Recent advances in sample preparation techniques for environmental matrix

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Abstract: Sample preparation is an essential step in analysis, greatly influencing the reliability and accuracy of result. In general, the analytical method involves processes such as sampling (collection of the samples), sample preparation (separation from the matrix, concentration, fractionation and, if necessary, derivatization), separation, detection and data analysis. Surveys show that more than 80% of analysis time is spent on sample collection and sample preparation. Classical sample preparation techniques are time consuming, labor-intensive and multi-stage operations. Each step, especially concentration, can introduce errors and losses especially when analyzing volatile compounds. Waste disposal of solvents is an additional problem, adding extra cost to the analytical procedure, extra charge for the environment and creates health hazards to the laboratory personnel. The development of modern sample preparation techniques has significant advantages over conventional methods in this Scenario. This review emphasis on some of modern sample preparation techniques like Solid phase extraction (SPE), Solid-Phase Micro extraction (SPME), Matrix solid phase dispersion (MSPD), Stir bar sorptive extraction (SBSE), Single-drop Microextraction (SDME) & Pressurized Fluid Extraction (PFE) for environmental matrices.

Key words: Sample Preparation, SPE, SPME, MSPD, SBSE, SDME & PFE

1. Introduction

Sample preparation is an essential step in analysis, greatly influencing the reliability and accuracy of result. Present analytical and separation methods can resolve practically all kinds of complex mixtures, from gases to biological macromolecules, with detection limits down to the femtogram range. In general, the analytical method involves processes such as sampling (collection of the samples), sample preparation (separation from the matrix, concentration, fractionation and, if necessary, derivatization), separation, detection and data analysis. Surveys show that more than 80% of analysis time is spent on sample collection and sample preparation. This is necessary because in most cases analytical instruments cannot handle the sample matrices directly. The whole analytical process can be wasted if an unsuitable sample preparation method has been employed before the sample reaches the chromatograph and the analyser.

Current sample preparation procedures using solvents (liquid–liquid extraction techniques (LLE)) are time consuming, labor-intensive and multi-stage operations. Each step, especially concentration, can introduce errors and losses especially when analysing volatile compounds. Waste disposal of solvents is an additional problem, adding extra cost to the analytical procedure, extra charge for the environment and creates health hazards to the laboratory personnel. The development of modern sample preparation techniques has significant advantages over conventional methods in terms of reduction in organic solvent consumption and in minimizing sample degradation. They also result in the elimination of undesirable and insoluble components from the extract. This review emphasis on some of modern sample preparation techniques like Solid phase extraction (SPE), Solid-Phase Microextraction (SPME), Matrix solid phase dispersion (MSPD), Stir bar sorptive extraction (SBSE), Single-drop Microextraction (SDME) & Pressurized Fluid Extraction (PFE) for environmental matrices.

2. Sample preparation techniques

2.1 Solid phase extraction (SPE)

Solid phase extraction is the very popular technique currently available for rapid and selective sample preparation. The versatility of SPE allows use of this technique for many purposes, such as purification, trace enrichment, desalting, derivatisation and class fractionation. The last few years have been characterized by a wide interest in this technique and many publications describing SPE methods have been published.
This method is based on the extracts containing target analytes through a column filled with the appropriate sorbent (which was previously conditioned by an appropriate solvent or solvent mixture), or passing of an appropriate solvent through the SPE column to which a suitable amount of sample was previously added. Using selective solvents, first the coextractants from the SPE column can be successfully eluted, and then the target analytes (Figure 1, A), or the elution of analytes can be direct, where undesirable coextractants derived from the sample matrix remain in the SPE column (Figure 1, B). The principle is similar to that of liquid-liquid extraction, involving a partitioning of solutes between two phases. However, instead of two immiscible liquid phases, as in LLE, SPE involves partitioning between a liquid and a solid (sorbent) phase. This sample treatment technique enables the concentration and purification of analytes from solution by sorption on a solid sorbent and purification of extract after extraction. Compared with the traditional methods, SPE has many attractive features. It is easy to operate, costs less, it has been automated and uses small amounts of solvent. SPE is the multifunctional techniques, since the purification and the concentration occur in the same step. Unfortunately, SPE has certain limitations, primarily related to lower yields (recovery), i.e. slightly lower sensitivity, in situations where there is "clogging" of the SPE column (blocking of the sorption centers by solid and oily components originating from the sample).

The most commonly used SPE sorbents in pesticide residues determination are: reverse phase octadecyl (C18), normal-phase aminopropyl (-NH\(_2\)) and primary-secondary amine (PSA), anion-exchanger three-methyl ammonium (SAX) and adsorbents such as graphitized carbon black (GCB). Normal-phase sorbents such as florisil (MgSiO\(_3\)), aluminum oxide (Al\(_2\)O\(_3\)) and silica (SiO\(_2\)) are usually used in combination with the previously mentioned sorbents. The SPE cartridge should be chosen depending on the physicochemical properties of pesticides that are searched for in a particular sample, and the nature of the sample matrix.

SPE was developed to determine and to monitor the content of organophosphorus pesticides in tomato crops from the agricultural areas. SPE technique with activated charcoal was used for purification and recovery of the pesticides methamidophos, acephate, malation and methyl parathion. The solvents used for sample extraction and elution were chosen after several comparative tests. Best results were achieved using ethyl acetate for extraction and dichloromethane - ethyl acetate (7:3) for elution. Average recoveries from the matrices fortified with 0.20 to 0.60 µg g\(^{-1}\) ranged from 85.2 to 100 % with overall coefficients of variation of 1.3 to 6.3 %. The limits of detection of the method varied between 0.04 and 0.12 ng g\(^{-1}\) as shown in below Table 1 & figure 2. Activated charcoal demonstrated to be efficient for tomato matrix purification and for quantitative recovery of the analytes.

