Sample Preparation For Pesticide Analysis in a Forensic Toxicology Laboratory: A Review

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Abstract

Pesticides used to protect agricultural crops are often involved in human poisoning throughout the world. Therefore, forensic toxicologists must face the challenge of detecting, identifying and quantifying pesticides in human specimens. Forensic toxicology methods are difficult to standardize, as every case is unique, matrices analyzed are highly complex the nature of the target analyte is unknown. Due to the extremely complex nature of matrices normally analyzed in forensic toxicology laboratories, it is not recommended to perform instrumental analysis directly. Therefore, a sample preparation stage is necessary to make sample suitable for instrumental analysis. The present paper focusses its attention in reviewing sample preparation methodology normally employed in forensic laboratories for pesticides analysis. Sample preparation is considered as the bottleneck of the analytical procedure as it represents approximately the 80% of the whole process. Furthermore, an accurate sample preparation plays a central role in forensic case's resolution as any error occurred during sample preparation cannot be corrected even by the best instrumental technique. Databases searches were performed in order to know how many papers were published from 2008 related to pesticide extraction from human samples in a forensic toxicology setting. From the reviewed literature, it can be stated that the most used method for extraction of pesticides from human samples of forensic interest still are liquid-liquid extraction, solid-phase extraction and solid-phase microextraction. Moreover, it can be drawn as a conclusion that these conventional extraction techniques are now evolving thanks to the availability of some new materials that can be employed as solvents or sorbents. These new techniques have found a wide application in pesticides extraction from environmental or food matrices. However, with the exception of QuECHERS their implementation in forensic laboratories are nowadays limited. When available, examples of their application in forensic laboratories will be presented in this paper.

Keywords

Forensic Toxicology; Human Poisoning; Pesticide; Sample Preparation

Introduction

Pesticides are widely used throughout the world to protect agricultural crops from the actions of insects, rodents, fungi and unwanted plants. According to FAO (Food and Agriculture Organization of the United Nations) over 650,000 tons of active ingredients of pesticides were used worldwide in 2015 [1]. Despite their beneficial effects on agriculture, pesticides are often involved in human poisoning. Humans can be exposed to pesticides potential adverse effects due to their absorption through the food chain [2]. Furthermore, significant health problems can be related to their use or misuse leading to accidental or intentional exposure [3,4]. Morbidity and mortality attributable to these chemicals vary from country to country. Fatalities range from less of 1% of the death poisoning in EU countries up to 70% of all violent deaths in Western Pacific and Southeast Asia [5]. Self-poisoning with pesticides is a major contributor to the global burden of suicide [6]. Mew et al. estimated that pesticide poisoning accounted for one-third of the world's suicides, approximately 260000 deaths per year [7]. In all cases of suspected poisoning caused by pesticide, forensic laboratories must face the challenge of detecting, identifying and quantifying those chemicals in human specimens. Human specimens are highly complex matrices where pesticides are normally at low concentrations. Moreover, quite frequently specimens received at forensic laboratories are in advanced stage of decomposition. The main objective of any forensic toxicologist is the separation of target analytes and reduction or elimination of possible interferences. Those are the most critical steps in the entire analytical process [4]. Also, forensic toxicologists must pursue techniques, protocols and devices to improve the sensitivity and the selectivity of the analytical methods, without compromising the reliabil-
ity of the results, the overall speed of the procedures and their cost [8]. Generally speaking, pesticide analysis protocols involve two main stages: sample preparation and the analytical method for their determination. Sample preparation, which involves both the isolation of the pesticides from the matrix components and the purification of the extract obtained, contributes highly to the total cost and compromises method’s reliability and accuracy. Moreover, sample preparation methodology conditions the qualitative and quantitative determination of target analytes, as any error that may occur during sample preparation cannot be corrected even by the best separation or detection method. The selection of sample preparation methodology is highly dependent on both analytes and sample nature [2]. Usually sample preparation includes also processes such as dilution, precipitation, filtration, and centrifugation. It has been estimated that all the processes comprised in sample preparation typically take 80% of the total analysis time [9]. Therefore, sample preparation is considered as the bottleneck of the entire procedure [3].

The development of methodologies, which cover all the analytes under study, is quite difficult as there are many different classes of pesticides with a wide range of physicochemical properties. It should be noted that the detailed description of pesticide physicochemical properties are beyond the scope of the present paper. As a consequence of the broad spectrum of pesticides properties and the lack of information of the substance involved in the poisoning, the implementation of multi-residue methods is nowadays the main applied strategy in forensic laboratories because it allows the proper control of a large number of pesticides in a unique analysis. A multi-residue method should be simple, cost-effective, and easy to perform, require the minimum amount of solvents and sample, and be able to cover a wide range of analytes [10]. Traditionally, two main types of extraction procedures have been used in forensic toxicology for pesticide analysis: liquid-liquid extraction (LLE) and solid phase extraction (SPE). Among the methodologies of extraction conventionally used in forensic toxicology, solid phase microextraction (SPME) must also be mentioned. In recent years, new forms of extraction have been developed and introduced in the field of pesticides analysis. Many of them comply with the requirements of green analytical chemistry [3,11,12]. Generally speaking, any of them gives the possibility of separating the analyte from the matrix, eliminating or reducing interferences from other components, and the enrichment of the analyte to a level allowing appropriate determination by the instrumental technique of choice [9].

In view of the above considerations, the main objective of this paper is to present the state of the art in sample preparation for pesticide analysis, through the discussion of the most relevant papers published in the literature in the last 10 years (from 2008 to the present moment). Scopus and Web of Science databases were searched using the following key words: “pesticide” and “sample preparation, obtaining a total of 1155 and 1694 documents, respectively. Search results were filtered in order to pay special attention to those documents related to extraction methods employed in forensic toxicology laboratories in cases of alleged human poisoning with pesticides. The filtering was made using key words such as “blood”, “urine”, “human poisoning”, “postmortem” and “forensic toxicology”. (Figure 1) shows the results obtained in each search. After comparing search results and eliminating repeated ones, a total of 85 documents were selected for revision. Furthermore, some innovative extraction techniques will be presented and when it is possible examples of their application in forensic laboratories will be discussed. It must be noted that traditionally, the implementation of innovations in pesticides extraction has been first developed for food matrices or environmental samples. Therefore, when no examples are available in the field of forensic toxicology, some examples of their applications in other fields will be presented. In order to facilitate the reading of the present paper, the information related to the extractive techniques is given in the different sections of the present paper whereas examples of their application to pesticides extraction from human samples will be displayed in tables. Different extraction procedures are shown in tables 2,4,5,6 and 8 along with the reference to the paper in which they were published. Therefore, details of each method can be obtained from the corresponding reference.

