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APPLICATION OF MULTIRESIDUE PROCEDURES IN PESTICIDES RESIDUES ANALYSIS

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Application of multiresidue procedures in pesticides residues analysis

Abstract - The fields of application of multiresidue procedures and the proper selection of compounds and samples to be analysed are discussed. The various processes of well established multiresidue procedures are reviewed and critically assessed in order to give guidance for the analysis of compounds being not included in current methods and to help laboratories to improve their own methodology.

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1 INTRODUCTION

The introduction of new pesticides, the extension of the activities of the field of residue analysis and the increasing requirements regarding time, cost and safety necessitate the regular revision and improvement of the analytical methods available. The main objective of the use of multiresidue procedures (MRPs) is to determine as many pesticides of different chemical structure as possible in various types of samples of known or more often of unknown origin in a single procedure.

The analysis of pesticide residues in samples of unknown origin consists of two phases: (1) rapid screening of residues possibly present, (2) identification (confirmation) and quantification of residues present. The requirements regarding the processes applied in the two phases of the determination may differ significantly. For example at the screening stage a recovery value as low as 30% may be acceptable if the overall sensitivity of the method enables the indication of the residue exceeding a certain limit, while for the quantitation the method is generally considered satisfactory if the recovery is over 70-80% with a relative standard deviation of 10% for repeatability.

The limits over which the residues should be detected depend on the purpose of the analysis and should be determined on a case by case basis. For quantitation of those components for which the recovery is lower than 70% an additional analysis with a specific method may be considered necessary if an accurate result is required (e.g. the residue detected approaches the legal limit).

The proper application of MRPs requires knowledge of extractability of different compounds from various types of samples, the distribution properties in solvent systems of different polarity, elution patterns in column chromatographic systems, the loadability and selectivity of chromatographic separation and the specificity and sensitivity of detection. Information obtained on compounds which are not extractable, not recoverable from purification steps, or not detectable is as important as the positive results in this regard.

For the determination of pesticide residues such a multitude of methods has been described that a complete survey of literature is very difficult for the analyst. However, by far the majority of these methods consist of a few working steps such as extraction with a

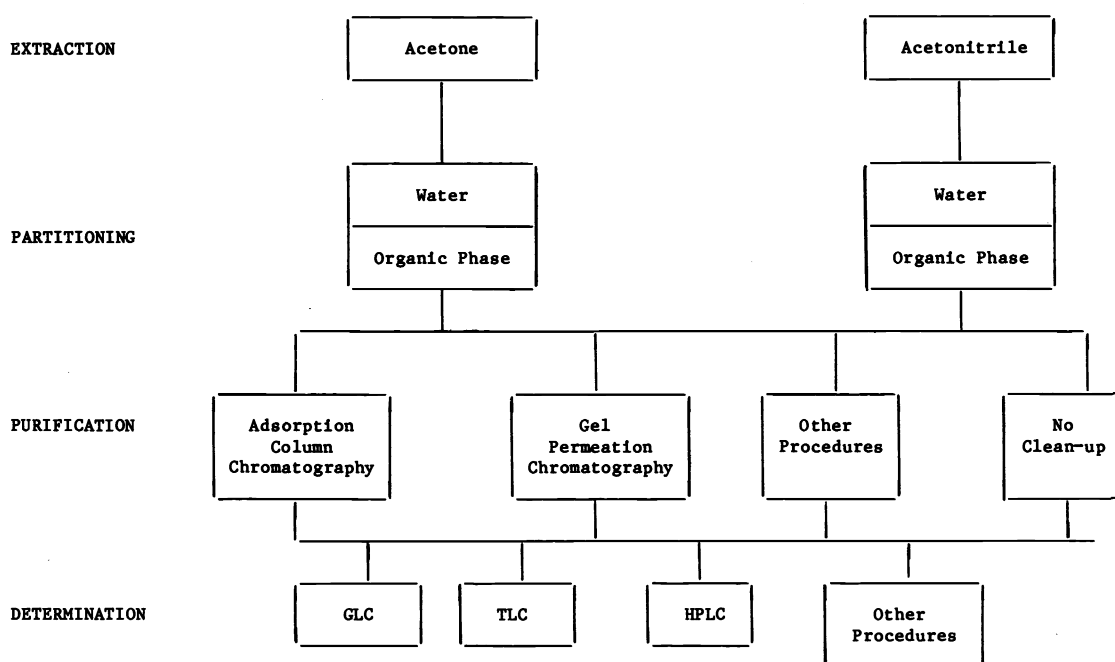

FIGURE 1. Very simplified scheme of the working steps in analysis of plant material

limited number of solvents, liquid-liquid partitioning, adsorbent column chromatography or gel permeation chromatography and the determination, mostly with GLC, TLC, or HPLC. For the various pesticide groups they differ only in some details (e.g. amount and activity of adsorbents, composition and polarity of solvent mixtures) selected according to the attributes of the residues to be analysed and the co-extractives to be separated. For this reason, an experienced analyst can modify the procedures by interchanging or modifying the individual steps in order to get the best result for the given purpose of the analysis.

The objective of this paper is to give some guidance on the application of the various processes used in the general scheme of MRPs (Figure 1) by comparing some well established methods covering a wide range of pesticides.

2 FIELDS OF APPLICATION OF MULTIRESIDUE PROCEDURES (MRPs)

The regular analysis of pesticide residues in various substrates is necessary to ensure the safe and efficient use of the parent compounds and to protect the consumers and the environment.

The main areas of residues analysis are:

(i) Disappearance studies following the application of individual compounds (supervised trials);

(ii) (determination of pesticide residue content of raw agricultural commodities moving in commerce;

(iii) determination of pesticide residues in food prepared for human consumption (total diet studies);

(iv) identification and quantitation of residues in environmental elements.

In supervised trials, mainly specific methods are used. These should be suitable for the determination of the quality and quantity of the parent compound and its major metabolites and/or degradation products individually in order to provide the necessary data for the establishment of Maximum Residue Limits (MRLs) (1).

In total diet studies and in certain environmental monitoring programmes all residue components of toxicological importance should be determined regardless of the way MRLs are expressed (metabolites may or may not be included). Therefore, in addition to the MRPs, which are mostly suitable for the determination of parent compounds only, specific methods are also needed for the analysis of selected metabolites.

Multiresidue procedures are the preferred methods for the analysis of samples derived from selective field surveys providing samples of known origin (2) or taken from commodities of unknown origin which generally form the lots moving in commerce. The residues included in the MRLs have to be analysed in these two situations as the main objectives of these studies are either the enforcement of MRLs or the collection of information on the residues in food items in relation to the MRL. The latter information may be used for the assessment of possible maximum exposure of consumers to pesticide residues or for setting up priority lists of pesticides and sampling plans for commodities which should be checked preferentially.

However, there is no single multiresidue method which could cover the wide variety of chemical-sample matrices which need to be analysed for regulatory control. In addition there are many compounds which cannot be recovered by MRPs at all; consequently individual methods have also to be used for the determination of their residues. Positive results should be confirmed in each case bearing in mind the various sources of possible interferences. Every analyst should be aware of the applicability and limitations of the methods used concerning the type of samples and compounds involved. In order to select the most suitable procedures, to provide the widest information on the samples within the shortest time and/or at the lowest cost, the number of active ingredients and metabolites to be analysed should be limited by careful selection.

3 SELECTION OF COMPOUNDS AND COMMODITIES FOR REGULATORY ANALYSIS

The protection of plants and harvested crops requires a wide range of active ingredients, the number of which exceeds 200 in many countries. As one pesticide may be used in/on several crops the number of possible pesticide/crop combinations is often over 1000. The Codex Committee on Pesticide Residues has recommended Maximum Residue Limits or is considering proposals for over 2000 pesticide residue/food combinations regarded as important in international trade (3). All these and many additional ones may have to be considered in imported commodities.

Based on the results of a large number of previous analyses and on other considerations, several countries have set up priority lists of compounds to be looked for in regulatory analysis. Some of the compounds detected most often or included in priority lists are given in Appendix 1. The number and kind of active ingredients registered or used in various countries varies widely, due to the different climatic and economic conditions, pest situation and growing practices. Therefore each country or group of countries having similar conditions needs to define their own priority lists. On the selection of compounds to be included in a priority list for regulatory analysis, information on the extent of practical application, the frequency of occurrence and level of residue in samples marketed and the toxicity of the residue has to be taken into account.

The primary source of information on the level and the behaviour of the residue in the commodities are the reports on supervised trials carried out before registration. A great number of these and other data are collected in the series of "Pesticide Residues in Foods, Evaluations" published by FAO yearly (4). The monographs on individual compounds summarize the most essential toxicological and residue data, metabolic pathway and evaluations of the results. Those data obtained from trials where the rate of application, the growing stage and the preharvest intervals were close or similar to the use patterns registered should be considered first.

The limited number of supervised trials are mainly concentrated on major crops and varieties and designed to indicate the probable maximum residues level at the time of harvest. Consequently, the data from such trials cannot generally give sufficient information on the residue distribution in lots treated on a large scale under average farming practice conditions. The exceptions are those compounds which can only be applied presowing or at the beginning of growing season and the use of which does not result in measurable residues at the time of harvest. For these compounds the results of supervised trials provide sufficient information and further regulatory control is usually not necessary.

For the other compounds the residue patterns reflecting the practical use and weather conditions have to be determined during the first few years after the commencement of the large scale use. Therefore, all these compounds should be included in the priority list and should be regularly checked in all commodities in which their use is permitted or proposed.

Having collected sufficient information on the residue distribution further selection can be made. It is proposed that the priority list should include compounds which

- are sold in large quantities and used extensively;

- may be applied close to harvest or marketing;

- lead to residues disappearing at an apparent half life of 4 or more days;

- themselves or their residues are highly toxic;
- lead either to residue levels at or over MRL with a frequency exceeding 2%, or to detectable residues in 20% or a higher percentage of the samples;
- are preferred by farmers and, either their use might be extended to those crops excluded from the registered uses or they might be applied closer to harvest than the pre-harvest intervals established permit (possible misuses).

In devising the sampling plans higher priority should be given to crops requiring intensive plant protection (e.g. apple, citrus fruits, tomato etc.) and which form an appreciable part of the diet.

4 PREPARATION OF SAMPLES FOR ANALYSIS

4.1 Grouping of samples

Differences in the material and the texture of the samples necesitate somewhat different processes for the extraction and especially for the cleanup of the concentrated extracts. Several methods classify samples for extraction into three groups: samples of medium and high water content, dry samples and fatty samples. Within the first group some methods (5, 6, 7) distinguish the samples having 5 to 15% or 15 to 30% sugar content. A more recent approach also takes into account the different cleanup requirements (8). From the samples of medium and high water content three sub-groups are proposed: root and bulb vegetables (e.g. carrots, onions); fruits and vegetables of low chlorophyll content (e.g. pome and stone fruits, berries, fruiting vegetables, citrus fruits); plants and crops of high chlorophyll content (e.g. leafy and legume vegetables). The universal applicability of gel column chromatography as a pre-cleanup step for different samples and compounds permits identification of fatty and non-fatty classes of samples only (19 h).

