On-line Solid-Phase Extraction for Liquid Chromatography-Mass Spectrometry

Analysis of Pesticides

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Abstract

Public concern about pesticides in food and water has increased dramatically in the last two decades. In order to guarantee consumers’ health and safety, analytical methods that could provide fast and reliable answers without compromising accuracy and precision are required.

Sample treatment is probably the most tedious and time-consuming step in many analytical procedures and, despite the significant advances in chromatographic separations and mass spectrometry techniques, sample treatment is still one of the most important parts of the analytical process for achieving good analytical results. Therefore, over the last years, considerable efforts have been made to simplify the stage and to develop fast, accurate and robust methods which allow the determination of a wide range of pesticides without compromising the integrity of the extraction process.

This review article intends to give a short overview of recently developed on-line solid-phase extraction, pre-concentration and clean-up procedures for the determination of pesticides in complex matrices by liquid chromatography-mass spectrometry techniques.
1. Introduction

Pesticides are substances employed in agriculture because of their capacity to protect crops against a wide range of threats from weeds, fungal diseases and insect pests, avoiding economic losses and increasing agricultural productivity and performance. However, in spite of these advantages, they must be carefully used because they can cause serious environmental pollution and serious health effects such as diseases of the nervous system, reproductive disorders, cancer or even genetic problems [1]. Because of the huge volumes of production of these compounds and their continuous worldwide use, some pesticides have become relatively persistent substances in the environment. Thus, residues of these compounds and/or their transformation products may easily leach from pesticide application point sources on soil surfaces or infiltrate from environmental waters (lakes or rivers) that were polluted as a result of direct application and/or runoff, and finally reach aquifers and alter ground-water quality which often are used as sources for drinking water supply [2,3]. Consequently, pesticides must undergo extensive efficacy, environmental and toxicological testing to be registered by governments for legal use in specified applications. The monitoring of pesticide residues in both food and water is nowadays a priority objective in order to get extensive evaluation of food and water quality and to avoid possible risks to human health. For instance, very low pesticide residue limits (10 μg/kg) in fruits and vegetables intended for baby food production have been established by the European Union [4].

Regarding pesticide pollution in environmental waters, the United States Environmental Protection Agency (US-EPA) has established maximum concentration levels (MCLs) for some pesticides in drinking water, such as atrazine (3.0 mg/L) (http://www.epa.gov/oppsrrd1/reregistration/atrazine/atrazine_update.htm). European legislation is more restrictive and the Council Directive 80/778/EEC [5] has set limits for pesticides at 0.1 μg/L for individual pesticides and 0.5 μg/L for the sum of all pesticides in water used for human consumption, while Directive 2008/105/EC [6] set environmental quality standards for priority substances and certain pollutants in surface waters, such as atrazine (0.6 μg/L) and diuron (0.2 μg/L).

Traditionally, gas chromatography-mass spectrometry (GC-MS) have been proposed for the analysis of pesticides [7-11], and frequently by using well established library searching routines. But today, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is becoming a powerful tool for pesticide residue analysis in
complex matrices [12-17] due to its selectivity and sensitivity, a substantial reduction of sample treatment steps compared with other methodologies such as GC-MS/(MS), and its reliable quantification and confirmation at the low concentration levels required by legislation. Moreover, the demands of high sample throughput in short time frames have given rise to high efficiency and fast or even ultra-fast LC methods, which are becoming also very popular for the analysis of pesticides. Among the several modern approaches in HPLC methods that enable the reduction of the analysis time without compromising resolution and separation efficiency, UHPLC methods either using sub-2µm totally porous particle-packed columns or partially porous core-shell columns (with sub-3 µm superficially porous particles) are among the most popular in the analysis of pesticides [18-20].

Nevertheless, due to the increased number of pesticides used worldwide and the variety and complexity of food and environmental matrices, the use of ultra-fast separations is not enough to develop fast analytical methods for the analysis of pesticide residues. Besides, multi-residue screening methods able to analyze not only target but non-target pesticide residues or even unknown compounds are demanded. Nonetheless, despite the advances in chromatographic separations and mass spectrometry techniques, sample treatment is still one of the most important parts of the analytical process and effective sample preparation is essential for achieving good analytical results [21]. Ideal sample preparation methods should be fast, accurate, precise and must keep sample integrity. Therefore, over the last years, considerable efforts have been made to develop modern approaches in sample treatment techniques for the analysis of pesticide residues without compromising the integrity of the extraction process.

