International Journal of Pharmacy and Chemistry
2020; 6(6): 65-76
http://www.sciencepublishinggroup.com/j/ijpc
doi: 10.11648/j.ijpc.20200606.11
ISSN: 2575-5730 (Print); ISSN: 2575-5749 (Online)

Review Article

A Review: Sample Preparation Methods for the Pesticide Residue Analysis in Food Samples

Shibiru Eticha

Department of Chemistry, Oda Bultum University, Chiro, Ethiopia

Email address:
shibeticha52@gmail.com

To cite this article:
Shibiru Eticha. A Review: Sample Preparation Methods for the Pesticide Residue Analysis in Food Samples. *International Journal of Pharmacy and Chemistry*. Vol. 6, No. 6, 2020, pp. 65-76. doi: 10.11648/j.ijpc.20200606.11

Received: September 16, 2020; **Accepted**: September 30, 2020; **Published**: November 11, 2020

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 some food samples. The methods include: Liquid-Liquid extraction (LLE), Solid-Phase extraction (SPE), Matrix Solid-Phase Dispersion (MSPD), Solid-Phase Micro-extraction (SPME), QuEChERS, and Liquid Phase Micro-extraction (LPME).

Keywords: Sample Preparation, Pesticide Residues, Food, Extraction, Clean-up

1. Introduction

Pesticides are chemicals used to control pest populations, reduce pest damage on crops, landscape or animals. Pesticides are present in fresh or processed animal foods, if animals have been fed with contaminated feed or water, or from practices involving pesticides in places where animals are living or in food-processing factories. Pesticides residues in foods of great public and regulatory concern have been insecticides such as Organochlorine, Organophosphorus, carbamates, pyrethroids and fungicides such as benzimidazoles.

Pesticide residues in food samples of vegetal origin are pesticides that are applied to the plants to attack invertebrate pests and plant diseases. Organic pollutants in foods of animal origin are classified as contaminants and residues. Contaminants are substances that are not added deliberately to the foods; but they can enter into foods during their production process, transformation, storage, packed, transport and practices. Residues are compounds that are occur in foodstuffs as the result of intentional usage of phytosanitary or veterinary products during plant or animal production. Therefore, pesticides found in foods of animal origins are belongs to the residues' groups.

Pesticide residues in food are unnecessary and a preventable contamination. The residue uptake through diet has raised public concern now day. Periodic residue monitoring is being performed in food products mainly for the regularly consumed food to avoid any significant risk to human health. Maximum Residue Level (MRL) presents a set of legally permitted maximum level for pesticide residue in food items.

Global scientific concerns have been raised regarding the potential toxicity of pesticides that have promoted their strict regulation in order to protect consumers, environment and the users of pesticides. Maximum Residue Levels values are defined as the highest levels of a pesticide residues that are legally tolerated in food when pesticides are applied correctly were established. Legislations were enacted in the USA, the European Commission (EC) and other countries to regulate pesticides residues in food item and products.

Food samples are challenge to Analytical Chemists to

determine residues of pesticides at trace levels to satisfy food safety regulations. Food samples from liquids to solids require different sample preparation techniques for accurate and reproducible results with Chromatographic techniques such as Gas Chromatography and Higher Performance Liquid Chromatography. A wide range of pesticides which are used legally for crop protection and their residue content in food must be accurately monitored for safe consumption.

The Gas Chromatography (GC) and Higher Performance Liquid Chromatography (HPLC) techniques with different types of detector systems can provide such analysis at trace levels to fulfill the maximum residue levels as per the food safety regulations. However, the accurate and reproducible results often depend upon the sample preparation techniques associated with the different food samples.

The aim of present paper is to review the application of some sample preparation in the analysis of pesticide residues in some food samples.

2. Sample Preparation Methods

2.1. Liquid –Liquid Extraction

It is based on the solubility of analyte in two immiscible solvents and is governed by the equilibrium distribution coefficient. The homogenized samples are extracted with an immiscible organic solvent and the extracts are then centrifuged, concentrated and purified before the final analysis. Its extraction efficiency depends on the equilibrium distribution or partition coefficient between the donor phase and the acceptor phase.

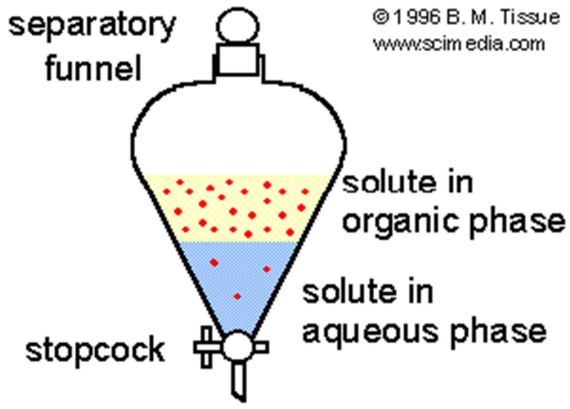

Figure 1. *Processes in Liquid -Liquid extractions.*

Acetonitrile is miscible in water, it is separated from the aqueous phase by salting-out effect and it is effective in extracting both polar and non-polar pesticide residues with small amounts of matrix co-extractives. Medium polarity solvents such as Ethyl Acetate decreases the polarity of a polar solvent or increase the polarity of non-polar solvent in the Liquid- Liquid extractions.

Chloroform is medium-polarity solvent used for the Liquid - Liquid extractions of pesticide residues. Acetone is also a medium-polarity solvent, it is not used for Liquid-Liquid extractions due to its difficult separation from the aqueous phase. Non-polar organic solvents such as Hexane, Cyclohexane and Petroleum are applied in the Liquid - Liquid extractions of non-polar analytes and as the modifiers of other non-polar solvents. It is necessary to clean-up the extracts for reducing the interferences after the initial Liquid-Liquid extractions.

Liquid-Liquid extraction with low temperature partitioning was optimized and validated for determination of the aldicarb, carbofuran and carbaryl in grape juice and chocolate milk beverages by high performance liquid chromatography combined with ultraviolet-visible detector [2]. It involved extraction with Acetonitrile, Liquid-Liquid partition at low temperature and analysis by high performance liquid chromatography combined with ultraviolet-visible detector and gives recovery percentages above 90%. Liquid-Liquid extraction with ethyl acetate was developed for the determination of mebendazole and its hydrolyzed and reduced metabolites in pork, chicken, and horse muscles and analyzed by liquid chromatography-tandem mass spectrometry method [1]. Limit of detection and limit of quantification for all analytes were 0.07μgkg^{-1} and 0.2 μgkg^{-1}, respectively.

Salting out Liquid-Liquid extraction method followed by high performance liquid chromatography with ultraviolet-visible detector has been reported for the analysis of atrazine, ametryn, terbutryn, carbaryl and chlorothalonil in beer, wine and Ethiopian honey wine [3]. Under the optimum experimental conditions, matrix-matched calibration curves were constructed using beer sample as the representative matrix and good linearity over wide concentration ranges were obtained with R^2 of 0.997. Limit of detection and limit of quantification were in the ranges of 1.3-3.9 and 4.5–12.8 μg L^{-1} and % RSD were less than 10%. The recoveries were in the ranges of 71-104%. The results of the study revealed that the developed salting out Liquid-Liquid extraction method is selective and efficient sample preparation procedure prior to quantitative analysis of the target analytes by high performance liquid chromatography with ultraviolet-visible detector.

However, in Liquid-Liquid extraction organic solvents used leading to a large amount of toxic residues, the formation of emulsions, which are difficult to break up and the difficulties for automation of the whole process make Liquid-Liquid extraction to be considered a tedious, time-consuming, and costly technique.

