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Modern Sample Preparation Techniques for Pesticide Analysis

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1. Introduction

Sample preparation is applicable to all sample matrices; for example biotechnological, biological, environmental, forensic and pharmaceutical as it presents tremendous benefits. The reduction of interferences helps to prevent an overload on separation columns and subsequently extends the durability of analytical columns. Selective extraction improves the detection sensitivity levels of analytes of interest and simplifies the subsequent interpretation of chromatograms and other analytical data (Fontanals et al., 2007). Automation of the extraction process results in faster analysis and hence reduced overall cost of analysis (Smith, 2003).

Traditional sample preparation techniques such as liquid-liquid extraction (LLE) and soxhlet extraction generated large volumes of solvent, were laborious, had low sample throughput and lacked selectivity (Baltussen et al., 1997; Moreno et al., 2007; Shimelis et al., 2007). Modern sample preparation techniques such as solid phase extraction (SPE) with its various commercially available sorbents and solid phase microextraction (SPME) have been driven by the move towards the consumption of micro-volumes of solvent to techniques that offer solventless extractions, automation, sample throughput, enrichment factor and selectivity (Wells and Lloyd, 2002; Hyötyläinen and Riekkola, 2008). These extraction approaches are usually easy to carry out and call for optimization of several parameters to enhance the performance of the overall analysis.

The key to rational choice of a sample preparation technique for a particular matrix is based on an understanding of the fundamental principles governing the kinetics of mass transfer within the extraction system (Pawliszyn, 2003). This chapter briefly describes the fundamental principles of some of the modern sample preparation techniques employed to liquid and solid matrices for pesticide analysis. Parameters to be considered with each technique such as sample matrix type i.e. liquid or solid and the physicochemical properties of the analyte(s) of interest are discussed. Optimisation parameters for each technique are also discussed.

2. Liquid phase microextraction (LPME)

This technique is a miniaturization of the traditional LLE and employs micro-volumes of solvent instead of the traditional tens or hundreds of milliliters (Mahugo-Santana et al., 2011). LPME is a two-phase system whereby analytes are transferred from an aqueous

sample solution (donor phase) into an organic solvent (acceptor phase). The organic phase is usually a micro-drop of 1-3 µL volume suspended from a syringe (single drop microextraction [SDME]) or present in the pores of a hydrophobic membrane (supported liquid membrane extraction [SLME]).
LPME is an equilibrium process and as such the distribution ratio (K) of an analyte in both the organic and aqueous sample can be described as

$$K = \frac{C_{org,eq}}{C_{aq,eq}} \quad (1)$$

Where
$C_{org,eq}$ = equilibrium concentration of an analyte in the organic phase
$C_{aq,eq}$ = equilibrium concentration of an analyte in the aqueous phase
The mass balance relationship becomes

$$C_{tot}V_{aq} = C_{org,eq}V_{org} + C_{aq,eq}V_{aq} \quad (2)$$

Where
C_{tot} = total concentration of analytes in the aqueous sample
V_{aq} = volume of the aqueous sample
V_{org} = volume of the organic phase
The enrichment factor can be calculated from equations (1) and (2)

$$EF = \frac{1}{\left(\frac{V_{org}}{V_{aq}} + \frac{1}{K}\right)} \quad (3)$$

Equation (3) shows that in order to obtain a high EF, a low organic-aqueous volume ratio and a high distribution coefficient are required. Conditions that are essential for LLME are;

- The immiscibility of the organic and aqueous phases
- Moderately polar and non-polar analytes that will be extracted with ease into the non-polar organic phase

In three-phase LPME, the analyte (X) is extracted from an aqueous sample solution (donor phase) through an organic solvent immobilized in a porous hydrophobic membrane(organic phase) into an aqueous solution (acceptor phase) contained within the membrane. Equation (4) describes the extraction process

$$X_{donor} \leftrightarrow X_{organic} \leftrightarrow X_{acceptor} \quad (4)$$

SLM requires that analytes be ionisable, hence limited to acidic or basic compounds and pH adjustment is critical for the diffusion of analytes through the membrane. SDME and membrane liquid phase microextraction are discussed further in sections 2.1 and 2.2.

2.1 Single drop microextraction (SDME)

The possibility of performing LLE in a micro-scale was introduced by Liu and Dasgupta (1996) when they extracted sodium dodecyl sulphate as an ion pair into a 1.3 µL drop of chloroform enclosed inside the aqueous drop. Later in the same year, the technique was termed solvent microextraction by Jeannot and Cantwell (1996). An 8 µL droplet of 1-octanol was suspended at the end of a Teflon rod in a stirred aqueous solution and after a fixed time, the Teflon rod was withdrawn from the aqueous solution and the octanol drop sampled with a micro-syringe for injection into the GC. Jeannot and Cantwell (1997) as well

as He and Lee (1997) independently improved the technique by the use of a micro-syringe, hence the name – single drop microextraction. The SDME syringe can be fully immersed into a solution (static SDME) (Fig. 1).

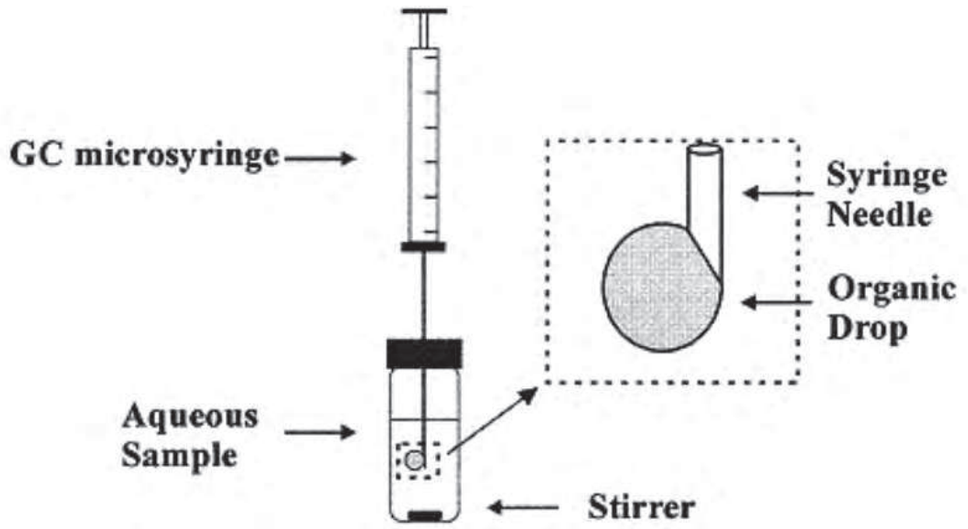


Fig. 1. Schematic representation of static single drop microextraction (static SDME) (Xu et al., 2007).

Alternatively, dynamic SDME can be carried out with a repeated movement of the syringe plunger (Fig. 2).

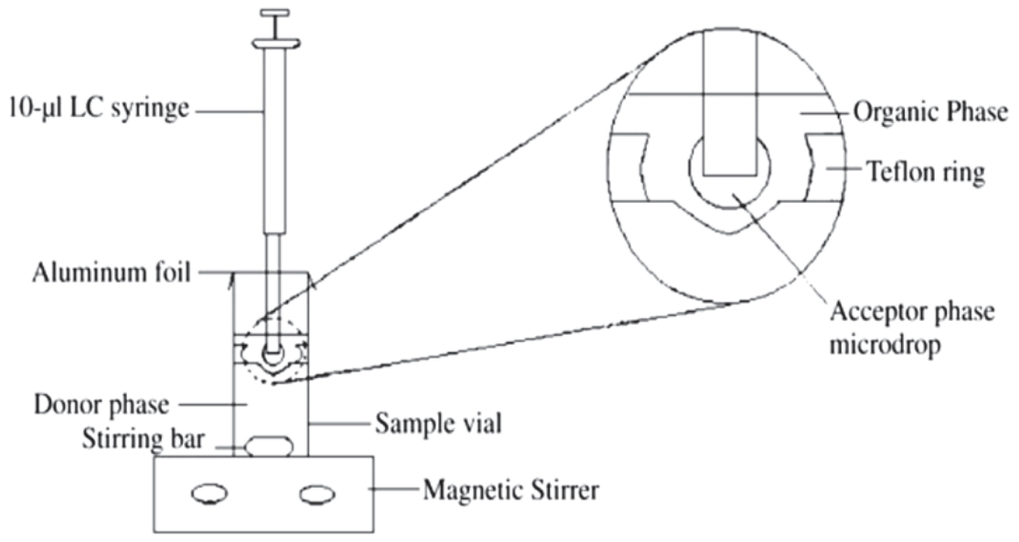


Fig. 2. Schematic representation of dynamic single drop microextraction (DSDME) (Lambropoulou, 2007).

Liu and Lee (2000) introduced a continuous flow microextraction in which an aqueous sample solution flows continuously past a microdrop of solvent (1-5 µL) suspended from a microsyringe. The organic microdrop interacts continuously with the sample solution and

hence allows enrichment factors much higher (1000-fold) than those obtained in static SDME. Table 1 lists the advantages and limitations of SDME.

