, providing the isolation, extraction, and concentration of the target analytes [3, 4].

Analysts generally use cartridge-type devices for SPE that contain packed solid particles of porous chromatographic material.

The mechanism of action of SPE is the interaction between the solid sorbent phase and the liquid sample solution containing the analytes that is percolated through the SPE bed.

*Corresponding author.
E-mail: stefano.dugheri@unifi.it

This interaction might happen through different kinds of bonding, depending on the physical-chemical properties of the solid sorbent, the fluid-carrier, and the analyte molecules. We can recognize four main types of interaction mechanisms that are responsible for the SPE separations. These are polarity, ionic/electrical charge, molecular size exclusion, and chemical reactivity. These interactions can all be present together and may act simultaneously or sequentially, depending on the chemistry of the SPE experiment under observation.

SPE techniques provide several significant benefits to chemical analysis. The substantial removal of non-target species in complex matrices, like a biological fluid, allows to isolate a small number of compounds of interest, and implicates sample purification. Secondly, SPE reduces the amount of substances introduced inside the analytical instrument, reducing the possibility of contamination and interference, such as ion suppression/enhancement in mass spectrometry applications (matrix effect). Thirdly, SPE allows to fractionate the sample matrix, i.e., it enables the removal of non-target matrix constituents, multiclass extraction and selective separation of the retained compounds by selective elution (i.e., varying the characteristics of the eluent). Finally, SPE offers the possibility to concentrate the analytes present at the level of traces in the matrix. If the target compounds have concentration levels lower than the instrumental sensitivity, a pre-concentration strategy can result in a relevant increase of the compound concentration level and thus of the analytical signal [5].

The first categorization of the SPE techniques that we propose is the following:

1. Normal-Phase: the solid stationary phase material is polar, and the sample matrix is non-polar;
2. Reversed-phase: includes any chromatographic method that uses a hydrophobic stationary phase;
3. Ion-Exchange and Mixed-Mode: refers to chromatographic methods that utilize more than one form of interaction between the stationary phase and analytes to achieve their separation;
4. MIP: is based on molecularly imprinted polymer (MIP);
5. Specialty techniques: like cartridges doped with a precise reagent to react specifically with a kind of analyte.

Innovative materials used for making sorbents increased the sensitivity and selectivity of SPE methods. Out of the many types of materials used, those more successful are mixed-mode stationary phases, restricted access media, immunosorbents, and MIPs [6].

Due to the many advantages of SPE techniques and its miniaturization, the interest around them has been arising and many studies have been published in recent years. Some of them focus on the green analytical chemistry and on how micro-extraction solventless techniques consent to make the sample pre-treatment greener [7] and are an alternative to liquid-liquid extraction [8]. Others studied green aspects joint to how to combine sample preparation techniques to overcome the disadvantages of individual methods [9]. Moreover, specific studies as reviews on extraction

techniques of liquid samples to liquid chromatography [10], on affinity monolithic chromatography [11] and on specific technologies, like pipette tip micro solid-phase extraction (μ SPE), spin-column micro solid-phase extraction, and thin-film solid-phase microextraction [12] or dispersive μ SPE [13] are available.

This study aims to provide the state-of-the-art of all the commercially available μ SPE techniques, dividing μ SPE products into categories and sub-categories, in order to consent a straightforward approach to the choice of the better-fitted technique and device for the sample pre-treatment. Each technique is described, including the devices used. Also, we give a survey on the historical development of μ SPE, from birth, to patent, and the current state-of-the-art, highlighting advantages, disadvantages, and possible future developments for the device itself and automation.

CATEGORIZATION OF SPE PHASES BY SORBENT MATERIAL

At the beginning of the twentieth century, Tswett invented chromatography. The first experiment was the separation of plant extracts using a glass column filled with CaCO_3 . The eluents used were mixtures of petroleum ether and ethanol. The first version of chromatography thus consisted in the separation of polar molecules dissolved in mixtures of non-polar and polar eluents applied onto a polar sorbent. Today, these conditions are those of column liquid chromatography in the “normal-phase” mode.

Current SPE applies these same conditions. Polar analytes are contacted with the SPE stationary phase using a carrier that favors retentive interaction between them and the sorbent surface.

Polar organic analytes exhibit functionalities that interact with the polar groups on the sorbent, and that provide the normal-phase separation. Dipolar interactions between the analytes and the sorbent such as, e.g., hydrogen bonding, dipole/dipole, and π - π , provide different levels of selectivity, allowing to achieve separations of compounds very similar in structure [14, 15]. Non-polar solvents enhance the retention of analytes by polar interactions. Polar solvents and aqueous solutions having high ionic strength, instead, facilitate the elution of the analytes from polar sorbents. Secondary dipolar interactions can play an essential role in the retention of amine- or hydroxyl-containing analytes. Non-polar solvents can also help to modulate such interactions [15].

Reversed-Phase SPE is considered the least selective separation mode, compared to others like normal-phase, or ion-exchange SPE. Apolar sorbents can hardly differentiate between molecules that are structurally similar. Non-dipolar interactions take place between the carbon-hydrogen bonds of the sorbent functional groups and the carbon-hydrogen bonds of the analytes. The forces responsible for such interactions are “Van der Waals” or “dispersion” forces. Many organic molecules are non-polar, so the weak interactions

due to dispersion forces can bind the analytes on the surface of sorbents made of non-polar materials. Reversed-phase SPE is thus handy to retain most hydrophobic analytes within the sample [14]. The sorbent most widely used for reversed-phase separations is octadecyl silane bonded to the silica substrate, called C18. Despite the very poor selectivity of C18 materials, these are the most popular sorbents existing at present. On C18 columns, polar solvents enhance the retention of hydrophobic analytes by favoring non-polar interactions. On the opposite, non-polar solvents promote the elution of hydrophobic analytes from C18-based sorbents [15].

Retention in Ion-Exchange SPE is due to electrostatic interactions between charged species. Ionic moieties on the sorbent bind analytes bearing the opposite charge on their molecular frame. Mixed-mode SPE sorbents instead retain the analytes by a combination of reversed-phase and ion-exchange interactions [14]. Retention by ion-exchange interaction is influenced by:

1. pH of the solvent, sample, and sorbent. The interaction that provides retention occurs between oppositely charged species.
2. The ionic strength of the solvent and the sample; usually, the ionic strength of the eluting solvent and the sample are controlled and kept as low as possible to prevent competition for the charged sites on the sorbent.
3. Equilibration of the sorbent with low selectivity counter-ions. The sorbent is conditioned before the application of the analytes to obtain optimal retention.

Elution of the analytes from ion-exchange sorbents is promoted by:

1. pH of the elution solvent. We can change the ionization of the analyte or the sorbent by adjusting the eluent pH. For instance, the pH of aqueous eluents modulates the retention of protonated amine-containing analytes [15].
2. The ionic strength of the elution solvent. Similarly to the effect of pH, we can weaken the electrostatic interaction between analyte and sorbent using an aqueous solution having appropriate ionic strength.
3. Elution solvent containing high selectivity counter-ions. Specific counter-ions can compete with the analyte for the binding sites on the sorbent, thus causing the analyte elution.

Table 1 summarizes the information given above for normal-phase, reversed-phase, and ion-exchange sorbents.

