



Review Article

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A Review on Modern Extraction Techniques for the Determination of Pesticide Residues in Plant, Soil and Food samples

Shakirbasha. S*, Vijayraj. S, Lohita. M, Edukondalu.T

Department of Pharmaceutical analysis, Sree Vidyanikethan College of Pharmacy, Sree sainath Nagar, A.
Rangampet, Tirupathi-517102, Chittoor (Dt), Andhra Pradesh, India.

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Abstract

Pesticides are widely utilized during cultivation to protect against pests and in post harvest treatments to prolong storage. During the last years much attention has been directed to control pesticide in plant, soil and food samples. Considering low concentration levels of pesticide residues in plant, soil and food matrices and the determination of these residues often requires extensive sample extraction and purification prior to the analysis. Due to intensive use of pesticides, their residues have become an unavoidable part of the environment, and they are often detected in all environmental segments and therefore their monitoring has been frequently performed throughout the world. As the presence of trace amounts of both pesticide residues and their degradation products could be potential health hazards, United Nation organization has formed specialized groups: World Health Organization (WHO) and Food Agriculture Organization (FAO), with the aim to establish restrictive measures to protect the environment against pollution. These organizations and their experts groups on annual meetings summarize international achievements in pesticides domain, establish legislation and make recommendations obligating member states to act in accordance with international standards. This review provides the information regarding pesticides and their residues in different samples.

Keywords: Pesticides, Harvest, Concentration, Residues, Degradation, Sample Preparation, Pollution etc.

Contents

1. Introduction	1408
2. Sample Extraction Techniques	1409
3. Gel Permeation Chromatography (GPC).	1414
4. Matrix solid phase dispersion (MSPD).	1415
5. Conclusion.	1415
6. Acknowledgement.	1415
7. References	1415

*Corresponding author

Shakirbasha. S

Department of Pharmaceutical Analysis
Sree Vidyanikethan College of Pharmacy,
Tirupathi-517102, Chittoor (Dt), A.P, India
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1. Introduction

As pesticides are a very heterogeneous group of compounds with different biological and physicochemical properties, the current trend in pesticide residues analysis is developing multi-residual methods that not only provide simultaneous determination of large number of pesticides, but also can be applicable to large numbers of samples of different origin. Although separation chemical analysis involves several stages (sample preparation, analyte separation i.e. quantification and data analysis), sample preparation step can be marked as "the most critical" one.

Traditional sample preparation methods (liquid-liquid extraction, Soxhlet extraction, etc.) are laborious, time consuming, expensive, requires large amounts of organic solvents and usually involve many steps, leading to loss of some analyte quantity. Additionally, consequences of hydrocarbon solvents use, such as ozone depletion and generation of considerable cancer waste, lead to reduction of not only their use but also their manufacture. As a result, modern sample preparation procedures, such as accelerated solvent extraction (ASE), supercritical fluid extraction (SFE), microwave assisted extraction (MAE), solid phase extraction (SPE), solid phase micro extraction (SPME), matrix solid phase dispersion (MSPD) extraction and QuEChERS (quick, easy, cheap, effective, rugged and safe), have been developed to overcome the drawbacks of the traditional approaches. It should be indicated that SFE, ASE and MAE are instrumental techniques, and often use SPE and SPME for purification of obtained extracts, and also its concentration in case of SPME. Overall, a comprehensive analytical procedure is carried out in the way that obtained results can be found within prior established concentration range. Whether the measurement will be performed at the desired concentration range is determined by the instrument sensitivity and the choice of sample preparation method. Multiple factors, related to the physical properties of not only tested active ingredients (volatility, solubility in water and organic solvents, stability, acid-base properties, etc.), but also sample matrix (water, lipids, pigments content etc.), must be considered during an experiment planning. The choice of sample treatment depends directly on mentioned factors, but also on analysis purpose i.e. on required detection method sensitivity (limits of detection) and quantification accuracy (whether the aim is to establish the exact concentration value regardless maximum residues limits (MRLs), or just to establish if result is above or under MRLs). Considering complexity of these issues, analytical procedure for each specific case should be chosen in order to minimize problems relating to analysis duration, consumption of solvents and other necessary reagents, and also to reduce number of involved analytical steps, which would minimize potential sources of errors. This paper describes the basic principles of modern extraction techniques, comparing their advantages and drawbacks, and their ability and applicability for pesticide residues determination, with special emphases on plant material and soil samples. The present chapter covers published methods and research articles, in which pesticide residues have been extracted from different samples, then cleaned-up, and isolated by chromatographic techniques to be identified and quantified by mass spectrometric methods. Lastly, future developments and perspectives in this field.

