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ANALYTICAL METHODS FOR THE CONGENER SPECIFIC DETERMINATION OF POLYCHLORINATED BIPHENYLS IN ENVIRONMENTAL AND FOOD SAMPLES

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Abstract

A method for the on-line extraction, clean-up, fractionation and congener specific determination of *ortho-* and non-*ortho*-PCBs by gas chromatography-mass spectrometry was developed and validated. The chromatographic clean-up and fractionation of PCBs was carried out on a silica multilayer column coupled to a supported carbon column. A solvent elution scheme for the efficient separation of *ortho-* and non-*ortho*-substituted polychlorinated biphenyls, in two different fractions was developed. Detection was by gas chromatography-mass spectrometry (GC-MS). The method was applied to the analysis of fruit and vegetable samples obtained from sites in South Wales including the vicinity of a chemical incinerator. In all cases where PCBs were detected, levels were ≤ 0.2 μg kg^{-1}. Concentrations expressed in toxic equivalents (TEQs) showed that the most important contributor to the total TEQ was the non-*ortho*-PCB-126.

On-line high performance liquid chromatography-gas chromatography (HPLC-GC) is described for the concurrent determination of *ortho-* and non-*ortho*-substituted polychlorinated biphenyls (PCBs) in commercial mixture and soil samples. A porous graphitic carbon column eluted with hexane:toluene and toluene was used for the HPLC separation of *ortho-* and non-*ortho*-PCBs. Fractions were transferred from the HPLC to the GC column. Cryogenic cold trapping was used for analyte focusing at the head of the GC analytical column. *Ortho-* and non-*ortho*-PCB congeners were determined using electron capture and mass spectrometric detection.

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Dedication

To my mother and late father, Thank you for everything.

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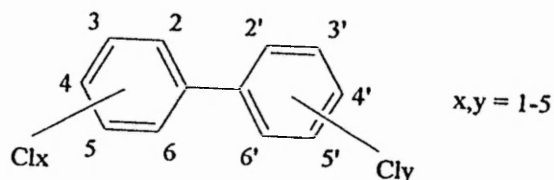
CHAPTER 1

Introduction

1.1. POLYCHLORINATED BIPHENYLS

1.1.1. Properties of the polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) consist of a class of compounds in which different numbers of chlorine atoms are substituted into the biphenyl ring, with a general formula $C_{12}H_xCl_y$, where x = 0-9 and y =10-x



Biphenyl has 10 positions (labelled 2-6 and 2'-6') available for chlorine substitution and different structural arrangements make possible 209 PCB compounds (Table 1.1). All possible 209 PCBs have been synthesized and analysed using capillary gas chromatography.[1] The term congener is applied to any of the 209 possible PCBs whilst isomers are those which have the same number of chlorine atoms, but differ in the arrangement of chlorines on the biphenyl ring. Each PCB congener is assigned an IUPAC number from 1 to 209, a scheme introduced by Ballschmiter and Zell.[2]

Commercial PCBs were produced by the catalytic chlorination of the biphenyl ring. A mixture of congeners needed for a specific use was obtained by collecting fractions during distillation of the chlorinated biphenyl mixtures over appropriate boiling range (Table 1.2).

TABLE 1.1. Distribution of PCB congeners by level of chlorination

Number of Cl atoms per biphenyl	Number of theoretically possible isomers
1	3
2	12
3	24
4	42
5	46
6	42
7	24
8	12
9	3
10	1
Total	209

The PCBs were marketed under a number of trade names including Askarel, Pyroclor, Aroclor (USA), Kaneclor and Santotherm (Japan), Clophen (Germany), Phenoclor and Pyrolene (France), Savol (USSR), Chemko (Czechoslovakia) and Fenclor (Italy). Each PCB formulation was assigned an identifying number. For most PCB Aroclors (such as Aroclor 1242), the first two numbers indicate the 12 carbons in the biphenyl ring and the last two numbers indicate the weight percentage of chlorine (i.e. 42% for Aroclor 1242). An exception is Aroclor 1016, which contains approximately 40% chlorine. Similarly, the Japanese equivalent, Kaneclor 300 contained an average of 3 chlorine atoms per biphenyl. All the formulations are mixtures of PCB congeners, but not all 209 PCBs are present in these formulations, because some positions on the biphenyl ring are more susceptible to chlorination than others. About 125 PCBs have been found in Aroclors, but the number of reported components of each Aroclor has varied,[3] (Table 1.2) depending on the types of analyses performed and quantities analyzed. In addition, the composition of any commercial formulation varies from batch to batch.

PCBs were first synthesized in 1881 by Schmidt and Schulz[4] and commercial production was started in 1929. The physical and chemical characteristics of PCBs, such as high stability, inertness and dielectric properties were extremely advantageous for many industrial purposes (Table 1.3).[5-9] Their worldwide use continued until they were discovered in the environment by Jensen[10] in 1966. Their production was restricted in the 1970s and eventually banned in the UK in 1980.

TABLE 1.2. Characteristics of commercial PCB formulations, Aroclors

Aroclor	Distillation Range, °C	Reported No. of compounds
1221	275-320	25
1232	290-325	34
1016	323-356	49
1242	325-366	74
1248	340-375	63
1254	365-390	116
1260	385-420	124
1262	390-425	Not available
1268	435-450	Not available

Source: Brinkman, U.A.Th. and De Kok, A., *Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxines and related products. Production , properties and usage,* Elsevier/North-Holland Biomedical Press, New York, 1980.

The cumulative world production of PCBs is estimated to be 2 x 10^9 Kgs since 1929.[11,12] Of this, about 31% is present in the global environment, whilst 65% is still in use in older electrical equipment and other products, or deposited in landfills and dumps, or in storage. Since this quantity is more than double the total PCB load that has escaped into the environment, the implication is that these land-stocked PCBs hold a crucial key to the forthcoming environmental pollution and possible biological effects of PCBs.

TABLE 1.3. Use of PCBs classified to grade of Aroclor

Use of polychlorinated biphenyls	Grade of Aroclor used
Electrical capacitors	1016 (1221, 1254)
Electrical transformer	1242, 1254, 1260
Vacuum pumps	1248, 1254
Gas-transmission turbines	1221, 1242
Hydraulic fluids	1232, 1242, 1248, 1254, 1260
Plasticizer in synthetic resins	1248, 1254, 1260, 1262, 1268
Adhesives	1221, 1232, 1242, 1248, 1254
Plasticizer in rubbers	1221, 1232, 1242, 1248, 1254, 1268
Heat transfer systems	1242
Wax extenders	1242, 1254, 1268
Dedusting agents	1254, 1260
Pesticide extenders, inks, lubricants, cutting oils	1254
Carbonless reproducing paper	1242

Source: Hutzinger, O., Safe, S. and Zatko, V., *The chemistry of PCBs*, CRC Press Cleveland, Ohio, 1974. Ch. 2, p.8.

1.1.2. Toxicity of polychlorinated biphenyls

PCB formulations elicit toxic and carcinogenic[13] responses which have been investigated in laboratory animals and found to depend on various factors including age, sex, species of test animals, route of administration, duration of exposure and chlorine content of the PCB mixture.[14] Hepatic damage, dermal disorders, reproductive toxicity, thymic atrophy, body weight loss, immunotoxicity and teratogenicity characterise the toxic responses of commercial PCB mixtures.[14]

The study of individuals occupationally and accidentally exposed to polychlorinated biphenyls has been used to determine their effects in humans. A broad spectrum of symptoms in humans can arise from occupational exposure to PCBs. These symptoms include possible hepatic damage, respiratory problems, elevated serum lipid levels and increased levels of some serum enzymes.

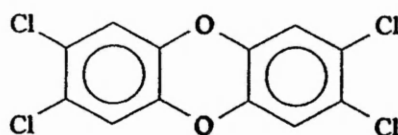
The possibility of adverse effects of PCBs on human health was highlighted by a poisoning outbreak in Japan[15-21] (Yusho incident) that affected people who had consumed a particular rice oil contaminated with PCBs and other contaminants including the related polychlorinated dibenzofurans. Those affected by the incident suffered from various symptoms such as chloracne, discolouration of gums, lethargy and pain in the joints, abnormal wax secretions from glands in the eyelids and swelling of the joints.[22] Moreover, children born to the affected patients showed retarded growth, abnormal tooth development and were undersized.[23] It was soon clear that PCBs used as a heat exchange fluid in the factory was accidentally mixed with the rice oil.[20] The causative agents of poisoning were considered to be contaminants of PCBs such as the polychlorinated dibenzofurans that were secondarily formed during the

heating of PCBs when rice was cooked in the oil. However, later studies demonstrated the presence of extremely toxic non-*ortho*-substituted PCB congeners such as 3,3',4,4'-tetrachlorinated biphenyl (PCB-77), 3,3'4,4'5-pentachlorinated biphenyl (PCB-126) and 3,3',4,4',5,5'-hexachlorinated biphenyl (PCB-169) in commercial PCB mixtures and biological samples in significant quantities,[24] which may have contributed to the observed health effects.

1.1.2.1. Isomers intrinsic in PCB toxicity

The toxicology of halogenated aromatics has been understood by taking 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) to serve as a model.[25] There is strong evidence that many of the toxic and biological effects elicited by this compound are mediated by an aryl-hydrocarbon (Ah) receptor mechanism of action.[26,27] The ligand - receptor complex is thought to initiate the synthesis of aryl hydrocarbon hydroxylase (AHH), ethoxyresorufin O-deethylase (EROD) and related enzymes that may be involved either in biotransformation, conjugation and removal, or bioactivation of certain planar lipophilic foreign compounds to toxic intermediates. Polychlorinated biphenyls elicit many of the toxic and biological responses[28-44] that resemble those caused by 2,3,7,8-TCDD and a similar correlation between toxic response potencies and Ah receptor binding affinities has been observed for PCBs.

PCBs which do not have chlorine substitution in the *ortho* position (non-*ortho*-PCBs) will show maximum coplanar conformation and hence resemble the structure of 2,3,7,8-TCDD.



2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Non-*ortho* congeners therefore exhibit a stronger ligand-receptor interaction because these interactions are stereoselective. Structure-binding relationships for PCBs have shown[26,27,45-48] that the most active compounds have four or more chlorine atoms at both *para* and *meta* positions in the biphenyl ring, but no chlorine atoms in *ortho* positions. Among the 209 theoretical PCB isomers and congeners, 20 members attain coplanarity due to non-*ortho* chlorine substitution in the biphenyl ring but only three in this group namely PCBs 77, 126 and 169 are considered toxic because they are approximate isostereomers of highly toxic 2,3,7,8-TCDD in their coplanar conformations. Some of mono-*ortho*-PCBs with similar structures to non-*ortho* exhibit lower competitive binding affinities for the Ah receptor.

1.1.2.2. Toxic Equivalents

Toxic equivalent factors (TEFs) are assigned to individual compounds to indicate their potency relative to that of 2,3,7,8-TCDD (which is assigned a factor of 1) if the compound comply with the following criteria.[49] The compound must:

(i) show a structural relationship to the PCDDs and PCDFs,

(ii) bind to the Ah receptor

(iii) elicit dioxin-specific biochemical and toxic responses

(iv) be persistent and accumulate in the food chain.

There is no international agreement yet on the TEFs for individual PCB congeners and the most recent TEFs for a limited number of non-*ortho*, mono-*ortho* and di-*ortho*-PCBs proposed by a WHO-ECEH and ICPS consultation, and reported by Ahlborg et al.[49] are presented in Table 1.4. The TEFs may be used as weighing factors to express the toxicity of these particular PCB congeners in terms of the equivalent amount of 2,3,7,8-TCDD. Therefore, the total "2,3,7,8-TCDD" like toxicity of a mixture of PCBs is the summation of the concentration of the individual toxic congeners times a relative potency factor (TEF) for each individual congener and is given as toxic equivalent (TEQ):

$$TEQ = \sum (PCB_i \times TEF_i) \qquad 1.10$$

where PCB_i and TEF_i are the concentration and toxic equivalent factor, respectively, of an individual congener for the n congeners in a mixture which exhibit TCDD-like activity.

Table 1.4. WHO/IPCS interim TEFs for human intake

IUPAC No.	Structure	TEF
Non-*ortho*		
77	3,3',4,4'	0.0005
126	3,3',4,4',5	0.1
169	3,3',4,4',5,5'	0.01
Mono-*ortho*		
105	2,3,3',4,4'	0.0001
114	2,3,4,4',5	0.0005
118	2,3',4,4',5	0.0001
123	2,3,4,4',5	0.0001
156	2,3,3',4,4',5	0.0005
157	2,3,3',4,4',5'	0.0005
167	2,3',4,4',5,5'	0.00001
189	2,3,3',4,4',5,5'	0.0001
Di-*ortho*		
170	2,2',3,3',4,4',5	0.0001
180	2,2',3,4,4',5,5'	0.00001

Source: Ahlborg, U.G., Becking, G.C., Birnbaum, L.S., Brouwer, A., Derks, H.J.G.M., Feely, M., Golor, G., Hanberg, A., Larsen, J.C., Liem, A.K.D., Safe, S.H., Schlatter, C., Waern, F., Younes, M., Yrjanheikki, E., *Chemosphere*, 1994,**28**, 1049.

1.1.3. Polychlorinated biphenyls in the environment and foods

The worldwide use of PCBs since they were first produced commercially in 1929, and their chemical properties such as lipophilicity and resistance to degradation has resulted in them becoming persistent and ubiquitous environmental contaminants[50-57] and has led to their accumulation in the food chain.[58-60]

Polychlorinated biphenyls have been detected in various environmental samples including air, water, snow, ice, fish, birds and mammals.[61-64] The discovery of PCBs in the open ocean atmosphere,[65] surface water, and subsurface water[66] confirm their penetration throughout the global environment. As far as the open ocean environment is concerned the contamination of PCBs is more prominent in the northern hemisphere than in the southern hemisphere.[67] A similar trend was observed in other environmental matrices like air, water, fish, plankton and marine mammals.[68] The maximum contamination was observed in the mid-latitudes of the northern hemisphere. This geographical distribution can be explained on the basis of extensive use and production of PCBs in this part of the globe and their environmental transport to cooler regions.

1.1.3.1. Sources, transport and fate in the environment

(a) Sources

The physical and chemical characteristics of PCBs such as high stability, inertness, and dielectric properties were extremely advantageous for many industrial purposes. Consequently, these versatile properties of PCBs led to their widespread application not only in industry but also to other human activities (Table 1.3). The uses of PCBs can be placed in three categories:

(i) Closed systems: PCBs are used as dielectrics in transformers and capacitors. PCBs in these systems can, at least in theory, be monitored in use, taken out of service, safely stored, and disposed of sensibly with minimal leakage. Closed systems make up 53% of the total of PCB applications.

(ii) Nominally closed systems : PCBs are used as hydraulic and heat transfer fluids as well as lubricants. Leakage of small quantities is expected. These systems make up 16% of the total of PCB applications.

(iii) Open-ended applications : PCBs have been used in the manufacture of plasticisers, adhesives, extenders, inks, copying paper and fireproofing. In these applications, PCBs are in direct contact with the environment and it is impossible to recover them once the product is scrapped. These make up 31% of the total PCB application.

There are a number of pathways by which polychlorinated biphenyls could be dispersed into the environment[58]:

(i) Accidental leaks and spillage from working industrial equipment (transformers, capacitors, etc.).

(ii) Incomplete combustion during incineration of PCB contaminated products.

(iii) Escape from facilities where PCBs are reclaimed from redundant equipment or processed into other products.

(iv) Leachate from landfill disposal sites and industrial discharge.

(v) The breakdown of scrap electrical components where PCBs have not been recovered.

(vi) Recycling of PCB-containing papers in the production of paper from scrap paper.

(vii) Disposal of sewage sludge.

(viii) Dispersive loses of all materials used in the open-ended applications.

(b) Transport and fate

The physio-chemical properties and chemical stability determine the way in which organic contaminants such as polychlorinated biphenyls are transported in the environment. The transport and fate of organic compounds in the global environment has been recently reviewed.[69]

The most important sinks for polychlorinated biphenyls are terrestrial and coastal sediments and sea water in the open oceans (Table 1.5). PCBs are mainly discharged to fresh water streams and coastal waters where they can be adsorbed on particles and carried to oceans. The exchange reactions between water and aquatic sediments and between water surface and atmosphere (particularly over the ocean) are of particular significance in the balance of source-transport-sink model of PCBs in the environment. The atmosphere does not represent a significant reservoir of PCBs, but it is considered as a major input pathway. From the atmosphere, PCBs are transferred

by wet and dry deposition over a wide land area. Material deposited on the land surface is largely re-evaporated or washed out into rivers. As the physco-chemical properties of individual congeners vary significantly, each process such as evaporation, wet and dry deposition and adsorption will be congener dependent and will result in different congener distributions in different phases.

Dispersed polychlorinated biphenyls are naturally removed from the environment mainly by the processes of photolysis and microbial degradation.[14,70-74] The degree of chlorination and the position of chlorine atom on the biphenyl molecule determine the microbial (aerobic and anaerobic) degradation of the PCBs.[14] Anaerobic degradation needs relatively high concentrations of PCBs as compared to aerobic degradation.[14] Volatilisation and other transport mechanisms remove significant amounts of PCBs from the environment. The chances of photolytic degradation are increased once PCBs are volatilised.

TABLE 1.5. Estimated PCB loads in the global environment

Environment	PCB load (t)	Percentage of PCB load	Percentage of world production
Terrential and coastal			
Air	500	0.13	
River and lake water	3500	0.94	
Seawater	2400	0.64	
Soil	2400	0.64	
Sediment	130000	35	
Biota	4300	1.1	
Total (A)	143000	39	
Open ocean			
Air	790	0.21	
Seawater	230000	61	
Sediment	110	0.03	
Biota	270	0.07	
Total (B)	231000	61.31	
Total load in the environment (A+B)	374000	100	31
Degraded and incinerated	43000		4
Land-stocked[a]	783000		65
Word production	1200000		100

[a]Still in use in electrical equipment and other products, and deposited in landfills and dumps.
Source: Tanabe, S. *Environ. Pollut.*, 1988, **50**, 5.

1.1.3.2. Environmental levels of PCBs in the UK

PCBs have been measured in many environmental matrices in the UK including air, water, soil, vegetation and human tissues. The contemporary flux of ΣPCBs to the UK surface has been estimated as being 17.5 tonnes per annum, compared with an estimated annual flux to the atmosphere of between 2.2 and 5 tonnes.[69] Primary emissions to the atmosphere have been calculated based on total PCB concentrations, whilst the deposition flux data have been calculated on a congener specific basis, and therefore the differences between PCBs emitted and deposited would be greater if the estimated deposition fluxes had been calculated on a total PCB flux basis. The emission estimates, however, ignore those PCBs present in the atmosphere which have revolatised from soils, which account for 88% of the ΣPCBs emitted.[57] After taking into account volatilisation from soils an estimated flux of between 33 and 42 tonnes will be emitted annually implying that the major sources of PCBs from the UK environment have been identified and that there is currently a net loss of these compounds away from the UK.

The analysis of dated sediment cores[75] and archived air filters, soils and vegetation[76-78] from the UK demonstrate that input of PCBs to the UK environment grew steadily following the onset of their commercial production, peaked in the early to mid-1960s, declined significantly following restrictions on their use in the 1970s and have since continued to decrease. Although PCB residues can be degraded selectively in soil and air they are amongst the more persistent and mobile of trace organics and they continue to be recycled through the UK environment by deposition and volatilisation between air, soil and water bodies.

Evidence of a decline in PCB concentrations in human tissues is not as clear due to the high persistence and potential for bioaccumulation. Even though the limited data available on PCB concentrations in human tissues (e.g. adipose tissue) does not suggest a significant decline in levels in recent years,[79] present day exposure to PCBs through foodstuffs is likely to be lower than in the past[80] as shown by a decline in the PCB concentration in other compartments of the UK environment. This is presumably because PCBs are very persistent in the fatty tissues where they are deposited and will therefore be slower to respond to the restrictions in PCB usage. Lower chlorinated PCBs have shorter half-lives in human tissues and are more readily degraded and metabolised from the body, whilst higher chlorinated congeners accumulate in the body throughout the life of the individual. For example, congener 28 has a half-life of about 3 years whilst congener 153 has a half-life of 27.5 years.[81] As a consequence, congener 28 has been predicted to reach a steady state in human tissues after 20 years (i.e. at 20 years of age the average UK individual will consume as much of congener 28 as is eliminated from the body), whilst congener 153 will accumulate in human tissues through out the lifetime of the individual.[80]

1.1.3.3. Accumulation of polychlorinated biphenyls in the environment and foods

Wildlife absorb polychlorinated biphenyls through injestion, inhalation of air and dermal contact. PCBs tend to accumulate in fats and oils because of their lipophilic nature and are biomagnified through the trophic levels.[74] The highest marine predator, dolphins, exhibited a bioaccumulation factor of (concentration of PCBs in organs to concentration of PCBs in water) as high as 10^7. Typical levels of polychlorinated biphenyls in the environmental compartments are given in Table 1.6.

Polychlorinated biphenyls may get into human bodies through occupational, accidental, or environmental (background) exposure. Background contamination is possible by inhalation of air and injestion of particles from air, dermal adsorption and food consumption.[58,59] Food is considered as the major root of polychlorinated biphenyls in humans.[58,59] Food can become contaminated with PCBs by three many routes, namely:

TABLE 1.6. Typical concentrations of PCBs in environmental compartments

Compartment	Typical concentrations
Air (rural)	0.05 ng/m^3
(urban)	1-5 ng/m^3
Surface soil	2-50 μg/kg
Vegetation	10 μg/kg
Water	2 ng/l
Man (adipose tissue)	1 mg/kg (wet weight)
(breast milk)	10 μg/l (wet weight)
Marine mammals (blubber)	5-50 mg/kg (wet weight)
Otter (fat)	5-200 mg/kg (wet weight)

Source: Jones, K.C., Burnett, V., Duarte-Davidson, R. and Waterhouse, K.S., *Chem. Brit.*, 1991, **27**, 435.

(i) Contamination of food or animal foodstuffs, directly (e.g. by an industrial accident like the Yusho poisoning in Japan).

(ii) Migration from packaging into food

(iii) Absorption from the environment by fish, birds, and other animals, vegetables and other plants through roots or air.

Levels of PCBs in daily products, particularly in cow's milk from the Soviet Union[82] and USA[83] and retail milk[60] and in the UK[84] have been reported in the literature. Extended surveys of polychlorinated biphenyls in food and human tissues from the UK were reported in 1983[58] and 1989.[59] In recent times PCBs have been identified in fruit and vegetables[85-88] and other plants.[89]

(a) Sources and uptake of PCBs into fruit and vegetables

(i) Sources

The environmental behaviour of PCBs, which is related to their physical and chemical characteristics, should be considered when assessing the possible sources of PCBs for uptake by fruit and vegetables. The vapour pressure of PCBs, which increases with decreasing congener chlorination level, makes it relatively easy for such compounds to reach the troposphere.[90] Atmospheric transport of PCBs has been reported to occur over considerable distances,[91] with air temperature (and hence vapour pressure) along the path of the air mass, being the major influence on ambient concentrations.

A high proportion of PCBs in ambient air occur in the vapour phase,[92] with little of the volatile material being absorbed by airborne particulate matter.[93,94] Since the less chlorinated PCBs are more volatile, significant amounts of these have been found in the vapour phase.[95-97] The distribution of PCBs between the vapour and particulate phases is likely to be an important factor in the uptake of such compounds by fruit and vegetables.

The extent of seasonal variability in ambient air concentrations of PCBs should be considered when assessing their possible sources. Such a trend is clearly apparent in published results from the survey of four UK urban areas,[98] with concentrations in urban air during July-September 1991 being typically at least 40% higher than those recorded during February or March of the same year.

(ii) Uptake routes

PCB accumulation in fruit and vegetables can occur by the following pathways: (1) root uptake and translocation to upper plant parts, (2) atmospheric deposition, both wet and dry, of contaminated particles onto exposed plant surfaces, (3) uptake of airborne vapours by aerial plant parts. Air-to-vegetation transfer of gaseous contaminants may be accomplished by either adherence to the leaf cuticle or by incorporation into plant tissue during respiration.[99] Vapour transfer of PCBs from soil to above ground parts of plants would be expected to occur to varying extents for all crops. For vegetables, such as courgettes which develop in contact with or in close proximity to the ground, it is possible that uptake of PCBs could also occur by direct adsorption from the soil and by splash-over of soil during heavy rain.[100-102]

The evidence currently available suggests that the adsorption or absorption of PCBs into plant routes and their subsequent translocation into other parts of the plant structure, is limited.[101,103,104-106] The hydrophobic nature of PCBs and their subsequent strong adsorption to soil particles renders them largely immobile and generally unavailable to plants. The transport of such contaminants from the roots to the above-ground parts of the plant to any substantial extent is thus considered unlikely.[107]

The relative significance of the various routes by which PCBs can be taken up in fruit and vegetables is likely to be influenced by a wide range of factors, and perhaps because of the difficulties of carrying out research in this area under field conditions, little data currently exist on the importance of different uptake pathways for PCBs. The complex nature of PCB congener mixtures further complicates these routes. Thus, there is a need for new methods for PCB determination in fruit and vegetables.

1.2. ANALYTICAL METHODS FOR THE DETERMINATION OF PCBs IN FRUIT, VEGETABLE AND SOIL SAMPLES

The analytical methods used in the determination of polychlorinated biphenyls in fruit, vegetable and soil samples generally include the following steps: extraction of the PCBs, isolation of the PCBs from the bulk of the co-extracted material, purification and determination by gas chromatography with electron capture or mass spectrometric detection.

1.2.1. Sample extraction

The aim of any extraction method is to separate the analyte of interest such as PCBs quantitatively, from the sample into a matrix which is more compatible with the rest of the analytical procedure.[14] PCBs are non-polar, semivolatile organics. Thus, they are highly soluble in non-polar solvents such as hexane and only slightly soluble in polar solvents such as water and acetonitrile. This property is advantageous for extraction from water containing samples, since the PCBs will readily partition from the water to most non-polar (i.e. immiscible) organic compounds. However, when the PCBs are dissolved in a non-polar matrix such as oil, the extraction process becomes much more difficult, if not impossible.

As with any partition scheme between two phases, PCB extraction relies on a favourable partition of the PCBs from the sample matrix into the extraction matrix. The more favourable the partition coefficient (see section 1.3.1), the higher the extraction efficiency. Efficiency is also improved by repetitious extractions. For most matrices, the partition coefficient is high and the traditional number of repetitions is 3.[14]

1.2.1.1. Liquid-solid partitioning

In this method, the PCBs are removed from the solid sample by partitioning with an organic solvent. The critical component of an extraction technique for fruit, vegetable or soil samples is the contact between the solvent and the matrix. This can be accomplished by physical shaking (e.g. manual shaking). Thorough mixing and maceration of chunks is an important pre-extraction step.

The general scheme used for extracting PCBs from fruit and vegetable samples is to make a homogenate with appropriate solvent prior to extraction.[85,86] Davies[85] homogenised a 100 g of fruit or vegetable mixed with 100 g of anhydrous Na_2SO_4, with 300 ml of dichloromethane for 10 minutes. The dichloromethane extract was then decanted through a 5 cm column of anhydrous Na_2SO_4 to remove extrained water. Jan and Adamic[86] washed fruit or vegetable samples and then chopped and blended the flesh in acetonitrile-hexane (3:1). The acetonitrile was removed by washing with water.

For soil samples the general scheme used for extraction is as follows: the soil sample is dried to constant weight, ground and sieved before being extracted with an appropriate solvent. Several workers[51-53,55,108,109] have used a scheme by which PCBs are soxhlet extracted with a suitable solvent or a solvent mixture. For example, hexane:acetone (41:59 v/v),[108,109] dichloromethane,[51,53] benzene:methanol (75:25, v/v),[55] and hexane:acetone (80:20, v/v)[52] have all been applied to the extraction of PCBs from soils. A number of workers have extracted PCBs from soil successfully by simply shaking sieved soil with organic solvents such as hexane:acetone.[110-112] Hansen et al.[113] and Fuoco et al.[114] extracted PCBs by homogenising soil with hexane:acetone

(1:1, v/v) using an ultrasonic water bath extractor. Other workers have extracted PCBs from soil using supercritical fluid extraction (SFE). Donnely et al.[115] extracted PCBs with the supercritical carbon dioxide at optimum extraction conditions. Yang et al.[116] used supercritical water at 50 atm instead of the usual supercritical carbon dioxide to extract PCBs from soil.

Bowadt and Johansson[117] and Morselli et al.[118] compared the extraction efficiencies for supercritical fluid extraction and soxhlet extraction. For soxhlet extraction,[117] soil was mixed with anhydrous sodium sulphate and extracted with 200 ml of hexane:acetone (2:3) or 250 ml hexane:acetone (4:1) or 250 ml hexane, whilst modified carbon dioxide with methanol was used for SFE. They concluded that SFE was better than soxhlet extraction because of the shorter time and less solvent required and also better precision obtained with this technique. Morselli et al.[118] soxhlet extracted soil spiked with PCB congeners using acetone:hexane (1:1) whilst SFE was performed with supercritical carbon dioxide. They found that the recovery of PCBs using SFE was higher than that obtained with soxhlet extraction.

1.2.2. Sample clean-up

The sample is cleaned-up to remove substances which interference with the determination of polychlorinated biphenyls. This is usually achieved by exploiting the difference in physical and chemical properties between PCBs and the unwanted constituents. The clean-up process may be expressed in terms of enrichment, where the ratio of PCBs to interferents is increased. In ideal circumstances, a clean-up will achieve 100% recovery of PCBs in one fraction with interfering compounds in other fractions.

The extent of the clean-up process depends on the requirements of the determination step.[14] A highly selective detector such as mass spectrometer requires less clean-up to remove other chlorinated organic compounds than would be required for electron capture detector (ECD).[14] On the other hand, low resolution electron impact mass spectrometry requires a more extensive clean-up to remove the matrix components before the determination of trace levels of PCBs.

1.2.2.1. Chemical degradation

Concentrated sulphuric acid oxidises or destroys many organic substances that may be present in the extract but does not affect PCBs. The sulphuric acid step usually involves a simple shaking of the sample extract with concentrated sulphuric acid[55,86,110-112] followed by washing with water. The acid treatment has been used in combination with chromatography over florisil[86,110,111] and porous graphitic carbon (PGC).[111] Alternatively, some workers[53,108,109,117] have used a column packed with sulphuric acid impregnated silica gel to remove bulk of the sample matrix from soil extracts. Stenhouse and Badsha[52] used a column packed with celite 545 impregnated sulphuric acid to clean soil extracts and Fuoco et al.[114] cleaned soil extracts with copper powder and mercury to remove sulphur.

1.2.2.2. Column chromatography on alumina, florisil, silica and silver nitrate

Adsorption column chromatography on alumina,[52,86,113] silica,[53] florisil,[108,109,110,114] and silver nitrate on silica[55] has been used to separate PCBs from other chlorinated compounds after bulk of organic substances have been removed by acid degradation,[52,53,55,86,108,109,110] or after sulphur[114] has been removed with mercury and copper powder in fruit and vegetable extracts[86] and soil.[52,53,55,86,108,109,110,114]

Alumina and florisil exhibit an excellent selectivity for planar aromatic molecules. Stenhouse and Badsha[52] separated PCBs in soil extracts from dioxins and furans on basic alumina column after a primary purification step on a mult-layer column packed with sodium sulphate, sulphuric acid/celite 545, sodium sulphate/sodium hydrogen carbonate, silica gel and eluted with hexane. Jan and Adamic[86] separated planar (mostly polychlorinated naphthalenes) components from PCBs in fruit and vegetable extracts on alumina and florisil with hexane. In a similar manner, Fuoco et al.[114] selectively eluted PCBs with n-hexane from other components on a florisil column. Creaser et al.[108,109,110] separated PCBs from dioxins and furans by eluting a florisil column with 2% DCM in hexane.

Polar compounds are more strongly retained on silica and usually a solvent such as hexane is used to remove PCBs, whilst more polar pesticides are retained on the column. Bracewell et al.[53] separated PCBs from other components on a combined silica/acid modified silica gel column with hexane whilst Ohsaki and Matsueda[55] separated non-*ortho*-PCBs from other compounds on silver nitrate/silica column with hexane.

1.2.2.3. Carbon adsorbents

Carbon column chromatography was first used by Smith[119] and Smith et al.[120] for the determination of polychlorinated dibenzofurans (PCDFs) and dioxins (PCDDs) in environmental samples. They used Amoco PX-21 activated carbon supported on glass fibres to isolate furans and dioxins from other components in salmon oil, fish and sediment extracts. This was achieved by eluting a column containing carbon supported on glass fibre with DCM:methanol:benzene (15:4:1) in the forward

direction to collect *ortho*-PCBs and other components. By eluting the column with toluene in the reverse-flow direction, PCDDs, PCDFs and non-*ortho*-PCBs, as well as other chemical classes such as polychlorinated naphthalenes (PCNs) polychlorinated biphenylenes and certain polynuclear aromatic hydrocarbons were recovered. Using a similar approach, carbon column chromatography[55,111,115,121-126] has been used in combination with other techniques (florisil, acid degradation, silver nitrate, silica, etc.) as a method for clean-up and fractionation of soil extracts and Aroclors.

Ohsaki and Matsueda[55] separated other PCBs and DDT analog from coplanar PCBs in soil, using carbon supported on silica with hexane:DCM (1:1). The coplanar PCBs were recovered separately from the column with toluene. Donnelly et al.[115] separated *ortho* from non-*ortho*-PCBs in soil and Aroclors on SK-4 carbon adsorbent by eluting the column with DCM:hexane (2:1) to collect di-*ortho* and some mono-*ortho*-PCBs. Inverting the column and eluting with toluene enabled non-*ortho* and some mono-*ortho*-PCBs to be recovered. Al-Haddad[111] managed to separate other PCBs from non-*ortho*-PCBs in soil and Aroclors on porous graphitic carbon column (PGC) using hexane as mobile phase.

Different types of carbon columns have been used in the separation of non-*ortho* from *ortho*-PCBs in Aroclors. Columns of activated carbon PX-21 dispersed on shredded polyurethane foam[121] have been used to separate non-*ortho*-PCBs. The *ortho*-PCBs were eluted from the columns in the first 60 ml of hexane:toluene whilst the non-*ortho*-PCBs were recovered in the 160-700 ml column eluates. Kannan et al.[122] separated non-*ortho* from *ortho*-PCBs on activated carbon column by eluting the column with hexane:DCM (80:20) to collect *ortho*-PCBs. The non-*ortho*-PCBs were

recovered from the column with benzene:ethyl acetate (1:1). Activated carbon AX-21 supported on silica gel[123,124] has also been used to separate non-*ortho*-PCBs from *ortho*-PCBs. Lazar et al.[123] succeeded in separating non-*ortho* from *ortho*-PCBs by eluting the column with hexane, DCM and then by inverting the column, the non-*ortho*-PCBs were recovered in toluene whilst Gardinali et al.[124] succeeded in obtaining di-, mono- and non-*ortho*-PCBs in separate fractions. The fraction containing *ortho*-PCBs was recovered with hexane:DCM (4:1), the mono-*ortho*-PCBs with DCM:toluene (9:1) and non-*ortho*-PCBs in toluene. Schwartz et al.[126] separated *ortho*, mono-*ortho* and non-*ortho*-PCBs on activated carbon dispersed on glass fibre in three fractions. The column was eluted with hexane:DCM (49:1) to collect bulk of *ortho*-PCBs, then with hexane:DCM (80:20) to recover mono- and di-*ortho*-PCBs. The non-*ortho* as well as mono-*ortho*-PCBs were collected in the third fraction with heptane. Kocan et al.[125] employed activated carbon AX-21 supported on Celite 545 for the separation of mono- and non-*ortho*-PCBs from *ortho*-PCBs. The *ortho*-PCBs were collected in the second fraction with cyclohexane:DCM:methanol (2:2:1) after discarding the first 4 ml fraction of the same solvent. The mono- and non-*ortho*-PCBs were recovered with toluene.

Different types of carbon columns and solvent systems have all been reported to effectively separate non-*ortho*-PCBs and dioxins and furans from *ortho*-PCBs and other planar interferences.[127]

1.2.2.4. Gel permeation chromatography

While chromatographic separation on e.g. alumina, Florisil and silica gel is based on

adsorption and partition phenomena, gel permeation or size-exclusion chromatography permits the separation of molecules due to their molecular size. Davies[85] separated PCBs from organochlorines and metabolites (chlordane, DDT, HCH, heptachlor, epoxide, dieldrin, endrin and methoxychlor) in fruit and vegetable sample extracts on ACB Autoprep automated gel permeating chromatograph.

1.2.3. Determination of PCBs

The determination of PCBs is carried out mainly by gas chromatography with electron capture detector (ECD) or mass spectrometric (MS) detection.

1.2.3.1. Gas chromatography separation

High resolution congener-specific analysis is usually the method of choice for the determination of PCBs in fruit, vegetable and soil samples. The most commonly used stationary phases for capillary column chromatography (section 1.3.1.1) are the non-polar 100% dimethylpolysiloxane (e.g. BP-1),[112] 5% phenyl-methylpolysiloxane (e.g. HP Ultra 2, DB-5, BP-5 etc),[51,52,113,115,117,118,128] 5% diphenyl, 94% dimethyl, 1% vinyl polysiloxane (SPB-5)[53,86,111] or the more polar 14% cyanopropylphenyl-methylpolysiloxane (OV-1701).[86] Alternatively, some researchers have used intermediate polarity phases such as 50%-phenyl-methylpolysiloxane (e.g. MPS-50, DB-17 etc).[55,116,117] Sometimes more than one columns have been used. Bowadt and Johansson[117] injected a soil extract on two parallel coupled columns, a diphenyl-dimethylpolysiloxane (similar to DB-17) and a series combination of 5% diphenyl-methylpolysiloxane (SIL-8) and a 1,7-dicar-*closo*-dodecarborane-dimethylpolysiloxane (HT-5). They also used two different columns, a bis (cyanopropyl) phenylpolysiloxane (SP-2331) and a 5% diphenyl-dimethylpolysiloxane

(DB-5) as well as a combination of 5% diphenyl-dimethylpolysiloxane and DB-dioxin (similar to DB-23 with phase 50% cyanopropyl-methylpolysiloxane) columns for soil extracts.

1.2.3.2. Detection

Sample components must be detected as they elute from the GC column. Mass spectrometric detection (section 1.3.2) with electron impact ionisation and selected ion monitoring has been widely used in the determination of PCBs in soil samples.[50,51,53,112,114,115,117] The advantage of the mass spectrometer is that it provides information on the molecular weight and the number of chlorine atoms in a PCB. This information significantly increases the likelyhood that the component has been correctly identified. The use of $^{13}C_{12}$ isotopes as internal standards (section 1.3.2.3) has been used to increase analytical confidence at very low levels of PCBs in environmental samples, especially for the non-*ortho*-PCBs , e.g. PCBs 77, 126 and 169. Other workers have preferred to use the electron capture detector (section 1.3.1.5) for the detection of PCBs in fruit and vegetable[85,86] samples and soil.[51;52,111,113-118,128]

1.2.3.3. Sample quantification

High resolution gas chromatography with capillary columns offers the advantage of separating a large number of polychlorinated biphenyls. Determination of a few selected congeners[50,51,90,86-88,111,115-117] facilitates the use of individual congeners as standards and makes identification certain and quantitation more precise.

1.3. CHROMATOGRAPHIC PRINCIPLES

1.3.1. Gas chromatography

Chromatography can be defined as a separation technique based on the different distribution of solutes between two phases, one which is fixed (stationary phase) and the other moving in one direction (mobile phase).

In gas-liquid chromatography, the stationary phase is a liquid that is stabilised on the surface of a solid support such as the internal wall of a fused silica capillary column by adsorption or chemical bonding. When a vapourised solute enters the column it is carried through by a chemically inert gas (mobile phase), and it immediately distributes itself between the stationary and mobile phases. The time taken for a component to elute from the column (retention time) depends on the relative affinity for the mobile and stationary phases. If the mobile phase flow is stopped at any time, the solute assumes an equilibrium distribution between the two phases. The concentration of the solute in each phase is given by the partition coefficient, K,

$$K = C_s/C_m \qquad 1.11$$

where C_s and C_m are the concentrations of the solute in the stationary and mobile phases, respectively.

1.3.1.1. Analytical columns

Column efficiency[129] can be expressed in terms of the number of theoretical plates, N, and the height equivalent to one theoretical plate, H, (HETP). The two parameters are related by the equation:

$$H = L/N \qquad 1.12$$

where L is the length of the column. The number of theoretical plates reflects the number of times the solute partitions between the two phases during its passage through the column and the height equivalent to one theoretical plate is the distance a solute travels while undergoing one partition.[130] As the number of theoretical plates becomes greater and as the plate height becomes smaller, the efficiency of chromatographic columns increases. The number of theoretical plates can be determined experimentally from the chromatogram using the equation:

$$\mathbf{N = 16(t_R/w_b)^2} \qquad \mathbf{1.13}$$

where t_R is the retention time of a solute and w_b is the peak width at base.

Resolution can be defined as a measure of the separation of two adjacent peaks (A and B) in a chromatogram.[131] Resolution may also be defined in terms of retention characteristics of the components, column efficiency (band broadening) and the selectivity or separating capabilities of a column by the equation 1.14. The retention characteristics are described by the retention factor (capacity factor), k', which is the capacity of a stationary phase to retain a component (equation 1.15), and the selectivity by the separation factor, α (equation 1.16).

$$\mathbf{Resolution = R_s = N^{0.5}(\alpha\text{-}1)\,(k')/4\alpha(k'+1)} \qquad \mathbf{1.14}$$

$$\text{where } k' = t'_R/\,t_M = (t_R - t_M)/\,t_M \qquad \mathbf{1.15}$$

$$\text{and } \alpha = k_B/k_A \qquad \mathbf{1.16}$$

where t_M is dead time; t_R and t'_R are retention time and corrected retention time, respectively; k_A and k_B are retention factors for components A and B, respectively.

Adjacent peaks are considered resolved if there is baseline separation, that is, the sum of their half widths is less than the separation between the peak maxima. The ratio of peak separation to half widths is therefore a measure of the resolution:

$$\mathbf{R_S = 2(t_{RB} - t_{RA})/(w_{bB} + w_{bA}) = 2\Delta t/(w_{bB} + w_{bA}) \approx \Delta t/w_{bB}} \qquad \mathbf{1.17}$$

Thus, for a satisfactory resolution the peak maxima separation, Δt, should be at least the width of the second peak.

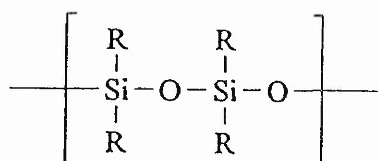
(a) Capillary columns

There are two main types of capillary columns: (a) packed columns containing solid support particles coated with stationary phase over the whole diameter of the column (micropacked) and (b) open tubular columns with an open and unrestricted flow path through the middle of the column.[130-133] The latter are divided into wall-coated open tubular (WCOT) coumns, support-coated open tubular (SCOT) columns and porous-layer open tubular (PLOT) columns. The inner surface of WCOT capillary column is coated with the stationary liquid phase.[132,133] In SCOT and PLOT columns, the inner surface of the capillary is coated with a layer of fine particle support material (such as diatomaceous earth), which in turn is coated with a thin film of stationary phase. The presence of the support material permits the column to carry several times as much stationary phase and hence large samples can be accommodated. Capillary columns

are fabricated from fused silica and offer the advantage of physical strength, lower reactivity and flexibility. Typical column dimensions are 10-50 m with internal diameters of 0.2-0.5 mm.

(b) Stationary phases

The function of the stationary liquid phase is to separate the sample components into discrete peaks. The stationary phase should have a reasonable chemical and thermal stability, and a reasonable column life over the operating temperature range. Polysiloxanes are the most important group of stationary phases for capillary columns.[131] The basic siloxane backbone can be represented by the following structure, in which R can be either methyl, vinyl, phenyl, 3,3,3-trifluoropropyl, cyanoethyl, or cyanopropyl groups.



Many polymers contain mixtures of the above functional groups (e.g. 5% phenyl 95% methyl polysiloxane, 50% phenyl 50% methyl polysiloxane, 25% cyanopropyl 25% phenyl 50% methyl polysiloxane etc.) and as a result stationary phases can cover a wide range of polarities. Phase polarity is very important since it determines the dissolving power of the stationary phase and consequently its ability to separate sample components. A non-polar stationary phase is best suited for analysis of non-polar compounds. For non-polar phases, the elution order for analytes of similar polarity usually correlates with boiling point. In modern capillary columns, stationary phases are polymerised *in situ* and bonded directly to the silica tube. Bond formation

occurs also within the stationary phase itself. Bonding the stationary phase to the inner surface of a capillary column permanently anchors the stationary phase on the surface. Bonded-phase columns are resistant to stripping (column bleed), and can even be back-flushed with liquid solvent without affecting the stationary phase film.

Table 1.7. Properties and characteristics of typical GC columns[a]

	Type of column			
	FSOT[b]	WCOT[c]	PLOT[d]	Packed
Length, m	10-100	10-100	10-100	1-6
Inside diameter, mm	0.1-0.5	0.25-0.75	0.5	2-4
Efficiency, plates/m	2000-4000	1000-4000	600-1200	500-1000
Sample size, ng	10-75	10-1000	10-1000	$10\text{-}10^6$
Relative pressure	Low	Low	Low	High
Relative speed	Fast	Fast	Fast	Low
Chemical inertness	Best———	———	———	—>Poorest
Flexible?	Yes	No	No	No

[a]From Skoog, D.A., West, D.M. and Holler, F.J., *Fundamentals of Analytical Chemistry*, 5th edn., Saunders, USA, 1988, p. 632.
[b]Fused-silica open tubular column
[c]Wall-coated open tubular column
[d]Porous-layer open tubular column (also called support-coated open tubular SCOT)

1.3.1.2. Carrier gas

The purpose of the carrier gas is to transport the sample through the column to the detector. Selecting the proper carrier gas is very important because it affects both column and detector performance. The mean mobile phase velocity (μ) is related to the column efficiency by the van Deemter equation, $H = A + B/\mu + C\mu$, where A results from the inhomogeneity of flow velocities and path lengths around packing particles, B defines the effect of longitudinal or axial diffusion, i.e random molecular motion within the mobile phase and C represents radial mass transfer resistance between adjacent stream lines of mobile phase. A van Deemter plot for the three most common carrier gases is shown in Figure 1.1. Virtually the same minimum plate height is achieved with each gas. The difference arises in the optimum linear velocity; although lower for nitrogen, the curves for helium and hydrogen are flatter. This means lower loss in efficiency when linear velocities higher than the optimum are used. The use of higher velocities can shorten the analysis time significantly. In this respect, hydrogen is the best choice followed by helium. The disadvantage of using hydrogen is the explosion hazard from leaks within the column oven.