2.2. Solid Phase Extraction

In Solid Phase extraction, the extracts are passed through the cartridge and adsorbed on the solid phase materials, which have been conditioned and activated with water or organic solvent before use. The interferences are removed by pre-washing by organic solvents while the analytes are retained on the adsorbents. After clean-up step, the analytes are eluted with other organic solvents to obtain clean extracts.

Solid Phase extraction precedes the selective retention of target analytes on adsorbent packed in a disposable extraction of mini-column.

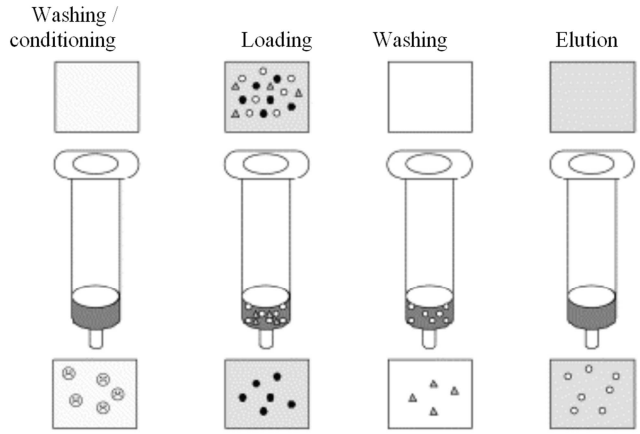

Figure 2. *Solid Phase Extraction operation Procedures.*

In Solid Phase extraction various types of adsorbents are used for the clean-up or preconcentration of pesticide residues in foods. C_{18} was used in Solid Phase extraction material to prevent the broadening of peaks in the on-line Solid Phase extraction-high performance liquid chromatography system. Amino propyl (NH_2) Solid Phase extraction cartridge used to eliminate lipid components at low-temperature. GCB retain and remove planar molecules. Silica-bond TMA Chloride (SAX)-PSA cartridge was selected to remove fatty acids, organic acids and sugars. Multi-walled carbon nanotubes were first developed as Solid Phase extraction adsorbents for the extraction of organophosphorus pesticides from fruit juices [9].

pH determines the stability of the analytes, the pH of extracts is crucial to ensure the high retention of pesticides on the adsorbent. Therefore, an appropriate pH is necessary to maintain the stability of pesticides and to increase the absorption of analytes on the solid phase. In order to ensure the stability of organophosphorus pesticides in apple, grape, orange and pine apple juices the pH was adjusted to 6.0 with 1.0 M NaOH [8].

Analytical method for the determination of imazaquin residues in soybeans was developed based on liquid-liquid partition strong anion exchange Solid Phase extraction [5]. The method was characterized by recovery > 88.4%, precision 6.7% RSD and sensitivity of 0.005 mg kg^{-1}. The proposed method was successfully applied to the analysis of imazaquin residues in soybean samples grown in an experimental field after treatments of imazaquin formulation.

The use of Solid Phase extraction in combination with high performance liquid chromatography with diode array detector was employed to determine bispyribacsodium residues in rice [10]. The liquid–liquid partition and anion exchange solid phase procedures that were developed provide effective extraction and cleanup methods for analysis feasibility, with recoveries between 83.98 to 98.51% with a RSD from 0.56 to 6.36% and sensitivity of 0.01 mg kg^{-1}, with main advantages of high precision, accuracy and good selectivity.

A sensitive and simple method for simultaneous analysis of acetochlor and propisochlor in corn has been developed [7]. The extraction of pesticides was performed with methanol: water and acetone, respectively, followed by solid phase extraction to remove co-extractives, prior to analysis by gas chromatography combined with electron capture detector. Primary secondary amine solid phase extraction cartridges were used for sample preparation. The recoveries of two pesticides ranged from 73.8% to 115.5% with RSD less than 11.1% and sensitivity of 0.01 mg kg^{-1}.

A new extraction with cleanup procedure with gas chromatography was developed for the determination of acephate, dimethoate, malathion, diazinon, quinalphos, chlorpyrifos, profenofos, α-endosulfan, β-endosulfan, chlorothalonil and carbaryl using 1-chloro-4-fluorobenzene as an internal standard in fruits and vegetables [4]. The extracting solvent with a mixture of acetone: ethyl acetate: hexane (10:80:10, v/v/v) and a eluting solvent of 5% acetone in hexane used with the RPC_{18} cartridge gave the best recovery for all of the investigated pesticides and minimized the interference from co-extract ants. The recoveries of 85-99% with RSD < 5.0% (n=3) for most of the pesticides at the 0.02 - 0.5 mg/kg level were obtained. The limit of detection was between 0.005 – 0.01 mg/kg and limit of quantification was 0.01 mg/kg. This analytical procedure was characterized with high accuracy and acceptable sensitivity to meet requirements for monitoring pesticides in crops.

A method for simultaneous determination of the organochlorine pesticides in milk and milk powder sample has been developed by gel permeation chromatography-solid phase extraction-gas chromatography-tandem mass spectrometry [6]. Limit of quantification of all organochlorine pesticides were 0.8μg/kg. With the exception of endrin, limit of quantification are significantly lower than maximum residue limits set by the European Union and China. The average recoveries were in the range of 70.1 to 114.7% at 3 spiked concentration levels (0.8, 2.0, and 10.0 μg/kg) with residual standard deviation lower than 12.9%. The developed method was successfully applied to analyze the organochlorine pesticides in commercial milk products.

Solid Phase extraction is simpler, more convenient, less solvent consuming and easier to automate and avoid the formation of emulsion. It can complete the whole sample preparation without any further treatments and provide the subsequent clean-up procedure of these extraction methods. But in Solid Phase extraction it is difficult to rapidly choose the appropriate adsorbents and elution solvents for the analysis of multi- residue pesticides with a very wide range of physio- chemical characteristics and the commercial Solid Phase extraction cartridges cannot be reused.

2.3. Solid Phase Micro-extraction

It is based on the partition equilibrium of analytes between the sample and the stationary phase. It combines sampling, extraction, concentration and injecting the sample into a single step. It is classified as direct-immersion solid phase micro-extraction (DI-SPME) and head-space solid phase micro-extraction (HS-SPME). Using the PDMS-DVB fiber direct-immersion-solid phase micro-extraction was carried out to determine pesticide residues in red wines at ambient temperature for 143 min with continuous stirring at 900 rpm

[8]. Then the pesticides were desorbed from the fiber with 1mL methanol by stirring for 13 min at 1000 rpm.

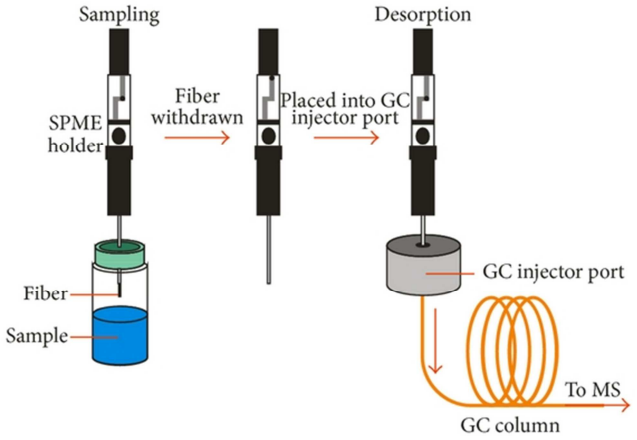

Figure 3. *Solid phase micro extraction.*

In headspace solid phase micro-extraction, solid phase micro-extraction fiber is put in the air above the liquid or solid sample.

