ANALYTICAL APPROACHES FOR SAMPLING AND SAMPLE PREPARATION FOR PESTICIDES ANALYSIS IN ENVIRONMENTAL, FOOD AND BIOLOGICAL SAMPLES

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Abstract. Residues of pesticides, especially organochlorine pesticides (OCPs) in sediment and aquatic biota have been an environmental concern since the 1960s. Widely used in agriculture in the past, most of OCPs are resistant to photochemical, biological and chemical degradation for a long period of time. For their determination in different environmental media, sampling and sample preparation represents a time consuming stage, but a key factor in the entire pesticides trace analysis procedure. Scientific efforts directed towards the sample pretreatment issue are focused on developing methods for enriching and isolating components present in complex sample matrices. Due to the differences of pesticides properties (volatility, polarity), to the complexity of the sample matrix and to the required degree of preconcentration, there is no unique strategy for the sample preparation. This paper presents different techniques available for samples preparation for pesticide analysis in environment, in food or in biological samples.

Keywords: pesticides, sampling, extraction, preconcentration, clean-up

1. Introduction

Pesticides and their metabolites have received particular attention in the last few years in environmental trace organic analysis because they are regularly

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detected in surface and ground waters especially throughout Europe and North America as a consequence of their widespread use for agricultural and non-agricultural purposes (Hennion and Pichon, 2001).

The determination of pesticides in food and environmental samples at low concentrations is always a challenge. Ideally, the analyte to be determined would be already in solution and at a concentration level high enough to be detected and quantified by the selected final determination technique (i.e., HPLC or GC) (Turiel and Martin Esteban, 2007). However, in environment, in food or in biological samples they are present at trace levels and despite the advanced techniques available for separation and quantification, no sample can be directly analyzed, therefore an extraction and concentration stage is required.

Even when the analyte is already in a liquid media (i.e. water, juice, serum), the presence at different concentration levels of the matrix-interfering compounds, imposes the need to overcome several difficulties related to the required selectivity and sensitivity of the analytical technique. Consequently, the selection of an appropriate sample preparation procedure involving extraction, concentration and cleanup steps becomes mandatory to obtain a final extract, enriched in the target analyte, as free as possible of the interfering compounds.

This contribution presents different sample treatment techniques currently available and most commonly used in specialized laboratories for the analysis of pesticides in environmental, food and biological samples. Depending on the nature of the sample (solid or liquid) and the specific application (type of pesticide, concentration level, multiresidue analysis), the final procedure might involve the use of only one, or a combination of several different described techniques.

2. General Procedures for Samples Preparation

Environmental analysis, like any other analytical process, must follow three major steps: (i) sampling and sample preparation for measurements; (ii) measuring; (iii) data processing (Chirila et al., 2006). Sampling for organic compounds analysis, like pesticides, is based on the principle to extract them from the sample matrix in a soluble and stable form (Draghici and Chirila, 2009).

One key problem in pesticide analysis comes from the diversity of their chemical functional groups with varying polarity and physicochemical properties. Sampling and sample preparation represents a time consuming step, very important in the whole procedure for trace analysis of pesticides. The methods for extraction and concentration of pesticides are mainly liquid-liquid extraction and solid-phase extraction. Table 1 presents comparative information

about the general pesticides extraction techniques from solid and liquid samples, that will be further presented.

TABLE 1. Comparison of general pesticides extraction techniques from liquid and solid environmental samples

Extraction method	Application	Cost	Time of extraction	Solvent Volume (mL)
Purge & trap	V (L, S)	High	30 min	-
Headspace	V (L, S)	Low	30 min	-
LLE	V, SV, NV (L)	Low	1 h	500
SPE	V, SV, NV (L)	Medium	30 min	50 – 100
SPME	V, SV, NV (L)	Low	30 min	-
Soxhlet / Soxtec	SV, NV (S)	Low	12 - 48 h	300 – 500
USE	SV, NV (S)	Medium	15-30 min	5 – 30
ASE (PSE)	SV, NV (S)	High	20-30 min	30
MAE	SV, NV (S)	Medium	15 min	40
SFE	SV, NV (L, S)	High	30 min	5 – 20

ASE – accelerated solvent extraction; LLE – liquid-liquid extraction; MAE – microwave assisted extraction; PSE – pressurized solvent extraction; SFE – supercritical fluid extraction; SPE – solid phase extraction; SPME – solid phase microextraction; USE – ultrasonic solvent extraction; L – liquid; NV – nonvolatile; S – solid; SV – semivolatile; V – volatile.