MIPs are highly cross-linked polymers engineered to extract a single analyte of interest or a class of structurally related analytes with an extremely high degree of selectivity [4]. In the synthesis of the MIPs, one template molecule, designed to imitate the analyte of interest, drives the formation of cavities or imprints that are sterically and chemically complementary to the shape of the molecules of interest. MIPs contain cavities able to bind the target analyte through multiple interaction points (ion-exchange, reversed-phase with polymer backbone, and hydrogen bonding). Hence, the MIPs binding sites entail a strong interaction

with the analyte requiring harsh eluting conditions but at the same time producing cleaner extracts. As a consequence of their extreme selectivity, MIPs provide low background noise, reduced matrix effect and thus lower limits of detection [4, 14].

Three approaches exist for the synthesis of MIPs: the covalent, non-covalent, and semi-covalent routes.

The covalent approach involves the formation of covalent bonds between the template and monomers before polymerization. After the polymerization, the template is removed from the polymer cleaving the corresponding covalent bonds. These bonds are then responsible for trapping the analytes inside the MIP. The high stability of this kind of binding yields a homogenous population of binding sites, minimizing the occurrence of non-specific cavities. However, this approach is not easy to design because it requires the use of an appropriate template-monomer complex in which the covalent bond switching happens under mild conditions.

In the semi-covalent approach, similarly to the covalent one, the template is covalently bound to a functional monomer. After polymerization, the MIP retains the target analytes by non-covalent bonds, like hydrogen bonding or ionic.

The non-covalent approach to MIPs synthesis relies on the formation of bonds by weak interactions such as hydrogen-hydrogen or dipolar/ionic. This one is the most common approach for the preparation of MIPs because it is experimentally straightforward. Various monomers are commercially available that can interact with a variety of templates. However, one limitation of this approach is that the template-monomer binding is an equilibrium process. So, to push the equilibrium towards the right, a high amount of monomer is used. The excess of monomer unbound is randomly incorporated into the polymer, leading to the formation of non-selective binding cavities [16].

Specialty techniques in SPE can modify the sorbent bed chemically to react with the sample while it passes through the cartridge. By this approach, it is possible to transform the analytes in species suitable for analysis.

For instance, special polymeric materials such as PS/DVB adsorb 2,4-Dinitrophenylhydrazine (DNPH). Cartridges packed with PS/DVB-DNPH thus can trap aldehydes in air, forming hydrazones on the sorbent bed. The UV-responsive hydrazone derivatives are then eluted and analyzed by HPLC-UV [5]. This technique is used to determine formaldehyde vapors in the workplace using a miniaturized cartridge [17, 18].

In Fig. 1, we propose a division in categories and sub-categories of the commercially available μ SPE products.

MEPS

Microextraction in packed syringe (MEPS) is a technique for miniaturized SPE that can be connected on-line to GC or LC without any modifications. The MEPS technique was invented by Mohamed Abdel-Rehim, patented in 2004 [19], and produced by SGE Analytical Science (Trajan Scientific

Table 1. SPE phase selection [1] and sorbent structures [2]

AQUEOUS MATRIX (biological fluids, water, aqueous extracts of tissues, etc.)						ORGANIC MATRIX (organic extracts of tissues, hexane, dichloromethane, etc.)		
REVERSED-PHASE			ION-EXCHANGE			NORMAL-PHASE		
Moderately polar to non-polar compound			Strong or weak cations/anions			Polar to moderately polar compounds		
NON-POLAR SORBENTS			ION EXCHANGE			POLAR SORBENTS		
C18	Octadecyl	$\begin{array}{c} \\ -Si- C_{18}H_{37} \\ \end{array}$	SCX	Benzenesulfonylpropyl	$\begin{array}{c} \\ -Si- C_3H_6(C_6H_5)SO_3^- \\ \end{array}$	CN	Cyanopropyl	$\begin{array}{c} \\ -Si- C_3H_6CN \\ \end{array}$
C8	Octyl	$\begin{array}{c} \\ -Si- C_8H_{17} \\ \end{array}$	PRS	Sulfonylpropyl	$\begin{array}{c} \\ -Si- C_3H_6SO_3^- \\ \end{array}$	20H	Diol	$\begin{array}{c} \\ -Si- C_3H_6OC_2H_4(OH)CH_2OH \\ \end{array}$
C2	Ethyl	$\begin{array}{c} \\ -Si- C_2H_5 \\ \end{array}$	CBA	Carboxymethyl	$\begin{array}{c} \\ -Si- CH_2COOH^- \\ \end{array}$	SI	Silica	$\begin{array}{c} \\ -Si- OH \\ \end{array}$
CH	Cyclohexyl	$\begin{array}{c} \\ -Si- C_6H_{12} \\ \end{array}$	DEA	Diethylaminopropyl	$\begin{array}{c} \\ -Si- C_3H_6NH^+(CH_2CH_3)_2 \\ \end{array}$	NH ₂	Aminopropyl	$\begin{array}{c} \\ -Si- C_3H_6NH_2 \\ \end{array}$
PH	Phenyl	$\begin{array}{c} \\ -Si- C_6H_5 \\ \end{array}$	SAX	Trimethylaminopropyl	$\begin{array}{c} \\ -Si- C_3H_6N^+(CH_3)_3 \\ \end{array}$	PSA	N-propylethylenediamine	$\begin{array}{c} \\ -Si- C_3H_6NHC_2H_4NH_2 \\ \end{array}$

CATEGORY	SUB-CATEGORY	CATEGORY	SUB-CATEGORY
IN-NEEDLE	MEPS (SGE Analytical Science)	SUPPORT COATED	Disk
	MonoTrap™ (GL Sciences Inc.)
	μSPEd Cartridges (E- PREP Analytical)		Rod
	Micro One-Way Valve		Thin Film SPME (Gerstel)
DISPERSIVE	SPME C18 (Supelco)	MINI-CARTRIDGES	Twister Back- Extraction TBE (Gerstel)
	96 Well Plate
ON-LINE	NeedlEx (Shinwa Chemical Industries Ltd.)		Pipette Tips

Fig. 1. The commercially available micro-solid-phase extraction (μSPE) techniques

Australia Pty Ltd 2019) [20]. The sampling system for MEPS is a conventional syringe modified to host the sorbent for the extraction of the sample. The syringe is a plastic vessel with a volume of 100–250 μL that contains about 2 mg of solid material packed as a plug placed between the barrel and the needle. Therefore the whole device is composed of the MEPS syringe and the cartridge, also called the BIN. The packing material of the BIN has a mean particle size of 45 μm. The plug of sorbent can be of any absorption material, such as silica-based [21], restricted access material (RAM), or molecular imprinted polymers (MIPs) [22]. So, the MEPS fits for reversed-phase, normal-phase, ion-exchange, or mixed-mode extractions.

MEPS is an adaptation of SPE into a miniaturized device integrated directly into the syringe used for sampling. Compared with SPE, in MEPS, the packing is used in a much smaller amount, typically having a void volume of less than 10 μL. Also, the design of the system that includes the packing into the syringe and not in a separate column reduces the sampling time and solvent consumption. SPE and MEPS are robust techniques built on the same principles; therefore transferring a method from traditional SPE to MEPS is straightforward. One crucial difference between SPE and MEPS is that, in SPE, the eluent flows only in one direction, i.e., downwards. In MEPS, instead, the solvent is eluted in two directions by moving up and down the syringe plunger. Therefore in MEPS, it is necessary to select the more appropriate number of washing and elution volumes by choosing the number of plunger movements in both steps of the extraction [20].

