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Clean-up and confirmation procedures for gas chromatographic determination of pesticide residues in contaminated waters. Part I☆

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ABSTRACT

The effects of sulphuric acid, potassium hydroxide and chromic acid on eleven organochlorine and ten organophosphorus pesticides were investigated. The treatments destroy some pesticides totally or partially, leave others unaltered and have a clean-up effect. These reactions can be used to confirm the presence of an identified pesticide. The clean-up extracts in the environmental samples facilitate the identification of the organochlorine and organophosphorus pesticides and their quantitative analysis. The results obtained for contaminated surface waters show the usefulness of these methods for multi-residue capillary gas chromatography without the need for other additional separative chromatographic steps to avoid interferences or the use of a highly sensitive mass spectrometer for confirmation.

INTRODUCTION

The correct identification of a particular pesticide residue in environmental samples is of major importance. The determination of pesticides in complex matrices presents difficulties because the biocides themselves, their metabolites, some contaminants and artifacts of synthesis often have similar physico-chemical properties and identical retention times when gas chromatography (GC) is used [1–4]. Therefore, false-positive results can be obtained. Several methods, such as chemical clean-up, sweep co-distillation and gel permeation, liquid–liquid partition and adsorption chromatography have been employed to remove co-extractants and minimize these difficulties in environmental contaminant analysis [5–10].

The efficacy of sulphuric acid clean-up has been studied and applied to organochlorine residue analyses of water samples [11], food commodities [12] and other samples [13–15]. Alkaline treatment of sample extracts provides the clean-up of co-extracted materials and dehydrochlorination to the corresponding alkenes of various organochlorine pesticides [16,17]. The same effect is achieved with a precolumn of sodium or potassium hydroxide on-line with the GC system [18–20]. Chromic acid has been used to remove interferences [21] and to distinguish polychlorinated biphenyls from DDT and its analogues [22–24].

The need for a rapid and inexpensive clean-up technique for routine multi-residue pesticide analysis of contaminated waters has led to the use of chemical treatments (concentrated sulphuric acid, ethanolic potassium hydroxide and chromium trioxide) of samples for organochlorine pesticide residue determination followed by GC with electron-capture detection (ECD) [25–27]. However, there are no refer-

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ences to their utilization in organophosphorus pesticide residue determination, with the exception of a recent report by Wan [28] in which the efficacy of sulphuric acid clean-up for the determination of four organophosphorus insecticides, such as malathion, fenitrothion, dimethoate and quinalphos, was evaluated in tea.

The purpose of this work was to determine the effect of different chemical treatments on organochlorine and organophosphorus pesticides in order to be able to use them in routine purification and confirmation in multi-residue analysis when ECD and alkaline flame ionization detection [nitrogen–phosphorus detection (NPD)] in capillary GC are simultaneously used. The clean-up procedures including treatments with sulphuric acid, potassium hydroxide and chromic acid were tested on extracts of surface water samples obtained by solid-phase extraction using octadecylsilica.

EXPERIMENTAL

Reagents

The organochlorine pesticides used were *o,p'*-DDE, *o,p'*-DDT, dieldrin, α-endosulfan, β-endosulfan, endosulfan sulphate, α-HCH, β-HCH, δ-HCH, isodrin and mirex and the organophosphorus pesticides used were disulfoton, ethion, fonofos, heptenophos, malathion, parathion-ethyl, parathion-methyl, phenthoate, sumithion and trithion. All of them, with purities between 95 and 99%, were purchased from Promochem (Wesel, Germany). Stock solutions were prepared in ethyl acetate and preserved at 4°C.

Ethyl acetate, *n*-hexane, ethanol and methanol (J.T. Baker, Phillipsburg, NJ, USA) were glass-distilled and free from interfering residues (as tested by GC following concentration by a factor of 100). Preparative octadecylsilica, 55–105 μm, was obtained from Waters–Millipore (Milford, MA, USA). Sulphuric acid, sp. gr. 1.84, and potassium hydroxide solution (2*M* in ethanol) were purchased from Merck (Darmstadt, Germany). To prepare chromic acid solution, 5 g of chromium(VI) oxide (Merck) were dissolved in 3 ml of water and 60 ml of glacial acetic acid (Merck) were added.

