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Review

Trace analysis of pesticides by gas chromatography

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Abstract

The analysis of pesticides is relevant to both food quality and the environment. Many laboratories are occupied with the analysis of pesticides in food, water or soil. Capillary gas chromatography is the technique most widely used in pesticide analysis. In present laboratory practice it serves as a screening method for over 300 pesticides. In this review we describe the role of gas chromatography as an analytical tool in combination with currently used or recently developed sample preparation techniques.

Keywords: Reviews; Sample preparation; Food analysis; Water analysis; Pesticides

Contents

1. Introduction

In this century great efforts have been made to improve agricultural productivity. At present more food is produced from a lower area of cultivated land with less labour than ever before.

Part of this development can be attributed to the use or to the application of sophisticated agricultural

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techniques involving extensive mechanisation, advanced agricultural practices and the selection of more appropriate plant varieties. The extensive use of pesticides also played an important role in the increase of the world food production. Pesticides are substances or mixtures of substances intended for preventing, destroying, repelling, or mitigating any pest. Pests can be insects, mice and other animals, unwanted plants (weeds), fungi, or microorganisms like bacteria and viruses. Though often misunderstood to refer only to insecticides, the term pesticide also applies to herbicides, fungicides, and various other substances used to control pests. In most countries, substances intended for use as a plant regulator, defoliant, or desiccant are included in the same regulatory framework. Chemical classification of pesticides can be based on functional groups in their molecular structure or their specific biological activity on plagues. Table 1 gives a classification of pesticides identified on the basis of their chemical structure, modes of action and analytical technique (LC or GC) used. The division in organochlorine, organophosphorus and organonitrogen compounds originates from their methods of analysis; in most manuals this subdivision is still used indicating which detector, e.g. electron-capture or flame-photometric, is used despite the fact that these detectors are nowadays gradually replaced by bench-top mass spectrometers [1]. With respect towards trace analysis for pesticide residues in all types of matrices, nowadays world-wide a total of approx. 500 compounds are registered as pesticides or metabolites of pesticides of which over 300 are amenable to gas chromatography, thus yielding gas chromatography to be the most widely used technique in the trace analysis of pesticides [2].

Legislation is in place in the European Union (EU) and the USA that regulates the admission of pesticides. In the USA the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), first pub-

Table 1
Chemical classification and biological activity and main method of analysis of pesticides

Chemical class of pesticide	Biological activity	Number of pesticides per group	Typical representative	Method of analysis: LC, GC–FPD, GC–NPD or GC–ECD
Inorganic compounds	Fungicide	7	Sulfur	LC
Organotin compounds	Fungicide, anti-foulants	5	Fentin	GC–FPD
Organophosporus compounds	Insecticide, acaricide	76	Parathion, diazinon	GC–FPD
Others	Insecticide, acaricide	4	Glyphosate	LC
Organonitrogen compounds				
N-methyl carbamates	Insecticide, acaricide	12	Aldicarb,	LC
Dinitro compounds	Herbicide, fungicide	6	Dinoseb	LC
Dithiocarbamates	Fungicide	9	Maneb, zineb	LC
Derivatives of benzimidazole	Fungicide	4	Carbendazim, thiabendazole	LC
Derivatives of aromatic amines	Herbicide	22	Diuron, isoproturon	LC
Triazines	Herbicide	9	Atrazine, simazine	GC–NPD
Quats	Growth regulator, herbicide	6	Paraquat, diquat	LC
Others	Herbicide, fungicide	91	Bentazon metolachlor, metazachlor	GC–NPD and LC
Organohalogen compounds				
Pyrethroids	Insecticide	12	Permethrin	GC–ECD
Carboxylic acids	Herbicide	9	Mecoprop, diclorprop	LC
Others	Insecticide, fungicide	44	DDT, iprodione	GC–ECD
Organosulfur compounds	Herbicide, acaricide	2	Ethofumesate	GC–FPD
Others	Growth regulator, insecticide	11	Dikegulac	LC
Fumigants	Insecticide, nematicide	10	Methyl bromide	GC–ECD

lished in 1947. Significant new dimensions were added in 1996 when the Food Quality Protection Act (FQPA) was enacted which amends FIFRA establishing new safety standards for pesticides in food emphasizing health protection for infants and children. Under FQPA all pesticide food uses must be safe; that is EPA must be able to conclude with 'reasonable certainty that no harm will result from aggregate exposure' to each pesticide from dietary and other sources. The FQPA prompted a comprehensive reassessment of all registrations and tolerances, to be finished in 2006.

In Europe a similar change of the legislative framework takes place. Until 1990 registration took place at the national level. From 1991, EU has legislation in place which fully harmonizes the registration of pesticides and tolerances throughout the community. The European Community also has strict legislation on the occurrence of pesticides in water intended for human consumption, the maximum concentration of a pesticide should not exceed 0.1 μg/l while the sum of all pesticides must be below 0.5 μg/l [3,4].

An important aspect of the safe use of pesticides is the possible occurrence of residues of these chemicals and their metabolites in food which has, in any stage of its growth or production, been treated with pesticides. For this reason regulation and, consequently, analysis of pesticide residues has been adopted after the second world war. Moreover, since the late 1970s, concern has extended to other routes of possible exposure to pesticides. Environmental criteria play an important role in the reregistration of older pesticides and the registration of new chemicals. New areas of concern are, amongst others, the environment, effects on non-target organisms and the exposure of man via other routes than the foodchain. These facts, finally lead to a growing concern about these undesired exposures and resulted in large environmental monitoring activities on a broad range of chemical substances.

Important areas in which monitoring takes place are obviously the commodities on which the pesticides are applied, mainly fruits and vegetables, but due to veterinary use or proliferation of pesticides further in the food chain also in products of animal

Table 2
Results of the European Community monitoring exercise in 1996 for each pesticide or pesticide group analysed for in apples, strawberries, tomatoes, lettuce and grapes[a] (the percentages are calculated from the sum of the total number of samples)

Pesticide	Commodity, MRL (mg/kg)	Sum of the total number of samples	Sum of the total number of samples	Sum of the total number of samples with residues	Samples with residues exceeding the MRLs
Acephate	Lettuce 1.0	9514	9367	117 (1.2%)	22 (0.2%)
Chlorpyriphos	Apples 0.5	11 924	11 614	310 (2.6%)	10 (0.1%)
Chlorpyriphos methyl	Strawberries 0.5	11 464	11 372	92 (0.8%)	3 (0.03%)
Methamidophos	Lettuce 0.2	9691	9598	93 (1.0%)	28 (0.3%)
Iprodione	Lettuce 10	11 905	10 394	1511 (12.7%)	13 (0.1%)
Procymidone	Lettuce 5	12 044	10 892	1152 (9.6%)	30 (0.3%)
Chlorothalonil	Lettuce 0.01	11 633	11 444	189 (1.6%)	34 (0.3%)
Benomyl group	Grapes 3	4258	3908	350 (8.2%)	50 (1.2%)
Maneb group[b]	Lettuce 5	4464	3831	687 (15.4%)	82 (1.8%)

[a] LODs were not reported.
[b] The compounds of the maneb group are dithiocarbamates.

