Detection of Pesticide Residues in Soil, Water, and Food

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Abstract. Pesticide is widely used in modern agriculture for increasing crop production. However, pesticides can contaminate the environment, including water, soil, and food, and cause damage to ecosystems and organism health. Analysis of pesticide residues in soil, water, and food samples aims at detecting presence of pesticides in these environmental samples and providing knowledge for further research and decision making. Common methods used in such analysis serve varying ends. Sample preparation methods like solid phase extraction and microextraction cleans the sample and enriches the analytes of interest. Chromatography, including gas chromatography and liquid chromatography, separates the analytes based on their chemical nature. Detectors like ultraviolet-visible spectra detectors and mass spectrometers analyze the compounds separated by chromatographs. They provide critical information on the analytes and allow for both identification and quantification of the pesticide residues in the sample. The article aims at providing a brief overview of the aforesaid methods in context of pesticide residue analysis. Their basic principles are demonstrated, and their strengths and weaknesses are briefly discussed. Applications of the methods are also presented through a number of published researches using such methods in pesticide analysis.

1. Introduction

Modern agriculture relies heavily on pesticides as a mean to sustain an adequate supply for the growing demands of crops around the world. Pesticide use is critical to increasing yield per unit area. Therefore, numerous kinds of pesticides are developed and widely used for controlling insects, fungi, bacteria, weeds, nematodes, rodents, et cetera. In spite of their benefits, pesticides, especially when used extensively, cause serious harms to the environment and organisms by contaminating soil, water, and food. Many pesticides are toxic to humans, and many cause acute and/or chronic health effects. Because of these potential consequences, reliable methods for analyzing pesticide residues are necessary for properly control pesticide use and protecting the environment and human health.

To this end, numerous techniques and devices have been developed. The first area of analytical methods lies in sample preparation. Due to the complexity of environmental samples, especially soil and food samples, sample preparation is often necessary for enriching and purifying the pesticide analytes. Solid-phase extraction and microextraction (SPE and SPME) are the most widely used techniques. They extract the analytes or remove the impurities by adsorbing certain compounds from the sample solution and making the adsorbents easy to separate from the rest of the sample.

Sample analysis then follow the separation methods. Due to its high sensitivity and selectivity, chromatography is the most frequently used method for separating analytes in a prepared sample. The most common chromatographic techniques used are gas chromatography (GC) and high performance liquid chromatography (HPLC).

However, common detectors provide limited sensitivity and information on the compounds present in the samples. Therefore, mass spectrometry (MS) is developed to better analyze the samples. GC-MS and LC-MS are then established from mass spectrometers coupled with chromatographs. MS identifies
compounds based on their mass-to-charge values, which are species-specific. Recently, ambient MS (AMS) has been developed as a family of MS techniques that allows for efficient analysis under ambient conditions without or with less chromatography. The most commonly applied AMS in pesticide analysis is the direct analysis in real time (DART) method, where a steam of excited nitrogen or helium gas ionizes the analytes for MS detection.

The purpose of this paper is to provide a brief introduction to the techniques used in pesticide residue analysis in environmental samples like water, soil, and food samples. The discussion focuses on further explaining the aforesaid methods, namely SPE and SPME; GC and LC; and MS and AMS along with their applications in actual environmental studies. Further research in the field would yield fruitful advancements in pesticide detection, and fields with deep potential include the combination of qualitative analysis with metabolomics to investigate the influences of harmful pesticides on plant growth or on human body.

2. Sample preparation: solid phase extraction and microextraction

Before being available for fine analysis, most liquid samples require sample preparation. Reasons for the preparations include: (a) to remove impurities, (b) to enrich the concentration of the target substance, and (c) to change the sample matrix (the solvent of the sample solution) to one suitable for sample analysis. While physical methods and liquid-liquid extraction (LLE) are common, more sophisticated methods like SPE and SPME are developed for better accuracy. In the following section, SPE and SPME will be discussed, along with their applications in pesticide analysis.

Solid phase extraction (SPE) was developed as a more effective alternative to LLE. In SPE, the sample is processed by a stationary phase made of material that can engage in bonding interaction to retain the target molecules. When the sample flows through the stationary phase, the target molecules are retained by the stationary phase. Then, the stationary phase is washed with an eluent that desorbs the analyte from the stationary phase by disrupting the interactions between them. SPE proved to be faster, cheaper, and more effective at analyte enrichment than LLE.

