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Gas Chromatography

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Introduction

A pesticide is usually defined as any organism or substance that is manufactured for direct or indirect control or prevention of any pest. Pesticides often alter the growth, development or characteristics of insects and plants. Most pesticides are synthetic chemicals that can be classified into six classes, according to their chemical type: organochlorine compounds, organophosphorus compounds, carbamates, phenoxyalkanoic acid derivatives, substituted ureas and triazines. Currently, several hundred pesticides are widely applied to a broad variety of crops to reduce losses from weeds, insects and diseases. Herbicides are employed in agriculture for pre- and post-emergent weed control of corn, wheat, barley and sorghum; they are also used on railways and roadside verges. In general, organochlorine compounds are resistant to hydrolysis, and those that undergo photochemical reaction tend to form compounds with a persistence comparable to, or greater than, their parent compounds. Some organochlorine pesticides have been banned due to their toxicity, persistence and bioaccumulation in environmental matrices. Owing to the environmental impact of pesticides, several priority lists, also called 'black' or 'red' lists, have been published to protect the quality of surface and tap water. Thirty-nine pesticides are listed in priority order in the 76/464 EEC (European Economic Community) Council Directive on pollution caused by certain dangerous substances discharged into the aquatic environment of the community. The US Environmental Protection Agency (EPA) has established drinking water regulations and health advisory levels for individual pesticides.

Since the publication of Rachel Carson's book *Silent Spring* in 1962, many countries have legislated for public health protection. Such regulations have ultimately focused on protecting the general public from pesticide residues. An illegal residue is defined as one that is above US EPA tolerance, one for which the 76/464/EEC Directive on the Quality of Water Intended for Human Consumption sets a maximum admissible concentration (MAC), or one that rises above the detection limit listed in the Department of Food and Agriculture's Multiresidue Pesticide Screen. The surveillance of various bodies of water is one of several important activities that provide information on present pollutant levels and future trends in waters. Monitoring pesticide residues in water is one of the important parameters in suveillance activity and environmental study. Regardless of the timing of the application, most pesticides applied to the soil are only lightly incorporated into the soil. Minimizing the risks of above-ground environmental contamination and reducing the hazard to nontarget organisms are very important for human health. Monitoring of pesticide residues in food of animal origin might also indicate a major source of exposure for humans. Therefore, pesticide residue determination has become a critical part of environmental analysis.

Many kinds of pesticides have been released into the environment, making it impossible to separate all pesticides in a single analysis. Thus, the analytical procedure for monitoring pesticide residues must be fast, easy, applicable to a number of different sample classes with only slight modification, and sufficiently sensitive and selective.

Many analytical procedures for analysing pesticide residues have been proposed in the development of multiclass, multiresidue; single-class, multiresidue; and single-residue methods, for a wide variety of sample types. Most pesticide residue analyses have been performed using multiresidue methods involving solvent extraction of the analytes from the sample matrix. Multidimensional analytical systems combining gas chromatography (GC) and high performance liquid chromatography (HPLC) with multiple detectors give many multiresidue methods. Qualitative and quantitative determination by GC with element-selective detectors and confirmation of results using an ancillary method such as mass spectrometry (MS) is the predominant method in pesticide residue analysis. The accuracy and precision of pesticide analyses depend on both sample preparation and instrumental performance. The chromatographic technique requires efficient isolation and concentration procedures. This study presents information on the analysis of pesticides using GC and introduces methods for sample preparation including various types of extraction techniques and derivatization procedures. The effectiveness of the techniques described are demonstrated by determining pesticide residues in real samples.

Sampling, Extraction, Clean-up and Derivatization

Pesticide residue analysis determines not only the parent compounds, but also their metabolites and degradation products. As many investigations have confirmed, determining pesticide residues in the range below ng mL^{-1} is difficult and extremely complex because of the need to isolate, accurately identify, and measure such minute quantities in large amounts of extraneous material. Gas chromatography is the most widely adopted technique in pesticide residue analysis. Pesticides that are amenable to direct analysis by means of GC should preferably be determined by means of this method because it separates well, is fast, and has available many selective and sensitive detectors. The sample generally cannot be analysed directly by using GC for pesticide residues; extraction is required to isolate the target contaminates from the sample matrix.