Advantages	Limitations
Simple to operate	Requires high manual dexterity
Requires inexpensive apparatus	The organic solvent has to have a high surface tension to allow it to hang at the end of a microsyringe
Uses micro-quantities of organic solvent	Long extraction times due to low stirring rates
Allows high throughput sampling and pre-concentration	Instability of the droplet i.e. the microdrop is affected by matrix components such as humic acids and suspended solids causing dissolution of the microdroplet

Table 1. Advantages and limitations of SDME (Psillakis and Kalogerakis, 2002).

Parameters to consider for optimization in SDME

- *Duration of extraction* – extended sample extraction times may cause swelling of the solvent drop size as the solvent absorbs analytes and water and may result in the instability of the drop.
- *Physical and chemical properties of the solvent* – the extracting solvent should be immiscible with water and not be volatile so as to remain stable during the extraction and give good reproducibility. The analytes should be more soluble in the extracting solvent than in the sample solution.
- *Drop size* – Solvent drop volumes between 1 and 2 μL are preferred as those larger than 2 μL are less stable and result in poor reproducibility (Wardencki et al., 2007).
- *Stirring rate* – The stirring rate directly affects mass transfer kinetics of the analytes from the sample solution to the extracting drop but high stirring rates may de-stabilize the solvent drop.
- *Temperature during extraction* – higher temperatures result in higher extraction efficiencies but may also cause solvent drop instability hence most extractions are carried out at room temperature (Lopez-Blanco et al., 2005).

SDME has been widely employed as a sample preparation technique for pesticide analysis and Table 2 shows some examples.

2.2 Membrane liquid phase microextraction

Further improvements of SDME were aimed at minimizing the instability of the microdrop by supporting it with a polymeric membrane (Jönsson and Mathiasson, 2001). This acts as a barrier between the aqueous and organic phases and allows the use of larger solvent volumes. Hollow fiber liquid phase microextraction membranes (HF-LPMEs) can be in the form of flat, rod or U-shaped formats and involve the use of a membrane placed between stagnant aqueous and organic phases (Fig. 3).

Supported liquid membrane (SLM) is a three-phase system and consists of two flat membranes sandwiched by an organic solvent-impregnated membrane (Fig. 4).

This format requires a pump to drive the donor phase through one membrane and the acceptor phase through the other membrane and the extraction process is described by equation (4).

Sample	Analyte	Solvent	Final analysis	Reference
Water	Organochlorines	Hexane	GC/ECD	Jager and Andrews, 2000.
Water	Organophosphorus pesticides	Isooctane	GC/NPD	Lopez-Blanco et al., 2003.
Water	α- & β- Endosulfan	Isooctane	GC/ECD	Lopez-Blanco et al., 2005.
Water	Organophosphorus pesticides	Toluene	GC/MS	Lambropoulou et al., 2004.
Water	Chloroacetanilide pesticides	Toluene	GC/μECD	Zhao et al., 2006.
Water, fruit juice	Organophosphorus pesticides	Toluene	GC/FPD	Ciao et al., 2006.
Orange juice	Organophosphorus	Toluene	GC/FPD	Ahmadi et al., 2006.

Table 2. Examples of SDME applications in pesticide analysis

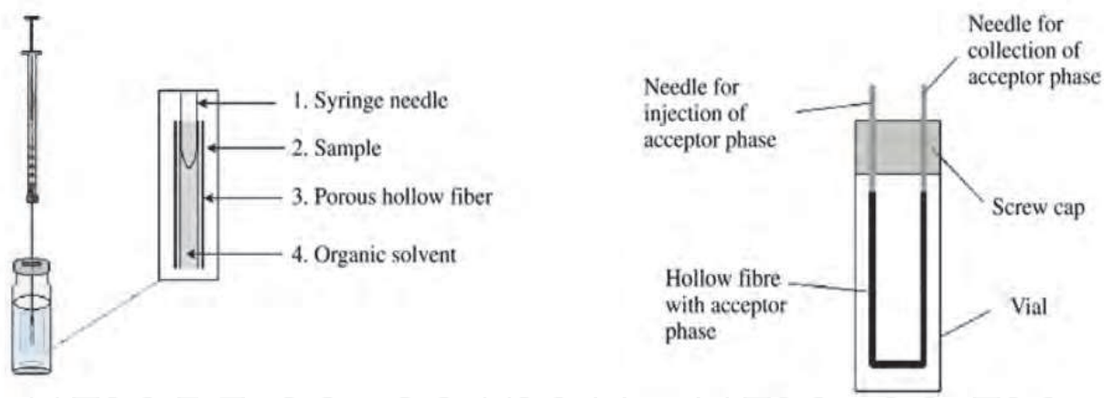


Fig. 3. Schematic representation of rod and U-shaped porous hollow fiber membranes.

Parameters to be optimized in SLME

- *Extracting solvent* – the extracting solvent should be immiscible with water and strongly immobilized in the pores of the membrane. In addition, the solvent should not be volatile as that would result in poor reproducibilities.
- *Pore size of the membrane* – This affects selectivity as some degree of size exclusion also occurs during extraction (Hyötyläinen and Riekkola, 2008).

3. Solid phase extraction (SPE)

Solid phase extraction (SPE) is based on selective retention of analytes on a sorbent and subsequent elution with a suitable solvent (Jakubowska et al., 2009). SPE generally involves four steps i.e.

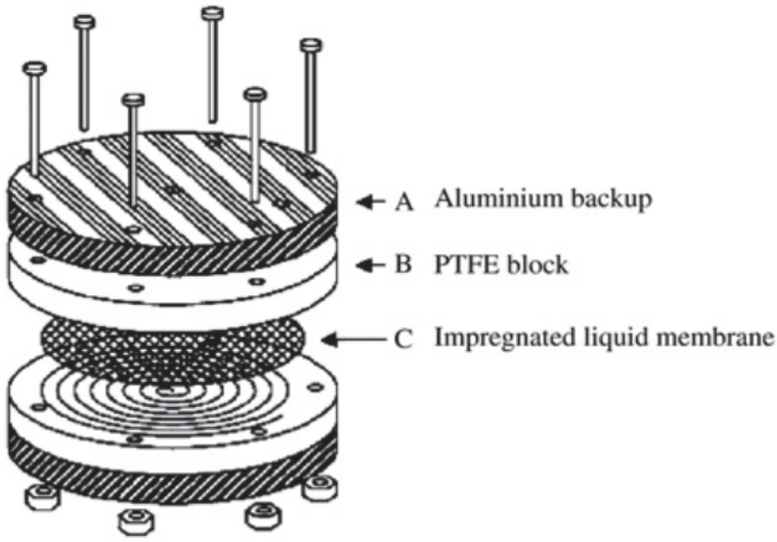


Fig. 4. Schematic diagram of a supported liquid membrane extraction (SLME).

1. *Sorbent conditioning step* – functions to activate or 'wet' the sorbent to prepare for its interaction with the analyte. This is especially necessary for hydrophobic sorbents that would not be activated by an aqueous sample. If the sorbent is not adequately conditioned, poor reproducibility and analyte recoveries may be obtained. If pH is critical for retention, then the conditioning solvent has to be adjusted to match the sample pH. The organic strength of the conditioning solvent also has to be matched to that of the sample to prepare for maximum retention of the analyte during the loading step.
2. *Sample loading step* – when the sample is added to the sorbent, sufficient residence time should be allowed for maximum interaction and avoid breakthrough. This is especially critical when employing ion exchange to provide adequate residence time of the sample solution in the sorbent since the analyte has to achieve an appropriate orientation for electrostatic retention with the sorbent functional groups.
3. *Washing step* – this serves to remove/minimize interferences retained on the sorbent and should not affect the retained analytes. The elution strength of the wash solvent should be higher than that of the sample solution but should be less than that of the elution solvent. This may entail employing a solvent with a higher organic phase content, ionic strength or different pH in comparison to the sample solution. However, a wash solvent that is too weak will not remove interferences but will result in co-elution of interferences with the analytes in the elution step. Hence optimization of this step is aimed at identifying the strongest solvent to ensure the highest recovery of analytes and minimal interferences. In mechanisms employing ion exchange, the pH of the wash solvent should be sufficient enough to disrupt the charged sites of interferences but not affect the analyte.
4. *Elution step* – the elution solvent should be strong enough to disrupt all analyte-sorbent interactions in order to obtain the highest recoveries. However the use of harsh solvents will not only strip analytes from the solvent but will elute strongly adsorbed interferences as well.

