Pure & Appl. Chem., Vol. 60, No. 9, pp. 1437–1448, 1988.
Printed in Great Britain.

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

ANALYTICAL CHEMISTRY DIVISION

COMMISSION ON MICROCHEMICAL TECHNIQUES AND TRACE ANALYSIS*

WORKING PARTY ON ORGANIC TRACE ANALYSIS

EXTRACTION, CLEAN-UP AND GROUP SEPARATION TECHNIQUES IN ORGANOCHLORINE TRACE ANALYSIS

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Extraction, clean-up and group separation techniques in organochlorine trace analysis

INTRODUCTION

This paper critically reviews and gives recommendations for the isolation/extraction and subsequent clean-up stages of analysis (Table 1) of persistent halogenated hydrocarbons, related pesticides and biocides which occur in the environment at the trace level (Table 2). Many analytical schemes currently in use for the determination of these compounds have been developed from specific methods of analysis for individual or related pesticide, herbicide and fungicide residues. These are well documented in reference texts such as The Pesticide Analytical Manuals (three vols) (ref. 1), the Manual for Environmental Analysis, EPA (ref. 2), the Guide to Chemicals in Crop Protection (ref. 3), the Pesticide Manual (UK) (Worthing and Walker) (ref. 4) and the Agrochemicals Handbook (Hartley and Kidd) (ref. 5). Recent developments in extraction and clean-up of organochlorines have been assessed in reviews by Getz and Hill (ref. 6), Onuska (refs 7 and 7b) and Erikson (ref. 8). The IUPAC Commission (VI. 5), Working Group on Pesticide Chemistry, have published a series of status reports (ref. 9) on the Development, Improvement and Standardisation of Methods, (ref. 10) and Improvements on Cost-effective approaches to Pesticide Analysis (ref. 11).

Many of these separation and clean-up protocols are well established and have now been applied to many areas of trace organic analysis (refs 12 and 13). This has broadened the scope of this field in terms of the number and type of compounds to be determined, and the range of concentration and limits of detection. These protocols have been incorporated in Master Analytical Schemes (MAS) for the analysis of the Priority Pollutants (refs 14, 15, 16 and 17) and are used for the determination of compounds included on the Black (List I) and Grey List (List II) European Communities Directive 76/464/EEC (ref. 18).

The development of the "Master Analytical Scheme" approach has led, in some degree, to compromise the optimum analytical conditions for the determination of the individual compounds. This has produced the "Black Box" Syndrome where compounds having some chemical similarities are treated in a uniform manner. Such multi-residue schemes are understandable on economic grounds, but the chemist should always be aware of the level of compromise. It is an inevitable consequence of the multi-residue scheme and, although an understandable development on economic grounds, the chemist should always be aware of the level of compromise. It is essential for any specific determination to optimise the method for the particular analyte, or group of analytes of interest eg Chlorobiphenyl congeners.

GOOD LABORATORY PRACTICE AND ANALYTICAL QUALITY CONTROL

In trace analysis the concentration of the determinand is often close to the limit of detection of the analytical system. The limit of detection for qualitative analysis is generally regarded as ~x2 baseline noise, with the limits for quantification being some 5-10 times greater. Background interference from the sample matrix, solvent and induced contamination will adversely affect the signal/noise ratio and must be kept to a minimum at each stage of sample treatment prior to detection. The general standards of good laboratory practice required to maintain the sample integrity and minimise the risk of contamination in organic trace analysis are discussed in a number of comprehensive texts (refs 12, 14, 16 and 19). Some specific points relevant to organochlorine residues analysis (refs 20, 21 and 22) are mentioned here.

The protocols used for sample preparation, prior to detection by either Gas Chromatography (GC) or Gas Chromatography-Mass Spectrometry (GC-MS) (Table 1), are crucial in maintaining the sensitivity of the system. These methods not only to filter out the gross matrix and the minor co-extractants, but also to separate classes of similar compounds which may be unresolved, even with high resolution chromatography eg Polychlorinated Camphenes (PCCs) and Polychlorinated Biphenyls (PCBs). Unless extreme care is taken the concentration of impurities will be amplified at each concentration step, as the solvent is evaporated, and the determinands of interest will be masked. These impurities may also increase the baseline signal and reduce sensitivity. These impurities may originate from the solvent or from contaminated equipment.

TABLE 1. Overview diagram for the analysis of trace organic compounds.