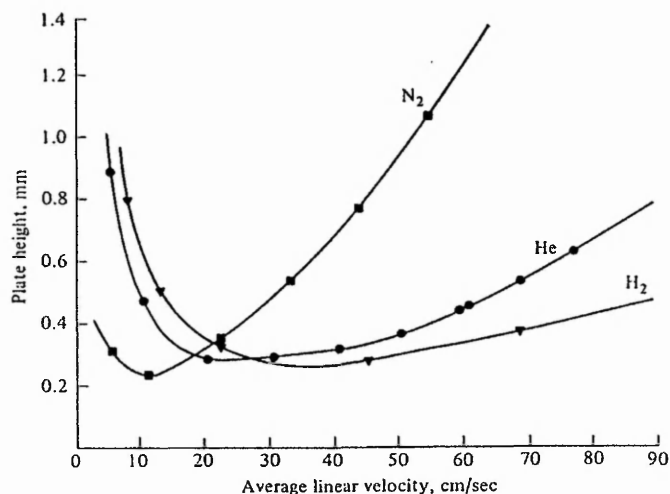


Figure 1.1. Variation of plate height with flow rate for three different carrier gases (from ref. 130)

1.3.1.3. Injection techniques

One of the qualities of chromatography is determined by the way the sample is introduced onto the column. The inlet system for gas chromatography must provide an efficient means of receiving the sample, instantaneously vapourising it, if not already a gas, and delivering the vapourised material as a discrete, narrow plug to the head of the column. The most important techniques for introducing the sample in capillary gas chromatography, are split, splitless and on-column injection.[134] Splitless and on-column injection are mostly used in analysing for compounds found at very low levels (e.g. PCBs in foods and the environment).

(a) Splitless injection

This injection system allows trace-level components to be determined. The entire sample, including the solvent (typically 1-2 μl), is injected into a vaporizer with the

vent line closed so that the sample is forced to enter the analytical column. The oven temperature is kept at 20-30 °C below the boiling point of the solvent and, as a result, the sample and solvent are condensed and focused in a small cold zone on the top of the column. The injector is subsequently vented to remove any residual sample and the column temperature is increased. Sample components are desorbed and released as a sharp band and the chromatography of the sample is commenced.

(b) On-column injection

In this system, the sample is deposited by a syringe into the column without prior heating or mixing with the carrier gas. Since, there is no contact of the sample vapour with a hot injector body, decomposition or discrimination of analytes due to thermal or catalytic effects is minimised. A narrow diameter needle is needed and the needle must fit inside the capillary column and therefore a special syringe is required.

1.3.1.4. Column Temperature

The temperature of the column is an important variable since the distribution of solutes between mobile and stationary phases is strongly temperature dependent. The optimum column temperature depends on the boiling point of the sample and the degree of separation required. It should be high enough to elute the compounds in a reasonable time without thermally decomposing the sample and without causing excessive column bleed, and low enough so that desired separation is achieved. For complex mixtures in which the sample components have a wide range of boiling points, temperature programming is widely used. A proper choice of the temperature program offers an increased separation of early eluting peaks, better detectability of

late eluting peaks (late eluting peaks become sharper and more easily measured) and shorter analysis time.

1.3.1.5. Detectors

A detector, located at the exit of the separation column, senses the presence of the individual components as they leave the column. The detector volume must be small to prevent the remixing of components separated on the column. Electron capture detector (ECD) and mass spectrometry are the most commonly used detectors in PCB analysis.

(a) Electron Capture Detector (ECD)

In the ECD the column effluent passes between two electrodes. One of the electrodes has on its surface a radioisotope (such as tritium or nickel-63) that emits high-energy electrons (beta particles) as it decays. These electrons bombard the carrier gas, such as nitrogen, resulting in the formation of a plasma of positive ions, radicals and thermal electrons by a series of elastic and inelastic collisions. The application of potential difference between the electrodes allows the collection of the thermal electrons that constitute the detector standing current or baseline signal when only carrier gas is passing through the detector. Molecules with high electron affinities, such as the PCBs capture the thermal electrons as they pass through the detector thus reducing the detector current generating an output signal proportional to the sample molecular concentration.

1.3.2. Mass spectrometry

In addition to the information obtained with an ECD, a mass spectrometer provides information on the molecular weight and the number of chlorine atoms in a PCB. The highly specific nature of mass spectrometry makes the technique a very selective gas chromatographic detector which is extremely useful for organic trace analysis.

Functionally, all mass spectrometers perform three basic tasks[130]:

(i) Creating gaseous ions and fragments from the sample

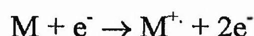
(ii) Sorting these ions according to mass to charge ratio

(iii) Measuring the relative abundance of ion fragments of each mass

The total effluent, for capillary gas chromatography-mass spectrometry, can be introduced to the ion source of the mass spectrometer. In this direct coupling interface, the end of the column passes into the ion source through a glass-lined stainless-steal capillary that is heated to the required temperature. Once inside the ion source sample molecules can be ionised by a number of different techniques.

1.3.2.1. Ionisation

The electron-impact ionisation (EI) technique is the most commonly used. Electrons produced from a heated filament are accelerated by an electric field through a chamber where they interact with vapourised sample molecules. The number of electrons is controlled by the filament temperature, whereas the energy of the electrons is controlled by the filament potential. Ions are formed by the exchange of energy during the collision of the electron beam and sample molecules.



$$M + e^- \rightarrow M^{+\cdot} + 2e^-$$

Where M is the sample molecule, e^- are high energy electrons emitted from the filament (normally 70 eV) and $M^{+\cdot}$ is the molecular ion (radical cation). If the molecular ion has sufficient internal energy following ionisation, fragment ions are formed. Operating with low energy electrons minimises fragmentation but drastically reduces ion yield. The ionised species are drawn out of the chamber, focused and injected into the mass analyser by a series of electrically charged plates.

1.3.2.2. Mass analysis

The function of a mass analyser is to separate the ions produced in the ion source according to their different mass to charge ratios. The most common forms of mass analysers used in GC-MS instruments are the single or double focusing magnetic sector and the quadrupole analysers.[135] Quadrupole devices were used exclusively in this work and are described in detail below. The most important parameter of a mass analyser is its resolving power. Two overlapping ion peaks of equal height, h, and of masses m and m+Δm are said to be resolved when the height, Δh, of the valley between them is 10% of the individual peak height. The resolution (called "10% valley resolution") is given by:

$$R = m/\Delta m \qquad 1.18$$

where m is the mass of the lighter peak An alternative definition (called "peak width at half height resolution") is based on the peak width measured at 50% peak height,[130]

where R = $m/w_{1/2}$. In general, spectrometers are classified as low resolution (R<2000), medium resolution (2000<6000) or higher resolution (R>6000).[131] Quadrupole devices fall into the low resolution category.

(a) Quadrupole mass analyser

The quadrupole mass analyser or mass filter consist of four short, parallel metal rods arranged symmetrically around the ion beam and placed between the ion source and the detector (Figure 1.2). The field within the analyser is created by coupling opposite pairs of rods together and applying radio frequency, V, and direct current, U, between the pairs of rods. This combination of electric forces constrains the ions to undergo complex trajectories along the rods.[136,137] At a selected U/V setting, only those ions with a certain m/z ratio have stable trajectories and are transmitted through the analyser, whilst ions of different m/z ratios are removed by collisions within one of the rods (Figure 1.2). A mass spectrum may be generated by scanning the values of U and V with a fixed U/V ratio at a constant radio frequency, or by scanning the radiofrequency and holding U and V constant. Commercial quadrupole analysers have a practical resolution limit of about 1000 (peak width at half height).

The quadrupole mass analyser has low inductance and relatively low capacitance and can therefore be scanned at high rates. The high scanning speed makes the quadrupole particularly suitable for coupling with a gas chromatograph. During operation in selected ion monitoring (SIM), the radiofrequency and direct current voltages are rapidly and sequentially switched between a number of values corresponding to masses of interest.

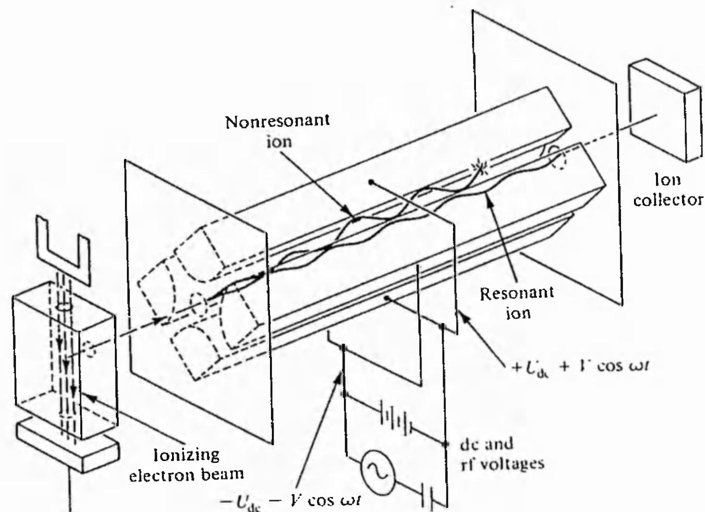


Figure 1.2. Schematic diagram of a quadrupole mass analyser (from ref. 130)

(b) Quadrupole Ion Trap Mass Spectrometer

The quadruple ion trap mass spectrometer (QITMS), is a mass filter where the quadrupole field is applied in three dimensions.[138] The trap, which consists of a central ring electrode insulated from two hyperbolic end caps positioned one on each side, is shown schematically in Figure 1.3. In contrast to conventional quadruples, the trap serves as both the ion source and the mass filter. Gaseous sample is introduced into the trap through a hole in the ring electrode, wherein ionisation is performed by electrons gated into the trap *via* one of the end caps. The geometry of the trap interior or ion volume is designed to provide a uniform field gradient, with the ring electrode radius (r_0) and the shortest distance between the trap center and the end caps (z_0) arranged such that $r_0^2 = 2z_0^2$.

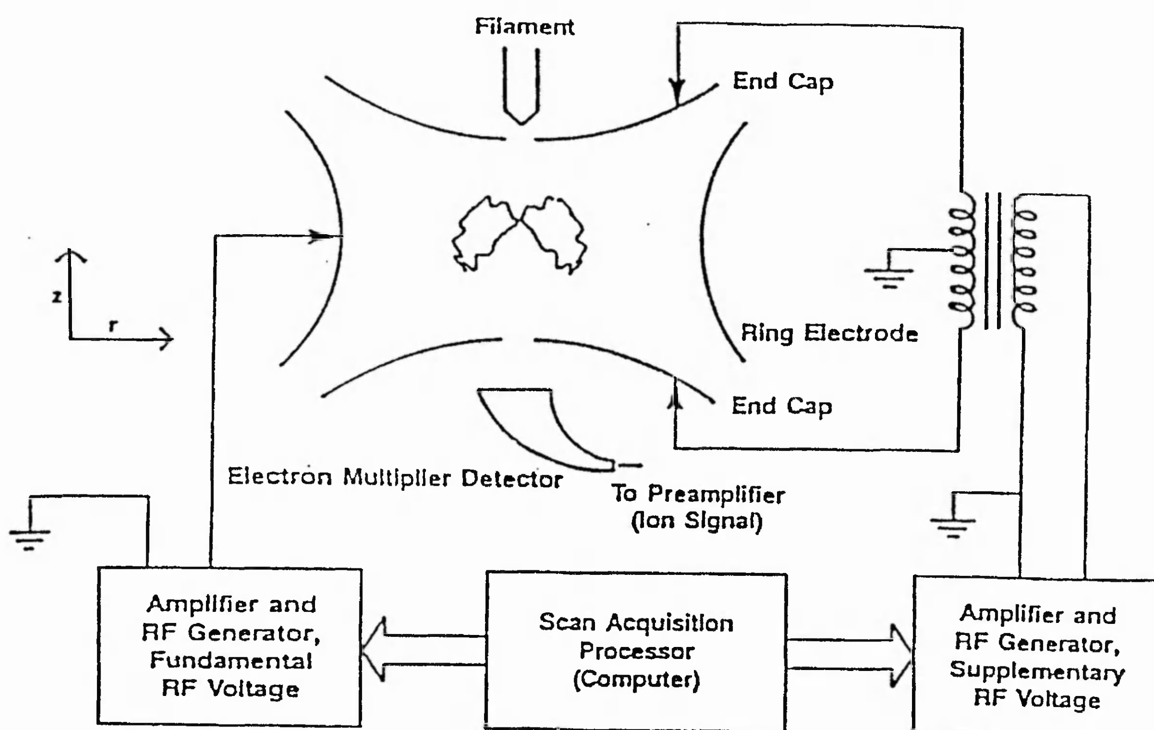


Figure 1.3. Schematic illustration of the ion trap assembly

The trap is operated using rf (V) or both rf (V) and dc (U) voltages, which can be varied to provide a rotationally symmetrical field in the space between the three electrodes, where the ions are trapped in a complex oscillating path. The voltages can be adjusted to trap ions of an individual m/z ratio, or over a m/z range. The stability of ions for given m/z ratios can be described in terms of a_z and q_z values, which relate the applied rf and dc voltages, to the ring electrode radius and the angular frequency of the rf defined as:

$$a_z = -16eU/m(r^2_o + 2z^2_o)\ \Omega^2 \qquad 1.19$$

$$q_z = 8eV/m(r^2_o + 2z^2_o)\ \Omega^2 \qquad 1.20$$

where U is the applied dc voltage, V is the applied rf voltage, r is the radius of the ring electrode, Ω is the angular frequency of the rf, m is mass and e is charge. If the rf is the only mode of operation, $a_z = 0$ and the position of the ion in the quadrupole stability diagram is given by the q_z parameter. Ions with q_z values over 0.91 are unstabilised and are ejected from the trap. Increasing rf amplitude (U) causes sequential destabilisation and ejection of the ions of increasing m/z ratios in the z direction as their q_z values reach 0.91. Half the ions become neutralised on one end cap, the rest leave through apertures in the other, and are detected and amplified by an electron multiplier. Compared to conventional quadrupoles, the pressure inside the trap for typical EI analysis is high at 10^{-3} torr. This is due to helium, which is introduced as a buffer gas to minimise ion scattering, keeping ions focussed in the trap before destabilisation. This improves both resolution and sensitivity.

1.3.2.3. Quantification in gas chromatography-mass spectrometry

Full mass scanning and selected ion monitoring are the two main methods to acquire mass spectral data for compounds introduced into the mass spectrometer *via* a gas chromatograph.[139,140] Full scan data is acquired when the spectrometer is set to scan the spectrum repetitively over the entire predefined mass range (e.g. m/z 50 to 600) allowing several complete spectra to be obtained for each chromatography peak. Selected ion monitoring (SIM)[140] is used to monitor the intensity of a small number of ions, which are characteristic of the compound being determined, by focusing the selected ions alternatively on to the detector for a fixed period of time. The advantage of full scanning is that it provides more analytical information (complete mass spectra) and consequently an increased confidence in the identification of

compounds. However, SIM can improve the sensitivity greatly because the detector records only the selected ions for the entire time the analyser is set to transmit those ions from the ion source to the detector (typically 50-100 ms per ion). The increased sensitivity of SIM is achieved at the expense of specificity when compared to full scanning mode. By carefully selection of the m/z values to be monitored, the use of at least two ions for each analyte and by careful checks on ion ratios, this disadvantage of SIM can be minimised.[139,141] SIM is usually the method of choice for trace organic analysis.

(a) Internal standards

The use of internal standards offers the highest precision in quantitative mass spectrometry. This is because any uncertainties introduced during mass spectrometric operation originating from variations in the source temperature and pressure, condition of the filament, etc. are controlled.[139] A known amount of internal standard is introduced into each sample and standard and the ratio of analyte peak response to internal standard peak response serves as the analytical parameter. Any unavoidable or accidental losses of sample (e.g. due to incomplete extraction or spillage) can be compensated for as long as internal standard and analyte to be quantified behave identically during extraction and clean-up. Internal standards should be added as early as possible during analysis and should structurally as similar as possible to the analyte. A stable isotope labelled analogue of the compound to be measured will have very similar physical and chemical properties to the unlabelled compound and will be distinguishable from the unlabelled compound by mass spectrometry because of the mass difference. Stable isotope internal standards are therefore analytically ideal

(although they have the disadvantage of high cost) and labelled internal standards based on $^{13}C_{12}$ analogy of the PCBs have been used in this work.

1.3.3. Introduction to high performance liquid chromatography

High performance liquid chromatography (HPLC) performs high efficiency separations and affords shorter analysis time when compared to low pressure liquid chromatographic techniques. HPLC is amenable to the analysis of non-volatile or thermally unstable analytes which cannot be analysed by GC, including ionic species, polar drugs, polysaccharides etc., as well as many volatile species. This technique consists of an injection system, a stationary phase contained within a column, a liquid mobile phase and a detection system. The stationary phase consists of tightly packed particles of 3 μm to 10 μm diameter. Pumps are usually of reciprocating piston type, syringe type or constant-pressure type. Compared to GC, HPLC separations are more strongly influenced by the mobile phase viscosity and the characteristics of the column packing.

Column efficiency is expressed as the reduced plate height, h, as obtained by Knox[142] given by:

$$\mathbf{h = B/\nu + A\nu^{0.33} + C\nu} \qquad \mathbf{1.21}$$

where h is the number of particle diameters responsible for one plate height and ν is the reduced velocity of the mobile phase, which relates the time taken to displace a solute molecule by a distance equivalent to one particle diameter. The relationships

described by this equation can similarly be demonstrated as a van Deemter-type graph, as shown previously.

1.3.3.1. Sample injection

The sample is introduced as a solution, usually in a solvent of the same composition as the mobile phase. The sample is typically 10-100 μl in volume with conventional 4.6 mm i.d. columns. A two-way switching valve is used to introduce the sample. In one position, the sample is introduced into a loop, and the other, mobile phase flushes out the loop and carries the sample to the column.

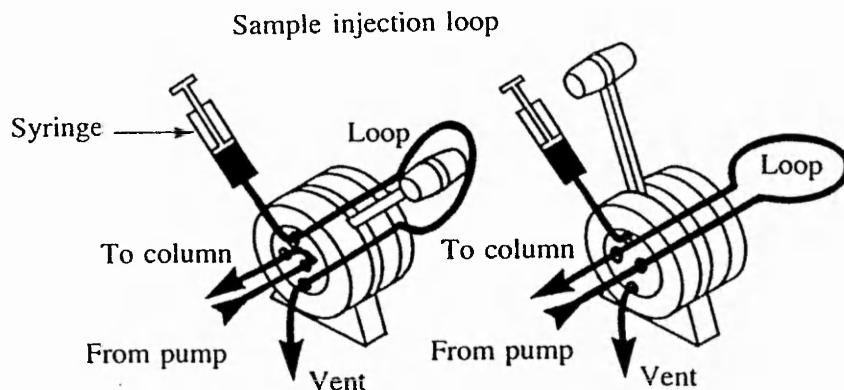


Figure 1.4. Schematic illustration of an HPLC sample injection loop (from ref. 130)

1.3.3.2. Analytical columns

A variety of columns are used, in HPLC, according to the nature of the sample. These include reverse phase, normal phase (adsorption and bonded phase), ion exchange (cation and anion) and size exclusion (gel permeation). Columns are constructed of heavy-wall, glass-lined metal tubing or stainless steel tubing to withstand high pressures and the chemical action of the mobile phase. The tubing can be between 1 mm and 4.6 mm i.d. and from 10-100 cm in length, packed under pressure with particles of the stationary phase bonded, or coated onto support. The stationary phase particles are usually spherical and typically 3-10 μm in size for analytical separations, with larger particles used for preparative work. Bonded and size exclusion phases have been used most widely for PCB determination.

In bonded phases, the phase is covalently bonded to silica particles and consist of an organic moiety covalently attached to silica with a siloxane type of bond (Si-O-Si-C). The reverse phase stationary phases are prepared by the attachment of hydrophobic groups, and include octadecasilyl (ODS), octyl (C_8), ethyl (C_2) and phenyl. Other side chains can be attached to render different selectivities, such as cyanopropyl and diol to give polar bonded phases for normal phase separations. Amino (NH_2) group side chains give a column for both normal and reversed phase separation, and weak cation exchange.

(a) Porous graphitic carbon

Porous graphitic carbon (PGC) is a chromatographic material[143] consisting of porous carbon spheres whose size can, in principle, be chosen from a few micrometers to a

few hundred micrometers. Because of its strength and ability to withstand the high-pressure gradients used in HPLC and in slurry packing procedures, PGC is the only carbon that can be used as a packing material for HPLC. The efficiency of PGC is comparable to that obtained with bonded phase silica gel for many compounds such as methylbenzenes, phenols, ethers, monosubstituted benzenes amines and acids using normal or reverse phase elution. The ability of PGC to separate aromatics on the basis of molecular planarity provides a high efficient and straight-forward method for the direct isolation of non-*ortho*-PCBs from other PCBs.[109,111]

(b) Size exclusion chromatography

With size exclusion chromatography (SEC), also called gel permeation chromatography, the accessibility of solutes to the interior of a porous stationary phase packing, is the basis for the separation according to molecular size/shape.

As the sample passes through the column, the solute molecules are sorted out. Very large molecules cannot penetrate into the open regions of the packing and hence are excluded. They travel mostly around the exterior of the packing and elute at the bed void volume of the mobile phase. Very small molecules diffuse into all or many of the pores accessible to them and with a larger column volume at their disposal, small molecules exit the column last. Intermediate size molecules can penetrate some passages but not others and, consequently, suffer retardations in their progress down the column and exit at intermediate times.

1.3.3.3. Mobile phases

High polarity solvents such as methanol/water and acetonitrile/water mixtures are used in reverse phase. The composition is optimised to achieve the separation either by eluting with a fixed concentration throughout (isocratic) or by operating a solvent gradient. With normal phase separations, using silica, PGC or bonded stationary phases, non-polar solvents such as hydrocarbons (e.g. hexane) are used. Mobile phases for exclusion chromatography are simple in that a single solvent can be used, since it is the size and shape of the analyte molecules which governs the retention.

1.3.4. Multidimensional separations

The ability of a chromatographic column to separate a complex mixture into its component is defined by the peak capacity, n_c. This value or quantity describes the number of peaks may be fitted side by side into the separation area of a chromatogram at a given analytical resolution[144,145] and it is the quantity which defines the advantages of multidimensional systems over the unidimensional separations. The quantity, n_c is a measure of the maximum number of peaks which may fit between retention times t_{R1} and t_{R2} at a minimum spacing x_o, i.e. a minimun distance between two peaks which still allows two components to be identified as separate peaks where peak width remains constant for a single chromatographic process.[146]

$$n_c = (t_{R2} - t_{R1})/x_o \qquad 1.22$$

The equation holds over any given interval of the chromatogram, provided the component density is consistent. When this is true over the whole chromatogram, then:

$$n_c = (t_{Rmax} - t_{R0})/x_o \qquad 1.23$$

where t_{Ro} is the retention time for an unretained component and t_{Rmax} is the maximum practical retention time. In the isocratic or isothermal mode, peak widths gradually increase with increasing retention time, and n_c is given by:

$$n_c = (N^{0.5}/4Rs)\ln(t_{R2}/t_{R1}) \qquad 1.24$$

(where N is the theoretical plate number) and for the entire chromatogram this gives:

$$n_c = (N^{0.5}/4Rs)\ln(t_{Rmax}/t_{R0}) \qquad 1.25$$

This can be written as,

$$n_c = \theta N^{0.5} \qquad 1.26$$

Thus for a single chromatographic process the peak capacity, i.e. the number of components that can be separated in a mixture is proportional to the square root of the number of theoretical plates in the analytical column.

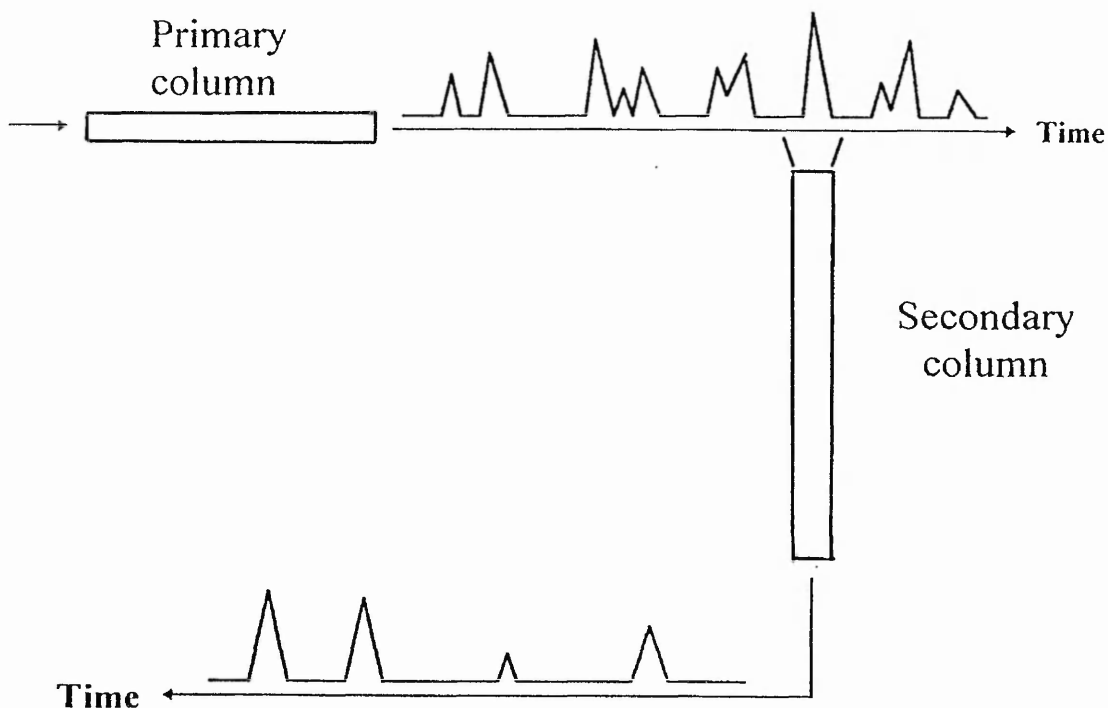


Figure 1.5. Illustration of a coupled column separation in which cuts taken from the effluent of the primary column are routed to a secondary column.

In multidimensional separations (Figure 1.5), components are separated on a primary column and then selected 'fractions' of the effluent from this column are fed into a secondary column (and, if desired, a tertiary column etc.). Components which coelute from the first column, with a particular retention mechanism, due to their retention properties being too similar, may therefore be separated on a second column by a different retention property.

Such multidimensional systems have the additional advantage over unidimensional separations because components which are not included in a fraction sent to the secondary column, cannot merge together again and coelute with those that are transferred.[147] The total peak capacity (n_{cc}) of a coupled column system has been

calculated as the sum of the individual peak capacities (n_i) of the individual columns, given by.[148]

$$n_{cc} \approx \Sigma n_i = n_c \text{ x number of columns} \qquad 1.27$$

where n_c is the mean peak capacity. It has been shown that if the interactions that determine the separation on the first column are totally independent from those of the second, then the total peak capacity is equal to the product of the individual capacities,[147] i.e.

$$n_{cc} = n_{c1} \text{ x } n_{c2} \text{ x } n_{c3} \text{........etc} \qquad 1.28$$

Therefore, if each separation mode has approximately the same peak capacity, then for j separations:

$$n_{cc} = n_c^j \qquad 1.29$$

Sagliano et al.[149] point out that the above relationship is very difficult to achieve with coupled column systems because of secondary effects which influence any chromatographic separation. If there is complete redundancy throughout the separation, then

$$n_{cc} = ((jN)^{0.5}/4R_s)\ln j(t_{Rmax}/t_{Rm}) \qquad 1.30$$

which approximates to

$$n_{cc} = j^{0.5} n_c \qquad 1.31$$

which basically describes the same relationship as equation 1.26 and illustrates that, for an for an equivalent number of theoretical plates to a single chromatographic column, a coupled multidimensional chromatographic system with columns having the same retention mechanism can separate $j^{0.5}$ more components in the available separation space of the chromatogram. However in practice, the capacity of the system falls between the extremes implied by equations 1.29 and 1.31. The total quantity of peak information produced, is therefore much less than the peak capacity the system is capable of delivering, but this in no way diminishes the power that the multidimensional approach affords; and because of ease with which a flowing coupled system lends itself to automation, it makes such approaches very attractive to the analyst involved in trace determination of substances in complex matrices.

1.3.4.1. On-line HPLC-GC: Methodology and literature review

Substantial sample preparation is frequently necessary, before a sample can be introduced into an instrument such as a gas chromatograph or a mass spectrometer for quantitative analysis. This is particularly true for trace analysis of environmental samples and foodstuffs where ppm or ppt detection limits may be required. The preliminary off-line clean-up steps employed, such as solid phase extraction may be expensive and time consuming. Therefore it is useful to be able to automate the process as far as possible. This can be done by development of an efficient on-line procedure. An example is the interfacing of HPLC with GC (HPLC-GC or LC-GC). this technique utilises HPLC to perform a selective preliminary clean-up, before a fraction containing the analyte(s) of interest is diverted to the GC for further selective

separation and quantification. Only a crude preliminary extraction is necessary. HPLC-GC has been the subject of many review articles and papers.[149-153]

(a) Interfacing of HPLC with GC

Two types of interfaces have usually been used for HPLC-GC, the on-column interface[151,154] (Figure 1.6) and the loop-type interface[151,155-157] (Figure 1.7). The interface should transfer the fraction of interest from the HPLC procedure to the GC system without any losses or contamination. It should also remove the bulk of the eluent and effect the refocussing of transferred compounds to obtain sharp peaks in the GC chromatogram. These demands become more important as the volume that is transferred to the GC becomes larger.

(i) On-column interface

The on-column interface incorporates an HPLC switching valve (Figure 1.6). In one position the column effluent is diverted to waste, and in the other the effluent is transferred to the GC column by the HPLC pump. The sample size is determined by the duration for which the valve is switched to the GC and the effluent flow rate. In both cases, the LC fraction is usually introduced into a pre-column or retention gap which is butt connected to the GC column inside the GC oven. This provides a reservoir into which the LC fraction can pass. The pre-column is usually a length of uncoated deactivated fused silica tubing, or a coated section may also be used.[158] The analytes within the sample fraction transferred to the GC can be focused at the head of the analytical GC column by a number of mechanisms which are described later.

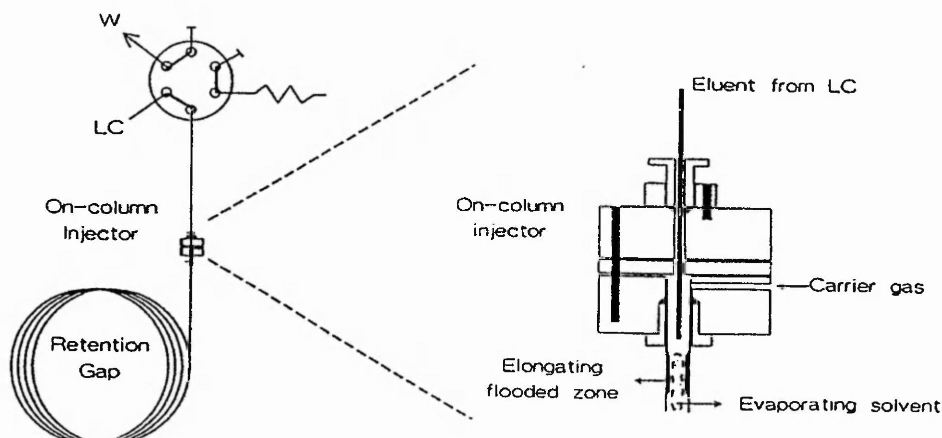


Figure 1.6. Scheme of the on-column interface, which comprises a switching valve, an on-column injector and a retention gap.

The length of the pre-column which becomes flooded during eluent introduction depends upon the wettability of the deactivated surface to the solvent used and the temperature.[159] Methyl-deactivated surfaces provide a suitably wettable surface for very non-polar solvents such as hexane, but a more polar surface is more suitable for more polar solvents.[159-161] Noy et al.[158] developed a system which incorporated a pre-column downstream of an in-line vapouriser within the GC oven which enabled the sample to be volatalised whilst maintaining the GC oven cool, allowing focusing of the analytes by cold trapping. Alternatively, instead of using a pre-column, a small glass reservoir (on-line concentrator) or packed glass tube, such as that used in a programmed-temperature vapourising (PTV) inlet may be used.[162-164] Large sample volumes (up to 100 μl or more) are slowly injected into a PTV inlet under cold conditions with the vent open. The solvent is evaporated, leaving non-volatile solutes

behind. The vent is then closed and the inlet heated sufficiently to vapourise the analytes, which pass into the column and are analysed.

(ii) Loop-type interface

The loop-type interface incorporates an HPLC switching valve fitted with a loop through which the effluent from the column passes. At the appropriate time the valve is switched, and GC carrier gas pressure conveys the sample into the GC. A modified loop-type interface has been reported which incorporated two loops,[165] such that two separate LC fractions could be collected and delivered to the GC.

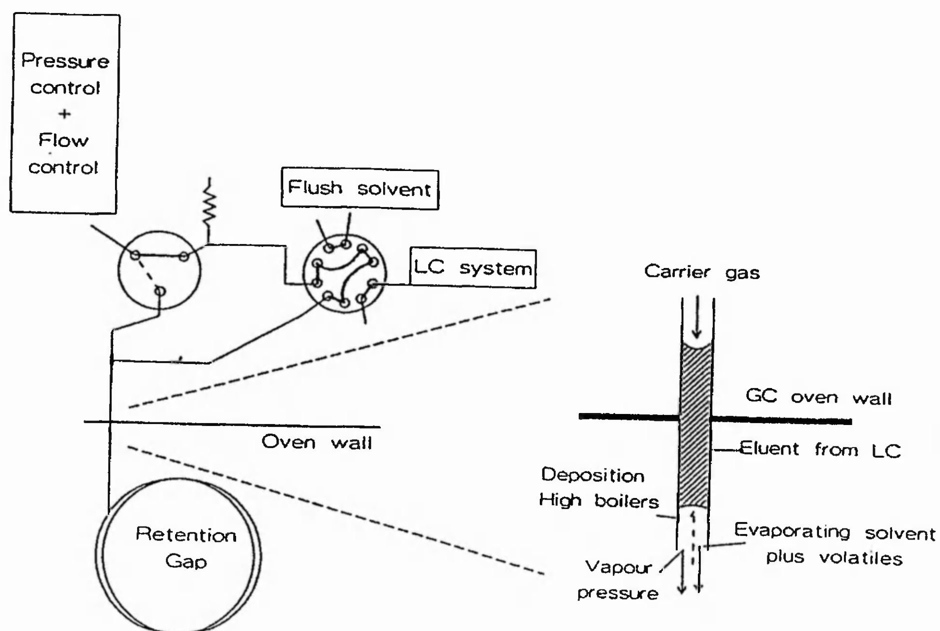


Figure 1.7. Scheme of the loop-type interface, which consists of two valves, flow control system and a retention gap.

(b) Mobile phase removal

When interfacing HPLC with GC, the removal of solvent vapour is a major concern. If the detector is able to tolerate the sample volume then the solvent vapour can be allowed to pass through the GC column. But if not, then there is need to get rid of the solvent without losing the analytes of interest. Therefore, a vent or early vapour exit (EVE) may be positioned upstream of the GC column,[159,166-168] to enable most of the solvent to be diverted way from the analytical column.

Depending on the analytes concerned, the solvent in the HPLC-GC system can be removed in one of the three ways.

(i) Fully concurrent evaporation

Fully concurrent evaporation[156,169] involves the introduction of the sample with the oven temperature above that of the boiling point of the solvent at the inlet pressure used, to allow immediate vapourisation on entry. This mode of evaporation is usually used with the loop-type interface or with less volatile analytes using an on-column interface.

(ii) Partially concurrent evaporation

Partially concurrent evaporation[168-171] involves the introduction of the sample at the temperature slightly below that of the adjusted solvent boiling point. This mode of operation allows the removal of some solvent during introduction to reduce analysis time, but leaves some behind to perform solvent focusing.

An alternative method involves the conventional retention gap technique,[154,172] where the pre-column is of sufficient volume to hold the complete sample without any appreciable evaporation. Sample introduction is performed below the boiling point of the solvent at the inlet pressure used, and analyte focusing is achieved by solvent effects.[173]

(c) Analyte focusing

Focusing of the analyte(s) can be achieved by solvent effects,[152,153] phase soaking and phase-ratio-focusing effects[174,175] (Figure 1.8) and cold trapping, depending on the volatility of the analytes concerned and the mode of solvent evaporation.

During solvent effect focusing, the sample is introduced into the retention gap (uncoated pre-column, from 1-50 m long) as a plug, which then spreads along the walls as a film. Solvent from the rear of the film evaporates such that the rear boundary of the film advances along the retention gap. Involatile analytes become deposited along the pre-column walls behind the evaporating film. Low boiling solutes become volatilised as solvent evaporates, but become trapped in the rear of the solvent film once more as they begin to advance along the retention gap and become concentrated as a tight band with the last portion of solvent. This type of focusing happens when using the retention gap solvent removal. The same process occurs in partially concurrent evaporation since some sample is left as a film on the walls of the pre-column so that when the delivery from the LC is stopped, solvent trapping occurs as described above. The phase soaking effect on the other hand takes place in the coated column just ahead of the flooded zone. In this region, the solvent

from the sample introduced saturates the stationary phase and in doing so retards the migration solutes allowing full reconcentration.

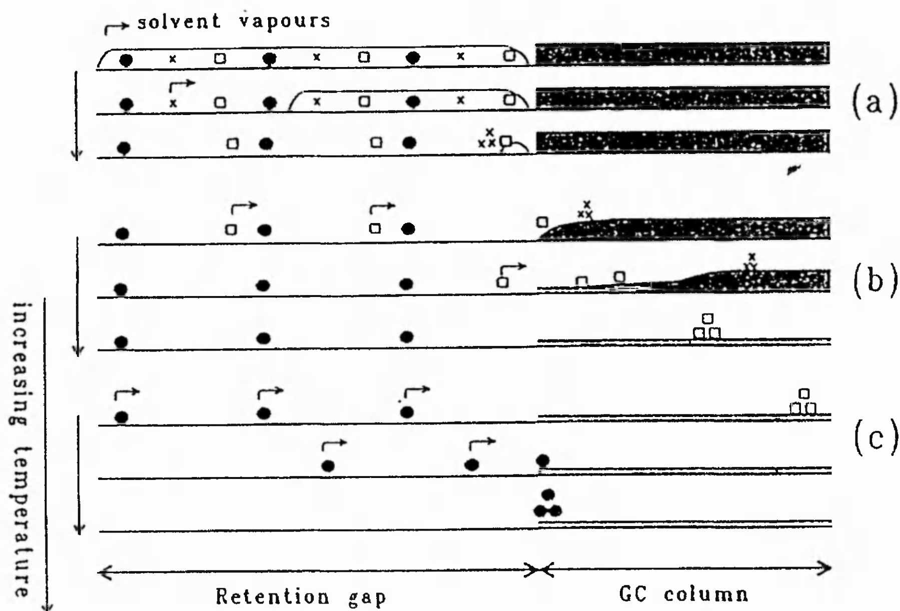


Figure 1.8. Reconcentration of volatile and non-volatile sample constituents by (a) the solvent effect, (b) the phase soaking effect and (c) the phase-ratio-focusing effect: X, volatiles; □, less volatiles; •, low volatility. Part (b) represents the situation immediately after analyte transfer from the LC column to the retention gap has been completed (the changing thickness of the gray-shaded stationary phase indicates that it has been swollen by the solvent, which is evaporating from the left to the right).

Phase ratio focusing is responsible for focusing high boiling solutes. The film of liquid in the retention gap causes less volatile compounds to remain spread over the retention gap wall. The difference in the migration speed in the retention gap and in the coated GC column leeds to reconcentration of the analyte. As soon as the front end of the zone reaches the stationary phase, its migration speed is greatly reduced, while the remaining part of the zone, which is still in the retention gap, continues to migrate at the initial, higher speed and catches up with the front zone. Alternatively

focusing may be achieved through internal cold trapping.[152,153] This is because the solute may be retained longer at a lower temperature in the cold trap than is possible in the coated column at the temperature required to transfer the solute from the pre-column.

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CHAPTER 2

Method development for the concurrent congener specific determination of *ortho* and non-*ortho* polychlorinated biphenyls in fruit and vegetable samples

2.1. INTRODUCTION

The non-*ortho* or planar PCB congeners are usually present in lower concentrations than other PCB congeners in the environment and food[1-10] although their contribution to the total-toxicity of PCB burden is high.[11-14] The determination of these PCBs is therefore crucial for environmental and food studies, but their analysis is usually complicated by two factors: (i) the relative low abundance of the toxic congeners compared to other PCBs and, (ii) unwanted interferences are usually present at much higher concentration in the samples than these analytes and are, in most cases, capable of completely shielding the signal from the analytes or giving false positive results on the analytical system used.[15,16] To solve these problems, it is necessary to establish analytical methods for better extraction, clean-up and to fractionate and quantify these *ortho-* and non-*ortho*-PCBs separately.

There has been considerable interest in developing analytical schemes to obtain data on selected PCBs, dioxins and furans from a single sample. For biological samples, the method of Smith, Staling and Johnson[17] is often used for dioxin and furan determination. This procedure has been automated[18,19] and equipment is commercially available.

Methods for the fractionation of non-*ortho*-PCBs in different matrices have been reviewed[4] and include chromatography on florisil.[10,20-22] basic alumina,[23-25] carbon[17,26-34] and silica gel.[1] The aim of this work was to develop a method for the congener specific determination of selected *ortho-* and non-*ortho*-polychlorinated biphenyls in fruit and vegetable sample matrices using an on-line clean-up procedure based on

Smith et al.[17] methodology and gas chromatography-mass spectrometry (GC-MS) analysis.

2.2. EXPERIMENTAL

2.2.1 PCB standards and other chemicals

All the native $^{12}C_{12}$ PCB standards in iso-octane or n-hexane were obtained either from Accu Standard Inc. (25 Science Park, New Haven, USA), Greyhould Chemical LTD (UK), National Research Council (Canada), or Laboratory of the Government Chemist (Teddington, Middlesex, UK).

Internal quantification standards: Isotopically labelled standards in iso-octane were obtained from Cambridge Isotope Laboratories (Woburn, Massachusetts, USA).

PCB windowdefining mixture: Aroclor mixture 1242:1254:1260 (1:1:1) was obtained from Promochem Ltd, UK., as oils.

Other reagents were obtained as follows:

Hexane (distol grade, Fisons Scientific Equipment, Loughborough, UK).

Dichloromethane (distol grade, Fisons Scientific Equipment, Loughborough, UK).

Toluene (distol grade, Fisons Scientific Equipment, Loughborough, UK).

Acetone (distol grade, Fisons Scientific Equipment, Loughborough, UK)

Methanol (distol grade, Fisons Scientific Equipment, Loughborough, UK)

Water (distilled)

Anhydrous sodium sulphate (AR, Fisons Scientific Equipment, Loughborough, UK): Washed with hexane, dried at 250 °C for 16 hours and stored at 130 °C in an oven.

Sodium hydrogen carbonate (AR, Fisons Scientific Equipment, Loughborough, UK): Washed with hexane and stored at 130 °C.

Silica gel 60 (Merck 7734, 70-230 mesh, UK): Washed with hexane and activated at 130 °C and stored in a tightly closed container.

Concentrated sulphuric acid (AR, specific gravity 1.84, Fisons Scientific Equipment, Loughborough, UK).

Sulphuric acid impregnated silica gel: Two parts of concentrated sulphuric acid by weight were added to three parts of activated silica gel in a conical flask and mixed by shaking until a free flowing sample was obtained.

Glass wool: Washed with hexane, dried and stored at 130 °C.

Amoco PX-21 active carbon (Amoco Research Corporation, UK)

Potassium hydroxide pellets (AnalaR grade, Fisons Scientific Equipment, Loughborough, UK)

Potassium silicate was prepared as follows[17]: Potassium hydroxide pellets (168 g) were dissolved in methanol (600 ml) in a one litre round bottomed flask. Activated silica gel (300 g) was added carefully and mixed at 55 °C for 90 minutes using a rotary evaporator (without applying vacuum). The mixture was then transferred to a glass column fitted with a sintered glass filter and washed with methanol (700 ml) and dichloromethane, DCM (500 ml). The washed potassium silicate was dried by purified air flushing and activated at 130 °C overnight. This was stored in a tightly closed conical flask.

All glassware was washed thoroughly with Decon 90 detergent, rinsed with distilled water, acetone and finally with hexane, prior to use.

2.2.2. Equipment

Rotary evaporator: Solvent evaporation for large sample volumes (>100 ml) was performed on a Buchi Rotary evaporator RE-121 fitted with a Buchi Waterbath 461 (Buchi, Switzerland). The water bath temperature was maintained at 40 °C and 60 °C and solvent evaporation was accomplished under reduced pressure. Between each sample the vapour duct unit was rinsed with hexane.

Vials were obtained from Chromacol Ltd, UK. Tapered screw top vials (1.1-STVG type) were used. Silicone/PTFE septa were used for sealing the vials.

2.2.3. Solvent reservoir and clean-up column

A glass column widened at the top served both as solvent reservoir and clean-up column. The top end (solvent reservoir) had dimensions of 100 mm x 70 mm i.d and

was adapted with a socket joint so that purified air could be used to force solvent through the system. The lower end (clean-up column) was constructed from a 250 mm x 25 mm i.d. glass tubing joined at the lower end to a 25 mm i.d. screw thread fitting and teflon end-piece (Omnifit). The clean-up column was packed from the bottom with:

- Glass wool
- 2 cm of anhydrous sodium sulphate
- 3 cm of potassium silicate
- 5 cm of silica gel
- 1 cm of sodium hydrogen carbonate
- 5 cm of 60:40 silica gel: sulphuric acid mixture
- 2 cm of anhydrous sodium sulphate

Prior to addition of the sample extract, the column was washed with 100 ml of hexane:DCM (80:20, v/v). The column was repacked for each sample.

Two solvent reservoirs were separately constructed from 100 mm x 70 mm i.d. glass tubings joined at the lower ends to 25 mm i.d. screw thread fittings (Omnifit) and teflon end-pieces. Their top ends were similarly adapted with large socket joints.

2.2.4. Carbon/glass fibre column preparation[35]

Amoco PX-21 active carbon was dried at 110 °C overnight, transferred into a beaker and a small amount of dichloromethane (DCM) just covering the carbon was added. The mixture was stirred gently and allowed to settle. Fine particles that remained in suspension were decanted into a beaker and the solvent was allowed to evaporate.

This was repeated until 50 mg of carbon was collected. Glass fibre filter paper (700 mg, Whatman GF/D) was cut into small square pieces of approximately 3 mm x 3 mm and homogenised in DCM for 15-30 seconds using an Ultra Turrax homogeniser. The carbon was then added and the mixture was thoroughly stirred with a glass rod until the carbon was uniformly distributed. The slurry was packed into a 100 mm x 10 mm standard glass column (Omnifit) between glass fibre discs (15-20 on each side), to give a total bed length of approximately 40 mm. During packing, the slurry was compressed firmly with a glass rod to remove air spaces. The column was washed with DCM and toluene prior to use.

After the fractionation of each sample extract or PCB standard mixture the column was regenerated by washing with:

- 100 ml toluene (reverse flow)
- 100 ml methanol (reverse flow)
- 100 ml hexane:DCM (80:20, v/v)(reverse flow)

The columns were connected according to Figure 2.1. The solvent flow switching valves were Hamilton miniature inert valves with 5 port housing. All components used to assemble and connect the columns together such as Teflon endpieces, flangeless gripper fittings, 1/4-28 thread tube end fittings, 1/4-28 thread compression nuts and Teflon tubing (1/16 OD) were purchased from Omnifit. An inert pressure regulator unit (Omnifit) was used for regulating the purified compressed air pressure at 0-50 psi.

