Journal of Chromatography, 555 (1991) 221–228
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 363

Simultaneous separation and determination of hydrocarbons and organochlorine compounds by using a two-step microcolumn

O. M. RODRIGUEZ
Department of Chemistry, University of Costa Rica, San Josè (Costa Rica)
and
P. G. DESIDERI*, L. LEPRI and L. CHECCHINI
Department of Public Health, Epidemiology and Environmental Analytical Chemistry, University of Florence, Via G. Capponi, 9-50121 Florence (Italy)
(First received December 21st, 1990; revised manuscript received April 3rd, 1991)

ABSTRACT

The simultaneous separation and determination of a mixture of hydrocarbons and organochlorine compounds was successfully carried out by using sorption chromatography on a two-step microcolumn of silica and aluminium oxide for their fractionation, and a dual detector system. In addition to the separation and identification of hydrocarbons and heterocompounds containing nitrogen, oxygen and sulphur atoms, separation and identification of chlorinated hydrocarbons (dichlorobenzenes, *p*-chlorotoluene, hexachlorobutadiene, 1,2,4-trichlorobenzene and 2-chloronaphthalene), pesticides (chlorpicrin, aldrin, lindane, α- and β-benzene hexachloride (BHC), endrin, dieldrin, endosulphan, methoxychlor) and herbicides (propanil, dichlobenil, trifluralin, difolatan) were achieved in mixtures containing polychlorinated biphenyl, strobane and chlordane.

INTRODUCTION

Investigations on the organochlorides [*i.e.* pesticides, benzene hexachloride (BHC) isomers and polychlorinated biphenyl (PCB) congeners] are of great importance in environmental studies since, as is well known, these compounds are persistent, have cumulative effects in human tissues, and occur widely in the environment [1–5].

Recent data [6–9] on the presence of untransformed 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDT) analogues in rain, snow, soil and peat samples indicate that, although their use is forbidden by law, there is a new input of DDTs even in regions where there is no evidence that direct applications are taking place.

The organochlorine compounds are a vast class of organic molecules which show similar chemical–physical properties. Pesticides for instance are chemically related to PCBs: this is a serious disadvantage to their identification and quantification because if simultaneously present in the same sample they will interfere with each

0021-9673/91/$03.50

other. A preliminary separation of these compounds is thus essential, and a great many papers on the subject are available in the literature; however, the problem has still not been definitely settled [10–11].

The present work investigates the possibility of extending the separation method already adopted for the analysis of hydrocarbons and heterocompounds containing nitrogen, oxygen and sulphur atoms [12] to mixtures that also contain organochlorine compounds. Our aim was to analyse the complex mixtures of organic compounds usually present in the environmental matrices [13–14] by using a single separation of the sample in homogeneous fractions by means of a two-step microcolumn coupled with gas chromatographic (GC) determinations using a dual detector system [flame ionization detection–electron-capture detection (FID–ECD)].

EXPERIMENTAL

Reagents and materials

The solvents used, *n*-hexane, methylene chloride, carbon tetrachloride, acetone and methanol, all supplied by Merck (Darmstadt, Germany), were for pesticide analysis. The hydrocarbons, pesticides, herbicides and chlorinated phenols were from Polyscience (IL, USA). The PCB standards, Arochlors 1016, 1232, 1242, 1248, 1260 and 1268, were from Supelco (St. Louis, MO, USA). Standards solutions of all compounds and several mixtures were prepared in *n*-hexane. The concentrations of hydrocarbons and of organochlorides were 0.2 mg/ml and 2 μg/ml for each compound, respectively. The volume of solution used for analysis was 50 μl. The silica gel 60 (70–230 mesh) and the basic aluminium oxide E type (70–230 mesh) supplied by Merck were activated at 120°C for 12 h.

Apparatus

A Carlo Erba (Milan, Italy) HRGC-5160 Mega Series gas chromatograph was used to identify simultaneously hydrocarbons and organochlorides in the different fractions. This instrument was equipped with both a flame ionization detector and an electron-capture detector, working in parallel by means of a split in the capillary column.

The injection was made using a cold split–splitless liquid injector according to the following temperature program: injection at 40°C, then an immediate increase of temperature to 300°C. After 30 s the split was automatically switched on. A capillary column (30 m × 0.25 mm I.D.), SPB-5 (Supelco), was used. The column temperature program was 40°C for 1 min then a linear increase to 300°C at 6°C/min. The carrier gas was hydrogen.

