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A Simplified Confirmatory Technique for Organochlorine Residues

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The identification and quantification of chlorinated pesticides in environmental samples are usually carried out by application of gas-liquid chromatography (GLC) or gas-liquid chromatography associated to mass spectrometry (GC-MS). Using the first technique, an accurate confirmation of the identity of different organochlorine compounds requires two or three gas chromatographic columns with a careful study of the retention ratios in the obtained chromatograms. Derivation techniques in GLC proved to be very useful processes for identity confirmation (e.g. Drozd 1975). Chemical derivation in liquid phase -off line technique- may be used successfully as confirmatory procedure after the first injection into the gas chromatographic system.

In this study, we combined two simple acid and alkaline treatments, which are generally used in an independent way by many workers as clean-up techniques (Larson et al. 1974; Särkkä et al. 1978; Sodergren 1973; Tanabe and Tatsukawa 1980; Tanabe et al. 1982), but are not fully utilized by residue laboratories in routine confirmatory analyses. This procedure, which is based on the different relative stabilities presented by some organochlorine pesticides when they are sequencially treated with concentrated sulfuric acid and ethanolic-potassium hydroxide solutions, does not require any modification in the gas chromatographic system, such as those necessary for in line techniques. This method was found to be fast, inexpensive and very useful in the identification of organochlorine pesticides in freswater, seawater and surface sediment samples (Sericano and Pucci 1984; Zubillaga et al. 1984).

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This simple procedure can be efficiently used in the study of PCB's present in a mixture of organochlorine compounds since they are not altered by these two treatments and their peaks will appear without important interferences in the final chromatogram (Westö and Noren 1970).

MATERIAL AND METHODS

In this study, we used one liter of seawater previously extracted with n-hexane-acetone (2:1) and n-hexane as is indicated below, discarding those extracts. The seawater sample was later fortified with eleven chlorinated pesticides (spike concentrations are given in parenthesis): α BHC (16.3 ng/l), γ BHC (15.2 ng/l), δ BHC (15.5 ng/l), aldrin (26.2 ng/l), dieldrin (25.0 ng/l), heptachlor (16.6 ng/l), heptachlor epoxide (21.3 ng/l), o-p' DDD (38.5 ng/l), p-p' DDD (37.5 ng/l), o-p' DDT (43.7 ng/l) and p-p' DDT (42.7 ng/l). The seawater extract was obtained by extraction with 30 ml of n-hexane-acetone (2:1) in order to achieve a complete recovery of the organochlorine compounds adsorbed on the particulate matter, shaking vigorously in a separatory funnel during 3 minutes. This operation was repeated twice more but only with 30 ml of n-hexane each time, collecting the three extracts in one fraction. The mixture containing the pesticides was later washed with sodium chloride 5% (w/v) aqueous solution and dried up with anhydrous sodium sulfate, which had been previously heated at 625 C for two hours to eliminate interferences and cooled before use.

Clean-up procedure was performed with partially deactivated alumina column. Alumina was prepared by activating aluminium hydroxide at 800 C for four hours; it was then partially deactivated with 5% by weight of distilled water (Holden and Marsden 1969). The organochlorine compounds were eluted with 20 ml of n-hexane and collected in one fraction. The extract was finally concentrated to 0.5 ml by means of a Kuderna-Danish concentrator equipped with three ball Snyder column. Thus, the "normal" chromatogram was obtained (Chromatogram A in Fig. 1). After this first injection, the sample extract was sequencially treated with concentrated sulfuric acid and ethanolic-potassium hydroxide solutions in order to obtain two "derivative" chromatograms (Chromatograms B and C respectively in Fig. 1).

Acid products were obtained by adding 1 ml of concentrated sulfuric acid to 0.5 ml of sample extract, shaking for 2 - 3 minutes in a well stoppered test tube. The hexane layer was allowed to separate for a few minutes and was then withdrew by means of a microlitre

syringe for further determination by gas-liquid chromatography.

Alkaline products were obtained by adding 1 ml of 2% ethanolic-potassium hydroxide solution to the extract remaining from the acid treatment. The test tube was held over an opening bath in such manner that gentle boiling occurs for 10 - 15 minutes or until the volume reached 0.5 ml. Two ml of ethanol-water (1:1) and 2 ml of n-hexane were added and shaken for about 1 minute. The hexane layer was allowed to separate and was withdrew for analysis.

Organochlorine pesticide identifications and quantifications were carried out on a gas chromatograph SIGMA 3 Perkin Elmer, fitted with an electron capture detector (^{63}Ni) and a 6 ft x 1/8 in, i.d., glass column packed with 8% OV-17 on Gas Chrom W-HP 100/120 mesh. Injector, column and detector temperatures were set at 220 C, 200 C and 300 C respectively. Nitrogen was used as carrier gas at a flow rate of 40 ml/min.

RESULTS AND DISCUSSION

Table 1 lists the relative stability of chlorinated pesticides towards reagents for acid and alkaline treatments and the final results. Figure 1 shows the three different chromatograms obtained through this methodology.

