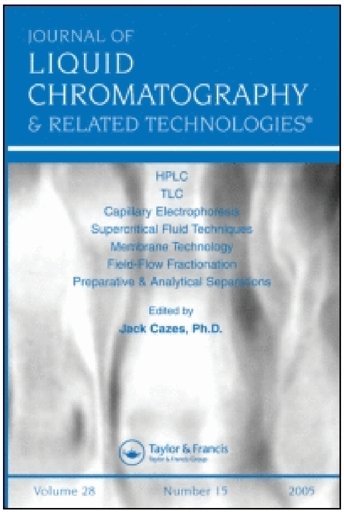

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ANALYSIS OF A MIXTURE OF POLYCHLORINATED BIPHENYLS, DDT AND ITS ANALOGUES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple and rapid method for separation and quantitative analysis of polychlorinated biphenyls (PCBs) and chlorinated pesticides (DDT and its analogues DDE and DDD in their o,p'- and p,p'-isomers) is described. The procedure consists of two steps:a)transformation of DDT and its analogues in o,p'- and p,p'-dichlorobenzophenone (DCBP); b)determination of the amount of PCBs and ΣDDT as DCBP by HPLC. Results obtained confirm that HPLC can be considered as an alternative or a supplementary methodology to conventional methods such as gas chromatography. The method is applied to marine organisms.

INTRODUCTION

PCBs are widely used in modern technology and are very persistent and cumulative environmental pollutants. They, along with DDE*, are reported to be the most abundant of the chlorinated aromatic pollutants in the ecosystem (1). It has been reported (2) that they are more stable than DDT and its metabolites, probably because the PCBs lack the C_2 residue linking the aromatic rings, which is the action site of most DDT biodegradation. The most relevant toxicological aspects resulting from exposure to the PCBs are chloroacne (3), liver injury (4), hydropericardial edema (5) and teratogenic effects (6). Therefore, the rapid analysis of PCBs is an important problem which has stimulated the development of many procedures employing TLC and GC. However, HPLC has only rarely been considered as a useful methodology to detect PCBs and ΣDDT (7-9). On the other hand, it is known that PCBs yield many individual peaks in GC analysis with widely different retention times. For this reason,

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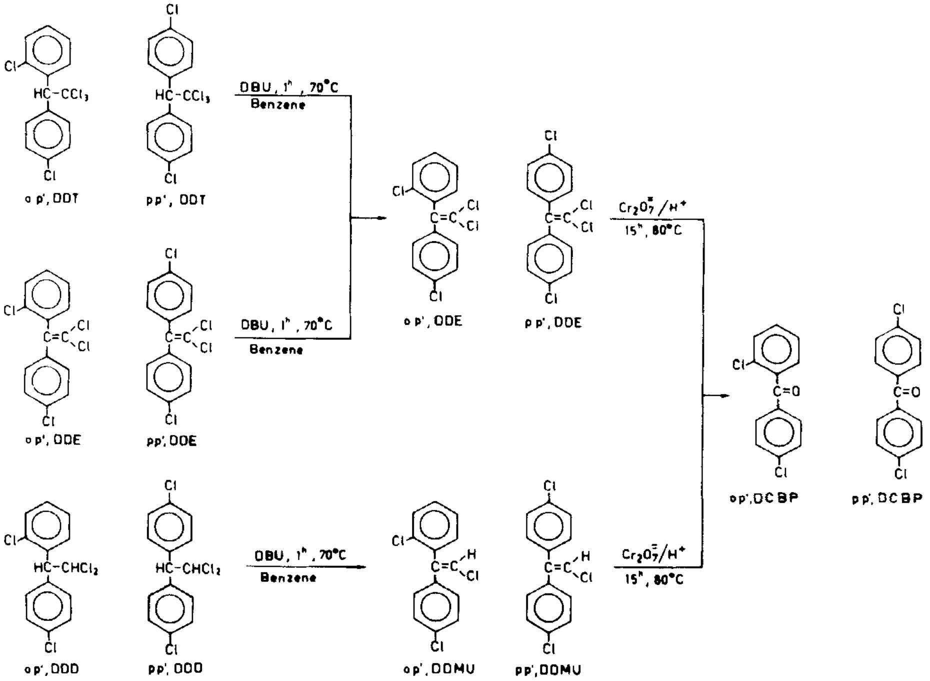
*DDE = 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene;DDT = 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane;DDD = 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane;DDMU = 1-chloro-2,2-bis(p-chlorophenyl)ethylene and isomers o,p'- respectively;DBU = 1,5-diazabicyclo-[5.4.0]undec-5-ene.