TABLE 1. Some clean-up procedures used for analysis of organochlorine (OC) and organosphosphorus (OP) pesticides in plant samples

First author	Pesti-cides	Anal. sample (g)	Extraction (ml)	Ali-quot	Dilution (ml)	Parti-tioning	Column Chromatography	Elution (ml)
AOAC (5a), PAM (6a) (Mills)	OC, OP	100	200 Acnit	-	600 W, 10 NaClsat.	100 PE	10 cm Florisil (2 cm i.d.)	200 PE/Eth 94+6 200 PE/Eth 85+15 200 PE/Eth 50+50
AOAC (5b), PAM (6b) (Storherr)	OP	100	200 Acnit	1/10	-	30 Dichlm	6 g C/MgO/Celite 1+2+4	120 Bz/Acnit 1+1
Panel (18)	OP	20	3x50 Acnit (2.5%)	-	500 Na_2SO_4	3x50 Dhlfm	-	-
Becker (19a)	OC, OP others	100	200 Ac	1/5	250 W, 25 NaClsat.	2x50 Dichlm	15 g Silica gel + 1 g C, 5 g Silica gel	140 Dichlm/Tol/Ac 10+2+2
Ambrus (8)	OC, OP others	50	150 Ac	-	450 Na_2SO_4 (0-4%)	100, 2x50 Dichlm	7 g C/MgO/Cel. 1+2+4	150 Dichlm
							8 g Alumina N (19% W)	30 Hex 30 Hex/Eth 7+3
							25 g Alumina B (16% W)	80 Hex 75 Hex/Eth 2+1
Luke (13,20)	OC, OP, others	100	200 Ac	80 ml	-	200 PE/Dichlm 1+1, 2x100 Dichlm	(10 cm Florisil, 2 cm i.d.)	(200 PE/Eth 85+ 15)
Specht (19j)	OC, OP others	100	200 Ac	200 ml	-	100 Dichlm	32 cm Bio Beads S-X3	175 Cyclh/EtAc 1+1
Ebing (19b,c)	OP	100	200 Ac	1/5	250 W, 35 NaClast.	2x50 Dichlm	375 ml Sephadex LH-20	450 Ethanol
Ebing (19d,e,f)	OC, OP	100	200 Ac	1/5	250 W, 35 NaClsat.	2x50 Dichlm	Sweep-Co-Distillation	
AOAC (5c),	OC, OP	25	125 EtAc	-	-	-	Sweep-Co-Distillation	
Watts (18,21)	OP	50	250 EtAc	-	-	-	10 gC/MgO/Cel. 1+2+4	150 Tol/Ac/EtAc 2+1+1

It should be emphasized that none of the subgroups is sufficiently uniform for the various samples to be treated similarly at cleanup. Depending on the method of detection some of the materials require additional procedures in order to remove interfering constituents. For instance the sulfur-containing compounds which interfere with the ECD determination must be removed from extracts of onion, leek, certain cabbages etc. with a specific procedure such as the silver nitrate/aluminium oxide column chromatographic method (9).

4.2 Preparation of portion of sample to be analysed

An analytical sample is that part of a laboratory sample which is analysed to provide information on the quality (the residue content) of the entire sample. The analytical sample has to be a fully representative portion of laboratory sample or a certain part of it depending on the purpose of the analysis. Thus the first step in the preparation of an analytical sample is to separate the appropriate portions of individual items to be analysed from the laboratory sample. As the residues are unevenly distributed in/on crops the portion of sample selected for the analysis should always be consistent if comparable results are sought. In order to enable the results obtained in various countries to be widely utilized the procedures recommended by the Codex Committee on Pesticide Residues (10,11) should be used as far as applicable for the objective of the analysis.

5 EXTRACTION AND LIQUID-LIQUID PARTITIONING

In the case of MRPs the extracting solvent must be suitable for the extraction of compounds with a wide range of polarity from various matrices containing different amount of water, fat, sugar and other substances. In order to provide suitable conditions for the transfer of residues from the samples to the extracting solvent the analytical sample needs to be disintegrated in high speed homogenizers or choppers in the presence of one solvent or a solvent mixture. The type of solvent and the blender used in extraction may influence materially the efficiency of extraction. The differences caused by the use of various equipment can be reduced by shaking the solvent sample mixture for an hour after homogenization (8). For the extraction of systemic and contact pesticide residues simultaneously the differences in the hydrolytic stability of the compounds, especially the sensitivity of some contact pesticides, have to be considered (12).

In MRPs the most widely used solvents are acetonitrile and acetone. Both ae miscible with water, consequently the actual extracting agent is their mixture with the water derived from the sample. Methods based on the extraction of plant materials with acetonitrile and acetone are summarized in Table 1. The cleanup procedures mainly used for fats are given in Table 2. Table 3 lists the compounds reported to be covered by the most widely used methods.

TABLE 2. Some clean-up procedures used for analysis of organochlorine pesticides in fats or lipid extacts from fatty foods

First author	g fat max.	Extraction, Partitioning	Column Chromatography	Elution (ml)
ADAC (5a), PAM (6d)	3	PE/Acnit	10 cm Florisil (2 cm i.d.)	200 PE/Eth 94+6 200 PE/Eth 85+15
de Faubert Maunder (22)	5	Hex/DMF	5 g Alumina N (7% W)	50 Hex
Specht (19h)	5	PE/DMF	30 g Florisil (5% W)	200 PE/Eth 94+6
Wood (23)	1	Column extr. with DMSO	5 g Alumina N (7% W) + 5 g Florisil (15% W)	100 Hex
Schulte (24)	1.5	Column extr. with Propc	10 g Alumina N (17.5% W)	30 PE
Stijve (19i)	1	(Soln in PE)	25 g Florisil (3% W)	300 PE/Dichlm 8+2
Telling (25,26)	0.5	(Soln in Hex)	22 g Alumina N (10% W)	150 Hex
Steinwandter (27,32)	0.5	(Soln in PE)	20 g Silica gel (30% W)	250 PE
Specht (19j)	0.3	(Soln in GPC solvent	32 cm BioBeads S-X3	100+65 Cyclh/EtAc 1+1
Stijve (28)	0.1	(Soln in PE)	3 g Florisil (3% W)	30 PE/Dichlm 8+2
Greve (29)	0.05	(Soln in Hex)	2 g Alumina B (10% W)	15 Hex

Notes: N = neutral, B = basic

Abbreviations for solvents used in all tables:

Ac : Acetone
Acnit : Acetonitrile
Bz : Benzene
Chlfm : Chloroform
Cyclh : Cyclohexane
Dichlm : Dichloromethane
DMF : Dimethylformamide
DMSO : Dimethylsuphoxide
EtAc : Ethyl acetate
Eth : Diethyl ether
Hex : Hexane
Meth : Methanol
PE : Petroleum ether
Propc : Popylene carbonate
Tol : Toluene
W : Water

Bz can be mostly replaced by Tol, Chlfm by Dichlm.

TABLE 3. Applicability of multiresidue procedures

Explanation of columns 1–7 in Table

1	AOAC/FDA:	Acnit/water/PE, Florisil, PE/Eth mixtures, 3 eluates
2	AOAC/FDA:	Acnit/water/PE, Florisil, Dichlm/Hex/Acnit mixtures 3 eluates
3	Becker:	Ac/water/Dichlm, Silica/carbon, 1 eluate
4	Ambrus:	Ac/water/Dichlm, mixed adsorbent, 1 eluate
5	Ambrus:	Ac/water/Dichlm, Alumina N, 2 eluates
6	Luke:	Ac/Dichlm, no clean-up (AOAC 1984 + PAM Table 201-I + PAM Section 232.4)
7	Specht:	Ac/Dichlm, GPC, Silica, 5 eluates

Explanation of signs:

Pesticide can be determined according to author's publication, other references or unpublished work:

++	well recovered, >70%	1, 2, 3, 4, 5 :
+	partially recovered, 40-70%	Number of eluate
/	hardly recovered, >40%	applies only to
-	not recovered	procedure 1, 2, 5, 7)

Detectors used:

E	ECD
F	FPD (P-mode)
P	thermionic (P-mode)
N	thermionic (N-mode)
S	FPD (S-mode)
H	Hall Electrol.Cond.Det.
L	by HPLC only
T	by TLC only

Structural analogies indicate that pesticide can probably be determined with the procedure (including column clean-up).

e, f, p, n, s, h	corresponds to detectors E, F, P, N, S, H.
c	from crude extract only (no column clean-up)
g	from GPC eluate only (applies only to procedure 7)
x	probably no recovery

	1	2	3	4	5	6	7
Organohalogen pesticides							
Aldrin	++E1	++E1	++E	++E	++E1	++H	++E1
Brompropylate	++E23	-				++H	++E34
Captafol	/E3	++E3	++E	++E		++H	++E3
Captan	/E3	++E3	++E	++E		++H	++E3
Chlorbenside	++E1	++E1			e	++H	/E3
Chlorbenzilate	++E23	++E1				++H	++E34
Camphechlor	++E1	++E1			e	h	++E1
Chlordane	++E1	++E1			e	++H	++E1
Chlordecone	+E23	-	-			h	++E4
Chlorfenprop-methyl						h	++E23
Chlorfenson	++E2	++E2			e	h	++E12
Chlorfensulphide				++E			e
Chloroneb	++E1	++E2			e	h	++E2
Chlorpropylate	++E23	/E3		++E		h	++E34
Chlorthal			++E	++E	++E1	h	++E2
Chlorthalonil					++E1	++H	++E2
Chlorthiamid							-
Cymoxanil				++E			++4
DDD (TDE)	++E1	++E1	++E		++E1	++H	++E1
DDE	++E1	++E1	++E		++E1	++H	++E1
DDT	++E1	++E1	++E		++E1	++H	++E1
Dichlobenil	+E2	+E2	++E		++E1		++E2
Dichlofluanid			++E			++H	++E23
Dichloran	+E23	++E2		/E	+E1	++H	++E2
Dichlorbenzamid							++E4
Diclofop-methyl						h	++E3
Dicofol	+E12	++E12	++E	++E	+E	++H	++E12
Dieldrin	++/2	++E2	++E		++E1	++H	++E2
α-Endosulfan	++E2	++E2	++E		++E1	h	++E12
β-Endosulfan	++E23	++Ew	-		e	++H	++E2
Endosulfansulfate	++E3	++E2	-			++H	++E2
Endrin	++E2	++E2			++E1	++H	++E2

TABLE 3. (continued)