Solid Phase Microextraction (SPME) was developed by Belardi and Pawliszyn in 1989 as a micro-scale adaptation of the SPE. SPME is a powerful method that allows for highly sensitive extraction of a dissolved substance. Thanks for their small sizes, SPME extractors can be placed into a capillary column or the sample itself. As in SPE, SPME concentrates the extracts by adsorbing the dissolved substance onto a stationary phase. SPME yields relatively concentrated, pure extracts, which are preferable for chromatography and mass spectroscopy (MS) analysis [1].

2.1 In-tube SPME

The most commonly applied design of SPME is the in-tube SPME method. This class of SPME methods have the sample solution flow through a column packed with special material to extract the analytes. In-tube SPME use packed columns (columns filled with discrete particles) or monolith columns (columns filled by a continuous, porous block) to perform extraction. When the sample flows through the column, the analytes are retained by specific monomers built in the column.

While packed columns were common, polymer monoliths are of particular interest in its application in processing environmental samples. Organic monoliths are synthesized in situ from functional monomers, crosslinkers, free radical initiators, and porogenic monomers [2]. Functional monomers interact with the analyte and retain it; crosslinkers provide structural support for the functional monomers and form the bulk of the monolith; free radical initiators promote polymerization of the former two monomers; porogenic monomers give the monolith its porous structure. Organic monoliths are relatively simple in synthesis and are stable under a wide scope of pH values [2]. They are widely used in analysis of pesticide residue in environmental samples.

2.2 Molecularly imprinted polymer monolith

Monoliths can be chemically modified to increase interaction with the particular analyte, thereby increasing its specificity. One such variant is molecularly imprinted polymer (MIP) monolith. In synthesizing an MIP monolith, a template molecule (often the analyte itself) is added to the mixture for monolith synthesis. The template molecules orient the functional monomers around them with aid from
the crosslinkers. After the synthesis, the template molecules are removed, leaving empty binding sites in the monolith. The binding sites are precise complementaries of the template/analyte molecule, lending the monolith a high level of specificity to the template/analyte molecule. Figure 1 gives a schematic illustration of the mechanism of the MIP.

![Figure 1](image)

Figure 1. Schematic illustration of the mechanism of the MIP [3].

The MIP technique has been applied to analysis of triazine herbicides (including atrazine and propazine) in environmental samples by Turiel et al. The researchers synthesized a MIP monolith inside a silica capillary to analyze triazine compounds in soil and food (potato and pea) samples. They used propazine (an azine) as the template, methacrylic acid as functional monomer, and ethylene glycol dimethacrylate as crosslinker; methanol was used as the eluent. Their samples after SPME were analyzed with the liquid chromatography-ultra violet technique [4].

Du et al. developed a pipette-tip SPME MIP monolith for analyzing levels of dissolved difenoconazole, a fungicide. The monolith is synthesized from methacrylic acid (MAA) as the functional monomer and Ethylene glycol dimethacrylate (EGDMA) as the crosslinker. Their design is shown in Figure 2. The sample is loaded in the syringe and processed by the monolith in the pipette tip as it flows through. Then the eluent is loaded, and the analyte retained in the monolith is eluted [5].

![Figure 2](image)

Figure 2. Scheme of the PT–MIPMME device [5].

2.3 Online and offline SPME

SPME methods can be classified into two major classes based on their coupling with sample analysis devices. Offline SPME is conducted separately from devices of further analysis: the samples prepared are removed from the extractor and then injected into a separate analyzer. In contrast, online SPME is directly coupled to a sample analysis device, including chromatography devices and mass spectrometers; the samples are directly injected into the analyzers after sample preparation.

Compared to offline SPME, online SPME is better automated, making processing a large number of sample viable and efficient. In addition, it provides higher stability and reproducibility.

On the other hand, offline SPME using organic monoliths offers a wider variety of formats, including syringes and pipette tips (the design in [5] by Du et al. discussed in 2.2 is an example). The monoliths
are directly synthesized inside these carriers. Offline SPME is able to process a relatively larger volume of sample, compared to online SPME. In addition, since offline SPME is not directly connected to a sample analyzer, the eluent is not limited by requirements of the analyzer; in online SPME, however, only solvents compatible with the analytical device are applicable.

3. Chromatography

After adequate sample preparation, analytical methods are then applicable to provide key information on the composition of the processed sample and the concentration of certain substances. Chromatography is one of the most widely used methods for sample analysis. Gas and liquid chromatography provide a quantitative analysis with good resolution. This advantage makes them the predominant methodologies of chromatography analysis of environmental samples.