2.1. PURGE AND TRAP EXTRACTION

Purge and trap extraction is used for organic non-polar volatile compounds to be further used for GC analysis. An inert gas is bubbled in the water sample, transferring the organic volatiles into the vapor phase. These are trapped in an active carbon and/or condensed. The trap containing the adsorbent is passed into a heated desorption chamber that allows desorption of the retained compounds. This is not always a fast process (as needed for GC) but cryogenic focusing may be used. It is very important to use highly pure purge gas. Purging water media may raise difficult problems because usually low water quantities are allowed in the column.

2.2. HEADSPACE EXTRACTION

Headspace extraction is used for the pollutants trapped in a matrix that cannot be introduced, as such, in a chromatographic system. There are two techniques, depending on the way to introduce the sample in the measuring equipment:

- **Static headspace technique** is probably the simplest solvent-free sample preparation technique, has been used for decades to analyze volatile organic compounds; the sample (liquid or solid) is placed in a vial; the vial is sealed, then heated and the volatile compounds are driven into the headspace; equilibrium between the headspace and the sample matrix is reached (Figure 1a); a portion of the vapor from the headspace is injected then into a GC;
- **Dynamic headspace technique** uses a carrier gas (helium) for eluting the volatile parts to a collector where they are adsorbed and concentrated; a thermal desorption follows in the collector, allowing the gas components to enter the GC (Figure 1b); the sample can be recovered by stripping.

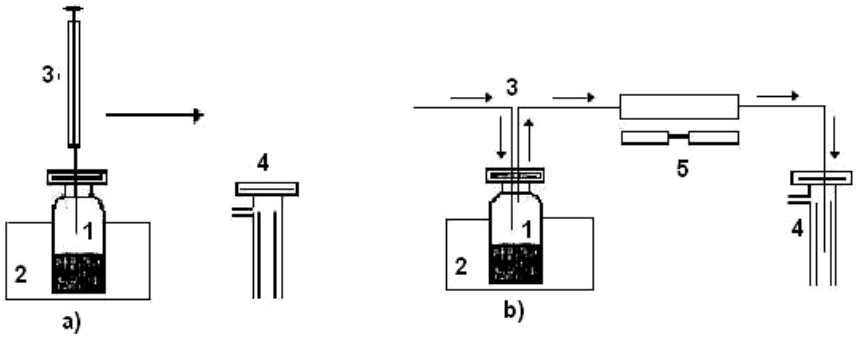

Figure 1. Headspace extraction techniques: a) static; b) dynamic.
1 – headspace vial; 2 – termostate; 3 – sample prelevator; 4 – GC injector;
5 – collector.

2.3. LIQUID-LIQUID EXTRACTION

Liquid-liquid Extraction (LLE) has been widely used for the extraction of pesticides from aqueous liquid samples and, although to a lesser extent, for the purification of organic extracts. LLE is based on the partitioning of target analyte between two immiscible liquids. The efficiency of the process depends on the affinity of the analyte for the solvents, on the ratio of volumes of each phase, and on the number of successive extractions. Hexane and cyclohexane are typical solvents for extracting non-polar compounds, such as organochlorine and some organophosphorus pesticides, whereas dichloromethane and chloroform are the common solvents used for the extraction of medium-polarity pesticides.

The use and evaporation of large volumes of solvent, often toxic and flammable, are the main drawbacks. Therefore, the trends in reducing the use of organic solvents in analytical laboratories and the low performances in extracting polar compounds explain the increasing replacement of LLE by liquid phase micro-extraction and/or solid-phase extraction.

Liquid Phase Micro Extraction (LPME) is a miniaturized implementation of conventional LLE in which only microliters of solvents are used instead of several hundred milliliters in LLE. The technique is quick, inexpensive and can be automated (Lambropoulou and Albanis, 2007).

There are two sampling modes that can be used with LPME: two-phase and three-phase. In two-phase LPME, the analytes are extracted from the aqueous sample solution (donor phase) into the organic solvent (acceptor phase) which either consist of a micro-drop (ca. 1–3 μL), suspended from the needle of a micro-syringe (**single drop micro-extraction – SDME**, Figure 2a and 2b) or it is present in the pores and/or inside the lumen of a hydrophobic membrane, respectively a **hollow fiber (HF LPME)**, Figure 3a and 3b.

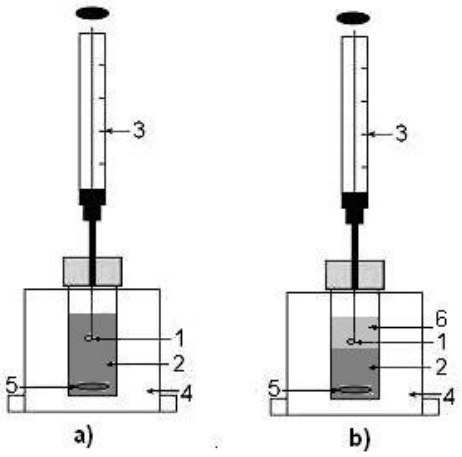

Figure 2. The schematic representation of SDME: a) two phase; b) three phase:
1 – solvent drop; 2 – aqueous phase; 3 – chromatographic micro-syringe; 4 – water bath; 5 – stir bar; 6 – organic solvent layer.