2. Sample Extraction Techniques

Now a days, in the determination of pesticides residues, pre-concentration and isolation of the analytes from the food, soil and plant samples by some types of sample preparation process would be critical for obtaining correct results. Various sample preparation techniques have been employed for this purpose. However, some of these methods are time consuming, have complicated procedures and use a large amount of organic solvents which are often toxic and flammable. Recently, chlorine solvent free and excellent performance techniques have been extensively studied as the substitution to this type of sample preparation processes.

a. Manual Sample Extraction Techniques

A comprehensive control of pesticide residues in food with multi residue methods is needed to protect people effectively. While the basic determination methods LC and GC allow a high sample throughput with determinations up to 100 analytes, conventional sample extraction methods are often demanding in terms of labour, time and cost. In the past few years, the manual sample extraction methods are easy to use and evolved an economical and environmentally compatible sample extracts for GC-MS and LC-MS. The advantages of these methods in comparison with classical clean-up methods are: high sample throughput due to a quick and easy few step procedure, low need of laboratory glasses, bench space and equipment, low consumption of solvents, broad range of pesticides can be determined and rugged method with high and safe recovery rates.

1. Solid-Liquid and Liquid-Liquid Extraction

In a recent review about pesticides analysis in plant, soil and food sample showed that in the last two decades¹⁻², the most widely used pesticide extraction techniques from these samples was solid-liquid extraction (SLE) and liquid-liquid extraction (LLE). The SLE procedure consists in grinding chopped samples or extracted fats several times at high speed in selected organic solvents. This technical procedure has been applied to meat and meat products animal fat, eggs and fish for extracting different kinds of pesticides. Similarly, LLE procedure consists in shaking liquid samples several times in selected organic solvents for extracting pesticide residues.

2. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was originally developed for extracting a wide range of pesticides in fruit and vegetables has become very popular since it was introduced in 2003 by Anastassiades and co-workers. The method is characterized by using the polar solvent acetonitrile for extraction of water containing matrices with addition of salts in order to get phase separation. Since its introduction, this method has been readily accepted by many pesticide residue analysts because of its low cost, fast, the accurate procedures are no time consuming. The authors questioned the conditions previously used for pesticide residues analysis, and through extensive experiments and novel use of MgSO₄ for salting out extraction/partitioning and dispersive solid-phase extraction (d-SPE) for cleanup, they devised a highly streamlined sample preparation method with excellent results for a wide range of pesticides in many types of samples. The original procedure consists in the sample

extraction by hand-shaking or vortex mixing with the 10 ml of acetonitrile (MeCN). Gram quantities of salts (4 g of MgSO₄ and 2 g of NaCl) are then added to the sample by mixing, to drive analytes partitioning between the aqueous residue and the solvent. After vortex mixing and centrifugation, clean-up and removal of residual water is performed using a d-SPE procedure (PSA adsorbent and anhydrous MgSO₄ are mixed with the sample extract), that requires less time than the traditional SPE and simultaneously removes residual water and many polar matrix components, such as organic acids, some polar pigments, and sugars. As a polar solvent, miscible with water, with sufficient dispersive (hydrophobic) properties to extract effectively both polar and non-polar pesticides, MeCN is chosen as the QuEChERS solvent. Use of this solvent in the QuEChERS method proved to be successful for extraction of several pesticides classes from different matrices. Studies showed that some pesticides gave lower recoveries depending on pH of the matrix³ realized that buffering at pH=5 during extraction gave the optimum balance to achieve acceptably recoveries (>70%) for pH-dependent pesticides, independent of the matrix.