Apparatus

GC was performed with a Konik 2000-C gas chromatograph (Sant Cugat de Vallés, Barcelona, Spain) equipped with a splitless injector, a ^{63}Ni high-temperature electron-capture detector, an alkaline flame ionization detector and a Spectra-Physics SP 4290 integrator. Two fused-silica capillary columns were required; the working column was a BP-5 0.25-μm bonded-phase (5% phenyl–methylsiloxane) column (25 m × 0.22 mm I.D.), provided by Scientific Glass Engineering (Ringwood, Australia), and the second column, used for confirmatory purposes, was a DB-17 0.25-μm bonded-phase (50% phenyl–methylsiloxane) column (30 m × 0.24 mm I.D.), provided by J & W Scientific (Folsom, CA, USA). Helium was used as the carrier gas at a flow-rate of 2.8 ml min^{-1}. The injector temperature was set at 285°C and both detector temperatures (ECD and NPD) were set at 300°C. Splitless injection was performed with the column oven at 50°C. The column temperature was maintained at 50°C for 0.8 min, increased at 30°C min^{-1} to 140°C, which was maintained for 2 min, then at 5°C min^{-1} to 280°C, the final temperature being held for 12 min.

Extraction procedure

Water (1 l) was poured into a separating funnel connected by means of a microcolumn (100 mm × 9 mm I.D.) with a No. 3 (coarse) frit containing 0.5 g of octadecylsilica.

The octadecylsilica was conditioned before use by adding methanol (10 ml) followed by distilled water (10 ml). The water samples were forced through the solid phase with aid of a vacuum in order to obtain a flow-rate of about 30–40 ml min^{-1}. The material adsorbed was eluted with 5 ml of ethyl acetate and 5 ml of *n*-hexane. The organic layer was concentrated to 0.6 ml using a gentle stream of nitrogen. Samples of 3 μl were injected into the gas chromatograph.

Differences between the retention times of compounds on the non-polar BP-5 capillary column and semipolar DB-17 capillary column enable the identities of the studied pesticides to be confirmed and the products of pesticide reactions after chemical treatments to be discriminated.

Aliquots of 0.2 ml of this final hexane solution were used to identify the peaks with sulphuric acid, potassium hydroxide and chromic acid, according to the treatment procedures.

Acid treatment procedure

An aliquot of 0.2 ml of organic extract was introduced into a 5-ml centrifuge tube and 0.2 ml of concentrated sulphuric acid was added. The tube was stoppered and shaken vigorously for 5 min, 2 ml of distilled water were added and the mixture was allowed to stand until the two layers had separated. The organic layer was decanted with a Pasteur pipette and then diluted to 0.2 ml with *n*-hexane.

Alkali treatment procedure

An aliquot of 0.2 ml of organic extract was mixed with 0.2 ml of potassium hydroxide solution. The mixture was shaken vigorously for 2 min in an automatic vibrator and left to stand for 15 min at room temperature. Distilled water (3 ml) was added and the two layers were separated. The organic layer was decanted with a Pasteur pipette and diluted to 0.2 ml with *n*-hexane.

Oxidative treatment procedure

An aliquot of 0.2 ml of organic extract was evaporated to dryness in a tube, 5 ml of chromic acid solution was added to the residue and the mixture was placed in a water-bath at 75–80°C for 15 min. It was then removed and cooled. A 20-ml volume of distilled water and 20 ml of *n*-hexane were added and the mixture was shaken for 15 s. The *n*-hexane layer was retained and washed with water until the organic layer became colourless. The *n*-hexane portion was then decanted and concentrated to 0.2 ml.