### Table 1. Recoveries of OPPs by SPE using sorbent activated charcoal, extraction with ethyl acetate and elution with 10 mL of CH\(_2\)Cl\(_2\) - ethyl acetate (7:3)

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Fortification level(µg g(^{-1}))</th>
<th>Recovery (%)</th>
<th>CV</th>
<th>LOD (ng g(^{-1}))</th>
<th>MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methamidophos</td>
<td>0.4</td>
<td>99.0</td>
<td>4.2</td>
<td>0.04</td>
<td>0.30</td>
</tr>
<tr>
<td>acephate</td>
<td>0.6</td>
<td>97.2</td>
<td>1.3</td>
<td>0.12</td>
<td>0.50</td>
</tr>
<tr>
<td>malation</td>
<td>0.20</td>
<td>100.0</td>
<td>0.8</td>
<td>0.07</td>
<td>3.00</td>
</tr>
<tr>
<td>methyl parathion</td>
<td>0.20</td>
<td>85.2</td>
<td>6.3</td>
<td>0.10</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Figure 1. The basic principle of SPE technique
Figure 2. Chromatogram showing OPPs recoveries after purification by SPE using activated charcoal, extraction with ethyl acetate and elution with $\text{CH}_2\text{Cl}_2$ - ethyl acetate (7:3). The peaks correspond to the pesticides methamidophos (1), acephate (2), methyl parathion (3), and malathion (4).

It can be confirmed that activated charcoal is an efficient sorbent of pigments from the tomato matrix and that it improved the recovery of pesticides of different polarities. In addition, the SPE technique has shown advantages such as simplification in the extraction and purification steps, lower solvent consumption, and time of analysis.

2.2 Solid-phase micro extraction (SME)

Solid-Phase Microextraction (SPME) is a very simple and efficient, solvent less sample preparation method. SPME has been widely used in different fields of analytical chemistry since its first applications to environmental and food analysis and is ideally suited for coupling with mass spectrometry (MS). All steps of the conventional liquid–liquid extraction (LLE) such as extraction, concentration, (derivatization) and transfer to the chromatograph are integrated into one step and one device, considerably simplifying the sample preparation procedure. It uses a fused-silica fibre that is coated on the outside with an appropriate stationary phase. The analytes in the sample are directly extracted to the fibre coating. The SPME technique can be routinely used in combination with gas chromatography, high-performance liquid chromatography and capillary electrophoresis and places no restriction on MS. SPME reduces the time necessary for sample preparation, decreases purchase and disposal costs of solvents and can improve detection limits. The SPME technique is ideally suited for MS applications, combining a simple and efficient sample preparation with versatile and sensitive detection. This review summarizes analytical characteristics and variants of the SPME technique and its applications in combination with MS [6, 8].

The concept of SPME may have been derived from the idea of an immersed GC capillary column. The SPME apparatus is a very simple device. It looks like modified syringe (Fig. 3) consisting of a fibre holder and a fibre assembly, the latter containing a 1–2 cm long retractable SPME fibre. The SPME fibre itself is a thin fused-silica optical fibre, coated with a thin polymer film (such as polydimethylsiloxane (PDMS)), conventionally used as a coating material in chromatography. There are two typical SPME applications, sampling gases (headspace (HS)) or sampling solutions. In either case the SPME needle is inserted into the appropriate position (e.g. through a septum into the headspace), the needle protecting the fibre is retracted and the fibre is exposed to the environment. The polymer coating acts like a sponge, concentrating the analytes by absorption/adsorption processes. Extraction is based on a similar principle to chromatography, based on gas–liquid or liquid–liquid partitioning [9].

Figure 3. Schematic diagram of a commercial SPME device.
After sampling, the fibre is retracted into the metal needle (for mechanical protection), and the next step is transfer of the analyte from the fibre into the chromatograph. Gas chromatography (GC or GC/MS) is one of the preferentially used techniques. In this case, thermal desorption of the analyte takes place in the hot GC injector. After inserting the needle into the injector, the fibre is pushed outside the metal needle. The other common option is analysis by HPLC (HPLC/MS). Mitani et al. [10] applied an automated on-line method for the determination of the isoflavones, daidzein and genistein in soybean foods by using in-tube SPME coupled to HPLC. The detection limits obtained were 0.4 - 0.5 ngmL^{-1} and the recoveries were above 97%. As shown below in Table 2 & 3 respectively.

**Table 2.** Linear regression and detection limits of isoflavones by in tube SPME -HPLC

<table>
<thead>
<tr>
<th>Compound</th>
<th>Range(ngmL^{-1})</th>
<th>Slope</th>
<th>Intercept</th>
<th>r</th>
<th>Detection limit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>In–tube- SPME</td>
</tr>
<tr>
<td>Daidzein</td>
<td>5- 200</td>
<td>0.0095</td>
<td>0.0161</td>
<td>0.99993</td>
<td>0.41</td>
</tr>
<tr>
<td>Genistein</td>
<td>5- 200</td>
<td>0.0106</td>
<td>-0.0003</td>
<td>0.99993</td>
<td>0.48</td>
</tr>
</tbody>
</table>

**Table 3.** Recoveries of isoflavones spiked to dried soybeans

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sample(mg)</th>
<th>Spiked (µgg^{-1})</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daidzein</td>
<td>5.0</td>
<td>4.0</td>
<td>112.1 ± 8.3</td>
</tr>
<tr>
<td>Genistein</td>
<td>5.0</td>
<td>4.0</td>
<td>97.8 ± 3.2</td>
</tr>
<tr>
<td>Daidzin</td>
<td>5.0</td>
<td>20.0</td>
<td>116.8 ± 6.7</td>
</tr>
<tr>
<td>Genistein</td>
<td>5.0</td>
<td>20.0</td>
<td>107.9 ± 3.1</td>
</tr>
</tbody>
</table>

**Figure. 4.** Chromatograms obtained from several food samples. (A) Dried soybeans (5 mg), (B) Soybean paste (500 mg), (C) adzuki beans, (D) Soy milk (100 mg). Samples were analyzed in 2 M HCl-CH₃OH (1:3) containing 0.05% BHT at 100°C. peak: 1, daidzein; 2, genistein; 3, β-naphthol, in tube SME-HPLC conditions.