Table 3
Distribution of pesticides detected in the US Pesticide Data Program 1995[a]

		Apples	Bananas	Carrots	Grapes	Green beans	Oranges	Peaches	Potatoes	Spinach	Sweet corn	Sweet peas
Total number of samples analysed		695	486	703	694	587	700	377	707	634	671	670
Total percentage of samples with residues detected[b]		95	62	71	80	57	84	92	83	83	[c]	16
Pesticide	Incidents											
Thiabendazole	1146	349	251	1	1	[c]	413	1	130	[c]	[c]	[c]
Iprodione	711	6	[c]	173	267	9	[c]	256	[c]	[c]	[c]	[c]
DDE	689	[c]	[c]	263	11	8		1	106	300	[c]	[c]
Endosulfans	508	46	[c]	25	28	139	14	29	137	87	1	2
Chlorpropham	503	[c]	[c]	[c]	1	19	[c]	1	482	[c]	[c]	[c]
Diphenylamine	493	489	1	[c]	1	[c]	[c]	1	1	[c]	[c]	[c]
Imazalil	468	[c]	73	[c]	[c]	[c]	395	[c]	[c]	[c]	[c]	[c]
Azinphos methyl	430	320	[c]	[c]	3	4	1	102	[c]	[c]	[c]	[c]
Captan	429	98	[c]	3	255	13	[c]	60	[c]	[c]	[c]	[c]
Permethrins	389	3	[c]	[c]	[c]	5	[c]	9	[c]	372	[c]	[c]
Chlorpyriphos	371	153	[c]	6	56	[c]	50	60	[c]	46	[c]	[c]

[a] Number of samples with detection per commodity. Total number of samples analysed in 1995: 6924.
[b] LODs dependent on matrix, analyte and detector used, range 0.001–0.150 mg/kg.
[c] Not analysed.

origin. In the environment water and soil are the main areas of interest.

For fruit and vegetables large monitoring programs are in place. Tables 2 and 3 show the results of monitoring programmes in the EU [5] and the USA [6]. Only few samples show to be non-compliant; in approx. 0.1 to 2% of samples tolerances were exceeded. The major source of positive findings in fruits and vegetables originates from insecticides or fungicides. Moreover most of the residues reported are compounds amenable to gas chromatography, thus emphasizing the role of gas chromatography to this field.

Monitoring activities of pesticides in products of animal origin is focused on the older banned pesticides which still widely as contaminants in the food chain. These compounds are still detected as high in the tropical web as human milk. Ubiquity of these can be seen from a survey on Dutch human milk, collected in 1993, for organochlorinated pesticides 90% percentile values were found of 0.01, 0.02, 0.05, 0.07, 0.08 and 0.99 mg/kg fat for γ-HCH, β-Hepo, p,p′-DDT, β-HCH, HCB and p,p′-DDE [7].

In the environmental field other pesticides occur, the major sources of pesticide pollution in the environment are industrial emission during production and large scale agricultural or household use. An example of industrial emissions is the occurrence of bentazone in the river Rhine in the late eighties. Nowadays agricultural use of herbicides (either household or agricultural seems to be the major source. As an example Table 4 shows the occurrence of pesticides in the river Rhine from the IAWR monitoring program [8].

Occurrence of pesticides in ground water largely depends on the physical and chemical properties of the compound involved. Therefore, classification and modeling of pesticides in order to estimate the potential threads in water is primarily based on solubility, persistence (DT_{50}), leachability (K_{oc}) parameters. A combination of both DT_{50} and K_{oc} is represented as the Groundwater Ubiquity Score (GUS), for GUS values higher than 2.8 indicate a high probability that a pesticide will be a contaminant, obviously the amount of pesticides used in a certain area plays a key role [9]. For soil a reverse reasoning can be used, persistent compounds that are not too mobile may be found.

In the early days of pesticide residue analysis, colorimetric methods were used, for example DDT was analysed in vegetables employing derivatization to yield a blue color with subsequent colorimetric determination [10,11]; other pesticides were analysed by similar methods [12]. Drawbacks of these methods are the impossibility to analyse more than one pesticide simultaneously. A first step towards multi-residue methods was based on thin layer chromatography (TLC) [13–15], which employed on-plate detection often based on biological activity such as cholinesterase inhibition or fungi-spores [16–18].

In this overview we describe the emergence of gas chromatography, originally in 1968 seen as a ‘supplemental tool’ [19], towards being an indispensable tool in 1972 [20]. Gas chromatography has strict

Table 4
Concentration values (μg/l; 90 percentile) of pesticides found in the Rhine from 1993 until 1997

Compound	1993	1994	1995	1996	1997
Atrazine	0.18	0.16	0.17	0.12	0.09
Desethylatrazine	0.07	0.09	0.08	0.08	<0.05
Simazine	0.09	0.09	0.06	0.05	<0.05
Diuron	0.07	0.11	0.07	<0.05	<0.05
Isoproturon	0.13	0.14	0.18	0.06	0.06
Chloridazon	0.07	0.07	0.09	<0.05	<0.05
Bentazon	0.05	0.06	0.07	0.07	0.06
Metazachlor	<0.05	<0.05	<0.05	<0.05	<0.05
Metolachlor	<0.05	<0.05	<0.05	<0.05	<0.05
Dichlorprop	0.05	0.05	0.07	0.06	0.06
Mecoprop	0.08	0.06	0.08	0.07	0.07

requirements for the sample introduction and therefore the second part of the overview will be devoted to sample preparation.

2. Gas chromatography

2.1. Separation

One of the great advantages of capillary GC as already predicted by Golay is the separation power [21] which finally resulted in the introduction of commercially available fused-silica capillary columns [22] as a great step forward with regard to the peak capacity.

As can be seen from Table 5, however, pesticides will not present challenging separation problems because only a few pesticides will be detected simultaneously in single samples. From the separation point of view capillary chromatography brought far more improvement for the determination of polychlorinated biphenyls (PCBs), for which the prevailing methodology was based on perchlorination of all congeners, yielding decachlorobiphenyl [23,24]. By using high resolution capillary columns, individual congeners could be determined, leading to the unambiguous determination of single congeners [25]. For pesticide analysis the benefits of capillary gas chromatography can be found in the gain in sensitivity due to the reduction in peakwidth.

In pesticide analysis GC can be seen as a screening method suitable for the 300^{+} not simultaneously present in single sample. Theoretically capillary GC is a faster technique than packed column GC, however in practice, this may appear to be different. For example targeted analysis for pyrethroids requires only a 15 min isothermal run on a packed column, thus yielding fast results in the decision whether or not a consignment should be retained at a port of entry, for e.g. a container-carrier loaded with fruits which has to checked for compliance. Full screening utilising temperature programmed capillary columns requires runs of at least 60 min for this application, hence slowing down the sample through-

Table 5
Number of samples with residues in the European Committee with more than one pesticide in 1996

Country	Number of samples	Number of different pesticides detected in a single sample								
		≥ 2	≥ 3	≥ 4	≥ 5	≥ 6	≥ 7	≥ 8	≥ 9	≥ 10
Belgium	932	231	129	66	28	12	3	1	1	
Denmark	1273	110	38	15	4	2	1	1		
Germany	4257		84	19	3	1				
Greece	1132	60	30	11	2					
France		984	410	141	55	10	2			
Ireland	505	120	53	24	8	1				
Italy	7194	246	52	4						
Luxemburg	212	40	16	4						
Netherlands	11 015	1374	540	181	60	32	8	4		
Portugal	600	79	31	6						
Austria		69	45	20	11	5	2			
Sweden	3908	759	358	165	67	23	9	4	2	1
United Kingdom	878	101	36	13	1					
Norway	2936	895	334	132	52	17	2			
European Union +Norway[a]	30 585	4015	1617	621	222	87	23	10	3	1
%		13.2	5.3	2	0.72	0.28	0.07	0.03	0.01	0.003
USA[b]	6924	1260	826	387	206	82	15	11	3	1
%		18.2	11.9	5.6	3	1.2	0.2	0.2	0.004	0.001

[a] Data obtained from the Monitoring for pesticide residues in the European Union and Norway – Report 1996. Data from Austria, Germany and France were not complete and are therefore not included in the summary as well as not in the calculations, data from Finland and Spain were not available at all.

[b] Data from the 5th annual summary of the Pesticide Data Program for January–December 1995, L. Hatamiya (Ed.), United States Department of Agriculture, Washington D.C., 1996.

put by a factor of 4, obviously with a considerable gain in resolution. Fast GC may be the answer to this problem, showing tremendous speed of analysis on very narrow bore capillary GC columns. The applicability for trace analysis of this technique is hampered by the high split ratios needed to obtain sharp initial injection bands, consequently leading to yet unacceptable losses in sensitivity [26,27].

