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Cent. Eur. J. Chem. • 10(3) • 2012 • 900-925
DOI: 10.2478/s11532-012-0034-1

Central European Journal of **Chemistry**

A review of sample preparation methods for the pesticide residue analysis in foods

Review Article

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Received 30 October 2011; Accepted 16 February 2012

Abstract: The pesticide residues in foods have received increasing attention as one of the most important food safety issues. Therefore, more strict regulations on the maximum residue limits (MRLs) for pesticides in foods have been established in many countries and health organizations, based on the sensitive and reliable analysis methods of pesticide residues. However, the analysis of pesticide residues is a continuing challenge mainly because of the small quantities of analytes as well as the large amounts of interfering substances which can be co-extracted with them, often leading to experimental errors and damage to the analytical instruments. Thus, extensive sample preparation is often required for the pesticide residue analysis for the effective extraction of the analytes and removal of the interferences. This paper focuses on reviewing the recent development in the sample preparation methods for the pesticide residue analysis in foods since 2006. The methods include: liquid-liquid extraction (LLE), supercritical-fluid extraction (SFE), pressurized-liquid extraction (PLE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), gel permeation chromatography (GPC), solid-phase extraction (SPE), molecularly imprinted polymers (MIPs), matrix solid-phase dispersion (MSPD), solid-phase micro-extraction (SPME), QuEChERS, cloud point extraction (CPE) and liquid phase micro-extraction (LPME), *etc*. Particularly their advantages, disadvantages and future perspectives will be discussed.

Keywords: *Sample preparation • Pesticide residues • Food • Extraction • Clean-up*

1. Introduction

Nowadays, more than 800 different kinds of pesticides are used for the control of insects, rodents, fungi and unwanted plants in the process of agricultural production. Although most of them leave the products or degrade in soil, water and atmosphere, some trace amounts of pesticide residues can be transferred to humans *via* the food chain, being potentially harmful to human health [1]. To ensure the acceptable risk levels of pesticide residues, the regulations on maximum residue limits (MRLs) for pesticide residues in foods have been established in many countries and health organizations, for example in the United States, Japan, European Union, and Food and Agriculture Organization (FAO). These legislative limits have become stricter than ever due to the concerns of food safety and the demands of trade barriers, driving the demand for more sensitive and reliable analysis methods for pesticide residues [2].

The analysis of pesticide residues in foods consists of sample preparation and the instrumental determination. Although the analytical instruments are developing rapidly [3], their detector noise, detection limits, and final quantification are usually influenced by the interferences from food matrices [4-7]. Thus, the sample preparation is the bottleneck for the effective and accurate analysis

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of trace pesticide residues [4,5]. The aim of the sample preparation is to isolate the trace amounts of analytes from a large quantity of complex matrices and eliminate the interferences from the food matrix as much as possible. Typical sample preparation steps include the sampling/homogenization, extraction, and clean-up. Among them, the extraction and clean-up steps play a critical role in the success of pesticide residue analysis. The traditional sample extraction methods, especially liquid-liquid extraction (LLE), have been widely used for pesticide residue analysis. However, these methods are laborious, time- and solvent-consuming, and subject to the loss of analytes due to the tedious experimental procedure.. Therefore, new extraction and clean-up methods have been introduced in the field of pesticide residue analysis in foods. These include: supercritical-fluid extraction (SFE), pressurized-liquid extraction (PLE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), gel permeation chromatography (GPC), solid-phase extraction (SPE), molecularly imprinted polymers (MIPs), matrix solid-phase dispersion (MSPD), solid-phase micro-extraction (SPME), QuEChERS, cloud point extraction (CPE) and liquid phase micro-extraction (LPME). The sample preparation for pesticide residue analysis in food matrices has been previously reviewed by Lambropoulou and Albanis [8]. Therefore, the current paper focuses on the research progress since 2006. This paper gives a brief review of the sample preparation methods available for the analysis of pesticide residues in foods. After introducing these methods, the emphasis will be on the recent developments and trends in the sample preparation of the pesticide residue analysis in foods.

2. Liquid-liquid extraction (LLE)

From the all sample preparation methods, LLE may be the oldest and the most common extraction method. Before the LLE, solid samples are transformed into fine and homogeneous particles by mechanical grinding, mixing, rolling, agitating, chopping, crushing, macerating, mincing, pressing, or pulverizing. The homogenized solid or liquid samples are repeatedly extracted with an immiscible organic solvent, and the extracts are then centrifuged, concentrated and/or purified before the final analysis. The recent applications of LLE for the determination of pesticide residues in different foods are listed in Table 1 [10-15].

In the LLE, the extraction efficiency of analytes depends mainly on the equilibrium distribution/partition coefficient between the donor phase and the acceptor phase, which requires matching the polarities of the extraction solvents and analytes according to the similarity principle. Although acetonitrile (MeCN) is miscible in water, it can be separated from the aqueous phase by salting-out effect, and it has been found to be effective in extracting both polar and/or non-polar pesticide residues with small amounts of matrix co-extractives [9]. Therefore, MeCN has been widely applied as the extraction solvent in pesticide residue analysis [10]. A medium polarity solvent, ethyl acetate (EtAc) can decrease the polarity of a polar solvent or increase the polarity of a non-polar solvent in the LLE procedure. A simple and practical LLE method with MeCN-EtAc (13:3, volume:volume ratio, will be used below) for the pesticide analysis in honey samples was optimized by Pinho *et al*. [12]. Another similar LLE method used a MeCN-H_2O-EtAc mixture (16:1:3) as a solvent for the analysis of pesticide residues in tomatoes [13]. In addition, Tahboub *et al*. [14] reported a reliable LLE for the simultaneous identification and quantification of organochlorine pesticides (OCPs) in honey using a EtAc-petroleum ether mixture (20:80) as a solvent. In addition to EtAc, chloroform is another medium-polarity solvent used for the LLE of pesticide residues. Liu *et al*. [15] developed a LLE with chloroform, which diminished the tedious clean-up procedure and the amount of water-soluble co-extractive compounds. Although acetone is also a medium-polarity solvent, it has not been used for LLE due to its difficult separation from the aqueous phase. Diethyl ether has seldom been used in the LLE for the analysis of pesticide residues owing to its low ignition point and its high tendency to form explosive peroxides. Other non-polar organic solvents, such as hexane, cyclohexane and light petroleum are occasionally applied in the LLE of non-polar analytes or as the modifiers of other non-polar solvents.

It is necessary to clean-up the extracts for reducing the interferences after the initial LLE. Nguyen *et al*. [10] used the centrifugation, low temperature purification (LTP) at - 20°C for 6 h, and dispersive solid phase extraction (d-SPE) with Florisil as the clean-up steps of the extracts of LLE. Similar to this procedure, the LLE extracts of the pesticides in honey were chilled to - 20°C for a few hours [11,12] to obtain the phase separation, after which the organic phase of the biphasic system was removed and cleaned up in a SPE cartridge. Pinho *et al*. [13] repeated clean-up steps similar to the ones above for the extracts of pesticides in tomatoes. Liu *et al*. [15] used chloroform for the clean-up of the QuEChERS extracts, which shortened the tedious procedure and avoided the co-extraction of the water-soluble matrix substances with the analytes.

LLE is a classic method for the routine sample preparation due to its simplicity, robustness and efficiency.

Table 1. LLE applications in the analysis of pesticide residues in foods.

Matrix	pesticides	Sample preparation	References
soybean oil	95 pesticides	Extraction: 5 mL MeCN, twice; Clean-up: freezed at -20°C for 6 h, d-SPE (25 mg Florisil + 100 mg $MgSO_4$).	[10]
palm oil	7 pesticides	Extraction: 10 mL MeCN, freezed at -20°C for 4 h; MSPD: 2 g PSA dispersant, 1 g GCB cartridge, 15 mL MeCN.	[11]
honey	OPPs, pyrethroids	Extraction: 6.5 mL MeCN + 1.5 mL EtA; Clean-up: freezed at -20°C for 6 h, SPE (2.0 g Florisil+1.5 g Na_2SO_4).	[12]
tomatoes	OPPs, pyrethroids	Extraction: 8.0 mL MeCN+0.5 mL H_2O+1.5 mL EtAc; Clean-up: freezed at -20°C for 6 h, 1.5 g Na_2SO_4.	[13]
honey	11 OCPs	Extraction: 3×30 mL n-hexane-EtAc (80:20); Clean-up: SPE (Florisil, 25 mL n-hexane-diethyl ether (80: 20).	[14]
fruits, meats vegetables,	20 polar pesticides	Extraction:10 mL MeCN; Further Extraction: 4 g $MgSO_4$, 1.0 mL $CHCl_3$.	[15]

Table 2. SFE applications in the analysis of pesticide residues in foods.

Matrix	Pesticides	Sample preparation	References
tomatoes	5 OPPs	Extraction: CO_2, extraction vessel (50°C, 320 bar), first separation vessel (40°C, 150 bar), second separation vessel (25°C, 60 bar).	[32]
olive oil	Paraquat, diquat	Extraction: 2 g diatomaceous earth, CO_2, 60°C, 40 MPa, 1.5 mL min^{-1}.	[33]

However, it still remains to be laborious, and time- and solvent-consuming. To overcome these drawbacks, more simple and faster sample preparation methods have been introduced for the analysis of pesticide residues in foods. SFE, PLE, MAE and UAE have seen rapid development and serve as the alternatives of LLE in the pesticide residue analysis in foods.

3. Supercritical-fluid extraction (SFE)

SFE has recently been developed for the fast extraction of target analytes from solid samples by supercritical fluids [16]. As supercritical fluids are different from distinct liquid and gas phases in their physiochemical properties, they can diffuse into the solid matrix and dissolve the analytes. Thus, supercritical fluids can serve as substitutes for organic solvents in the sample preparation for the pesticide residue analysis (Table 2) [17,18].

Recent literature [19-30] reveals that carbon dioxide (CO_2) is the most commonly used supercritical fluid in the pesticide residue analysis in foods, because of its moderate critical temperature and pressure, non-flammability, low toxicity, high purity at low cost, and easy evaporation from the extracts [20,31]. Organophosphorus pesticides (OPPs) were extracted with lycopene and other carotenoids from tomato samples by SFE using supercritical CO_2 [32].

As supercritical CO_2 is effective in the extraction of non-polar or low-polarity compounds, it can remove the non-polar interfering compounds from the original samples and leave the polar pesticide residues in the extraction chamber. Supercritical CO_2 was applied for the on-line clean-up of paraquat and diquat in olive oil [33]. The best recoveries were achieved at 60°C by using a flow-rate of 1.5 mL min^{-1}, while the peaks of the analytes were not resolved at temperatures lower than 50°C, and the recoveries of the analytes declined at temperatures higher than 80°C.

Compared to the traditional solvent extraction, SFE can offer cleaner extracts with lower solvent consumption, less extraction time, and potentially more efficient and selective extraction from complex matrices. Especially, supercritical fluids with a low critical temperature can be employed for the extraction of thermally unstable analytes [34]. However, the non-polarity of supercritical CO_2 allows it to extract only non-polar or low-polarity analytes. For the extraction of moderately polar or polar pesticide residues, it is necessary to add organic solvent modifiers to enhance the polarity of extraction solvents.

4. Pressurized-liquid extraction (PLE)

PLE, the Dionex name for accelerated solvent extraction (ASE), is among the most widely used extraction methods of solid and semi-solid samples [35]. Since

PLE is always performed at high pressures and temperatures, the solvents tend to penetrate the solid samples at a high rate and supply a fast and efficient extraction [36]. The recent applications of PLE for the analysis of pesticide residues in different food matrices are listed in Table 3 [35,37-43].

During packing the sample in the extractor, selected drying materials are chosen as a desiccant to reduce the moisture and a disperser to increase the permeation of the solvents into the sample matrices. Na_2SO_4 has often been selected as the drying material to eliminate the co-extracted water [37,38,42]. In addition, diatomaceous earth [35], extrelut 20 [39], silica [43], hydromatrix [40], or acidic alumina [41] have been mixed with the food matrix to prevent the aggregation of sample particles to yield efficient extraction.

It is critical to match the polarity of the solvent to those of the analytes in PLE. Although hexane and hexane-acetone mixture (50:50) both matched the polarity of OCPs and yielded similar recovery values, the latter was finally selected as the extraction solvent to avoid the excessive extraction of pigments from green leafy vegetables [35]. The extracts of *n*-hexane, dichlomethane (DCM), *n*-hexane-DCM, *n*-hexane-acetone and *n*-hexane-EtAc were transparent and visually clean, however, the *n*-hexane-EtAc mixture was chosen as the extraction solvent due to the high recoveries of three pyrethroids, a carbamate and two OPPs in seaweeds [37]. From cyclohexane, DCM and EtAc, EtAc as an extraction solvents provided the best recoveries of 70-117% for six insecticides [38]. Wu *et al*. [39] chose MeCN as the extraction solvent because its extracts contained less fat than those of cyclohexane-EtAc or hexane-acetone.

Temperature is an important factor influencing the extraction efficiency of analytes in PLE, as it can decrease the viscosity of solvents and promote the diffusion of the analytes to the solvents. Tanaka *et al*. [38] demonstrated that the recoveries increased when temperature was increased from 60°C to 100°C, while the recoveries of the most pesticides stayed nearly constant at temperatures between 100 and 150°C. Although Barriada-Pereira *et al*. [35] obtained similar recoveries of OCPs at the temperature range 80-120°C, 110°C seems to be optimal temperature for the extraction of majority of the target compounds.