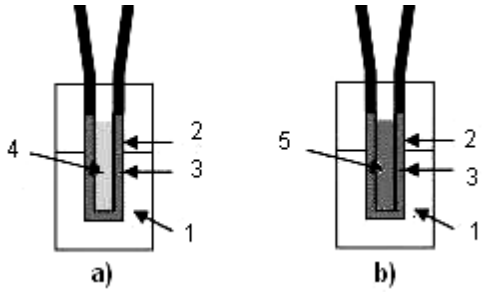

Figure 3. HF – LPME a) two – phase; b) three – phase: 1 – sample; 2 – hollow fiber; 3 – supported liquid membrane; 4 – acceptor phase (organic solvent); 5 – acceptor phase (aqueous solution)

Basically, in liquid phase micro extraction using hollow fiber membranes (HF-LPME) technique, the piece of porous polypropylene hollow fiber is impregnated with a water-immiscible solvent and the analytes are extracted by passive diffusion from the sample into the hydrophobic organic solvent supported by the fiber (two phase HF-LPME). On the other hand, the analytes can be extracted through the organic solvent immobilized in the pores of the fiber and further into a new aqueous phase in the lumen of the fiber (three phase HF-LPME) (Plaza-Bolanos et al., 2008).

Liquid Membrane Extraction Techniques, supported liquid membrane, (SLME), and micro porous membrane liquid–liquid (MMLLE) extractions are based on the use a hydrophobic membrane, containing an organic solvent, which separates two immiscible phases. These extraction techniques are a combination of three simultaneous processes: (1) extraction of analyte into organic phase; (2) membrane transport (3) re-extraction in an acceptor phase. SLME and MMLLE have been successfully applied for enrichment of phenoxy acid, sulfonylurea, and triazine herbicides from environmental water samples.

2.4. SOLID-LIQUID EXTRACTION

Solid–liquid extractions processes are based on the extraction of the analytes from a liquid sample in a solid material (solid phase extraction), or are based on the extraction of the analytes from solid samples with liquid solvents.

2.4.1. Extraction from Liquid Samples

In **Solid Phase Extraction (SPE)**, a liquid phase (liquid sample or liquid sample extracts) is loaded onto a solid sorbent (polar, ion exchange, non-polar,

affinity), which is packed in disposable cartridges or enmeshed in inert matrix of an extraction disk. Those compounds with higher affinity for the sorbent will be retained on it, whereas others will pass through it unaltered. Subsequently, if target analytes are retained, they can be eluted using a suitable solvent with a certain degree of selectivity (Chirila and Draghici, 2011). Understanding the mechanism of interaction between the sorbent and the analyte is a key factor on the development of a SPE method, since it will ease choosing the right sorbent from the wide variety of them available in the market: polar (silica, alumina, florisil), non-polar (n-alkyl-bonded silica, styrene-divinylbenzene based polymers, graphitized carbon), ion-exchange, affinity (immunosorbents, molecularly imprinted polymers).

SPE has demonstrated to be a very useful procedure for the extraction of a great variety of pesticides in food and environmental analysis. However, although in a lower extent than LLE, this technique still requires the use of toxic organic solvents and its applicability is restricted to liquid samples.

Solid Phase Micro Extraction (SPME), introduced in 1989 (Belardi and Pawliszyn 1989) eliminates these drawbacks. The SPME device contains a melted silica fiber, coated with an adsorptive material which adsorbs the pesticides from liquid samples or those contained in headspace. After adsorption, the silica fiber is extracted from the flask and then is coupled to a GC injector, where the analytes are thermally desorbed, cryogenic focused at the entrance of the column, then separated. The SPME process is presented in Figure 4.

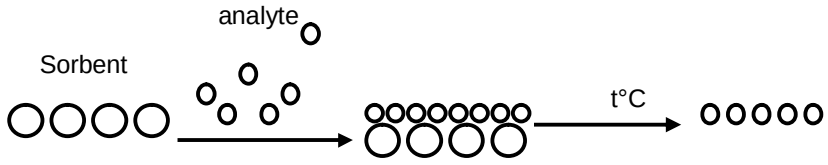

Figure 4. Solid phase micro extraction process

The SPME can use **split-splitless** or **on column injectors.** A proper selection of the SPME sorbent is a key factor in the success of the analysis. In general, the polarity of the fiber should be as similar as possible to that of the analyte of interest. In this sense, there are nowadays a great variety of fibers commercially available that cover a wide range of polarities: carbowax/DVB for polar compounds, or polydimethylsiloxane (PDMS) for hydrophobic compounds.

