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Review

Analysis of organic micropollutants in the lipid fraction of foodstuffs

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ABSTRACT

An overview is given of current techniques for the analysis of organic micropollutants that accumulate in the fatty fraction of foodstuffs, such as pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, polychlorinated dibenzodioxins and polychlorinated dibenzofurans. Isolation and clean-up are considered to be of great importance in the field of residue analysis. In general, problems are related to the low levels of the individual compounds at which they usually occur and the complexity of extraction and clean-up procedures for isolating and separating analytes from matrix components and other contaminants. Therefore, special attention is focused on sample pretreatment and on coupled chromatographic techniques, showing developments towards multi-residue methods, miniaturization and automation of analytical procedures. Coupling of chromatographic techniques with spectroscopic techniques is also considered as an important tool for identification and confirmation purposes.

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1. INTRODUCTION

In the last few decades, considerable effort has been put into the development of analytical schemes for the determination of persistent halogenated hydrocarbons and pesticides in foodstuffs. The production and use of certain organic compounds in agriculture and the unintended formation of certain contaminants during chemical and combustion processes have led to a world-wide occurrence of these compounds in the biosphere. One result is that some foodstuffs may become contaminated by trace amounts of these compounds. Some groups of these compounds exhibit a high degree of persistence and predominantly accumulate in the lipid fractions of the human food chain, by which food has become a major route of exposure for humans.

In order to assess the daily exposure to organic contaminants in food by the general population, several field studies have been performed on the occurrence of polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polychlorobiphenyls (PCBs) and, more recently, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in food [1–3]. Problems in chemical analysis are usually related to the low levels of individual compounds and the complexity of extraction and clean-up procedures to isolate the analytes from the food components, caused by the presence of major and minor components (matrix, other contaminants) which potentially disturb reliable identification and quantification. Several solutions have been proposed to decrease limits of detection (LOD) to the appropriate levels at which the analytes occur and to eliminate major sources of interference such as lipids, waxes and animal sterols in tissue extracts and carotenes in extracts from vegetable materials [4].

In order to identify and quantify residues of the different classes of organic compounds, well defined schemes have been established for the analysis of individual components or specific groups of compounds. To ensure and improve the reliability and comparability of analytical work in this field, these analytical schemes are well documented in reference texts, such as the *Pesticide Analytical Manual* (FDA) [5], the *Manual for Analytical Quality Control* (EPA) [6], the *Guide to Chemicals in Crop Protection* (1982) [7], *The Pesticide Manual* (UK) [8], *Analytical Methods for Residues of Pesticides* (Netherlands) [9], *Manual of Pesticide Residue Analysis* [10], *Official Methods of Analysis of the AOAC* [11] and *The Agrochemicals Handbook* [12]. Current methods for extraction, clean-up and group separation techniques in organochlorine trace analysis have been recently reviewed by the IUPAC Commission on Microchemical Techniques and Trace Analysis [4]. Further, several national and international frameworks (CEN, EC–BCR, WHO, FAO) frequently evaluate the analytical performance of laboratories performing analyses of specific analyte–matrix combinations by means of inter-laboratory comparison studies. This all may have led to a high degree of comparability of procedures applied in the analysis of foodstuffs and an acceptable level of reliability of the analytical results obtained.

In the last few years, several trends can be observed in the trace analysis of organic compounds. First, a growing tendency towards multi-residue methods for the simultaneous identification and quantification of several groups of compounds exhibiting similar environmental and/or toxic properties, such as pesticides, PCBs, PCDDs and PCDFs, can be observed. In this regard, developments usually involve adapting and modifying conventional techniques. Second, coupling of chromatographic and detection techniques, either off-line or on-line, miniaturization and automation of both sample pretreatment and analytical procedures have become of growing interest in the organic analytical field. Most of this work is primarily directed to have access to less time-consuming, miniaturized and automated procedures, providing useful tools for less expensive routine analyses in field studies and regulatory practice. Finally, other contaminants which pose a potential hazard after environmental and human exposure have become of interest. For some of

these compounds (*e.g.*, planar and mono-*ortho*-PCBs, polar pesticides, di- and tetrachlorobenzyltoluenes), a more profound development seemed to be necessary, as modifying available master schemes did not provide the necessary sensitivity and selectivity to determine these compounds at the levels at which they usually occur.

This paper critically reviews recently developed methods for the isolation/extraction and subsequent clean-up and determination of persistent halogenated hydrocarbons and pesticides in the lipid fractions of foodstuffs. Papers were selected by means of a literature search covering the period from 1985 until December 1991; for the historical perspective older references have also been included. The search was restricted to chromatographic techniques for the analysis of the following classes of compounds: pesticides (*e.g.*, OCPs), PCBs (including planar and mono-*ortho*-substituted congeners), polycyclic aromatic hydrocarbons (PAHs), PCDDs and PCDFs. Other reviews have been consulted to offer a broader view on current trends in the analytical field. Table 1 gives an overview of the analytical techniques currently employed by routine laboratories for the determination of various contaminants in fatty foods.

2. SAMPLE PRETREATMENT

Field studies dealing with food contaminants are usually performed either on total diet samples or on individual food products. Wells [4] reviewed current methods for the isolation of trace organics from several types of matrices.

In dietary intake studies [13], collected samples usually refer to pooled samples of food products (meat, fruit, vegetables, drinks, etc.) that an individual person has consumed during one day (24 h). In most studies, collected samples are initially freeze-dried to remove the water content and subsequently blended [14] in the presence of an organic solvent mixture such as acetone–pentane (1:1, v/v) or Soxhlet extracted [15] with organic solvents such as pentane or hexane to isolate the lipid fraction of the sample.

Methods for the isolation of lipid fractions from individual food products depend on the type of sample. Butter, fats and oils are generally assumed to be homogeneous, and normally do not require extensive extraction procedures. Aliquots of such samples can be dissolved in *n*-hexane or light petroleum to the desired concentration.

Meat products having a lipid content of *ca.* 10 wt.% or lower are initially blended and homogenized. Next, a representative test sample is ground with anhydrous sodium sulphate, until a free-flowing powder is obtained. This mixture can then be extracted by using either blending techniques (see earlier), a cold column extraction technique (elution of a column packed with a dried mixture with an organic solvent or solvent mixture) [16–18] or a Soxhlet extraction technique [15].

Milk can either be freeze-dried or chemically dried with anhydrous sodium sulphate, followed by Soxhlet extraction with organic solvent, or subjected to a liquid–liquid extraction procedure consisting of mixing with sodium oxalate and ethanol or methanol, followed by (repeated) extraction steps with a combination of organic solvents such as acetone–pentane [9] or diethyl ether–light petroleum [11].

Vegetable materials usually have a high water content and will dehydrate prior to analysis unless extracted immediately after sampling. Vegetables are therefore crushed, chopped and gently dried at 40–50°C prior to storage and analysis. Isolation procedures include grinding with coarse sea sand, blending, mixing with a more polar solvent (acetone) and subsequent partitioning with dichloromethane or hexane (applicable for matrices with a high sugar content) [19] or mixing with a more polar solvent (acetone) followed by either shaking or Soxhlet extraction [4].

The methods described above represent current techniques for isolation and are still widely in use at laboratories performing trace analyses of organic contaminants in foodstuffs. These methods have frequently served as the starting point for the variety of clean-up and separation methods described in the following sections. More recently, other methods for isolation/extraction, *e.g.*, solid-phase extraction, liquid–liquid extraction and supercritical fluid extraction, have been introduced. A brief description of the latest developments and a short evaluation of their applicability are presented. Sample purification schemes serve two purposes: removal of gross levels of co-extractants and separation of the organochlorine residues into groups, based on

TABLE 1

OVERVIEW OF ANALYTICAL TECHNIQUES FOR DIFFERENT CLASSES OF ORGANIC MICROPOLLUTANTS CURRENTLY IN USE IN ROUTINE LABORATORIES