As shown above in Fig. 4, daidzein and genistein are useful tool for the screening and determination and they were detected from several food samples. Peak of these compounds were identified from absorption spectrum analysis with DAD. Menezes-Filhoa et al. [11] developed a method for the simultaneous analysis of 14 pesticide residues (clofentezine, carbofuran, diazinon, methyl parathion, malathion, fenithion, thiabendazole, imazalil, bifenthin, permethrin, prochloraz, pyraclostrobin, difenoconazole and azoxyystrobin) in mango fruit, based on SPME coupled to GC–MS. Different parameters of the method were evaluated. The best results were obtained using polyacrylate
fiber and direct immersion mode at 50°C for 30 min, along with stirring at 250 rpm and desorption for 5 min at 280 °C. The method was validated using mango samples spiked with pesticides at concentration levels ranging from 33.3 to 333.3 µg kg⁻¹. The average recoveries (n = 3) for the lowest concentration level ranged from 71.6 to 117.5%, with RSD between 3.1 and 12.3%, respectively. Detection and quantification limits ranged from 1.0 to 3.3 µg kg⁻¹ and from 3.33 to 33.33 µg kg⁻¹, respectively as shown below in Table 4. SPME produces relatively clean and concentrated extracts, and is ideal for MS applications. This technique does not suffer from the plugging or channeling problems encountered with SPE. It also completely eliminates use of organic solvents. A relatively long equilibration time (up to 1 h) is needed, and methods such as sample stirring, sample sonication, fiber vibration, and fiber rotation have been used to reduce this absorption time [12, 13].

### Table 4. Analytical figures of merit obtained and maximum residue levels of the pesticides

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Regression equation</th>
<th>R²</th>
<th>Linear range (µg kg⁻¹)</th>
<th>LOD (µg kg⁻¹)</th>
<th>LOQ (µg kg⁻¹)</th>
<th>MRL (µg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clofentezine</td>
<td>2061.50x + 60577</td>
<td>0.9967</td>
<td>3.33–1665.00</td>
<td>1.00</td>
<td>3.33</td>
<td>–</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>42.54x−1295.4</td>
<td>0.9906</td>
<td>33.33–1665.00</td>
<td>10.00</td>
<td>33.33</td>
<td>–</td>
</tr>
<tr>
<td>Diazinon</td>
<td>39.58x−5.217</td>
<td>0.9987</td>
<td>16.65–1665.00</td>
<td>5.00</td>
<td>16.65</td>
<td>–</td>
</tr>
<tr>
<td>Methyl parathion</td>
<td>453.69x−12124</td>
<td>0.9960</td>
<td>16.65–1665.00</td>
<td>5.00</td>
<td>16.65</td>
<td>–</td>
</tr>
<tr>
<td>Malathion</td>
<td>395.65x−22928</td>
<td>0.9907</td>
<td>6.66–1665.00</td>
<td>2.00</td>
<td>6.66</td>
<td>–</td>
</tr>
<tr>
<td>Fenthion</td>
<td>3917.3x + 165795</td>
<td>0.9966</td>
<td>3.33–1665.00</td>
<td>1.00</td>
<td>3.33</td>
<td>50</td>
</tr>
<tr>
<td>Thiabendazole</td>
<td>267.40x + 17590</td>
<td>0.9948</td>
<td>33.33–1665.00</td>
<td>10.00</td>
<td>33.33</td>
<td>2000</td>
</tr>
<tr>
<td>Imazalil</td>
<td>114.65x−3382.2</td>
<td>0.9980</td>
<td>33.33–1665.00</td>
<td>10.00</td>
<td>33.33</td>
<td>1000</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>6792.7x−286761</td>
<td>0.9973</td>
<td>6.66–1665.00</td>
<td>2.00</td>
<td>6.66</td>
<td>100</td>
</tr>
<tr>
<td>Permethrin</td>
<td>1537.10x−106775</td>
<td>0.9903</td>
<td>16.65–1665.00</td>
<td>5.00</td>
<td>16.65</td>
<td>–</td>
</tr>
<tr>
<td>Prochloraz</td>
<td>335.83x−852.13</td>
<td>0.9960</td>
<td>6.66–1665.00</td>
<td>2.00</td>
<td>6.66</td>
<td>200</td>
</tr>
<tr>
<td>Pyraclostrobin</td>
<td>1689.80x + 4841.3</td>
<td>0.9991</td>
<td>16.65–1665.00</td>
<td>5.00</td>
<td>16.65</td>
<td>100</td>
</tr>
<tr>
<td>Difenoconazole</td>
<td>4357.3x + 29790</td>
<td>0.9986</td>
<td>3.33–1665.00</td>
<td>1.00</td>
<td>3.33</td>
<td>200</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>1857x−19938</td>
<td>0.9929</td>
<td>6.66–1665.00</td>
<td>1.00</td>
<td>6.66</td>
<td>500</td>
</tr>
</tbody>
</table>

### 2.3 Matrix Solid-phase Dispersion (MSPD)

Matrix solid-phase dispersion (MSPD) has been cited as the extraction method employed in over 250 studies. It has proven to be an efficient and somewhat generic technique for the isolation of a wide range of drugs, pesticides, naturally occurring constituents and other compounds for a wide variety of complex plant and animal samples. MSPD combines aspects of several analytical techniques, performing sample disruption while dispersing the components of the sample on and into a solid support, thereby generating a chromatographic material that possesses a particular character for the extraction of compounds from the dispersed sample. In the MSPD process, a sample (liquid, semi-solid or solid) is placed in a glass or agate mortar containing an appropriate bonded-phase or other solid support material such as octadecylsiloxane (ODS) and derivatized silica (C18) or other suitable support materials Figure 5 [14].

This technique enables extraction of analytes from samples dispersed homogeneously in a solid support, usually Florisil or C18. The homogenized mixture is placed in a column in which the adsorbent works as an abrasive compound breaking the physical structure of the sample and enabling its fractionation and adsorption of the compounds of the matrix. The column is finally eluted with an appropriate solvent and the extract can then be analyzed directly. Interferences, for example pigments or other polar compounds, are retained on the adsorbent and so sample extraction and clean-up are performed in the same step with good recovery and reproducibility, reducing the analysis time and the amount of solvent used [15, 16].