Clean-up is also necessary to isolate the target pesticides from interfering co-extractives before injection into a GC column. In pesticide extraction, classical solvent extraction method is the conventional method. Conventional extraction and matrix solid-phase dispersion methods have been used for organochlorine pesticides (OCPs) in fish muscle. Carbon dioxide is used as an extractant in high pressure Soxhlet extraction for determining organochlorine pesticides in olive oil, vegetable and fish tissue samples. Solid-phase extraction is extensively employed for the trace enrichment of pesticide residues from complex matrices. Common adsorbents are charcoal and porous polymers. Charcoal is widely used to extract low relative molecular mass polar pesticides and their metabolites, which are highly retained in water.

The homogeneous structure of polymers results in greater reproducibility in enrichment experiments. The most widely used types of polymers are 2,6-diphenyl-*p*-phenylene oxide (Tenax GC), acrylate polymers (Separon SE, XAD-8), ethylvinylbenzene–divinylbenzene (Porapak Q) and styrene–divinylbenzene copolymers (Polysorbs, Amberlite, XAD-2, PRP-1). The bonded silicas, including C_{18}, C_8, cyano and amino, have been widely applied for the preconcentration of various types of pesticides from water samples. Octadecyl-bonded SPE has been proposed by the US EPA in Method 525.

A wide range of polarities of pesticide residues can be separated from sample coextractants by optimizing the SPE elution solvent. Homogeneous sample pulp, prepared from vegetables and fruits, is adsorbed on the surface of activated Florisil, which is then extracted in a glass column with ethyl acetate or methylene chloride mixed with acetone. Kadenczki found a recovery rate of over 80% for pesticide residues, including carbamate, organochlorine, organophosphate, synthetic pyrethroid, triazine and miscellaneous pesticides. The recovery is also independent of the sample material. Snyder investigated the use of a supercritical fluid extraction method to extract 12 organochlorine and organophosphate pesticides from four soils: sand, clay, top soil and river sediment. The soils were extracted at a pressure of 350 atm and a temperature of 50°C with supercritical CO_2 modified with 3% methanol. The recovery of pesticides was greater than 85% for each of the matrices.

Solid-phase microextraction (SPME) has been introduced by Pawliszyn and his group. The mechanism of SPME is mainly based on adsorbing analytes from aqueous solutions onto a fused silica fibre coated with a polymeric adsorbent. An equilibrium occurs of analyte concentration between the sample and the solid-phase fibre coating. Extracted compounds are then thermally desorbed in the injector of the gas chromatographic system. Sampling in the SPME method can be done rapidly, directly, and without any solvent. Pawliszyn selected a 100 μm poly(dimethylsiloxane) (PDMS) fibre to extract ametryn, parathion, prometryn, simetry and terbutryn pesticides. Different modes of absorption were evaluated included static, magnetic stirring, fibre vibration and flow-through extraction cell methods. Montury has investigated using the SPME technique with 100 μm PDMS, to determine the residues of insecticides and fungicides commonly used for vineyard protection at the level of 20 $\mu g\,L^{-1}$.

Carbamates and some pesticides that have high polarity and low vapour pressure are generally not amenable to direct GC analysis. Derivatization is a convenient means of obtaining better chromatographic separations with increasing volatility and detector sensitivity, particularly with the electron capture detector (ECD). A chemical derivatization technique is also a highly effective procedure for confirming a pesticide residue's identity. By using this approach, the resulting derivatives examined by GC provide specific identification. Dehydrochlorination with KOH or NaOH is used to confirm the residues of DDT and its analogues. Reductive dechlorination with $CrCl_2$ is applied to identify heptachlor, endrin, metabolites of endrin, and some organophosphorus pesticides. For determining carbamate pesticides, silylation and acylation are the most widely applied derivatization procedures. Some halogenated silylating reagents are chloromethyldimethylchlorosilane, bromomethyldimethylchlorosilane, and 1,3-bis(chloromethyltetramethyldisilazane). The acylation reagents are trichloroacetic anhydride, pentafluoropropionic anhydride, trichloroacetyl chloride, heptafluorobutyric anhydride and trifluoroacetic anhydride.