On the other hand, Lehotay modified the method to use even stronger acetate buffering conditions. Both versions of methods went through extensive laboratory trials and successfully met statistical criteria for acceptability by independent scientific standards organizations. So the acetate-buffering version becomes AOAC Official Method 2007. (Lehotay, 2007) and the citrate-buffering version being named as Standard EN 15662 Method. There is an abundance of the QuEChERS applications for pesticides determination in different plant samples. Thus, for example, QuEChERS provides satisfactory results for determination of 229 pesticides in lettuce and orange⁴⁻⁵, 109 in rice⁶, 160 in tomato, pear and orange⁷, 140 in cucumber and orange⁸⁻¹⁰, 118 in vegetables juice¹¹, 138 in apples, bananas, pears, apple juice, peas, creamed corn, squash and carrots¹²⁻¹⁴, 150 in tomato, strawberry, potato, orange, and lettuce¹⁵, 300 in tomato, apple, lettuce, cucumber, carrot, mushroom, grapes, lemon, pepper, pear, potato and cabbage¹⁶, 69 in zucchini, melon, cucumber, tomato, garlic, lettuce and pepper¹⁷, 46 in onion, spinach, potato, carrot, cucumber, cabbage and tomato¹⁸, 150 in grapes¹⁹, 148 in onion, spinach, potato, carrot, peas and tomato, 73 OPPs and carbamates in rice, tree nuts and citric fruits and 14 OCPs in apricot, plum, cherry, nectarine, pear and apple²⁰⁻²¹.

3. Matrix solid phase dispersion (MSPD)

Matrix solid-phase dispersion (MSPD) is a new SPE-based extraction and clean-up technique developed for pesticide multi-residue analysis. The MSPD method is based on the homogenization of a viscous, solid or semi-solid sample with an abrasive solid support material in a glass mortar, in order to perform the complete disruption and dispersal of the sample. After blending, the sample is transferred into a column and analytes are eluted with appropriate solvent. Complete disruption of the sample and its dispersion over the support surface greatly enhance surface area for the sample extraction. Furthermore, interferences are retained on the adsorbent and in that way, extraction and clean-up are performed simultaneously, reducing the analysis time and the amount of solvent used.

A rapid, sensitive and accurate MSPD method combined with accelerated solvent extraction has been developed by for selective determination of sixteen organochlorine pesticide residues in fish samples by GC-MS. 2 g fresh fish muscle was dispersed with 10 g anhydrous sodium sulfate and 2 g acid alumina thoroughly, and loaded into the stainless-steel extraction cell containing 6 g of acid alumina and 10 g anhydrous sodium sulfate. The adequate extraction efficiency was showed using dichloromethane-hexane (3:7, v/v) mixture as solvent.

Not only the lipids, but also other co-extracts, which peaks mostly located in the forepart of chromatograms and maybe interfere the identification or quantitation of analytes, were eliminated exhaustively, while analytes were extracted selectively. The performance of proposed method was evaluated and validated: the detection limits were 0.008-0.05 µg kg⁻¹ (1.9-5.0, RSD%) and recoveries were 91.0-104.1% spiked at 10 µg kg. Reversed-phase materials such as C8 and C18-bonded silica are the most commonly used adsorbents, because their lipophilic properties enable good disruption, dispersion and retention of lipophilic species²²⁻²³. Several methods based on use of these adsorbents have been used for determination of several pesticides classes including OPPs and OCPs in tomato, lettuce, orange, lemon, grapefruit, pear and plum²⁴⁻²⁵, carbamates in orange, onion, grape and tomato²⁶⁻²⁸, eight fungicides in orange, apple, tomato, carrot and zucchini²⁹, thirteen fungicides and insecticides in pepper, lettuce, tomato, orange, apple and pear²⁹⁻³¹, five fungicides in orange, lemon, banana, pepper, chard and onion³²⁻³³. Besides C8 and C18-bonded silica, florisil has been successfully used for determination of OCPs in tomato juice³⁴, lemon, orange, apple, mango, grapes, banana, carrot, potato, onion, cucumber, tomato, cabbage, spinach and wheat. [35].

