Chapter 3

Contemporary Analytical Techniques for pesticide Residues in Food

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Pesticides may occur in foods in concentrations called trace levels. Trace levels are generally at concentrations of parts per million, that is, one microgram of pesticide per gram of food or less. Measuring such small amounts of pesticides in the presence of enormous amounts of other chemicals that occur naturally in food is a challenge because those chemicals may interfere with measurement, A variety of analytical methods (see ch. 6) are currently used to detect pesticide residues, and all contain certain basic steps in application. The basic steps of an analytical method include the following:

- sample *preparation:* preparation of the sample to be analyzed by chopping, grinding, or separating plant parts;
- *extraction:* removal of a pesticide residue from the sample's other components;
- cleanup *(isolation):* removal of constituents that interfere with the analysis of the pesticide residue of interest, this step includes partitioning and purification;
- *determination–separation:* separation of components, individual pesticides, and sample coextractives according to differential partitioning between a solid or nonvolatile solvent and a liquid or gas carrier that moves through a column (liquid and gas chromatography) or along a coated plate (thin layer chromatography); and
- *determination—detection:* production of a response that measures the amount of the components moving through the column, allowing detection and quantification of each pesticide.

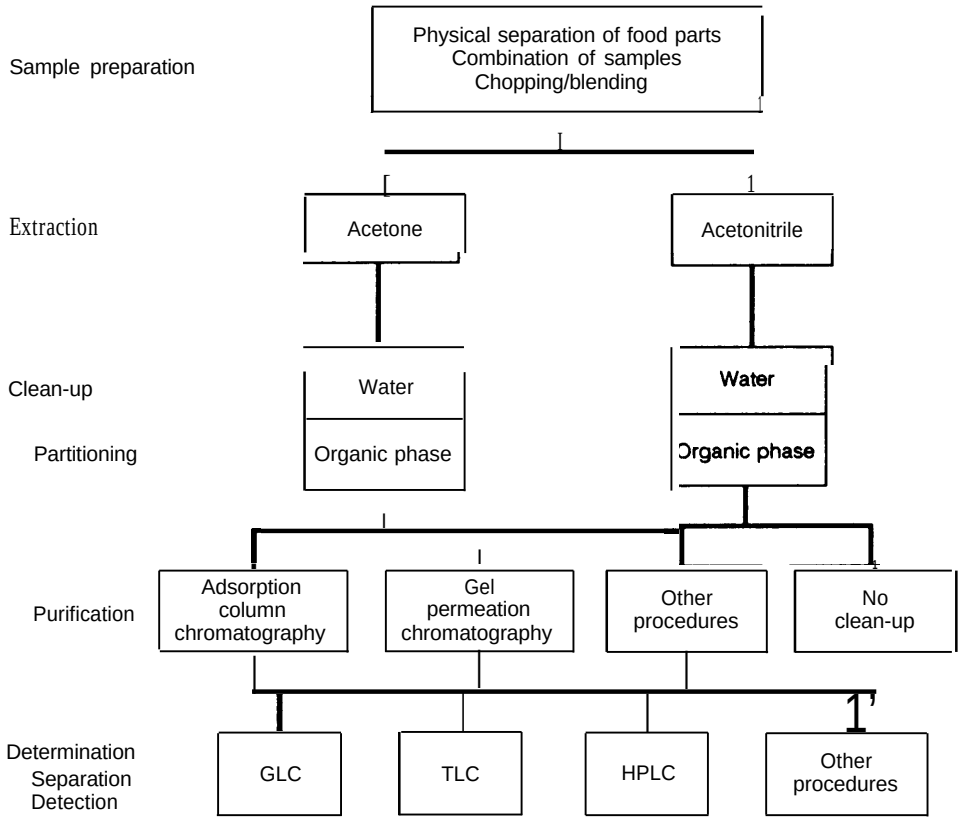
How these steps interact within any particular method is shown in figure 3-I (l). The cleanup step in figure 3-1 has two parts, partitioning and purification, and the extracting solvent is either acetone or acetonitrile.

This chapter describes existing and new technologies currently used to analyze pesticide residues in food and notes how these technologies can improve the analytical steps described above.

SAMPLE PREPARATION

The first step to analyzing a food sample is to chop, grind, or otherwise separate plant or animal parts. The samples must be handled in such a way as to avoid the loss of volatile pesticide residues and to prevent contamination of the sample with other pesticides or interfering chemicals. If only the edible portion of the sample is to be analyzed, it must be removed from non-edible portions. If several different edible portions of a food are analyzed separately, the portions must be separated from each other in each sample. If, however, several samples are combined to provide a representative composite sample from which one or more subsamples are to be taken for analysis, all samples must be handled in an identical manner to avoid inaccurate results in analyzing the subsamples. Chopping or grinding followed by blending and mixing are manipulations designed to produce a homogeneous composite sample from which subsamples can be taken and to disrupt the gross structural components of the food to facilitate extracting pesticides from the sample. Performing this step can be time-consuming and labor intensive.