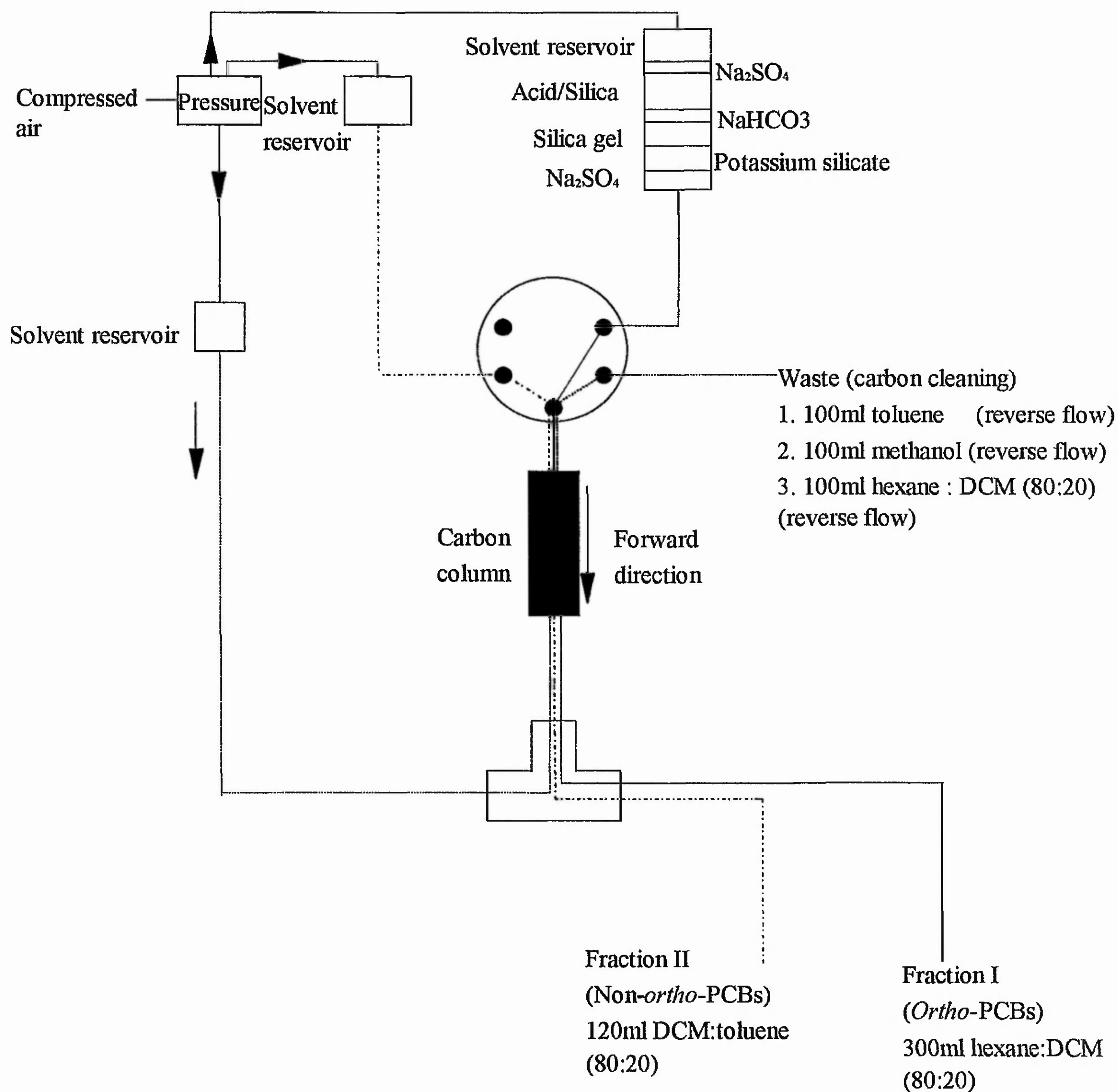

Figure 2.1. Schematic diagram for clean-up and separation of *ortho* and non-*ortho*-PCBs in fruit and vegetable samples

2.2.5. Method development

2.2.5.1. GC-MS validation

(a) Aroclor 1242:1254:1260 (1:1:1) mixture for PCB window definition

Aroclor PCB standard mixture (151 ng μl^{-1}, 1242:1254:1260) was run on GC-MS in selected ion monitoring by injecting 2 µl into the GC (see section 2.2.5.1 (d) for GC-MS conditions).

(b) NTU-SMS0018 PCB standard mixture

A standard mixture solution, NTU-SMS0018 containing PCBs (PCBs-28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156,157, 167, 169, 180 and 189) at *ca* 1.5 ng μl^{-1} each was spiked with an internal standard solution containing $^{13}C_{12}$-PCBs (PCBs-28, 52, 77, 101, 126 138, 169 and 180) at 2 ng μl^{-1} each. Sample aliquots were analysed by GC-MS in selected ion monitoring (SIM) mode by injecting 2 µl into the GC. Unspiked aliquots of this solution were sealed in glass ampoules, weighed and dispatched to two other laboratories for analysis.

(c) *NTU-SMS0046 PCB standard mixture*

A PCB standard mixture, NTU-SMS0046 containing 41 *ortho*-PCBs (PCBs- 4, 18, 28, 31, 33, 41, 44, 47, 49, 51, 52, 60, 66, 74, 87, 99, 101, 105, 110, 114, 118, 123, 138, 141, 151, 153, 156, 157, 167, 180, 183, 185, 187, 189, 191, 193, 194, 201, 203, 206 and 209) and 5 non-*ortho*-PCBs (PCB-37, 77, 81, 126 and 169) at *ca* 50 pg μl^{-1} each was run on the GC-MS in selected ion monitoring (SIM) mode by injecting 2 µl into the GC.

(d) GC-MS conditions

Analysis was accomplished using an HP5971 mass spectrometer (Hewlett-Packard, Wilmington, Delaware, USA) operated in electron impact mode (EI) at 70 eV with selected ion monitoring (SIM), coupled to an HP5890 Series II gas chromatograph (Hewlett-Packard, Wilmington, Delaware, USA). The ions chosen for the selected ion monitoring (SIM) of the PCBs are listed in Table 2.1. The dwell time per ion was 80 msec and the number of cycles/sec was 0.882. The GC-MS interface was maintained at 280 °C. The GC separation was carried out on a DB-17 column (J&W Scientific Inc., 60 m x 0.2 mm i.d., 0.25 μm film thickness) operated either in splitless or on-column mode. The oven temperature was programmed as follows: 85 °C for 1 minute; ramp 15 °C min^{-1} to 200 °C, 4 min; ramp 2 °C min^{-1} to 250 °C; ramp 5 °C min^{-1} to 280 °C, 20 min. Helium was used as carrier gas at a flow rate of *ca* 1 ml min^{-1}.

Table 2.1. PCB Ions monitored.

Congener group	Ions monitored	
	$^{12}C_{12}$	$^{13}C_{12}$
Cl_3-PCB	256, 258	268, 270
Cl_4-PCB	290, 292	302, 304
Cl_5-PCB	326, 328	338, 340
Cl_6-PCB	360, 362	372, 374
Cl_7-PCB	394, 396	406, 408
Cl_8-PCB	428, 430	440, 442
Cl_9-PCB	464, 466	476, 478
Cl_{10}-PCB	498, 500	510, 512

2.2.5.2. Acceptance and quality control criteria

Acceptance criteria for the positive identification and quantification of polychlorinated biphenyls were similar to those applied to the analysis of dioxin and furans[36] and those reported by Kuehl et al.[37] for the determination of non-*ortho*-PCBs.

(a) Criteria for congener identification

(i) Simultaneous (+/-1s or +/-1scan) response for all relevant channels

(ii) Isotope ratio within +/-15% of theoretical value

(iii) Signal-to-noise ratio > 3:1

(iv) Identical retention time (+/-2s or +/-2scans) for analyte and matching internal

standard (where appropriate)

(v) Satisfactory demonstration of GC column performance

(b) Criteria for acceptance for quantification

(i) All identity confirmation criteria met

(ii) Recovery of internal quantification standard within 50 and 120%

(iii) Signal-to-noise ratio for internal quantification standard > 20:1

2.2.5.3. Quality control

(a) Maintenance of optimum GC-MS performance

To maintain column and detector performance, a standard solution, NTU-SMS0046 containing 41 *ortho*-PCBs (PCBs- 4, 18, 28, 31, 33, 41, 44, 47, 49, 51, 52, 60, 66, 74, 87, 99, 101, 105, 110, 114, 118, 123, 138, 141, 151, 153, 156, 157, 167, 180, 183, 185, 187, 189, 191, 193, 194, 201, 203, 206 and 209) and 5 non-*ortho*-PCBs (PCB-37, 77, 81, 126 and 169) at *ca* 50 pg μl^{-1}, was run once a day before analysis of the sample fractions. This was to check for the sensitivity of the MS and the resolution of the analytical. A method blank was conducted at the beginning of every batch of five samples and measured sample response to be significantly greater than that of the method blank.

2.2.5.4. Quantification

(a) Sample quantification

The relative response factor, R*, is first determined as follows.

$$R^* = Ws \times Ris/Wis \times Rs$$

where R* is the relative response factor

Ws is the mass (concentration) of the reference standard of analyte

Rs is the response to Wis

Wis is mass (concentration) of the internal standard

Ris is the response to Wis

Applying to the case of the assay of unknown quantity of analyte, Wu:

$$Wu = R^* \times Wis \times Ru/Ris$$

Whence Wu can be calculated.

(b) Recoveries for method development

%Recoveries of the internal standards were calculated as fpllows.

%Recovery = Ris(c) x R'rs(n) x 100/R'is(n) x Rrs(c)

where Ris(c) is response of internal standard in cleaned-up sample

R'rs(n) is response of recovery standard in cleaned-up sample

R'rs(n) is response of recovery standard in standard mixture

Rrs(c) is response of internal standard in standard mixture

2.2.5.5. Efficiency of on-line fractionation of *ortho-* and non-*ortho*-PCBs in a PCB standard mixture on carbon/glass fibre column.

The apparatus and the solvent system applied to this method development is a modification of the system described previously by Krokos et al.[33] A PCB standard mixture containing 40 ng each of *ortho-* (PCBs 28, 52, 101, 118, 138, 153 and 180)

and non-*ortho*- (PCBs 77, 126 and 169) PCBs was added to the top of the clean-up column. A selected solvent mixture was placed in the reservoir and the system was pressurised with compressed air (4 psi) to give a flow rate of approximately 2 ml min^{-1} and fractions were collected.

Each fraction was treated as follows: The solvent was evaporated on a rotary evaporator to approximately 4 ml and transferred in portions, together with three rinses with hexane (approximately 0.4 ml), to a tapered 1.1 ml screw top vial and gently blown down under a steady stream of purified compressed air. Each rinse was reduced to a smaller volume under a steady stream of air before the next was added. Following the last transfer, the solvent was evaporated to near dryness and 50 μl of isooctane was added. The fractions were subsequently analysed using gas chromatography-mass spectrometry (GC-MS) in selected ion monitoring mode (SIM) by injecting 2 μl into the GC.

2.2.5.6. Intra-laboratory method validation for spiked apple homogenate

Apples (161 g) bought from a local supermarket were cut into small pieces and homogenised with 200 g of distilled water.[38] The resulting slurry was subdivided into four equal portions and stored in a freezer. A portion of the slurry was spiked with an internal standard solution containing $^{13}C_{12}$-labelled *ortho*- (28, 52, 101 and 138) and non-*ortho*- (77, 126 and 169) polychlorinated biphenyls at 16 ng each. Anhydrous sodium sulphate (100 g) was added and the slurry was extracted with 3 x 40 ml hexane. The combined extracts were applied to the top of the clean-up (multicolumn) column. The first solvent (300 ml of hexane:DCM (80:20, v/v)) was placed on top of

the multicolumn and the second solvent reservoir was filled with the second solvent (120 ml of DCM:toluene (80:20, v/v). The multicolumn was then pressurised with air (4-5 psi) to give a flow rate of approximately 2 ml min^{-1}. The valve (Figure 2.1) was positioned so that the first solvent mixture (hexane:DCM) eluted the sample through the multicolumn, followed by the carbon column in the forward direction and was subsequently collected in a 500 ml round bottom flask (***ortho*- substituted PCB fraction**).

The valve was switched so that the DCM:toluene eluating solvent would bypass the clean-up multicolumn. The reservoir containing this second solvent mixture was pressurised with air (3 psi, 2 ml min^{-1} flow rate) to elute the carbon column in the forward direction and this eluate was finally collected in a 250 ml round bottom flask (**non-*ortho*-substituted PCB fraction**). The carbon was regenerated according to section 2.2.4.

Each fraction was treated as follows: The solvent was evaporated on the rotary evaporator to approximately 4 ml by maintaining the water bath temperature at 40 °C for the *ortho*-PCB fraction and at 60 °C for the non-*ortho*-PCB fraction, transferred to a 1.1 ml screw top vial with a number of hexane rinses and gently blown down under a steady stream of compressed air until just dry. 50 µl of recovery standard solution containing $^{13}C_{12}$-PCB-180 at 400 pg μl^{-1} in iso-octane was added. The hexane rinses were separately blown down to near dryness in the same vial. The fractions were analysed by GC-MS with selected ion monitoring.

2.3. RESULTS AND DISCUSSIONS

2.3.1. Method development

2.3.1.1. Gas chromatography-mass spectrometry

(a) Gas chromatography

Gas chromatography of PCBs is so complex that no single column is presently available which can achieve the elution of each congener as a fully resolved peak. Different types of GC columns have been used for PCB determination, but in this study, DB-17 (50% phenylmethyl polysiloxane phase) was used because of its ability to separate most of the PCBs of interest.

Figure 2.2 shows a PCB standard mixture, NTU-SMS0046, containing 41 *ortho*-polychlorinated (PCB 4, 18, 28, 31, 33, 41, 44, 47, 49, 51, 52, 60, 66, 74, 87, 99, 101, 105, 110, 114, 118, 123, 138, 141, 151, 153, 156, 157, 167, 180, 183, 185, 187, 189, 191, 193, 194, 201, 203, 206 and 209) and 5 non-*ortho*-polychlorinated (PCB-37, 77, 81, 126 and 169) biphenyls at 100 pg level each under GC-MS analysis. PCBs 118 and 123 were not fully separated on the DB-17 GC column. These two PCBs are both *ortho* substituted, have the same number of chlorine atoms, and hence the same molecular weight, and therefore cannot be separated by the mass spectrometer. The separation of these congeners was used in the optimisation of the GC oven temperature programme and was constantly monitored to confirm the quality of the chromatographic separation. The valley between the two PCBs was required to be less than 70 % of the average peak heights of the two PCBs (Figure 2.3) before analysis was carried out.

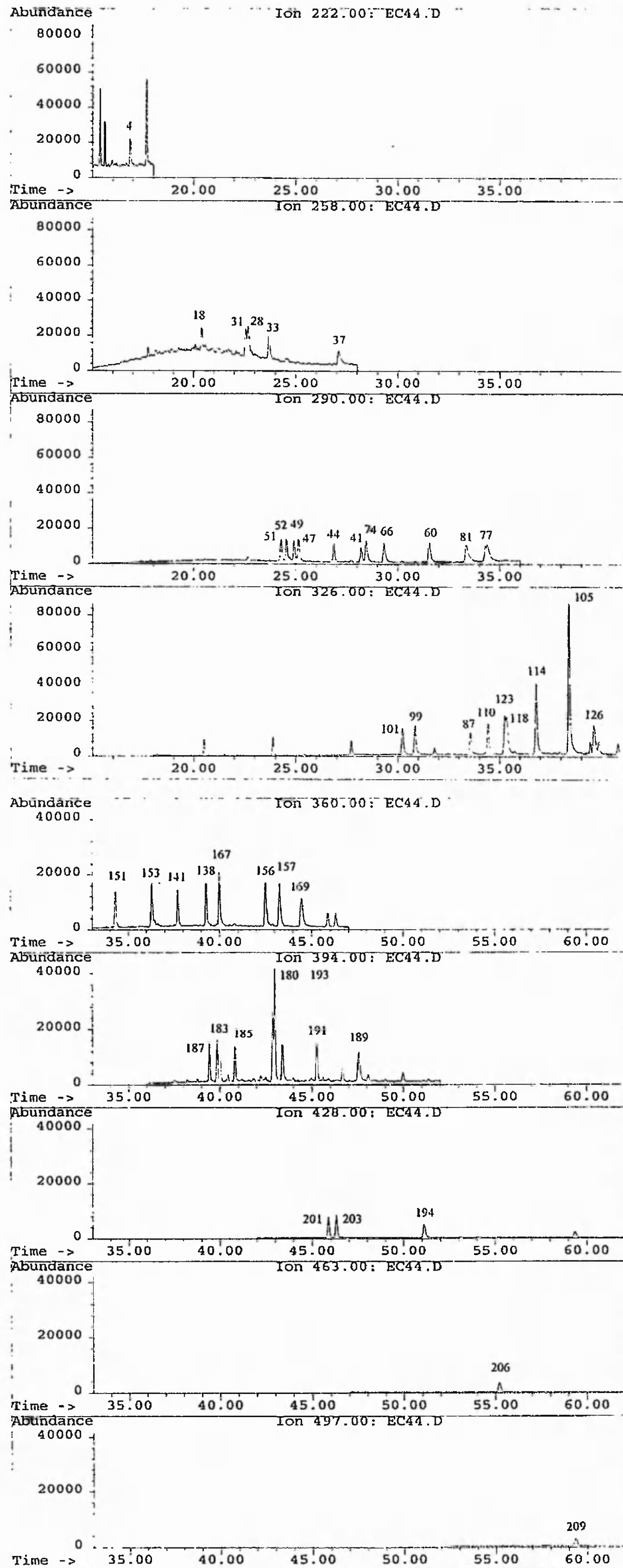

Figure 2.2. Separation and detection of *ortho-* and non-*ortho*-PCB congeners in NTU-SMS0046 by GC-MS

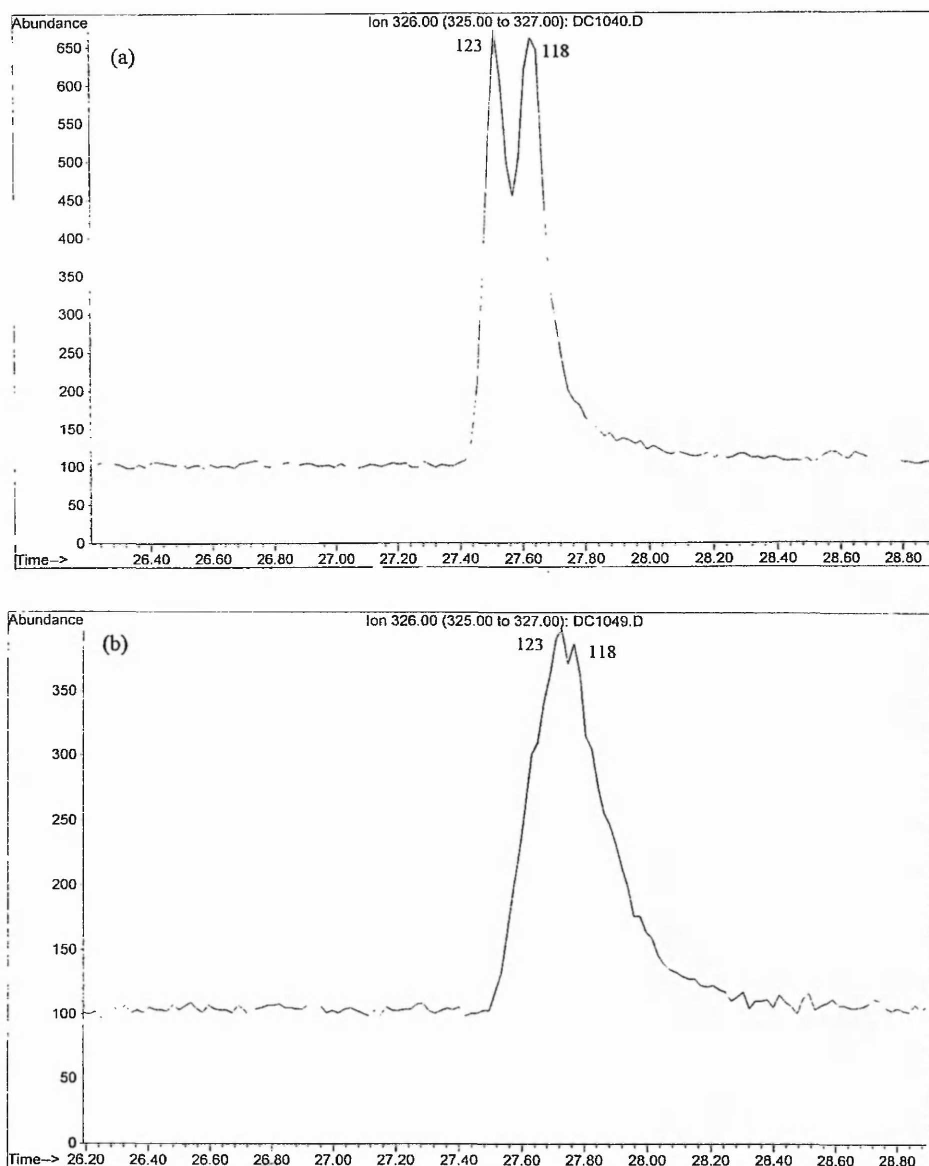

Figure 2.3. The performance of the GC column on the resolution of PCB-118 and PCB-123, (a) 69% valley and (b) 97% valley

Several groups[10,37,39,40] have addressed the problem of accurate quantification of PCB-77 in the presence of PCB-110 because these congeners coelute on most GC columns. Although these congeners are collected in two different fractions during carbon column chromatography (explained in section 2.3.1.2), potential problems can arise if trace amounts of PCB-110 left in the non-*ortho* fraction. The two congeners have different number of chlorines and can be separated by mass spectrometry. However,[28,29] an interference arising during the coelution of PCB-77 (four chlorines) and 110 (five chlorines) due to a fragment ion at m/z 290 (loss of HCl) from the molecular ion of PCB-110) may result in a falsely enhanced area for congener 77 (m/z 290 is a quantification ion for PCB-77). This can be seen in Figure 2.2 in which the PCB-77 peak appears as a broad peak probably due the coelution with the fragment ion m/z 290 from PCB-110 after losing HCl. Krokos et al.[33] established the extent of this interference by repetitive analyses of a standard solution containing PCB-110. It was found that m/z 290 represented only *ca* 2% of the molecular ion. Consequently, after separation on carbon and analysis by GC-MS the overall interference from PCB-110 in the quantification of PCB-77 was less than 0.002% and considered to be insignificant. Rood and Hastings[41] have reported the partial resolution of PCB-77 and PCB-110 on a DB-5ms (similar to 5% phenyl 95% methyl phase) GC column.

PCBs 180 and 193 were only partly resolved (Figure 2.2) because both PCBs are heptachlorinated *ortho*-substituted congeners and hence could not be separated on the carbon column (explained in section 2.3.1.2). However, PCB 193 is usually not detected in the environment and hence this dual peak can be quantified as a single component.

(b) Mass spectrometry

For classes of chlorinated compounds, such as PCBs, the most important electron ionisation mass spectral feature is the molecular ion ($M^{+\cdot}$) cluster produced by the natural abundance of ^{35}Cl and ^{37}Cl isotopes.[42] GC-MS analyses were therefore carried out in selected ion monitoring (SIM) mode, monitoring the two most abundant ions in the molecular ion cluster of each homologue and internal standard.

Before any analysis could be conducted, an ion detection programme for SIM had to be developed. For each homologue group four ions were monitored; two for the native congeners and two for the $^{13}C_{12}$ internal standards. During the gas chromatographic determination of PCBs, there is an overlap in the retention times of the different homologue groups. For example, hexachlorinated biphenyls elute from DB-17 column before the last congener of the pentachlorinated group have eluted (Figure 2.4). If the retention times of the first and last eluting congener of each homologue group are determined, the SIM programme can be set to collect all congeners for each chlorination group. This is particularly important where homologue group total concentrations are to be determined. Aroclor 1242:1254:1260 (1:1:1) was used as a window defining mixture (Figure 2.4) because it contains most of the PCBs found in the environment and the collection windows for SIM were set accordingly (Table 2.2). During acquisition of the window defining mixture the mass spectrometer was operating in full scan mode. Because of the overlap in retention times of the PCBs with different chlorination numbers (Figure 2.4), a SIM programme (Table 2.2) was set so that three different chlorination numbers were monitored in one acquisition group. For example, dichloro, trichloro and tetrachlorinated PCBs , trichloro, tetrachloro and pentachlorinated PCBs and so

on as shown in Table 2.2. The time when to start and stop MS acquisition of a particular group was determined using Figure 2.4. For example, Group 1 (Table 2.2), which consists of dichloro-, trichloro- and tetrachlorinated PCBs was set to start at 13 min, because the dichloro-PCBs started eluting just before this time, and stop at 18 min to exclude the dichloro fragment ion clusters ($M-70^{+\cdot}$) produced by the tetrachlorinated PCBs (explained in the next paragraph) The time to start and stop MS acquisition for the remaining groups was similarly set (Table 2.2).

Higher chlorinated PCBs undergo a characteristic loss of two chlorines to produce $(M-70)^{+\cdot}$ ion clusters which partially overlap the $M^{+\cdot}$ ion cluster produced by a congener containing two less chlorine atoms.[43] This is illustrated in Figure 2.4, where for example, fragments from the pentachloro group are detected in the chromatogram of the trichloro group. During the analysis of samples, fragment of ions produced by coeluting PCB congeners of high chlorination can interfere. Therefore, to verify a positive assignment of the level of chlorination the observed relative abundance of isotope cluster components must be close to the expected (theoretical) relative abundance.

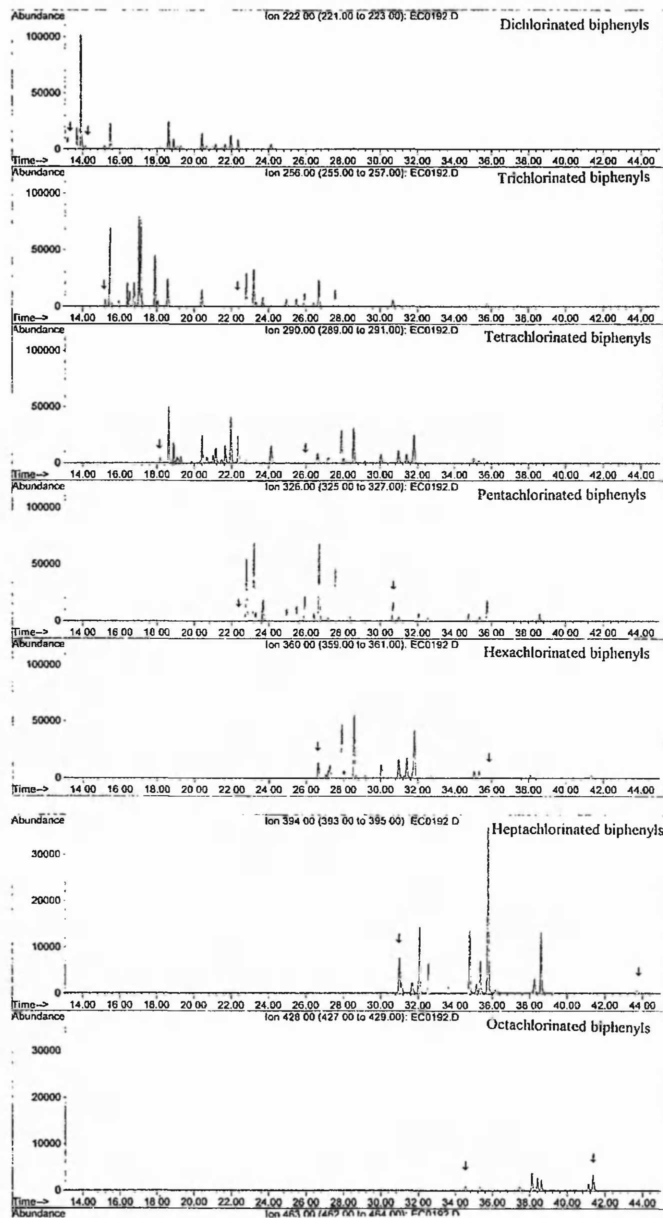

Figure 2.4. GC-MS single ion chromatograms of Aroclor 1242:1254:1260 (1:1:1) (first and last eluting PCB congeners of each homologue group and are marked)

Table 2.2. Ion detection programme for PCBs during SIM GC-MS

Group	Norminal Mass (m/z)				Start time (min)	Stop time (min)
1	222 (2)*	256 (3)	**268** (3)**	290 (4)	13	18
	224 (2)	258 (3)	**270 (3)**	292 (4)	13	18
2	256 (3)	290 (4)	**302 (4)**	326 (5)	18	22.4
	258 (3)	292 (4)	**304 (4)**	328 (5)	18	22.4
3	290 (4)	326 (5)	**338 (5)**	360 (6)	22.4	26
	292 (4)	328 (5)	**340 (5)**	362 (6)	22.4	26
4	326 (5)	360 (6)	**372 (6)**	394 (7)	26	32
	328 (5)	362 (6)	**374 (6)**	396 (7)	26	32
5	360 (6)	394 (7)	**406 (7)**	**440 (8)**	32	36
	362 (6)	396 (7)	**408 (7)**	**442 (8)**	32	36
6	394 (7)	428 (8)	**440 (8)**	464 (9)	36	65
	396 (7)	430 (8)	**442 (8)**	466 (9)	36	65
7	428 (8)	464 (9)	**476 (9)**	498 (10)	36	65
	430 (8)	466 (9)	**478 (9)**	500 (10)	36	65
			510 (10)		36	65
			512 (10)		36	65

*Number in brackets: number of chlorine atoms
Numbers in **bold: masses for the internal standards

(c) GC-MS validation with NTU-SMS0018

To establish the GC-MS precision, a standard PCB solution NTU-SMS0018 containing the 18 PCB congeners (PCB-28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180 and 189) was prepared and aliquots analysed in Nottingham and by two independent analytical laboratory A[44] and laboratory C.[45] The results of the analyses are given in Table 2.3. An examination of the data indicates a general good agreement between the three laboratories. The mean inter-laboratory precision for the sum of seven and eighteen congeners as expressed by the coefficient of variation (COV), was 6.3% and 7.7% respectively, is well within acceptable levels and confirms the satisfactory performance of GC-MS system although a wider range of precessions was observed for individual congeners particularly those without corresponding $^{13}C_{12}$ labelled internal standards or quantification standards.

Table 2.3. Analysis of an 18 PCB congener standard solution (NTU-SMS0018) : a comparison of inter-laboratory precision.

Targeted congener (pg µl)	Laboratory A[a]	B[b]	C[c]	Mean	COV(%)
PCB-28	80	72	84	79	7.8
PCB-52	83	90	41	71	37
PCB-77	69	82	77	76	8.6
PCB-81	77	125	88	97	26
PCB-101	88	90	73	84	11
PCB-105	170	65	133	123	43
PCB-114	88	79	146	104	35
PCB-118	84	84	115	94	19
PCB-123	60	41	101	67	46
PCB-126	78	88	108	91	17
PCB-138	85	90	75	83	9.2
PCB-153	83	124	90	99	22
PCB-156	71	45	78	65	27
PCB-157	59	44	78	62	25
PCB-167	65	61	79	68	14
PCB-169	150	144	145	147	2.2
PCB-180	84	84	82	83	1.4
PCB-189	67	37	120	75	56
Σ7[d]	587	634	560	594	6.3
Σ18[e]	1543	1475	1713	1577	7.7

[a]from reference 44, [c]from reference 45 and [b]B is this work
[d]Σ7 is sum of the congeners 28, 52, 101, 118, 138, 153 and 180.
[e]Σ18 is the total concentration for 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180 and 189.

2.3.1.2. Efficiency of on-line fractionation of *ortho*- and non-*ortho*-PCBs on carbon/glass fibre column

The ability of activated carbon to separate aromatic molecules according to structural characteristics was investigated by the fractionation of a PCB mixture containing non-*ortho*, mono-*ortho* and di-*ortho* PCBs through the carbon/glass fibre column. The solvent composition used was hexane:DCM (80:20, v/v).

The multicolumn, connected to the carbon/glass fibre column, was spiked with a PCB standard mixture containing non-*ortho* (PCBs 77, 126 and 169), mono-*ortho* (PCBs 28 and 118) and di-*ortho* (PCBs 52, 101, 138, 153 and 180) PCBs and eluted fractions were collected and analysed by GC-MS. The results obtained are presented in Figure 2.5. From the solvent system studied (hexane:DCM, 80:20), it is clear that the non-*ortho* substituted congeners needed a larger elution volume than the *ortho*-substituted congeners. Figure 2.5 shows that di-*ortho* and mono-*ortho* substituted PCBs required up to 250 ml of solvent to elute completely from the column, whilst none of the non-*ortho* substituted congener eluted even when up to 400 ml solvent was used. This is because active carbon, being a porous material and prepared by partial oxidation of organic substances, has a very large adsorptive power. The adsorptive power is primarily due to this highly developed porous structure.[46] There are two different forms of activated carbons or charcoals that exist: graphitised (non polar) charcoals that are prepared by high temperature activation and oxidised (polar) charcoals which result from low temperature oxidation. Most commercial active carbons fall between the two extremes of graphite and oxidised charcoal. They contain some surface oxygen groups (-OH, =O, C-O-C, COOH etc.), but tend to resemble graphite in terms of sample selectivity.

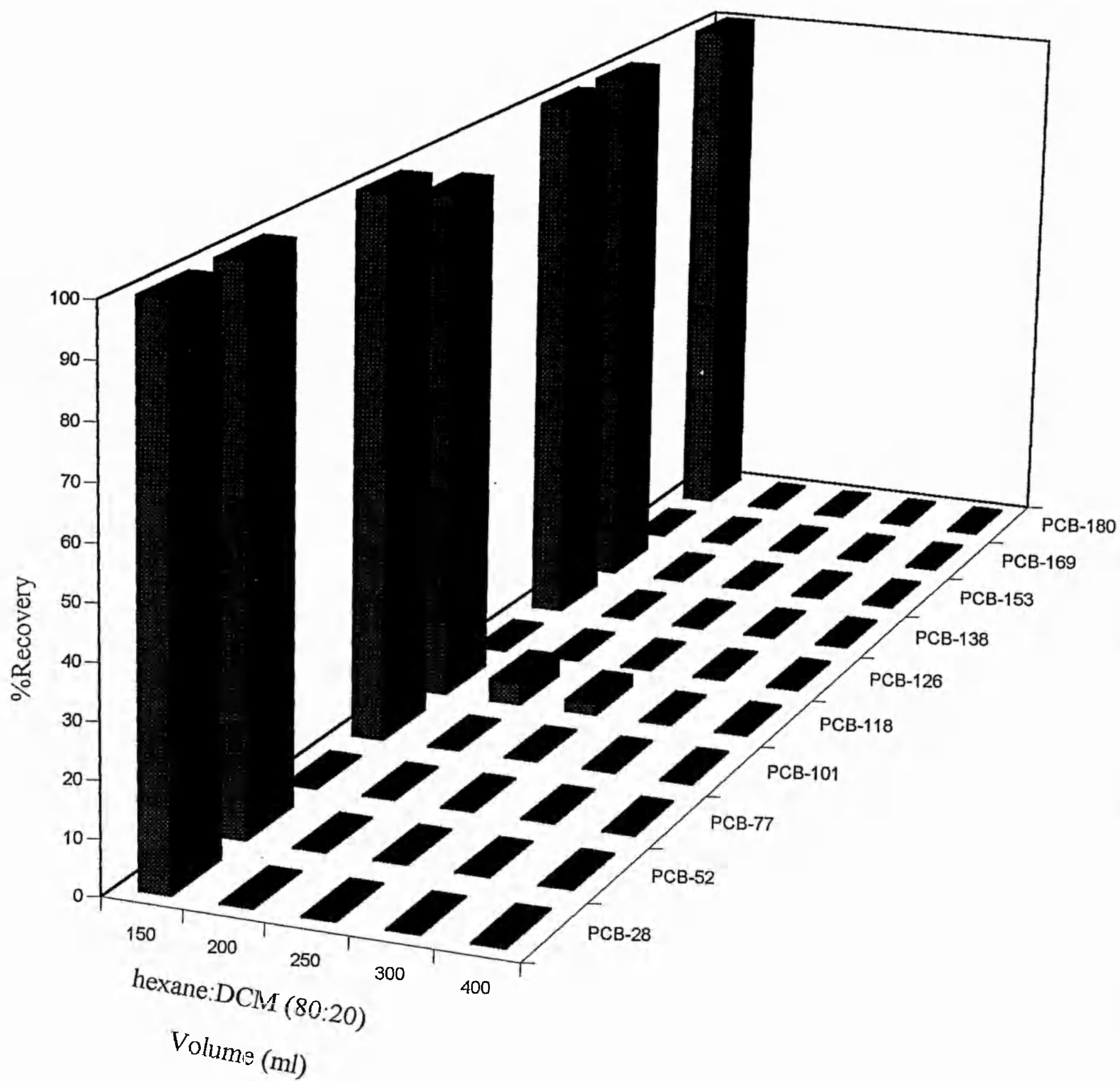

Figure 2.5. Fractionation of *ortho-* and non-*ortho*-PCBs on carbon/glass fibre column using hexane:DCM (80:20, v/v)

The more closely matched are the planes of the adsorbate molecule and the underlying adsorbent surface, the stronger is the interaction between adsorbate and adsorbent in adsorption chromatography. Planar aromatic molecules or molecules which can most easily adopt a planar configuration, such as non-*ortho*-PCBs, will be adsorbed more strongly onto carbon than corresponding non-planar (di-*ortho*-PCBs) molecules (or those molecules that can attain a planar configuration only with difficulty, such as mono-*ortho*-PCBs), as their carbon skeleton is just matched by the underlying carbon atoms of the graphite-like surface. Mono-*ortho*-PCBs, on the other hand, will be adsorbed more strongly onto carbon than corresponding di-*ortho*-PCBs. Thus, a tetrachloro non-*ortho* substituted congener (PCB-77) was found to have a much larger retention volume than a heptachloro-*ortho* substituted congener (PCB-180) and mono-*ortho*-PCB-118 was retained more on carbon than di-*ortho*-PCB-101 even though both are pentachlorinated (Figure 2.5)

Steric hindrance or intramolecular steric effects of an adsorbate molecule can reduce adsorption energy by preventing a flexible molecule from adopting a preferred configuration (e.g. planar) for adsorption.[47] It has been shown,[48] that for chlorinated biphenyls, the steric hindrance increases with increasing *ortho* substitution and as the preferred conformation is driven further from planarity, the adsorption energy is reduced significantly. Consequently, it is the degree of *ortho* substitution that primarily determines the retention characteristics of PCB congeners on carbon by controlling the ability of the congener to adopt a planar configuration. Thus, mono-*ortho*-PCB-118 showed a larger retention volume than di-*ortho*-PCB-101 even though both are pentachlorinated PCBs. This can be considered as a primarily substitution effect.

Other factors may be involved when the adsorption of molecules such as PCBs on activated carbon is considered. It is widely accepted[47] that for the adsorption of non-polar molecules onto adsorbents like carbon, dispersion forces contribute essentially all the adsorption energy. The energy of dispersion interaction increases by increasing surface tension, molecular polarizability and molecular area of the adsorbed sample molecule. Therefore, the number of electronegative substituents can also influence the retention of a PCB congener on carbon. For two congeners having the same number of *ortho-* substituted chlorines, the higher chlorinated one will be more strongly retained. Finally, the substitution pattern affects the electron density of the compound since *ortho-* and *para*-substitution can lead to more polarizable π-electrons than *meta*-substitution and hence greater polarizability and adsorption strength. This can be regarded as a secondary substitution effect.

The retention behaviour of PCB congeners is not always straightforward and can involve one or more of the mechanisms discussed previously. From a practical point of view however, it is important to know that PCB congeners have individual retention behaviour on activated carbon, a behaviour which is primarily controlled by the degree of *ortho* chlorine substitution.

(a) Effect of eluting solvent

After collecting *ortho*-PCBs, the solvent was then changed from hexane:DCM (80:20, v/v) to DCM:toluene (80:20, v/v). The carbon/glass fibre column alone was eluted with DCM:toluene (80:20, v/v) and fractions were collected and analysed by GC-MS. The recoveries are shown in Figure 2.6. The results obtained when hexane:DCM

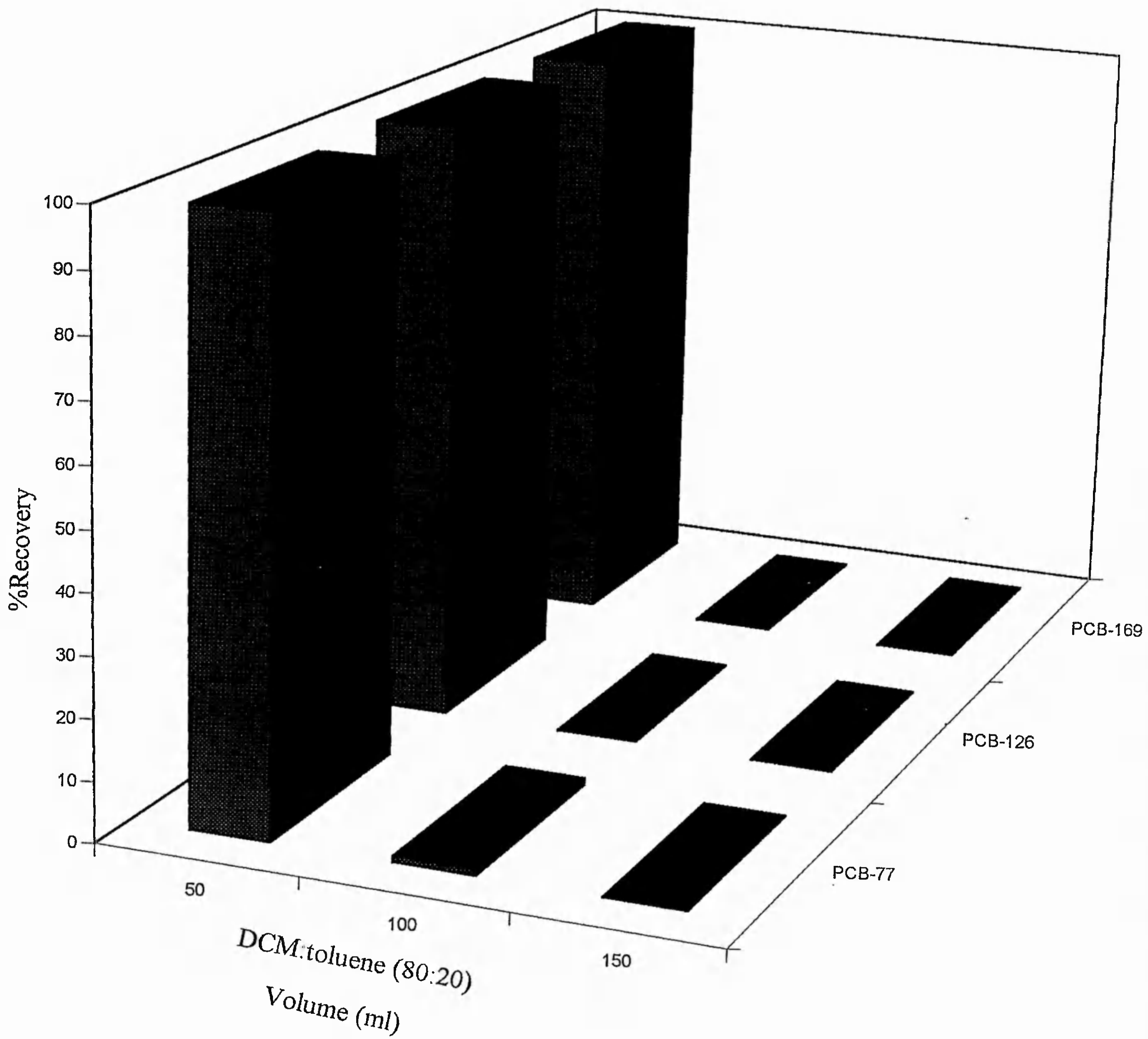

Figure 2.6. Fractionation of non-*ortho*-PCBs on carbon/glass fibre column using DCM:toluene (80:20,v/v)

(80:20, v/v) and DCM:toluene (80:20, v/v) were used agree with the reported solvent strength sequence of Table 2.4. It can be seen in Figure 2.5 that all the *ortho*-PCBs, but none of the non-*ortho* PCBs, were eluted on the carbon/glass fibre column with 400 ml of hexane:DCM (80:20, v/v). When the solvent was changed to DCM:toluene (80:20, v/v), 99%, 100% and 100% of the non-*ortho* PCBs 77, 126 and 169, respectively, retained on the carbon column were eluted in the first 50 ml of the solvent mixture (Figure 2.6) because of the greater solvent strength of the DCM:toluene mixture.

It is assumed by current models of the adsorption process in liquid-solid chromatography[47,49] that the adsorption sites of the adsorbent are completely covered by either solute or solvent molecules and that retention of a solute requires displacement of an equivalent number of adsorbed solvent molecules. The stronger the interaction between a solvent molecule and the adsorbent surface, the more difficult it will be for a solute to displace the solvent, and thus the earlier the solute will elute due to the limited interaction with the adsorbent. For adsorption on charcoal, aromatic solvents are much stronger than corresponding aliphatic solvents because of optimum interaction between their planar surface and the underlying planar graphite-like surface of active carbon. Furthermore, the higher surface tensions and molecular polarizabilities of aromatic compounds also favour their adsorption on charcoal.

Table 2.4. Solvent strength in liquid-solid chromatography for carbon as adsorbent[a]

Solvent	ε^{o} (carbon)
Methanol	0.00
Acetonitrile	0.04
Ethanol	0.05
Ethyl acetate	0.09
n-Hexane	0.10
n-Heptane	0.13
Dichloromethane	0.13
Butyl chloride	0.13
n-Octane	0.14
Tetrahydrofuran	0.14
n-Nonane	0.17
Benzene	0.20
Xylene	0.24

[a]From ref. 47

As adsorption on carbon surfaces is essentially controlled by dispersion forces, polarity will be of secondary importance. Adsorption on charcoal decreases with increasing solvent strength, possibly because of stronger interactions between polar solvent molecules than between solvent and adsorbent molecules.[47]

The strength of a solvent is expressed by the parameter ε^{o} (adsorption energy of solvent per unit area of the standard active surface) which defines the effect of solvent upon the adsorption of a given sample[50] (an increase of ε^{o} means a stronger solvent). A list of ε^{o} for carbon[47] as adsorbent is given in Table 2.4. It can be observed that for a homologue series (for example n-alkanes; n-hexane to n-nonane), ε^{o} increases with increasing carbon number. This can be attributed in part to the higher area which the molecule covers on the adsorbent surface. Benzene and xylene are the strongest solvents of the series. It would be expected that toluene would have an ε^{o} value between 0.20 and 0.24 (ε^{o} values for benzene and xylene respectively) as its molecular size falls between that of benzene and xylene.

(b) Choice of the solvent volume

Having established the retention characteristics of individual PCB congeners on the PX-21 carbon/glass fibre column for solvent mixtures containing hexane, DCM and toluene, the next step was to develop a solvent system for the efficient fractionation of *ortho*- and non-*ortho* substituted PCBs. The solvent used for the elution of the first fraction (*ortho*-substituted PCBs) should be sufficient in strength to ensure a quantitative removal of these PCBs of interest from the carbon column, but leave the

non-*ortho*-PCBs behind on the carbon, and that the second fraction (non-*ortho*-PCBs) should be recovered using a reasonable amount of the second solvent.