**Liquid-Liquid Extraction**

From all the sample preparation methods, LLE may be the oldest and the most common extraction method. The sample is treated with sequential volumes of a selected organic solvent or a variety of solvents that increase in polarity and as a result, the targeted analyte or group of analytes is obtained in different extract fractions [3]. In the LLE, the extraction efficiency of analytes depends mainly on the equilibrium distribution/partition coefficient between the donor phase and the acceptor phase, which requires matching the polarities of the extraction solvents and analytes according to the similarity principle. (Table 1) summarizes the properties of different organic solvents and their eventual application to pesticide extraction [3,13]. LLE is a classic method for the routine sample preparation due to its simplicity, robustness and efficiency, which has been applied to the extraction of organochlorine, organophosphorus and carbamate compounds in alleged cases of pesticide poisoning [14-16]. LLE exhibits some main disadvantages, as it is a laborious procedure that requires large volumes of organic solvents. These solvents are usually toxic, thus being harmful for both the analytical chemists and the environment. Furthermore, there is a high possibility for analyte loss, sample contamination and low sensitivity. However, LLE is still used in pesticide determination in forensic toxicology laboratories. (Table 2) shows several examples of LLE methods applied in cases of suspected lethal poisoning with pesticides, published in the last 10 years.

**Solid Phase Extraction**

SPE is nowadays the most widely used sample preparation method for the pesticide residue analysis, was first introduced in the mid-1970’s [3]. SPE usually proceeds by the selective retention of analytes from an aqueous phase on a sorbent packed in a disposable column. During the SPE, the extracts are passed through the column, which have been previously conditioned and acti-
vated with water and/or organic solvent. Then the interferences are removed by washing by organic solvents while the analytes are still retained on the adsorbents. After this step, the analytes can be subsequently eluted with other organic solvents to obtain clean extracts and then are determined by appropriate analytical technique [26]. Therefore, when developing an analytical method is of the utmost importance the selection of the appropriate sorbent, solvents and pH conditions. A large number of sorbents have been developed and commercialized in order to be able to extract pesticides with a wide range of physicochemical properties. They can be classified as according to their mechanism of extraction [27-28]. (Table 3) summarizes the different kind of sorbents available. In addition to the sorbent, the appropriate elution solvent or a mixture of elution solvents play an important role in increasing the clean-up efficiency, since the solvents can disrupt the interaction between the target analytes and the adsorbent by eluting the analytes from the adsorbents. As pH determines the stability of the analytes, the pH of extracts is crucial to ensure the high retention of pesticides on the adsorbent. Therefore, an appropriate pH is necessary to maintain the stability of pesticides and to increase the absorption of analytes on the solid phase. SPE experimental procedure is simpler, less solvent consuming and easier to automate than LLE. Furthermore, SPE can avoid the formation of emulsion that is typical in LLE and provides a fast sample throughput. Therefore, it has replaced LLE for sample preparation in most forensic laboratories. (Table 4) shows several examples of SPE methods applied in cases of suspected poisoning with pesticides. Molecularly Imprinted Polymers (MIPs) are stable polymers with molecular recognition abilities, provided by the presence of a template during their synthesis and thus are excellent materials to provide selectivity to sample preparation [45]. Although several sorbents that employ MIPs has been developed for phenylureas, sulfonylureas, triazines or organophosphorus pesticides until now no evidence of their application in forensic toxicology has been found [46-49]. In recent years SPE underwent a number of modifications, which has resulted in the development of new extraction techniques such as Microextraction by Packed Sorbent (MEPs), Matrix Solid Phase Dispersion (MSPD), Stir-Bar Sorptive Extraction (SBSE), SPME and Dispersive Solid-Phase Extraction (d-SPE). The main features of these new techniques will be discussed later in this paper.
| Pesticide                  | Matrix                  | Solvent                                                                 | Instrumental technique                  | Reference |
|---------------------------|-------------------------|-------------------------------------------------------------------------|-----------------------------------------|-----------|
| Dimethoate                | Blood                   | Toxitubes TM                                                            | GC-MS                                   | [17]      |
|                           | Urine                   |                                                                         |                                         |           |
|                           | Liver                   |                                                                         |                                         |           |
|                           | Brain, Kidney           |                                                                         |                                         |           |
| Tolfenpyrad + metabolites | Plasma                  | Ethyl acetate at pH 9.2, Ethyl acetate:ter-butyl methyl ether            | GC-MS, LC-Q-TOF-MS                      | [18-19]   |
| Methidation               | Blood, Urine            | Extralut (LLE)                                                          | GC-MS                                   | [20]      |
|                           | Urine                   | Ethylacetate pH 9                                                      |                                         |           |
|                           | Stomach contents        |                                                                         |                                         |           |
| 8 Organophosphorus        | Blood                   | Toluene: Chloroform (4:1 v/v)                                           | GC-MS                                   |           |
| 3 Carbamates              | Blood, Bile             | n-hexane+ acetonitrile                                                 | GC-MS                                   | [21]      |
| Carbofuran                | Blood, Urine            | Derivatization with p-toluene sulphonyl chloride, Ethyl acetate at acidic pH | LC-MS/MS                                |           |
| Pesticide (multiresidue)  | Blood                   | Dichlorometane/ethyl acetate/acetone (50/30/20)                         | GC-MS/MS, LC-ESI-MS/MS                 | [5]       |
| Pyrethroid insecticides:  | Blood, Urine            | n-hexane:acetone (8:2 v/v)                                             | GC-MS                                   | [24]      |
| alfa-cypermethrin, deltamethrin |                   |                                                                         |                                         |           |
| Glyphosate, Gluphosinate  | Blood                   | Modified Quick Polar Pesticide Extraction Method (Methanol) + Oasis PRiME HLB | LC-MS/MS                                | [25]      |
| Bialaphos                 |                         |                                                                         |                                         |           |

Table 2: LLE methods applied to suspected cases of lethal poisoning with pesticides.

**Microextraction**

Traditional extraction methods employ large volumes of solvents. In the last decade, these methods have been oriented toward the development of efficient, economical, and miniaturized sample preparation methods searching for techniques that use lower volumes of less toxic solvents and generate fewer residues. The current trend of miniaturization of sample preparation and minimization of the use of solvents has led to the development of new sample preparation techniques, known as microextraction methods [9]. There are two main types of microextraction methods: Liquid-Phase Microextraction (LPME) and SPME [50]. These methods are environmental friendly procedures, which reduce the negative impact on the environment and on the health of analytical chemists. Besides, the reduction of the amount of organic solvents employed during the extraction process translates into reduced costs in waste treatment and solvents used [51].

**Solid Phase Microextraction**

Fiber SPME: In 1989, fiber SPME was first introduced as a SPE development by Pawliszyn [52]. Fiber SPME is a sample preparation technique that utilizes fibers made of fused silica covered with appropriate solid phase. Similar to the SPE, fiber SPME is based on the partition equilibrium of analytes between the sample and the stationary phase. It is a simple two-stage technique, which includes the adsorption of the analytes on the solid phase and then desorption prior to their introduction into analytical instrumentation. Desorption is thermal when Gas chromatography (GC) is the chromatographic technique employed. If liquid chromatography is the analytical technique used for separation, desorption occurs by Liquid Chromatography (LC) mobile phase solvent elution [53]. There are two main kinds of SPME modes, the first of which is the Direct-Immersion Solid Phase Microextraction (DI-SPME). Another SPME mode is called Headspace Solid Phase Microextraction (HS-SPME). Different from the DI-SPME, the SPME fiber is put in the air above the liquid or solid sample and is used to extract volatile and semi volatile analytes. The type and the property of the coating have an effect on the efficiency of extraction and the final sensitivity of the analysis. The most commonly used sorbent is Polydimethylsiloxane (PDMS) which is an immobilized liquid at the extraction temperature, thermostable to around 300°C, chemically neutral, with no active centers and a non-polar material efficient for the extraction of non-polar compounds [54-55]. Other commercially available coatings are: Divinylbenzene (DVB), Carboxen (CAR), Poly...
Table 3: Mechanism of extraction and sorbents employed in pesticide analysis.

