FOOD ANALYSIS BY HPLC

15
Analysis of Pesticide Residues in Food by HPLC

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INTRODUCTION

Worldwide, pesticide-monitoring programs are having increased demands placed upon them by the public perception that food supplies are unsafe. Such allegations, real or unreal, need to be answered. In order to be accurate, efficient, and cost effective in analyzing food pesticide residues, several types of analytical instruments are employed, one of which is high performance liquid chromatography (HPLC).

Ever since 1971, when HPLC was first used for residue analysis of abate in pond water (1), the technique has grown tremendously in popularity because of certain advantages over other instruments. Like gas chromatography (GC), HPLC can be completely automated from start to finish including interfacing with other instruments (mass spectrometer and infrared photometer). However, unlike GC, compounds analyzed by HPLC do not have to be volatile or stable to heat. Volatility can become important when trying to analyze large and/or polar pesticide molecules such as rotenoids, dithiocarbamates, phenoxy herbicides, metabolites, rodenticides, and peptide/proteins. Heat stability comes into play with many of the carbamate, urea, and organophosphate pesticides.

This chapter will give some basic information on pesticide chemistry, food sampling, extraction, clean-up, derivatization, separation, and detectors as applied to pesticides, followed by a section on food pesticide residue applications. Although numerous books and review articles have been written on HPLC analyses of pesticides (2–15), none have focused entirely on food applications.

HPLC THEORY VS. PESTICIDE ANALYSIS

The ultimate goal of a chromatographer in residue analysis is the separation of one or more pesticides from a complex mixture in the shortest time and with the greatest sensitivity. In theory, resolution basically depends upon the interaction of the

solutes between the mobile and stationary phases. A chromatographer has two major ways of controlling resolution—retention and band-broadening. Retention is the easiest means for the chromatographer to control separation. This is done by manipulating capacity and selectivity. These factors can be changed by using different mobile phases, stationary phases, and temperatures.

Band-broadening is the other method of maintaining resolution. The two simplest measures of controlling spreading of analytes is by (a) using a column that has a large N (number of theoretical plates), which means a column well packed with 3–5 μm packing, and (b) preventing extra column band-broadening. Extra column effects can be accomplished by using the shortest length and smallest diameter tubing for critical HPLC connections. Such connections are between the injector and column and column to detector. Keeping dead volume low prevents diffusion.

As can be seen, it is important to know what variables can cause changes in resolution so that one can obtain the best separation. To gain this knowledge, an understanding of HPLC theory is important. However, equally important is the hands-on experience of analyzing pesticide residue samples along with some knowledge of pesticide chemistry. Analysis of food for pesticides is so complex because of the matrix that HPLC experience becomes extremely valuable in developing the best method in terms of cost, simplicity, sensitivity, and reproducibility. If the reader wishes to learn more concerning HPLC theory the Refs. 16–24 will be very helpful.

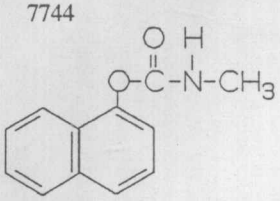
PESTICIDES

Definition and Nomenclature

Pesticides are chemical compounds (synthetic or naturally occurring) that are used to control pests. Pests can be as small as microorganisms or as large as mammals. Because of the wide range of pests to control, pesticides are divided into the following subdivisions based on pests: acaricides, algicides, bactericides, fungicides, growth regulators, herbicides, insecticides, molluscicides nematicides, repellents, and rodenticides. Approximately 1300 pesticides are used worldwide, with most being insecticides, fungicides, and herbicides. Each country has an organization(s) to control and monitor the use of pesticides along with setting tolerances for food.

The names associated with pesticides can be very confusing since there are so many. First is the common name selected by the appropriate professional scientific society and approved by the International Organization for Standardization. Next is the proprietary name (also called the trade or brand name), which comes from the manufacturer or the formulator. A pesticide may have as many as six or seven proprietary names, which can be very confusing. Thus, this is why a common name is assigned to every pesticide. The last name, which is the most important to the chromatographer, is the chemical name because the chemical structure can be drawn from the information provided. Chemical structures can tell the chromatographer how to approach the development of a method. The chemical name is presented according to principles of nomenclature used by IUPAC. An example of this naming process can be seen in Table 1 for carbaryl.

