

Review

Recent Applications of Solid Phase Microextraction Coupled to Liquid Chromatography

Carlo Zambonin *  and Antonella Aresta

Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", via E. Orabona 4, 70124 Bari, Italy; antonellamaria.aresta@uniba.it

* Correspondence: carlo.zambonin@uniba.it

Abstract: Solid phase microextraction (SPME) is one of the most popular sample preparation methods which can be applied to organic compounds allowing the simultaneous extraction and pre-concentration of analytes from the sample matrix. It is based on the partitioning of the analyte between the extracting phase, generally immobilized on a fiber substrate, and the matrix (water, air, etc.), and has numerous advantages such as rapidity, simplicity, low cost, ease of use and automation, and absence of toxic solvents. Fiber SPME has been widely used in combination with various analytical instrumentation even if most of the work has been done coupling the extraction technique with gas and liquid chromatography (GC and LC). This manuscript presents an overview of the recent works (from 2010 to date) of solid phase microextraction coupled to liquid chromatography (SPME-LC) relevant to analytical applications performed using commercially available fibers or lab-made fibers already developed in previous papers, and to improved instrumental systems and approaches.

Keywords: SPME-LC; applications; biological; environmental; food; review



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1. Introduction

The demand for modern, sensitive, and accurate analytical methods for the determination of compounds at low concentration levels in complex matrices, such as biological, food, and environmental, is always increasing. The main steps of an analytical process are sampling, sample preparation, instrumental analysis, and data processing, and any of them may deeply affect the analytical performance. Today, analytical instrumentation has reached an advanced level of technology and performances unimaginable until a few years ago, that allow selective and sensitive analytes determination even in complex extracts. On the other hand, sample preparation is usually the major source of inaccuracy and imprecision on analysis and remains a critical and time-consuming component of the workflow. It is devoted to the transfer of analytes from sample matrices to simplified final extracts compatible with the analytical instrument to be employed and is certainly a prerequisite for the attainment of reliable results. Consequently, any improvement on chemical analysis greatly depends also on the technological advances of modern sample preparation techniques.

Solid phase microextraction (SPME) is an evolving technique for the extraction of organic compounds from gaseous, aqueous, and solid matrices. The principle of SPME involves the equilibration of the analytes between a polymeric phase, generally coated onto a fiber, and the sample matrix. It has numerous advantages over other sample preparation techniques such as liquid–liquid (LLE), solid-phase (SPE), and supercritical fluid extraction (SFE), i.e., rapidity, low cost, ease of use and automation, and ability to perform in situ measurements. Furthermore, no harmful solvents are needed.

Since its introduction [1], many variants of the SPME technique have evolved and it can be found in different geometries, in particular in-tube [2], hollow fiber [3], thin-film [4], coated-tip [5], and magnetic nanoparticles [6]. However, the fiber geometry, where a fiber is

covered by a specific sorbent to extract target compounds from the sample matrix by direct immersion (DI-SPME) or from the headspace (HS-SPME) [7], remains the most widespread.

Fiber SPME has been widely used in combination with various analytical techniques: gas and liquid chromatography (GC, LC) [8–12], capillary electrophoresis (CE) [13], mass spectrometry (MS) [10–12], inductively coupled plasma MS (ICP-MS) [14], and ICP-optical emission spectrometry (ICP-OES) [15]. However, most applications have been performed with gas chromatography (GC) and, to a lesser extent, liquid chromatography (LC).

Of course, SPME-GC applications are limited to the analysis of volatile and thermally stable compounds, while SPME-LC can be used for the analysis of thermally labile compounds with poor volatility. Nevertheless, for many years, most efforts towards applications and practical implementations of SPME have privileged coupling with GC. The main reason for that is the different nature between the desorption steps used in LC and GC. In fact, solid phase microextraction devices are easily interfaced with GC injectors since the thermal desorption process follows the same steps used for standard GC injections. On the contrary, the SPME-LC coupling requires a slower liquid desorption process, due to slower diffusion kinetics. Desorption is generally performed off-line, in a vial, or on-line, in the chamber of a dedicated interface, using two different modes: in the flowing mobile phase (dynamic mode) or in an appropriate solvent mixture for a specific time before injection is performed (static mode). Furthermore, the on-line liquid desorption process must be carefully optimized to avoid the decrease of the chromatographic efficiency.

Despite the problems, SPME-LC has gained a good degree of popularity over the years, as demonstrated by dedicated articles that have extensively reviewed its state of the art in the past [8,9]. Back then, most of the reviewed works were focused on applications of the technique to the determination of various analytes in environmental, biological, and food matrices, as well as on partitioning studies, thanks to its peculiar non-exhaustive extractive features.

On the contrary, the many additional works published on the subject over the past decade have followed differentiated trends. Most of them have been focused on the search, discovery, and application of novel materials [16–21] to be used as extractive phases. In fact, a wide range of new sorbents has been developed and recently reviewed for both SPME-GC and LC applications, including carbon-based [22], metal organic frameworks [23], molecular imprinted polymers [24], ionic liquids [25–27], immunosorbents, sol-gel-based compounds [28,29], and nanomaterial-based [30], among others.

On the other hand, a substantial part of the fiber SPME-LC literature of the past 10 years has not yet been reviewed and is the object of the present work. The purpose of this review is therefore to provide an overview of the main recent applications performed with the traditional SPME-LC approach using the fiber geometry. The manuscript is deliberately focused on analytical applications mainly performed using commercially available fibers, in order to provide a collection of works immediately usable by professionals and/or a starting point for scientists for developing future applications. Some interesting, selected works dealing with the development of new instrumental systems and approaches are also reported. This work could represent a very useful tool for those interested in SPME-LC fast and practical applications.

2. Applications

2.1. Biological

2.1.1. Fluids and Tissues

Liquid chromatography can be considered as the technique of choice currently used for drugs and metabolites analysis in biological fluids and tissues. However, biological matrices, such as serum, plasma, and tissues, are complex mixtures incompatible with a direct injection into conventional LC supports and a sample preparation step that limits the number of interferences co-extracted with the analytes is always needed, especially when working with LC coupled to electrospray mass spectrometry (ESI-MS) to prevent

ionization issues. The commonly used extraction techniques (SPE, LLE, ultrafiltration, dialysis) generally work off-line, therefore being complex and tedious, and a substantially time-limiting step in the analytical process. Furthermore, they often require protein precipitation procedures that can induce analyte losses due to the known phenomenon of co-precipitation. SPME-LC has proven to be a good fast and sensitive approach for the determination of semi- or non-volatile analytes in biological samples, also for its ability to determine free and/or total drug concentration, depending on the extraction conditions in the sample under investigation [31].