The solid support and sample are manually blended together using a glass or agate pestle, a step that takes about 30 seconds. When blending is complete, the sample is then packed into an empty column or on top of a solid-phase extraction (SPE) sorbent without any further drying or cleanup prior to elution. The column is often an empty syringe barrel or a cartridge with a stainless-steel or polypropylene frit, cellulose filter or a plug of...
silanized glass wool at the bottom. A second frit or plug is often placed on top of the sample before compression with a syringe plunger. The main difference between MSPD and SPE is that the sample is dispersed throughout the column and not retained in only the first few millimeters. As regards elution, there are two possibilities: (a) the target analytes are retained on the column and interfering compounds are eluted in a washing step, followed by the target analytes being eluted by a different solvent; or (b) the interfering matrix components are selectively retained on the column and the target analytes directly eluted. Finally, additional cleanup is performed or the sample is directly analyzed. Sometimes, the MSPD column is coupled on line with an SPE column or, as in several recent applications; the SPE sorbent is packed in the bottom part of the MSPD column to remove interfering matrix components.

Figure. 5. MSPD Extraction Procedures

Several factors have been examined for their effects in the MSPD extraction. These include:

a. The effects of average particle size diameter, where as expected, very small particle sizes (3 - 10 µm) would lead to extended solvent elution times and the need for excessive pressures or vacuum to obtain an adequate flow. A blend of silicas possessing a range of particle sizes (40 - 100 µm) works quite well and such materials also tend to be less expensive.

b. The character of the bonded-phase. Depending on the polarity of the phase chosen various effects on the results may be observed. Applications requiring a lipophilic bonded-phase employ C18 and C8 materials interchangeably.

c. The use of underivatized silica or other solid support materials. Use of unmodified or underivatized solids, such as sand to blend samples do not work in exactly the same manner as originally described for the bonded-phase solid support, such as ODS. Silica-based support materials (derivatized silica, silica gel, sand, florisil) are still being used almost exclusively in MSPD. Blasco et al. have demonstrated the use of an activated carbon fiber for the isolation of dithiocarbamates from fruits, vegetables and cereals.

d. The best proportion ratio of sample to solid support material. The most often applied is 1 to 4, respectively, but it can vary from application to application. This ratio is dependent on the method employed. Both smaller and greater ratios have been used successfully.

e. Chemical modification of the matrix or matrix solid support blend. Addition of chelating agents such as acids and bases at the time of blending would affect the distribution and elution of target analytes from the sample. The solution profile of matrix components is likewise affected.

f. The optimum choice of eluent and the sequence of their application to a column. The Elution solvent sequence is to isolate the analyte or further clean the column of interfering substances with each solvent step. MSPD columns permit isolation of analytes with different polarities or the entire chemical classes of compounds in a single...
solvent, making MSPD amenable to multi residue analysis on a single sample. Several recent studies have reported the use of hot water as an eluting solvent as well as the addition of pressure, which is known as pressurized-liquid extraction (PLE) or accelerated solvent extraction (ASE). Such applications demonstrate the potential to make extraction methods based on MSPD free of hazardous solvents and even less expensive to perform [19].

- The elution volume. It has been observed that for an 8 ml elution of a 2 g MSPD column blended with 0.5 g sample, the target analytes usually elute in the first 4 ml, which is approximately one column volume. This will vary for each application and should be examined to reduce the use of solvent and the unintended co-elution of potential interferences.

h. The effect of the sample matrix itself. All the components of the sample are dispersed throughout the column, covering much of the bonded-phase solid support surface, creating a new phase that can have dramatic effects on isolation in going from one matrix to another [20].

Kristenson et al. [21] developed a miniaturized automated MSPD method for extracting pesticides from apples, pears and grapes. Only 25 mg of sample and 0.1 ml ethyl acetate were used and the extracts were analyzed by GC-MS without any further purification. In terms of recovery, C18, C8 and silica were compared for use as dispersants. The best results were obtained by using C18. The LODs were 4 - 90 µg kg⁻¹. Bogialli et al. also [22] developed a simple, rapid and specific method for analyzing seven widely used carbamate insecticides in fruits and vegetables. After matrix deposition on crystobalite (sand), the analytes were extracted with water, heated to 50 – 100 °C. At 50 °C, recoveries were between 76 to 99 %.

A method based on MSPD and GC was proposed by Hu et al. [23] for the determination of OC and pyrethroid insecticides in tea leaves. After evaluating various extraction conditions, it was found that the best compromise in terms of recovery and cleanup was the use of Florisil as the dispersant and hexane-dichloromethane (DCM) as the extractant. LODs of the method ranged between 2 and 60 ng g⁻¹, which are lower than the MRLs set by the EU.

A macro matrix solid-phase dispersion (MSPD) method was developed [24] to extract 266 pesticides from apple juice samples prior to gas chromatography– mass selective detection (GC-MSD) determination. A 10 g samples was mixed with 20 g diatomaceous earth. The mixture was transferred into a glass column. Pesticide residues were leached with a 160 mL hexane-dichloromethane (1:1) at 5 mL min⁻¹. Two hundred and sixty-six pesticides were divided into three groups and detected by GC–MSD under selective ion monitoring. The proposed method takes advantage of both liquid–liquid extraction and conventional MSPD methods.

The 266 pesticide samples were divided into three groups, namely, 97 pesticides in G0 group, 86 in G1 group and 83 in G2 groups. Several temperature programs have been used for chromatographic fractionation of the three groups, and the final temperature study has worked out favorably on the pesticides. Ninety-two chromatographic peaks were extracted from the 97 pesticides in G0 group (Fig. 6), 84 chromatographic peaks from G1 group (Fig. 7) and 80 from G2 group (Fig. 8). The chromatographic peaks, such as parathion-methyl and chlorpyrifos-methyl in G0 group, dimefuron and isopropalin in G1 group, and captan and phosfolan in G2 group.

Figure. 6. The total ion chromatography of 97 pesticides in G0 group
It was concluded MSPD has become a well-established sample-preparation technique in food analysis. In many of the studies reported MSPD was compared with other extraction techniques for a variety of pesticide compounds and matrices. The performance of MSPD was usually similar or superior. It has several advantages, including simplifying and speeding up the sample-treatment process, reducing the use of large amounts of toxic solvents, eliminating emulsion formation, and increasing reliability, selectivity, and sensitivity. The primary advantage of MSPD is that sample extraction and clean-up are performed in the same step by use of small amounts of adsorbent and solvent, thus reducing cost and analysis time. Solvent evaporation remains a problem, however, and literature reports of on-line coupling of MSPD to LC or GC instruments are scarce.