In order to obtain the recoveries of the three chemical treatments (acid, alkali and oxidative), the chromatograms of treated samples were compared with those of the pesticides not subjected to the different treatments.

RESULTS AND DISCUSSION

In previous studies [29–32], the efficiency of octadecylsilica solid-phase extraction for these and other pesticides from tap, surface and sea waters was studied.

Figs. 1 and 2 show the chromatograms of standard mixtures of organochlorine and organophosphorus pesticides before and after chemical treatment, respectively. The results obtained with chemical treatments in terms of

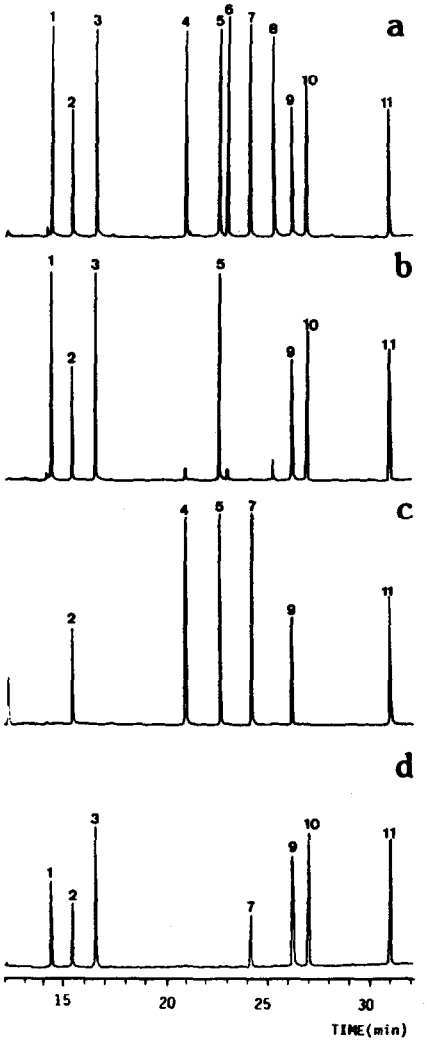

Fig. 1. Chromatogram of working organochlorine pesticide solution obtained with the BP-5 capillary column and ECD: (a) without any treatment; (b) after acid treatment; (c) after alkali treatment; (d) after oxidative treatment. 1 = α-HCH; 2 = β-HCH; 3 = δ-HCH; 4 = isodrin; 5 = o,p'-DDE; 6 = α-endosulfan; 7 = dieldrin; 8 = β-endosulfan; 9 = o,p'-DDT; 10 = endosulfan sulphate; 11 = mirex. For GC conditions, see Experimental.

