Journal of Chromatography, 149 (1978) 161–168

CHROM. 10,767

SEPARATION OF CHLORINATED DIBENZO-*p*-DIOXINS FROM CHLORINATED CONGENERS

R. J. DOLPHIN and F. W. WILLMOTT
Philips Research Laboratories, Redhill, Surrey (Great Britain)

SUMMARY

Chlorinated dibenzo-*p*-dioxins can be separated from commonly interfering compounds by high-performance liquid chromatography on a microparticulate alumina column. This separation forms the basis for a suggested rapid screening method for the determination of dioxins in environmental samples.

INTRODUCTION

Some of the polychlorinated dibenzo-*p*-dioxins (PCDDs) are highly toxic compounds and in particular 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, tetra) is one of the most powerful small-molecule toxins known to man[1]. These compounds have been found as impurities in chlorinated phenols[2] and in the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)[3]. TCDD is formed by a side-reaction occurring during the industrial production of 2,4,5-trichlorophenol and its subsequent conversion into 2,4,5-T or hexachlorophene (Fig. 1). The chlorinated phenols and their products are widely used both domestically and industrially as wood preservatives, bactericides, fungicides, herbicides, etc., providing a number of possible routes for their eventual entry into human foodstuffs. More serious, but usually more localized, environmental contamination has occurred following accidents at chemical plants. For example, in July 1976 an explosion occurred at a trichlorophenol plant in Seveso, Northern Italy[4] and an estimated 2 kg of TCDD was discharged over an area inhabited by over 2000 people. In such cases a rapid screening method is needed to measure TCDD in many different matrices, *e.g.*, water, crops and soil, at low concentrations, so that the extent of contamination can be determined. Furthermore, in cases of serious pollution, where evacuation has been carried out, the levels of TCDD must be monitored to indicate when relocation of the population is possible.

The analysis of environmental samples for chlorinated dibenzo-*p*-dioxins is complicated by interfering chlorinated congeners such as polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), polychlorinated dibenzofurans (PCDFs) and organochlorine pesticides, from both natural and industrial sources.

The high probability of interference is apparent from the large number of theoretically possible compounds of similar structure (Fig. 1), *e.g.*, 210 PCBs, 75 PCNs, 75 PCDFs and 75 PCDDs. The similar chromatographic behaviour of these