Due to the low selectivity of the extraction solvent, some interfering components may be co-extracted with the target pesticides from foods during the PLE procedure. To avoid the disturbance of interfering compounds and protect the analytical instrument, it is necessary to perform some clean-up steps before the final determination. When the extracts of green leafy vegetables were cleaned up by Florisil, silica gel and acidic alumina, a weak cloudiness and a strong color were observed, while transparent and colorless extracts and satisfactory recoveries were achieved with the clean-up by graphitized carbon black (GCB) [38]. In addition, the mixture of GCB and Florisil could provide colorless extracts of seaweeds, high recoveries and weak chromatogram background, which could not be achieved by Florisil only [37].

PLE not only greatly reduces the consumption of extraction solvents and operation time, but also improves the reproducibility and the recoveries. Furthermore, it has some obvious advantages such as high-level automation, high extraction efficiency, good selectivity, improved safety and good environmental compatibility. Even when compared with the SPE [35,44,45] and gel permeation chromatography (GPC) [46,47], it has more advantages due to low time-consumption and good reproducibility. However, PLE demands specific instrumentation and a high extraction temperature, which may result in the degradation of thermally labile compounds.

5. Microwave-assisted extraction (MAE)

As a fast extraction method, MAE was applied for the first time for the extraction of organic pollutants in 1986 [48]. Recently, it has been successfully used for the analysis of a wide range of pesticides in many food matrices (Table 4) [35,49-56]. Different from the conventional extraction methods, the analytes are selectively heated by microwaves and transferred from the sample to the organic solvents while the food contents are not extracted.

The polarity of the extraction solvent should closely match to those of the analytes. From four solvents, cyclohexane, *n*-hexane, *n*-octane and *n*-heptane, the last one was chosen as an organic solvent for the MAE of OCPs and pyrethroids in Chinese teas giving the best extraction efficiency for the studied pesticides [49]. In the extraction of OCPs from vegetables, hexane-acetone mixture (50:50) gave fewer co-extracts than hexane [35]. Since hexane, acetone, DCM and EtAc were not suitable for the on-line chromatographic analysis, Chen *et al*. [50] preferred MeCN-H_2O (95:5) to 100% MeCN for the determination of OCPs in grain samples due to cleaner extracts.In addition, acetone [51], acetone-MeCN (50:50) [52], MeCN-DCM (90:10) [53], and Polyoxyethylene 10 Lauryl Ether [54] have been chosen

Table 3. PLE applications in the analysis of pesticide residues in foods.

Matrix	pesticides	Sample preparation	References
vegetables	11 OCPs	Extraction: 0.075 g diatomaceous earth, hexane-acetone (50:50), 110°C, 10 MPa, 5 min, purge (N_2, 60 s), one cycle; Clean-up: SPE (GCB, 10 mL hexane-EtAc (80:20)).	[35]
seaweeds	6 pesticides	Extraction: 1.6 g Florisil + 0.4 g GCB + 1 g Na_2SO_4, *n*-hexane-EtAc (80:20), 100°C, 10 MPa, 2 min, one cycle.	[37]
vegetables	8 insecticides	Extraction: 10g Na_2SO_4+12 g GCB, EtAc, 100°C, 11 MPa, 10 min, purge (N_2, 5 s), one cycle.	[38]
meats	109 pesticides	Extraction: 5 g Extrelut 20, MeCN, 80°C, 1500 psi, 5 min, purge (N_2, 100 s), two cycles; Clean-up: GPC (40 g Bio-Beads S-X3, cyclohexane-EtAc (1:1), 5 mL min^{-1}, 254 nm); Further clean-up: 0.2 g PSA.	[39]
honey	12 insecticides	Extraction: 20 g Silica, EtAc, 75°C, 1500 psi, 7 min, purge (N_2, 60 s), two cycles.	[43]
tobacco	49 pesticides	Extraction: 3 g hydromatrix, acetone, 100°C, 1500 psi, 3 min, purge (N_2, 60 s), 3 cycles; Clean-up: SPE (Florisil, 5 mL *n*-hexane-acetone (20:80)).	[40]
fruits	12 insecticides	Extraction: 20 g acidic alumina, EtAc, 75°C, 10 MPa, 7 min, purge (N_2, 60 s), two cycles.	[41]
honeys	3 OCPs	Extraction: Na_2SO_4, acetone-CH_2Cl_2 (1:1), 100°C, 10 MPa, 3 min, 2 mL min^{-1}, 3 cycles; Clean-up: SPE (aluminum + silica, 20 mL CH_2Cl_2-hexane (1:1)).	[42]

Table 4. MAE applications in the analysis of pesticide residues in foods.

Matrix	pesticides	Sample preparation	References
vegetables	11 OCPs	Extraction: 0.075 g diatomaceous earth, 15 mL hexane-acetone (1:1), 1 min ramp from 100 to 800 W, 4 min hold at 800 W, 0W for 2 min; Clean-up: SPE(GCB, 10 mL hexane-EtAc(80:20)).	[35]
Chinese teas	OCPs, pyrethroids	Extraction: 20 mL *n*-heptane, 200 W, 2 min; Clean-up: SPE(1.0 g Na_2SO_4+0.4 g activated carbon+0.5 g Florisil, 10 mL *n*-hexane-acetone (1:1).	[49]
grain	6 OCPs	Extraction: 10 mL MeCN-H_2O (95:5), 80 W, 10 min; Clean-up: SPE (C_{18}, 3 mL MeCN, 4 mL MeCN-H_2O (20:80)).	[50]
vegetables	Thiophanatem-ethyl, carbendazim	Extraction: 10 mL acetone, 30 s, 50% power; Clean-up: hexane (10+10+5 mL), DCM (10+10+5 mL), 1 g Na_2SO_4.	[51]
Fruits, vegetables	72 pesticides	Extraction: 0.1 mL acetic acid +15 mL acetone-MeCN (1:1), 2 min ramp from 100 to 300 W, 300 W (3 min), 2 min ramp from 300 W to 100 W, 100 W (2 min); Clean-up: d-SPE (100 mg PSA + 100-200 mg $MgSO_4$).	[52]
olive oil	10 OPPs	Extraction: 5 mL MeCN-DCM (90:10), 250 W (2 min), 700 W (8 min); Clean-up: SPE (500 mg GCB, 3 mL DCM).	[53]
seaweed	6 OCPs	Extraction: 10 mL 5% Polyoxyethylene10 Lauryl Ether, 300 W (14 min); Clean-up: SPME (60 min absorption, 10 min desorption at room temperature) or SPE (C_{18}, 2 mL methanol).	[54]
olive, avocado oil	9 OPPs	Extraction: 15 mL MeCN, 150 W, 13 min; Clean-up: SPE (C_{18}, 5 mL MeCN).	[55]
low-fat food	16 OCPs	Extraction: 8 mL MeCN-H_2O (95:5), 100°C, 10 min; Clean-up: SPE (2 g Florisil, 5 mL n-hexane-acetone (9:1), 2 times).	[56]

as extraction solvents for the MAE of pesticides in different food matrices due to the high selectivity and high recoveries towards the target pesticides.

High temperature or microwaves are believed to accelerate the extraction procedure and enhance the recoveries of analytes. The recoveries increased when the microwave output power ranged from 20 W to 80 W, while the recoveries remained nearly constant within the power range 80-100 W [56]. However, excessively high power could result in the degradation of pesticides and a decrease in recoveries [51].

Extraction time also influenced the total recoveries. Chen *et al*. [50] found that the optimal extraction times of OCPs varied. Singh *et al*. [51] suggested that more co-extractives were obtained when the extraction time was longer than 30 s. Furthermore, lower extraction efficiencies were obtained when the extraction was carried out for longer than 4 min at 100 W or for 2 min at 200 W and 400 W [49], due to the possible degradation of analytes.

The main advantages of MAE are the low required temperature, high extraction efficiency, complete automation, and the possibility of simultaneous extraction of different kinds of analytes. However, the MAE lacks selectivity, resulting in the co-extraction of interfering compounds and it requires additional clean-up before the chromatographic determination. Furthermore, poor extraction efficiencies can usually be observed for the non-polar target compounds.

Table 5. UAE applications in the analysis of pesticide residues in foods.

Matrix	pesticides	Sample preparation.	References
honey	organophosphates	Extraction: 100 mL HCl (0.1 mol L^{-1})+100 μL Triton X-114 (100g L^{-1}), 85°C, 5 min, 3500 rpm, 5 min; ultrasound-assisted back-extraction: 60 μL hexane, 5 min.	[58]
tomato	OPPs	Sonication extraction: 5 mL acetone, 35 min; DLLME: 60.0μL chlorobenzene+1mL acetone.	[59]
fish	OCPs	Ultrasonic assisted-MSPD: 0.1 g Na_2SO_4+0.4 g C_{18}, 1.5 mL MeCN, 37 kHz, 40°C, 10 min; LLE: 0.85 mL MeCN+ 35 μL $CHCl_3$, 40°C, 5 min.	[60]
fruits	15OPPs, 9 triazines	ultrasonic-assisted-MSPD: 700 mg C_8, 700 μL EtAc, 35 kHz, 1 min.	[61]

6. Ultrasound-assisted extraction (UAE)

In addition to the extraction methods above, UAE was developed for the demand of fast extraction and high extraction efficiency. During the UAE, bubbles produced by acoustic cavitations not only facilitate the disruption of the cell walls of food samples, but also promote the solvent penetration to cell walls, which both facilitate the release of target analytes [57]. Providing more efficient contact between the solid sample and extraction solvents, UAE is a good alternative for the classic sample preparations for the extraction of organic compounds from food matrices. In recent years, UAE has attracted much attention in the sample preparation of the pesticide residue analysis in the foods (Table 5) [58-61].

According to the similarity principle, the selection of solvents in the UAE depends on the polarity of the target analytes. An ultrasound-assisted back-extraction of OPPs in honey samples was performed by adding hexane into the coacervate phase [58]. Due to low toxicity, low cost and miscibility with water, acetone was selected as the extraction solvent for the UAE of OPPs in tomatoes [59], which also served as the disperser solvent in the following liquid-liquid micro-extraction (DLLME). From acetone, MeCN and methanol, Rezaei *et al*. [60] chose MeCN as the extraction solvent for the extraction of OCPs in fish. Ramos *et al*. [61] carried out the ultrasonic-assisted matrix solid phase dispersion for the determination of pesticides in fruits, followed by the elution of the solid phase extraction tube by EtAc.

The extraction efficiency depends on the extraction time. The sonication time of 30 min could yield higher average recoveries for OPPs than 15 min [61]. Bidari *et al*. [59] found that the recoveries of all analytes increased with longer sonication times up to 30 min while the extraction efficiency kept constant at time range of 30-40 min. However, Rezaei *et al*. [60] obtained lower recoveries when the sonication time was longer than 10 min due to the possible degradation of some analytes. Moreover, Fontana *et al*. [58] suggested that the sonication time of 5 min was enough to avoid the continuous decrease of the extraction efficiency of pesticides in ultrasound-assisted back-extraction.

Although high sonication temperature can greatly promote the UAE, it may influence the stability of analytes and the recoveries. Rezaei *et al*. [60] found that the sonication temperature higher than 40°C would lead to the degradation of the analytes. Therefore, the sonication temperature of 40°C was chosen as the optimal temperature for the procedure.

The UAE offers many advantages including lower solvent consumption, lower temperature and shorter extraction time for, useful in the extraction of thermolabile and unstable compounds. Compared to the other assisted extraction methods such as MAE, the ultrasonic device is cheaper and its operation is much easier. However, it is important to strictly control the extraction parameters, especially the sonication time and sonication temperature to avoid the degradation of the analytes.

7. Gel permeation chromatography (GPC)

Usually, SFE, PLE, MAE and UAE are used as the initial extraction methods for the complex food samples due to the existence of numerous co-extractives. A powerful clean-up method, GPC, also known as size exclusion chromatography, was used for the first time in 1970's for the extraction and clean-up of pesticides [62,63].

The separation mechanism of GPC is based on the molecular size (Fig. 1). In GPC large molecules elute from the gel, followed by smaller molecules. Therefore, GPC is applied as a universal clean-up procedure for the separation of pesticide multi-residues from complex and volatile non-polar co-extractives (Table 6) [39,46,64-75]. Especially, it is an established method for the fractionation and/or clean-up of fatty matrices of both plant and animal origin including leeks [65], tea [73], fat vegetable matrices

Table 6. GPC applications in the analysis of pesticide residues in foods.