Basically, the adsorbent choice depends on analyte polarity and interferences which could be co-extracted from sample matrix. A MSPD and gas chromatography-mass spectrometry were combined to determine procymidone, malathion, bifenthrin and pirimicarb in honey³⁶. The best results were obtained using 1.0 g of honey, 1.0 g of silica-gel as dispersant sorbent and acetonitrile as eluting solvent. The method was validated by fortified honey samples at three concentration levels (0.2, 0.5 to 1.0 mg kg⁻¹). Average recoveries (n=7) ranged from 54 to 84%, with relative standard deviations between 3.7 and 8.5%. Detection and quantification limits attained by the developed method ranged from 0.02 to 0.08 mg kg⁻¹ and 0.07 to 0.25 mg kg⁻¹ for the honey, respectively.

b. Instrumental-based Sample Extraction Techniques

A number of instrumental-based extraction procedures have been developed to isolate pesticides residues from food of animal origin, including microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), pressurised liquid extraction (PLE), solid-phase extraction (SPE) and solid-phase micro-extraction (SPME) systems. Advantages in using such technology include the potential for automation, more selective isolation of residues through tuning of instrument parameters and online clean-up of samples. Disadvantages include the limited number of commercially available instruments, additional extraction costs and instrumental downtime.

1. Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) is a technique based on usage of the microwave energy, and where compounds can be extracted more selectively and rapidly, with similar or better recovery compared to conventional extraction processes. The MAE effects a direct migration of the desired components out of the matrix, as a result of selective energy application into the matrix. High method efficiency is a result of the matrix macrostructure destruction³⁷⁻³⁸. During the MAE of plant material, microwave rays travel freely through the solvent and interact selectively with the free matrix water causing localized heating. The result is non-uniform temperature rise with more pronounced effects where the free water is in larger proportions. The result is a volume expansion within the systems. The walls of these systems cannot accommodate the high internal pressures and rupture spontaneously, allowing the organic contents to flow freely toward the relatively cool surrounding solvent that solubilises them rapidly³⁹. Considering the complexity of plant material and its non-uniformity regarding different amount of free water, the MAE advantage can be noticed. Particularly, by providing different microwave energy levels, it is possible to selectively rupture some systems over others, thus it is possible to develop schemes that will effect the selective extraction of the given systems contents. MAE is also a promising technique for soil samples, particularly owing its possibility to control temperature, pressure and microwave energy, as well as to perform a few extractions simultaneously⁴⁰.

For method optimization, several variables such as volume and solvent composition, extraction temperature and time, are usually studied. In order to heat a solvent, part of it must be polar with high dielectric constant to absorb microwave energy efficiently. Non polar solvents with low dielectric constants can be also used, by adding certain amount of polar solvent that absorbs the microwave radiation and passes it on to other molecules⁴¹. For example, hexane and toluene can be modulated by the addition of small amounts of acetone or methanol⁴².

The main advantages of microwave pre treatment are the low temperature requirement, high extraction rate, automation and the possibility of simultaneously extracting different samples at the same time without interferences. However, solvent choice is limited, care must be taken not to overheat the sample, additional clean-up of the samples is generally necessary prior to analysis and MAE is not amenable to automation. The first use of MAE technique for pesticide residues determination (parathion and bromophos in maize, soya bean, fava bean, walnut, cotton seed and soil), was reported by Ganzler et al. (1986)⁴³. The results show that MAE is a viable alternative for determination of atrazine and OPPs in orange peel, carbendazim, diethofencarb, azoxystrobin, napropamide and bupirimate in strawberries⁴⁴.

2. Supercritical fluid extraction (SFE)

This technique uses supercritical fluid (SF) as an extraction tool for "drawing out" the organic compounds from solid matrices. Commonly used for this purpose is CO₂, as it has relatively low critical temperature (31.1 °C) and low critical pressure (7.38 MPa)⁴⁵, it is not reactive and is accessible in a high degree of purity at low cost. Changes in temperature and pressure at which the supercritical CO₂ is held will increase or decrease the "strength" of solvent and thus the selectivity of extraction performed. At constant temperature which exceeds critical temperature, the supercritical CO₂ will be able to extract analytes of low polarity at low pressure, and high polarity analytes at high pressure. SFE with CO₂ is usually performed at pressures that are not high enough to achieve efficient extraction of polar compounds. In such conditions, the supercritical CO₂ is a good extraction medium for non-polar compounds and moderately polar ones, such as PAHs, PCBs, organochlorine (OCPs) and organophosphorus (OPPs) pesticides, etc..