Things to consider before SPE;

- Log P – this is the octanol-water partition coefficient and is an indicator of the hydrophobicity of an analyte. If the Log P is positive, the analyte is hydrophobic and hence hydrophobic sorbents such as C_8 and C_{18} should be employed to obtain sufficient analyte retention
- pKa – this describes the extent of ionisability of the analyte. When analytes contain acidic or basic functional groups with low (<1.5) or high (>9.5) pKa values, then weak anion or cation exchange sorbents are required. The charge states of weak ion exchange sorbents are more easily altered by solution pH to strengthen or disrupt the analyte-sorbent interaction. When pka values are between 2 and 8, strong or weak ion exchange sorbents can be employed and it is possible to alter the charge state of either the analyte or sorbent through solvent pH. Mixed mode sorbents, consisting of a polymeric hydrophobic backbone with ionisable functional moieties make use of both hydrophobic and ionic interactions and are popular in modern SPE especially in the pharmaceutical industry. In the environmental industry, mixed mode sorbents are employed to selectively extract both acidic and basic pesticides. The solution pH should be adjusted such that both the analyte and sorbent are ionized during sample loading and then re-adjusted to neutralize either the analyte or sorbent for the elution step.

In addition to the 4 basic steps of SPE protocol, extra steps such as soaking and sorbent drying can be included in the SPE protocol to help improve recoveries. The soaking step may be employed to enhance interaction between the analyte and the sorbent and can be applied to the conditioning or loading steps. The drying step may be necessary after the wash step especially if the elution step involves the use of an organic solvent that is immiscible with the aqueous sample solution. The drying step would serve to eliminate aqueous films on the sorbent surface and allow interaction between the organic elution solvent and the analyte-sorbent bonds. Even if the elution solvent is miscible with the sample solution, a drying step may be necessary to reduce the duration of subsequent solvent evaporation of the eluate. This is necessary when the eluate has to be concentrated down by evaporation or if the analytes have to be re-constituted in a different solvent that may be more compatible with the analytical instrument. SPE is widely popular with liquid samples and boasts of several advantages listed in Table 3.

Advantages	Limitations
Less time consuming than traditional LLE	Considerable variation of the performance of the same type of sorbent e.g. C_{18}, amongst the different manufacturers
Eliminates the formation of emulsions characteristic of LLE	Lot-to-lot variation of sorbents from the same manufacturer
Low volumes of solvent used	Sample clean-up of large sample volumes is not always possible
High sample throughput with SPE manifolds or 96-well plates	SPE cartridge materials may absorb analytes and increase interferences in the analysis.
Can be automated	
Availability of several commercial SPE sorbents in various formats	

Table 3. Advantages and limitations of SPE

There are several published applications of SPE in pesticide analysis employing mostly C_{18} and polymeric sorbents (Martel and Zeggane, 2002; Jimenez et al., 2001). New types of sorbents such as graphitised carbon sorbents have demonstrated high recoveries for polar pesticides that were poorly recovered with the classical C_{18} (Hennion, 2000). Magnetic nanoparticles have also found applications in environmental analysis (Ding et al., 2010). Carbon ferromagnetic nanocomposites containing a hydrophobic sublayer and a hydrophilic surface are employed in dispersive SPE. This eliminates packing columns and the magnetic particles can easily be removed from the sample matrix by the use of a magnet (Khajeh, 2009; Zhao et al., 2008; Liu et al., 2009; Ji et al., 2009). Adeyemi and co-workers (2011) have employed electrospun nanofiber sorbents for the pre-concentration of DDE with subsequent desorption by hot water extraction, thus eliminating the use of organic solvent for elution. The wide applicability of SPE in sample preparation has led to the development of more specialized SPE sorbent materials such as molecularly imprinted polymers (MIPs), immunosorbents (ISs) and restricted access materials (RAMs) (Hennion, 1999) that are discussed in sections 3.1, 3.2 and 3.3.

3.1 Molecularly imprinted polymers (MIPs)

Molecularly imprinted polymers (MIPs) possess recognition sites that are adapted to the 3-dimensional shape and functionalities of the analyte of interest. They have demonstrated high specificity by being capable of repeated binding and re-binding to analytes of interest in the presence of other closely related compounds hence their incorporation as SPE sorbents (Caro et al., 2006; Kandimalla, 2004; Qiao et al., 2006). In aqueous mobile phases, MIPs display reversed phase interactions. MIP sorbents are robust, resistant to a wide range of pH, solvents and temperatures (Yin et al., 2006). The greatest limitation of MIPS is the incomplete removal of analytes during the elution step hence they are susceptible to poor recoveries and carry-over after repeated use.

3.2 Immunosorbents (ISs)

Immunosorbents contain covalently bonded immobilized antibodies or antigens that have a strong affinity for their corresponding antigens or antibodies (Majors, 2007). ISs have been widely employed for sample preparation in the pharmaceutical and food industries and have subsequently found applications in the environmental industry (Delaunay et al., 2000; Stevenson, 2000; Delaunay-Bertoncini et al., 2001). Class specific ISs are commercially available for herbicides. In a comparison of three anti-atrazine ISs by Delaunay-Bertoncini and co-workers (2003), anti-ametryn IS bound to all atrazines in the study while anti-atrazine IS was found to be specific to the chloroatrazines and anti-dichloroatrazine IS was specific to tertbutylatrazine and cyanazine. Finding the appropriate solvent for elution of analytes from immunoaffinity sorbents can be a challenge since organic solvents denature antibodies hence the use of competitive binding agents, pH and temperature variation (Hennion and Pichon, 2003). An elution solvent of MeOH:Water (1:1 v/v) gave the highest recovery of diazinon (93%) on an anti-diazinon IS (Prince et al., 2001).

3.3 Restricted access materials (RAMs)

These were initially employed for the extraction of low molecular drugs from biological fluids but have since found use in the extraction of herbicides from surface waters (Simpson, 2000; Boos and Grimm, 1999; Hogendoorn and van Zoonen, 2000). RAMs function by

preventing/restricting access of macro molecules such as proteins to regions of the sorbent surface where analyte retention occurs (Fig. 5)

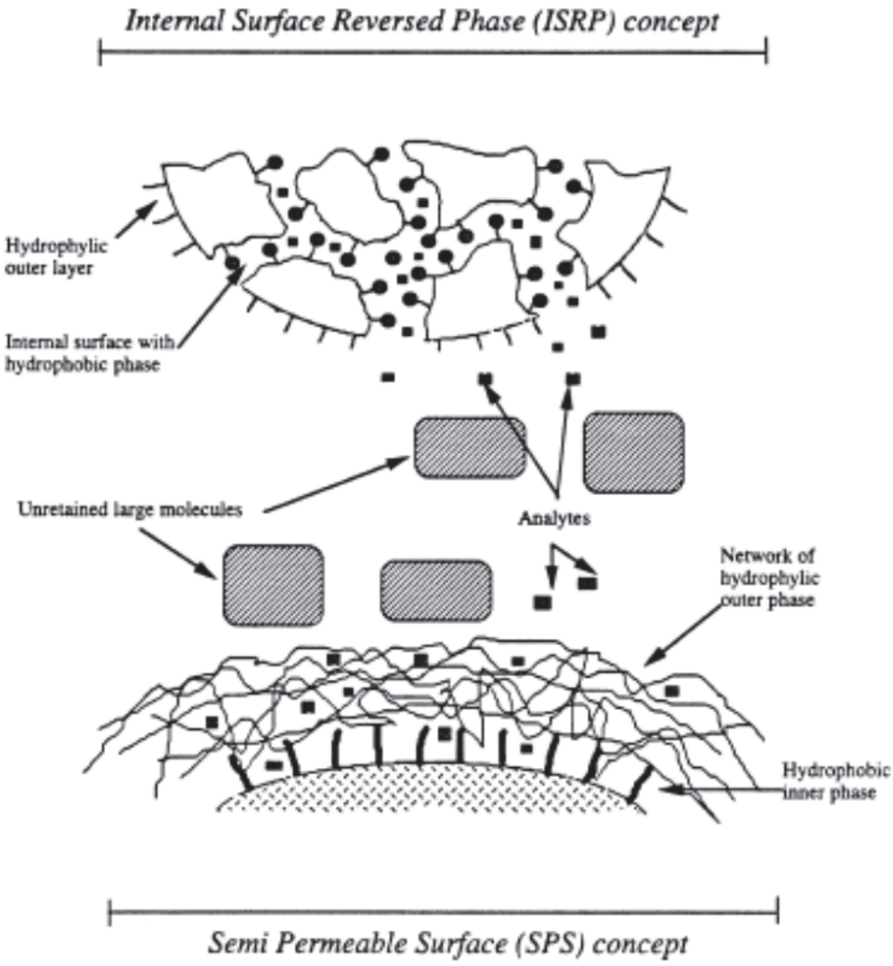
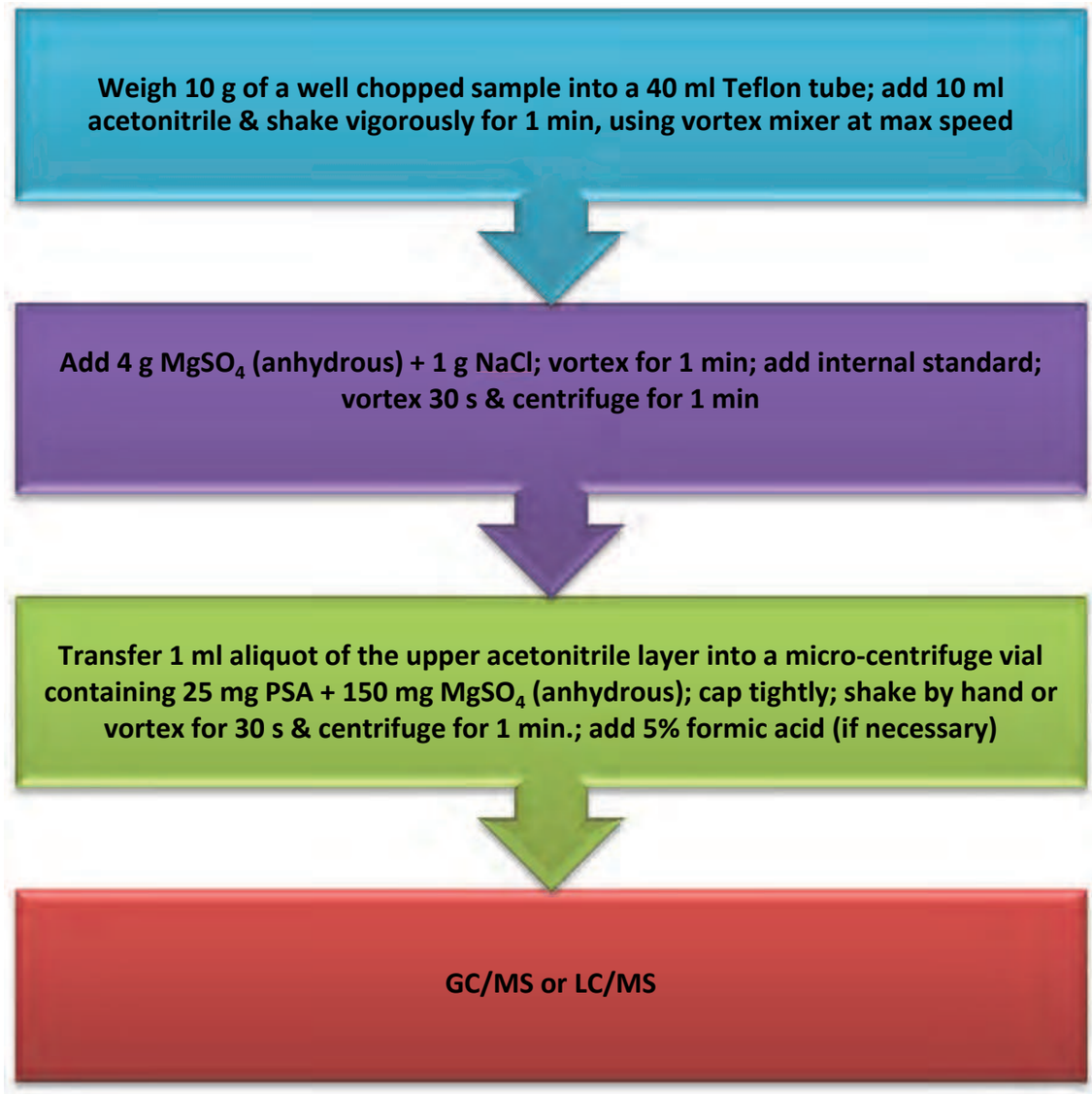