In 2003, Anastassiades et al. [22] developed an ideal sample extraction and clean-up procedure named QuEChERS, acronym of “Quick, Easy, Cheap, Effective, Rugged, and Safe” which has become particularly popular to determine moderately polar pesticides residues in various food matrices [23,24]. However, sample manipulation is still required when employing QuEChERS. The use of on-line solid-phase extraction (SPE), which minimizes sample manipulation and provides both high preconcentration factors and recoveries, is becoming very popular in the analysis of pesticide residues in environmental water samples [25-27]. It is an increasingly powerful and rapid technique used to improve the sample throughput and overcome many of the limitations associated with the classical off-line SPE procedure. Other more
SPE-based selective approaches such as the use of molecularly imprinted polymers (MIPs) have also been proposed for the analysis of pesticides [25].

In this manuscript, the state-of-the-art of on-line SPE methodologies for the LC-MS analysis of pesticide residues in complex matrices such as food and environmental water samples will be reviewed. We are aiming to give a short overview of recently developed on-line SPE extraction, pre-concentration and clean-up procedures, so the advantages and disadvantages of on-line SPE versus off-line SPE procedures, the most frequently used SPE sorbents for the clean-up and preconcentration of pesticides, and applications of on-line SPE to pesticide residue analysis will be described by means of relevant application examples.

2. On-line SPE versus off-line SPE

The choice of the proper sample treatment methodology in pesticide residue analysis depends on several aspects, such as the sample matrix composition and the physical-chemical properties of the target pesticides. Taking into consideration that these compounds are usually found at very low concentration levels (ng/L-µg/L), sample treatment undoubtedly represents one of the most challenging parts of the overall analytical method for obtaining adequate preconcentration and clean-up efficiencies [17].

SPE is probably still the most frequently used procedure in sample preparation for pesticide, veterinary and other residues in food and environmental samples since it offers many benefits and advantages over other sample preparation methods. In fact, SPE is fast, robust and highly versatile, offering users a reliable tool to successfully perform different analytical tasks, such as purification, trace enrichment, desalting, derivatization, solvent exchange and class fractionation. In principle, SPE is analogous to liquid-liquid extraction (LLE) since it involves partitioning, but in this case between a liquid (sample matrix or solvent with analytes) and a solid sorbent phase with compounds that are extracted from the sample and adsorbed onto the sorbent material, thus providing concentrated sample extracts that are almost free of interfering matrix components [24].