Table 1 Pesticide Nomenclature System—Carbaryl

Common name	Carbaryl
Proprietary name	Sevin, Hexavin, Karbaspray, Ravyon, UC 7744
Structural formula	
Chemical name	l-Naphthyl methylcarbamate
Empirical formula	$C_{12}H_{11}NO_2$

Chemistry

As discussed above, pesticides can be subdivided according to the organisms that they control. Also, they can be divided into groups based upon their chemical structure. This last grouping is the most important to chromatographers. Of the more than 100 elements known to man, pesticides are comprised of just 21 (Table 2). Only seven are extensively found in pesticide structures: carbon, hydrogen, oxygen, nitrogen, phosphorus, chlorine, and sulfur.

The major chemical categories of pesticides are as follows: chlorohydrocarbons, organophosphates, carbamates, nitrophenols, azo and hydrazines, diphenyl-

Table 2 Elements That Comprise Pesticides

Name of element	Symbol
Arsenic	As
Boron	B
Bromine	Br
Cadmium	Cd
Carbon	C
Chlorine	Cl
Copper	Cu
Fluorine	F
Hydrogen	H
Iron	Fe
Lead	Fb
Magnesium	Mg
Manganese	Mn
Mercury	Hg
Nitrogen	N
Phosphorus	P
Sodium	Na
Sulfur	S
Tin	Sn
Zinc	Zn

carbinols, fluorinated, formamidines, organometallic, mercaptans and thioethers, sulfoxides, sulfones and sulfonates, thiocarbonates, dithiocarbamates, heterocyclic sulfur, hydrocarbons, alcohols and phenols, carboxamides, carboximides, amine derivatives, pyridine heterocycles, imidazoles, triazoles, benzimidazoles and thiophanates, pyrimidines, quinozalines, S-alkyl dialkylcarbamothioates, ureas, carboxylic acids and derivatives, dinitroanilines, and triazines. Carbamates and organophosphates are the two largest groups. It should be mentioned that this type of classification crosses boundary lines. For example, not all organophosphates are insecticides—some of them may be fungicides. An example of the structure of each chemical type is given in Table 3. As is shown, there is quite a variety of structures, which ones would expect based on the wide range of pests that are controlled.

A chromatographer working on food-pesticide analysis must be familiar with not only the structures of major chemical classes of pesticides but also their chemical and physical properties. Knowledge of these properties is essential if the analyst is to be able to develop the best residue methods using the appropriate equipment. Not all pesticides are best analyzed by HPLC. A researcher must be able to determine which equipment to use for what compound, and this can be aided by knowing pesticide chemistry. To further enhance your understanding of pesticide chemistry, see Ref. 25.

SAMPLING

Sample Taking

Pesticide residue data is meaningless unless the sample is taken, handled, and prepared properly. Taking samples is very complex and should be performed by a trained person. The main objective is to obtain an exact miniature replica of the authentic product. To maintain this homogenicity, a certain protocol must be followed. When dealing with food, certain quantities that should be sampled. For example, in a homogeneous sample like processed food, approximately 1 kg should be sampled. For units that are not homogeneous, it depends upon the size of each unit (see Table 4 for the exact size). These field samples should be taken in a random manner.

Sample Handling

As soon as the sample is taken, it should be marked properly with all pertinent information and then put into a proper storage container to preserve its original integrity. This is usually low temperature to prevent chemical change. Once at the place of analysis, the sample should either be inspected for possible signs of spoilage or pesticide application such as wettable powders, etc., with all observations recorded. The sample should be extracted immediately, if possible. If not, then fresh samples should be quick frozen, but first washed lightly if any dirt is present. Handling of processed samples will depend upon the stability of the process. For example, frozen products will have to remain frozen, while dry or thermal products should be stored in a dry atmosphere at room temperature.

Sample Preparation

If the sample is homogeneous, then a subsample can be removed and extracted for pesticide residues. However, if the sample is nonhomogeneous, then it will have to