Fig. 5. Schematic representation of the principle of restricted access materials (Poole, 2003).

Restricted access to the retentive part of the sorbent is achieved by either size exclusion in internal surface reversed sorbents or a chemical diffusion barrier such as polymer network on the outer sorbent surface in semi-permeable surface materials. 60-90% recoveries were obtained for acidic pesticides in organic matter - rich soil cleaned online with RAM-C_{18} internal reverse phase GFF-II columns (Hogendoorn et al., 2001).

4. QuEChERS

The quick, easy, cheap, effective, rugged and safe (QuEChERS) technique was developed by Anastassiades and colleagues (2003) for the simultaneous extraction/isolation and clean-up of pesticides in food matrices. Since its inception, the technique has gained popularity as a multi-class pesticide extraction method that combines several steps of sample preparation into basically three steps involving extraction, dispersive SPE clean-up and solvent exchange. Scheme 1 shows the general steps in the QuEChERS technique;



Scheme 1. The main steps in the QuEChERS protocol.

- *Extraction* – a chopped sample is extracted with acetonitrile. The sample must be thoroughly homogenized & sufficient water must be added to dry samples & this volume should reflect in the final calculations. $MgSO_4$ and NaCl are added to ensure that water is separated from acetonitrile.
- *Cleanup* – dispersive SPE is carried out to remove a majority of the matrix. C_{18} & primary secondary amine (PSA) help to remove a fatty matrix – more may be added to remove fatty acid co-extractives in cereals and grains at the expense of ~20% loss of certain polar pesticides (Lehotay et al., 2005). The inclusion of graphitic carbon black (GCB) in the d-SPE assists to remove pigments such as chlorophyll from dark green vegetables even though it reduces recoveries of some planar pesticides (Anastassiades et al., 2003). Both column based SPE and d-SPE were compared using C_{18}, PSA and GCB and d-SPE was found to be more effective and flexible (Table 4).

	Traditional column based SPE	Dispersive SPE
Advantages	• Ensures better sample clean-up	• Ensures larger and more reproducible recoveries of analytes with acidic or basic properties (e.g. acephate, carbendazim, imazalil, methamidophos, pymetrozine and thiabendazol), • Does not require SPE apparatus, cartridges, vacuum, pretreatment of sorbent, channelling, drying out, collection tube, flow control, elution solvent, dilution of extract or solvent evaporation steps, d-SPE is therefore quicker and cheaper • Uses less sorbent, smaller amounts of sample and less equipment; d-SPE is thus a cheaper and easier technique • Provides better interaction with the extract for clean-up
Disadvantages	• Requires plastic cartridges containing 250–2000 mg of a sorbent material and vacuum manifolds, • Requires a larger sample, • Requires column preconditioning, solvent evaporation steps, manual operation and multiple solvents, • Generates solvent waste fractions	• Can only be used when the SPE sorbent removes matrix components and not the analytes

Table 4. Comparison of column based SPE with dispersive SPE (Wilkowska and Biziuk, 2011).

- *Solvent exchange* - ensures that the final solvent is compatible with the analytical instrument and may be used to concentrate analytes in order to reach lower detection limits. Solvent exchange may also function to discriminate against undesired compounds in the final extract eg changing the solvent from acetonitrile to hexane:acetone (9:1) in green tea extracts excluded caffeine & polyphenols (due to their poor solubility in the final solvent system) (Anastassiades et al., 2003).

After the introduction of the multiclass - multiresidue QuEChERS method, it was realised that recoveries of some "problematic" pesticides such as chlorothalonil, folpert and tolylfluanid were not improved. These pesticides are base-sensitive, unstable and were poorly recovered by any existing multiresidue method (Maštovská and Lehotay, 2004). This led to modifications of the original method to an acetate-buffering version adopted as the AOAC Official Method 2007.01 (Lehotay, 2007) and the citrate-buffering version adopted as the European Committee for Standardisation (CEN) Standard Method EN 15662 (Lehotay et al., 2010). When the acetate and citrate buffered versions were compared alongside the unbuffered original method on apple-blueberry sauce (mixture of fruits), peas (a green vegetable) and limes (acidic fruit), all gave good recoveries with the acetate buffered method giving the highest recoveries. However, recoveries for the "problematic" pesticides were consistently low amongst the three methods but better with the acetate buffered method (Lehotay, 2010).

QuEChERS has not only proved to be a useful sample preparation technique for pesticides in different classes of fruits and vegetables including those with a high fat and pigment content, but has found applications in the determination of pesticide residues in other food types such as fish (Mpofu et al., 2011), veterinary drugs in animal tissue (Stubbings and Bigwood, 2009), hormone esters in muscle tissue (Costain et al., 2008). A version of QuEChERS has been suggested by Pinto and colleagues (2010) for the extraction of organochlorines in soils. The flexibility of QuEChERS to various matrices will continue to open its potential to many more applications beyond pesticides (Wilkowska and Biziuk, 2011).

5. Solid phase microextraction (SPME)

Solid phase microextraction (SPME) was introduced by Arthur and Pawliszyn (1990) and has found applications in the environmental, food and medical industries have since followed its commercialization in 1993. The SPME device (Fig. 6) is based on a fused – silica fiber, coated with a thin layer of polymeric sorbent or immobilised liquid that is encased in a steel needle within a syringe-like arrangement.

The SPME device can be immersed directly into gaseous or liquid samples or suspended in the headspace above liquid or solid samples (HS-SPME). The principle behind SPME is based on a partition mechanism and the establishment of an equilibrium between the analyte adsorbed on the fiber and analyte in the sample matrix. The partition coefficient, K, is described as;

$$K = \frac{C_e}{C_s} \tag{5}$$

Where

C_e *and* C_s are the equilibrium concentrations of the analyte in the extracting sorbent and sample matrix, respectively.