Two main different SPE approaches are currently available: off-line and on-line procedures. Off-line SPE is a very simple technique to use that employs economical and uncomplicated equipment formed by disposable extraction columns, microplates and cartridges available in a wide range of reservoir volumes, formats and sorbents. Figure 1
shows the general SPE process which basically consists in four different steps: a) the solid sorbent is wetted by an appropriate solvent in order to activate the functional groups on its surface (conditioning step); b) sample is percolated through the sorbent (loading step); c) clean-up by washing the sorbent with a solvent of low elution strength to eliminate matrix components that have been retained on the solid sorbent (washing step); and d) elution of analytes of interest by employing an appropriate solvent with a higher elution strength (elution step). Off-line SPE methodology has been extensively used to extract pesticides and, despite the fact that today there is a general trend towards on-line methods, it is probably still the most widely used configuration in sample preparation for environmental and food analysis in general [28-30]. Off-line SPE approach remains a useful technique for analyzing pesticides in complex matrices, because of its greater flexibility and whenever elution solvent is not compatible with the subsequent method of analysis [24]. For instance, a simple and sensitive method for the simultaneous determination of 103 pesticide residues in tea by using LC-MS/MS was recently developed and validated by Huang et al. [31]. The residual pesticides were extracted from spiked and real tea samples with ACN and then purified using Carb-NH$_2$ SPE cartridges. Good recoveries ranging from 65 to 114% with intra-day precisions lower than 19.6% were obtained. An off-line SPE gas chromatography–mass spectrometry was also proposed by Ma et al. [32] for the determination of organophosphorus pesticides (dichlorovos, methyl parathion, malathion, and parathion) in underground water. The method showed limits of detection for spiked water samples in the range of 4–10 ng/L together with satisfactory recoveries for all analytes (59.5-94.6%). Another example highlighting the versatility and usefulness of off-line SPE procedure has been reported by Kouzayha et al. [33]. In this study, the authors developed a new multi-residue method based on off-line SPE modality for determination and quantification of 67 pesticides in water samples. The use of a centrifugation technique in both the drying and elution steps allowed good recoveries (higher than 65-68% for the 67 analyzed pesticides) with relative standard deviations lower than 12.3%. However, off-line SPE procedure also presents several weaknesses which include: time consuming and labor-intensive steps, requirement of large amount of organic solvents, and possible loss of analytes during the evaporation steps or sample manipulation that may lead to sample contamination, and less accuracy and precision with respect to the on-line mode. These aspects, together with the demands of high sample throughput in short time frames, have given rise to high efficiency and fast or
even ultra-fast analytical methods that employ automated and/or on-line SPE procedures [34]. In this context, on-line SPE modality has become very popular over the last few years for the analysis of pesticides in complex samples [25]. In fact, on-line SPE has several advantages over off-line methods such as high sample throughput, reduced sample manipulation, improved precision, and low reagent consumption. Furthermore, on-line systems are especially beneficial when the amount of sample is limited, or when very high sensitivity is required because of the low concentration of target molecules in the sample. Although SPE technique can be easily coupled on-line to liquid chromatography (LC) and gas-chromatography (GC) systems, analytical methods which combine SPE with LC are the most frequently used approach. Different systems and configurations are available [24]. As an example, Figure 2 shows the most common on-line SPE configuration which normally involves the implementation of a small SPE column within the injection loop of a six-port rotary valve. The SPE column should usually be as small as possible (i.e., 30 x 2 mm i.d. or 8 x 3 mm i.d.) in order to prevent band broadening leading to poor resolution and chromatographic performance. Furthermore, SPE column sorbent must be obviously compatible with the one of the analytical column. As an example, Viglino et al. [35] used an automated on-line SPE method combined with LC-electrospray-tandem mass spectrometry (LC-ESI-MS/MS) with positive electrospray ionization for the simultaneous analysis of pharmaceuticals, pesticides and some metabolites in drinking, surface and wastewater samples. The total analysis time, including the period required to flush the C18 SPE cartridge with organic solvent and reconditioning the LC column, was only 20 min, thus overcoming some of the limitations associated with the classical off-line SPE (i.e., tedious and time consuming sample procedure) and making possible the development of a faster method together with high preconcentration factors and recoveries. On the other hand, Díaz-Plaza et al. [36] reported an on-line coupling reversed-phase liquid chromatography–gas chromatography method for organophosphorus, organochlorine and triazine pesticides using the through oven transfer adsorption desorption (TOTAD) interface with subsequent simultaneous electron-capture and nitrogen–phosphorus detection by post-column splitter. The use of fully automated on-line RP LC/GC has also been reported for the determination of four organophosphorus pesticides (dimethoate, methidathion, chlorpyriphos and fenitrothion) in olive oil [37,38]. However, the majority of reports on the application of on-line SPE describe pesticides monitoring in aqueous environmental samples with only a few that have been applied to food analysis. Examples of the latter
include analysis of mepiquat and chlormequat in pears, tomatoes, and wheat flour [39], and N-methylcarbamates (oxamyl, dioxacarb, metolcarb, carbofuran, carbaryl and isoprocarb) and their metabolites in apple, pear and cucumber samples [40].

If on-line SPE mode has several advantages over traditional off-line SPE, it also has limitations and drawbacks. For instance, certain drawbacks of the on-line approach are the possibility of memory effects and progressive deterioration of the column sorbent when the SPE column is re-used. The latter may lead to important changes in the SPE selectivity and capacity. Furthermore, on-line SPE approach also possesses less flexibility regarding the choice of eluting solvents since usually it must be the same as the mobile phase used in the analytical column. Finally, in most cases, even though the use of an automated on-line instrument is quite straightforward, experienced personnel are required for method development and eventual trouble-shooting [41].