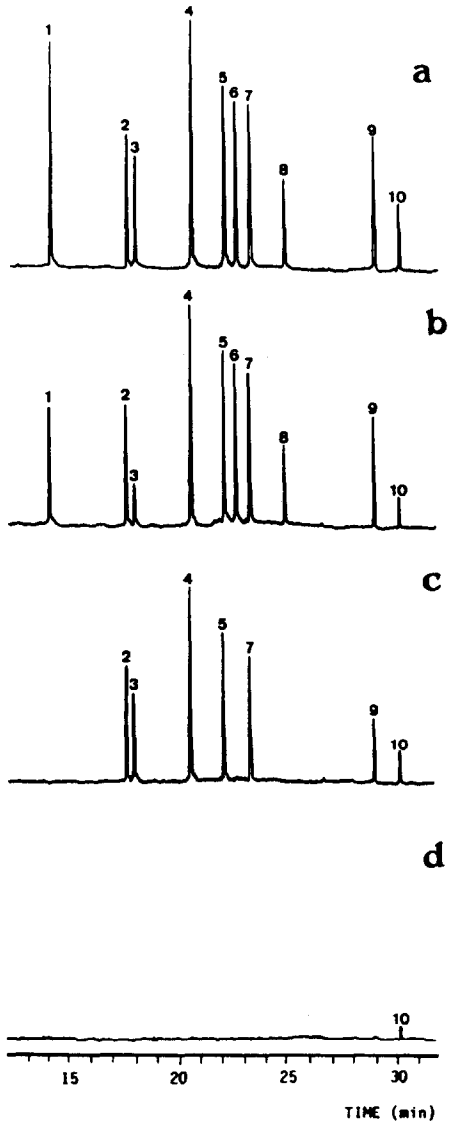

Fig. 2. Chromatogram of working organochlorine pesticide solution obtained with the BP-5 capillary column and NPD: (a) without any treatment; (b) after acid treatment; (c) after alkali treatment; (d) after oxidative treatment. 1 = Heptenophos; 2 = fonofos; 3 = disulfoton; 4 = parathion-methyl; 5 = malathion; 6 = sumithion; 7 = parathion-ethyl; 8 = phenthoate; 9 = ethion; 10 = trithion. For GC conditions, see Experimental.

either the decrease or disappearance of the chromatographic signal of the pesticides or the appearance of reaction products help to confirm and quantify the pesticides.

After the chemical treatments, the results obtained by GC using ECD for organochlorine and NPD for the organophosphorus pesticides are given in Table I. They are particularly valuable for monitoring residues in environmental waters. Table I shows the average recoveries and relative standard deviations (R.S.D.s) (n = 6) obtained when the standard aqueous solutions of organochlorine and organophosphorus pesticides were subjected to the previously described treatment procedures with sulphuric acid, potassium hydroxide and chromic acid.

According to Table I, the degradation of the studied pesticides after sulphuric acid treatment can be separated into three categories:

(I) no reaction occurred (less than 25% destroyed): o,p'-DDE, o,p'-DDT, endosulfan sulphate, α-HCH, β-HCH, δ-HCH, mirex, ethion, fonofos, malathion, parathion-ethyl, parathion-methyl, phenthoate, sumithion and trithion;

(II) partial reaction (25–75% destroyed): disulfoton, heptenophos and trithion;

(III) extensive reaction (more than 75% destroyed): dieldrin, α-endosulfan, β-endosulfan and isodrin.

For the ethanolic potassium hydroxide treatment the pesticides can also be divided into the same three categories:

(I) no reaction occurred (less than 25% destroyed): o,p'-DDE, o,p'-DDT, dieldrin, β-HCH, isodrin, mirex, disulfoton, fonofos, malathion, parathion-ethyl and parathion-methyl;

(II) partial reaction (25–75% destroyed): ethion and trithion;

(III) extensive reaction (more than 75% destroyed): α-endosulfan, β-endosulfan, endosulfan sulphate, α-HCH, δ-HCH, heptenophos, phenthoate and sumithion.

Finally, the pesticides after chromic acid treatment can also be divided into the same three categories:

(I) no reaction occurred (less than 25% destroyed): o,p'-DDT, endosulfan sulphate and mirex;

(II) partial reaction (25–57% destroyed): dieldrin, α-HCH, β-HCH and δ-HCH;

(III) extensive reaction (more than 75% destroyed): o,p'-DDE, which produces a new