Identification of organic compounds

Eight substances were selected as reference standards for the hydrocarbons and eight for organochlorides. For the organochlorine compounds the chosen substances were: chlorobenzene, hexachloroethane, dacthal and the hexyl, octyl, decyl, palmityl and stearyl esters of trichloroacetic acid. The standards used in the identification of hydrocarbons were the *n*-alkanes *n*-C_8, *n*-C_{12}, *n*-C_{16}, *n*-C_{20}, *n*-C_{24}, *n*-C_{28}, *n*-C_{32} and *n*-C_{34}. The identification of organic compounds was realized by using a Lotus 1-2-3 spreadsheet program based on the retention index calculated with respect to the reference standards [15].

Fractionation

Mixtures containing the organochlorides and the hydrocarbons were separated into five fractions indicated by roman numbers, I, II, III, IV and V, using the sorption chromatography on a two-step microcolumn of silica gel and aluminium oxide. The microcolumn was formed by a first semi column of silica gel and by a second of aluminium oxide, separated by a glass frit [12].

Every fraction was then concentrated to 100 μl at 25°C under a stream of nitrogen. The volume injected into the GC column was 1 μl.

RESULTS AND DISCUSSION

Fig. 1 shows the complete scheme of separation of the different classes of organic compounds studied. A standard mixture of PCBs, hydrocarbons, chlorinated hydrocarbons, pesticides, herbicides, fatty acid esters, phthalic acid diesters with 1–12 carbon atoms and other polar compounds (alcohols, ketones, aldehydes, phenols and chlorophenols) was eluted on the silica gel semicolumn with 2 ml of *n*-hexane; in this way the aliphatic hydrocarbons and chlorinated hydrocarbons were completely recovered in fraction I (see Table I).

The highest-molecular-weight alkanes could not be eluted from a mixed column of silica gel and aluminium oxide using *n*-hexane since such compounds are strongly adsorbed on aluminium oxide. By combining the two semi columns and eluting with solvent mixtures of increasing polarity a further fractionation of the compounds was obtained. The following modifications to the original scheme [12] were made:

(1) The eluent *n*-pentane was substituted with *n*-hexane without apparent variations of the hydrocarbons in the different fractions.

(2) *n*-Pentane–carbon tetrachloride (8:2, v/v) and *n*-pentane–methylene chloride (9:1, v/v), used to separate aromatic hydrocarbons with one and two rings into two fractions [12], were substituted by a solution of *n*-hexane–methylene chloride (9:1, v/v). This meant that such compounds were found in a single fraction (see Fig. 1). This replacement was necessary because carbon tetrachloride interferes negatively

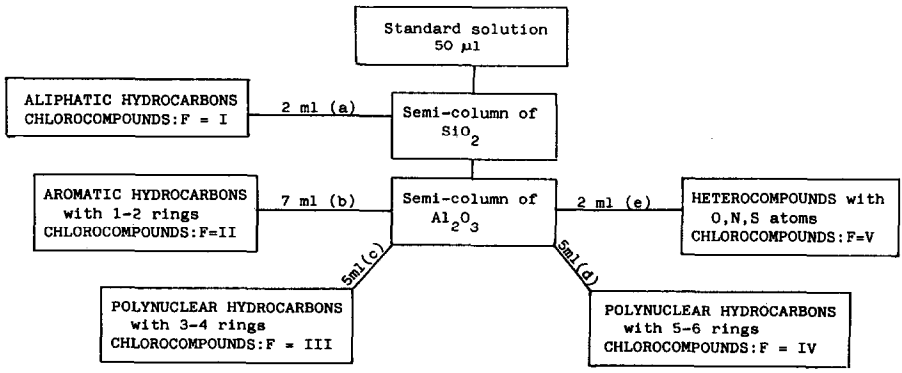

Fig. 1. Fractionation scheme. Eluents: (a) *n*-hexane; (b) *n*-hexane–methylene chloride (9:1, v/v); (c) *n*-hexane–methylene chloride (7:3, v/v); (d) *n*-hexane–methylene chloride (2:8, v/v); (e) acetone–methanol (2:1, v/v). For the identification of chlorocompounds, see Table I.