3. Sorbents used for on-line SPE

Different types of sorbents are currently available to carry out SPE of pesticides. To achieve optimal SPE extraction performances, one of the key factors is therefore the choice of the proper type of sorbent since its nature strongly controls parameters of primary importance such as selectivity, affinity and capacity [42]. This choice obviously depends on the physical-chemical properties of the target pesticides as well as on the nature of the sample matrix. In fact, the SPE extraction performances are determined by the interaction of pesticides with the chosen sorbent as well as of the interaction of sample matrix with both sorbent and pesticides [24]. Several sorbents have so far been employed for SPE extraction of pesticides, including alumina, silica gel, Florisil, ion-exchange resins, octadecyl-, octyl-, phenyl-silica based sorbents and graphitized black carbon (GBC) [43]. Schenck et al. [44] have recently evaluated the clean-up and extraction efficiencies of pesticide residues in fresh fruits and vegetables using GBC, octadecysilyl, strong anion exchange, aminopropyl, and primary secondary amine (PSA) SPE columns. As a result, aminopropyl and PSA sorbents were found to provide the most effective clean-up, removing the greatest number of sample matrix interferences whereas GBC removed most of the visible plant pigment in the extracts but also showed a lower ability to remove fatty acid matrix interferences. Furthermore, the authors revealed that using an acetone extraction followed by a PSA clean-up, both polar and non-polar pesticides present in samples at 1.0 ng/g could be recovered. However, in general, polar-SPE (silica, alumina, Florisil) is more suitable for the clean-
up procedure of most apolar pesticides (i.e., organochlorine and some organophosphorus compounds) from organic extracts. On the other hand, octadecyl-bonded silica (C18) allows retention of a wide variety of analytes with different polarities. In the last few years, other novel material technology, such as monoliths [45], molecularly imprinted polymers (MIP) [45,46], immunoaffinity columns (IAC), and hydrophilic polymeric sorbents have also been introduced as SPE sorbents. For instance, MIPs are synthetic and intelligent materials with an artificially generated three-dimensional network that is able to specifically rebind a target molecule [45-49]. MIP has the advantages to be cost-effective, and chemically and thermally stable without storage limitations and stability problems regarding organic solvents [50,51]. Recently, magnetic or magnetically modified adsorbents materials [52] as SPE sorbents have been shown to allow high degree of clean up and enrichment factors in the analysis of pesticide residues in environmental samples. The most widely used magnetic nanoparticle (NP) for residue analysis is probably C18 fabricated Fe3O4 core-shell NPs that have proved to be suitable for the preconcentration or cleaning up of both non polar and moderately polar pesticides [53]. Graphene-based SPE has also been proposed for the determination of organophosphorus pesticide residues in apple juices, showing average recoveries of the analytes at two spiked levels (5 and 20 ng/mL) for real-sample analysis of 69.8-106.2% [30]. However, only few of these SPE sorbents have been applied in the on-line mode. The fact that many solid phases, such as GBC, are not pressure resistant is one possible reason. Hypercrosslinked sorbents, which can be effectively packed in precolumns, allows to overcome this problem thus offering an alternative for the on-line enrichment of polar pollutants from aqueous samples [54]. Among all the possible sorbents for the SPE extraction, on-line SPE using hydrophilic lipophilic balanced (HLB) sorbent is probably one of the most popular analytical choices. For instance, the direct coupling of an on-line Oasis HLB SPE cartridge to LC–MS/MS has been developed by Stoob et al. [55] for the analysis of neutral (triazines, phenylureas, amides, chloracetanilides) and acidic (phenoxyacetic acids and triketones) pesticides in natural water. Absolute extraction recoveries from 85 to 112% were obtained for the different analytes. Detection limits for environmental samples between 0.5 and 5 ng/L were obtained, and matrix induced ion suppression smaller than 25% was also observed. Recently Togola et al. [56] highlighted the advances of on-line SPE coupled with ultra-high performance liquid chromatography (UHPLC)-MS/MS for determining the fate of 18 pesticides and their degradates in aquatic organisms exposed
in mesocosms. Limits of quantification from 15 to 25 ng/L for pesticides and metabolites have been obtained, with linearity range up to 1 μg/L. On the other hand, on-line Hypersil GOLD C18 column has also been showed to achieve detection limits in the range of 2 to 24 ng/L for the compounds of interest by using only 1 mL of filtered water sample. Good recoveries from 87 to 110% in surface as well as wastewater samples were obtained whereas matrix effects observed for some compounds was lower than 25% [35].

Finally, it is apparent that the development of new selective materials for a given application as well as of universal sorbent suitable for every purpose will continue to be a major part of the scientific research and technological innovation in the field of SPE, with special attention to the development of high pressure resistant sorbents able to be coupled on-line with fast or even ultra-fast analytical methods (i.e., UHPLC).

4. Applications of on-line SPE to pesticide residue analysis

On-line SPE coupled to LC-MS(ESI)-MS methods are becoming very popular for the preconcentration and analysis of pesticide residues especially in water samples, and many examples of their application varying in number of pesticides and the type of SPE sorbent used can be found in the literature. In Table 1, a selection of some of these examples published in the last years is presented [35,57-63]. Columns and SPE cartridges based on C18 can be found among the most commonly used sorbents for the on-line SPE preconcentration of pesticides in water samples [35,63-65]. For instance, Hernández et al. [65] reported the use of an C18 SPE cartridge (10x2 mm) for the on-line SPE-LC-MS/MS analysis of 29 pesticides (1 fungicide, 16 insecticides, 10 herbicides and 2 acaricides) and 6 metabolites in environmental water samples by the direct injection of only 1.3 mL of filtered water sample, with a total analysis time of 18 min. Figure 3 shows, as an example, the chromatograms of two positive water samples. Recently, Sun et al. [63] proposed the use of a C18 SPE column (7.5x4.6 mm, 10 μm particle size) for the on-line SPE LC-MS/MS analysis of 9 emerging pesticides in lake water and seawater samples at trace level. To enhance method sensitivity, the authors proposed the use of a large enrichment water sample volume (50 mL), allowing the detection of these compounds at 1-10 ng/L.