In many applications, MEPS is more robust than fiber-based microextraction techniques.

In particular, SPME suffers from poor physical-chemical stability in relation to the sample nature. All biological fluids rapidly modify the fiber coating after direct immersion. On the contrary, plasma, urine, and other complex matrices do not damage MEPS sorbents. Also, MEPS showed higher extraction recoveries (60–90%) in comparison with SPME (1–10%). Finally, MEPS allows the treatment of minute

sample volumes (10 μL) compared to either headspace- or direct immersion-SPME [21].

This MEPS technique combines extraction, pre-concentration, and clean-up in a single device. The syringe for microextraction can be used in different ways, like manually or in fully automated on-line instruments. Its characteristics, combined with its compatibility with autosamplers, make the MEPS format ideal for a digital LC-elution or GC analysis [20, 22]. One example of an autosampler that is compatible to use with MEPS is the eVol® XR digital analytical syringe. This digitally controlled positive displacement dispensing system can be programmed to operate in full automation. It performs with high precision and accuracy a wide variety of liquid handling procedures taking only about 1 min processing for each sample [22, 23].

μSPEd cartridges

The μSPEd cartridges were brought into the market in 2014 by EPREP PTY LTD. They contain the micro one-way valve that is technological innovation patented in 2015 by Ernest Frederick Dawes, Peter Alexander Dawes, Reno Cerra, and Andrew Minett [24, 25].

Figure 2 shows the μSPEd cartridge cross-section view. The cartridge houses an efficient pressure-driven one-way check valve that allows an ultra-low dead volume connection. The check valve also provides a single way flow path through the sorbent bed in every step of the extraction protocol. The valve design allows to draw the sample into the syringe body avoiding transit through the sorbent bed by pulling the plunger. Then the sample passes through the sorbent bed by merely pushing the syringe plunger. The whole microextraction system is incorporated into a single cartridge, not requiring additional tubes and fittings. So, smaller particles can be used, offering a higher surface area, and favoring a more efficient separation, improving extraction efficiency. μSPEd cartridges are washable and reusable depending on the sample matrix and the standard requirements of the operating procedure. After use, the operator rinses the cartridge with about 100 μL of solvent.

Fig. 2. μ SPEd cross-section view [3]

The type and volume of the solvent depending on the sample matrix complexity. Since the analytes retained in the sorbent bed are not disturbed by the solvent aspiration, the elution concentration is higher, leading to an increase of sensitivity. Furthermore, the risk of carryover is low, and the extraction of analytes poorly retained in the sorbent is efficient.

The μ SPEd cartridge is comparable to a short HPLC column (length <1 cm, 3 μ m sorbent particle size) due to the small sorbent particles, instead of the 50 μ m diameter particles traditionally used in SPE, the efficient pressure-driven one-way check valve, and ultra-low dead volume connection. A constant and high-pressure (up to 1,600 psi) single-direction flow through the small particle size sorbent is allowed, retrieving more efficient extractions of the target analytes. Similarly to HPLC, several sorbents are currently available, consisting of materials that range from C18 to polymers such as polystyrene divinylbenzene (PS/DVB). Furthermore, μ SPEd permits to perform the sample extraction and chromatographic separation within the cartridge. So it can be connected to the conventional detection system, skipping the intermediate chromatographic separation in an LC column and simplifying the apparatus layout.

The standard eVol[®] syringe and the new eXact Digital Syringe Driver (EPREP) are fully compatible with μ SPEd cartridges. The EPREP system can cope with higher backpressures than the standard eVol[®] syringe. Such characteristics can be very advantageous for the extraction of complex

samples, such as biological fluids, that often cause sorbent clogging. However, unlike the cordless eVol[®], the eXact requires a continuous power connection to operate [26–28].

The use of a laboratory workstation enables the automation of the μ SPEd cartridges allowing to program sequences of operations.

SPME C18

In 1995 the inventor of the SPME Janusz B. Pawliszyn developed a new interface to couple the SPME sampling technique with HPLC systems [29]. In 2009, Supelco marketed innovative fiber coatings for SPME consisting of C18-functionalized silica. The new fibers were resistant to common HPLC solvents, suitable for polar and non-polar analytes, and reproducible.

The fiber core is made of a flexible metal alloy with shape memory properties, so it “keeps the memory” of its shape, reacquiring it when it is overheated, and a diameter larger than typical SPME fibers. This new fiber is used as a plunger and fiber core, simplifying the construction of the device and reducing the cost of the probe. Fibers designs changed. Probes made of a special polymer contained a fiber coated with C18-functionalized silica. The polymer was a unique material patented, biocompatible, and mechanically stable after exposure to organic solvents. The new design of the fiber enables the assembly to pierce into a catheter shunt, vial septa, or the fiber can be exposed for direct immersion into a liquid sample. This technique is minimally invasive and allows one to sample specimens *in vivo*, opening up research applications of bioSPME [30, 31].

In *in vivo* applications, for single-use, the fiber price is low enough to enable research on bioSPME [32].

The polymer housing prevents the contamination of the sorbent by contact with macromolecules. The small molecules of the analytes in the sample pass through the polymer and interact with the C18 phase. The free-form analytes in the sample can flow through the polymer, whereas macromolecules-bound analytes can not. Therefore, the fiber can be directly immersed in biological fluids, like whole blood, serum, plasma, and urine, without fouling the sorbent.

The sample pretreatment does not require protein precipitation or desalting. In some cases, the regulation of pH and ionic force of the sample solution can improve extraction efficiency. Sample extraction times depend on the equilibrium process involving the transfer of the analytes from the matrix to the fiber coating. Usually, extraction time ranges from a few minutes up to 1 h. The extraction time, like all other SPME operating conditions, is best studied using multivariate tools, as recently reviewed [33].

After exposure to the sample solution, a small amount of organic solvent (~100 μ L) is sufficient to desorb the analytes from the fibers and the extract is then injected into the HPLC system for analysis.

SPME is easily automated using the GERSTEL Multi-Purpose Sampler (MPS), enabling reproducible and efficient concentration of many types of analytes covering a broad polarity range.

An innovative configuration of SPME fibers recently produced by Supelco with a barcode label, the Multi-Fiber Exchange (MFX) accessory, allows the fully automated exchange of SPME fibers within a sequence using the SPME Fast Fit Assemblies (FFA) [34–36].

NeedlEx

In 2004, Hiroo Wada, Kenzo Kotera, Hisashi Matsuura, Kiyokatsu Jinno, and Woshihiro Saito patented the NeedlEx, a needle for μ SPE, packed with a novel copolymer, useful as an adsorption-desorption medium [37].

This new device enables wieldy sample extraction. The unique needle can be attached to a gas sampling system, and the target compounds are selectively extracted/concentrated by aspirating the sample. The extraction is very rapid and reproducible. Sealing both ends of the needle using Teflon plugs permits the storage of the sample for approximately ten days. The NeedlEx is reusable on average for 25 to 30 extractions [38].

In 2008, Hiroo Wada patented a needle designed for simultaneous entrapment and derivatization of aldehydes and ketones [39]. The new needle for microextraction contains a fiber-packing coated with 2,4-Dinitrophenylhydrazine (DNPH). After sampling, a small volume of organic solvent flowed through the needle is sufficient to desorb the DNPH derivatives before analysis [38].

