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Review

Chromatographic methods for the determination of pesticides in foods

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ABSTRACT

Chromatography is the most important technique available to the analyst dealing with the determination of pesticide residues in food, feed and environmental samples. Numerous methods for pesticide residues in foods have been developed in the past few years, and this paper reviews some of the most important procedures. A great variety of chromatographic methods, such as solid-phase extractions, column chromatographic clean-up methods, thin-layer, gas, high-performance liquid and supercritical fluid chromatography, and their coupling with sensitive and selective detection methods are surveyed.

CONTENTS

1. INTRODUCTION

In modern agricultural food production, good results can be obtained only with the help of pesticides, but these materials contaminate the environment and some more or less persistent pesticide residues remain on the surface of or inside the products. Methods for pesticide residue determinations for the qualification of food products are under rapid development. The residue limits given by the World Health Organization are becoming even lower and lower creating an ever increasing demand for more selective and sensitive methods. The great variety of pesticides need multi-residue methods but their applicability is limited by the different nature of the food materials. These multi-residue methods allow the simultaneous determination of herbicides, insecticides, fungicides and others in one extract. As the concentration of the pesticide residues in food material is in the nanogram, picogram or sometimes femtogram per gram range, their evaluation can be carried out only by extremely selective and sensitive detection methods.

The determination of pesticide residues in food

materials consists in sample preparation and the determination. The role of chromatography is very important in both of these steps. Chromatography can be used as a preliminary concentrating procedure which can be continued with column chromatography as a part of the clean-up. Moreover, chromatography also plays a very important role in the determination step. Gas (GC), supercritical fluid (SFC), high-performance liquid (HPLC), thin-layer (TLC) and overpressure thin-layer chromatography (OPTLC) and combinations of these methods with other techniques, e.g., mass spectrometry (GC–MS, HPLC–MS), will be discussed here.

2. SAMPLE PREPARATION: EXTRACTION AND CLEAN-UP

The sample preparation consists of the extraction and clean-up steps, which are influenced by the final determination. If the determination is selective enough, the clean-up need not be so thorough, but less selective detection methods need more efficient clean-up steps.

Large numbers of methods have been developed for the determination of pesticides in different food materials. These multi-residue methods offer the possibility of the detection and determination of organochlorine (OC), organophosphate (OP) and carbamate insecticides, triazine and thiocarbamate herbicides, dithiocarbamates and other fungicides and other contaminants, sometimes in one extract.

The method of the Association of Official Analytical Chemists (AOAC) [1] is representative of the internationally recognized multi-residue methods that allow the determination of numerous OC and OP insecticides, carbamates and other pesticides in fatty and non-fatty food samples and in foods with high or low sugar contents, etc. According to this method, extraction is carried out with acetonitrile, followed by liquid–liquid partition and Florisil column clean-up when OC insecticides are to be determined in fatty foods and sweep co-distillation for OP insecticides. The AOAC multi-residue method was extended to the determination of thiocarbamate herbicides in food samples of plant origin [2]. In several samples of maize products (Table 1) some co-extracts were present, which disturbed the determination. Interferences could be eliminated by

further clean-up, steps, e.g., coagulation with ammonium chloride and urea solutions followed by Florisil column chromatography (Fig. 1).

Several other methods use sweep co-distillation [3,4] in the determination of OP insecticides. Size-exclusion chromatography [5–7] and column chromatography on charcoal and mixed columns [8–10] have also been used as clean-up methods in OP determinations. Recently published methods use disposable cartridges. For example, an Extrelut-20 cartridge was applied for the clean-up of fruit and vegetable extracts containing eighteen OP insecticides (Table 1) [11]. OP insecticides were determined in tea extracts, a sulphuric acid treatment being necessary before the Florisil column clean-up (Table 1) [12]. Twenty-three persistent OC insecticides were investigated in lipid-rich food samples. Negative interfering peaks were observable in the gas chromatograms when Florisil column clean-up was used alone. These interferences were eliminated with a sulphuric acid treatment on a solid matrix column (Table 1) [13].

A Carbopack B cartridge and sulphonic acid-type silica-based cation-exchange (SCX) columns were applied for the clean-up of triazine-containing vegetable extracts. HPLC determination gave the recovery data shown in Table 1 [14].