Polymeric sorbents are also commonly employed for the on-line SPE preconcentration of pesticides, such as PLPR-s (macroporous spherical particles of polystyrene and divinylbenzene), which is usually employed for the analysis of medium
to highly polar pesticides [58,60-62,66-69], or Strata-X SPE (polymeric sorbent with N-vinylpyrrolidine functional groups) [59]. For example, Köck-Schulmeyer at al. have been using PLPR-s sorbents for the on-line SPE LC-MS/MS monitoring pesticides in wastewater treatment plants, river waters and groundwaters from Catalonia (Spain) [61,62,69]. With the proposed method, and using a quadrupole-linear ion trap MS instrument, limits of detection down to 5 ng/L (groundwater samples) with good accuracies and precisions were achieved.

An interesting application is the one recently reported by Huntscha et al. [57] using a home-made single mixed-bed multilayer cartridge with 4 extraction materials for the on-line SPE LC-MS/MS multi-residue analysis of 88 polar organic micropollutants with a broad range of chemical properties (pesticides, biocides, pharmaceuticals, corrosion inhibitors, and many of their transformation products) in ground, surface and wastewater samples. The SPE cartridge was prepared in-house by filling an empty stainless steel cartridge with 10 mg Oasis HLB (hydrophilic-lipophilic balance, Waters) as the first material in the enrichment flow direction. As second material, 10 mg of a mixture of Strata X-AW, Strata X-CW (both from Phenomenex) and Isolute ENV+ (Biotage) in a ratio of 1:1:1.5 (X-AW:X-CW:ENV+) was used. Although hydrophobicity is, in general, the driving force for the enrichment of compounds on typical reversed-phase materials such as alkyl-modified silica or poly(styrene-divinylbenzene) polymers, the introduction of new polymeric sorbents with novel functional groups in the polymeric structure extended the applicability of SPE to more hydrophilic compounds. For instance, the use of Oasis HLB proposed by Huntscha et al. [57], which provides lipophilic (divinylbenzene-rings) and hydrophilic (N-vinyl-pyrrolidine) groups, allows to achieve retention of both non-polar and polar compounds. For most of the compounds evaluated, limits of quantification were below 10 ng/L in groundwater (n=71) and surface water (n=70) and below 100 ng/L in wastewater (n=70).

As previously commented, novel material technology such as molecularly imprinted polymers are beginning to be used in on-line SPE procedures for sample preparation [51], and some examples describing its application to the analysis of pesticides can be found in the literature. For instance, Koeber et al. [70] proposed a novel and highly selective on-line sample clean-up procedure based on the use of MIPs as SPE material for the analysis of triazines in river water samples. The method comprises the combination of a restricted access material (RAM) and a MIP allowing a
selective sample preparation to be achieved in an on-line mode. The RAM column combines size exclusion and adsorption chromatography, reducing the concentration of matrix molecules present in the river water by a cutoff of 15 kDa, while the MIP column selectively retains the triazine analytes whereas the residual matrix is not retained and separated completely, removing then all matrix and non-target compounds. As an example, Figure 4a shows the chromatograms obtained for a solution of a humic acid (150 mg/L) after being analyzed by LC-UV without sample clean-up (1), after an on-line RAM SPE clean-up procedure (2), and after the proposed on-line RAM-MIP SPE clean-up procedure (3), showing the strong capacity to reduce matrix components of this method. In Figure 4b the selectivity of the multidimensional on-line RAM-MIP SPE clean-up platform is demonstrated, showing that only molecules with a triazine structure are recognized and enriched. Total analysis time, including on-line sample clean-up, was lower than 15 min, achieving limits of detection in the range 10-50 ng/L (1.5 mL of sample analyzed) with acceptable recoveries (51-102%). In addition, the performance of the MIP material did not change even after more than 300 enrichment and desorption cycles.