	1	2	3	4	5	6	7
Organohalogen pesticides (continued)							
Fenson	e	e			e	h	++E2
Fluorodifen						h	e
Folpet	+E23	++E23	++E	++E		++H	++23
Halacrinate							
Heptachlor	++E1	++E1	++E		e	h	++E1
Heptachlor-epoxide	++E1	++E2	++E		++E1	++H	++E12
HCB	++E1	++E1	-		e	h	++E1
α-HCH	++E1	++E1	++E	++E	++E1	++H	++E1
β-HCH	++E1	++E1	++E	++E	++E1	++H	++E1
γ-HCH (Lindane)	++E1	++E1	++E	++E	++E1	++H	+EE1
δ-HCH	++E12	++E1				h	++E1
Isobenzan	e	e			e	h	++E1
Isodrin	++E1	++E1			e	h	++E1
Kelevan							++E34
Methoxychlor	++E1	++E2	++E	++E	++E1		++E2
Mirex	++E1	++E1			e	++H	++E1
Nitrofen	++E2	++E2			++E1		++E12
Nitrothal-isoprop.				++E	++E1		+E234
Oxyclordane	++E1	++E1					++E12
Pentachloranilin	++E1	++E1	-				++E1
Perthane	++E1	++E1	++E		e	h	++E12
Tecnazene	++E1	++E1	++E		e	h	++E1
Quintozene	++E1	++E1	++E		e	++H	++E1
Tetradifon	++E2	++E2	++E		++E1	++H	++E2
Tetrasul	++E1	++E1	++E	++E	++E1		++E1
Tolyfluanid			++E				++E23
Binapacryl	++E2	e					++E2
Dinobuton	e			++E	++E1		++E23
Dinocap	++E2	++E2		++E	++E1		++E2
Dinocton							e
Dinofenate							e
Dinoseb acetate				++E			++E2
Benfluralin	++E1	++E2		++E	++E1		++E1
Butralin							e
Fluchoralin							++E12
Isopropalin							++E1
Nitralin	+E3	++E3					++E23
Pedimethalin							++E2
Profluarlin							++E1
Trifluralin	++E1	++E2		++E	++E1		++E1
Organophosphorus pesticides							
Acephate						++	++F5
Azinpohos-ethyl	+P3	++P3	++P			++F	++P3
Azinphos-methyl	-	-	++P	+P	+P1	++F	++P3
Bensulide	++P3	/P3					+E123
Bromophos*	++P1		++P	++P	++P1	++?	++P12
Bromophos-ethyl*	++P1		++P		P		++P12
Butonate				+P	+P1		
Carbophenothion	++P1	+P2	++P		p	++F	+P2
Chlorfenvinphos*	-	-	++P	++P	p	++F	++P34
Chlormephos							++P12
Chlorpyrifos*	++P1	+P2	++P	++P	++P1	++F	++P12
Chlorpyrifos-methyl*	p	p	++P	++P	++P		++P12
Chlorthion*	++P1						
Chlorthiophos*			++P			PPF	++P2
Coumaphos	=	++P3					++gF
Crotoxyphos	-						++F4
Crufomate	-						++F45
Cyanofenphos			++P				++F23
Cycloate				++	++ 12		
Demeton	-					++	++gF
Dialifos	++P2	++P2	+P			++F	++F23
Diazinon*	++P2	++P3	++P	++P	++P1	++	++P3
Dichlofenthion*	++P1		++P				+++12
Dichlorvos*	-	-	++P	++P	+P1	++F	+F34
Dicrotophos	-					++F	++P5
Dimefox							++P5
Dimethoate	-	-	++P	++P	-	++	++P45
Dioxathion	-	+P2	++PO				+++F234
Disulfoton*	+P1	-	++P	++P	++P1		+F2
Ditalimfos			++P	++P	++P1		++P3

TABLE 3. (continued)

	1	2	3	4	5	6	7
Organophosphorus pesticides (continued)							
EPN	++P2	++P2				++F	++FP2
Ethion*	++P1	++P2	++P			++	++P2
Ethoprophos			++P	++P	++P1		++F34
Etrimfos			++P	++P	+P1		++F3
Famophos							++F3
Fenamiphos						++F	++gF
Fenchlorphos*	++P1	++P2	++P		p	++F	++P12
Fenitrothion*	++P2	++P2	++P	++P	++P1	++F	++P2
Fensulfothion	-	-	++P			++F	++F45
Fenthion*	+P12	-	++P	++P	++P1	++F	+F2
Fonofos	++P1	+P2	++P	++P	++P1	++F	++F23
Formothion			++P				++P3
Heptenophos			++P	++P			++F34
Iodfenphos*	p				p		++P12
Isofenphos			++P				++gF
Leptophos	++P1	++P2				++F	++F12
Malathion*	++P23	++P3	++P	++P	+P1	++	++P3
Malaoxon*	-	-	++P			++F	++P4
Menazon							-
Mephosfolan						++F	++F45
Methacrifos				++P			
Methamidophos				—		++	++P5
Methidathion	/P3	++P3	++P	++P	++P1	++F	++P3
Mevinphos	-		++P			++	++P4
Monocrotophos	-	-		++P		++	++F5
Naled*	-	-	++P			++F	++F34
Omethoate	-	-				++F	++P5
Parathion*	++P2	++P2	++P	++P	++P	++	++P23
Paraoxon*	-	-	++P			++F	++P4
Parathion-methyl*	++P2	++P2	++P	++P	++P1	++	++P23
Paraoxon-methyl*	-	-					++FP234
Phenkapton*	++P1		++P	++P	++P1		++FP12
Phenthoate				++P	++P1	++S	++P23
Phorate	++P1		++P	++P	++P1	++	++gF
Phosalone	++P3	++P23	++P			++F	++P3
Phosmet	-	+P3		+P	++P1	++F	++P3
Phosphamidon	-	-	-	++P	-	++?	++F45
Phoxim				++P		++F	++F2
Pirimiphos-ethyl							++F3
Pirimiphos-methyl			++P	/P	++P1		++P3
Profenophos*			++P			++F	++F34
Prothiofos*			++P				++P1
Prothoate							f
Pyrazophos			++P	++P	+P1	++F	++P3
Quinalphos							++F34
Sulfotep	++P12	+P2	++P				++P23
Sulprofos						++F	++gF
Temephos							-
TEPP							
Terbufos	+P1						++gN
Tetrachlorvinphos*	-	-	++P	++P	++P1	++F	++FP34
Thiometon							++gF
Thionazin	+P23		++P			++F	++P3
Triamiphos			-				++FP3
Triazophos			++P			PPF	++P34
Trichloronat*							++P12
Trichlorfon*				++P	-	++F	++F5
Vamidothion							+gF
Pyrethrins, Pyrethroids							
Pyrethrins			++E				++E34
Cypermethrin					++E1	++	++E2
Decamethrin					++E1		++E2
Fenvalerate					++E1	++	++E23
Permethrin					++E1	++H	++E23
Resmethrin							++2
Tetramethrin							++E3

*Pesticide can also be determined by E.

TABLE 3. (continued)

	1	2	3	4	5	6	7
Carbamate insecticides and herbicides							
Aldicarb	x	x	++cL	++T	++T1		
Aminocarb			++cL				
Barban							++EN23
Bufencarb							
Butocarboxim							
Carbanolate							
Carbaryl			++c:	+N	++N1		n
Carbetamide							
Carbofuran				++N	++N12		n
Chlorbufam							++N12
Chlorpropham	++E2	++E2	++cL	++N	++N	++N1	++EN23
Desmedipham				-	++T1		
Dioxacarb				++N	++N2		++N4
Ethiofencarb				++N			
Formetanate							
Landrin							
Mercaptodimethur				++cL			
Methomyl	x	x	++cL	-		++H	
Oxamyl	x	x		-			
Phenmedipham	x	x	++cL	-	++T2		
Pirimicarb				++N	/N3	++	++N4
Promecarb			++cL				
Propham			++cL			++N	
Propoxur			++cL	++N		++N	++N34
Thiofanox							
Butylate				++N	++N12		n
Diallate							++E23
Eptam	+ 2			++N	++N12		n
Ethiolate							
Pebulate							
Sulfallate	++ 1	++ 2					++gE
Thiobencarb				+N	++N1		ns
Vernolate	+ 2						n
Urea herbicides							
Benzthiazuron	x	x					
Buturon	x	x	++cL				++ 23
Chlorbromuron	x	x	++cL	++N			++gE
Chloroxuron	x	x	cl	++N	++N12		++E34
Chlortoluron	x	x	cl	+	++ 12		n
Cycluron	x	x					++N45
Difenoxuron	x	x	cl		++N2		n
Diflubenzuron	x	x					
Dimefuron	x	x					
Diuron	-	-	cl	+N	n		n
Fenuron	x	x	++cL		n		n
Isonoruron	x	x					
Isoproturon	x	x	cl	+N	+N		
Linuron	x	x	cl	++N	++N12	++N	++N34
Methabenzthiazuron	x	x		++N	++N1		++N4
Metmercapturon	x	x		++N	++N2		N
Metobromuron	x	x	++cL	++N	++N12		++N234
Metoxuron	x	x	++cL	-			
Monolinuron	x	x	++cL	++	++ 12		++N34
Monuron	-	-	++cL		n		n
Neburon	-	-	++cL		n		++E4
Triazine herbicides							
Ametryne			++N	-	++N12	++S	++gN
Atrazine	/N3	-	++N	++N	++N2		++N34
Aziprotryne			++N	++N	++N12	s	++gN
Cyanazine	-			++N	++N2		++N4
Cyprazine					n		n
Desmetryn			++N		n	s	+N45
Dipropethryn					n	s	++gN
Methoprotryn			++N		n		++N45
Prometryn	+N3	-	++N	++N	++N12	++S	++gN
Propazine	+N23	-	++N		n		++N34
Simazine	-	-	++N	/N	++N2		++N34
Terbumeton				++N	++N2		n
Terbuthylazine	+N23			++N	++N12		++N34
Terbutryn			++N	++N	++N12	s	++gN
Trietazine					n		++N34

TABLE 3. (continued)