Based upon the results (Figures 2.5 and 2.6), the solvent system which was proposed for the separation of *ortho*- and non-*ortho*-substituted polychlorinated biphenyls into different fractions was:

Fraction I 300 ml of 20% DCM in hexane

(di-*ortho* and mono-*ortho* substituted PCBs)

Fraction II 120 ml of 20% toluene in DCM

(non-*ortho* substituted PCBs)

The fractionation efficiency of the carbon/glass fibre column, using the proposed solvent system, was tested by spiking the column with a PCB standard mixture containing non-*ortho* (PCBs 77, 126 and 169), mono-*ortho* (PCBs 28 and 118) and di-*ortho* (PCBs 52, 101, 138, 153 and 180) PCBs and eluting with 300 ml of 20% DCM in hexane to collect mono-*ortho* and di-*ortho*-PCBs followed by 120 ml of 20% toluene in DCM to collect non-*ortho*-PCBs and fractions analysed by GC-MS. The results are shown in Figure 2.7. Figure 2.7 shows that the *ortho*- and non-*ortho*-PCBs are clearly separated by the carbon/glass fibre using the solvent systems proposed without any cross-over.

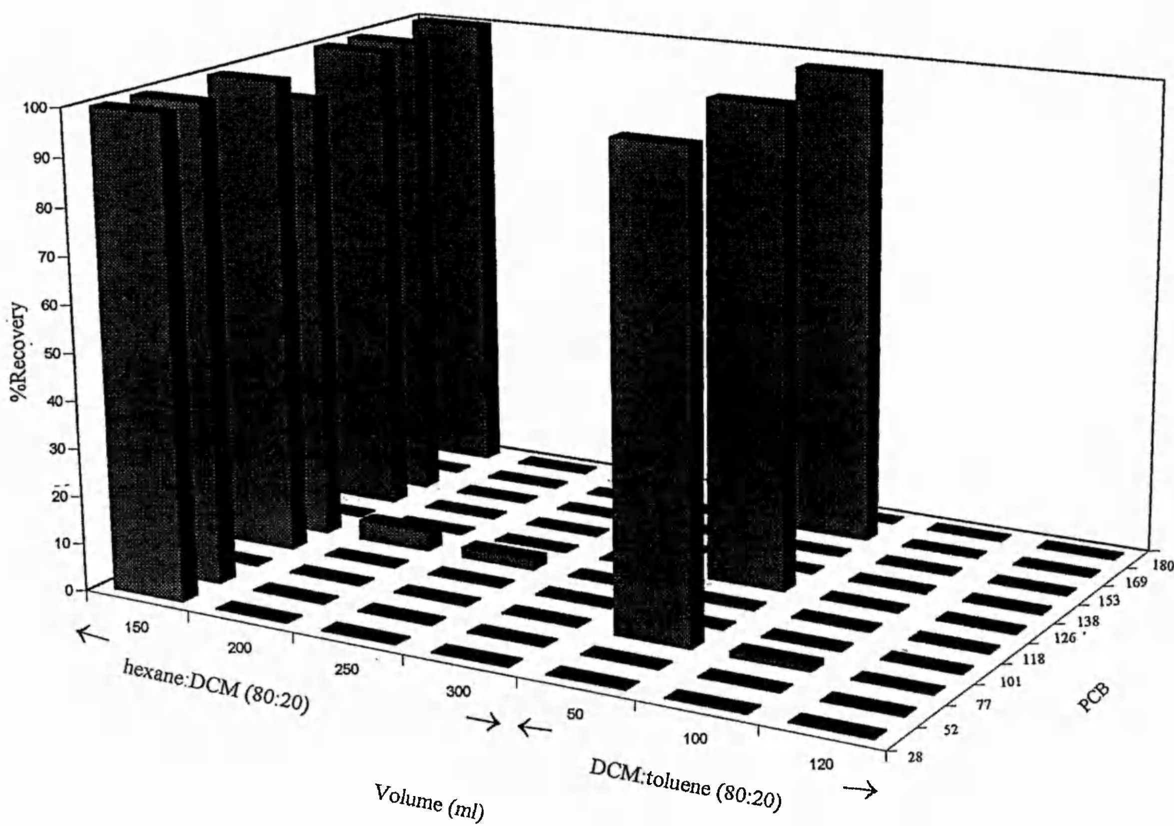

Figure 2.7. Fractionation of *ortho*- and non-*ortho*-PCBs on carbon/glass fibre column using hexane:DCM (80:20, v/v) and DCM:toluene (80:20,v/v)

2.3.1.3. Efficiency of on-line fractionation of *ortho* and non-*ortho*-PCBs in NTU-SMS0018 PCB standard

Although the method was originally optimised for on-line separation of the 10 *ortho*- and non-*ortho* (PCBs 28, 52, 77, 101, 118, 126, 138, 153, 169 and 180) polychlorinated biphenyls, a PCB standard mixture, NTU-SMS0018, containing 18 *ortho*- and non-*ortho* (PCBs 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180 and 189) polychlorinated biphenyls (Table 2.5), was used to evaluate the efficiency of the entire method for the fractionation of *ortho*- and non-*ortho*-substituted PCB congeners.

The original unfractionated NTU-SMS0018 PCB standard shows all eighteen congeners under GC-MS analysis (Figure 2.8). This sample was successfully separated into the two fractions using the carbon/glass fibre column. The single ion chromatograms for the GC-MS analysis of fraction I (Figure 2.9) contains PCB congeners with one or more chlorine atoms in the *ortho* position of the biphenyl ring and are clearly separated on the DB-17 analytical column. Figure 2.10 shows the single ion chromatograms of fraction II of the non-*ortho*-PCB congeners. Figures 2.9 and 2.10 show also that there is no cross over of *ortho*- and non-*ortho*-PCBs in the two fractions for the eighteen congeners.

The separation of the *ortho*- and non-*ortho*-PCBs on the carbon/glass fibre column and their further separation into single peaks on the GC column confirms the efficiency of the on-line clean-up method which can be applied to a large number of PCB congeners so long there is no coelution on the GC column.

Table 2.5. Selected PCBs for which the method has been optimised.

Congeners No. (IUPAC numbers)	Chlorine substitution
28	2,4,4'-trichlorinated biphenyl
52	2,5,2',5'-tetrachlorinated biphenyl
77[a]	3,4,3',4'-tetrachlorinated biphenyl
81[a]	3,4,4',5-tetrachlorinated biphenyl
101	2,4,5,2',5'-pentachlorinated biphenyl
105	2,3,3',4,4'-pentachlorinated biphenyl
114	2,3,4,4',5-pentachlorinated biphenyl
118	2,4,5,3',4-pentachlorinated biphenyl
123	2,3,4,4'5-pentachlorinated biphenyl
126[a]	3,3',4,4',5-pentachlorinated biphenyl
138	2,3,4,2',4,5-hexachlorinated biphenyl
153	2,4,5,2',4',5'-hexachlorinated biphenyl
156	2,3,3',4,4',5-hexachlorinated biphenyl
157	2,3,3',4,4',5-hexachlorinated biphenyl
167	2,3'4,4'5,5'-hexachlorinated biphenyl
169[a]	3,3'4,4'5,5'-hexachlorinated biphenyl
180	2,3,4,5,2',4',5'-heptachlorinated biphenyl
189	2,3,3',4,4',5,5'-heptachlorinated biphenyl

[a]Non-*ortho*-substituted polychlorinated biphenyls

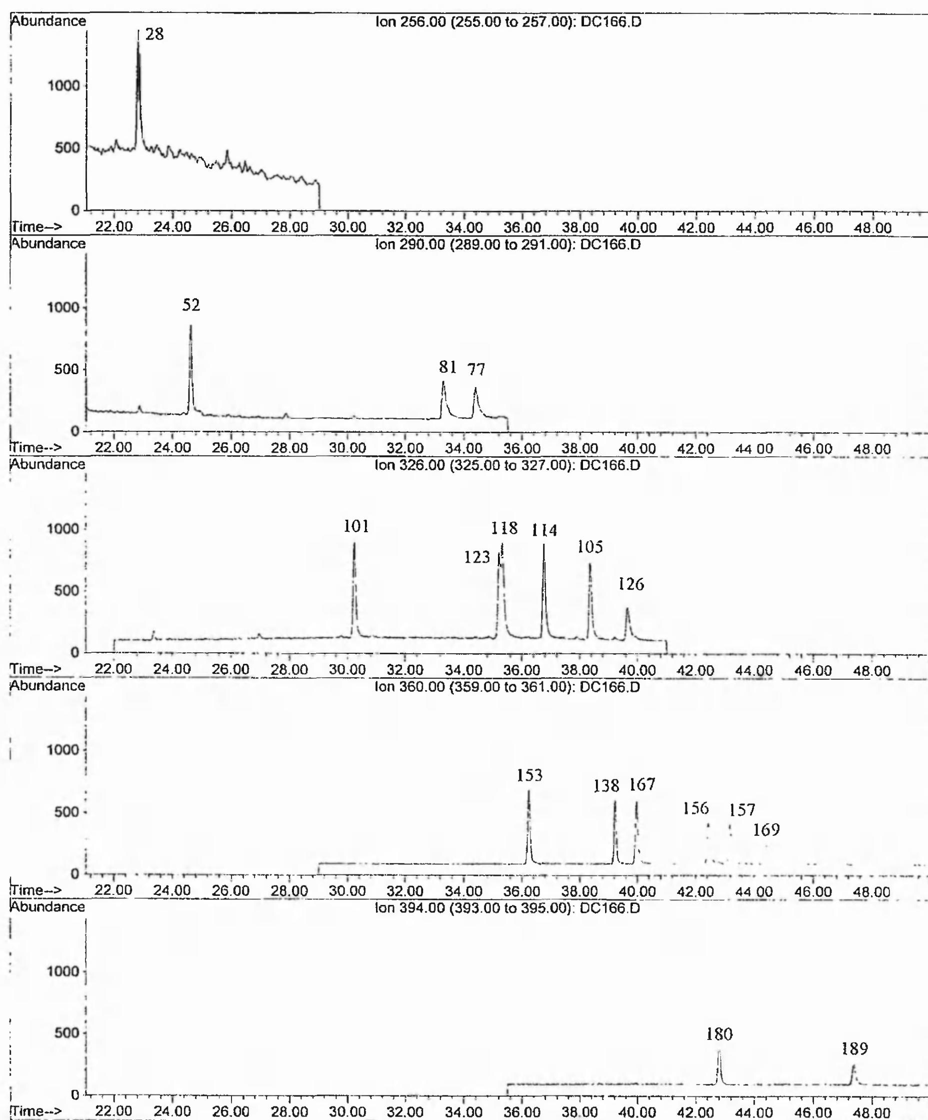

Figure 2.8. Separation and detection of *ortho-* and non-*ortho*-PCB congeners in unfractionated NTU-SMS0018 by GC-MS

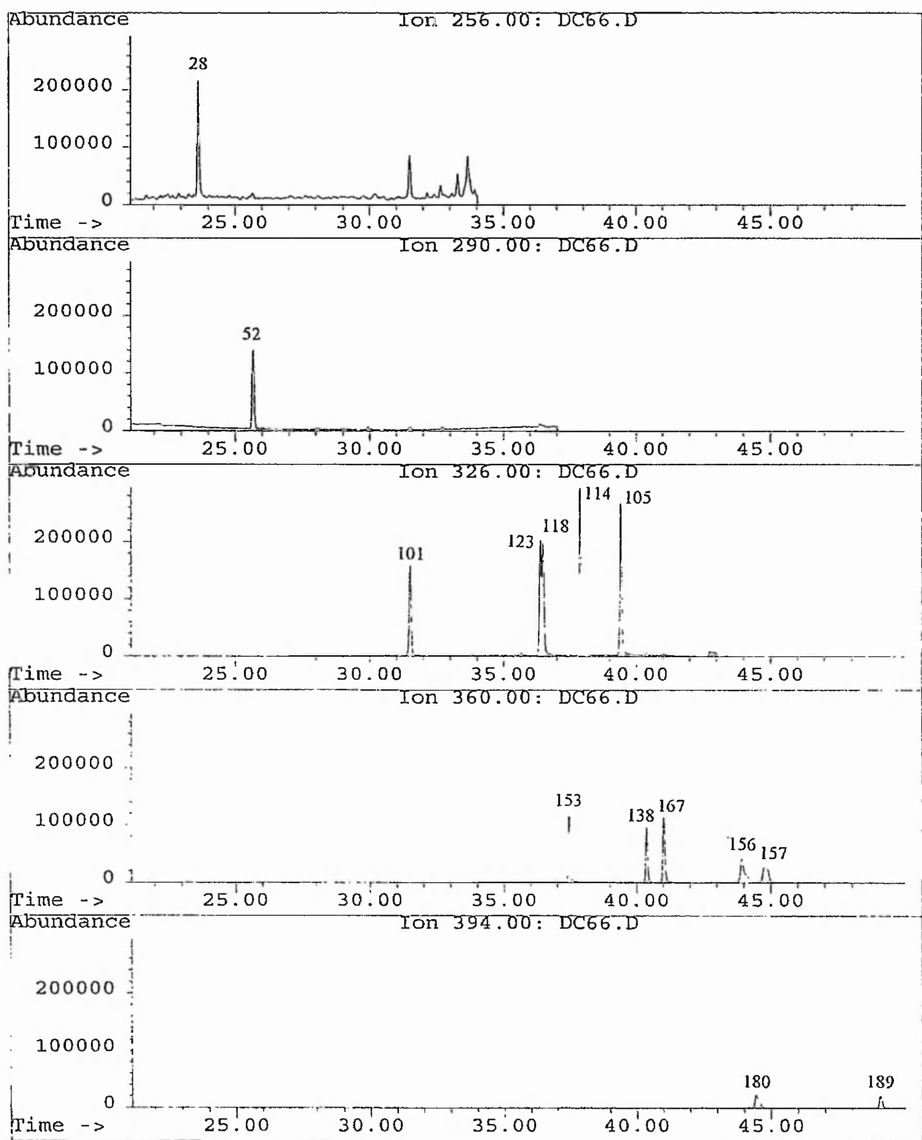

Figure 2.9. Separation and detection of *ortho*-PCB congeners in Fraction I of NTU-SMS0018 by GC-MS

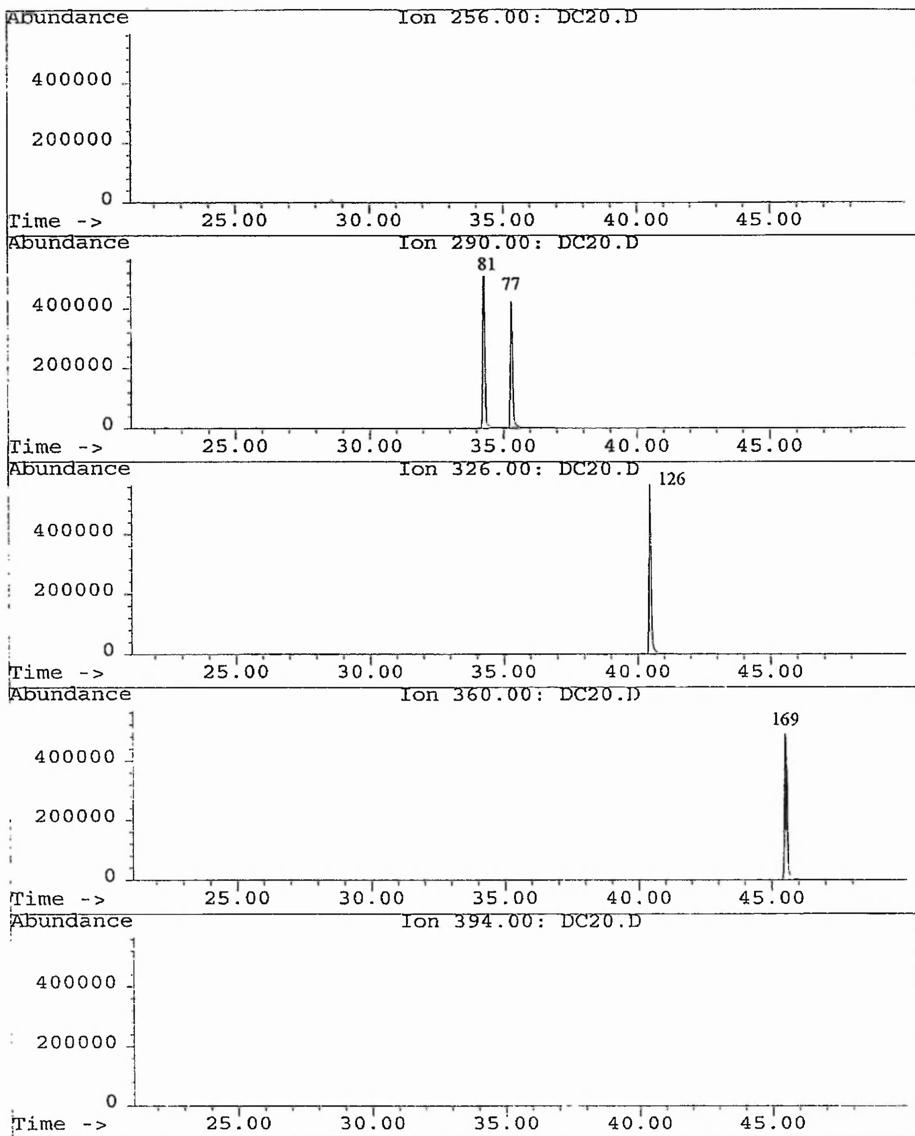

Figure 2.10. Separation and detection of non-*ortho*-PCB congeners in Fraction II of NTU-SMS0018 by GC-MS

2.3.1.4. Intra-laboratory method validation

In order to test the extraction efficiency and performance of the complete on-line clean-up (Figure 2.1) with GC-MS analysis for the determination of fruit and vegetable samples, apple was chosen as a representative matrix and apple samples spiked with an internal standard solution containing $^{13}C_{12}$-labelled *ortho-* (28, 52, 101 and 138) and non-*ortho-* (77, 126 and 169) polychlorinated biphenyls were analysed. Figure 2.11 shows the single ion chromatograms of the apple extract of some PCB congeners identified. It can be seen in Figure 2.11 that the extracts were generally clean (clean-up explained later) and the congeners identified were adequately separated from closely eluting PCBs. The results of the analyses (Table 2.6) also show that the recoveries for the $^{13}C_{12}$-PCB internal standards investigated ranged from 54-65% for *ortho*-substituted-PCBs and 88-94% for non-*ortho* substituted PCBs. The intra-laboratory precision (coefficient of variation, %COV) for the method varied from 3.1-21% (mean 9.5%) and these were considered acceptable for the complete method. The lower chlorinated PCB congeners were observed to have lower recoveries than the higher chlorinated congeners. During this experiment, recoveries for PCBs 28 and 52 (55 and 54%, respectively) in sub samples were lower than for the other PCBs, probably due to their volatility and subsequent loss during the blow down process. With isotope dilution mass spectrometry, the percentage of recovery is of less importance than is the case in external standardisation, since the calculation of the concentration of the native analytes compensates for errors arising from variations in recoveries. However, a low recovery can result in a high limit of detection and for this reason the required minimum recovery was approximated 50% for subsequent sample analyses.

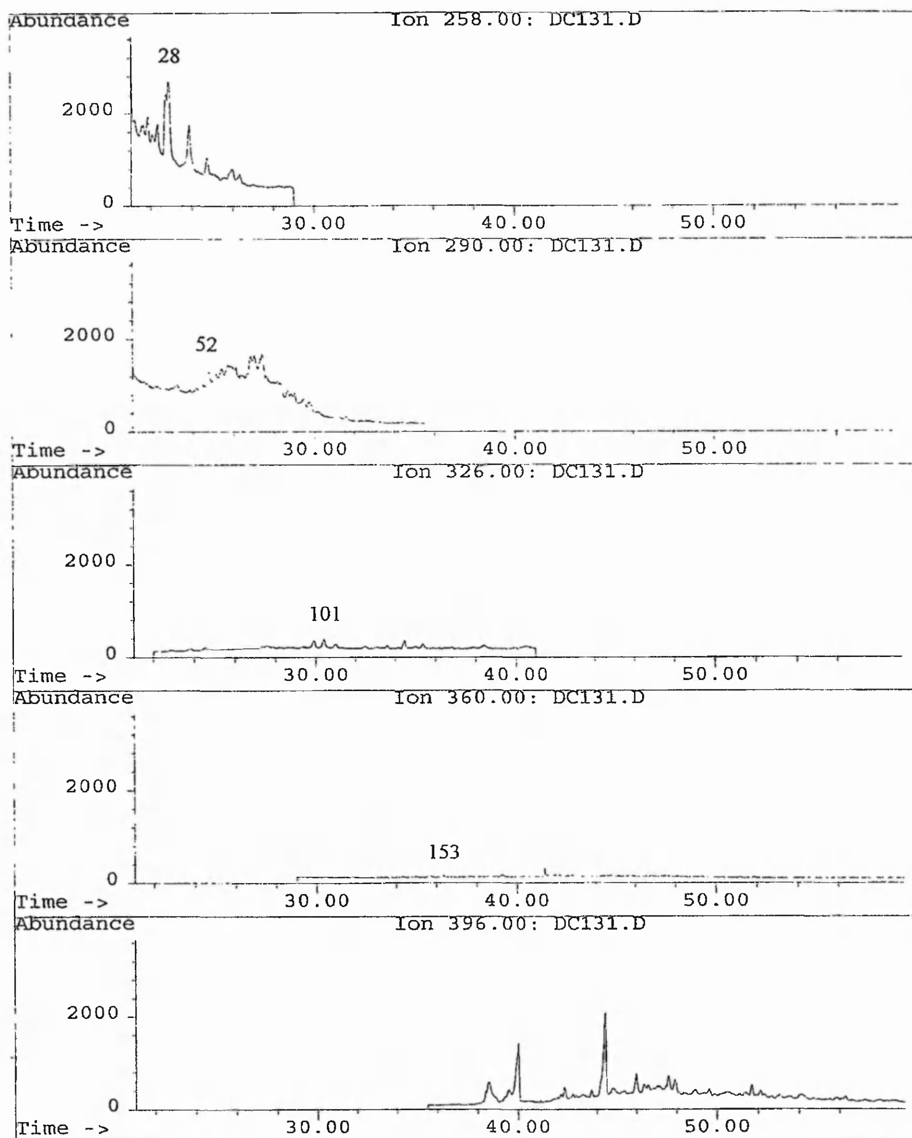

Figure 2.11. Typical GC-MS chromatograms of some PCBs identified in apple

Table 2.6. Recoveries and Precision for $^{13}C_{12}$-PCBs in apple extracts

	PCBs						
	28	52	77	101	126	138	169
Recovery (%)							
Sub-sample							
1	56	58	116	63	103	a	90
2	55	57	71	59	80	70	81
3	53	56	88	57	100	65	89
4	57	50	103	53	78	68	92
Av. recovery (%)	55	54	94	58	90	65	88
Precision							
%COV	3.1	6.5	21	6.7	14	9.5	5.6

[a] Not determined

The recoveries for the *ortho*-PCBs were lower than the non- *ortho*-PCBs (Table 2.6). Figures 2.9 and 2.10 show that there is no carry over of the *ortho*-PCBs into the non-*ortho* fraction to explain these low recoveries. The non-*ortho*-PCBs may have been retained better on the glass walls of the flask and vials during rotary evaporation and blow down, repectively, whilst the *ortho* congeners remained in the solvent and could easily have evaporated during these two processes.

In summary, the results obtained during this investigation demonstrate that for the determination of PCBs in apple: (a) acceptable recovery was obtained for all the PCB congener groups, (b) there were greater losses of low chlorinated *ortho*-PCB congeners (PCB-28 and PCB-52) relative to the higher chlorinated congeners and for *ortho*-PCBs relative to the non-*ortho*-PCBs and (c) the precision (repeatability) of the on-line clean-up method was higher enough to be considered acceptable.

However, it is very difficult to validate an extraction procedure with complete confidence, as the absolute extraction efficiency of the native analytes present in the matrix cannot be determined. The use of isotopically labelled internal standards provides an assessment of the recoveries through the clean-up procedure, but the recovery of a spiked compound may not be the same as the recovery of the corresponding endogenous compound.[51]

In order to clean-up apple samples, the extracts were applied to the top of the clean-up column (multicolumn) (Figure 2.1) and passed through the adsorbents in the following order, acid impregnated silica gel, silica gel, potassium silicate and finally carbon/glass fibre column. The first solvent mixture (300 ml of 20% DCM in hexane)

was used to clean-up the extract on the silica based adsorbates and finally separate *ortho*-PCBs from non-*ortho*-PCBs on the carbon column. At this stage *ortho*-PCBs have been eluted whilst the non-*ortho*-PCBs are still retained on the carbon column. The second solvent mixture (120 ml of 20% toluene in DCM) selectively elutes PCBs from the carbon. The role of the first solvent is very critical. It must have the right composition to allow the separation of *ortho*-PCBs from non-*ortho*-PCBs.

Spiking the carbon/glass fibre column with apple extracts without prior clean-up on the multicolumn could have caused difficulties with the analysis. Smith et al.[17] reported that application of extracts of biological samples directly to the carbon/glass fibres adsorbent could lead to unacceptable high back pressures as a result of the adsorption of biogenic substances on the carbon. Pretreatment of the extract with the strongly basic adsorbent potassium silicate and silica gel protects the adsorptive capacity of the activated carbon and removes acidic and highly polar coextracts. Alkali metal silicate adsorbents (such as potassium silicate) have been demonstrated[17,52-54] to retain acidic substances such as phenolic and carboxylic acid compounds, acidic components of lipids, hydroxyl PCBs and hydroxydiphenyl ethers. Biogenic substances which are not retained by the potassium silicate/silica gel combination exhibit very low affinity for activated carbon[17] and are subsequently eluted in the first fraction along with the *ortho*-PCBs. Other coextracts are removed by the use of sulphuric acid impregnated silica gel (40%, w/w). Sulphuric acid impregnated silica gel is very effective in removing different types of compounds by reactions of dehydration, acid-catalysed condensations, oxidation and sulphonation.[17,54] On reaction with the adsorbent a number of organic compounds are converted into more polar species which are subsequently retained on silica. Silica

exhibits an affinity for polar compounds mainly due to the interaction of the hydroxyl groups of silica gel with the polar functional groups of the compounds.

2.4. CONCLUSIONS

Gas chromatographic conditions were optimised for the separation of PCB congeners and performance criteria set for the resolution of an Aroclor test mixture and particularly the separation of PCB-118 and PCB-123. Quantification of the analytes of interest was based on the use of isotopically labelled $^{13}C_{12}$ internal standards using selected ion monitoring mass spectrometry.

The degree of *ortho* chlorine substitution was observed to control the retention characteristics of polychlorinated biphenyls on the carbon column (Amoco PX-21). Elution of a mixture containing *ortho-* and non-*ortho*-PCBs showed that retention on carbon increases with decreasing chlorine atoms substitution on the *ortho* (2,2',6,6') positions. The composition of eluting solvent was optimised to control the elution of the PCB congeners. Eluting the carbon column with (i) 300 ml hexane:DCM (80:20,v/v) and (ii) 120 ml DCM:toluene (80:20, v/v) separated the *ortho-* and non-*ortho*-PCBs into two different fractions and the efficiency of fractionation was demonstrated by the absence of cross over of the PCBs in each fraction.

Validation of the overall on-line clean-up method for *ortho-* and non-*ortho*-substituted congeners in apple was achieved by the separation of *ortho-* and non-*ortho*-PCBs and by replicate analysis of apple homogenate spiked with $^{13}C_{12}$ internal standards. The method demonstrated a good precision which when expressed as %COV ranged from 3.1-21% (mean 9.5%). The method described would be applicable to the concurrent determination of *ortho* substituted polychlorinated biphenyls and non-*ortho* substituted polychlorinated biphenyls in fruit and vegetable samples.

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CHAPTER 3

The determination of selected *ortho* and non-*ortho* polychlorinated biphenyl congeners in fruit and vegetable samples

3.1. INTRODUCTION

A number of surveys of the PCB concentrations in the foodstuffs have been carried out in various parts of the world since they were discovered in wildlife samples in 1966.[1] One of the earliest monitoring programmes was conducted by Westoo and Noren[2] and Westoo et al.[3] in which less than 1.0 μg Kg^{-1} of total PCB was found in samples of butter, margarine, vegetable oils, eggs, beef, lamb, chicken bread, biscuits and baby food. Similar investigations by Kolby,[4] Mes et al.,[5] Himberg[6] and Theelen et al.[7] have followed. However, few studies have included measurements of PCB concentrations in fruit and vegetables because of the lipophilic nature of PCBs, and thus very few data exist on which estimates of dietry intake can be based.

The most comprehensive UK investigation which encompassed fruit and vegetables sought to estimate the total dietary intake of PCBs by the general population.[8] Retail samples of cereals, meat and fish, fats, fruit and preserves, root vegetables, 'other vegetables' and milk, obtained between 1970 and 1976 from a wide range of urban locations across the UK were analysed in this study. No PCBs were detected at the limit of detection for total PCB of 10 μg Kg^{-1} level, in any of the fruit and preserve, root vegetables or other vegetables samples analysed.

In Ontario, a study was undertaken during 1985 to determine the dietary intake of PCBs in fresh foods, including root vegetables (such as potatoes), fresh fruit, leafy and other above ground vegetables.[9] No PCBs were detected at 0.1 μg Kg^{-1} total PCB level, in either the leafy vegetable composite (which composed lettuce, cabbage and tomatoes) or the root vegetable composite (in which potatoes and carrots were

major constituents). A survey by Klein[10] in Germany focused primarily on lipid-rich foods, but also included some with plant origins such as cereals and potatoes. A total of 106 potato samples were analysed but none contained total PCB concentrations above the limit of detection of 2 μg Kg^{-1} fresh mass. A WHO review of the 1980-1983 data[11] concluded that the total PCB concentrations in fruit and vegetable samples were usually in the range 0.5-5 μg Kg^{-}.[1] However, this range reflects the relatively high detection limits of the analytical methods used in the surveys.

Although a trend towards congener specific analyses of PCBs in food is evident in more recent studies, these have focused exclusively on fatty foods.[6,7,12-19] Few published data on the concentrations of individual PCB congeners in fruit and vegetable samples are currently available. The on-line clean-up method developed in chapter 2 was therefore applied to the analysis of a range of foodstuffs with detection limits significantly lower than reported previously.

3.2 Experimental

The on-line clean-up method developed in chapter 2 was applied to the determination of PCBs in fruit and vegetable samples.

3.2.1. Fruit and vegetable sampling[20]

Sampling was carried out by Staff at Environmental and Risk Assessment Unit, School of Environmental Sciences, University of East Anglia, Norwich, UK. Samples of fruit and vegetables were taken from the Panteg district of Pontypool and from three other areas, namely, the Gower peninsula (South Wales), Celedigion district (mid-Wales) and South Herefordshire, as part of the monitoring programme by the Welsh Office to asses the extent of public exposure to PCBs, dioxins and furans in the Panteg area resulting from consumption of locally grown produce. The sampling programme was carried out during the first week of September, 1993. Details of the sites from which samples of each type of fruit and vegetable were taken are given in Table 3.1.

Lettuce and cabbage samples were cut at the base of the stem. Any dead or decaying leaves that were clearly unfit for human consumption were removed and any residual soil remaining on the lower plant removed by gently washing with distilled water. Courgettes were also harvested by cutting at the base of the stem and any soil adhering to the underside removed in a similar way. Potato samples were carefully harvested to divest them of as much attached soil as possible.

Each sample was weighed in a double layer of hexane-washed aluminium foil and placed in a polypropylene sealable box (Stewart Ltd.). With the exception of cabbage

and lettuce, all samples were stored at -15 °C at the University of East Anglia, Norwich prior to preparation for distribution. Lettuce and cabbage samples were delivered directly to the analytical laboratories whilst still fresh.

Table 3.1. Sites sampled for each type of fruit or vegetable

Sample Code	Location
APPLES	
VS	Pontyfelin House, Lower New Inn
VP	Dinmore, Herefordshire
WN	Ciliaueron, Ceredigion
VR	Llangennith, Gower
LETTUCE	
VA	Pontyfelin House, Lower New Inn
VB	Afon Close, Lower New Inn
VI	Dinmore, Herefordshire
VJ	Caswell, Gower
COURGETTES	
VD	Afon Close, Lower New Inn
VE	Dinmore, Herefordshire
POTATOES	
VZ	Pontyfelin House, Lower New Inn
WA	Llangennith, Gower
CABBAGE	
VK	Afon Close, Lower New Inn
VM	Pontyfelin House, Lower New Inn

3.2.2. Sample preparation[20]

To ascertain the optimum ratio of fruit (or vegetable) sample to added water necessary to achieve a free-flowing and homogeneous mixture capable of being effectively sub-divided into representative aliquots, preliminary experiments were carried out on apples, courgettes and potatoes obtained from a local supermarket. The percentage of added water so determined for apples, courgette and potato was 50%, 25% and 25%, respectively which was then used to prepare the homogenates.

After removal from the freezer, the sample was allowed to thaw completely in its container, taking care to ensure that any condensate or fluid produced during the thawing process was retained. The fully thawed tissue and associated fluids, plus the appropriate volume of distilled water was homogenised in a Waring blender for five minutes at low speed and one minute at high speed. The homogenate was then immediately transferred into hexane pre-washed glass bottles and stored at -15 °C prior to analysis. The skin and inside of whole apple sample VS were separately prepared as homogenates, using the procedure described for the whole apple samples.

Samples of cabbage and lettuce were washed to remove any soil/animal life and blotted dry, prior to being weighed. They were then homogenised in hexane:acetone (3:1 v/v) with a solvent volume four times greater than the mass of the sample. The homogenate was then filtered, and the liquid phase transferred to a separating funnel. The acetone/water layer was separated from the hexane layer, which was then stored in bottles prior to analysis. The samples analysed in this survey are shown in Table 3.2.

Table 3.2. Matrices of fruit and vegetable samples

Code	Matrix	Nature of sample
VS	Apple	W
VS	Apple	H
WN	Apple	W
VR	Apple	H
VP	Apples	H
VM	Cabbage	E
VK	Cabbage	E
VD	Courgette	H
VE	Courgette	H
VA	Lettuce	E
VB	Lettuce	E
VI	Lettuce	E
VJ	Lettuce	E
VZ	Potato	H
WA	Potato	H

W = whole fruit or vegetable
H = Homogenate
E = Extract

3.2.3. Inter-laboratory method validation for fruit and vegetable samples

The samples were analysed by the on-line procedure described and by two other laboratories, Laboratory A and Laboratory C, using their standard methods.[21,22] The following PCBs were determined, mono-*ortho* (PCBs 105, 114, 118, 123, 153, 157, 167 and 189), di-*ortho* (PCBs 28, 52, 101, 138 and 180) and non-*ortho* (PCBs 77, 81, 126 and 169) polychlorinated biphenyls.

(a) Homogenate samples

Apple, courgette and potato homogenate samples were treated exactly as described in section 2.2.5.6. except that the non-*ortho* fraction was reconstituted into 20 μl of iso-octane after blow down. The fractions were then analysed by GC-MS (see section 2.2.5 for GC-MS conditions).

(b) Extracted samples

These samples were spiked with internal standards and directly applied to the top of the multicolumn and then treated as described in section 2.2.5.6 and the non-*ortho* fraction was finally reconstituted into 20 μl of iso-octane after blow down. The two fractions were analysed by GC-MS.

3.3. Results and discussion

3.3.1. Detection limits, blanks and inter-laboratotry validation of method

The method developed in chapter 2 was applied to the determination of PCBs in fruit and vegetable samples.

3.3.1.1. Detection limits

The limit of detection (LOD) is usually defined as the lowest concentration of an analyte that gives a signal significantly different from the analytical blank or background signal. The LOD was estimated from the amount of a PCB congener standard needed to provide a signal-to-noise ratio of 3:1 (Table 3.3), since blank background interference was not observed for *ortho*- and non-*ortho*-PCBs in this investigation. Limits of detection for the PCB congener determined in this study were <10 ng kg^{-1}. During the analyses of sample extracts, there may be variations in recovery, instrumental sensitivity, background noise and the presence of interferences which can lead to considerable variation in the ability to detect low concentrations.[23] In this work the analyte was reported as present only if a signal-to-noise ratio of >3:1 was achieved and all the acceptance criteria (section 2.2.5.2) were met.

3.3.1.2. Blanks

A reagent blank, run through the entire analytical procedure was processed every five extractions. Blank samples corresponding to both *ortho* and non-*ortho* fractions gave undetectable levels of all the eighteen *ortho* and non-*ortho* substituted PCB congeners determined in fruit and vegetable samples (Figures 3.1 and 3.2).

Table 3.3. Limits of detection for *ortho* and non-*ortho* substituted congeners (ng/kg)

	Limit of detection[a]													
	Apple			Cabbage		Courgettes		Lettuce				Potatoes	
	VP	VS	WN	VK	VM	VD	VE	VA	VB	VI	VJ	VZ	WA
Ortho-PCBs													
PCB-28	2.0	2.0	4.0	1.0	1.0	2.0	3.0	0.3	1.0	0.2	1.0	2.0	2.0
PCB-52	1.0	2.0	3.0	1.0	1.0	2.0	3.0	0.2	1.0	0.2	1.0	1.0	2.0
PCB-101	4.0	2.0	4.0	1.0	1.0	3.0	4.0	0.4	1.0	0.2	1.0	2.0	2.0
PCB-105	4.0	3.0	1.0	1.0	1.0	3.0	4.0	0.4	1.0	0.2	1.0	2.0	2.0
PCB-114	4.0	3.0	4.0	1.0	1.0	2.0	3.0	0.5	1.0	0.3	1.0	2.0	2.0
PCB-118	5.0	4.0	4.0	1.0	1.0	3.0	4.0	0.4	1.0	0.3	1.0	2.0	2.0
PCB-123	5.0	4.0	4.0	1.0	1.0	3.0	4.0	0.5	1.0	0.3	1.0	3.0	3.0
PCB-138	3.0	2.0	2.0	1.0	0.5	2.0	2.0	1.0	1.0	0.4	1.0	2.0	3.0
PCB-153	2.0	2.0	2.0	0.5	0.3	2.0	2.0	0.5	1.0	0.5	0.5	2.0	2.0
PCB-156	3.0	2.0	3.0	1.0	1.0	1.0	2.0	1.0	2.0	0.7	1.0	3.0	3.0
PCB-157	4.0	3.0	3.0	1.0	1.0	1.0	1.0	1.0	1.5	0.8	1.0	4.0	4.0
PCB-167	2.0	2.0	2.0	0.5	0.4	2.0	2.0	0.5	1.0	0.8	1.0	2.0	3.0
PCB-180	5.0	4.0	4.0	1.0	1.0	3.0	4.0	1.0	3.0	0.3	1.0	3.0	3.0
Non-*Ortho*-PCBs													
PCB-77	3.0	1.0	3.0	1.0	0.3	2.0	1.0	0.4	1.0	0.2	1.0	1.0	2.0
PCB-81	2.0	2.0	2.0	0.4	0.3	1.0	1.0	0.2	1.0	0.2	1.0	1.0	2.0
PCB-126	3.0	1.0	2.0	1.0	0.3	1.0	1.0	0.2	1.0	0.2	1.0	2.0	2.0
PCB-169	6.0	5.0	2.0	1.0	1.0	1.0	1.0	0.5	1.0	0.2	1.0	2.0	3.0
Amount used (g)	41	52	48	190	250	78	60	175	130	420	200	50	48

[a]The limit of detection was defined as the amount of standard needed to provide a signal-to-noise ratio of 3:1

Blanks containing significant amounts of non-*ortho* PCBs (especially PCB-77) have been reported in the literature[24-28] and appropriate quality assurance/quality control criteria have been adopted. For example, as a consequence of the non-zero blank values, the concentration of an analyte was only reported if the concentration exceeded two[27] or four[25] times the blank value. This was not the case in this investigation since both *ortho-* and non-*ortho-* fraction blanks were contamination free probably because in the on-line method there are less manipulation stages during the analysis which can lead to sample contamination. Weistrand et al.[29] observed high

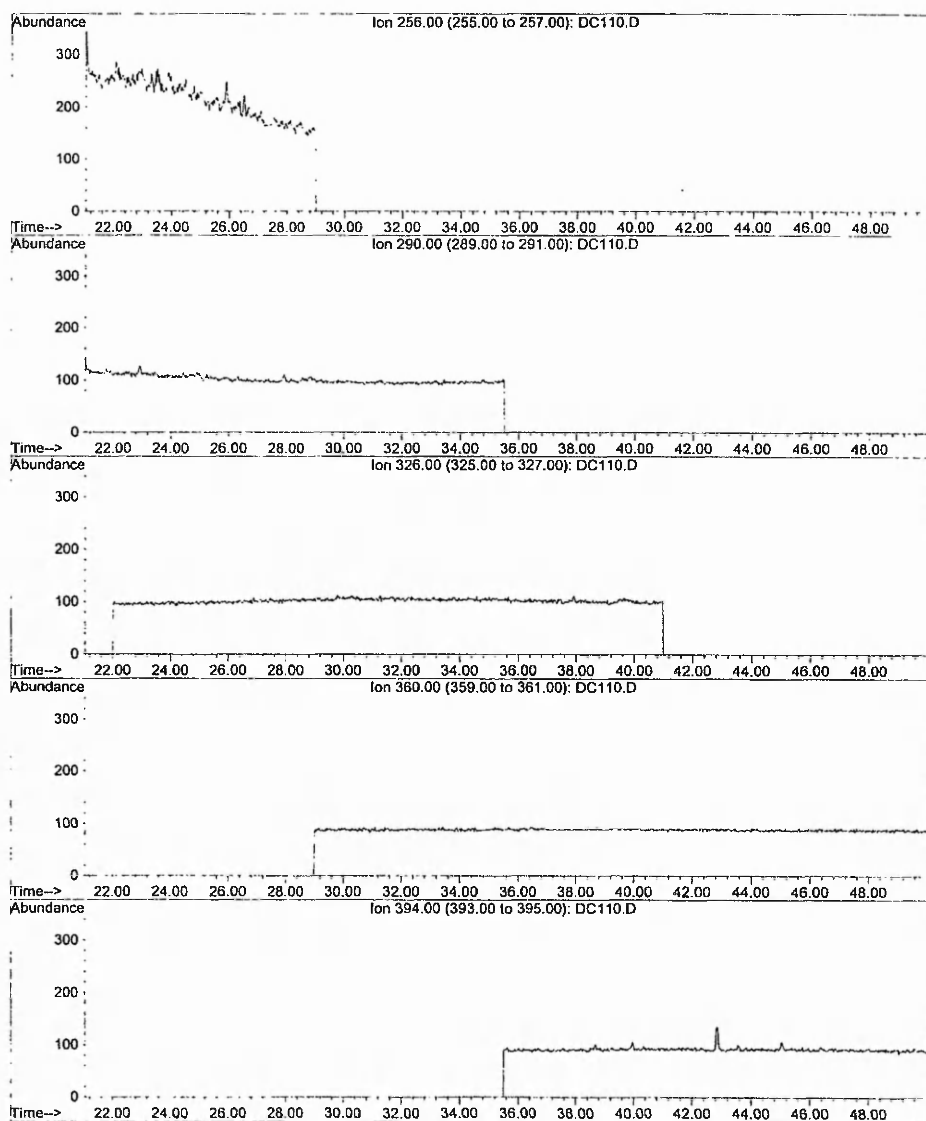

Figure 3.1. GC-MS blank run for the entire on-line clean-up method corresponding to the *ortho* fraction

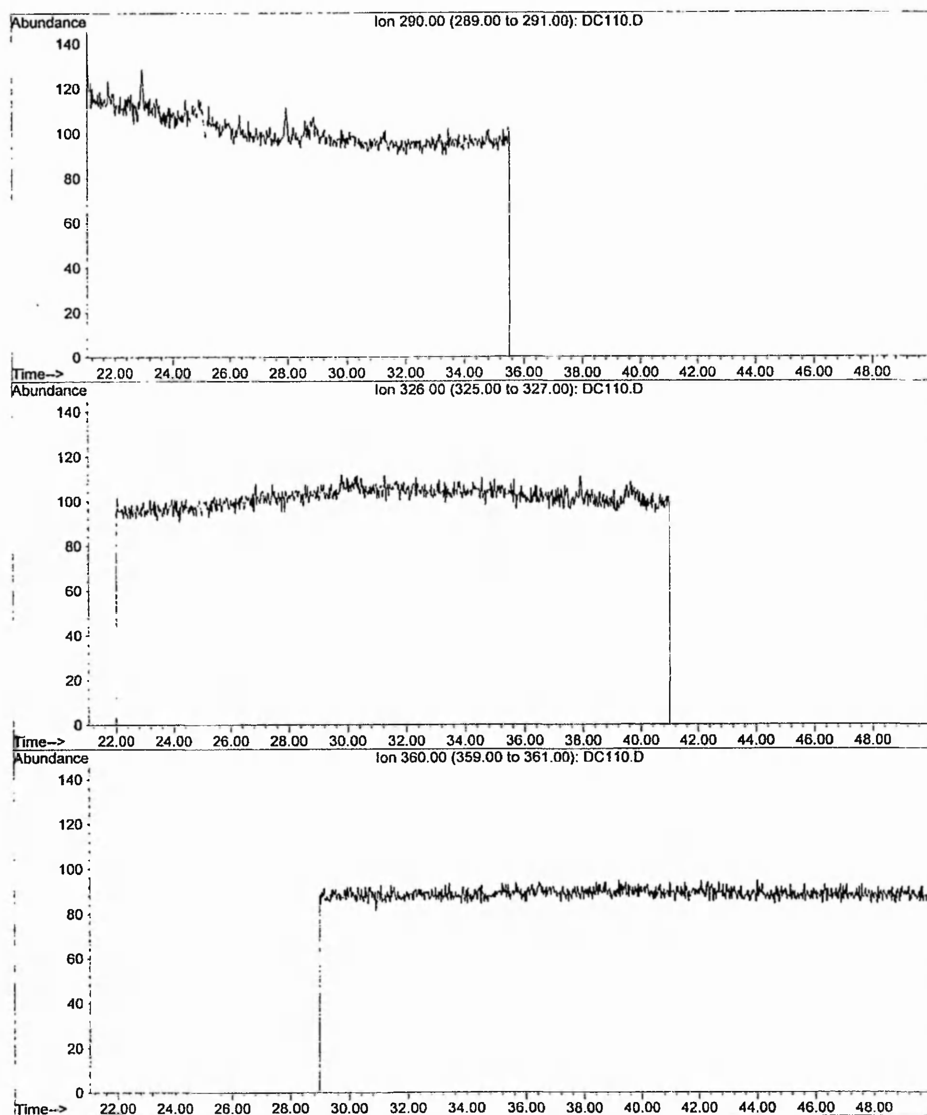

Figure 3.2. GC-MS blank run of the entire on-line clean-up method corresponding to the non-*ortho* fraction

levels of PCBs and naphthalenes in the blanks during the analyses of organochlorine compounds. The laboratory air was suspected to be contaminated and the source was found to be the capacitors and wire coatings placed next to the laboratory. This problem occurred probably because the method they used was not enclosed and therefore contamination could have occurred at any stage of the analysis, particularly those stages involving solvent evaporation and transfer of the extract between clean-up procedures. The possibility of this problem occuring in the on-line method is minimal.