Stage 1	Sampling
Separation of the sample from its environment	
Stage 2	Isolation/extraction
Separation of the determinand from its major matrix	
Concentration step 1	
Stage 3	Clean-up
Separation of the determinands from other co-extracted major/minor components	
Concentration step 2	
Stage 4	High performance/ resolution chromatography
Separation of the determinands from similar compounds, and/or isomeric structures	
Stage 5	Specific detection
Electron capture detector/mass spectrometry	

The following points have been included as essential safeguards.

1. Laboratory cleanliness is essential. Polish on benches or floors should be kept to a minimum. Workers should avoid using hand creams. Skin contact with solvents should be strictly avoided to prevent sample contamination and to safeguard human health. Contamination may arise from cosmetics, medication and domestic horticultural formulations brought into the laboratory by workers or visitors.

2. Only good quality glass or stainless steel should be used for sample storage and manipulation. Solid sample containers should be lined with clean aluminium foil, and liquid sample or organic solution/solvent containers should be lined with PTFE.

3. Plastic and rubber tubing, particularly PVC type, should be avoided. All organically based equipment which cannot be cleaned with the glassware should be checked for its suitability prior to use.

4. Glassware should be cleaned in a biologically active cleaner eg Haemosol or Decon-90, rinsed thoroughly in distilled water and heated in an oven at 200-300°C.

5. The use of solvents to wash or rinse labware should be avoided. It is expensive and often leads to contamination problems by adsorption. There is also an increased health risk unless all such manipulations are carried out in a fume hood or similar protection.

6. All solvent operations should be covered eg reflux condensers, gravity fed adsorption columns. Stoppers should be kept in the solvent bottles when not in active use. Exposure, even for a short period of time can cause significant contamination from the atmosphere, particularly from phthlate esters.

7. High quality solvents are commercially available, but a sample of each batch should always be tested by taking the same volume and concentration step used in the method and examining the concentrate by GC. This procedure should be part of the routine analytical quality control (AQC). Further purification, if necessary, is best achieved over lithium aluminium hydride or sodium metal in an efficient, all glass distillation system (ref. 23).

8. Quality control of each stage of the analysis should be maintained on a regular batch basis, with each batch containing a complete method blank, a laboratory standard and, an external standard. Where available, a Standard Reference Material should be analysed to confirm that the optimum analytical conditions are being maintained. This may be done on a less frequent basis eg once per month.

At present the organic SRMs available are limited in the range of determinands and matrices offered (refs 24, 25 and 26). However, within each laboratory it is possible to use a sample from a large batch which has been homogenised and analysed. Alternatively, the standard addition technique may be used in conjunction with a cleaner sample, containing a low concentration of the determinands of interest. In addition, each laboratory should participate in an inter-laboratory analytical exercise for the particular determination being made. This approach will identify the magnitude of any laboratory bias in the analytical technique used, and by discussion and re-analysis help, to highlight any major causes of error.

Sampling

Consideration of sampling strategies and techniques, although important has not been considered in this report beyond air sampling, since that is an intrinsic part of the "extraction" procedure. No discussion has been given for example, to the size of sediment/soil particles, part of the plant, root, stem, leaf or choice of tissue.

Sampling and extraction

Air Air sampling for volatile organics and particulates (refs 27, 28, 29 and 30) is relatively easy since the matrix is free from large concentrations of other organics. It is, therefore, possible to complete the sampling and enrichment in a single step. However, the concentration of organics in air, particularly volatile halohydrocarbons is usually low and a high volume sampling (5-10 m^3) is necessary. Air is filtered through glass fibre filters of known pore size (eg 100 μm) to remove particulates and the organic aerosols are passed through an adsorption tube. Tenax and charcoal are the two most commonly used adsorbents, but polyurethane foam, florisil and silica gel have also been used. The adsorption tubes are usually cooled with solid CO_2 or liquid nitrogen and, after sampling, the trapped materials are desorbed by rapid heating (~300°C) in a fast gas stream into the chromatographic system. The desorb volatile components can be cryofocused in a cold trap before eluting into the GC to reduce the band width of the solute. Alternatively the determind(s) can be eluted with carbon disulphide, acetone of toluene and injected using a syringe. Thermal desorption in a conventional oven (ref. 29) or by microwave (ref. 31) may diffuse the determinands if heating is too slow or the thermal capacity of the adsorption tube too large. However this bandspreading can be corrected by further cryofocusing prior to analysis. If the sample contains a large number of determinands then solvent desorption is preferable as this allows further group separation prior to GC or GC-MS analysis. The main disadvantage of solvent desorption is the loss in sensitivity (~ x 100) by sample dilution, but may be necessary when charcoal is used as the adsorbent since it is difficult to quantitatively remove adsorbed trace organics from the charcoal using thermal desorption techniques.