	1	2	3	4	5	6	7
Other pesticides							
Alachlor	-	/E3					++EN3
Allidochlor	-	-					++E3
4-Aminopyridin							
Amitraz							++gN
Anilazine	++E2	++E2					++E3
Benodanil							++gE
Bentazon				-	-		
Benzoylpropethyl	-	-					++E23
Benzoximate							-
Bifenox							++E23
Bitertanol							++N4
Bromacil	-	-	++E				++E4
Bromofenoxim							
Brompyrazon							++N45
Bupirimate			++N	++N	++N		
Carboxin				++	++ 1	+N12	
Chinomethionate					++ 1	++S	++ 23
Chloridazone							++N45
Dazomet							
Dimethachlor			++E				++N34
Diphenamid	-				++ 1		++N34
Diphenylamine							++gN
Dithianon							
Drazoxolon							
Endothal							
Ethirimol							-
Ethofumesate				++	++ 23		
Fenarimol				++	+	++EN4	
Fenazaflor							++E2
Fenfuram							
Flamprop-isopropyl							
Flamprop-methyl							
Fluotrimazol							++EN34
Imazalil							++N5
Iprodione			++E	++E	++E	++	++E3
Isocarbamid							
Isomethiozin							++gN
Lenacil							++N4
Metalaxyl			++N	++			++N4
Metamitron							++N45
Methazole							
Methfuroxam							
Metolachlor							++E34
Metribuzin				++	++ 12	++S	++gN
Molinate				++	/ 1		++gN
Napropamid				++			++N34
Nitrapyrin							++E1
Norflurazon							++E4
Nuarimol				+E	+E		
Oxadiazon	++E2			++	++ 1		++EN3
Oxycarboxin							
Procymidon			++E				++EN3
Propachlor	-				++ 1		++E3
Propanil	-	+E3		++E			++E34
Propargite					++ 1	++S	++E23
Propiconazol				+E			++N45
Propyzamid			++E	+	+ 1	++N	++E3
Pyridinitril							
Rabenzazol							++N3
Terbacil	-	/E23					++N4
Thiochinox							
Triadimefon			++	++	++ 12		++FN34
Triadimenol							++N45
Trichlophenidin							
Triforine							
Vinclozolin			++	++	++ 1	++H	++EN23

TABLE 3. (continued)

Pesticides which cannot be recovered by the multiresidue procedures cited			
Azocyclotin	Bromoxynil	Chlormequat	Amitrole
Cyhexatin	2,4-D	Diquat	Antrachinon
Fenbutatin oxide	2,4-D	Difenzoquat	Aramite
Fentin	Dalapon	Morfamquat	Asulam
Benomyl	Dicamba	Paraquat	Benzadox
Carbendazim	Dichlorprop		Chlorflurenol
Fuberidazol	Dinoseb	Ferbam	Daminozide
Thiabendazol	Dinoterb	Mancozeb	Dodine
Thiophanate-methyl	DNOC	Maneb	Etephon
	Fenoprop	Metam	Flurenol
	Ioxynil	Metiram	Glyodin
	MCPA	Methylmetiram	Glyphosate
	MCPB	Nabam	Guazatine
	Mecoprop	Nema	Maleic hydrazide
	Medinoterb	Propineb	Metaladehyde
	Naphthylacetic acid	Thiram	Methyl bromide
	Naphthylacetamide	Vondozeb	Nicotine
	Pentachlorophenol	Zineb	Piperonyl butoxide
	2,4,5-T	Ziram	Rotenone
	2,3,6-TBA		Sethoxydim
	TCA		Sulphur
			Tridemorph
			Trifenmorph

It should be borne in mind that many of the methods are suitable for the determination of additional pesticides which were not in use at the time of the publication of the methods. Using the tabulated data an estimation can be made on the applicability of certain methods or processes for the analysis of new compounds based on the similarities of chemical structure and physico-chemical properties.

The merit of acetonitrile is that much lipophilic plant material such as fats and waxes is not extracted. The extract therefore contains only a minor load of co-extractives. Disadvantages are high cost, toxicity, difficulties in purification and some difficulty in removing it, if necessary, before the final determination.

In comparison, acetone can be obtained commercially in higher purity grades, is more volatile and can be used with commodities of high sugar content because it does not form a two phase system with water in the presence of sugar (13). However, it yields extracts containing appreciably more co-extracted plant substances which have to be removed during the cleanup.

With both solvents, crude extracts are obtained which contain the water extracted from the plant matrix. The extracts cannot be evaporated directly to dryness, as pesticide residues would be lost via distillation with water. Residues are therefore transferred into a low boiling solvent immiscible with water. The low polarity petroleum ether or the moderately polar methylene chloride are used almost exclusively for this purpose, with or without previous dilution of the crude extract with additional water.

Partitioning with petroleum ether is an excellent cleanup step for analysis of non-polar residues (e.g. organochlorines, PCBs, some low polarity fungicides and herbicides), for only low polarity co-extractives (fats, waxes, carotenoids) are transferred into the petroleum ether with the pesticides.

Partitioning with methylene chloride is much less effective for cleanup, but will be necessary for sufficient recovery of the more polar pesticides which are not soluble in petroleum ether. In more recent methods, the crude extract is not diluted with water, but is diluted with methylene chloride only, yielding the co-extracted water as a separate aqueous layer. This is optimal for maximum recovery of nearly all (even highly water-soluble) pesticides, but has little if any cleanup effect.

Some other solvents have been used, mainly for the extraction of organophosphorus compounds. The major advantage of ethyl acetate (21) and butanol is the fact that one can directly use an aliquot of dried extract because of the limited solubility of water in ethyl acetate. The extraction procedure is therefore extremely quick, no partition step is required and it gives cleaner extracts than with acetone (15). Benzene, methanol (14) and chloroform have also been used in some methods but their use is limited by their toxicity.

Although the efficiency of the extraction with mixtures of water-immiscible and water-miscible solvents is very good, they are not widely used, possibly because of emulsion problems and difficulties in obtaining representative aliquots for the analysis (16).

Techniques for dry crops after they have been ground to powder include extraction with acetonitrile containing 35% water (5,6) or with dichlormethane (8). Powdered material can be efficiently extracted with chloroform/methanol 1+1 in a column after mixing it with Celite 545 but the adoption of the method is limited by the toxicity of the solvents.

Extraction in a chromatographic column was also found to be very efficient for a wide range of compounds and samples with medium and high water content (17). The sample is homogenized with the addition of a small amount of water, if required. A portion of the pulp is mixed with Florisil, activated at 450°C, to provide a constant ratio of Florisil to water of 1.67. The free-flowing dry mixture of sample pulp and Florisil is transferred onto a column over a 5 mm layer of anhydrous sodium sulphate. The pesticides are extracted with 100 ml of dichloromethane/acetone mixture, 9+1. The concentrated extract is clean enough to determine organophosphorus compounds directly with the phosphorus selective TID, although in other cases additionally cleanup is necessary.

For the extraction of acidic pesticides (e.g. chlorphenoxy herbicides or phenols), the use of a slightly alkaline solution is the best. Partitioning from organic phase into a slightly alkaline aqueous solution, acidification and re-extraction into an organic layer is a very efficient tool for separating residues from co-extractives.

The efficiency of extraction is a very important parameter of any method. Special attention should be paid to testing the extraction efficiency for any new compounds which are intended to be analysed by a given method. It must be emphasised that the recovery of a pesticide from fortified samples does not give accurate information on the extraction efficiency and can only be used for testing the percentage loss of the compound added to the sample. The efficiency of extraction may be checked by analysing field-treated samples with a specific method of known efficiency and with the method to be tested.

6 FURTHER CLEANUP

6.1 Adsorbent column chromatography

Most multiresidue methods include a cleanup using adsorption columns, in particular Florisil, alumina and carbon. Reproducible results depend both on the material used and on some external conditions. For example, separation is influenced by quality, quantity and particle size of the sorbent and its activity, the relative humidity of the air, polarity and composition of the eluting mixture, activation or deactivation of the sorbent by the solvents, temperature, nature of the residues and the co-extractives and loading of the column. Most adsorbent columns will achieve a good cleanup only when they are eluted with solvent mixtures of low polarity, eluting less polar residues and leaving more polar co-extractives behind on the column. The more the eluting solvent polarity is increased, the greater will be the portion of interfering substances eluted and the less effective the cleanup. Solvents of high polarity used for carrying the sample into the column can deactivate the adsorbent immediately and may change the elution profile of some compounds (12). Therefore the type and amount of solvent applied in a method should not be altered without adequate checking if similar results are sought.

6.1.1 Florisil

Of all the sorbents used in residue analysis, Florisil has gained the greatest attention. Although it is sometimes used as activated material (130°C), it is more often used in deactivated form (addition of 2-7% water). As Florisil retains some lipids preferentially (25 g Florisil with 3% water will retain 1 g fat), it is particularly well suited for the cleanup of fatty foods. When a Florisil column is eluted with solvent mixtures of low polarity, non-polar residues are recovered almost quantitatively. The eluates are very clean for GLC with ECD as well as for TLC detection on silver nitrate coated plates. The most widely used eluants are mixtures of petroleum ether with a low percentage of diethyl ether or dichloromethane. There have been many attempts to improve cleanup efficiency by more complex solvent mixtures, but without particular success. Florisil is one of the most useful absorbents for cleanup in the analysis of organochlorines and PCBs in fatty foods. Miniaturization is readily possible. A major disadvantage is, however, that activity may vary from one batch to another, so that Florisil needs always to be standardized very carefully, otherwise poor cleanup or recovery will result.

For the analysis of plant material, Florisil is of minor importance, although it has been often recommended in the framework of multiresidue procedures for fruits and vegetables. The main reason is that the cleanup is poor when more polar pesticides need to be eluted from the column and, in addition, even well-deactivated Florisil will decompose several pesticides (e.g. the phthalimide fungicides); will oxidise organophosphates with thio-ether groups or will adsorb the oxons of some organophosphates irreversibly. Examples of elution possibilities of some organochlorine and organophosphorus compounds are given in Tables 4 and 5.

Table 4. Elution behaviour of some organochlorine compounds on Florisil

Author	AOAC (5a)		Specht (19h)	Mestres (30)		Stijve (19i)
Florisil quantity	20 g		30 g	5 g		25 g
Water content (g/100 g)	-		5	5		3
Solvents	PE/Eth 94+6	PE/Eth 85+15	PE/Eth 94+6,	PE	PE/Eth 8+2	PE/Dichlm 8+2
Quantity (ml)	200	200	200	50	50	300
HCB	+		+	+		+
Lindane	+		+	+		+
Aldrin	+		+	+		+
Heptachlor	+		+	+		+
p,p'-DDE	+		+	+		+
p,p'-DDT	+		+	+		+
PCB	+		+	+		+
Heptachlor-epoxide	+		+		+	+
Methoxychlor	+		+		+	+
Dieldrin		+	+		+	+
Endrin		+	+		+	+

Notes: + : quantitative recovery

TABLE 5. Elution behaviour of some organophosphorus compounds on 20 g activated Florisil

Author	AOAC (5a)			Mills (5a)		Backman (65)	
Solvents	PE/Eth 94+6	85+15	94+6	Dichlm/Hec/Acnit 50+ 49.65 +0.35	50+ 48.5 +1.5	Bz/Eth Ac 2+1	
Quantity (ml)	200	200	200	200	200	50	100
Carbophenothion	+			(+)		+	
Ethion	+			+		+	
Fenchlorphos	+			+		+	
Disulfoton	(+)			-	-	+	
Chlorpyrifos	+			+		+	
Fenthion	(+)	(+)		-	-	+	
Parathion		+		+		+	
Fenitrothion		+		+		(+)	(+)
Parathion-methyl		+		+		(+)	+
Diazinon		+			+	(+)	+
Malathion		(+)	+		+		+
Azinphos-ethyl			(+)		+		+
Dimethoate				-	-		+
Dichlorvos	-	-	-	-	-		(+)
Paraoxon				-	-		
Malaoxon				-	-		

Notes: Recovery + : quantitative
(+) : only partially
- : no

6.1.2 Alumina

In many cases, Florisil can be replaced by alumina, particularly for the analysis of fatty foods. Basic alumina shows similar elution and cleanup characteristics for the removal of lipids but does not exhibit the problems of varying activity to the same degree. Miniaturized methods are also available. As with Florisil, experience has shown that alumina can remove some special types of plant co-extractives but it cannot be unequivocally recommended for cleanup of plant material. Basic alumina will readily decompose some organophosphates, and some more polar pesticides are not or only partially recovered from neutral or acidic alumina (Table 6). To achieve maximum efficiency, one should carefully consider optimum conditions in relation to both substrates and pesticides to be analysed.