Matrix	pesticides	Sample preparation	References
animal origin	109 pesticides	Extraction: ASE (5 g Extrelut 20, MeCN, 80°C, 1500 psi, 5 min, purge (N_2,100 s), two cycles); GPC: 40 g Bio-Beads S-X3, cyclohexane-EtAc (1:1), 5 mL min^{-1}, 254 nm; Further clean-up: 0.2 g PSA.	[39]
olive oil	26 pesticides	Extraction: 3 mg Na_2SO_4, 2 mL *n*-hexane+10 mL MeCN; GPC: styrene-divinylbenzene copolymer, DCM, 5 mL min^{-1}, 220 nm and 254 nm.	[64]
leek	102 pesticides	Extraction: 3 mg Na_2SO_4, 80 mL acetone + 60 mL DCM; GPC: Bio-Beads S-X3, cyclohexane- EtAc (1:1), 2 mL min^{-1}; Further clean-up: SPE (GCB, 30 mL acetone-EtAc (1:1)).	[65]
agricultural products	97 pesticides	Extraction: 10 mL MeCN, 1 g NaCl+4 g $MgSO_4$, 150 mg $MgSO_4$ + 50 mg PSA; GPC: Shodex CLNpak EV-200 AC column, acetone-cyclohexan(3:7), 0.1 mL min^{-1}.	[66]
animal liver	OCPS,OPPs	Extraction: 20 mL EtAc, 3 times; GPC: polystyrene-divinylbenzene, cyclohexane-EtAc (1:1), 5 mL min^{-1}; MSPD: 2 g C_{18} dispersant, 2 g Florisil cartridge, 10 mL EtAc.	[67]
animal tissues	660 pesticideS	Extraction: 20 g Na_2SO_4, 35 mL cyclohexane- EtAc (1:1); GPC: Bio-Beads $S\text{-}X_3$, cyclohexane- EtAc (1:1), 5 mL min^{-1}, 254 nm.	[68]
chicken, pork, lamb muscle	OCPS,OPPs	Extraction: 20 mL EtAc, 3 times, 3 g Na_2SO_4; GPC: styrene-divinilbenzene copolymer, cyclohexane-EtAc (1:1), 5 mL min^{-1}.	[69]
Pesticides, PAHs	Olive, olive-pomace oils	Extraction: 2 mL *n*-hexane + 10 mL MeCN, 3 mg Na_2SO_4; GPC: styrene-divinylbenzene copolymer, DCM, 5 mL min^{-1}, 220 nm and 254 nm.	[70]
100 pesticides and contaminants	animal feed	Extraction: 20 mL EtAc; GPC: Enviro Gel column, cyclohexane-EtAc (1:1), 5 mL min^{-1}; Further clean-up:100 mg PSA.	[71]
111 pesticides	fruit-based baby food	Extraction: 100 mL EtAc, 75 g Na_2SO_4; GPC: PL gel column, cyclohexane-EtAc(1:1), 1 mL min^{-1}.	[72]
102 pesticides	Chinese teas	Extraction: 4 mL acetone-EtAc-*n*-hexane (1:2:1); GPC: cosmosil packed column, cyclohexane- EtAc (1:1), 3 mL min^{-1}; Further clean-up: SPE (GCB, 6 mL acetone- EtAc (1:2).	[73]
32 pesticides	virgin olive oil	Extraction: 10 mL MeCN saturated in *n*-hexane, 3 times; GPC: Envirogel GPC columns, cyclohexane-EtAc (1:1), 5 mL min^{-1}.	[74]
32 pesticides	olives	Extraction: 100 mL light petroleum; GPC: Envirogel GPC columns, cyclohexane-EtAc (1:1), 5 mL min^{-1}.	[75]
65 pesticides	avocado	Extraction: 50 mL cyclohexane-EtAc (1:1); GPC: polystyrene-divinylbenzene, cyclohexane-EtAc (1:1), 5 mL min^{-1}.	[46]

[46], olive oil [64,70,74,75], agricultural products [66], animal origin [39], animal liver [67], animal tissues [68], poultry muscle [69], animal feed [71] and fruit-based baby foods [72]. Since the relative molar mass of most lipids ranges from 600 to 1500 g mol^{-1} while those of most synthetic pesticides are within 200 and 400 g mol^{-1}, the lipid molecules are too large to pass through the pores of the polymer and are eluted the first from the column by the mobile phase [46,64,67-75]. Although DCM was believed to be the most efficient mobile phase among the eight studied organic solvents (MeCN, methanol, EtAc, DCM, diethyl ether, cyclohexane, *n*-hexane and petroleum ether) [64,70], it could damage the GPC columns and reduce their lifetime. Therefore, the mobile phase for the GPC system have been replaced with the less harmful mixtures of EtAc-cyclohexane (1:1) [39,46,67-69,71,72,74,75], acetone-cyclohexane (3:7) [66], and hexane-EtAc [73].

Before the GPC procedure, the ASE with MeCN [39], QuEChERS with MeCN [66], LLE using *n*-hexane-MeCN [64,70,74] and acetone-DCM [65], liquid-solid extraction using EtAc [67,71,72] and cyclohexane-EtAc [46,68] were used for the extraction and initial clean-up of analytes. However, some additional clean-up steps are necessary to reduce the amount of co-extracted interferences and the damage to the chromatographic system. For example, SPE column or cartridge packed with different materials were reported in many references to further clean-up the extracts after GPC procedure, such as in [65,67,73]. In addition, the GPC eluate was further purified by the matrix solid phase disperse treatment with PSA to obtain a clean extract [39]. Although the SPE material PSA may result in the degradation of some pesticides, it does not affect the majority of the GC amenable pesticides under the optimized amount of PSA. Therefore, PSA was used for the removal of the fatty acids and other compounds with hydroxy groups [71].

As the best suitable method for the multi-residue analysis of pesticides, GPC is generally recommended for the clean-up of extracts obtained from biological samples. However, there are some limitations, *e.g.* the GPC requires special equipments, whose cost greatly limits its popularization. Furthermore, more efforts are

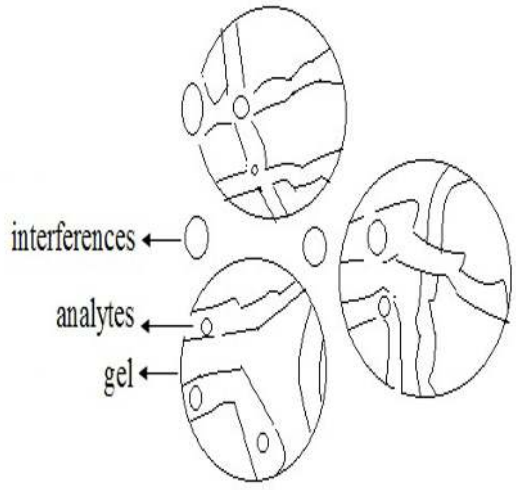

Figure 1. Schematic diagram of GPC.

still needed to reduce the analysis time and experimental cost, such as the consumption of extraction solvent and the gel column.

8. Solid phase extraction (SPE)

SPE is the most widely used sample preparation method for the pesticide residue analysis in foods, first introduced in the mid-1970's [76]. During the SPE, the extracts are passed through the cartridge and adsorbed on the solid phase materials, which have been previously conditioned and activated with water and/or organic solvent before use. Then the interferences are removed by pre-washing by organic solvents while the analytes are still retained on the adsorbents. After this clean-up step, the analytes can be subsequently eluted with other organic solvents to obtain clean extracts [77]. Because SPE requires small solvent volumes, and common experimental equipment, and provides simple experimental procedure, and a rapid sample throughput, it has been widely accepted as an alternative to LLE for the sample preparation, especially for the clean-up and enrichment of organic compounds in water samples. The applications of SPE for the sample clean-up in pesticide residue analysis in food matrix are listed in Table 7 [13,50,52,53,65,78-88].

SPE procedure is proceeds usually through the selective retention of target analytes on an adsorbent packed in a disposable extraction mini-column. To ensure sufficient absorption of analytes, various types of adsorbents have been developed for the clean-up/preconcentration of pesticide residues in foods. C_{18} was used as the SPE material to prevent the broadening of peaks in the on-line SPE-HPLC system [50]. After an initial removal of most lipid components of fish using a low-temperature clean-up at -24°C, Chen *et al*. [78] eliminated the remaining lipids by an aminopropyl (NH_2) SPE cartridge. Although GCB could retain and remove planar molecules such as pigments and sterols commonly present in crops, the Silica-bond TMA Chloride (SAX)-PSA cartridge was selected to remove fatty acids, other organic acids and various sugars from peach and lettuce extracts [80]. GCB-PSA dual-layer SPE was applied for the removal of fatty acid matrix components from several food matrices [81]. Xie *et al*. [82] obtained very clean eluates using activated carbon and Oasis HLB SPE cartridges for the extraction and clean-up steps, respectively. Although Envi-Carb or NH_2-LC could not separately absorb both the pigment contents and the polar matter (*e.g*. sugar, protein, *etc*.) of the berry matrix, their coupled column could provide the best clean-up effect and recoveries for both non-polar and polar pesticides [79]. In addition to theSPE materials above, multi-walled carbon nanotubes were first developed as SPE adsorbents by Ravelo-Pérez *et al*. [87] for the extraction of OPPs from fruit juices. It is obvious that the adsorbent capacity of the carboxylated single-walled carbon nanotubes was considerably better than the multi-walled carbon nanotubes [88]. Recently, a fast clean-up step based on d-SPE has been developed on the basis of the principle of QuEChERS method [13,52,85,86], which not only takes less time and consumes less labor and solvents, but also avoids the channeling, the breakthrough of analytes or matrix, and the preconditioning of SPE cartridges. So far, C_{18} combined with PSA [85,86], GCB coupled with PSA [52], and PSA associated with Florisil [13] have been selected as the d-SPE adsorbent to provide a good clean-up and high recoveries.

In addition to the adsorbent, the appropriate elution solvent or a mixture of elution solvents plays an important role in increasing the clean-up efficiency, since the solvents can disrupt the interaction between the target analytes and the adsorbent by eluting the analytes from the adsorbents. After the clean-up of OPPs in olive oil by GCB SPE cartridge, 3 mL DCM was chosen as the optimal elution to avoid the excessive elution of co-extracted oil [53]. Compared with methanol and MeCN, EtAc was applied for the elution of multi-class pesticide residues in virgin olive oils from a carboxylated single-walled carbon nanotubes cartridge [88]. Based on an earlier experiment [89], a MeCN-toluene solvent mixture (3:1) was used for the elution of the pesticide multi-residues from a NH_2 cartridge [78], a GCB cartridge coupled with NH_2-LC cartridge [79] and a dual-layer GCB-PSA SPE cartridge [81]. Balinova *et al*. [80] carried out the initial elution of pesticide multi-residues in crop from GCB by

Table 7. SPE applications in the analysis of pesticide residues in foods.

Matrix	pesticides	Sample preparation	References
fish	21 pesticides	Extraction: 20 mL MeCN, 5 g NaCl, freezed at -20°C for 20 min; Clean-up: SPE (NH_2 cartridge + Na_2SO_4, 25 mL MeCN-toluene (3:1).	[78]
berry fruits	88 pesticides	Extraction: 20 mL MeCN, 5 g NaCl; Clean-up: SPE (GCB + NH_2-LC + Na_2SO_4, 25 mL MeCN- toluene (3:1), 2 mL min^{-1}).	[79]
crops	25 pesticides	Extraction: 20 mL acetone or 40 ml acetone-water (8:2); Clean-up: SPE (GCB, 10 mL EtAc-methanol (8:2), 1 mL min^{-1}); Further clean-up: 250 mg Bond Elute SAX-250 mg PSA, 1mL EtAc-methanol (8:2).	[80]
leek	102 pesticides	Extraction: 3 mg Na_2SO_4, 80mL acetone+60mL DCM; GPC: Bio-Beads S-X3, cyclohexane-EtAc (1:1), 2 mL min^{-1}; Further clean-up: SPE (GCB, 35 mL acetone-EtAc (1:1)).	[65]
food	29 pesticides	Extraction: 100 mL MeCN or 20 mL MeCN; Clean-up: SPE (GCB-PSA, 14 mL MeCN-toluene (3:1))	[81]
olive oil	9 OPPs	Extraction: 5 mL MeCN-DCM (90:10), 2 min (250 W), 8 min (700 W); Clean-up: SPE (GCB, 3 mL DCM).	[72] [53]
agricultural samples	6 neonicotinoid pesticides	Extraction: MeCN, Na_2SO_4; Clean-up: SPE (activated carbon, 5 mL MeCN); Further clean-up: SPE (Oasis HLB, 5 mL methanol, 2 times).	[82]
wines	46 pesticides	Clean-up: SPE (Oasis HLB, 5 mL methanol, 2 times).	[83]
royal jelly	9 pesticides	Extraction: 10 mL MeCN-H_2O (1:1); Clean-up: SPE (C_{18}, 2 mL EtAc, 2 mL *n*-hexane).	[84]
grain	5 OCPs	Extraction: 10 mL MeCN-H_2O (95:5), 80 W, 10 min; Clean-up: SPE (C_{18}, 3 mL MeCN, 4 mL MeCN-H_2O (20:80)).	[50]
bovine milk	44 pesticides	Clean-up: d-SPE (C_{18}-PSA, 1 mL methanol).	[85]
fruits, vegetables	72 pesticides	Extraction: 0.1 mL acetic acid +15 mL acetone- MeCN (1:1), 2 min ramp from 100 to 300 W, 300W (3 min), 2 min ramp from 300W to 100W, 100W (2 min); Clean-up: d-SPE (100 mg PSA + 100-200 mg $MgSO_4$).	[52]
wines	160 pesticides	Extraction: 10 mL MeCN, 0.5 g disodium hydrogencitrate sesquehydrate, 1 g trisodium citrate dihydrate, 4 g $MgSO_4$, 1 g NaCl; Clean-up: PSA + C_{18}.	[86]
soybean oil	95 pesticides	Extraction: 5 mL MeCN, 2 times; Clean-up: freezed at -20°C for 6 h, d-SPE (25 mg Florisil + 100 mg $MgSO_4$).	[13]
fruit juices	8 OPPs	Clean-up: SPE (MWCNTs, 20 mL DCM).	[87]
olive oils	25 pesticides	Clean-up: SPE (single-walled carbon nanotubes, 3.0 mL methanol).	[88]

10 mL EtAc-methanol (8:2), and the second clean-up step was executed by a SAX-PSA SPE cartridge using EtAc-methanol (8:2) as an eluent. After the adsorption of the neonicotinoid insecticides on activated carbon SPE cartridge and the elution by MeCN, Xie *et al*. [82] loaded the eluates on a HLB SPE cartridge and eluted them with methanol rather than with MeCN for a further clean-up. Using the Oasis HLB SPE cartridges, Economou *et al*. [83] obtained higher recoveries of multi-class pesticide residues in wines by the elution with methanol than with EtAc or MeCN. Chen *et al*. [50] found that the recoveries of OCPs loaded on the SPE cartridge decreased with increasing MeCN concentration in the aqueous solution. When the concentration of MeCN was equal to or lower than 20%, the recoveries ranged from 95% to 103%.