Figure 3-1. -Simplified Scheme of the Steps in the Analysis of Plant Material for Pesticide Residue



SOURCE: Modified from A Ambrus and H P Thier, "Allocation of Multiresidue Procedures in Pesticide Residue Analysis," *Pure and Applied Chemistry* 56(7): 1035-1062, July 1986

EXTRACTION

Extraction is performed with a solvent to remove the pesticide residue of interest from other components of the sample. In most analytical laboratories, a solvent such as acetone or acetonitrile is used to extract pesticides from 250 grams or less of the food to be analyzed. The solvent is blended with the food, and smaller amounts can be further homogenized using an ultrasound generator. Salts, such as sodium chloride or sodium sulfate, can be added to absorb water. Or additional water can be added, if desired, so that the resulting aqueous solution can be partitioned with a water-immiscible solvent in a subsequent cleanup step.

Extraction times vary from a few minutes to several hours, depending on the pesticide to be analyzed and the sample type. Problems that occur during the extraction process include incomplete recovery and emulsion formation. Incomplete recovery generally can be remedied by selecting a more efficient solvent. Emulsions, the production of a third phase or solvent layer that confuses the partitioning process, can usually be broken down by adding salt to the sample/solvent combination. Residual amounts of the extracting solvent or partitioning solvent should not be allowed to reach the detector if it is an element-specific detector and the sol-

vent contains that specific element. These problems can be solved by proper solvent selection or by removal of the interfering solvent during the cleanup process.

Supercritical fluids (SFs) may provide a new technique for extracting pesticides. SFs are fluids that are more dense than gases but less dense than liquids. SFs are not yet used in regulatory methods to analyze pesticide residues in food but are gaining favor among analytical chemists and food engineers for the ability to extract a wide variety of chemicals from many sample types. SFs have many advantages over conventional solvents. They yield high recoveries of the extracted chemical in a short time, sometimes as quickly as 10 to 30 minutes at temperatures only slightly above ambient (40 to 500 C). Such temperatures prevent thermal decomposition of the extracted chemical. Since some degree of extraction selectivity can be created by choosing an appropriate pressure, this feature may allow the chemist to separate compounds that may interfere during extraction. Removal of the SF from the dissolved chemical in the gas form is easily accomplished (19). The residual chemical of interest can then be dissolved in a conventional solvent and carried through one of the conventional chromatographic analyses (discussed later). Much remains to be done to explore the usefulness of supercritical fluid extraction (SFE) for rapidly and efficiently extracting pesticides from foods.

CLEANUP

Cleanup or isolation removes the constituents that interfere with the analysis of the pesticide residue of interest. Cleanup is usually achieved by a combination of partitioning' and purification, and the latter is usually accomplished by preparative chromatography. The degree of cleanup required is determined by the efficiency with which the partitioning solvent can remove pesticides from the sample extract while leaving behind mutually occurring interferences, Special modification techniques may improve the efficiency of cleanup as well as the efficiency of detection (16).

The preparative chromatography typically used for purification is of the: 1) adsorptive, or 2) gel permeation (or size exclusion) type. Adsorption chromatography is based on the interaction between a chemical dissolved in a solvent and an adsorptive surface. Particles of the chromatographic material are placed in large glass columns (30 cm x 2 cm), the sample is deposited in a solvent on the top of the column and eluted with various types of organic solvents. Separation occurs when the pesticide elutes in fractions different than the sample coextractives. Table 3-1 summarizes the materials that have been used with these two types of preparative chromatographic modes, giving some of their distinguishing features.

Gel permeation (or size exclusion) chromatography is a technique that separates compounds from one another on the basis of differences in molecular size. Preparative-sized columns similar to those used in adsorption chromatography are used, and samples are placed at the top of the column and then eluted with a solvent; larger molecules elute before smaller ones in an ordered fashion. The ordering by size in gel permeation is a result of small holes designed into the particles placed in the column that retard the movement of smaller molecules through the column; such sizing cannot occur on adsorption columns.

The advantages of gel permeation over adsorption chromatography are that no loss of pesticide occurs on the column, either by irreversible adsorption or by chemical reactions. A disadvantage is that a medium-pressure piston-type pump is required to deliver solvent to the column, making a sample injection valve nec-

'Partitioning is the process of distributing the pesticide between two immiscible solvents so that the pesticide will appear in one phase and potential interferences in another, which then can be discarded.

Table 3-1.—Materials Used for the Preparative Chromatography of Pesticide Residues in Food

Florisil

1. A diatomaceous earth adsorbent; retains some lipids preferentially; particularly suited for cleanup of fatty foods.
2. Good for cleanup of nonpolar pesticides, such as the chlorinated hydrocarbons; produces very clean eluants, removes most interferences when eluted with nonpolar solvents.
3. Difficult to use for fruits and vegetables when moderately polar to polar pesticides are present.
4. Prone to vary from batch to batch.
5. Sometimes oxidizes organophosphates with thio-ether linkages; adsorbs some oxons irreversibly.
6. Most widely used material in the United States.