| Mechanism of extraction | Phase                                                                 |
|-------------------------|----------------------------------------------------------------------|
| Adsorption              | Silica gel without bonded phase                                      |
|                         | Alumina ((A,B,C)                                                     |
|                         | Florisil                                                              |
|                         | Graphitized carbon black                                              |
| Normal                  | Silica-bonded phases                                                 |
|                         | Cyano                                                                |
|                         | Diol                                                                 |
|                         | Amino                                                                |
| Reverse                 | Silica-bonded phases                                                 |
|                         | Aminopropyl                                                          |
|                         | Cyanopropyl                                                          |
|                         | Octadecyl (C8)                                                       |
|                         | Octadodecyl (C18)                                                    |
|                         | Phenyl                                                               |
| Reverse                 | Polymeric phases                                                     |
|                         | Polystyrene-divinilbencene (PS-DVB)                                  |
|                         | N-vinyl pirroldone                                                   |
|                         | Hydrophobic-Lipophilic balanced (HLB)                                 |
| Ion exchange            | Anion or Cation exchange                                             |
|                         | Quaternary amine bonded silica                                       |
| Mixed-mode              | Non-polar + ion exchange                                             |
|                         | Polymeric strong/weak cation-exchange                                |
|                         | Polymeric strong/weak anion-exchange                                 |
| Restricted access       | Size-exclusion and reversed-phase mechanisms                         |
| Immunosorbent           | Molecular recognition by antibodies                                  |
| Molecular imprinted     | Selective sorbent polymer with recognition sites for specific pesticides|

ethylene Glycol (PEG) and Carbowax (CW) in different thicknesses and combinations [56]. Sometimes, stationary phases combine polar and non-polar materials in order to achieve isolation and enrichment of mixtures of compounds of different groups, which have wide polarity range. The most commonly used mixed-phase sorbents are: CAR/PDMS, PDMS/DVB, DVB/CAR/PDMS and Carbopack Z/PDMS [56]. In order to make it possible to analyze certain substances with GC and to improve the efficiency of extraction, analyte derivatization combined with SPME is used. Performing derivatization in the sample before SPME increases the extraction efficiency due to greater volatility and lipophilicity of the derivative compounds. In turn, derivatization on the fiber can occur simultaneously with extraction or after. The advantage of this procedure is that trace amounts of volatile substances can be captured on the fiber as their derivatives and thus measured with very high sensitivity. SPME combines the sampling, extraction, concentration and injecting the sample into a single sample preparation procedure. Therefore, it is a simple, one-step and solvent-free method of extraction, which fulfills the requirements of green analytical chemistry. SPME main advantages are good analytical performance, simplicity, and low cost. HS-fiber-SPME has also the advantage of minimizing damage done to the fiber by aggressive compounds of the matrix. Thus, it is possible to analyze more than 100 samples with the same fiber [57]. However, SPME is still laborious because the equilibrium between the sample solution and the fiber may take a long time and needs many rigorous extraction conditions. Furthermore, the fibers used in SPME are expensive and fragile. Moreover, HS-fiber-SPME shows low adsorption equilibrium and enrichment effect for the compounds with high boiling points. Although the extraction efficiency usually increases with increasing extraction temperatures, excessively high temperature could result in analyte signal reduction [55]. Pragst, in 2007, reviewed SPME applications in analytical toxicology [58]. He found that SPME application to pesticide extraction in human samples was essentially limited to organophosphorus compounds [59]. More recently, some SPME methods for the determinations of pesticides in clinical and forensic settings have been published. (Table 5) summarizes those articles.
| SPE mode-phase                | Analyte                                      | Matrix     | Instrumental technique | Reference |
|------------------------------|----------------------------------------------|------------|------------------------|-----------|
| **Mixed mode:** Bond Elut Certify (C18+ SCX) | Fenamidol                                    | Gastric content | HPLC-DAD              | [29]      |
|                              |                                              | Liver      | LC-ESI-MS              |           |
|                              |                                              | Kidney     |                       |           |
| **Reversed phase:** Oasis WAX™ | 14 pesticides different properties          | Blood      | LC-IT-MS              | [30]      |
| **Cation exchange:** Oasis-MCX™ | 47 volatile pesticides                       | Blood      | GC-MS                 | [31]      |
| **Polymeric phase:** Focus™ | 14 polar pesticides                          | Blood      | LC-MS                 | [31]      |
| Polymeric Weak Cation exchange, Strata-X-AW™-96 well plate | Dialkylphosphate metabolites of organophosphorous pesticides | Urine | LC-ESI-MS/MS | [33] |
| Polymeric Weak Cation Exchange Strata-X-CW | Atrazine and its metabolites | Urine | LC-ESI-MS/MS | [34] |
| HLB- Oasis™ | 8 Organophosphorus pesticides | Blood | GC-MS | [35] |
| Oasis™-96 well plate | 12 metabolites of organophosphorous, pyrethroid and herbicides | Urine | LC-ESI-MS/MS | [36] |
| Adsorption+ Normal phase: Carbon/PSA | Non-polar pesticides: organochlorine, organophosphate and pyrethroid | Hair | GC-MS | [37] |
| **Polymeric phase:** PS-DVB | Hydrophilic metabolites of non-polar pesticides | Hair | GC-MS/MS | [37] |
| Normal phase+ anion exchange: SAX/PSA | Polar pesticides (carbamates, neonicotinoid, azoles) and metabolites without nucleophilic groups | Hair | UPLC-LC-MS/MS | [37] |
| **Reversed phase:** C18 cartridges | 4-chloro-2-methyl phenoxyacetic acid (MCPA) | Urine | GC-MS | [38] |
| LLE+ Normal Phase SPE: Florisil | 11 anticoagulant rodenticides | Stomach contents | HPLC-DAD/ Fluorescence | [39] |
| **Reversed phase:** Sep-Pak C18 | Organophosphorus pesticides | Blood | GC-MS, CG-µ-ECD | [40] |
| **Mixed mode:** Bond Elut Certify (C18+ SCX) | Benzoylurea insecticide: Flufenoxuron | Blood | LC-ESI-MS/MS | [41] |
|                              |                                              | Gastric contents |                       |           |
| **Mixed mode:** Bond Elut Certify (C18+ SCX) | Rotenone                                      | Blood      | Blood                 | [42]      |
| HLB- Oasis™ | Organochlorine                               | Serum      | GC-MS/MS              | [43]      |
| Polymeric reversed phase Sola TM | Chlorfenvinphos                               | Liver      | Blood                 | [44]      |

**Table 4:** Examples of SPE methods applied in cases of lethal poisoning with pesticides.
Micro-Solid-Phase Extraction (µ-SPE):

Micro-Solid-Phase Extraction (µ-SPE) was first proposed in 2006 [68] as a promising alternative to multi-step SPE. µ-SPE is a simple, environment-friendly and efficient sample preparation method for complex samples. It is also known as porous membrane-protected SPE. µ-SPE consists of a solid sorbent or a liquid phase packed in small bags of porous polypropylene, whose edges are heat sealed after packaging. Analytes diffuse through the polypropylene pores and are retained in the solid sorbent or liquid acceptor phase. After that, analytes are eluted from solid phase with the appropriate solvent, whereas if a liquid phase is used is necessary to withdraw the acceptor solvent with a microsyringe [53]. The µ-SPE sorbent influences the extraction efficacy. Features such as pore size, surface area, adsorption capacity and desorption kinetics should be characterized prior to their employment in a specific method [69]. Advantages of µ-SPE are: minimized usage of solvent, high analyte enrichment easy manipulation, less time consumption and low cost [53]. Furthermore, it does not have the drawbacks of traditional SPE such as the requirement of high back pressure and the relatively long extraction time [69].