MonoTrap™

In the early 1990s, Merck proposed monolithic columns as an alternative to HPLC columns packed with particulate materials. Monoliths, in principle, are ideal packings for LC because they are very stable structures, both mechanically and chemically. The monolithic rods used for HPLC had pores highly interconnected, very permeable to mobile phases. These mechanical properties provide high mass transfer also at high flow rates, and the column backpressure is low [40]. On the contrary, small particle size stationary phases do not allow high flow rates due to the high backpressure. With regard to HPLC, short columns are not a viable solution because of two reasons. First, particulate packings have always a dead volume that originates from the incomplete filling of the available space inside the column housing. Secondly, short columns eluted at high flow rates may lose efficiency due to the formation of preferential paths of flow (channeling).

These same considerations also apply to SPE. New SPE systems and formats overcome the limitations of particle packings. Various designs exist now, such as disks containing sorbents having small particle sizes, or fibers, stirrers, and capillary tubes coated with thin membranes. However, monolithic sorbents fulfill the requirements of modern SPE materials that must be of rapid use, sensitive, accurate, and fit for miniaturized devices operating in an automatic mode. Monoliths can concentrate highly diluted samples, so they are ideal for the pretreatment of environmental, food, and biological samples.

The classification of monolithic materials refers to the type of retention mechanism between the sorbent and the analytes. Hence, we can recognize hydrophobic, ion-exchange- and immunoaffinity-based monolithic materials.

In 2002, Karin Cabrera et al. patented monolithic sorbents for preparative HPLC [41].

In 2014, Atsushi Sato, Hiroyuki Terashima, and Yoshiyuki Takei patented a new method and apparatus for adsorbing samples based on a monolith adsorbent [42].

MonoTrap™ is a state-of-the-art silica monolithic hybrid adsorbent having properties based on silica, activated carbon, and C18 functional group. The monolithic system has a large surface area, thanks to the porosity of the silica. For this reason, the monolith can operate at high flow rates with very resistance to flow. The sorbent does not require pre-conditioning, allows fast extraction by surface adsorption, and complete desorption of the analytes using small solvent volumes.

Sampling with MonoTrap™ is possible by headspace, direct contact, and passive diffusion.

The monolithic material can provide two different chemistries of adsorption. One is like silica gel, more suitable for adsorption of non-polar compounds; while the second one is similar to silica modified with activated carbon as an enhancer of adsorption surface to aid the retention of more polar components. Both types of monoliths are functionalized with C18 groups and are available in disk and rod formats [43].

MonoTrap™ desorption requires about 200 μ L of organic solvent. The system is reusable several times after flushing. In the case of rod-shaped MonoTrap™, solvent extraction is also possible inside the auto-sampler vials [44].

Twister back-extraction TBE

In 1999, Baltussen proposed the Stir Bar Sorptive Extraction (SBSE) technique [45]. Initially, this was a miniaturized solventless extraction technique considered as a mere modification of SPME. The tool used for the extraction is a magnetic stir bar closed in a glass shell covered with a sorbent layer. The first sorbent used was a polydimethylsiloxane (PDMS) layer with a thickness of 0.5–1 mm.

The SBSE mainstream application was the extraction of polar analytes from water samples [46]. In 2002, the SBSE invention and its applications were patented in the US [47].

SBSE allows the extraction and enrichment of the analytes simultaneously, providing very high sensitivity [46].

The research groups of UFZ Leipzig Halle GMBH, in close cooperation with Gerstel, developed the application of the twister extraction to allow its applicability to liquid chromatography. They proposed the Twister Back-Extraction (TBE) procedure: it takes place putting the stir bar and the sample into a vial. The bar stirs the sample solution until the concentration of the target analytes in the sample reaches the equilibrium with that in the PDMS coating [48], thus it is a non-exhaustive technique. After extraction, the operator cleans the stir bar and puts it into a clean vial where the desorption of the target compounds with a proper

solvent takes place. After the back extraction, the solvent containing the target compounds is injected in interfaced instruments with appropriate detection modes TBE is the most efficient way of using the SBSE applied to liquid chromatography and high performance liquid chromatography [48]. The principles of this technique are the same as SPME with PDMS-coated fibers, but the volume of the extraction phase is 50–250 times more massive [49].

Moreover, the Twister Back Extraction procedure is one of the sample preparation steps available with the GERSTEL MultiPurpose Sampler MPS (MPS), so it can be carried out fully automated.

Thin-film TFME

In the early 2000s, Inge Bruheim, Xiaochuan Liu, and Janusz Pawliszyn started to develop a new sampling device, the Thin-film microextraction (TFME), to reach higher extraction efficiency and sensitivity. At the beginning of the process, the sorbent was a rolled membrane. This device consists of a house-shape thin film of PDMS mounted on a stainless wire for support. It can be used both for active and passive sampling, after conditioning and baking-out [50].

The idea behind this new system is that the amount of analyte extracted depends on the sorbent geometry of the sampling device. Therefore, changing the geometry from the traditional cylinder (fiber or rod) coated with a layer of sorbent to a thin, flat, support having larger surface provides higher loading capacity [51].

TFME affords efficiency and sensitivity higher than SBSE and fiber SPME in similar sampling times. This improvement is due to the larger surface area to the extraction-phase volume ratio. Using a large surface area, thin-film sorbents, and small sample volume, rapid quantitative extraction is achievable. The drawback of the technique is that the higher volume of the extraction phase is challenging to manage in standard injection and desorption systems. Therefore, TFME requires dedicated large-volume injection systems [52].

Gerstel introduced TFME on the market, suggesting to use thermal desorption for transferring the analytes into the instrument for analysis. The company developed on purpose a specific automated thermal desorption unit (TDU) that is compatible with modern injection systems. However, solvent-desorption is also a viable option for recovering the analytes from the film-shaped sorbent. The procedure involving TFME, solvent-desorption, and liquid injection is a valid alternative using instruments that do not include a TDU and it is suitable for high-boiling point substances [53].

96-Well plate

In the early '50s, epidemic influenza in Hungary led to a shortage of laboratory equipment, including pipettes and test tubes. To overcome the shortage, the scientist and inventor Gyula Takátsy worked to find a quick, reliable, and low-cost method to identify the influenza virus. So, he constructed the first microplate. The device consisted of six