3.3.1.3. Inter-laboratory method validation for fruit and vegetable samples

To establish the inter-laboratory precision, apple, cabbage, lettuce and potato samples were analysed for PCBs by the on-line method described here and by two independent laboratories, Laboratory A and Laboratory C using their standard methods.

The results for the on-line method and the two independent laboratories are shown in Tables 3.4 for apple and potato homogenate samples. In each case the reported levels reflected the differences in the limits of detection for each of the laboratories, which ranged from 0.1 $\mu g\ Kg^{-1}$ for laboratory A to $<0.01\ \mu g\ Kg^{-1}$ for the on-line clean-up, since congeners not detected were considered to contribute a concentration equivalent to the LOD for the purpose of calculating the sum of seven ($\Sigma 7$) and eighteen ($\Sigma 18$) congeners. Examination of the data indicates a satisfactory agreement between the on-line method and the other laboratories for the individual PCB congeners and for $\Sigma 7$ and $\Sigma 18$ congener data. Furthermore, the on-line method has the lowest LOD for PCBs. The ability of the method to detect PCB congeners at very low levels

compared to the other two independent laboratories allowed concentrations to be detected for fruit and vegetable samples.

Table 3.4. Inter-laboratory comparison of the PCB congeners in fruit and vegetable samples

(a) PCBs in apple homogenate sample VP (μg/Kg)

PCB	Laboratory		
	A	B[a]	C
28	<0.3	0.1	0.2
52	<0.6	0.2	0.1
77	<0.1	<0.014	0.1
81	<0.1	<0.016	<0.01
101	<0.1	0.1	0.3
105	<0.1	0.01	0.03
114	<0.1	<0.004	<0.01
118	<0.1	0.03	<0.08
123	<0.1	<0.005	0.02
126	<0.1	<0.016	0.01
138	<0.1	0.02	0.09
153	<0.1	0.02	0.14
156	<0.1	<0.003	<0.01
157	<0.1	<0.004	0.01
167	<0.1	<0.003	<0.01
169	<0.1	<0.03	0.07
180	<0.1	<0.005	0.04
189	<0.1	<0.01	0.1
[b]Σ7	1.4	0.4	0.9
[c]Σ18	2.5	0.5	1.2

B[a] this work
[b]Σ7 is the sum of congeners 28, 52, 101, 118, 138, 153 and 180 concentrations
[c]Σ18 is the total concentration for 28, 52, 77, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180 and 189

(b) PCBs in potato homogenate sample WA (μg/Kg)

PCB	Laboratory		
	A	B[a]	C
28	<0.1	0.06	0.09
52	<0.1	0.08	0.04
77	<0.1	<0.029	0.01
81	<0.1	<0.024	<0.01
101	<0.1	0.04	0.1
105	<0.1	<0.015	<0.01
114	<0.1	<0.026	<0.01
118	<0.1	0.03	0.03
123	<0.1	<0.02	<0.01
126	<0.1	<0.06	0.05
138	<0.1	<0.1	0.04
153	<0.1	<0.2	0.06
156	<0.1	<0.05	<0.01
157	<0.1	<0.1	<0.01
167	<0.1	<0.08	<0.01
169	<0.1	<0.04	0.06
180	<0.1	<0.06	0.02
189	<0.1	<0.07	0.1
Σ7	0.7	0.5	0.4
Σ18	1.7	1.0	0.7

B[a] this work

[b]Σ7 is the sum of congeners 28, 52, 101, 118, 138, 153 and 180 concentrations

[c]Σ18 is the total concentration for 28, 52, 77, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180 and 189

3.3.2. PCB concentration in fruit and vegetables

3.3.2.1. Distribution of PCBs in apple

Table 3.5 shows the PCB concentrations detected on the skin and the inside flesh of an apple sample (VS), in the whole apple sample and an apple homogenate sample from the same batch, determined by the on-line method. The homogenate sample was also determined by other two independent laboratories (Table 3.6). The results (Table 3.5) show that there is a significantly elevated concentration of PCBs on the apple skin compared to the flesh, which is consistent with the accumulation of airborne PCBs in the wax coating of the apple. The sum of PCB concentrations in skin and flesh determined by the on-line method (Table 3.5) agrees with the levels in the apple homogenate determined by the on-line method and for the apple homogenate determined by the other two laboratories (Table 3.6). This indicates also the good agreement between the on-line clean-up method and the other two laboratories for the individual PCB congener and for Σ7 and Σ18 congener data and confirms the reliability of the on-line method. Whole apple results show poorer agreement presumably reflecting the much greater sample to sample variation in PCB concentrations for individual apples compared to that observed for the apple homogenates.

3.3.2.2. PCB concentrations in fruit and vegetables

The *ortho-* and non-*ortho* substituted fractions were analysed by GC-MS in SIM mode as described in section 2.2.5. Typical chromatograms for *ortho-* and non-*ortho* fractions for an apple homogenate sample extracts (sample VS) are presented in Figures 3.3 and 3.4, respectively. Extracts were generally clean and all congeners

Table 3.5. Comparison of PCB concentrations in flesh, skin, whole apple and apple homogenate of apple sample VS

(a) Laboratory B[a]

PCB (μg/Kg)	skin	flesh	whole	homogenate
28	0.249	0.041	0.290	0.18
52	0.211	0.026	0.237	0.28
77	0.056	0.002	0.058	0.05
81	<0.001	<0.002	<0.003	<0.003
101	0.247	0.020	0.267	0.43
105	0.038	0.004	0.042	0.07
114	<0.004	<0.004	<0.008	<0.01
118	0.120	0.015	0.135	0.19
123	<0.002	<0.002	<0.004	<0.01
126	<0.004	<0.001	<0.005	<0.003
138	0.348	0.014	0.362	0.38
153	0.328	0.015	0.343	0.23
156	0.026	<0.02	<0.046	0.03
157	<0.007	<0.01	<0.017	<0.003
167	0.030	<0.009	<0.039	0.015
169	<0.001	<0.007	<0.008	<0.007
180	0.189	<0.02	<0.209	0.28
189	<0.01	<0.02	<0.03	<0.006
[b]Σ7	1.7	0.15	1.85	2.0
[c]Σ18	1.9	0.20	2.10	2.2

[a]This work
[b]Σ7 is the sum of congeners 28, 52, 101, 118, 138, 153 and 180 concentrations
[c]Σ18 is the total concentration for 28, 52, 77, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180 and 189

Table 3.6. Comparison of PCB concentrations in whole apple and apple homogenate of apple sample VS

PCB	Laboratory A		Laboratory C	
(μg/Kg)	whole	homogenate	whole	homogenate
28	<0.6	<0.1	0.4	0.3
52	<0.6	<0.1	0.1	0.06
77	<0.1	<0.1	0.05	0.06
81	<0.1	<0.1	<0.01	<0.01
101	<0.6	<0.2	1.6	0.7
105	<0.1	<0.1	0.2	0.03
114	<0.1	<0.1	<0.01	<0.01
118	<0.2	<0.1	0.4	0.1
123	<0.1	<0.1	0.07	<0.01
126	<0.1	<0.1	0.05	0.09
138	<0.4	0.3	1.2	0.5
153	<0.3	0.8	1.6	0.7
156	<0.1	<0.1	0.04	0.01
157	<0.1	<0.1	<0.01	<0.01
167	<0.1	<0.1	0.09	0.03
169	<0.1	<0.1	0.05	0.08
180	<0.2	<0.1	0.6	0.2
189	<0.1	<0.1	0.1	0.09
Σ7	2.9	1.7	5.9	2.6
Σ18	4.0	2.8	6.6	3.0

Σ7 is the sum of congeners 28, 52, 101, 118, 138, 153 and 180 concentrations
Σ18 is the total concentration for 28, 52, 77, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180 and 189

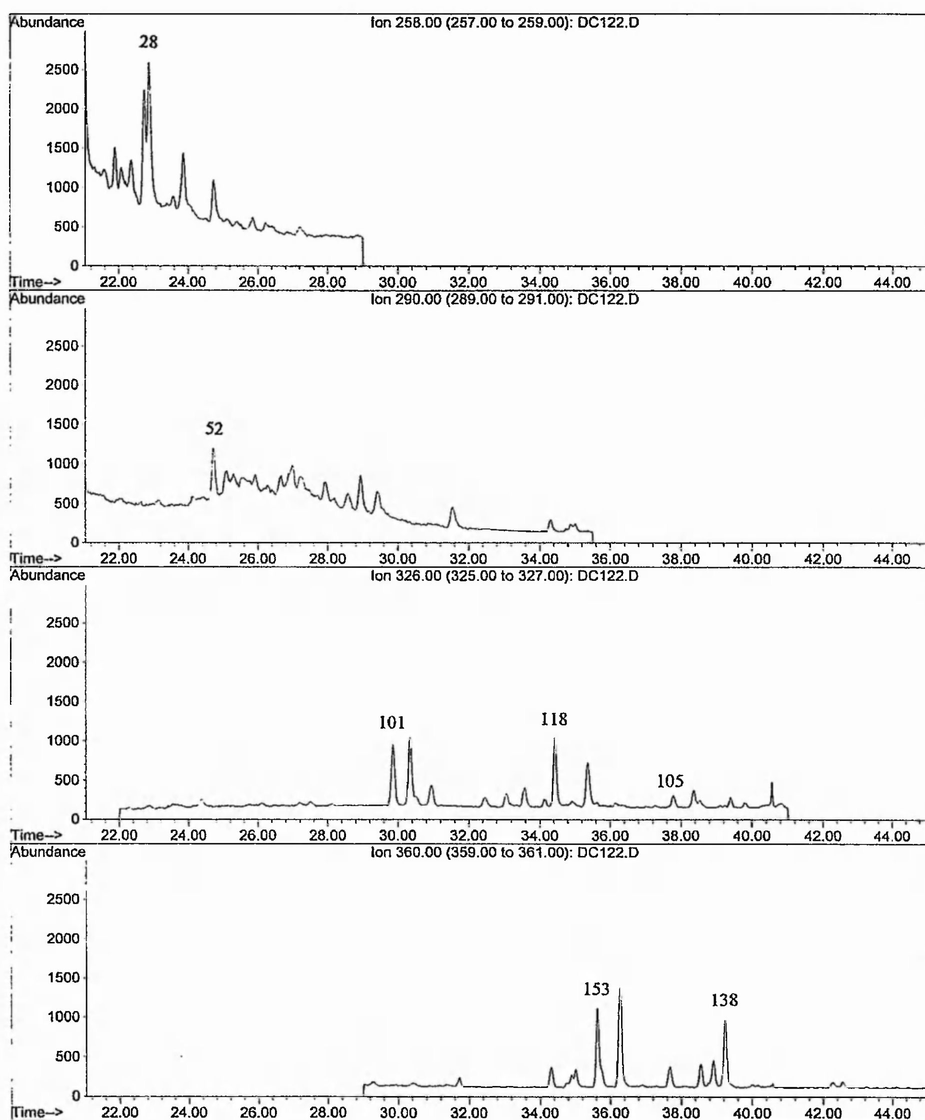

Figure 3.3. Typical GC-MS chromatograms of some *ortho*-substituted PCBs identified in apple homogenate sample VS

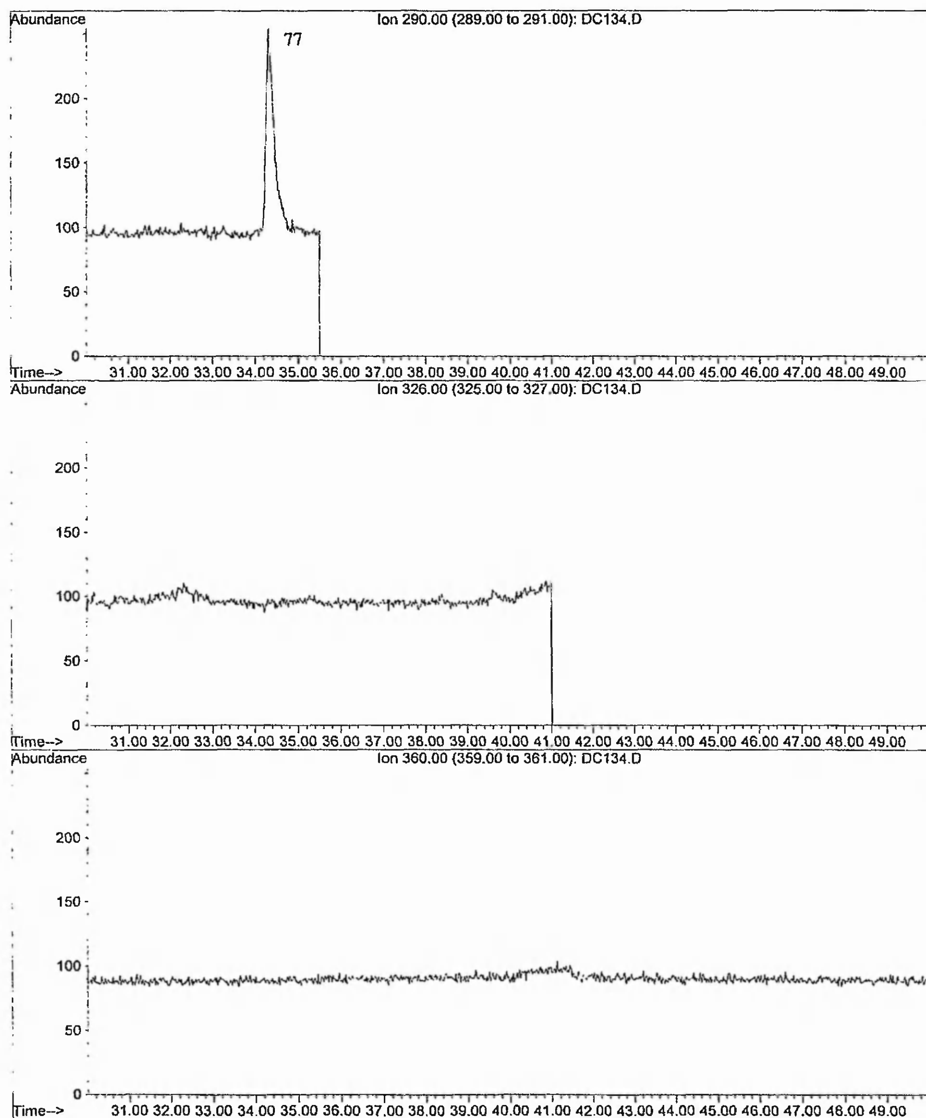

Figure 3.4. GC-MS chromatograms for non-*ortho* PCBs identified in apple homogenate sample VS

determined were adequately separated from closely eluting congeners. In some cases though, interferences present in one of the two channels monitored for PCB-180 made it difficult to quantify this congener. In this case, the area of only one channel was used for quantification as shown in Figure 3.5 in which the channel for m/z 394 for apple homogenate sample VS could not be used for quantification because of the large interfering peaks present at the retention time of PCB-180.

Details of the PCB concentrations found in fruit and vegetable samples are presented in Tables 3.7-3.11. Inspection of these results reveals that in many samples individual congeners were often below the LOD. The major congeners present in fruit and vegetable samples, in most cases were PCBs 28, 52, 101, 118, 138 and 153. In all cases where PCBs were detected, levels were ≤0.2 μg kg^{-1}. Such low values present analytical problems in reliably quantifying concentrations, and may make it difficult to establish trends in the data. However, it is worth noting that PCBs 28 and 52 most consistenly recorded concentrations above 0.1 μg kg^{-1}. This predominance of the lower chlorinated PCBs for fruit and vegetable samples is also found in high volume air samples[30] and so the results are consistent with atmospheric deposition being an important contamination pathway. This provides further evidence to support the results in Table 3.5 which presents the outcome of separate PCB analyses conducted on apple skin and flesh. It is readily apparent that the concentrations in the skin were greater and the contrasts appear to be particularly pronounced for congeners 28, 52, 101, 118, 138, 153 and 180.

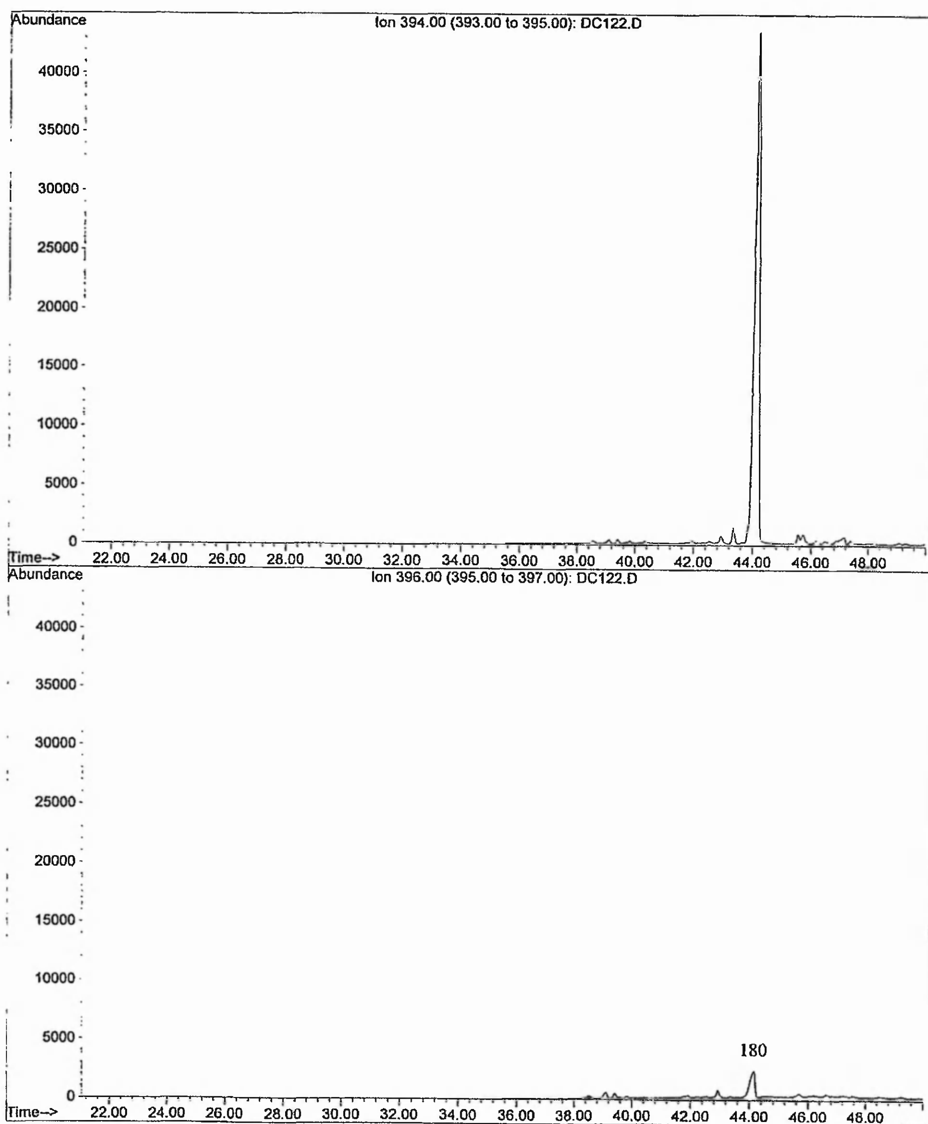

Figure 3.5. GC-MS chromatograms for (a) channel m/z 394 and (b) channel m/z 396 of apple extract

Table 3.7. PCBs in apple samples

PCB (µg/kg)	VP[a]	VR[a]	VS[a]	VS[b]	Wn[b]
28	0.1	0.1	0.18	0.29	0.2
52	0.2	0.02	0.28	0.237	0 1
77	<0.014	<0.001	0.05	0.058	<0.004
81	<0.016	<0.001	<0.003	<0.003	<0.01
101	0.1	0.02	0.43	0.267	0.04
105	0.01	0.01	0.07	0.042	0.007
114	<0.004	<0.01	<0.01	<0.008	<0.005
118	0.03	0.03	0.19	0.135	0.03
123	<0.005	<0.001	<0.01	<0.004	<0.009
126	<0.16	<0.002	<0.003	<0.005	<0.004
138	0.02	0.004	0.38	0.362	0.01
153	0.02	0.01	0.23	0.343	0.01
156	<0.003	<0.005	0.03	<0.046	<0.003
157	<0.004	<0.005	<0.003	<0.017	<0.005
167	<0.003	<0.002	0.015	<0.039	<0.006
169	<0.03	<0.006	<0.007	<0.008	<0.012
180	<0.005	<0.009	0.28	<0.209	<0.023
189	<0.01	<0.01	<0.006	<0.03	<0.027
[c]Σ7	0.4	0.1	2.0	1.85	0.4
[d]Σ18	0.5	0.2	2.2	2.10	0.5

[a]homogenate
[b]whole apple
[b]Σ7 is the sum of congeners 28, 52, 101, 118, 138, 153 and 180 concentrations
[c]Σ18 is the total concentration for 28, 52, 77, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180 and 189

Table 3.8. PCBs in cabbage extract samples

PCB (μg/kg)	VK	VM
28	0.06	0.06
52	0.1	0.04
77	<0.01	<0.002
81	<0.01	<0.008
101	0.02	0.06
105	0.008	0.014
114	<0.002	<0.001
118	0.011	0.02
123	<0.003	<0.004
126	<0.01	<0.009
138	0.02	0.05
153	0.03	0.06
156	<0.005	<0.002
157	<0.005	<0.004
167	<0.002	<0.003
169	<0.01	<0.012
180	<0.04	<0.018
189	<0.008	<0.018
Σ7	0.3	0.3
Σ18	0.4	0.3

Σ7 is the sum of congeners 28, 52, 101, 118, 138, 153 and 180 concentrations
Σ18 is the total concentration for 28, 52, 77, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180 and 189

Table 3.9. PCBs in courgette homogenate samples

PCB (μg/kg)	VD	VE
28	<0.01	0.1
52	0.04	0.2
77	<0.011	<0.14
81	<0.011	<0.02
101	0.02	0.09
105	0.004	<0.006
114	<0.017	<0.052
118	0.01	0.04
123	<0.01	<0.013
126	<0.009	<0.12
138	0.03	0.02
153	0.008	0.02
156	<0.014	<0.021
157	<0.019	<0.015
167	<0.029	<0.017
169	<0.005	<0.2
180	<0.022	<0.033
189	<0.022	<0.017
Σ7	0.1	0.5
Σ18	0.3	1.2

Σ7 is the sum of congeners 28, 52, 101, 118, 138, 153 and 180 concentrations
Σ18 is the total concentration for 28, 52, 77, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180 and 189

Table 3.10. PCBs in lettuce extract samples

PCB (μg/kg)	VA	VB	VI	VJ
28	0.04	0.05	0.03	0.02
52	0.03	0.01	0.006	0.01
77	<0.002	<0.004	<0.002	<0.01
81	<0.002	<0.015	<0.001	<0.016
101	0.03	0.01	0.003	0.004
105	0.007	<0.002	<0.001	0.003
114	<0.003	<0.006	<0.001	<0.002
118	0.02	0.007	0.02	0.003
123	<0.002	<0.004	<0.001	<0.004
126	<0.003	<0.002	<0.001	<0.012
138	0.04	0.02	<0.002	0.002
153	0.06	0.04	<0.002	0.002
156	<0.004	<0.006	<0.001	<0.002
157	<0.002	<0.006	<0.003	<0.002
167	<0.002	<0.008	<0.002	<0.002
169	<0.002	<0.003	<0.001	<0.025
180	0.03	0.05	<0.002	<0.04
189	<0.016	<0.084	<0.01	<0.04
Σ7	0.2	0.2	0.1	0.1
Σ18	0.3	0.3	0.2	0.2

Σ7 is the sum of congeners 28, 52, 101, 118, 138, 153 and 180 concentrations
Σ18 is the total concentration for 28, 52, 77, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180 and 189

Table 3.11. PCBs in potato homogenate samples

PCB (μg/kg)	VZ	WA
28	0.04	0.06
52	0.09	0.08
77	<0.027	<0.029
81	<0.033	<0.024
101	0.09	0.04
105	<0.018	<0.015
114	<0.006	<0.026
118	0.03	0.03
123	<0.025	<0.02
126	<0.059	<0.06
138	0.024	<0.1
153	0.03	<0.2
156	<0.091	<0.05
157	<0.077	<0.1
167	<0.081	<0.08
169	<0.07	<0.04
180	<0.06	<0.06
189	<0.056	<0.07
Σ7	0.4	0.5
Σ18	0.9	1.0

Σ7 is the sum of congeners 28, 52, 101, 118, 138, 153 and 180 concentrations
Σ18 is the total concentration for 28, 52, 77, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180 and 189

The results also show that the PCB concentrations in those samples directly exposed to the atmospheric deposition are not obviously different from those in vegetables grown underground. This reflects the fact that root vegetables come into contact with cultivated surface soil.

The highest PCB values, although still very low, were found in apples (VS), the samples with the greatest PCB concentrations being from Pontyfelin House, which is near an incinerator. These samples therefore reflect the higher concentration in air detected in this area. The individual PCB and total of seven and eighteen congener levels are generally low in the Panteg area and are similar to other areas in the UK.[31] There is little evidence to suggest that human exposure to PCBs in fruit and vegetables, grown in the Panteg district is likely to result in intakes of PCBs, that are significantly higher than those from background locations.

(a) Congener profiles

The presence of polychlorinated biphenyls in fruit and vegetable samples reflects their origin in the environment which arise, almost without exception, from the use of commercial formulations. After their release into the environment, the technical mixtures can undergo further modification, in respect of the proportion of individual compounds present, as a result of photodagradation or hydrolysis, preferential solutbility, adsorption or evaporation or mixing with other commercial PCB products manufactured at different level of chlorination. The variable partitioning of the congeners between different trophic levels, as well as the favoured biological degradation of certain congeners make the composition of PCBs in samples quite different from that of commercial mixtures.

The environmental fate of PCBs is dependent on several factors which include the degree of chlorination and the position of chlorine substituents on the biphenyl ring.[32-41] The most bioaccumulating PCB congeners have five to seven chlorine atoms per molecule. The more highly chlorinated congeners are generally less available to organisms both because they are more tightly bound with soils and sediments and because they are usually present in lower quantities in the environment.

3.3.2.3. TEQs for fruit and vegetable samples

Using concentrations from Tables 3.7-3.11 for the PCB congeners determined during this investigation, TEQs for the fruit and vegetable samples were calculated and are presented in Table 3.12 along with the percentage contribution of each congener. In cases where a PCB congener concentration was reported as limit of detection, this value was considered to contribute a concentration equivalent to the LOD for the purpose of calculating the TEQ values. Therefore, the TEQs calculated in this investigation represent the maximum attainable values and will affect the percentage contribution of each congener to the total TEQ value. This implies that the percentage contribution of each congener (Table 3.12) does not reflect a true but a maximum percentage contribution to the total TEQ value. The TEFs values for mono-*ortho*-PCB-28 and di-*ortho*-PCBs 52, 101, 138 and 153 and non-*ortho*-PCB-81, are considered to be very small[42] and hence were taken as zero.

From Table 3.12, the important congeners in general, in terms of TEQ, are the non-*ortho* substituted congeners PCBs 77, 126 and 169 (total contribution of 96.1%) of which the most important one is PCB-126 which contributes approximately 82.7%. The TEQ percentage value for PCB-126 (82.7%) agrees with Krokos[43] (87%) for

retail British milk samples. The mono-*ortho*-PCBs contributed a small percentage (3.38%) to the total TEQ and the di-*ortho* congeners made a little contribution to the TEQ. Among the mono-*ortho* congeners, PCBs 114, 118 and 156 (0.63, 0.74 and 0.81%, respectively) are the most important, in this investigation. There is no TEQ data on fruit and vegetables to compare with data, which is one of the most detailed for this class of food.

Table 3.12. TEQs for polychlorinated biphenyls in Welsh fruit and vegetable samples and percentage contribution of individual congeners to TEQ

		Contribution (%) of individual congeners to TEQ																	
A	B	28	52	77	81	101	105	114	118	123	126	138	153	156	157	167	169	180	189
Apple																			
VP	1.2	0	0	0.4	0	0	0.1	0.1	0.2	0.03	83	0	0	0.1	0.1	0.002	16	0.003	0.1
VR	0.3	0	0	0.2	0	0	0.4	1.8	1.1	0.04	71	0	0	0.9	0.9	0.01	21	0.03	0.4
VS[a]	0.5	0	0	5.6	0	0	1.6	1.1	4.2	0.2	67	0	0	3.3	0.3	0.03	16	0.6	0.1
VS[b]	0.7	0	0	4.3	0	0	0.6	0.6	2.0	0.1	75	0	0	3.4	1.3	0.1	12	0.3	0.5
WN	0.5	0	0	0.4	0	0	0.4	1.9	0.6	0.2	74	0	0	0.3	0.5	0.01	22	0.04	0.5
Cabbage																			
VK	1.1	0	0	0.5	0	0	0.1	0.1	0.1	0.03	90	0	0	0.2	0.2	0.002	9	0.04	0.1
VM	1.0	0	0	0.1	0	0	0.1	0.5	0.2	0.01	87	0	0	0.1	0.2	0.003	12	0.02	0.2
Courgettes																			
VD	1.0	0	0	0.6	0	0	0.04	0.9	0.1	0.1	91	0	0	0.7	1.0	0.03	5.1	0.02	0.2
VE	14	0	0	0.5	0	0	0	0.2	0.03	0.01	85	0	0	0.1	0.1	0.001	14	0.002	0.01
Lettuce																			
VA	0.3	0	0	0.3	0	0	0.2	0.5	0.6	0.1	91	0	0	0.6	0.3	0.01	6.1	0.1	0.5
VB	0.5	0	0	0.8	0	0	0.1	1.2	0.3	0.2	80	0	0	1.2	1.2	0.03	12	0.2	1.6
VI	0.1	0	0	0.8	0	0	0.1	0.4	1.7	0.1	83	0	0	0.4	1.3	0.02	8.3	0.02	0.8
VJ	1.5	0	0	0.3	0	0	0.02	0.1	0.02	0.03	82	0	0	0.02	0.1	0.001	17	0.03	0.3
Potatoes																			
VZ	6.7	0	0	0.02	0	0	0.03	0.04	0.04	0.03	88	0	0	0.7	0.07	0.01	11	0.01	0.1
WA	6.5	0	0	0.2	0	0	0.02	0.2	0.05	0.03	92	0	0	0.1	0.8	0.01	6.0	0.01	0.1
Average		0	0	1.0	0	0	0.2	0.6	0.7	0.1	83	0	0	0.8	0.5	0.02	12	0.1	0.3

A = Sample code
B = TEQ (ng/kg whole sample), TEFs from Table 1.4 have been used for the calculations of TEQs
[a]Homogenate apple sample
[b]whole apple sample

3.4. CONCLUSIONS

The on-line method developed for the fractionation of polychlorinated biphenyls on the carbon column was successfully applied to the determination of *ortho* and non-*ortho* substituted PCBs in fruit and vegetable samples. Limits of detection varied from sample to sample and were generally ≤ 0.1 μg kg^{-1}.

Concentrations of single PCBs found in fruit and vegetables in many cases were below the detection limits for the on-line method and the other two laboratories and this can present a problem when comparing data. The determination of PCBs in fruit and vegetable samples and especially at low levels present in these homogenates is a very demanding analytical challenge, but where significant numbers of congeners were above the detection limit (for example in some apple samples) then generally good agreement was observed between the on-line method developed and the other two laboratories. PCBs in fruit and vegetables contribute little to daily intake for the samples analysed. However a more significant contribution might be expected to result from the consumption of locally grown food in areas with highly elevated concentration of PCBs in air arising from a point source.

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CHAPTER 4

Multidimensional high performance liquid chromatography-gas chromatography determination of PCBs

4.1. INTRODUCTION

A wide variety of multistep analytical procedures have been reported for the trace determination of PCBs (chapter 1, section 1.2) in environmental and food samples. These procedures are characterised by a series of discrete open column or low pressure chromatographic separation, usually linked by manual transfer, evaporation or other steps. Sample pre-treatment methods of this type have, however, been found in inter-laboratory tests to be the most problematic area in PCB determination[1,2] requiring the highest level of care to avoid poor precision, analyte loss and the introduction of interferences. Sample pretreatment method should therefore be as simple as possible for routine purposes requiring a minimum of sampling and handling steps.

The use of on-line procedures linking low resolution separation steps, such as those described in chapters 2 and 3, and automated sample clean-up procedures[3-5] offers one approach to improving the quality of the clean-up. An alternative approach is to employ on-line high resolution separation technique such as high performance liquid chromatography (HPLC) and capillary gas chromatography (GC). In the multidimensional high performance liquid chromatography-gas chromatography (HPLC-GC) method the sample is separated on the HPLC column and the fraction of interest is transferred on-line directly to the GC capillary column for further separation (section 1.3.4). The HPLC fraction can be introduced to the GC through an on-column or a loop-type interface and a solvent vapour exit[6-8] is needed to protect the GC detector from massive solvent input. This should remove the eluent vapours as early as possible without solute loss. The solvent vapour exit can be located at the

entrance to the GC pre-column, after the pre-column or between the separation column and the GC detector inlet.

There are two methods for evaporating the solvent; the partially concurrent and fully concurrent solvent evaporation techniques.[6-10] In the partially concurrent solvent evaporation technique, the temperature of the GC oven is kept below the eluent boiling point at the carrier gas inlet pressure. In the fully concurrent solvent technique the temperature of the GC oven is kept above the eluent boiling point at the carrier gas inlet pressure. These procedures are described in detail in section 1.3.4.1.

On-line HPLC-GC has been applied to the determination of PCBs in human blood plasma, coal tar, water, fish, sediment and vegetable oil. These methods were employed to determine total PCB, or either of the *ortho* or non-*ortho* substituted PCB fractions, but not both, in a single run. Grimvall et al.[8] isolated non-*ortho*-PCBs in plasma from other PCBs and matrix components by HPLC on a dinitroanilino-propyl silica column and collected a heart-cut fraction that was transferred on-line through a loop-type inter-face to the GC column (5% phenylmethylpolysiloxane phase) using mass spectrometric detection. The *ortho*-PCBs were analysed by off-line GC-ECD following HPLC fractionation. Cortes et al.[11] determined total PCB concentration in coal tar by transferring a PCB fraction on-line, from a silica HPLC column, to the GC column (polyethylene glycol phase) with flame ionisation detection. In an alternative approach, Noroozian et al.[12] determined total PCB in water by transferring an extract directly from the injector to the GC column (100% dimethylpolysiloxane phase) *via* a loop-type interface. Hyvonen et al.[13] was able to separate, *ortho*-PCBs in fish on an

HPLC cyano phase and transfer them on-line through a loop-type interface to the GC column (100% dimethylpolysiloxane phase), whilst Grob et al.[14] analysed for total PCB in the same matrix by isolating the PCB fraction from other components on a silica HPLC column and transferring the fraction on-line through a loop-type interface to the GC column (100% methylpolysiloxane phase). Marris[15] was able to separate *ortho*-PCBs from non-*ortho*-PCBs and other components of sediment samples on a silica HPLC column. The *ortho*-PCBs were then transferred on-line, *via* an on-column interface, to the GC column (100% dimethyl polysiloxane phase) for analysis. Size exclusion chromatography (SEC)[16] has also been used to fractionate PCBs in vegetable oil, which were then transferred on-line, through an on-column interface, to the GC column (5% phenyl 1% vinylmethylpolysiloxane phase).

The aim of this work was to investigate the applicability of HPLC-GC for the concurrent on-line fractionation and determination of both *ortho*- and non-*ortho*-substituted PCB congeners using a porous graphitic carbon (PGC) HPLC column. PGC is an HPLC phase which has been shown to be a highly efficient medium for the separation of these PCB classes.[17,18] The method is demonstrated for the analysis of commercial PCB mixture and soil samples.

4.2. EXPERIMENTAL

4.2.1. PCB standards and other chemicals

Native $^{12}C_{12}$ PCB standards in iso-octane or n-hexane were obtained either from Accu Standard Inc. (25 Science Park, New Haven, USA), Greyhould Chemical LTD (UK), National Research Council (Canada), or Laboratory of the Government Chemist (Teddington, Middlesex, UK).

Internal quantification standards: Isotopically labelled PCB standards in iso-octane were obtained from Cambridge Isotope Laboratories (Woburn, Massachusetts, USA).

Neat Aroclor 1254 (98%) was obtained from Promochem, UK.

Pyrene was purchased from Aldrich Chemical Co. Ltd., Dorset, U.K (Analytical reagent grade)

Hexane (distol grade, Fisons Scientific Equipment, Loughborough, UK).

Toluene (distol grade, Fisons Scientific Equipment, Loughborough, UK).

Acetone (distol grade, Fisons Scientific Equipment, Loughborough, UK)

Anhydrous sodium sulphate (AR, Fisons Scientific Equipment, Loughborough, UK). Washed with hexane, dried at 250 °C for 16 hours and stored at 130 °C in an oven.

Sodium hydrogen carbonate (AR, Fisons). Washed with hexane and stored at 130 °C.

Silica gel 60 (Merck 7734, 70-230 mesh, UK). Washed with hexane and activated at 130 °C and stored in a tightly closed container.

Concentrated sulphuric acid (AR, specific gravity 1.84, Fisons Scientific Equipment, Loughborough, UK).

Sulphuric acid impregnated silica gel. Two parts of concentrated sulphuric acid by weight were added to three parts of activated silica gel in a cornical flask and mixed by shaking until it was free of lumps.

Glass wool. Washed with hexane, dried and stored at 130 °C.

Potassium hydroxide pellets (AnalaR grade, Fisons Scientific Equipment, Loughborough, UK)

Potassium silicate was prepared as described in section 2.2.1.

Water (distilled)

4.2.2. Equipment

Rotary evaporator: Solvent evaporation was performed on a Buchi Rotary evaporator RE-121 fitted with a Buchi Waterbath 461 (Buchi, Switzerland). The water bath temperature was maintained at 40 °C and solvent evaporation was accomplished

under reduced pressure. Between each sample the vapour duct unit was rinsed with hexane.

Blow down: For solvent reduction of small sample volumes (<4 ml), solvents were blown down in a 1.1 tapered vials without any heating, under a steady stream of purified compressed air (air pressure at 2 psi). Charcoal and molecular sieves were used to purify the air.

Vials: All vials were obtained from Chromacol Ltd., UK. 1.1 ml tapered screw top vials (1.1-STVG type) were used. Silicone/PTFE septa were used for sealing the vials.

4.2.3. HPLC-GC instrumentation

The HPLC-GC system is reported in detail elsewhere[19,20] and is briefly described below.

4.2.3.1. HPLC instrumentation

The HPLC consisted of an LKB 2150 dual piston pump (LKB, Bromma, Sweden) and a Waters 455 variable wavelength UV detector (Waters Associates, Milford, MA, USA) fitted with a 12 µl flow cell. The column was 10 cm x 3 mm i.d. porous graphitic carbon (PGC, Hypercarb PH 100, Wellington House, Waterloo Street West, Macclesfield, Cheshire SK11 6PJ, England, 7-µm particle size) eluted with 5% toluene in hexane or 100% toluene mobile phase at 300 µl min^{-1}. The injection valve was a Negretti-Zambra 6-port fitted with a 20 µl loop.

4.2.3.2. HPLC-GC switching interface

Switching between the HPLC and the GC was achieved by using a Valco C10W 10-port switching valve (Valco Instruments Co. Inc., Houston, TX, USA) as part of an on-column interface (Figure 4.1). Effluent eluting from the UV detector was directed to one port of the valve using stainless steel tubing (45 cm x 0.25 mm i.d). An adjacent port was fitted with a length of uncoated deactivated fused silica transfer line (45 cm x 0.22 mm i.d). The coupling was secured by using a conventional stainless steel ferrule over a 0.5 cm x 0.25 mm i.d. PTFE tubing sleeve, through which the fused silica transfer line was passed. The other end of the transfer line was threaded through the septum of the GC split/splitless injector port and into the uncoated pre-column to a depth of *ca* 10 cm. The pre-column was secured *ca* 5 cm inside the injector, thus the end of the transfer line was *ca* 5 cm inside the GC oven. The switching of the valve allowed the effluent from the HPLC column to be directed to waste, or to the GC to deliver appropriate fraction. This arrangement also allowed the forward flushing of the transfer line with mobile phase, as well as backflushing with carrier gas. The supply of the carrier gas from the PCSS module to the GC injector port was connected *via* the Valco C10W valve in both switching positions.

4.2.3.3. Pneumatic column switching system

The pneumatic column switching system (PCSS, SGE UK Ltd., Milton Keynes) was installed in a Carlo Erba Fractovap 4160 GC (Carlo Erba Strumentazione, Milan, Italy) and is shown schematically in Figure 4.1. Detailed arrangements for the solvent early vapour exit (EVE) and the supply of liquid CO_2 to the cold trap within the HPLC-GC system is shown in Figure 4.2. Helium was used as carrier gas at a flow

rate of 1 ml min^{-1} in the analytical column. The carrier gas connection to the GC split/splitless injector was *via* the inlet pressure regulator of the PCSS module and a Valco C10W switching valve (see Figure 4.1). Liquid CO_2 was direct from the cylinder (BOC Ltd., UK) with no pressure regulator, using 0.5 mm i.d. stainless steel tubing. The solvent early vapour exit was a length of uncoated deactivated fused silica (*ca* 20 cm x 0.25 mm i.d). This tubing diverted the effluent from the solvent early vapour exit to waste through an activated carbon trap.

The inlet of the mid-point double 'T' union was connected to the split/splitless injector of the GC by a 10 m x 0.53 mm i.d uncoated deactivated fused silica pre-column (Hewlett-Packard Ltd., UK). A 0.23 meter length of 0.53 mm i.d. x 1.5 µm film thickness DB-5 wide bore column (J&W, Folson, CA, USA) was butt connected between the outlet of the pre-column and the inlet of the double 'T' for some experiments. The double 'T' was connected to the mid-point split 'T' using a 13 cm x 0.53 mm i.d., 0.1 µm film thickness length of BP-1 wide bore column (SGE International, Pty., Ltd., Australia), threaded through the cryogenic cold trap 'T' piece, which enabled the cryogenic trapping of analytes on the BP-1 column section (see Figure 4.2). The inlet end of the GC capillary column (30 m x 0.25 mm i.d. 0.20 µm film thickness DB-17 (J&W, Folson, CA, USA) was connected to the vacant arm of the mid-point split 'T' piece and was threaded inside the wide bore BP-1 column section to a depth of *ca* 1-2 mm. The outlet end of the GC capillary column was connected to an electron capture detector (ECD-400, Carlo Erba Strumentazione, Milan, Italy) utilising nitrogen as make-up gas (310 kPa) held at 330 °C or to a

quadrupole ion trap mass spectrometer (Finnigan MAT, ITMS). The PCSS module used to control the switching operations is shown in Figure 4.3.

(a) Quadrupole ion trap mass spectrometer (QITMS) conditions

The outlet end of the GC capillary column was threaded through the GC/MS transfer line held at 280 °C and into the QITMS. The pressure inside the vacuum manifold at 120 °C was 2.0×10^{-5} torr (uncorrected). Full scanning electron ionisation (EIMS) was performed over the mass range m/z 234-425 with ionisation time of 1000 μs.