Silver nitrate coated alumina can be of special value for eliminating interfering sulfur-containing substances from kale, onions etc. for ECD detection. In this case alumina acts mainly as support for reactive silver nitrate (9).

TABLE 6. Elution behaviour of different pesticides on neutral alumina (8)
8 g alumina (19% water): eluents: 1: 30 ml hexane, 2: 30 ml hexane-diethylether 7+3

Organochlorines	Organophosphates	Methylcarbamates	Other compounds
Aldrin	Bromophos	Carbaryl	Butylate[a]
DDT, DDD, DDE	Chlorpyrifos	Carbofuran[a]	Carboxin
Dieldrin	Diazinon	Dioxacarb[b]	Chinomethionate
Endosulfan	Disulfoton	[x]Pirimicarb	Chlorpropham
Endrin	Ditalimfos		Chlorthal
Heptachlor-epoxide	Fenitrothion	**Ureas**	Cycloate[a]
α-HCH	Fenthion	Chloroxuron[a]	Desmedipham
β-HCH	Fonofos	Chlortoluron[a]	Dichlobenil
Lindane	Parathion-methyl	Linuron[a]	Diphenamid
Tetradifon	Phecapton	Metobromuron[a]	EPTC[a]
Tetrasul	Phorate	Monolinuron[a]	Ethofumesate[a]
	Phosmet	[x]Methabenzthiazuron	Metribuzin[a]
Pyrethroids	Pirimiphos-methyl		Nitrothal-isopr.
Cypermethrin	Tetrachlorvinphos	**Triazines**	Oxadiazon
Decamethrin	[x]Azinphos-methyl	Ametryn[a]	Phenmedipham
Fenvalerate	[x]Butonate	Atrazine[b]	Piperonyl butoxide
Permethrin	[x]Dichlorvos	Cyanazine[b]	Propachlor
	[o]Dimethoate	Prometryn[a]	Propargite
Dinitro-compounds	[x]Etrimfos	Simazine[b]	Trifluralin
Dinobuton	[x]Malathion	Terbumeton[b]	Vinclozolin
Dinocap	[x]Methidathion	Terbuthylazin[a]	[x]Aldicarb
[o]Dinoseb	[o]Phosphamidon	Terbutryn[a]	[o]Benomyl
[o]DNOC	[x]Pyrazophos		[x]Dichloran
	[o]Trichlorfon		[o]Difenzoquat
			[x]Triadimefon

Notes: No mark: the pesticide elutes in fraction 1 with recovery higher than 80%

a : the pesticide appears in both fractions

b : the pesticide elutes in fraction 2

x : recovery lower than 80%

o : recovery lower than 40% or not recovered

6.1.3 Silica gel

In general, silica gel is less efficient than alumina as a cleanup adsorbent and will not adequately separate pesticides from plant co-extractives. Its importance in the analysis of plant material lies in its use for the fractionation of certain residues according to their polarity without appreciable losses (10), thus yielding additional information to GLC data (Tables 7 and 8). Low polarity eluates are simultaneously cleaned up, so that GLC detection with the ECD is possible. Special attention is drawn to the use of silica gel deactivated with 10-30% of water for the removal of lipids from organochlorine compounds in fat analysis. Eluates obtained with petroleum ether are very clean and may even be suitable for TLC estimation.

TABLE 7. Examples for the separation of pesticides having similar GLC relative retention times (RRT) on a silica gel column (31).

GLC column: 3% OV-22 on Gas Chrom Q (100-120 mesh), temp. 180°C. Column chromatography: 5 g Silica gel (Woelm no. 02747), containing 5% water. Elution fractions: I: 10 ml Hex; II: 16 ml Hex/Bz 4+6; III: 16 ml Bz; IV: 20 ml Bz/EtAc 1+1; V: 50 ml EtAc.

Compound pairs	Relation or RRTs	Silica gel column fraction	Compound pairs	Relation or RRTs	Silica gel column fraction
Bromophos	1.06	II	Dioxacarb	1.06	IV (10%) + V (90%)
Malathion		IV	Bromophos		II
Carbaryl	1.05	IV	Phosphamidon II	1.01	V
Fenitrothion		III	Parathion-methyl		III
Fenthion	1.06	III	Prometryne	1.0	IV (90%) + V (10%)
Bromophos		II	Parathion-methyl		III
Dioxacarb	1.01	IV (10%) + V (90%)	Propachlor	1.03	IV
Fenthion		III	Chlorpropham		III

TABLE 8. Elution behaviour of some organochlorines on silica gel (32). Adsorbent: 15 g Silica gel (Merck no. 7734) containing 10% water. Elution: 10 ml fractions of PE/Dichlm 8+2

Pesticide	% Recovery in fractions									
	1	2	3	4	5	6	7	8	9	10
HCB	95	5	-	-	-	-	-	-	-	-
α-HCH	-	25	75	-	-	-	-	-	-	-
β-HCH	-	-	26	54	20	-	-	-	-	-
γ-HCH	-	40	60	-	-	-	-	-	-	-
δ-HCH	-	-	-	-	5	35	45	15	-	-
Heptachlor	36	64	-	-	-	-	-	-	-	-
Heptachlor-epoxide	-	-	50	50	-	-	-	-	-	-
Dieldrin	-	-	-	-	-	9	37	41	13	-
Endrin	-	-	-	-	-	7	26	47	20	-
o,p'-DDT	30	70	-	-	-	-	-	-	-	-
p,p'-DDT	20	80	-	-	-	-	-	-	-	-
p,p'-DDE	41	59	-	-	-	-	-	-	-	-
p,p'-DDD	-	70	30	-	-	-	-	-	-	-

6.1.4 Magnesia

Magnesium oxide is valuable in some situations for removing some interfering co-extractives from plant extracts known to contain sulfurous material. It is not used as such for basic cleanup but only for additional cleanup (see also mixed adsorbents).

6.1.5 Carbon

Unlike other adsorbents mentioned above, carbon shows different elution characteristics due to its lipophilic nature. It adsorbs preferentially non-polar, lipophilic and high molecular weight substances. It is particularly suitable for cleanup of extracts with high chlorophyll content (vegetables) but no so effective for the removal of plant wax in

the analysis of organophosphates. Efficiency is, however, affected by type and pre-treatment of the carbon, so that results reported in the literature are often not directly comparable. As finely-divided carbon columns have poor flow characteristics, the material is diluted with diatomaceous earth used in granular form or is used in adsorbent mixtures.

6.1.6 Mixed adsorbents

There have been many attempts to combine the different properties of the hydrophilic adsorbents and the lipophilic carbon. Some of them have gained some (mostly national) importance, such as the silica/carbon column or mixtures of carbon with magnesia and celite etc. Choice of eluting mixtures appears to be rather accidental and without systematic approach other than observation and experience (Table 9).

6.2 Gel permeation chromatography (GPC)

The most universally applicable cleanup step is GPC. In the analysis of plant extracts it separates efficiently the relatively small pesticide molecules from the surplus of natural and higher molecular weight plant constituents. Searation is generally performed by using DVB-linked polystyrene gels, mostly Bio Beads S-X2 or S-X3. It is suitable for organo-chlorine, organophosphorus and nearly all other types of pesticides and does not involve any losses by adsorption. For elution several solvent mixtures have been recommended (Table 10) among which cyclohexane/ethyl acetate 1+1 has proved to be suitable for cleanup

TABLE 9. Examples for clean-up of organophosphates with mixed adsorbants

Adsorbent mass (g)	Char-coal	MgO	Celite 545	Others	Eluting solvent (ml)	Ref.
2.9	0.7	-	-	2.2 cellulose	200 Chlfm, 200 Bz	57
6.0	1	2	4	-	200 Dichlm/Ac 2+1	13
6.0	1	2	4	-	120 Bz/Acnit 1+1	5b
7.0	1	2	4	-	150 Dichlm	8
10.0	1	2	4	-	150 Tol/EtAc/Ac 2+1+1	18
14.0	1	2	4	-	300 Bz/EtAc 3+1	21
20.0	1	-	8	4 attaclay 10 florisil 10 sodium sulphate	100 Ac, 100 Dichlm	59

TABLE 10. Examples for the separation of residues by GPC with Bio Beads S-X3 in an automatic device

Eluting mixture	Column length (cm)	Pesticides	Fraction (ml)	Ref.
EtAc/Tol 3+1	27	Organochlorines Organophosphates Phenoxy esters	100 - 150 90 - 130 90 - 130	60
	35	Organochlorines	120 - 220	61
Cyclh/Dichlm 85+15	30	Organophosphates	80 - 270	62
	35	Methyl carbamates	110 - 205	58
	35	Organochlorines	120 - 220	63
Cyclh/EtAc 1+1	32	Organochlorines Organophosphates Other pesticides	100 - 170 100 - 170 100 - 160	19j
Hex/Dichlm 1+1	20	Organochlorines Organophosphates	70 - 120 60 - 110	64

of more than 300 pesticides (19 j). Dextran gels have also been used for cleanup of organophosphorus compounds but they require much more time and solvent and exhibit additional interactions between gel and pesticides.

Under the conditions used for plant extracts, GPC with Bio Beads can be applied in the analysis of fats and oils effectively removing lipids before analysis of organochlorines and less polar organophosphates. Another valuable feature is that GPC can be carried out in an automatically controlled device. This versatile technique can be used as a general cleanup step for almost any type of pesticide and substrate and may be supplemented if necessary by an specific purification.