rows of twelve wells housed into a block of methacrylate. At the same time, he also invented the first microplate-based automated tool, the Microtiterator. This innovative tool allowed scientists to mix and transfer pre-defined volumes of solutions in serial dilution testing [54]. Takátsy tried to patent his invention, but the authorities did not understand the importance of the Microtiterator, and they denied the authorization to patent the invention. Therefore, Takátsy published his inventions [55]. After the publication in English [56], the microplate-based method gained popularity. In the '60s, John Sever, while leading a Rubella vaccine program at the National Institute of Health (NIH), teamed up with engineer Frank Cooke to work towards manufacturing an automated loop system including microplates. They created thus the microtiter plates. So, for the first time, it was possible to manage eight to twelve automatic loops and droppers, moving them from row to row in a plate. It was an efficient system, but it was still manual. Tom Astle invented the Autotiter a few years later. He provided the scientific community with a fully automated serial dilution system. It resulted in being a worthy clinical lab instrument, able to perform thousands of hemagglutination inhibition tests during a trial for a Rubella vaccine. In 1976, the London Centers for Disease Control (CDC) began to use microplates for ELISA (Enzyme-Linked Immunosorbent Assay) diagnostics and quality control techniques. It became one of the most common applications for microplates, leading to a significant increase in the request for instruments performing ELISA tests. This observation prompted instrumentation manufacturers to develop microplate readers like the Multiskan photometer. This instrument was a pioneer version of the microplate reader commercially available today [54]. In 1996, the SPE system also included the microplate version of the sampling system. It consisted of a block installing a standard 96-well microtitre plate containing ninety-six SPE cartridges. It was the automated 96-well plate SPE and provided essential improvements in the parallel sample preparation techniques [57, 58], enhancing sample throughput. The Pfizer company patented this system [59], and the Porvair Filtronics company started the commercial production under license. In 1998, the Society of Biomolecular Screening (SBS) instituted a committee to standardize the dimensions of the microplate. Since then, microplate manufacturers modified the production equipment so that the tools complied with the new standards [54]. In 2004, the American National Standards Institute (ANSI, Washington, DC, USA) outlined a uniform standard for the dimensions of the multiwell plate format, making it suitable for robotic automation [60]. So, 96-well plates have become widespread for many different sample preparation methods [61].

A well plate is an option for the use of vials or tubes in analytical methods. Multi-well plates analytical systems are faster compared to those using vials or tubes. They do not involve crimping or handling of vials, so all the wells can be filled continuously, without interrupting the instrument process. The knowledge of the techniques using multi-well plates is fundamental to select the most appropriate

instrument out of the many options available. Depending on the type of detection, for instance, it is necessary to select plates of different colors. Optical absorbance and luminescence detectors require white plates. Fluorescent biological assays can be performed using black plates, and both pigments prevent crossover from one well to another. Colorimetric assays, cell culture, and sample storage usually exploit transparent plates. Besides the color, the shape of the plates can vary too. Wells can be C-, F-, U-, or V-shaped. Each geometry has a specific use depending on method requirements. The internal surface of the wells can be treated for immunological and cell culture studies. 96-well plates for SPME allow organic solvent desorption of the analytes from the sorbent before HPLC analysis and can be fully automated [62].

Dispersive

In 2003, Anastassiades et al. [63] introduced a new SPE technique, named dispersive solid-phase extraction (DSPE), to carry out rapidly and efficiently the target analytes clean-up and extraction from food and environmental sample.

The underlying principle of this technique is the addition of the sorbent material into a fixed volume of extract to remove the matrix interference. After extraction, centrifugation of the suspension allows the separation of the sorbent material from the supernatant solution. This procedure in-batch avoids the extraction of the sample using an SPE column. It involves small amounts of sorbent and solvent, saves time and labor [64].

Hence, DSPE helps to avoid common shortcomings in conventional column-SPE, like channeling, clogging and analyte loss due to breakthrough [65]. Furthermore, since extraction techniques are surface-dependant processes, DSPE allows to increase the sorbent surface available for analytes adsorption. However, at the microscale level, when using minute amounts of solvent, DSPE suffers from the limitation on the amount of extractant because this reduces the extraction capacity [65, 66]. However, owing to the advantages of DSPE, it appears in the list of the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) techniques [64].

The automation of DSPE is challenging since it requires centrifugation. Sample filtration is a viable option, as in the new filter vial DSPE concept. Still, the filter material inevitably retains some analytes. This significant drawback limits the applicability of DSPE because when processing large numbers of samples, automation is desirable [65]. An alternative way is the use of magnetic sorbent phases, viz. performing a magnetic SPE (MSPE) [3].

In DSPE, it is crucial to disaggregate to the maximum extent the sorbent into the sample solution to maximize the area of contact. Therefore, the sorbent solution is mechanically stirred, sonicated, or irradiated with microwaves during extraction. To increase the sorbent dispersion and interaction with the target compounds, also solvents, oxidants, or functionalizing agents are used.

As for other extraction techniques, before the instrumental determination, it is necessary to desorb the analytes from the sorbent thermally or chemically [67].

The Anatune CF-100 centrifuge is an implementation of the GERSTEL MPS autosampler used to separate the sorbent automatically after extraction in the micro-scale version of DSPE for QuEChERS. The GERSTEL MAESTRO Software, integrated with the software of the chromatograph, controls the process [68].

To avoid centrifugation, Zhu et al. developed a novel in-syringe DSPE method that exploits the advantages of fibers as adsorbents in packed SPE (pSPE) [69].

Electrospun fibers have dimensions in the sub-micron scale. These types of fibers are ideal sorbents for pSPE with low backpressure. For this reason, the in-syringe DSPE technique consists of the use of a barrel of a special syringe containing electrospun fibers as sorbent.

According to Shi et al. [70], dispersive micro-solid phase extraction is a miniaturized version of matrix solid-phase dispersion (MSPD) based on hydrophobic magnetic nanoparticles. MSPD was proposed in 1989 by Barker et al. [71] as an efficient and general technique for the isolation of a wide range of compounds. In 1993, MSPD was patented [72] and found application as an analytical technique for the preparation, extraction, and fractionation of solid, semi-solid, and highly viscous biological samples [73].

In 2015, Morris et al. developed an automated clean-up SPE column (c-SPE), based on zirconia-coated silica. The technique found application in the Instrument Top Sample Preparation (ITSP) mini-cartridges, on a robotic XYZ instrument autosampler [74]. Cartridge-based SPE (c-SPE) usually provides better clean-up than dispersive-SPE (DSPE). However, in batch applications, DSPE is cheaper, and faster than c-SPE, especially DSPE in the filter-vial mode, which cleans and filters extracts in autosampler vials. On the opposite, on-line, automated, SPE techniques that perform simultaneous clean-up and separation, prevent analyte degradation. The risk of analyte degradation is consistent in batch routines, when several hours can pass between sample extraction and analysis [75].

The application of c-SPE instead of DSPE allowed high sample throughput and recoveries in samples containing a large number of pesticides in complex matrices with high levels of lipids. The use of this type of cartridges can be fully automated, for example, using the PAL RTC autosampler [76], as shown in Fig. 3.

Pipette tips

The need for automated SPE has led to the development of extraction devices having new formats to meet with the autosamplers already present in many laboratories. Commonly, the liquid-handling systems use pipette tips to dispense liquid samples and solvents rapidly. So, a natural evolution of the existing technology is the use of sorbent-filled pipette tips for SPE.

Fig. 3. μ SPE controlled elution from low particle size sorbent bed [4]

In 1998, Ansys Technologies (Lake Forest, California) introduced the sorbent-impregnated disk SPE pipette tip format [77].

The SPE pipette format can fit multichannel pipetting devices. Also, the flow through the SPE system is bidirectional. The advantage of this feature is that extraction and dispensing of liquid samples is possible from the bottom or the top of the sampling device.

Automated XYZ cartesian systems do not require any modification to fit SPE in pipette format. In such systems, the disposable pipette tips eliminate the risk of carryover and cross-contamination, which are instead common findings in 96-well plate designs. One limitation of the disk SPE pipette tip format, however, is that the small cross-sectional area and the disk are prone to plugging sampling complex matrices [78].