3.1 Gas Chromatography, DFPD, and its application in pesticide analysis

Gas Chromatography was developed by James and Martin in 1952, and has quickly developed to be one of the most common analytical methods in modern laboratories [6]. Gas chromatography enjoys an easy inflow of the mobile phase, since constant pressure and flow can be achieved relatively easily with gases. In addition, the carrier gases are generally cheaper than the liquid solvents used in LC. However, for vaporization, GC requires thermally stable analytes with low boiling points and small molecular masses. GC has wide application in the field of environmental science and effectively analyzes samples from water samples, food samples, and soil samples.

Dual flame photometric detector (DFPD) is commonly applied to GC analytes in pesticide analysis. As its name suggests, it has two streams of flames, the first of which degrades the analytes, and the second produces light emissions from the degraded products. The detector is sensitive to sulfur, phosphorus, and chlorine in the compounds, all three of which are common in pesticides.

Fosu-Mensah et al. applied GC in their study on organophosphorus pesticide residues (chlorpyrifos, diazinon, pirimiphos-methyl) in the environment in Ghana [7]. Samples were collected from Dormaa West District, an agrarian region of Ghana where pesticides are widely used for growing cocoa. The researchers used a silica capillary column produced by BGB Company with nitrogen as carrier gas and VF-1701 ms, a coating designed for analyzing trace residues of pesticides. The heating of the sample was programmed to rise from 70°C to 200°C then to 250°C.

In another study, researchers An and Shin developed a reliable method for determining concentrations of six compounds (cyanazine, uniconazole, difluufenican, vernolate, bromxynil, and asulam), each from a major class of pesticides. They applied GC-ECD and GC-MS as their method of detection. The researchers used an HP-5MS column, using 14% phenyl and 86% dimethylpolysiloxane as the stationary phase. Their method was tested on rice, apples, and soybeans, and the recovery rates and limits of quantification (LOQ) of the six pesticides were determined [8].

3.2 High Performance Liquid Chromatography and its application in pesticide analysis

Liquid chromatography is similar to gas chromatography in principles, with a mobile phase carrying the sample through a stationary phase. Despite the technical complications, HPLC enjoys crucial advantages. Compared to GC, HPLC has less requirements for the analyte molecules. The size limit, thermal stability, and volatility are less demanding in HPLC, since larger molecules can dissolve at moderate temperatures for HPLC.

Because of its wider range of applicable analytes, HPLC is widely applied in analyses of pesticide residues in environmental samples. In their study on pesticide residues in farmland soil samples from the city of Batu, Tea et al. applied HPLC in sample analysis. The researchers evaluated the levels of diazinon and chlorantraniliprole (two insecticides). Factors like eluent flow rate and wavelength recorded in the UV-Vis were adjusted to optimize the HPLC process, with a 60:40 acetonitrile-water solution as the eluent. With no significant peaks corresponding to the two insecticides, the researchers concluded that the samples were free of detectable amounts of residues of the two insecticides [9].

In another research conducted by Salleh et al., HPLC was applied to develop a method of analyzing organophosphorus pesticides in samples treated with online SPME. SFCO₂ (supercritical fluid CO₂) was used as the eluent, and is dissolved along with the analytes in a 80:20 methanol-water solution (the
mobile phase). The pesticides, including bensulide, diazinon, and chlorpyrifos, were analyzed using a UV-Vis detector, and the researchers made optimizations to the SPME and HPLC methods. They concluded that UV-Vis detectors offer a relatively high LOD but could be replaced with a mass spectrometer for better sensitivity. The recovery rate of pesticide analytes were also measured with a river water sample [10].

4. Mass spectrometry

While UV-Vis, FID, and other detectors coupled with chromatographic devices provide methods for identifying compounds in samples, mass spectrometry (MS) offers a more sensitive and informative way to analyze the processed samples. Similar to the other detectors, MS devices are directly connected to the chromatographic columns.

4.1 Principles of mass spectrometry

MS detects charged molecules and measures their mass-to-charge ratio. Therefore, an ionization process is necessary for the detection of electrically neutral substances. The most common process used is the electrospray ionization (ESI) developed by John B. Fenn, who won the 2002 Nobel Prize for this invention [11]. Figure 3 shows the process.