Dispersive Micro-Solid-Phase Extraction (DMSPE):

Dispersive Micro-Solid-Phase Extraction (DMSPE) consists of the dispersion of solid extracting sorbent in the sample solution. In the resulting suspension, analytes are adsorbed on the sorbent particles. Suspension is then centrifuged and the sorbent is collected. Finally, the analytes are desorbed with an appropriate volume of an organic solvent [12]. Compared with classic SPE, the analytes are in better contact with the sorbent particles and this provides higher extraction efficiency and faster extraction procedures. In general, DMSPE is a simple and fast microextraction technique, compatible with various detection methods, that provides high recovery values with low sorbent and organic solvent requirements [53]. DMSPE applicable materials are characterized by high capacity, large surface area and high dispersibility in liquid samples. Such materials include carbon nanomaterials, such as carbon nanotubes (CNTs), graphene and fullerene and inorganic nanoparticles, such as magnetic nanoparticles (MNPs). The advantage of magnetic nanoparticles is that they can be efficiently removed from the liquid phase using a magnet, thus eliminating the centrifugation step.

Magnetic Solid-Phase Extraction (MSPE):

Magnetic Solid-Phase Extraction (MSPE) was introduced in 1999 as an efficient preparation and preconcentration technique of different organic and inorganic analytes, based on magnetic nanoparticles [12]. MSPE uses a magnetic sorbent which is dispersed in the sample solution by ultrasonication or vortexing. Analytes are adsorbed on the surface of the sorbent particles. Then a magnetic field is applied in order to separate magnetic sorbent particles, avoiding filtration or centrifugation steps. Analytes are eluted from the sorbent particles using an appropriate solvent. Obtained extracts can be analyzed by an instrumental technique [70]. The choice of the appropriate combination of sorbent material and elution solvent is crucial for the efficient extraction of the analytes in MSPE and depends both on the physicochemical properties of the analytes and the nature of the matrix analyzed [53]. A variety of solids have already been used as sorbents, being the nanoparticles containing magnetite (Fe3O4) most popular material because of their low toxicity and low price. Other materials are: magnetite/maghemite (Fe2O3) coated with silica or cellulose, aminoolkyl silylated magnetite and magnetic cores coated with alumina, zirconium, chitosan, polycrystalline and alginate [71]. MSPE presents little interferences due to the diamagnetic nature of the majority of matrix impurities. MSPE reduces the time taken for analysis in compar-

| SPME fiber | Pesticide              | Matrix  | Instrumental technique | Reference |
|------------|------------------------|---------|------------------------|-----------|
| 100µm PDMS | Methyl-parathion        | Liver   | HS-SPME-GC-MS          | [61]      |
| 100µm PDMS | Fluoroacetamide         | Blood   | DI-SPME-GC-MS          | [62]      |
| 65µm CW/ DVB | Parathion              | Blood   | DI-SPME-GC-MS          | [63]      |
| Polyacrylate | Organochlorine        | Serum   | HS-SPME-GC-MS          | [64]      |
| PDMS       | Paraquat (after reduction NiCl2-NaBH4) | Plasma   | HS-SPME-GC-MS          | [65]      |
| PDMS       | Organochlorine         | Urine   | HS-SPME-GC-MS          | [66]      |
| PDMS/DB    | Carbaryl + metabolites | Plasma   | HS-SPME-GC-MS          | [67]      |

Table 5: SPME application to pesticide analysis in human samples.
ison to traditional SPE by reducing the steps in the extraction procedure. It also minimizes organic solvent waste, presents a high enrichment factor and avoids the high back pressure caused by packed SPE cartridges. In addition, magnetic sorbents can be regenerated and used in another extraction process.

**Microextraction by Packed Sorbents (MEPS):**

Microextraction by Packed Sorbent (MEPS) resulted from the miniaturization of classic solid-phase extraction (SPE). In this case, the sorbent is packed directly inside a microsyringe. Usually, few milligrams of sorbent material are packed as a plug inside a liquid-handling syringe, between the needle and the syringe barrel [12]. There is another type of MEPS known as needle set-up. In this case the sorbent material plug is placed on the syringe needle, providing easier handling when the material inside the BIN (barrel insert and needle) needs to be renewed or replaced. Sorbent materials include any commercially available SPE based materials, such as: reversed-phase (C2, C8, C18), normal phase, ion-exchange, mixed or organic monolithic sorbents. A typical MEPS application includes sorbent conditioning, sample loading, washing and analyte elution. Unlike SPE, the two-direction flow potential (up and down) in MEPS provides repetition of each step and sufficient sorbent conditioning, improved sample-sorbent contact, sample loading and preconcentration and enhanced analyte elution. This characteristic enables sample extraction, preconcentration and clean-up in a single device [53,72]. In general, MEPS is a fast, simple and inexpensive green bioanalytical technique, with reduced sample, solvent and sorbent requirements. Washing and elution steps can be carried out with 20–50 µL of organic solvents and 1-4 mg of reusable sorbent material that is enough for efficient analyte extraction. MEPS can be semi or fully automated, with on-line coupling capability and autosampler compatibility for LC and GC analysis. As a result, MEPS could replace SPE cartridges in any of the existing SPE methods [72]. Santos and coworkers have recently published a fully validated method for the determination of six organophosphorus pesticides in blood by MEPS (C18). MEPS extracts were analyzed by GC-tandem mass spectrometry (GC-MS/MS). Authors considered MEPS as a promising extraction technique in clinical and forensic laboratories as the procedure is fast and reliable [73].