Environmental Applications

The maximum levels of pesticide residue allowed in the European Union (EU) are 0.1 $\mu g\,L^{-1}$ in drinking water and 1–3 $\mu g\,L^{-1}$ in surface water. Pico presented a procedure for analysing water samples using online, solid-phase extraction gas chromatography. A cartridge packed with styrene–divinylbenzene polymer was used in sampling and ethyl acetate was used as the elution solvent. Organophosphorous pesticides and several triazines in tap water were tested using a GC flame photometric (FPD), flame ionization (FID), or nitrogen-phosphorous (NPD) detector. When a 10 mL sample of the tap water was analysed, the detection limit for all pesticides was lower than 0.1 $\mu g\,L^{-1}$. NPD and FPD are very much more sensitive and selective than FID. Hence, the SPE-GC-NPD system has been used widely for the analysis of pesticides, particularly organophosphorus pesticides in surface water samples. Tan compared solvent extraction using hexane and Freon TF (trichlorotrifluoroethane) and solid-phase extraction using octadecyl (C_{18})-bonded porous silica, to determine the presence of organochlorine pesticide residues in water. It was concluded that the recoveries and precision using the SPE method for detecting organochlorine pesticides were poorer than those using the extraction method.

We have developed a methylation post-derivatization method on the fibre following SPME with GC-MS to analyse acidic herbicides from an aqueous sample. The acidic herbicides included 2-(4-chloro-2-methylphenoxy)propionic acid (MCPP), 2-methoxy-3,6-dichlorobenzolic acid (dicamba), 4-chloro-2-methylphenoxyacetic acid (MCPA), 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP), 2,4-dichlorophenoxyacetic acid (2,4-D), 2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2-(*sec*-butyl)-4,6-dinitrophenol (dinoseb), and 2-(2,4-dichlorophenoxy)butyric acid (2,4-DB). A polyacrylate (PA)-coated SPME fibre yielded a higher extraction efficiency than that obtained with PDMS. The selected ion monitoring (SIM) mode in MS was used to quantitatively analyse the sample. **Figure 1** displays the mass chromatogram of the 100 $\mu g\,L^{-1}$ acidic herbicides solution determined in this way.

Detection limits at the level of 10–30 $ng\,L^{-1}$ are achieved for all herbicides by using this technique. Linearity is obtained over a wide range, with pre-

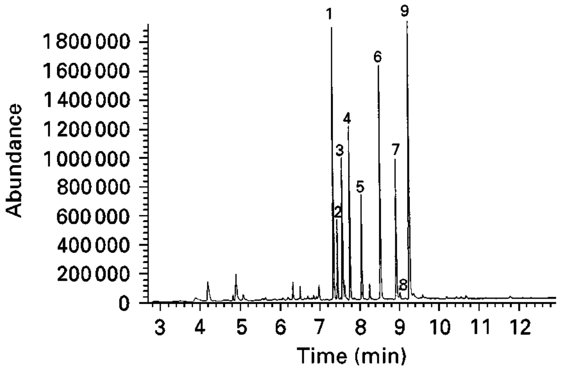

Figure 1 Mass chromatogram of 100 μg L^{-1} acidic herbicides solution by post-derivatization following SPME with PA fibre in GC-MS analysis. Peaks: 1, MCPP; 2, dicamba; 3, MCPA; 4, 2,4-DP; 5, 2,4-D; 6, 2,4,5-TP; 7, 2,4,5-T; 8, dinoseb; 9, 2,4-DB.

cision below 12% relative standard deviation (RSD). In addition, various degradation compounds of acidic herbicides in basic solution including 2,4-dichlorophenol, 2,4,5-trichlorophenol and 4-chloro-3-methylphenol have been detected.