As pH determined the stability of the analytes, the pH of extracts is crucial to ensure the high retention of pesticides on the adsorbent. Therefore, an appropriate pH is necessary to maintain the stability of pesticides and to increase the absorption of analytes on the solid phase. In order to ensure the stability of OPPs, Ravelo-Pérez *et al*. [87] adjusted the pH of apple, grape, orange and pine apple juices to 6.0 with 1.0 M NaOH.

Karazafiris *et al*. [84] loaded the supernatant of MeCN extracts of Royal jelly on a Varian Bond Elut C_{18} cartridge and then used 2 mL EtAc and 2 mL *n*-hexane as eluents. Ravelo-Pérez *et al*. [87] eluted the OPPs from a carbon nanotube-based cartridge with 20 mL DCM, which proved to be quick, cheap, accurate and highly selective for the analysis of this group of pesticides in fruit juices. A d-SPE method was utilized by Dagnac *et al*. [13,52,85,86] for the simultaneous analysis of pesticide multi-residues, which were extracted by an organic solvent, dried by salts, and then mixed with dispersive materials for the chromatographic determination.

SPE is simpler, more convenient, less solvent-consuming and easier to automate than LLE, and it can effectively avoid the formation of emulsion often encountered in LLE. When compared to SFE, PLE, MAE and UAE, SPE usually can complete the whole sample preparation without any further treatments and provide the subsequent clean-up procedure of these extraction methods. Furthermore, the SPE procedure is more convenient and cheaper than the GPC. Although SPE has become a well- established routine method for the clean-up or concentration of pesticides in food samples,

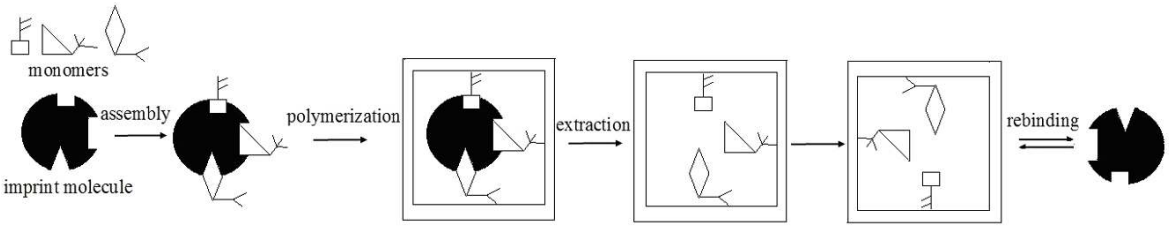

Figure 2. Schematic diagram of the synthesis of MIPs.

some features still need further improvements. First, it is difficult to rapidly choose the appropriate adsorbents and elution solvents for the analysis of pesticide multi-residues with a very wide range of physicochemical characteristics.Second, the commercial SPE cartridges can not be reused, which will greatly increase the experimental budget.

9. Molecularly imprinted polymers (MIPs)

In addition to the SPE materials mentioned above, molecularly imprinted polymers (MIPs) have been utilized. As shown in Fig. 2, MIPs are obtained by copolymerizing a monomer with a cross-linker in the presence of a template molecule (print molecule). After the template is washed away, the polymer contains recognition sites that are complementary in size, shape and chemical functionality to the template molecules. As the MIPs can selectively rebind with the template (analyte) and its analogous structures, they present specific molecular recognition ability and high binding affinity to the template molecules. Therefore, the MIPs can be used as adsorbents for the selective recognition and enrichment of the pesticide residues in foods (Table 8) [90-94].

Using suspension polymerization, polymers of methacrylic acid were highly cross-linked with ethylene dimethacrylate in the presence of the template of carbaryl molecule and its metabolite to obtain their molecular imprinted polymers, which were packed into a pre-column to isolate the analytes from complex matrices without extensive sample preparation and clean-up [90]. Djozan *et al*. [91] fabricated a monolithic SPME fiber from atrazine-imprinted polymers. The fiber is thermally and chemically stable and flexible enough to be placed in home-made SPME syringe for the extraction of atrazine and its structural analogues. In addition to these MIPs, a novel chemiluminescence sensor was synthesized using precipitation polymerization for the determination of glyphosate [92]. The glyphosate-molecularly imprinted microspheres were then modified on glass sheets, which were placed at the bottom of the microplate for the recognizer of glyphosate. Lv *et al*. [93] introduced non-covalent MIPs, which were obtained by using dimethoate as the template molecule, methyl methacrylate as the functional monomer and tetrahydrofuran as the porogen. To avoid the drawbacks of the conventional MIPs, another molecular imprinting method, surface-imprinting at silica adsorbents has been introduced [94]. The high density imprinted layer-coated silica nanoparticles were prepared and used as dispersive solid-phase extraction materials for the selective recognition of chlorpyrifos from complex matrices.

After the extraction with MIPs, the pesticide residues can be analyzed by a universal and unspecific determination method. Hantash *et al*. [90] reported for the first time a method for a selective extraction of carbaryl and its metabolite in apple, followed by the analysis by a universal and unspecific detection method such as LC-UV. Similarly, the chlorpyrifos-imprinted/non-imprinted nanoparticles were dispersed in the sample solutions, cleared up with chloroform, dispersed in methanol/acetic acid, and the desorption solution was then converted into methanol and analyzed with HPLC [94]. Lv *et al*. [93] found that the MIPs have shown selective recognition and high affinity to its corresponding template, and the eluates could also be analyzed by HPLC. In addition, the final desorption solution can be analyzed by GC. After the direct immersion of the atrazine-imprinted SPME fiber in rice and onion samples, the fiber was removed from the vial and washed with methanol and distilled water before the final GC analysis [91].

The sample preparation methods mentioned in previous chapters deal with the analysis of different kinds of pesticide residues and MIPs are developed for the analysis of analogous pesticides. Even though MIPs have successfully been used for the pesticide residue analysis in food samples, it still has many limitations including incomplete template removal, small binding capacity, low affinity and irregular material shape. Therefore, MIPs demand low cost, easy preparation, a more homogeneous binding site population, high affinity for the target analytes and good physicochemical stability over a wide range of experimental conditions and solvents.

Table 8. MIPs applications in the analysis of pesticide residues in foods.

Matrix	pesticides	Sample preparation	References
apple	carbaryl and its metabolite	Pre-column: carbaryl-imprinted polymer, MeCN-phosphate buffer (0.5 M, pH 7.0) (40:60).	[90]
rice, onion	triazine	SMPE: pH 7, atrazine-MIP and NIP fibers, direct immersion, room temperature, 500 rpm, 25 min, methanol and distilled water; Thermal desorption: 250°C,1 min.	[91]
foodstuff	glyphosate	Multimode Reader: chemiluminescence-molecular imprinting sensor, cleaned with doubly distilled water, $KMnO_4$+HCl+Tween-80.	[92]
tea leaves	dimethoate	Extraction: 600 mL hexane, 3 min; MISPE:100 mg dimethoate-MIP and NIP fibers, Tetrahydrofuran-acetic acid (95:5).	[93]
Green vegetables	chlorpyrifos	d-SPE: 20 mg chlorpyrifos-imprinted/nonimprinted layer-coated silica nanoparticles, cleared up with 2 mL $CHCl_3$, 1 mL methanol-acetic acid (9:1).	[94]

Table 9. MSPD applications in the analysis of pesticide residues in foods.

Matrix	pesticides	Sample preparation	References
fish	6 OCPs	Ultrasonic assisted-MSPD: 0.1 g sample + 0.1 g Na_2SO_4+0.4 g C_{18}, 1.5 mL MeCN, 37 kHz, 40°C, 10 min; homogeneous liquid-liquid extraction: 0.85 mL MeCN+ 35μL $CHCl_3$, 40°C, 5 min.	[60]
Onion	5 pesticides	MSPD: 0.5 g sample+1 g C_{18}, 10 mL MeCN.	[98]
oranges	carbendazim	MSPD: 0.5 g sample+0.5 g C_{18}+0.2 g sea sand, 10 mL DCM.	[99]
Porcine tissue	fluoroquinolones, OCPs, carbamates	MSPD: 0.5 g sample+2 g C_{18} dispersant, 1.5 g Na_2SO_4+0.25 g C_{18} cartridge, 6 mL *n*-hexane, 8 mL MeCN.	[100]
biota samples	OCPs, PBDEs	MSPD: 0.1 g sample + 0.4 g C_{18} dispersant, 0.1 g Florisil cartridge, 1.2 mL MeCN; SPME: PDMS- DVB fiber, 2 min.	[101]
bovine	OPPs	MSPD: 0.5 g sample + 2 g C_{18} dispersant, 5 mL MeCN; Clean-up: (0.5 g silica gel column, 15 mL MeCN) or (1.0g Florisil, 5 mL MeCN).	[102]
coconut	eight pesticide	MSPD: 0.5 g sample + 1 g C_{18} dispersant, 1 g Na_2SO_4 + 1 g Florisil cartridge, 40 mL MeCN- *n*-hexane (85:15).	[103]
eggs	OCPs, OPPs, PCBs	MSPD: 0.5 g sample + 2 g C_{18} + 1 g $MgSO_4$ dispersant, 2 g Florisil cartridge,1.5 mL MeCN- *n*-hexane (85:15), 8.5 mL EtAc.	[104]
animal liver	OCPs, OPPs	Extraction: 20 mL EtAc, 3 times; GPC: polystyrene-divinylbenzene, cyclohexane-EtAc (1:1), 5 mL min^{-1}; MSPD: 0.5 g sample + 2.0g C_{18} dispersant, 2 g Florisil cartridge, 10 mL EtAc.	[67]
fruits	OPPs, triazines, pyrethroids	MSPD: 0.5 g sample + 0.5 g C_8 dispersant, 0.7 mL EtAc.	[105]
fruits	15 OPPs□ 9 triazines	ultrasonic-assisted-MSPD with sonoreactor: 700 mg sample + 700 mg C_8, 700 μL EtAc, 35 kHz, 1 min.	[61]
olive oil	105 pesticides	MSPD: 1.0g sample + 2 g Bondesil-NH_2 dispersant, 2 g Florisil cartridge, 5 mL MeCN, 2 times.	[108]
olives	104 pesticides	MSPD: 1.0g sample + 2 g Bondesil-NH_2 dispersant, 2 g Florisil cartridge, 5 mL MeCN, 2 times.	[109]
propolis	3 OPPs	MSPD: 1mL sample+2 g $Al_2(SO_4)_3$ dispersant, 2 g water- deactivated Florisil cartridge, 30mL CH_2Cl_2-EtAc (9:1).	[96]
cattle feed	Pyrethroid, OCPs	MSPD: 0.5 g sample+ 2 g alumina+0.2 g Na_2SO_4 dispersant, 2 g Florisil cartridge, 5 mL EtAc.	[97]
palm oil	7 pesticides	Extraction: 10 mL MeCN, freezed at -20 °C for 6 h; MSPD: 2 mL sample + 2 g PSA dispersant, 1 g GCB cartridge, 15 mL MeCN.	[11]
plant	4 HCH	MSPD: 5 g sample+0.5 g Florisil+1 g $MgSO_4$+0.5 g NaCl dispersant, 2 g alumina + 0.5 g Na_2SO_4 cartridge, 40mL *n*-hexane-EtAc (7:3).	[106]
fruit juices	12 pesticides	MSPD: 1.0 mL sample +1 g diatomaceous earth dispersant, 10 mL DCM.	[107]

10. Matrix solid-phase dispersion (MSPD)

Matrix solid-phase dispersion (MSPD) was introduced for the sample preparation by Barker *et al*. in 1989 [95]. In contrast to the common SPE methods, MSPD combines the extraction and clean-up procedure into a single step. Generally, the MSPD method consists of the following steps: sample homogenization, cellular disruption, exhaustive extraction, fractionation, and the clean-up by adsorbents. Fig. 3 demonstrates the MSPD procedure. The samples are homogenized, transferred in a glass or agate mortar and dispersed on an adsorbent and/or a drying agent by the grinding effect of a glass or agate pestle, during which the physical structure of the sample is broken by the abrasive force of the adsorbent

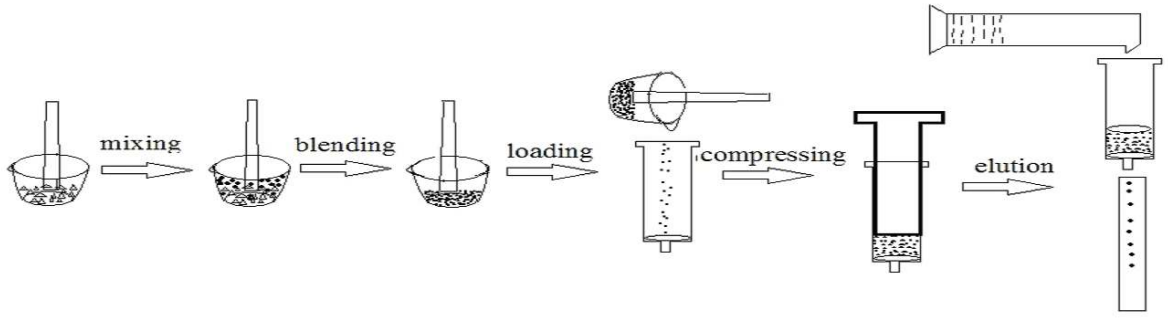

Figure 3. Schematic diagram of the operation of MSPD.

and ground into homogeneous and fine particles. The blended materials are then poured in a column that is empty or packed by other clean-up materials. The top and end of the column are covered by two frits to retain the entire sample and adsorbent. To obtain a good elution, it is necessary to avoid the channels in the column and over-compressing the material during packing. In the next step, the mortar and pestle are flushed with the elution solvent and the washings are applied to the column before the subsequent elution. Following the development of SPE, MSPD has become a well-established sample preparation method for the analysis of pesticide residues in food matrices (Table 9) [11,60,61,67,98-109], such as fruit and vegetables, oil, biota samples, eggs and fish.