6.3 Other cleanup procedures

There are other cleanup steps available which can be applied only to certain groups of pesticides and substrates. For example steam distillation, treatment with sulfuric acid or calcium silicate (Calflo E) is suitable for most organochlorine compounds; for organophosphates or methyl carbamates treatment with a coagulating solution. These may offer ways for solving special cleanup problems but their applicability is rather limited. They are not normally components of multiresidue procedures but could be incorporated if required.

Sweep co-distillation has been frequently recommended in the frame of multiresidue procedures. In principle, it is suitable for a broad range of pesticides and substrates (fat, plant extracts). It is not very time-consuming and does not need special adsorbents nor large volumes of solvent; instead, it needs a high gas flow. As this technique can give rise to problems in recovery and affect the stability of some sensitive pesticides, it has been adopted in only a few laboratories that are experienced in the field.

6.4 No cleanup

Due to the high selectivity and/or sensitivity of some GLC detectors, crude extracts can be analysed in principle without any cleanup. This is, however, only possible for organophosphorus or organonitrogen pesticides with the FPD or the Hall electrolytic conductivity detector and for some fruits with the thermionic detectors operated in phosphorus mode. Co-extractives may, however, rapidly shorten the life-time of the GLC column. In practice, this mode of of operation can only be recommended if the load capacity of the detector and the column has been carefully determined and the working parameters (capacity and inertness of the column, selectivity and sensitivity of the detector) are regularly observed and controlled.

7 DETERMINATION OF RESIDUES

7.1 GLC analysis

At present gas-liquid chromatography employing specific detectors (ECD, TID, FPD, Hall electrolytic conductivity detector) is the most widely used technique for the identification and quantitation of the compounds in the sample extract.

7.1.1 Application of packed columns

Although many packings have been recommended in the literature, most separations can be achieved by using not more than 5 stationary phases of differing polarities. These 5 could be selected from non-polar silicones (e.g. SE-30, OV-101): moderately polar silicones (e.g. OV-17, OV-210); polar silicones (e.g. OV-225); polyethers (e.g. Carbowax 20M); polyesters (e.g. NPGS, DEGS); the examples being approximately in the order of increasing polarity.

Table 11 shows chromatographic conditions applied in some MRPs. The most important parameter in the selection of supports, packing and chromatographic equipment is inertness. Because of the high sensitivity of many residues to the surface activity only the most inert supports (e.g. Gaschrom Q, Chromosorb W HP) are recommended for use with a minimum coating of 3% liquid phase. It is essential to use pyrex glass injectors and columns washed with hydrochloric acid and treated with dimethyl dichlorosilane. Acid washed pyrex wool should be used for closing the packings but quartz wool gives better results. The inertness of the column varies depending on the contaminating substances injected and on the quality of the solvents and carrier gas used. Therefore the regular control and the maintenance of the column inertness are advisable. The injection of a carbaryl/propham mixture was found to be valuable for testing the inertness of the column. If the response ratio of carbaryl/propham is equal to or higher than 0.5 at a 5 ng level with a nitrogen specific detector the inertness of the system is suitable for the analysis of labile pesticides (33). If the first few cm of the packing and the quartz wool are changed regularly the life of columns can be reasonably extended. The inertness of the column can be improved by the injection of few µl Silyl 8 column conditioner or a similar agent. It was found necessary to change the quartz wool used after silylation (33).

TABLE 11. Gas chromatographic conditions applied in some MRPs

First author	Column size length x i.d. [cm]	Packing[a]	Column temp. °C	Carrier gas flow rate ml/min	Compounds
AOAC [5a] FDA [6h]	183 x 0.4	10% DC-200 Chrom W-HP 80-100 mesh	180 200	80 120	general purpose
FDA [6h]	183 x 0.4	10% DC-200 15% QF-1 1:1 Chrom W-HP, 80-100 mesh	200	120	general purpose
	183 x 0.4	15% OV-210 Chrom W-HP	190	80	HCB, BHC isomers confirmation
	183 x 0.4	2% DEGS Gas Chrom Q, 80-100 mesh	165-210	60	confirmation
Ambrus [31]	90 x 0.2 45 x 0.3	3% OV-22 Gas Chrom Q 100-120 mesh	140 [1 min] 10°/min, 240 [2 min]	14-35[b]	general purpose
		3% OV-101 Gas Crhom Q 100-120 mesh	148 [1 min] 10°/min, 240 [2 min] 180 or 200		general purpose
		1.95% SP-2401 + 1.5% SP-2250 Supelcoport 100-120 mesh	180 or 200		chlorinated hydrocarbons
		3% NPGS Gas Chrom Q 100-120 mesh	140-220		confirmation
Ambrus [31]		3% SE-30 Gas Chrom Q 100-120 mesh	160-240		pyrethroids confirmation
Becker [19a]	183 x 0.2	3% SE-30 Chrom W-AW-DMCS 80-100 mesh	210	30	chlorine containing pesticides triazines
	183 x 0.2	2% FS-1265 Chrom W-AW-DMCS 60-80 mesh	210	45	OP
Ebing [19f]	210 x 0.17	4% OV-1 Chrom W-HP-DMCS 0.12-0.15 mm	140 [18 min], 8°/min, 285 [2 min]	20	OP
	210 x 0.17	4% OV-17 Chrom W-HP-DMCS 0.12-0.15 mm	170 [18 min], 8°/min, 315 [6 min]	20	OP
Ebing [19b]	206 x 0.19	4% OV-17 Chrom W-HP-DMCS 0.12-0.15 mm	200, 4°/min, 230	70	OP
Eichner [19e]	380 x 0.2	5% QF-1 Chrom W-AW-DMCS 80-100 mesh	200	30	chlorine containing pesticides
Luke [13]	120 x 0.2	2% DEGS Chrom W-AW 80-100 mesh	180	60	) carbamates) triazines) organochlorine) organosulfur OP) miscellaneous) organonitrogen) pesticides
	120 x 0.2	2% DEGS + 0.5% H_3PO_4	180	25-30	
	30.5 x 0.2	2% DEGS + 0.5% H_3PO_4 Chrom W-AW 80-100 mesh	120	25-30	

TABLE 11. (continued)

First author	Column size length x i.d. [cm]	Packing	Column temp. °C	Carrier gas flow rate ml/min	Compounds
Luke [13]	120 x 0.2	2% OV-101 Chrom W-HP 100-120 mesh	200	60	) carbamates) triazines) organochlorine) organosulfur OP
	76.2 x 0.2	4% SE-30 + 6.5% OV-210 Chrom W-HP 80-100 mesh	200	60	) miscellaneous) organonitrogen) pesticides
Specht [19h]	180 x 0.4	5% OV-101 Gas Chrom Q 70-100 mesh	180 [5 min], 2°/min, 200°C [5 min]	90	organochlorine and OP
	180 x 0.4	1.5% OV-17 + 1.95% QF-1 Chrom W-HP 100-120 mesh	200 [5 min], 2°/min, 220 [5 min]	90	organochlorine and OP
	120 x 0.4	2.5% XE-60 Chrom G-AW-DMCS 80-100 mesh	190	90	organochlorine and OP
	180 x 0.4	2% DEGS + 0.5% H_3PO_4 Chrom W-HP 100-120 mesh	180	90	organochlorine and OP
Specht [19h]	200 x 0.2	15% QF-1 + 10% DC-200 1:1 Gas Chrom Q 70-100 mesh	200	60	organochlorine and OP
Stijve [19i]	160 x 0.3	1.5% OV-17 + 1.95% QF-1 Chrom W-DMCS 100-120 mesh	210	40	organochlorine and OP
	160 x 0.3	2% DEGS + 0.5% H_3PO_4 Chrom W-HP 100-120 mesh	185	40	organochlorine and OP

Notes: [a]. particle sizes 60-80, 80-100, 100-120 mesh are equivalent to 0.25-0.177, 0.177-0.15, 0.15-0.125 mm respectively

[b]. flow rate is selected to achieve optimum performance of the specific detectors

Most of the methods recommend chromatographic columns of unnecessary large capacity which results in long analysis time (50 to 98 min for certain compounds) (16) and loss of sensitivity of the detection. These disadvantages may be partly compensated by increasing carrier gas flow rate and temperature. However, the former may adversely affect the detector performance while the latter increases the decomposition of labile compounds. Narrow (i.d. 2 mm) and short (50-120 cm) columns eliminate the disadvantages mentioned above (13,31). With appropriate selection of particle size of packing and inner diameter of the column the greatest number of effective plates per unit time can be achieved at an optimal flow rate for the specific detector used (33).

Because of the great number of pesticides which can be analysed by GLC, even if 180-200 cm long columns of large capacity are used and the interferences from plant materials and reagents are eliminated, any single peak on the chromatogram may represent more than one pesticide. The detected compounds must be investigated further, at least on another column of different polarity. On the other hand, it is rare to find in an extract more than one compound having similar biological effects except for chlorinated hydrocarbons. Thus the use of slow large capacity columns has no advantage over the quick short columns.

7.1.2 Application of capillary columns

The efficiency of capillary columns and the relative speed of analysis (number of effective plates generated within unit time) are much better than those of short packed columns which explains the rapid widespread acceptance of their use. The main advantage of GLC on capillary columns is that it yields particularly narrow and high peaks. For this reason, pesticide residues can be identified with greater reliability and may be quantitated with better sensitivity. At the same time, an excellent separation of pesticides from each other and from co-extractives can be obtained. In residue analysis, wall coated open tubular (WCOT) columns are used almost exclusively. The liquid phase is deposited on the inner walls of the capillary (10-50 m long, 0.2-0.5 mm i.d.) as an 0.05-1 μm thin, continuous and uniform film throughout the column.

As column materials, borosilicate glass or fused silica are suitable. In earlier years most glass columns commercially available were very expensive and not sufficiently deactivated for residue analysis. Workers in this field often made the capillaries in their own laboratory by using a glass drawinging machine and carried out the pretreatment and coating operations themselves. Unlike glass columns, fused silica capillaries cannot be drawn in the laboratory. The columns commercially available are very thin but are covered by a protective polyimide coating which makes them very flexible. When rolled up, their ends remain straight and are always ready for installation; a section broken off can be reused immediately. A further advantage is that the inner surface can be easily and thoroughly deactivated.

Workers with limited experience with the capillary technique are recommended to use coated fused silica columns commercially available. In recent years their performance has been improved considerably and there are now several types which meet the high demands of residue analysis. The most important aspect is deactiation which plays such an important role in trace analysis.

Particularly promising are commercial capillaries with immobilised stationary phases, where the coating is cross-linked and bound to the surface by covalent chemical bonds, e.g. Durabond (J & W), CB phases (Chrompack), Ultra (Hp), Mega (Erba) etc. Phases of this kind exhibit very low bleeding and can even be rinsed with some solvents to removing extraneous deposits which may lead to tailing peaks after some routine use.