Table 1. Relative stability of chlorinated pesticides to acid and alkaline treatments.

Chlorinated pesticide	Acid treatment	Alkaline treatment	Result
Aldrin	U	U	Aldrin
Dieldrin	D	-	-
α BHC	U	D	-
ɣ BHC	U	D	-
δ BHC	U	D	-
Heptachlor	U	U	Heptachlor
Hept. epoxide	U	U	Hept. epoxide
p-p'DDT	U	D→p-p'DDE	p-p'DDE
o-p'DDT	U	D→o-p'DDE	o-p'DDE
p-p'DDD	U	D→p-p'DDMU	p-p'DDMU (n.p.)
o-p'DDD	U	D→o-p'DDMU	o-p'DDMU (n.p.)

U = unchanged.
D = decomposed (products were not detected).
D→ = dehydrochlorination to the corresponding olefin.
n.p. = not present in the chromatograms.

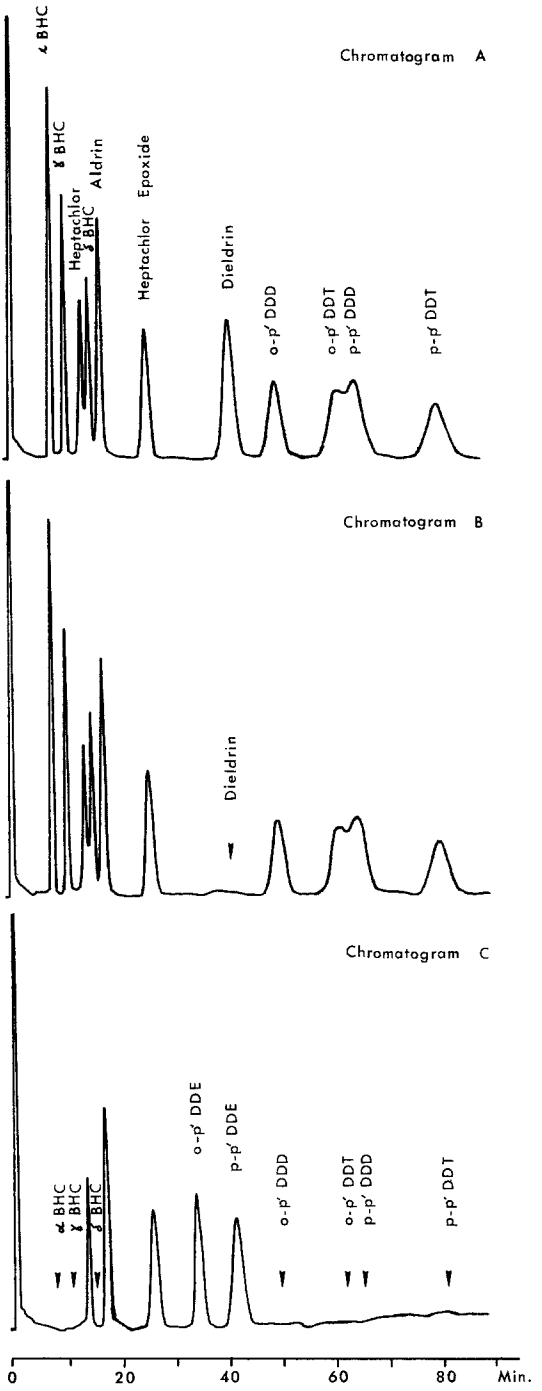

Figure 1. "Normal" (A) and "derivative" chromatograms (B and C).

By the acid treatment, dieldrin was decomposed to products which were not detected in the related chromatogram (B) while the other organochlorines remained unchanged. Thin layer chromatography gives at least three major spots when this pesticide is treated with concentrated sulfuric acid (Kurhekar et al. 1981). Those products were collectively designed as "dieldrin ketone" (Maybury and Cochrane 1973).

In the treatment with ethanolic-potassium hydroxide solution, the hexachlorocyclohexane isomers (α , γ and δ) were also derivated to compounds which were not present in the corresponding chromatogram (C) while the saturated DDT metabolites (o-p' DDD and p-p' DDD) and o-p' DDT and p-p' DDT were almost quantitatively converted to the respective DDT olefins (o-p' DDMU, p-p' DDMU, o-p' DDE and p-p' DDE); however, the peaks corresponding to o-p' DDMU and p-p' DDMU were not detected. Thus, the final chromatogram (C) shows the presence of o-p' DDE, p-p' DDE, which appeared with the same relative retention time of dieldrin presented in the "normal" chromatogram, and the peaks of heptachlor, heptachlor epoxide and aldrin, which were not affected by both treatments.

The first injection into the gas chromatographic system gave the "normal" chromatogram (A) showing the presence and quantitative distribution of the compounds studied. The second and third injections gave two "derivative" chromatograms (B and C) as a result of the acid and alkaline treatments respectively. The combination of these three chromatograms as well as the information obtained through the behavior of the organochlorine pesticides when treated with concentrated sulfuric acid and ethanolic-potassium hydroxide solutions provides sufficient data for quantification and accurate identity confirmation of the compounds considered.

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