Alumina

1. Basic alumina can be substituted for Florisil for the cleanup of fatty foods.
2. Does not vary from batch to batch as much as Florisil,
3. Will decompose some organophosphates.
4. Does not effectively separate some plant materials from the pesticide.

Silica gel

1. Particularly useful for isolation of certain polar pesticides without losses.
2. Will not adequately separate some plant coextractives from some pesticides.
3. Will separate some organochlorine pesticides from animal fat well enough to permit thin layer chromatography.

Carbon

1. Unlike other absorbents, carbon has different elution characteristics due to its lipophilic nature; absorbs preferentially nonpolar and high molecular weight pesticides.
2. Removes chlorophyll well from vegetables but not waxes.
3. Strongly affected by pretreatment; results in literature often not comparable.
4. Difficult to maintain flow rates in columns.

SOURCE: Office of Technology Assessment, 1988.

Photo credit: Food Safety and Inspection Service Laboratory, Athens, GA

After putting the sample through an alumina packed column, solvent is added to elute the pesticides off of the packing in the column.

essary. The required equipment is more expensive than that used in adsorption chromatography, although such equipment is available in an automated package.

The cleanup step is often a limitation in pesticide residue methods because it generally consumes a large amount of the total analysis time and restricts the number of pesticides that are recovered in some cases, as a result of losses in chromatography, partitioning, and other cleanup steps. New technologies such as solid phase extraction (SPE) (also known as accumulator or concentrator columns) can speed up cleanup as well as extraction. The SPE packing materials or cartridges retain the pesticide when the extract is passed through without retaining potential interferences extracted from the food. The SPE cartridge is a small plastic, open-ended container filled with adsorptive particles of various types and adsorption characteristics. The pesticide is then eluted and carried forward to an appropriate determinative step. Conversely, SPE may be used to cleanup the extract by retaining coextractives and allowing the pesticide to pass through.

SPE technology is particularly attractive for use in pesticide residue analytical methods, since it often eliminates the need for expensive and environmentally sensitive solvents. These cartridges also have the following advantages: batch sample processing capabilities, small size, adaptability to robotic technology, low cost, and ready availability from many sources. SPEs have the disadvantages of being unproven for many pesticides, unable to handle large sample sizes, and generally are ineffective for extracting water-soluble pesticides and metabolites. As more types of absorbents become available, however, the last disadvantage may be remedied. Using this type of cleanup sometimes results in losses of pesticides that cannot then be determined chromatographically, but under such circumstances, cleanup steps should be minimized or eliminated. SPE is be-

ing used by industry and private laboratories but is not yet routinely used by regulatory agencies to a significant extent, SPE cartridges are being used by several FDA laboratories to clean-up extracts before the detection step to protect the column used in high performance liquid chromatography (HPLC).

DETERMINATION-CHROMATOGRAPMK SEPARATION

After a pesticide has been extracted and isolated from the sample, it is further separated from other coextractives, usually by either gas chromatography or liquid chromatography or, less frequently, by thin layer chromatography.

Gas Chromatography (GC) Separations

Historically, gas chromatography has been the dominant technique of separation, with at least 40 years of development and refinement. Most multiresidue methods (MRMs) used by the FDA and USDA and most single residue methods (SRMs) are based on GC.

Separations of pesticides and sample coextractives occur in analytical columns within a gas chromatography; the columns are usually made of glass and are either the type that is packed or the wall-coated, open tubular type known as capillary.

A column filled with particles is called a packed column and has an internal diameter of about 2 millimeters; a column with a thin film on the wall and an internal diameter of about 0.1 millimeter is called a capillary column. Packed columns are typically 2 meters or less in length whereas capillary columns are typically 10 meters and longer, sometimes reaching 50 meters.