In the early 2000s, Brewer patented the disposable pipette extraction (DPX) system [79]. The DPX is another alternative to traditional SPE that combines efficient and rapid extraction with significantly reduced solvent and time consumptions.

The first commercial DPX contained a C18 sorbent derived from the chromatographic technology. However, at present, different phases are available. DPX is a dispersive μ SPE technique based on a disposable pipette tip containing sorbent loosely packed, kept in place by upper and lower permeable septa. DPX enables mixing the sorbent with sample solutions. The tips do not necessarily require conditioning before the mixing of the sample and the sorbent. Extraction efficiency is not affected by channeling at any flow rate. Extractions can be fully automated, reducing sample manipulation, and thus improving precision and sample throughput [80].

Figure 4 illustrates the disposable pipette extraction process. It is possible to equilibrate the sorbent with the solvent before extraction. The process starts drawing the sample into the pipette tip in direct contact with the solid phase. Turbulent air bubble mixing leads to a suspension of sorbent in the sample, assuring optimal contact and efficient extraction. Then, the extracted sample is discharged,

typically after 30 s. If necessary, the sorbent is washed to remove unwanted residues/non-target interfering substances. Finally, extracted analytes are eluted into a vial for the following instrumental analytical step.

DPX methods are fully and easily automated using the GERSTEL MultiPurpose Sampler (MPS), which can introduce the extract into a GC/MS or LC/MS system. Other sample preparation steps, like derivatization or addition of internal standard are possible can be done placing the samples in the MPS autosampler and activating the sequence table from the MAESTRO software [81].

On-line

Besides the offline mode, the SPE technique is suitable for coupling simultaneous extraction and analysis. On-line SPE enables partial or total automation of the analytical process, reducing analysis time, decreasing analyte loss, increasing sensitivity, and improving analytical precision. Thus, the combination of on-line SPE to LC leads to fast and reliable methods, and often allows to reduce sample volumes while maintaining high sensitivity [68, 82].

Fig. 4. Disposable pipette extraction process [5]

In the early 1980s, sample enrichment involved the use of pre-columns. The use of pre-columns in HPLC systems was the first type of coupling of on-line SPE and LC [83]. In 1990, the first system incorporating an SPE cartridge in an LC column was patented [84].

The most common approach to couple on-line SPE with LC is through column switching. To this purpose, a small, typically 2–15 mm long and 1–4.6 mm i.d., pre-column used as SPE column is connected to a conventional LC analytical column through a switching valve. The possible configurations of column switching involve various numbers of pre-columns, switching valves, and pumps. Figure 5 shows a simple column switching configuration. When the switching valve is in “Configuration (a),” the sample is injected into the SPE column, previously conditioned using appropriate solvents. At the same time, the analytical column is equilibrated with the chromatographic mobile phase. After the elution of the interfering compounds, the valve is switched in “Configuration (b).” The analytes are thus eluted from the SPE column either in the direct-flushing mode, *a*), or back-flushing mode, *b*). Then, they are transferred into the analytical column that separates the analytes before detection. During the separation, the valve is switched into its initial position (“Configuration (a)”), and the SPE column is rinsed so that it can be ready for the next sample injection with no carryover effects [82].

While the process and the method of on-line SPE-LC are similar, the sorbents used in the on-line SPE are different. Traditional SPE sorbents include the same materials used for analytical columns. Chemically functionalized silica, ion-exchange, mixed-mode, and specialty materials. These materials in SPE, however, show limited selectivity. Moreover, most matrix constituents can also be concentrated on them, together or in competition with the target analytes, altering the extraction process, separation, and detection. More selective sorbents exist. Specialty monolithic, restricted access material (RAM), molecularly imprinted polymer (MIP), and immunoaffinity extraction (IAE) sorbents are only a few examples.

Monolithic materials attracted considerable attention in recent years as a new class of non-particulate sorbent [82], as described above in Section “MonoTrap™”. These sorbents

are also called continuous beds (phases). Monolithic columns are one piece of silica rod or organic polymer with flow-through pores. The smaller pores entail large surface areas for sufficient separation capacity, while the larger ones reduce flow resistance [48]. These materials overcome problems of large void volume between the packed particles and slow mass transfer, typical in conventional particulate material [82].

Restricted access materials designed to remove macromolecules by size-exclusion showed great potential for bioanalysis. These materials have a pore size that provides size exclusion and hydrophilic shielding preventing large molecules from entering the inner region of the sorbent. At the same time, the porosity is such that lets the small molecule analytes access the internal region of the sorbent, where retention takes place [48]. Accordingly, only small molecules can penetrate the pores and interact with a RAM stationary phase. In contrast, large molecules are excluded from the stationary phase and are washed away. RAM pre-columns are used in bioanalysis to remove proteins, but also to treat environmental samples, such as water or sediment [82]. The benefits of MIPs are the high specificity, physical-chemical stability, and low cost of preparation, besides the potential application to a wide range of target molecules [82].

Silica-based materials binding antibodies form the so-called immunoaffinity extraction (IAE) sorbents. These materials provide high selectivity towards specific classes of target compounds. Immobilized antibodies onto a pressure-resistant support make a material that can fit into an extraction column. They can remove a specific analyte from complex matrices affording high selectivity and sensitivity [48].

IAE sorbents designed for proteins or viruses are more selective than those for small molecules. The cross-reactivity of antibodies has been exploited and enhanced in IAE sorbents, which can isolate structurally related analytes. One IAE-based method allows for the selective determination of classes of organic pollutants in groundwater and surface water. This method was proposed as an economical, time-saving, and user-friendly alternative to the US Environmental Protection Agency (EPA) reference method [82].

Fig. 5. Representation of on-line extraction system with *a*) direct-flushing column switching, and *b*) back-flushing column switching [6]

Two other types of on-line μ SPE still not commercially available, but being actively studied may also be mentioned. These are in-tube SPME and magnetic beads. In-tube SPME is a variation of SPME that uses a section of a GC capillary column. The extraction system so obtained can be coupled to a commercial HPLC autosampler, developed by Eisert and Pawliszyn in 1997 [85].

Magnetic beads are prepared by the same process used for obtaining magnetic particles. The magnetic particles, coupled to a wide variety of molecules, yield the beads. The magnetic beads were patented by Mark Steven Chagnon in 1984 [86], and on bead injection was proposed by Scampavia et al. in 1999 [87].

CONCLUSIONS

This review aims to provide the state-of-the-art of μ SPE techniques.

The division here proposed of the techniques in categories and sub-categories follows the characteristics of the μ SPE and has no correspondence to previous reports. Some classifications reported earlier differ from ours because of the principles considered. For example, Hiroyuki Kataoka [88] and Wenkui Li et al. [48] proposed classifications of μ SPE based on the technique of the extraction.

Jialiang Pan et al. [89] proposed a classification for the solid phase formats for on-line solid-phase-based extraction.

Our division regards configurations of μ SPE and not the principles behind the mechanism of action. Furthermore, we considered only commercially available products. In the category “on-line,” we included only μ SPEs that exist both in-line and on-line.