Alternatively a single stage adsorption technique can be used by passing the sample through a cryoscopically cooled (liquid nitrogen or solid CO_2) low melting point solvent. Melcher et al. (ref. 32) and Bidleman (ref. 33) have extensively reviewed sampling techniques and extraction procedures for OCs in gases, vapours and on particulates in air.

Water The analytical techniques developed for the determination of halogenated trace organics in water and waste water have, to some extent, been consolidated in the protocols developed for the analysis of the Priority Pollutants (refs 15 and 17) and the "EEC Black List" and "Grey List" (EEC 1976). There are many symposia reports (refs 34, 35, 36, 37 and 38) on this topic as workers had adapted the basic methods for their own application. For sampling and separation purposes these organics have been classified as volatiles or extractables. This does not account for the substantial quantity of organics, some at the trace level, which are not separated by these techniques and are beyond the scope of this report (ref. 39). The division between volatiles and extractables is not finite and some overlap occurs.

Volatiles In a recent review, McIntyre and Lester (ref. 37) concur that the purge and trap method (refs 15 and 17) is currently the most widely used and accepted technique. Based on the method of Bellar and Lichtenberg (ref. 40), the sample (5-10 ml) is purged with high purity helium (40-50 ml min^{-1}) at room temperature for 10 minutes and the volatiles are trapped at room temperature on tenax, silica gel or charcoal. The trap system is switched on-line to the GC using a 6-port valve and the trap is desorbed (180°C for four minutes). Further cryofocusing in the fused silica capillary column, prior to

analysis is possible if sample diffusion occurs at ambient temperature. The trap may be re-used following conditioning (200°C for 10 minutes) and the purge container is also re-usable simply by cleaning with solvent washed distilled water between samples to prevent cross contamination. This technique can be used for waste water, final effluents, and sewage sludges, if the solids are diluted to <5000 mgl^{-1} (ref. 15) prior to purging, and a second trap or foam beaker (ref. 41) is used to prevent contamination of the trap by the emulsion.

Direct aqueous injection onto Tenax GC, Porapak or Chromosorb polymers can also be used for more contaminated samples (refs 42 and 43). However there is no advantage in using this method unless all of the samples contain a high concentration of volatile components.

Head space analysis has also been applied to the determination of trace organics in potable and river water. Although detection levels of <1 μgl^{-1} have been reported the variability in vapour pressures tends to give much less reliable results at these lower levels.

Extractables There are three general techniques used for the isolation of the less volatile organohalides from water (1) steam distillation, (2) adsorption onto porous polymers and resins and (3) solvent extraction. Steam distillation (ref. 44), although useful for particular application, is prone to variable results and not applicable to all compounds in this class.

Amberlite XAD resins (refs 15, 45, 46) Chromosorb W coated with n-undecane and Carbowax 4000 (ref. 47), and polyurethane foam, coated with DC 200 (ref. 48) have all been used to concentrate low concentrations of Organochlorines (OCs) from large volumes (~20l) of river and rain water. Of these the Amberlite XAD resins have been investigated more fully and are, in general, more reliable (ref. 49). Howere there are a number of disadvantages in use. It is difficult to maintain clean resins with a sufficiently low background bleed from the polymer. The solvent extraction techniques necessary to achieve this are tedious. However, it has been achieved to some extent by automation (refs 15 and 50), but not to a level that the resins can be used reliably for large volumes of relatively uncontaminated waters where this technique is, in theory, most applicable. Adsorption efficiency, even with this limited class of compounds is variable and breakthough volumes at high volumes/low concentration still requires further investigation. The efficiency is also effected by suspended solids which tend to clog the leading surface of the resin. For routine analysis of organohalogen residues liquid-liquid extraction (LLE) is still currently a preferred technique.

The organochlorine residues are suitable for extraction from water, and waste water by LLE (refs 12, 15 and 17). Samples are not usually filtered and final effluents, with a high level of suspended solids, can usually be diluted prior to extraction. The solvents most widely used are n-hexane (refs 51, 52 and 53) and dichloromethane in two or three 30 ml aliquots per litre of sample. There are various techniques for the actual extraction using the conventional separating funnel, stirring (for batch extraction) and flow-under continuous LLE; the latter being more reproducible, quicker, much less inclined to form emulsions, and operable as multiple systems. Emulsions if formed can be blended by stirring, filtration, centrifugation and or by the addition of anhydrous sodium sulphate to separate the wet solvent layer. The acidic components may be extracted together with the neutral OCs by adjusting the water to pH 2 and then using a separation scheme with aluminia (ref. 53) or separating at the extraction stage by making an initial extraction at pH 11 for the base-neutral components.