2.4. Stir-bar Sorptive Extraction (SBSE)
Stir bar sorptive extraction (SBSE) was developed by Baltussen et al. \[25\] to overcome the limited extraction capacity of SPME fibers. A glass stirrer bar is coated with a potentially thick bonded absorbent layer (polydimethylsiloxane – PDMS) to give a large surface area of stationary phase, leading to a higher phase ratio and hence a better recovery and sample capacity (Figure 9). The advantages of sorptive extraction using PDMS include predictable enrichment, the absence of displacement effects, inertness, and rapid thermal desorption at mild temperature. Stir bar sorptive extraction of a liquid sample is performed by placing a suitable amount of sample in a headspace vial. The stir bar is added and the sample is stirred, typically for 30 - 240 min. The extraction time is controlled kinetically, determined by sample volume, stirring speed, and stir bar dimensions and must be optimized for a given application.

SBSE is applied to the extraction of aqueous samples containing low concentrations of organic compounds. For samples containing high concentrations of solvents, the solutions should be diluted before extraction. For the extraction of highly non-polar solutes, an organic modifier is added to minimize wall adsorption. Thus, the optimization of the organic modifier concentration is necessary. After extraction, the stir bar is removed, then placed on a clean tissue paper, rinsed with distilled water to remove water droplets, and introduced in a thermal desorption unit. This step will avoid the formation of non-volatile material during the thermal desorption step.
Rinsing would not cause any solute loss, because the adsorbed solutes are present inside the PDMS phase. After thermal desorption, the stir bars can be reused. Typically, the lifetime of a single stir bar is approximately 20 to 50 extractions, depending on the matrix [26].

Since SBSE using PDMS coating is similar to liquid-liquid extraction using a non-polar solvent, the technique is mainly used for non-fatty matrices (< 3% fat). The analysis of pesticides in fruits and vegetables [27, 28] has been described. After homogenization, the fruit and vegetable samples are extracted using a water miscible solvent. An aliquot of the extract is diluted with water and followed by SBSE.

Both LC-MS desorption and thermal desorption GC-MS have been used. Sandra et al. [27] used SBSE with thermal desorption capillary GC-MS for the screening of pesticides (OPPs and OCPs) in fruits, vegetables and baby food. A 10 mm stir bar coated with 0.5 mm PDMS was used. The recoveries for spiked samples ranged between 43-75%. The coupling of SBSE with RTL-GC-MS operated in the scan mode could monitor simultaneously about 300 pesticides present in fruits, vegetables and baby food. The detection limits from mg kg\(^{-1}\) to the sub µg kg\(^{-1}\) level were obtained.

Juan-Garcia et al. [28] also studied the detection of fungicide residues in grapes by LC-MS. Two procedures based on SPE and SBSE have been assessed for extracting these compounds in grapes. The recoveries obtained by SPE in samples spiked at the LOQ level ranged from 60 to 100% with RSDs from 7 to 17%. With the SBSE the recoveries obtained from samples spiked at the LOQ level were between 15 and 100% and the RSDs between 10 and 19%. The LOQs of most compounds are better via the SPE (0.003 – 0.01 mg kg\(^{-1}\)) than by SBSE (0.01 mg kg\(^{-1}\) for all fungicides).

Guan et al. [29] prepared a novel poly (phthalazine ether sulfone ketone) (PPESK) film and coated this on stir bars with a thickness of 250 µm for sorptive extraction. The PPESK coated stir bar has high thermo stability (290°C) and a long life time (50 repeated uses). The extraction properties of this stir bar were evaluated for the extraction of both polar and semi-polar analytes, including organochlorine compounds and organophosphorus pesticides. The PPESK stir bar showed higher affinity towards polar compounds than that of a PDMS coated stir bar and a higher sample load compared with the corresponding PPESK fiber. It was applied to the determination of organophosphorus pesticides in juices by gas chromatographic analysis. Limits of detection for organophosphorus pesticides were in the range of 0.17–2.25 ng L\(^{-1}\) and 2.47 10.3 ng L\(^{-1}\) in grape and peach juice, respectively, using the flame thermionic detector (FTD), with precisions of less than 20% RSD.

Although SBSE is widely applied in environmental and food analysis, it has also some limitations or drawbacks. One of the drawbacks is related to the fact that the coated stir-bar cannot be directly desorbed in a simple split/splitless injection port of a gas chromatograph. Hence the analyte has to be back extracted into a suitable solvent, which adds an additional step to the overall analytical method, or a specially designed Thermal Desorption Unit (TDU) has to be used. Moreover, operations like removing the stir-bar from the sample, rinsing and drying are usually performed manually, which are laborious and can introduce errors. Automation of these steps is possible but this increases the cost and complexity of the hardware involved. However, the most important limitations of SBSE are related to the coating of stir-bars. The non polar PDMS is at present the only polymer commercially available as a coating for stir-bars. Recovery of polar analytes is poor and often in situ derivatisation is applied to increase extraction yields. Stir-bars coated with materials with better affinity to polar compounds would improve SBSE flexibility and selectivity while maintaining its concentration capability. New approaches or concentrating materials are therefore required to overcome the above-mentioned limitation and to extend the range of applications. Up to now, developments of novel stir-bars have been reported with limited references. One of the methods developed was to use dual-phase-coated stir-bars, which combine two or more sampling materials with different concentration capabilities. These new stir-bars consist of a short PDMS tube at both ends with two magnetic stoppers, whose inner
cavity is packed with different types of adsorbents such as activated carbon. Dual-phase stir-bars with carbon have been shown to improve the recovery of volatile and polar compounds compared to the conventional PDMS stir-bar [30].

A method based on stir bar sorptive extraction (SBSE) coupled to thermal desorption (TD) and retention time locked (RTL) GC-MS for determination of five groups of 85 pesticides – organochlorine organochlorine, carbamate, organ phosphorous, pyrethroid and others - in non-fatty food, e.g. vegetables, fruits and green tea is describe. Figure 10 shows a comparison of the mass chromatograms (m/z 163) obtained for extraction of a methanol extract of spinach sample using A: Fivefold dilution (single SBSE); B: combined twofold and fivefold dilution (dual SBSE); C: mass spectrum of cypermethrin 3 obtained for B. Cypermethrin 1,2,3,4 was determined at 3.9 μg kg⁻¹ [31].