REFERENCES

- Dugheri, S.; Mucci, N.; Bonari, A.; Marrubini, G.; Cappelli, G.; Ubiali, D.; Campagna, M.; Montalti, M.; Arcangeli, G. *Acta Chromatogr.* **2020**, *32*, 69–79.
- Dugheri, S.; Mucci, N.; Bonari, A.; Marrubini, G.; Cappelli, G.; Ubiali, D.; Campagna, M.; Montalti, M.; Arcangeli, G. *Acta Chromatogr.* **2020**, *32*, 1–9.
- Speltini, A.; Sturini, M.; Maraschi, F.; Profumo, A. *Trends Environ. Anal. Chem.* **2016**, *10*, 11–23.
- Speltini, A.; Scalabrini, A.; Maraschi, F.; Sturini, M.; Profumo, A. *Anal. Chim. Acta* **2017**, *974*, 1–26.
- Arsenault, J. C. *Beginner's Guide to SPE: Solid-Phase Extraction*; Waters Corporation: Milford, **2014**.
- Hennion, M. C. *J. Chromatogr. A* **1999**, *856*, 3–54.
- Spietelun, A.; Marcinkowski, Ł.; de la Guardia, M.; Namieśnik, J. *J. Chromatogr. A* **2013**, *1321*, 1–13.
- Plotka-Wasyłka, J.; Szczepańska, N.; de la Guardia, M.; Namieśnik, J. *TrAC Trends Anal. Chem.* **2015**, *73*, 19–38.
- Sajid, M.; Plotka-Wasyłka, J. *TrAC Trends Anal. Chem.* **2018**, *103*, 74–86.
- Bielicka-Daszkiewicz, K. *J. Liq. Chromatogr. Rel. Technol.* **2016**, *39*, 477–87.
- Vergara-Barberán, M.; Carrasco-Correa, E. J.; Lerma-García, M. J.; Simó-Alfonso, E. F.; Herrero-Martínez, J. M. *Anal. Chim. Acta* **2019**, *1084*, 1–20.
- Seidi, S.; Tajik, M.; Baharfar, M.; Rezazadeh, M. *TrAC Trends Anal. Chem.* **2019**, *118*, 810–27.
- Chisvert, A.; Cárdenas, S.; Lucena, R. *TrAC Trends Anal. Chem.* **2019**, *112*, 226–33.
- Supelco Sigma-Aldrich Solid Phase Extraction Products [on line] Available at: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Supelco/General_Information/t402150.pdf (accessed Dec 02, 2019).
- Simpson, N.; Van Horne, K. C. *Sorbent Extraction Technology Handbook*; Varian: Lake Forest, **1993**.
- Tamayo, F. G.; Turiel, E.; Martín-Esteban, A. *J. Chromatogr. A* **2007**, *1152*, 32–40.
- Dugheri, S.; Bonari, A.; Pompilio, I.; Colpo, M.; Mucci, N.; Montalti, M.; Arcangeli, G. *Acta Chromatogr.* **2017**, *29*, 511–4.
- Dugheri, S.; Bonari, A.; Pompilio, I.; Colpo, M.; Mucci, N.; Arcangeli, G. *Saf. Health Work* **2018**, *9*, 479–85.
- Abdel-Rehim, M., United States Patent US 2004/0241874 A1, **December 2, 2004**.
- Moein, M. M.; Abdel-Rehim, A.; Abdel-Rehim, M. *TrAC Trends Anal. Chem.* **2015**, *67*, 34–44.
- Trajan Scientific Australia Pty Ltd. **2019**. TRAJAN MEPS [on line] Available at: <http://www.sge.com/products/meps> (accessed Dec 04, 2019).
- Abdel-Rehim, M. *J. Chromatogr. B* **2004**, *801*, 317–21.
- Trajan Scientific Australia Pty Ltd. **2019**. TRAJAN eVol [on line] Available at: <http://www.sge.com/products/evol> (accessed Dec 04, 2019).
- Dawes, E. F.; Dawes, P. A.; Cerra, R.; Minett, A., United States Patent US 2015/0352543 A1, December 10, **2015**.
- Alexandrou, L. D.; Spencer, M. J.; Morrison, P. D.; Meehan, B. J.; Jones, O. A. *Sci. Tot. Environ.* **2015**, *512*, 210–4.
- Pereira, J. A. M.; Gonçalves, J.; Porto-Figueira, P.; Figueira, J. A.; Alves, V.; Perestrelo, R.; Medina, S.; Câmara, J. S. *Analyst* **2019**, *144*, 5048–74.
- Porto-Figueira, P.; Figueira, J. A.; Pereira, J. A.; Câmara, J. S. *J. Chromatogr. A* **2015**, *1424*, 1–9.
- EST Analytical EST Analytical E-prep SPE Cartridges. [on line] Available at: <https://www.estanalytical.com/eprep-%C2%B5speed-cartridges/> (accessed Dec 04, 2019).
- Chen, J.; Pawliszyn, J. B. *Anal. Chem.* **1995**, *67*, 2530–3.
- Shirey, R.; Aurand, C. *Reporter* **2010**, *28*, 3–5.
- Supelco Sigma-Aldrich SPME LC (BioSPME). [on line] Available at: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/promo_NOT_INDEXED/Brochure/1/biocompatible-spme-pip.pdf (accessed Dec 04, 2019).
- Vuckovic, D.; Shirey, R.; Chen, Y.; Sidisky, L.; Aurand, C.; Stenerson, K.; Pawliszyn, J. B. *Anal. Chim. Acta* **2009**, *638*, 175–85.
- Marrubini, G.; Dugheri, S.; Cappelli, G.; Arcangeli, G.; Mucci, N.; Appelblad, P.; Melzi, C.; Speltini, A. *Anal. Chim. Acta* **2020**, *1119*, 77–100.
- Gerstel Gerstel AutomATED SPME-Analysis Multi Fiber Exchange MFX. [on line] Available at: <https://www.srainstruments.it/wp-content/uploads/2018/04/MFX-SPME-GERSTEL.pdf> (accessed Dec 10, 2019).