The dispersants of MSPD are supposed to break the physical structure of the sample, extract the analytes from the sample and supply clean-up material for the sample matrix. Some adsorbents, such as C_{18}, C_8, silica, Florisil, diatomaceous earth and $Al_2(SO_4)_3$ have been used as dispersants of MSPD. Among them, C_{18} is still the most widely used dispersant in the MSPD procedure [60,67,98-104], as well as C_8 [61,105]. As neutral alumina, silica gel, Florisil and C_{18} were not suitable for the extraction of the polyphenolic of Uruguayan propolis, Pérez-Parada *et al*. [96] proposed $Al_2(SO_4)_3$ as a MSPD dispersant to enhance the distribution of sample on the adsorbent and the elimination of residual water. When the dispersant of C18 was used for the MSPD extraction of multiclass pesticides from palm oil, it produced an extracts with maximal interferences for most of the pesticides studied. However, the dispersing sorbent of PSA assisted with the clean-up sorbent of GCB could obtain the colorless extracts with minimal interferences and satisfactory recoveries [11]. Although diatomaceous earth, reused C_{18} and fresh C_{18} all presented satisfactory recoveries for the MSPD extraction of pesticides from onion, the reused C_{18} was chosen as the MSPD dispersant because it did not need any treatment before its use [98]. Using Florisil as a dispersant, Pinho *et al*. [11] and Abhilash *et al*. [106] conducted the MSPD extraction of pesticide residues in vegetables and fruits to obtain high recoveries and clean chromatographic background.

It is extremely important to select the ratio between the sample and the sorbent to ensure the formation of fine particles and effective dispersion of the sample on the sorbent. The normal ratio between the sample and the sorbent typically ranges from 1:1 to 1:4. The ratios between onion and reused C_{18} of 1:2 and 1:3 presented better recoveries than the ratio of 1:1 and 1:4. To save the sorbent material and facilitate the packing of the column, the ratio 1:2 was chosen for the MSPD [98]. As egg matrix is a fatty and highly viscous sample, Bolaños *et al*. [104] found more matrix content in the final extract with the ratio of 1:4 than that with 1:2 ratio. However, Wang *et al*. [100] could not achieve complete MSPD and high reproducibility with 0.5 g sample matrix and 1.0 g C_{18}, leading to the use of 0.5 g sample mixed with 2.0 g C_{18}. Furthermore, Frenich *et al*. [67] found that the ratios of 1:4 supplied higher extraction efficiencies than 1:2 ratio. Therefore, the 1:4 ratio may be practical in most cases although it is less economic.

The nature and volume of the elution solvent is important for the efficient desorption of pesticides from the adsorbent and the absorption of interferences on the SPE column. A large variety of solvents, for example MeCN, methanol, EtAc, DCM or mixtures of them, have been tested in the MSPD. In the elution of selected pesticides in fruits from diatomaceous earth, Radišić *et al*. [107] demonstrated that EtAc could not give satisfactory recoveries, and the elution by methanol gave a turbid extract, so DCM was chosen as the elution solvent. The *n*-hexane-EtAc mixture (70:30) not only retained a large number of plant co-extracts on the Florisil column, but also allowed successful elution of the hexachlorocyclohexane isomers in plant matrix [106]. In the elution of pesticide residues from Florisil column using DCM-EtAc, the ratio of 9:1 was selected as a compromise in the quantitative elution of pesticides and matrix co-extractives in propolis tinctures [96]. As hexane could not effectively elute the pyrethroid and

OCPs from alumina column, EtAc was found to be suitable for the elution [97]. During the elution of OCPs and OPPs from C_{18}, the recoveries with EtAc were better than those with cyclohexane, MeCN and EtAc-cyclohexane [67]. Considering quantitative recoveries, good reproducibility and clean-up of interfering compounds, Silva *et al*. [103] revealed that the elution by MeCN saturated with *n*-hexane was better than that of MeCN-EtAc (1:1). In contrast to acetone and methanol, MeCN provided maximum elution efficiencies for the OCPs from C_{18} column [60]. Although MeCN gave lower recoveries than EtAc, it was selected as the elution solvent owing to its suitable relative standard deviation (RSD) and good chromatographic resolution [98]. As hexane, acetoacetate and MeCN-acetic acid could not efficiently elute the analytes, MeCN was used for the elution of the OPPs and N-methyl carbamates from C_{18} [100]. Although 9 mL 80% or 4 mL 100% MeCN proved to complete the elution of OPPs from C_{18}, the latter was adopted due to the minimal consumption of MeCN [102].

During the MSPD, some matrices are extracted and scattered on the dispersant, and they may be eluted with the analytes by the solvent. To avoid the disturbance of the interferences, occasionally an additional clean-up step is necessary and done by packing the co-column materials at the bottom of the adsorbent column. To remove the polyphenolic co-extractives, Florisil was used as co-sorbent material below the blended mixture of $Al_2(SO_4)_3$ and propolis [96]. Gilbert-Lópeza *et al*. [108,109] used a commercially available SPE cartridge containing Florisil in an extra clean-up step to remove the residual matrix of olives and olive oil eluted from the aminopropyl sorbent. As C_{18} led to abnormally high recoveries for some pesticides, Florisil was selected as the extra clean-up material and it proved to provide the most effective recoveries for all target analytes [97]. During the clean-up of bovine extracts from the MSPD column packed with C_{18}, the silica in the bottom of the MSPD phase and the C_{18} co-column both supplied lower recoveries due to the washing interferences. Florisil gave even lower recoveries due to its potential to retain polar analytes. However, the silica adsorbent packed in an independent cartridge could provide satisfactory recoveries [102]. Sobhanzadeh *et al*. [11] gained a clean extract and low recoveries of multiclass pesticides in palm oil with the clean-up sorbent of Florisil, which could be done by the clean-up adsorbent of GCB.

In addition to the methods above, a MSPD using 0.5 g sample mixed with 0.5 g C_{18} and 0.2 g sea sand [99] allowed the detection of carbendazim at a 5.0×10^{-9} M level to meet the legal requirements. Bolaños *et al*. [104] introduced a new pesticide multi-residue method for the simultaneous analysis of OCPs, OPPs, and polychlorinated biphenyls in eggs, based on the C_{18} adsorbent and the elution by 1.5 mL *n*-hexane-MeCN (15:85) and 8.5 mL EtAc. A miniaturized MSPD was developed by Moliner-Martineza *et al*. [101] using C_{18} as dispersant, Florisil as fat retainer, and MeCN-water as elution solvent for the analysis of legislated OCPs and polybrominated diphenylethers in biota samples. Processing the small quantity of food samples not only proved to be easier and faster than the conventional MSPD procedure, but it also greatly reduced the amounts of sample, dispersant and solvent volume. Using C_8 as dispersant and EtAc as extraction solvent, an ultrasonic-assisted MSPD method was used in the analysis of OPPs and triazines in fruits [61], which could meet the low detection limits except for dimethoate and disulfuton.

MSPD simultaneously performs the disruption of sample and the dispersion of sample components on a solid support, thereby generating a chromatographic material suitable for the extraction of analytes from the dispersed sample. MSPD has evident superiority when it is applied to solid, semi-solid, and liquid foodstuffs, because it avoids the troublesome pre-treatment process. Compared to the traditional LLE and SPE, the primary advantage of MSPD is that both the sample extraction and the clean-up procedure are performed in one step using small amounts of adsorbent and solvent. Thus it not only simplifies and speeds up the sample preparation process, but also reduces the consumption of large amounts of toxic solvents, avoids emulsion formation, shortens the analysis time and increases the reliability, selectivity and sensitivity of pesticide residue analysis. However, it is hard to ensure the repeatability of the homogenizing and grinding procedure because of the hand-made operation, which may lead to experimental errors and instability. Thus, significant technology innovations should be introduced in the MSPD process.

11. Solid phase micro-extraction (SPME)

Modern trends in the analysis of pesticide residues require the simplification of sample preparation, as well as the minimization of organic solvent and operation time. In 1989, solid phase micro-extraction (SPME) was first introduced as a SPE development by Pawliszyn *et al*. [110], and it has been rapidly commercialized by Supelco in the past years. Similar to the SPE, SPME is based on the partition equilibrium of analytes between the sample and the stationary phase. This means that

Table 10. SPME applications in the analysis of pesticide residues in foods.

Matrix	pesticides	Sample preparation	References
red wines	11 pesticides	DI-SMPE: PDMS-DVB, 3 g NaCl (30%), pH 9.5, 900 rpm, 143 min, ambient temperature; Desorption: 1.0 mL methanol, 1000 rpm, 13 min.	[112]
fruit juice	54 pesticide	DI-SMPE: PDMS-DVB, ambient temperature, 10 min; Desorption: 250°C, 5 min.	[113]
bovine milk	30 pesticides	DI-SMPE: PDMS-DVB or CAR-PDMS, 20% NaCl, 30 min; Desorption: 270°C or 290°C for 5 min.	[114]
tomatoes	7 pesticides	DI-SMPE: PDMS-DVB, 30% NaCl, pH 9.5, 900 rpm, 143 min; Desorption: 1.0 mL methanol, 1000 rpm, 13 min.	[115]
fruit juices	carbamate and phenylurea	DI-SMPE: PDMS-DVB and CW/TPR, 30% NaCl, 20°C, 90 min; Desorption: methanol-water (70:30), 15 min.	[116]
baby foods	fungicides	DI-SMPE: PDMS-DVB, pH 5.0, 60°C, 40 min; Desorption: 240°C, 4 min.	[117]
cucumber watermelon	pyrethroids	DI-SMPE: PDMS-DVB, pH 3.0, 65°C, 1000 rpm, 30 min; Desorption: MeCN-water (25:75), 5 min.	[118]
vegetables	organochlorine pyrethroid	DI-SMPE: PMPS-OH, 1000 rpm, 40°C, 30 min; Desorption: 280°C, 4 min	[119]
tea	13 pesticides	DI-SMPE: Single-walled carbon nanotubes coated fibers, 15% NaCl, pH 5.5, 50°C, 40 min; Desorption: 180°C, 3 min.	[120]
tomato strawberry	OCPs	DI-SMPE: polypropylene hollow fiber membrane, 2.91 g NaCl, 59°C, 60 min; Desorption: toluene- hexane (60:40), 10 min.	[121]
mangoes	14 pesticide	DI-SMPE: Polyacrylate, 50°C, 250 rpm,30 min; Desorption: 280°C, 5 min.	[122]
rice, onion	10 pesticides	DI-SMPE: copolymerization of atrazine-molecular imprinted polymer, pH 7.0, room temperature, 250 rpm, 25 min; Desorption: 250°C, 1 min.	[91]
honey	OPPs, carbamates	DI-SMPE: CW/TPR, 120 min; Desorption: methanol-water (70:30), 15 min.	[43]
cow milk	OPPs	HS-SMPE: PDMS-DVB, 90°C, 600 rpm, 45 min; Desorption: 250°C, 5 min.	[123]
tea	36 pesticides	HS-SMPE: PDMS, 70°C, 60 min; Desorption: 270°C, 2 min.	[124]
cucumber strawberry	OPPs, OCPs	HS-SMPE: PDMS, 60°C, 800 rpm, 30 min; Desorption: 240°C, 10 min	[125]
fruits vegetables	8 pesticides	HS-SMPE: PDMS, 60°C, 800 rpm, 30 min; Desorption: 240°C, 10 min	[126]
fish tissue	OCPs	HS-SMPE: PA, 80°C, 45 min; Desorption: 240°C, 5 min.	[127]

the analytes are absorbed on the solid phase and then desorbed either by thermal energy of the GC injection port, or by the solvent elution of HPLC mobile phase during the subsequent chromatographic determination. SPME combines the sampling, extraction, concentration and injecting the sample into a single sample preparation procedure [111]. Greatly reducing the consumption of organic solvent and complicated procedures, SPME proves to be a valuable alternative analytical method to many traditional procedures. Therefore, there is increasing interest toward the SMPE in the pesticide residue analysis in food samples (Table 10) [43,91,112-127].