Retention data of pesticides can be found in several manuals [2,28,29]. The selection of columns depends on the nature of the pesticides to be separated. For example, for the separation of organochlorine and pyrethroid pesticides a non-polar stationary phase like DB-1 (or OV-1) and DB-5 (or BPX-5) is used. For the separation of somewhat more polar compounds like organophosphorus compounds OV-17 (or DB-1701) can be applied, especially in combination with FPD. A polar stationary phase, e.g. DB-wax, is suitable for more polar compounds such as methamidofos but its application to some detection modes is limited due to bleeding. For the screening methods non-polar stationary are generally preferred due to their robustness.

2.2. Detection

The value of GC for pesticide analysis can be found in the availability of selective and sensitive detectors. In the 1960s the real breakthrough of GC in pesticide residue analysis was induced by the introduction of electron capture detection, enabling simultaneous analysis of various chlorinated pesticides at detection levels hundred times lower than the available flame detectors. Early electron capture-detectors consisted of a titanium foil on which ^{3}H was embedded. The temperature limit of these foils was only 225°C thus limiting the oven temperatures during gas chromatographic separation. Moreover cleaning the detector at elevated temperatures is impossible, leading to rapid adulteration. The more thermostable ^{63}Ni source gradually replaced the ^{3}H source type since operation temperatures can be used up to 400°C. Electron-capture detection (ECD) only solved part of the problem, halogenated pesticides (DDT, hexachlorocyclohexanes, hexachlorobenzene) could be detected sensitively and selectively, but pesticides without halogens such as organophosphorus insecticides still lacked a sensitive detector in GC.

The success of the ECD prompted the development and application of other selective detection principles for non-halogenated pesticides. From flame ionisation detection (FID), nitrogen–phosphorus detection (NPD) was discovered by the observation that an alkali salt in the flame of a FID system enhanced the ionisation of N and P compounds, which finally led to the first detector with low detection limits and good selectivity over (interfering) carbon compounds [30,31]. Long-term stability however can be a problem in routine analysis when the detector bead, which consists of a rubidium salt, deteriorates.

Flame photometric detection (FPD) is based on element specific luminescence produced when sulfur or phosphorus compounds are burnt in a hydrogen-rich flame. These emission bands of S_2 for sulfur and HPO for phosphorus species can be detected at 394 and 526 nm, respectively. Although selectivity is excellent for the determination of phosphorus and sulfur compounds quenching can occur due to high carbon levels and a non-linear detector response in the case of sulfur. Recent developments in detector technology resulted in the introduction of a pulsed flame photometric detector (P-FPD) which has shown improved performance compared to the conventional FPD regarding sensitivity, selectivity and multi-element capability. Compared to the conventional FPD which is hampered by the fact that sulfur flame emission bands are extended in the region in which phosphorus is measured, the P-FPD shows a time profile for sulfur and phosphorus flame emissions which are different. The P-FPD has the capability to distinguish between these two signals by using electronic gating to eliminate the sulfur response without a significant decrease in the phosphorus signal [32–34].

2.3. Post column hyphenation

As GC is mainly used as a multi-residue screening technique and the ubiquity of pesticides is low there has always been a strong demand for confirmation of positive findings. Originally confirmation was sought in the use of alternative columns or detectors or even alternative techniques such as thin layer or liquid chromatography [35]. Nowadays, post-column hyphenation renders the confirmatory information

needed. One of the oldest examples is the microwave induced atomic emission detector (AED); applications on organophosphorus pesticides can be found in the literature as early as 1965 [36], after a slumber of more than 20 years commercial equipment is presently slowly emerging again in the last decade [37,38]. However, for multi residue screening the application of AED detection is hampered by the fact that the present instruments are not capable to cover all elements in a single run, hence full screening needs several GC-runs [39,40]. The coupling of GC to Fourier transform infrared spectrometry (FTIR) renders a highly selective combination. Early instruments were based on a light pipe design [41] while later cryofocussing of the GC chromatogram led to lower determination limits, more relevant to trace analysis. Nevertheless present instrumentation is rather complex rendering it unsuitable [42].

The confirmation of non-compliant samples in, e.g. trade conflicts, has always been important for residue laboratories and GC–MS has always been seen as one of the most conclusive techniques [35]. The application of mass spectrometric (MS) detection in gas chromatography for pesticide residue analysis initially was inhibited by the fact that direct coupling of packed columns, most commonly used in the early days of pesticide analysis, was incompatible with the vacuum in the ionisation chamber of the mass spectrometer, due to the high carrier gas flow used for packed columns. Developments finally led to a complicated jet separation system in order to selectively remove the small carrier gas molecules. Direct coupling of GC to MS became feasible with the introduction of capillary columns. The first benchtop GC–MS systems, based on quadrupole mass analyzers were introduced in the early 1980s, but for pesticide residue analysis, those at that time expensive instruments lacked sensitivity and tuning these instruments was tedious, rendering them not yet applicable for routine analysis. The introduction of ion trap detectors (ITD) coupled to GC, in the early 1990s, showed to be more applicable for routine application for the analysis of food [43,44] as well as water [45–48]. An important feature of the ion-trap detector is that there is no loss in sensitivity when going from full scan data acquisition to single ion monitoring data. This renders this detector to be well suited for broad screening purposes as encountered in pesticide analysis. Moreover, enhanced selectivity and confirmation can be obtained in the MS/MS-mode of the ITD. A drawback of the instrument is that sensitivity is dependent on the amount of ions present in the trap, thus additional requirements for either calibration procedures (matrix-modified) or clean-up are required. In recent years both ion trap detectors and benchtop quadrupole instruments were improved in both their detector design and operation and acquisition software, leading to the wide spread use of bench-top mass spectrometers in routine laboratories. From proficiency testing data it seems that nowadays both types of instruments yield comparable performance. As compared to ECD LODs are reported to be comparable [49], obviously with much more qualitative reliability.

2.4. Sample introduction

Conventional injection techniques either using a hot liner as evaporation-chamber [50–52] or on-column injection [53–55] usually involve the injection of 1–5 μl of a sample extract into the GC [2–6]. In pesticide residue analysis liner based injection techniques are used more often because the liner serves as a trap for involatiles originating from the sample since internal surface is much larger compared to a retention gap [56]. The elevated temperature during a split/splitless injection may, however, result in decomposition of the more thermolabile pesticides due to the combination of active sites in the liner and elevated temperature. Active sites can be formed when sample extracts contain traces of water which hydrolysis the deactivated surface or even worse will leach the glass surface of the GC liner at injection temperatures over 200°C. For example in the determination of p,p′-DDT active sites or dirty liners will result in the transformation of DDT to TDE or DDE [57]. The problem also arises in the determination of organophosphorus pesticides, e.g. acephate and methamidophos are well known examples of compounds susceptible to active sites in the injector system [28]. Similar problems have been observed in the application of on-column injection. Although conditions are milder compared to the hot splitless injector, it is less robust since the capacity of a retention gap towards involatile sample

components which will remain in the injection area is lower than that of a liner. With on-column injection, involatile matrix components will quickly spoil the retention gap resulting in excessive peak-tailing [58] and periodic replacement of the retention gap will be necessary. With splitless injection, on the other hand, peak tailing caused by a 'dirty liner' will not show up in the final chromatogram due to the solvent refocussing effect in the colder GC oven during injection. Hence, splitless injection is generally preferred in the analysis of pesticides due to its robustness. Quantitative reliability can be enhanced by using matrix matched calibration. Due to the low occurrence of pesticides blank extracts can be obtained easily. These extracts spiked with calibration solution can serve as a correction on matrix influenced responses [59].

2.5. Precolumn hyphenation

As will be demonstrated in the application section in this overview, the injection of larger volumes of extract is of great importance for the automation of sample preparation. On-column introduction in combination with an GC autosampler with low injection speed by means of gas-pressure has been applied for the automated introduction of aliquots up to 10 μl on a routine basis. In this set-up all evaporating solvent vapors (*n*-hexane) were vented through the entire GC-system including the detector [60]. However, developments in LC–GC hyphenation required target fractions exceeding 10 μl. Higher volumes can be injected by using a GC precolumn system equipped with an early vapor exit consisting of a T-splitter between the precolumn system and the analytical column [61]. In this set-up introduction time can be reduced while the column and the detector are protected from the excess of solvent.