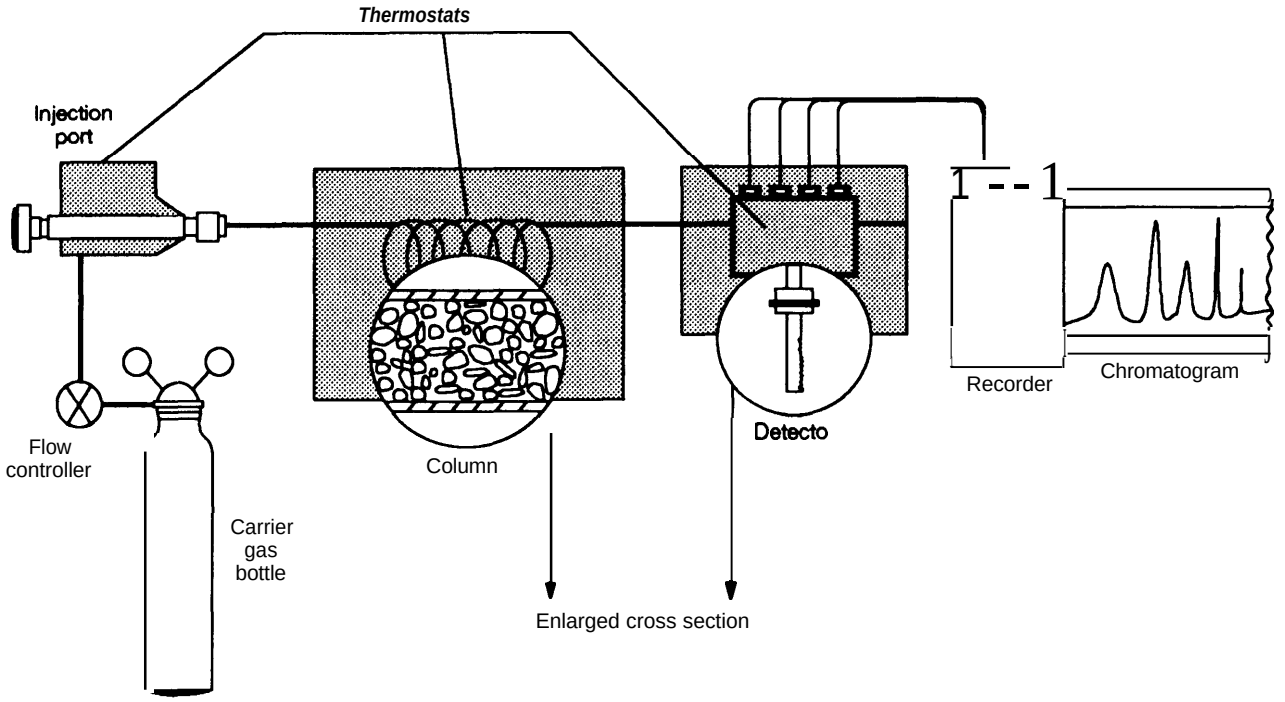
A sample of food extract, about 10 microliters or less and either cleaned up or not, is placed at the beginning of the column where the solvent is flash evaporated along with the pesticide. A gas, called the carrier gas, is continually flowing through the column, moving the pesticide along, which partitions between the particles packing the column or the thin film of involatile liquid on the wall of the column if it is a capillary column. The relative affinity of the pesticide for the particles or the thin film determines when it elutes from the column, at which time it goes through the detector where a response is generated and printed out on a recorder. The continuous trace of such responses is called the chromatogram (figure 3-2), Chromatographic peaks appear on the chromatogram; their position on the chromatogram is called the retention time. Quantifications are performed by measuring the area under the peak and comparing its area to that of varying amounts of analytical standards (figure 3-3).

Historically, the packed column has been used by most pesticide residue analytical chemists. As a result, a vast amount of retention data' exists for pesticides on packed columns. One way of expressing retention data is the use of "relative retention time" (rrt) for a particular pesticide/column combination. The rrt values are then used to identify an unknown by comparing the rrt to that of a standard. Chlorpyrifos is typically used as the standard for chlorinated hydrocarbon and organophosphate pesticides. The lack of rrt data for capillary columns is a constraint to their use.

Until the mid-1970s, capillary chromatography was used *only* when packed columns could not fully resolve the many components in the sample undergoing analysis, Today, the availability of a varied and growing selection of capillary columns has increased their popularity, A conventional capillary chromatogram has been more time-consuming to develop (requiring as much as 30 to 45 minutes) than packed column chromatograms (requiring less than 30 minutes) (3). However, the availability of the

[2]Retention data are retention time (time required to elute a compound from a chromatographic column) and retention volume (volume of carrier gas required to elute a compound from a GC column].

Figure 3-2.-Schematic Diagram of a Gas Chromatographic System



SOURCE: H M McNair and EJ Bonelli, *Basic Gas Chromatography* (Berkeley, CA: Consolidated Printers, 1969)

wide bore capillary column has reduced time. Table 3-2 summarizes retention data for seven pesticides on a packed column and on a wide bore capillary column.

A new generation of hardware gives flexibility in the use of "guard columns, " pieces of deactivated but uncoated fused silica tubing used to guard the analytical column from contamination by deposition of involatile food coextractives. Such guard columns could enable capillary column chromatography of relatively unclean food extracts that heretofore could only have been chromatographed on packed columns (2).