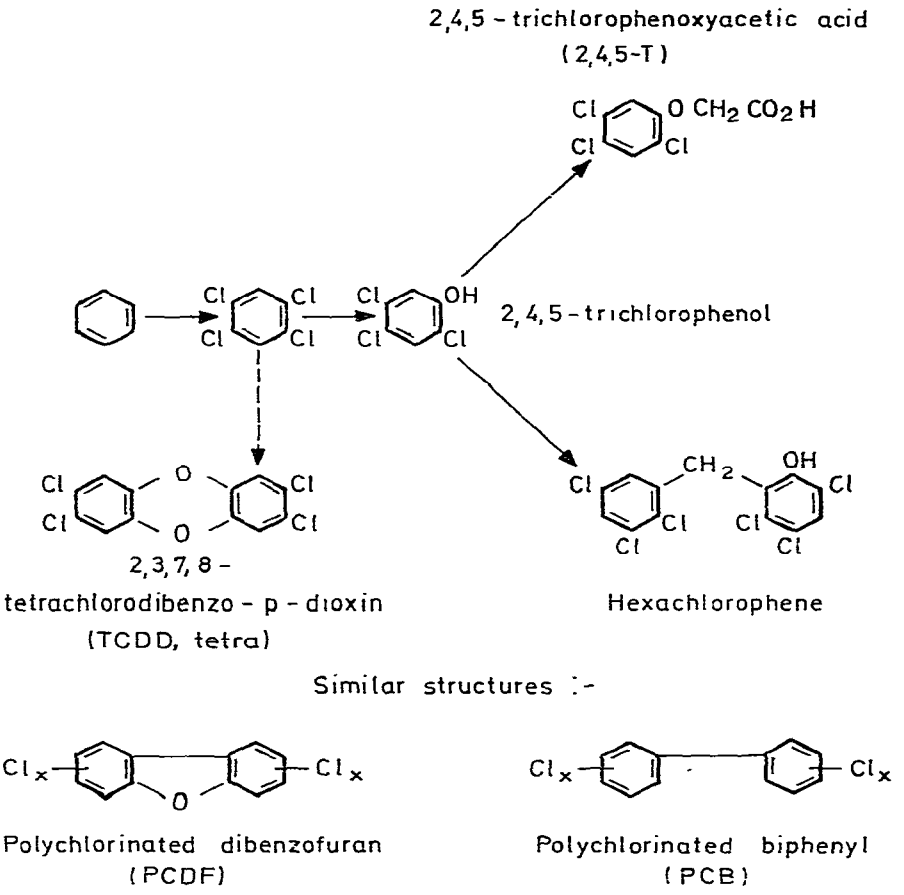

Fig. 1. Origin of TCDD and structures of related compounds.

compounds presents difficulties in determining TCDD by gas chromatography (GC) unless prior clean-up and separation from similar congeners is carried out. These steps are usually performed by open-column liquid or thin-layer chromatography (TLC) prior to analysis by GC followed by electron-capture (EC) detection.

In multi-residue pesticide methodology, open-column chromatography on Florisil[5] and silica gel[6] is commonly used to separate PCBs and PCNs from DDT and related compounds. A combination of silica and alumina columns[7,8] gives an improved separation into several groups of similar compounds. However, the separation of *p,p'*-DDE from all PCBs is difficult[8] except under very closely controlled conditions of adsorbent activity. Differentiation between PCBs and PCNs is also difficult as they have very similar retention characteristics on silica, and GC combined with mass spectrometry (GC–MS) is required in order to identify specific compounds. When only the total PCB or PCN concentration is required, perchlorination[9] to octachlorobiphenyl and octachloronaphthalene, respectively, which are clearly separated, and GC with EC detection provides a very sensitive method as these highly chlorinated compounds have a very high EC response. The situation is further complicated because

PCDDs are also eluted with PCBs and PCNs from Florisil and silica gel. However, PCDDs can be separated from PCBs by open-column adsorption chromatography[10,11] or TLC[12] on alumina.

In addition to being used for sample preparation, high-performance liquid chromatography (HPLC) has also been used for the direct determination of PCDDs in chlorophenols and related compounds[13,14]. In these types of samples the number of interfering compounds is small compared with those possible in environmental samples and, apart from isolating the non-phenolic impurities, no further sample preparation is necessary. The use of HPLC for PCDD determinations has so far been limited to quality control of commercial products down to concentrations of *ca.* 1 ppm. Brinkman and co-workers[15,16] have characterized PCBs and PCNs by HPLC on microparticulate silica columns.

In this paper we show how the PCDDs can be separated from other very similar groups of compounds by HPLC on microparticulate alumina. This separation could form the basis of a rapid screening method for the determination of TCDD in environmental samples. An important advantage of HPLC is the reduced sample preparation compared with GC[17] and the elimination of sample volume reduction with its inherent risk of losing volatile compounds.

EXPERIMENTAL

All separations were carried out on a Pye Unicam LC3 liquid chromatograph equipped with a variable-wavelength UV detector. For high sensitivity work the Pye Unicam liquid chromatography electron-capture detector (LC/EC) was employed.

The mobile phase, *n*-hexane (HPLC grade, Rathburn Chemicals, Walkerburn, Great Britain), was used without further purification.

The microparticulate silica column was slurry packed using 5-μm LiChrosorb SI 60 (BDH, Poole, Dorset, Great Britain). Pre-packed microparticulate alumina (10-μm Alox T; Chrompack U.K., London, Great Britain) and bonded-phase (10-μm Partisil-PAC; Whatman, Maidstone, Great Britain) columns were also used.

Standards were obtained from Analabs (North Haven, Conn., U.S.A.) (PCDDs, PCDFs, PCBs, PCNs) and Applied Science Labs. (State College, Pa., U.S.A.) (organochlorine pesticides). Standard solutions were prepared in either the mobile phase, AnalaR-grade chloroform or benzene (BDH). The most toxic PCDDs and PCDFs are those which have halogens in at least three of the four lateral ring positions and at least one non-halogenated ring position. The octachlorinated compounds and the parent molecules have relatively low toxicities and in this work, where we primarily wished to illustrate group separations, we have used only these compounds for safety reasons. Nevertheless, care should be exercised in handling these compounds as appreciable amounts of toxic impurities such as TCDD may be present.