Table 3 Chemical Categories of Pesticides

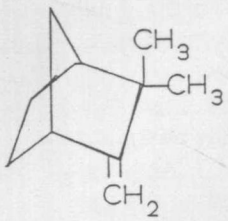
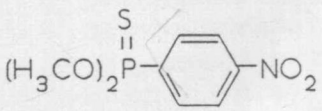
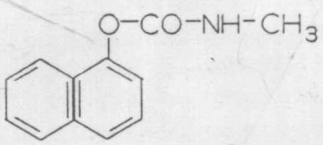
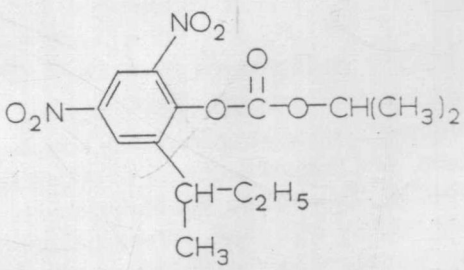
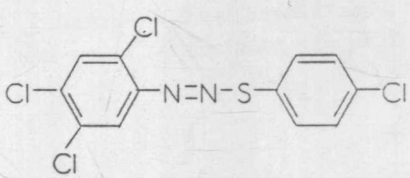
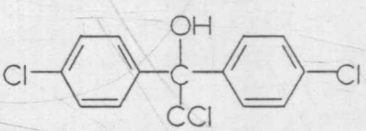
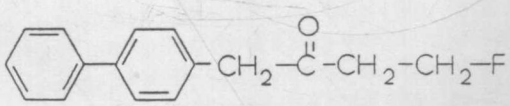
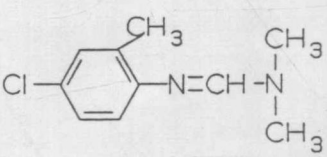
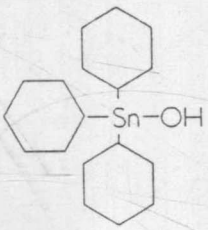
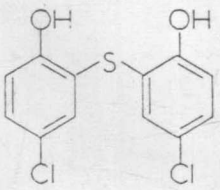
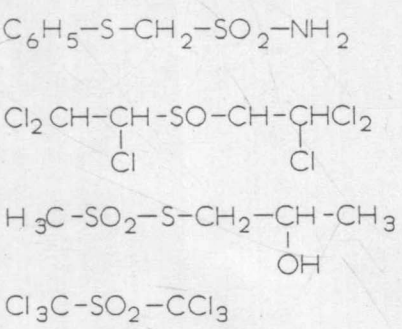
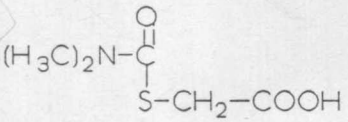
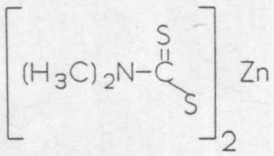
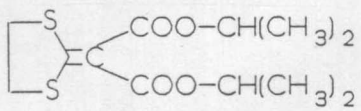
Name	Structures
Chlorohydrocarbons	
Organophosphates	
Carbamates	
Nitrophenols	
Azo and Hydrazines	
Diphenylcarbinols	
Fluorinated	
Formamidines	

Table 3 (Continued)

Name	Structures
Organometallic	
Mercaptans and thioethers	
Sulfoxides, sulfones, and sulfonates	
Thiocarbonates	
Dithiocarbamates	
Heterocyclic sulfur	

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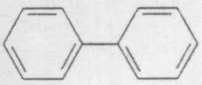
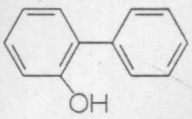
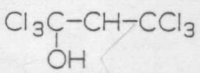
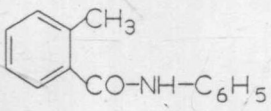
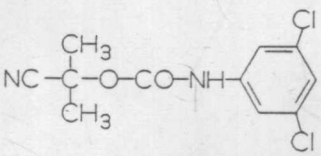
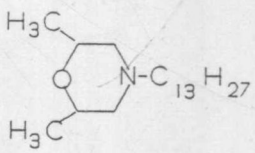
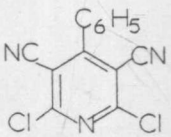
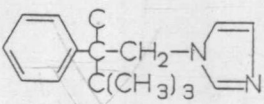
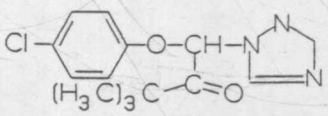
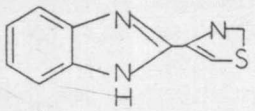
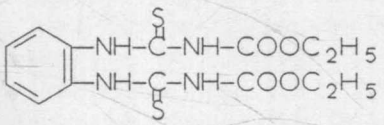
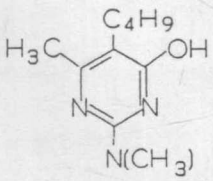
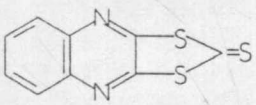
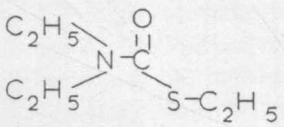
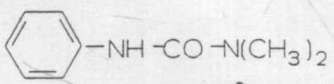
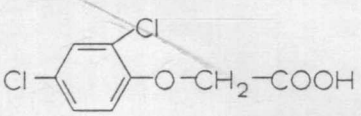
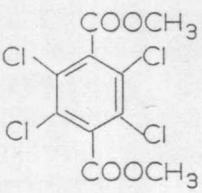
Hydrocarbons	
Alcohols and phenols	 
Carboxamides	
Carboximides	
Amine derivatives	
Pyridine heterocyclics	
Imidazole and triazole	 