At equilibrium, a linear relationship exists between the number of moles of an analyte adsorbed on the fiber and the concentration of the analyte in the aqueous phase. The relationship is represented by the equation;

$$n_e = \frac{KV_eV_sC_s}{KV_e + V_s} \quad (6)$$

Where
n_e = the number of moles of the analyte extracted into the extracting sorbent
V_e = volume of analyte in the extracting sorbent
V_s = volume of analyte in the sample solution (Arthur and Pawliszyn, 1990; Lord and Pawliszyn, 2000).

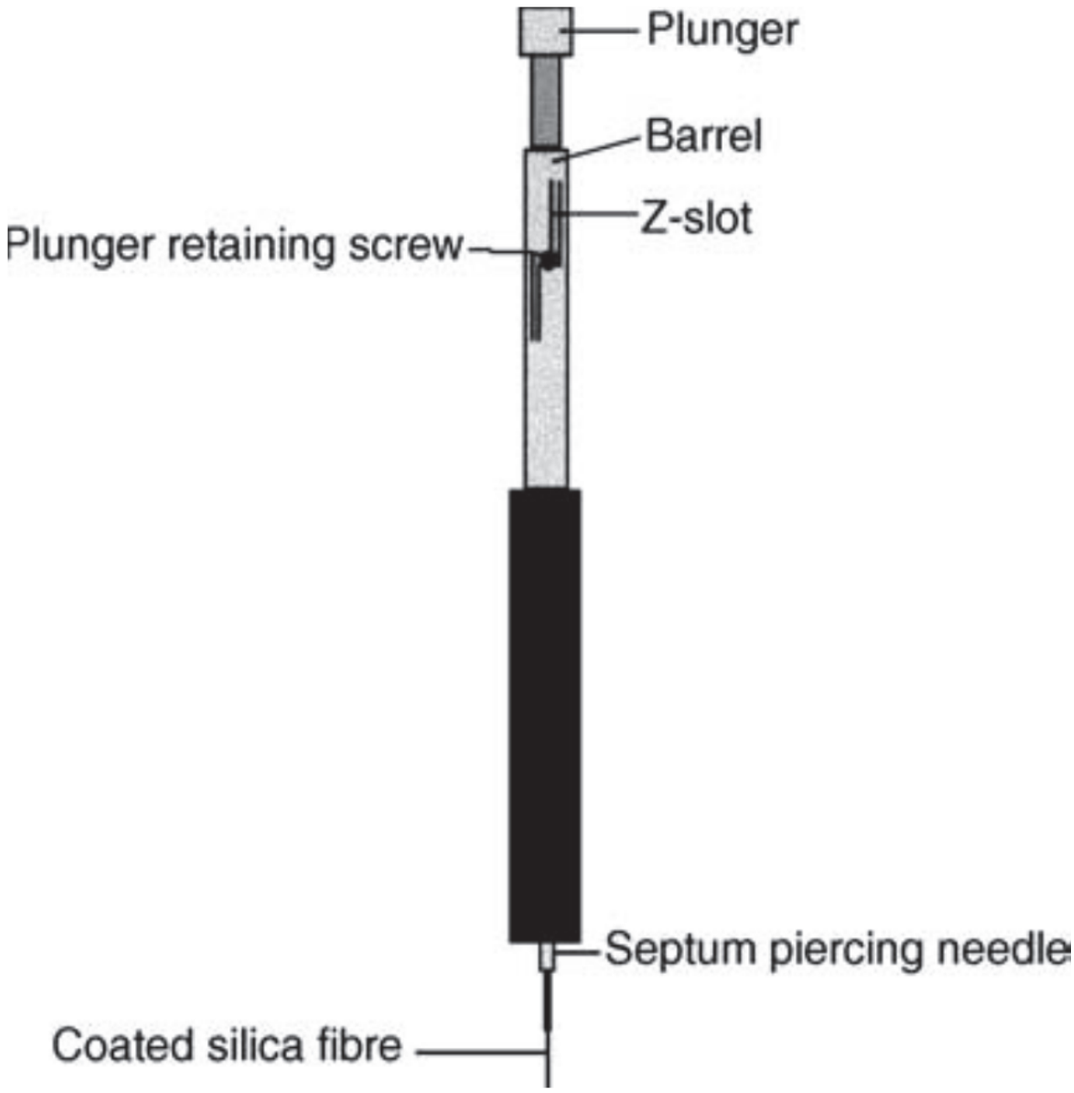
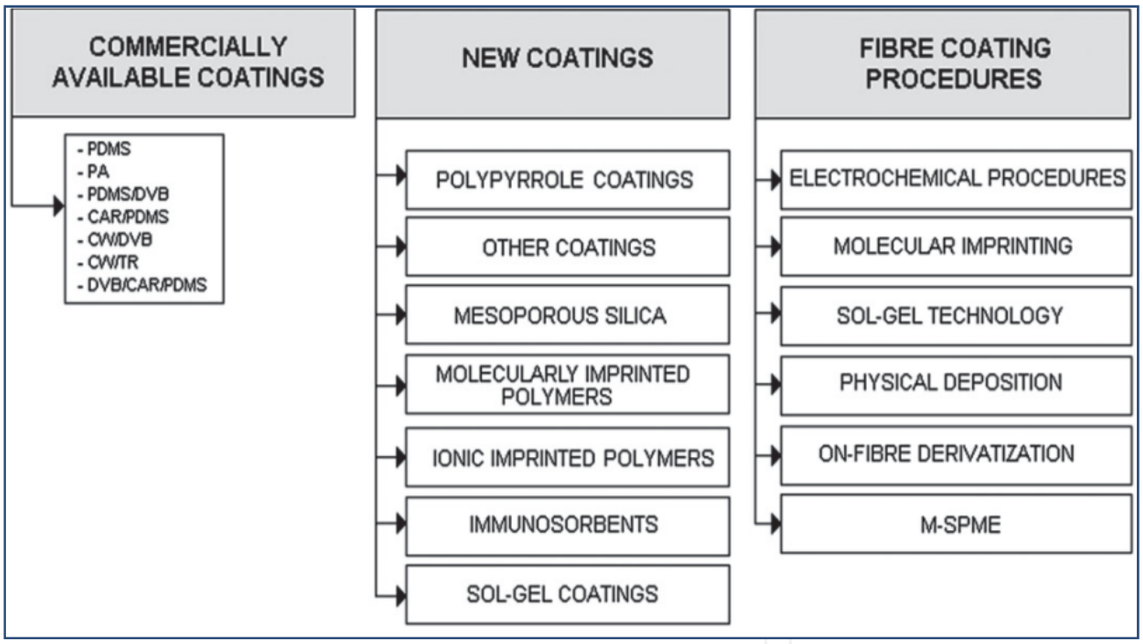


Fig. 6. Schematic diagram of a SPME device (King et al., 2003).

Advantages	Limitations
Ease of operation	Fragility of the fibers
Solventless extraction	Poor extraction efficiency of polar analytes contained in polar matrices
Short extraction time	Limited choice of commercially available fibers
Compatibility with GC	
Possibility of automation	

Table 5. Advantages and limitations of SPME (Zhang et al., 1994).

The limited choice of commercially available fibers has led to the in-house fabrication of various fiber types. Scheme 2 shows the different types of commercially available fibers, in-house fabricated fibers and the techniques employed for their synthesis.



Scheme 2. A diagrammatic representation of the various SPME sorbent and the techniques employed for their synthesis (Spietelun et al., 2010).

Parameters to consider during the optimization of SPME

- *Mode of extraction* – volatile analytes are often sampled in the headspace mode as this eliminates simultaneous adsorption of interferences and safe-guards against deterioration of the fiber coating experienced during direct immersion (Kataoka et al., 2000).
- *Sample volume* – lower sample volumes have been found to favour higher extraction efficiencies since equilibrium is reached faster without overloading the fiber coating (Pawliszyn, 1997; Yang and Peppard, 1994).

- *Temperature and extraction time* – A higher extraction temperature facilitates transport of analytes from the sample solution to the headspace, however excessive temperature may result in premature desorption of analytes from the fiber. Longer extraction times allows more analyte adsorption on the fiber but when all sites on the fiber are occupied, longer extraction times will not improve extraction efficiency but may result in desorption (Zhang and Pawliszyn, 1999).
- *Salting out effect* – Increasing the ionic strength of the sample solution suppresses the solubility of hydrophobic analytes in the sample solution hence promoting their adsorption onto the fiber. This approach has been widely employed in the extraction of pesticides (Wu et al., 2000; Hwang and Lee, 2000; Fernandez et a., 2001).

SPME sorbent type	Sorbent thickness (μm)	Final analysis	Type of pesticides	Reference
PDMS/DVB	65	GC	Organochlorines	Mmualefe et al., 2009.
PDMS	7, 30, 100	GC/HPLC	Organochlorines & organophosphorus	Batlle et al., 1999; Lipinski, 2000.
PA	85	GC	Triazines & organophosphorus Nitrogen containing & herbicides	Batlle et al., 1999; Eisert andLevsen, 1995. Boyd-Boland and Pawliszyn, 1995.
CW/DVB	65, 70	GC	Herbicides	Batlle et al., 1999; Hernandez et al., 2000.