RESULTS AND DISCUSSION

PCDDs are eluted with PCBs and PCNs from a silica gel clean-up column and Fig. 2 shows that a similar result is obtained by HPLC on microparticulate silica. In general, the retention time of these compounds increases with decreasing chlorine substitution in the parent molecule (this effect is opposite to that observed in GC).

Hence, the retention limits of each group of compounds, *e.g.*, PCBs, is given by the retentions of the unsubstituted and fully substituted compounds, *e.g.*, biphenyl and decachlorobiphenyl. The retention range of the PCBs overlaps that of both the PCNs and the PCDDs and unless this separation is followed by MS, correct identification of PCDDs is unlikely. On the other hand, the silica–dry *n*-hexane system is excellent for resolving commercial PCB and PCN mixtures, *e.g.*, Aroclors and Halowaxes, respectively, into their individual components[15,16] or for identifying commercial mixtures according to their characteristic fingerprint patterns.

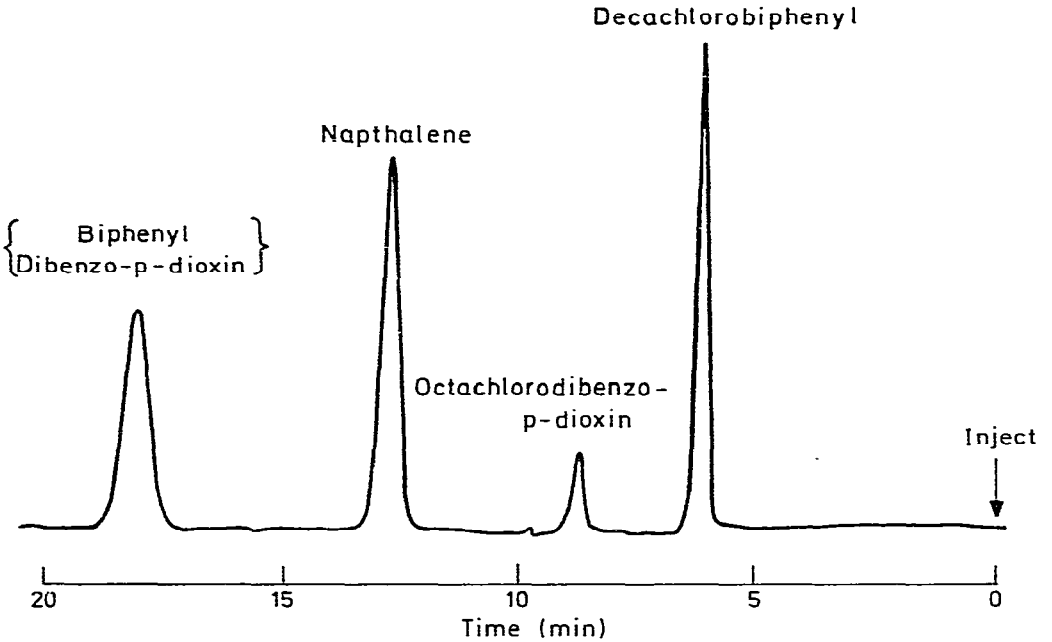

Fig. 2. Chromatogram of dioxins and similar compounds on microparticulate silica. Column: 5-μm LiChrosorb SI 60, 210 × 4.9 mm I.D. Mobile phase: *n*-hexane, flow-rate 1.0 $cm^3 \cdot min^{-1}$. Detector: Pye Unicam LC3, wavelength 220 nm, attenuation 0.025 absorbance unit.

Similar results to those on silica were obtained on a polar bonded phase (Partisil-PAC) for normal-phase HPLC (see Fig. 3). Figs. 3b and 3c show typical chromatograms for a PCN (Halowax 1099 containing *ca.* 50% of chlorine) and a PCB (Aroclor 1268 containing *ca.* 68% of chlorine), which compare favourably with those on silica gel in refs. 15 and 16. However, there is no improvement over silica as far as a group separation of PCBs and PCNs from PCDDs is concerned.