Class of compounds	Sample pretreatment techniques		Analysis	References
	Extraction	Clean-up		
PAHs	Saponification Solvent extraction Dimethylformamide–water partitioning Caffeine complexation	Adsorption chromatography on silica Adsorption chromatography on alumina Adsorption chromatography on XAD-2 Molecular partitioning on Sephadex LC-20	HPLC–fluorescence/UV GC–FID–MS	1,4,93,94, 130
PCDDs, PCDFs (planar PCBs)	Solid-phase (column) extraction Solvent extraction Soxhlet extraction Liquid–liquid partitioning	Adsorption chromatography on silica Adsorption chromatography on carbon Adsorption chromatography on alumina Adsorption chromatography on Florisil Gel permeation chromatography	GC–MS	2,4,58–61, 68,131
PCBs (planar)	Saponification Solvent extraction Liquid–liquid partitioning Soxhlet extraction	Adsorption chromatography on silica Adsorption chromatography on carbon Adsorption chromatography on alumina Liquid–liquid partitioning Treatment with sulphuric acid Gel permeation chromatography HPLC–Hypercarb column (PGC)	GC–ECD GC–MS	24,64,66, 68–69,81,82
PCBs (planar and mono-*ortho*)	Saponification Solvent extraction Liquid–liquid partitioning Soxhlet extraction	Treatment with sulphuric acid Carbon on silica (LC) Gel permeation chromatography HPLC fractionation–PYE column	GC–ECD, GC–MS GC–GC–ECD	24,63,83,84
PCBs	Solvent extraction Liquid–liquid partitioning Soxhlet extraction Saponification Acid hydrolysis	Liquid–liquid partitioning Adsorption chromatography on silica Adsorption chromatography on Florisil Adsorption chromatography on alumina Gel permeation chromatography Adsorption chromatography on carbon HPLC (silica)	GC–ECD GC–NPD/FDP GC–MS (confirmation)	4,24,132–136
Pesticides	Solvent extraction Liquid–liquid partitioning	Adsorption chromatography on silica Adsorption chromatography on Florisil Adsorption chromatography on alumina Gel permeation chromatography Sweep co-distillation	GC–ECD GC–NPD/FPD GC–MS (confirmation)	4–12,26,34– 49, 82,83

their solid–liquid adsorption characteristics [4]. In the 1970s and 1980s, a number of useful conventional treatments for the removal of interfering co-extractants and organic macromolecules were introduced. However, some of these methods, *e.g.*. saponification with ethanolic potassium hydroxide for specific analysis of PCBs [20,21] and treatment with sulphuric acid (either by shaking or by using acid-impregnated silica) [22,23], are known to lead to a loss of other analytes such as chlorinated pesticides, an observation recently pointed out again by Wells [4] and De Voogt *et al.* [24]. As the scope of this review is directed towards advances in multi-residue analysis, only the less destructive methods will be described, *e.g.*, methods based on the use of activated carbon, magnesium silicate (Florisil), alumina and/or silica, and those implementing high-performance liquid chromatographic (HPLC) techniques and gel permeation chromatography (GPC). Recent reviews on the application of these techniques have been written by Erickson [25], Wells [4] and De Voogt *et al.* [24].

2.1. Liquid–liquid extraction

Liquid–liquid extraction is a well established technique used for the isolation of organic micropollutants from food samples. Isolation and clean-up techniques applied in the determination of pesticides in fatty foods were reviewed recently by Walters [26].

Typically, homogenized, representative subsamples are extracted once, or several times, with a water-immiscible solvent mixture such as hexane–acetone or light petroleum–diethyl ether. The analytes of interest, together with a wide variety of other lipophilic compounds, and the total lipid fraction are extracted in this way. After drying by filtration over anhydrous sodium sulphate, the crude extract is evaporated to dryness. After this procedure, the residue, consisting of the lipid fraction of the foodstuff, can be kept in a refrigerator or a freezer prior to further analysis. As this procedure isolates the fat matrix and the fat-soluble residues, an extensive clean-up and fractionation will be involved in further analysis. More selective isolation methods include liquid–liquid partitioning steps. When the foodstuff is of a solid nature, a (sub)sample is melted, macerated or blended with an aliquot of an apolar solvent (mixture) or depending on the texture, salt and/or sand is added before the (multiple) extraction with an apolar solvent (mixture) takes place [9]. Subsequently, solvent partitioning can be carried out by adding acetonitrile to the extract. The compounds of interest dissolve in the acetonitrile layer, while the fat remains mainly in the hexane or light petroleum layer. Because of the presence of residual fat a further clean-up of the acetonitrile extract is still necessary in most instances. The method can be applied to virtually all solid foodstuffs of animal and vegetable nature.

Prapamontol and Stevenson [27] developed a single-step extraction method for milk with ethyl acetate–acetone–methanol (2:4:4) by ultrasonification. Owing to the polar nature of this solvent mixture compared with conventionally used solvent, the amount of co-extracted fat was reduced significantly. This resulted in a considerable simplification of the subsequent sample clean-up process. The eleven organochlorine pesticides tested could be recovered quantitatively owing to the breaking down of the milk fat globules that can otherwise trap fat-soluble compounds.

A technique that is often used for the isolation of contaminants that are difficult to extract with conventional liquid–liquid extraction is Soxhlet extraction. In this way, continuous extraction of a sample with an appropriate solvent mixture, at elevated temperatures, if necessary for several days, can be performed without the need for much attention.

Huckins [28] described the use of semi-permeable membrane bags during Soxhlet extraction for separating the component fractions from the lipid matrix of foodstuffs. During extraction, components such as PCBs, PAHs, PCDDs and PCDFs are allowed to pass through the semi-permeable membrane of the polyethylene bag, while the lipid matrix is retained in the bag. An extraction time of only a few hours instead of the normal duration of 10–20 h in the original operation with toluene was sufficient for the quantitative recovery of all components of interest. Zebühr *et al.* [29] recently introduced a multi-residue procedure, including this isolation/extraction method followed by an automated HPLC clean-up method, for the analysis of residues of PCBs (including planar and mono-*ortho*-PCBs), PAHs, PCDDs and PCDFs.

Recently, automated equipment was introduced

for automated sequential trace enrichment of dialysates (ASTED); some of the applications published so far involve the determination of veterinary drugs and food additives in foodstuffs [30,31].

Recent developments in liquid–liquid extraction show a trend towards smaller sample sizes, resulting in savings on solvents and other chemicals, and also resulting in faster clean-up procedures [32]. The state of the art in the field of extraction methodology for pesticides was reviewed by Steinwandter [33]. However, it should be noted that a reduction in the amount of sample handled can only be achieved if the analytical method is sensitive enough to detect the reduced amount of the analytes. In some instances the efficiency of the analytical procedure can be enhanced by the use of on-line coupled techniques which totally transfer a small amount of the sample to the chromatographic system. Examples of such techniques are described in Section 3.

2.2. Adsorption chromatography

Adsorption chromatography has been used for over 35 years for the clean-up and fractionation of food sample extracts. Commonly used sorbents include magnesia, Florisil, silica and alumina; for some applications these sorbents have also been modified with, *e.g.*, silver nitrate or sulphuric acid. These materials show a polar behaviour, thus retaining the lipid fraction on elution with organic solvents of low polarity. This means that these sorbents are only suitable for apolar analytes, because more polar analytes will co-elute with the lipid fraction.

In earlier work in the USA and Canada, much effort was put into the development of methods involving magnesia or the synthetic magnesium silicate Florisil [34–36]. On the basis of this methodology numerous multi-residue applications were developed over the years [37,38]. It is interesting that these methods, developed more than 30 years ago, are still included in the official AOAC and FDA manuals [5,11].

Method development in Europe was focused more on clean-up procedures involving alumina and silica sorbents. For the separation of the fat from analytes of interest alumina has been extensively studied [39–42]. For the fractionation of the analytes, once the fat has been removed, either by alumina chromatography or another technique such as GPC or liquid–liquid partition, to a certain extent silica can be used for further clean-up [43–49]. These methods usually involve a liquid–liquid extraction technique, isolating the fat. Subsequently, the fat is separated from the organochlorine compounds of interest by alumina column chromatography, and after this the separation of the PCBs from the organochlorine compounds, necessary for a reliable gas chromatographic quantification, can be achieved by column chromatography over silica. A relatively new application of this procedure was described by Fürst *et al.* [50,51] for the determination of tetrachlorobenzyltoluenes (TCBTs; Ugilec) in fish samples. The most critical step in these schemes is the alumina chromatography; in order to obtain an efficient separation of the lipid fraction from the relevant compounds, the alumina must be deactivated to an appropriate degree by the addition of water. If too much water is added, fat retention will be insufficient. On the other hand, if the water content is too low, the analytes will not elute from the column within a reasonable time. Owing to the poor batch-to-batch reproducibility of these sorbent materials, exact adjustment of the chromatographic conditions is tedious and the hygroscopic properties of the materials make the storage of a prepared amount troublesome.

Major drawbacks of the techniques described above is that they are laborious and difficult to automate, combined with high solvent consumption. Therefore, several workers have investigated the feasibility of miniaturization of the chromatographic systems involved [52,53]. Miniaturization can result in the use of solid-phase extraction cartridges instead of the conventional glass chromatographic columns. Commercially available polypropylene tubes, typically filled with 100–1000 mg of sorbent, are being used. A wide range of materials such as ion exchangers, silica and alumina and reversed-phase type chemically bonded silicas have been tested. For the separation of analytes from lipids, the polar sorbents, conventially used in adsorption chromatography, are being used almost exclusively. The more recently developed phases such as chemically bonded silicas and modified carbons have the potential for clean-up purposes at least in specific application areas.

Because the activity of the sorbent cannot be ad-

justed batchwise off-line, an on-line adjustment has to be carried out. The extraction/clean-up procedure is as follows: the sorbent is conditioned by washing with an appropriate solvent, an aliquot of the crude sample extract is brought on to the column, the column is washed to eliminate matrix interferences and finally the analytes of interest are eluted. Clean-up of the extract also takes place because part of the interferences remains immobile on the sorbent during the elution step. Clean-up of milk extracts for the analysis of PCBs and organochlorine pesticides by means of solid-phase extraction cartridges has been studied by several workers [27,54,55]. An additional advantage of the use of solid-phase extraction cartridges is that procedures can be automated by the use of modified LC autosamplers [56].