DDT and PCBs peaks overlap; this makes it necessary to remove PCBs PCBs from the samples prior to analysis. This problem is generally overcome by chromatography of cleaned-up extracts from chromatography on Florisil (10), silicic acid-Celite (11), activated coal (12), or silica gel (13) before analysis by GC. To simplify the analysis of a heterogeneous mixture of PCBs, it has been suggested that these should be converted to a single derivative. Usually, perchlorination is the preferred route (14).

The aim of this work was to investigate the use of HPLC to determine the contamination of environment by PCBs in the presence of DDT and its metabolites.

MATERIALS AND METHODS

A Perkin-Elmer Series 3B liquid chromatograph, equipped with an LC-75 Spectrophotometric Detector at 254 nm and an LC Autocontrol, was used. The stainless-steel column (Perkin-Elmer Silica-A) was 25 cm long and had an i.d. of 0.26 cm. Chromatograms were recorded and integrated with a Perkin-Elmer Sigma 10B Chromatography Data Station. The mobile phase consisted of n-hexane/$CHCl_3$ (95:5, 12:2 v/v) and n-hexane/Et_2O (9:1, 95:5 v/v). As a standard, PCBs sample Aroclor 1242, purchased from Monsanto (St.Louis, Mo.,USA), was used. The chlorinated pesticides were all from Aldrich-Europe (Beerse,Belgium). The dehydrohalogenation and oxidation reactions were carried out according to Miles (15) but using different reaction times (Scheme 1). Standard curves were obtained by plotting peaks areas against the amount of compound injected. All separations were carried out at ambient temperature.



Scheme 1.

RESULTS AND DISCUSSION

A procedure to avoid the preliminary removal of PCBs from DDT and its analogues was set up to speed up the analysis. The chemical transformation of DDT and its metabolites in DCBP is performed by dehydrohalogenation, followed by oxidation. Analysis of the reaction products by HPLC leads to elimination of a chromatographic step; PCBs, recovered unalterated from the reactions, are in fact eluted as a single peak. Moreover, under the experimental conditions used, PCBs are completely separated from DCBP (Fig. 1).