Open-column adsorption chromatography on alumina has become a standard technique for the pre-treatment of environmental samples before analysis for PCDDs by GC. In broad agreement with these classical methods, Fig. 4 shows chromatograms of PCNs, PCBs and PCDDs obtained on a microparticulate alumina–*n*-hexane HPLC system. In contrast to the silica–*n*-hexane and Partisil-PAC–*n*-hexane systems (Fig. 2), the retention increases with increasing chlorine content of the compound. The PCB (A1268) and PCN (H1051) mixtures are those which have the highest chlo-

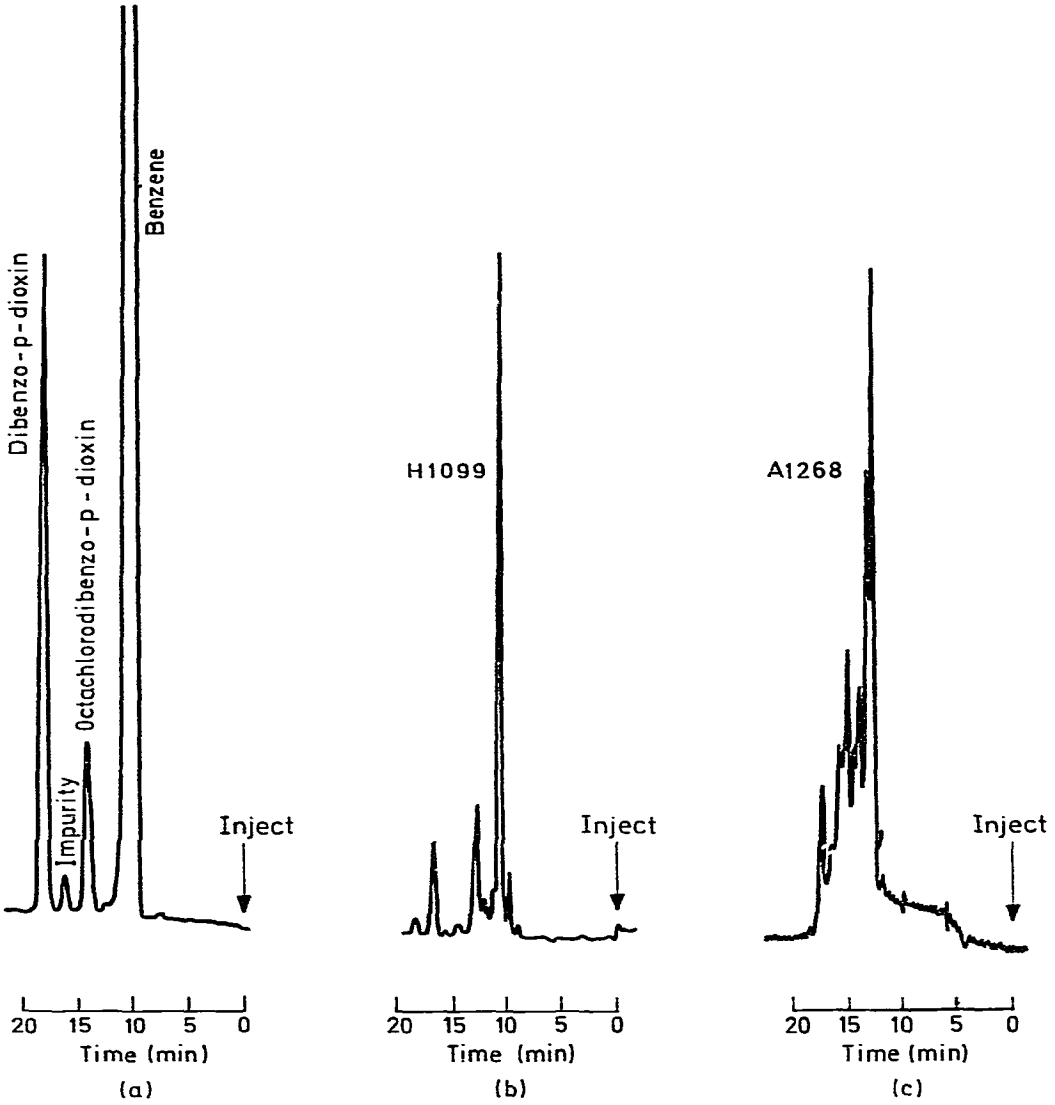

Fig. 3. Chromatograms of (a) dioxins, (b) PCN (Halowax 1099) and (c) PCB (Aroclor 1268) on a bonded-phase column. Column: 10-μm Partisil-PAC, 250 × 4.6 mm I.D. Mobile phase: *n*-Hexane, flow-rate 0.6 $cm^3 \cdot min^{-1}$. Detector: Pye Unicam LC3, wavelength 220 nm, attenuation 0.2 absorbance unit.