The basis of carbon chromatography was laid by Stalling and co-workers [18,57,58], who described the potential of activated carbon chromatography for the specific fractionation of planar aromatic molecules. The retention of solutes is based on the coplanarity of closely situated aromatic systems and is increased by electronegative substituents (chlorine, bromine, nitro) on the aromatic systems.

A major drawback of finely divided carbon as a packing material is the high back-pressure. Stalling *et al.* [57] described a method for dispersing finely divided carbon, Amoco PX-21, on the surface of shredded polyurethane foam, improving the recoveries and separation of strongly adsorbed planar compounds. Later, carbon dispersed on glass fibres was used to fractionate non-ionic chlorinated pesticides and polar PCBs from planar PCBs and PCDD/Fs [57]. Clean-up through a series of silica-based adsorbents is necessary before application of the activated-carbon adsorbents.

A modified version of this procedure was reported by Liem *et al.* [59] for determination of PCDD/Fs in eel and milk, and Beck and co-workers [60,61] also applied a slightly modified carbon adsorbent for the determination of PCDD/Fs and PCB 77 in human milk and tissue.

Miyata *et al.* [62] used active carbon on silica for the fractionation of polar PCBs from planar PCBs and PCDD/Fs with elution with toluene at 80°C for the analysis of Yusho oil and tissues of patients with Yusho disease. Mixing of activated carbon with silica [carbon AX-21–silica (1:1)] for use in low-pressure LC in the clean-up of fish samples was performed by Hong and Bush [63]. Stepwise elution with different eluents resulted in several fractions containing 2–4 *ortho*-substituted PCBs, mono- and non-*ortho*-PCBs and PCDD/Fs. Norén *et al.* [64] utilized a mixture of activated charcoal (SP-1) and Chromosorb W in a final step in sample preparation for the determination of planar PCBs in milk.

In several studies activated carbon was used without modification or mixing, often resulting in a lengthy clean-up (using several eluents and large elution volumes) and broad and tailing elution profiles as a result of the inhomogeneity of the active sites on the activated carbon [65–67]. To reduce the large volumes of different solvents, Liem *et al.* [68] introduced Carbosphere activated carbon, which has a high loading capacity for fat and a low affinity for lipids and which is used in a reflux unit. For the isolation of PCDD/Fs from extracts of milk only 40 ml of toluene is used in a back-reflux mode whereas others use 200 ml of solvent. The same method can be used for the determination of planar PCBs in milk and other food matrices (fish, meat, butter, cheese), in which the analytes are recovered after direct refluxing with toluene for 1.5 h [69].

2.3. *HPLC sample clean-up*

Adsorption chromatographic methods, using alumina, magnesium or silica-based material, as described in the previous section are laborious and automation is difficult. HPLC and solid-phase extraction techniques are more suitable for the development of automated techniques. HPLC has additional advantages because first, it has a high separation potential compared with SPE or classical column chromatography and second, the separation process can be followed directly by UV or refractive index (RI) detection. Gillespie and Walters [70] introduced a procedure using a semi-preparative silica HPLC column (250 × 9.2 mm I.D., packed with 6-μm porous spherical particles) to separate OCPs and PCBs from butter fat. Solutions containing 0.4 g/ml of fat in hexane were injected on to this column. With a mobile phase of dichloromethane–hexane (20:80) at a flow-rate of 4 ml/min, 300 mg of fat were well resolved from five different organochlorine pesticides together with the PCBs. The elution of these compounds, however, required

148 ml of mobile phase. The method was compared with the official AOAC method [11], which is based on Florisil column chromatography. LC was found to be superior with respect to the time of analysis and the separation efficiency.

Dolphin *et al.* [71] introduced LC column switching for the automated analysis of OCPs in milk extracts. A precolumn (50 × 2.1 mm I.D., packed with 5-μm Partisil) was used for the retention of the fat and for the separation of the more polar pesticides (*e.g.*, β-HCH, heptachlor epoxide and dieldrin), whereas an analytical column (150 × 3.1 mm I.D., 10-μm Partisil) resolved the early-eluting compounds (*e.g.*, HCB, DDT complex and α-HCH). For both columns the mobile phase was *n*-hexane. The pesticides were detected with an electron-capture detector coupled directly to the LC system. Fairly high limits of determination (0.1 ppm level) were reported. This procedure is less suitable for the PCBs because LC cannot provide an adequate separation of the individual congeners.

A similar procedure was used for sample pretreatment prior to gas chromatography (GC) for the determination of organochlorine pesticides and PCBs in human milk [55]. On-line LC–GC is less suitable for the OCPs as no group separation for this heterogeneous group of compounds is available by LC. Hence no simple heart-cutting technique will be available for these compounds. Therefore, an off-line LC procedure was developed in which both the PCBs and the OCPs can be handled in a single clean-up procedure. In this work a completely automated clean-up with an LC system involving column switching for the separation of OCPs and the PCB fraction in fat extracts prepared from human milk was described. With this procedure it was possible to obtain separated fractions of OCPs and PCBs prior to capillary GC–electron-capture detection (ECD) with a capacity of 20 samples per day.

The availability of automated HPLC is one of the factors that led to the development of gel permeation chromatography (size exclusion or GPC) as we know it today. GPC is a powerful preparative chromatographic clean-up procedure which can be used prior to HPLC and GC analysis [45,72,73]. The original applications used Bio-Beads SX2 and cyclohexane as eluent with a 270 × 20 mm I.D. column, allowing injections of up to 500 mg of fat. Nowadays, commercially available auto-preparative systems use Bio-Beads SX3 and toluene–ethyl acetate (1:3) as eluent with an increased loading capacity of up to 1–2 g of fat per injection.

GPC has several advantages over atmospheric pressure chromatography. First, the same column can be used for the clean-up of large series of samples. Second, the clean-up procedure itself can be monitored with a UV or RI detector. This leads to a technique that can easily be automated. Separation with GPC is based on molecular size rather than on boiling point and/or polarity, thus adding selectivity to the analytical procedure, as the smaller molecules show the highest retention. It is an ideal technique for the separation of macromolecules such as lipids and pigments from lower molecular mass organochlorine contaminants.

A drawback is the relatively low separation power of GPC in the lower molecular mass range, resulting in large eluate volumes and in the impossibility of performing fractionation. In addition, GPC seems to be less suitable for automated routine analyses of planar PCB congeners and the toxic PCDDs and PCDFs, as even the most sensitive GC–mass spectrometric (MS) procedures demand sample amounts of at least 5 g of fat or more. Increasing the loading capacity by enlarging the GPC column dimensions will inevitably result in a large throughput of solvents and adsorbents, introducing high costs and higher risks of contamination [24]. For these groups of compounds, activated carbon (see the previous section) and the use of the recently introduced 2-(1-pyrenyl)ethyldimethylsilylated silica column in HPLC [74] seem to be more appropriate. In the applications discussed in this section HPLC is used as a preparative technique. This facilitates the use of more exotic materials compared with analytical HPLC, because bad peak shapes are less a problem.

As noted in Section 2.2, carbon-based materials have a fair potential in the clean-up of PCBs and PCDD/Fs. A new development in carbon-based chromatographic materials is porous graphitic carbon (PGC). PGC is an amorphous glassy carbon containing micropores and mesopores. On a colloidal level it has a strong sponge-like structure, capable of withstanding considerable shearing forces, rendering it suitable for HPLC [75]. The surface area is about 150 m^2/g, the mean pore volume 2.0 cm^3/g and the particle porosity 70%. The advan-

tages of PGC over activated carbon are that it typically uses a single eluent instead of a step gradient and sharp peaks can be obtained, because of the homogeneous nature of active sites of PGC. Disadvantages are that PCDD/Fs have to be recovered by backflushing the column, giving broad peaks (200 ml of hexane, or a smaller volume of toluene) and that preliminary pretreatment of extracts is necessary, as PGC has a relatively low capacity for coextracted organics, causing overloading of the columns [76]. Much research has been carried out on the properties of PGC itself [75–77] and in relation to silica and organic polymers as packing materials for HPLC [78–80]. With PGC, a non-polar adsorbent, so that the solute retention is based on the balance between the non-specific intermolecular interactions, polar solvents have lower elutropic strengths; dichloromethane and dimethylformamide are the strongest solvents. Commercial PGC columns are now available, Creaser and Haddad [76] were the first to use a Shandon Hypercarb column (7 μm, 50 × 4.7 mm I.D.) for the HPLC separation of pesticides, lower chlorinated PCBs, planar PCBs and PCDD/Fs using hexane as mobile phase (5 ml/min). An improved separation could be