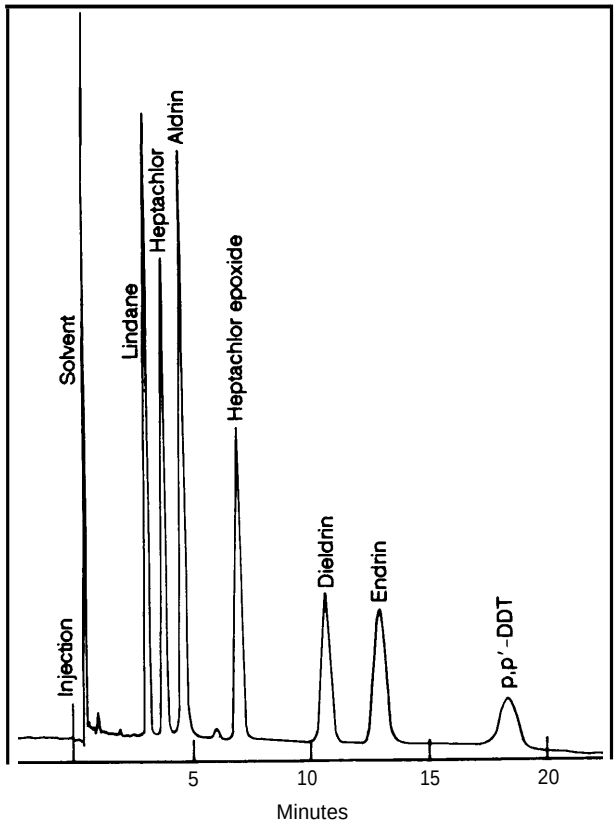
High Performance Liquid Chromatography (HPLC) Separations

HPLC for the analysis of pesticide residues is a fairly recent occurrence, but it is becoming the second most frequently used technique after GC. GC depends upon the volatilization of the pesticide, whereas HPLC is dependent on the ability of the chemical to be dissolved in a suitable solvent.

Separations occur on the analytical column packed with uniformly sized and shaped particles with a liquid film of varying polarities or adsorptive sites. A small volume of sample is deposited on the top of the column, and solvent is pumped through at high pressure. As the solvent moves through the column, the pesticide distributes itself between the particles (stationary phase) and the solvent (mobile phase); the pesticides that have a higher affinity for the stationary phase exit the column last (figure 3-4).

Stationary phases are commercially available that can selectively retain any molecular structure—polar, nonpolar, ionic, or neutral; separations can even be made to occur as a function of molecular size (gel permeation). Chemical derivatizations, the synthesis of a chemical derivative of the pesticide, therefore are not required for separations by HPLC. They are used to label molecules that do not respond to con-

Figure 3-3. -Chromatogram of a Gas Chromatography



During sample analysis, the results of GC and HPLC chromatographic separation and detection steps appear on the chromatogram as peaks. The time it takes a peak to appear is used to identifv the pesticide. The quantity of the pesticide can be determined-by measuring the area under the peak.

SOURCE: Alltech Associates, Inc, Applied Science Labs, State College, PA, 1988

ventional analytical detectors. Such labeling usually enhances the detectability of the molecule. Sometimes labeling is done "post column, " i.e., after elution from the chromatographic column, as for the N-methylcarbamates and carbamoyl oximes (13). Such labeling permits measurements of these classes of pesticides in the presence of other potential interferences as a result of the specificity of the reaction.

HPLC is not as efficient as capillary gas chromatography for separator purposes because the chromatographic peaks are broader. However, HPLC columns are more efficient than packed GC columns when columns of equal

Table 3-2.—Comparison of Retention Data for Seven Chlorinated Hydrocarbon Pesticides on a Packed Column and on a Wide Bore Capillary Column

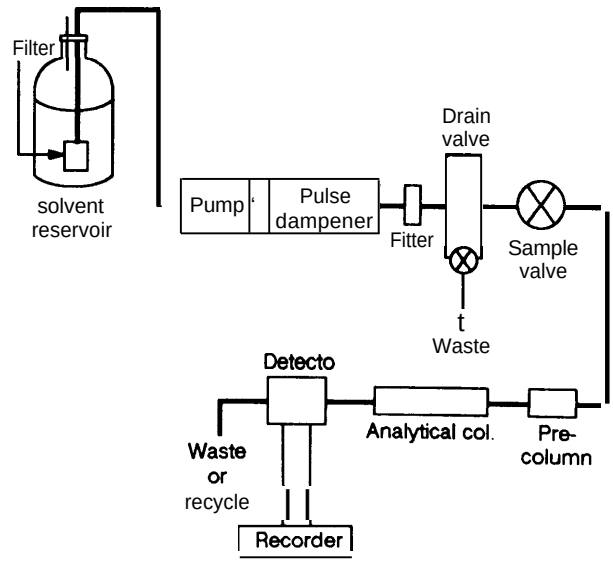
	Retention times (minutes)[b]	
Pesticide[a]	Packed column[c]	Capillary column[d]
Lindane	2.5	0.7
Heptachlor	3.9	1.1
Aldrin	4.8	1.5
Heptachlor epoxide	6.7	1.9
Dieldrin	9.9	2.8
Endrin	12.0	3.2
P,P' DDT	14.1	4.5
Total analysis time[e]	15.0	4.7

[a] Both columns exhibit comparable resolution between peaks, R s > 1.0.
[b] Retention time (time required to elute a compound frOm a chromatographic column) and retention volume (volume of Carrier gas required to elute a compound from a GC column).
[c] Glass column, 1.8 M x 0.4 cm; PT 40/. SE-30 + 6% OV-210 On Gas Chrom Q, 80/100; 2000C; electron capture detector; nitrogen carrier, flow 90 ml/min; 2 x 10⁻ grams for each pesticide.
[d] Open tubular column, 10 M x 0.053 cm; RSL/200, 1.2 microns thick; 200 °C; electron capture detector; nitrogen carrier, flow 5 ml/min, 15 ml/min makeup; unknown amounts of pesticide.
[e] Represents time at which all pesticides have passed through the Column.