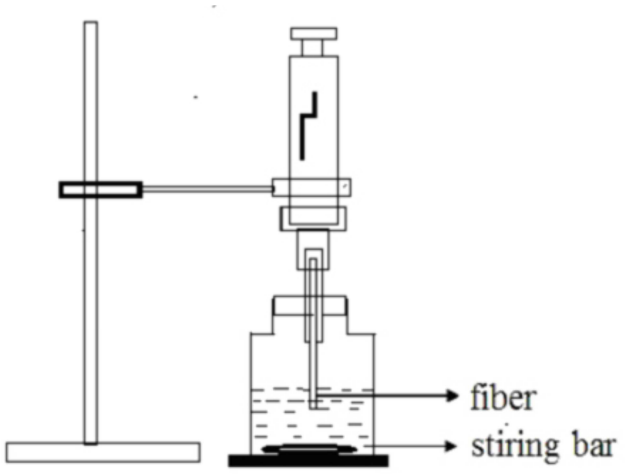

Figure 4. *Schematic diagram of direct-immersion solid phase micro-extraction.*

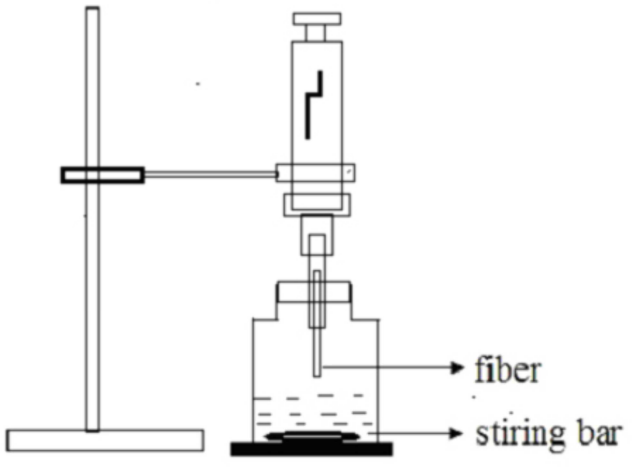

Figure 5. *Schematic diagram of HS-SPME.*

The addition of NaCl and the adjustment of pH were important for the solid phase micro-extraction. However, the ionic strength and pH have no significant effect on the final results during the solid phase micro-extraction. In addition, volume of solution, the extraction temperature and equilibration time are also important in solid phase micro-extraction procedures.

Headspace solid phase micro-extraction overcomes the problem of matrix interference, it shows low adsorption equilibrium and enrichment effect for the compounds with high boiling points. The extraction efficiency increased with increasing extraction temperatures, excessively high temperature could result in a drop the relative signals the analytes. Headspace solid phase micro-extraction and direct-immersion solid phase micro-extraction using PDMS and PA fibers observed that the extraction efficiencies of Headspace solid phase micro-extraction were better than those of direct-immersion solid phase micro-extraction and the PA fiber showed slightly better extraction efficiency than the PDMS.

A direct immersion solid phase micro-extraction with GC-ECD method using a 100 µm PDMS fiber was developed for trace determination of chlorinated pesticides in tomato samples [39]. The Limit of detection ranged from 0.5 to 664 8 µg/kg, and the limit of quantification from 5 to 30 µg/kg with good linearity ranging from 0.97 to 0.9985. Organochlorine pesticides such as lindane, heptachlor, aldrin, dieldrin and endrin from milk samples were investigated by using headspace solid phase micro-extraction with GC-ECD. A fiber coating DVB/Car/PDMS exhibited the best extraction efficiency towards the target pesticides.

Solid phase micro-extraction with gas chromatography-tandem mass spectrometry method was developed to quantify pesticides in fortified white wine and fortified red wine [29]. The analytical method showed good linearity, presenting correlation coefficients (R^2) ≥ 0.989 for all compounds. The limit of detection and limit of quantifications are in the ranges of 0.05-72.35 and 0.16-219.23 µg/L, respectively, were obtained. LOQs are below MRL set by European Regulation for grapes.

A fast and robust method was developed for the determination of triazole fungicides in fruit samples using direct-immersion solid phase micro-extraction coupled to gas chromatography with time-of-flight mass spectrometry detection [13]. Under optimized conditions, the method was linear for over 4 orders of magnitude in concentration, with R^2 greater than 0.99 for all test compounds in both matrices. Method reproducibility, as determined by analysis of spiked grapes and strawberries, was better than ±20%. The LOQs ranged from 0.25 to 5 ng g^{-1} for both matrices, well below the MRLs allowed for those compounds in both matrices. The method was successfully applied in the analysis of commercial samples of grapes and strawberries.

Solid phase micro-extraction has inherent high sensitivity and the absence of solvents and sample pretreatment

required, thus minimizing the sample manipulation and contamination. The main disadvantages of solid phase micro-extraction are poor fiber-to-fiber reproducibility and poor precision and ruggedness on the determination. The technique is limited to relatively semi-volatile or volatile compounds, and matrix-effects showed up in complex matrices.

2.4. Matrix Solid-phase Dispersion

It contains extraction and clean-up into a single step. It consists of: sample homogenization, cellular disruption, exhaustive extraction, fractionation, and clean-up by adsorbents. It is common sample preparation method for the analysis of pesticide residues in food samples such as fruit, vegetables, oil, biota samples, eggs and fish.

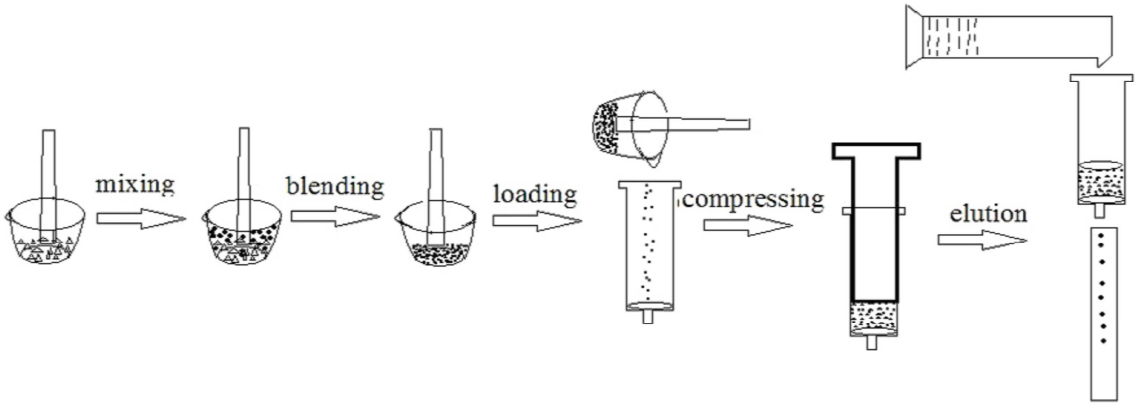

Figure 6. *Schematic diagram of the operation of Matrix solid-phase dispersion (MSPD).*

The dispersants in matrix solid-phase dispersion break the physical structure of the sample, extract the analytes from the sample and supply clean-up material for the sample matrix. Adsorbents, such as C_{18}, C_8, silica, Florisil, diatomaceous earth and Al_2 $(SO_4)_3$ are used as dispersants in matrix solid-phase. C_{18} is commonly used dispersant in the matrix solid-phase dispersion. It is important to select the ratio between the sample and the sorbent for the formation of fine particles and effective dispersion of the sample on the sorbent. The normal ratio between the sample and sorbent ranges from 1:1 to 1:4. To save the sorbent material and to facilitate the packing of the column, the ratio of 1:2 was chosen for the matrix solid-phase [15].

The nature and volume of the elution solvent is important for the desorption of pesticides from the adsorbent and the absorption of interferences on the SPE column. Solvents such as MeCN, methanol, EtAc, DCM and mixtures of them, are used in the matrix solid-phase. In the elution of pesticide residues from Florisil column DCM-EtAc (9:1) was selected as elution solvents of pesticides and matrix co-extractives in propolis tinctures [11]. Hexane was not used effectively to elute the pyrethroid and organochlorine pesticides from alumina column, but EtAc was to be suitable for the elution of the pyrethroid and organochlorine pesticides from alumina column [14].

