A short review of sample preparation methods for the pesticide residue analysis in fatty samples

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Abstract. The Arctic region is one of the most vulnerable regions in terms of ecology. Transboundary transfers of toxic substances such as pesticides and polychlorobiphenyls (PCBs) can adversely affect its ecosystem. These substances can be preserved in environmental objects for decades. The danger of pesticides is associated with their high resistance to biodegradation and high migration ability [Waid, 1987, Moiseenko, 2009, AMAP 2015]. Low average annual temperatures in the Arctic region also contribute to their long decomposition. Pesticides and PCBs through food chains can get into the human body. In addition, many organochlorine pesticides and PCBs are highly toxic and have a pronounced effect on public health (Borlakoglu, Haegele, 1991, AMAP, 2015).The main problem in pesticides analysis is the large amounts of interfering substances which can be co-extracted with them, such as lipids. They can lead to experimental errors and analytical instruments damage. Thus, extensive sample preparation is often required for the pesticide residue analysis for the effective extraction of the analytes and removal of the interferences. Typical sample preparation steps include the sampling/homogenization, extraction, and clean-up procedure. The methods include: liquid-liquid extraction (LLE), supercritical-fluid extraction (SFE), accelerated solvent extraction (ASE), microwave-assisted extraction (MAE), gel permeation chromatography (GPC), solid-phase extraction (SPE), QuEChERS.

1. Introduction

The Arctic region is one of the most vulnerable regions in terms of ecology. Transboundary transfers of toxic substances such as pesticides and polychlorobiphenyls (PCBs) can adversely affect its ecosystem. These substances can be preserved in environmental objects for decades. The danger of pesticides is associated with their high resistance to biodegradation and high migration ability (Waid, 1987, Moiseenko, 2009, AMAP 2015). Low average annual temperatures in the Arctic region also contribute to their long decomposition. Pesticides and PCBs through food chains can get into the human body. In addition, many organochlorine pesticides and PCBs are highly toxic and have a pronounced effect on public health.

Pesticides are one of the most toxic substances in the environment and consequently represent a risk for ecosystems and human health [M. Furio, F. Bernardes, M. Pazin, L.C. Pereira, D. Junqueira Dorta. Impact of Pesticides on Environmental and Human Health, Toxicology Studies - Cells, Drugs and Environment InTech (2015)].

It is very important to keep pesticide residues in the environment low. Also, it is necessary to control pesticide residues in food and feed for public health reasons. This has been achieved through the establishment of maximum residue levels (MRL), i.e., the highest pesticide levels legally tolerated after their correct application in food products [Regulation No. 396/2005 of the European Parliament

and of the Council of February 23, 2005 on Maximum Residue Levels of Pesticides in or on Food and Feed of Plant and Animal Origin and Amending Council Directive 91/414/EEC].

Determination of pesticide residues in biota or in any other high fat content food sample, expected to contain low ng/g levels, requires the development of advanced multi-residue analytical methods with high sensitivity and selectivity instrumental technologies, such as gas chromatography (GC) or liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) [Y. PicóPesticides and Herbicides: Residue Determination. Encyclopedia of Food and Health; J.J. Villaverde, B. Sevilla-Morán, C. López-Goti, J.L. Alonso-Prados, P. Sandín-España. Trends in the analysis of pesticide residues to fulfil the European Regulation (EC) No.1107/2009].

2. Pesticides extraction methods

2.1. Traditional extraction methods

These extraction methods are the oldest and the most common ones for pesticides analysis in plant, soil and food samples [1].

The homogenized solid or liquid samples are repeatedly extracted with an immiscible organic solvent, and the extracts are then centrifuged, concentrated and purified before the final analysis.

The extraction efficiency of analytes depends mainly on the equilibrium distribution/partition coefficient between the donor phase and the acceptor phase, which requires matching the polarities of the extraction solvents and analytes according to the similarity principle [2].

The main disadvantages of traditional sample preparation methods are as follows: they are laborious, time consuming, expensive, require large amounts of organic solvents, and include many steps that may lead to loss of some analyte quantity.

So, simpler and faster sample preparation methods have been introduced for the analysis of pesticide residues.