Stir Bar Sorptive Extraction (SBSE) is similar with SPME, based on the partitioning of target analytes between the sample (mostly aqueous-based liquid samples) and a stationary phase-coated stir bar. The experimental procedure followed in SBSE is quite simple. The liquid sample and the PDMS-coated magnetic stir bar are placed in a container. Then, the sample is stirred for a certain period of time (30–240 min) until no additional recovery for target analytes is observed, even when the extraction time is further increased. Finally, the stir bar is removed and placed in a specially designed unit in which thermal desorption and transfer of target analytes to the head of the GC column takes place.

2.4.2. Extraction from Solid Samples

Solid–liquid extraction is probably the most widely used procedure in the analysis of pesticides in solid samples and includes various extraction techniques based on the contact of a certain amount of sample with an appropriate solvent. The steps that take place in a solid–liquid extraction procedure are: (i) solvent penetration inside the pores of the samples' particles; (ii) desorption of the analytes bound to matrix active sites; (iii) diffusion of the analytes through the matrix; (iv) dissolution of the analytes in the extracting solvent; (v) diffusion of the analytes through the solvent and (vi) recovery of external solvent containing analytes. The final extraction efficiency is influenced by the proper selection of the solvent to be used and also by other parameters such as pressure and temperature. Working at high pressure facilitates the solvent to penetrate sample pores (step 1) and, in general, increasing temperature increases solubility of the analytes on the solvent. Moreover, high temperatures increase diffusion coefficients (steps 3 and 5) and the capacity of the solvent to disrupt matrix–analyte interactions (step 2). Depending on the strength of the interaction between the analyte and the sample matrix, the extraction will be performed in soft, mild, or aggressive conditions. Table 2 shows a summary and a comparison of advantages and drawbacks of the different solid–liquid extraction techniques most commonly employed in the analysis of pesticides in food and environmental samples.

Supercritical Fluid Extraction (SFE) uses as solvents supercritical fluids which can be considered as hybrid between liquids and gases, and possess ideal properties for the extraction of pesticides from solid samples. Supercritical fluids have in common with gases the ability to diffuse through the sample, which facilitates the extraction of analytes located in not easily accessible

pores. In addition, the solvation power of supercritical fluids is similar to that of liquids, allowing the release of target analytes from the sample to the fluid.

TABLE 2. Solid–Liquid Extraction Techniques

Technique	Description	Advantages	Drawbacks
Shaking	samples and solvent are placed in a glass vessel; shaking can be done manually or mechanically	simple fast (15–30 min) low cost	filtration of the extract is necessary dependent of matrix type moderate solvent consumption (25–100 mL)
Soxhlet	sample is placed in a porous cartridge and solvent returns continuously by distillation–condensation cycles	standard method no further filtration of the extract necessary independent of kind of matrix low cost	time-consuming (12–48 h) high solvent volumes (300–500 mL) solvent evaporation needed
USE	samples and solvent are placed in a glass vessel and introduced in an ultrasonic bath	fast (15–30 min) low solvent consumption (5–30 mL). Bath temperature can be adjusted low cost	filtration of the extract is necessary dependent of kind of matrix
MAE	sample and solvent are placed in a reaction vessel; microwave energy is used to heat the mixture	fast (~15 min) low solvent consumption (15–40 mL) easily programmable	filtration of the extract is necessary addition of a polar solvent is required moderate cost
PSE	sample is placed in a cartridge and pressurized with a high temperature solvent	fast (20–30 min) low solvent consumption (30 mL) Easy control of extraction parameters (temperature, pressure) high temperatures achieve	initial high cost dependent on the kind of matrix

USE – ultrasound - assisted extraction; MAE, microwave-assisted extraction; PSE, pressurized solvent extraction

Carbon dioxide is the most used in SFE because it can be obtained with high purity, it is chemically inert, and its critical point (31.1°C and 71.8 atm) is easily accessible. Its main drawback is its nonpolar character, limiting its applicability to the extraction of hydrophobic compounds. In order to

overcome, at least to a certain extent, this drawback, the addition of a small amount of an organic solvent modifier (i.e., methanol) has been proposed and permits varying the polarity of the fluid, thus increasing the range of extractable compounds. However, the role of the modifier during the extraction is not well understood. Once target analytes are in the supercritical fluid phase, they have to be isolated for further analysis, which is accomplished by decompression of the fluid through a restrictor by getting analytes trapped on a liquid trap or a solid surface. With a liquid trap, the restrictor is immersed in a suitable liquid and thus, the analyte is gradually dissolved in the solvent, while CO_2 is discharged into the atmosphere. In the solid surface technique, analytes are trapped on a solid surface (i.e., glass vial, glass beads, solid-phase sorbents) cryogenically cooled directly by the expansion of the supercritical fluid or with the aid of liquid N_2. Alternatively, SFE can be directly coupled with gas chromatography or with supercritical fluid chromatography, and its successful online coupling depends on the used interface, which determines the quantitative transfer of target analytes to the analytical column (Zougagh et al., 2004).