Determination of penoxsulam, tricyclazole, propanil, azoxystrobin, molinate, profoxydim, cyhalofop-butyl and deltamethrin and 3, 4-dichloroaniline, the main metabolite of propanil in rice, was performed by an optimized matrix solid-phase alumina using acetonitrile as the elution solvent and analyzed by HPLC-DAD [19]. Linear regression coefficients (r^2) were above 0.9948. LOD and LOQ varied from 0.002 to 0.200 mg kg^{-1} and 0.006 to 0.600 mg kg^{-1}, respectively. Recoveries were investigated between (74–127%) with RSD below 12%.

Matrix solid-phase dispersion has been developed, optimized and validated for the analysis of cypermethrin pesticide residues in samples of cows' milk and analyzed by GC-MS [12]. Milk (0.25 g) was fortified with cypermethrin and blended with 1 g each of C_{18} silica and Na_2SO_4, used to trap fats and water, respectively. The homogenized material was transferred to a commercial solid phase extraction cartridge containing 1 g activated Florisil with 5 mL acetonitrile. Cypermethrin was eluted under vacuum with 5x 9 x2 mL acetonitrile and the extract was concentrated to 1 mL and analyzed by gas chromatography–mass Spectrometry. The LOD and LOQ of the method were 0.025 and 0.08 mg kg^{-1}, respectively.

Matrix solid-phase dispersion for extraction of carbendazim residue from wheat grain and determined by HPLC-DAD [19]. The mean recovery rate for fortified samples was 87.3% with a RSD of 2.9%. The LOQ was 0.04 μg g^{-1}. Matrix solid-phase dispersion performs the disruption of sample and the dispersion of sample components on a solid support and generate a chromatographic material for the extraction of analytes from the dispersed sample. It is applied to solid, semi-solid, and liquid food samples.

Matrix solid-phase dispersion was developed to determine trichlorfon, pyrimethanil, methyl parathion, tetraconazole, thiabendazole, imazalil, and tebuconazole in papaya and mango using gas chromatography-mass Spectrometry with selected ion monitoring [18]. Matrix solid-phase dispersion was reported to determine dimethoate, malathion, lufenuron, carbofuran, 3-hydroxycarbofuran, thiabendazole, difenoconazole and trichlorfon in coconut pulp using gas chromatography–mass spectrometry with selected ion monitoring [16].

The method was validated using coconut pulp samples

fortified with pesticides at different concentration levels (0.25–1.0 mg kg^{-1}). Average recoveries ranged from 70.1% to 98.7%, with RSD between 2.7% and 14.7%, except for lufenuron and difenoconazole, for which recoveries were 47.2% and 48.2%, respectively. LOD and LOQ for coconut pulp ranged from 0.02 to 0.17 mg kg^{-1} and from 0.15 to 0.25 mg kg^{-1}, respectively.

Matrix solid-phase dispersion is rapid, inexpensive and can be carried out under mild extraction conditions (room temperature and atmospheric pressure) and provides acceptable yield and selectivity and thus, in turn, decreases environmental contamination and improves worker safety. Matrix solid-phase dispersion technique is not easily automated and could be time-consuming for a large number sample size. Although the Matrix solid-phase dispersion extracts are clean enough for direct instrumental analysis, a further cleanup step is often required, particularly with fatty matrices.

2.5. QUCHERES

It is based on the micro-scale extraction using MeCN, water absorption and liquid-liquid partition utilizing $MgSO_4$ and NaCl, and clean-up step of d-SPE employing primary-secondary Amine adsorbent. It avoids blending, filtration, large volume of solvent transfers, evaporation/condensation and necessary solvent exchanges for the chromatographic determination. The abbreviation of QuEChERS stands for quick, easy, cheap, effective, rugged and safe.

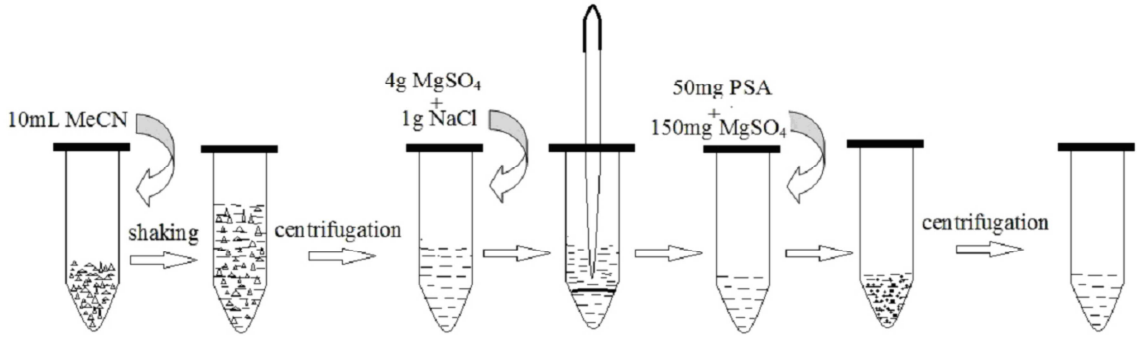

Figure 7. *Schematic diagram of the common procedure of QuEChERS.*

Selecting the extraction solvent in QuEChERS directly determines the extraction efficiency. MeCN is widely used extraction solvent for the QuEChERS procedure. Although MeCN is miscible with water, it can be easily separated from water by the salting-out effect and centrifugation. Different from the common QuEChERS, the dry ice-partitioning QuEChERS method for the determination of 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 [24].

High $_P$H influence the stability of base sensitive pesticides and the final recoveries, certain buffer solutions are advised to avoid the degradation of these pH-dependent pesticides during the QuEChERS procedure. The addition of acetic acid sodium acetate buffer solution to the MeCN extracts guarantees the stability of base-sensitive pesticides and supplies high recoveries.

After the extraction or 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 in foods. C_{18} adsorbent was added to remove the lipophilic co-extracts of the MeCN extracts from low-fat baby food matrices. GCB was 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 which was found to retain the pesticides with planar ring structures in the complex matrix. An extra SPE cartridge loaded with GCB- amino propyl silanized silica gel was adopted for the complementary clean-up step of QuEChERS method to remove the pigments from tea [21].

A modified QuEChERS method was developed and validated for determination of pesticide multi-residues in green tea by LC-MS [26]. Lead acetate was first time used together with PSA and GCB to eliminate tannin, caffeine and other pigments in tea and thus reduced the matrix effects. The method was compared to the original QuEChERS method as well as A. O. A. C. QuEChERS method. The method showed good performance in the concentration range from 0.01 to 1 mg kg^{-1}. All pesticides could be quantified at and lower than 0.01 mg kg^{-1}. Recoveries were from 70 to 120% and repeatability were <15% RSD depending on the compounds.

The level of pesticide residues in consumed fruits and vegetables in Kuwait were analyzed for the presence of pesticides using QuEChERS multi-residue extraction, followed by Gas Chromatography-Mass Spectrophotometer [23]. Out of a total of 150 samples of pesticide residues above MRL were detected in 21% of the samples and 79% of the samples contained residues below the MRL. Multiple residues were present in 40% of the samples and four samples were contaminated with more than four pesticide residues. Imidacloprid, deltamethrin, cypermethrin, Malathion, acetamiprid, monocrotophos and diazinon exceeded their MRLs. Aldrin, was detected in one apple sample, below the MRL.

Concentrations of pesticides residues in honey were determined from the major honey producing forest in Ghana.