The efficiency of supercritical CO₂ can be improved by adding small amounts of modifiers, which identity is often more important than their concentration, since the major role of a modifier is to interact with the sample matrix to promote desorption into the fluid⁴⁶. Some of the common solvents such as acetone and methanol⁴⁷ are now mostly used as used both with and without modifiers. The basic principles and possibilities of applying the SFE technique for determining pesticide residues in samples of different origin can be found in the reports generated by Gilbert-Lopez et al. (2009)⁴⁸⁻⁴⁹.

In general, SFE usually lasts less than two hours, and the further analysis can be accomplished in several ways. According to one, SF with analytes is passed through a capillary that is immersed in an appropriate solvent. While in the capillary, SF exists, but after leaving the capillary it becomes a gas (the pressure falls below the critical

pressure). The largest part of this gas passes through the solvent, while the extracted analytes are retained in the solvent (the degree of retention depends on the solvent, i.e. the solubility of the analyte in it). Read phonetically Also, the flow of SF can be directed to a solid sorbent, which will then bind analytes, and its elution by appropriate solvent, analysts translate into a solution suitable for further analysis. Also, the flow of SF could be directed directly to capillary column of the gas chromatograph (GC), thus obtaining the "on-line" SFE. This approach enables analytical scheme with the highest sensitivity for a limited amount of sample available for analysis.

SFE is usually an efficient extraction method, primarily applicable to solid samples. However, as well as its numerous advantages (efficacy, selectivity, short extraction times, low solvent volumes) it also has serious drawbacks (difficult optimisation, high apparatus and maintenance cost, high blank and noise levels). In the case of pesticide residue analysis, recoveries for several compounds were unacceptable. Indeed, SFE techniques have not tended to be widely used for pesticide analysis in food from animal origin. An analytical procedure using supercritical fluid extraction (SFE) and capillary gas chromatography with electron-capture detection and confirmation of pesticide identity was performed by gas chromatography–mass spectrometry in selected-ion monitoring mode was developed to determine simultaneously residues of different pesticides (organochlorine, organophosphorus, organonitrogen and pyrethroid) in honey samples. Best efficiency was achieved at 400 bar using acetonitrile as modifier at 90 °C.

Compared with the conventional methodology, the main advantages of SFE are that the chances of sample contamination are greatly diminished as sample handling is minimized and the use of organic solvents is reduced. A much lower solvent evaporation, a simplified clean-up step, higher power diffusion and solubility are the other advantages of SFE. The recent studies have shown that SFE methods, followed by additional purification of the obtained extracts, meet the strict criteria of the pesticide residues analysis. Thus, Ono et al. showed that SFE method combined with purification on the SPE columns (C18 and Envicarb/NH₂), could be used for determination of 242 pesticides⁵⁰, in spinach, 245 in green beans and 263 in orange. Rissato et al. (2005) concluded that the SFE preparation of potato, tomato, lettuce and apple samples, combined with the extracts cleanup on amino propylene columns, could be used for determination of 37 pesticides⁵¹.

3. Accelerated solvent extraction (ASE)

Accelerated solvent extraction (ASE), also known as pressurized liquid extraction (PLE), is relatively new sample preparation technique, that uses small amounts of water and organic solvents, and is based on the extraction under elevated temperature (up to 200 °C) and pressure (up to 20 MPa) for short time periods, resulting in better extraction efficiency. This technique utilizes solvents that are raised to the near supercritical region, where they show better extraction properties. At high temperatures, the rate of extraction increases because the viscosity and the surface tension decreases, while its solubility and diffusion rate into the sample increase. Pressure keeps the solvent below its boiling point and forces its penetration into the pores of the sample. The combined use of high pressures (500-3000 psi) and temperatures (50-200 °C) provides a faster extraction process (5- 10 min) that requires smaller amounts of solvent compared with traditional extraction, thus decreasing the dilution of the sample⁵². The time required for extraction is practically independent of the sample mass and the efficiency of extraction is mainly dependent on temperature.