2.5. Single-drop Micro extraction (SDME)

Recently, alternative but SDME related concepts have been introduced for sample extraction. The use of a single droplet for extraction purposes was first recommended in the mid-1990s [32]. Figure 11 shows one possible embodiment of the SDME technique employing a microsyringe. The syringe needle is used to pierce the septum of a closed container. When the tip of the needle is in the desired position (in the aqueous phase or in the headspace) a hanging droplet of solvent is exposed to the matrix by depressing the plunger of the syringe. After extraction is completed, the droplet is withdrawn into the syringe barrel by lifting the plunger. The extracted samples can then be submitted directly to GC analysis. Thus the system Requires two discrete parts: the first for extraction and the second for injection.

Figure 10. Comparison of mass chromatogram (m/z 163) obtained for extraction of a methanol extract of a spinach sample using: A: twofold dilution (single stir bar); B: combined twofold and fivefold dilution (simultaneous analysis of two stir bars); C: mass spectrum of cypermethrin 3 obtained for B; cypermethrin 1,2,3,4 in B was determined at 3.9 μg kg⁻¹.
The properties of the analyte and its matrix will determine whether direct immersion (DI-SDME) or headspace (HS-SDME) extraction is appropriate. Thus, one must consider the volatility (boiling point), ionization (for acids and bases) and polarity of the analyte and matrix. HS SDME is appropriate for most polar and non-polar, lower molecular weight, volatile and semi volatile compounds. DI-SDME extraction is appropriate for non-polar or moderately polar higher molecular weight, semi volatile chemicals. There are some important restrictions on the selection of a particular extracting solvent. When extracting from an aqueous solution, the solvent needs to be water immiscible. The solvent needs to have a boiling point high enough that it will not evaporate, but also appropriate for the chromatographic system. It needs to have a high enough viscosity to cling onto the tip of a syringe needle, but not so viscous that the diffusion rate of the analyte into the drop affects extraction time significantly. The intermolecular attraction characteristics of the solvent must also be compatible with the analyte being extracted. Toluene appears to be the most commonly used acceptor phase, because it has high solubility for the target analytes, is immiscible in water and stable enough over the extraction time. Based on this solvent as the acceptor phase, several methods were validated and applied to the determination of OP and OC pesticides in solid samples [33]. Carbon tetrachloride has also been successfully applied to the extraction of OP pesticides [34] by Ahmadi et al. This solvent is, however, more prone to dissolve or become dislodged when long extraction periods are used. Isooctane and n-hexane have been also used for the determination of OP and OC pesticides. In the SDME procedure, solvent volumes lower than 3 µL are commonly used, due to the instability of the micro drop at higher values as well as to the good compatibility with the GC instruments. SDME involves dynamic partitioning of the target compounds between the acceptor phase and the sample solution, and the extraction efficiency depends on the mass transfer of analyte from the aqueous phase to the organic solvent phase. Since the mass transfer is a time-dependent process, a graph representing the relationship between peak area and extraction time is typically reported. Generally, extraction yield increases over relatively long exposure times. Since SDME is not an exhaustive extraction technique, it is not always practical to match extraction time with extraction equilibrium, because the potential for solvent loss due to dissolution increases with time. Therefore, extraction times are rarely set at equilibrium but rather at a point where sensitivity and precision are maximized over an acceptable experimental time. For pesticide analysis, extraction times of 15-30 min are usually selected [35, 36].

Alternative method HS-SDME, which can be employed for the determination of pesticide residues in the food matrices, was evaluated by [37]. Headspace analysis enables more complex matrices to be extracted than by direct analysis, and it can also be applied for the determination of semi-volatile analytes in complex food matrices, such as fruits and vegetables. HS-SPME and SPE are more efficient than HS-SDME in the present system because it has better linearity, precision, LOD, and LOQ as shown in Table 5. However, the HS-SDME method requires more elaborate manual operations, whereas the HS-SPME is easier to perform. Overall, both HS-SDME and HS-SPME techniques represent powerful alternatives to the conventional extraction method due to their speed, simplicity, cost, and sol-vent-free nature compared to SPE.

The results of linearity and precision studied are summarized in Table 5. For HS-SPME, the correlation coefficient \( r^2 \) ranged from 0.9969–0.9990; for SPE, the \( r^2 \) ranged from 0.9981–0.9996. However, for HS-SDME, the values ranged from 0.9834–0.9966. Overall, the repeatability expressed as the RSD was found to be satisfactory for HS-SPME ranging from 1.30–5.93% with a mean value.
of 3.87%; for SPE, values ranged from 0.70–2.69% with a mean value of 1.63%. However, the RSD values of HS-SDME were less precise and varied between 5.88–15.15% with a mean value of 10.62%. An additional consideration for the HS-SPME and HS-SDME extraction techniques is that higher RSDs are expected when, as in this study, extractions are carried out under non-equilibrium conditions. It is evident that, with HS-SPME and SPE, better precision and linearity are obtained for all the investigated pesticides compared to HS-SDME. Agitation is a critical parameter in SDME procedures.

The mass transfer of the target compounds to the organic solvent can be enhanced by agitation of the sample solution, thereby reducing the time required to attain thermodynamic equilibrium. However, excessive agitation could result in a dislodgement of the acceptor phase and equilibrium. However, excessive agitation could result in a dislodgement of the acceptor phase and thus most of the studies have been performed without or with a small amount of salt addition.\[38\]

Xiao et al.\[33\] developed a single-drop microextraction (SDME) procedure for the analysis of organophosphorus pesticides (OPPs) in fruit juice by gas chromatography (GC) with flame photometric detection (GC-FPD). Two types of SDME mode, static and cycle-flow SDME were evaluated. The enrichment factors for six OPPs in static SDME were nearly 100-fold (except for dichlorvos 23-fold), which were much better than that in cycle-flow SDME. Therefore, static SDME with tributyl phosphate (TBP) as the internal standard was selected for the real sample analysis. A 100-fold dilution of fruit juice samples is adequate to determine levels of most pesticides below the MRLs because of the low limits of detection of the method. The recoveries for the spiked juice samples were from 77.7 to 113.6%.