2.5. SOLID-SOLID EXTRACTION

Matrix Solid Phase Dispersion (MSPD), introduced by Barker (Barker et al., 1989), is based on the complete disruption of the sample (liquid, viscous, semisolid, or solid), while the sample components are dispersed into a solid sorbent. Most methods use C8- and C18-bonded silica as solid support. Other sorbents such as Florisil and silica have also been used although to a lesser extent. Experimentally, the sample is placed in a glass mortar and blended with the sorbent until a complete disruption and dispersion of the sample on the sorbent is obtained. Then, the mixture is directly packed into an empty cartridge as those used in SPE. Finally, analytes are eluted after a washing step for removing interfering compounds. The main difference between MSPD and SPE is that the sample is dispersed through the column instead of only onto the first layers of sorbent, which typically allows the obtainment of rather clean final extracts avoiding the necessity of performing a further cleanup.

MSPD has been successfully applied for the extraction of several pesticides from fruit juices, honey, oranges, cereals, and soil, and the obtained results, compared with those obtained by other classical extraction methods, has been found superior in most cases. The main advantages of MSPD are the short extraction times needed, the small amount of sample, sorbent, and solvents required, and the possibility of performing extraction and cleanup in one single step.

3. Air Sampling and Samples Preparation for Pesticides Determination

The atmosphere is known to be a good pathway for the worldwide dissemination of pesticides. Pesticides can enter into the atmosphere by ‘‘spray drift’’ during application, post application volatilization from soils and leaves, and by wind erosion when pesticides are retained to soil particles and entrained into the atmosphere on windblown particles. Pesticides are present in the atmosphere in the gas phase (from volatilization processes) and in the particle phase (including aerosols) (Millet, 2007).

Due to the very low concentrations of pesticides in the ambient air, appropriate sampling and pre-concentration techniques are necessary to achieve the sensitivity of the analytical instruments. The most common sampling techniques for pesticides in ambient air can be grouped into two categories: active and passive (diffusive) samplers.

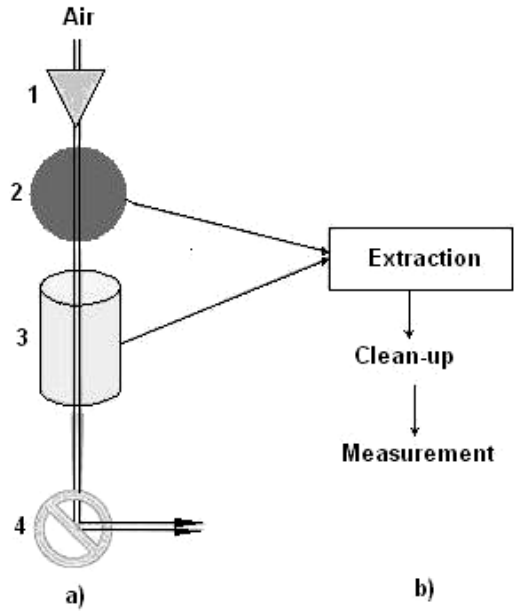

Figure 5. Schematic diagram of a typical active air sampling and the analysis steps of filters and adsorbents: a) – sampling; b) – sample preparation; 1 – sample inlet;
2 – filter; 3 – sorption material; 4 – aspiration pump (adapted from Yusa et al., 2009).

Active sampling is carried out by pumping the air through sorbents, glass fiber or quartz filters. The pesticides in the particulate phase are retained in the filter, whereas those present in the gas phase are trapped by the adsorbent (Figure 5).

Before analysis, the filters and sorption materials are submitted to extraction and clean-up steps.

Passive air sampler (PAS) is based on a device that collects chemicals from the atmosphere without the aid of a pump, and consists in an accumulating medium that has a high-retention capacity for the target analytes. Such samplers allow for integrative (time-averaged concentrations, TWA) sampling in locations where active samplers would not be practical over long periods, due to lack of electricity supply in remote locations. Nevertheless, passive samplers are able to collect only the free gaseous phase pollutants and the duration of sampling ranges from few weeks to several months, significantly larger than the usual time required for active samplers. After sampling, the adsorbed analytes are desorbed off the adsorbent by solvent or thermal desorption.

A range of PAS are available for different chemical species sampling from air. Most commercially available passive/diffusive samplers have a planar or axial shape (Figure 6).