Table 3 (Continued)

Name	Structures
Benzimidazoles and thiophanates	
	
Pyrimidines	
Quinoxalines	
S-Alkyl dialkylcarbamothioates	
Ureas	
Carboxylic acids and derivatives	
	

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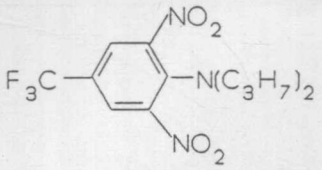
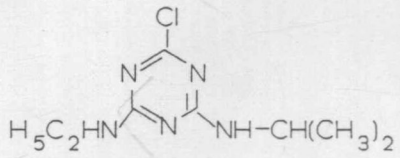
Dinitroanilines	
Triazines	

be composited and processed in such a way as to make it homogeneous without destroying the residues. The best homogenizers for most products are bowl cutters, meat mincers, or blenders, whereas for dry materials the best homogenizers are ball or hammer mills. Once homogenization is complete, then a subsample of usually 100 g is extracted for pesticide residues. Most often two subsamples are extracted. It is also best to freeze a couple of aliquots in case of mishaps.

As mentioned earlier, the analyst must know the chemical properties of pesticides. A case in point, dithiocarbamates undergo rapid degradation upon intense comminution so they have to be handled differently. Therefore, the analyst must always keep current and also refer to the *Pesticide Analytical Manual* (26), the Codex Committee on Pesticide Residues (27) and/or *Manual of Pesticide Residue Analysis,* Volume 1 (28), and/or sampling techniques for pesticides (29).

EXTRACTION, CLEAN-UP, AND DERIVATIZATION

Extraction

Extraction of pesticide residues from food depends primarily upon the type of food and the pesticide. Food types generally take on four forms for pesticide analysis:

Table 4 Size of Laboratory Sample

Type of product	Sample size
Homogeneous products	0.5–1 kg
Units less than 25 g	approx. 1 kg (at least 50 units)
Units 25–100 g	1–3 kg (at least 30 units)
Units 100–250 g	2–5 kg (at least 15 units)
Units more than 250 g	at least 10 units

These values are given as a guide. In some instances (e.g. cereals, tea, melons), laboratory samples of sizes differing from those given above may be required.

Source: Ref. 28.

liquid (milk and juice), solid high water content (fruits and vegetables), solid low moisture (dried foods), and high fat foods (meat, eggs, and fish). As for pesticides, the polarity is the key. Polarities range from nonpolar, intermediate polar, to polar.

Liquid foods can be the easiest to extract if one uses solid-phase extraction. Solid-phase extraction has most often been employed for clean-up purposes, but recently has been shown to be effective in the extraction of liquid foods. For example, West and Day (30) extracted the herbicide fluridone from milk using two C18 Sep-Pak cartridges (Waters Associates, Inc.). Bushway (31) was able to extract carbaryl from fruit juices using a C18 Sep-Pak.

All other food-residue extractions including some liquids are performed by mechanical means with solvents, usually an organic solvent except for some extremely polar or volatile pesticides and metabolites like paraquat, dicamba, naptalam, 2-aminobenzimidazole (2AB), glyphosate, 2-benzimidazolecarbamate (MBC), 2-imidazoline, thiabendazole, methyl 2-phenylphenol, and biphenyl, whereby water with acid or base is used (32–39). Organic solvents used depend upon the polarity of the pesticides and the matrix. The most popular solvents employed are acetone, petroleum ether, benzene, heptane, hexane, ether, ethyl acetate, methylene chloride, chloroform, methanol, and acetonitrile (single use or in various combinations). Acetone seems to be the most versatile extraction solvent. In fact, Luke et al. (40–42) have developed several extraction methods using acetone, acetone/water, or acetonitrile. At last count these methods have been successful at extracting over 200 pesticides in a number of different foods (43). Although these methods were developed for GC analysis, the extraction procedure will work for HPLC. Three other solvents—methanol, methylene chloride, and ethyl acetate—also have been very efficient at food pesticide removal, especially for carbamates, phenylurea herbicides, and imidazole fungicides (6,7,44–58).

The mechanical means of food extraction also varies (shaker, blender, Soxhlet, ultrasonic bath, ploytron, reflux, and omni-mixer). Soxhlet extraction is the least used today, with the polytron being the most employed device. With solvents being expensive and toxic, it is important to use as little as possible and thus this requires the use of a very small homogeneous sample (2–5 g). Thus, a polytron is the mechanical device used for such an extraction procedure.