TABLE I

RECOVERY PERCENTAGE OF CHLORINATED COMPOUNDS WITH DUAL COLUMN FRACTIONATION[a]

Compound	Fraction				
	I	II	III	IV	V
Chlorinated hydrocarbons					
Chlorotoluene	100	–	–	–	–
2-Chloronaphthalene	90	10	–	–	–
Dichlorobenzene	100	–	–	–	–
1,2,4-Trichlorobenzene	100	–	–	–	–
Hexachlorobutadiene	100	–	–	–	–
Hexachlorobenzene	100	–	–	–	–
Arochlors (PCBs)					
1016	10	90	–	–	–
1232	10	90	–	–	–
1242	10	90	–	–	–
1248	15	85	–	–	–
1254	35	65	–	–	–
1260	40	60	–	–	–
1262	80	20	–	–	–
1268	100	–	–	–	–
Pesticides					
Chloropicrin	100	–	–	–	–
Aldrin	85	15	–	–	–
Heptachlor	–	100	–	–	–
o,p'-DDT	–	100	–	–	–
p,p'-DDT	–	100	–	–	–
1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p*-DDE)	–	100	–	–	–
o,p'-DDE	–	100	–	–	–
1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p*-DDD)	–	–	100	–	–
o,p'-DDD	–	55	45	–	–
Chlordane	–	40	60	–	–
Strobane	–	35	65	–	–
α-BHC	–	–	100	–	–
Lindane (γ-BHC)	–	–	100	–	–
β-BHC	–	–	–	100	–
Methoxychlor	–	–	–	100	–
Endrin	–	–	–	100	–
Dieldrin	–	–	–	100	–
Endosulphan	–	–	–	75	25
Herbicides					
Trifluralin	–	–	100	–	–
Dichlobenil	–	–	–	100	–
Propanil	–	–	–	–	100
Difolatan	–	–	–	–	100
Chlorinated phenols					
2,3-Dichlorophenol	–	–	–	–	80
2,4,5-Trichlorophenol	–	–	–	–	–
2,3,4,5-Tetrachlorophenol	–	–	–	–	–

[a] The recoveries are average values from five determinations. The absolute error for these measurements is 5%. The standard deviations are between 2 and 5%.

with the ECD of organochlorides. In any case the identification and quantitation of all the aromatic hydrocarbons with one and two rings posed no problems, as this mixture is easily resolved by GC.

(3) Methanol used for polar heterocompounds was substituted with a mixture of acetone and methanol (2:1, v/v) to cut down the interference while using ECD.

A careful inspection of the scheme clearly shows that the organochlorides are divided according to their chemical characteristics in all the five fractions. The organochlorine compounds present in the different fractions together with the relative percentages are reported in Table I.

Fraction I

This fraction shows a complete recovery of the chlorinated hydrocarbons with the sole exception of 2-chloronaphthalene, but even for this component the recovery is quite good.

The Arochlors show instead a non-homogeneous behaviour because the relative percentage of the various mixtures increases with the degree of chlorination, reaching 100% only for Arochlor 1268. Of the pesticides, only chlorpicrin and aldrin are present in this fraction, both in very high percentages.

The chromatographic analysis of this fraction shows that the chlorinated hydrocarbons are visibly separated from all the other eluted compounds and so they can be always determined without problems regardless of the complexity of the mixture. The Arochlor compounds on the other hand, generally interfere with each other and