2.2. Microwave-assisted extraction (MAE)

Microwave-assisted extraction is a fast extraction method, it was applied for the first time for the extraction of organic pollutants in 1986 [3]. It was successfully used in determination of pesticides in food matrices [4], [5], [6], [7] and was accepted as US EPA method 3546.

The principle of microwave-assisted extraction is based on the usage of microwave energy. Compounds can be extracted more selectively and rapidly, with similar or better recovery compared to traditional extraction methods. High efficiency is the result of the matrix macrostructure destruction [8].

This is an automated method where a sample (1 – 30 g) is put into the vessel with a polar and non-polar solvent and a stir bar. Extraction is performed with elevated pressure and temperature for 30 minutes. However, excessive power can lead to degradation of pesticides and a decrease in recoveries [9].

The main advantages of MAE are full automation, low required temperature, high extraction efficiency and the possibility of simultaneous extraction of various types of analytes. This method needs additional clean-up because it has also co-extracted interfering compounds, and it also has poor extraction for non-polar pesticides [2].

2.3. Supercritical-fluid extraction (SFE)

Another fast extraction method is SFE. This method uses supercritical fluids as extractants for target analytes from solid samples [10].

Supercritical fluids are different from distinct liquid and gas phases in their physiochemical properties. They behave like gases, although they have the density of liquids and, as a result, high diffusivity, low viscosity, good penetration capability and adjustable density. They can diffuse into the solid matrix and dissolve the analytes [11].

Different studies reveal that carbon dioxide (CO2) is the most commonly used supercritical fluid in the pesticide residue analysis, because it has moderate critical temperature (31°C), low critical pressure (73 kPa), is non-flammable, has low toxicity, can be easily evaporated from the extracts and is accessible in a high degree of purity [12].

SFE steps:

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

One of the biggest advantages of supercritical-fluid extraction is that it can give clean extracts with low co-extractives, which is important for complex matrices analysis. Compared with traditional extraction methods, SFE has lower solvent consumption, less extraction time and better efficiency. Especially, supercritical fluids with a low critical temperature can be employed for the extraction of thermally unstable analytes [13]. The non-polar 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. For the extraction of moderately polar or polar pesticide residues, it is necessary to add organic solvent modifiers.

2.4. Accelerated solvent extraction (ASE)

Accelerated solvent extraction (ASE), also known as pressurized liquid extraction (PLE) or pressurized fluid extraction (PFE) or pressurized solvent extraction (PSE), is a method that was introduced by Dionex corporation in 1995 [14] and is one of the most widely used extraction methods of solid and semi-solid samples [15]. This method uses elevated temperature (40 – 200°C) and elevated pressure (up to 20 MPa) to keep solvents in a liquid state. The temperature is increased above the boiling points. Due to increased temperature and pressure, the solubility of analytes is increased, whereas the viscosity of the solvent is reduced and, therefore, the diffusion of the analyte into the solvent is improved. It increases the extraction efficiency of analytes from their matrix, and reduces the extraction time [16].

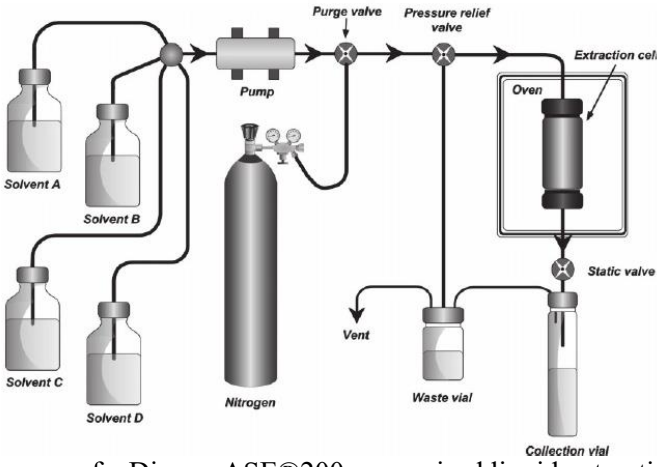

Figure 1. Diagram of a Dionex ASE®200 pressurised liquid extraction system.

Prior to the extraction, a sample is placed into a stainless steel extraction cell. Different drying materials can be added to reduce moisture and dispersion in order to increase the permeation of the solvents into the sample matrices [2]. A desiccant, such as sodium sulphate [17], diatomaceous earth [15], cellulose, extrelut 20 [18], silica [19] or acidic alumina [20] can be added directly to the extraction cell to prevent the aggregation of sample particles to yield efficient extraction.