Commercially coated columns, however, are rather expensive and may sometimes not offer the optimal solution for a specific separation problem (e.g. in PCB analysis). In such cases it is advisable to start with blank glass or fused silica columns and to coat them with the selected phase in one's own laboratory.

When injecting 1-2 µl of the solution to be analysed, the resulting volume of solvent vapour is so large that with the usual carrier gas flow of only 2-3 ml/min, pesticides would arrive at the column not as a small band but only as a diffuse zone. For analysis of the

TABLE 12. Examples for GLC conditions for separating some pesticide groups with glass or fused silica capillary columns

Pesticide group	Length m	i.d. mm	Station. phase	Column temp. °C	°C/min	Detector	Ref.
Organochlorine pesticides, PCB	60	0.33	SE-30	180-260	2	ECD	34
	50	0.3	SE-30/SE-52	150-230	2	ECD	35
	40	0.3	SE-30	150-200	1	ECD	36
	30	0.3	OV-61	140-230	5	ECD	37
	20	0.25	OV-17	80-220	7	ECD	38
Organophosphorus pesticides	50	0.35	SE-30	200-290	4	FPD	39
	25	0.3	DEGA	100-250	8	TID	40
	20	0.3	SE-54	120-200	4	MS	41
Methylcarbamate insecticides	18	0.31	SE-52	170-190	4	TID	42
	12	0.3	SE-54	130-145	3	TID	43
Triazine herbicides	12	0.3	Carbowax 20M	120-220	15	TID	44
	22	0.25	SE-52	110-220	2	TID	45
Phenylurea herbicides	15	0.3	SE-52[a]	90-210	8	TID/ECD	45
	15	0.3	SE-52, OV-73		4	FID/TID	47
Pyrethroids	25	0.23	OV-101	50-210	25	ECD	48
	15	0.32	OV-1	180-245	3	ECD	19g
Fungicides	35	0.22	SE-30	100-250	5	ECD	50
Ethylenthiourea	30	0.3	FFAP	180	-	TID	51
	8	0.25	Carbowax 20M	60-220	30	FPD	52
Phenoxy herbicides[b]	60	0.27	SE-30	130-230	4	ECD	53
Triphenyltins[c]	12	0.22	OV-101	40-250	10	FPD	54
Benomyl[d]	50	0.25	OV-101	150	-	TID	55

Notes: [a]: as isocyanates [c]: as methyl derivatives

[b]: as pentafluorobenzylic esters [d]: as carbendazim acetate and 2-AB acetate

organochlorine compounds with the ECD, sensitivity is usually high enough for a split system to be used which results in a proportion (e.g. one tenth) of the injected solution as a sharp band on to the column. In other cases, however, e.g. for total diet studies or when working with other selective detectors, the highest sensitivity may be required. Recommended techniques include injection at low temperature followed by a rapid temperature rise to start separation; the on-column injection with a very thin needle using a special valve inlet; or the moving-needle injector evaporating the solvent on a needle tip before introducing it into the carrier gas stream.

Table 12 shows some examples for separation conditions described in the literature for the analysis of pesticide residues in food matrices. As can be seen, methyl silicone gums such as SE-30 and similar phases (SE-52, SE-54, OV-1701) when used with the ECD provide particularly good separations for the organochlorine compounds and PCBs. Less viscous phases are not as satisfactory for some risk remains that some volatile components may enter the ECD contaminating it seriously. Non-polar as well as more polar columns are suitable for organophosphates, triazines etc. They can be combined with all types of selective detectors including mass spectrometry. In most cases a temperature programme will provide the best separation conditions for the numerous representatives of a certain pesticide group. Extracts injected on to capillary columns should be reasonably clean although there are no special cleanup requirements. The same multiresidue cleanup procedures used for GLC with packed columns are satisfactory.

The loadability of capillary columns with pesticides plus co-extractives depends on the thickness of the film of stationary phase. Optimum film thickness for residues is about 0.1 µm. Heavier coating will result in peak broadening and is not recommended for best separation and sensitivity. Even for 0.1 µm films, loadability is still sufficiently high in most cases if extracts have been properly cleaned up. Overloading will be easily perceptible for it results in a typical peak distortion (usually called "leading", slowly ascending and sharply descending peaks). If overloading occurs, a smaller injection volume or a diluted solution should be used.

7.1.3 Detection of the residues

Of the GLC detectors presently available the electron capture (ECD), thermionic (TID, AFID), flame photometric (FPD), and Hall electrolytic conductivity (HECD) detectors are used exclusively in the MRPs. The mass spectrometer, the most specific detector for GLC, is applicable for screening of few compounds or for confirmation.

The AFID, FPD and HECD are element selective while ECD detects all compounds having electron absorbing properties such as nitro, 1,2-diketo, and halogen derivatives. All detectors have undergone continuous development, resulting in improved selectivity, sensitivity and stability but their limitations have to be borne in mind when they are used in MRPs. The performance of the detectors depend considerably on the construction and on the operating conditions and it may vary even when detectors of same make are compared. Some changes in the detector parameters also occur as a function of operation time.

Application of ECD requires properly cleaned sample extracts which can only be achieved with solvents and adsorbents of high purity. In addition to the cleanup, special attention should be given to the interpretation of the results due to the variation of recovery and detector response from compound to compound.

The AFID is specific to phosphorus and nitrogen. In case of heated bead detector the ratio of P/N sensitivity can vary between 10 and 50 depending on the construction and operating conditions. As the plant extracts usually contain naturally occurring nitrogen compounds, the cleanup needs to be almost as rigorous as in case of ECD, in contrast to the earlier detectors which contained an alkali salt tip over the flame. Therefore the direct injection of concentrated plant extracts is rarely possible in case of heated bead AFID in spite of its excellent selectivity expressed in terms of response ratio of phosphorus and hydrocarbon compounds.

The FPD is one of the most reliable GLC detectors. Its performance is mainly influenced by the design, oxygen/hydrogen ratio and by the total oxygen and carrier gas flow. Carbon compounds produce peaks in the microgramme range only, therefore the interference from carbon compounds is not usually a problem. It was found that interference from S in the P mode is more likely to occur than from P in the S mode, unless working at S levels near the detection limit (84). For example, broccoli, Brussels sprouts, cauliflower, onions, peas and radish give significant peaks with the FPD in the P mode at a sensitivity range of about 0.1-5 ng residues (13). Hence, the positive response in the P mode should always be confirmed.

The Hall extrolytic conductivity detector detects Cl, N, or S with good selectivity, but its sensitivity is lower than in case of other detectors. Its performance largely depends on the operating conditions and it is rather difficult to achieve and especially to maintain the optimal operating conditions. It is used routinely in relatively few laboratories.

7.2 Thin-layer chromatographic analysis

Because of the rapid development in the instrumentation of gas chromatography and more recently the high-pressure liquid chromatography providing higher sensitivity and separation power, thin-layer chromatography is used only occasionally in many laboratories. However, its advantages, namely the versatility, speed and low cost could be utilized more in many laboratories where the required facilities of GLC and HPLC are not available. In addition, TLC is an excellent means for confirmation of results obtained by GLC and HPLC methods. Table 13 gives some examples for the applicability of TLC methods.

TABLE 13. Application of TLC in MRPs

First author	Layer	Development system	Visualization	Compounds
AOAC (5a) FDA (6e)	Alumina G	Heptane/Ac 98+2	$AgNO_3$/2-phenoxyethanol	organochlorines
	Alumina G	15-20% DMF in Eth, Methylcyclohexane	tetrabromophenolphthalein ethyl ester, $AgNO_3$, citric acid	thiophosphates
FDA (6g)	Silic AR	2,2,4-Trimethylpen tane/Ac/Chlfm 70+25+5	p-nitrobenzylpyridine, tetra-ethylenpentamine	OP
Ambrus (56)	Silica gel H	EtAc or Dichlm	chlorine vapor, o-tolidine + potassium iodide	carbamates, ureas, triazines, misc.
	Silica gel H	EtAc or Dichlm	NaOH + p-nitrobenzenediazonium fluoroborate	carbamates
	Silica gel H	EtAc or Dichlm	bioassay with fungi spores (*Aspergillus niger* L.)	fungicides
	Silica gel H	EtAc or Dichlm	horse blood serum, acetyl thio-choline iodide + 2,6-dichloro-phenol indophenol	OP, carbamates
	Silica gel H	EtAc or Dichlm	human blood plasma α-naphthyl acetate, fast blue salt B	OP, carbamates
	Alumina, $AgNO_3$-impreg.	EtAc or Dichlm	UV radiation	halogen containing compounds
	Alumina G	EtAc or Dichlm	p-dimethylaminobenzaldehyde	ureas, some carbamates
Stijve (19i)	Alumina N or Alumina 60-F_{254}	PE or PE/Ac 99+1	$AngNO_3$ + UV	organochlorines
	Silica gel H	Hex/Dichlm/Eth 8+1+2	human blood plasma, β-naphthyl acetate, fast blue salt B	OP
Canadian Man. (7)	Silica gel	Hex/Ac 99+1 or Hex/Bz 9+1 to 1+1	$AgNO_3$ + UV	organochlorines
DDR-Manual (66)	Silica gel G	Bz/Ac 95+5 or 66+34	bovine liver extract, β-naphthyl	OP, carbamates
Thier (67,68)	Silica gel G	Hex/Ac/Eth 8+2+1	2,6-dichloro/dibromo/quinone chloroimine, formic acid	OP
	Silica gel G	PE/Eth 95+5	$AgNO_3$/2-phenoxyethanol	phenoxy herbicides (methyl esters)
Onley (69)	Alumina GF	Bz/Chlfm/Meth 25+9+1	HCl, dimethylaminobenzaldehyde	ureas
El-Dib (70)	Silica gel	Hex/Ac 7+3	dimethylaminobenzaldehyde	methyl carbamates
Delley (71)	Silica gel	Tol/Ac 85+15	chlorine vapor, potassium iodide + starch	triazines
Abbott (72)	Silica gel	Chlfm/Ac 9+1	brilliant green, bromine capor	triazines
Sundararajan (49)	Alumina, $AgNO_3$-impreg.	Bz/Hex 55+45	UV radiation	pyrethroids

It has been demonstrated that by the proper application of TLC methods reproducible results can be obtained even at the 0.05-0.1 mg/kg residue level for many pesticides. It is recommended to spot reference compounds on each plate at the limit of detection in order to indicate whether the optimum condition for the detection and elution has been achieved or not. In the latter case the reference spot may not be properly visible or its Rf value differs from the usual one (56). The reference compound should be selected from those which are seen on the plates only when the detection has been carried out properly.

It was found that the elution order of 120 pesticides tested did not change regardless the relative humidity of the air in the developing chamber or the mode of saturation of the chamber (12) though the latter especially, greatly affects the Rf values. Consequently spotting two or three compounds of different Rf values on the plates and comparing their Rf values to those found in the sample significantly increases the probability of correct identification of the residues which is very important at the screening stage. Particularly suitable are commercially available factory-coated plates which are easier to handle, yield more defined spots and permit better quantification.