![Figure 3: Electrospray Ionization](image)

**Figure 3.** Electrospray Ionization [12].

4.2 Ambient MS

While standard MS techniques offer good sensitivity, it requires pre-separation of the sample analytes, which can be a costly and inefficient process. Ambient MS (AMS) was developed as a variant of MS that circumvents the requirement and achieves greater efficiency. In AMS, the sample is ionized under room conditions, with no encasement or separation. The mostly used ionizer design of AMS in environmental analysis is the direct analysis in real time (DART) design. The DART uses a stream of excited or charged gas stream (usually helium, argon, or nitrogen) to ionize a pre-treated sample.

4.3 Application of MS and AMS in pesticide analysis

Because of their high sensitivity and precise identification, normal MS and AMS are widely used with GC and HPLC in environmental analysis on pesticides. Suganthi et al. developed a LC-MS protocol for analyzing neonicotinoid insecticides (including acetamiprid, clothianidin, imidacloprid, et cetera) in soil samples. Samples were prepared with the QuEChERS method (a specific form of SPE), and components were separated with LC. The ion fragments produced by each analyte pesticide were predicted, and the limits of detection and quantification (LOD and LOQ) for each pesticide were calculated from a test run using a standard solution. The procedure was tested with five soil samples collected from sugarcane fields and glass houses, and results indicated a quantifiable amount of imidacloprid and clothainidin present [13]. Samarghandi et al. applied GC-MS in their study on various pesticide residues in water sources in Kermanshah Province of Iran. The samples were collected from drinking water sources like springs and wells in cities around the province. The samples were analyzed with GC-MS with nitrogen as the carrier
gas. The MS results indicated the presence of organophosphorus and pyrethroid insecticides, butachlor and glyphosate (herbicides), and difenoconazole (a fungicide) present in some of the samples. Quantified results warned of unhealthy levels of difenoconazole in cities like Kangawar and Songhor [14]. Li et al. applied DART AMS with offline SPE in their research on triazine herbicide analysis. The samples were treated with MIL-101(Cr), a chromium-containing metal-organic framework that adsorbs the analytes. After elution with isopropanol, the enriched analyte solution is collected, and an approximate 2μL sample is collected by dipping a glass capillary into the sample. The capillary is placed between the DART ionizer and the AMS of the ToF design. The researchers optimized the SPE and the AMS processes, and tested their method on water samples collected from Weiming Lake, Kunming Lake, and Deep Jade Pool Park in Beijing, China. Results showed the presence of triazines like gestamine, prometon, and desmetryn in all three water samples [15]. In another study on triazine analysis, Wang et al. applied DART AMS with online polymer monolith SPME. Single-wall carbon nanotubes were added to a polymer monolith made from methacrylic acid (MAA) and ethylene dimethacrylate (EDMA) to improve analyte extraction. The monolith is connected to a syringe and a pinhead, placed between the DART ionizer and the ToF AMS. Upon desorption, the analytes are exposed to the excited nitrogen gas produced by the DART and are ionized for AMS analysis (Figure 4). Compared to traditional MAA-EDMA monoliths, the new monolith with SWNT yields 100% to 300% greater analyte extraction and compensates for the relatively lower sensitivity of DART AMS. The SPME and AMS processes were optimized, and the analysis protocol was tested with lake water samples and orange juice. The researchers detected quantified simazine residues in the lake water samples, and an unquantifiable amount of prometon was found in the orange juice [16].

**Figure 4.** Online coupling of SPME with DART-MS [16].

5. Conclusion
The paper has discussed the principles of the most commonly used methods in pesticide residue analysis: sample preparation with SPE and SPME, analyte separation with GC and HPLC, and component analysis with MS and AMS. Published applications of these techniques are also reviewed, in which calibration curves with strong linear correlation ($R^2>0.99$) and good recovery rates (80%–120% were obtained). While the field is already equipped with sensitive, well documented analytical methods, future research is still valuable. DART-MS has high efficiency in sample analysis, but its current usage limits the sensitivity of the method. Therefore, DART-MS can potentially couple with HPLC to yield efficient and sensitive analysis, as the separating effect of the HPLC can compensate for the limited
precision of DART-MS, with a near ten-fold improvement in detection sensitivity. Furthermore, new chromatographs are a potential field for research. Current chromatographic techniques are limited to a linear column, and future explorations in 2D chromatography can gain value since the 2D variant promises greater separation than its 1D precursor does. In addition, a number of other AMS ion sources besides DART-MS have been developed, and they have potential value in pesticides analysis and environmental monitoring.

6. References
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