Phenylurea herbicides were studied in the presence of aniline-type compounds on a graphitized carbon black (Carbopack B) cartridge connected with a strong cation exchanger. This method was compared with the use of a C_{18}-bonded silica cartridge. The recoveries were established by reversed-phase (RP) HPLC with UV detection (Table 1) [15]. Better recoveries were reported using a C_{18}-bonded silica cartridge compared with Carbopack B in the determination of 24 basic–neutral and eleven acidic pesticides in water samples (Table 1) [16] by RP-HPLC. The extraction is seven times quicker than that with Carbopack B because it does not need any pH adjustment. Nevertheless, Carbopack B seems to be more adaptable in field use.

Rapid and selective on-column extraction of OC pesticide residues from milk samples was carried out. The extraction system minimizes the fatty co-extractives and gives almost quantitative recoveries of pesticides (Table 1) [17].

TABLE 1

EXTRACTION AND CLEAN-UP PROCEDURES

Compound	Sample	Extraction	Clean-up	Recovery (%)	Method of analysis	Ref.
EPTC Butylate Molinate Lindane p,p'-DDT	Deep-frozen peas Potatoes Beans	Acetonitrile	Florisil	73–100	TLC densitometry	2
p,p'-DDE Trichlorphon Dimethoate Mevinphos Methylparathion	Maize Maize flour Maize grits Shelled grain		Coagulation Florisil			
Chlorpyrifos metabolites	Banana pulp	Acetone	Silica gel (15% water) Charcoal–MgO– Celite (1:2:4)		TLC	9
OC, OP Carbamates Triazines	Apple Spinach Carrot	Acetone	Charcoal–magnesia– diatomaceous earth (1:2:4)		TLC	10
Methacriphos Fonofos Fenchlorphos Dimethoate Parathion-methyl Parathion Metidathion Diazinon Ten other OP	Broccoli Cauliflower Onion Radish Peach Tomato	Acetone	Extrelut-20 CH_2Cl_2–light petroleum (1:3)	75–107	GLC	11
Malathion Fenitrothion Quinalphos Dimethoat	Tea	Toluene–MeOH (3:1)	Sulphuric acid Florisil Toluene–acetone (98:2)	96–97	GLC 6% OV-101 Chromosorb W (80–100 mesh), NPD	12
23 Persistent OC	Human milk Cow milk Vegetable oil		Florisil Extrelut-1 Sulphuric acid	89–117 (49)	OV-17 + QF-1 (1.5% + 1.95%) Chromosorb W, ECD	13
Simazine Simetryne Atrazine Prometon Ametryn Propazine Prometryn	Water Vegetables Lettuce Spinach Chicory Endive Kale	$CH_3CN–H_2O$ (6:4)	Carbopack B CH_2Cl_2–MeOH (6:4) (a) CH_2Cl_2–MeOH (6:4) (b) CH_2Cl_2 (c) CH_2Cl_2– CH_3CN (6:4)	 65–100 82–86 96–98	RP-HPLC, UV LC-18-DB	14
17 Triazines and carbamates	Water		(a) Carbopack B Amberlite CG-120-I (b) C_{18}-bonded silica	92 6.3–69.6	RP-HPLC LC-18	15

TABLE 1 *(continued)*

Compound	Sample	Extraction	Clean-up	Recovery (%)	Method of analysis	Ref.
(a) 24 Basic–neutral pesticides	Drinking water		Carbopack B	95	RP-HPLC LC-18	16
(b) 11 Acidic pesticides			C_{18}-bonded silica	76		
HCB, α-HCH	Milk	Light petro-	Chem-Elut CE	77	GLC, ECD	17
β-HCH	Milk powder	leum–CH_3CN–	1010		OV-17 + QF-1	
Endrin		EtOH (100:25:5)	Florisil		(1.5% + 1.95%)	
Heptachlor epoxide				94–113	Chromosorb W HP	
Dieldrin						
p,p'-DDE						
p,p'-DDT						

3. METHODS FOR THE DETERMINATION OF PESTICIDE RESIDUES

3.1. Thin-layer chromatography

For the determination of pesticides in food and feed samples, at least two independent methods are necessary to decide whether the sample is appropriate for consumption or not. Pesticide-like interfering co-extractives can cause serious problems. In ambiguous cases alternative methods are applied. TLC can serve as one of the alternative methods when the qualitative results are quantified by *in situ* densitometry. The detection methods applied should have two orders of magnitude higher sensitivity than the given residue limit of the compound being studied.