**Stir Bar Sorptive Extraction (SBSE):**

Stir Bar Sorptive Extraction (SBSE) has been considered as an efficient sample preparation technique since 1999 when was first introduced [74]. Stir bar has three essential parts: a magnetic stirring rod, a thin glass jacket that covers the magnetic stirring rod and a layer of sorbent into which the analyte is extracted. The sorbent material is usually PDMS [75]. PDMS-covered magnetic stirring rods that are commercially available (Twister®) are 10–40 mm long and are coated by 55-219 µL of PDMS liquid phase. The 10 mm rods are best suited for stirring 10-50 mL samples, while the 40 mm rods work best for sample volumes up to 250 mL. The sorption process is essentially a liquid-liquid partition process, therefore not only the surface area but also the total amount of the extraction phase is involved in sorptive extraction [9]. Other commercially available coatings in SBSE are ethylene glycol-silicone and polyacrylate. However, these coatings are very limited, expensive, have low thermal and chemical stability, short lifetime and bad extraction selectivity when used to extract compounds with similar polarity [9]. The two basic steps that SBSE process includes are extraction and desorption. There are two types of SBSE techniques, direct immersion (DI-SBSE) and headspace (HS-SBSE). In DI-SBSE, the stirring rod is placed in an appropriate volume of a liquid sample contained in a vial, and the sample is stirred until equilibrium is reached. After extraction the rod can be easily removed with tweezers, rinsed with water and then dried with a clean paper tissue. In the literature, most applications are performed in immersion mode [76]. In HS-SBSE the stirring rod is placed above the liquid or solid sample in a special device that keeps the rod in the headspace. After HS sampling, it is also recommended that the polymer coated stir bar be rinsed with distilled water and gently wiped with clean tissue paper. The merits of this approach are the long lifetime of the stir bar, the high selectivity and the fact that no sorption of non-volatile compounds is carried out [76-77]. With regard to the extraction step, variables such as extraction time and temperature, pH adjustment, sample and acceptor phase volumes, stirring speed, additions of an inert salt, organic modifier and derivatization agents must be established [78]. Desorption is another principal step of SBSE. After the extraction, the analytes retained by the sorptive element are desorbed via thermal desorption (TD) which is followed by GC analysis or liquid desorption (LD) which is followed by HPLC, capillary electrophoresis (CE) or GC with large-volume injection [76]. SBSE simultaneously combines extraction and concentration of the analytes in a single step. SBSE advantages are simplicity, sample clean-up ability, high extraction efficiency, good reproducibility and high sensitivity. Furthermore, SBSE allows reutilization, can be easily combined with modern analytical instrumentation and coupled with derivatization procedures [75]. SBSE is a solvent less, rapid and environmentally friendly sample preparation technique, which complies with the principles of green analytical chemistry. The improved sensitivity of this technique allows the minimization of the sample amount needed for the analysis [12]. The main drawback of this technique is the lack of stir bar coatings with high affinity towards polar or less polar analytes, which restricts the wide application of SBSE. The development of new coatings is the most needed improvement in order to increase the selectivity and expand the applicability of SBSE allowing the effective extraction of polar compounds [75]. In 2003, SBSE was successfully applied to development of multi-residue method for the determination of a wide range of pesticides in food matrices [79]. Later, a dual SBSE method intended for the extraction of both polar and apolar pesticides from food matrices was published. Previous to SBSE, samples were extracted with methanol and diluted with water. Developed method showed good linearity and sensitivity for 85 pesticides [80]. There is scarce applica-
Liquid Phase Microextraction

LPME is a miniaturized extraction method of recent development [87]. LPME has emerged from LLE in order to overcome its main disadvantages: high consumption of time and solvents and its tedious application. LPME is a simple and low-cost sample preparation technique. It combines into a single step, extraction, concentration, and sample introduction. During the LPME, analytes are extracted from an aqueous phase (donor phase) to several microliters of water-immiscible solvent (extractant or acceptor phase) [12]. As in LLE, the choice of the solvent is a critical parameter for LPME applications. The solvent should have good affinity to the targeted analytes, low solubility in water, good stability during the whole extraction procedure and excellent chromatographic behavior [12]. LPME has been carried out under different extraction modes, which can be classified into three main categories: single-drop microextraction (SDME), hollow-fiber LPME (HF-LPME), and dispersive liquid-liquid microextraction (DLLME).

Single-Drop Microextraction:

Single-drop microextraction (SDME) was the first liquid-phase microextraction mode to be developed [4]. SDME consists of the distribution of analytes between a microdrop of an immiscible organic solvent (acceptor phase) and an aqueous sample (donor phase). The microdrop usually constitutes of 1-10 µL of an organic solvent [11]. SDME methodology is based on two-phase and three-phase distribution systems

• Direct immersion SMDE (DI-SDME) is the simplest two-phase system:

A micro-drop of extraction solvent is set at the tip of a microsyringe needle and immersed in the sample solution. After a period of magnetic stirring, the distribution equilibrium is established between the sample and the microdrop of extraction solvent. Finally, the microdrop is retracted back into the microsyringe and injected for the subsequent determination. Most of all, the extraction solvent must have low water solubility and high boiling point [3]. Since the organic solvent is a critical factor in SDME, high purity water immiscible solvents, such as dichloromethane, trichloromethane, carbon tetrachloride, hexane, cyclohexane and xylene, usually constitute the microdrop. 1-butanol, 1-octanol, isoctane, toluene, dodecane and undecane are also used because of their compatibility with GC systems [11].

• Headspace SMDE (HS-SDME) is the simplest three-phase system:

Where the microdrop is suspended in the vapors, which consist of the volatile compounds formed over the aqueous sample. HS-SDME is free of matrix interferences [9].

In revised literature, there is not any example of application of this technique to the determination of pesticides in forensic samples. However, it has been applied successfully to the analysis of pesticides in different matrices (Table 6). SDME is a simple, cost effective, environmental friendly microextraction technique that lacks carry-over through each analysis, since the microdrop is renewed for each extraction cycle. It is applicable in various matrices, can be fully automated and is compatible with chromatographic injection systems (LC and GC) [12]. However, drop volume fluctuation and stability are the main drawbacks of SDME. Intense conditions, such as high stirring speeds, extended extraction period and high temperatures, as well as suspended particles in dirty samples, affect the drop stability [89].
Hollow Fiber-Liquid Phase Microextraction:

To avoid the drop instability in SDME, HF-LPME was introduced as another type of LPME method in 1999 [93]. HF-LPME is a technique in which analytes are firstly extracted into a supported liquid membrane sustained in the pores of hydrophobic hollow-fiber (HF), and later into an acceptor solution placed inside the lumen of the fiber. The porous membrane of the hollow fiber separates the donor and the acceptor phases. Thus, the acceptor solution in hollow fiber is effectively protected within the fiber and can avoid the instability of the drop of the extraction solvent [12]. Finally, the acceptor phase is removed with a microsyringe and injected into the appropriate analytical instrument [11]. Before its use, HF is usually preconditioned by soaking into an immiscible organic solvent like 1-octanol, toluene, n-hexane, o-xylene. Once conditioned, HF can be used to extract analytes from aqueous sample. According to the number of phases involved, two HF-LPME configurations can be distinguished [11].

Microporous membrane liquid–liquid extraction:

There are two phases involved: one is a donor phase (aqueous sample) and the other is the acceptor phase (organic phase). The organic phase is placed inside the fiber to form the liquid membrane. This mode is suitable for more hydrophobic analytes and compatible with direct GC injection.

Supported liquid membrane extraction:

There are three phases involved: one of them is a donor phase (aqueous sample), the intermediate phase is a liquid membrane on the fiber wall formed by an organic solvent and the third one is the acceptor phase (second aqueous solution). This mode is suitable for the extraction of polar analytes and compatible with HPLC, CE and atomic absorption spectrometry. HF-LPME is an effective, low-cost microextraction technique that can be automated and further miniaturized. Compared to SDME, it presents enhanced solvent stability when increased sampling time and temperature is required. There are no carry-over and memory effects between each extraction due to the disposable fibers. Additionally, the fiber pores provide improved selectivity by preventing the extraction of high molecular weight molecules [11].