Extracts are concentrated using standard Kuderna-Danish apparatus (ref. 17) or in a stream of clean, dry air and, following any further clean-up or group separation, reduced to a volume of 100 ul provided an internal standard is used. This gives a concentration of 20,000 for a 2l sample. A micro extraction procedure (ref. 54) has been devised to extract one litre of water with 200 ul of hexane, avoiding solvent concentration, and would serve for a rapid screening method. Small cartridges of C-18 Reverse Phase HPLC (Sepak) packing material have also been used for small volumes of relatively contaminated waters. The sample (10-50 ml) is syringed through the cartridge and eluted with a solvent such as methylene chloride. Generally the sample volumes required for the measurement of the OCs in water at the trace level are too large for these techniques.

Solids Sewage sludge, sediments and soils all vary in their moisture content depending on the location of the sample. The samples should be dried in a heated air cabinet which is isolated from any likely sources of cross contamination. The drying conditions reported vary from 105°C for 4 h (ref. 55) to 50°C for 24 h (ref. 56). In this Laboratory slurry mixes are dried at 40°C for 48 h. Lower temperatures are preferable to minimise any co-distillation of the OCs with the water. Loss at this stage is minimal as the OCs remain bound to the organic waxes and lipids associated with the solids.

Hexane is not a sufficiently polar solvent to quantitatively extract many of the OC residues from sediment. Acetone (60%): hexane (40%) (ref. 57) and methylene chloride (66%): methanol (33%) (ref. 56) mixtures have both been used in soxhlet extraction and give good recoveries. Grimalt et al., (ref. 56) also showed that sonication at room temperature with the methylene chloride and methanol, 2:1, gave comparable results to soxhlet extraction. Wegman and Hofstee (ref. 57) shook soil (30 g) with acetone (100 ml) for 30 minutes and left the mixture standing overnight to extract OCs. Buchert (ref. 23) used a mixture of hexane-acetone and methanol. Although there is currently no common protocol for the extraction of solids, a binary mixture of either hexane:acetone or hexane:methylene chloride is suitable with soxhlet extraction or shaking. One advantage of using acetone is that it is easily removed by washing the extract with water prior to further clean-up/separation procedures.

Czuczwa and Hites (ref. 58) used a mixture of methylene chloride and propan-2-ol to extract sediments containing PCDDs and PCDFs and benzene in a soxhlet to extract fly ash. Ballschmiter (ref. 59) has recommended a reflux extraction technique with a higher boiling point mixture of toluene and methoxyethanol to remove the PCDDs more effectively. The more rigorous extraction conditions required for the fly ash reflect the physical differences and the binding of the PCDDs and PCDFs to the substrate.

Plant materials Most fresh plant material, like sediments, has a high water content and will dehydrate prior to analysis unless extracted directly after sampling. Plants should, therefore, be crushed, chopped and dried gently at 40-50°C prior to storage and analysis. The plant material should be ground with coarse sea sand or blended prior to extraction. Some less stable pesticides (eg dithiocarbamates) are destroyed by such processes, but all OC compounds reported in these schemes are stable to such manipulations.

Matrices with a high sugar content require a reasonably polar solvent for extraction. Ferreira and Fernandes (ref. 60) used acetone followed by partitioning into methylene chloride and Sissons and Telling (ref. 61) used an acetone-hexane extraction. Providing that the solvent is sufficiently polar to rupture and penetrate the plant cell walls then either shaking or soxhlet extraction will remove most organochlorine residues efficiently.

Body tissue and fluids Visceral or adipose tissue should initially be blended or homogenised. This is easier with softer tissues such as heart, gill or liver, but difficulties may be encountered with more heterogeneous tissue. The resultant fluid should be ground with anhydrous sodium sulphate and coarse silica until a free flowing powder is obtained. Three main methods of extraction have been used. Cold extracts using blending techniques (refs 62 and 63) require a more polar solvent than n-pentane or n-hexane. Mes found that methanol:dichloromethane 1:1 gave >80% recovery from adipose tissue. Other solvents or solvent mixtures eg benzene, benzene:acteone did not significantly improve recovery for this tissue examined (ref. 64).