35. Supelco Gerstel SPME Fast Fit Fiber Assemblies (FFA). [on line] Available at: https://www.gerstel.com/pdf/SPME_FFA_Flyer_low_EN.pdf (accessed Dec 10, 2019).
36. Dugheri, S.; Bonari, A.; Pompilio, I.; Mucci, N.; Montalti, M.; Arcangeli, G. *RASĀYAN J. Chem.* **2016**, 9, 657–66.
37. Wada, H.; Kotera, K.; Matsuura, H.; Jinno, K.; Saito, Y., United States Patent US 2004/0091400 A1, **May 13, 2004**.
38. Saito, Y.; Ueta, I.; Ogawa, M.; Jinno, K. *Anal. Bioanal. Chem.* **2006**, 386, 725–32.
39. Wada, H., Europe Patent EP 1 985 986 A1, **October 29, 2008**.
40. Svec, F.; Tennikova, T. B.; Deyl, Z. *Monolithic Materials Preparation, Properties and Applications*; Elsevier: Amsterdam, **2003**.
41. Cabrera, K.; Lubda, D.; Schulte, M.; Meudt, A.; Ludemann-Hombourger, O.; Wieland, G.; Czerny, K.; Delp, A.; Dicks, E.; Kraus, A., United States Patent US 6,398,962 B1, **June 04, 2002**.
42. Sato, A.; Terashima, H.; Takei, Y., United States Patent US 8,795,410 B2, **August 05, 2014**.
43. Chromatography Today Chromatography Today A new approach for sample adsorption and extraction of volatile and non-volatile components. **2010**. [on line] Available at: <https://www.chromatographytoday.com/news/solid-phase-extraction-spe/34/hichrom-limited/a-new-approach-for-sample-adsorption-and-extraction-of-volatile-and-non-volatile-components/10960> (accessed Dec 05, 2019).
44. GL Sciences GL Sciences MonoTrap™ Monolithic Material Sorptive Extraction. [on line] Available at: <https://www.glsiences.com/solutions/food/eap3f1000000kac4-att/MonoTrap.pdf> (accessed Dec 05, 2019).
45. Baltussen, E.; Sandra, P.; David, F.; Cramers, C. *J. Microcolumn Sep.* **1999**, 11, 737–47.
46. Ochiai, N.; Sasamoto, K.; David, F.; Sandra, P. *J. Chromatogr. A* **2016**, 1455, 45–56.
47. Sandra, P.; Baltussen, E.; David, F., United States Patent US 2002/0098594A1, **July 25, 2002**.
48. Li, W.; Jian, W.; Fu, Y. *Sample Preparation in LC-MS Bioanalysis*; WILEY, **2019**.
49. David, F.; Sandra, P. *J. Chromatogr. A* **2007**, 1152, 54–69.
50. Risticvic, S.; Niri, V. H.; Vuckovic, D.; Pawliszyn, J. *Anal. Bioanal. Chem.* **2009**, 393, 781–95.
51. Vuckovic, D.; Cudjoe, E.; Musteata, F. M.; Pawliszyn, J. *Nat. Protoc.* **2010**, 5, 140–61.
52. Bruheim, I.; Liu, X.; Pawliszyn, J. *Anal. Chem.* **2003**, 75, 1002–10.
53. Jiang, R.; Pawliszyn, J. *TrAC Trends Anal. Chem.* **2012**, 39, 245–53.
54. BioTek Lab Manager Evolution of Microplate Technology. [on line] Available at: https://cdn2.hubspot.net/hubfs/547446/LabManager/Downloads/Infographics/Microplate%20Evolution/LM_Microplate-EVO-WEB-1.pdf (accessed Dec 10, 2019).
55. Farkas, E. *Current Contents/Life Sciences* **1992**, No. 30, 10–10.
56. Takatsy, G. Y. *Acta Microbiologica* **1955**, 3, 191–202.
57. Kaye, B.; Herron, W. J.; Macrae, P. V.; Robinson, S.; Stopher, D. A.; Venn, R. F.; Wild, W. *Anal. Chem.* **1996**, 68, 1658–60.
58. Allanson, J. P.; Biddlecombe, R. A.; Jones, A. E.; Pleasance, S. *Rapid Commun. Mass Spectrom.* **1996**, 10, 811–6.
59. Merson, J. R.; Bojanic, D., UK Patent 2243446A, **October 30, 1991**.
60. Majors, R. E. *LC GC North America* **2004**, 22, 1062–72.
61. Hutchinson, J. P.; Setkova, L.; Pawliszyn, J. *J. Chromatogr. A* **2007**, 1149, 127–37.
62. Staff Chromessentials.com Chromatography Essentials - 96-well plate 50 Years plus Strong. [on line] Available at: <https://chromessentials.com/96-well-plate/> (accessed Dec 10, 2019).
63. Anastassiades, M.; Lehotay, S. J.; Štajnbaher, D.; Schenck, F. J. *J. AOAC Inter.* **2003**, 86, 412–31.
64. Fontana, A. R.; Camargo, A.; Martinez, L. D.; Altamirano, J. C. *J. Chromatogr. A* **2011**, 1218, 2490–6.
65. Tang, S.; Chia, G. H.; Chang, Y.; Lee, H. K. *Anal. Chem.* **2014**, 86, 11070–6.
66. Cruz-Vera, M.; Lucena, R.; Cárdenas, S.; Valcárcel, M. *Anal. Method.* **2011**, 3, 1719–28.
67. Valcárcel, M.; Cárdenas, S.; Lucena, R. *Analytical Microextraction Techniques*; Bentham Science Publishers: Cordoba, **2017**.
68. Pfannkoch, E. A.; Stuff, J. R.; Whitecavage, J. A.; Foster, F. GERSTEL Automated QuEChERS Extraction for the Determination of Pesticide Residues in Foods using Gas Chromatography/Mass Spectrometry. [on line] Available at: <https://www.gerstel.com/pdf/p-gc-an-2011-05.pdf> (accessed Dec 13, 2019).
69. Zhu, G. T.; He, X. M.; Cai, B. D.; Wang, H.; Ding, J.; Yuan, B. F.; Feng, Y. Q. *Analyst* **2014**, 139, 6266–71.
70. Shi, Z. G.; Lee, H. K. *Anal. Chem.* **2010**, 82, 1540–5.
71. Barker, S. A.; Long, A. R.; Short, C. R. *J. Chromatogr. A* **1989**, 475, 353–61.
72. Barker, S. A.; Long, A. R., United States Patent 5,272,094, **December 21, 1993**.
73. Barker, S. A. *J. Biochem. Biophys. Method.* **2007**, 70, 151–62.
74. Morris, B. D.; Schriener, R. B. *J. Agri. Food Chem.* **2015**, 63, 5107–19.
75. Lehotay, S. J.; Han, L.; Sapozhnikova, Y. *Chromatographia* **2016**, 79, 1113–30.
76. Agilent Technologies JSB United Kingdom and Ireland. [on line] Available at: http://www.go-jsb.co.uk/resources/media/application_notes/Agilent_Manchester_2017_QuEChERS_GBoehm_final.pdf (accessed Feb 13, 2020).
77. Majors, R. E. *LC GC Int.* **1998**, 16, 344–5.
78. Majors, R. E. *LC GC North America* **2001**, 19, 678–87.
79. Brewer, W. E., United States Patent 6,566,145 B2, **May 20, 2003**.
80. Soares, F. Q.; Alvarenga, B. F.; Ruggiero, M. A.; Casanova, M. C.; Lima, E. M.; Rabelo, D.; Chaves, A. R. *Acta Chromatogr.* **2019**, 31, 250–4.
81. Gerstel Gerstel Automated Disposable Pipette Extraction DPX. [on line] Available at: http://www.gerstel.com/pdf/S00135-030-02-DPX_Flyer_en.pdf (accessed Jan 08, 2020).
82. Chen, L.; Wang, H.; Zeng, Q.; Xu, Y.; Sun, L.; Xu, H.; Ding, L. *J. Chromatogr. Sci.* **2009**, 47, 614–23.
83. Koch, D. D.; Kissinger, P. T. *Anal. Chem.* **1980**, 52, 27–9.
84. Fogelman, K. D.; Engel, S. J.; Price, T. E.; Augenblick, K.; Ganz, A., Europe Patent EP0446970B1, **August 11, 1990**.
85. Eisert, R.; Pawliszyn, J. *Anal. Chem.* **1997**, 69, 3140–7.
86. Chagnon, M. S.; Groman, E. V.; Josephson, L.; Whitehead, R. A., Europe Patent EP0125995A2, **May 10, 1984**.
87. Scampavia, L.; Ruzicka, J. *Anal. Chem.* **1999**, 71, 257A–63A.
88. Kataoka, H. *Anal. Sci.* **2011**, 27, 893–893.
89. Pan, J.; Zhang, C.; Zhang, Z.; Li, G. *Anal. Chim. Acta* **2014**, 815, 1–15.