With this solvent safety factor in mind, there are two new emerging techniques for pesticide extraction. One is superfluid extraction (SFE) (59). Although SFE does not usually extract the analyte without interfering substances, it does remove the analyte without toxic solvents with usually a better extraction efficiency than the classical methods (60–63). Once extracted, then a clean-up step can be applied using a mini-column or some other technique that uses very little solvent (Fig. 1) (47). Presently, SFE is primarily interfaced with capillary supercritical chromatography (SFE), but there is no reason why it cannot be used in conjunction with HPLC.

On-line extraction (64–67) is the other new method. Although it does employ solvents, the advantages are fewer solvents and less handling because of no filtration, no separatory funnel, and total extraction time of 1–2 min (67).

Clean-Up

It is very uncommon in HPLC to be able to analyze pesticides in food without exercising some form of clean-up. For the most part, methods without clean-up are

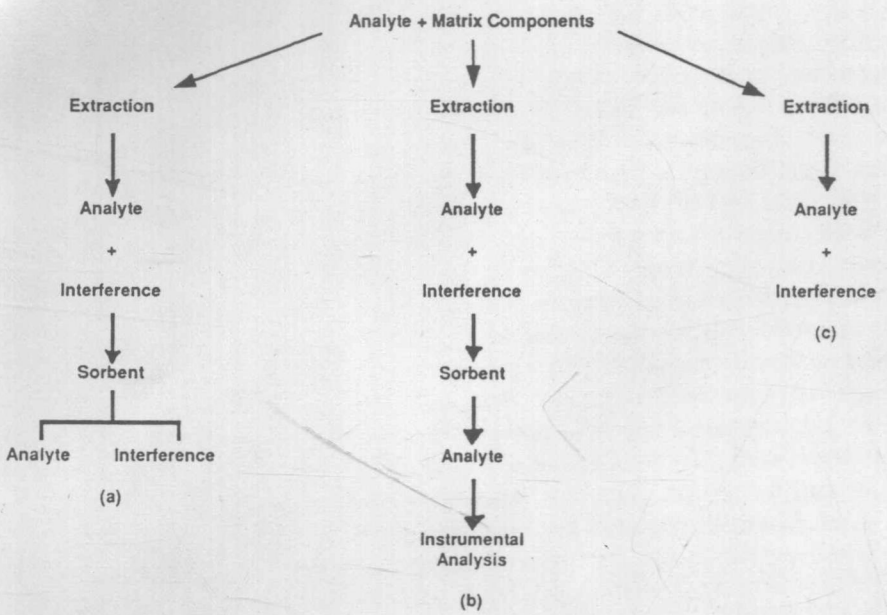


Figure 1 Generalized supercritical fluid methods for extraction and analysis involving separation of interfering components. (From Ref. 59.)

either determining pesticides at a high residue level, analyzing one pesticide, determining pesticides in a liquid food, or using a selective detector (31,50,68–85).

Liquid-Liquid Partitioning

Partitioning is the oldest clean-up step and still is the most used. It involves moving the analyte in and out of water and organic solvents in order to remove interfering compounds. This is accomplished by the aid of a separatory funnel. For example, a pesticide is extracted from a potato with acetone. This mixture is added to a separatory funnel followed by the addition of hexane:methylene chloride (1:1 mixture). The pesticide is partitioned into the hexane:methylene chloride fraction leaving polar interfering substances in the aqueous phase. A more complicated example would be a pesticide that will ionize in acid. One can do an acid-base type partitioning between water and organic solvents.

Although some pesticides in certain foods can be sufficiently cleaned up by partitioning (6,7,25,27,42,43,57,74–103), it is usually just the first phase of clean-up followed by one or more steps to further remove interferences.

Distillation

Distillation is a clean-up technique that has been primarily applied to spectrophotometric or gas chromatographic methods. However, it has been employed in HPLC for two nonpolar fungicides—biphenyl and 2 phenylphenol (37). These postharvest fungicides have been analyzed in citrus fruit by distillation followed by HPLC. Also, the herbicide naptalam and its metabolite 1-naphthylamine were removed by distillation from peaches, asparagus, and cranberries (33).