The cold column extraction technique initially reported by Ernst (ref. 65) has been effectively adopted (refs 66 and 67) by passing hexane:acetone (2:1) through the gravity fed column packed with the dried tissue mixture. Stalling (ref. 68) used a similar technique with dichloromethane as the solvent. The main disadvantage of this method is the relatively large solvent volume which is required when larger tissue samples are extracted. This has been partially overcome by using the soxhlet extraction system (refs 51 and 69). In such cases it was possible to reduce the polarity of the solvent by using n-hexane, without decreasing extraction efficiency for the OCs. One disadvantage of the hot extraction technique is that a small percentage of the extracted fat can precipitate after cooling. Therefore it is necessary to remove the appropriate aliquot for analysis and lipid determination immediately on cooling.

Body fluids, eg blood, bile, stomach contents, milk can be "dried" and ground into a free flowing powder using sodium sulphate, which is then treated as a normal tissue preparation. If the fluid bulk is too great the sample should be treated as a liquid and extracted with hexane:propan-2-ol (80:20). The extraction mixture should be placed in an ultrasonic bath for five minutes prior to dilution with water and stirred gently to minimise the formation of emulsions (ref. 71).

Butter, fats, oils Relatively homogeneous lipid based materials do not normally require extraction and may be dissolved in n-hexane or petroleum ether to the desired concentration and cleaned-up using one of the techniques outlined below.

CLEAN-UP AND GROUP SEPARATION

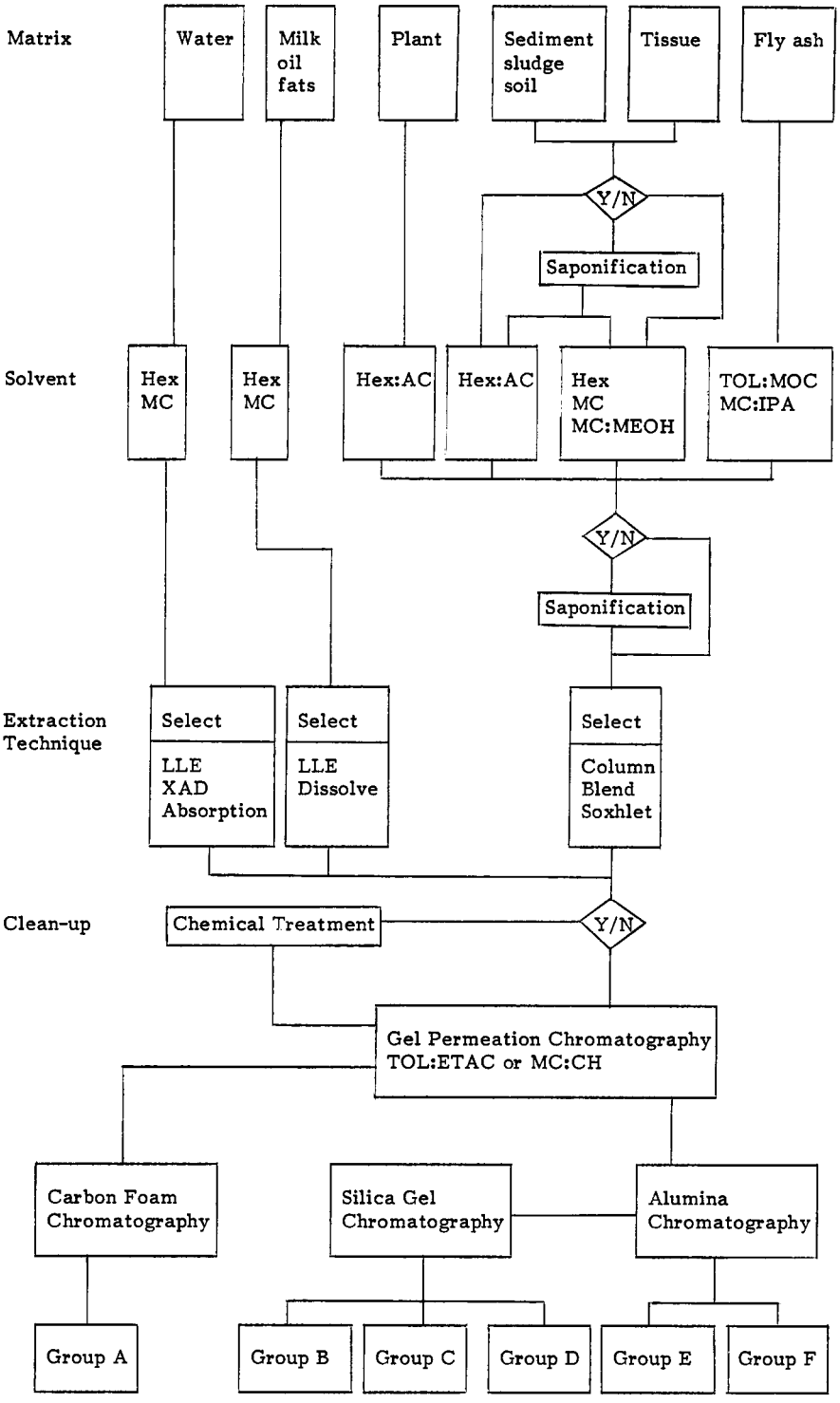
Sample manipulation following extraction is often similar for a wide range of matrix types and each extract may be considered for a variety of clean-up/separation procedures.