The liquid chromatography-tandem mass spectrometry (LC-MS/MS) determination of the antifibrinolytic drug tranexamic acid in plasma samples extracted by SPME, using a commercial biocompatible C18 coating, was accomplished by Bojko et al. [32]. Good accuracy and precision were obtained, and the matrix effect was minimized making the sample compatible with ESI detection. A comparison between the new method and standard techniques of protein precipitation and ultrafiltration showed no significant differences among the performances of the approaches. The potential of the procedure was demonstrated by the successful analysis of clinical plasma samples, determining the analyte at concentration levels that exceeded the capability of current assays employing sample preparation using protein precipitation followed by LC/MS analysis. In another study, the SPME-LC-UV simultaneous determination of clenbuterol, salbutamol, and ractopamine residues using polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibers and on-line desorption was reported by Dua et al. [33], and its application to real samples demonstrated by the analysis of pig samples (muscle and liver), while Gonzalez and co-workers proposed a one-step simple extraction (using two fibers, carboxen-polydimethylsiloxane (CAR/PDMS) and polyacrylate (PA)) of BTEX (benzene, toluene, ethylbenzene, and xylenes and its metabolites) from spiked tooth followed by SPME-HPLC or HS-SPME-GC/MS determination [34]. In particular, the SPME-LC procedure showed the typical advantages of SPME (simple, rapid, accurate, solvent-free), good precision, linearity, and sensitivity, even if limits of detection (LODs) were slightly lower than those obtained with alternative approaches due to the limitation of the UV detector. The method was successfully applied in human teeth as environmental biomarker of BTEX and metabolites.

SPME (biocompatible C18 fibers) coupled to LC-MS/MS (triple quadrupole, QqQ) was also used to determine selected antibiotics and their metabolites in human blood and tissue taken from afflicted areas [35]. Samples of 25 patients under treatment were successfully analyzed to estimate pharmacokinetic profiles. Interestingly, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI/TOF-MS), coupled off-line with LC, was also used for identification and quantification of the analytes, showing results comparable to those obtained with LC-MS/MS. Another pharmacokinetic study in plasma for methylphenidate, a stimulant for the human central nervous system, was successfully performed coupling SPME (PDMS fibers) and thermal desorption-electrospray ionization/mass spectrometry (TD-ESI/MS) [36]. The applicability of the approach was demonstrated by the validation of the relevant results by means of LC-MS/MS.

Post-mortem human blood and bone marrow aspirate samples have been the target of a rapid and simple method for the determination of 25 common psychotropic drugs of different classes by SPME and liquid chromatography–quadrupole time-of-flight mass spectrometry (LC-QqTOF-MS) [37]. All the validation parameters were within the acceptable range for toxicological analyses. Scanning electron microscope images of used fibers showed some few networks (likely proteins) that did not affect the performances of the fiber itself despite the intensive use, indicating that it can be safely employed for multiple extractions.

2.1.2. In Vivo Sampling

Another crucial branch of biological applications is represented by in vivo sampling procedures. They should allow the preservation of the sample to ensure that the information eventually obtained are effectively representative of the situation of the living organism

under investigation. Fiber SPME perfectly fits this requirement thanks to its syringe-like format that allows a faster and simpler sampling, compared to alternative methods (i.e., microdialysis or ultrafiltration), followed by retraction into the holder for storage, protection, and transportation of the extracted compounds.

Togunde et al. developed three original SPME-LC-MS/MS applications for the analysis of target pharmaceuticals (carbamazepine, diazepam, nordiazepam, and others) in fish samples. In the first study [38], a method for the determination of the bioconcentration of trace contaminants in fish bile was first validated in rainbow trout after laboratory exposures and then applied to fathead minnow caged upstream and downstream of a local wastewater treatment plant. The method performances in field were demonstrated by the detection of some analytes detected in fish bile at the downstream location. In the second one [39], the same approach was applied to the *in vitro* and *in vivo* study of kinetic desorption rate constants of the analytes in agarose gel model used to simulate tissue matrix and fish muscle (dorsal muscle), respectively, using a triple quadrupole-linear ion trap (Qtrap) mass spectrometer as LC detector. The third work [40] was focused on the development and evaluation of a new improved *in vivo* SPME device (PDMS coating), that enabled a simplified sampling procedure without the need of fish anesthetization, as demonstrated by the *in vivo* determination of the target drugs in the muscle tissue of wild river fishes.

In situ SPME (mixed-mode fibers, octadecyl and benzenesulfonic acid groups) with LC-MS/MS detection was proposed as a green *in vivo* method for the direct underwater sampling of a very wide range of different small compounds from corals, so that the organisms can remain in their natural habitat [41]. More than 280 species were identified, mostly eicosanoids, steroids, prenol lipids, fatty acids, glycerolipids, and glycerophospholipids, likely because of the sampling location, near tentacles, biological membranes of corals.

Three SPME (reverse phase amide fiber, RPA) calibration methods for *in vivo* application, i.e., on-fiber standardization, dominant pre-equilibrium desorption, and diffusion-based interface model were compared by Yeung et al. [42] using fenoterol as a drug model and LC-MS/MS detection. The methods were also validated against SPME equilibrium extraction external calibration and protein precipitation. The study demonstrates that the diffusion-based model was the most convenient for future *in-vein* blood SPME investigations, to sample the circulating blood of animals without the need to withdraw representative samples. In fact, the same research group subsequently developed, using the same RPA fiber, a new *in vivo* SPME-LC-MS/MS procedure [43] to determine the pharmacokinetics of fenoterol and methoxyfenoterol in rat blood using the diffusion-based calibration interface model. The results obtained were in good agreement with those obtained by a traditional approach of blood withdrawal followed by protein precipitation, indicating the utility of the proposed method.

A protocol for the *in vivo* determination of benzodiazepines and metabolites in beagles by SPME-LC-MS/MS was validated by Lord and co-workers [44], using a polypyrrole wire coating exposed to the bloodstream through a SPME probe directly inserted into a peripheral vein by means of a standard medical catheter. A similar approach was employed for the development of two more applications performed using C18 biocompatible extractive coating: a further pharmacokinetic study in mice [45] to determine free and total concentrations of carbamazepine and a derived active metabolite, and the *in vivo* monitoring of the concentration of eicosanoids [46], known lipid mediators involved in the development of inflammatory conditions, in the blood of rats subjected to induced inflammation. In both cases, the results obtained were comparable with those obtained with conventional sample pre-treatment methods or literature data, demonstrating once again that *in vivo* SPME represents a quite reliable technique. Roszkowska et al. [47] successfully developed a SPME procedure for the determination of the anticancer drug doxorubicin using homogenized lamb's lungs as a surrogate matrix that was subsequently applied to the LC-MS/MS analysis of pig lung biopsies obtained via *in vivo* lung perfu-

sion. A future perspective of this application can be the possibility to perform repeated non-invasive samplings in the organ continuously measuring the concentration levels of drugs, to eventually optimize a personal dosage for each patient.

2.1.3. Metabolomic

SPME, including the *in vivo* format, can be also a very suitable means for metabolomic studies. Metabolomics, which is defined as the comprehensive analysis of metabolites in a biological specimen, is currently one of the most powerful analytical approaches for studying the functional status of biological systems in numerous research fields. As metabolites are the end-products of anabolic and catabolic pathways within living organisms, their comprehensive profiling may considerably help to understand many molecular mechanisms. As a matter of fact, metabolomics is deeply influenced by the sample pre-treatment procedure; it is crucial to maintain the metabolic integrity of biological samples so that metabolomic profiles can really be the direct expression of the biochemical status of a given organism.