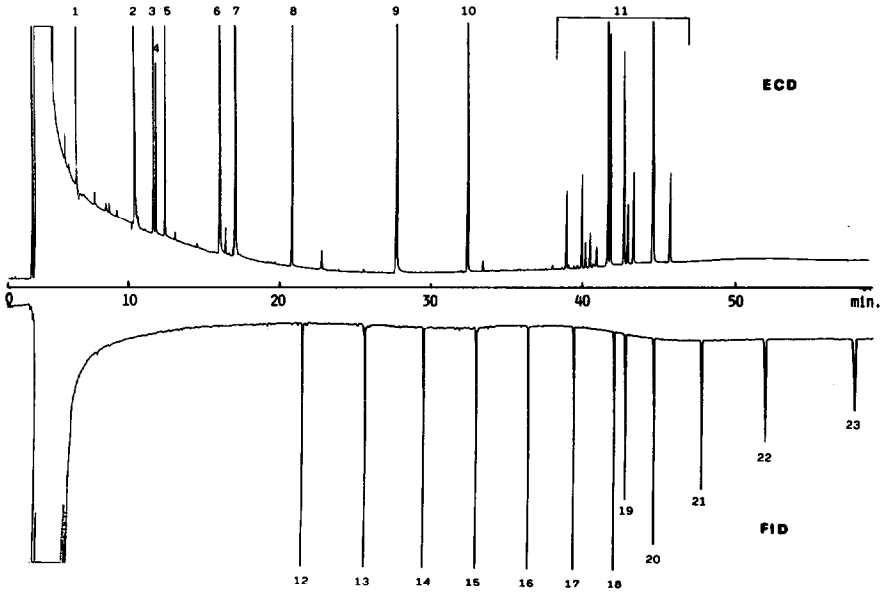

Fig. 2. Fraction I,. GC–ECD: 1 = chlorpicrin; 2 = *p*-chlorotoluene; 3 = 1,3-dichlorobenzene; 4 = 1,4-dichlorobenzene; 5 = 1,2-dichlorobenzene; 6 = 1,2,4-trichlorobenzene; 7 = hexachlorobutadiene; 8 = 2-chloronaphthalene; 9 = hexachlorobenzene; 10 = aldrin; 11 = Arochlor 1268. GC–FID: 12 = n-C_{14}; 13 = n-C_{16}; 14 = n-C_{18}; 15 = n-C_{20}; 16 = n-C_{22}; 17 = n-C_{24}; 18 = n-C_{26}; 19 = squalane; 20 = n-C_{28}; 21 = n-C_{30}; 22 = n-C_{32}; 23 = n-C_{34}.

with aldrin, with the exception of Arochlor 1268, for which no interferences occur. Fig. 2 shows the simultaneous determination of chlorinated compounds, chlorpicrin, aldrin and Arochlor 1268 (GC–ECD) and of aliphatic hydrocarbons (GC–FID).

Fraction II

The second fraction has some limitations from the analytical point of view because most of its components are found in other fractions too. Chlordane, strobane and PCBs show a very complex GC pattern that seriously interferes with the determination of the other pesticides contained in 100% recovery percentages in this fraction. As a consequence these pesticides can be determined only when the intererences are absent.

Fraction III

In this fraction the PCBs are missing. This is why the possible interferences in the determination of the pesticides present and of trifluoralin are associated with the presence of chlordane and strobane. When chlordane and strobane are absent the determination of the trifluoralin and pesticides is easily achieved even if their concentrations in the analysed mixture are at ppt (10^{-12}) levels. This fraction, on the other hand, allows the determination of strobane and chlordane even when they are simultaneously present in the mixture. It is interesting to note that the α- and γ-BHC isomers were completely recovered in this fraction, while the β isomer was not eluted from the column.

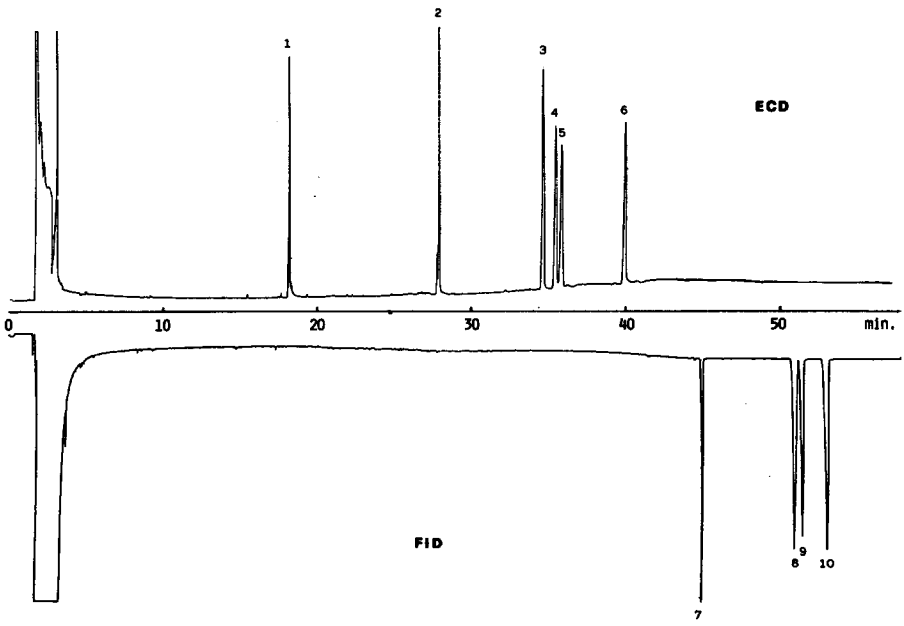

Fig. 3. Fraction IV. GC–ECD: 1 = dichlobenil; 2 = β-BHC; 3 = dieldrin; 4 = endrin; 5 = endosulphan; 6 = methoxychlor. GC–FID: 7 = benzo[a]pyrene; 8 = indeno [1,2,3-*cd*]pyrene; 9 = dibenzo[*a,h*]anthracene; 10 = benzo[*g,h,i*]perylene.