OP, OC and carbamate-type compounds were investigated by the extended AOAC method for the determination of thiocarbamates. Possible interferences were checked. The detection of OP compounds was carried out with the chromogenic agent 4-(4'-nitrobenzyl)pyridine (NBP), that of OC compounds with silver nitrate–2-phenoxyethanol and that of thiocarbamates with 2,6-dibromobenzoquinone N-chloroimine (DBI) and N,2,6-trichlorobenzoquinone N-chloroimine (TBI) in acidic solution (Table 2) [2,18]. Possible interferences were investigated. The sensitivity of these detection systems was studied on different TLC supports. Reversed-phase TLC on alumina G and adsorption chromatography on the same support was compared with the use of Polygram Cel 300. The latter gave the most sensitive reaction (0.05 µg per spot) in OC detection. Only the halogen-containing chlorfenvinphos gave a positive reaction; the other OP and thiocarbamates did not react. In analyses for OP compounds, sensitive detection was achieved on Silufol and Polygram SIL G, plates with 0.05 µg per spot sensitivity. The chromogenic reagents DBI and TBI detected not only the thiocarbamates but also some other sulphur-containing OP compounds with 0.02–0.05 µg per spot sensitivity. These detection methods were also checked on different supports such as Kieselgel 60,
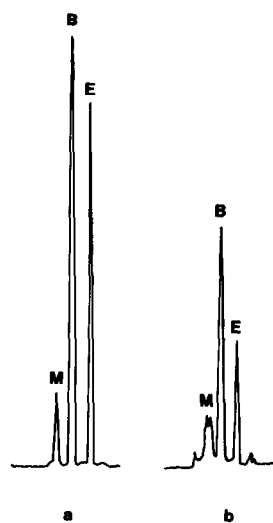

Fig. 1. Densitogram of thiocarbamates in deep-frozen beans by AOAC method [2]: (a) with coagulation; (b) without coagulation. E = EPTC; B = butylate; M = molinate.

TABLE 2

THIN-LAYER CHROMATOGRAPHY OF PESTICIDES

Compound	Sample	TLC			Recovery (%)	Ref.
		Plate	Eluent	Detection		
EPTC Butylate Molinate Pebulate Cycloate	Potatoes Beans Peas Maize Flour	Silufol Polygram Sil G	Hexane–diethyl ether– acetone (7.5:2:0.5)	DBI TBI in CH$_3$COOH	80–100	2
p,p'-DDT p,p'-DDE	Grits Grains	RP alumina G	CH$_3$CN–acetone– MeOH–H$_2$O (40:18:40:2)	AgNO$_3$– 2-phenoxy ethanol		
Mevinphos Dimethoate Fenthion Chlorfenvinphos Trichlorphon		Alumina G Polygram Cel 300 Silufol Polygram Sil G Kieselgel 60 Silica gel 60– Kieselguhr	Hexane–diethyl ether– acetone (7.5:2:0.5) Hexane–diethyl ether (8:2)	NBP		
Carbofuran	Goose and pig feed	Silica gel G	CCl$_4$–EtOH–acetone (4:1:1)	TBI in NaOH solution		21
Herbicides Insecticides Fungicides Phenylureas Modified ureas Carbamates N-Heterocyclic compounds	Drinking water		Automated multiple development		70–120	22
Dursban Metathion Dimethoate Phosalone	Apple	Silica gel G	Hexane–acetone (9:2)	Indoxyl acetate		24
Insecticides Acaricides Fungicides	Fresh apple Processed apple	Two-dimensional TLC Silica gel GF$_{254}$	(1) Cyclohexane– acetone (10:1) (2) Light petroleum– benzene–ethanol (65:30:5)	UV 255, 366 nm Bromophenol blue		25

silica gel 60–Kieselguhr and Silufol plates [2]. The best and most reproducible results were obtained on Silufol plates (Table 2).

The chemical processes responsible for the detection of the thiocarbamate herbicides were also studied. The detection of thiocarbamate herbicides was carried out in acidic solutions, giving yellow spots on a white background. With DBI some side-reactions were taking place, a higher halogen content being observable in the mass spectrum of the coloured compound. Surprisingly, chlorine was also present in the product formed in the reaction of DBI. Here 2,6-dihalo- and 2,3,6-trihalobenzoquinone imine derivatives were observed (Fig. 2). These groups of the mixed halogenated benzoquinone derivatives were separated by flash chromatography using xylene or mesitylene as eluents. Here some charge-transfer complex formation is assumed. The 2,6-dihalobenzoquinone imine derivatives, having very small differences in their polarity, were separated by OPTLC using cyclohexene as mobile phase [19]. In the TLC detection of thiocarbamate herbicides,

X₁ X₃ diagram: O=⟨benzene ring⟩=N–S–R with X₂
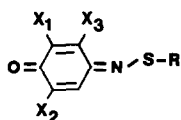
$X_1 = Br, Cl$

$X_2 = Br, Cl$

$X_3 = H, Br, Cl$

$R = alkyl\ chain$

Fig. 2. Coloured compounds formed in the TLC detection of thiocarbamates with TBI and DBI.