In vivo solid-phase microextraction was proposed by Vuckovic and co-workers [48] as a sampling method for metabolomics studies of living systems using liquid chromatography-mass spectrometry and mice as a model system. The results showed that it was possible to extract more than 1500 untargeted metabolites with different polarities using a single mixed-mode coated fiber. This sampling approach is potentially able to capture unique metabolites and detect changes in metabolic profiles associated with any kind of modification occurring in the organism under investigation. For instance, two recent different *in vivo* metabolomic studies were carried out in the muscle tissue of living trout by Roszkowska et al. [49,50] using SPME fibers with biocompatible coatings and LC coupled to high resolution mass spectrometry (HRMS). Lipidic profiles were obtained [49] and compared to those obtained with an *ex vivo* SPME study of samples stored for a prolonged period, to determine the influence of sample storage, and to those obtained by solid liquid extraction. The great potential of the new approach was evident, since it enabled the characterization of a variegated lipidome, even detecting compounds not determinable with *ex vivo* SPME or SLE. Furthermore, the changes in the metabolic profile associated to short- and long-term exposition to sublethal doses of benzo[a]pyrene (BaP) [50] were determined in the muscle tissue of living fish by SPME-LC-HRMS, focusing on interesting dose and time-dependent alterations observed in the exposome by case-control model. The results indicated that BaP exposition was able to decrease the number of detected compounds.

In vivo SPME, using a mixed-mode (C18 with benzenesulfonic acid) coating, coupled to LC-MS allowed the detection of more than 50 oxylipins, brain mediators involved in key processes, under normal physiological conditions in awake moving rats [51]. Using the same well established experimental set-up as *in vivo* microdialysis, the approach permitted to obtain detailed oxylipin profiles in real time, eliminating post-mortem changes randomly observed in oxylipin concentrations.

SPME-LC metabolomic studies were also performed *ex vivo* in biological fluids. A comprehensive study [52] reported the extraction of 36 different metabolites with a wide range of polarities in biological fluids by comparing the performances of more than 40 fiber coatings. Mixed-mode coatings, polar-enhanced polystyrene–divinylbenzene, and phenylboronic acid resulted to be the most suitable for the simultaneous extraction of the largest number of heterogeneous compounds and, consequently, for untargeted metabolomic profiling applications. The mixed-mode SPME coated fiber coupled to LC-HRMS was then employed to develop a fast method for the metabolomic profiling of human plasma. A comparison with ultrafiltration and solvent precipitation clearly showed the numerous advantages of the new approach in terms of metabolite coverage, method precision, and ESI ionization suppression.

A new SPME-LC-qTOF-MS method for the solventless extraction of human milk lipidome has been reported by Garwolinska et al. [53]. Almost 70 lipids were detected and identified through an automated search of a lipid database, with the goal to properly understand the health benefits of human milk and to improve the quality and composition of artificial formulas.

In a further work, SPME directly coupled to ESI-QqQ mass spectrometer provided similar ions profiles as LC-MS/MS for the screening of secondary metabolites in actinobacteria solid medium [54]. In this case, SPME was performed by means of nitinol wires coated using a slurry composed by hydrophilic–lipophilic balance (HLB) particles (5 µm) and polyacrylonitrile (PAN).

2.1.4. Partitioning Studies

Binding of chemicals to dissolved organic carbon, sediment, proteins, or other components may reduce the free concentration and thus the bioavailability or effectivity of the chemical. Therefore, knowledge of the extent of binding or partitioning of the chemical of interest is essential in several scientific disciplines and much research is focused on measuring partition coefficients or binding affinities. For highly bound compounds, the concentration of the free fraction is frequently low and difficult to detect. SPME is the perfect solution for these applications due to its ability to determine chemicals in aqueous matrices even at very low concentration and to extract only a minimum amount of ligand (negligible depletion), so that the free concentration in the solution will not change significantly, and the equilibrium between the bound and free form will remain undisturbed. Thus, when partitioning studies involving semi- or non-volatile molecules must be performed, SPME-LC coupling is a good alternative to more conventional approaches. For instance, a method for the determination of the free fraction of chlorpromazine in medium containing bovine serum albumin (BSA) by SPME with commercial polyacrylate fibers and LC-UV detection was developed [55] and used to measure the protein binding of chlorpromazine and to calculate the chlorpromazine-bovine serum albumin (BSA) affinity constant. A similar analytical challenge, i.e., the study of the binding of human serum albumin (HSA) with carbamazepine, was faced by Bojko et al. [56] by SPME coupled to LC-UV or LC-MS, and by spectroscopic fluorescence, and nuclear magnetic resonance (¹H NMR). The multi-instrumental approach provided synergic information that allowed for the identification of high and low binding sites for carbamazepine, while SPME-LC-MS was used to determine *in vivo* the free concentrations of carbamazepine and to verify *in vitro* binding predictions at therapeutically relevant doses. Drugs-bovine serum albumin interactions were also the object of an SPME (C18 fibers) study [57] devoted to quantifying the protein binding of diclofenac, 2,4-dichlorophenoxyacetic acid (2,4-D), ibuprofen, naproxen, torasemide, warfarin, and genistein to BSA, phospholipid liposomes, fetal bovine serum (FBS), and cells.

2.2. Food

Food safety and quality control are great concerns for national and international regulatory committees and the scientific community. Therefore, food sample analysis constitutes one of the most important challenges in analytical chemistry and numerous scientists are highly focused on the enhancement of the speed and reliability of analytical processes, that should also be easy to perform, be cost-effective, require small amounts of solvents, provide a high selectivity, and be able to simultaneously detect multiple analytes. However, food-based matrices are complex and characterized by a wide range of chemical compositions that influence the performance of chemical analytical measurements, making sample preparation a crucial step before final analysis. SPME-LC represents a useful tool to face some of the analytical problems related to food.

A comparison of the performances of four procedures, including QuEChERS, SPE, pressurized liquid extraction (PLE), and SPME, for the extraction of different organophosphorus (OPPs) and carbamates (CMs) insecticides from honey samples before LC-MS/MS analysis was reported by Blasco and co-workers [58]. All the approaches were successfully able to recover the selected analytes and the results obtained applying the four extraction techniques to real honey samples were similar.

The five methods that will be described below have been developed using LC-UV as an analytical technique. This aspect could be considered by many as a liability due to the low selectivity and sensitivity of the technique, especially if compared to LC-MS. However, looking from a different perspective, it should be considered an added value, due to the low cost and reliability of a standard instrumentation that can be easily found in most laboratories. In fact, if a SPME-LC-UV analytical method can detect the target compounds at the concentration levels typically encountered in real matrices, even below the required maximum residue levels (MRLs), it could represent a very useful tool for cost-effective determinations. An SPME-LC-UV protocol (PDMS fiber) has been developed [59] and validated in terms of linearity, accuracy, precision, and sensitivity for the simultaneous determination of ten pesticides in mango fruits. The method was proposed as a fast screening prior to LC-MS analysis, even if it proved to be sensitive enough to detect some of the target compounds in mango samples at concentration levels below the Brazilian MRLs. A further SPME-LC-UV screening method for the simultaneous determination of six triazoles (diniconazole, fluquinconazole, flusilazole, myclobutanil, tebuconazole, and tetraconazole) in grape and apple fruit samples (peel, pulp, juice, and rain under the fruit trees) was fully validated by Bordagaray et al. [60]. The procedure was applied to real samples previously sprayed with commercial products containing the analytes and, eventually, myclobutanil and tebuconazole were found in the peel and rain, but not in pulp and juice samples. Aresta and co-workers successfully developed three different fast and simple SPME-LC-UV applications in various food matrices using commercially available fibers, on-line desorption, and LC isocratic conditions. The first work [61] reported the simultaneous determination of benzoic acid, salicylic acid, and some of its derivatives in fava beans, blueberries, kiwi, tangerines, lemons, oranges, and fruit juice (lemon and blueberry) samples, using a PDMS/DVB fiber, that was also the coating used in the development of another procedure for the simultaneous determination of major isoflavones in soy milks and blended rice/soy beverages [62]. In the latter study, the binding constants of isoflavones with serum albumin bovine were also defined to evaluate their bioavailability and to study the interaction with the main constituents in plants. A further method for the determination of free and total (after enzymatic deconjugation of piceid) concentration of trans-resveratrol in wines, spirits, and grape juices [63], was carried out using a polyacrylate fiber. The analyte was found in all the considered samples at different levels.