There are two main kinds of SPME modes, the first of which is the direct-immersion solid phase micro-extraction (DI-SPME), illustrated in Fig. 4. Using the PDMS-DVB fiber, Ravelo-Pérez and coworkers [112] carried out the DI-SPME of pesticide residues in red wines at ambient temperature for 143 min with continuous stirring at 900 rpm. Then the pesticides were desorbed from the fiber with 1.0 mL methanol by stirring for 13 min at 1000 rpm. Similar to this DI-SPME method above, the PDMS-DVB fiber was used for the extraction of pesticides from fruit juice [113], bovine milk [114], tomatoes [115], fruit juices [116], baby foods [117] and cucumber and watermelon [118]. In addition to the PDMS-DVB fiber, CW-TPR fiber [116], hydroxyl-terminated polymethylphenylsiloxane (PMPS-OH) [119], single-walled carbon nanotubes coated fiber [120], polyacrylate fiber [122], and atrazine-molecular imprinted polymer [91] were recently developed for the DI-SMPE of pesticide residues in food samples. The simultaneous liquid-liquid micro-extraction and polypropylene microporous membrane SPE of OCPs in tomato and strawberry samples was reported by Bedendo *et al*. [121], during which the analytes were concentrated onto the microporous membranes containing water with 1-octanol. Although the DI-SPME with CW-TPR was accurate for the extraction of the selected pesticides from honey, it could not be serve as a quantification method due to its low recovery of some pesticides when compared to QuEChERS, SPE and PLE [43].

Another SPME mode is called headspace solid phase micro-extraction (HS-SPME). Different from the DI-SPME, the SPME fiber is put in the air above the liquid or solid sample, demonstrated in Fig. 5. Schurek *et al*. [124] optimized the HS-SPME conditions for the pesticide multi-residue analysis in tea samples. Using a PDMS fiber, the analytes were extracted at 70°C for

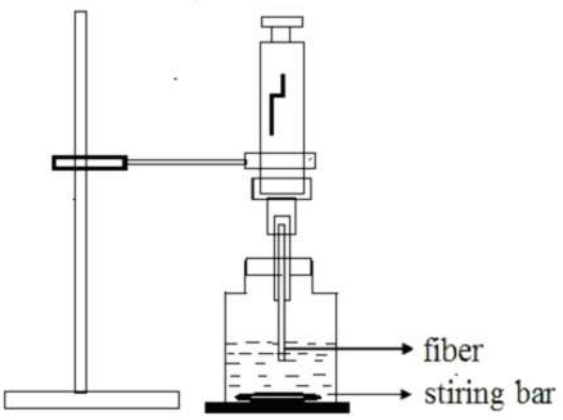

Figure 4. Schematic diagram of DI-SPME.

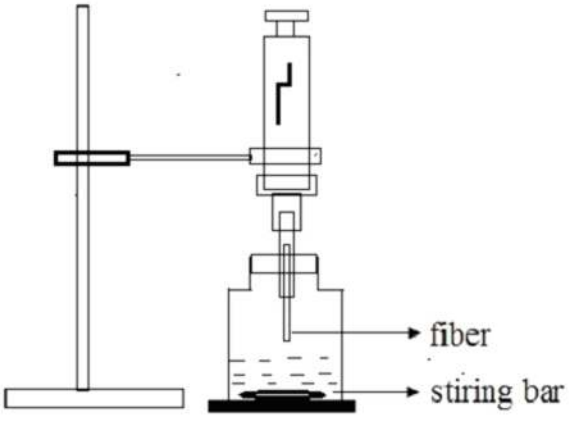

Figure 5. Schematic diagram of HS-SPME.

60 min and thermally desorbed from the fiber by a hot splitless GC injection port at 270°C for 2 min. A similar HS-SPME was also executed using a PDMS-coated fiber for the evaluation of pesticide residue contents in fruits and vegetables [125,126]. Rodrigues *et al*. [123] exposed the PDMS-DVB fiber to the headspace of the cow milk. After the extraction and pre-concentration of analytes, the fiber was then inserted directly into the GC injector for desorption. HS-SPME using a PA fiber was introduced for the enantioselective determination of the organochlorine pesticide bromocyclen in fish tissue [127].

Although DI-SPME is able to reach fast adsorption equilibrium, it is easily disturbed by matrix impurities. The factors affecting the DI-SPME were usually optimized. Ravelo-Pérez *et al*. [115] found that acetone provided higher recoveries with a clean electropherogram than MeCN and EtAc during the ultrasound-assisted extraction. Additionally, PDMS, PDMS-DVB, CAR-PDMS and PA fibers supplied similar recoveries while CW-DVB fiber was the least efficient one for pesticides. In view of the efficient extraction of all the target analytes, PDMS-DVB and CAR-PDMS were the most suitable coatings. The addition of NaCl and the adjustment of pH [112,116,120] were also important for the extraction. However, the ionic strength and pH seemed to have no significant effect on the final results during the SPME extraction [113]. Furthermore, Fernandez-Alvarez *et al*. [114] found that 1:10 dilution ratio was necessary to reduce the solution viscosity and adding acetone was helpful to modify the distribution constant between the fiber and the sample in the fortified solutions. In addition, the volume of solution [116], the extraction temperature [116,117,119,120] and equilibration time [116,117,119,120] were also discussed during different SMPE procedures.

Although HS-SPME can overcome the problem of matrix interference, it shows low adsorption equilibrium and enrichment effect for the compounds with high boiling points. Although the extraction efficiency usually increased with increasing extraction temperatures, excessively high temperature could result in a drop the relative signals the analytes [6]. Fidalgo-Used *et al*. [127] investigated the HS-SPME and DI-SPME using PDMS and PA fibers in detail, and observed that the extraction efficiencies of HS-SPME were better than those of DI-SPME and the PA fiber showed slightly better extraction efficiency than the PDMS. When the extraction efficiency increased with the extraction temperature at the range from 25°C to 80°C, here HS-SPME was chosen to be carried out at an extraction temperature of 80°C. Although no considerable improvement of pesticide extraction was observed with adding various concentrations of NaCl, the equilibrium conditions were obtained at 45 min and at the pH values of 6 - 7.

Compared with the SPE, SPME is a simple, one-step, automated and solvent-free method of extraction. The main advantages of SPME are good analytical performance, simplicity, and low cost. Furthermore, it does not suffer from the plugging or channeling problems encountered with SPE. However, SPME is still laborious because the equilibrium between the sample solution and the fiber may take a long time and need many rigorous extraction conditions. Furthermore, the fibers used in SPME are expensive and fragile.

12. QuEChERS

The most extensively applied sample preparation method of the ones discussed above in the pesticide residue analysis in food samples is SPE. Another, more recent and now widely used sample preparation method named QuEChERS was introduced in 2003 [128]. This method is based on the micro-scale extraction

Table 11. QuEChERS applications in the analysis of pesticide residues in foods.

Matrix	pesticides	Sample preparation	References
sugarcane juice	7 herbicides	Extraction: 10 mL MeCN, 4 g $MgSO_4$+1 g NaCl; d-SPE: 0.2 g PSA+0.6g $MgSO_4$.	[129]
honey	OPPs, carbamates	Extraction: 3 mL MeCN, 6g $MgSO_4$+1.5 g NaCl; d-SPE: 50 mg PSA + 150 mg $MgSO_4$.	[43]
baby food	4 OPPs	Extraction: 10 mL MeCN, 4 g $MgSO_4$+1 g NaCl; d-SPE: 150 mg $MgSO_4$ +50 mgPSA + 0.1-0.3 g C_{18}.	[130]
grapes, musts, wines	27 pesticides	Extraction: 10 mL MeCN, 4 g $MgSO_4$ + 1 g NaCl; d-SPE: 0.15 g $MgSO_4$ + 0.05 g PSA + 0.05 g C_{18}	[131]
olive oil	105 pesticides	Extraction: 10 mL MeCN, 4 g $MgSO_4$ + 1 g NaCl, 0.25 g PSA + 0.25 g C_{18} + 0.25 g GCB + 0.75 g $MgSO_4$.	[108]
olives	104 pesticides	Extraction: 10 mL MeCN, 4 g $MgSO_4$ + 1 g NaCl; d-SPE: 0.25 g PSA + 0.25 g C_{18} + 0.25 g GCB + 0.75 g $MgSO_4$.	[109]
plant matrices	212 pesticides	Extraction: 10 mL MeCN, 4 g $MgSO_4$ + 1 g NaCl.	[132]
paprika	168 pesticides	Extraction: 30 mL MeCN + 10 mL H_2O, 10 g dry ice; d-SPE: 62.5 mg PSA + 18.5 mg GCB + 37.5mg $MgSO_4$.	[133]
fruits, vegetables	32 pesticides	Extraction: 15 mL MeCN + 15 mL Hac(1%), 6g $MgSO_4$ + 1.5 g NaAc; d-SPE: 50 mg PSA + 50 mg C_{18} + 150 mg $MgSO_4$.	[134]
cooked foodstuff	41 pesticide	Extraction: 15 mL MeCN + 0.15 mL HAc, 6 g $MgSO_4$ + 1.5 g NaAc; d-SPE: 0.3 g PSA + 0.9 g $MgSO_4$.	[135]
leeks	20 OPPs	Extraction: 15 mL MeCN+0.15 mL HAc, 4 g $MgSO_4$ + 2 g NaAc; d-SPE: 100 mg PSA + 40 mg GCB + 600 mg $MgSO_4$.	[136]
fruits, vegetables	150 pesticide	Extraction: 15 mL MeCN + 0.15 mL HAc(1%), 6g $MgSO_4$ +1.5 g NaAc; d-SPE: 50 mg PSA + 50 mg C_{18}+ 7.5 mg GCB + 150 mg $MgSO_4$.	[137]
wheat, white flour, bran.	24 pesticides	Extraction: 10 mL MeCN + 0.1mL HAc, 3 g $MgSO_4$ + 1.7 g NaAc + 1 g sodium citrate; d-SPE: 0.5 g C_{18} + 0.6 g $MgSO_4$.	[138]
tea	65 pesticide	Extraction: 10 mL MeCN + 0.1mL HAc, 4 g $MgSO_4$ + 1.5 g NaAc; SPE: GCB-NH_2 , 20 mL MeCN-toluene(3:1) (1% acetic acid).	[139]
rice	herbicides, fungicides insecticides	Extraction: 15 mL MeCN + 0.15 mL HAc, 7g $MgSO_4$ + 1 g NaAc; d-SPE: 1 g $MgSO_4$.	[31] [140]
Soya grain	169 Pesticides	Extraction: 20 mL MeCN + 0.2 mL HAc, 2 g $MgSO_4$ + 2.5 g NaAc; d-SPE: 2.0 g $MgSO_4$.	[9]
fruit juices	90 pesticides	Extraction: 10 mL MeCN + 0.1 mL HAc, 4 g $MgSO_4$+1.0 g NaAc.	[141]
farming foodstuff	140 pesticides	Extraction: 10 mL MeCN, 1 g Na_3Citrate dihydrate+0.5 g Na_2HCitrate sesquihydrate+4.0 g $MgSO_4$+1 g NaCl, d-SPE: 0.15 g PSA+0.95 g $MgSO_4$.	[142]
fruit	14 OCPs	Extraction: 10 mL MeCN, 1 g Na_3Citrate dihydrate+0.5 g Na_2HCitrate sesquihydrate+1.0 g NaCl+ 4.0 g $MgSO_4$; d-SPE: 0.15 g PSA+0.9 g $MgSO_4$.	[143]
bananas	11 pesticides	Extraction: 10 mL MeCN, 1 g Na_3Citrate dihydrate + 0.5 g Na_2HCitrate sesquihydrate + 1 g NaCl+4 g $MgSO_4$; d-SPE: 0.125 g PSA + 0.75 g $MgSO_4$.	[144]

using MeCN, the water absorption and liquid-liquid partition utilizing $MgSO_4$ and NaCl, and the clean-up step of d-SPE employing primary-secondary amine (PSA) adsorbent. The detailed operation procedure of QuEChERS is presented in Fig. 6. Combining the conventional extraction, isolation and clean-up procedures into one step, this method greatly avoids blending, filtration, large volume of solvent transfers, evaporation/condensation and/or necessary solvent exchanges for the chromatographic determination. The abbreviation QuEChERS stands for quick, easy, cheap, effective, rugged and safe [61], describing the advantages over the traditional LLE. Thus it has gained significant popularity in the sample preparation of the pesticide residue analysis in food matrix (Table 11) [9,43,108,109,129-144].