Then the extraction cell is placed onto a carousel and automatically drawn into the oven and filled with the solvent. Next the sample can be exposed to the solvent for a predetermined amount of time, which is referred to as a static extraction. Once the static extraction is complete, the system pumps fresh solvent through the cell and pathway and purges with nitrogen.

Before analysis the extraction conditions must be optimized. The type of the solvent is very important. Apart from the used type of the solvent, the main parameters, which influence ASE efficiency, are extraction temperature and time [21].

ASE is an automated method with higher reproducibility over traditional methods. It can be used with a range of sample sizes (1–100 g). While the traditional methods take hours for extraction, ASE takes 15- 30 minutes; instead of hundreds of milliliters of solvent, ASE requires 10 - 30 milliliters depending on the application.

ASE has been successfully used for pesticides determination in samples of different origin [22]. ASE is widely used by government agencies and laboratories worldwide. Most of the POPs (pesticides and herbicides, PAHs and semi-volatile compounds of PCBs) are determined by U.S. EPA method 3545A.

The main advantages of this method are: automation, high extraction efficiency, good selectivity, improved safety and good environmental compatibility. However, PLE demands specific instrumentation and a high extraction temperature, which may result in the degradation of thermally labile compounds [2].

3. Pesticides clean-up methods

3.1. Gel permeation chromatography (GPC)

GPC is a method based on the principle of size exclusion. This efficient clean-up method was for the first time used in 1970s for the extraction and clean-up of pesticides [23].

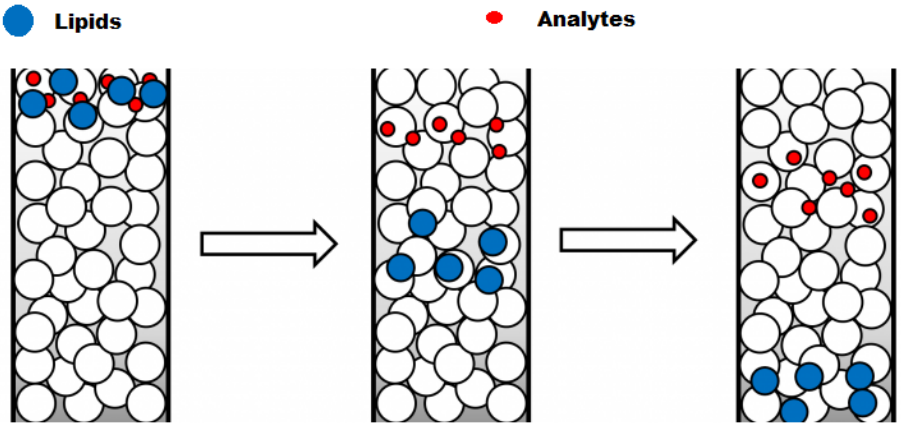

Figure 2. Size exclusion in the column.

The GPC separation mechanism is based on the molecular size where large molecules are eluted from the gel, followed by smaller molecules. The lipid molecules are too large to pass through the pores of the polymer and are eluted first from the column in the mobile phase.

GPC is a universal clean-up method for multi-residue pesticide analysis in a complex matrix: animal origin [18], animal liver [24], animal tissues [25]. Also, the further clean-up step can be held after GPC, for example SPE [26].

Different mobile phases can be used for the GPC clean-up: ethyl acetate - cyclohexane [27], acetone-cyclohexane and hexane-ethyl acetate [26].

GPC is one of the best clean-up methods for samples with high fat concentration, especially for biological samples. However, GPC requires expensive special equipment, which limits method

popularization. Furthermore, the analysis time and experimental cost, such as extraction solvent consumption and the gel column need to be reduced.

3.2. Solid phase extraction (SPE)

SPE is one of the most commonly used sorbent techniques in analyzing pesticide residues, first introduced in the mid-1970s [28].

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).

It is important to activate the sorbent. If the sorbent is not adequately conditioned, poor reproducibility and analyte recoveries may be obtained. Enough time for maximum interaction between sorbents and analytes should pass.

SPE is a fast and simple method, requiring small solvent volumes and common experimental equipment.