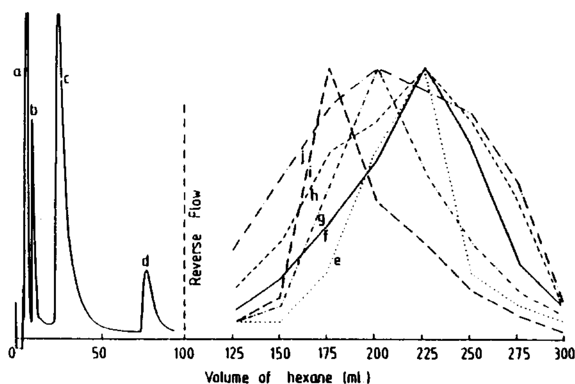

Fig. 1. Separation of PCBs and pesticides from PCDDs and PCDFs on porous graphitic carbon (50 × 4.7 mm I.D.; eluent, hexane, 5 ml/min). (a) PCBs (Aroclor 1254 + 1260) and pesticides; (b) 3,3′,5,5′-T_4CB and 3,4′,5-T_3CB; (c) 3,3′,4,4′-T_4CB and 3,4,4′,5-T_4CB; (d) 3,3′,4,4′,5,5′-H_6CB; (e) 1,2,3,4,6,7,8-H_7CDD and O_8CDD; (f) 1,2,3,7,8-P_5CDD, 1,2,3,7,8-P_5CDF, 1,3,7,8-T_4CDD, and 1,2,7,8-T_4CDD; (g) 1,2,3,4,7,8-H_6CDD; (h) 1,2,3,6,7,8-/1,2,3,7,8,9-H_6CDD and 1,2,3,4,8,9-H_6CDD; (i) 1,2,3,4,6,7,8-H_7CDD; (j) O_8CDF. 0–100 ml, UV detection (245 nm); 100–300 ml, GC–ECD analysis of discrete fractions. From Creaser and Al-Haddad [76].

achieved with acetonitrile–water (80:20). A typical LC trace of PCBs and PCDD/Fs on Hypercarb is shown in Fig. 1.

Tuinstra *et al.* [81] demonstrated the use of the Hypercarb column for the determination of planar PCBs in horse fat. Samples were extracted and subsequently cleaned with GPC and alumina, before HPLC separation using cyclohexane–dichloromethane (1:1) (2 ml/min) as eluent for the first fraction (0–30 min) of OCPs and non-planar PCBs (Nos. 28, 52, 101, 105, 118, 138, 153 and 180) and switching to toluene for the second fraction (30–60 min) containing the planar PCBs (Nos. 77, 126 and 169). A large difference in the results between analysis with GC–ECD and GC–MS was found owing to interferences during ECD, which needs more clean-up.

Böhm *et al.* [82] also used HPLC fractionation with a Hypercarb column (10 × 4.7 mm I.D.) for the determination of planar PCBs in food using PCB 169 as internal standard, which may lead to a loss of relevant information as PCB 169 has been detected in a variety of foodstuffs [68] and in human milk [69]. GC–ECD was used in the analysis step instead of the more sensitive GC–high-resolution MS instrumentation.

Another new packing material with a potential similar to PGC for the separation of planar components is 2-(1-pyrenyl)ethyldimethylsilylated silica (available from Cosmosil as 5-PYE, 5 μm, 150 × 4.6 mm I.D.). PYE was found to be intermediate between silica and PGC with respect to the selectivity based on electronic and steric interactions with the fused-ring aromatic systems on the stationary phase, whereas carbon chromatography is based on charge-transfer interactions. The pyrenyl group on the PYE phase possesses fused aromatic systems with sixteen π-electrons, which may be regarded as a small part of a graphite surface and therefore it provides a much greater effect of a planar aromatic structure on retention than silica [80]. Advantages over carbon columns are the higher efficiency, less tailing elution profiles, as can be seen from Fig. 2, no irreversible adsorptions and a better batch-to-batch reproducibility.

Fig. 2 demonstrates the use of the PYE column for the isolation of mono-*ortho*- and planar PCBs from biological samples (fish and animal) as performed by Haglund *et al.* [74,83]. Hexane was used

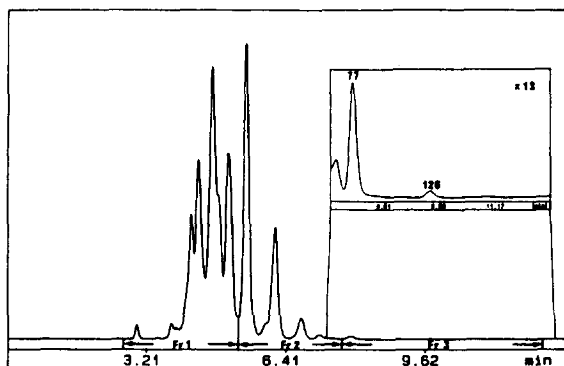

Fig. 2. HPLC showing the fractionation of Clophen A50 on a 150 × 4.6 mm I.D. PYE column. The numbered peaks correspond to 3,3',4,4'-T_4CB (IUPAC77) and 3,3',4,4',5-P_5CB (IUPAC 126). For chromatographic conditions, see Haglund *et al.* [74]. From Haglund *et al.* [74].

as the mobile phase, resulting in two fractions containing the bulk PCBs and mono-*ortho*-PCBs (Nos. 105, 118, 156, 157 and 159) in a small volume of 2.5 ml; planar PCBs were eluted in the backflush mode with a higher flow-rate (1.2 ml/min). Windows were defined with PCB 118 and 77, the first eluting congeners for mono-*ortho*- and planar PCBs, respectively. Retention in HPLC is reduced by matrix effects of non-hydrolysed lipids, which could be removed by GPC. To obtain optimum performance of the column it is necessary to remove the lipids almost completely. The same method was used for the determination of planar PCBs in fish [84].

2.4. *Supercritical fluid extraction*

Sample pretreatment with supercritical fluid extraction (SFE) is a relatively new technique in comparison with the other techniques discussed. The advantages of SFE over conventional liquid extraction methods are that it saves time and extraction solvents, it opens ways to more efficient extractions, selectivity is enhanced and it can easily be coupled to other chromatographic techniques. These advantages originate from the properties of supercritical fluids. First, the selectivity of extraction procedures can be tuned by the varying solvating power of the solvent as a function of its density, so that discrete fractions of analytes can be isolated from a sample by density and pressure programming. Second, rapid mass transfer during extraction is facilitated by the low viscosity and high solute diffusivities due to the liquid- and gas-like behaviour of supercritical fluids, thus improving the efficiency of the extraction process.

At present, carbon dioxide (CO_2) is most often used as the extraction solvent because of its moderate critical temperature (31°C) and pressure [73 atm (1 atm = 101.325 kPa)], it is non-flammable, non-toxic and relatively inexpensive and there are no waste problems. Modifiers can simply be added to adjust the solvating power of the fluid beyond the range that is accessible by density programming.

Until now, only few applications of SFE of fatty foods have been published. Generally, two different approaches can be distinguished in the application of SFE for sample pretreatment of fatty foods: using SFE to extract as many components and fat from the matrix as possible followed by an additional on- or off-line clean-up [85–90]; and selective extraction of only the components of interest by a proper choice of parameters [87,88,91]. An example of these two approaches was given by King [89], who studied the behaviour of porcine fat spiked with traces of DDT by varying the pressure. At low pressures up to 95 atm only a negligible yield of the lipid background was recovered. On increasing the pressure to 204 atm a finite yield of fat was observed combined with 75% recovery of DDT, and further extraction at 300 atm gave a significant amount of fat and some additional DDT. From these experiments it can be deduced that it should be possible to extract most of the DDT from lipid interferences if the extraction is performed at *ca.* 100 atm.

Hopper and King [90] used extreme extraction conditions (69 MPa, 80°C and 5 l/min of CO_2) to extract pesticides and co-extracting lipids from butter fat and peanut butter. Extraction was followed by an additional clean-up with GPC and Florisil. Good results were reported for the sample preparation by mixing the sample with an extraction enhancer (pelletized diatomaceous earth) to remove moisture and to prevent channelling during extraction.

Nam *et al.* [88] performed extractions of several pesticides from spiked dairy products, which were mixed with Florisil and sulphate, suspended on silanized glass-wool and homogenized, under conditions of 150 atm and 50°C for 30 min followed by clean-up with GPC. For thiophosphate and phen-

oxy ester herbicides good recoveries were found (70–95%); for triazines and carbamates the recoveries were lower (50–70%) owing to their polarity. Improvements were achieved by the use of a modifier (chloroform or methanol).

For the extraction of PAHs from spiked fish samples and 2,3,7,8-TCDD from spiked liver, higher pressures were necessary (170 atm and 50°C for 30 min); addition of a small amount of toluene to the extraction cell increased the extraction efficiency. The recoveries for TCDD were better than 70% at levels of 50 ppt–1 ppb (ppt = parts per 10^{12}; ppb = parts per 10^9).

Selective extraction procedures were performed by Nam *et al.* [88] using off-line SFE to extract chlorinated pesticides and PCBs from milk. The spiked samples (1–20 ppb) were mixed with Florisil and sulphate, suspended on silanized glass-wool and homogenized. Extraction was done at 160 atm and 50°C for 30 min and samples were trapped in hexane. The extraction efficiencies obtained with SFE were comparable to those obtained with liquid extractions.