Liquid/Solid

Liquid/solid clean-up procedures encompass such techniques as open column chromatography and column switching. Open column includes size exclusion (GPC), absorption/partition (alumina, silica, florisil, and all bonded-type phases), and ion exchange either in large or miniature column form. Most HPLC pesticide food residue analyses employ one or more of the open column clean-up procedures (6,7,39,46,47,56,58,70,104–169). The trend today is to use the miniature columns, which can also concentrate the analyte. In many instances both concentration and clean-up are needed. Such columns can be either made from pipettes or purchased from several manufacturers with the two biggest brands being Sep-Pak and Bond Elut. Only recently have small GPC columns been introduced, but then only with molecular weight cutoffs of 10,000 daltons or greater.

A survey of column clean-up methods for food residues is given in Table 5. Twenty-four different column materials or combinations have been used, with florisil being the most prevalent followed by alumina, ion exchange, and C18. It is not surprising that most methods use an absorption-type clean-up since many of the HPLC procedures use reverse-phase analytical columns. Although GPC alone was not employed much, it was in combination with others materials like silica, charcoal, and alumina. As smaller molecular weight cutoffs are developed for the commercial cartridges, one will see more use of GPC for no other reason than to extend the life of analytical columns.

Column Switching

Column switching has been extensively used in GC and has found some use in HPLC, especially the petroleum and pharmaceutical industries (170). Although it was used for food residue analysis in the early and mid 1980s (157,170–174), it has begun a recent resurgence. There are advantages to column switching that should increase its use for food residues. These are (a) to gain resolution and selectivity, (b) for trace enrichment, (c) to protect sensitive detectors such as electrochemical detectors from food co-extractives, and (d) to maintain column equilibrium by removing unwanted compounds (158,159).

Configuration of column switching can take numerous forms (170,174–178). The classification system is based on the way samples are transferred from one column to another, which leads to four switching techniques: direct, indirect, reverse, and loop transfers (174). Recent applications to the food residue area have basically exercised two of these: (a) direct and (b) indirect transfers.

Direct transfer was utilized by Cessna (58,123) in determining linuron in saskatoon berries and asparagus and analyzing maleic hydrazide in parsnips (179). He employed two analytical columns and a guard column, all of which were reverse-phase. A 10 ppb limit of quantification with an 86% recovery was obtained.

The indirect column switching technique has been applied most often. In all instances a precolumn with an analytical column was used. The precolumn acted as a concentrator and clean-up device. Haddad et al. (126) analyzed five pyrethroids and the synergist piperonyl butoxide in rice. Glyphosate was determined in cereals, potatoes, asparagus, and strawberries by Tuinstra and Kienhuis (76). Goewie and Hogendoorn (52,137) have been very successful using column switching for food. First they were able to analyze 3 *N*-methylcarbamate pesticides (carbaryl, carbofuran, and propoxur) in total diet samples which have very complex matrices.

Table 5 Survey of Column Clean-up Methods Used for HPLC Analysis of Food-Pesticide Residues

Column material	Number of methods	Number of non-commercial columns	Number of commercial columns	Ref.
Florisil	19	17	2	56,58,104–106, 110,113,114,116, 117,121,123,130, 135,158,160,179, 193
C18	7	0	7	107,120,122,137, 154,161,169
GPC	2	1	1	108,165
Florisil-alumina-silica	1	1	0	109
Alumina-charcoal	1	1	0	111
Alumina	7	7	0	70,72,112,119, 136,139,153
Ion exchange	8	8	0	34,36,115,119, 141,142,143,148
Carbopack-ion exchange	1	0	1	124
Florisil-alumina	1	0	1	126
GPC-silica	3	4	0	127,144,147, 168
C18-silica	2	0	2	128,149
GPC-charcoal	1	1	0	140
Silica	5	1	4	131,138,151,166, 167
Amino	2	0	2	133,164
Diol	1	0	1	134
Charcoal-Celite	3	3	0	46,47,129
Carbon-clay	2	1	1	145,159
Silicic acid	1	1	0	146
C18-florisil	1	1	1	150
GPC-alumina	1	1	0	152
C8	1	0	1	155
Extrelut	2	0	2	125,132
C18-alumina	1	1	1	163
Extrelut-alumina	1	1	1	156

Second, the herbicides cyanazine and bentazone were quantified in sugar maize by on-line multicolumn chromatography.

Derivatization

Derivatization plays an extremely important role in food-pesticide residue analysis by HPLC. The primary reason for performing derivatization is to obtain sensitivity, but it can also be important in selectivity (removal of interfering compounds by nondetection). There are two major types: precolumn and postcolumn. Several excellent reviews have been written on each (180–193).

Precolumn

Currently, precolumn derivatization is the least used, but may be subject to change in the future. As the word indicates, this kind of derivatization takes place before the chromatography, forming a UV or fluorescent substance. The following conditions should be met (194): (a) must know the reaction stoichiometry and product structure, (b) must be a relatively fast reaction with the derivative stable in solution, and (c) reagent(s) and derivative(s) must be stable during the chromatographic process.