2.3. Environmental

To determine pollutants and/or the fate and impact of their potentially harmful degradation products in matrices of environmental interest, sensitive analytical methods to be applied to aqueous-based matrices that do not require the use of organic solvent are required. Furthermore, environmental samples are normally complex, and sample pre-treatment should be able to minimize interference arising from undesired interfering compounds. Considering that many contaminants are not suitable for GC analysis because of their physico-chemical properties, the SPME-LC approach represents an attractive choice to solve many of these analytical problems.

To allow the SPME extraction and simultaneous LC-MS/MS determination of 16 drugs having acid and basic properties, urban wastewaters samples were split in two aliquots that were adjusted at pH 3 and 11, respectively, and extracted by two different Carbowax/Templated Resin (CW-TPR) fibers [64]. The fibers were then consecutively transferred in the desorption chamber for desorption in static mode and subsequent analysis. This original "dual SPME" approach permitted the quantification of all the analytes in influ-

ent and effluent wastewater treatment plant samples by means of a single chromatographic run. Another environmental application was focused on the SPME extraction (CW-TPR fiber) and LC-UV determination of chloramphenicol in tap and sea water [65]. Since the introduction of drugs into municipal sewage-treatment plants is often due to human excretion, the method was also applied to the determination of the analyte to urine samples. The optimized procedure required a simple sample pre-treatment, isocratic elution, and provided enough sensitivity for the analyte determination in the considered samples.

An interesting study has considered for the first time the issues potentially caused in ESI-MS ionization by interferences extracted by the SPME fibers from complex matrices (environmental and biological) [66], finding significant enhancement or suppression of ionization in all the samples under investigation. Then, an isotopic internal standard method was developed and suggested as a tool to correct matrix effects in samples where its occurrence is unavoidable. A further work proposed a homemade interface to couple SPME (PDMS fiber) to LC-UV for the reliable and simultaneous determination of the antibacterial triclosan, triclocarban, and four transformation products [67] in river water samples. More recently, the performances of three fibers (acrylate, nylon, and polydimethylsiloxane) for the extraction of endocrine disruptors (EDCs), triclosan, bisphenol A, and 17 α -ethinylestradiol, from seawater and for bioavailability studies were compared [68]. LC-MS/MS was used as analytical device, and acrylate fibers were demonstrated to be the most suitable for the analytes.

A fast SPME-LC-MS/MS method (about 35 min of pre-treatment time) for the determination of tetrabromobisphenol A and hexabromocyclododecane, two currently used brominated flame retardants (BFRs), was developed [69] using spiked natural waters and subsequently applied to the analysis of tap water, even if the analytes were not found in the real matrix. Finally, the ultra-trace determination of sudan dyes in wastewater has been very recently reported [70] using two different extraction protocols, namely SPME and on-line SPE, in combination with LC-UV. As far as the SPME approach is concerned, using a PDMS/DVB coating, it was able to detect sudan I and II at sub- μ g/l levels while sudan III and IV were not detectable at these levels likely due to strong binding to dissolved organic matter.

2.4. Technical Improvements

Since the beginning, SPME has grown and evolved continuously, as demonstrated by the huge number of publications relating not only to its applications or to the search of new coatings and geometries, but also to experimental setups and novel coupling approaches. Many SPME-LC works have been recently published in the attempt to improve the performances of the whole SPME-LC analytical procedure.

Back to 2010, the idea to use electrochemistry as a concentration approach led to the development of a method to electrochemically assist SPME extraction of parathion in water [71]. A pencil lead electrode simultaneously played the role of electrode and fiber. The extraction occurred with the application of a suitable reductive potential to the electrode/fiber. A static/dynamic desorption step was subsequently performed in the commercial SPME-LC desorption chamber, followed by LC-APCI-MS/MS analysis at trace levels.

In the attempt to fasten the desorption process, a heated lab-made interface chamber was introduced by Alves et al. and adopted in the development of a SPME-LC protocol for the determination of anticonvulsants in human plasma samples [72]. The method was fully validated and demonstrated to be adequate for therapeutic drug monitoring.

Modifications on the SPME-LC interface and on the commercial PAN/C18 fiber assembly were also made by Chen et al. [73], to avoid fiber coating damage and solvent/mobile phase leaks. The interface original tubes, replaced to minimize extra column volume, together with the optimized desorption and elution conditions, produced a good chromatographic efficiency. The system was tested by analyzing polycyclic aromatic hydrocarbons (PAH), proving to be robust and reliable.

Analytes extracted by the SPME fibers from complex matrices were desorbed into solvent droplets in a digital microfluidics (DMF) device prior to LC-MS analysis in a further study [74] focused on the improvement of the desorption process. The brand new SPME-DMF approach was successfully applied to the sensitive determination of free steroids in urine. A relevant pre-concentration of the analytes was observed, due to the low volumes inherent to DMF, indicating the applicability of the device to the determination of different analytes in complex matrices at trace levels.

A microfluidic open interface was designed and used to directly couple biocompatible SPME to tandem mass spectrometry [75]. Using this approach, the authors have optimized a rapid method for the determination of the antifibrinolytic drug tranexamic acid in patients undergoing cardiac surgery and experiencing renal dysfunction, demonstrating the ability of this interface to perform an ultrafast monitoring of its concentration during administration (30 s per sample, 15 min with sample preparation) and then correcting the dosage in real time. A cross validation against a more conventional technique, such as standard thin film SPME-LC-MS/MS, confirmed the reliability of the procedure.

Kayali-Sayadi et al. [76] proposed a modification of the commercial SPME-LC interface to perform both vibrations-assisted extraction and desorption, directly placing the fiber in the chamber usually dedicated to the desorption step. Extraction in dynamic mode was made by continuously supplying the sample matrix using a syringe, while a mixed static/dynamic mode was chosen for desorption. Selected PAH were determined in water samples at low concentration levels by LC with fluorescence detection and the results were found not significantly different from those obtained with the 550.1 EPA method.

3. Conclusions

SPME has undoubtedly become an established and ever-growing extraction technique. Its coupling with liquid chromatography has always been problematic due to the criticalities associated with the desorption process. Nevertheless, the SPME-LC applications to biological (Table 1), food, and environmental (Table 2) samples produced in recent years with commercial or already described fibers, reported in this review, are quite considerable and, added to the numerous SPME-LC works dedicated to the development of new extractive materials that will likely bring to a fast increase of commercial fibers, make clear the impact that this coupling continues to have in the analytical chemical context.