PLE can be performed in both static and dynamic (flow through) modes, or a combination of both. In static mode, the sample is enclosed in a stainless steel vessel filled with an extraction solvent, and following extraction the remaining solvent is purged with N₂ into a collection vial. Flow-through systems continuously pump solvent through the sample, but this has the disadvantage of using larger volumes of solvent and of diluting the extract. A desiccant, such as sodium sulphate, diatomaceous earth or cellulose can be added directly to the extraction cell or sorbent materials can be used to provide in situ clean-up. The extraction conditions must be optimised and this can be done using statistical "experimental design" procedures to minimize the number of experiments⁵³. Comparing PLE to LLE or Soxhlet extraction, the advantage of reducing solvent consumption and extraction time contrast with the disadvantage of using very expensive and specialized equipment. ASE has been successfully used for pesticides determination in samples of different origin. In 2007, Barriada-Pereira described it as a satisfactory method for determining 21 OCPs in lettuce, tomato, spinach, potato, tomato and bean⁵⁴.

4. Solid phase micro extraction (SPME)

Solid phase micro extraction, one of the newest extraction techniques, is widely used in the pesticide residues analysis in samples of different origin, due to the fact that purification and concentration of the sample extract (analytes of interest) are running simultaneously. The basic part of the SPME system is SPME syringe that visually resembles on the chromatographic, except for the fact that it contains a 1 cm long fiber located within a syringe needle, which is made of an appropriate polymer deposited on the holder of fused silica. Micro extraction process is based on the redistribution of analytes between micro extraction fiber and sample matrix, i.e. on the selective

sorption of target analytes in the active layer of the fiber and direct desorption in the chromatograph injector (thermal in the case of the GC, i.e. by solvent elution in the case of LC - liquid chromatography). The basic principle of analytes micro extraction from the solution is shown in Figure.

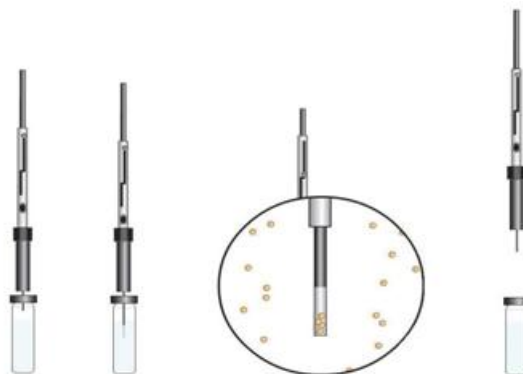


Figure 1: Procedure for micro extraction of analytes from solution

Before the analysis, the fiber is drawn into a metal tube of the SPME syringe. After breaking through the vial septum in which a certain sample amount was previously inserted, the fiber is pulled out from the syringe i.e. it is exposed to the sample by lowering the syringe plunger. After specific time, the fiber with the sorbed analytes is drawn into the needle, which is then pulled out from the vial. Analytes desorption from the fiber is performed by introducing a SPME syringe needle into the injector of the chromatographic system. SPME is an equilibrium technique, where analytes are distributed between the three phases: sample, gas phase and fiber. The fiber does not extract all analytes present in the sample, but by the proper calibration, this technique can be used for successful quantification⁵⁵. The amount of analytes that would be adsorbed on the fiber will depend on the thickness and polarity of the active fiber layer, sampling mode (direct sampling – micro extraction from solution, »DM-SPME« and headspace sampling – micro extraction from gas phase, »HS-SPME«), the nature of the sample and the analyte (analyte polarity, its molecular weight, pH value, nature of matrix), the mode and speed of the sample mixing, the SPME duration, the temperature at which it is performed, and so on.

Dual-layer solid-phase extraction, a primary–secondary amine (PSA) in combination with graphitized carbon black (GCB), was evaluated for sample cleanup during multi residue pesticide screening of agricultural and food products. The determination was made by GC-MSD. The retention of fatty acids by the PSA sorbent was quantified and the effect of the elution solvent on the retention of fatty acid on the SPE cartridge was evaluated. The use of stronger elution solvents to elute certain pesticides from graphitized carbon was shown to interfere with the capacity of PSA to bind fatty acids.

The proposed method was tested using GCB/PSA dual-layer SPE to clean-up food matrices (milk, orange juice spinach and bacon) and to simultaneously screen multiple fortified pesticides with a wide range of physico-chemical properties. With a few exceptions, pesticide recoveries were between 85 and 110%, and sample-to-sample differences of less than 5% were achieved, demonstrating the versatile suitability of the dual-layer SPE to sample clean-up. The research results indicate that the most often used SPME fibers in the pesticide residues analysis (PDMS and PA) are a good choice for determination of: phenyl urea in carrot, onion and potato⁵⁶. OCPs in soils and OPPs in strawberries and cherries⁵⁷⁻⁵⁸ and samples of banana, apple, grapefruit, orange, lemon, kiwi, pineapple, pear, peach, apricot, grapes, melon, lotus, plum, mango, cherry and coconut.