GLOSSARY: Gas Chrom Q—a white diatomaceous earth that has been screened, acid and base washed, neutralized, and silanized (support for liquid phase); OV-210—50% trifluoropropyl, methyl silicone (liquid phase of gas chromatographic column packing material); PT—pretested; R=s 1.0—Resolution (the true separation of two consecutive peaks) of greater than 1 second; RSL/200—poly-diphenyldimethylsiloxane (liquid phase); SE-30—methyl silicone gum (liquid phase of gas chromatographic column packing material).

SOURCE: Alltech Associates, Inc. "Catalog #150," Avondale, PA, 1988,

Figure 34.-Schematic Diagram of a High Performance Liquid Chromatographic System



SOURCE: J M Miller, *An Introduction to Liquid Chromatography for the Gas Chromatographer* (Bridgewater, NJ: GOW-MAC Instrument Company)

length are considered. HPLC columns usually last longer because they are not subjected to the extremely high temperatures that GC columns are.

Thin Layer Chromatography (TLC) reparations

This technique is based upon partitioning a pesticide between a solvent and a thin layer of adsorbent, which is usually silica or alumina oxide that has been physically bonded to a glass or plastic plate. Samples are applied in a solvent as spots or bands at one edge of the plate and the plate is then placed in a tank containing a solvent. The solvent migrates up the plate by capillary action, taking the pesticide with it and depositing it at a given distance up the plate. The time required for TLC plate development may range from a few minutes to several hours depending on the pesticide, the solvent, and the adsorbent. Following complete development, the plate is then removed from the tank and the spots or bands left by the migration of the solvent are detected using one of several techniques.

As a separator technique, TLC is much less efficient than either GC or HPLC because the resolution separated by TLC is approximately less than one-tenth of that found using a packed GC column to produce the same separation. Consequently, TLC as a separator technique has largely been replaced by GC and HPLC, On the other hand, interest exists in using TLCS to develop rapid, semiquantitative methods (see ch. 6).

Supercritical Fluid Chromatography (SFC) Separations

SFS may provide a new technique for chromatographic separation in the regulatory analysis of pesticide residues in food. with SFs as the solvent phase, SFC can chromatography chemicals that cannot be handled by gas chromatography because of their involatility or thermal instability. Because the chemical undergoing chromatography diffuses more readily in the SF than in the liquid used for HPLC, the solvent can be pumped at a higher velocity, resulting in shorter analysis times. A fringe benefit is that many detectors designed for GC can also be used in SFC. Detectors that have been shown to be effective are the flame ionization, the nitrogen-phosphorus, and the atomic emission spectrometric as well as the UV absorbance detector.

Extraction and chromatographic separation using SFS was recently demonstrated for the analysis of sulfonylurea herbicides (8). This technique, called SFE/SFC, was capable of producing chromatographic responses from extracts of sand, soil, wheat kernels, whole wheat flour, wheat straw, and from a cell culture medium. No recoveries or concentration levels were given.

Such a coupled extraction and analysis using supercritical fluids warrant further examination as a rapid means of analyzing pesticide residues in foods, if automation in general and robotics in particular can be used for sample insertion into the instrumentation.

DETERMINATION-DETECTION

This final step of analysis produces a response that can be used to measure the amount of pesticides moving in the column. There are numerous types of detectors. These detectors operate under various principles and have the bility, in some cases, to detect only certain classes of chemicals.

Gas Chromatography Detectors

Concurrent with improvements in gas chromatographic column technology have been major improvements in detectors. These improvaments resulted in a growing number of detector types becoming available, increases in detec-

tor sensitivity due to improved design and enhanced electronic stability, and a trend toward detector miniaturization, which makes them more amenable for use in capillary chromatography.

Historically, only five detectors have been used, They are the electron capture detector (EC D), Hall microelectrolytic conductivity detector (HECD), the thermionic detectors (NPD and AFID), and the flame photometric detector. Table 3-3 summarizes their characteristics.

Of these detectors, the ECD was the first to be used for pesticide residue analysis. ECD measures the loss of detector electrical current produced by a sample component containing electron-absorbing molecule(s). Being very sensitive for measuring halogenated pesticides, its value lies in the analysis of chlorinated hydrocarbon pesticides such as aldrin, dieldrin, and DDT. Its sensitivity to such compounds has made ECD attractive for the analysis of polychlorinated biphenyls (PCBs) as well. ECD also responds to portions of organic molecules, other than halogens, which have a large electron affinity, and for that reason the detector sometimes has difficulties analyzing some unclean crop extracts. Recent improvements in related electronics and the incorporation of a high-temperature radioactive source have made the technology less susceptible to fouling from crop coextractives.