DBI gave more sensitive detection but TBI gave more reproducible results. It should be mentioned that oxidative metabolites of S-ethyl dipropylthiocarbamate (EPTC), butylate and other thiocarbamates also gave positive reactions with TBI and DBI [20]. Some sulphur-containing pesticides and thio- and dithiophosphates gave positive reactions with good sensitivity [2]. This method was developed for the in situ densitometric evaluation of thiocarbamates and other pesticide in food extracts (Fig. 1).

The same reagents can also be used for carbofuran detection in alkaline solution. Contaminated goose and pig feeds were analysed after dichloromethane extraction. The residues were reacted with an alkaline solution of TBI and the blue indophenol derivative obtained was separated on silica gel G plates (Table 2) [21].

A straightforward method for drinking water analysis using high-performance TLC was published. Numerous pesticides (Table 2) were extracted by solid-phase extraction on a C_{18}-modified silica cartridge. A correct pH adjustment helped the separation of the pesticides. Automated multiple development (AMD) was applied in TLC. The limitation of the method is the adjustment of the pH during the elution. The TLC results should be checked by GC or HPLC [22].

Another extremely specific detection method was developed for endosulfan and phosphamidon residues. The plates were sprayed with cobalt acetate in alkaline solution and subsequently with tolidine in acidic media. Neither OC insecticides (endrin, aldrin, dieldrin, DDT), OP insecticides (malathion, parathion, dimethoate, quinalphos, phorate, fenitrothion) nor carabamate insecticides (baygon, carbaryl and carbofuran) gave any coloured spots. Further, no reaction was reported with amino acids,

peptides and proteins present as co-extractives. This reagent is five times more sensitive than ethanolic diphenylamine and o-tolidine or o-dianisidine with UV irradiation [23].

Some sulphur-containing OP insecticides in apples were determined by GC and TLC determination. Indoxyl acetate was applied as developer (Table 2) [24]. Insecticides, acaricides and fungicides were determined by TLC in fresh and processed apples. After two-dimensional TLC separation, eighteen pesticides were detected by UV irradiation and spraying with bromophenol blue solution (Table 2) [25].

The enzyme inhibition method gives the most sensitive detection for pesticides on TLC plates. These reactions are very sensitive and selective. A recently published review deals with the enzyme inhibition method using selected enzymes and substrates in different combinations [26].

Pesticide detection by enzyme inhibition can be thought of as fluorescent detection of the compounds. After hydrolysis, the enzyme converts the substrate into a fluorescent derivative so the background becomes fluorescent and the inhibition spots of the pesticides develop. Changing the substrates and the esterases can enhance the sensitivity. Some enzymes, such as acetylcholine esterases in pig liver, human plasma, horse serum and beef liver, with substrates such as indoxyl acetate, 5-bromoindoxyl acetate and butyrylthiocholine can give very sensitive and selective detection with nanogram per spot or higher sensitivity. OP insecticides and carbamate-type compounds were detected by this method [18].

Some other biological detection methods should be mentioned here. Inhibition of the Hill reaction is useful in the detection of photosynthesis-inhibiting herbicides. This procedure gives as high a sensitivity for herbicide detection as the acetylcholine esterase-inhibiting insecticide tests [18].

Some fungicide tests were published earlier which offer very sensitive detection for compounds having fungicidal activity. Culvularia lunata, Phytium ultimum, Cladosporium cucumerium and numerous other fungal strains were applied in these tests [18].

Pesticides can be revealed by liquid crystals according to recently published results. The developed TLC plates were covered with a porous foil which was impregnated with liquid crystals. When the foil was pressed on to the plate, the presence of

pesticides disturbed the structure of the liquid crystals in the foil. This caused a change in the light transmittance of the layer, permitting the determination of the spot areas [27].

3.2. Gas chromatography

The GC analysis of pesticides is of great importance nowadays. Numerous methods have been developed for large numbers of insecticides, fungicides, herbicides, etc., showing very different chemical behaviour [28–30].