Initially the sample must be transferred into the appropriate solvent for the next preparation stage, eg n-hexane, or cyclohexane for Gel Permeation Chromatography. Care should be exercised when concentrating samples from a more polar solvent, as co-extracted material may precipitate and carry some of the OCs in the precipitate. This loss can be avoided by reducing the concentration step prior to clean-up. Extracts containing acetone should be washed by stirring with water, removing the acetone/water layer and adding the necessary volume of hexane (~50-100 ml). Lipid based materials including sediments must be measured for their extractable lipid content (1) to allow the final OC residue content to be expressed on an extractable lipid basis (as well as a fresh weight/volume basis) and (2) to prevent lipid overload of the adsorption clean-up columns (ref. 69). The lipid measurement obtained by evaporation of the solvent in a weighed dish is an estimate of the extractable residue, rather than an accurate value for the lipid content.

The following sample preparation procedures have two functions: (1) the removal of gross levels of co-extractants, and (2) to separate the OCs into groups based on their solid/liquid adsorption characteristics. As well as the lipids, waxes and animal sterols from tissue extracts, elemental sulphur and organosulphur compound in sediments and soils and carotenes mainly in plant extracts have been identified as major sources of interference.

TABLE 2. An overview of clean-up and separation techniques for extractable organochlorine residues

See Glossary for abbreviations of solvents and the compounds in each of the group separations



Chemical clean-up

Most chemical clean-up techniques reported have used fairly harsh acidic conditions, based on concentrated sulphuric acid or chromic acid. Anhoff and Josefsson (ref. 70) used these acids and Raney-nickel to remove lipids and elemental sulphur. Veierov and Aharanson (ref. 72) also used concentrated sulphuric acid Suzuki et al. (ref. 73) used 40% sulphuric acid and 4% potassium permanganate to supplement their Florisil clean-up. A number of methods have been used to remove elemental sulphur (ref. 70). A useful non-destructive technique with tetrabutylammonium sulphite was developed by Jensen et al. (ref. 74). More recently Babkina et al. (ref. 75) have used concentrated sulphuric acid and aqueous sodium sulphite with tetrabutylammonium sulphate on sediment extracts.

Other methods involving chromic acid, perchlorination or dehydrochlorination have been used to assess the total PCB/OC concentration, but these are not considered here since individual components are not identified but are transformed to give a total OC group based on either the fully chlorinated or hydrocarbon skeleton.

Sulphuric acid can be used successfully to clean-up extracts for the determination of the more robust organochlorines. Most recent reports use variations and adaptations of the early methods. Compounds in the Drin group (Aldrin, Endrin, Dieldrin) are destroyed by this treatment, as are most other oxidisable organics.

Alternatively, the co-extracted lipid material may be modified by saponification with ethanolic potassium hydroxide prior to separation on alumina or silica gel (ref. 76). This has the advantage of removing the lipid hydrolysis products by back extraction into an aqueous phase and reducing to lipid loading to any secondary clean-up column. Saponification of tissue prior to extraction may also improve the recovery for some organochlorines.

Sweep co-distillation

The potential of sweep co-distillation in the routine clean-up of fatty tissue has only recently been practically realised. Since the first report by Storherr and Watts (ref. 77) there have been a number of developments (ref. 78) and commercial applications (ref. 79). The recent designs allow up to 10 fat samples (1 g of fat) to be injected into a heated (235°C) fractionation tube containing silanised glass beads. The OCs are distilled in a stream of pure nitrogen into a cold florisil (deactivated with 1% water) trap on the outside of the oven, which is subsequently detached and eluted with hexane or hexane-diethyl either to remove the groups of OCs. Until recently (ref. 80) this technique was unreliable because of sample loss by degradation and adsorption. However improved silanisation techniques on the glass packing now allow excellent recoveries from 1 g of fat at 0.02-0.1 $mgkg^{-1}$ range for OCs and 0.100 $mgkg^{-1}$ for total PCBs. Good yields were also obtained for the Organophosphorus (OPs) such as Fenchlorophos and Chlorophyriphos, and for pentachlorophenol, which clearly indicate the future possibilities for the current systems.