The injection of water or even water-saturated polar organic solvents using an on-column injection with retention gaps will eventually lead to deterioration of the silanised surface [62]. However, when the deactivation layer is damaged, dynamic deactivation is possible by the addition of a high boiling alkane as a co-solvent [63]. Even without high boiling alkanes, the solvent alone can shield active sites in a retention

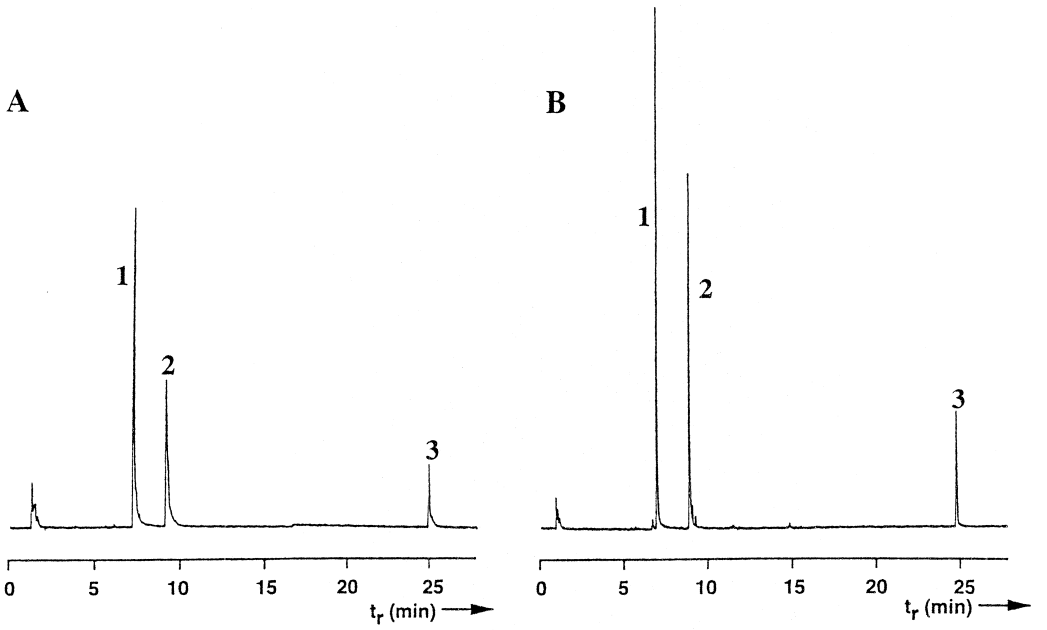

Fig. 1. Deactivation properties of a *n*-hexane–acetone solvent mixture (3:1; v/v). Injection of pesticides on an active retention gap. Large volume on-column injection. Left: Conventional 1 μl on-column injection of a mixture of 1: acephate (0.2 ng), 2: methamidophos (0.2 ng) and 3: vamidothione (0.1 ng). Right: Large volume injection (50 μl) containing the same absolute amounts of pesticides. Flooded zone just below the length of the retention gap.

gap. Large volume injection also alleviates some of the problems encountered in normal scale injections. Fig. 1 clearly shows the improvement in peak shape when using an active retention gap on which a conventional 1 μl on-column injection (left) of a mixture of acephate, methamidophos and vamidothione. The second chromatogram shows an injection of 50 μl which is performed from the same solution 50 times diluted. The flooded-zone almost equals the length of the retention gap, thus depositing the sample further down-stream, minimizing the interaction of the components with the surface.

Parallel to the developments in on-column methodology, the first programmed temperature injectors (PTVs) with liner internal diameters of 1 mm were developed and were capable of splitting the solvent to waste (vent) whilst minimizing the loss of analytes [64,65]. Speed controlled introduction in PTV injection was performed by Staniewski et al. [66] but the breakthrough for PTV injection was initiated by the availability of liners with larger internal diameters with a capacity of at least 100 μl without the need of speed controlled injection [67]. A PTV injector in combination with an autosampler using a stepper-motor, originally developed for large-volume on-column introduction [68], allowed automated large-volume introduction to be performed on a routine basis. However, injection without loosing the more volatile components cannot be achieved without careful optimisation and additional (cooling) devices.

Although the first applications of large volume injection were performed with the on-column injection technique, the use of PTVs is increasing probably for the same reasons that in conventional splitless injection is preferred over on-column injection.

3. Applications

3.1. General considerations

Several aspects play a role in the design of a sample preparation method:

1. GC-requirements; matrix-interferences should not jeopardize the injection system, the column or the detector.
2. The sensitivity requirement; reliable data should be generated at the level of the maximum allowable concentration.
3. Homogeneity; the laboratory sample must be representative for the consignment of food, or the compartment of the environment studied, thus hampering miniaturisation in the processing of solid samples.

Important application areas for pesticide residue analysis are foodstuffs, soil and water. Limits of quantification to be achieved generally amount from 20 μg/kg up to several mg/kg for foodstuffs, for soils usually 1–20 μg/kg is required while, in Europe, for water intended for human consumption 0.1 μg/l is the maximum allowable concentration for a pesticide, while the sum of all pesticides may not exceed 0.5 μg/l. As a rule of thumb most GC detectors are able to adequately quantify 10 pg of analyte. Based on this assumption Table 6 shows the equivalent sample amounts that should reach the detector for the alternative application field. It is clear that food requires the lowest sample amounts, while the analysis of water requires at least a 1000-fold higher amount. Soil is in the middle position requiring higher concentration factors with a relatively high interfering carbon load. On the other hand the carbon load of food samples is much higher. Due to both occurrence of different pesticides and sample type food-applications can be subdivided in products of plant or animal origin. Products of animal origin often contain fat (triglycerides) which cannot be injected into the GC without damage to the injection system leading to additional demands in sample preparation. Below the applications of pesticide analysis in water, soil and food are described.

3.2. Water

Basically water serves two purposes: (i) as a

Table 6
Equivalent sample amounts that should reach the detector for the different application fields

	Food	Soil	Water
MRL (μg/kg)	10–10 000	1–20	0.1
Minimal sample amount required for 10 pg analyte (mg)	1–0.001	10–0.5	100