Murphy and Richter [91] demonstrated the extraction of aldrin from soybean oil; selection of the optimum pressure was performed on the basis of recovery of the aldrin and co-extraction of lipids. A pressure just below that for the optimum recovery gave less matrix interferences. Good recoveries were found for all spiked matrices and were not improved by adding a modifier to the extraction cell.

SFE is a promising technique for the extraction of all kinds of matrices. Selective extraction procedures are preferred, where precise control of the extraction pressure is used to facilitate enrichment of the component from co-extractants from the matrix.

3. CHROMATOGRAPHIC TECHNIQUES

3.1. Liquid chromatographic techniques

3.1.1. Liquid chromatography

Today GC still appears to be the major analytical technique for residue analysis owing to its high separation power (capillary column), the availability of selective and sensitive detectors and, perhaps the most important factor, most of the laboratories involved in residue analysis are, for historical reasons, better equipped with GC systems. However, the application of HPLC is growing, especially for the analysis of pesticides that cannot be analysed directly by GC owing to poor volatility, high polarity and/or thermal instability of the compounds. Currently special reversed-phase (RP) columns have been introduced for the analysis of groups of compounds, *e.g.*, PAHs, carbamates, phenylurea herbicides and nitrophenols.

Brodsky and Ballschmiter [92] demonstrated the potential of HPLC as a confirmatory step in the isomer-specific determination of PCBs. They compared six different stationary phases of modified silica gel (Nucleosil $5C_{18}$ and 5CN, Hypersil ODS C_{18}, Sepralyte Diphenyl, Vydac 201 TP C_{18} polymer and R Sil HLDA C_{18}) for their separating properties by determining the retention indices of 87 PCB congeners in technical PCB mixtures. The highest selectivity was obtained with Nucleosil $5C_{18}$ with 75–90% methanol as eluent. Certain PCB congeners (*e.g.*, with IUPAC Nos. 132 and 153), usually difficult to separate in conventional GC, could be separated by using LC. It was concluded that LC can be used for confirmatory purposes.

Vaessen *et al.* [93,94] evaluated methods and associated problems observed in inter-laboratory comparison studies on the determination of PAHs in samples of coconut oil and green kale. As all the participating laboratories (fourteen) were considered to be experienced, results from this study represent the current state-of-the-art of PAH methodology in the field of food analysis. An overview is given of current techniques for the extraction, clean-up and analysis employed in PAH analysis. HPLC and glass capillary GC were the main techniques used for the separation and determination of PAHs. For the HPLC analysis of PAHs, Vydac columns provide the best answer to this analytical problem at present.

3.1.2. LC–LC coupling

An important feature of HPLC is the application of (pre)column switching, which offers the possibility of integrating sample preparation and clean-up in the chromatographic procedure. The use of multi-dimensional chromatographic procedures in LC also alleviates the major drawback of the technique *i.e.*, the lower separation power compared with capillary GC. The first papers on residue analysis of

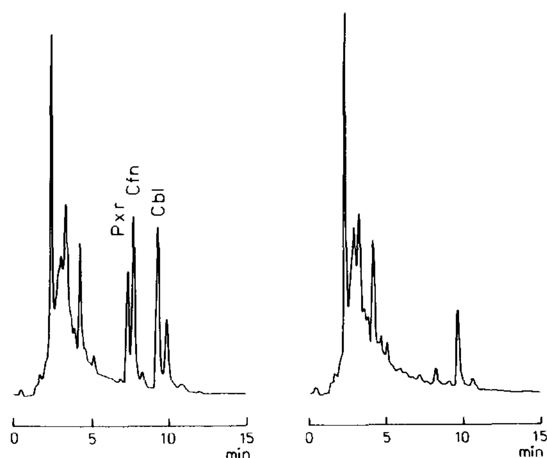

Fig. 3. Analysis of a 1-ml aliquot of total diet extract (5 g/ml). (a) Spiked with propoxur (Pxr, 5.5 μg/kg), carbofuran (Cfn, 6.4 μg/kg) and carbaryl (Cbl, 7.5 μg/kg). (b) Blank. LC column-switching technique according to Goewie and Hogendoorn [99].

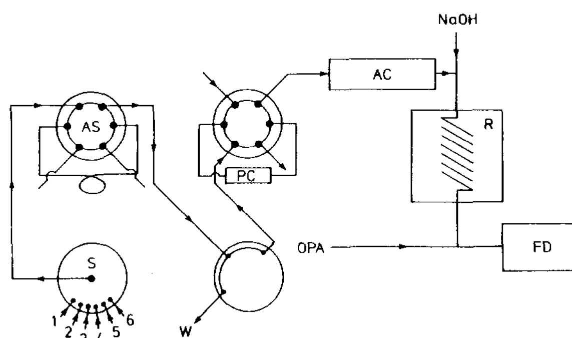

Fig. 4. Schematic diagram of the equipment for on-line precolumn switching HPLC analysis and postcolumn reaction used for the determination of N-methylcarbamate pesticides. S = Selector valve (low-pressure); AS = autosampler; PC = high-pressure switching valve with precolumn; W = high-pressure selector valve with "waste" line; AC = analytical column; NaOH = hydrolysis reagent; R = reactor coil; OPA = o-phthaldialdehyde, reagent mixture; FD = fluorescence detector. From Goewie and Hogendoorn [99].

pesticides with RP-LC column switching dealt with the preconcentration of analytes from aqueous samples, such as river water, soft drinks or serum [95–97]. For these applications precolumns of 2–10 mm × 2–3 mm I.D., packed with 5–10-μm C_{18} particles, were optimum with regard to loadability and performance. With this approach a manual extraction procedure is avoided. An obvious limitation is that the application field is limited to aqueous samples. Even with those samples a filtration step must always precede the on-line procedure in order to prevent clogging of the precolumn. As a consequence, the part of the analyte adsorbed on solid particles present in the sample must be analysed separately in order to obtain the total analyte content of the sample. Owing to their low separation power, these small precolumns are less suitable for clean-up purposes, as they cannot provide enough separation between analytes and sample interferences.

More recent studies using HPLC column switching for pesticide residue analysis have established that the use of longer (pre)columns in combination with off-line extraction/concentration results in a considerable selectivity enhancement [98].

An example of the use of precolumn clean-up of duplicate diet samples for N-methylcarbamates was given by Goewie and Hogendoorn [99]. The method was based on the improved HPLC method of Krause, extended with an automated column switching. Fig. 3 shows a typical chromatogram of the procedure and the experimental set-up is shown in Fig. 4.

3.1.3. LC–GC coupling

As stated before, capillary GC still remains the major analytical technique in residue analysis. A major drawback of capillary GC is that it has, contrary to LC, a low tolerance towards involatile and polar sample constituents, because these interferences cannot be removed effectively from a GC sys-

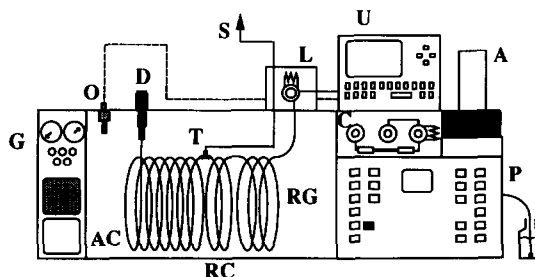

Fig. 5. Schematic presentation of the equipment used for LC–GC analysis. A = Autosampler; P = HPLC pump; C = LC column with switching valves; U = UV–VIS HPLC detector; G = gas chromatograph; L = loop-type interface; O = on-column interface: RG = retention gap; RC = retaining precolumn; AC = analytical column; S = solvent vapour unit; D = GC detector.

tem. For this reason, GC usually requires extensive clean-up procedures using solid–liquid chromatography. HPLC is far less critical in accepting dirty samples, and therefore coupling of HPLC with capillary GC seems to yield a very powerful combination. Especially the introduction of interfaces capable of transferring relatively large volumes of the LC eluate (typically 1 ml) greatly facilitated the development of LC–GC, as standard LC dimensions and flow-rates can be combined with capillary GC [100]. Equipment for LC–GC is commercially available now. Fig. 5 shows a schematic diagram of such an instrument. Maris *et al.* [101] reported on the use of a LC–GC interface for the enrichment of PCBs and pesticides from aqueous and sediment samples at the ppt level. Their system consisted of a micro-RP-LC column directly coupled to the capillary GC system through an interface consisting of an on-column injector and a retention gap.

Interfacing LC to GC can yield fast, reliable and automated analytical techniques. Most of the interfaces used today are based on concurrent or partially concurrent solvent evaporation. Concurrent solvent evaporation is a very powerful technique because it allows the injection of an, in principle, infinite volume of liquid into a capillary GC system. Many applications of LC–GC are based on the transfer of a heart-cut from a normal-phase liquid chromatogram, hence these techniques are extremely selective owing to the fact that the total chromatographic process is multi-dimensional.