Samples were collected and extracted using the QuEChERS Method and analyzed for pyrethroids, organochlorine and organophosphate pesticide residues by GC-ECD [22]. Aldrin, γ-HCH, β-HCH, Σendosulfan, cyfluthrin, cypermethrin, deltamethrin, permethrin, methoxychlor, ΣDDT, chlorpyrifos, fenvalerate, malathion, dimethoate and diazinon were all detected at the concentration of 0.01 mg/kg, while cyfluthrin and permethrin were detected at mean concentrations of 0.02 and 0.04 mg/kg, respectively. All the pesticide residues were detected were below their respective MRLs set by the European Union.

A method for the simultaneous determination of pesticides, bio pesticides and mycotoxins in wheat, cucumber and red wine was developed based on modified QuEChERS procedure [25]. It was based on a single extraction with acidified acetonitrile, followed by partitioning with salts and analyzed by UHPLC-MS/MS. Recoveries of the spiked samples were in the between 70 and 120%. RSD lower than 20% except for picloram and quinmerac. LOQ were lower than 10 gkg $^{-1}$. The developed method was successfully applied to the analysis of organic food products, detecting analytes belonging to the three types of compounds.

Modified QuEChERS and (GC–MS) was developed and validated for the determination of permethrin**,** primicarb**,** dichlorvos**,** diazinone**,** fenpropathrin**,** carbaryl**,** chlorpyrifos, malathion, chlortalonil, brompropilate, propargit, tetradifone, phosalone, iprodion and endosulfane from different classes of tomatoes [20]. The recovery ranged from 83.84 to 119.73% and the RSD was below 20.54%. The LODs were between 1.63 to 10.5 mg/kg and LOQs were between 5.43 to 35 mg/kg. An amount of 31.81% of samples showed contamination above MRLs with pesticides and13.6% of samples had contamination with diazinone and 18.18% of samples with chlorpyrifos.

QuEChERS sample preparations are simpler and less time-consuming procedure and lower organic solvent consumption. Since QuEChERS 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.

2.6. Liquid Phase Micro-extraction

It is miniaturized liquid phase extraction method. In Liquid Phase Micro-extraction, the analytes is shifted from an aqueous phase (donor phase) to water-immiscible solvent (extract ant or acceptor). In the sample preparation procedure, the Liquid phase micro-extraction **(**LPME) can be classified into three main categories: single-drop micro-extraction (SDME), hollow-fiber Liquid Phase Micro-extraction (HF-LPME) and dispersive liquid-liquid micro-extraction (DLLME).

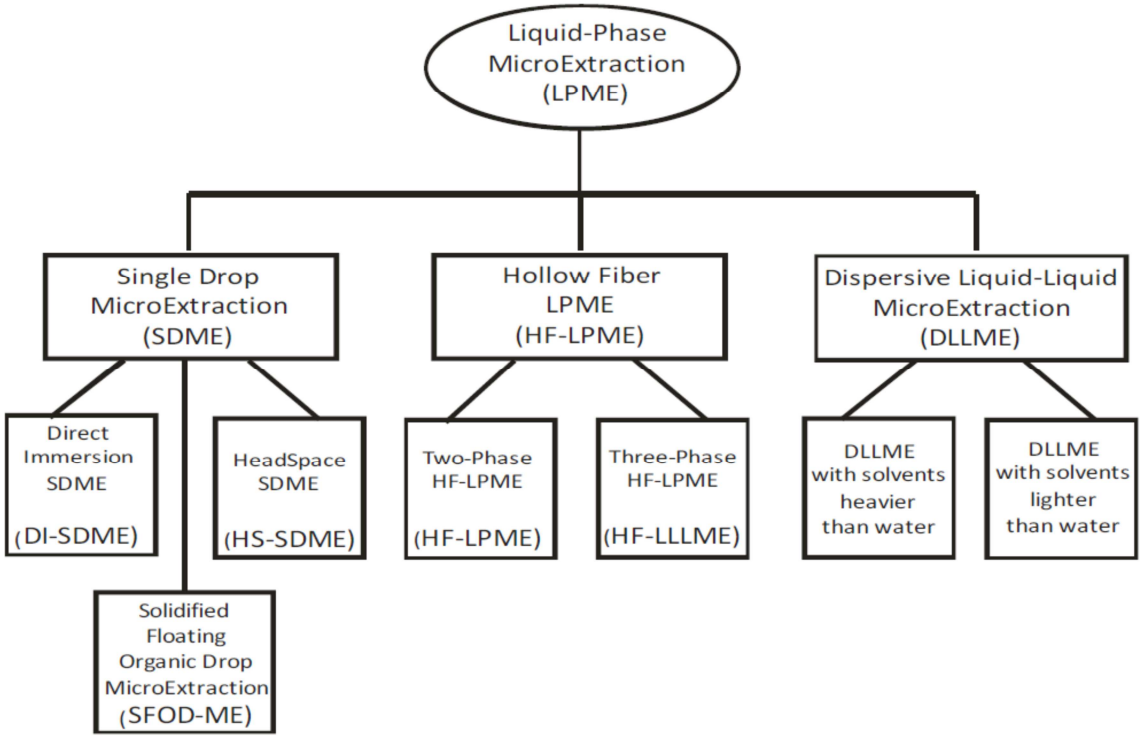

Figure 8. *Variants of liquid-phase micro extraction (LPME).*

In single-drop micro-extraction a micro-drop of extraction solvent is set at the tip of a micro syringe needle and immersed in the sample solution. After a period of magnetic stirring, the equilibrium is established between the sample and the micro-drop of extraction solvent. Finally, the micro-drop is retracted back into the micro syringe and injected for the subsequent determination. Most of all, the extraction solvent must have low water-solubility and high boiling point [27].

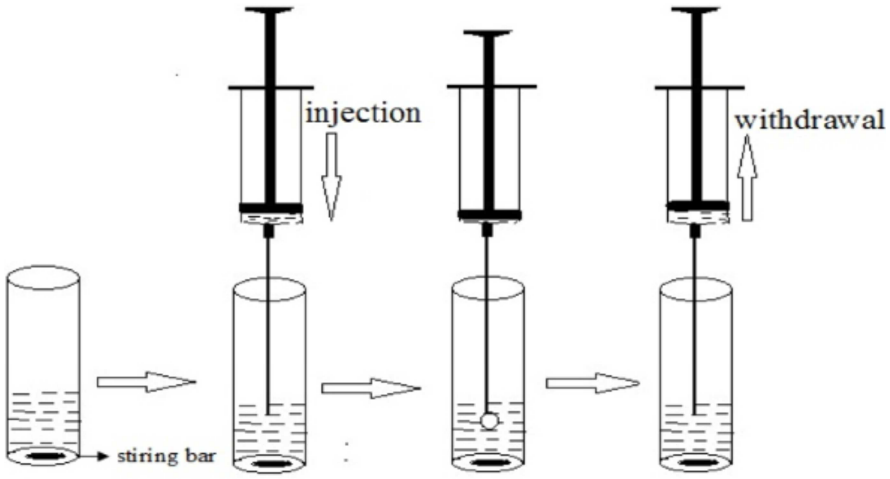

Figure 9. *Schematic diagram of SDME.*

Single-drop micro-extraction method was developed for the determination of metribuzin, vinclozolin, fosthiazate, procymidone, fludioxonil, kresoxim-methyl, fenhexamid, iprodione, bifenthrin, cyhalothrin, indoxacarb and azoxystrobin in tomatoes by gas chromatography equipped with a micro electron capture detector [30]. For all pesticides studied, with the exception of pyrethroids, single-drop micro-extraction exhibited good analytical characteristics. The enrichment factors of the single-drop micro-extraction procedure applied in tomato extracts ranged from 0.7 for bifenthrin to 812 for fenhexamid whereas, the concentration factors for the whole SDME studied ranged from 50.1 for bifenthrin and cyhalothrin to 52 for fenhexamid. The recoveries ranged from 67 to 90%.