5. Solid phase extraction (SPE)

SPE is one of the most commonly used sorbent techniques in analyzing pesticide residues. This method is based on the omission of extracts containing target analytes through a column filled with the appropriate sorbent (which was previously conditioned by an appropriate solvent or solvent mixture), or passing of an appropriate solvent through the SPE column to which a suitable amount of sample was previously added. Using selective solvents, first the coextractants from the SPE column can be successfully eluted, and then the target analytes (Figure 1, A), or the elution of analytes can be direct, where undesirable coextractants derived from the sample matrix remain in the SPE column (Figure 1, B). Compared with the traditional methods, SPE has many attractive features. It is easy to operate, costs less, it has been automated and uses small amounts of solvent. SPE is the multifunctional techniques, since the purification and the concentration occur in the same step. Unfortunately, SPE has certain limitations, primarily related to lower yields (recovery), i.e. slightly lower sensitivity, in situations where there is "clogging" of the SPE column (blocking of the sorption centers by solid and oily components originating from the sample).

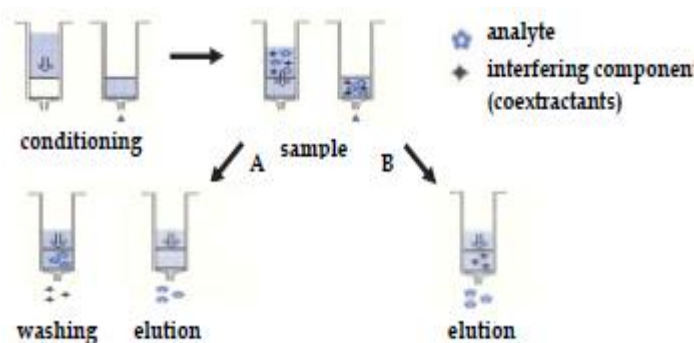


Figure 2

Sample clean-up

Matrix constituents can be co-extracted and later co-eluted with analysed components and can consequently interfere with analyte identification and quantification. Moreover, co-extracted compounds, especially lipids, tend to adsorb in GC systems such as injection port and column, resulting in poor chromatographic performance. A thorough clean-up minimizes such matrix issues, improves sensitivity, permits more consistent and repeatable results, and extends the capillary column lifetime; Several approaches have been attempted to eliminate co extracted interference from extracts, including SPE, d-SPE, GPC and ultra-filtration.

6. Dispersive Solid-phase Extraction (d-SPE)

The amount of co-extractants obtained after the extraction process selected for pesticide determination is a relevant parameter in routine laboratories not only because it can affect the performance of the method but also for the maintenance of the analytical equipment⁵⁹. The complexity of food matrices decrease the life time of chromatographic columns and can even cause problems in the ionization and detection systems of the analytical instrument. It is therefore necessary to select a methodology that allows the analysis of the largest number of analytes but not disregarding this factor (Pareja et al., 2011). During the QuEChERS development, (Anastassiades et al., 2003; Ma et al., 2010) proposed a powerful clean-up method, called dispersive solid phase extraction (d-SPE), which is based on a clean-up with primary secondary amine (PSA)⁶⁰. PSA is a good adsorbent for organic acids, pigments, and other polar impurities from the samples. So it shows great beneficial potentiality in detection of pesticides residues in food stuffs. Dispersive-SPE is a clean-up technique that involves mixing sorbent with a sample that has been pre-extracted with acetonitrile solvent. It is typically part of the QuEChERS method where it follows the bi-polarity extraction step. The appropriate sorbent adsorbs matrix co-extractives on to its surface, leaving analytes of interest in the solvent. MgSO₄ is added to provide additional clean-up by removing residual H₂O and some other compounds via chelation. Afterwards, the mixture is centrifuged and the resulting supernatant can be analyzed directly or can be subjected to a concentration and/or solvent exchange step if necessary.