TABLE I

RECOVERIES OF ORGANOCHLORINE AND ORGANOPHOSPHORUS PESTICIDES AFTER CHEMICAL TREATMENTS

Pesticides	Working solution ($\mu g l^{-1}$)	Recovery [% ± R.S.D. ($n = 6$)]			Detection limit ($ng l^{-1}$)
		H_2SO_4	KOH	Cr(VI)	
Organochlorine compounds					
o,p'-DDE	0.25	95 ± 3	98 ± 1	0[a]	3.6
o,p'-DDT	0.25	88 ± 9	78 ± 8	83 ± 11	3.5
Dieldrin	0.30	0	97 ± 5	27 ± 6	2.1
α-Endosulfan	0.25	4	0	0	3.5
β-Endosulfan	0.25	10	0	0	3.7
Endosulfan sulfate	0.25	106 ± 7	0	89 ± 9	3.9
α-HCH	0.20	91 ± 3	0	42 ± 12	1.9
β-HCH	0.20	86 ± 4	86 ± 7	55 ± 10	2.1
δ-HCH	0.20	92 ± 9	0	48 ± 15	1.9
Isodrin	0.30	5	98 ± 3	0	2.5
Mirex	0.40	101 ± 6	97 ± 8	91 ± 2	6.7
Organophosphorus compounds					
Disulfoton	0.50	29 ± 11	78 ± 8	0	3.0
Ethion	0.50	83 ± 12	51 ± 9	4	3.6
Fonofos	0.50	94 ± 4	90 ± 5	0	4.0
Heptenophos	1.00	53 ± 2	0	0	10.4
Malathion	1.20	97 ± 2	79 ± 9	0	15.6
Parathion-ethyl	1.00	97 ± 3	79 ± 7	0	10.4
Parathion-methyl	1.00	97 ± 4	83 ± 11	0	7.8
Phenthoate	1.00	94 ± 4	0	0	6.2
Sumithion	0.50	93 ± 6	0	0	3.0
Trithion	1.00	47 ± 15	52 ± 10	17 ± 2	10.4

[a] Degradation product dichlorobenzophenone.

degradation peak, α-endosulfan, β-endosulfan, isodrin and all organophosphorus pesticides; *o,p'*-DDE is converted into *o,p'*-dichlorobenzophenone, but is not seen because of the chromatographic conditions employed.

Generally, our results agree with the few studies found in the literature [18,21,26,27], except for *o,p'*-DDT [18,27] and β-HCH [18] with the alkali treatment. In order to clarify this discrepancy, the alkali treatments described in refs. 18 and 27 were carried out at 100°C for a longer time.

On the other hand, in the alkali treatment previously described, if 5 *M* potassium hydroxide solution is employed instead of 2 *M* potassium hydroxide, *o,p'*-DDT and β-HCH are destroyed.

Other chemical treatments reported in the literature are different and their results are not comparable.

The oxidative treatment is the most destructive of the three treatments studied, followed by the alkali treatment. The acid treatment is the least destructive. Consequently, the three treatments described in this paper can be used to confirm the peak of pesticides, taking into account the different behaviours observed for each of them and the fact that they are rapid, simple and applicable to many samples.

Application to water analysis

The content of organochlorine and organophosphorus pesticides in surface water samples was determined by using octadecyl-bonded

porous silica glass microcolumn extraction, followed by chemical treatment with sulphuric acid, potassium hydroxide and chromic acid for confirmation of peaks. The treatment procedures remove the interferences by destroying some of the organic matter contained in contaminated waters. Therefore, Florisil or alumina column adsorption chromatography was found to be unnecessary.

Fig. 3 shows the ECD and NPD chromatograms corresponding to the analysis of a surface water sample collected from a predominantly agricultural area in which pesticide residues are fairly common.

The results of the analysis of twenty surface water are given in Table II. The pesticides were identified by using the retention times obtained with two different polarity capillary columns. The concentration of pesticides was calculated before any chemical treatment, by comparing the peak areas with those of standards injected under identical conditions. The confirmation is