Determinations of organochlorine pesticides from vegetable samples coupling SDME with gas chromatography–mass spectrometry were developed [38]. Parameters such as organic solvent, exposure time, agitation and organic drop volume were controlled and optimized. It was applied for the determination of OCPs in vegetable samples with a linearity range of 0.05–20 $ngmL^{-1}$ for α BHC and dicofol, 0.5–20 $ngmL^{-1}$ for dieldrin and 2,2-bis(4-chlorophenyl)-1,1-dichloroethane (DDD) or 0.5–50 $ngmL^{-1}$ for 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene and 2-(2-chlorophenyl)-2 (4-chlorophenyl)-1,1,1-trichloroethane. The determination limit at an S/N of 3 ranged from 0.05 $ngmL^{-1}$ for, BHC to 0.2 $ngmL^{-1}$ for dicofol, dieldrin. The recoveries were from 63.3 to 100%, with RSD from 8.74 to 18.9%.

Determination of ethoprophos, diazinon, parathion methyl, fenitrothion, malathion, isocarbophos and quinaphos in orange juice was developed [31]. The orange juice was simply centrifuged and diluted with water, extracted by single-drop micro-extraction and analyzed by gas chromatography equipped with a flame photometric detection. Fortification tests were conducted for concentrations between 10 and 500 µg/L; mean relative recoveries for each pesticide were all above 76.2% and below 108.0%. Limits of detection of the method for orange juice were below 5 µg/L for all target pesticides. The RSD varied between 4.6 and 14.1%. The proposed method is acceptable in the analysis of organo phosphorus pesticides in juice matrices.

To avoid the drop instability in single-drop micro-extraction hollow-fiber liquid phase micro-extraction was introduced as another type of liquid phase micro-extraction. Hollow-fiber liquid phase micro-extraction omits the clean-up step, eliminates the solid phase extraction step, simplifies the sample preparation procedure, decreases the solvent consumption and lowers the cost of analysis [29]. In Hollow-fiber liquid phase micro-extraction 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 [41].

A sensitive and reliable extraction method based on two-phase hollow fiber liquid phase micro extraction followed by gas Chromatography-mass spectrometry has been developed for determination of pyrethroid pesticides in Vegetable juice, Apple juice, Peach juice, Orange juice and Kiwi juice [37]. The parameters affecting the extraction efficiency were studied via rotatable-centered cube central composite design. The optimization results showed that speed of agitation, extraction time and ionic strength were significantly important in the extraction process. A response surface equation was derived, and the statistical parameters of the derived model were obtained as R^2=0.9862 and F=142.46. The response surface plots revealed a separation optimum with 480 rpm speed agitation, extraction time of 41 min and NaCl concentration of 3% (w/v). Limit of detection were obtained in the range of 0.02–0.07 ng/mL and limit of quantification were between 0.08 and 0.10 ng/mL.

Liquid phase micro extraction based on polypropylene hollow fibers was evaluated for the extraction of thiabendazole, carbendazim and imazalil from orange juices [32].

Analytes were extracted in their neutral state through a supported liquid membrane of 2-octanone into 20µL of a

stagnant aqueous solution of 10 mM HCl inside the lumen of the hollow fiber. Subsequently, the acceptor solution was directly subjected to CE for the analysis. With recoveries that ranged between 17.0 and 33.7%. The Analytical performance of the method was evaluated by LC/MS, with better sensitivity permitted the detection below the μgL^{-1} level. The RSDs ranged between 3.4 and 10.6%. Linearity was obtained in the range 0.1–10.0μgL^{-1}, with r=0.999 and 0.998 for TBZ and IMZ, respectively. Limit of detection were below 0.1μgL^{-1} and it has been demonstrated the suitability of three-phase Liquid phase micro extraction for the extraction of pesticides from citrus juices, suppressing any pretreatment step such as filtration or removal of the solid material from the sample, that may potentially involve a loss of analyte.

A new method based on phase hollow fiber liquid phase micro extraction has been developed for the determination of organophosphorus pesticides and some of their metabolites, in two commercial cereal-based baby foods and one wheat flour prior to Gas Chromatography-Nitrogen Phosphorus detection [39]. Samples were first extracted by ultrasound-assisted extraction with ACN containing 1.25% (v/v) of formic acid. After evaporation and reconstitution in Milli-Q water, the hollow fiber liquid phase micro extraction procedure, using 1-octanol as extraction solvent, was applied followed by a desorption step in ACN, which clearly improved the performance of the technique.

The effects of sample pH, ionic strength, stirring rate, extraction temperature and time as well as the desorption procedure were investigated. The limit of detection were between 0.29 and3.20 µg/kg. The extraction of Milli-Q water, as an example of the applicability of the procedure to aqueous samples, allowed achieving limit of detection in the range 0.01–0.04 µg/L.

Hollow fiber liquid phase micro extraction technique was used for the extraction procedure for the determination of organophosphorus pesticides in fish tissue [43]. In this study organophosphorus pesticides were first extracted with acetone from fish sample, the organic extract after rotatory evaporation was then redissolved with water–methanol (95:5, v/v) solution, followed by polyvinylidene difluoride (PVDF) hollow fiber liquid phase micro extraction. Good linearity were observed in the range of 20–500 ng/g, limit of detection were in the range of 2.1-4.5 ng/g.

Dispersive liquid-liquid micro extraction 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 extract ant 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 collected at the bottom of the tube through centrifugation.

Dispersive liquid- liquid micro extraction possess shorter extraction time, quicker and the absence of a clean-up procedure, lower consumption of organic solvent, low limits of detection, good repeatability, high enrichment factor and good recovery within a short time [42].

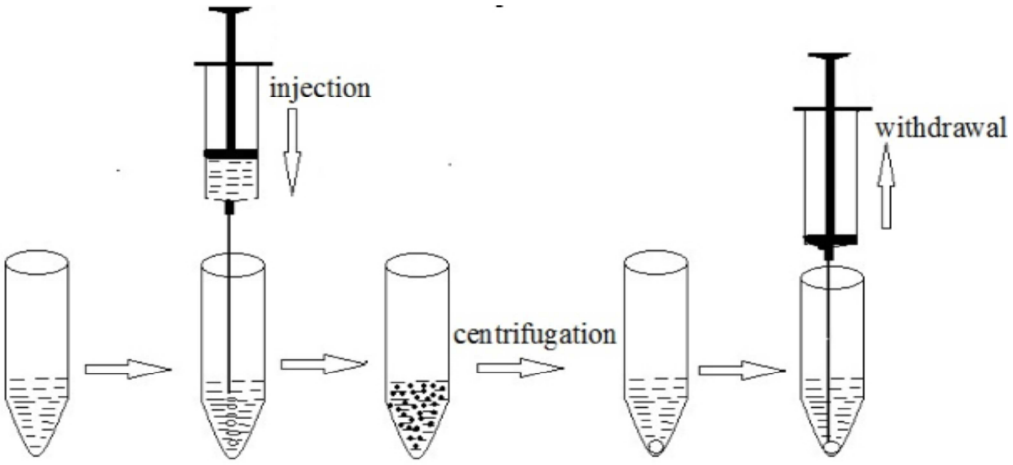

Figure 10. *Schematic diagram of Dispersive liquid- liquid micro extraction.*

In dispersive liquid- liquid micro extraction, the organic solvents should have higher density than water, low water solubility, high extraction capability of target compounds and good chromatographic behavior. Tetrachloroethane, chlorobenzene, carbon tetrachloride and C_2Cl_4 have been used as extraction solvent [35].