Table 6. Applications of commercially available SPME sorbent types in pesticide analysis.

6. Pressurized liquid extraction (PLE)

Pressurized liquid extraction (PLE), also referred to as pressurized fluid extraction (PFE), pressurized solvent extraction (PSE) or accelerated solvent extraction (ASE), is a technique that was introduced by Dionex corporation in 1995 (Richter et al., 1996). The principle of the technique is based on using elevated temperatures (50 – 200 °C) and pressures (50-150 atm) to extract analytes from solid or semi-solid samples within short periods of time (5 – 15 min).

The Dionex ASE® 200 system consists of a solvent delivery component controlled by an HPLC pump, nitrogen gas purge valve, a carousel for extraction cells and collection vials as well as a waste vial (Fig. 7)

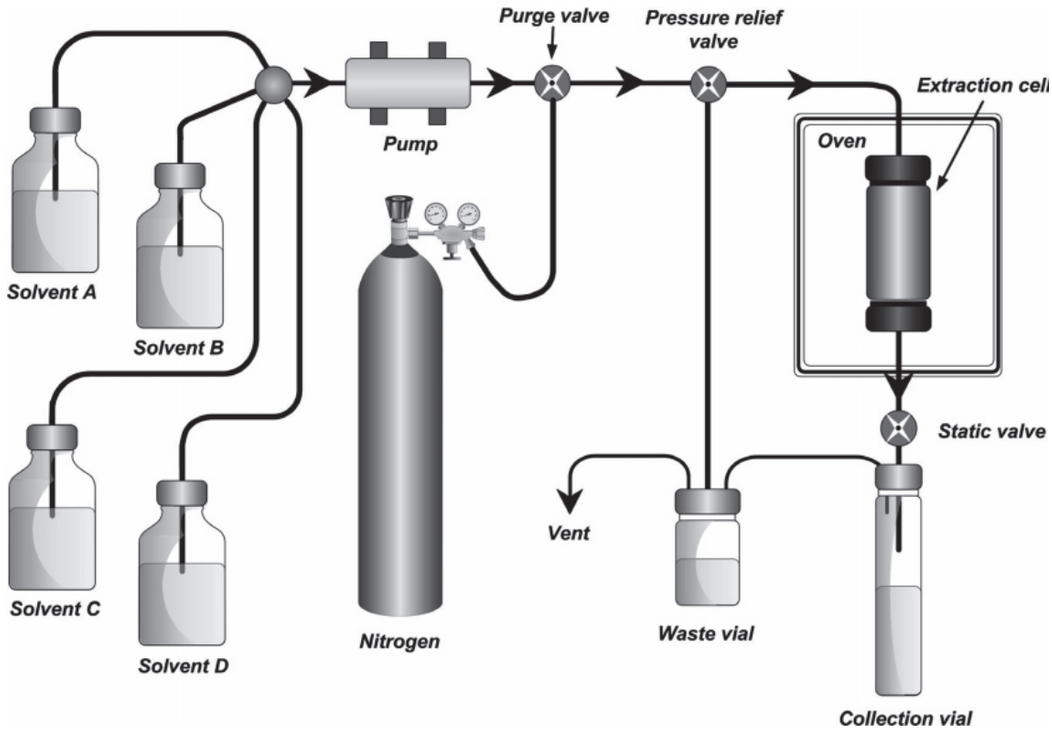


Fig. 7. Diagram of a Dionex ASE® 200 pressurised liquid extraction system

Prior to extraction, a solid/ semi-solid sample is placed into a stainless steel extraction cell lined with a filter paper disk on the outlet end to prevent passage of solid matter from the cell into the collection vial. The extraction cell is then placed onto a carousel and automatically drawn into the oven and filled with solvent. During extraction, the cell is heated, causing thermal expansion of the solvent and hence an increase of pressure inside the cell. The static and pressure relief valves function to regulate pressure inside the cell during static extraction by adding more solvent or opening the static valve to let solvent out of the extraction cell, whichever one is needed to maintain the desired pressure.

After static extraction, some of the solvent inside the extraction cell can be replaced by fresh solvent for a subsequent extraction cycle. This flush volume can vary from 5 to 150 % of the extraction cell. The introduction of fresh solvent increases the concentration gradient between the extraction solvent in the cell and the surface of the sample matrix resulting in improved mass transfer and consequently better extraction efficiency compared to a single cycle extraction (Richter et al., 1996). Finally, pressurized nitrogen purges the remaining solvent from the cell and lines to a collection vial. Parameters to optimize in PLE include;

- *Temperature (60 – 200 °C)* - the increased temperatures disrupt the strong solute-matrix interactions caused by van der Waals forces, hydrogen bonding and dipole interactions of the solute molecules and active sites on the matrix. When the solvent is in contact with the matrix, the thermal energy in the heated solvent assists to desorb analytes from the matrix by overcoming cohesive (solute-solute) and adhesive (solute-matrix) interactions. This decreases the activation energy required for the desorption process (Richter et al., 1996; Mockel et al., 1987).

- *Pressure (50 – 100 bar)* - The high pressures employed in PLE maintain the solvent in its liquid state even at temperatures above its atmospheric boiling point. The high pressure increases the solvation power and speeds up the extraction kinetics of solvents by forcing solvent into the pores of the matrix that normally would not be in contact with solvent at atmospheric pressure. This helps solvate analytes trapped in matrix pores that have been "sealed" with water or air bubbles. The pressurized flow in PLE also assists to solubilize air bubbles surrounding analytes that are found on the surface of the matrix as well (Richter et al., 1996).

For polar solvents such as water, increasing the temperature lowers the dielectric constant thus making it suitable for the extraction of less polar compounds (Turner et al., 2006). The dielectric constant of water at 25 °C is ~80, making it an extremely polar solvent. Increasing the temperature of water to 250 °C while applying sufficient pressure to maintain it in its liquid state reduces the dielectric constant to 27 which is midway between those of methanol (ε = 33) and ethanol (ε = 24) at 25 °C (Miller and Hawthorne, 1998). As a result, water at higher temperatures is more "miscible" or "soluble" in organic solvents and is often referred to as pressurised hot water extraction (PHWE). Table 7 shows some examples of applications of PLE in the extraction of pesticides.

Sample	Analyte	Extraction solvent	Reference
Baby food	Malathion, chlorpyrifos, 4,4'-DDE, 4,4'-DDT.	Acetonitrile or ethyl acetate	Chuang et al., 2001.
Oranges and peaches	Benzimidazoles and azoles, organophosphorus, carbamates, neonicotinoids & acaricides	Ethyl acetate	Blasco et al., 2005.
Fresh vegetables	Wide range of pesticides	Ethyl acetate/acetone (3:1 v/v)	Garrido et al., 2005.
Fresh pear, cantaloupe, potato and cabbage	Wide range of pesticdes	Acetone/ dichloromethan e (3:1 v/v)	Adou et al., 2001.

Table 7. Examples of PLE in sample preparation for the determination of pesticides.

7. Supercritical fluid extraction (SFE)

This technique employs fluids in their supercritical states for the extraction of solid samples. Supercritical fluids behave like gases although they have the density of liquids and as a result, they have a high diffusivity, low viscosity, good penetration capability and adjustable density (Goncalves et al., 2006). In comparison to soxhlet extraction, supercritical fluid extraction (SFE) offers several advantages such as shorter extraction times, lower solvent consumption (hence environmentally friendly), suitability for thermally labile compounds and reduced working temperature (Brachet et al., 2000).

In SFE, a solid or semi-solid sample is placed in a pressure vessel (Fig. 8) and extracted with a re-circulated stream of supercritical fluid which is well mixed with the sample matrix to allow analytes to transfer to the fluid. At the end of the extraction, the extract is collected in a vial or cartridge.

SFE involves five sequential steps;

1. Wetting of the matrix with supercritical fluid.
2. Partitioning of the analyte from the matrix into the supercritical fluid
3. Diffusion of analytes from the matrix.
4. Elution of the analyte from the extraction cell
5. Collection of the analytes.

Wetting of the sample with supercritical fluid is especially important when the sample matrix contains water. Partitioning of non-polar analytes from the matrix is a relatively fast process if supercritical carbon dioxide is employed. For analytes that are strongly bound to the matrix, a higher temperature or addition of an organic solvent is required. The second step depends on factors such as diffusion of the analyte between the matrix active sites and the ability of the supercritical fluid to displace the analyte from these sites. This initial desorption step is often the rate determining step in the SFE of most environmental samples (Bowadt and Hawthorne, 1995). All three steps contribute to the overall extraction efficiency.

SFE has been widely employed as a sample preparation tool for pesticide analysis such as in sediments (Mmualefe et al., 2008), wheat and maize (Norman and Panton, 2001) and in vegetables (Ono et al., 2006).