Some of the interesting capillary GC (cGC) methods will be discussed here. The earlier recommended AOAC method for the extraction of pesticide residues from non-fatty foods was based on an extraction with acetonitrile or acetonitrile–water. It was modified in 1985 by an acetone extraction. This was given as an official final action method in 1986 [30]. The efficiencies of these methods were checked

by cGC with different detection modes. In fruit samples OP and OC insecticides were extracted by two different methods and determined by cGC using different detectors. The results were checked by GC–MS (Table 3) [31]. Lemon essential oil extracts were obtained either by pressure extraction of the peel half-cups or by excoriation of the whole fruit using an on-line extraction system (Table 3) [32]. According to another method, nine halogen-containing pesticides were determined on a dimethylpolysiloxane-coated capillary column with electron-capture detection (ECD) after extraction and a short-column Florisil clean-up (Table 3) [33]. Rice and soybean samples were studied by cGC in point of the determination of the α-BHC and carbaryl content with ECD and nitrogen–phosphorus-specific detection (NPD) (Table 3) [34]. About 20 pesticides were investigated after three successive extractions in fruit and vegetable products from Spain (Table 3) [35].

TABLE 3

GAS CHROMATOGRAPHIC DETERMINATION OF PESTICIDES

Compound	Sample	Extraction	Clean-up	Recovery (%)	Detection	Determination	Ref.
Dimethoate Lindane Fenitrothion Malathion Chlorpyrifos Methidathion Tetradifon Tetradithion Phenthoate	Fruit	(1) CH_3CN–H_2O (2) Acetone (3) Acetone– MeOH (4) CH_3CN, Na_2SO_4	Partition Sep-Pak C_{18}	(1) 82–140 (2) 87–129 (3) 81–130 (4) 81–129	ECD NPD FPD GC–MS	Chromosorb W SPB-5 cGC	31
Parathion-methyl Parathion-ethyl Methidathion Quinalphos Diazinon Fenitrothion Malathion Bromophos-ethyl	Lemon oil	Pressure extraction On-line extraction system			NPD	cGC	32
Chorpyrifos Dichlofluanid Dichloran Endosulfan γ-HCH Procymidon Vinclozolin	Pepper Cucumber	EtOAc Na_2SO_4	Florisil	>80	ECD	Dimethylpoly- siloxane cGC	33

TABLE 3 *(continued)*

Compound	Sample	Extraction	Clean-up	Recovery (%)	Detection	Determination	Ref.
α-BHC Carbaryl	Rice Soy bean	Acetone–MeOH	Bio-Beads S-X₃ Cyclohexyl- amine– CH₂Cl₂ (1:1)	>83 >81	ECD NPD	BP-1 cGC	34
19 Pesticides	Melon Sweet pepper Cucumbers Lettuce Zucchini					HP-17 BP-1 SPB-1	35
C-, P-, Cl-, F-, N-, S-containing pesticides Cl-, F-, P-containing pesticides	12 Agricultural commodities		No clean-up		AES ECD, ED, NPD, FPD	cGC	36
Bromoxynil	Onion	80% EtOH NaOH, hydrolysis	NaCl satd. soln. Diethyl ether Florisil	94–117	Derivati- zation CH₂N₂	HP-1 cGC	37
Triadimenol	Fruit Cereal	Acetylation	Florisil Light petro- leum–diethyl ether (96:4) Light petro- leum–EtOAc (3:2)	83–96	TID	5% OV-101 Chromosorb W HP	38
Paraquat Diquat	Potatoes Rapeseed	Deriv. NaBH₄	Partition	86–100	NPD GC–MS	5% Apiezon L 3% KOH on Interton Super	39
Ethoprop	Mint hay Oil Spearmint Peppermint	Hexane	Charcoal Florisil		GC GC–MS	7% OV-17 Chromosorb W HP DB-5 capillary	40
Carbaryl Captan Dichloran Dimethoate Methamidophos Phosmet	Apple Peach Tomato Potato			73–120	GC–CI-MS	DB-1 Methyl fused silica	41
Dicamba	Dried tobacco	Hexane–diethyl ether (1:1)	Derivati- zation		On-line LC–GC UV ECD	LC Spherisorb S-5-W OV-16-OH ID fused silica	45
OC	Butter	Melting Na₂SO₄			LC–GC ECD	Biol-Sil ODS-10 Capillary SE-52	46

The applicability of detectors were compared with atomic emission spectrometric (AES) detection in the GC of twelve agricultural products. The extracts were prepared according to the procedure of the California Department of Food and Agriculture and no clean-up was used. AES was used in the C-, P-, Cl-, N- and S-selective modes and showed higher selectivity in the determination of chlorine-, fluorine- and phosphorus-containing pesticides than other detection methods (Table 3) [36].

The selectivity of determination can be enhanced by derivatization. An extract of bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) was first hydrolysed and subsequently converted with diazomethane into its methylated form. The derivative-containing extract was cleaned up on a Florisil column and determined by cGC (Table 3) [37].