rine content in their respective groups. Hence, with reference to Fig. 4, it can be seen that as A1268 and H1051 do not interfere with the analysis of PCDDs, the microparticulate alumina–*n*-hexane system performs a very useful separation of PCDDs from all of the common PCB and PCN mixtures. Although this column gives a good group separation, it is not as good as either the silica or polar bonded columns for separation of the PCB–PCN mixtures into their individual components. This can be seen in Fig. 4, where both the PCB and PCN mixtures give only a single peak.

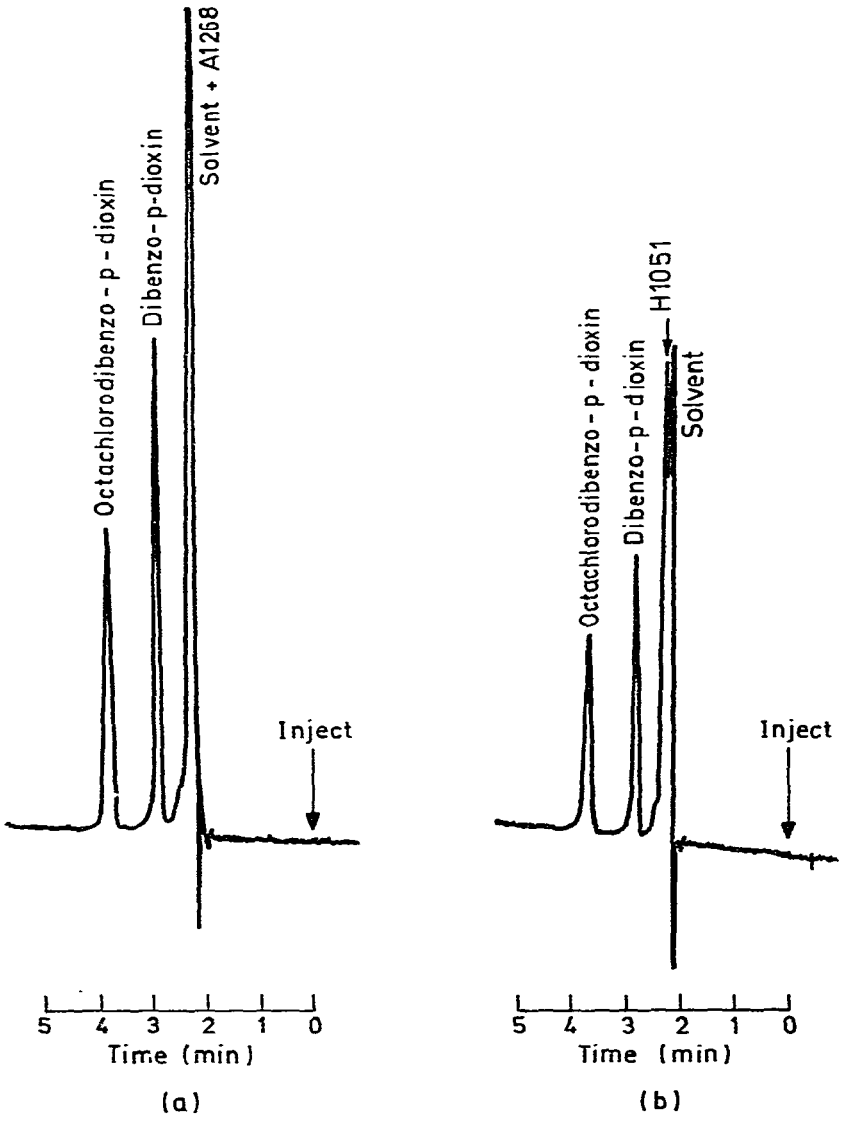

Fig. 4. Separation of dioxins from (a) PCB (Aroclor 1268) and (b) PCN (Halowax 1051) on a microparticulate alumina column. Column: 10-μm Alox T, 250 × 4.6 mm I.D. Mobile phase: *n*-Hexane, flow-rate 1.8 $cm^3 \cdot min^{-1}$. Detector: Pye Unicam LC3, wavelength 270 nm, attenuation 0.04 absorbance unit.