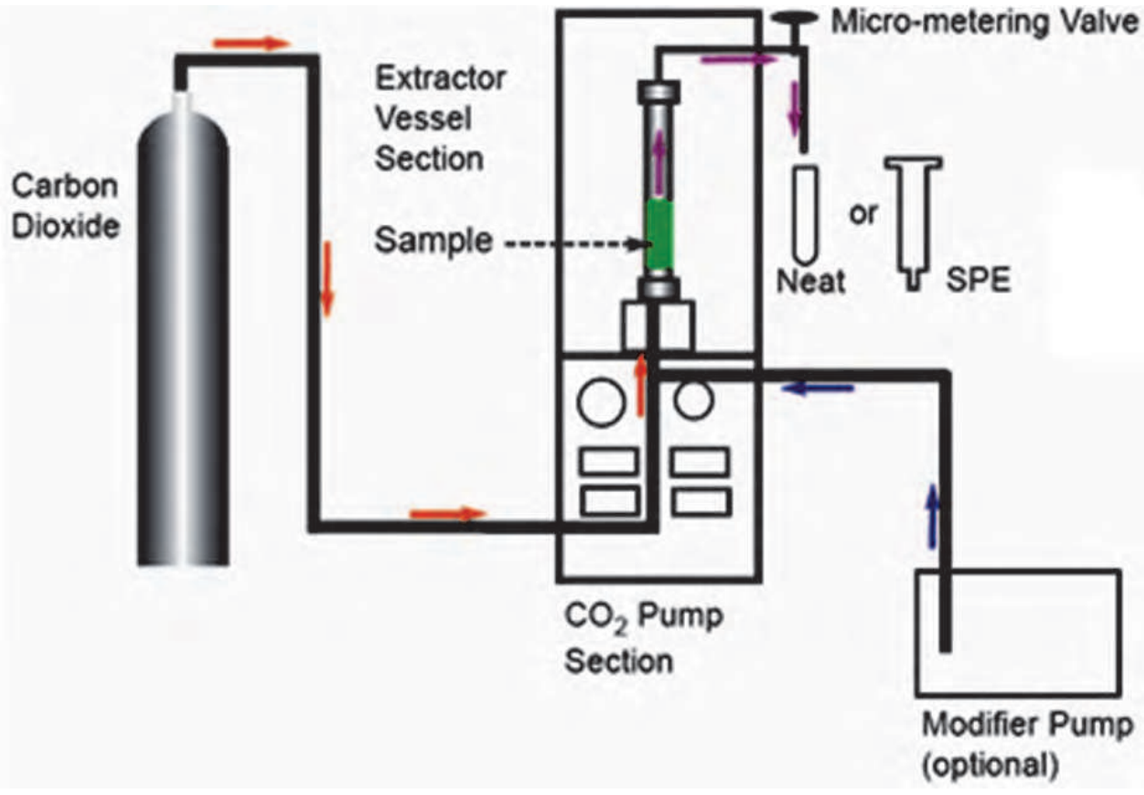


Fig. 8. Diagram of a Spe-ed™ Prime SFE by Applied Separations Inc. (2008)

8. Conclusions

Sample preparation continues to evolve with the search for techniques that offer higher selectivity to handle highly complex matrices and hence improve recoveries and reproducibility of results. Sample throughput, ease of operation and cost of analysis are critical parameters when choosing sample preparation techniques in the industry. Limitations of any technique open opportunities for analytical chemists to come up with alternative strategies or invent completely new techniques to conquer challenges of the existing technique.

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10. References

Adeyemi, D., Mokgadi, J., Darkwa, J., Anyakora, C., Ukpo, G. & Torto, N. (2011) *Chromatographia*, 73, 1015.

Adou, K., Bontoyan, W.R. & Sweeny, P.J. (2000). *J. Agric. Food Chem.*, 49, 4153.

Ahmadi, F., Assadi, Y., Hosseinin, S.M.R.M. & Rezaee, M. (2001). *Analyst*, 126, 1674.

Anastassiades, M., Lehotay, S.J., Stajnbaher, D. & Schenck, F.J. (2003). *J. AOAC Int.,* 86(22), 412.

Arthur, C.L. & Pawliszyn, J. (1990). *Anal. Chem*., 62, 2145.

Baltussen, E., Janssen, H.-G., Sandra, P. & Cramers, C.A. (1997). *J. High Res. Chromatogr.* 20, 395.

Batlle, R., Sánchez, C. & Nerín, C. (1999). *Anal. Chem.,* 71, 2417.

Blasco, C., Font, G. & Pico, Y. (2005). *J. Chromatogr. A*, 1098, 1-2, 37.

Boos, K.-S. & Grimm, C.-H. (1999). *Trends Anal. Chem*., 18, 175.

Bowadt, S. & Hawthorne, S.B. (1995). *J. Chromatogr. A*, 703, 549.

Boyd-Boland, A.A. & Pawliszyn, J. (1995). *J. Chromatogr. A*, 704, 163.

Brachet, A., Christen, P., Gauvrit, J.Y., Longeray, R., Lanteri, P. & Veuthey, J.L. (2000). *J. Biochem. Bioph. Meth*., 43, 353.

Caro, E., Marcé. R.M., Borrull F., Cormack P.A.G. & Sherrington, D.C. (2006). *Trends Anal. Chem*., 25, 143.

Chen, Y., Guo, Z., Wang, X. & Qui, C. (2008). *J. Chromatogr. A*, 1184, 191.

Chuang, J.C, Hart, K., Chang, J.S., Boman, l.E., van Emon, J.M. & Reed, A.W. (2001). *Anal. Chim. Acta*, 444, 1, 87.

Ciao, Q., Hu, B., Yu, C., Xia, L. & Jiang, Z. (2006). *Talanta*, 69, 848.

Clifford, A.A., Burford, M.D., Hawthorne, S.B., Langenfeld, J.J. & Miller, D.J. (1995). *J. Chem. Soc.Faraday Trans.* 91, 1333.

Costain, R.M., Fesser, A.C., McKenzie, D., Mizuno, M. & Macneil, J.D. (2008). *Food Add. Contam.*, 21, 1.

Delaunay, N., Pichon, V. & Hennion, M.-C. (2000). *J. Chromatogr. B*, 745, 15.

Delaunay-Bertoncini, N., Pichon, V. & Hennion, M.-C. (2001). *Chromatographia*, 53, S224.

Ding, J., Gao, Q., Luo, D., Shi, Z.-G. & Feng, Y.-Q. (2010). *J. Chromatogr. A*, 1217, 7351.

Hernandez, F., Beltran, J., Lopez, F.J. & Gaspar, J.V. (2000). *Anal. Chem*., 72, 2313.