Dispersive Liquid-Liquid Microextraction:

Dispersive liquid-liquid microextraction (DLLME) was introduced in 2006 and has attracted great attention due to its wide range of applications [3]. DLLME employs a ternary component solvent system composed of an aqueous solution containing the analytes, a water-immiscible extraction solvent and a water-immiscible disperser solvent. When the disperser and extractant are mixed and rapidly introduced into the aqueous solution, a cloudy solution appears, indicating the equilibrium between the droplets of the extraction solvent and the aqueous sample. The equilibrium is reached within a few seconds due to the very large surface area of the micropores in the dispersed state. The extraction solvent is normally collected at the bottom of the tube through centrifugation [94,95]. Table 7 shows solvent characteristics. The type and volume of disperser solvent can significantly affect the volume of the sedimented phase, which in turn, influences the extraction efficiency. In order to help the dispersion process, vortex agitation is proposed. Sometimes the use of ultrasonic stirring or a cationic surfactant, instead of a solvent, has been proposed in order to disperse the extraction solvent. In order to remove the centrifugation step, which is considered one of the most time-consuming steps in this method, demulsified DLLME has been proposed. After the introduction of the solvent mixture and consequently emulsion formation, another portion of dispersive solvent (serving as demulsifier) is introduced, leading to the breakup of the emulsion. Compared with the conventional sample preparation methods, DLLME showed advantages as: the shorter extraction time, it is inexpensive, quicker and easier to operate, the absence of a clean-up procedure, lower consumption of organic solvent, low limits of detection, good repeatability, high enrichment factor and good recovery within a short time. Moreover, it can be coupled either with GC or LC. Nevertheless, this technique has some limitations, which are related mainly to the requirements posed for the extraction and disperser solvents [94-96]. From literature reviewed, it can be concluded that most of the applications of DLLME are intended for the isolation of...
different kinds of pesticides from water [94,97]. As an example a fast and cost effective DLLME-GC-MS methodology for routine determination of pesticides in a single analytical run can be cited. This procedure uses a mixture of methanol and tetrachloroethylene as a dispersive solvent for extracting from water 34 pesticides with quite different physicochemical properties [96]. A modification of DLLME known as dispersive liquid-liquid microextraction with solidification of a floating organic drop has been developed. After performing DLLME, samples are cooled and the solidified drop of solvent is collected from the vial, melted and analyzed. This technique has been applied to determination of chlorpyrifos in water [98]. In 2013, Qui and coworkers developed a method for the determination of rodenticide bro-madiolone in plasma using DLLME as extraction technique. An ionic liquid 1-hexyl-3-methylimidazolium hexafluorophosphate was used as extraction solvent, methanol as dispersant and analysis was performed by HPLC. Authors stated that it is a simple, rapid, accurate and practical method with optimal sensitivity, linearity, precision and recoveries [99]. Jain and Singh reviewed the applications of DLLME in forensic toxicology. They found several papers in which DLLME was applied to the extraction of organochlorine (endosulfan and its metabolites), pyrethroid (cypermethrin) and triazole pesticides (myclobutanil, uniconazole, penconazole and hexaconazole) for matrices such as blood, plasma, urine and liver [100].

| Type of solvent | Features | Examples of solvents |
|----------------|----------|----------------------|
| Extractant     | Higher density than water | Carbon disulfide |
|                | Low water solubility      | Tetrachloroethane |
|                | High extraction capability | Chlorobenzene     |
|                | Good chromatographic behavior | Carbon tetrachloride |
|                | High-melting point        | 1-Undecanol       |
|                | Lower density than water  | 1- Dodecanol      |
|                | Aqueous miscible          | Hexadecane        |
| Dispersive     | Polar                   | Acetonitrile      |
|                | Large volume             | Methanol           |

Table 7: Main characteristics of solvent employed in DLLME [3].

**QuEChERS**

QuEChERS is a type of sample preparation method that was first reported in 2003. The abbreviation QuEChERS stands for quick, easy, cheap, effective, rugged and safe, describing the advantages over the traditional LLE [101]. This method is based on the micro-scale extraction using acetonitrile, water absorption and liquid-liquid partition utilizing MgSO4 and NaCl and the clean-up step of d-SPE employing primary-secondary amine (PSA) adsorbent [101]. Although acetonitrile is miscible with water, it can be easily separated from water by the salting-out effect and centrifugation. Furthermore, acetonitrile not only yields higher recoveries and less interference than other solvents such as acetone and methanol, but it also offers slightly better limit of detection and reproducibility than acetone [13]. As high pH may influence the stability of some base-sensitive pesticides and the final recoveries, the use of certain buffer solutions is advised in order to avoid the degradation of these pH-dependent pesticides during the QuEChERS procedure [13]. QuEChERS method combines the conventional extraction, isolation and clean-up procedures into one step. It omits blending, filtration, large volume of solvent transfers, evaporation and solvent exchanges usually applied for the chromatographic determination. Furthermore, QuEChERS provides reliable quantitative results for a wide scope of pesticides with different polarity and volatility in different matrices meeting low detection limits [3]. QuEChERS procedure has been extensively applied to pesticide extraction from food matrices and environmental samples during the last few years. The majority of the methods developed are oriented to multi-class pesticide analysis [102]. The excellent results achieved for food matrices have lead both AOAC and European Commission to establish standard methods for pesticide determination using QuEChERS methodology [103,104]. However, QuEChERS application to human samples in a forensic setting is not, according to reviewed literature, so extended. Some examples of these applications are given in (Table 8).

**Matrix Solid-Phase Extraction**

Matrix solid-phase dispersion (MSPD) was introduced in 1989 [68]. In contrast to the common SPE methods, MSPD combines the extraction and clean-up procedure into a single step. Generally, this method consists of the following steps: sample homogenization, cellular disruption, exhaustive extraction, fractionation and the clean-up by adsorbents [3]. MSPD simultaneously performs the disruption of sample and the dispersion of sample components on a solid support, thereby generating a chromatographic material suitable for the extraction of analytes from the dispersed sample. The dispersants used in MSPD are C18, C8, silica, Florisil, diatomaceous earth and Al2 (SO4)3. Among them, C18 is still the most widely used dispersant in the MSPD procedure. It is extremely important to select the ratio between the sample and the sorbent to ensure the formation of fine particles and effective dispersion of the sample on the sorbent. The normal ratio between the sample and the sorbent typically ranges from 1:1 to 1:4 [3]. The nature and volume of the elution solvent is important for the efficient desorption of pesticides from the adsorbent and the absorption of interferences on the SPE column. A large variety of solvents, for example acetonitrile, methanol, ethyl acetate, dichloromethane or mixtures of them, have been tested in the MSPD. Compared to the traditional LLE and SPE, the primary advantage of MSPD is that both, the sample extraction and the clean-up procedure are performed in one step using small amounts of adsorbent and solvent. Thus, it not
only simplifies and speeds up the sample preparation process, but also reduces the consumption of large amounts of toxic solvents, avoids emulsion formation, shortens the analysis time and increases the reliability, selectivity and sensitivity of pesticide residue analysis. However, it is hard to ensure the repeatability of the homogenizing and grinding procedure because of the handmade operation, which may lead to experimental errors and instability [111]. MSPD has found particular application as an analytical process for the preparation, extraction and fractionation of solid, semi-solid and/or highly viscous biological samples. Therefore, it has been extensively applied to the analysis of pesticides in food matrices. However, there are few applications of this technique to the extraction of matrices like blood, plasma or urine [112]. In reviewed literature, no example of MSPD application to the extraction of pesticides from human samples in a forensic setting has been found.