Dispersive liquid- liquid micro extraction procedures using room temperature ionic liquids such as [C_6MIM] [PF_6], 1, 3-dibutylimidazolium hexafluorophosphate and [C_8MIM] [PF_6] have been developed for quantifying trace amounts of pesticides [34].

A new dispersive liquid- liquid micro extraction method based on the solidification of a floating organic droplet using the extraction solvent of dodecan-1-ol was reported [33]. 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.

Dispersive liquid- liquid micro extraction followed by gas chromatography-nitrogen phosphorus detection was developed for the extraction, preconcentration and determination of penconazole, hexaconazole, diniconazole, tebuconazole, and difenoconazole in honey samples [36].

Parameters such as type and volume of the extraction and disperser solvents, temperature, salt addition and pH were evaluated. Under the optimum extraction conditions, the method resulted in limit of detection and limit of quantification within the range 0.05 – 0.21 ng g^{-1} in honey (15 - 70 ng L^{-1} in solution) and 0.15–1.1 ng g^{-1} in honey (45 - 210 ng L^{-1} in solution), respectively. Enrichment factors and extraction recoveries were in the ranges of 1943 – 1994 and 97 – 100%, respectively. The method precision was evaluated at 1.5 ng g^{-1} of each analyte and the RSD were found to be less than 4% for intra-day and less than 6% for inter-days. The method was successfully applied to the analysis of honey samples and difenoconazole was determined at ng g^{-1} levels.

Simple, inexpensive, reliable and environmentally friendly sample preparation method based on solidification of an ionic liquid after performing dispersive liquid–liquid micro extraction has been developed for the extraction and preconcentration of carbamate pesticides (carbofuran, methiocarb, carbaryl and thiodicarb) followed by determination with high performance liquid Chromatography-diode array detector [40]. Secondary amine is mixed with a carboxylic acid to prepare an ionic liquid with a low melting point. Then, the prepared ionic liquid is used as an extraction solvent in the micro extraction procedure. Limit of detection and limit of quantification in the ranges of 0.32–0.51 and 1.09–1.72 ng mL^{-1}, respectively, were obtained. Enrichment factors and extraction recoveries were obtained in the ranges of 173–227 and 69–91%, respectively. Finally, the proposed method was successfully used in the determination of the selected carbamate pesticides in some fruit juice and vegetable samples at ng mL^{-1} level.

Simple, rapid and efficient method has been developed for the determination of sulfonylurea herbicides (SUHs) in commercial grape and apple juice samples, using dispersive liquid–liquid microextraction coupled with capillary high-performance liquid chromatography with diode array detection [28].

Parameters that influence the extraction efficiency, such as the type and volume of extraction and disperser solvents, sample pH and salt addition, were investigated and optimized. Under the optimum conditions, limits of detection and quantification of the method were in the ranges of 2–9 and 8–29 μg L^{-1}, respectively, lower than the MRLs set by the European Union for the raw fruits, such as grape and apple. The intra and inter-day relative standard deviations varied from 1.0 to 8.2 and 1.8 to 9.8%, respectively, with recoveries between 72.0 and 109.5% for commercial grape (both white and red) and apple juice samples, showing satisfactory accuracy for the determination of sulfonylurea herbicides (SUHs) in fruit juices.

3. Conclusion

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 limit of detection. 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. Sample preparation methods such as: solid phase extraction, matrix solid-phase dispersion, solid phase micro-extraction and liquid phase micro-extraction 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.

References

[1] Ji Sun Lee, Soo Hee Cho, Chae Mi Lim, Moon Ik Chang, Hyun Jin Joo, Hojae Bae, Hyun Jin Park. (2017). A Liquid Chromatography - Tandem Mass Spectrometry Approach for the Identification of Mebendazole Residue in Pork, Chicken and Horse. *12 (1)*. doi: 10.1371/journal.pone.0169597.

[2] Simone M., Goulart, Renata D. Alves, Washington X. de Paul José Humberto de Queiroz, Antônio A. Nevesa and Maria Eliana L. R. de Queiroz. (2012). Determination of Carbamates in Beverages by Liquid-Liquid Extraction with Low Temperature Partitioning and Liquid Chromatography. *J. Braz. Chem. Soc, 23*, 1154-1165. doi: 10.1590/S0103-50532012000600022.

[3] Tesfa Bedassa, Negussie Megersa and Abera Gure. (2017). Salting-out Assisted Liquid-Liquid Extraction for the Determination of Multiresidue Pesticides in Alcoholic Beverages by High Performance Liquid Chromatography. *Science Journal of Analytical Chemistry, 5 (3)*, 38-45. doi: 10.11648/j.sjac.20170503.11.

[4] Asha LAL, Guanhuat TAN, and Meekin CHAI. (2008). Multiresidue Analysis of Pesticides in Fruits and Vegetables Using Solid-Phase Extraction and Gas Chromatographic Methods. *Analytical sciences, 24, 231-236.*

[5] Guo C, Hu JY, Chen XY, Li JZ. (2008). Analysis of Imazaquin in Soybeans by Solid-phase Extraction and High-performance Liquid Chromatography. *Bull. Environ. Contam, 80*, 173–177.

[6] Guocan Zheng, Chao Han, Yi Liu, Jing Wang, Meiwen Zhu, Chengjun Wang, and Yan Shen (2014). Multiresidue analysis of 30 organochlorine pesticides in milk and milk powder by gel permeation chromatography-solid phaseextraction-gas chromatography-tandem mass spectrometry. *J. Dairy Sci., 97*, 6016–6026. Retrieved from dx.doi.org/ 10.3168/jds.2014-8192.

[7] Hu JY, Zhen ZH, Deng ZB. (2011). Simultaneous Determination of Acetochlor and Propisochlor Residues in Corn and Soil by Solid Phase Extraction and GasChromatography with Electron Capture Detection. *Bull. Environ. Contam. Toxicol., 86*, 95-100.

[8] Ravelo-Pérez, J. Hernández-Borges, M. Rodríguez-Delgado. (2008). *J. Chromatogr. A, 1211, 33*.

[9] Van der Lee, G. van der Weg, W. A. Traag, G. J. M. Hans. (2008). *J. Chromatogr. A, 1186, 325*.

[10] Wu S, Mei J. (2011). Analysis of the Herbicide Bispyribac-sodium in Rice by Solid Phase Extraction and High Performance Liquid Chromatography. *Bull. Environ. Contam., 86*, 314–318.

[11] Pérez-Parada, M. C.-A. (2011). *J. Chromatogr. A 2011, 1218, 5852*.

[12] Adriana N. Macedo, Ana R. A. Nogueira, Silvia Helena Govoni Brondi. (2009). Matrix Solid-Phase Dispersion Extraction for Analysis of Cypermethrin Residue in Cows' Milk. *chromatographia, 69*, 571–573.

[13] Érica A. Souza-Silvaa, Viorica Lopez-Avilab, Janusz Pawliszyna. (2013). Fast and robust direct immersion solid phase microextraction coupled with gas chromatography–time-of-flight mass spectrometry method employing a matrix compatible fiber for determination of triazolefungicides in fruits. *Journal of Chromatography A*. Retrieved from www.elsevier.com/locate/chroma.

[14] Fernandez-Alvarez, M. Llompart, J. P. Lamas, M. Lores, C. Garcia-Jares, R. Cela, T. Dagnac. (2009). *J. Chromatogr. A, 1216, 2832*.

[15] Chai, G. H. Tan. (2009). *Food Chem, 117, 561*.

[16] Maria Geovania Dantas Silva, Adriano Aquino, Haroldo Silveira D ˆ orea, Sandro Navickiene ´. (2008). Simultaneous determination of eight pesticide residues in coconut using MSPD and GC/MS. *Talanta 2008, 76*, 680–684. doi: 10.1016/j.talanta.2008.04.018.