The fungicides triadimenol [1-(4-chlorophenoxy)-3, 3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanol] and bitertanol   [1-(biphenyl-4-yloxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanol] can be determined in acetylated form after extraction. The derivative-containing sample was passed through a Florisil column and determined by GC (Table 3) [38].

Diquat and paraquat are rapid-acting herbicides. These compounds were determined in potatoes and rapeseed after conversion into their volatile derivatives by hydrogenation with sodium tetrahydroborate. Another possibility is the dequaternization of these bipyridinium herbicides by pyrolysis. This approach was followed in GC-MS studies of biological samples (Table 3) [39].

Ethoprop was determined in mint hay and oil by GC. Ethoprop residues were present in the oil samples in 100-fold higher concentrations than in the mint hay according to GC-MS studies (Table 3) [40]. Carbamate and OP residues were investigated by cGC-MS method. Twelve pesticides and two metabolites were determined in 25 different samples each of four different food materials. A computer program allowed a search for several hundred target ions. This cGC-CI-MS methodology can be applied with convenience in routine analyses by regulatory agencies (Table 3) [41]. OP insecticides were measured in biological samples without any interferences [42]. A review on monitoring pesticides in food, feed and environmental samples has been published [43].

The first example of coupled LC-GC in pesticides determinations was reported for atrazine in 1987 [44]. More recently dicamba was studied in dry tobacco samples by on-line coupled HPLC-GC. In this procedure no liquid-liquid partitioning is necessary. After simple extraction, the dicamba residue is converted with diazomethane into its ester. Normal-phase HPLC serves for clean-up and the final determination is carried out by GC. The main problem here is the concurrent evaporation of the eluent during transfer of the extract between the two systems. This problem was overcome as reported (Table 3) [45]. OC pesticides were determined in fat samples by this on-line HPLC-GC method (Table 3) [46].

Another technique was reported for the determination of 30 polychlorinated biphenyl congeners using two capillary columns with non-linear multi-level calibration. The capillary columns, which were operating with parallel coupling, exhibited different polarities. Various seal extracts were analysed in this way [47].

3.3. Supercritical fluid chromatography

The SFC method is very useful when GC and HPLC are inappropriate [48]. In addition, more effective separations can be achieved with SFC than with GC from the point of view of the number of effective plates or separation speed. Modified mobile phases used as supercritical fluids and packed or capillary columns give a choice for separations of pesticides of different natures. The best detection method is MS, which has high selectivity and sensitivity. The determination of less volatile analytes, e.g., labile insecticides and herbicides, is the most interesting application of SFC-MS. Some examples of this rapidly developing method used in pesticide analysis will be discussed here.

A splitless injection method was developed for interfacing microbore or high flow-rate capillary SFC with MS detection [48]. Eight pesticides were examined on a microbore column with carbon dioxide elution. The polar pesticides could be separated with much better efficiencies when 1% of methanol was added to the carbon dioxide mobile phase. Peak tailing was eliminated and retention was also reduced (Table 4) [48].

A high flow-rate interface for SFC-MS determination of OP insecticides has been published recently. This interface allows a pressure-programmed

separation on microbore HPLC columns. For analysis for thermally unstable compounds of high molecular mass and low volatility, a lower temperature separation method was necessary. SFC–CI-MS was very useful in the determination of eight OP insecticides. These compounds were determind on an amino-phase microbore column. Better resolution was obtained when 2% (v/v) of 2-propanol was added to the carbon dioxide mobile phase on the column mentioned above. A C_{18}-bonded non-polar phase column gave less effective separations of these OP compounds under the same analytical conditions, e.g., pressure programming and addition of 2-propanol to the mobile phase (Table 4) [49].

Eight non-volatile triazine and triazole herbicides were also investigated. A modified HPLC system with UV detection was used, eluting first with carbon dioxide alone, but it was found that a much better separation was achieved with gradient elution using carbon dioxide containing 2.4–33% of methanol (Table 4) [50].

Packed capillary SFC of OP insecticides with phosphorus-selective detection was reported recently. The carbon dioxide eluent was modified with methanol or 2-propanol, leading to the determination of the OP insecticides in onion and tomato samples. Linearity was obtained over a four orders of magnitude range (Table 4) [51].

Several thermally labile pesticides (ureas and OP compounds) were measured by capillary column SFC without any modification of the mobile phase using ECD. Picograms and high femtograms were given as detection limits for nitro- and halogen-containing pesticides, respectively [52].

3.4. High-performance liquid chromatography

HPLC is suitable for compounds having low volatility or those which are thermally unstable. HPLC is just as important as GC in pesticide residue analyses. Some recently published methods for analyses for fungicides, herbicides, insecticides and growth regulators are summarized here.