The Hall detector can be set to measure chlorine (and other halogens), nitrogen, or sulfur. When set for chlorine, the detector is especially useful for simplifying the detection of halogenated pesticides because nonhalogens are not detected, thus producing a simpler chromatogram to interpret. Similarly, when the detector is set for one mode, it *will* not detect pesticides that require one of the other settings. This detector is more selective than the ECD, but the ECD is more sensitive. In addition, the Hall detector does not need as clean an extract as the ECD, and therefore its use can lead to faster methods by allowing reductions in cleanup. A drawback is that the Hall detector requires more maintenance than the ECD.

Somewhat less sensitive than the ECD, but essentially nonresponsive to nonhalogenated compounds, the Hall electrolytic conductivit$_y$ detector also has improved over the last few years. In fact, it has replaced the ECD in some laboratories where extreme sensitivity is not required. The Hall detector can also be set up for nitrogen and sulfur containing compounds.

Both the NPD and AFID measure the presence of nitrogen and phosphorus atoms in the pesticide, with little response resulting from other types of atoms in the molecules. At this time, the NPD has all but replaced the AFID in most residue laboratories due to its more sim-

Table 3-3.—Gas Chromatography Detectors Used for Pesticide Residue Analysis

Type	Selectivity	Approximate limit of detection	Sample destruction	Reliability	Examples of pesticides detected
Alkali Flame (AFID)	Organic P, N	10-12 g P 10^{-10} g N	Yes	Fair	Triazine herbicides (atrazine)
Electron Capture Detector (ECD)	Electronegative Containing Groups	10^{-13} g Cl/see as lindane	Yes	Fair	Organochlorines (methoxychlor)
Flame Photometric	Organic P, S	10^{-12} g P/see $2x10^{-12}$ g S/see	Yes	Excellent	Organophosphate (malathion)
Hall Electrolytic Conductivity Detector	Organic Cl, S, N	1 -$2x10^{13}$ g CL/see 5-$10x10^{-13}$ g S/see 1-$2x10^{-12}$ g N/see	Yes	Fair	Organochlorlnes (aldrin)
Nitrogen-phosphorus Detector (NPD)	Organic P, N	<$0.2x10^{-12}$ g P/see <$0.4x10^{-12}$ g N/see	Yes	Good	Organophosphates (parathion)
Mass Selective Detector (MSD); Ion Trap Detector (ITD)	everything except carrier	10^{-11} g; MID	Yes	Good	All pesticides

SOURCE Office of Technology Assessment, 1988

ple operation as well as more reproducible responses from individual detectors.

The flame photometric detector measures sulfur or phosphorus. It is a rugged detector, highly stable, and very selective since it does not detect compounds other than organophosphates and those containing sulfur. The flame photometric detector is less sensitive for phosphorus than the NPD and less sensitive for sulfur than the Hall detector. However, it is useful for the analysis of unclean food extracts.

Conventional mass spectrometers (MS) have been used by some pesticide residue laboratories as gas chromatography detectors and to a lesser extent as high performance liquid chromatography detectors. Their cost ($150,000 and higher) has limited their use. MS is normally used when special techniques are necessary to confirm the identity of a particular pesticide, when conventional detectors cannot detect the pesticide, or for unidentified analytical responses (discussed in ch. 6). Usually an MS is set to the multiple ion detection mode in order to gain sensitivity; the alternative would be to obtain full spectral scans on each chromatographic peak, which is always less sensitive. The use of MS is growing, especially with the development of the more portable and less costly mass selective detector (MSD).

The MSD and ion trap detector (ITD) may become more routinely used for pesticide residue analysis as improvements in their computer software are made and their scan parameters become more suitable for chromatography. Both detectors operate on the principle of mass spectrometry. They differ primarily in the manner in which ions are filtered and in the software that is available for controlling the scan parameters and data acquisition. Both can be set to monitor one or more ions during the development of a chromatogram, and both can take full scans (mass spectra) of chromatographic peaks. Consequently, these detectors can be used to acquire quantitative and structural data on chromatographic peaks; both are compatible with capillary columns.

A large and significant difference exists in the way in which selected ions can be monitored during the chromatography, however. Only the MSD can be programmed to change which ions are being monitored during the chromatogram; this allows the instrument to be set so that as suspected pesticides elute from the column, the ions that give the greatest response and are characteristic of the molecule can be sequentially monitored. The ITD does not have this capability. Both detectors have the disadvantage that if nothing is known about the nature of the sample, they cannot be programmed for selected ion monitoring.