Sample material carried over into the concentrated extract influences both the separation and the detectability. Overloaded plates cannot be used for either qualitative or quantitative determination. The loadability of the layer depends on the mode of detection and to some extent on the developing solvent.

Table 14 gives some examples from the results of a systematic study carried out with different plant materials under various chromatographic conditions applying the extract obtained with the column chromatographic extraction method (17) described in Section 5. In addition to the described procedure the samples were extracted in a column which contained 7 g of mixed adsorbent (see Table 9, Ref.8) under the Florisil and sodium sulfate layer. The preparation of detecting reagents and the developing methods were similar to those described in Ref. 56. The samples selected were considered to represent the most difficult cases in various sample groups, so the results obtained can be used as a guide for the other samples as well. However, it has to be emphasized that the co-extractives from different samples may have different Rf ranges and intensity and therefore, the applicability of the given method has to be checked for each sample.

TABLE 14. Loadability of TLC plates: Grams of sample aliquots which can be spotted from extracts obtained with column chromatographic extraction on Florisil (CE) (17) or with additional mixed adsorbents (CE+MA)

Crop	Develop. solvent	p-DAB		$AgNO_3$/UV		Enzyme		Fungi		Cl_2/o-Tol.		Fluorobor.	
		CE	CE+MA	CE	CE+MA	CE	CE+MA	CE	CE+MA	CE	CE+MA	CE	CE+MA
Onion	Dichlm	0.2	1	0.5	1	0.1	0.2	<0.2	<0.2	0.5	1	0.2	0.5
	PE/Eth 1+2	-	-	0.5	1	0.05	0.1	<0.2	<0.2	0.5	1	0.2	0.5
	EtAc	0.2	0.5	0.05	0.05	0.05	0.1	<0.2	<0.2	0.1	0.5	0.1	0.2
Carrot	PE/Eth 1+2	-	-	1	2	0.1	0.5	<0.2	<0.5	0.2	0.5	0.25	2
	EtAc	0.1	0.5	1	2	0.1	0.5	0.5	0.5	0.1	0.25	0.1	0.5
Lemon	Dichlm	0.1	0.5	1	2	0.2	0.2	<0.2	0.5	0.1	0.25	0.1	0.5
	PE/Eth 1+2	-	-	1	2	0.5	0.5	<0.2	0.2	0.2	0.5	0.1	0.5
	EtAc	0.1	0.5	1	2	0.2	0.5	<0.2	<0.2	0.2	0.5	0.2	0.5
Spinach	Dichlm	0.25	0.5	0.25	1	0.05	0.25	0.5	1	0.25	1	0.2	1
	PE/Eth 1+2	-	-	0.5	1	0.1	0.5	0.25	2	0.1	2	0.05	1
	EtAc	0.1	0.5	<0.1	1	0.1	0.5	0.25	2	0.1	1	0.1	1
Cabbage	Dichlm	0.5	2	0.2	1	0.1	0.2	0.2	<0.5	0.5	1	1	2
	PE/Eth 1+2	-	-	0.5	1	0.1	0.2	0.2	<0.5	0.2	2	0.2	2
	EtAc	0.1	1	0.2	1	0.05	0.2	<0.2	<0.5	0.2	0.5	0.2	1
Raisin	Dichlm	0.2	0.5	2	2	0.05	0.1	0.2	1	0.2	0.4	0.2	0.5
	PE/Eth 1+2	-	-	1	2	0.05	0.1	0.5	0.5	0.2	0.5	0.2	0.5
	EtAc	0.2	0.5	2	2	0.05	0.1	0.5	0.5	0.2	0.5	0.1	0.4

		Pesticides used with solvents:		
	Layer	Dichlm	PE/Eth 1+2	EtAc
p-DAB: p-dimethylaminobenzaldehyde + HCl	Alumina	Diuron		Diuron
$AgNO_3$/UV: $AgNO_3$ impreganted in the layer + UV	Alumina	Atrazine	Aldrin, Dieldrin	Captafol
Enzyme: human blood plasma, α-naphthyl acetate, Fast Blue Salt B	Silicagel H	Triazophos	Mevinphos, Carbaryl	Dioxicarb
Fungi: spore suspension of *A. niger*	Silicagel H	Folpet	Carbedazim	Folpet
Cl_2/o-Tol.: o-tolidine and potassium iodide	Silicagel H	Chlorpropham	Secbumeton, Atrazine	Atrazine
Fluorobor.: NaOH + p-nitrobenzene diazonium fluoroborate	Silicagel H	Carbaryl	Dioxacarb, Carbaryl	Carbaryl

Note: Detecting reagents, layers and pesticides used

7.3 HPLC analysis

In residue analysis, the use of HPLC is particularly useful for pesticides which are not directly amenable to GLC determination, such as those that lack thermal stability, e.g. methylcarbamate insecticides (73,74,75) or phenylurea herbicides (76,77); cannot be analysed directly (phenoxyacetic acids, 78) or are not sufficiently volatile (e.g. some benzimidazole fungicides, 79,80) without further derivatisation. There are a number of examples that demonstrate that HPLC is equally as suitable as GLC for some additional compound classes such as sythetic pyrethroids, organophosphates and triazines mainly because of simpler cleanup procedures and faster analyses. In many cases HPLC offers better separation and faster and more accurate analysis than it is possible with TLC. However it is much more expensive, requires regular maintenance and is not as universally applicable as TLC. The main drawback of HPLC is that detectors currently available are much less sensitive and selective than in GLC.

The UV detector with variable wavelength is mostly used in multiresidue analysis. Its selectivity is best for pesticides with absorption maxima at high wavelengths. The sensitivity depends largely on the extinction coefficients of pesticides and co-extractives present but usually the detection limit will not be better than 0.1-0.5 mg/kg even if up to 100 µl are injected for analysis.

The more efficient fluorescence detection was originally suitable only for individual pesticides exhibiting proper fluorescence of the molecule, but is now of increasing application for post-column derivatisation in multiresidue analysis of methylcarbamates using dansyl chloride (81) or o-phthalaldehyde/2-mercaptoethanol (82).

Separation is performed on 5-10 µm silica gel and usually reversed-phase or other surface modified material is used. As a rule, reversed-phase conditions are particularly versatile for residue analysis of low polarity pesticides because of broad applicability and low risk of irreversible contamination of the column by co-extractives. Separation qualities of column packings commercially available may, however, differ widely according to the brand. Very promising is the combination of columns with different separation properties by column-switching techniques (83).

When a broad spectrum cleanup procedure is used (e.g. gel permeation chromatography) the cleaned extract may well be used for both GLC and HPLC determinations, each of them covering different groups of pesticides. It should be kept in mind, however, that most current cleanup methods have been elaborated and proved for GLC analysis with selective detectors and will not necessarily offer the best performance when used with HPLC. As in GLC all identifications based on HPLC will need a confirmation by another suitable procedure.

CONCLUSIONS

Most of the multiresidue procedures considered in this paper were developed some years ago and are capable of identifying and determining a large number of pesticide residues. In the meantime, many of them have undergone some standardization by national or international bodies. The advantage of the use of standardized processes is evident. The comparison and assessment of various processes indicate, however, that the procedures used in analytical practice are not necessarily restricted to the pesticides specified by the authors but may cover many more compounds. This is particularly true for pesticides introduced in recent years. There is therefore a great demand for information on which of the pesticides of current importance will be included in a certain method. Thus it would be for the benefit of the analysts if the demonstrated extension of standardized procedures was published, regularly providing information on both positive as well as negative results.

A study of the results of monitoring programmes indicates that most of the compounds detected can easily be determined by MRPs. This approach may mislead those who are not familiar with the preconditions of the analytical programme and are interested only in the results and the actual residue situation. Exclusive use of MRPs will therefore draw attention to a specific number of compounds and will not provide information on the existence or significance of other pesticide residues. For this reason the MRPs should never be applied uncritically in routine analysis and the pesticides specified in market control, selective field survey and environmental monitoring programmes should not be selected according to the analytical possibilities of MRPs but rather by considering actual use patterns and practice, the probability of the appearance of a residue and its toxicity. The detailed list of compounds which were looked for should be specified in every report.

Most standardized MRPs were developed on the basis of partitioning and adsorption column chromatography clean-up steps. In the meantime, gel permeation chromatography has proven its efficiency as an excellent basic cleanup step removing the surplus of co-extractives or lipids in the crude sample extract. The aliquots of an extract precleaned by GPC can be used directly for certain GLC or TLC analyses or can be further purified with specific processes to meet the requirements of the various pesticides and sample matrices.

Since speed and low cost of analysis (46) are priorities in most laboratories the most promising way of achieving this is the use of miniaturized methods for cleanup involving a basic GPC precleaning step.

The mode of detection of residues may vary from one laboratory to another depending on the facilities available. The degree of sample concentration and cleanup requirement depend strongly on the mode and condition of detection. The operating conditions have to be optimized individually in each laboratory. The actual detecting parameters govern the kind and sequence of cleanup steps, and every laboratory has to establish its own internal procedure, including confirmation which is preferably based on standardized processes of widely used, internationally accepted methods.

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APPENDIX

Compounds frequently reported to be present in food commodities

Organohalogen compounds

aldrin
DDT, DDD, DDE and isomers
dieldrin
endosulfan
HCH isomers
hexachlorobenzene
lindane
quintozene
captafol
captan
chlorothalonil
dichlofluanid
dicloran
dithianon
folpet

Dithiocarbamates

Triazines

atrazine

Carbamates

carbaryl
chlorpropham
ethiofencarb
pirimicarb
propham
propoxur

Other pesticides

benomyl
carbendazim
chinomethionate
chlormequat
daminozide
dichloran
dinobuton
dinocap
dinoseb-acetate
diquat
dodine
ethephon
etrimphos
fenarimol
imazalil
iprodione
metalaxyl
nitrothal-isopropyl
paraquat
pendimethalin
phosphine
procymidone
propargite
propiconazol
thiabendazol
tolylfluanid
trichlorphenidin
vinclozolin

Organophosphorus compounds

acephate
azinphos-methyl
azinphos-ethyl
bromophos
bromophos-ethyl
carbophenothion
chlorfenvinphos
chlorpyrifos
dialifos
diazinon
dichlorvos
dimethoate/omethoate
ditalimfos
ethion
fenitrothion
fenthion
malathion
methamidophos
methidation
mevinphos
parathion
parathion methyl
phosalone
phosphamidon
pirimiphos-methyl
sulfotep
tetrachlorvinphos
triazophos
trichloronate

Synthetic pyrethroids

cypermethrin
deltamethrin
fenvalerate
permethrin

Other residues from pesticides

bromide ion