A method was published for the determination of four fungicides in must and wine samples. The

TABLE 4

SUPERCRITICAL FLUID CHROMATOGRAPHY OF PESTICIDES

Compound	Sample	SFC conditions	Column	Detection	Ref.
Alachlor BMPC Propachlor Propoxur Linuron Carbofuran Carbaryl Diuron		CO_2 CO_2–MeOH (1%) 50–100°C 400–450 bar	Microbore C_{18} silica Capillary (SE-54)	SFC–CI-MS	48
Chlorpyriphos-methyl Chlorpyriphos Iodofenphos Leptophos Methidathion Tetrachlorvinphos Phosmet Famphur		CO_2, 75°C, 410 bar 25 bar/min. 2% 2-propanol–CO_2	Amino phase Microbore	SFC–MS	49
Triazine Triazole Herbicides	Cherries	CO_2–MeOH, gradient elution (2–33%)	Deltabond Cyanopropyl	SFC UV	50
Phoxim Dimethoate Azinphos methyl	Onion Tomato	CO_2–2-propanol CO_2–MeOH	Packed Capillary	SFC TID	51

degradation process of these fungicides was followed during the winification process. Sample preparation is very simple: the must and wine samples are extracted with benzene and the organic layer is evaporated to dryness, the residues being analysed by RP-HPLC. Water–methanol eluents gave reproducible results when buffer was applied; an acetonitrile–water gradient system gave reproducible results without buffers. During the alcoholic fermentation, carbendazim and metalaxil levels decreased. In red wine extracts interfering co-extractives were observed (Table 5) [53].

A multi-residue method for fungicides in fruit and vegetables involving solid-phase extraction (SPE) was published. Fungicides eluted from an SPE cartridge were determined by GC or HPLC. The rise in the baseline was due to captafol decomposition under GC conditions. More reproducible results were obtained when determination was carried out by HPLC. UV detection was affected by some interferences but fluorimetric detection gave reproducible results for several samples (Table 5) [54].

Bensulfuron-methyl residues in rice grain and straw samples were investigated by HPLC with photoconductivity detection. Different sample preparation methods were published for grain and straw samples (Table 5) [55]. The qualification of feed samples is equally important. A method was published for hexazinon and monuron residues in alfalfa tissues. The recovery was checked after each sample preparation step. The best recoveries were obtained according to the method given in Table 5. For HPLC analysis a precolumn was also applied [56].

Cyanazine and bentazone herbicide residues in sugar maize and surface water were examined using HPLC and on-line clean-up column switching (Table 5) [57]. Phenoxy acid herbicides were determined in water samples. The compounds studied were converted with 9-anthryldiazomethane into their derivatives, which were detected with a fluorescence detector. The recoveries were > 95% (Table 5) [58].

Some recently published HPLC methods deal with the analysis of insecticides in food samples. Carbamate (carbaryl) in fruit juice samples can be determined by HPLC. Residues were collected on an SPE cartridge (Table 5). However, the fruit matrix does not allow detection limits for carbaryl as low as in water, being at the low-ppb (parts per 10^9) level [59].

Flufenoxuron, a slow-acting growth regulator, was determined in apples and kiwi fruit by HPLC. Residue recoveries were 81–117% (Table 5) [60]. Ethiofencarb, a systemic insecticide, and its oxidative metabolites (sulphoxide and sulphone) were identified in lettuce by HPLC with 90–103.1% recoveries. The method is simple and does not need any derivatization [61].

HPLC combined with thermospray mass spectrometry (TSP-LC-MS) in the positive- (PI) and negative-ion (NI) modes was used for the determination of six pesticides and their photodegradation products in water samples. The pesticides were representatives of carbamates, chlorotriazines, phenylureas and OP compounds. A combination of the PI and NI modes allows the identification of numerous photodegradation products (Table 5) for different pesticides. Some of the degradation products could be detected only in the PI or NI mode, so for precise information on toxic metabolites or degradation products both detection methods should be used (Table 5) [62].

4. CONCLUSIONS

Recently published methods for the determination of pesticide residues have been reviewed. The most important chromatographic sample preparation and determination methods have been discussed. Newer chromatographic methods applied in pesticide residue determinations such as solid-phase extraction and supercritical fluid chromatography are becoming increasingly important. Gas chromatography and high-performance liquid chromatography provide the basis of numerous determination methods alone or in combination with very sensitive and selective detection methods such as mass spectrometry. Thin-layer chromatography combined with densitometry is also applied as an alternative method because of its simplicity.