Table 1. Biological applications of SPME-LC.

| Application | Analyte | Matrix | Coating | Desorption | Detector | LOQ | Ref |
|--------------------|--------------------|-------------------------|-----------------|-----------------|------------------|-------------------|------|
| Fluids and tissues | tranexamic acid | human plasma | C18 | off-line | MS/MS, QqQ | 1.5 µg/mL | [32] |
| | β2-agonists | pig tissues | PDMS/DVB | on-line | UV | 0.2–0.5 µg/L | [33] |
| | BTEX | human teeth | CAR-PDMS and PA | off-line | UV/DAD | - | [34] |
| | antibiotics | human blood/tissue | C18 | off-line | MS/MS, QqQ | 0.085–0.135 µg/mL | [35] |
| | methylphenidate | human plasma | PDMS | on-line thermal | direct TD-ESI/MS | below 0.2 ng/mL | [36] |
| | psychotropic drugs | human blood/bone marrow | C18 | off-line | MS/MS, QqTOF | 5.60–42.80 ng/mL | [37] |
| In vivo | psychotropic drugs | fishes bile | PDMS | off-line | MS/MS | - | [38] |
| | psychotropic drugs | trout muscle | PDMS | off-line | MS/MS, Qtrap | 0.40–0.97 (ng/g) | [39] |
| | psychotropic drugs | trout muscle | PDMS | off-line | MS/MS | 0.40–0.97 ng/g | [40] |
| | untargeted | coral | mixed-mode | off-line | MS/MS, Orbitrap | - | [41] |

Table 1. Cont.

| Application | Analyte | Matrix | Coating | Desorption | Detector | LOQ | Ref |
|---------------------|--------------------------------|---|---------------------|------------|----------------|---------------|------|
| | fenoterol | for future in-vein studies | reverse phase amide | off-line | MS/MS, QqQ | - | [42] |
| | fenoterol and methoxyfenoterol | rats blood | reverse phase amide | off-line | MS/MS, QqQ | 1 ng/mL | [43] |
| | benzodiazepines | beagles blood | polypyrrole wire | off-line | MS/MS | - | [44] |
| | carbamazepine and metabolite | mouse blood | C18 | off-line | MS/MS, QqQ | 1 ng/mL | [45] |
| | eicosanoids | rats blood | C18 | off-line | MS/MS; QqQ | 0.4–0.8 ng/mL | [46] |
| | doxorubicin | pig lung | mixed-mode | off-line | MS/MS, QqQ | 2.5 µg/g | [47] |
| | untargeted | mouse blood | mixed-mode | off-line | MS | - | [48] |
| | lipidome | trout muscle | mixed-mode | off-line | HRMS, Orbitrap | - | [49] |
| | BaP induced metabolites | trout muscle | mixed-mode | off-line | HRMS | - | [50] |
| Metabolomic | oxilipins | rats brain | mixed-mode | off-line | HRMS | - | [51] |
| | untargeted | human plasma | mixed-mode | off-line | HRMS, Orbitrap | - | [52] |
| | lipidome | human milk | C18 | off-line | MS/MS, QqTOF | - | [53] |
| | untargeted | actinobacteria | HLB-PAN | off-line | MS/MS, QqQ | - | [54] |
| Application | Analyte | Binding host | Coating | Desorption | Detector | LOQ | Ref |
| | chlorpromazine | BSA | PA | off-line | UV | - | [55] |
| | carbamazepine | HSA | C18 | off-line | UV or MS | - | [56] |
| Partitioning | various drugs | BSA, FBS, phospholipid liposomes, cells | C18 | off-line | MS/MS, Qtrap | - | [57] |

Table 2. Food and environmental applications of SPME-LC.

| Application | Analyte | Matrix | Coating | Desorption | Detector | LOQ | Ref |
|----------------------|-----------------------|------------------------|----------|------------|-----------------|-----------------------|------|
| | OPPs/CMs insecticides | honey | CW/TPR | on-line | MS/MS | (CCα) 1–55 ng/g | [58] |
| | pesticides | mango | PDMS | on-line | UV | 2.0–10.0 µg/kg | [59] |
| | triazoles | grape/apple | PDMS/DVB | on-line | UV | (LOD) 0.08–0.3 mg/kg | [60] |
| | salicylates | fruits/vegetables | PDMS/DVB | on-line | UV | 0.007–0.095 µg/mL | [61] |
| | isoflavones | soy milk and beverages | PDMS/DVB | on-line | UV | 0.016–0.166 µM | [62] |
| | trans-resveratrol | Wine/grape juices | PA | on-line | UV | 1.6–3.7ng/mL | [63] |
| | pharmaceuticals | wastewater | CW/TPR | on-line | MS/MS, ion trap | 0.005–0.05 ng/mL | [64] |
| | chloramphenicol | tap/sea water | CW-TPR | on-line | UV | 0.3/0.7 ng/mL | [65] |
| | - | Environm./Biological | C18 | off-line | MS/MS | - | [66] |
| Environmental | antibacterials | river | PDMS | on-line | UV | (MDL) 0.12–0.73 ng/mL | [67] |
| | EDCs | seawater | Acrylate | off-line | MS/MS, QqQ | - | [68] |
| | BFRs | tap water | PDMS/DVB | on-line | MS/MS, Qtrap | (LOD) 0.01–0.04 ng/mL | [69] |
| | sudan dyes | wastewater | PDMS/DVB | on-line | UV | 0.2 and 0.5 µg/L | [70] |

As regards the desorption phase, several users and developers have turned to off-line desorption mode, which provides longer fiber lifetime due to the lower probability of damages in the interface and is still able to guarantee good results in terms of sensitivity. The disadvantage of this approach is a loss in terms of rapidity of analysis, which is not

indispensable for many partitioning, in vivo, and/or metabolomics applications. The on-line desorption mode is also widely used, as demonstrated by several applications carried out with the commercial interface as well as by studies dedicated to its improvement.