A new on-line SPE sample treatment system, turbulent flow chromatography (TFC), which combines high-throughput and high reproducibility by means of separating analytes from various matrices with reduced sample handling [21], has also been proposed for the analysis of pesticides [71-74]. Within these systems, the sample can be injected directly onto a narrow diameter column (0.5 or 1.0 mm) packed with large particles (30-60 µm) at a high flow rate (higher than 1 mL/min) helping creating a very high linear velocity inside the turbulent flow column. Under turbulent flow conditions the improved mass transfer across the bulk mobile phase allows for all molecules to improve their radial distribution, however, under these conditions a laminar zone around the stationary phase particles still exists, where diffusional forces still dominate the mass transfer process. Molecules with low molecular weight diffuse faster than molecules with a high molecular weight, forcing large molecules to quickly flow to waste while retaining the small analytes [21]. Asperger et al. [72] proposed the use of an on-line SPE TFC-LC-MS/MS system for the rapid analysis of eleven trace level priority pesticides in surface and drinking water. The use of TFC columns (50x1 mm, 30-50 µm particle size) enables fast on-line SPE at high sampling flow-rates (5 mL/min). Both polymeric (Oasis HLB) and carbon based (Hypercarb) TFC columns allow complete extraction of pesticides with good recoveries from water volumes up to
50 mL. Figure 5 shows the chromatogram obtained for the analysis of 10 mL sample of river water by on-line TFC SPE-LC-APCI-MS/MS. Afterwards, the same group proposed the combination of two different TFC columns (polymer based column followed by a carbon based column) for the rapid extraction of pesticides from small water sample volumes (10 mL) at high flow rates (5 mL/min) [71]. Analytes are eluted with purely organic eluent from the TFC column, and the effluent is re-mixed with water previous to LC column to provide efficient analyte focusing. A short monolithic LC column is then used for the chromatographic separation to achieve a fast on-line TFC-LC-APCI-MS/MS analysis. This approach allowed quantitative results for nearly 30 pesticide species (triazines, phenyl ureas and organophosphorous pesticides among others) in less than 14 minutes, with a method well reproducible, robust and extremely sensitive achieving LODs between 0.1 and 1 ng/L. Recently, Quinete et al. [74] also described the application of an on-line TFC SPE-UHPLC-APCI-MS/MS method for the analysis of chlorinated pesticides, such as endosulfans, at part per trillion levels, by injecting 20 mL water samples into a Turboflow HTLC C18 XL column.

On-line SPE-LC-MS/(MS) approaches have also been described for the clean-up and preconcentration of pesticides from food products after an extraction step from the solid sample matrix and reconstitution in an adequate solvent [39,73,75,76]. For instance, chlormequat and mepiquat residues have been analyzed in pear, tomato and wheat flour samples by direct injection of the food extract onto an on-line SPE (Prospekt) coupled with LC-ESI-MS/MS using a strong cation-exchange resin as SPE sorbent [39]. Surrogate standards (d9-chlormequat, d6-mepiquat) were employed to compensate for recovery losses and potential MS/MS signal suppression. A limit of quantification for both cationic analytes at, or below, 5 μg/kg was obtained, with good intra- and inter-assay precisions with mean variability values lower than 7%. In another work, Vichapong et al. [76] analyzed carbamate residues in food and environmental samples by on-line SPE-LC in a reversed-phase C18 bead (25-40 μm). Fruit and vegetable samples were extracted with acetonitrile, and after solvent removal in a rotary evaporator, the residue was dissolved with water and analyzed by the proposed method.

Turbulent flow chromatography coupled to LC-MS/MS has also been recently proposed for the analysis of pesticide residues in grapes, baby food and wheat flour matrices [73]. Sample extracts were injected into the on-line TFC SPE-LC-MS/MS system consisting of a polymeric based cyclone MCX-2 (50x0.5 mm) TurboFlow column for sample preparation and a C18 Hypersil Gold (150x4.6 mm, 5μm) column.
for analytical separation. Limits of detection between 0.8 and 6.0 ng/g for baby food and 0.8-10.3 ng/g for the other matrices, with acceptable precisions (RSDs lower than 22%) and good sample recoveries (67-124%), were obtained within a total analysis time of 13 min.

5. Concluding remarks and future perspectives

The use of on-line SPE preconcentration approaches are becoming very popular for the analysis of pesticides in complex matrices, especially when dealing with environmental water samples, because of their many benefits and advantages over other sample treatment procedures such as high sample throughput, reduced sample manipulation, improved precision and low reagent consumption. It is also very advantageous when a limited amount of sample is available or high sensitivity is required. However, when developing on-line SPE LC-MS/MS methodologies for the analysis of pesticides some limitations and drawbacks must also be considered. The most important one relies in the re-utilization of the on-line SPE sorbents, so it is important to evaluate the possibility of memory effects and, especially, the deterioration of the SPE sorbent because of matrix components, in particular when complex matrices are analyzed. Despite all this, many on-line SPE sorbents commercially available today are very stable and, together with adequate washing programs in the SPE procedure, many SPE cycles can be carried-out without observing any problem. Another consideration to take into account when developing on-line SPE preconcentration is that elution solvents must be compatible with the LC-MS/MS method, being in general the same mobile phase used for the chromatographic separation.