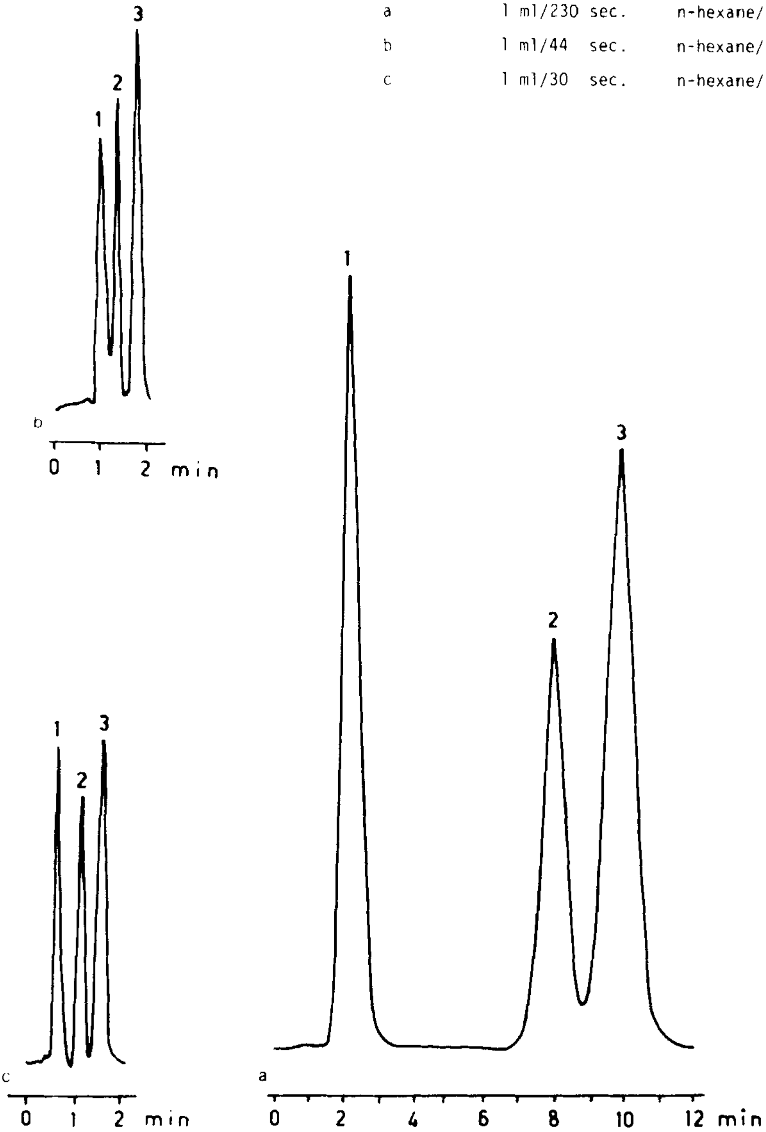
The most important parameters characterizing the method have been calculated.

The minimum detectable amounts (Fig. 2) of PCBs, p,p'-DCBP and o,p'-DCBP were 2.57 ng, 0.62 ng and 1.30 ng respectively.

The standard deviation, evaluated from eight repeated injections with four different volumes of standard mixture, was in the range of ± 2%.

PCBs and DCBP could be determined in a concentration range of 10-100 ng with no loss in linearity (Fig. 3).

In four different experiments the average recovery was



Experiment	Flow	Eluent
a	1 ml/230 sec.	n-hexane/$CHCl_3$ (12:2)
b	1 ml/44 sec.	n-hexane/ Et_2O (9:1)
c	1 ml/30 sec.	n-hexane/ Et_2O (95:5)

Figure 1. Chromatograms of standard mixtures of PCBs (1), p,p'-DCBP (2) and o,p'-DCBP (3).

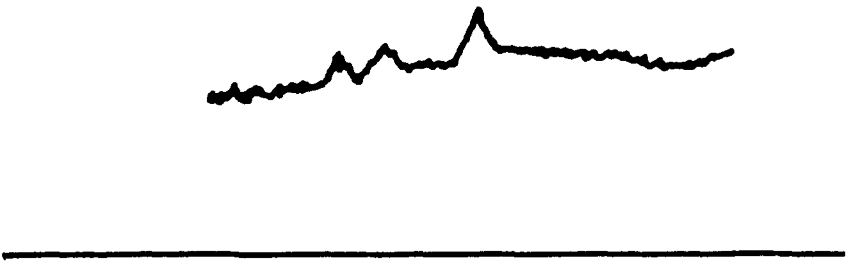


Figure 2. Minimum detectable sample.