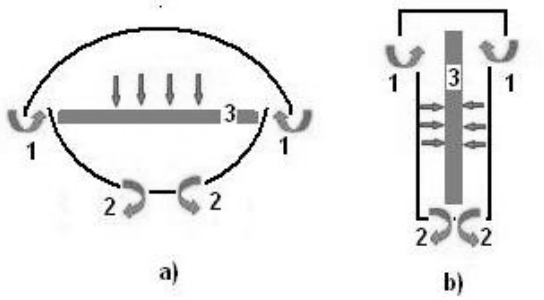

Figure 6. Schematic diagram of passive air samplers: a) planar; b) axial;
1 – air inlet; 2 – air outlet; 3 – adsorption surface.

The used **adsorbent materials** are mainly polyurethane foam (PUF) disks, semi-permeable membrane devices (SPMDs), polymer-coated glass (POG), and styrene – divinylbenzene resin (XAD) (Yusa et al., 2009). In the dynamic air sampling techniques there are used glass-fiber filters (GFF) or quartz-fiber filters (QFF) followed by the adsorption on materials like XAD or PUF. The types of materials used for passive air sampling devices (PAS) and sampling duration are presented in Table 3.

SPMDs comprise a low density polyethylene (LDPE) bag, of 70–90 μm wall thickness, filled with triolein (1,2,3-tris-*cis*-9-octadecenoyl glycerol).

Standard devices are 106 cm long, 2.54 cm wide, and contain 1 mL of triolein. The operation of a SPMD as passive sampler is based on the diffusion of compounds through the polymeric membrane bag and their accumulation in the lipophilic solvent. POG samplers are created by applying a thin polymeric stationary phase (ethylene vinyl acetate-EVA) to a solid glass surface. XAD have been previously used to collect a variety of pesticides including diazinon, chlorpyrifos, disulfoton, fonofos, mevinphos, phorate, terbufos, cyanazine, alachlor, metolachlor, simazine, atrazine, deethyl atrazine, deisopropyl atrazine, molinate, hexachlorobenzene, trifluralin, methyl parathion, dichlorvos, and isofenphos.

TABLE 3. Types of passive air samplers (adapted after Kosikovska and Biziuk, 2010)

Nr.	Type	Sampling duration
1.	XAD-2	5 – 12 months
2.	PUF disks	4 weeks – 4 months
3.	PDMS	14 days
4.	POG	7 days
5.	SPMD	7 days

Solid Phase Micro Extraction (SPME) has significant advantages over the traditional methods: is a solvent-free technique, convenient coupling with field analytical instruments, cost effectiveness and simplicity of operation. Depending on the purpose of the study, sampling time with SPME can range from a few seconds to days, for assessment of short-term and long-term exposures, respectively. For peak concentration, the SPME fiber is exposed to the sample. If a TWA concentration is needed, the fiber is kept retracted in the needle (Wang et al., 2009). Table 4 presents examples of air samples preparation for pesticides analysis in studies published during the last years.

TABLE 4. Examples of air samples preparation for pesticides analysis

Location, samplers, materials	Extraction, solvent	Clean-up	Measurement	Reference
Luxemburg, passive, XAD-2	PSE, acetonitrile	SPME-PDMS	GC/MS-MS	Schummer et al., 2012
Algeria, passive, PUF	Soxhlet, hexane/diethylether	Not specified	GC/MS-MS	Moussaoui et al., 2012
Algeria, active, GFF	Direct, methylene chloride	Not specified		
Spain, active, QFF	MAE, ethyl acetate,	GPC, hexane	GC/EI/MS-MS	Coscola et al., 2011

	dichloromethane			
France, greenhouse, active, SPME/PDMS	Not necessary	Not necessary	GC/MS-MS	
France, greenhouse, active, glass cartridge with PUF	Soxhlet, hexane/diethylether	Kuderna Danish concentration, alumina, hexane	GC/MS	Wang et al., 2009

GFF – glass fiber filter; GPC – gel permeation chromatography; MAE – microwave assisted extraction; QFF – quartz fiber filter; PDMS – polytimethylsiloxane; PSE – pressurized solvent extraction; PUF – polyuretane foam; SPME – solid phase microextraction; XAD – styrene-divinylbenzene resin.

4. Water Samples Preparation for Pesticides Determination

The need for detecting pesticides at trace levels means that a water sample must be reduced many times in such a volume that a small aliquot of the final sample may provide adequate sensitivity for detection. The concentration magnification is achieved through phase transfer by using liquid-liquid extraction or solid-phase extraction. Many other methods may be considered as variations of the traditional LLE and SPE methods (Figure 7).