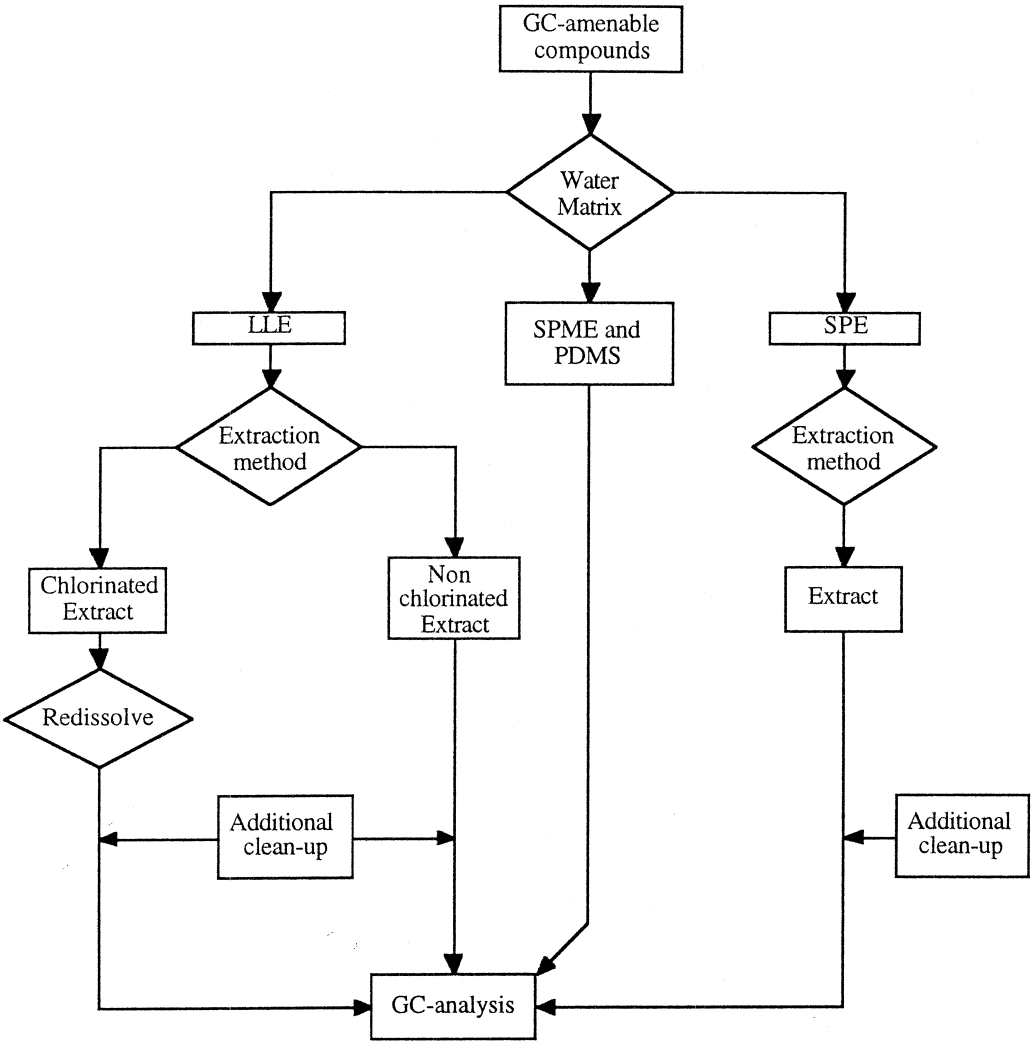

Fig. 2. Sample preparation in the analysis of water. LLE: liquid–liquid extraction, SPME: solid-phase micro extraction, PDMS: sorption on polydimethylsiloxane and SPE: solid-phase extraction.

source for our drinking water and (ii) as the living environment for aquatic organisms. From concern over both issues the demand for trace analysis of pesticides in water has emerged. The concern over water quality in Europe led to stringent regulations for drinking water [3,69]. Limits in the USA or World Health Organisation guidelines usually indicate higher levels, however. On the other hand, lower levels may be indicated since, ecotoxicological studies have shown that some pesticides can be hazardous to aquatic organisms even in the low ng/l range [70]. An overview of currently used approaches to water analysis is depicted in Fig. 2. The two major pathways are: (i) liquid–liquid extraction and (ii) solid-phase extraction (SPE).

3.3. Liquid–liquid extractions

Using liquid–liquid extraction, typically a volume of 500–1000 ml is extracted with either *n*-hexane or

dichloromethane which was followed by an evaporation step to dryness when needed and subsequently GC analysis of several microliters was performed [71]. The *n*-hexane extraction will selectively yield the non-polar pesticides, while the dichloromethane extraction will cover a wider polarity range but obviously also include more matrix interferences. The major drawbacks of liquid–liquid extraction are the low sample throughput due to manual concentration steps and the large amounts of organic solvents used creating a waste problem. One way to avoid the excess organic waste is to reduce the volume of organic solvent, by extracting 1000 ml of sample with 1 ml solvent [72]. Disadvantages of this procedure are unfavorable phase-ratios which may render low extraction efficiencies, and the requirement that the extracting solvent should be completely immiscible with the water sample, which is difficult to achieve with the more polar solvents that will dissolve in the sample.

The emergence of large volume injection techniques opens new perspectives towards avoiding the use of large amounts of organic solvents. Miniaturised liquid–liquid extraction can also be performed by flow injection technology yielding a favorable phase-ratio. The flow contains segments of organic solvent and aqueous sample and extraction is performed through axial dispersion in a PTFE coil, followed by separation of the aqueous phase from the organic phase by a flow injection type phase separator [73,74]. The on-line approach can also be used for interfacing reversed-phase LC with capillary GC by on-line extraction of the analytes from the aqueous LC mobile phase into solvents of lower polarity [75,76]. On-line approaches can be very attractive, but it should be mentioned that alternative somewhat less complicated approaches are also feasible. Batch wise extraction of 1 ml organic solvent and 1 ml water sample will render the same analytical performance using only standard laboratory glassware. Automation of such a system, can be performed by means of an LC autosampler and has been shown for organophosphorus pesticides in surface water [77]. Manual and automated procedures were reported to be comparable for the analysis of triazines in water [78]. Water is to some extent soluble in suitable polar solvents like ethyl acetate or methyl *tert*-butyl ether (MTBE) while dichloromethane, in which the solubility of water is low, is poorly compatible with flame-type detectors.

3.4. Solid phase extraction

Original applications of SPE used modified silica on which a volume of water of typically 0.4 to 5 l was enriched on a 100 mg to 1 g cartridge [79,80]. Originally C-8 or C-18 solid phases were applied, but for the more polar compounds breakthrough occurs. Therefore, more hydrophobic sorbents such as XAD [81,82] and styrene–divinylbenzene (SDB) [83] have been applied, leading to successful isolation of the more polar compounds. A drawback of these solid phases is the coextraction of interferences leading to a need for more selective sorbents. Selectivity can be enhanced by chemical modification of the resin [84]. An even more selective approach is the use of immuno-affinity chromatography, in this approach antibodies are coupled to the solid-phase leading to highly specific retention [85,86]. However, for GC applications one should realise that enlargement of the scope of an extraction method has its limits since very polar compounds will rarely be amenable to gas chromatography. Also the more selective materials may be superfluous because of the high inherent selectivity of the GC separation and detection. Sorbents commonly used in combination with GC methods are SDB [87] and PLRP [88]. As an alternative for SPE cartridge, membrane extraction disks are available on the market. These extraction disks typically contain some 90% of a polymeric or alkyl-bonded silica material in a PTFE mesh [89]. The advantage of extraction disks is that samples can be loaded at high flow-rates with less chance of clogging. However, due to the dimensions of the equipment desorption requires more organic solvent [90]. Due to the large number of analyses required, the elimination of manual steps is needed. The developments made in the introduction of larger sample volumes into GC, as described above, makes it possible to combine miniaturised sample pretreatment methods on line with GC.

However, straightforward downscaling of a conventional extraction procedure is hampered by the fact that routinely applied extraction solvents are not

compatible with GC due to their polarity and volatility.

3.5. Automated solid-phase extraction

On-line application of solid-phase extraction methodology in combination with GC originated from coupled LC–GC. In these applications small volumes from the LC eluent were transferred through a modified GC-autosampler [91,92]. Similar to the development in liquid–liquid extraction large volume injection also had its benefits in the automation of solid-phase extraction procedures. On-line coupling of SPE to GC applied to chlorinated compounds was first achieved by Noroozian et al. [93] as an logical continuation of their work on coupled LC–GC [94]. In these applications desorption took place with *n*-hexane which was a convenient solvent for the GC-introduction but hampered the desorption of more polar compounds. For these compounds Vreuls at al. used more polar solvents such as ethyl acetate or *n*-propanol [95]. These solvents require careful optimisation of the transfer procedure since early transfer leads to the injection of water, while too late a transfer would lead to the loss of analytes. Traces of water do not pose a problem because the solvents mentioned above will evaporate azeotropically. Another solution to the drying problem is the use of extraction disks as used by Kwakman et al. [96]. In this application organophosphorus pesticides were preconcentrated on three 0.5 mm thick, 4.2 mm extraction disks packed in a cartridge. This cartridge can be dried efficiently with a nitrogen purge at ambient temperature for 10–15 min.

Similar to off-line applications of SPE, the application of co-polymer based sorbents such as PLRP-S and SDB have become increasingly popular. The stronger sorption characteristics are shown in the determination of nitrogen/phosphorus containing pesticides with GC-NPD. In this application 2 ml of sample is enriched on a 10×2 mm I.D. precolumn packed with a PLRP-S stationary phase. The sample is desorbed with 500 μl MTBE–ethyl acetate [63]. The actual enrichment factor achieved by SPE in this application is only a factor of 4, in fact the SPE cartridge is utilised to achieve a phase-switch between water and a solvent more suitable for GC-injection. A major advantage of these small sample volumes is that breakthrough of polar pesticides is circumvented, thus extending the scope to more polar compounds.