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References

1. Arthur, C.L.; Pawliszyn, J. Solid phase microextraction with thermal desorption using fused silica optical fiber. *Anal. Chem.* **1990**, *62*, 2145–2148. [[CrossRef](#)]
2. Moliner-Martinez, Y.; Herráez-Hernández, R.; Verdú-Andrés, J.; Molins-Legua, C.; Campíns-Falcó, P. Recent advances of in-tube solid-phase microextraction. *Trends Anal. Chem.* **2015**, *71*, 205–213. [[CrossRef](#)]
3. Li, J.; Wang, Y.B.; Li, K.Y.; Cao, Y.Q.; Wu, S.; Wu, L. Advances in different configurations of solid-phase microextraction and their applications in food and environmental analysis. *Trends Anal. Chem.* **2015**, *72*, 141–152. [[CrossRef](#)]
4. Seidi, S.; Tajik, M.; Baharfar, M.; Rezazadeh, M. Micro solid-phase extraction (pipette tip and spin column) and thin film solid-phase microextraction: Miniaturized concepts for chromatographic analysis. *Trends Anal. Chem.* **2019**, *118*, 810–827. [[CrossRef](#)]
5. Bagheri, H.; Piri-Moghadam, H. Recent advances in capillary microextraction. *Trends Anal. Chem.* **2015**, *73*, 64–80. [[CrossRef](#)]
6. Ozturk, E.E.; Bozyigit, G.D.; Buyukpinar, C.; Bakirdere, S. Magnetic Nanoparticles Based Solid Phase Extraction Methods for the Determination of Trace Elements. *Crit. Rev. Anal. Chem.* **2020**. [[CrossRef](#)]
7. Gionfriddo, E.; Souza-Silva, E.A.; Pawliszyn, J. Headspace versus Direct Immersion Solid Phase Microextraction in Complex Matrixes: Investigation of Analyte Behavior in Multicomponent Mixtures. *Anal. Chem.* **2015**, *87*, 8448–8456. [[CrossRef](#)]
8. Zambonin, C.G. Coupling solid-phase microextraction to liquid chromatography: A review. *Anal. Bioanal. Chem.* **2003**, *375*, 73–80. [[CrossRef](#)]
9. Lord, H.L. Strategies for interfacing solid-phase microextraction with liquid chromatography. *J. Chromatogr. A* **2007**, *1152*, 2–13. [[CrossRef](#)]
10. Souza-Silva, E.A.; Jiang, R.; Rodríguez-Lafuente, A.; Gionfriddo, E.; Pawliszyn, J. A critical review of the state of the art of solid-phase microextraction of complex matrices I. Environmental analysis. *Trends Anal. Chem.* **2015**, *71*, 224–235. [[CrossRef](#)]
11. Souza-Silva, E.A.; Gionfriddo, E.; Pawliszyn, J. A critical review of the state of the art of solid-phase microextraction of complex matrices II. Food analysis. *Trends Anal. Chem.* **2015**, *71*, 236–248. [[CrossRef](#)]
12. Souza-Silva, E.A.; Reyes-Garcés, N.; Gómez-Ríos, G.A.; Boyaci, E.; Bojko, B.; Pawliszyn, J. A critical review of the state of the art of solid-phase microextraction of complex matrices III. Bioanalytical and clinical applications. *Trends Anal. Chem.* **2015**, *71*, 249–264. [[CrossRef](#)]
13. Santos, B.; Simonet, B.M.; Ríos, A.; Valcárcel, M. On-line coupling of solid-phase microextraction to commercial CE-MS equipment. *Electrophoresis* **2007**, *28*, 1312–1318. [[CrossRef](#)] [[PubMed](#)]
14. Li, P.; Zhang, X.Q.; Chen, Y.J.; Lian, H.Z.; Hu, X.; A sequential, P. solid phase microextraction system coupled with inductively coupled plasma mass spectrometry for speciation of inorganic arsenic. *Anal. Methods* **2014**, *6*, 4205–4211. [[CrossRef](#)]
15. Zheng, C.; Hu, L.; Hou, X.; He, B.; Jiang, G. Headspace Solid-Phase Microextraction Coupled to Miniaturized Microplasma Optical Emission Spectrometry for Detection of Mercury and Lead. *Anal. Chem.* **2018**, *90*, 3683–3691. [[CrossRef](#)]
16. Xu, J.; Zheng, J.; Tian, J.; Zhu, F.; Zeng, F.; Su, C.; Ouyang, G. New materials in solid-phase microextraction. *Trends Anal. Chem.* **2013**, *47*, 68–83. [[CrossRef](#)]
17. Yin, L.; Xu, J.; Huang, Z.; Chen, G.; Zheng, J.; Ouyang, G. Solid-Phase Microextraction Fibers Based on Novel Materials: Preparation and Application. *Prog. Chem.* **2017**, *29*, 1127–1141.
18. Piri-Moghadam, H.; Alam, M.N.; Pawliszyn, J. Review of geometries and coating materials in solid phase microextraction: Opportunities, limitations, and future perspectives. *Anal. Chim. Acta* **2017**, *984*, 42–65. [[CrossRef](#)]
19. Hashemi, B.; Zohrabi, P.; Shamsipur, M. Recent developments and applications of different sorbents for SPE and SPME from biological samples. *Talanta* **2018**, *187*, 337–347. [[CrossRef](#)]

20. Lashgari, M.; Yamini, Y. An overview of the most common lab-made coating materials in solid phase microextraction. *Talanta* **2019**, *191*, 283–306. [[CrossRef](#)] [[PubMed](#)]
21. Sajid, M.; Khaled Nazal, M.; Rutkowska, M.; Szczepańska, N.; Namieśnik, J.; Płotka-Wasyłka, J. Solid Phase Microextraction: Apparatus, Sorbent Materials, and Application. *Crit. Rev. Anal. Chem.* **2019**, *49*, 271–288. [[CrossRef](#)]
22. Guo, J.; Park, S.J.; Meng, L.Y.; Jin, X. Applications of carbon-based materials in solid phase micro-extraction: A review. *Carbon Lett.* **2017**, *24*, 10–17.
23. Rocío-Bautista, P.; Pacheco-Fernández, I.; Pasán, J.; Pino, V. Are metal-organic frameworks able to provide a new generation of solid-phase microextraction coatings?—A review. *Anal. Chim. Acta* **2016**, *939*, 26–41. [[CrossRef](#)]
24. Li, G.; Row, K.H. Recent Applications of Molecularly Imprinted Polymers (MIPs) on Micro-extraction Techniques. *Sep. Purif. Rev.* **2018**, *47*, 1–18. [[CrossRef](#)]
25. Yu, H.; Ho, T.D.; Anderson, J.L. Ionic liquid and polymeric ionic liquid coatings in solid-phase microextraction. *Trends Anal. Chem.* **2013**, *45*, 219–232. [[CrossRef](#)]
26. Patinha, D.J.S.; Silvestre, A.J.D.; Marrucho, I.M. Poly (ionic liquids) in solid phase microextraction: Recent advances and perspectives. *Prog. Polym. Sci.* **2019**, *98*, 101148. [[CrossRef](#)]
27. Yavir, K.; Konieczna, K.; Marcinkowski, L.; Kloskowski, A. Ionic liquids in the microextraction techniques: The influence of ILs structure and properties. *Trends Anal. Chem.* **2020**, *45*, 219–232.
28. McLean, M.; Malik, A. *Comprehensive Sampling and Sample Preparation*; Pawliszyn, J., Ed.; Elsevier: Amsterdam, The Netherlands, 2012; pp. 311–329.
29. Kabir, A.; Furton, K.G.; Malik, A. Innovations in sol-gel microextraction phases for solvent-free sample preparation in analytical chemistry. *Trends Anal. Chem.* **2013**, *45*, 197–218. [[CrossRef](#)]
30. Murtada, K. Trends in nanomaterial-based solid-phase microextraction with a focus on environmental applications—A review. *Trends Environ. Anal.* **2020**, *25*, 00077. [[CrossRef](#)]
31. Zambonin, C.; Aresta, A.; Palmisano, F. Determination of the immunosuppressant mycophenolic acid in human serum by solid-phase microextraction coupled to liquid chromatography. *J. Chromatogr. B* **2004**, *806*, 89–93. [[CrossRef](#)] [[PubMed](#)]
32. Bojko, B.; Vuckovic, D.; Cudjoe, E.; Hoque, E.; Mirmaghi, F.; Wasowicz, M.; Jerath, A.; Pawliszyn, J. Determination of tranexamic acid concentration by solid phase microextraction and liquid chromatography–tandem mass spectrometry: First step to in vivo analysis. *J. Chromatogr. B* **2011**, *879*, 3781–3787. [[CrossRef](#)] [[PubMed](#)]
33. Dua, W.; Zhang, S.; Fua, Q.; Zhao, G.; Changa, C. Combined solid-phase microextraction and high-performance liquid chromatography with ultraviolet detection for simultaneous analysis of clenbuterol, salbutamol and ractopamine in pig samples. *Biomed. Chromatogr.* **2013**, *27*, 1775–1781. [[CrossRef](#)] [[PubMed](#)]
34. González, J.L.; Pell, A.; López-Mesas, M.; Valiente, M. Simultaneous determination of BTEX and their metabolites using solid-phase microextraction followed by HPLC or GC/MS: An application in teeth as environmental biomarkers. *Sci. Total Environ.* **2017**, *603–604*, 109–117. [[CrossRef](#)]
35. Szultka-Mlynska, M.; Pomastowski, P.L.; Buszewski, B. Application of solid phase microextraction followed by liquid chromatography-mass spectrometry in the determination of antibiotic drugs and their metabolites in human whole blood and tissue samples. *J. Chromatogr. B* **2018**, *1086*, 153–165. [[CrossRef](#)]
36. Wang, C.H.; Su, H.; Chou, J.H.; Huang, M.Z.; Lin, H.J.; Shiea, J. Solid phase microextraction combined with thermal-desorption electrospray ionization mass spectrometry for high-throughput pharmacokinetics assays. *Anal. Chim. Acta* **2018**, *1021*, 60–68. [[CrossRef](#)] [[PubMed](#)]
37. Majda, A.; Mrochem, K.; Wietecha-Postuszny, R.; Zapotoczny, S.; Zawadzki, M. Fast and efficient analyses of the post-mortem human blood and bone marrow using DI-SPME/LC-TOFMS method for forensic medicine purposes. *Talanta* **2020**, *209*, 120533. [[CrossRef](#)]
38. Togunde, O.P.; Oakes, K.D.; Servos, M.R.; Pawliszyn, J. Determination of Pharmaceutical Residues in Fish Bile by Solid-Phase Microextraction Couple with Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS). *Environ. Sci. Technol.* **2012**, *46*, 5302–5309. [[CrossRef](#)] [[PubMed](#)]
39. Togunde, O.P.; Oakes, K.; Servos, M.; Pawliszyn, J. Study of kinetic desorption rate constant in fish muscle and agarose gel model using solid phase microextraction coupled with liquid chromatography with tandem mass spectrometry. *Anal. Chim. Acta* **2012**, *742*, 2–9. [[CrossRef](#)]
40. Togunde, O.P.; Lord, H.; Oakes, K.D.; Servos, M.R.; Pawliszyn, J. Development and evaluation of a new in vivo solid-phase microextraction sampler. *J. Sep. Sci.* **2013**, *36*, 219–223. [[CrossRef](#)]
41. Bessonneau, V.; Bojko, B.; Pawliszyn, J. In situ chemical exploration of underwater ecosystems with microsampling/enrichment device. *J. Chromatogr. A* **2014**, *1328*, 113–117. [[CrossRef](#)]
42. Yeung, J.C.Y.; Vuckovic, D.; Pawliszyn, J. Comparison and validation of calibration methods for in vivo SPME determinations using an artificial vein system. *Anal. Chim. Acta* **2010**, *665*, 160–166. [[CrossRef](#)]
43. Yeung, J.C.Y.; de Lannoy, I.; Gien, B.; Vuckovic, D.; Yang, Y.; Bojko, B.; Pawliszyn, J. Semi-automated in vivo solid-phase microextraction sampling and the diffusion-based interface calibration model to determine the pharmacokinetics of methoxyfenoterol and fenoterol in rats. *Anal. Chim. Acta* **2012**, *742*, 37–44. [[CrossRef](#)] [[PubMed](#)]
44. Lord, H.; Zhang, X.; Musteata, F.M.; Vuckovic, D.; Pawliszyn, J. In vivo solid-phase microextraction for monitoring intravenous concentrations of drugs and metabolites. *Nat. Protoc.* **2011**, *6*, 896–924. [[CrossRef](#)]