On-line coupled LC–GC was reviewed extensively by Grob [100]. The technique is based on transferring a heart-cut from an LC column to a capillary GC system. PCBs can be determined in sediments with a relatively short sample pretreatment [99]. Grob *et al.* [102] reported an LC–GC method for the determination of PCBs in fish. In this study fat was well separated from the PCBs but the LC separation was insufficient to separate the OCPs from the PCB fraction. In these studies, however, resolution between OCPs and PCBs was not ideal because six OCPs co-eluted with the PCBs. Grob *et al.* [103] recently discussed the capacity of silica columns for retaining fat.

Barcorolo [104] used a modified ODS silica phase with isooctane as the mobile phase for the determination of organochlorine pesticides in fat-containing samples. Pesticides elute rapidly under these conditions whereas the fat is well retained by the stationary phase. The column was regenerated by rinsing with *n*-hexane.

One of the most important clean-up methods in residue analysis is gel permeation chromatography (GPC). Miniaturized GPC seems to be a logical candidate for LC–GC applications. The selectivity of this method lies in the separation of the analytes in one, relatively small, fraction from macromolecular interferences from the samples. Miniaturized GPC with a 0.32 mm I.D. fused-silica capillary column packed with 5-μm Rogel with a pore size of 5 nm has been shown to separate PCBs from triglycerides [105]. However, the application shown (10% of PCBs in oil) is far from the levels needed for practical purposes. A major problem in coupling GPC with GC is still the problem of tailing triglyceride peaks, destroying the capillary GC column. In a recent paper, Grob and Kalin [106] claimed that a large part of the peak tailing is caused by the LC injection valves, and therefore suggested putting the injection valves off-line to the main flow scheme of the LC–GC system.

Some developments in interfacing LC to GC can be expected from programmed-temperature vaporizer (PTV) split inlet [107–110]. With this method, a solvent purge technique is applied to preseparate volatile material, including the solvent, from high-boiling solutes in the injector. During the initial split-open period, solvent and low-boiling compounds are allowed to evaporate, while high-boiling components remain cold-trapped in the injection liner. Next, the splitter is closed and the inlet is rapidly heated, allowing the trapped components to enter the column. Grob [111] recently discussed the potential of PTV injectors for LC–GC interfacing.

3.2. Gas chromatographic techniques

The growing tendency towards multi-residue methods combining a high sample throughput with high levels of reproducibility and accuracy have led to improvements in column technology and knowledge concerning the retention behaviour of specific component groups (*e.g.*, PCBs, PCDD/Fs). In addition, improvements can be observed in multi-dimensional GC, LC–GC and SFE–GC, showing their potential for application in food analysis. In this section, recent developments are described.

In residue analysis, specific GC detection techniques such as electron-capture detection (ECD), nitrogen–phosphorus or thermionic detection (NPD) and flame photometric detection (FPD) are used. Numerous applications can be found for the detection of PCBs and the large group of nitrogen- and/or phosphorus-containing pesticides, including triazines and organophosphorus esters, and sulphur-containing compounds. Good overviews are given in the biannually published reviews in *Analytical Chemistry* [112,113]. The field of GC detection is more or less stable. The only noticeable recent development is the availability of a commercial instrument for atomic emission detection (AED), which is able to detect different specific elements including carbon, phosphorus, nitrogen and metals, such as tin and lead in organometallic compounds. A tendency towards the combined use of several different detection techniques to allow proper identification can be observed. The following sections refer only to recent developments in column technology in GC, the use of multi-dimensional GC and on-line SFE–GC techniques. Progress in the use of spectroscopic techniques is described in Section 4.

3.2.1. Gas chromatography

The inability of non-polar columns to separate all analytes of interest in a single GC run [114,115] and the thermal instability of highly polar stationary phases [115,116] has led several workers to the application of other and more stable stationary phases, *e.g.*, liquid crystal capillary columns [114,117,118], OH-terminated stationary phases [116] and columns coated with graphitized carbon black (GCB) [119,120]. Advances in column technology can be observed especially for the isomer-specific analysis of PCBs, PCDDs and PCDFs. The chemical and physical properties of pesticides are heterogeneous and therefore no real separation problems are encountered in this field.

Larsen *et al.* [115] recently tested commercially available columns for the analysis of non-*ortho*- and mono-*ortho*-PCB congeners by comparing the retentions of 140 PCB congeners in GC–ECD and GC–MS analyses on six narrow-bore fused-silica columns: SIL-5 (dimethyl), SIL-8 (5% diphenyl–dimethyl), SIL-19 (14% cyanopropylphenyl–1% vinyldimethyl), SIL-88 (biscyanopropylphenyl), HT-5 (1,2-dicarba-*closo*-dodecaboranedimethyl) and SIL-8–HT-5 (SIL-8 and HT-5 coupled in series). Of these, the HT-5 column offers the highest maximum temperature range allowing rapid analyses. It was concluded that no single GC column was able to separate all toxic PCBs from co-eluting congeners. Even the classical methylphenyl (5%)–polysiloxane phases for PCB analysis (*e.g.*, SE-54, DB-5, SIL-8), appeared to be an inferior choice for planar PCB analysis. The best choice for planar PCB analysis was the SIL-8–HT-5 combination, whereas the best overall performance (clear separation for 37 of 52 potentially toxic PCBs) was found for the non-polar dimethylsiloxane phase.

An alternative column for planar PCB analysis was recently introduced by Fischer and Ballschmiter [121,122]. They showed that, within a mixture of 176 PCBs, the planar PCB congeners can be clearly eluted last within the group of homologues by using a 50-m capillary coated with an 86% dimethyl–14% cyanopropylphenyl polysiloxane phase (*e.g.*, OV-1701, SB Octyl 50).

In the field of PCDD/F analysis, Schmid and Schlatter [116] compared the separation characteristics of glass capillaries coated with five different polysiloxanes, SP-2330 (10% methyl–90% 3-cyanopropyl), OV-240-OH (OH-terminated 67% methyl–33% 3-cyanopropyl), OV-225-OH (OH-terminated 50% methyl–25% phenyl–25% 3-cyanopropyl), OV-17-OH (OH-terminated 50% methyl–50% phenyl) and PS 247.5 (OH-terminated 100% methyl). The comparison showed a high coating efficiency with immobilized polysiloxane coatings, allowing the baseline resolution of all PCDD/F isomers with short columns. The OV-225-OH polysiloxane offered baseline separation of all congeners of interest and was also able to distinguish homologous groups and was therefore chosen as the stationary phase with the most suitable selectivity.

Ryan *et al.* [114] presented an extensive study on the GC–ECD separation of all 136 tetra- to octa-PCDD/Fs on nine stationary phases, including non-polar (DB-1, 100% methyl; DB-5, 5% phenyl), medium-polarity (DB-17 and OV-17, 50% phenyl–50% methyl; DB-210, trifluoropropyl), polar (DB-25, CPS-1, SP-2331, CP-Sil88, cyanopropyl) and others (SB-smectic, liquid crystalline). Except for certain pairs of PCDDs with 1,2,4-substitution, most of 136 PCDD/Fs can be readily separated by the use of a combination of two or more conven-

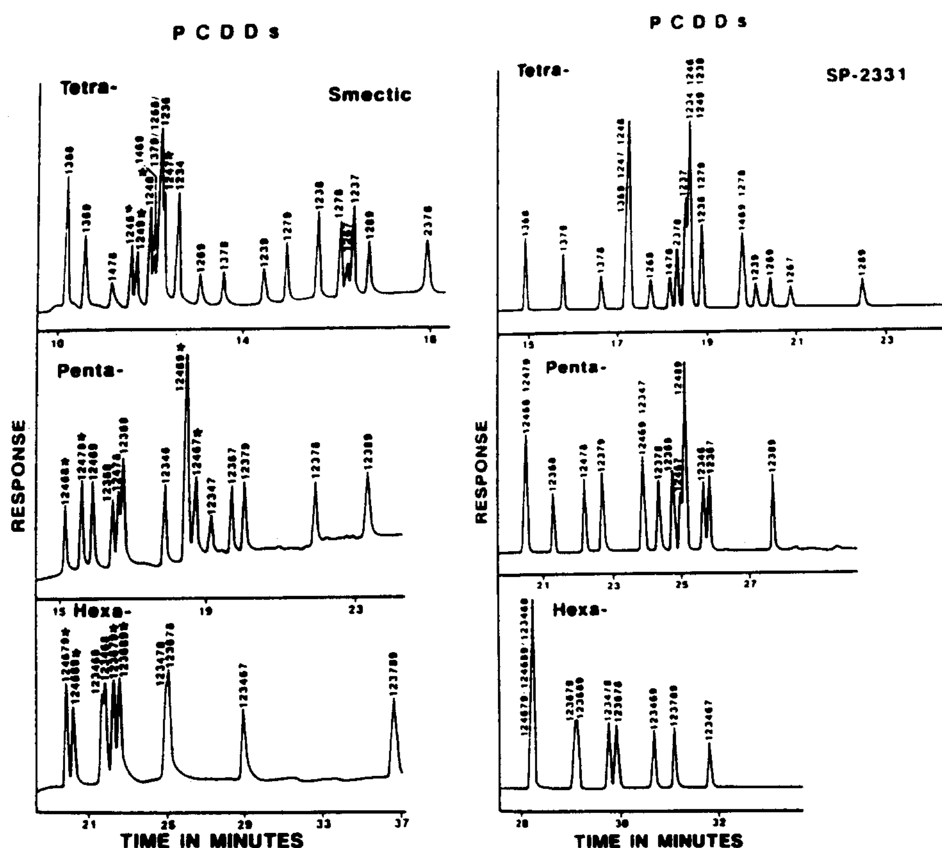

Fig. 6. GC–ECD trace of the separation of 22 T_4CDDs, 14 P_5CDDs and 10 H_6CDDs on an SP-2331 (left) and a liquid crystalline smectic (right) fused-silica capillary column. Congeners marked wih asterisks are pairs which cannot be unequivocably assigned. From Ryan *et al.* [114].

tional GC phases. The newly developed smectic liquid crystalline phase is unique in its resolving power, including the 1,2,4-substituted PCDDs. The elution order of PCDD/Fs on various smectic columns can be different, depending on the thermal history of the column. As a typical example, Fig. 6 shows GC–ECD traces of the 22 tetra-, 14 penta- and 10 hexa-CDD congeners on an SP-2331 and a liquid crystalline smectic column [114].