The capacity factors of some other polychlorinated compounds are given in Table I, where it can be seen that most of the common organochlorine pesticides are outside the retention range of the PCDDs. The capacity factors for the hexachlorocyclohexane (HCH) isomers were measured using an LC/EC detector as HCH has no UV response. Table I also suggests that *p,p'*-DDE could be separated from PCBs, PCNs and PCDDs. This separation is shown in Fig. 5, and this system could be used

TABLE I

CAPACITY FACTORS ON MICROPARTICULATE ALUMINA WITH DRY *n*-HEXANE SOLVENT

Compound	*Capacity factor*	*Compound*	*Capacity factor*
p,p'-DDE	1.2	Octachlorodibenzofuran	1.5
p,p'-DDT	1.4	Octachlorodibenzo-*p*-dioxin	1.8
Dibenzofuran	1.4	α-HCH	2.3
Dibenzo-*p*-dioxin	1.4	β-HCH	2.9
		Dieldrin	7.2

to determine *p,p'*-DDE in those circumstances where interference due to PCBs and PCNs occurs in standard pesticide analysis methods.

HPLC can be used in the quantitative analysis of PCDDs in a number of ways. At the higher concentrations found in a simple matrix, *e.g.*, commercial chlorophenols, analysis can be carried out on a single column with either a UV or a LC/EC detector,