Methods using the precolumn procedure have been used in the past. For example, Newsome (38,112) employed *p*-nitrobenzylchloride, a UV derivatizing agent (183), for the determination of two degradation products (ethylene thiourea (ETU) and 2-imidazoline) of ethylenebis-dithiocarbamate (EBDC) fungicides. *para*-Nitrobenzyl chloride was also successfully applied by Tafuri et al. (55) in the derivatization of thiabendazole in fruits. Carbofuran and two of its metabolites were analyzed in foods by reacting the parent compounds with dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride) (157,184). Lawrence also used dansyl chloride to derivatize carbaryl so that it could be analyzed in potatoes (103). Dansyl chloride was popular because it is nonfluorescent, but will react to make highly fluorescent compounds. Furthermore, side products from the reaction with dansyl chloride are minimal. One other fluorescent agent, 9-fluorenylmethylchloroformate, has been used by Roseboom and Berkhoff (141) to derivatize glyphosate and its metabolite in straw.

Another type of precolumn treatment should be mentioned here, which is base hydrolysis of the pesticides before analysis, although it technically is not a derivatization step. This method has been used by Olek et al. (88) whereby they hydrolyzed carbamates for electrochemical detection.

As the author mentioned earlier, the future of precolumn derivatization may be bright with the development of on-line techniques (190). Nonautomation has been the biggest disadvantage of precolumn derivatization since it creates long preparation times and inconsistent reproducibility. An example of such an automated precolumn scheme is shown in Figure 2. Although this is for amino acids, there is no reason why the same scheme could not be modified and used for pesticide residue analysis.

Postcolumn

Postcolumn derivatization occurs after the analyte(s) have been separated. Initially, this technique had problems because of band-broadening, but once that was solved, it has been the derivatization procedure of choice for residues.

Postcolumn reactors can be classified into four groups: open-tubular, packed bed, segmented stream, and hollow-fiber membranes (190). The simplest and thus most popular is the open-tubular reactor. It is comprised of a tube (straight or coil) made of PTFE, glass, quartz, or stainless steel. Because of peak-broadening, tubular reactors are usually comprised of a coiled knitted piece of PTFE tubing. The design is such that it is best for fast kinectics (1 min or less), although the tube can be designed for slow reactions (several minutes) (190).

Packed-bed reactors or solid-phase reactors consist of a short piece of tube with some type of packing material. The first solid reactors contained a stainless steel tube with inert glass porous beads which totally served to deter band-

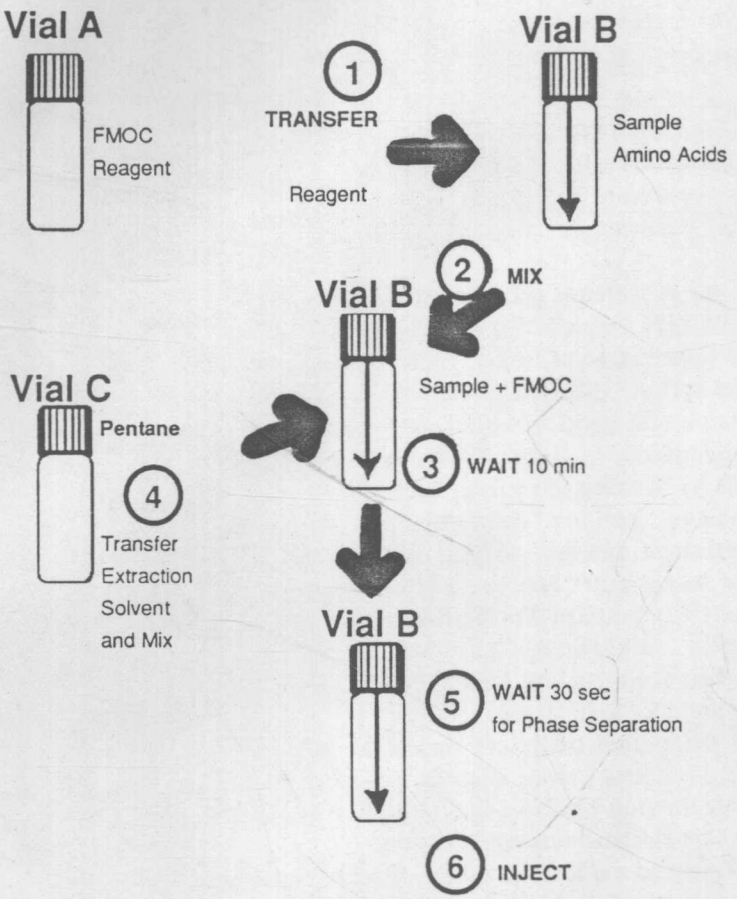


Figure 2 Automated precolumn derivatization sequence of amino acids with 9-fluorenylmethyl chloroformate (FMOC), marked by Varian. (From Ref. 190.)