Naikwadi and co-workers [117,118] synthesized homopolymeric liquid crystal polysiloxane stationary phases for the separation of 2,3,7,8-substituted PCDDs, especially 2,3,7,8-TCDD. The liquid crystals have high thermal stability, low volatility (no column bleed) and are suitable for thin-layer formation in capillary columns. The separation of geometric and positional isomers gives an elution order following the length-to-breath ratio (L/B) and planarity of the solutes, so 2,3,7,8-substituted PCDDs will elute later than the other TCDDs according to the elongated structure from the lateral substitions. For furans this was not seen, presumably owing to their non-planarity. According to the authors, unique selectivity can be achieved for the separation of anthracene from phenanthrene and benzo[*a*]pyrene from benzo[*e*]pyrene.

Another column technology was found for columns coated with graphitized carbon black (GCB), a monoatomic, highly homogeneous, non-specific adsorbent whose surface is formed by basal faces of graphite. As adsorption is mainly based on the geometry and polarizability of molecules, GCB has a different elution profile from conventional liquid phases. Disadvantages of GCB are the limited me-

chanical strength of carbon particles and that, in comparison with liquid phases, higher oven temperatures need to be used owing to the high adsorption potential of carbon. Improvements were suggested by modifying the adsorption and chromatographic properties [119,120]. No applications have been published yet on the use of this type of column for the analysis of (chlorinated) aromatics in food.

3.2.2. GC–GC coupling

Multi-dimensional gas chromatography (MDGC, GC–GC) can be used as a complementary technique to resolve difficulties in the separation of co-eluting compounds in single-column GC systems. The technique is an alternative to additional sample pretreatment steps (which could lead to a further loss of analytes), and when lengthening the GC column or changing the polarity of the stationary phase (requiring time-consuming parallel injections) lead to only minor improvements in resolving power.

In GC–GC, techniques such as heart cutting (selective transfer of a group of components from the first column into the second for further separation) and backflusing (for cleaning up the first separation column from uninteresting late-eluting components by reversing the direction of flow without applying excessive temperatures) are used [123]. By using two separation systems, two sets of retention data are obtained, offering more confidence in the identification of a certain component. Initial attempts at GC–GC often suffered from bad peak shapes and a loss of sensitivity compared with conventional GC. Nowadays, the introduction of cryofocusing immediately after the transfer of cuts from the first into the second column (valve-directed switching systems) and modifications to the construction and the materials (*e.g.*, metal instead of graphite) used in the coupling device of double-oven equipped valveless systems (Sichromat 2) have led to improvements in the application of GC–GC in trace analyses of complex mixtures [124,125].

Until recently, there were hardly any practical applications in use in pesticide residue laboratories. This can be partly attributed to the fact that the technique was introduced just before the widespread introduction of capillary GC with fused-silica capillary columns. A revival of interest in GC–GC (coupling of non-chiral and chiral columns) might come as a result of new trends in the pesticide market towards the production of optically active enantiomers instead of racemic mixtures. Stronger application of GC–GC can be found in the analysis of organic micropollutants such as PCBs, PCDDs and PCDFs [125,126].

Stan and Cristall [127] reported the application of two-dimensional capillary GC with effluent splitting to three selective detectors (ECD, NPD and FPD) in the separation of γ-HCH, propachlor, propyzamide and chloro- and bromopropylate from interfering matrix compounds in samples of onions and other foodstuffs. They used a double-oven GC–GC system with a 50-m SE-54 column in the first and a 30-m DB-17 column in the second oven.

Duinker *et al.* [126] used a 25-m SE-54 column in the first oven and a 30-m OV-210 column in the second oven and applied the heart-cutting technique to separate the planar PCB congeners and, in addition, some of the mono-*ortho*-substituted analogues from closely eluting congeners. Sippola and Himberg [128] used a system consisting of a (standard) GC set-up, employing two columns (SIL-8 and HP-FFAP) connected by an SGE valve column-switching system. The technique was capable of separating the toxic PCB congeners from the complex matrix and decreased the need for sample purification.

3.2.3. SFE–GC coupling

As mentioned in Section 2.4, only a few real applications of SFE have been published and many papers demonstrate the potential of coupled SFE techniques for fatty food matrices by qualitative experiments [86,129].

Murphy *et al.* [91] reported an on-line SFE–GC–flame ionization detection (FID) determination of spiked aldrin in soybean oil. A commercial system was used, in which the chromatographic column was interfaced through a stainless-steel tee located in the oven. The restrictor, connected to the extraction cell, deposited the solutes from the extraction cell in the decompression interface region in the tee. After extraction, carrier gas was allowed to sweep the solutes from the interface to the column. By varying the extraction conditions, broader peak shapes occurred using longer extraction times and the separation of aldrin from the interfering matrix peak became worse.

A special type of coupled SFE was used by Murugaverl and Voorhees [85], who called it SFSPE–SFC, an on-line supercritical fluid extraction in combination with clean-up using solid-phase extraction followed by SFC. The SFSPE–SFC method differs from SFE–SFC in that the solid-phase trap is used for both trapping and clean-up; collection and clean-up are accomplished in one step prior to introduction of the analyte into the analytical column. Spiked fats mixed with about three parts of C_{18} sorbents were placed in the extraction cell or on top of the clean-up column and were selectively extracted, retaining the sample matrices while eluting and depositing the analytes of interest in the cryogenic trap, which could be flushed to deposit the fraction in the SFC system. Column packings were evaluated which could retain lipids and allow analytes to pass through in CO_2, which seems possible for many commercially available materials (silica, C_{18}, CN, NH_2, C_2). In spiked samples of diuron, alachlor, carbaryl in soybean oil and bendiocarb in lard, lipids were completely separated from pesticides in the first fraction (extraction for 20 min). Using a larger amount of sample (4–8 mg), some interference is shown by endogenous compounds (fatty acids) co-extracted with the pesticides. Ramsey *et al.* [86] used a similar kind of procedure, based on the differences in polarity of the extracted endogeneous material and the analytes, which could only be used for more polar analytes.

4. SPECTROSCOPIC TECHNIQUES

Through the years, spectroscopic techniques have been introduced in many laboratories for residue analyses of organic micropollutants in foodstuffs. The reason of the use of these techniques is either to obtain more selectivity and sensitivity in the trace analysis of complex mixtures or to confirm the identity of analytes following dedicated analytical techniques, usually GC or HPLC. Until recently, the well established technique of GC–MS was used almost exclusively in this field. This technique combines high sensitivity and selectivity with diagnostic structural information. However, its application range is limited to gas chromatographable compounds. Other, more recent techniques are GC coupled to Fourier transform infrared spectroscopy (GC–FT-IR) and LC–MS.

4.1. Liquid chromatography–mass spectrometry

LC-MS was first introduced in the early 1970s with the development of the moving belt interface (MBI) [137–139]. Other LC–MS techniques have been developed in the 1980s in which problems associated with the handling of relatively large solvent streams from the LC column into the mass spectrometer source have been solved in different ways. Among these, the direct liquid introduction (DLI) interface [140,141], the particle beam interface (PBI) [142] and more recently the thermospray (TSP) interface [143] have found widespread use in modern analytical chemistry. Application ranges of these interfaces differ widely from low-polarity compounds by, *e.g.*, MBI, DLI and PBI to polar compounds by TSP and electrospray. Owing to the lipophilic properties of micropollutants in biological samples and foodstuffs, LC–MS is not frequently used in this field, as LC is not often the principal method of analysis. An exception is made for PAHs and their metabolites, which are routinely analysed in some laboratories by HPLC [144,145].

The particle beam interface (equivalent terms: MAGIC, LINK) is probably the most appropriate technique for HPLC–MS of lipophilic compounds. In this interface, the LC effluent is converted into a monodisperse aerosol, the solvent is subsequently removed by a momentum separator and the heavier analyte particles are transported to the MS source where they can be ionized by electron impact (EI) or chemical ionization (CI) for structure analysis or quantitative determination. The technique is compatible with normal-bore HPLC flow-rates and good sensitivities can be achieved for low- to medium-polarity compounds.