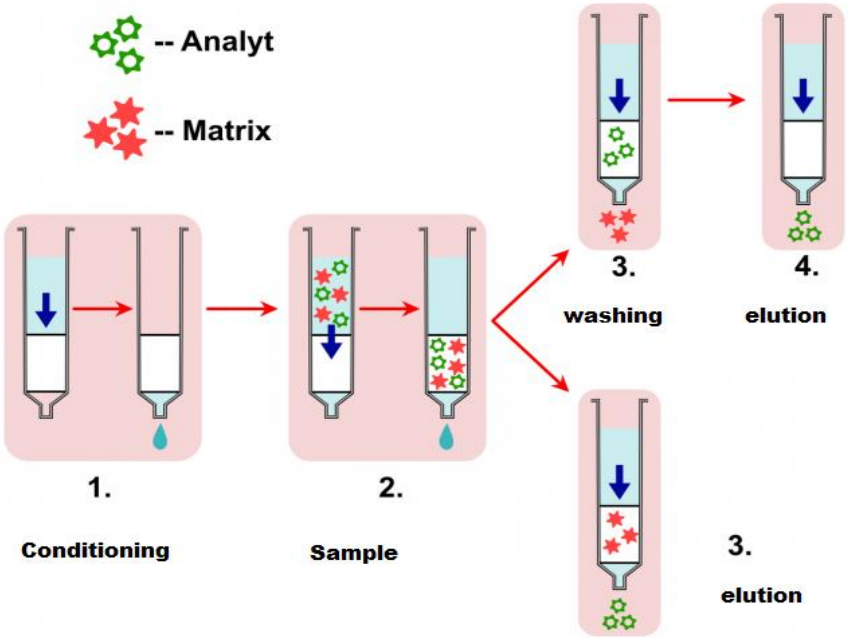

Figure 3. Solid phase extraction steps.

The most commonly used SPE of sorbents in pesticide residues determination are: reverse-phase octadecyl (C18), normal-phase aminopropyl (-NH2) and primary-secondary amine (PSA), anion-exchanger three-methyl ammonium (SAX) and adsorbents, such as graphitized carbon black (GCB). Normal-phase sorbents, such as florisil (MgSiO3), aluminum oxide (Al2O3) and silica (SiO2), are usually used in combination with the previously mentioned sorbents.

The SPE cartridge should be chosen depending on the physicochemical properties of pesticides that are searched for in a particular sample, and the nature of the sample matrix.

There are different combinations of SPE such as dual-layer GCB-PSA for the removal of lipids from food matrices [29], C18 combined with PSA [30], GCB with PSA and other.

SPE is simple, fast and more convenient than traditional extraction methods, it uses less solvent and is easier to automate. When compared to SFE, ASE and MAE, SPE usually can complete the whole sample preparation without any further treatment and provide the subsequent clean-up procedure of these extraction methods. SPE clean-up is cheaper than the GPC and other methods.

3.3. QuEChERS extraction and clean-up

Another popular sample preparation method is QuEChERS. It was introduced in 2003 [31]. This method is based on the micro-scale extraction using the following components: acetonitrile, the water absorption with salts (MgSO4 and NaCl) to aid partitioning of the analytes from the aqueous to the organic layers and buffers help to control the pH-protecting sensitive analytes, and the clean-up step of dispersive-solid phase extraction (d-SPE) employing primary-secondary amine (PSA) and C18 adsorbent.

Different versions of QuEChERS use different dispersive SPE sorbents. Typically, PSA is mixed with C18 for fatty samples, and with gravitons carbon black GCB for foods with high levels of chlorophyll or carotenoids. Acetonitrile is the most widely used extraction solvent for the QuEChERS procedure because it gives higher recoveries and less interference than other solvents, such as acetone and methanol [32].

There are several QuEChERS methods variantions: original unbuffered QuEChERS, AOAC Official Method 2007.01 using acetate buffering and European Committee for Standardization Standard Method EN 15662 calling for citrate buffering [32]. The next step after the extraction of acetonitrile in QuEChERS procedure is a d-SPE clean-up step with PSA adsorbent [19] and C18 to clean extract from fatty acids and other organic acids and lipophilic coextracts.

There are a lot of modified versions of QuEChERS method. QuEChERS is a simple and fast procedure, has lower organic solvent consumption compared with traditional methods.

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