Gel permeation

Gel permeation chromatography (size exclusion) has been applied to the clean-up of fatty tissue extracts by Stalling and Tindle (ref. 81) to great effect. Since the early applications and automation (refs 68 and 81 and references there-in) there have been few major changes in concept. Most workers since then (refs 76, 82, 83 and 84) have applied this technique and basic chromatographic conditions to clean up a wide variety of animal and vegetable oils for OCs.

The original applications used Bio-Beads SX2 and cyclohexane as an eluate with a 20 x 270 mm column allowing up to 500 mg of fat per injection. The separation and maximum loading (1500 mg) was improved, and solvent volume reduced (ref. 68) by using Bio-Beads SX-3 and toluene-ethyl acetate (1+3) as eluate. Auto preparative systems are commercially available and has been further improved by using a UV detector as a feed back control on-column separation and efficiency (ref. 85). The larger columns can be reduced in size and hence reduce the volume of expensive solvent, if the higher fat capacity is not required.

Partition clean-up

Liquid-liquid partition clean-up using acetonitrile (ref. 86), dimthyl sulphoxide (ref. 87) and dimethylformamide-hexane (ref. 88) were amongst the earliest methods developed to remove lipid materials. With care, quantitative recoveries were obtained although most workers (reflected in the papers published) have relinquished these techniques in favour of chromatographic separations, which can be more carefully controlled, miniaturised and automated.

Adsorption chromatography

There have been four materials which are widely used in column-liquid chromatographic sample preparation for OC analysis. Methods using florisil (refs 89 and 90) have been developed, primarily in the USA and have been incorporated in the Pesticide Analytical Manual Vol 1 (ref. 1). Alumina and silica (refs 51, 53, 69, 91 and 92) were originally used as alternatives because of the unreliable nature of the florisil activity. However this problem has now largely been overcome and both materials are used with equal success (ref. 93).

Alumina has also been mixed/impregnated with other compounds to aid clean-up. Holmes (ref. 94) used a mixed alumina/silver nitrate column to remove carotenoids, elemental sulphur, and some interferring organosulphur compounds, impregnated alumina with sulphuric acid to improve lipid removal and Gilliespie (ref. 95) has used alumina in a blending technique to remove lipid.

Although alumina and florisil are still used extensively for clean-up, GPC can handle substantially more lipid and therefore the adsorption columns have tended to revert to their other role of OC group separation (refs 53 and 68).

Carbon-foam has been used very successfully in the separation of very closely related OCs (ref. 68), groups such as PCDDs, PCDFs and PCBs and Toxaphene. Tai et al. (ref. 96) and Wells (ref. 53) have used silica to separate the PCB group and toxaphene, and Janssen et al. (ref. 97) have reported separating PCBs and PCNs using active charcoal. The combination of the alumina and silica columns by de Voogt et al. (ref. 98) has greatly reduced the analysis time without any loss in group separation.

There is a vast literature reporting "New", "Improved" (ref. 99), "Simplified" (refs 89, 90, 100 and 101) and "Rapid" techniques in the separation of OCs using these adsorption techniques. Most schemes are designed for specific separation of OCs required to solve a particular problem and use one or more of the above adsorbents of varying activity (3-10%) and with increasing polarity of solvent. The common factors controlling good separation by these techniques are:

1. Mesh size of adsorbent and column dimensions.
2. Controlled activity of the adsorbent which is usally kept in a sealed container and protected by an anhydrous sodium sulphate plug in use.
3. Dried solvents; either by addition of a desiccant eg anhydrous sodium sulphate, active silica or more thoroughly by distillation over sodium metal.
4. Limited lipid loading (preferably removed prior to the separation step.
5. Controlled adsorbent performance by calibration with eluting a known mixture and using external standards with each sample batch.

AUTOMATION

In most laboratories automation of the sample preparation procedure is considerably less advanced than for the final GC detection and data handling. This is primarily because of the diverse nature of the samples and the range of techniques used. The specific separation procedures required for each group of determinands and sample matrix has tended to evolve in an empirical way from a number of different laboratories. Although there is a common thread throughout these techniques the diversity of each method often makes a single automated system impractical.