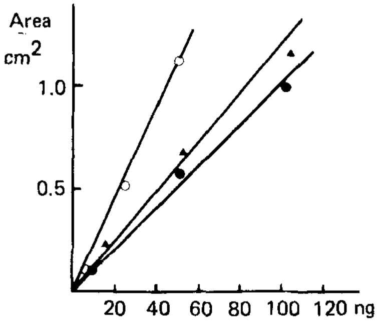


Figure 3. Calibration graphs for: ○ p,p'-DCBP, ● o,p'-DCBP, ▲ PCBs.

82.3 ± 1.2% for PCBs, 87.5 ± 1.8% for p,p'-DCBP and 88.2 ± 2.2% for o,p'-DCBP.

After standardization with pure samples, the method was applied to marine organisms, which are excellent indicators of the contamination level of the environment. Two species of organisms were used: tuna (*Thunnus thynnus thynnus* from Catania bay) and snail (*Murex brandaris* from Castellabate bay); the specimens were immediately frozen and kept at -20°C until use.

The tuna samples were purified as already described (16), including the chromatographic step and then subjected to the dehydrohalogenation and oxidation reactions; snail extracts, obtained as already reported (17), were freed from fats by repartition with acetonitrile, and directly submitted to the dehydrohalogenation and oxidation reactions.

The results (Fig. 4) show that the chromatographic step can, indeed, be by-passed.

The nature of compounds responsible for the individual peaks was confirmed by co-injection of the above extracts with reference samples.

The concentrations of PCBs and ΣDDT are reported in Table 1. Reported values, calculated from standard curves obtained with external standard, are consistent with those determined by other procedures (16), thus demonstrating the excellent sensitivity and specificity of the method.

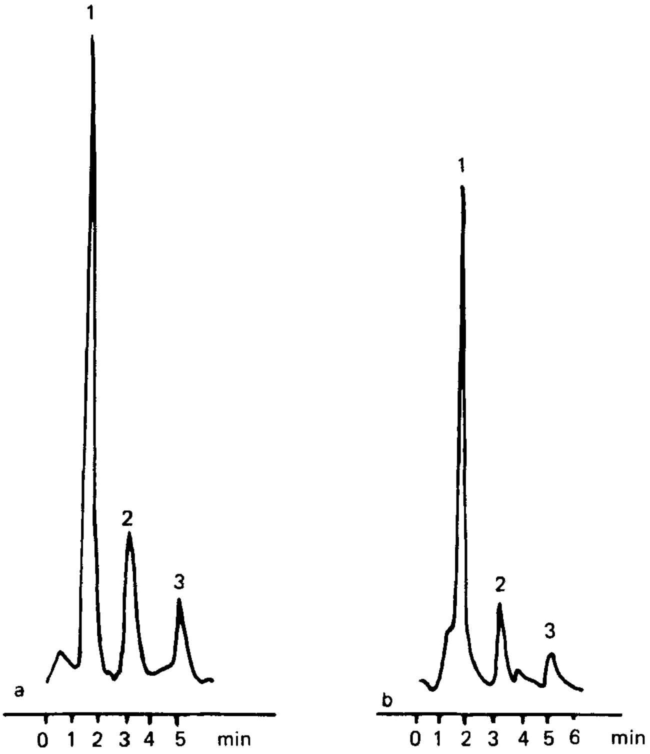


Figure 4. Separation of PCBs (1) from p,p'-DCBP (2) and o,p'-DCBP (3) in snail (a) and tuna (b).

TABLE 1

Concentrations of PCBs and ΣDDT in marine organisms expressed as ng g^{-1} of dry tissue.

Species	PCBs	ΣDDT
tuna	42.1	4.6
snail	63.6	5.2

The results show that HPLC can be an excellent procedure for the analysis of a mixture of PCBs and chlorinated pesticides. By this method, the need to separate PCBs from DDE is eliminated; this is the most significant interfering substance in the analysis. Moreover, PCBs eluted as a single peak can be easily determined. Furthermore, preparation of the sample is more rapid than in the case of GC, while the most important factors that influence the validity of the method are comparable with those of GC.

ACKNOWLEDGEMENTS

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