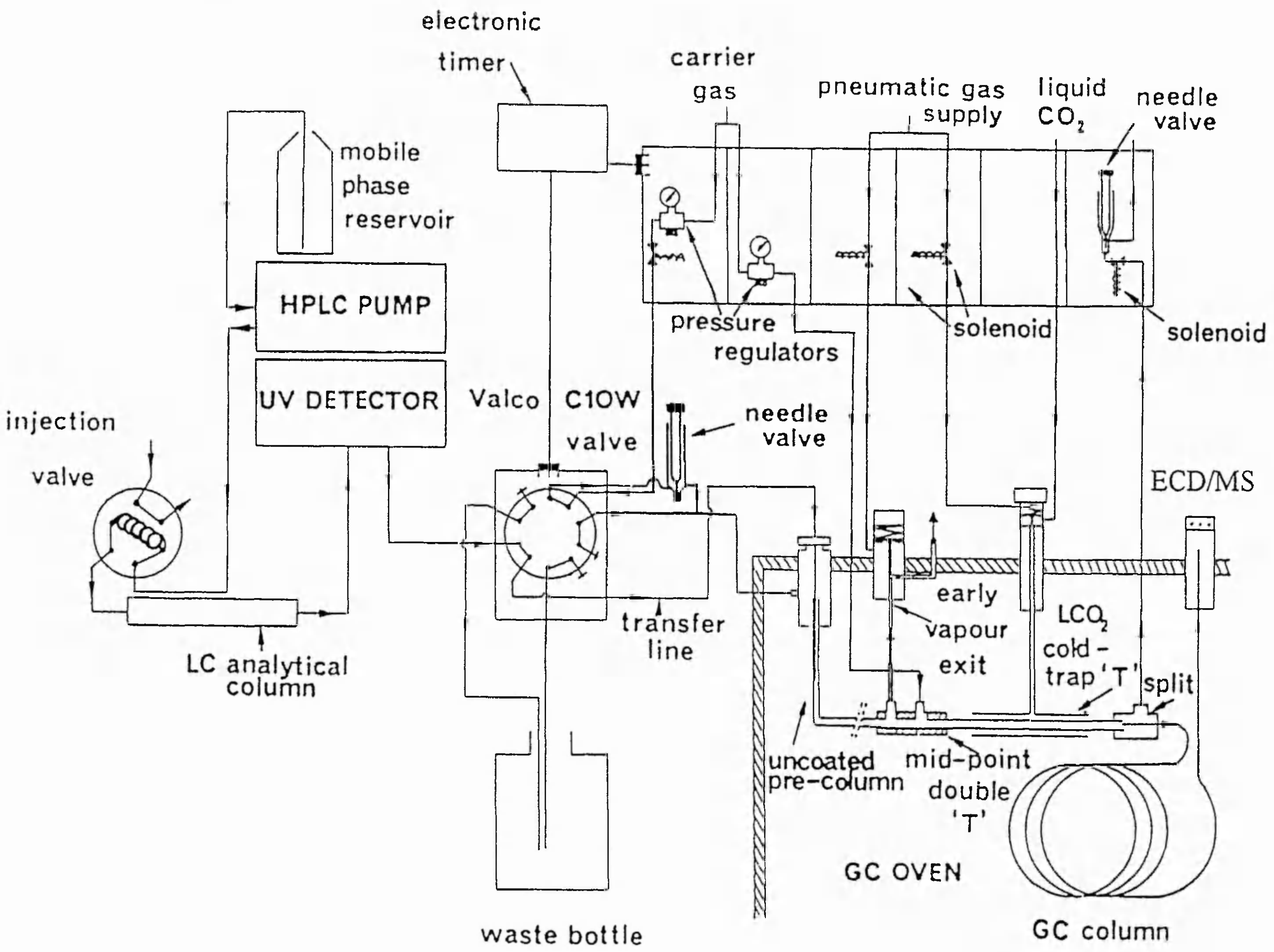


Figure 4.1. Schematic illustration of the HPLC-GC system

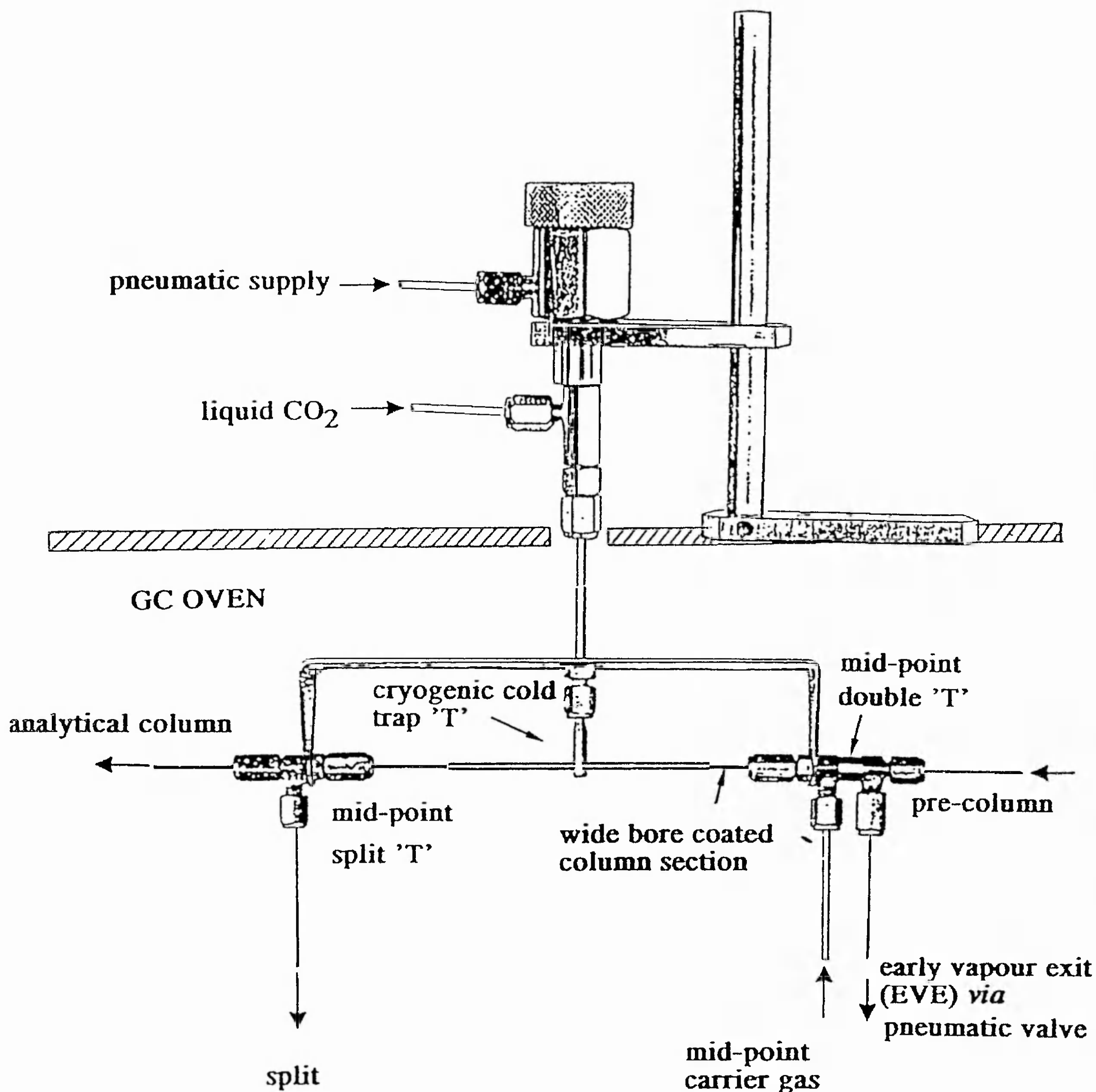
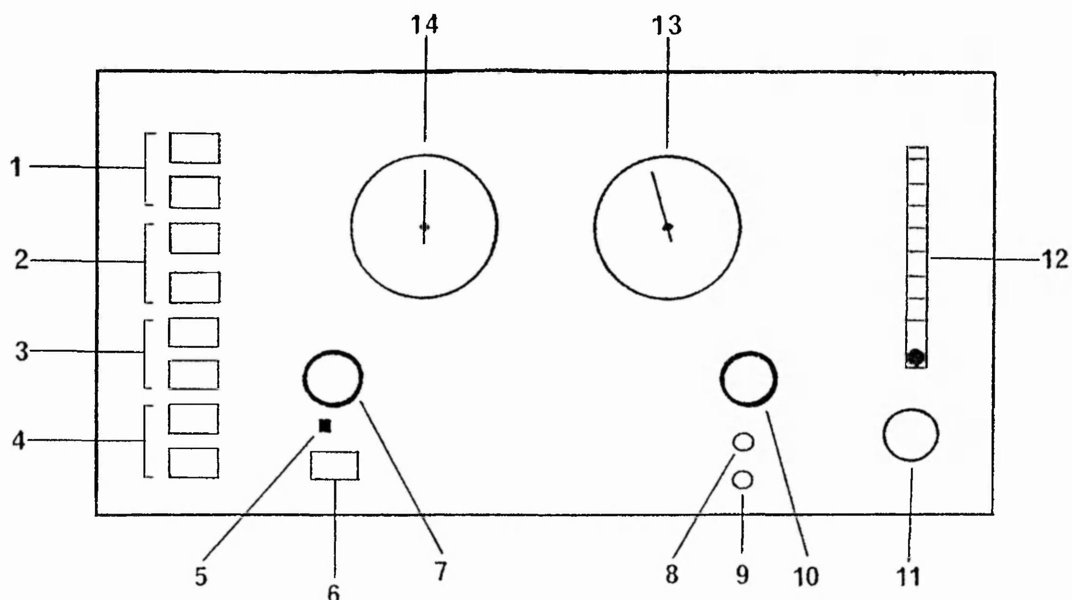


Figure. 4.2. Detailed illustration of the mid-point double 'T', cryogenic cold trap 'T' and mid-point split 'T' arrangement of the HPLC-GC system within the GC oven



1) Open/closed control of early vapour exit (EVE).
2) Open/closed control of mid-point split.
3) Carrier on/backflush control for inlet carrier gas (permanently on).
4) On/off control of cryogenic cold trap.
5) Remote control enable switch.
6) Power on/off.
7) Inlet carrier gas pressure controller.
8) Mid-point split needle valve.
9) Needle valve (redundant).
10) Mid-point double 'T' carrier gas pressure controller.
11) Make-up gas flow control to EVE pneumatic valve.
12) EVE make-up gas flow meter.
13) Mid-point double 'T' pressure gauge (pressure at EVE).
14) Inlet carrier gas pressure gauge.

Figure 4.3. The pneumatic column switching system module as used for control of HPLC-GC switching

4.2.3.4. Pressure balancing

Pressure balancing was carried out as follows; the early vapour exit (EVE) and the mid-point split were closed and the pre-column pressure regulator and the mid-point carrier supply were switched off. The knob of the mass flow regulator (Porter VCD-1000 series) which controls the flow in pre-column was opened completely. The inlet pressure (head pressure) was increased to 70 kPa, using the pre-column pressure regulator, to give a flow rate of 1 ml min^{-1} at the end of the analytical column. The mid-point pressure reached about 62 kPa. The inlet pressure was reduced to zero and the knob of the mass flow regulator was closed. The inlet pressure was increased to 178 kPa (i.e 108 kPa above that achieved previously). The knob of the mass flow regulator was opened slowly until 1 ml min^{-1} flow was once again achieved at the end of the analytical column. The early vapour exit was opened and at this point, the mid-point pressure was observed to drop to zero. The mid-point pressure was adjusted to its original value (i.e. 62 kPa). The flow at the mid-point split was then adjusted to 10 ml min^{-1} and the flow at the end of the analytical column readjusted to 1 ml min^{-1} using the mass flow regulator. With the analytical column connected to the ECD and the GC oven at 90 °C, a series of hexane injections (150 μl) were made and transferred to the pre-column using the HPLC pump at 300 μl min^{-1} with the EVE open during the transfer. The time between hexane introduction to the pre-column and closure of the EVE was increased until a reasonable residual solvent front was detected eluting from the analytical column, which was achieved 30 sec after hexane transfer. The fused silica tubing (*ca* 20 cm x 0.25 mm i.d.) connecting the EVE exit of the double 'T' to the pneumatic valve, allowed a flow of *ca* 100 ml min^{-1} to pass

i.e. sufficient to comfortably exceed the flow of carrier gas through the pre-column, plus the vapour formed from the evaporating mobile phase.

4.2.4. Preliminary optimisation of the HPLC-GC system operation

The PGC column was disconnected from the Valco C10W valve and the UV detector and replaced with a stainless steel tube. To demonstrate the system operation, a blank run was made by delivering hexane to the GC for 30 seconds at a flow rate of 300 μl min^{-1} (i.e. 150 μl) by switching the Valco C10W valve. The solvent transfer and the GC programme were started simultaneously. The EVE was closed half a minute after solvent transfer. The initial GC temperature of 90 °C was maintained throughout the period of transfer. The oven was programmed to 210 °C (4 min) at 15 °C min^{-1} and then to 260 °C (10 min) at 2 °C min^{-1}. A sample of pyrene in hexane (20 μl, 5 μg μl^{-1}) was transferred exactly as for the blank run. Solvent was eliminated by partially concurrent evaporation (at 90 °C, the oven temperature was above the boiling point of hexane (69 °C) at atmospheric pressure, but at the inlet pressure (178 kPa), the boiling point would be >100 °C because a change of pressure of +0.1 atmosphere can be expected to change a boiling temperature by about +3 °C[21]).

4.2.5. Determination of PCB elution profiles on PGC column with different solvent systems

With the PGC column connected between the Valco C10W valve and the UV detector, a PCB standard mixture, NTU-SMS0046 containing 41 *ortho* (PCB 4, 18, 28, 31, 33, 41, 44, 47, 49, 51, 52, 60, 66, 74, 87, 99, 101, 105, 110, 114, 118, 123, 138, 141, 151, 153, 156, 157, 167, 180, 183, 185, 187, 189, 191, 193, 194, 201, 203,

206 and 209) and 5 non-*ortho*-(PCB 37, 77, 81, 126 and 169) polychlorinated biphenyls at *ca* 1 ng each was chromatographed on the PGC column using hexane/toluene solvent systems at a flow rate of 300 μl min^{-1} and discrete HPLC fractions were collected in 1.1 ml screw top vials. Each fraction was blown down to almost dryness, reconstituted into 20 μl of iso-octane and analysed by GC-MS as described in chapters 2 and 3.

4.2.6. Determination of conditions for the on-line HPLC-GC for PCB standards

A standard containing $^{12}C_{12}$-PCB *ortho* (PCB 28, 52, 101, 118, 138, 153 and 180) and non-*ortho* (PCB 77, 126 and 169) polychlorinated biphenyls at *ca* 8 ng each was introduced into the PGC column and eluted with hexane/toluene mobile phase mixtures at 300 μl min^{-1}. The appropriate fractions containing *ortho*- and non-*ortho*-PCBs were diverted to the GC *via* the on-column interface, and the solvent evaporated by partially concurrent evaporation. A 0.23 m length of 0.53 mm i.d., 1.5 μm film thickness DB-5 column was butt connected to the end of the uncoated pre-column (10 m x 0.53 mm i.d.) to help retain the analytes on the pre-column. The analytical GC column was a 30 m x 0.25 mm i.d. x 0.25 μm film thickness DB-17 (J&W, Folsom, CA, USA), with carrier gas at 1 ml min^{-1} flow rate with N_2 as make up gas (310 kPa) and an ECD temperature of 330 °C.

4.2.6.1. HPLC-GC conditions for *ortho*-PCBs

The sequence of events within the HPLC-GC system, relative to the GC temperature programme for the determination of the *ortho*-PCBs, is shown in Figure 4.4. The GC temperature programme was: 90 °C, hold 8.5 min, programmed to 200 °C at 30 °C

min^{-1}, hold 5.5 min, then to 280 °C at 2 °C min^{-1}, hold 10 min. The programme was started on sample introduction. The PGC column was eluted with hexane:toluene (95:5, v/v) and the appropriate fraction (eluting between 2-6 min), was diverted to the GC with the EVE and mid-point split open. The HPLC pump was stopped after 24 min. The mobile phase was vented through the EVE at an oven temperature of 90 °C. The cold trap was switched on 1.5 min after completion of transfer of the mobile phase to the pre-column, the EVE was closed at the same time and the oven temperature was immediately ramped to 200 °C at 30 °C min^{-1}. The split was closed, the EVE opened and cold trap switched off after 5.5 min.

4.2.6.2. HPLC-GC conditions for non-*ortho*-PCBs

The sequence of events for the HPLC-GC system, relative to the GC temperature programme, for the determination of non-*ortho*-PCBs are shown in Figure 4.5. The GC temperature programme with cryogenic focusing was: 130 °C, hold 19 min ramp to 200 °C at 30 °C min^{-1}, hold 13 min, then to 280 °C at 2 °C min^{-1} for 10 min. The programme was started on the re-start of the HPLC pump. The first fraction (between 14-18 min) containing PCBs 77 and 126, eluted after changing to toluene mobile phase, was diverted to the GC with the EVE and mid-point split open. Toluene was eliminated by partially concurrent evaporation at 130 °C. The cold trap was switched on and the EVE closed at 18.25 min. The HPLC pump was stopped at 19 min and the oven temperature was ramped to 200 °C at 30 °C min^{-1} to transfer PCBs 77 and 126 to the pre-column. At 28 min, the EVE was re-opened and the HPLC pump re-started with diversion of the second HPLC fraction containing PCB-

169 (28-30 min). The EVE was closed at 32.25 min. The split was closed, EVE re-opened and cold trap switched off after 35.5 min.

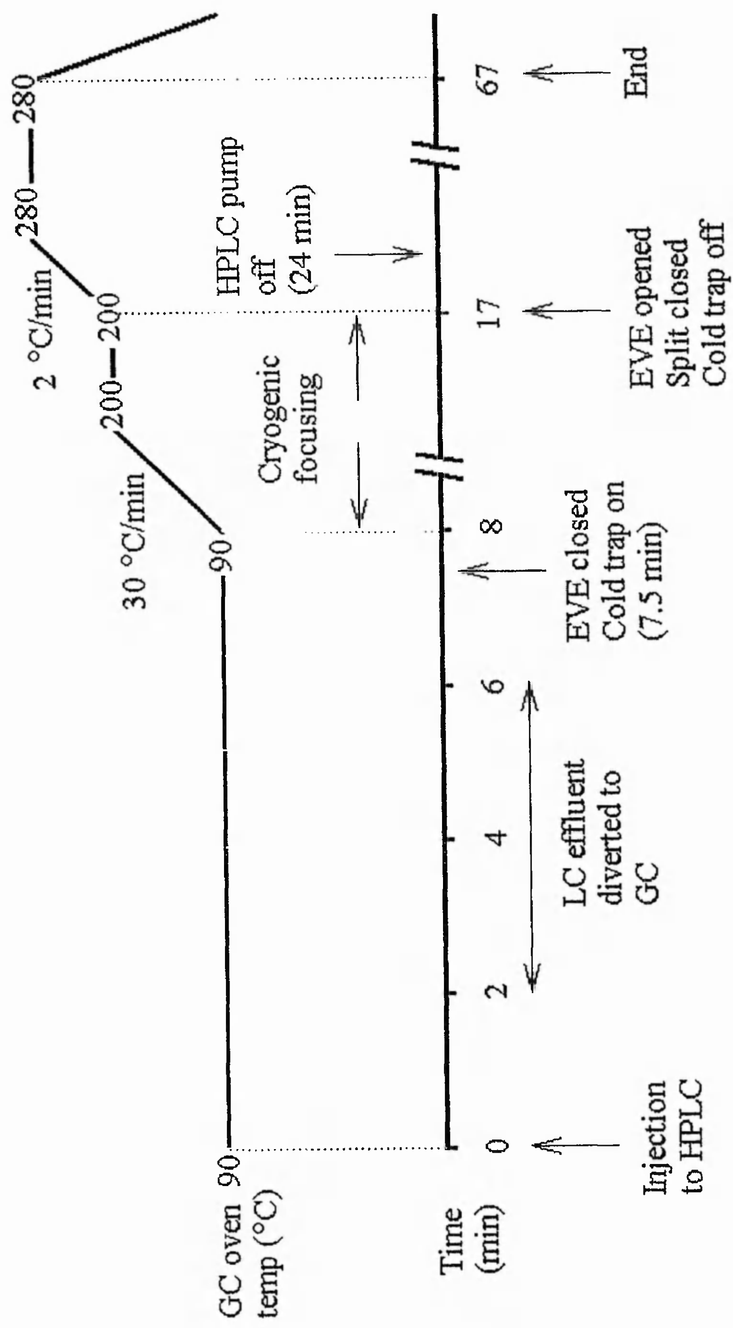


Figure 4.4. Sequence of events within the HPLC-GC system relative to the GC temperature programme for the *ortho*-PCBs

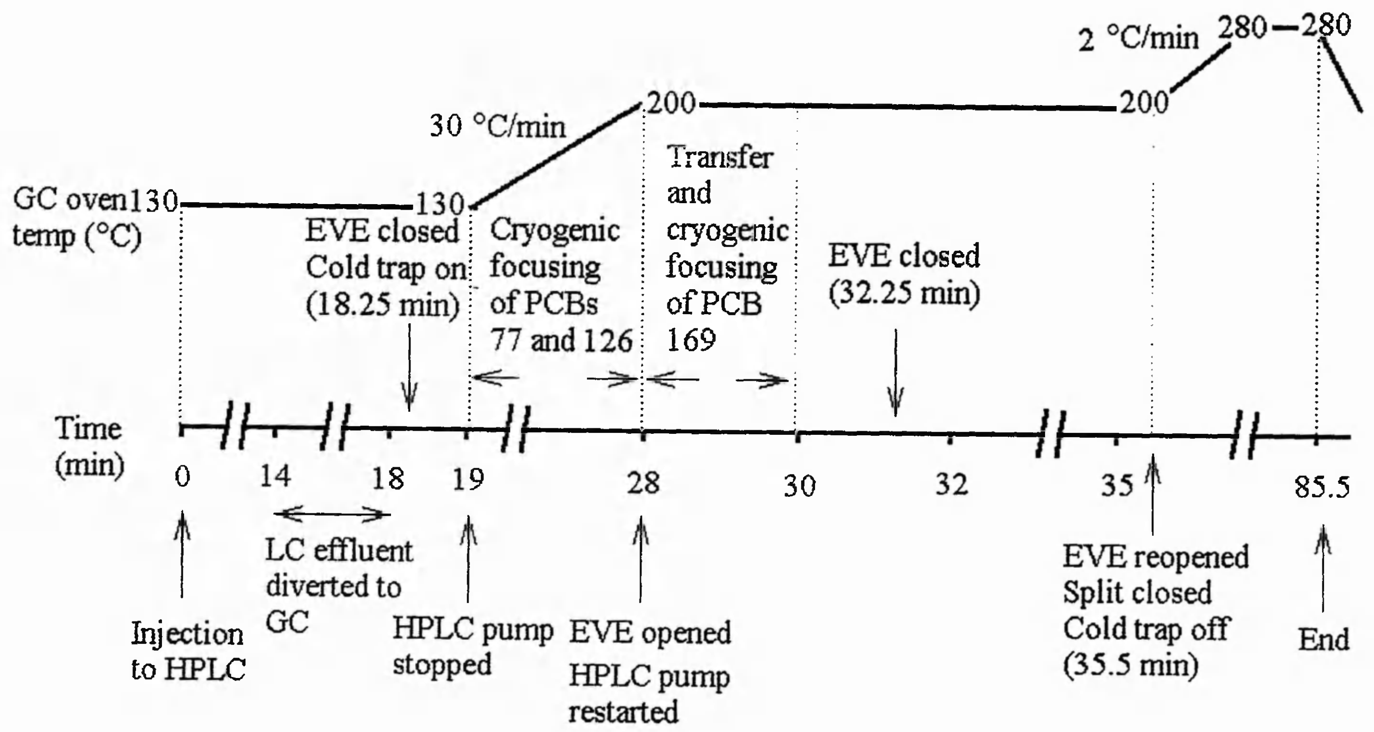


Figure 4.5. Sequence of events within the HPLC-GC system relative to the GC temperature programme for the non-*ortho*-PCBs

4.2.7. Method validation

The validation of the method was carried out using a PCB standard mixture containing *ortho-* (PCB-28, 52, 101, 118, 138, 153 and 180) and non-*ortho-* (PCB-77, 126 and 169) PCBs at *ca* 10 ng and 6.6 ng each, respectively. The PCB standard mixture was injected into the PGC column and the column was eluted with hexane:toluene (95:5, v/v) and the *ortho* fraction (eluting between 2-6 min) was diverted to the GC and analysed as explained in section 4.2.6.1. The HPLC pump was stopped after 24 min and solvent changed to toluene. The HPLC was restarted 55 min later (time taken from the transfer of *ortho* HPLC fraction to the GC up to the end of the GC run) and the non-*ortho* fractions (eluting between 14-18 and 20-21 min) were diverted to the GC and analysis carried out as explained in section 4.2.6.2.

4.2.8. Sample preparation

4.2.8.1. Aroclor 1254 standard

Neat Aroclor mixture (0.0135 g) was dissolved in 2 ml hexane:toluene (95:5, v/v) and a portion (100 μl) of the solution was diluted to 1.3 ml with the same solvent. An aliquot of the diluted Aroclor 1254 mixture (100 μl) was added to 0.9 ml of $^{13}C_{12}$-PCB internal standard solution containing *ortho* (28, 52, 101, 138, and 180) and non-*ortho* (77, 126 and 169) polychlorinated biphenyls, at *ca* 400 pg μl^{-1} for quadrupole ion trap detection. For ECD analysis, the diluted Aroclor 1254 (100 μl), was further diluted to 1.0 ml in hexane:toluene (95:5, v/v). A 20 μl aliquot of each of the diluted Aroclor 1254 samples was injected into the HPLC-GC system and analysed as described in sections 4.2.6.1 and 4.2.6.2.

4.2.8.2. Soil

Soil was dried to constant weight at 23-25 °C. Large stones were removed and the soil ground using a pestle and motor and sieved to 2 mm mesh. A sub-sample (20 g) was spiked with internal standard solution containing $^{13}C_{12}$-labelled *ortho* (28, 52, 101, 138 and 180) and non-*ortho* (77, 126 and 169) polychlorinated biphenyls (200 μl, 400 pg μl^{-1} each). The soil was then extracted with 3 x 40 ml of hexane and the combined extracts were transferred to a multilayer column (glass column, 25 cm x 25 mm i.d., packed from the bottom with glass wool, anhydrous sodium sulphate (1 cm), potassium silicate (3 cm), silica gel (5 cm), sodium hydrogen carbonate (1 cm), sulphuric acid/ silica gel mixture (5 cm, 40:60) and anhydrous sodium sulphate (1 cm)) which had previously been cleaned with 100 ml of hexane (see section 2.2.3). The multilayer column was eluted with 300 ml of hexane. The solvent was evaporated on a rotary evaporator to *ca* 4 ml, transferred into a 1.1 ml screw top vial and gently blown down under a steady stream of purified compressed air until just dry and then reconstituted into 100 μl of hexane:toluene (95:5, v/v). A 20 μl aliquot was injected into the HPLC-GC system.

4.2.9. Detection

Identification using ECD detection was based on retention times determined by analysing a standard mixture containing PCBs of interest under the same conditions used to analyse the sample. Concentrations of the PCBs of interest in the sample were determined by comparing peak heights for samples and standards. For ion trap mass spectrometry identification and quantification of PCBs were performed using internal standardisation as described in chapter 2, sections 2.2.5.2 and 2.2.5.4.

4.3. RESULTS AND DISCUSSIONS

4.3.1. Preliminary optimisation of HPLC-GC system operation

The HPLC analytical column was replaced with a length of stainless steel tubing for the preliminary studies of the HPLC-GC system. A solution of pyrene in hexane was used to test the efficiency of analyte transfer and solvent volatilisation/removal in the HPLC-GC interface. The HPLC-GC-EDC chromatograms for 20 μl injections of an hexane blank and a standard containing pyrene in hexane (5 μg μl^{-1}) are shown in Figures 4.6 (a) and (b), respectively. The two figures demonstrate the efficient removal of mobile phase in the interface and that pyrene was successfully transferred to the GC column. The longer retention time peak in both the blank and the spiked runs probably arose from an impurity in the solvent. A small amount of the solvent was allowed to pass to the GC column by closing the early vapour exit after 30 sec to ensure that the analyte was fully transferred preventing the loss of pyrene. The PGC column was reconnected between the HPLC pump and the uv detector for the investigations of the HPLC-GC behaviour of the PCBs

4.3.2. Elution profiles of PCBs on the PGC column

The PGC elution characteristics using several hexane/toluene solvent mixture were investigated to establish the best conditions for separation of *ortho* and non-*ortho* substituted PCBs into fractions which could separately be transferred into the GC from the HPLC. Results are presented in Tables 4.1-4.6 for the individual PCB congeners in PCB standard NTU-SMS0046 containing 41 *ortho*-(PCB 4, 18, 28, 31, 33, 41, 44, 47,49, 51, 52, 60, 66, 74, 87, 99,101, 105, 110, 114, 118, 123,138, 141, 151, 153, 156, 157,167, 180, 185, 187, 189, 191, 193, 194, 201, 203, 206 and 209)

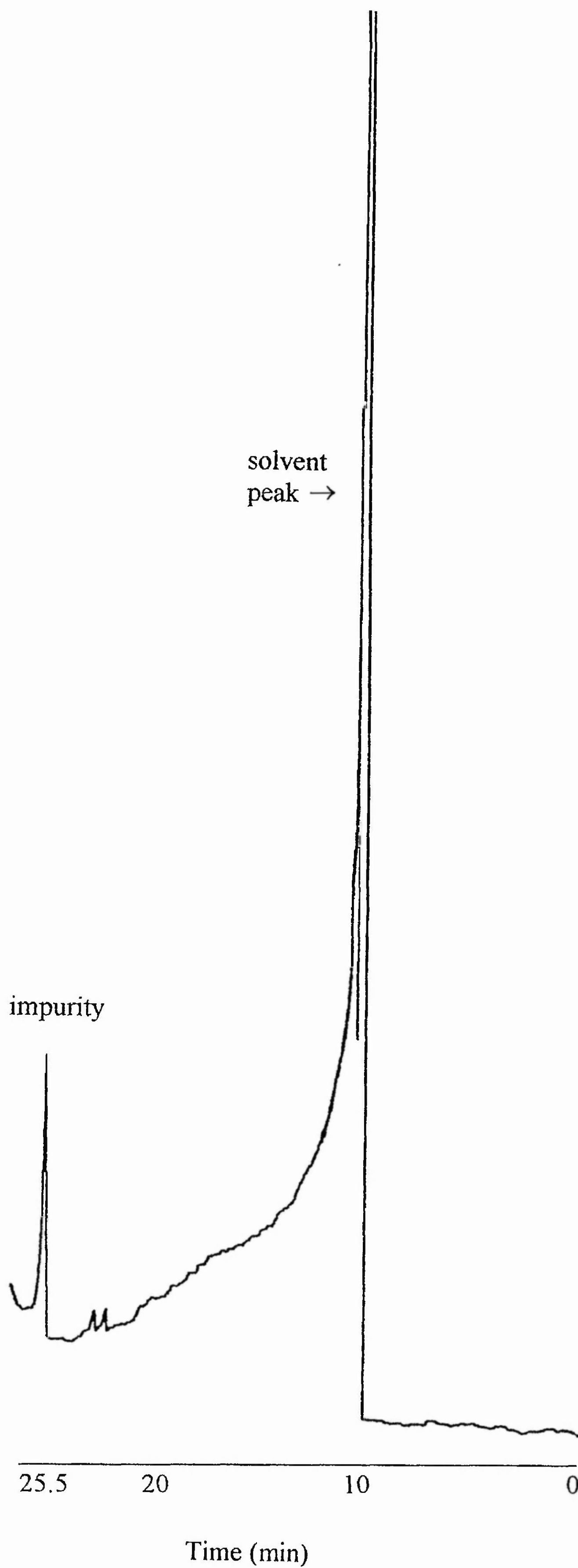


Figure 4.6 (a). HPLC-GC-ECD (att: 16) chromatogram of 150 μl fraction of hexane blank

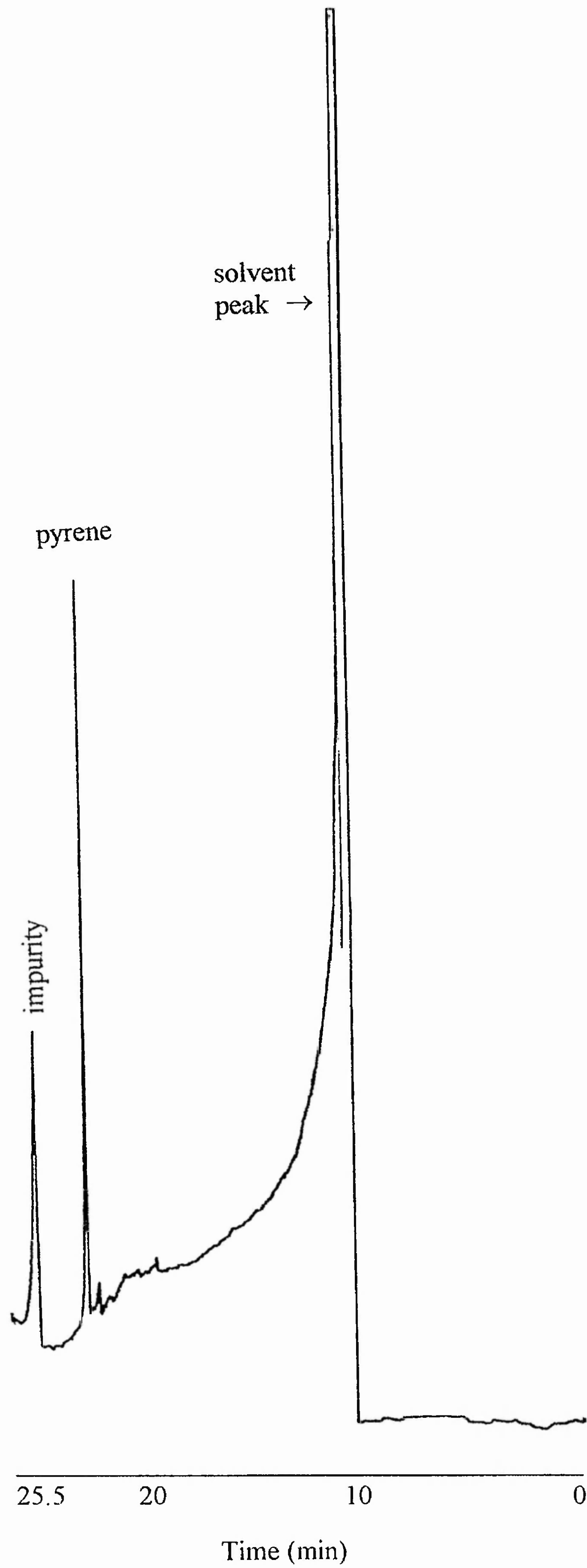


Figure 4.6 (b). HPLC-GC-ECD (att: 16) chromatogram of 100 μg of pyrene in 150 μl hexane fraction

and 5 non-*ortho*- (PCB 37, 77, 81, 126 and 169) polychlorinated biphenyls at *ca* 1 ng each.

The strong retention of the PCBs by the PGC column when hexane was used as the mobile phase resulted in an unacceptably large elution volume (0.6-30 ml). The *ortho*-PCBs (Table 4.1) requiring up to 98 min to elute from the HPLC at the flow rate of 0.3 ml min^{-1} which was required for the efficient solvent removal in the HPLC-GC interface, whilst none of the non-*ortho*-PCBs eluted within this time. However, addition of a small amount of toluene to the hexane greatly reduced retention on the PGC column. With a mixture of 5% toluene in hexane (Table 4.2), an elution volume of 1.8 ml was required for the *ortho*-PCBs because of the toluene increased strength of the solvent mixture. In contrast, the non-*ortho*-PCBs of interest (PCBs-77 and 126), eluted between 12.3 ml and 36 ml (i.e. between 41 min and 120 min) (Table 4.2) whilst PCB-169 was not observed to elute. The greater retention of the non-*ortho*-PCBs is attributed to their capability to assume a planar configuration more readily than *ortho*-PCBs, thus allowing non-*ortho*-PCBs a stronger interaction with the planar PGC surface (see section 2.3). The role of sample structure and solvent effects on the retention of PCBs on active carbon have been determined and discussed in chapter 2, (section 2.3) and it was noted that these tend to resemble the observed behaviour on PGC in terms of sample selectivity.

The retention volume for the non-*ortho*-PCBs was reduced to between 4.5 ml and 8.1 ml (transfer time of 12 min) with 50:50, v/v hexane:toluene (Table 4.3). Increasing the toluene to 80% (Table 4.4) made little difference to the observed

Table 4.1. Normalised recoveries (%) of selected PCB congeners on PGC column after eluting with 100 % hexane

Congener	Volume hexane (ml) 0.0-0.6	0.6-1.2	1.2-1.8	1.8-2.4	2.4-6.9	6.9-30	30-51
PCB-4	0	100	0	0	0	0	0
PCB-18	0	100	0	0	0	0	0
PCB-28	0	0	0	98	2	0	0
PCB-31	0	0	73	27	0	0	0
PCB-33	0	0	0	99	1	0	0
PCB-37[a]	0	0	0	0	0	0	0
PCB-41	0	98	2	0	0	0	0
PCB-44	0	100	0	0	0	0	0
PCB-47	0	88	12	0	0	0	0
PCB-49	0	97	3	0	0	0	0
PCB-51	0	100	0	0	0	0	0
PCB-52	0	89	11	0	0	0	0
PCB-60	0	0	0	0	100	0	0
PCB-66	0	0	0	0	100	0	0
PCB-74	0	0	0	99	1	0	0
PCB-77[a]	0	0	0	0	0	0	0
PCB-81[a]	0	0	0	0	0	0	0
PCB-87	0	94	6	0	0	0	0
PCB-99	0	100	0	0	0	0	0
PCB-101	0	94	6	0	0	0	0
PCB-105	0	0	0	0	57	43	0
PCB-110	0	0	0	100	0	0	0
PCB-114	0	0	0	0	100	0	0
PCB-118	0	0	0	96	4	0	0
PCB-123	0	0	0	0	100	0	0
PCB-126[a]	0	0	0	0	0	0	0
PCB-138	0	87	13	0	0	0	0
PCB-141	0	60	40	0	0	0	0
PCB-151	0	100	0	0	0	0	0
PCB-153	0	100	0	0	0	0	0
PCB-156	0	0	0	0	100	0	0
PCB-157	0	0	0	0	100	0	0
PCB-167	0	0	0	99	1	0	0
PCB-169[a]	0	0	0	0	0	0	0
PCB-180	0	76	24	0	0	0	0
PCB-183	0	100	0	0	0	0	0
PCB-185	0	100	0	0	0	0	0
PCB-187	0	100	0	0	0	0	0
PCB-189	0	0	0	0	100	0	0
PCB-191	0	100	0	0	0	0	0
PCB-193	0	0	0	100	0	0	0
PCB-194	0	0	100	0	0	0	0
PCB-201	0	98	2	0	0	0	0
PCB-203	0	96	4	0	0	0	0
PCB-206	0	100	0	0	0	0	0
PCB-209	0	100	0	0	0	0	0
Time (min)	0-2	2-4	4-6	6-8	8-23	23-100	100-170

[a]Non-*ortho*-substituted-PCBs

Table 4.2. Normalised recoveries (%) of selected PCB congeners on PGC column after eluting with 5 % toluene in hexane

Congener	Volume hexane:toluene (95:5) (ml)				
	0.0-0.6	0.6-1.2	1.2-1.8	7.2-12.3	12.3-36.0
PCB-4	0	100	0	0	0
PCB-18	0	100	0	0	0
PCB-28	0	100	0	0	0
PCB-31	0	100	0	0	0
PCB-33	0	100	0	0	0
PCB-37[a]	0	0	0	100	0
PCB-41	0	100	0	0	0
PCB-44	0	98	2	0	0
PCB-47	0	100	0	0	0
PCB-49	0	100	0	0	0
PCB-51	0	100	0	0	0
PCB-52	0	100	0	0	0
PCB-60	0	0	100	0	0
PCB-66	0	84	16	0	0
PCB-74	0	45	55	0	0
PCB-77[a]	0	0	0	0	100
PCB-81[a]	0	0	0	0	100
PCB-87	0	100	0	0	0
PCB-99	0	100	0	0	0
PCB-101	0	100	0	0	0
PCB-105	0	0	100	0	0
PCB-110	0	72	28	0	0
PCB-114	0	0	100	0	0
PCB-118	0	0	100	0	0
PCB-123	0	0	100	0	0
PCB-126[a]	0	0	0	0	100
PCB-138	0	100	0	0	0
PCB-141	0	100	0	0	0
PCB-141	0	100	0	0	0
PCB-153	0	100	0	0	0
PCB-156	0	0	100	0	0
PCB-157	0	0	100	0	0
PCB-167	0	0	100	0	0
PCB-169[a]	0	0	0	0	0
PCB-180	0	76	24	0	0
PCB-183	0	100	0	0	0
PCB-185	0	100	0	0	0
PCB-187	0	100	0	0	0
PCB-189	0	0	100	0	0
PCB-191	0	100	0	0	0
PCB-193	0	0	100	0	0
PCB-194	0	100	0	0	0
PCB-201	0	100	0	0	0
PCB-203	0	100	0	0	0
PCB-206	0	100	0	0	0
PCB-209	0	100	0	0	0
Time (min)	0-2	2-4	4-6	24-41	41-120

[a]Non-*ortho*-substituted-PCBs

Table 4.3. Normalised recoveries (%) of selected PCB congeners on PGC column after eluting with hexane:toluene (95:5) and then with 50 % hexane in toluene

	Volume (ml)						
	hexane:toluene (95:5)	hexane:toluene (50:50)					
Congener	0.6-1.8	0-0.9	3.6-4.5	4.5-5.4	5.4-6.3	6.3-7.2	7.2-8.1
PCB-4	100	0	0	0	0	0	0
PCB-18	100	0	0	0	0	0	0
PCB-28	100	0	0	0	0	0	0
PCB-31	100	0	0	0	0	0	0
PCB-33	100	0	0	0	0	0	0
PCB-37[a]	0	100	0	0	0	0	0
PCB-41	100	0	0	0	0	0	0
PCB-44	100	0	0	0	0	0	0
PCB-47	100	0	0	0	0	0	0
PCB-49	100	0	0	0	0	0	0
PCB-51	100	0	0	0	0	0	0
PCB-52	100	0	0	0	0	0	0
PCB-60	100	0	0	0	0	0	0
PCB-66	100	0	0	0	0	0	0
PCB-74	100	0	0	0	0	0	0
PCB-77[a]	0	0	0	100	0	0	0
PCB-81[a]	0	0	87	13	0	0	0
PCB-87	100	0	0	0	0	0	0
PCB-99	100	0	0	0	0	0	0
PCB-101	100	0	0	0	0	0	0
PCB-105	100	0	0	0	0	0	0
PCB-110	100	0	0	0	0	0	0
PCB-114	100	0	0	0	0	0	0
PCB-118	100	0	0	0	0	0	0
PCB-123	100	0	0	0	0	0	0
PCB-126[a]	0	0	0	0	94	6	0
PCB-138	100	0	0	0	0	0	0
PCB-141	100	0	0	0	0	0	0
PCB-151	100	0	0	0	0	0	0
PCB-153	100	0	0	0	0	0	0
PCB-156	100	0	0	0	0	0	0
PCB-157	100	0	0	0	0	0	0
PCB-167	100	0	0	0	0	0	0
PCB-169[a]	0	0	0	0	0	0	100
PCB-180	100	0	0	0	0	0	0
PCB-183	100	0	0	0	0	0	0
PCB-185	100	0	0	0	0	0	0
PCB-187	100	0	0	0	0	0	0
PCB-189	100	0	0	0	0	0	0
PCB-191	100	0	0	0	0	0	0
PCB-193	100	0	0	0	0	0	0
PCB-194	100	0	0	0	0	0	0
PCB-201	100	0	0	0	0	0	0
PCB-203	100	0	0	0	0	0	0
PCB-206	100	0	0	0	0	0	0
PCB-209	100	0	0	0	0	0	0
Time (min)	2-6	0-3	12-15	15-18	18-21	21-24	24-27

[a]Non-*ortho*-substituted-PCB

Table 4.4. Normalised recoveries (%) of selected PCB congeners on PGC column after eluting with hexane:toluene (95:5) and then with 15 % hexane in toluene

	Volume (ml)						
	hexane:toluene (95:5)	hexane:toluene (15:85)					
Congener	0.6-1.8	0-0.9	3.6-4.5	4.5-5.4	5.4-6.3	6.3-7.2	7.2-8.1
PCB-4	100	0	0	0	0	0	0
PCB-18	100	0	0	0	0	0	0
PCB-28	100	0	0	0	0	0	0
PCB-31	100	0	0	0	0	0	0
PCB-33	100	0	0	0	0	0	0
PCB-37[a]	0	100	0	0	0	0	0
PCB-41	100	0	0	0	0	0	0
PCB-44	100	0	0	0	0	0	0
PCB-47	100	0	0	0	0	0	0
PCB-49	100	0	0	0	0	0	0
PCB-51	100	0	0	0	0	0	0
PCB-52	100	0	0	0	0	0	0
PCB-60	100	0	0	0	0	0	0
PCB-66	100	0	0	0	0	0	0
PCB-74	100	0	0	0	0	0	0
PCB-77[a]	0	0	72	28	0	0	0
PCB-81[a]	0	0	95	5	0	0	0
PCB-87	100	0	0	0	0	0	0
PCB-99	100	0	0	0	0	0	0
PCB-101	100	0	0	0	0	0	0
PCB-105	100	0	0	0	0	0	0
PCB-110	100	0	0	0	0	0	0
PCB-114	100	0	0	0	0	0	0
PCB-118	100	0	0	0	0	0	0
PCB-123	100	0	0	0	0	0	0
PCB-126[a]	0	0	0	91	9	0	0
PCB-138	100	0	0	0	0	0	0
PCB-141	100	0	0	0	0	0	0
PCB-151	100	0	0	0	0	0	0
PCB-153	100	0	0	0	0	0	0
PCB-156	100	0	0	0	0	0	0
PCB-157	100	0	0	0	0	0	0
PCB-167	100	0	0	0	0	0	0
PCB-169[a]	0	0	0	0	24	76	0
PCB-180	100	0	0	0	0	0	0
PCB-183	100	0	0	0	0	0	0
PCB-185	100	0	0	0	0	0	0
PCB-187	100	0	0	0	0	0	0
PCB-189	100	0	0	0	0	0	0
PCB-191	100	0	0	0	0	0	0
PCB-193	100	0	0	0	0	0	0
PCB-194	100	0	0	0	0	0	0
PCB-201	100	0	0	0	0	0	0
PCB-203	100	0	0	0	0	0	0
PCB-206	100	0	0	0	0	0	0
PCB-209	100	0	0	0	0	0	0
Time (min)	2-6	0-3	12-15	15-18	18-21	21-24	24-27

[a]Non-*ortho*-substituted-PCBs

Table 4.5. Normalised recoveries (%) of selected PCB congeners on PGC column after eluting with hexane:toluene (95:5) and then with 5 % hexane in toluene

	Volume (ml)						
	hexane:toluene (95:5)	hexane:toluene (5:95)					
Congener	0.6-1.8	0-0.9	3.6-4.5	4.5-5.4	5.4-6.3	6.3-7.2	7.2-8.1
PCB-4	100	0	0	0	0	0	0
PCB-18	100	0	0	0	0	0	0
PCB-28	100	0	0	0	0	0	0
PCB-31	100	0	0	0	0	0	0
PCB-33	100	0	0	0	0	0	0
PCB-37[a]	0	100	0	0	0	0	0
PCB-41	100	0	0	0	0	0	0
PCB-44	100	0	0	0	0	0	0
PCB-47	100	0	0	0	0	0	0
PCB-49	100	0	0	0	0	0	0
PCB-51	100	0	0	0	0	0	0
PCB-52	100	0	0	0	0	0	0
PCB-60	100	0	0	0	0	0	0
PCB-66	100	0	0	0	0	0	0
PCB-74	100	0	0	0	0	0	0
PCB-77[a]	0	0	92	8	0	0	0
PCB-81[a]	0	0	97	3	0	0	0
PCB-87	100	0	0	0	0	0	0
PCB-99	100	0	0	0	0	0	0
PCB-101	100	0	0	0	0	0	0
PCB-105	100	0	0	0	0	0	0
PCB-110	100	0	0	0	0	0	0
PCB-114	100	0	0	0	0	0	0
PCB-118	100	0	0	0	0	0	0
PCB-123	100	0	0	0	0	0	0
PCB-126[a]	0	0	0	97	3	0	0
PCB-138	100	0	0	0	0	0	0
PCB-141	100	0	0	0	0	0	0
PCB-151	100	0	0	0	0	0	0
PCB-153	100	0	0	0	0	0	0
PCB-156	100	0	0	0	0	0	0
PCB-157	100	0	0	0	0	0	0
PCB-167	100	0	0	0	0	0	0
PCB-169[a]	0	0	0	0	100	0	0
PCB-180	100	0	0	0	0	0	0
PCB-183	100	0	0	0	0	0	0
PCB-185	100	0	0	0	0	0	0
PCB-187	100	0	0	0	0	0	0
PCB-189	100	0	0	0	0	0	0
PCB-191	100	0	0	0	0	0	0
PCB-193	100	0	0	0	0	0	0
PCB-194	100	0	0	0	0	0	0
PCB-201	100	0	0	0	0	0	0
PCB-203	100	0	0	0	0	0	0
PCB-206	100	0	0	0	0	0	0
PCB-209	100	0	0	0	0	0	0
Time (min)	2-6	0-3	12-15	15-18	18-21	21-24	24-27

[a]Non-*ortho*-substituted-PCBs

Table 4.6. Normalised recoveries (%) of selected PCB congeners on PGC column after eluting with hexane:toluene (95:5) and then with 100% toluene

	Volume (ml)						
	hexane:toluene (95:5)	toluene 100%					
Congener	0.6-1.8	0-0.9	3.9-4.2	4.2-4.5	4.5-4.8	5.1-5.4	6.0-6.3
PCB-4	100	0	0	0	0	0	0
PCB-18	100	0	0	0	0	0	0
PCB-28	100	0	0	0	0	0	0
PCB-31	100	0	0	0	0	0	0
PCB-33	100	0	0	0	0	0	0
PCB-37[a]	0	100	0	0	0	0	0
PCB-41	100	0	0	0	0	0	0
PCB-44	100	0	0	0	0	0	0
PCB-47	100	0	0	0	0	0	0
PCB-49	100	0	0	0	0	0	0
PCB-51	100	0	0	0	0	0	0
PCB-52	100	0	0	0	0	0	0
PCB-60	100	0	0	0	0	0	0
PCB-66	100	0	0	0	0	0	0
PCB-74	100	0	0	0	0	0	0
PCB-77[a]	0	0	0	80	20	0	0
PCB-81[a]	0	0	72	25	3	0	0
PCB-87	100	0	0	0	0	0	0
PCB-99	100	0	0	0	0	0	0
PCB-101	100	0	0	0	0	0	0
PCB-105	100	0	0	0	0	0	0
PCB-110	100	0	0	0	0	0	0
PCB-114	100	0	0	0	0	0	0
PCB-118	100	0	0	0	0	0	0
PCB-123	100	0	0	0	0	0	0
PCB-126[a]	0	0	0	0	0	100	0
PCB-138	100	0	0	0	0	0	0
PCB-141	100	0	0	0	0	0	0
PCB-151	100	0	0	0	0	0	0
PCB-153	100	0	0	0	0	0	0
PCB-156	100	0	0	0	0	0	0
PCB-157	100	0	0	0	0	0	0
PCB-167	100	0	0	0	0	0	0
PCB-169[a]	0	0	0	0	0	0	100
PCB-180	100	0	0	0	0	0	0
PCB-183	100	0	0	0	0	0	0
PCB-185	100	0	0	0	0	0	0
PCB-187	100	0	0	0	0	0	0
PCB-189	100	0	0	0	0	0	0
PCB-191	100	0	0	0	0	0	0
PCB-193	100	0	0	0	0	0	0
PCB-194	100	0	0	0	0	0	0
PCB-201	100	0	0	0	0	0	0
PCB-203	100	0	0	0	0	0	0
PCB-206	100	0	0	0	0	0	0
PCB-209	100	0	0	0	0	0	0
Time (min)	2-6	0-3	13-14	14-15	15-16	17-18	20-21

[a]Non-*ortho*-substituted-PCBs

retention volumes compared to 50% toluene in hexane, and so the concentration of toluene was increased to 95% (Table 4.5). The elution volume was reduced to 2.7 ml (elution time of 9 min) and the elution volume was further reduced to 2.1 ml (elution time of 7 min) with 100% of toluene (Table 4.6).