Several SPE sorbents have been evaluated for the on-line SPE LC-MS/MS analysis of pesticides. However the increased necessity in the multi-residue analysis of pesticides with a wide range of physicochemical properties is making necessary the simultaneous combination of sorbents with different stationary phases in order to achieve simultaneously good recoveries for different pesticide families, being this one of the main topics that may be exploited in the future.

Novel on-line SPE strategies such as the use of restricted access materials and molecularly imprinted polymer sorbents, as well as turbulent flow chromatography approaches are beginning to become popular when dealing with some particularly difficult applications in the analysis of pesticides. MIPs are cost-effective, stable regarding presence of organic solvents and very selective to the target pesticides to be
analyzed, which would make them appropriate for some specific applications, in particular for those families of compounds not correctly preconcentrated when dealing with multi-residue analysis. As regards TFC, this novel strategy has a powerful capacity to remove matrix interferences while keeping the advantages of on-line approaches such as reproducibility, robustness and sensitivity. All this novel on-line SPE strategies will be sure exploited in the near future for the analysis of pesticides.

Most of the on-line SPE LC-MS/MS applications dealing with the analysis of pesticides are focused in environmental water analysis because of the simplicity of sample manipulation. However, several interesting applications of these on-line approaches to the analysis of pesticides in food samples are also reported in the literature.

Finally, the new advances in manufacturing SPE sorbents more pressure resistant will facilitate the coupling of on-line SPE approaches to UHPLC-MS/MS methodologies, making more attractive the application of these methodologies for the analysis of pesticides in both environmental waters and food samples.

**Conflict of interest statement**

The authors have declared no conflict of interests.
6. References


Figure captions

Figure 1. Scheme of a general SPE process.

Figure 2. Scheme of a common on-line SPE configuration with a small SPE column within the injection loop of a six-port rotary valve.

Figure 3. On-line SPE-LC-ESI-MS/MS chromatograms from positive water samples. (a) ground water containing 9 ng/L of pyrimicarb (273>72), 650 ng/L of bromacil (259>203), 95 ng/L of terbutryne (242>186), 13 ng/L of diuron (233>72) and 10 ng/L of terbuthylazine (230>174), and (b) surface water containing 520 ng/L of carbendazim (192>160), 150 ng/L of dimethoate (230>199), 27 ng/L of terbumeton (226>170), 170 ng/L of terbacil (215>159) and 25 ng/L of methidathion (303>145). Reprinted with permission from ref. [65]. Copyright 2001 Elsevier.

Figure 4. (a) Reduction of interfering matrix components. A solution of a humic acid (150 mg/L) injected into different LC system configurations and the extract analyzed using UV/visible detection (λ=220 nm): (1) LC column only, (2) on-line RAM-LC coupling, and (3) on-line RAM-MIP-LC coupling. (b) Selectivity of the multidimensional on-line RAM-MIP SPE clean-up platform. The upper chromatogram was obtained by injecting a standard mixture of different pesticides (LC-MS system). The lower chromatogram was obtained after enrichment by RAM-MIP. Reprinted with permission from ref. [70]. Copyright 2001 American Chemical Society.

Figure 5. Total ion chromatogram and extracted MRM traces of eight pesticides obtained from on-line TFC SPE-LC-APCI-MS/MS analysis of a 10 mL river water sample (River Parthe, Leipzig, Germany). Reprinted with permission from ref. [72]. Copyright 2002 Elsevier.
Table 1. Selection of on-line SPE methods for the analysis of pesticide residues in water samples by LC-MS