However GPC methods have been successfully automated for some time (ref. 68) and can cope with a wide range of sample types and lipid loading. Its primary application has been in the separation of lipids and there is currently no information on the removal of other known interferring compounds eg sulphur in sediments. Sweep co-distillation systems (ref. 79) are currently available and process samples in batches of 10. It is likely that there will be a number of further developments in this technique.

Recently Gretch and Rosen (ref. 102) have reported an automated sample clean-up for multi-residue analysis using a continuous flow system and florisil columns. They report a similar precision and recovery to the manual method using 20% less solvent in a third of the time.

Clearly, there must be some justification for the financial outlay and technical expertise required for automation. However in an area of trace analysis which is growing so rapidly and which at present is so labour intensive, that justification is generally not too hard to find.

MINIATURISATION IN CLEAN-UP

Hemmingway (ref. 11) has covered this topic in a recent report to IUPAC for pesticide residue analysis and many of his comments are pertinent to this report. With the greater use of internal standards the final sample volume can be substantially reduced to ~100 µl or even less. However this is currently limited to at least ~200 µl if the GC or GC-MS is fitted with an autosampler. Reduction in the initial sample quantity may pose serious problems when subdividing a relatively heterogenous matrix. For example, sediments have a wide particle size range and invariably contain pebbles and debris which makes it difficult to reduce the sample size below 20-30 g. This can also occur when sampling adipose or visceral tissue, where analysis of the whole organ might be preferred.

During the clean-up and subsequent stages of analysis even greater care in manipulation would be required to prevent loss and contamination of the smaller volumes. Errors caused by the manipulation of volatile solvents at the macro scale are sufficiently problematic that miniaturisation without a closed system and automation would only add to variance in the results already experienced with current techniques.

GLOSSARY

Solvents

Abbreviation	Common Name	IUPAC Name
HEX	Hexane	Hexane
ACE	Acetone	Propan-2-one
DCM	Dichloromethane	Dichloromethane
TOL	Toluene	Methlybenzene
MOE	Methoxyethanol	Methoxyethane-1-ol
IPA	Isopropanol	Propan-2-ol
ETAC	Ethyl acetate	Ethyl acetate

Group separation (see table 2)

Group A	
PCDD	Polychlorodibenzoidioxin
PCDF	Polychlorodibenzofuran
PCN	Polychloronaphthalene
Group B	
CB	Chlorobenzene
PCB	Polychlorobiphenyls
Heptachlor	1,4,5,6,7,8,8-heptachlor-3a,4,7,7a tetrahydro-4, 7-methanoindene
Aldrin	(1R, 4S, 4aS, 5S, 8aR)-1, 2, 3, 4, 10, 10-hexachloro 1, 4, 4a, 5, 8, 7a-hexanhydro- = 1 , 4:5, 8-dimethanonaphthalene
DDE	1,2-dichloro-2,2-bis (4-chlorophenyl) ethylene
Mirex	dodecachloropentacyclo[5,30,0,2,6,0]-decane
PCT	Polychloroterphenyls
Group C	
Chlordene	1,2,4,5,6,7,8,8-octachloro-3,4,7,7a-tetrahydro-4,7-methanoindene
DDT	1,1-dichloro-2,2-bis(4-chlorohenyl)-ethane
Group D	
Hexachlorophene	
Toxaphene (PCC)	Polychlorinated campheme
Group E	
a & g HCH	a & g hexachlorocyclohexane
Chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoidene
DDD	1,1,1-trichloro-2,2-bis(4-chlorophenyl)-ethane
Dieldrin	(1R,4S,4aS,5R,6R,7S,8S,8aR)-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8, 8a- = octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene
Endrin	(1R,4S,4aS,5S,6S,7S,8R,8aR)-1,2,3,4,10,10-hexachloro-1.4.4a.5.6.7,8, 8a- = octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene
Endosulphan	(1,4,5,6,7,7-hexacholro-8,9,10-trinorborn-5-en-2,3-ylenebis-methylene) sulphite
Chloroanalides	
Group F	
b-HCH	b-hexachlorocyclohexane
Permethrin	3-phenoxybenzyl (1RS,3RS; 1RS,3SR)-3-(dichlorovinyl)-2,2- = dimethylcyclopropane carboxylate
Group G	
Chlorophenols	
Chlorosulphonamides	

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