An approach for the extraction of 9 kinds of organochlorine pesticides (OCPs) from vegetable samples (cabbage, cauliflower, Chinese cabbage) coupling single-drop microextraction with gas chromatography–mass spectrometry was presented by Zhang et al.\[39\]. An effective extraction was achieved by suspending a 1.00 µL mixed drop of p-xylene and acetone (8:2, v/v) to the tip of a micro syringe immersed in a 2 mL donor aqueous solution and stirred at 400 rpm. The relative recoveries were from 63.3 to 100%, with repeatability ranging from 8.74 to 18.9% (R.S.D.). In contrast to some common acceptor solvents, a novel combination of liquids comprising p-xylene and acetone showed better extractions and lower detection limits (0.05 ng mL\(^{-1}\)) for organochlorine pesticides.

Amvrazia et al.\[40\] evaluated the single-drop microextraction (SDME) technique coupled with GC-NPD and GC-ECD for the determination of 14 types of multi-class pesticides in vegetables (tomato and courgette). The optimum sample preparation was achieved with the use of a mixture of acetone/H\(_2\)O (10/90, v/v) in a donor sample solution and subsequent SDME using a toluene drop (1.6 µL) under mild stirring for 25 min. The efficiency of the extraction process was studied in fortified tomato and courgette samples and the matrix effect assessment performed showed that quantification should be performed using a standard curve of spiked vegetable samples since certain matrix components as observed in the tomato analysis, may enhance pesticide recoveries via SDME. The proposed method showed good linearity, limits of detection at the sub-µg kg\(^{-1}\) level and high precision (RSD <15%) and was applied successfully in real vegetable samples showing that

<table>
<thead>
<tr>
<th>Compound</th>
<th>HS-SPME</th>
<th>SPE</th>
<th>HS-SDME</th>
<th>r²</th>
<th>precision(RSD%, n =5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazinon</td>
<td>10–1000</td>
<td>100–10000</td>
<td>1000–100000</td>
<td>0.9985</td>
<td>0.9996</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>10–1000</td>
<td>100–10000</td>
<td>1000–100000</td>
<td>0.9977</td>
<td>0.9991</td>
</tr>
<tr>
<td>Malathion</td>
<td>50–5000</td>
<td>500–50000</td>
<td>5000–500000</td>
<td>0.9973</td>
<td>0.9981</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.5–50</td>
<td>5–500</td>
<td>50–5000</td>
<td>0.9969</td>
<td>0.9986</td>
</tr>
<tr>
<td>Quinalphos</td>
<td>50–5000</td>
<td>500–50000</td>
<td>5000–500000</td>
<td>0.9972</td>
<td>0.9992</td>
</tr>
<tr>
<td>α-Endosulfan</td>
<td>0.1–20</td>
<td>1–200</td>
<td>10–2000</td>
<td>0.9982</td>
<td>0.9987</td>
</tr>
<tr>
<td>Profenofos</td>
<td>1–100</td>
<td>10–1000</td>
<td>100–10000</td>
<td>0.9990</td>
<td>0.9996</td>
</tr>
<tr>
<td>β-Endosulfan</td>
<td>1–100</td>
<td>10–1000</td>
<td>100–10000</td>
<td>0.9990</td>
<td>0.9987</td>
</tr>
</tbody>
</table>
SDME can be a promising way for sample preparation in pesticide residue analysis. Due to its simplicity, ease of implementation, and insignificant startup cost, SDME is accessible to virtually all laboratories. However, it has some limitations, for example: (a) in its most basic form, direct immersion mode it requires careful and elaborate manual operation because of the problem of drop dislodgment and instability; (b) the SDME is affected by the presence of humic acids or suspended solids indicating that it has a limited advantage in complex matrices, in which extra filtration of the sample is necessary; (c) notwithstanding the acceptable analytical performance mentioned above, the sensitivity and the precision of SDME methods can be further improved. The main issue lies with the adverse consequences of prolonged extraction time and fast stirring rates, since they may result in drop dissolution and dislodgement; (d) SDME is not yet suitable as a routine online pre-concentration procedure. Although some progress has been made to automate SDME, cost considerations will mean that the approach will not be widely accessible.

2.6. Pressurized Fluid Extraction (PFE)

This technique, also named accelerated solvent extraction (ASE) or pressurized liquid extraction (PLE), is a solid-liquid extraction process performed in closed vessels at relatively elevated temperature, usually 80 to 200°C, and elevated pressures, between 10 and 20 MPa conditions for short time periods (5-10 min). Therefore, PFE is quite similar to supercritical fluid extraction (SFE) but CO₂ is replaced by organic solvents to mitigate potential polarity problems. Extraction is carried out under pressure to maintain the conventional organic solvents in its liquid state, but extracting at temperatures well above their atmospheric boiling points. Therefore, the solvent is still below its critical condition during PFE but has enhanced solvation power and low viscosities and hence allows higher diffusion rates for the analytes. In this way the extraction efficiency increases, minimizing the amount of solvent needed and expediting the extraction process. The time required for extraction is independent of the sample mass and the efficiency of extraction is mainly dependent on the temperature. Figure 12 shows a schematic diagram of a PFE system.

Both static and flow through extraction systems can be used. In the static extraction mode, the sample is loaded in an inert cell and pressurized with a solvent heated above its boiling point for some time. The extract is then automatically removed and transferred to a vial. In the flow through extraction mode, a fresh solvent is continuously introduced to the sample. This improves the extraction efficiency but, the extract is subsequently diluted. The extract is pushed into the collection vial by a second aliquot of solvent inserted into the extraction cell and this second aliquot is then collected into the same vial by pushing it with an inert gas flow. The whole process takes approximately 15-20 min.

In PFE, the pressure is applied to maintain the solvent in its liquid state. This reduces the number of parameters that need to be optimized to achieve efficient extractions. The main parameters to consider now are temperature and time and this reduces the time devoted to method development and optimization of the extraction procedure.