Fraction IV

This fraction contains a few important pesticides (β-BHC, endrin, dieldrin, methoxychlor, endosulphan) and the herbicide dichlobenil. These compounds are completely separated and easily identified, as the GC–ECD trace in Fig. 3 shows. The GC–FID trace in the same figure shows the simultaneous determination of four-ring polynuclear aromatic hydrocarbons. Fraction IV is very important because the above-mentioned compounds can be identified even in very complex mixtures containing PCBs, chlordane and strobane.

Fraction V

This fraction contains the other two herbicides examined (propanil and difolatan) and the 2,3-dichlorophenol, although this was not completely recovered; the loss in recovery can be ascribed to its affinity for the aluminium oxide support that prevents its complete elution from the column. The increase in the number of the chlorine atoms in the ring enhances the acidity of the compound and a greater affinity for the basic alumina semicolumn results.

In fact, neither 2,4,5-trichlorophenol nor 2,3,4,5-tetrachlorophenol is eluted from the column. This fraction is the only one of the five in which there occurs interference due to the simultaneous presence of organochlorides and heterocompounds containing oxygen, nitrogen and sulphur atoms, detectable with ECD.

CONCLUSIONS

The proposed fractionation method is an implementation of that successfully applied to the analysis of real complex mixtures of hydrocarbons and heterocompounds [12], taking also into account the organochlorides. It offers good potential for the fractionation and determination of a great variety of organic compounds.

Only some eluents and their total volumes were changed from those used previously, without any effect on the identification and determination of the hydrocarbons and heterocompounds containing nitrogen, oxygen and sulphur atoms.

These results were not obtained using the single column of silica gel or aluminium oxide. In fact, silica gel is commonly used to clean up samples containing organochlorides, which are very slightly retained even when eluted with hydrophobic solvents such as *n*-hexane [16–18]. This support cannot be used if the organochlorine compounds must be divided into several fractions eluting with mixtures of increasing polarity (*i.e.* *n*-hexane–methylene chloride). On the other hand, using aluminium oxide a quantitative recovery of aliphatic hydrocarbons with high molecular weight is not possible because of its strong affinity for these compounds.

REFERENCES

1 R. W. Risebrough, P. Rieche, S. G. Herman, D. B. Peakall and M. N. Kirven, *Nature (London)*, 220 (1968) 1099–1102.
2 F. J. Biros, A. Walker and A. Medbery, *Bull. Environ. Contam. Toxicol.*, 5 (1970) 317–323.
3 M. R. Bleavins and J. Aulerich, *Residue Rev.*, 90 (1983) 57–67.
4 M. Splinder, *Residue Rev.*, 90 (1983) 1–34.
5 A. L. Jensen, *Environ. Pollut. Ser. A*, 34 (1984) 73–82.
6 R. A. Rapaport, N. R. Urban, P. D. Capel, J. E. Baker, B. B. Looney, S. J. Einsenreich and E. Gorham, *Chemosphere*, 14 (1985) 1167–1173.

7 R. Tatsukawa, *Eisei Kagaku*, 31 (1985) 1–7.
8 H. Shiraishi, N. H. Oilkington, A. Otsuki and K. Fuwa, *Environ. Sci. Technol.*, 19 (1985) 585–589.
9 L. Morselli, S. Zappoli, A. Donati, *Ann. Chim. (Rome)*, 79 (1989) 677–688.
10 D. R. Thielen, G. Olsen, *Anal. Chem.*, 60 (1988) 1332.
11 J. Sherma, *Anal. Chem.*, 61 (1989) 153R.
12 P. G. Desideri, L. Lepri, D. Heimler, S. Giannessi and L. Checchini, *J. Chromatogr.*, 284 (1984) 167–178.
13 P. G. Desideri, L. Lepri, M. Canovaro and L. Checchini, in L. Pawloski, E. Mentasti, W. L. Lacy and C. Sarzanini (Editors), *Chemistry for Protection of the Environment 1987*, Elsevier, Amsterdam, 1988.
14 P. G. Desideri, L. Lepri and L. Checchini, *Ann. Chim. (Rome)*, 79 (1989) 589.
15 S. B. Tejada and J. E. Sigsby Jr., *J. Chromatogr. Sci.*, 26 (1988) 494.
16 H. Hidaka, S. Tanabe and R. Tatsukawa, *Agric. Biol. Chem.*, 47 (1983) 2009.
17 D. E. Gaskin, R. Frank and M. Holdrinet, *Arch. Environ. Contam. Toxicol.*, 12 (1983) 211.
18 B. D. Gardner, *S. Afr. J. Science*, 80 (1984) 43.