broadening within reaction times of 0.5 to 4 min. However, recently it was shown that this type of reactor would be more versatile if the packing material was a catalyst (metal, ion exchange, immobilized enzyme). The packing actually takes part in the derivatization reaction which not only cuts back on peak-broadening, but eliminates the need for a pump and a reagent that cannot be reused.

Another reactor is the segmented stream. It was designed for continuous flow analyzers. Initially the stream was segmented with air or other gas bubbles to prevent the dispersion of sample plugs allowing the needed residence time. It has also been shown that the stream could be segmented using a nonmiscible organic solvent. In fact, liquids give the system more stability since they do not respond to small fluctuations in pressure, temperature, or flow, but still do not affect peak-broadening up to residence times of 20 min. However, the tube in this type of reactor can drastically influence band-broadening. Glass seems to work well.

Finally is the hollow-fiber membrane reactor: porous and hollow fiber membranes are used to deliver the reagent. This does away with a pump and mixing tee and thus reduces the extra column band-broadening.

Once it has been established which type of reactor to employ, then it must be decided what kind of reaction is to take place in the reactor. The reaction type totally depends upon the analyte(s) to be quantified. There are several classes of reactions available, and they can be put into two main groups: physicochemical and derivatizations (183). Included in the physicochemical reactions are (a) electrochemical and redox, (b) hydrolytic, (c) micellar-enhanced, (d) photochemical, (e) physical interactions, and (f) thermo-initiated while derivatization reactions are (a) ion-pair, (b) ligand-exchange and complexation, and (c) true chemical derivatizations.

Postcolumn reaction for pesticide analysis by HPLC was initiated by Moye et al. (195) in 1977. This group of researchers was the first to apply the reaction of a primary amine with 2-mercaptoethanol (MERC) and *o*-phthalaldehyde (OPA) to form a fluorescent complex to *N*-methylcarbamate insecticides. The carbamates are hydrolyzed by base (postcolumn) to form methylamine, which in turns reacts with MERC and OPA to form the fluorescent complex. Krause refined the method in 1978 (196) and 1979 (197), which led to the development of the first methods for determination of carbamates in food (45,161). These procedures have been tested collaboratively (46,47) and have been shown to be very reproducible.

Other scientists have also modified the carbamate postcolumn technique (133,140,150). Most modifications have been in the tedious clean-up procedure. The best change was devised by deKok et al. (133), whereby they employed an aminopropyl Bond Elut solid-phase extraction column for clean-up. This eliminated the GPC and Nuchar/celite clean-up step. Sixteen different fruits, vegetables, and grains and 21 pesticides and 10 metabolites can be quantified with this simple clean-up.

Solid-phase reactors have been developed for carbamates (164,198–200), but only one has been successfully utilized for food samples. DeKok et al. (164) employed an inexpensive material (magnesium oxide) for the catalyst and was able to analyze 22 carbamates and 10 metabolites in several crops.

Since the first food methods for carbamate in the early 1980s, there have been many other postcolumn procedures developed. For example, the herbicide glyphosate and its metabolite, aminomethyl-phosphonic acid, can undergo hydrolysis to primary amine formation, which then can be reacted with the same postcolumn system as the carbamates (36,76,143,149).

Krause (48) has used a postcolumn base hydrolysis step to analyze six aryl *N*-methylcarbamates in four crops (apples, cabbage, grapes, and tomatoes). These carbamate pesticides form phenols when hydrolyzed, which can be detected easily and with much sensitivity and selectivity by electrochemical detection.

Also, Krause and August (129) were able to analyze three organophosphates (azinphos methyl, azinphos ethyl, azinphos methyl oxygen analog) in broccoli and peaches by postcolumn reaction with base. Base hydrolysis causes these pesticides to form anthranilic acid, which is a natural fluorescent substance.

Photochemical reactions postcolumn have also been applied successfully to residue analysis (191,193). The photochemical reaction is produced usually by a mercury lamp giving off UV light at 254 nm. Once the pesticides have been photolyzed, they are either derivatized by MERC and OPA or the ions produced are directly sent to a conductometric detector. Methods using photolysis and derivatization for food have been developed by Luchtefeld (58,84) and Miles and