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Sample</th>
<th>On-line SPE sorbent</th>
<th>Sample volume</th>
<th>Recoveries</th>
<th>LC conditions</th>
<th>MS conditions</th>
<th>LOQs</th>
<th>Ref.</th>
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<td>88 polar organic micropollutants including pesticides</td>
<td>Ground, surface and wastewater</td>
<td>Home-made single mixed-bed multilayer cartridge with 4 extraction materials: 10 mg Oasis HLB, 10 mg of Strata X-AW, Strata X-CW and Isolute ENV+ in a ratio 1/1/1.5</td>
<td>50 mL (adjusted to pH 7)</td>
<td>80-120%</td>
<td>Atlantis T3 column (150 x 3.0 mm i.D., 3µm particle size) Gradient elution A) 5 mM ammonium acetate in water B) methanol with 0.1% formic acid Flow-rate: 300 µL/min</td>
<td>Triple quadrupole instrument ESI in polarity switching mode SRM acquisition mode</td>
<td>0.1-200 ng/L</td>
<td>[57]</td>
</tr>
<tr>
<td>37 pesticides</td>
<td>Surface water</td>
<td>Macroporous spherical particles of polystyrene and divinylbenzene (PLRP-s) with dimensions 10 x 2 mm</td>
<td>1500 µL (two injections of 750 µL)</td>
<td>60-129%</td>
<td>Poroshell 120 EC-C18 column (50 x 4.6 mm i.D., 2.7 µm particle size) Gradient elution A) 5 mM ammonium acetate and 0.01% formic acid in water B) Acetonitrile Flow-rate: 350 µL/min</td>
<td>Triple quadrupole instrument ESI (positive ionization) SRM acquisition mode</td>
<td>0.3-33 ng/L</td>
<td>[58]</td>
</tr>
<tr>
<td>20 biocides and pesticides</td>
<td>Surface and wastewater</td>
<td>Strata-X extraction cartridge (polymeric sorbent with N-vinylpyrrolidone functional groups), 20 x 2.1 mm, 33 µm particle size</td>
<td>20 mL</td>
<td>65-90%</td>
<td>Xbridge-C18 column (50 x 2 mm i.D.) Gradient elution A) water with 0.1% formic acid B) methanol with 0.1% formic acid Flow-rate: 300 µL/min</td>
<td>Triple quadrupole instrument ESI (positive and negative ionization mode) SRM acquisition mode</td>
<td>3-100 ng/L</td>
<td>[59]</td>
</tr>
<tr>
<td>22 medium to highly polar pesticides</td>
<td>Groundwater Wastewater</td>
<td>PLRP-s cartridge (10 x 2 mm), and HySphere Resin GP (polydivinilbenzene, 10-12 µm particle size).</td>
<td>5 mL</td>
<td>75-178%</td>
<td>Purospher Star RP-18 end-capped column (125 x 2.0 mm i.D., 5 µm particle size) Gradient elution A) water B) acetonitrile Flow-rate: 300 µL/min</td>
<td>Quadrupole-linear ion trap instrument ESI (positive and negative ionization mode) SRM acquisition mode</td>
<td>0.02-3.91 ng/L a</td>
<td>[60,61] Groundwater [62] Wastewater</td>
</tr>
<tr>
<td>9 pesticides</td>
<td>Sea and lake water</td>
<td>C18 SPE column (7.5 x 4.6 mm i.D., 10 µm particle size)</td>
<td>50 mL</td>
<td>47-92%</td>
<td>Shimadzu VP-ODS column (250 x 4.6 mm i.D., 5µm particle size) Gradient elution A) water with 0.1% formic acid and 5 mM ammonium formate B) acetonitrile with 0.1% formic acid Flow-rate: 1 mL/min</td>
<td>Ion-trap mass analyzer instrument ESI (positive ionization mode) SRM acquisition mode (only 1 transition)</td>
<td>2-10 µg/L a</td>
<td>[63]</td>
</tr>
<tr>
<td>Pesticides and pharmaceuticals</td>
<td>Drinking, surface and wastewater</td>
<td>C18 Hypersil GOLD column (20 x 2.1 mm i.D., 12 µm particle size)</td>
<td>1.5 mL</td>
<td>87-110%</td>
<td>C18 Hypersil Gold column (50 x 2.1 mm i.D., 3 µm particle size) Gradient elution A) water with 0.1% formic acid B) methanol Flow-rate: 200 µL/min</td>
<td>Triple quadrupole instrument ESI (positive ionization mode) SRM acquisition mode</td>
<td>2-24 ng/L</td>
<td>[35]</td>
</tr>
</tbody>
</table>

a LODs
Figure 1
Figure 2
Figure 3
Figure 5

TIC (11 MRM)

Simazine (c = 11.6ng/l)

Atrazine (c = 5.1ng/l)

Isoproturon (c = 3.9ng/l)

Diuron (c = 8.8ng/l)

Terbutylazine (c=0.9ng/l)

Chlortoluron (c=0.8ng/l)

Prometryne (c=3.6ng/l)

Chlorfenvinphos (c=1.8ng/l)