Durand has studied the confirmation of chlorotriazine pesticides, their degradation products, and organophosphorus pesticides in soil samples, using GC-MS with electron impact and positive and negative chemical ionization. Sample pretreatment of soil samples was carried out via Soxhlet extraction for 12 h with methanol. Glass columns filled with 2 g of Florisil effected clean-up. The elution solvent was hexane. Residue levels of chlorotriazine pesticides and organophosphorus pesticides were determined in soil samples at levels from 5 ng g^{-1} to 9 μg g^{-1}. Snyder compared supercritical fluid extraction with classical sonication and Soxhlet extraction for organophosphorus and organochlorine pesticides from soils. Supercritical fluid extraction was found to have the best overall precision over either the Soxhlet or the sonication extractions, and was also less labour-intensive. We have compared different methods of mass spectrometry including GC-MS (EI, CI), GC-MS-MS, and GC-ECD to determine ethion from soil. The largest recoveries (over 96%) were achieved with a 1 : 1 mixture of methanol and acetone as extraction solvent for 3 h in Soxhlet extraction. The limit of detection of ethion for all techniques is below the nanogram level. The mass chromatograph of GC-MS-MS selected reaction monitoring (SRM, m/z 384 → m/z 231) in electron impact ionization mode, of a soil sample containing 0.5 ng g^{-1} ethion, is illustrated in **Figure 2**. The best ethion detection limit (170 pg g^{-1} of soil) was obtained using this technique.

Agricultural Applications

Leoni described a multiresidue method for the quantitative analysis of 28 organophosphorus pesticides in vegetable and animal foods using GC. The pesticides were extracted with different solvents from either fatty or nonfatty foods. Clean-up was carried out on active carbon-celite and on disposable minicolumns of bonded-phase silica, according to fat and pigment content. A wide-bore column (SPB-5) with flame photometric detection, operated in phosphorus mode, was chosen for GC analysis. The recovery tests obtained were 81–85% for apples, whole milk, pasta and eggs, and 89% for olive oil. Schenck described a procedure for screening organochlorine and organophosphorus pesticide residues in eggs using an SPE clean-up and GC detection. The pesticides were extracted from the eggs using acetonitrile. A tandem C_{18} and a Florisil SPE column were used for extract clean-up with recoveries ranging from 80% to 90%. The limit of quantitation was 0.005 μg mL^{-1} for organochlorine pesticides and 0.01 μg mL^{-1} for organophosphorus pesticides. Torres proposed a multiresidue extraction method based on matrix solid-phase dispersion (MSPD) for the extraction and GC screening of 18 insecticides including aldrin, ethion, and dicofol, from oranges. The limits of detection were from 2 to 171 μg kg^{-1}, which is about 10 times lower than the maximum residue level established by the European Community. Sulfuric acid pretreatment for the simultaneous GC screening of organochlorine and organophosphorus pesticides in herbal essential oils was studied by Yoon. A mixed phase of 17% (v/v) of sulfuric acid to organic solvent (hexane/ethyl ether 9 : 1, v/v) and a reaction time of 30 s was used to carry out steam distillation to extract pesticides from herbs; this was followed by GC-FID. The recovery of the studied pesticides was in the range of 75% to 111% for organochlorine pesticides

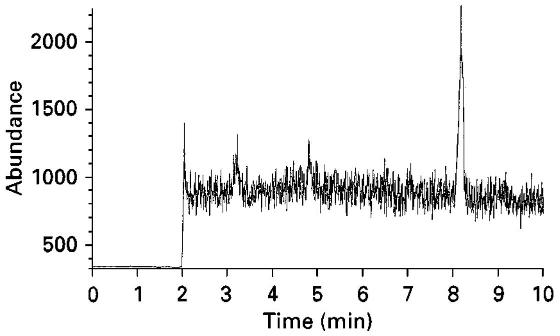

Figure 2 Mass chromatogram of a soil sample containing 0.5 ng g^{-1} ethion produced by using GC-MS-MS selected reaction monitoring (m/z 384 → 231) in EI mode.