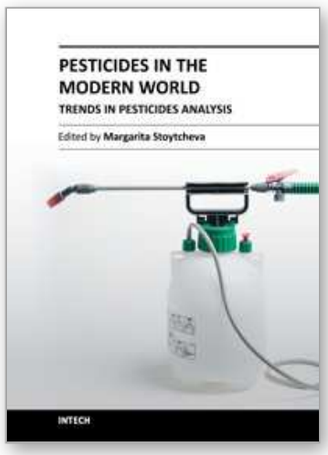
Fernandez, M., Padron, C., Marconi, L., Ghini, S., Colombo, R., Sabatini, A.G. & Girotti, S. (2001). *J Chromatogr A*, 922, 257.
Fontanals, N., Marce, R.M. & Borrull, F. (2007). New materials in sorptive extraction techniques for polar compounds. *J. Chromatogr. A*, 1152, 14.
Garrido, F.A., Martinez, S.I., Martinez, V.J.L. & Lopez-Lopez, T. (2005). *Anal. BioAnal. Chem.*, 33, 7-8, 1106.
Goncalves, C., Carvalho, J.J., Azenha, M.A. & Alpendurada, M.F. (2006). *J. Chromatogr. A*, 1110, 6.
He, Y. & Lee, H. K. (1997). *Anal. Chem.*, 69, 4634.
Hennion, M.-C. (1999). *J. Chromatogr. A*, 856, 3.
Hennion, M.-C. & Pichon, V. (2003). *J. Chromatogr. A*, 1000, 29.
Hogendoorn, E. & van Zoonen, P. (2000). *J. Chromatogr. A*, 892, 435.
Hogendoorn, E.A., Huls, R., Dijkman, E. & Hoogerbrugge, R. (2001). *J. Chromatogr. A*, 938,1-2, 23.
Hwang, B.H. & Lee, M.R. (2000). *J Chromatogr. A*, 898, 245.
Hyötyläinen, T. & Riekkola, M.-L. (2008). *Anal. Chim. Acta*, 614, 27.
Jager, L.S. & Andrews, A.J. (2000). *Analyst*, 125, 1943.
Jakubowska, N., Zygmunt, B., Polkowska, Z., Zabiegala, B. & Namiesnik, J. (2009). *J. Chromatogr. A*, 1216, 422.
Jeannot, M.A. & Cantwell, F.F. (1996). *Anal. Chem.*, 68, 13, 2236.
Jeannot, M.A. & Cantwell, F.F. (1997). *Anal. Chem.*, 69, 2, 235.
Ji, Y.S., Yin, J.J., Xu, Z.G., Zhao, C.D., Huang, H.Y., Zhang, H.X. & Wang, C.M. (2009). *Anal. BioAnal. Chem.* 395, 1125.
Jimenez, J.J., Bernal J.L., de Nozal, Ma., J., Toribio, L. & Arias E. (2001). *J. Chromatogr. A,* 919, 1, 147.
Jönsson, J. & Mathiasson, L. (2001). *J. Sep. Sci.*, 24, 495.
Kandimalla,V.B. & Ju, H. (2004). *Anal. BioAnal. Chem.*, 380, 587.
Khajeh, M. (2009). *Int. J. Environ. Anal. Chem.*, 89, 479.
King, A.J., Readman, J.W. & Zhou, J.L. (2003). *Environ. Geochem. Health*, 25, 69.
Lambropoulou, D.A, Psillakis, E., Albanis, T.A. & Kalogerakis, N. (2004). *Anal. Chim. Acta,* 516, 205.
Langenfeld, J.J., Hawthorne, S.B., Miller, D.J. & Pawliszyn, J. (1995). *Anal. Chem*. 67, 1727.
Lehotay, S.J. (2007). *J. AOAC Int.*, 90, 485.
Lehotay, S.J., Maštovská, K. & Yun, S.J. (2005). *J. AOAC Int.*, 88, 630.
Lehotay, S.J., Son, K.A., Kwon, H., Koesukwiwat, U., Fu, W., Maštovská, K., Hoh, E. & Leepipatpiboon, N. (2010). *J. Chromatogr. A*, 1217, 2548.
Lipinski, J. & Fresenius, J. (2000). *Anal. Chem.*, 367, 445.
Liu, H. & Dasgupta, P.K. (1996). *Anal. Chem.*, 68, 11, 1817.
Liu, Y., Li, H.F. & Lin, J.M. (2009). *Talanta*, 77, 1037.
Lopez-Blanco, C., Gomez-Alvarez, S., Rey-Garrote, M., Cancho-Grande, B. & Simal-Gandra, J. (2005). *Anal. BioAnal. Chem.,* 383, 557.
Lopez-Blanco, M.C., Blanco-Cid, S., Cancho-Grande, B. & Simal-Gandara, J. (2003). *J. Chromatogr. A*, 984, 245.
Mahugo-Santana, C., Sosa-Ferrera, Z., Torres-Padrón, M.E. & Santana-Rodríguez, J.J. (2011). *Trends Anal. Chem.*, doi: 10.1016/j.trac.2011.01.011.
Majors, R. (2007). *LC-GC Asia Pacific*, 10, 3.

Martel, A.C. & Zeggane, S. (2002). *J. Chromatogr. A*, 954, 173.
Maštovská, K. & Lehotay, S.J. (2004). *J. Chromatogr. A*, 1040, 259.
Miller, D.J. & Hawthorne, S.B. (1998). *Anal. Chem*. 70, 1618.
Mmualefe, L.C., Torto, N., Huntsman-Mapila, P. & Mbongwe, B. (2009*). Microchem. J*., 91, 239.
Mmualefe, L.C., Torto, N., Huntsman-Mapila, P. & Mbongwe, B. (2008). *Water SA*, 34, 405.
Mockel, H.J., Welter, G. & Melzer, H. (1987). *J. Chromatogr*., 388, 255.
Moreno, D.V., Ferrera, Z.S. & Rodriguez, J.J.S. (2007). *Microchem. J*., 87, 139.
Mpofu, C., Torto, N., Mmualefe, L.C., Obuseng, V.C., Sichilongo, K., Mosepele, K. & Mbongwe, B. (2011). (submitted manuscript).
Norman, K.N.T. & Panton, S.H.W. (2006). *J. Chromatogr. A*, 907, 1, 247.
Ono, Y., Yamagami, T., Nishina T. & Tobino T. (2006). *Anal. Sci*., 22, 11, 1473.
Pawliszyn, J. (2003). *Anal. Chem*., 75, 2543.
Pawliszyn, J. (Ed.), Sampling and Sample Preparation Techniques for Field and Laboratory, Elsevier, Amsterdam, The Netherlands, 2002.
Pawliszyn, J. in Solid Phase Microextraction: Theory and practice, Wiley-VCH Inc., New York, 1st edn, 1997.
Pinto, C.G., Laespada, M.E., Martins, S.H., Ferreira, A.M., Pavón, J.L. & Cordero, B.M. (2010). *Talanta*, 81, 1-2, 385.
Poole, C.F. (2003). *Trends Anal. Chem*., 22, 6, 362.
Prince, A.E., Fan, T.S., Skoczenski, B.A. & Bushway, R.J. (2001). *Anal. Chim. Acta*, 444, 37.
Psillakis, E. & Kalogerakis, N. (2002). *Trends Anal. Chem*., 21, 54.
Qiao, F., Sun, H., Yan, H., Row, K.H. (2006). *Chromatographia*, 64, 625.
Eisert, R., Levsen, K. & Fresenius. J. (1995). *Anal. Chem*., 351, 555.
Rahiminejad, M., Shahtaheri, S.J., Ganjali, M.R., Rahimi-Forushani, A. & Golbabaei, F. (2009). *Iran J. Environ. Health Sci. Eng*., 6, 2, 97.
Richter, B.E., Jones, B.A., Ezzell, J.L., Porter, N.L., Avdalovic, N. & Pohl, C., (1996). *Anal. Chem*., 68, 1033.
Shimelis, O., Yang, Y.H., Stenerson, K., Kaneko, T. & Ye, M. (2007). *J. Chromatogr. A*, 1165, 18.
Simpson, N.J.K. (Ed.), Solid-Phase Extraction: Principles, Strategies and Applications, Marcel Dekker, New York, USA, 2000.
Smith, R.M. (2003). *J. Chromatogr. A*, 1000, 3.
Spietelun, A., Pilarczyk, M., Kloskowski, A. & Namieśnik, J. (2010). *Chem. Soc. Rev*., 39, 4524.
Stevenson, D. (2000). *J. Chromatogr. B*, 745, 39.
Stubbings, G. & Bigwood, T. (2009). *Anal. Chim. Acta*, 637, 1-2, 68.
Turner, C., Turner, P., Jacobson, G., Almgren, K., Waldeback, M., Sjoberg, P., Karlsson, E.N. & Markides, K.E. (2006). *Green Chem*., 8, 949.
Wardencki, W., Curylo, J. & Namieśnik, J. (2007). J. *Biochem. Bioph. Meth*., 70, 275.
Wells, D., Lloyd, T. (2002). Automation of Sample Preparation for Pharmaceutical and Clinical Analysis, in: Pawliszyn, J. (Ed.), Sampling and Sample Preparation for Field and Laboratory, 1st ed. Elsevier, Amsterdam, pp. 837.
Wilkowska, A. & Biziuk, M. (2011). Food Chem., 125, 803.
Wu, J., Xie, W. & Pawliszyn, J. (2000). *Analyst*, 125, 2216.
Xu, L., Basheer, C. & Lee, H.K. (2007). *J. Chromatogr. A*, 1152, 1-2, 184.
Yang, X. & Peppard, T. (1994). J. Agric. Food. Chem., 42, 1925.
Yin, J., Wang, S., Yang, G., Yang, G. & Chen, Y. (2006). *J. Chromatogr*. B, 844, 142.

Zhang, Z. & Pawliszyn, J. (1999). *Anal. Chem.,* 65, 1843.
Zhang, Z.Y., Yang, M.J. & Pawliszyn, J. (1994). *Anal. Chem.*, 66, 844A.
Zhao, E., Shan, W., Jiang, S., Liu, Y. & Zhou, Z. (2006). *Microchem. J.*, 83, 105.
Zhao, X.L., Shi, Y.L., Cai, Y.Q. & Mou, S.F. (2008). *Environ. Sci. Technol.*, 42, 1201.

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Pesticides in the Modern World - Trends in Pesticides Analysis
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The book offers a professional look on the recent achievements and emerging trends in pesticides analysis, including pesticides identification and characterization. The 20 chapters are organized in three sections. The first book section addresses issues associated with pesticides classification, pesticides properties and environmental risks, and pesticides safe management, and provides a general overview on the advanced chromatographic and sensors- and biosensors-based methods for pesticides determination. The second book section is specially devoted to the chromatographic pesticides quantification, including sample preparation. The basic principles of the modern extraction techniques, such as: accelerated solvent extraction, supercritical fluid extraction, microwave assisted extraction, solid phase extraction, solid phase microextraction, matrix solid phase dispersion extraction, cloud point extraction, and QuEChERS are comprehensively described and critically evaluated. The third book section describes some alternative analytical approaches to the conventional methods of pesticides determination. These include voltammetric techniques making use of electrochemical sensors and biosensors, and solid-phase spectrometry combined with flow-injection analysis applying flow-based optosensors.

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