Off-line SPE in combination with large volume GC injection may also be attractive. The flexibility of off-line systems with their capability of different flushing steps for conditioning, sample loading, rinsing and sample desorption has advantages over the less flexible on-line set-up. Autosamplers capable of handling SPE cartridges or extraction discs have been applied for the determination of pyrethroid insecticides in surface water [97] and triazines in surface water [98]. The same equipment can be utilised for other purposes by utilising silica cartridges, for the automated clean-up of 18 electron-capturing compounds from surface water with high organic carbon content [99].

New developments in water analysis involve sorption-thermal desorption techniques. An exponent is solid-phase microextraction (SPME) as developed (and patented) by Pawliszyn et al [100–103]. Other techniques based on similar principles have been published by Mol et al. [104,105]. They used open tubular extraction coupled to PTV-GC for the analysis of organochlorine compounds. Recently polydimethylsiloxane (PDMS) is used in packed liners.

A relatively new approach involves involves preconcentration on these packed liners followed by thermal desorption in the GC injector [106].

3.6. Fruits and vegetables

Food analysis is the oldest application, therefore, at least some consensus exists on the general approach in sample treatment. Worldwide, two extraction procedures are applied; (i) acetone followed by partitioning with a mixture of dichloromethane and light petroleum (the Luke method) and (ii) extraction with ethyl acetate in the presence of sodium sulfate. These procedures have been harmonized at the national level [2,29,107–109]). While nowadays, slowly, international standards emerge on the level of general principles, there is no consensus on a single analytical procedure [110,111]. The international standards published so far have an open character and their set-up is modular describing alternative extraction, clean-up and instrumental steps. The user may combine alternative steps as

required for the specific problem at hand. Fig. 3 shows an example of the modular approach as applied in the Dutch National Manual [2]. More detailed standardisation is not seen as useful due to the wide scope in terms of pesticide-matrix combinations. In samples encountered in common laboratory practice some combinations are more likely than others; e.g. organophosphorus pesticides in fresh fruits such as strawberry and grapes or fungicides on storable crops such as carrots and potatoes. That is why laboratories have a tendency to fine-tune their methodology towards the combinations most frequently found. Yet harmonized methods and proficiency testing programs are of the utmost importance in generating comparable results in international trade. The extraction procedures mentioned above apply to non-fatty samples, defined as samples with a fat percentage of lower than 5%. The acetone extraction, according to Luke et al., starts with blending the sample in solvent followed by partition with a mixture of dichloromethane and petroleum ether [112,113]. A disadvantage of the Luke extraction method is the application of dichloromethane which is less desirable in view of present views on the environmental impact of chlorinated solvents. In Germany dichloromethane partition is gradually replaced by extraction with ethyl acetate–cyclohexane (1:1; v/v) [114]. Acetone was preferred because it is completely miscible with water, thus allowing a good penetration in the aqueous part of the crop.

In the mid-1980s ethyl acetate extraction was introduced. In this case the sample was blended in

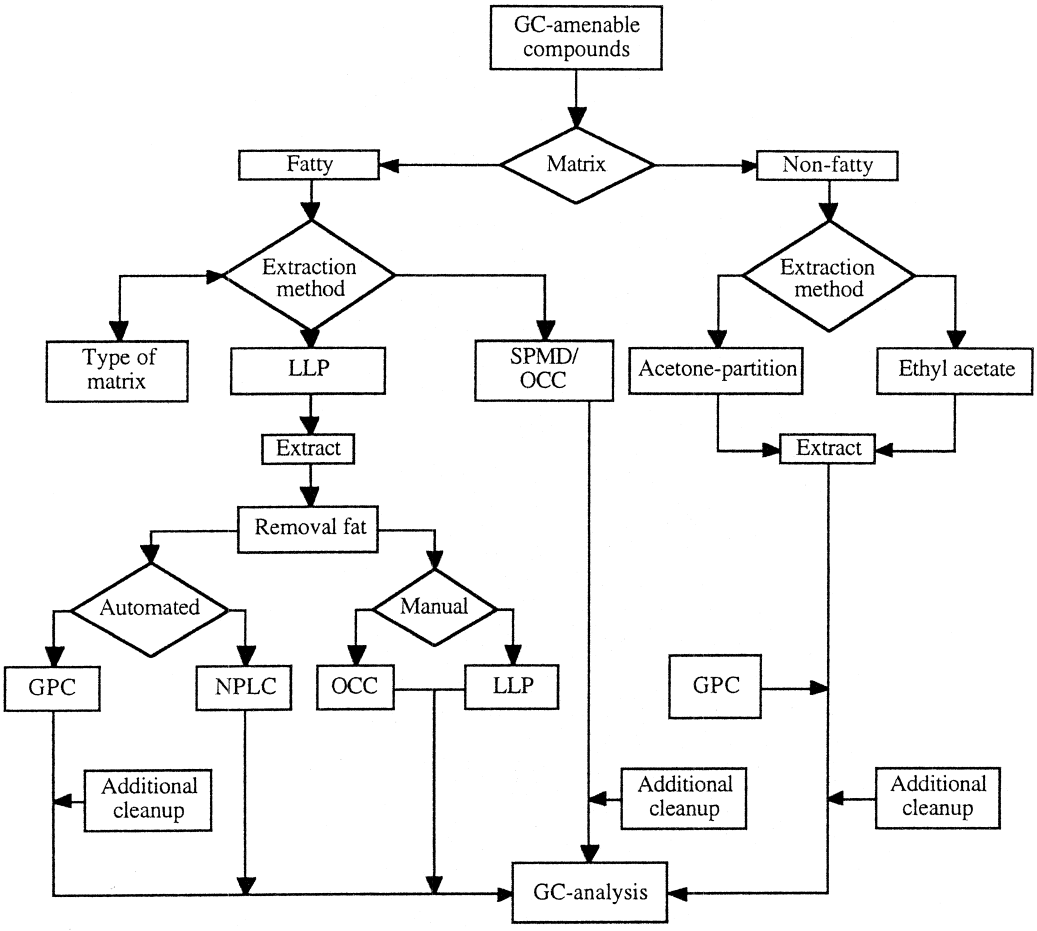

Fig. 3. Modular set-up in sample pretreatment in the Dutch National Manual. GPC: gel permeation chromatography, LLP: liquid–liquid partition, OCC: open column chromatography, NPLC: normal-phase liquid chromatography and SPMD: solid-phase matrix dispersion.

the presence of sodium sulfate [2,115]. The advantage of extraction with ethyl acetate is that the procedure is claimed to be less laborious, whilst yielding comparable results [2,109]. Ethyl acetate seems to be sufficiently miscible with water to allow good penetration into the plant cells and its polarity is sufficient to extract the more polar pesticides. Ethyl acetate is not completely miscible with water, hence after extraction no extra partition step is required, the water is simply removed by the excess of sodium sulfate.

In many instances clean-up steps can be omitted because the maximum residue limit (MRL) is in the mg/kg range or the compound can be detected sensitively. Obviously for more difficult sample types such as onions and leek, which contain, e.g. excessive amounts of sulfur, or for analytes with poor detectability at lower MRLs gel permeation chromatography (GPC) is generally applied. The application of GPC clean-up offers the widest scope [116], although small interfering molecules may be not removed from the final extract. In order to remove these interferences and to perform separation on polarity rather than size, GPC clean-up followed by mini silica gel fractionation can be applied [117]. On the other hand, when adsorption chromatography is used anyway, a single clean-up over Florisil [118,119] or silica [120] may be more convenient because it comprises only one step. In a recent document from the Codex Committee on Pesticide Residues an inventory was made of methods commonly used in government laboratories or other laboratories involved in the determination of tolerance compliance [121]. From this inventory it seems that an important part of the laboratories still perform their analysis on the basis of the conventional selective detectors; for organochlorine compounds often a clean-up step by either adsorption chromatography or gel permeation chromatography is included while organophosphorus and organonitrogen compounds are often analysed directly after extraction without clean-up, for MS-based methods there is a tendency of omitting the clean-up step.