5. ABBREVIATIONS

GC	Gas chromatography
cGC	Capillary gas chromatography
SPE	Solid-phase extraction
HPLC	High-performance liquid chromatography
TLC	Thin-layer chromatography

TABLE 5

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF PESTICIDES

Compound	Sample	Extraction	Clean-up	HPLC column	Eluent	Detection	Ref.
Carbendazim Metalaxyl Folpet Propiconazole	Must Wine	Benzene	No clean-up	Spherisorb ODS-1 (C$_{18}$)	CH$_3$CN–H$_2$O gradient	UV	53
Dichloram Chlorothalonil Vinclozolin Triadimefon Anilazine Captan Folpet Procymidone Iprodion o-Phenylphenol Biphenyl	Grape Apple Tomato Pear Cucumber Strawberry Orange Potato	Acetone SPE (C$_{18}$) (Supelco)	MeOH–H$_2$O (40:60)	Bondapak (C$_{18}$)	MeOH–buffer (60:40)	Fluorimetric	54
Bensulfuron-methyl	Rice Grain	CH$_2$Cl$_2$	C$_{18}$ Bond Elut (SPE)	Zorbax Sil	Hexane–isopropanol–MeOH–CH$_3$CN–CH$_3$COOH–H$_2$O (750:125:100:25:2:1)	Photoconductivity	55
	Rice straw	0.5% CH$_3$COOH CH$_2$Cl$_2$	Several partition steps		As above (720:110:110:55:2:1)		
Hexazinone (monuron)	Freeze-dried alfalfa	H$_2$O–MeOH	Hexane, CHCl$_3$	Precolumn CO:Pell ODS-18 R-Sil C$_{18}$	MeOH–H$_2$O (1:1)	UV, 254 nm	56
Bentazon Cyanazine Two metabolites	Sugar Maize Surface water	CH$_2$Cl$_2$ 6 M HCl CH$_2$Cl$_2$–CH$_3$CN		RP-18 Hypersil ODS	MeOH Phosphate buffer	UV, 299 nm	57

Compounds	Sample	Extraction	Cleanup	Column	Mobile phase	Detection	Ref.
2,4-D MCPA MCPP MCPB	Water	Hexane–EtOAc (20:80)	Silica gel Hexane–EtOAc (95:5), derivatization	RP TSK-gel ODS-120T	CH_3CN–H_2O–THF (72:25:3)	Fluorescence	58
Carbaryl	Apple Cherries Pineapple Orange Banana Cranberry Grape Juice, etc.	C_{18} Sep-Pak CH_3CN–H_2O		Ultramex C_{18}	MeOH–H_2O–CH_3CN (40:45:15)	UV, 224 nm	59
Flufenoxuron	Fresh fruit Apple Kiwi fruit	MeOH–H_2O	MeOH–hexane	Zorbax ODS HPLC RP-8	CH_3CN–H_2O (74:26)	UV, 254 nm	60
Ethiofencarb Ethiofencarb sulphoxide Ethiofencarb sulphone	Lettuce	CH_2Cl_2		RP-18 C_8–H	H_2O–CH_3CN (1:1), (6:3) (7:3), (6.5:3.5)	UV, 190 nm	61
Aldicarb Carbaryl Linuron Fenitrothion Cyanazine	Water			LiChrospher 100 RP-18 Spherisorb ODS Polyglosil 500-7	MeOH–H_2O (1:1) + 0.05 M ammonium acetate MeOH–H_2O (7:3) + 0.05 M ammonium formate	TSP-LC–MS, PI, NI	62
Parathion-methyl				C_{18}	CH_3CN–H_2O (1:1) + 0.05 M ammonium acetate		

OPTLC	Overpressure thin-layer chromatography
AES	Atomic emission spectrometric detection
AMD	Automated multiple development
ECD	Electron-capture detection
ED	Electrochemical detection
NPD	Nitrogen–phosphorus-selective detection
FPD	Flame photometric detection
TID	Thermionic detection
CI-MS	Chemical ionization mass spectrometry
EI-MS	Electron impact mass spectrometry
TSP-LC–MS	Thermospray-liquid chromatography–mass spectrometry
PI	Positive ion mode
NI	Negative ion mode
OC	Organochlorine
OP	Organophosphate
DBI	2,6-Dibromobenzoquinone N-chloroimine
TBI	N,2,6-Trichlorobenzoquinone N-chloroimine
NBP	4-(4′-Nitrobenzyl)pyridine
EtOAc	Ethyl acetate
EtOH	Ethanol
MeOH	Methanol
THF	Tetrahydrofuran

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