45. Vuckovic, D.; de Lannoy, I.; Gien, B.; Yang, Y.; Musteata, F.M.; Shirey, R.; Sidisky, L.; Pawliszyn, J. In vivo solid-phase microextraction for single rodent pharmacokinetics studies of carbamazepine and carbamazepine-10,11-epoxide in mice. *J. Chromatogr. A* **2011**, *1218*, 3367–3375. [[CrossRef](#)] [[PubMed](#)]
46. Bessonneau, V.; Zhan, Y.; De Lannoy, I.A.M.; Saldivia, V.; Pawliszyn, J. In vivo solid-phase microextraction liquid chromatography–tandem mass spectrometry for monitoring blood eicosanoids time profile after lipopolysaccharide-induced inflammation in Sprague-Dawley rats. *J. Chromatogr. A* **2015**, *1424*, 134–138. [[CrossRef](#)] [[PubMed](#)]
47. Roszkowska, A.; Tascon, M.; Bojko, B.; Goryński, K.; dos Santos, P.R.; Cypel, M.; Pawliszyn, J. Equilibrium ex vivo calibration of homogenized tissue for in vivo SPME quantitation of doxorubicin in lung tissue. *Talanta* **2018**, *183*, 304–310. [[CrossRef](#)]
48. Vuckovic, D.; de Lannoy, I.; Gien, B.; Shirey, Y.R.; Sidisky, L.; Dutta, S.; Pawliszyn, J. In Vivo Solid-Phase Microextraction: Capturing the Elusive Portion of Metabolome. *Angew. Chem.* **2011**, *50*, 5344–5348. [[CrossRef](#)] [[PubMed](#)]
49. Roszkowska, A.; Yu, M.; Bessonneau, V.; Bragg, L.; Servos, M.; Pawliszyn, J. Tissue storage affects lipidome profiling in comparison to in vivo microsampling approach. *Sci. Rep.* **2018**, *8*, 1–10. [[CrossRef](#)]
50. Roszkowska, A.; Yu, M.; Bessonneau, V.; Bragg, L.; Servos, M.; Pawliszyn, J. Metabolome Profiling of Fish Muscle Tissue Exposed to Benzoapyrene Using in Vivo Solid-Phase Microextraction. *Environ. Sci. Technol. Lett.* **2018**, *5*, 431–435. [[CrossRef](#)]
51. Napylov, A.; Reyes-Garces, N.; Gomez-Rios, G.; Olkowicz, M.; Lendor, S.; Monnin, C.; Bojko, B.; Hamani, C.; Pawliszyn, J.; Vuckovic, D. In Vivo Solid-Phase Microextraction for Sampling of Oxylipins in Brain of Awake, Moving Rats. *Angew. Chem.* **2020**, *59*, 2392–2398. [[CrossRef](#)]
52. Vuckovic, D.; Pawliszyn, J. Systematic evaluation of solid-phase microextraction coatings for untargeted metabolomic profiling of biological fluids by liquid chromatography–Mass spectrometry. *Anal. Chem.* **2011**, *83*, 1944–1954. [[CrossRef](#)] [[PubMed](#)]
53. Garwolinska, D.; Hewelt-Belka, W.; Namiesnik, J.; Kot-Wasik, A. Rapid characterization of the human breast milk lipidome using a solid-phase microextraction and liquid chromatography–Mass spectrometry-based approach. *J. Proteome Res.* **2017**, *16*, 3200–3208. [[CrossRef](#)] [[PubMed](#)]
54. Acquaro Junior, V.R.; Pereira Rodrigues, J.; Beraldo Moraes, L.A. Solid phase microextraction as a powerful alternative for screening of secondary metabolites in actinomycetes. *J. Mass Spectrom.* **2019**, *54*, 823–833. [[CrossRef](#)]
55. Broeders, J.J.W.; Blaauboer, B.J.; Hermens, J.L.M. Development of a negligible depletion-solid phase microextraction method to determine the free concentration of chlorpromazine in aqueous samples containing albumin. *J. Chromatogr. A* **2011**, *1218*, 8529–8535. [[CrossRef](#)]
56. Bojko, B.; Vuckovic, D.; Pawliszyn, J. Comparison of solid phase microextraction versus spectroscopic techniques for binding studies of carbamazepine. *J. Pharm. Biomed. Anal.* **2012**, *66*, 91–99. [[CrossRef](#)]
57. Henneberger, L.; Mühlenbrink, M.; Fischer, F.C.; Escher, B.I. C18-Coated Solid-Phase Microextraction Fibers for the Quantification of Partitioning of Organic Acids to Proteins, Lipids, and Cells. *Chem. Res. Toxicol.* **2019**, *32*, 168–178. [[CrossRef](#)]
58. Blasco, C.; Vazquez-Roig, P.; Onghena, M.; Masia, A.; Picò, Y. Analysis of insecticides in honey by liquid chromatography–ion trap–mass spectrometry: Comparison of different extraction procedures. *J. Chromatogr. A* **2011**, *1218*, 4892–4901. [[CrossRef](#)]
59. Filho, A.M.; dos Santos, F.N.; de Paula Pereira, P.A. Multi-residue analysis of pesticide residues in mangoes using solid-phase microextraction coupled to liquid chromatography and UV–Vis detection. *J. Sep. Sci.* **2011**, *34*, 2960–2966. [[CrossRef](#)] [[PubMed](#)]
60. Bordagaray, A.; García-Arrona, R.; Millan, E. Development and application of a screening method for triazole fungicide determination in liquid and fruit samples using solid-phase microextraction and HPLC-DAD. *Anal. Methods* **2013**, *5*, 2565–2571. [[CrossRef](#)]
61. Aresta, A.; Zambonin, C. Simultaneous determination of salicylic, 3-methyl salicylic, 4-methyl salicylic, acetylsalicylic and benzoic acids in fruit, vegetables and derived beverages by SPME-LC-UV/DAD. *J. Pharm. Biomed. Anal.* **2016**, *121*, 63–68. [[CrossRef](#)]
62. Aresta, A.; Di Grumo, F.; Zambonin, C. Determination of major isoflavones in soy drinks by solid-phase micro extraction coupled to liquid chromatography. *Food Anal. Methods* **2016**, *9*, 925–933. [[CrossRef](#)]
63. Aresta, A.; Cotugno, P.; Massari, F.; Zambonin, C. Determination of trans-resveratrol in wines, spirits and grape juices using solid-phase micro extraction coupled to liquid chromatography with UV diode array detection. *Food Anal. Methods* **2018**, *11*, 426–431. [[CrossRef](#)]
64. Unceta, N.; Sampedro, M.C.; Abu Bakar, N.K.; Gómez-Caballero, A.; Aránzazu Goicolea, M.; Barriola, R.J. Multi-residue analysis of pharmaceutical compounds in wastewaters by dual solid-phase microextraction coupled to liquid chromatography electrospray ionization ion trap mass spectrometry. *J. Chromatogr. A* **2010**, *1217*, 3392–3399. [[CrossRef](#)]
65. Aresta, A.; Bianchi, D.; Calvano, C.D.; Zambonin, C.G. Solid phase microextraction-liquid chromatography determination of chloramphenicol in urine and environmental water samples. *J. Pharm. Biomed. Anal.* **2010**, *53*, 440–444. [[CrossRef](#)] [[PubMed](#)]
66. Zhang, X.; Oakes, K.D.; Luong, D.; Metcalfe, C.D.; Servos, M.R. Solid-Phase Microextraction Coupled to LC-ESI-MS/MS: Evaluation and correction for matrix-induced ionization suppression/enhancement for pharmaceutical analysis in biological and environmental samples. *Anal. Chem.* **2011**, *83*, 6532–6538. [[CrossRef](#)] [[PubMed](#)]
67. Shen, J.Y.; Chang, M.S.; Yang, S.H.; Wu, G.J. Simultaneous and rapid determination of triclosan, triclocarban and their four related transformation products in water samples using SPME-HPLC-DAD. *J. Liq. Chromatogr. Relat. Technol.* **2012**, *35*, 2280–2293. [[CrossRef](#)]
68. Santos, D.M.; Williams, M.; Kookana, R.; de Marchi, M.R.R. Solid Phase Microextraction (SPME) Fibers: In situ measurements of endocrine disrupting chemicals in seawater. *J. Braz. Chem. Soc.* **2018**, *29*, 888–894. [[CrossRef](#)]