### Non-Conventional Materials for Sample Preparation

In the last 10 years, research on new materials for sample preparation has been oriented in two directions, the development of both, new sorbent materials capable of retaining polar pesticides and sorbents with better selectivity or specificity towards target analytes [8,26]. The newly developed materials are mostly the consequence of the progress of two different and often interrelated fields of knowledge, nanotechnology and biochemical sciences [8]. These new materials can be applied to different sample preparation techniques that have been previously discussed. According to reviewed literature, these nonconventional materials have been mostly applied to pesticide extraction from water, while their application to human samples is scarce.

### Surfactant Modified Sorbents

Surfactant-modified sorbents were described as SPE materials for isolation of organic compounds as early as 1996. These sorbents use surfactant at concentrations slightly below the critical micellar concentration (CMC). Surfactant molecules are sorbed on the surface of mineral oxides such as alumina, silica, titanium dioxide and ferric oxyhydroxides, forming monolayers or bilayers [113]. Two different sorbent structures known as hemimicelles and admicelles may be formed. On hemimicelle-based sorbents, the hydrophobic tail of the surfactant is exposed to the sample, so the corresponding sorbents have higher affinity towards non-polar analytes. As for sorbents containing admicelles on the surface, the portion of the coacervates exposed to the solution comprises the ionic tails of the surfactant exposed to the sample, so the corresponding sorbents have higher affinity towards non-polar analytes. As for sorbents containing admicelles on the surface, the portion of the coacervates exposed to the solution comprises the ionic tails of the surfactant molecules, rendering the materials more suitable for polar species [8]. In these materials a process known as adsolubilization takes place. Due to this process analytes are sorbed by micellar surface aggregates. The advantages of surfactant modified sorbents are high extraction yields, easy elution of analytes, high breakthrough volumes and high flow rate for sample loading [26]. Furthermore, due to their amphoteric nature these sorbents are capable of extracting pesticides of extremely wide range of polarities [114]. There are two papers dealing with the examples of the application of these materials to pesticide determination. First, Moral et al in 2008, successfully employed SPE cartridges packed with SDS/tetra- tanyl ammonium chloride (TBACl) hemimicelles and admicelles adsorbed on c-alumina to isolate 17 pesticides of varied acidity.

| Pesticide | Matrix | Extraction | Instrumental technique | Reference |
|-----------|--------|------------|------------------------|-----------|
| Disulfoton+ 5 metabolites | Blood | Modified QuEChERS | LC-MS/MS | [105] |
| | Urine | | | |
| Pesticide metabolites | Urine | Enzymatic hydrolysis+ QuEChERS [87] | UHPLC-Orbitrap-MS | [106] |
| 215 pesticides | Blood | QuEChERS [87] | LC-TQ/TOF/MS | [103] |
| | Gastric contents | | | |
| 4 pesticides | Blood | QuEChERS [4] | HRMS full scan | [108] |
| Organochlorine | Plasma | MiniQuEChERS | GC-MS/MS | [10] |
| Organophosphorous | | | | |
| Pyrethroids | | | | |
| Herbicides | | | | |
| 7 pesticides (organochlorine+ organophosphorous) | Blood | Fe3O4 magnetic particles + QuEChERS | GC-MS | [4] |
| 28 pesticides | Hair | QuEChERS | GC-MS | [109] |

Table 8: Examples of QuEChERS application to pesticide extraction from human samples.
and several classes (triazines, phenylureas, carbamates, azocompounds, anilides, chloroacetanilides, organophosphates, phenols, phenoxy and arylxylo acids) from natural water samples before HPLC analysis [115]. Second, in 2012, Luque and Rubio, also used alumina modified with admicelles of SDS and TBACl to extract pesticides having different structural groups from river and underground water samples. They reported high extraction efficiencies even using low volumes of solvents in the elution step. Also, no degradation of most of the sorbed analytes was observed, even after three months of storage of the cartridges [116].

Aptamers

Aptamers are short (up to 110 base pairs), single stranded, synthetic oligonucleotides that can fold in characteristic shapes capable of binding with high specificity to target molecules; recognition arises from hydrogen bonding, van der Waals forces and dipole and stacking interactions. Specific aptamers targeting specific analyte can be prepared using the process known as SELEX (Systematic Evolution of Ligands by Exponential Enrichment) in which a random pool of sequences of oligonucleotides is doped with the analyte and sequences that bind to the target are selected through iterative cycles of separation of aptamer/target complexes, isolation of template aptamers and amplification by PCR [117]. The overall process is automatable and reasonable amounts of highly specific aptamers for the desired target analytes can be obtained. There is still a very limited number of aptamer-modified sorbents prepared and characterized for use as SPE materials. However, there are many research opportunities related to the study of new aptamer-based molecular recognition sorbents as their preparation is fast and relatively inexpensive [8].

Carbon-Based Nanostructured Materials

Graphene and Other Graphitized Derived Materials:

Graphene was discovered in 2004 and it became a nanomaterial of special interest in sample preparation due its physicochemical properties. It is considered the basic building block of all graphitic forms and possesses a single layer of carbon atoms in a closely packed honeycomb two-dimensional lattice [118]. Graphene has a large adsorption capability thanks to the morphology of nanosheets that is accessible for molecular adsorption in both surfaces and to the large surface area. The delocalized-electron system provides good affinity for compounds with aromatic rings. Other properties are such as high fracture stretch and good mechanical and thermal stability. Graphene is easily prepared from graphite without sophisticated apparatus. In a first step graphite is oxidized to graphite oxide, which is further exfoliated to graphene oxide. Finally, a reduction step converts graphene oxide to graphene [119]. The main difference between graphene oxide and graphene is their polarity as graphene oxide in its surface structure contains hydroxyl, carboxyl and epoxy polar groups. Graphene and graphene oxide show excellent sorbent characteristics, including good compatibility with organic solvents, reproducibility, reusability, no impact of sorbent drying and high sorption capacity [118]. Due to these properties, graphene and graphene oxide sorbents have been used in different sample preparation methods, such as SPE, SPME, SBSE bars, matrix-solid phase dispersion and microextraction by packed sorbents [71]. In recent years a growing trend in the number of graphene and graphene oxide sorbents has been observed, especially in environmental fields. However, these materials have a high potential to be applied in the analysis of biological matrices [120]. There are some limitations to the application of graphene as SPE sorbent. It has been observed that miniscule graphene may escape from the cartridge and aggregation of graphene can occur when graphene or its oxide was directly used as SPE sorbent. To circumvent these problems, graphene oxide was linked to the amino groups of an amino-terminated silica adsorbent in organic phase [118]. Graphene has also been used in the coating of SPME fibers. One of its applications has been the determination of six pyrethroid pesticides, showing much better results than those obtained with PDMS and PDMS/DVB fibers [17]. Graphene coated SPME fibers have also been successfully used for the extraction of carbamate insecticides and triazine herbicides from water samples [121, 122]. Some authors have successfully applied graphene fibers in HS-SPME mode for the extraction of organochlorine pesticides from water samples [123]. Another kind of graphene-based materials, which have been applied to the extraction of several types of pesticides, are magnetic nanoparticles. Several authors employed them in the extraction of as triazine herbicides, carbamate and neonicotinoid insecticides and triazole fungicides from liquid samples [124-127].