Selecting the extraction solvent is critical in QuEChERS, as it directly determines the extraction efficiency. Although MeCN is miscible with water, it can be easily separated from water by the salting-out effect and centrifugation. Furthermore, MeCN not only yields higher recoveries and less interference than other solvents such as acetone and methanol [134,140], but it also offers slightly better limit of detection (LOD) and RSD than acetone [9]. Up to now, MeCN has been regarded as the most widely used extraction solvent for the QuEChERS procedure. Different from the common QuEChERS, Lee *et al*. [133] introduced the dry ice-partitioning QuEChERS method for the determination of 168 pesticides in paprika, creatively using dry ice to promote the separation of the upper MeCN layer without the salting-out effect and to avoid the possible degradation of thermal effect produced by the addition of $MgSO_4$ and NaCl.

As high pH may influence the stability of some base-sensitive pesticides and the final recoveries, certain

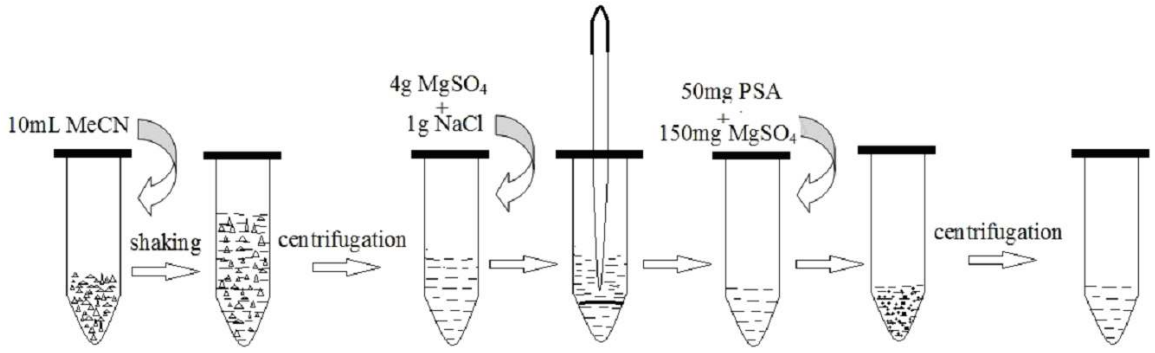

Figure 6. Schematic diagram of the common procedure of QuEChERS.

buffer solutions are advised to avoid the degradation of these pH-dependent pesticides during the QuEChERS procedure [9,134-141]. The addition of acetic acid-sodium acetate buffer solution to the MeCN extracts not only guaranteed the stability of base-sensitive pesticides, but also supplied adequately high recoveries [136,139]. The acetate-buffered versions of QuEChERS also showed higher recoveries and applied to the wider scope of pH-dependent pesticides than the original QuEChERS method [135,137]. In addition to the acetate-buffered QuEChERS, the citrate-buffered QuEChERS method has also been adopted for the pesticides multi-residues analysis in different foods [142-144], presenting in most cases good repeatability and recoveries. Lehotay *et al*. [134] investigated the difference between the original unbuffered QuEChERS method and two inter-laboratory validated versions, which were the AOAC Official Method 2007.01 using acetate buffering and European Committee for Standardization Standard Method EN 15662 calling for citrate buffering. The results showed that the acetate-buffered QuEChERS using MeCN was superior to the other tested methods. Furthermore, Pareja *et al*. [140] comprehensively studied four different QuEChERS methods, including the original QuEChERS, citrate-buffered QuEChERS, citrate-buffered QuEChERS without clean-up and acetate-buffered QuEChERS without clean-up. Among them, the last sample preparation method without the extra clean-up step provided cleaner chromatograms, less matrix effect and better results.

After the extraction/partition of MeCN, a d-SPE clean-up step with PSA adsorbent is always included in the conventional QuEChERS procedure, expected to retain fatty acids and other organic acids that are ubiquitous in foods [43,129]. As the addition of PSA could not complete the clean-up of extracts, another C_{18} adsorbent was added to remove the lipophilic coextracts of the MeCN extracts from three low-fat baby food matrices [130]. In addition, the GCB was also used as the clean-up material due to its intensive removal of the high content of fat, pigments and sterols in complex foodstuff extracts including olives, olive oil, leeks, fruits and vegetables [108,109,136,137], which was also found to retain the pesticides with planar ring structures in the complex matrix. An extra SPE cartridge loaded with GCB-aminopropylsilanized silica gel was adopted for the complementary clean-up step of QuEChERS method to remove the pigments from tea [139]. However, the clean-up material of PSA can be omitted under certain circumstances. As a result of the weak matrix effect arising from the low pH in the acetate-buffered QuEChERS procedure, Pareja *et al*. [140] abandoned the clean-up step with PSA for the analysis of pesticide residues in polished rice. The d-SPE procedure was excluded to reduce the matrix effect [9] and enhance the selectivity [141]. Furthermore, Lacina *et al*. [132] also gave up this d-SPE procedure in the identification/quantification of multiple pesticide residues in food plants due to its adsorption and/or degradation of base-sensitive analytes.

Compared to the classic LLE, the advantages of QuEChERS are simpler and less time-consuming procedure, and lower organic solvent consumption. Since QuEChERS method largely simplifies the extraction and clean-up step during the sample preparation and provides reliable quantitative results, it has a bright future in pesticide residues analysis in foods.

13. Cloud point extraction (CPE)

To enhance the sensitivity of the analysis, the analytes are always extracted and concentrated to a small amount of injection solution. This consumes a large volume of organic solvent, and requires long experimental procedures and costly equipment. To perfect the extraction methods, the cloud point extraction (CPE) or micelle-mediated extraction (MME) was introduced by Watanabe and co-workers in 1976 [145]. When the concentrations of non-ionic surfactants are increased

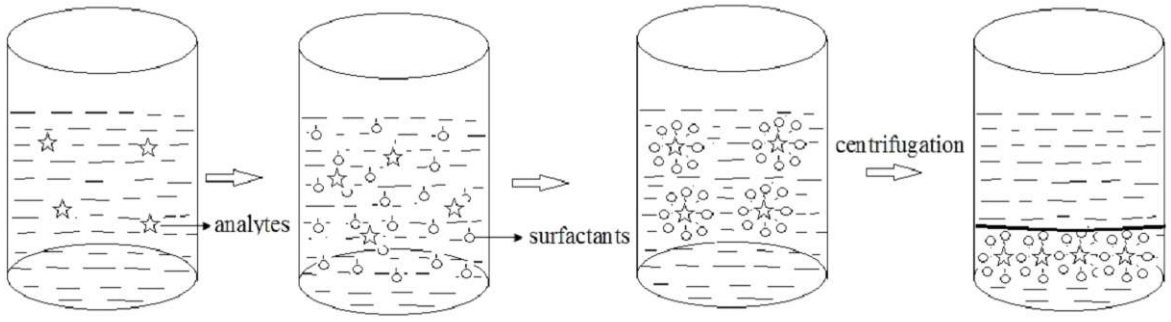

Figure 7. Schematic diagram of CPE.

above critical micelle concentration, they form micelles and become cloudy at the cloud-point temperature, which is usually higher than its critical temperature. The cloudy solution subsequently phase-separates into the aqueous phase and the surfactant-rich phase, where the latter one contains the analytes in very small volume. Fig. 7 explains in detail the procedure above. As the concentrated analytes are too viscous for the chromatographic injection, they can be diluted with a minimum volume of organic solvent or mobile phase. The uses of CPE in the pesticide residue analysis in foods have been listed below (Table 12) [146-151].

As different surfactants present different extraction efficiencies, it is necessary to optimize the surfactants for sufficient extraction of analytes. When PEG 4000 and PEG 10000 were tested for the CPE of OPPs from fruit juice, PEG 4000 supplied poor recoveries and PEG 10000 produced a viscous extracts, which were hard to carry out the back-extraction process. However, PEG 6000 could avoid these disadvantages and was used as the extraction solvent [146]. Although Triton X-114 had some UV absorption, it adsorbed on the chromatographic column and could be eluted after each chromatographic separation. Therefore, Triton X-114 did not interfere with the separation and detection of carbamate insecticide residues in fruits [147]. Compared to several anionic surfactants, such as sodium dodecyl sulfonate and sodium decyl sulfate, sodium dodecyl sulfate gave a surfactant-saturated phase with low absorbance at wavelengths above 270 nm [148]. From the four surfactants, PEG 4000, Tween 20, Triton X-100 and Triton X-114, PEG4000 was not suitable for the CPE due to restraining albumin precipitation. Triton X-100 and Triton X-114 had an advantage over Tween 20 in terms of the recoveries. After taking the impurities and viscosity of the enrichment phase into account, Triton X-100 was selected as the extracting solvent [150]. As the cloud point temperature for Triton X-100 is 65°C, this high temperature can lead to a hydrolysis of the organic compounds during the CPE procedure. Therefore, Triton X-114 and PEG-6000 were used for CPE separation of sulfonylurea herbicides in the sample preparation due to their relatively low cloud point. Triton X-114 gave higher recoveries than PEG-6000 [151].

As pH directly controls the ionization state of the analytes and the existence of analytes in the surfactant-rich (micelle) phase, it is one of the most critical factors in the CPE. It has been well known that the uncharged forms of the analytes bind better to the micelles than their charged counterparts [150]. As an example of weak acidic compounds, sulfonylurea herbicides can be ionized in solutions with high pH and therefore, pH 2.0 was the optimal value for the CPE considering the recoveries [151]. When the pH range of 3.5-8.0 was investigated, the recoveries of most OPPs were reduced with decreasing pH due to the hydrolysis in alkaline solution [146]. Zhou *et al*. [149] found that the recoveries of four carbamate pesticide derivatives increased rapidly with the increase of pH from 7.5 to 9.5 and then remained constant at the pH range of 9.5-11.

With increasing equilibration temperature, the volume of the surfactant-rich phase tends to decrease and as a result the concentration of the analytes in the surfactant phase increases. Therefore, an optimum equilibration temperature of CPE is needed for the CPE. The use of PEG 6000 surfactant could lead to the dehydration of micelle at room temperature and therefore the room temperature was selected to avoid the hydrolysis of OPPs at elevated temperatures [146]. Zhou *et al*. [149] found that a significant decrease in the extraction efficiency occurred above 30°C while the maximum extraction efficiency was observed at the range of 25-30°C, due to the the instability of the derivatives. Although the cloud point temperature of Triton X-114 surfactant is 25°C, a high equilibration temperature of 45°C is the optimal for the CPE procedure for the high recoveries [147]. During the CPE with Triton X-100, an increase in the recoveries was observed at the temperature range of 30-50°C while the recoveries gradually decreased at temperatures higher than 50°C [150]. When the

Table 12. MIPs applications in the analysis of pesticide residues in foods.

Matrix	pesticides	Sample preparation	References
fruit juice	9 OPPs	Extraction: 3.0 mL PEG 6000 (20%), 2 g Na_2SO_4, room temperature, 15 min incubation, 4000 rpm, 5 min centrifugation.	[152][146]
fruit	carbamate	Extraction: 1.5% Triton X-114, 7% NaCl, 45 °C, 20 min incubation, 3500 rpm, 20 min centrifugation.	[153] [147]
vegetables	carbaryl	Extraction: 0.3 g Sodium dodecyl sulfate + 5 mL HCl, ice bath, 2 min incubation, 3000 rpm, 1 min centrifugation.	[154] [148]
Corn	Carbamate	Extraction: 4% Triton-100, 18% Na_2SO_4, pH 9.5, 25°C, 10 min incubation, 3500 rpm, 10 min centrifugation.	[155] [149]
milk	sulphonamides	Extraction: Triton X-100, 65μL $NH_3.H_2O$ + 0.243 g Na2SO4+ 0.4 mL n-butyl alcohol, 50°C, 20 min incubation, 20 min centrifugation (6953.125 g).	[156] [150]
Rice	Metsulfuron-Methyl, Chlorsulfuron, Bensulfuron-Methyl	Extraction: 1.5% Triton X-114, 12% Na_2SO_4, pH 2.0, 50°C; 15 min incubation.	[157] [151]

temperature of CPE with Triton X-114 was higher than 55°C, the extraction efficiency decreased, while the recoveries slowly increased at the temperature range 50-55°C. Considering the recovery and stability of all analytes, an equilibration temperature of 50°C was used for the CPE [151].

Since the addition of salts may increase the density and the ionic strength of the aqueous phase, it can facilitate the separation of the cloudy solution into two phases and shift the analytes from the water-rich phase to the surfactant-rich phase. However, it can also make the surfactant-rich phase sticky, hampering the following analysis. Compared to NaCl, the CPE with Na_2SO_4 not only supplied higher recoveries, but also consumed less time to achieve the phase separation [146]. Furthermore, the CPE with Na_2SO_4 could be completed at room temperature and it yielded the highest recoveries of a variety of tested salts (Na_2SO_4, NaCl, $(NH_4)_2SO_4$, Na_2CO_3, and $C_6H_5Na_3O_7$) [149]. The extraction efficiencies increased with increasing Na_2SO_4 concentration from 8% to 12% and decreased with increasing Na_2SO_4 concentration from 12% to 16%, suggesting that the additional surface charge may alter the molecular architecture of the surfactant and affect the micelle-formation process when the salt concentration is high [151]. When Na_2CO_3 was used for the CPE, most analytes tended to degrade in its basic conditions. Although the same molar concentration of Na_2SO_4 was more effective than NaCl in terms of less reagent consumption and the ease of phase separation, the surfactant-rich phase obtained from Na_2SO_4 became cloudy when dissolved in methanol or mobile phase. Therefore, NaCl was the best choice from NaCl, Na_2CO_3 and Na_2SO_4 [147].