[17] Matrix Solid-Phase Dispersion Extraction for Analysis of Cypermethrin Residue in Cows' Milk. (2009). *Chromatographia, 69*, 571–573.

[18] Sandro Navickiene, Adriano Aquino and Débora Santos Silva Bezerra. (2010). A Matrix Solid-Phase Dispersion Method for theExtraction of Seven Pesticides from Mango and Papaya. *chromatographic science*. doi: 10.1093/chromsci/48.9.750.

[19] Tsochatzis ED, Menkissoglu-Spiroudi U, Karpouzas D G, Tzimou-Tsitouridou R. (2010). *Anal. Bioanal. Chem., 397*, 2181-2190.

[20] Elham JAHANMARD, Fatemeh ANSARI, Mansour FEIZI. (2016). Evaluation of Quechers Sample Preparation and GC Mass Spec-trometry Method for the Determination of 15 Pesticide Residues in Tomatoes Used in Salad Production Plants. *Iran J Public Health, 45*, 230-238. Retrieved from http://ijph.tums.ac.ir.

[21] Chen, P. Y. Cao, R. J. Liu,. (2011). *Food Chem*, 125, 1406.

[22] Godfred Darko, Jonah Addai Tabi, Michael Kodwo Adjaloo, and Lawrence Sheringham Borquaye1. (2017). Pesticide Residues in Honey from the Major Honey Producing Forest Belts in Ghana. *Journal of Environmental and Public Health*. Doi: doi.org/10.1155/2017/7957431.

[23] Mustapha F. A. Jallow, Dawood G. Awadh, Mohammed S. Albaho, Vimala Y. Devi and Nisar Ahmad. (2017). Monitoring of Pesticide Residues in Commonly Used Fruits and Vegetables in Kuwait. *International journal of Enviromental Research and public Health*.

[24] Viñas, N. Campillo, N. Martínez-Castillo, M. Hernández-Córdoba. (2009). *J. Chromatogr. A*, 1216, 40.

[25] R. Romero-Gonzáleza,, A. Garrido Frenicha, J. L. Martínez Vidala, O. D. Prestesb, S. L. Grioc. (2011). Simultaneous determination of pesticides, biopesticides and mycotoxins in organic products applying a quick, easy, cheap, effective, rugged and safe extraction procedure and ultra-high performance liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A, 1218*, 1477–1485. Retrieved from www.elsevier.com/locate/chroma.

[26] S. C. TRANa, H. TH. LEa and T. H. THAI-NGUYENb. (2015). Determination of pesticide multi-residues in green tea using a modified quechers extraction and liquid chromatography tandem mass spectrometry technique. *Acta Alimentaria, 44 (3)*, 409–419. doi: 10.1556/066.2015.44.0012.

[27] A. Garbi, V. S. (2010). *Talanta, 82, 1286*.

[28] Abera Gure, Francisco J. Lara, Negussie Megersa, Monsalud del Olmo-Iruela & Ana M. García-Campaña. (2014). Dispersive Liquid–Liquid Microextraction Followed by Capillary High-Performance Liquid Chromatography for the Determination of Six Sulfonylurea Herbicides in Fruit Juices. *Food Analytical Methods, 7*, 1468-1476. doi: 10.1007/s12161-013-9775-5.

[29] E. Sobhanzadeh, N. K. A. Bakar, M. R. B. Abas, K. Nemati. (2011). *J. Hazard. Mater., 186, 1308.*

[30] Elpiniki G. Amvrazi a, Asimina T. Papadi-Psyllou a & Nikolaos G. Tsiropoulos. (2010). Pesticide enrichment factors and matrix effects on the determination of multiclass pesticides in tomato samples by single-drop microextraction (SDME) coupled with gas chromatography andcomparison study between SDME andacetone-partition extraction procedure. *International Journal of Environmental Analytical Chemistry, 90*, 245-259. doi: 10.1080/03067310903166699.

[31] Ercheng Zhao, Lijun Han, Shuren Jiang, Qiuxia Wang, Zhiqiang Zhou. (2008). Application of a single-drop microextraction for the analysis of organophosphorus pesticides in juice. *Journal of Chromatography A, 1114,* 269–273.

[32] Francisco Barahonaa, Astrid Gjelstadb, Stig Pedersen-Bjergaardb, Knut Einar Rasmussenb. (2010). Hollow fiber-liquid-phase microextraction of fungicides from orange juices. *Journal of Chromatography A, 1217,* 1989–1994. doi: 10.1016/j.chroma.2010.01.077.

[33] G. Matsadiq, H. H. (2011). *J. Chromatogr. B, 879, 2113*.

[34] J. H. Zhang, H. X. Gao, B. Peng, S. Q. Li, Z. Q. Zhou,. (2011). *J. Chromatogr. A, 1218, 6621*.

[35] J. J. Du, H. Y. Yan, D. D. She, B. M. Liu, G. Y. Yang,. (2010). *Talanta, 82, 698*.

[36] M. A. Farajzadeh, M. R. A. Mogaddam, H. Ghorbanpour. (2014). Development of a new microextraction method based on elevated temperature dispersive liquid-liquid microextractionfor determination of triazole pesticides residues in honey bygas chromatography-nitrogen phosphorus detection. *Journal of chromatographia A*. doi: http://dx.doi.org/doi:10.1016/j.chroma.2014.04.067.

[37] Majid Arvand a, Elahe Bozorgzadeh a, Shahab Shariati. (2013). Two-phase hollow fiber liquid phase microextraction for preconcentration of pyrethroid pesticides residues in some fruits and vegetable juices prior to gas chromatography/mass spectrometry. *Journal of Food Composition and Analysis, 31*, 275–283. Retrieved from www.els evier.c om/lo cat e/jfca.

[38] Maosheng Zhang, Jiarong Huangc, Changlin Wei c, Binbin Yu a, Xiaoqing Yang d, Xi Chena. (2007). Mixed liquids for single-drop microextraction of organochlorine pesticides in vegetables. *Talanta*, 599–604.

[39] Miguel Ángel González-Curbelo, Javier Hernández-Borges, Teresa María Borges-Miquel, Miguel Ángel Rodríguez-Delgado. (2013). Determination of organophosphorus pesticides and metabolites incereal-based baby foods and wheat flour by means ofultrasound-assisted extraction and hollow-fiber liquid-phasemicroextraction prior to gas chromatography with nitrogenphosphorus detection. *Journal of Chromatography A*. doi: doi.org/10.1016/j.chroma.2013.05.081.

[40] Mir Ali Farajzadeh, Mahdi Bamorowat and Mohammad Reza Afshar Mogaddam. (2016). Development of a dispersive liquid–liquid microextraction method based on solidification of a floating ionic liquid for extraction of carbamate pesticides from fruit juice and vegetable samples. *RSC Adv, 6, 112939*. doi: 10.1039/c6ra20103b.

[41] S. Pedersen-Bjergaard, K. E. Rasmussen. (2008). *J. Chromatogr. A, 1184, 132*.

[42] S. C. Cunha, J. O. Fernandes, M. B. P. P. Oliveira. (2009). *J. Chromatogr. A, 1216, 8835*.

[43] Xiaojin Sun, Fang Zhu, Jiabin Xi, Tongbu Lu, Hong Liu, Yexiang Tong, Gangfeng Ouyang. (2011). Hollow fiber liquid-phase microextraction as clean-up step for the determination of organophosphorus pesticides residues in fish tissue by gas chromatographycoupled with mass spectrometry. *Marine Pollution Bulletin, 63*, 102–107. doi: 10.1016/j.marpolbul.2011.03.038.