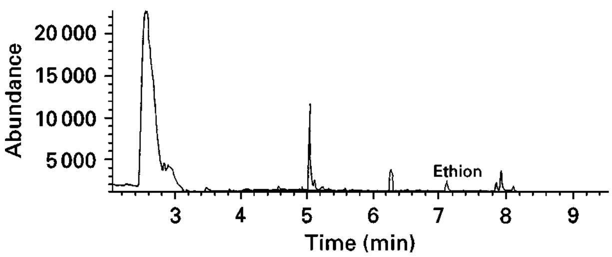

Figure 3 Mass chromatogram of a real orange sample for monitoring ethion in GC-MS analysis. A C_{18} SPE cartridge was used to extract ethion from oranges. A mixed solvent of hexane and dichloromethane, in a ratio of 1 : 1, was used as the elution solvent. A capillary fused silica DB5-MS column was used in the GC-MS analysis.

and 72% to 116% for organophosphorus pesticides. A SPME method was developed and validated by Simpli'cio to determine the level of organophosphorus pesticides, including diazinon, fenthion and triazophos, in fruits and fruit juice using an FPD in phosphorus mode. Limits of detection of the method for fruit and fruit juice matrices were below 2 μg kg^{-1} for all pesticides. Monitoring of ethion residues and its degradation compounds in oranges, using C_{18} SPE cartridges for extraction followed by GC-MS and GC-MS-MS, was studied in our laboratory. **Figure 3** presents the mass chromatogram of a real orange sample obtained using selected ion monitoring (m/z 384 of ethion).

Next, a comparison was made of GC-MS and GC-MS-MS with various ionization modes. The EI mode in GC-MS with selected ion monitoring was the best method, obtaining a 0.96 μg g^{-1} limit of detection. Various extraction methods, including Soxhlet extraction, SPE and SPME, were used to study the extraction of 19 organochlorine pesticides found in Chinese herbal medicines. GC-ECD and GC-MS were evaluated to determine the presence of the pesticides. **Figure 4** presents the analysis of 200 ng g^{-1} of spiked pesticides by using SPME-GC-ECD. The limits of detection for all organochlorine pesticides are below ng g^{-1}.

Biological Applications

Petty investigated a method for the analysis of nine organochlorine pesticides in wildlife urine. The urine samples collected from a single domestic dog were spiked with a standard mixture containing lindane, aldrin, chlordane, DDE, endrin and DDT. The extraction of organochlorine pesticides from urine was performed on a C_{18} SPE column. The method's limits of detection ranged from 1.4 to 2.7 μg L^{-1} when using an ECD.

Lott proposed a multiresidue isolation technique using matrix solid-phase dispersion (MSPD) and GC-ECD for screening 14 organochlorine pesticides in crayfish hepatopancreas. In the MSPD extraction, 0.5 g of the homogenized fish tissue was blended with 2 g of C_{18} silica, and the resulting mixture was then transferred to a 10 mL syringe-barrel column that contained 2 g of activated Florisil. Pesticides were eluted by gravity flow with 8 mL of acetonitrile. Two microlitres of the elute was directly analysed by GC. The results showed that the MSPD technique combined with GC-ECD enabled the successful extraction and determination of the 14 organochlorine pesticides at 125–2000 ng g^{-1} levels.

Diserens has proposed the extraction and clean-up for multiresidue determination of pesticides in lanolin. Lanolin, a waxy material extracted from wool, is one of the raw materials used in cosmetic products. The diatomaceous earth in an Extrelut column was used to adsorb a light petroleum solution containing lanolin. The acetonitrile extract was passed through an SPE cartridge containing a C_{18} column. Organophosphorus pesticides were determined by GC using FPD in the phosphorus mode. Organochlorine pesticides were determined by GC-ECD after extraction and clean-up in a Florisil column. The limits of detection for the various pesticides are about 5 μg kg^{-1} for organochlorine compounds and 50 μg kg^{-1} for organophosphorus pesticides.