3.7. Products of animal origin

Products of animal origin may have been contaminated by pesticides through the foodchain or by the use of pesticides as veterinary drugs. Target compounds are in most instances those pesticides that accumulate in the fat. Therefore, the polarity range of the pesticides is more limited than in fruit and vegetables. DDT, for example, is still a topic of major concern [122–124]. Basically the first step is the isolation of the fat from the matrix by either extraction (milk, eggs) or by rendering the fat (meat). The fat can be redissolved in a suitable solvent and has to be separated from the analytes. For the removal of the triglyceride matrix, various methods based on adsorption chromatography have been applied.

Clean-up methods based on Florisil [125,126], alumina or silica gel [127,128] have been used extensively. A more efficient removal of the fat matrix can be achieved by modification of silica gel with e.g. sulfuric acid [129]. However, less stable pesticides such as DDT, endrin and organophosphorus compounds may decompose thus hindering a universal application of this procedure. A disadvantage of both alumina and silica is their change in activity over a period of time. The activity is adjusted by the addition of water. The activity adjustment must be done by recovery measurement of critical compounds rendering this procedure to be laborious. Moreover, batch-to-batch reproducibility of these sorbents is usually poor which means that the whole procedure must be repeated after a change of batches.

Normal-phase HPLC is the more sophisticated alternative for adsorption chromatography. An important advantage is that the separation process can be monitored with the UV detector thus simplifying the adjustment procedure. Gillespie and Walters used a semi-preparative silica LC column for the separation of organochlorine and organophosphorus pesticides from butterfat [130]. Hogendoorn et al. used a normal-phase LC with column switching in order to obtain both group separation between pesticides and PCBs and removal of the fat-matrix [60]. If group separation is not required column-switching can be omitted [131]. The application range of this procedure to organophosphorus pesticides has been reported by Serrano et al. [132]. On-line GC application has been reported by Cortes and van der Hoff [133,134], yielding fully automated clean-up procedures.

For the separation of the fat matrix from the analytes GPC is an obvious candidate due to the

difference in molecular size between triglycerides and pesticides. It has the same advantages towards automation as HPLC, combined with a much wider scope since essentially pesticides are eluted in one fraction. After the introduction of the technique by Stalling et al. [135] the full potential of the method was adequately shown by Specht et al. [114,117]. A disadvantage of these applications were the fraction volumes in which the pesticides eluted, miniaturization of the GPC-column from typically 10 mm I.D. to 2.0 mm I.D. was reported by van Rhijn [136]. This work resulted in a reduction of the elution volumes GPC from 150 ml to typically 10 ml. On-line coupling of GPC to GC requires even smaller target fractions, Vreuls reported an on-line application for organophosphorus pesticides in olive-oil using a PLGel stationary phase with 5 μm particles instead of the commonly used Bio-Beads SX-3 [137]. All analytes could be transferred in a 2.5 ml fraction. Coupling of GPC high-performance adsorption chromatography in an automated system has been reported by Rimkus et al. [138].

Supercritical Fluid Extraction (SFE) has been successfully applied in the analysis of PCBs and polychlorinated dibenzodioxins and polychlorinated dibenzofurans; selective extraction is possible in the presence of a fat-retainer such as modified silica [139] or basic alumina [140], in order to reduce the coextraction of triglycerides [141]. Pesticides, however, usually comprise a broader polarity range, consequently polarity or solubility modifiers are required in SFE, resulting in a percentage of over 5% of co-extraction of the total lipid sample material, containing approx. 5 mg fat per ml to be introduced into GC [142].

In solid-phase matrix dispersion (SPMD) the fat matrix is thoroughly mixed with an SPE sorbent. The sorbent-sample mixture is put in a chromatographic tube after which the mixture is eluted to obtain the pesticide fraction. These developments with new adsorption materials may be an attractive alternative route for sample preparation in fatty samples [143,144].

The Codex Committee on Pesticide Residues also made an inventory of methods commonly used in government laboratories for products animal origin [145]. Basically two methods are widely in use; (i) adsorption chromatography over florisil according to AOAC method 970.52 [111], or (ii) GPC according to Specht et al. [114].

3.8. Soil

Contrary to food, extraction of soil samples is a topic in which new approaches are published more frequently. The interaction between the matrix and the analytes is stronger than in food so that bound residues can be formed, with different extraction behavior than the non-bound fraction. Therefore, in order to obtain comparable results an extraction procedure is required capable of liberating the bound residues of these analytes. Usually the liberation of the bound fraction requires prolongated contact time of the sample with the extraction fluid, and enhancement of contact by shaking, sonication or elevated temperatures. Regarding methodology for extraction; sonication with methanol [146] or mixtures of acetone and hexane [147] as well as liquid–solid extraction procedures have been reported. These procedures were either time consuming or labor intensive and therefore more automated procedures were required to elevate these disadvantages.

Soxhlet extraction has been applied for 30 years, and although time consuming it is more or less seen as the most exhaustive procedure. Due to the high recovery rates it is seen as a reference method for soil extraction [148].

In the application of SFE, extraction is performed by supercritical CO_2, with solubility of the analytes in supercritical CO_2 tuned by changing the density of the fluid. This is generally obtained by optimisation of the CO_2 pressure and the temperature of the extraction cell [149]. However, for quantitative extraction of moderately polar pesticide residues in soil, a modifier such as methanol has to be applied in order to obtain satisfactory results [150–152]. SFE has shown to be an extraction technique with which selectively one group of compounds can be isolated from soil, however, similar to the application of SFE to animal material, its potential as a broad screening technique has not been shown yet.

More recently, sophisticated equipment has been developed which combines elevated extraction temperature and high solvent pressure to increase the extraction efficiency. The microwave-assisted sol-

vent extractor (MASE) is based on a static extraction and microwave energy is applied to a closed vessel system with sensors in order to reach a preset temperature–pressure equilibrium. Polar solvents are needed to absorb the microwave energy and generate heat. This extraction technique has been introduced for soil analysis in 1986 using an ordinary domestic magnetron for the extraction of organophosphorus compounds [153]. Laboratory versions became available just some years ago. Optimisation of the extraction parameters using a statistical approach, maximum likelihood modeling, for fresh and aged pesticide residues in sand, clay and peat soils showed only strong temperature dependence on the extraction efficiency [154]. Results show that this extraction technique is applicable for a broad polarity range, for organochlorinated pesticides [155], organophosphorus compounds [156] as well as for more polar sulfonylurea herbicides [157]. Fig. 4 shows the chromatograms of yet unpublished results for the on-line LC–GC-ECD analysis of a sand, sea-clay and peat sample extracted with MASE after spiking with ten organochlorinated pesticides at levels of 1.0, 1.0 and 0.3 μg/kg. These chromatograms clearly show that in sand and clay, determination can be performed to sub-μg/kg levels while clean-up for peat samples is clearly not sufficient.

In pressurised liquid extraction (trade name: ASE) a high-pressure LC pump is used to program solvent pressure and composition on the sample cell which can be temperature-programmed. In comparison studies with Soxhlet extraction applied to organochlorinated pesticides, organophosphorus compounds and herbicides in different soils, good agreement has been found between results of both extraction procedures [158]. Based on these results, this extraction method has been determined by the Environmental Protection Agency (EPA) as equal performance in comparison to Soxhlet extraction [159].

At this moment, progress is still needed in development of extraction methodology for pesticides. The development of a unified procedure is severely hampered by the fact that soil composition varies from sand, with a low organic matter content, to heavy loamy soils, with higher organic matter contents. Furthermore, very little investigation is performed in the extraction of the most polar pesticides and their metabolites. Therefore, no consensus is reached on generally accepted standardised methods (Fig. 5).