TABLE II

PESTICIDES PRESENT IN SURFACE WATER SAMPLES FROM THE VALENCIA AREA

Sample No.	Pesticide possible	Concentration (ng/l)	Chemical treatment[a]			Confirmation
			Acid	Alkali	Oxidative	
1	Ethion	10	+	+	−	Positive
	Sumithion	8	+	+	−	Positive
2	Fonofos	9	+	+	−	Positive
3	β-Endosulfan	55	+	−	−	Negative
4	Parathion-ethyl	34	+	+	−	Positive
	Trithion	49	+	+	−	Positive
5	Parathion-ethyl	25	+	+	−	Positive
6	Parathion-ethyl	39	+	+	−	Positive
	Ethion	10	+	+	−	Positive
7	Parathion-ethyl	35	+	+	−	Positive
8	α-Endosulfan	24	−	−	−	Positive
	β-Endosulfan	18	−	−	−	Positive
	Parathion-ethyl	176	+	+	−	Positive
	Sumithion	30	+	−	−	Positive
9	o,p′-DDE	10	+	+	−	Positive
10	α-Endosulfan	352	−	−	−	Positive
	β-Endosulfan	112	−	−	−	Positive
	Disulfoton	103	+	+	−	Positive
	Parathion-ethyl	125	+	+	−	Positive
11	Parathion-ethyl	21	+	+	−	Positive
	Trithion	30	+	+	−	Positive
12	Disulfoton	57	+	+	−	Positive
	Ethion	21	+	+	−	Positive
	Trithion	23	+	+	−	Positive
13	Ethion	6	+	+	−	Positive
	Parathion-methyl	84	+	+	−	Positive
14	Trithion	585	+	+	−	Positive
15	Trithion	26	+	+	−	Positive
16	Ethion	7	+	+	−	Positive
17	Sumithion	6	+	−	−	Positive
18	Parathion-methyl	81	+	+	−	Positive
19	Malathion	16	+	+	−	Positive
20	Sumithion	23	+	−	−	Positive

[a] + = Unaltered; − = destroyed.

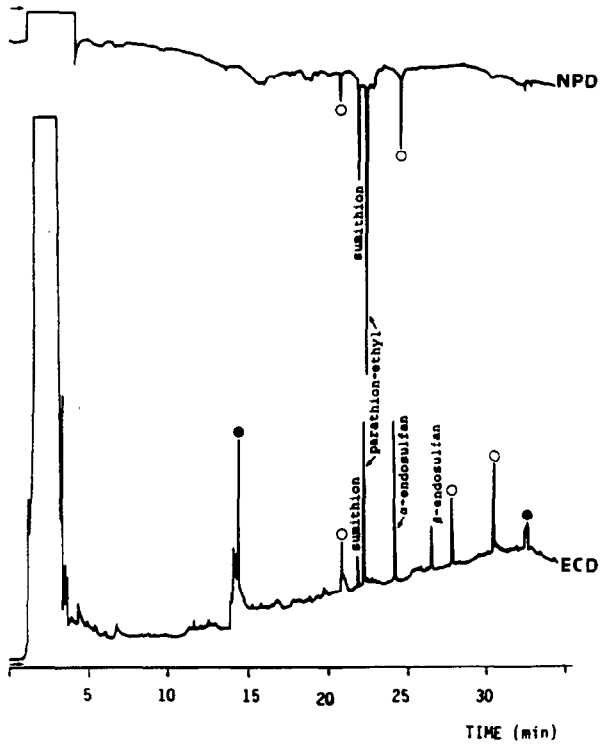

Fig. 3. Chromatogram corresponding to water sample No. 8 from the Valencia area (see Table II), obtained with parallel (ECD, NPD) detection using the BP-5 capillary column prior to any chemical treatment. ● = Unknown peak also in blank; ○ = unknown peak destroyed in all treatments. The peak identified as sumithion was destroyed in the oxidative and alkali treatments. The peak identified as parathion-ethyl was destroyed in the oxidative treatment. The peaks identified as α-endosulfan and β-endosulfan were destroyed in all treatments. See text for operating conditions.

positive when the results obtained by comparing the three chemical treatments are the same as those obtained with standards (Table I), and negative when at least one treatment differs from the standard.

As seen in Table II, the organochlorine pesticides *o,p'*-DDE, α-endosulfan and β-endosulfan were confirmed in surface water after the treatments, except for one sample in which the β-endosulfan gave a false-positive result.

The results obtained for all of the organophosphorus pesticides in the surface water samples analysed were confirmed by chemical treatments. The presence of organophosphorus compounds as the most important pesticides is due to the fact that the water samples were collected in an intensive agricultural area where non-persistent organophosphorus pesticides are predominantly used.

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