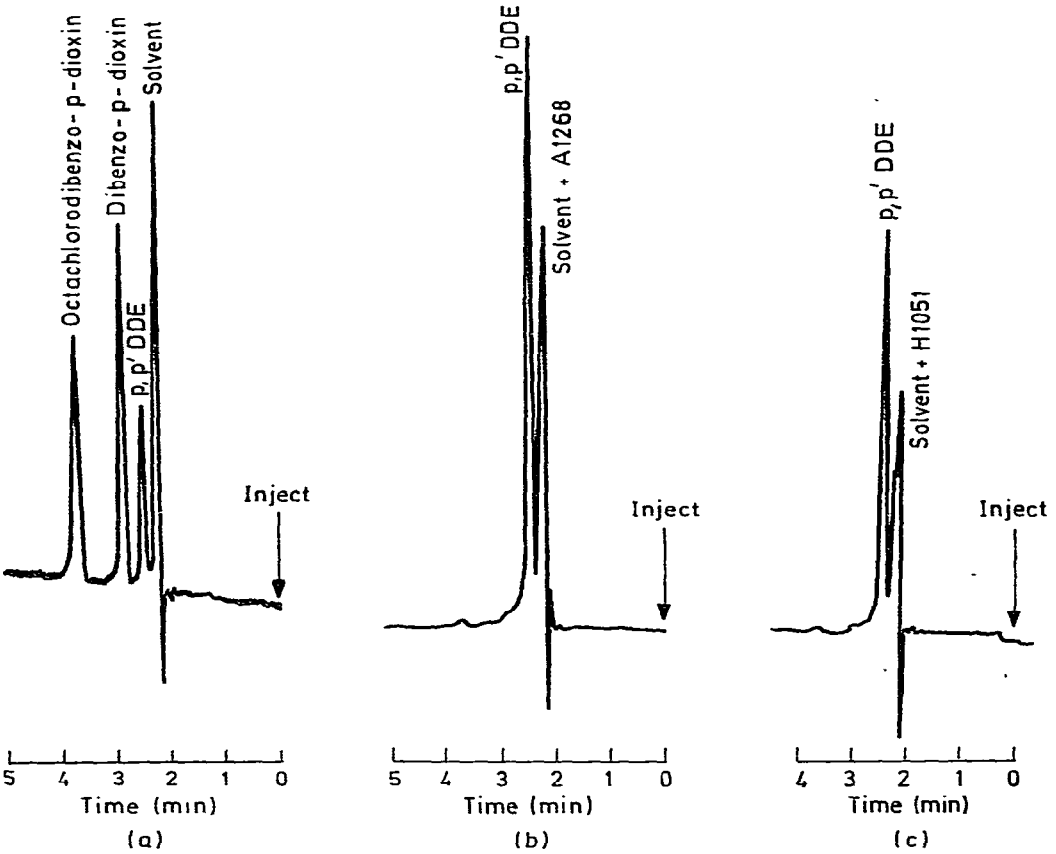

Fig. 5. Separation of *p,p'*-DDE from (a) dioxins, (b) PCB (Aroclor 1268) and (c) PCN (Halowax 1051). Conditions as in Fig. 4.

depending on the sensitivity required. For more complex matrices, *e.g.*, surface water, soil and foodstuffs, a series combination of alumina and silica columns can be used to give improved selectivity and lower detection limits. In this work we have shown that a microparticulate alumina column gives an excellent group separation of PCDDs from most other chlorinated congeners. Furthermore, silica and polar bonded-phase columns resolve chlorinated compounds according to the degree and position of substitution. By linking these two columns of different characteristics, a selected group of compounds separated on the first (*e.g.*, alumina) column can be transferred to a second column (*e.g.*, silica or polar bonded phase) for further separation into individual components. Column switching is common in GC and the advantages of "heart cutting", back-flushing, etc., as pointed out by Deans[18], are just as applicable in HPLC.

Maximum sensitivity of the method is obtained when large sample volumes are used together with the LC/EC detector[17,19]. Additional qualitative information may be given by using the UV and LC/EC detectors in series.

Table I shows that chlorinated dibenzofurans may interfere with the lower substituted dioxins, although it is probable that the most toxic PCDDs (all of which have three or more chlorine atoms) will be separated from the PCDFs. No methods have been reported for an improved separation of PCDDs from PCDFs. For such instances where the lower substituted PCDDs and PCDFs are to be quantified, then HPLC must be followed by MS analysis. HPLC can also be used solely for group separation prior to solvent reduction of the fraction collected (to increase concentration) followed by GC–MS analysis.

REFERENCES

1 A. Poland and E. Glover, *Environ. Health Perspect.*, 5 (1973) 245.
2 D. Firestone, J. Ress, N. L. Brown, R. P. Barron and J. N. Damico, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 85.
3 T. Ramstad, N. H. Mahle and R. Matalon, *Anal. Chem.*, 49 (1977) 386.
4 A. Hay, *Nature (London)*, 262 (1976) 636.
5 P. A. Mills, *J. Ass. Offic. Anal. Chem.*, 44 (1961) 170.
6 V. Leoni, *J. Chromatogr.*, 62 (1971) 63.
7 D. F. Goerlitz and L. M. Law, *J. Ass. Offic. Anal. Chem.*, 57 (1974) 176.
8 D. E. Wells and S. J. Johnstone, *J. Chromatogr.*, 140 (1977) 17.
9 O. Hutzinger, S. Safe and V. Zitko, *Int. J. Environ. Anal. Chem.*, 2 (1972) 95.
10 H. R. Buser, *Anal. Chem.*, 49 (1977) 918.
11 M. L. Porter and J. A. Burke, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 1426.
12 D. T. Williams and B. J. Blanchfield, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 1429.
13 W. B. Crummett and R. H. Stehl, *Environ. Health Perspect.*, 5 (1973) 15.
14 C. D. Pfeiffer, *J. Chromatogr. Sci.*, 14 (1976) 386.
15 U. A. Th. Brinkman, J. W. F. L. Seetz and H. G. M. Reymer, *J. Chromatogr.*, 116 (1976) 353.
16 U. A. Th. Brinkman, A. de Kok, H. G. M. Reymer and G. de Vries, *J. Chromatogr.*, 129 (1976) 193.
17 R. J. Dolphin, F. W. Willmott, A. D. Mills and L. P. J. Hoogeveen, *J. Chromatogr.*, 122 (1976) 259.
18 D. R. Deans, *Chromatographia*, 1 (1968) 18.
19 F. W. Willmott and R. J. Dolphin, *J. Chromatogr. Sci.*, 12 (1974) 695.