So far, most of the PLE applications reported in the literature were performed in the static mode followed by a brief post-extraction dynamic flush with the organic solvent. In this approach, the selected solvent is pumped to fill the cell containing the sample, which is kept for a specified time at the selected pressure and temperature. Next, the extraction solvent is transferred to a collection vial and the sample and the connective tubing are rinsed with a small volume of solvent; finally purged with nitrogen to guarantee complete removal of the solvent from the PLE system. In the dynamic PLE, the pressurized extraction solvent, this can be at room temperature or be preheated to the
selected temperature \cite{46}, is continuously pumped through the extraction cell at a constant flow-rate (typically 0.33 – 2.5 mL min\(^{-1}\)) for a specified period of time. According to Fick’s law of diffusion, continuous contact between the sample and fresh solvent should accelerate the mass transfer. Consequently, the extraction efficiency should be enhanced and the extraction time reduced \cite{44}. However, the results reported until now do not seem to support this conclusion. For example, Popp et al. \cite{47} used two 10-min static extractions with toluene at 14 MPa and 200°C for the determination of dibenzo-p-dioxins and furans (PCDD/Fs) in fly ash, while Bautz et al.\cite{44} proposed 30-min dynamic PLE with toluene/methanol (3:1, v/v) at 1 mL min\(^{-1}\), at 15 MPa and 200°C, while all other experimental conditions were rather similar in both cases. Despite the 10 more min used in the latter procedure, the PLE efficiency for the extraction of the 2, 3, 7, 8-hepta- and octa-CDD/Fs was found to be 30 – 50% higher with the static PLE method. Studies \cite{48, 49} have shown that a combination of both extraction modes can result in improved extraction.

Table 6. Recoveries of PCDD/Fs and dioxin-like PCBs (Co-PCBs) from Certified Reference Materials BCR 529 using Soxhlet and PLE

<table>
<thead>
<tr>
<th>PCDD/Fs &amp; Co-PCBs Isomers (IUPAC number)</th>
<th>BCR certificate value (ng kg(^{-1}))</th>
<th>Soxhlet (n = 3)</th>
<th>PLE (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ng kg(^{-1}))</td>
<td>Certified value (%)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>2,3,7,8-TCDF (F1)</td>
<td>78 ± 13</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1,2,3,7,8-PeCDF (F2)</td>
<td>140 ± 30</td>
<td>247</td>
<td>176</td>
</tr>
<tr>
<td>2,3,4,7,8-PeCDF (F3)</td>
<td>360 ± 70</td>
<td>555</td>
<td>154</td>
</tr>
<tr>
<td>1,2,3,4,7,8-HxCDF (F4)</td>
<td>3400 ± 500</td>
<td>4352</td>
<td>128</td>
</tr>
<tr>
<td>1,2,3,6,7,8-HxCDF (F5)</td>
<td>1090 ± 150</td>
<td>1184</td>
<td>109</td>
</tr>
<tr>
<td>2,3,4,6,7,8-HxCDF (F6)</td>
<td>370 ± 40</td>
<td>518</td>
<td>140</td>
</tr>
<tr>
<td>1,2,3,7,8,9-HxCDF (F7)</td>
<td>22 ± 10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-HpCDF (F8)</td>
<td>–</td>
<td>13652</td>
<td>–</td>
</tr>
<tr>
<td>1,2,3,4,7,8,9-HpCDF (F9)</td>
<td>–</td>
<td>1984</td>
<td>–</td>
</tr>
<tr>
<td>OCDF (F10)</td>
<td>–</td>
<td>60674</td>
<td>–</td>
</tr>
<tr>
<td>2,3,7,8-TCDD (D1)</td>
<td>4500 ± 600</td>
<td>3955</td>
<td>88</td>
</tr>
<tr>
<td>1,2,3,7,8-FeCDD (D2)</td>
<td>440 ± 50</td>
<td>666</td>
<td>151</td>
</tr>
<tr>
<td>1,2,3,4,7,8-HxCDD (D3)</td>
<td>1200 ± 300</td>
<td>1171</td>
<td>98</td>
</tr>
<tr>
<td>1,2,3,6,7,8-HxCDD (D4)</td>
<td>5400 ± 900</td>
<td>5041</td>
<td>93</td>
</tr>
<tr>
<td>1,2,3,7,8,9-HxCDD (D5)</td>
<td>3000 ± 400</td>
<td>2045</td>
<td>68</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-HpCDD (D6)</td>
<td>–</td>
<td>44920</td>
<td>–</td>
</tr>
</tbody>
</table>

Pressurized liquid extraction (PLE) applying three extraction cycles, temperature and pressure, improved the efficiency of solvent extraction when compared with the classical Soxhlet extraction. Polychlorinated-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like PCBs (coplanar polychlorinated biphenyls (Co-PCBs)) in two Certified Reference Materials [DX-1 (sediment) and BCR 529 (soil)] and in two contaminated environmental samples (sediment and soil) were extracted by ASE and Soxhlet methods. Unlike data previously reported by other authors, results demonstrated that ASE using n-hexane as solvent and three extraction cycles, 12.4MPa (1800 psi) and 150°C achieves similar recovery results than the classical Soxhlet extraction for PCDFs and Co-PCBs, and better recovery results for PCDDs as shown below in table 6. ASE extraction, performed in less time and with less solvent proved to be, under optimized conditions, an excellent extraction technique for the simultaneous analysis of PCDD/PCDFs and Co-PCBs from environmental samples \cite{50}.

3. Conclusions

It is well known that sample preparation is one of the most critical steps in the determination of trace pollutants in different environmental matrices. Modern sample preparation techniques should be not only simple, reliable, cheap and take into account chemical laboratory waste problems, but also must be similar to common analytical techniques, in order to minimize errors. For these
reasons, modern trends in analytical chemistry are towards the simplification and miniaturization of sample preparation, and the minimization of sample size and organic solvent used. Those sample preparation techniques used as alternative to conventional extraction methods, offering advantages with respect to extraction time, solvent consumption, extraction yields and reproducibility. Moreover they are amenable to high degree of automation, and several parameters can be controlled at a time. A good extraction can be achieved in shorter period of time, and the recovered extract can have improved yield and quality than that prepared by a conventional method. Methods like PLE & SDM are better suited for the extraction of heat labile and volatile compounds, which is not the case with the conventional methods. SPME and SDM are becoming attractive alternatives to SPE in terms of liquid samples, while PLE, MSPD are good alternatives for samples involving solid samples.

4. References
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