3. Gel Permeation Chromatography (GPC)

GPC is a method based in the principle size exclusion. This mode of purification is widely used in the area of pesticide residue analysis, which is considered a good technique for the separation of low molecular mass compounds (up to 400 μ m) such as pesticides from high molecular mass compounds such as lipids (600 to 1500 μ m). GPC systems comprises a LC pump a fraction collector and a detector (optional). The columns are made from polymeric porous microspheres, which enables the separation of compounds according to their molecular weights. Using this principle, pesticide fraction is separated from the high molecular weight lipids fractions⁶¹⁻⁶². In order to reach a higher sample throughput, replaced slow GPC purification with high-capacity disposable silica (HCDS) columns containing 28 g of acidic, 16 g of basic, and 6 g of neutral silica; this allowed up to 4 g of lipids for each sample to be retained.

The HCDS column is added to the classic set of columns and is the first one in contact with the sample. Such a column system has found application in purifying extracts from samples characterized by a high lipid content, e.g., poultry, fish and eggs (Beyer and Biziuk). A clean-up step by GPC was applied to remove fat and other matrix compounds from meat samples from chicken, pork and lamb. GPC was applied as a non destructive and semiautomatic clean-up method to determine residues of 109 pesticides (including isomers) in the samples of pork, beef and chicken and fish matrices by Wu et al. In this study, 300 mm x 10 mm i.d glass columns packed with Bio-Beads S-X3 was used. The flow rate was 5 mL min⁻¹ and the first fraction from 0 to 8 min of the eluant (about 40 mL) contained the lipids and was discarded. The samples were analysed with GC-MS in the selected ion monitoring mode (SIM) using one target and two qualitative ions for each analyte.

4. Matrix solid phase dispersion (MSPD)

Matrix solid-phase dispersion (MSPD) is a new SPE-based extraction and clean-up technique developed for pesticide multi-residue analysis. The MSPD method is based on the homogenization of a viscous, solid or semi-solid sample with an abrasive solid support material in a glass mortar, in order to perform the complete disruption and dispersal of the sample. After blending, the sample is transferred into a column and analytes are eluted with appropriate solvent. Complete disruption of the sample and its dispersion over the support surface greatly enhance surface area for the sample extraction. Furthermore, interferences are retained on the adsorbent and in that way, extraction and clean-up are performed simultaneously, reducing the analysis time and the amount of solvent used. Reversed-phase materials such as C8 and C18-bonded silica are the most commonly used adsorbents, because their lipophilic properties enable good disruption, dispersion and retention of lipophilic species⁶³⁻⁶⁴. Several methods based on use of these adsorbents have been used for determination of several pesticides classes including OPPs and OCPs in tomato, lettuce, orange, lemon, grapefruit, pear and plum⁶⁵. Besides C8 and C18-bonded silica, florisil has been successfully used for determination of OCPs in tomato juice. lemon, orange, apple, mango, grapes, banana, carrot, potato, onion, cucumber, tomato, cabbage, spinach and wheat⁶⁶⁻⁶⁷.

5. Conclusion

The sample extraction step, which accounts for about two-thirds of the total analysis time, is still the weakest link and the time-determining step in the whole analytical procedure. It is also the primary cause of errors and discrepancy between laboratories. However, in the recent past, improvements in the sample preparation techniques for different environmental samples have led to modification of the existing methods and development of new techniques, in order to save time and reduce use of chemicals and thus improve the overall performance of analytical process. As a result, several rapid, low cost, environmentally friendly, and readily automated methods of extraction are now available. Besides, because of the complexity of the matrices, extraction is usually followed by very specific clean-up procedures to achieve accurate sample quantification, so the new methods are modified in order to achieve a compromise between cost, selectivity, and sensitivity. Reduced solvent methods, including supercritical fluid extraction (SFE), solid phase extraction (SPE), solid phase micro extraction (SPME), microwave assisted extraction (MAE), accelerated solvent extraction (ASE), QuEChERS and matrix solid phase dispersion (MSPD) have grown in their maturity, which increased application of these techniques in pesticide analysis of plant and soil matrices. Although the composition of environmental matrices such as plants and soil varies from place to place, which requires application of different approaches and strategies, the development of a uniform procedure is highly encouraged. Future developments in all areas of analytical sample preparation are expected to continue to be application-driven in a quest for improved recovery, higher sample throughput, and reduced consumption of organic solvent with capability to provide accurate results.

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