When compared with the other extraction methods, CPE has a large number of advantages. It requires a very small amount of relatively nonflammable and nonvolatile surfactant and provides simple operation. It also combines the efficient extraction and enrichment in a single step. Therefore, it has been developed as an interesting alternative to the extraction systems. However, some surfactants in CPE may disturb the HPLC-UV determination of analytes.

14. Liquid phase micro-extraction (LPME)

Over the past years, a miniaturized liquid phase extraction method called liquid phase micro-extraction (LPME) has been developed [152]. During the LPME, the analytes is shifted from an aqueous phase (also known as donor phase) to several microliters of water-immiscible solvent (also known as extractant or acceptor) [153]. In the sample preparation procedure, the LPME of the pesticides in foods can be classified into three main categories: single-drop micro-extraction (SDME), hollow-fiber LPME (HF-LPME) and dispersive liquid-liquid micro-extraction (DLLME), the practical applications of which are summarized in Table 13 [59,154-158,161-173].

SDME was developed in 1996 by Jeannot and Cantwell [152]. Similar to the common LLE, the LPME methods also combine extraction, concentration and sample introduction in one step, being an alternative to the traditional sample preparation method due to the use of negligible amounts of toxic solvents and low consumption of experimental operation time [157]. During the SDME procedure, a micro-drop of extraction solvent is set at the tip of a microsyringe needle and immersed in the sample solution. After a period of magnetic stirring, the distribution equilibrium is established between the sample and the micro-drop of extraction solvent. Finally, the micro-drop is retracted back into the microsyringe and injected for the subsequent determination. Most of all, the extraction solvent must have low water-solubility and high boiling point [156]. Zhang *et al*. [154] and Amvrazi *et al*. [155] selected mixed drop of *p*-xylene-

Table 13. LPME applications in the analysis of pesticide residues in foods.

Matrix	pesticides	Sample preparation	References
vegetables	9 OCPs	Extraction: 1.0 μL acetone-*p*-xylene (2:8), 400 rpm, 30 min.	[154]
vegetables	14 pesticides	Extraction: 1.6 μL toluene, 350 rpm, 25 min.	[155]
wine	6 insecticides	Extraction: 2.0 μL isooctane, 180 rpm, 11.5 min.	[156]
grapes, apples	20 pesticides	Extraction: 1.6 μL toluene, 250 rpm, 25 min.	[157]
grape juice	6 pesticides	Extraction: 30 μL *n*-hexanol-*n*-hexane (50:50), 1% NaCl, 17 min.	[158]
fish tissue	8 OPPs	Extraction: PVDF hollow fiber, 30 μL *o*-xylene, 500 rpm, 30 min.	[161]
beverages	50 pesticides	Extraction: 1-octanol, 90 rpm, 45 min.	[162]
orange juices	3 fungicides	Extraction: 20 μL 2-octanone, 1000 rpm, 30 min.	[163]
beverage	organosulfur pesticides	Extraction: 5 μL o-xylene, 20 s.	[164]
beverage	6 organosulfur pesticides	Extraction: 10.0 μL carbon tetrachloride, 0.8 mL methanol, 3000 rpm centrifugation (15 min).	[164]
fruit juice	triazophos carbaryl	Extraction: 15 μL tetrachloroethane, 1 mL MeCN, 3500 rpm centrifugation (3 min).	[165]
tomato	13 OPPs	Extraction: 60 μL chlorobenzene, 1.0 mL acetone, 5000 rpm centrifugation (4 min).	[59]
Peach juices, pulps peels	PCB, OCP, pyrethroid	Extraction: 8 μL dodecan-1-ol, 0.4 mL acetone, 4000 rpm centrifugation (2 min).	[166]
apple juice	24 pesticides	Extraction: 100 μL carbon tetrachloride, 400 μL acetone, 1 min shaking, 5000 rpm centrifugation (2 min).	[167]
pear juice	cypermethrin permethrin	Extraction:30 μL C_2Cl_4, 3.5 mL methanol, 2 min ultrasound, 4000 rpm centrifugation (5 min).	[168]
watermelon cucumber	6 OPPs	Extraction: 20μL chlorobenzene, 1mL MeCN, 4000 rpm centrifugation (3 min).	[169]
tea	10 OPPs	Extraction: 24 μL *n*-hexane, 2 mL MeCN, 42 °C, 45 min stirring (1000 rpm).	[170]
bananas	8 pesticides	Extraction: 88 mg $[C_6MIM][PF_6]$,714 μL methanol, 4000 rpm centrifugation (20 min).	[171]
fruit	4 OPPs	Extraction: 50 μL 1,3-dibutylimidazolium hexafluorophosphate, 0.6 mL methanol, 4000 rpm centrifugation (5 min).	[172]
honey	4 pyrethroids	Extraction: 55 μL $[C_8MIM][PF_6]$, 200 μL methanol.	[173]

acetone (8:2) and a drop of toluene as the extraction solvents of the SDME, respectively. In addition to the common SDME, Farajzadeh *et al*. [158] reported a novel sample preparation method based on a dynamic single drop in a narrow-bore tube, in which a micro-drop extraction solvent mixture of *n*-hexanol-*n*-hexane (50:50) assisted by an air bubble was repeatedly passed through a narrow-bore closed end tube containing the aqueous sample.

To avoid the drop instability in SDME, HF-LPME was introduced as another type of LPME method in 1999 [159]. Compared to the traditional sample preparation methods for the analysis of pesticides in food samples, HF-LPME method omits the clean-up step, eliminates the SPE step, simplifies the sample preparation procedure, decreases the solvent consumption and lowers the cost of analysis [10-12]. During the HF-LPME, analytes are firstly extracted into a supported liquid membrane sustained in the pores of a hydrophobic hollow-fiber, and later into an acceptor solution placed inside the lumen of the fiber. As the sample donor and the acceptor phases are separated by the porous membrane of the hollow fiber, the acceptor solution in hollow fiber was effectively protected within the fiber to avoid the instability of the drop of the extraction solvent [160]. Xiong *et al*. [164] immersed the needle tip coated with the hollow fiber in *o*-xylene to impregnate the pores of the hollow fibers and then the *o*-xylene in the syringe was injected to the lumen of hollow fiber, which was more robust and more suitable than DLLME for the analysis of complicated matrix samples.

Based on the previous LPME studies, DLLME was developed as a new micro-extraction method in 2006. DLLME employs a ternary component solvent system composed of an aqueous solution containing the analytes, a water-immiscible extraction solvent and a water-miscible disperser solvent. When the disperser and extractant are mixed and rapidly introduced into the aqueous solution, a cloudy solution appears, indicating the equilibrium between the droplets of the extraction solvent and the aqueous sample. The extraction solvent is normally collected at the bottom of the tube through centrifugation. Compared with the conventional sample preparation methods, this method showed advantages of the shorter extraction time, quicker and easier operation, the absence of a clean-up procedure, higher enrichment factors, lower consumption of organic solvent, low limits of detection, good repeatability, high enrichment factor and good recovery within a short time [59,167,169]. In DLLME, the organic solvents should have higher density than water, low water solubility, high extraction capability of target compounds and

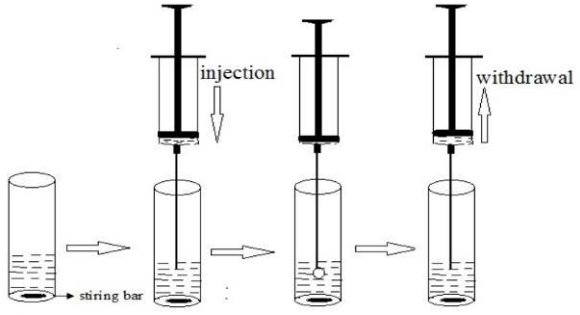

Figure 8. Schematic diagram of SDME.

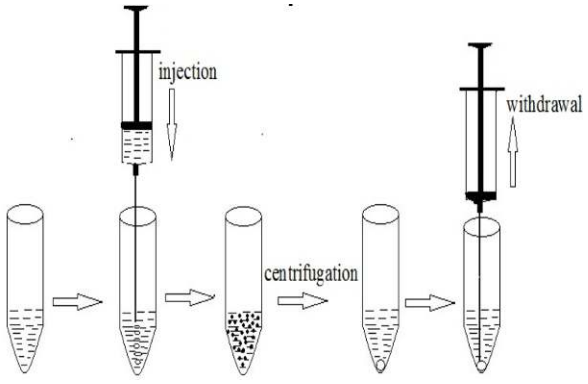

Figure 9. Schematic diagram of DLLME.

good chromatographic behavior. Tetrachloroethane [165], chlorobenzene [59], carbon tetrachloride [167], C_2Cl_4 [168] and chlorobenzene [169] have been used as extraction solvent. However, the *n*-hexane, that has lower density than water, has been introduced for the DLLME [170]. Additionally, some DLLME procedures using room temperature ionic liquids such as [C_6MIM][PF_6] [171], 1, 3-dibutylimidazolium hexafluorophosphate [172] and [C_8MIM][PF_6] [173] have been developed for quantifying trace amounts of pesticides. Matsadiq *et al*. [166] also reported a new DLLME method based on the solidification of a floating organic droplet using the extraction solvent of dodecan-1-ol. In the process, the dodecan-1-ol rose to the surface of an aqueous solution and turned into solid organic droplets floating on the surface due to the cooling by the ice bath. Although a cloudy solution was rapidly formed with the ternary component solvent system, Du *et al*. [169] reported an ultrasound-assisted DLLME for the simultaneous analysis of cypermethrin and permethrin residues in pear juice, during which the ternary component solvent mixture was emulsified by ultrasound to form cloudy solution. Furthermore, Zhang *et al*. [173] presented a comparative study of the performance of conventional, ultrasound-assisted, and temperature-controlled ionic liquid DLLME and found that ultrasound-assisted ionic liquid-DLLME provided the highest extraction efficiency.

LPME methods have taken an important role in the sample preparation because of their inherent advantages over conventional procedures. They have a high level of linearity over a wide range of analyte concentrations, they consume small volumes of solvents and samples, have a reduced risk for human health and the environment, and achieve low LOD due to good enrichment factors as a result of the limited extractant-to-sample volume ratios. In particular, they have been successfully applied for the analysis of food samples despite their complexity. DLLME has been applied more often for the analysis pesticides in foods than SDME and HF-LPME, possibly due to the fact that it is an easier method. According to the detailed revision of the literature, there are a large number of organic solvents available for LPME applications, which extend the potential of the method. In near future, it is very probable that this method will be increasingly applied in many analytical fields, especially in the complicated food analysis.

15. Conclusions

The analysis of pesticide residues in food matrices has become a necessity in viewpoint of food safety, and it requires that the pesticide residues should be efficiently extracted from the food matrix for the final determination. Because of the complexity of the food matrices, the clean-up steps of extracts are necessary before the final determination. The ideal sample preparation method should be a compromise between cost, accuracy, selectivity and sensitivity. Unfortunately, the traditional liquid solvent extractions frequently fail to meet these goals, being time-consuming, labor-intensive, complicated and expensive. They also produce considerable quantities of waste and provide an insufficient LOD. Often, many physically and chemically different compounds need to be determined rather than one or a single class of analytes, and therefore it is necessary to develop sample preparation methods for the analysis of pesticide multi-residues in food matrices. Some most frequently used sample preparation methods, such as SFE, PLE, MAE and UAE require an additional clean-up step. Other sample preparation methods, such as SPE, MSPD, SPME and LPME can finish the extraction and clean-up in one step, which not only reduces the consumption of organic solvent and operation time, but also simplifies the experimental procedure and decreases the experimental errors. Driven by the advances in science and technology and the quest for analytical results, in future the sample preparation methods are expected to continue developing rapidly.

Abbreviations

ASE, accelerated solvent extraction;
CW-TPR, carbowax-templated resins;
CPE, cloud point extraction;
DCM, dichloromethane;
DI-SPME, direct-immersion solid phase micro-extraction;
DLLME, dispersive liquid-liquid micro-extraction;
d-SPE, dispersive solid phase extraction;
EtAc, ethyl acetate;
GC, gas chromatography;
GPC, gel permeation chromatography;
GCB, graphitized carbon black;
HS-SPME, headspace solid phase micro-extraction ;
HCH, hexachlorocyclohexane;
HF-LPME, hollow-fiber LPME;
HPLC, high performance liquid chromatography;
LOD, limit of detection;
LPME, liquid phase micro-extraction;
MAE, microwave-assisted extraction;
MeCN, acetonitrile;
MRLs, maximum residue limits;
MSPD, matrix solid phase dispersion;
MIPs, molecularly imprinted polymers;
OCPs, organochlorine pesticides;
OPPs, organophosphorus pesticides;
PA, polyacrylate; PAHs, polycyclic aromatic hydrocarbons;
PBDEs, polybrominated diphenylethers;
PCBs, polychlorinated biphenyls;
PDMS-DVB, polydimethylsiloxane-divinylbenzene;
PLE, pressurized-liquid extraction;
PMPS-OH, hydroxyl-terminated polymethylphenylsiloxane;
PSA, primary-secondary amine;
PVDF, polyvinylidene difluoride;
RSD, relative standard deviation;
SDME, single-drop micro-extraction;
SFE, supercritical fluid extraction;
SPE, solid phase extraction;
SPME, solid phase micro-extraction;
UAE, ultrasound-assisted extraction.

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