We have evaluated GC-MS with selected ion monitoring for determining organochlorine pesticides in fish. Petroleum ether was used to extract pesticide residue from 2 g of fish tissue. The gas chromatogram

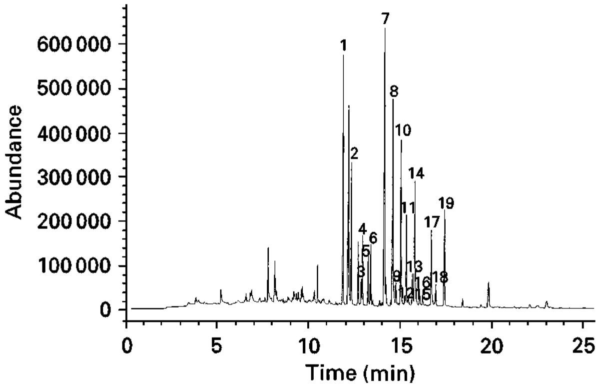

Figure 4 Gas chromatogram of spiked 200 ng g^{-1} 19 organochlorine pesticides in a herbal medicine formula analysed by SPME GC-ECD. Peaks: 1, α-BHC; 2, γ-BHC; 3, β-BHC; 4, heptachlor; 5, δ-BHC; 6, aldrin; 7, heptachlor epoxide; 8, endosulfan I; 9, *p,p′*-DDE; 10, dieldrin; 11, endrin; 12, *o,p′*-DDT; 13, *p,p′*-DDD; 14, endosulfan II; 15, *p,p′*-DDT; 16, endrin aldehyde; 17, endosulfan sulfate; 18, Methoxychlor; 19, endrin ketone.

from a shad fish captured from the Taiwan Strait gave limits of detection for the organochlorine pesticides in fish below ng g^{-1}. The concentration of p,p'-DDE was detected at 90 ng g^{-1}.

Future Prospects

In recent years, new and improved methods and technologies to analyse pesticide residues have rapidly evolved. Analytical methods for most residue pesticides have detection and quantification limits at the low parts per billion to parts per trillion for water analysis, and low parts per million to parts per billion for other samples such as crops, soils and biological matrices. Many preconcentration methods have been developed in clean-up procedures, including exhaustive solvent extraction, automated Soxhlet extraction, microwave-assisted extraction, MSPD extraction, SPE using microcolumns, cartridges, and Empore discs, and SPME. Qualitative and quantitative determination by GC with element-selective detectors, and confirmation of results using MS, continues to be the predominant technique for multiresidue pesticide analysis. MS has been widely used for confirmation of trace pesticide identification and quantitation. Tandem mass spectrometry (MS-MS) is replacing the conventional approaches to confirmation and GC added to the MS-MS will increase the specificity obtained. Now that ion trap mass spectrometry has been introduced, pesticide residue confirmation can be obtained through experiments involving MS-MS or MS^n on compounds of high relative molecular mass, to yield a pyramid of related product ions. With mass spectrometry developments the MS-MS technique will become inexpensive, and eventually the GC-MS-MS technique will be the conventional means of analysing pesticide in most classes of sample matrices.

See also: **II / Chromatography: Gas:** Detectors: Mass Spectrometry; Detectors: Selective. **Extraction:** Analytical Extractions; Solid-Phase Extraction; Solid-Phase Microextraction; Supercritical Fluid Extraction. **III / Herbicides:** Gas Chromatography; Solid-Phase Extraction; Thin-Layer (Planar) Chromatography. **Pesticides:** Extraction from Water; Supercritical Fluid Chromatography; Thin-Layer (Planar) Chromatography.

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Supercritical Fluid Chromatography

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Introduction

Supercritical fluid chromatography (SFC) is a useful tool in the analysis of pesticides and herbicides. Typically, this is done with liquid or gas chromatography and there are basic advantages and disadvantages to each of these method of analysis. For LC, the liquid phase offers the unique advantage of a wide range of solubilities but detection interfaces tend to be the limiting factor. GC has the ability to be more easily interfaced to specific detection capabilities but has significant limitations in the area of solubility; compounds must be able to be readily volatilized. For agriculturally active compounds, the lack of a universal detector for HPLC limits the scope of its applicability to compounds which do not contain UV chromophores. GC is limited to thermally stable volatile compounds since they must move through the