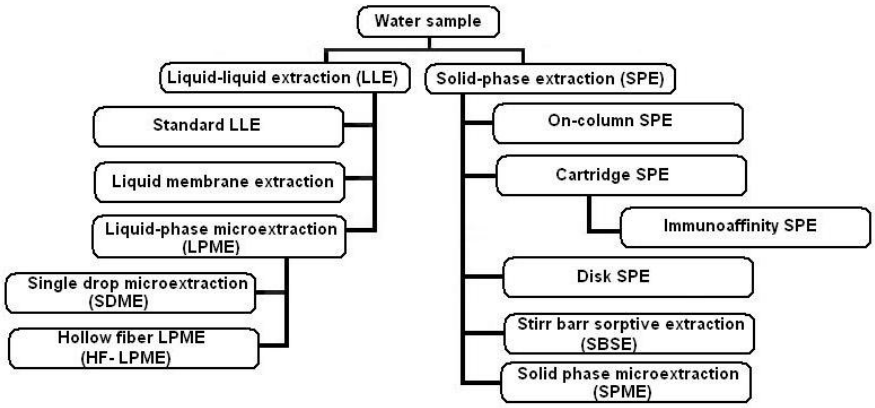

Figure 7. Sample preparation methods for pesticides analysis in water (adapted after Gan and Bondarenko, 2007)

For example, micro-LLE or single-drop extraction can be considered as a miniaturization of the standard LLE procedure. Variations of cartridge SPE include SPE disks and SPME. The available methods can also be classified based on the mechanisms used for pesticide detection. However, as detection methods are usually common among different sample matrices and are not limited only to water, this chapter will mostly focus on sample preparation methods for water analysis, with exceptions made only for immunoassays and capillary electrophoresis (CE) because of their significant deviations from conventional chromatographic methods (Gan and Bondarenko, 2007). Table 5 presents examples of water samples preparation for pesticides analysis.

TABLE 5. Examples of water samples preparation for pesticides analysis.

Water type, location	Sampling and preparation	Measurement	Reference
Lake water, USA	passive – SPE, extraction in methanol, evaporated to 1 mL, filtered, adjusted to 1mL with ethyl acetate	GC-MSD	Charlestra et al., 2012
River water, Spain	100 mL water spiked with standard mixture; 5 mL extracted with automated on-line SPE	LC-MS/MS	Köck-Schulmeyer et al., 2012
River water and tap water, Brazil	SDME - toluene	GC-MS	Pinheiro et al., 2011
River water, Botswana	HS-SPME	GC-ECD	Mmualfe et al., 2009
River water, Iran	SDME – hexyl acetate and derivatization	GC-MS	Saraji and Farajmand, 2008
Groundwater, Spain	SPME; acetonitrile/water	HPLC-PIF-FD	Parilla Vasquez et al., 2008a
Surface water, Europe	passive with Chemcatcher devices, extraction in acetonitrile/methanol, evaporation to dryness, redissolved in acetonitrile	GC-MSD	Schafer et al., 2008

FD – fluorescence detection; HS – headspace; MSD – mass selective detector; PIF – photochemically induced fluorimetry; SDME – single drop microextraction; SPE – solid-phase extraction; SPME – solid phase microextraction.

5. Soil, Biota and Food Samples Preparation for Pesticides Determination

Traditional pesticide residues analysis requires a large amount of organic solvent for sample extraction and a series of steps for preconcentration and clean-up, which are complicated, tedious and expensive (Dobrinas et al., 2004).

The most commonly used methods for solid sample preparation include pressurized solvent extraction (PSE), solid phase extraction (SPE), supercritical fluid extraction (SFE), solid phase microextraction (SPME), headspace – solid phase microextraction (HS-SPME), liquid phase microextraction (LPME), microwave assisted extraction (MAE), matrix solid phase dispersion (MSPD) etc. In addition, Quick Easy Cheap Effective Rugged Safe (QuEChERS) is another very popular analytical method in pesticide residue analysis, while a direct solid sample introduction is also available, and will further be presented.

5.1. QuEChERS METHOD

The recently introduced (Anastassiades et al., 2003) QuEChERS method avoids the use of nonpolar solvents inducing LLP by addition of $MgSO_4$ and NaCl to acetonitrile extracts, which leads to removal of majority of water and highly polar matrix components, yet achieving high recoveries of wide range of both GC- and LC-amenable pesticides. Additional SPE clean-up is performed to remove mainly sugars and fatty acids.