69. Yu, Y.; Zhu, X.; Zhu, J.; Li, L.; Zhang, X.; Xiang, M.; Ma, R.; Yu, L.; Yu, Z.; Wang, Z. Rapid and simultaneous analysis of tetrabromobisphenol A and hexabromocyclododecane in water by direct immersion solid phase microextraction: Uniform design to explore factors. *Ecotox. Environ. Safe.* **2019**, *176*, 364–369. [[CrossRef](#)]
70. Aresta, A.; De Vietro, N.; Zambonin, C. Ultra-trace determination of sudan I-IV in waste waters by solid-phase microextraction or on-line solid-phase extraction followed by high performance liquid chromatography. *Anal. Lett.* **2020**, *53*, 2559–2570. [[CrossRef](#)]
71. Yang, T.J.; Lee, M.R. Electrically assisted solid-phase microextraction combined with liquid chromatography–mass spectrometry for determination of parathion in water. *Talanta* **2010**, *82*, 766–770. [[CrossRef](#)]
72. Alves, C.; de Lima Gomes, P.C.F.; dos Santos-Neto, A.J.; Rodrigues, J.C.; Lanças, F.M. Determination of anticonvulsants in human plasma using SPME in a heated interface coupled online to liquid chromatography (SPME-LC). *Anal. Methods* **2012**, *4*, 1519–1524. [[CrossRef](#)]
73. Chen, Y.; Sidisky, L.M. Improvement of solid phase microextraction fiber assembly and interface for liquid chromatography. *Anal. Chim. Acta* **2012**, *743*, 61–68. [[CrossRef](#)] [[PubMed](#)]
74. Choi, K.; Boyacı, E.; Kim, J.; Seale, B.; Barrera-Arbelaez, L.; Pawliszyn, J.; Wheeler, A.R. A digital microfluidic interface between solid-phase microextraction and liquid chromatography–mass spectrometry. *J. Chromatogr. A* **2016**, *1444*, 1–7. [[CrossRef](#)] [[PubMed](#)]
75. Looby, N.T.; Tascon, M.; Acquaro, V.R.; Reyes-Garcés, N.; Vasiljevic, T.; Gomez-Rios, G.A.; Wasowicz, M.; Pawliszyn, J. Solid phase microextraction coupled to mass spectrometry via a microfluidic open interface for rapid therapeutic drug monitoring. *Analyst* **2019**, *144*, 3721–3728. [[CrossRef](#)]
76. Kayali-Sayadi, M.N.; Polo-Díez, L.M.; Rios-Acevedo, J.J. On-line HPLC/SPME Interface using dynamic extraction. *J. Phys. Conf. Ser.* **2020**, *1541*, 012009. [[CrossRef](#)]