4. Conclusion

The large majority of pesticide determinations are performed in either food, soil or water samples. Food analysis is by far the oldest application field and therefore a limited number of multi-residue methods are currently in use. Basically ethyl acetate or acetone extraction are the methods of choice. Clean-up steps are optional but GPC is the most versatile and widely used technique. The availability of inexpensive and sensitive bench-top mass spectrometers has contributed to the development of multi-residue methods with a scope of approximately some 300 pesticides with a variety of chemical and physical properties. Though the available methods today suffice, new legislation on, e.g. baby-food or the emergence of ‘eco-food’ on the market, claiming essentially pesticide free commodities may well lead to the need for far more sensitive methods applying larger concentration factors and consequently more rigorous clean-up. An example of legislation driven developments is the water area, the EU drinking water directive prompted the development of methods at a lower detection level, while the scope of these methods was more aimed at polar herbicides than at the insecticides and fungicides that food analysis originally focused on. This means that advanced large volume technology has found more basis in the environmental field than in food analysis. At present methodology for water seems to converge to solid-phase extraction in combination with large volume GC, either on- or off-line. The analysis of soil is still hampered by the fact that the matrix soil is extremely variable, e.g. a method that works on a sandy soil may not render acceptable results on a peat soil. For this reason the development of efficient, cost-effective and selective extraction methods tends to the direction in soil analysis.

Challenges in pesticide analysis today are the introduction of pre-column hyphenation in food analysis in order to meet the sensitivity requirements of tomorrow and the development of selective and efficient extraction methods. For the water applications the importance of GC will probably decrease

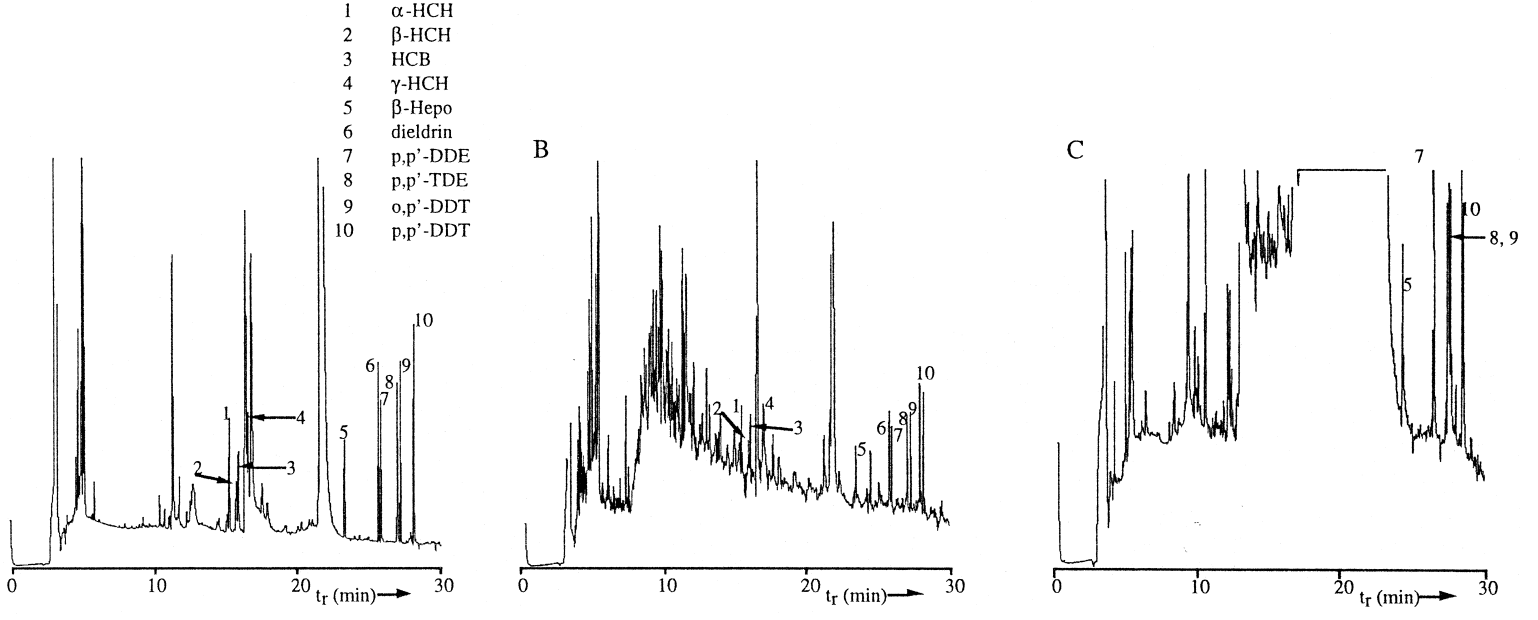

Fig. 4. GC chromatogram obtained after LC–GC/ECD analysis of a sand sample spiked at a level of 1.0 μg α-HCH/kg (att. ×128) (A), a sea-clay sample spiked at a level of 0.3 μg α-HCH/kg (att. ×128) (B) and a peat sample spiked at a level of 1.0 μg α-HCH/kg (without dieldrin) (att. ×64) (C). Peak identification see (A).

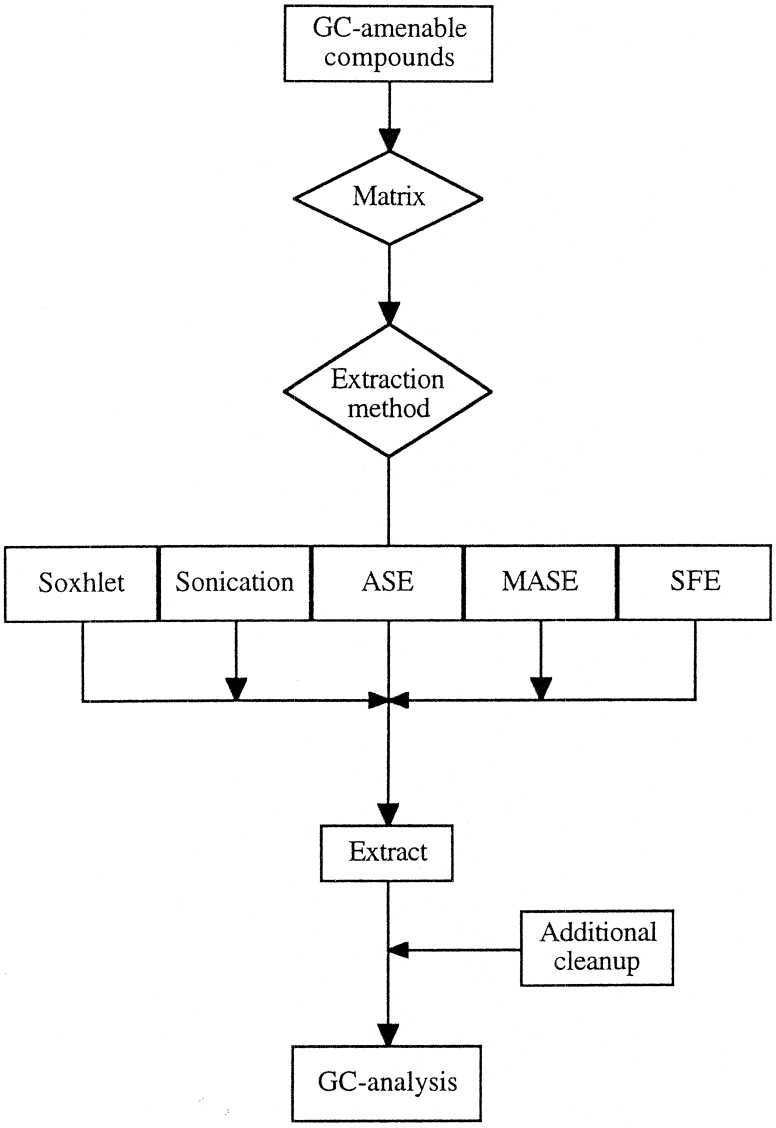

Fig. 5. Sample preparation in the analysis of soil and sediment. ASE: accelerated solvent extraction, MASE: microwave assisted solvent extraction, and SFE: supercritical fluid extraction.

because new problem areas will probably include the more polar transformation products. To this respect LC/MS will gain importance for this area.

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