High Performance Liquid Chromatography Dectectors

The HPLC detectors used for pesticide residue analysis are the UV absorption, fluorometer, conductivity, and electrochemical. A summary of the characteristics of those HPLC detectors is presented in table 3-4. The fixed wavelength UV absorbance detector is used frequently for trace analysis of pesticides. Many pesticides absorb UV light at the wavelength of mercury discharge (254 nanometers) and can be detected in very small quantities. Unfortunately, many food coextractives do so as well, making this detector nearly useless for trace analysis in foods.

An alternative is the variable wavelength detector, which can be tuned to a wavelength that is absorbed by the pesticide but not by the food coextractives. Several successes have been observed using the variable wavelength detector for “unclean” food extracts, including oxamyl and methomyl on strawberries (15). A newer version of the variable wavelength detector, the photo-diode array detector, is capable of molecular identification for the suspected pesticide because it is capable of taking a complete absorption spectrum on a chromatographic peak during the chromatogram. Recent versions of this detector approach the limits of detection observed for contemporary variable wavelength detectors.

The fluorometer is a highly sensitive HPLC detector for some pesticides. Typically, it is used for pesticides with aromatic molecular structures such as alachlor or paraquat. This detector, however, has limited application to

Table 3.4.—Detectors Used In High Performance Liquid Chromatography Analysis of Pesticide Residues

Type of device	Units	Full-scale sensitivity at + 1 noise	Sensitivity to favorable sample	Temperature sensitivity
UV Absorption	AU[1]	0.001	5×10^{-10} g/ml	Negligible
Fluorometer			10^{-10} g/ml	—
Conductivity	umho[2]	0.05	10^{-8} g/ml	2 % /°c
Amperometric (Electrochemical	A[3]	5×10^{-9}	10^{-10} g/ml	1%/oC

[1] AU = absorbance units
[2] mho = unit of conductivity; 1mho=ohm^{-1}
[3] A = amperes

SOURCE: Office of Technology Assessment, 1988.

the detection of most pesticides—those that do not fluoresce appreciably. Two ways exist to avoid this dilemma: labeling the pesticides with fluorescent molecules before chromatography by HPLC or by forming postcolumn fluorescent derivatives (11, 12). Another recent approach is to form fluorescent molecules from pesticides by photolyzing them in a photoreactor (7) and then measuring their fluorescence.

For compounds having photo-ionizable functional groups, the photoconductivity detector is especially advantageous over UV detectors. It has been well studied and used by FDA and other laboratories for residue analysis. The electrochemical detector is also under study for its potential to improve detection of electroactive functional groups,

Detection Techniques for Thin Layer Chromatography

The spots or bands produced after the development of a thin layer plate are detected using one of several techniques such as visualization under UV light. Another technique uses reagents to produce colors resulting from chemical reaction that is specific for the pesticide/reagent combination. Amounts of pesticide can be judged semiquantitatively by comparison with standards that are developed on the same plate as the unknowns. An extensive review of how this technique can be applied to pesticide residues in foods has been published recently (17).

A popular color reaction used to visualize and quantify pesticides separated by TLC is produced by a cholinesterase enzyme-linked chemical reaction (9, 10, 18). Thin layer chromatograms are developed in a tank in the normal way, removed from the tank and sprayed with a solution of the cholinesterase enzyme. The plate is then sprayed with a solution of the color-generating reagent; where inhibition of the enzyme by the pesticide occurs, the reagent is not hydrolyzed, and coloring does not occur in those areas of the plate occupied by the cholinesterase-inhibiting pesticide. Such an enzyme inhibition approach was used in the development of a postcolumn detector for the analysis of carbamate pesticides by HPLC (13). Both techniques are capable of analyzing nanogram amounts of insecticide. More recently, there have been several applications of the enzyme-linked Hill reaction for detecting photosynthesis-inhibiting herbicides, such as the triazines, phenyl ureas, and anilides following TLC separation (4, 5, 6, 14).

CONCLUSION

The techniques currently used in the analysis of pesticide residues in food permit precise and accurate detection and quantification of trace levels of hundreds of these chemicals. The relatively recent development of SFE and SPE promises to increase the efficiency of pesticide removal from food material and reduce analysis time. Technological advances in GC column

packing material, composition, wall coatings and size, and detectors have improved not only the sensitivity but also the specificity of the analyses performed. The detection and quantification in foods of an increased number of polar and thermally labile pesticides and metabolizes have been made possible by the increased use of HPLC, a technique that has also been improved in recent years. SFC may further enhance the ability to detect this group of pesticides. All of these techniques will continue to be refined not only as discrete and sequential steps in analytical method development, but also as equivalent and parallel steps to increase the number of pesticides that can be determined in a single sample by a single method (e.g., multi-detector systems, e.g., Luke procedure; see ch. 6).

However, as techniques are improved by changes in instrument and hardware design, bringing about more sensitive, selective, and reproducible devices, their costs usually increase, particularly when automated sample handling and data manipulation are included. These additional costs translate into higher costs to implement contemporary pesticide methodologies for food.

CHAPTER 3 REFERENCES

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*This reference paper is contained in appendix 13.