4.2. Gas chromatography–Fourier transform infrared spectroscopy

GC–FT-IR is a relatively new technique which is recognized as very suitable for confirmation analysis of complex mixtures. Present commercially available interfaces are the light-pipe interface [146], the cryotrapping technique [147] and the matrix isolation technique in solid argon [148]. The last two techniques allow on-line analysis and spectrum averaging on the stored chromatogram trace for increased sensitivity or spectrum quality. The sensi-

tivities of both techniques are comparable down to the sub-nanogram level on-column in the full spectra mode [149]. The utility of GC–FT-IR in micropollutant analysis lies predominantly in its complementary structural information to mass spectral data for the unambiguous identification of unknowns, particularly for positional isomers. Schneider *et al.* [150] and others [151,152] have used GC–FT-IR for the analysis of chlorinated pesticides, dioxins, PCBs and PAHs in a variety of foodstuffs. Mossoba *et al.* [153] and Powell and Compton [154] used GC–FT-IR for the analysis of trace components in alcoholic beverages and foods.

4.3. Gas chromatography–mass spectrometry

As noted, GC–MS is the preferred technique where possible because of its unsurpassed separation efficiency (GC) combined with high sensitivity and specificity (MS). In regulatory practice, GC–MS is often the principal quantification method, but is frequently also used for qualitative confirmatory analysis. An example of the former is the analysis of PCDDs and PCDFs. At present, GC–MS is the only technique able to provide the required sensitivity and selectivity for trace level analyses of PCDD/Fs in biological samples. The retention parameters from GC provide isomer specificity, whereas the MS parameters provide class and homologue specificity. Clement and Tosine [155] have recently published a comprehensive review on the GC–MS analysis of PCDD/Fs. Methods include the use of non-polar and polar fused-silica capillary columns combined with low-resolution (LR) or high-resolution (HR) MS and tandem MS–MS techniques [156,157] for improved selectivity. As with dioxins, GC–MS is becoming the method of choice for trace-level analyses of PCBs in biological samples, particularly for planar and mono-*ortho*-substituted PCBs [84]. Analytical methods for PCBs include sample isotope dilution, extraction, clean-up and GC with low- or high-resolution MS. The analytical procedures for PCBs are almost identical with those used for PCDD/Fs [81]. The detection limits for PCDD/Fs and PCBs vary with the sample matrix and sample size, down to the sub-ppt level on a fat basis in biological samples and foodstuffs [69].

In contrast to quantitative analysis, confirmatory analysis is preferably performed in the full-scan mode for identification. A new, interesting technique for confirmatory analysis is ion trap detection (ITD) MS. ITD instruments contain a three-dimensional quadrupole ion storage trap first developed by Paul and Steinwedel [158] and further optimized and commercialized by Finnigan MAT [159]. In the ITD source, ions are generated and stored during the entire sample ionization time and are subsequently mass analysed. This technique provides unique sensitivity in the scanning mode, providing good-quality spectra in the low picogram range in both EI and positive-ion CI modes. A benefit of the ITD instrument is its relatively low cost. A disadvantage, however, is that the sensitivity may vary considerably when real samples are analysed owing to overloading of the trap by, *e.g.*, a high GC baseline or the presence of co-eluting interferences. This will result in a shortening of the ionization/accumulation time and hence in a lower sensitivity.

Identification and confirmation with conventional MS in the full-scan mode usually require much larger samples than are needed for single (selected) or multiple ion recording analysis (SIR). Sensitivities in the scanning mode are typically one to two orders of magnitude lower than in SIR. Therefore, SIR is frequently used for confirmatory purposes by monitoring a few ions from the analyte spectrum. However, to obtain acceptable results, protocols must have appropriate criteria for the number of ions that must be monitored, intensity ratios, the retention time and the use of different ionization techniques [160].

As noted, modern GC–MS instruments offer the opportunity of positive- and negative-ion chemical ionization (PCI, NCI) for improved molecular mass information and/or increased sensitivity. Methane and ammonia are most commonly employed as reagent gases. PCI has been frequently used for pesticide analysis. Many of these compounds undergo strong fragmentation under EI with low-abundant molecular ions. Methane or ammonia CI often generate quasi-molecular ions, $[MH]^+$. Cairns *et al.* [161] have constructed a molecular mass list of the majority of pesticides as an aid for the identification of suspected pesticide residues.

In the analysis of PAHs, CI will generally not provide more additional information than EI, because EI spectra usually contain abundant molec-

ular ions. Difficulties with the identification of PAHs lies predominantly in the lack of diagnostic differences in the EI spectra of isomers. Additional information of GC retention parameters is often needed for identification. The selectivity for isomeric PAHs can be altered by the use of other ionization techniques. Hilpert [162] showed that the sensitivities of different PAHs to negative-ion CI differ greatly, which provided an easy method to discriminate between isomeric PAHs and alkylated PAHs. For example, fluoranthenes were sensitive in NCI, whereas pyrenes were almost transparent. Sim *et al.* [163] compared LC (moving belt) and GC, both combined with MS, for the determination of PAHs. Further complementary separation methods were not needed for complete separation and identification of a complex mixture. The higher column selectivity in HPLC was used for the determination of isomeric compounds. Differentiation between isomers was studied by Brotherton and Gulick [164] using hydrogen PCI. Others used charge transfer in the presence of argon–methane mixtures in the source [165].

NCI, particularly electron-capture negative-ion CI [166], has been found to be useful for increased sensitivity for the analysis of many micropollutants. The sensitivity of compounds under NCI often parallels that of the ECD in GC. In the NCI process, near-thermal electrons are generated in the source under high-pressure CI conditions (0.2–1 Torr), and rapidly react in relatively high yields with suitable molecules to produce M^- ions. Further fragmentation may occur, depending on the structure and the experimental and instrumental conditions [167]. The highest sensitivity is usually achieved for compounds with conjugated or aromatic structures with a sufficient number of halo substituents. Hexachlorobenzene (HCB), for example, is extremely sensitive in NCI. Stan and Kellner [168] examined 72 organophosphorus pesticides under PCI and NCI conditions and found 59 to be more sensitive in NCI and 13 in PCI.

Other polychlorinated micropollutants such as PCBs and PCDD/Fs can be analysed using NCI-MS. Characteristics of the NCI mass spectra of PCDD/Fs have been extensively studied [169]. The fragmentation and sensitivity depend on the degree of chlorination and the substitution pattern. For instance, 2,3,7,8-TCDD was found to be less sensitive than the other TCDDs [170] and PCDFs were more sensitive than PCDDs. Significant differences in spectra were observed depending on the operating conditions, the most critical being the source temperature [167] and the presence of oxygen in the ion source [171]. Traces of oxygen lead to the formation of O^- and O_2^- ions, which can react with molecular ions to give $[M\text{-}19]^-$ by displacement of Cl by O [172].

In summary, research over the last few decades has resulted in the development of impressive methodologies for the characterization, identification and highly sensitive determination of micropollutants in foodstuffs by spectroscopic techniques. Trace-level organochlorine pesticides can be detected and identified by capillary GC combined with EI-, PCI- and NCI-MS. Current methods for PCDD/Fs and PCB analysis are well established and are highly sensitive and specific at the sub-ppt level. Their performance is unmatched by other analytical techniques. Future research to improve GC–MS analyses of brominated or mixed bromo–chloro PCDD/Fs will primarily focus on the isomer-specific separation of the much larger number of toxic congeners. LC–MS and GC–MS methods using different ionization techniques are available for the identification of PAHs in complex samples, but discrimination remains difficult. A significant contribution to the solution of this problem is foreseen by the use of GC–FT-IR, which will find increased application in the field of micropollutant analysis, particularly for the identification of isomers.

5. CONCLUSIONS

Although the determination of apolar organic micropollutants in lipid materials is a well established field with its first publications originating from the 1950s, much research is still needed for the development and improvement of residue analysis. The methodology described in this paper is used by governmental laboratories for risk assessment and regulatory practice. For this reason, method development should mainly be focused on improvements of the sample throughput and reliability. High plate numbers in a novel miniaturized chromatographic column may seem to be very attractive from a theoretical point of view, but the final outcome that

counts is whether the system will be able to produce a large amount of reliable data in a minimum amount of time.

Over the years, attention has shifted from the OCPs to PAHs and PCBs, ultimately resulting in major efforts in the late 1980s to develop sensitive methods of analysis for PCDDs and PCDFs. At the same time chromatography has developed strongly, and a major trend towards coupled techniques can be observed. Coupling of chromatographic techniques with other chromatographic techniques (LC–GC, SFE–GC, GC–GC, LC–LC) greatly alleviates the problem of manual sample pretreatment and enhances selectivity and sensitivity. Coupling of chromatographic techniques with spectroscopic techniques (GC– and LC–MS, GC– and LC–FT-IR) gives a new dimension to the original chromatographic techniques. The use of GC–MS has greatly facilitated the development of ultra-trace-level methods for PCDDs, PCDFs and for the toxicologically related planar PCBs.

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