Therefore, to transfer the first group (*ortho*-PCBs) from the HPLC to the GC, 5% toluene in hexane (Table 4.2) was considered more suitable than 100% hexane (Table 4.1) because of the longer transfer time (98 min) required for pure hexane. Using this mobile phase, a volume of 1.2 ml eluting between 2 and 6 min would be required to transfer all *ortho*- and mono-*ortho*-PCBs to the GC pre-column. For the second group (the non-*ortho*-PCBs) several solvent systems (Tables 4.2-4.6) were tested and 100% toluene (Table 4.6) was considered most suitable because of the shorter elution time for these PCBs (between 14-21 min; 4.2-6.3 ml). These observations were used to establish the elution sequence for HPLC-GC analysis and a detailed description of the HPLC-GC procedure for *ortho*- and non-*ortho*-PCBs is given in section 4.3.3.

4.3.3. HPLC-GC analysis of a PCB standard mixture

The sequence of events for the HPLC-GC determination of *ortho*-PCBs in a PCB standard mixture, relative to the GC temperature programme is shown in Figure 4.4. The programme was started on sample introduction. The PGC column was eluted with hexane:toluene and the appropriate fraction, was diverted to the GC-ECD/MS using the on-column type interface between the HPLC and the GC. Partially concurrent evaporation was employed for removal of the mobile phase in the GC pre-column. The EVE was opened during sample introduction from the HPLC, permitting

the venting of solvent vapour away from the analytical column, with the PCBs analytes condensing on the pre-column in a similar manner to the system described by Noy et al. [22]

It was necessary to connect a short length of coated DB-5 column (23 cm x 0.53 mm i.d. 1.5 μm film thickness) to the end of the uncoated pre-column to retain the PCBs and prevent their loss through the EVE and mid-point split during transfer from the HPLC to the pre-column. Closure of the EVE, after almost complete solvent evaporation, allowed the PCBs remaining on the pre-column to be transferred to, and focused on, the length of coated column held within the cryogenically cooled 'T' piece cold trap. This trap, was capable of maintaining sub-ambient temperatures within the hot GC oven (200 °C) for peak focusing. The supply of carrier gas to the injector and hence the uncoated pre-column was *via* a pressure regulator (part of PCSS) positioned upstream of a mass flow regulator. This arrangement was necessary to ensure that a positive flow of carrier gas was maintained through the pre-column during sample introduction despite the back pressure exerted by the evaporating mobile phase. After transfer of the PCBs from the pre-column to the cryogenic cold trap, the early vapour exit was re-opened to flush the pre-column.

The splitter, positioned between the cryogenic cold trap and the head of the analytical column, enabled the flow rate through the pre-column (not compatible with the analytical column at *ca* 10 ml min^{-1}) to be maintained whilst the early vapour exit was closed. After transfer of the PCBs from the pre-column to the cryogenic cold trap, the early vapour exit was re-opened to flush the pre-column before the transfer of the

next HPLC fraction. Initial attempts to analyse PCBs without cryogenic focusing resulted in problems with chromatographic band broadening, because the low volatility of the PCBs made it difficult to focus these analytes at the head of the analytical column. Cryogenic focusing therefore provided a convenient method to obtain good chromatographic peak shapes for the PCBs and this approach ensured that GC resolution was not sacrificed by the process of transferring these analytes with a wide range of volatilities to the GC analytical column. The PCBs were retained quantitatively on the cold trap even with the split open and closure of the liquid CO_2 supply to the cold trap and the splitter, allowed the analysis to proceed on the analytical column. A typical HPLC-GC-ECD chromatogram for the *ortho*-PCB standard fraction is shown in Figure 4.7.

The HPLC-GC analysis of an *ortho*-PCB fraction of a PCB standard mixture containing $^{13}C_{12}$/$^{12}C_{12}$-PCB (PCB-28,52,101,118,138,153 and 180) at *ca* 16 ng each using mass spectrometric detection is presented in Figures 4.8-4.12. Figure 4.8(a) shows the single ion chromatogram of the trichlorinated $^{12}C_{12}$ PCBs present in the standard mixture. Figure 4.8(b) shows the single ion chromatogram of the corresponding $^{13}C_{12}$ internal standard and Figure 4.8(c) shows the mass spectrum obtained at the retention time of the internal standard. The fragmentation pattern observed in the EI mass spectra of these PCBs shows the molecular ion cluster, with the dominant ions corresponding to the presence of three chlorine atoms. The intensities of the molecular ion cluster peaks, for the trichlorinated biphenyls and its $^{13}C_{12}$-analogue resemble closely the theoretical isotopic ratios of 100:99:33:4. The

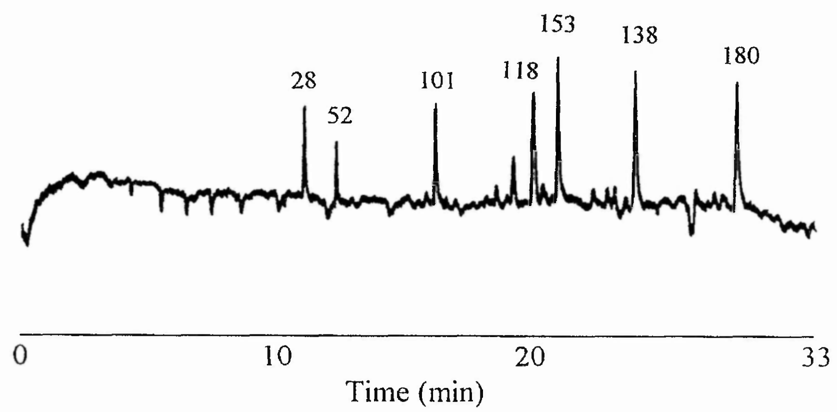
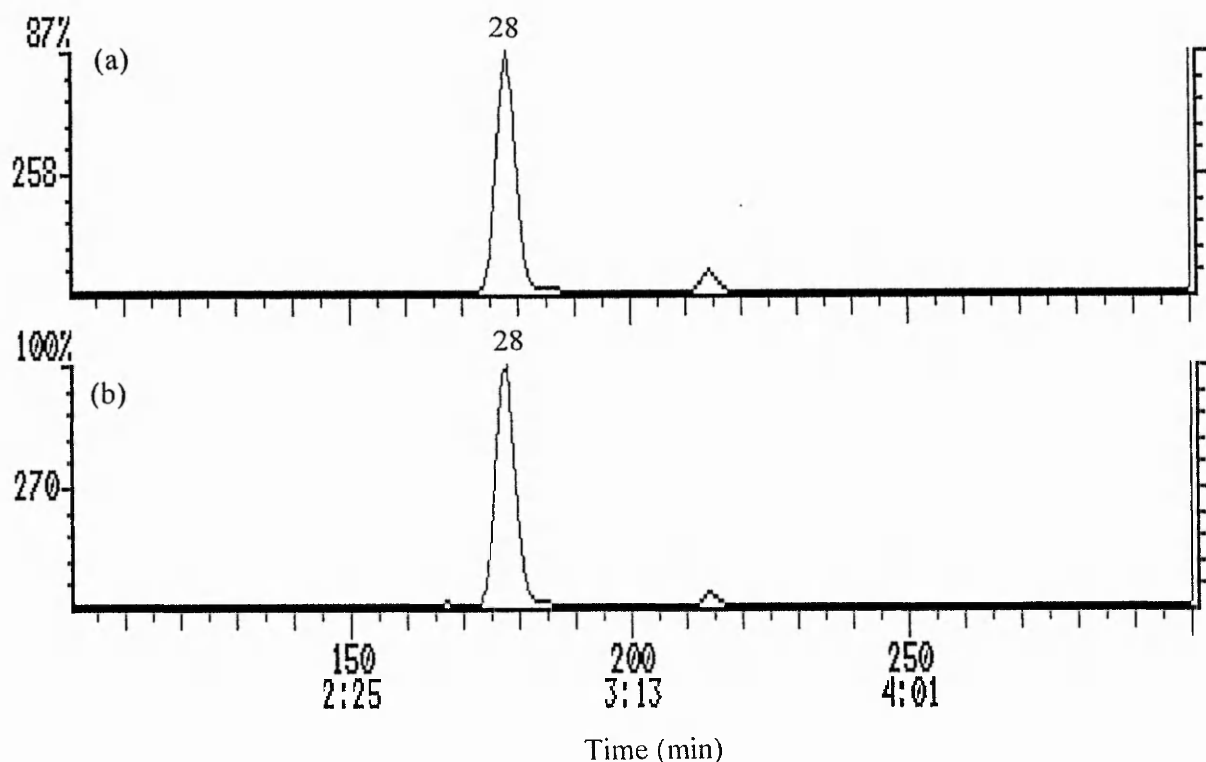


Figure 4.7. HPLC-GC-ECD (att:16) chromatogram showing the *ortho*-PCB standard fraction

results for the tetra-, penta-, hexa- and heptachlorinted biphenyls in the standard mixture are similarly presented in Figures 4.9-4.12. The PCB chromatographic peaks are well resolved from each other in all cases where there is more than one PCB congener with the same chlorination number. The base peaks for the mass spectra of the tetra-, penta-, hexa- and heptachlorinated $^{12}C_{12}$PCBs are the molecular cluster ions observed at m/z 292, 326, 360 and 394, respectively. The intensities of molecular clusters for tetra-, penta-, hexa- and heptachlorinated biphenyls are consistent with the expected isotopic ratios for the molecular ion clusters of 76:100:49:11:1, 61:100:66:22:4, 51:100:82:36:9 and 44:100:98:54:18, respectively. The mass spectrum shown was obtained at the retention time of the $^{13}C_{12}$-PCB internal standard for each chlorination number. The advantage of MS over ECD as a detector in trace analysis is that reliable results are obtained for the case of a single-component GC-peaks and for co-eluting congeners of different molecular weights[23] but the sensitivity of the ECD method is higher than positive ion EIMS especially for PCBs of high chlorine content. The disadvantage of using EIMS ionisation technique in PCB analysis arises from fragmentation of the molecular ion.[24] The main fragment recorded in the EI mass spectra of all the PCBs is loss of a Cl_2 from the molecular ion.[24-26] Therefore, the fragment-ion signal can overlap with the molecular ion clusters of a lower mass congeners.

The sequence of events for the concurrent HPLC-GC determination of the non-*ortho*-PCBs is shown in Figure 4.5. The GC temperature programme was started at the simultaneously with the restarting of the HPLC after changing the mobile phase from hexane:toluene (95:5, v/v) to toluene, following the analysis of the *ortho*-PCB



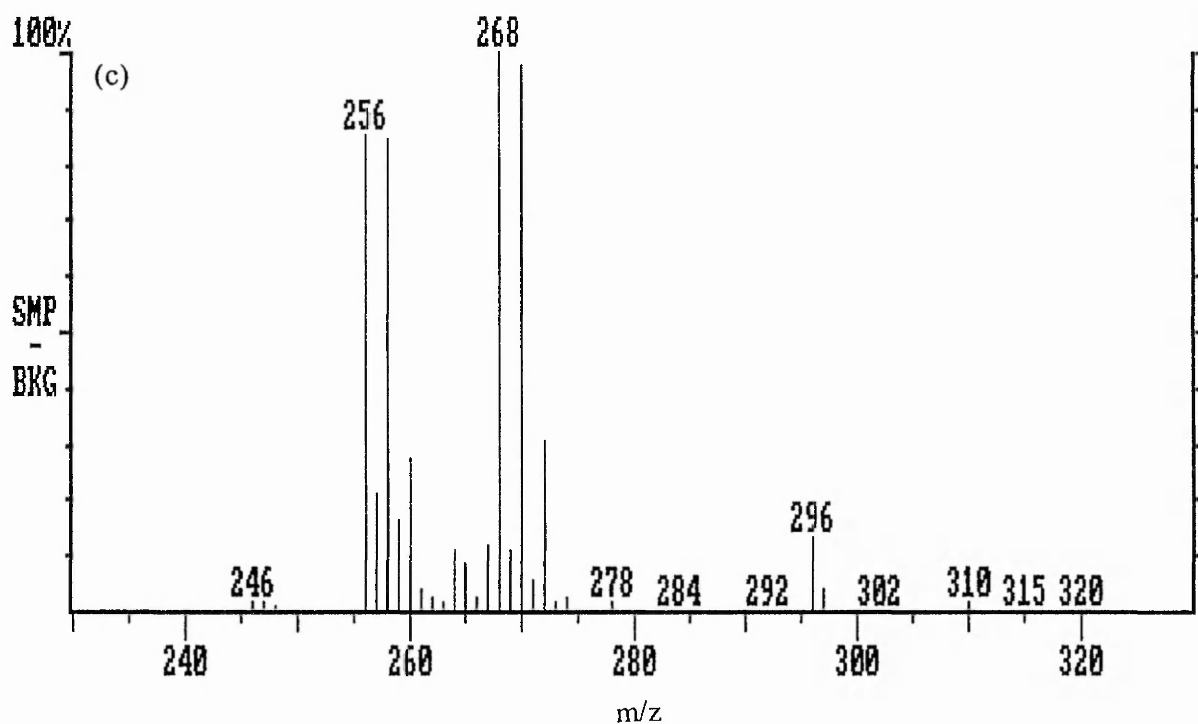
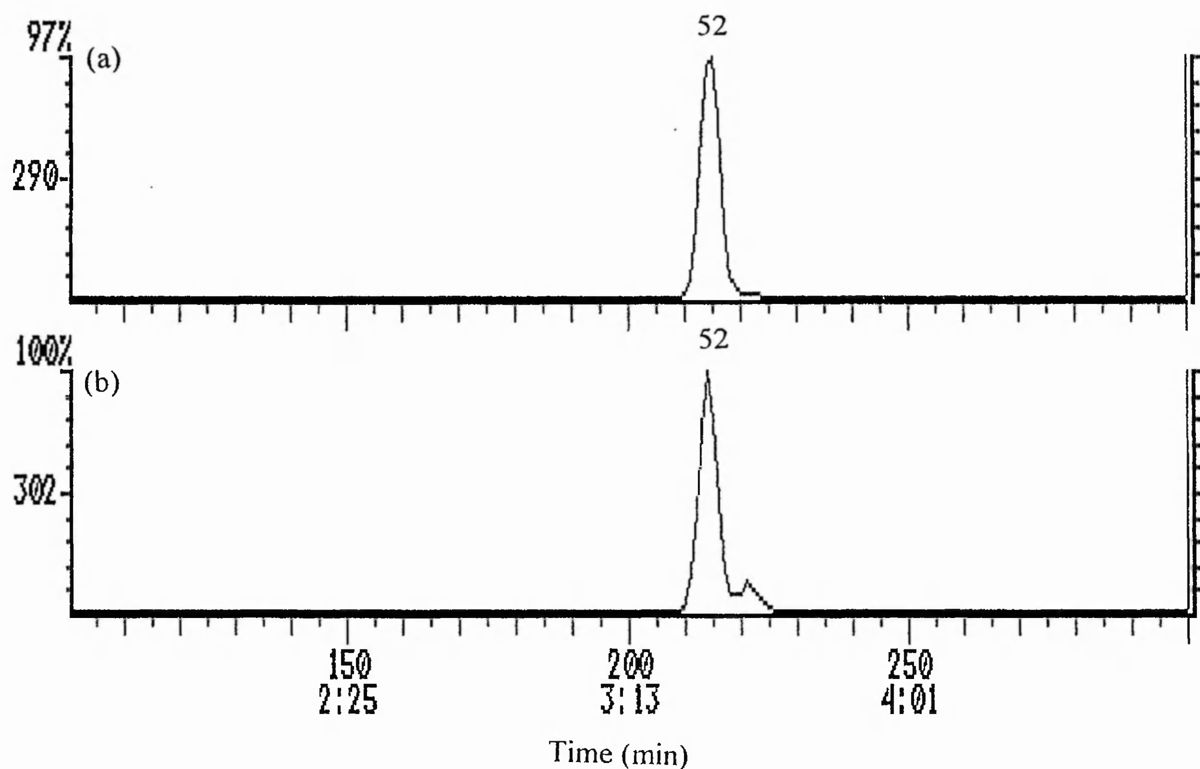


Figure 4.8. Trichlorinated PCBs in the *ortho* fraction of the PCB standard mixture: (a) selected ion chromatogram for $^{12}C_{12}$-PCB, (b) selected ion chromatogram for $^{13}C_{12}$-PCB internal standard and (c) mass spectrum obtained at the retention time of the internal standard



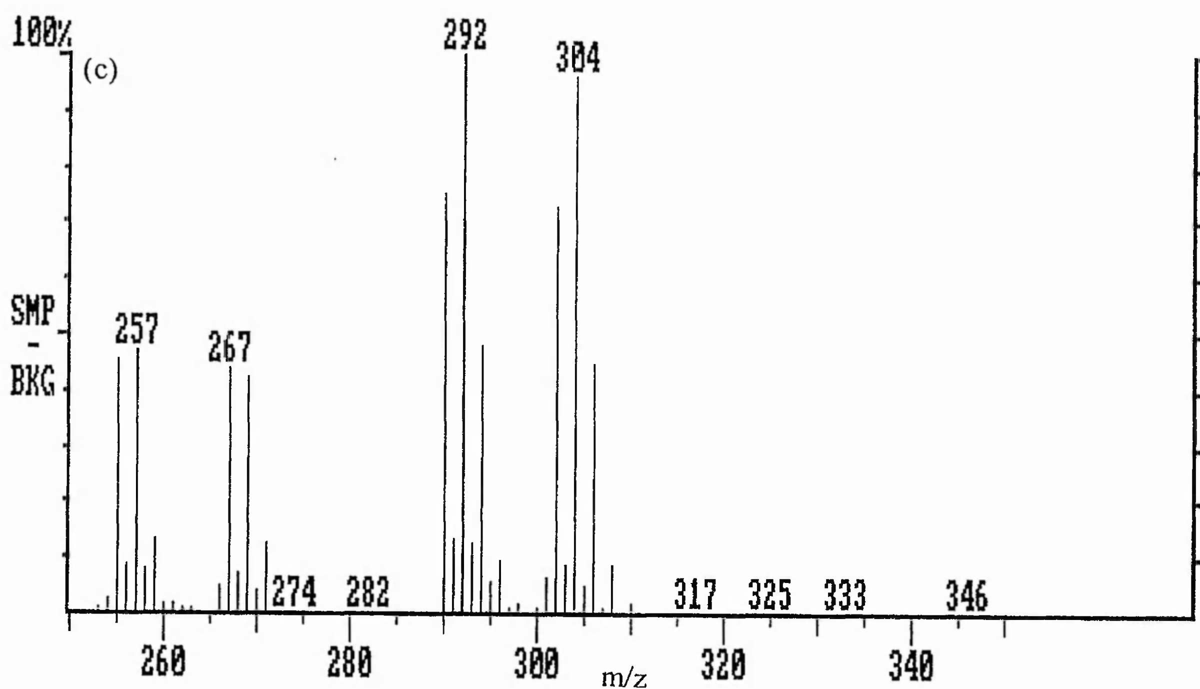
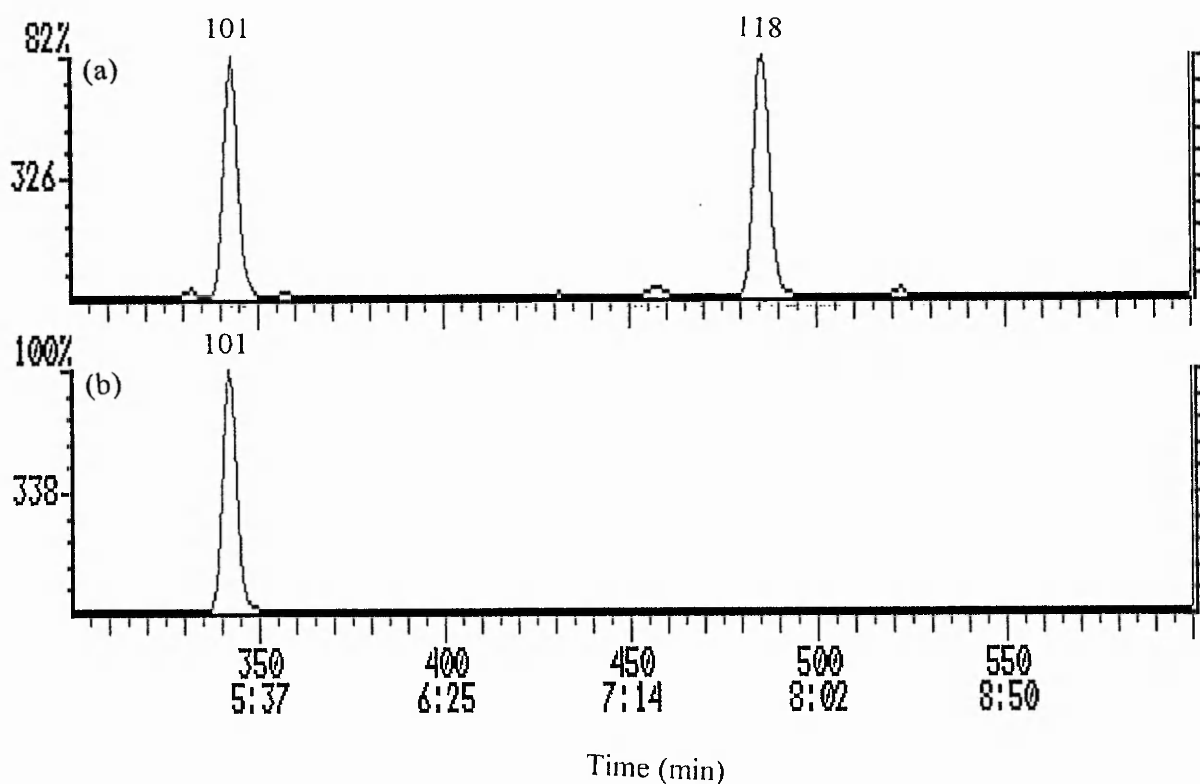


Figure 4.9. Tetrachlorinated PCBs in the *ortho* fraction of the PCB standard mixture: (a) selected ion chromatogram for $^{12}C_{12}$-PCB, (b) selected ion chromatogram for $^{13}C_{12}$-PCB internal standard and (c) mass spectrum obtained at the retention time of the internal standard



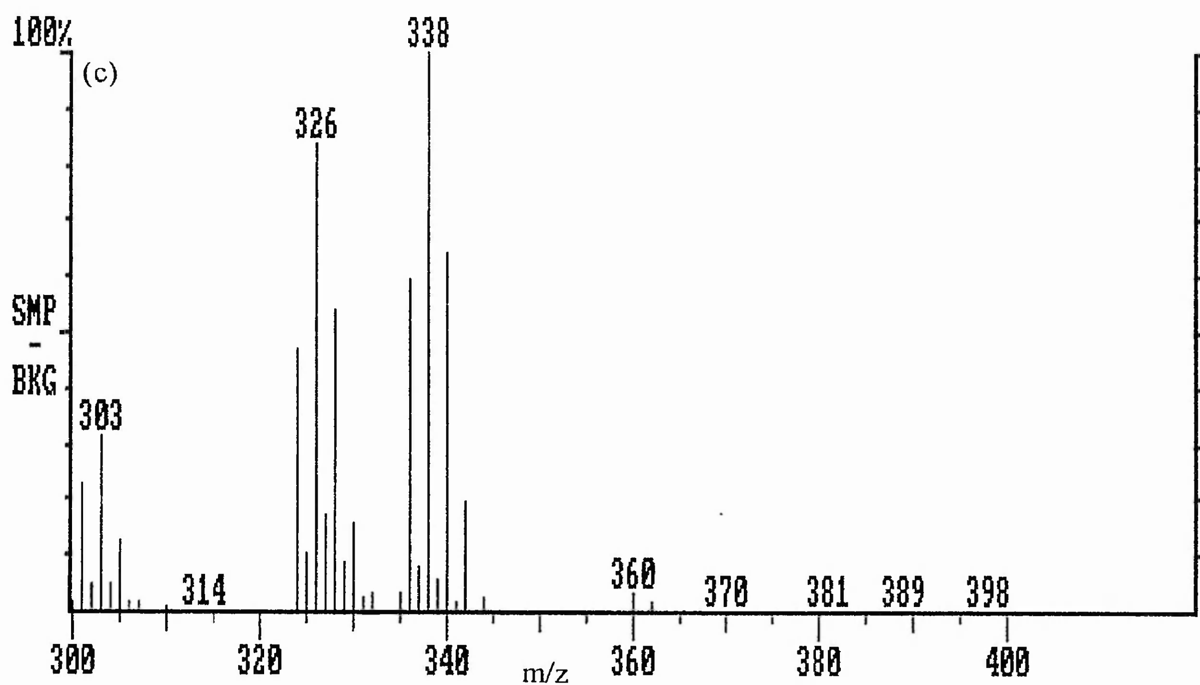
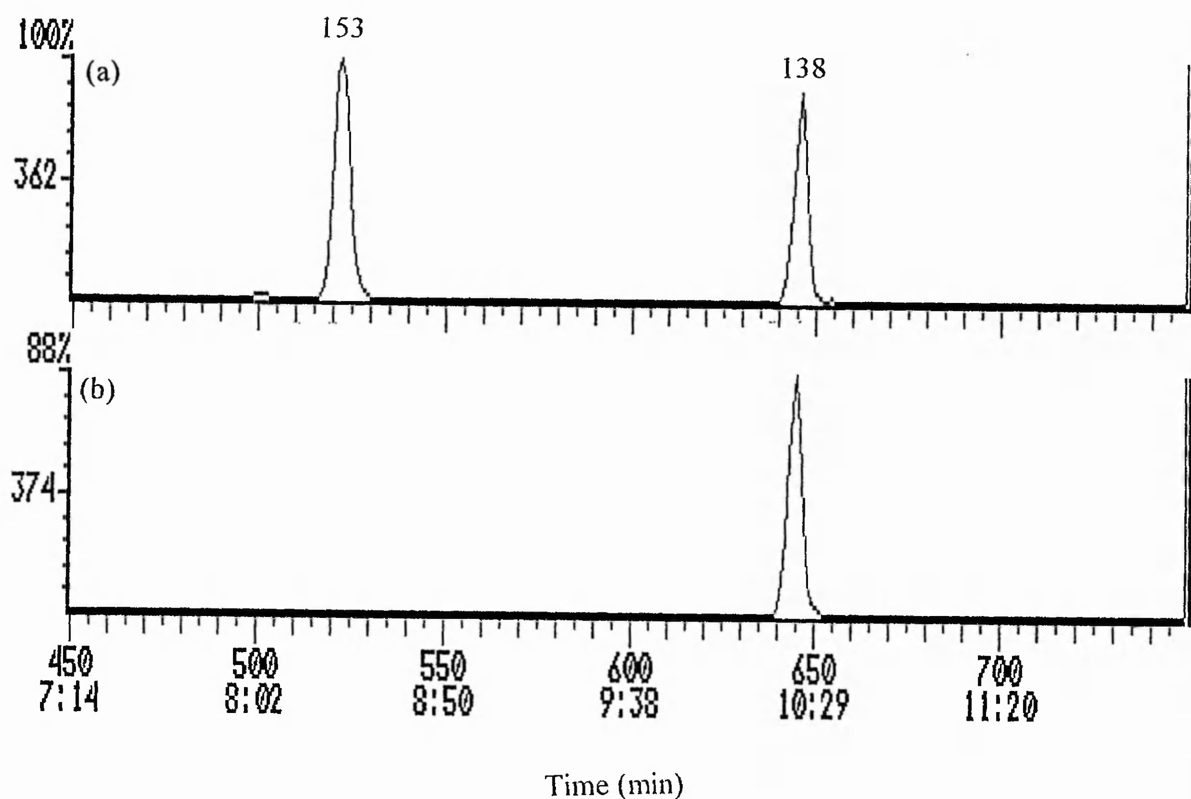


Figure 4.10. Pentachlorinated PCBs in the *ortho* fraction of the PCB standard mixture: (a) selected ion chromatogram for $^{12}C_{12}$-PCB, (b) selected ion chromatogram for $^{13}C_{12}$-PCB internal standard and (c) mass spectrum obtained at the retention time of the internal standard



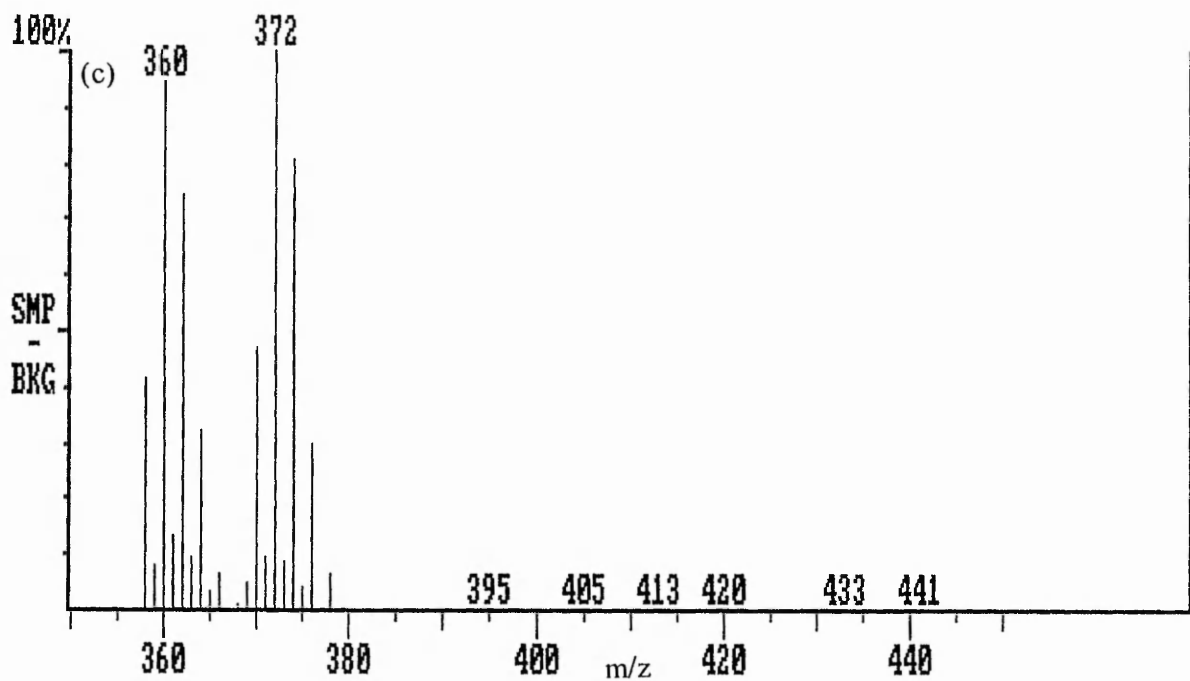


Figure 4.11. Hexachlorinated PCBs in the *ortho* fraction of the PCB standard mixture: (a) selected ion chromatogram for $^{12}C_{12}$-PCB, (b) selected ion chromatogram for $^{13}C_{12}$-PCB internal standard and (c) mass spectrum obtained at the retention time of the internal standard

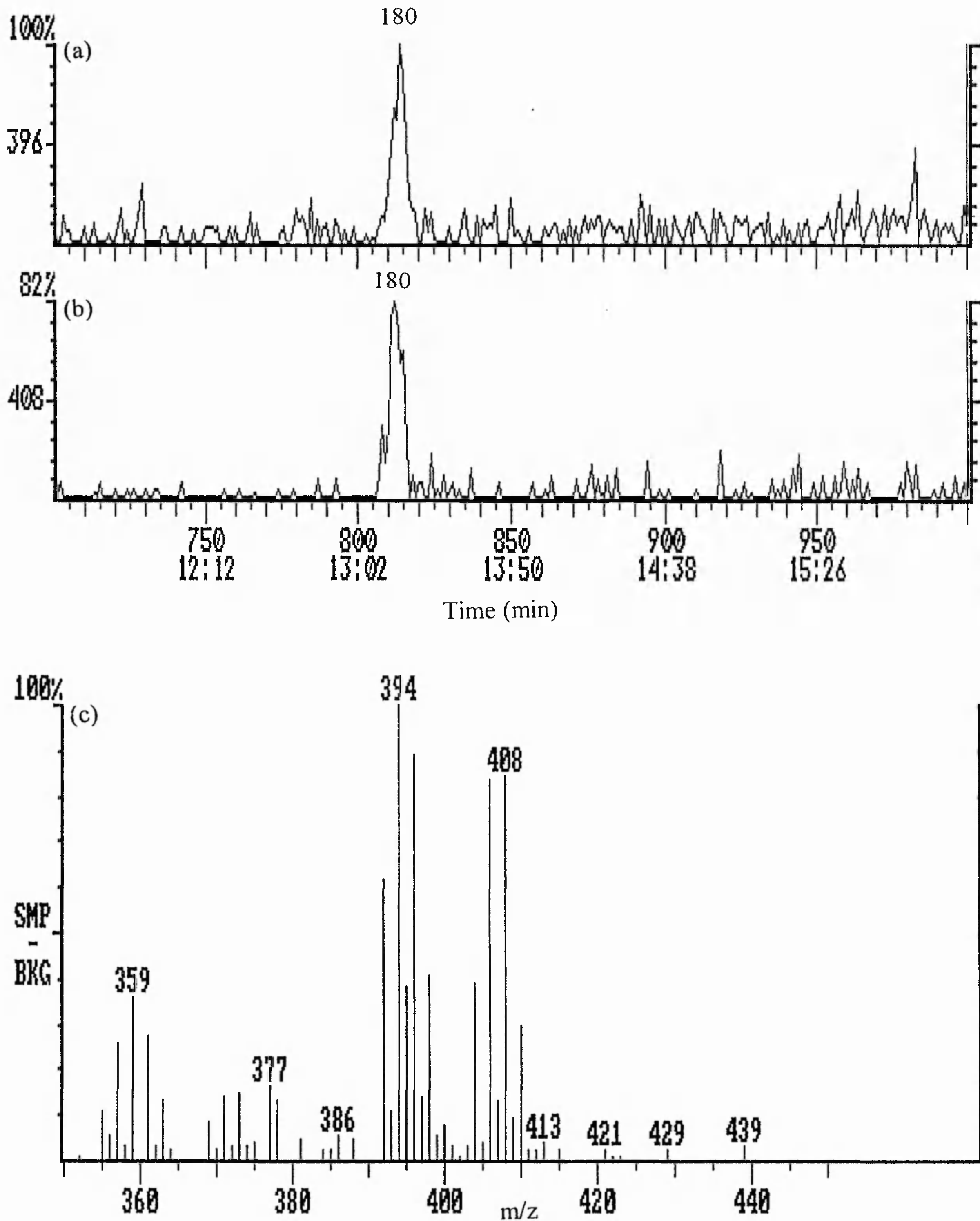


Figure 4.12. Heptachlorinated PCBs in the *ortho* fraction of the PCB standard mixture: (a) selected ion chromatogram for $^{12}C_{12}$-PCB, (b) selected ion chromatogram for $^{13}C_{12}$-PCB internal standard and (c) mass spectrum obtained at the retention time of the internal standard

fraction. Partially concurrent evaporation of the mobile phase was used for the non-*ortho*-PCB fraction.

Transferring the non-*ortho*-PCBs to the pre-column in a single 7 min fraction (14-21 min) which included all three non-*ortho*-PCBs (Table 4.6) revealed problems in retaining the earliest eluting non-*ortho* congener (PCB-77). It was found that whilst PCB-169, the last eluting PCB on the PGC column was being transferred from HPLC to the GC, more than 90% of PCB-77, the first eluting congener was lost through the EVE and split. Therefore, the non-*ortho*-PCBs were transferred from the HPLC to the GC in two fractions (Figure 4.4); the first fraction eluting between 14-18 min, containing PCBs-77 and 126, was transferred to the GC from the HPLC and retained on the length of coated column held within the cryogenically cooled 'T' piece cold trap until the second fraction, containing PCB-169 eluted after 20-21 min (Table 4.6) and was also transferred to the cryogenic trap. The three non-*ortho* PCB congeners were then released and chromatographed together to the analytical GC column. A typical HPLC-GC-ECD chromatogram for the non-*ortho*-PCB standard fraction using this approach is shown in Figure 4.13.

The precision of the on-line HPLC-GC method was estimated by injecting a PCB standard mixture containing *ortho* and non-*ortho*-PCBs onto the PGC column and diverting appropriate fractions from the PGC column to the GC-ECD. Peak heights for the PCBs were highly reproducible (Table 4.7) and the mean precision, expressed as %COV, of the system was 6.8% and 7.7% for *ortho*- and non-*ortho*-PCBs, respectively.

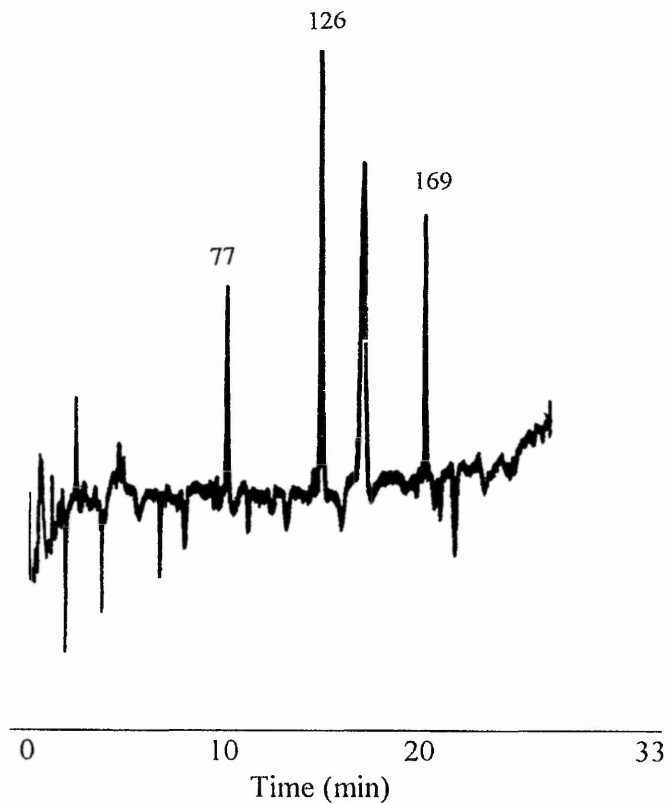


Figure 4.13. HPLC-GC-ECD (att:16) chromatogram showing the non-*ortho*-PCB standard fraction

The on-line HPLC-GC method precision compares well with the off-line method (chapter 2) where the mean precision was 6% and 13% for *ortho*- and non-*ortho*-PCBs, respectively.

Table 4.7. Peak heights (cm) and precision for *ortho* and non-*ortho*-PCB congeners

	PCB								
Experiment	28	52	77[e]	101	118	126[e]	138	153	169[e]
a	-	-	3.2	-	-	6.0	-	-	3.3
b	15.2	13.9	3.1	16.3	16.4	5.5	16.8	17.1	3.3
c	13.1	14.4	2.9	15.1	13.8	4.8	15.2	15.0	2.9
d	13.9	13.1	2.9	14.9	14.2	4.9	14.8	15.1	3.0
Av.	14.1	13.8	3.0	15.4	14.8	5.3	15.6	15.7	3.0
Precision									
%COV	7.5	4.8	5.0	4.9	9.5	11	6.8	7.5	7.0

[e]Non-*ortho*-PCBs

4.3.4. HPLC-GC analysis of a commercial PCB mixture

For the HPLC-GC analysis of PCBs in a commercial mixture, *ca* 1.0 μg of Aroclor 1254 mixture was chromatographed on the PGC column and the fraction eluting between 2-6 min, containing *ortho*-PCBs was transferred on-line to the GC-ECD/MS for analysis.

The HPLC-GC-ECD chromatogram for the *ortho*-substituted polychlorinated biphenyls in Aroclor 1254 is shown in Figure 4.14. Six of the seven *ortho*-PCBs (PCB 52,101, 118, 138,153 and 180) of interest in this study, were identified in Aroclor 1254 by comparing the retention times with that of a standard mixture. These PCBs are sufficiently resolved from the other PCB congeners present in the mixture. The Aroclor 1254 chromatogram resembles that reported by Draper et al.[27,28] even though a slightly less polar DB-5 column was used as the GC analytical column in their determinations.

The experiment was repeated using ion trap detection and the HPLC-GC-MS chromatograms for the native $^{12}C_{12}$-PCB, the $^{13}C_{12}$-PCB internal standard and the mass spectrum obtained at the retention time of the internal standard for each chlorination level for tri- to heptachlorinated *ortho*-PCBs in Aroclor 1254 are presented in Figures 4.15-4.19. The interfering ion with m/z 265 in Figure 4.15(c) could not be assigned because it co-eluted with the PCBs and other interfering compounds thereby making it impossible to obtain its clean mass spectrum. In each case the intensities of the molecular ion clusters for each chlorination number resembles closely the isotopic ratios observed for the PCB standard mixture. The

quantitative data for the *ortho*-PCBs 28, 52, 101, 118, 138, 153 and 180 in the Aroclor 1254 sample are presented in Table 4.8.

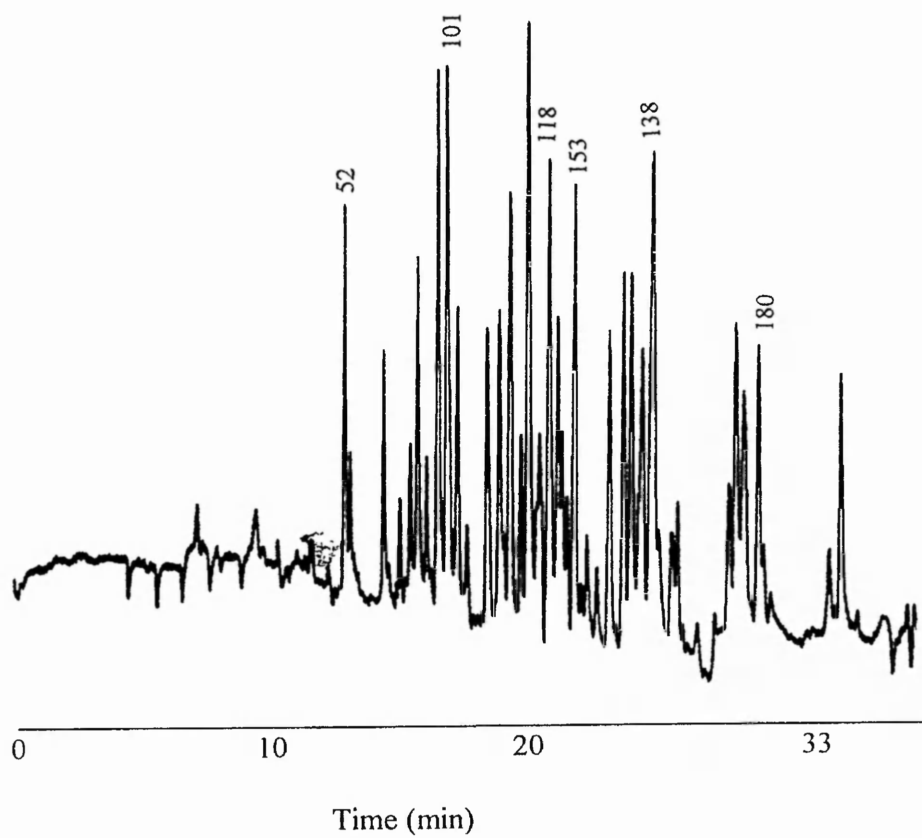
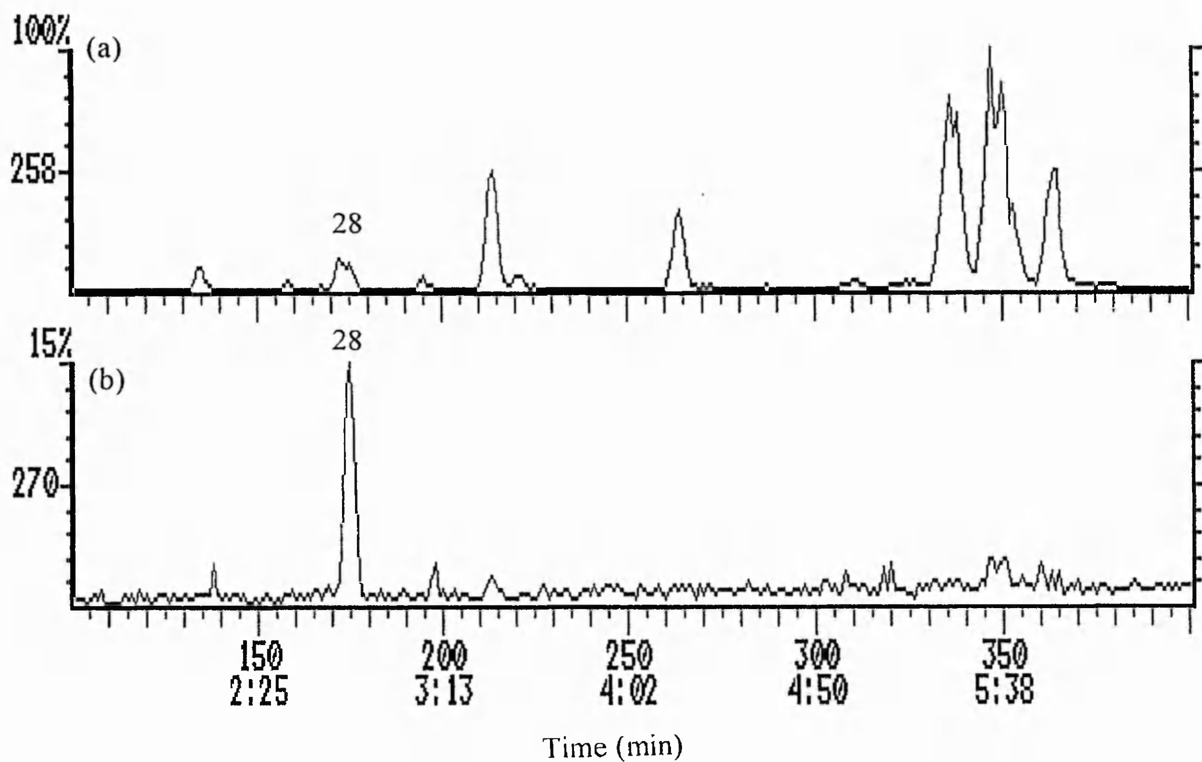


Figure 4.14. HPLC-GC-ECD (att:16) chromatogram showing the *ortho*-PCB fraction in Aroclor 1254

The accuracy of these determination can be evaluated by comparing them with the results of Draper et al.[27,28] and the multidimensional GC data of Schulz et al.[29] There is generally good agreement between the different laboratories for the 28, 52, 101, 118, 138 and 153 congeners, but the 180 level observed for the HPLC-GC method is higher than that previously reported. Variations can be attributed to Aroclor batches differing in chemical composition, but it is not known to what extent this contributed to the observed differences.