QuEChERS is a sample preparation approach entailing solvent extraction of high-moisture samples followed by clean up using d-SPE. Basically, the sample is firstly extracted with a water-miscible solvent (for example, acetonitrile–ACN) in the presence of high amounts of salts (for example, sodium chloride and magnesium sulfate) and buffering agents (for example, citrate) to induce liquid separation and stabilize acidic and basic labile pesticides, respectively. Upon shaking and centrifugation, an aliquot of the organic phase is subjected to further clean up using dispersive – solid phase extraction (d-SPE), by adding small amounts of bulk SPE packing sorbents to the extract. After sample clean up, the mixture is centrifuged and the resulting supernatant can be directly analyzed, or can be subjected to another concentration, when solvent exchange step if necessary. The sorbents used for d-SPE depend on the matrix compounds to be removed, as following:

- non-polar sorbents (C_{18} or C_8) are retaining lipids from the ACN extract, on which the majority of subsequent studies have demonstrated that it does not negatively affect pesticide recoveries, but helps to obtain cleaner extracts;
- graphitized carbon black (GCB) remove sterols and pigments and provide a greater degree of clean-up, giving less colored extracts;
- primary secondary amine (PSA) has been found as the most effective sorbent for removal of various matrices, significantly reducing matrix-enhancement effect.

The schematic diagram of QuEChERS method is presented in Figure 8.

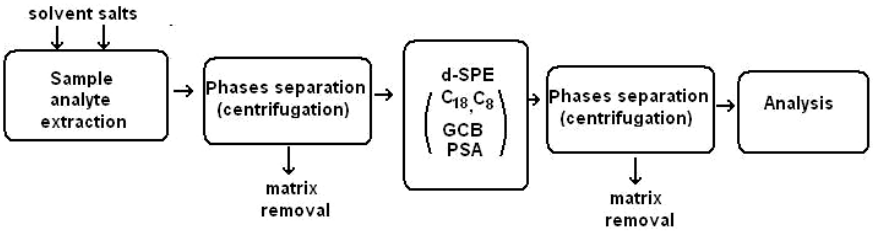

Figure 8. Schematic diagram of QuEChERS sampling method.

This type of solvent extraction method have shortened the whole analytical time and enhanced the extraction efficiency. However, these methods are not simple and quick enough with regard to the problem that more and more samples needed to be analyzed (Pareja et al., 2011, Gonzales-Curbelo et al., 2012).

5.2. DIRECT SOLID SAMPLE INTRODUCTION

In 2005, Zhang and coworkers have developed a new method for pesticide residue analysis in vegetables. Sample without any pretreatment was directly introduced into the split/splitless injector for GC–MS determination. This method was proven to be quick, convenient and accurate. It also worked well for rapid detection of pesticide residues in food and large scale screening of samples in field detection. In addition, the GC split/splitless injector was simply modified to quickly remove oxygen and low boiling point matrices of the sample by adding a venting gas line on the original pneumatic system. No sample pretreatment was needed and the sampling procedure required less than 5 min. The injector's modification can be conducted on portable GC. Hence this method is potential for field analysis of pesticide residues in crops and large scale screening of samples (Ng and Zang, 2011).

A known quantity of crushed solid sample having a diameter >1.5 mm is added in the middle of a glass liner and supported by a little bit glass wool. Finally the glass liner is installed back to the injector for GC analysis. The solid sample is disposable after each test. Next sample could be transferred into the liner for another experiment immediately.

Table 6 presents examples of solid samples preparation for pesticides analysis in papers published in last years.

TABLE 6. Examples of solid samples preparation for pesticide analysis.

Sample	Sample preparation	Measurement	Reference
Tea	QuEChERS + LLE	GC/MS-MS	Cajka et al., 2012
Lettuce	SPME	HPLC/DAD	Melo et al., 2012
Seaweeds	MSPD	GC/MS	Garcia-Rodriguez et al., 2012
Fruits	QuEChERS	GC/MS	Cieslik et al., 2011
Plant materials for medicine	QuEChERS	GC/ECD	Xu et al., 2011
Vegetables	Ultrasonic extraction dichloromethane	GC/MS	Latif et al., 2011
Crops	Direct solid sample introduction	GC/ECD	Ng and Zang, 2011
Cow milk	HS-SPME	GC/MS	Rodrigues et al., 2011
Bovine meat and liver	MSPD	HPLC/DAD	Garcia de Llasera and Reyes-Reyes, 2009
Tea	HS-SPME	GC×GC/TOF/MS	Schureg et al., 2008
Cucumber, watermelon	SPME	HPLC/PIF/FD	Parilla Vasquez et al., 2008b

FD – fluorescence detection; PIF – photochemically induced fluorimetry; TOF – time of flight.

6. Conclusions

This paper presents an overview of the available sample pretreatment techniques to be used for pesticides analysis from all types of gaseous, liquid and solid samples. Actual trends in analytical techniques are the simplification and miniaturization of sample preparation as well as the minimization of the solvents volumes used. There are several novel micro-extraction techniques which improve the sample preparation steps. These require the design and formulation of new materials capable to provide selective extraction of the organic pollutants, like pesticides are.

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