Electrospun Nanofibers (CNFs):

Carbon nanofibers (CNFs) are solid carbon fibers with lengths in the order of a few microns and diameters below 100 nm, but with high specific areas [118]. CNFs morphology, physical and chemical properties can be selected by optimizing operational parameters in their production. Surface properties can be modified with chemical treatments to satisfy some special needs. Polar groups, such as carboxyl, hydroxyl and carbonyl can be introduced to carbon nanofibers surface by treatment with the concentrated nitric acid [8]. Electrospinning is the most versatile technique for CNFs fabrication [128]. Therefore, CNFs properties make them interesting materials for the development of new sorbents, which can be used in different sample preparation methods. For example, electrospun nanofiber-coated SPME fiber has demonstrated superior extraction efficiencies over commercially available SPME fiber [118]. Another example of CNFs application is the development of extraction method for organophosphorus pesticides extraction from water samples at trace levels using new SPE sorbent made of a polystyrene [129] and polyacrylonitrile nanofibers [130].

Carbon nanotubes (CNTs):

CNTs are allotropic forms of carbon comprising tubular structures that can be considered a graphene sheet in the shape of a cylinder capped by fullerene-like structures [118]. There are
two forms of CNTs layouts: single-walled carbon nanotubes (SWCNTs) formed by a single rolled graphite lamella in a cylinder with a diameter up to 3 nm and multi-walled carbon nanotubes (MWCNTs) formed by several CNTs concentrically arranged around a common axis. MWCNTs diameter is up to 100 nm [131]. CNTs main features are: a super-large specific surface area, an outstanding thermal and chemical stability, large sorptive mass capacity and the possibility of large-scale production [17]. Another interesting feature of CNTs is that their surfaces can be chemically modified, resulting in functionalized adsorbents with distinctive properties [8]. Due to its properties, CNTs have been the most used carbon-based nanomaterials in sample preparation. Therefore, hundreds of research articles and tens of critical review papers are available in the scientific literature [118]. CNTs have been widely applied as SPE sorbents for the extraction of organophosphorus pesticides in water and food [17], sulfonylurea herbicides and phenoxy alkanoic acid herbicides (dicamba and 2,4,5-T) [8]. CNTs-coated SPME fibers often possess porous morphology and their mechanical and thermal stability as extraction performances have made them satisfactory for application in analytical chemistry [9]. Similar to other materials CNTs can be used as a support for MIPs (Molecular imprinted polymers) or magnetic particles [118].

Ionic Liquids

Ionic liquids (ILs) are liquid salts at room temperature (25°C) whose melting point is lower than 100°C. ILs are organic cations derived from Lewis bases, such as nitrogen and phosphorous donor atoms and polyatomic anions containing different inorganic or organic structures [71]. Their physicochemical features are: low vapor pressure, high viscosity, inflammability, thermal stability and miscibility with water and organic solvents. However, the application of ILs to pesticide sample extraction presents some drawbacks, such as: their high economic cost, lack of definition of some physicochemical properties and incompatibility with GC analysis [120]. ILs have found a wide range of applications in analytical chemistry. First of all, they represent an alternative to the conventional organic solvents with the advantage of being environmentally friendly. ILs are also used in the synthesis of new materials for SPE extraction and the fabrication of GC and LC stationary phases. The bonding and immobilization of ILs to an inert support is the major limitation of the fabrication of ILs SPE materials and chromatographic phases. Nowadays, silica is the most widely used support is silica [71, 120]. As SPE materials, ILs present a high enrichment factors. ILs-based silica SPE sorbents have been applied to the extraction of 12 sulfonylurea herbicides in environmental water, obtaining higher selectivity than commercially available C18 cartridges [132]. Due to their high thermal stability and the easiness of their functionalization, ILs have been employed as coating materials for SPME. ILs-SPME fibers have been successfully used in the analysis of mixtures containing mainly hydrophobic and semi-polar compounds [133]. Furthermore, a new type of HF has been prepared by injecting nanoparticles synthesized employing ILs into a polypropylene HF. Thanks to its high preconcentration factors, this HF could be used in the analysis of small amounts of pesticides present in hair samples [134]. Unfortunately, the application of ILs to the extraction of pesticides from biological samples is at the moment scarce, according to reviewed literature.

Future Trends

The ideal sample preparation method should be a compromise between cost, accuracy, selectivity and sensitivity. Unfortunately, the traditional extraction techniques frequently fail to meet these goals. The achievement of faster, more dependable methods with suitable sensitivity and selectivity depends on enhancements to all steps regularly included in analytical methods. Of all the steps included in analytical methods, sample treatment procedures are frequently related to loss of accuracy and precision. Therefore, the improvement of sample extraction methods is a recurrent topic in analytical chemistry research [8]. In order to accommodate the demands of green analytical chemistry, traditional procedures have been evolving into more sophisticated and environmentally friendly ones. The current trend in analytical chemistry is moving towards simpler, miniaturized, automated and solvent-free sample preparation procedures, while maintaining satisfactory extraction efficiency [96]. The replacement of classical extraction procedures with microextraction techniques allows significant decrease in the amount of sample and reagents used, while sustainability and efficacy of methods are improved. Therefore, it is expected that microextraction techniques will find increasing applications in the sample preparation step of complex biological samples such as saliva, blood, plasma, serum, urine, hair and cerebrospinal fluid [12]. The future perspectives for green microextraction techniques depend on the development of new sorbent materials, the incorporation of even less toxic solvents, further miniaturization, full automation and online coupling capability with analytical instruments. A further trend in sample preparation is the development of multi-class methods as pesticides available in the market present a wide range of physicochemical properties. This trend is supported by the fact that newer instrumental techniques such as tandem mass spectrometry or high-resolution mass spectrometry favor the use of multi-class methods. Multi-class methods provide more information of the different analytes potentially present in a sample, resulting in a higher sample throughput, as well as reduced consumption of materials and ultimately lower cost [50].

Conclusion

According to scientific literature, traditional methods of extraction, such as LLE, SPE and SPME are still the most used ones in forensic toxicology laboratories. Among the new analytical methods QuEChERS is the one the most developed and implemented in forensic toxicology laboratories. The implementation of multi-class methods is of special interest in forensic toxicology laboratories, as it is quite frequent that a general unknown screening must be performed in order to detect, identify and quantify the pesticide responsible for the poisoning. It should be noted that forensic samples are unique and usually available in

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small amounts. Therefore, the comprehensive implementation of microextraction techniques for pesticide analysis is of special interest.

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