The concentrations of non-*ortho* substituted polychlorinated biphenyl congeners in Aroclor 1254 have been reported. The methods used in these determinations involved the off-line fractionation of non-*ortho*-PCBs on PGC,[18] carbon dispersed on shredded polyurethane foam[30] or activated carbon[31,32] before GC analysis. In the method used in this investigation involving HPLC separation on PGC, the HPLC-GC-ECD chromatogram of the non-*ortho*-PCB fraction in Aroclor 1254 is shown in Figure 4.20. The quantitative data for non-*ortho* PCBs 77,126 and 169 is given in Table 4.9. The accuracy of these determination can be evaluated by comparing results to those obtained by the off-line fractionation of the non-*ortho*-PCBs.[18,30-32] Acceptable agreement with the earlier values was found (Table 4.9) for PCB-126 and PCB-169 , although the result for the 77 congener was low probably because Aroclor batches are known to vary in composition.



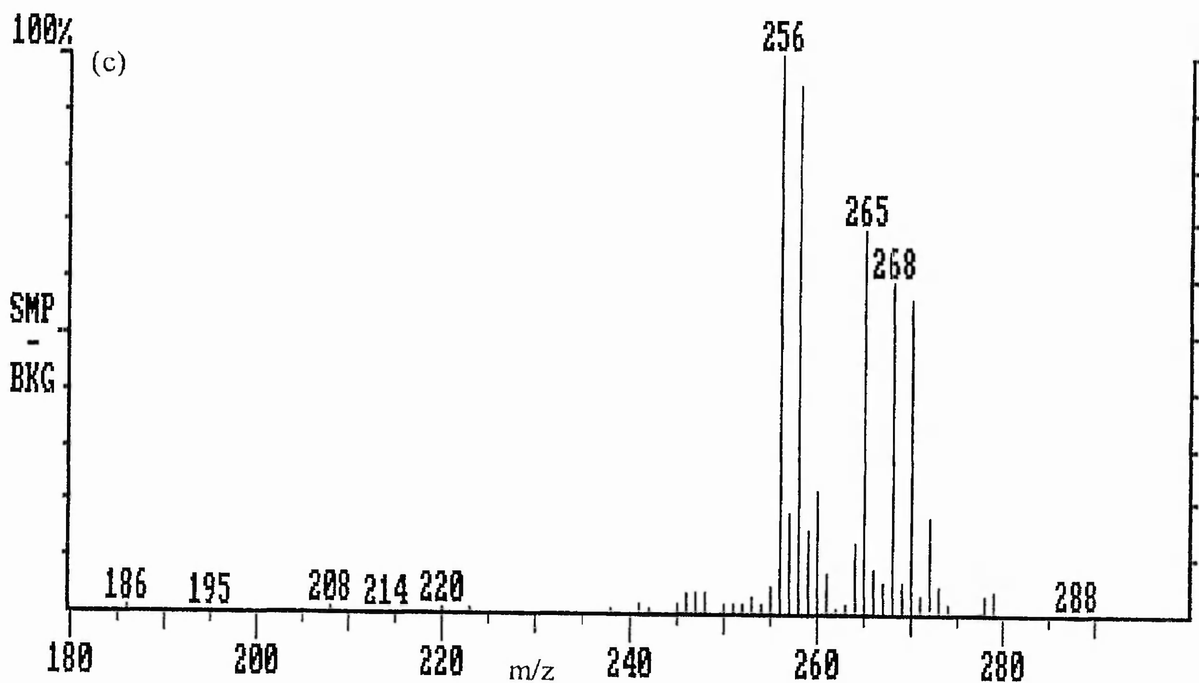
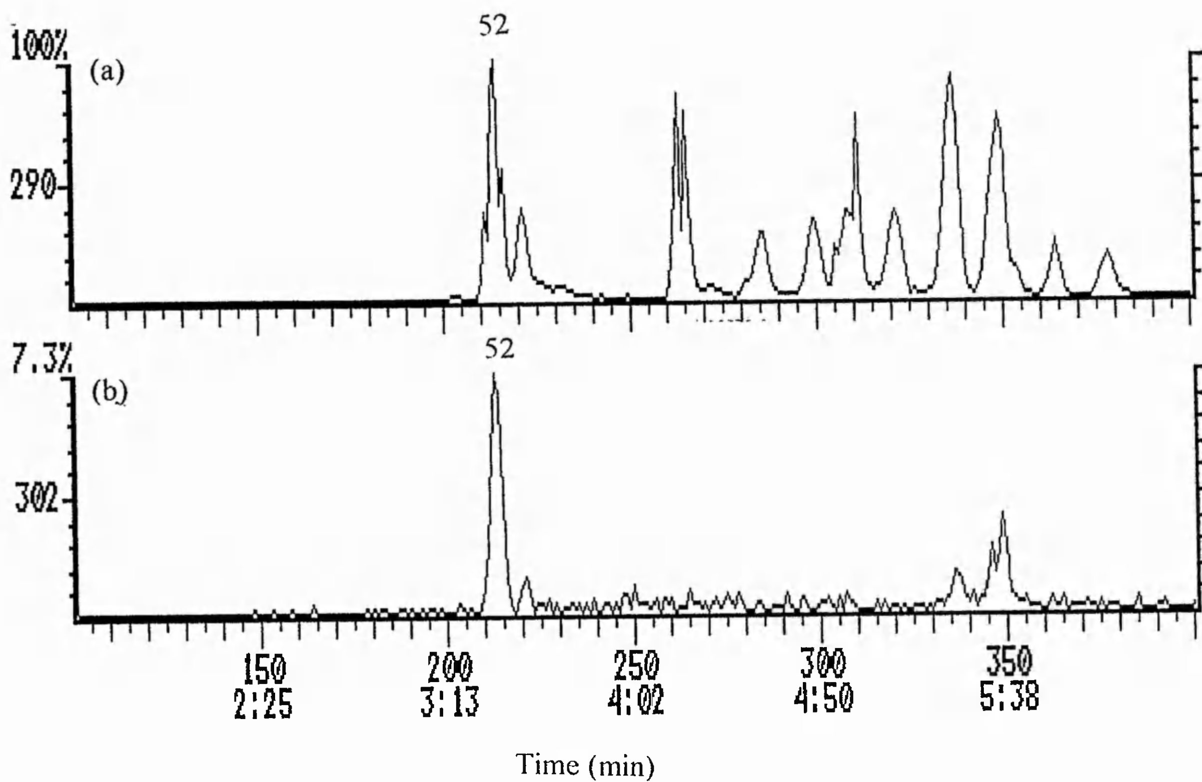


Figure 4.15. Trichlorinated PCBs in the *ortho* fraction of Aroclor 1254 mixture: (a) selected ion chromatogram for $^{12}C_{12}$-PCB, (b) selected ion chromatogram for $^{13}C_{12}$-PCB internal standard and (c) mass spectrum obtained at the retention time of the PCB-28 internal standard



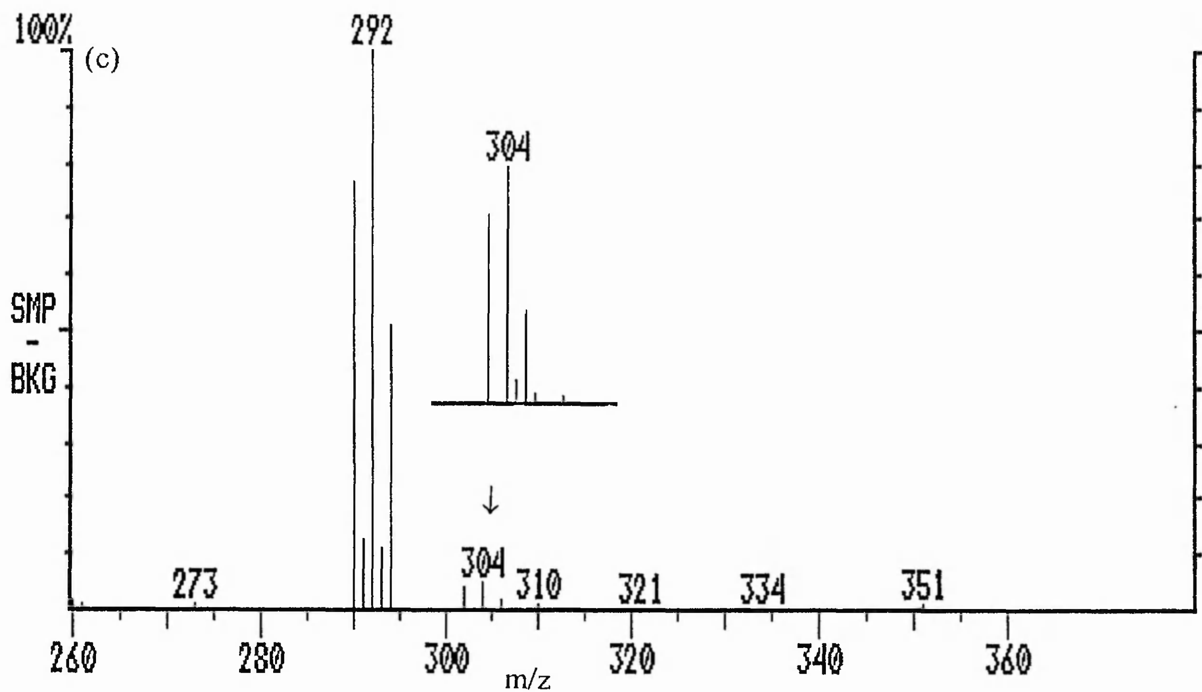
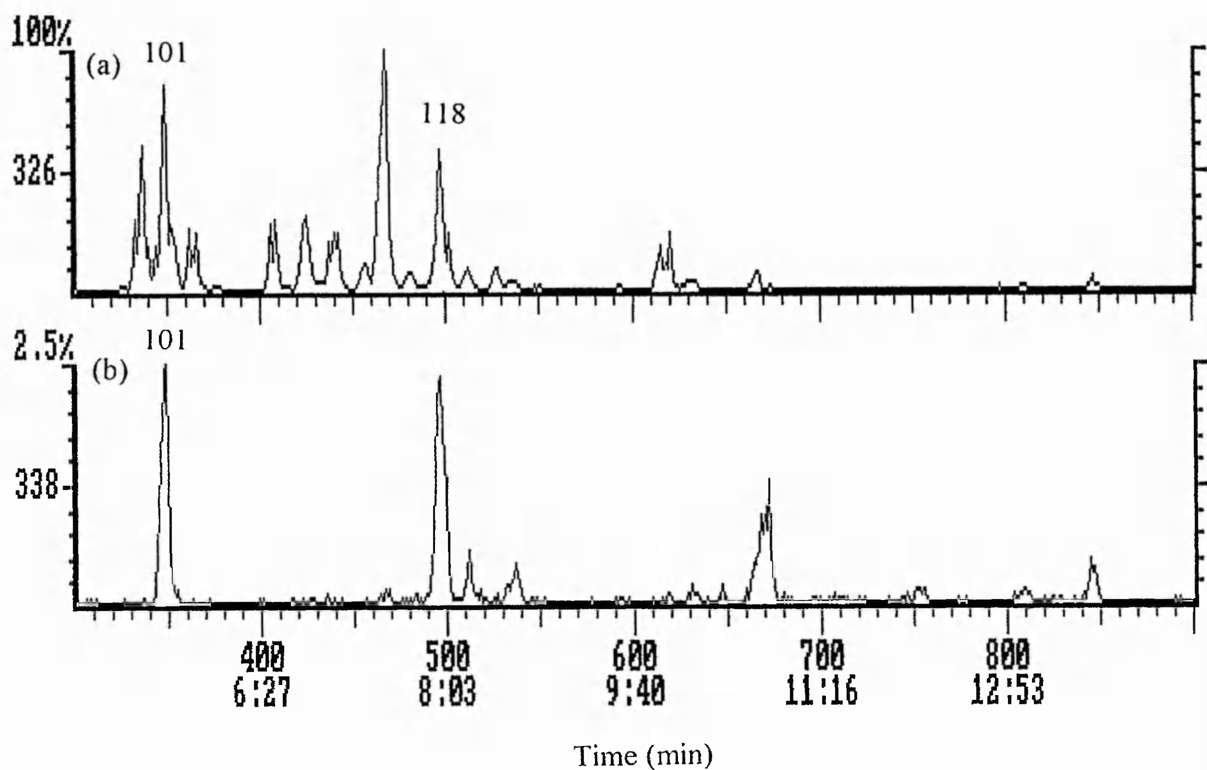


Figure 4.16. Tetrachlorinated PCBs in the *ortho* fraction of Aroclor 1254 mixture: (a) selected ion chromatogram for $^{12}C_{12}$-PCB, (b) selected ion chromatogram for $^{13}C_{12}$-PCB internal standard and (c) mass spectrum obtained at the retention time of the PCB-52 internal standard



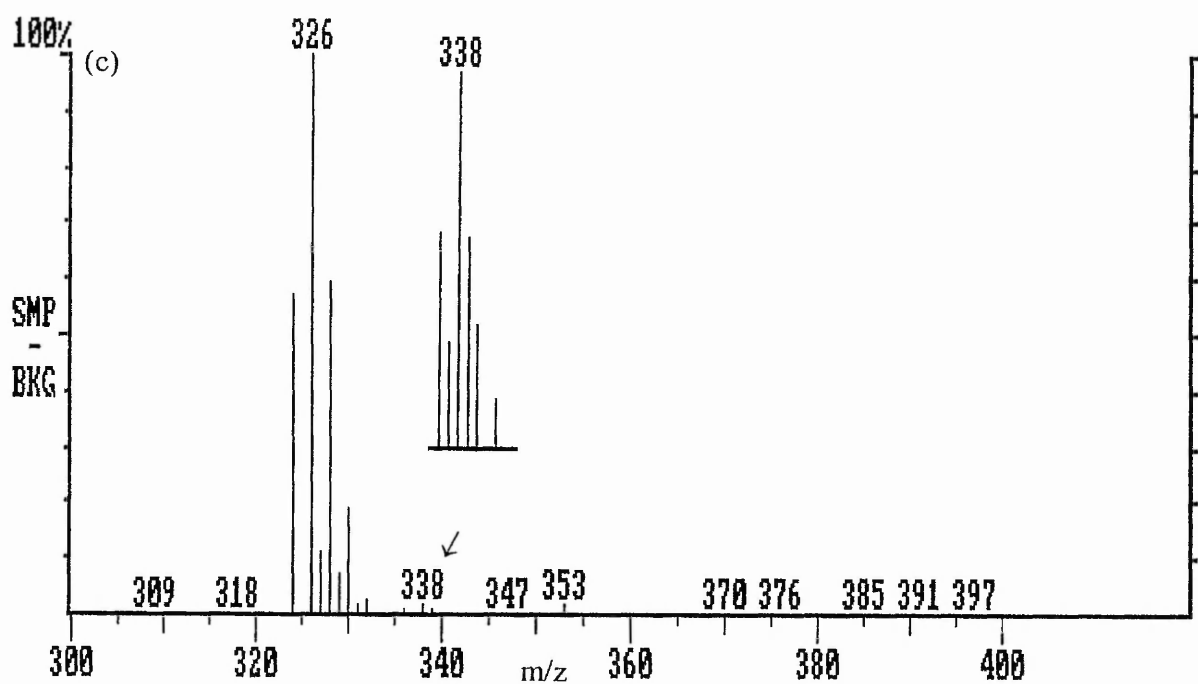
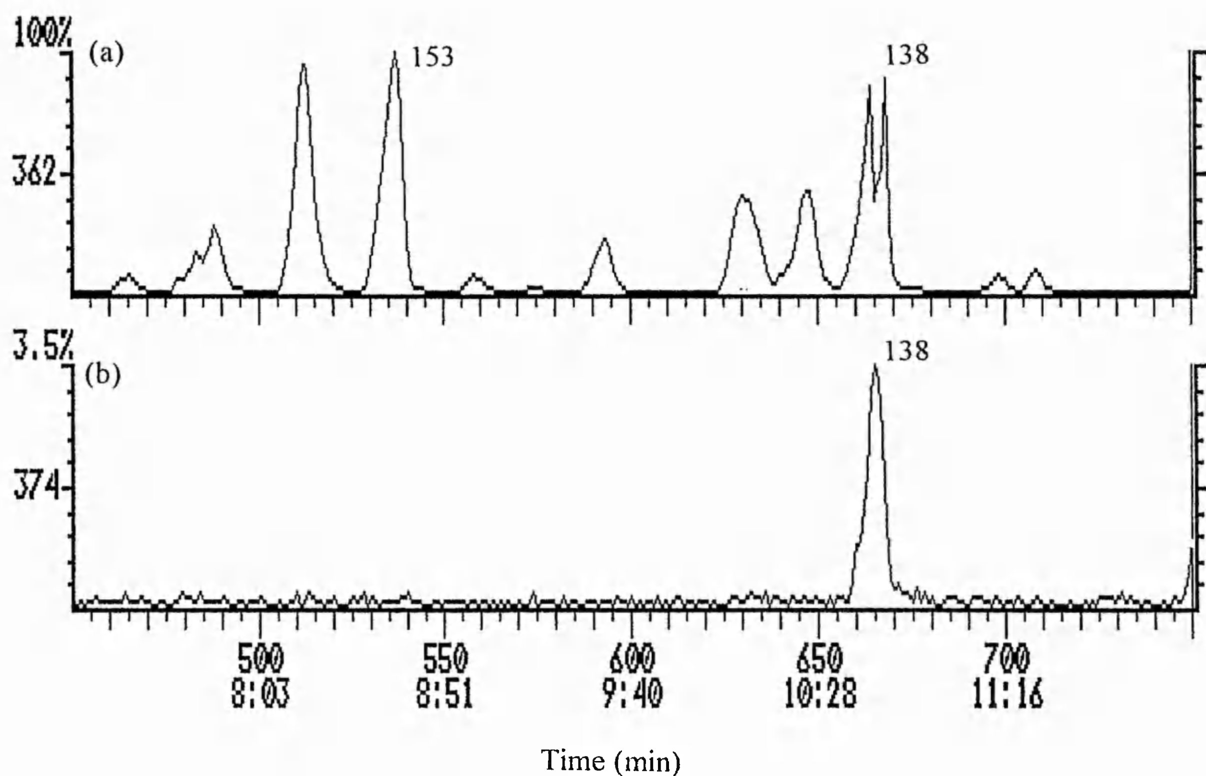


Figure 4.17. Pentachlorinated PCBs in the *ortho* fraction of Aroclor 1254 mixture: (a) selected ion chromatogram for $^{12}C_{12}$-PCB, (b) selected ion chromatogram for $^{13}C_{12}$-PCB internal standard and (c) mass spectrum obtained at the retention time of the PCB-101 internal standard



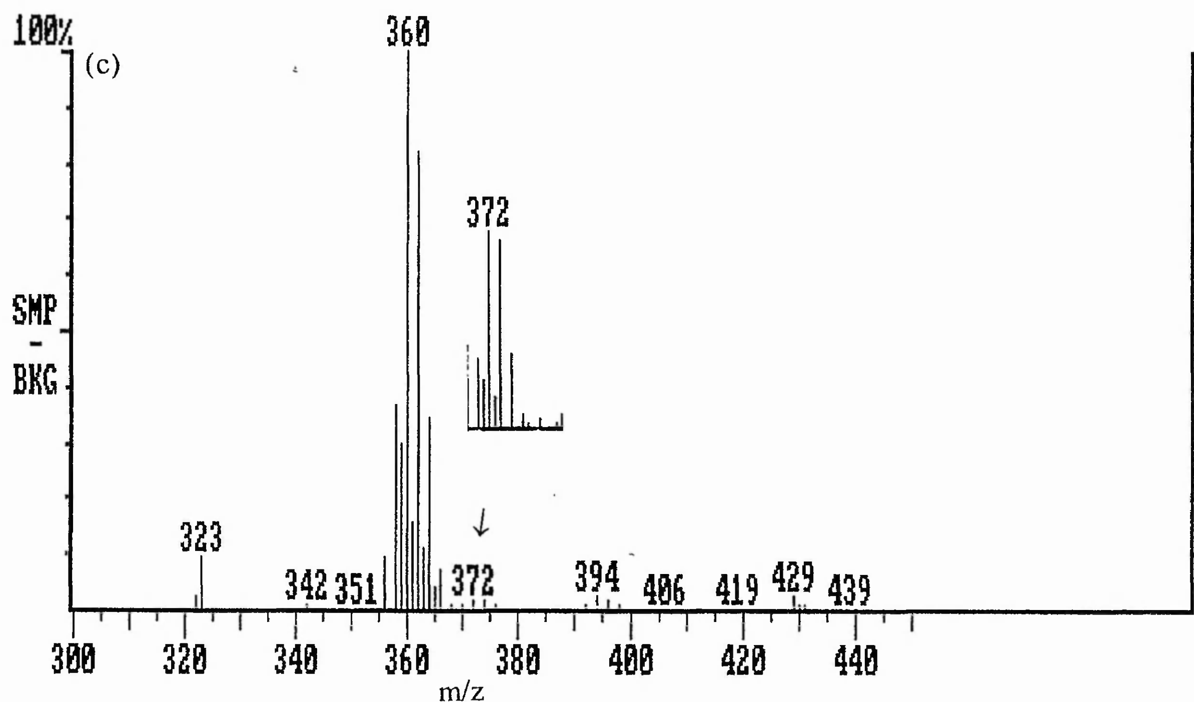
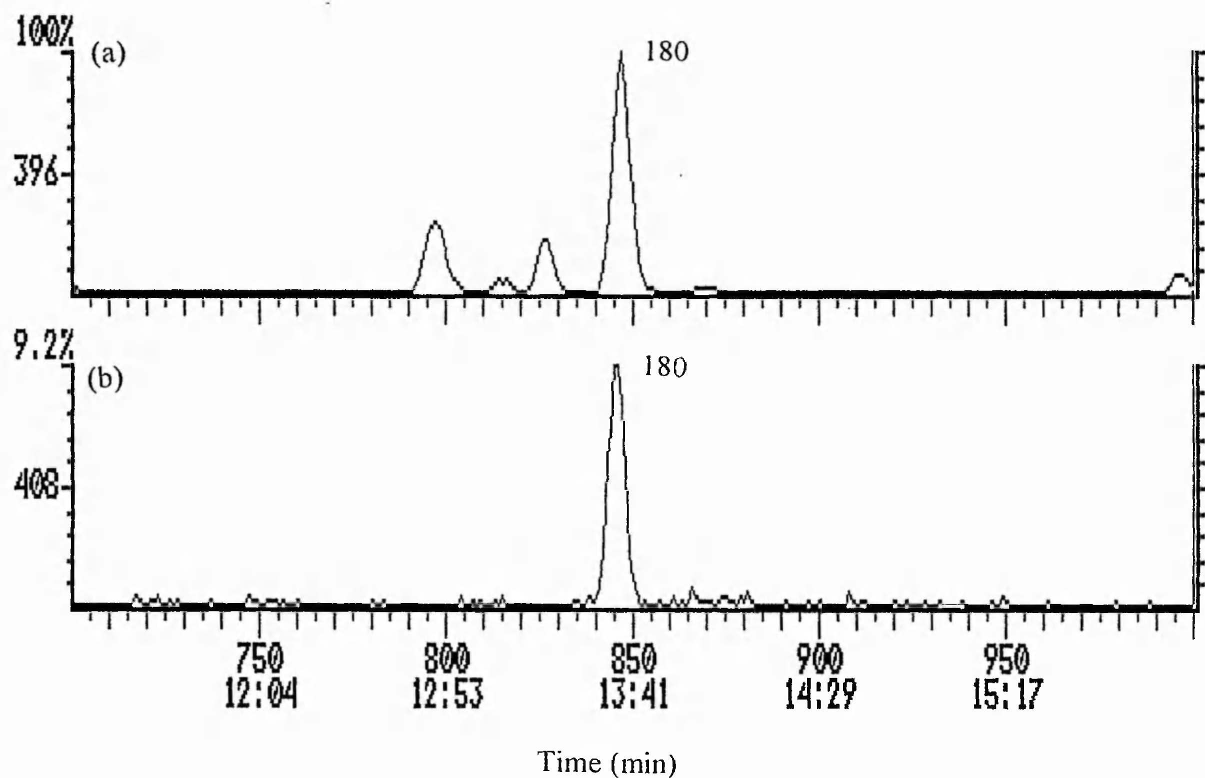


Figure 4.18. Hexachlorinated PCBs in the *ortho* fraction of Aroclor 1254 mixture: (a) selected ion chromatogram for $^{12}C_{12}$-PCB, (b) selected ion chromatogram for $^{13}C_{12}$-PCB internal standard and (c) mass spectrum obtained at the retention time of the PCB-138 internal standard



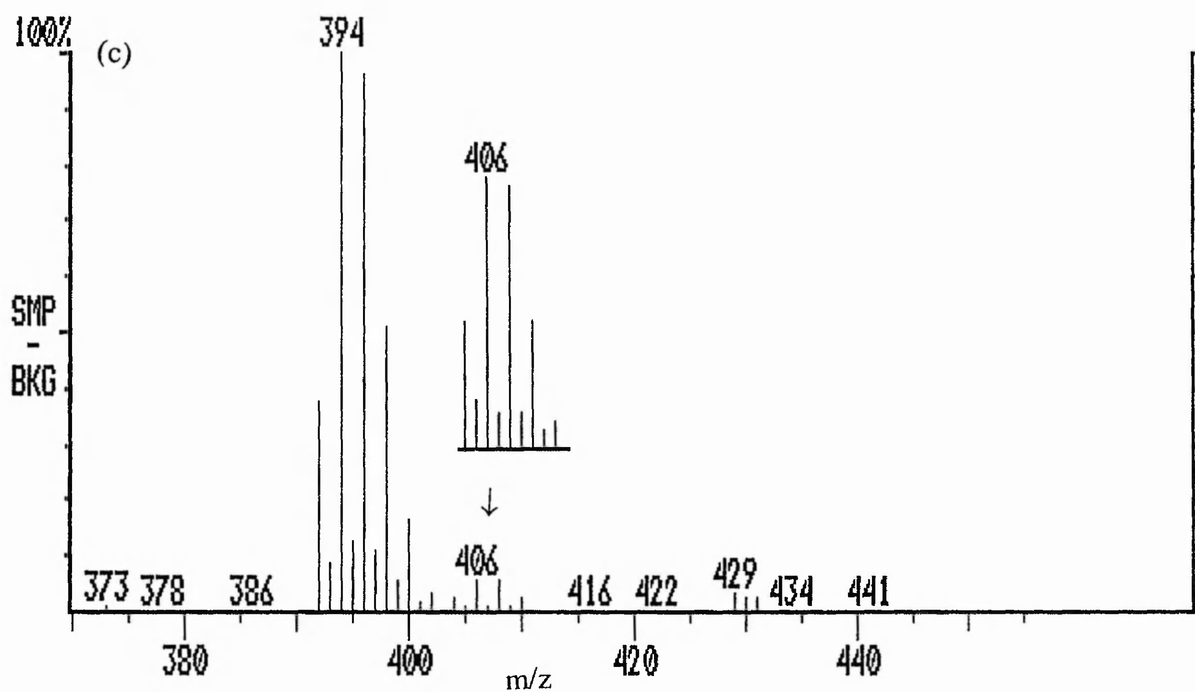


Figure 4.19. Heptachlorinated PCBs in the *ortho* fraction of Aroclor 1254 mixture: (a) selected ion chromatogram for $^{12}C_{12}$-PCB, (b) selected ion chromatogram for $^{13}C_{12}$-PCB internal standard and (c) mass spectrum obtained at the retention time of the PCB-180 internal standard

Table 4.8. *Ortho*-PCB congener composition (wt%) of selected PCBs in Aroclor 1254

	PCB						
Laboratory	28	52	101	118	138	153	180
This work	1.1[c]	7.2	25	20	8.5	4.9	6.1
Draper et al[a]	nd	7.8	13	11	9.5	5.3	0.9
Schulz et al[b]	nd	nd	7.9	6.4	3.2	4.3	nd

[a]from reference 27
[b]from reference 29
[c]wt% of sum of PCB-28 and PCB-31
[nd]Not detected

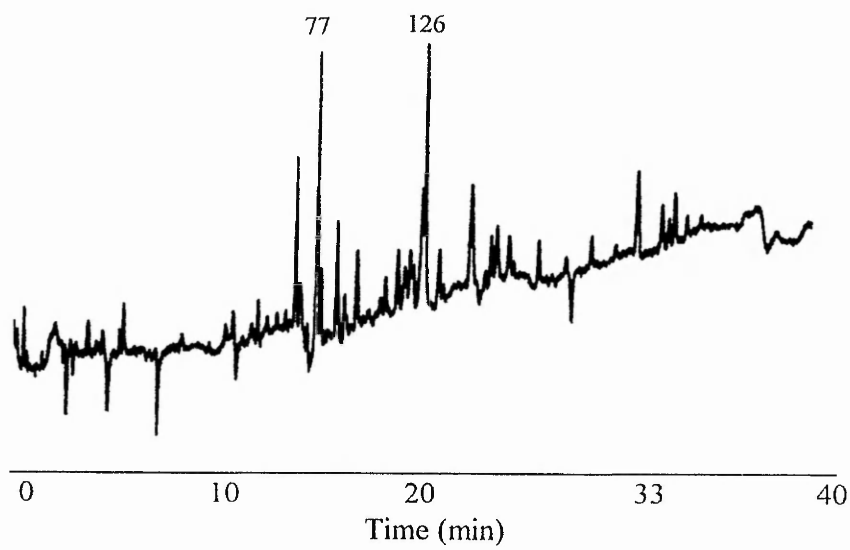


Figure 4.20. HPLC-GC-ECD (att:16) chromatogram for the non-*ortho*-PCB fraction in Aroclor 1254

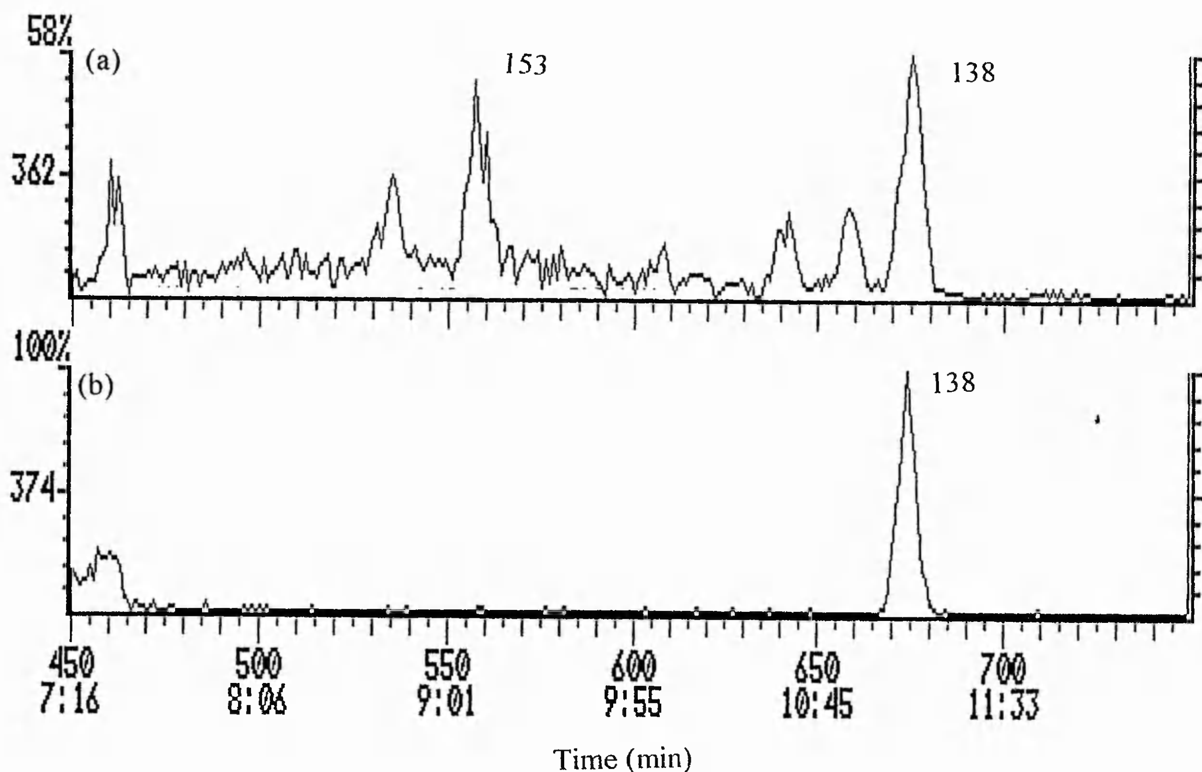
Table 4.9. Concentration ($\mu g\ g^{-1}$) of non-*ortho* substituted PCBs in Aroclor 1254

Laboratory	PCB 77	PCB 126	PCB 169
This work	97	40	<10
Al-Haddad[a]	240	80	<0.007
Huckins et al.[b]	210	<250	<0.4
Kannan et al.[c]	616	38.3	0.51
Schwartz et al.[d]	220	33	<1

[a]from reference 18, [b]from reference 30, [c]from reference 31 and [d]from reference 32

4.3.5. HPLC-GC analysis of a soil sample

Polychlorinated biphenyls were also identified in a soil sample obtained from the viscinity of a chemical incinerator in South Wales. For the isolation of PCBs-28, 52, 101, 118, 138, 153 and 180, the soil extract was cleaned-up on a multilayer column and chromatographed on the PGC column. The fraction eluting between 2-6 min was transferred on-line from the PGC HPLC column to the GC-MS for analysis. The higher chlorinated PCBs (PCBs-138,153 and 180) were identified at concentrations of 3, 2, and 5 $\mu g\ kg^{-1}$. The GC chromatograms for the native $^{12}C_{12}$-PCBs, the $^{13}C_{12}$-PCB internal standard and the mass spectrum obtained at the retention time of the internal standard for the hexa- and heptachlorinated PCBs are presented in Figures 4.21 and 4.22. The base peaks intensities of the molecular clusters for hexa- and heptachlorinated PCBs resemble closely the theoretical isotopic ratios as already



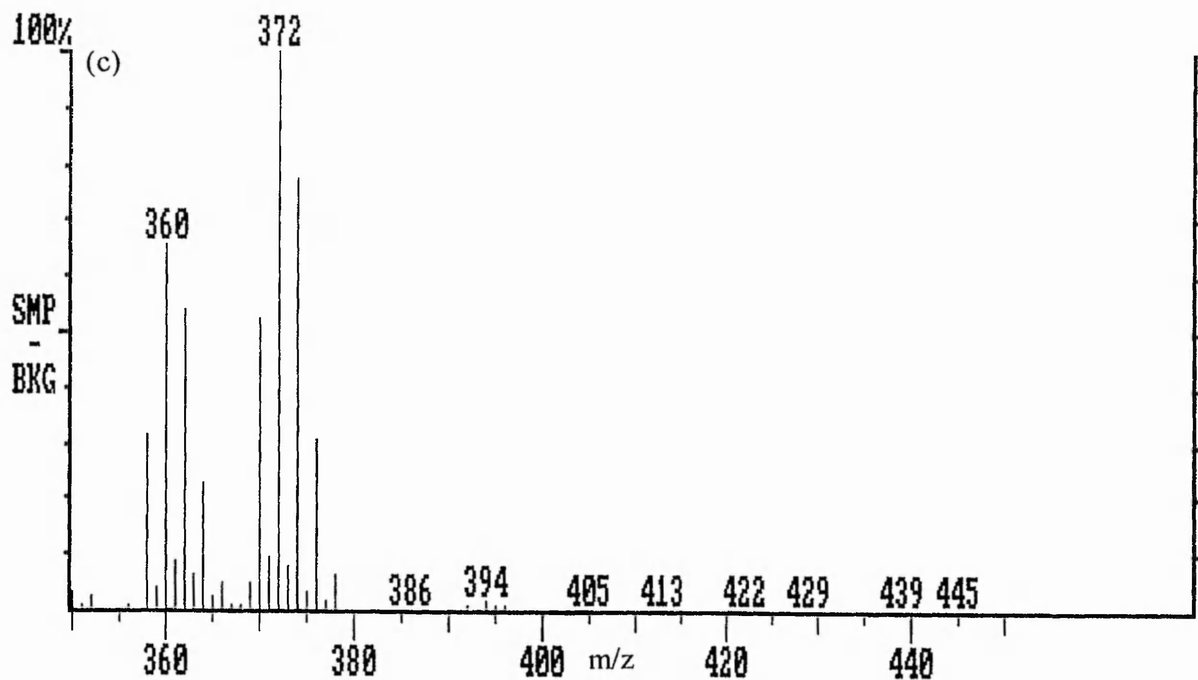
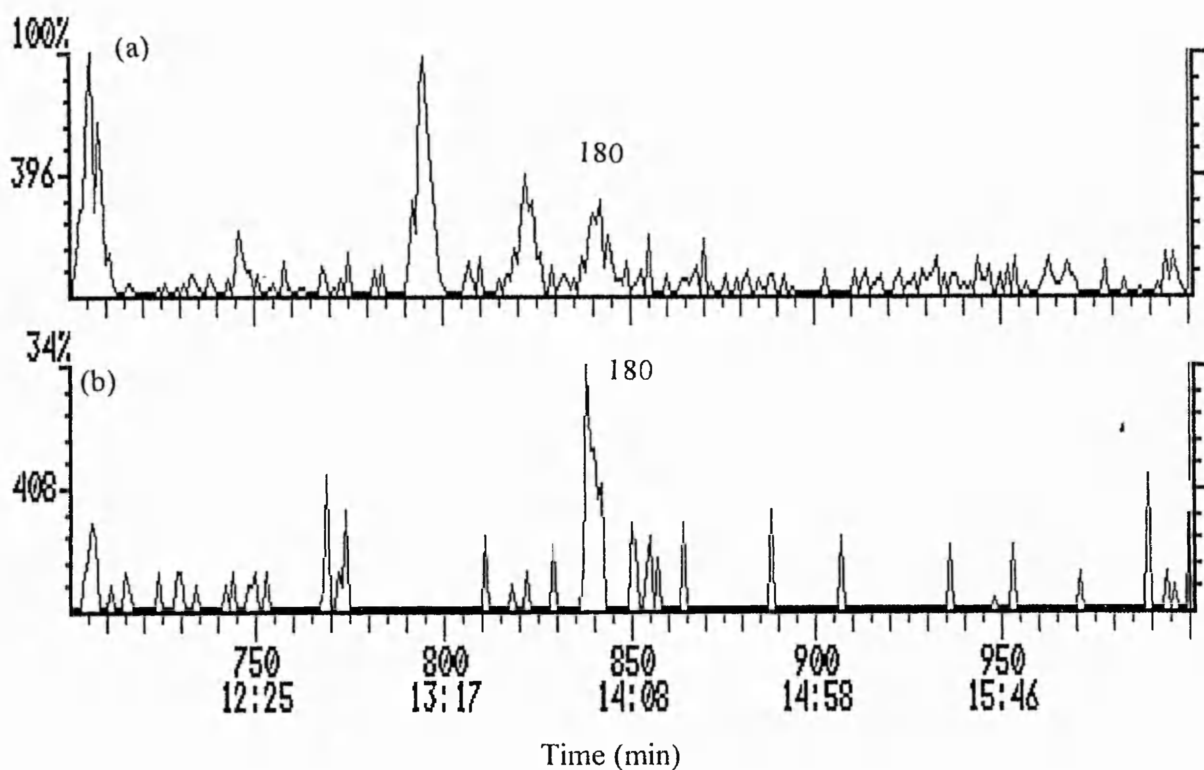


Figure 4.21. Hexachlorinated PCBs in the *ortho* fraction of a soil sample: (a) selected ion chromatogram for $^{12}C_{12}$-PCB, (b) selected ion chromatogram for $^{13}C_{12}$-PCB internal standard and (c) mass spectrum obtained at the retention time of the internal standard



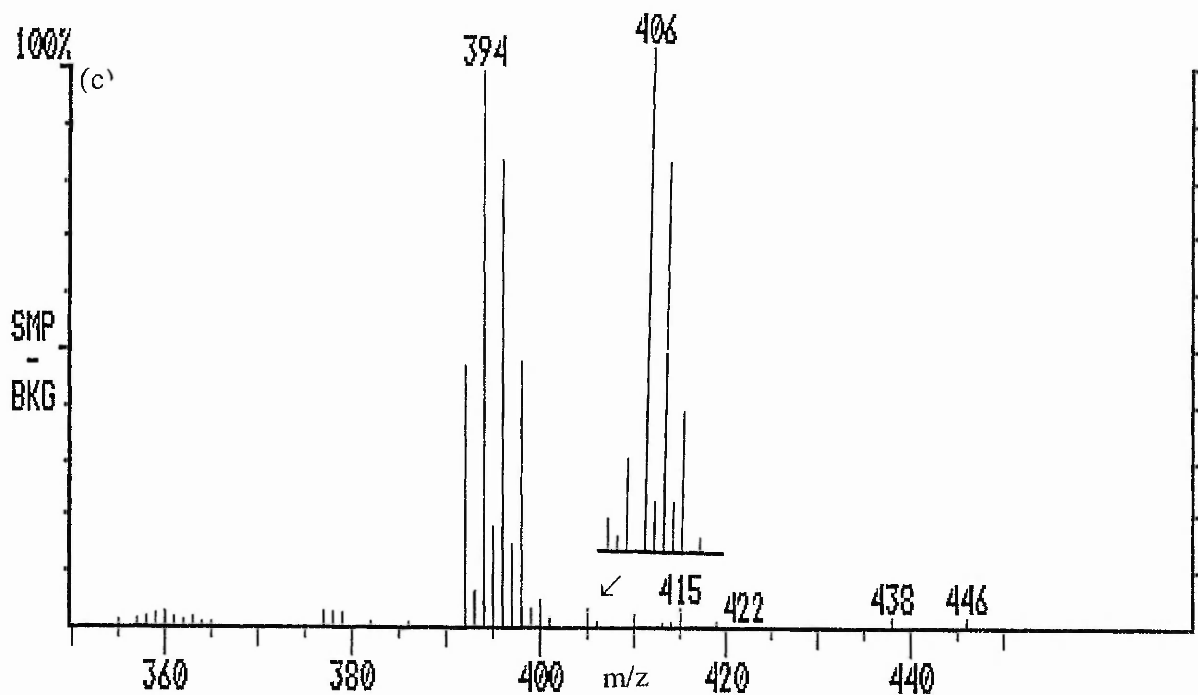


Figure 4.22. Heptachlorinated PCBs in the *ortho* fraction of a soil sample: (a) selected ion chromatogram for $^{12}C_{12}$-PCB, (b) selected ion chromatogram for $^{13}C_{12}$-PCB internal standard and (c) mass spectrum obtained at the retention time of the internal standard

observed for the PCB standard mixture. The lower chlorinated PCBs (PCBs-28, 52, 101 and 118) were not identified because of the high level of mineral transformer oil present in the sample. This particular soil sample (CJ) was analysed independently as part of a survey of the South Wales incinerator[33] and was one of the soil samples that showed the highest PCB concentrations and background interferences. A problem encountered with the HPLC-GC analysis of such highly contaminated soil samples was that the pre-column was contaminated easily by matrix components. However, provided the condition of the pre-column was monitored, the removal of *ca* 50 cm from the head of the pre-column was all that was required to restore the original performance of the HPLC-GC analysis.

4.4. CONCLUSIONS

The retention characteristics of PCBs on carbon have been discussed (see chapter 2, section 2.3) and are mainly determined by the degree of *ortho* chlorine substitution on the biphenyl ring. Similar retention characteristics were observed for PGC. Elution of the PGC column with hexane:toluene (95:5, v/v) enabled the *ortho*-PCBs to be separated from the non-*ortho*-PCBs and to the GC pre-column as a fraction eluting between 2-6 minutes at a flow rate of 0.3 ml min^{-1}. By changing the solvent from hexane:toluene (95:5, v/v) to pure toluene, the non-*ortho*-PCBs where eluted from the PGC column and diverted to the GC in two fractions. The first one containing PCBs 77 and 126 being diverted between 14-18 minutes and the second one between 18-20 minutes containing PCB-169. These fractions were combined on the cold trap before being together onto the analytical column.

The overall performance of the HPLC-GC system for *ortho-* and non-*ortho* substituted polychlorinated biphenyl congeners was determined by replicate analyses of a PCB standard mixture. The method demonstrated a good repeatability with mean precision (expressed as %COV) of 6.8% and 7.7% for *ortho-* and non-*ortho-*PCBs, respectively.

The HPLC-GC system developed was successfully applied to the determination of *ortho* and non-*ortho*-PCBs in soil and Aroclor 1254. Concentrations (wt%) of *ortho-*PCBs of interest in Aroclor 1254 ranged from 1.1% to 25% and from <10 $\mu g\ g^{-1}$ to 97 $\mu g\ g^{-1}$ for non-*ortho*-PCBs of interest.

Compared to conventional PCB determination methods, the on-line HPLC-GC technique reduces the analysis time by decreasing the number of sample clean-up steps. Additionally, the on-line technique reduces the risk of sample contamination and loss during the transfer from HPLC to GC.

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Appendix 1

Publications

1. On-line clean-up for the determination of *ortho-* and non-*ortho*-substituted polychlorinated biphenyls in fruit and vegetable samples by gas chromatography-mass spectrometry. C. S. Creaser and D. Chewe, presented at SAC '95, University of Hull, 1995.

2. Method development for the congener specific determination of *ortho-* and non-*ortho*-substituted polychlorinated biphenyls in fruit and vegetable samples. C. S. Creaser and D. Chewe, *Organohalogen compounds,* 1995, **23**, 89.

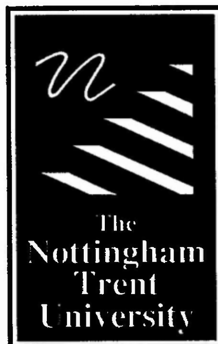
3. PCB and PCDD/DF concentrations in fruit and vegetable samples from urban and rural areas in Wales and England. C. D. Foxall, A. A. Lovett, C. S. Creaser and D. Chewe, *Organohalogen compounds,* 1995, **26**, 25.

4. PCB and PCDD/DF concentrations in egg and poultry meat samples from urban and rural areas in Wales and England. C. D. Foxall, A. A. Lovett, C. S. Creaser and D. Chewe, *Organohalogen compounds,* 1996, **28**, 160.

5. PCB and PCDD/DF congeners in locally grown fruit and vegetable samples from urban and rural areas in Wales and England. C. D. Foxall, A. A. Lovett, C. S. Creaser and D. Chewe, *Chemosphere,* 1997, **34**, 1421.

6. Validation of a congener specific method for *ortho-* and non-*ortho-* substituted polychlorinated biphenyls in fruit and vegetable samples. C. D. Foxall, A. A. Lovett, C. S. Creaser and D. Chewe, *Chemosphere,* 1997, **35**, 1399.

7. Concurrent determination of *ortho-* and non-*ortho*-substituted biphenyls by combined high performance liquid chromatography-gas chromatography. C. S. Creaser and D. Chewe, *J. Chromatogr.*, submitted for publication.



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