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Influence of the solid-phase extraction process on calibration and performance parameters for the determination of pesticide residues in water by gas chromatography

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Abstract

Twenty-six organophosphorus, organochlorine and other electron-capture detection-sensitive pesticides were extracted from water in a single step using a C_{18} solid-phase extraction cartridge, eluted with ethyl acetate and isooctane and determined by gas chromatography with electron-capture and flame photometric detection. The calibration equation for the extraction method was calculated for a twentyfold concentration range, including the EC limit of 0.1 μg/l. The linearity, precision, sensitivity and detection limit of the method were studied, applying the statistical model of linear regression. A lack of linearity was observed for fenthion, deltamethrin and trifluralin, but the proposed method was suitable for other pesticides studied. The limits of detection range from 20 to 120 ng/l applying the calibration graph and from 1 to 40 ng/l based on a signal-to-noise ratio of 3:1.

Keywords: Solid-phase extraction; Water analysis; Pesticides

1. Introduction

The control of the presence of organic contaminants in water is a priority owing to their environmental impact. The European Community (EC) has limited the maximum pesticide concentrations to be found in water to 0.1 μg/l [1].

Pesticides in water and other environmental matrices have been separated using different techniques such as solid-phase extraction (SPE) cartridges [2–7], SPE disks [6–10], solvent extraction [11,12] and finally determined by chromatographic or special techniques [13–15].

From an analytical point of view, it is of great interest to know the recovery behaviour of different pesticides from water at concentrations close to the mentioned upper limit of 0.1 μg/l, especially when recoveries for several pesticides have been reported to be dependent on the pesticide concentration [10] and on the sample volume processed [2,7,10,16].

A multi-residue method using solid-phase extraction (SPE) cartridges to clean up and concentrate water samples has been developed with final determination by gas chromatography with electron-capture detection (ECD) and flame photometric detection (FPD), techniques commonly available in most analytical laboratories. The aim of this work was to calibrate the whole extraction method and to study how it is affected by pesticide concentrations in water. To do this, a linear regression method was applied to data obtained from extracting Milli-Q water samples

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spiked with pesticide mixtures at five concentrations ranging from 5 to 400 ng/l.

2. Experimental

2.1. Reagents

Pesticides were selected according to their use in the studied area or to their high persistence and all of them had purities >98.5% (except fenthion): trifluralin, alachlor, folpet, oxyfluorfen, bromopropylate, dicofol, fonofos, fenthion (91.9%), chlorpyriphos, phosmet, azinphos-methyl and phosalone (Dr. Ehrenstorfer, Augsburg, Germany), triallate (Monsanto, St. Louis, MO, USA), *p,p'*-DDE and *p,p'*-DDT (Rohm & Haas, Philadelphia, PA, USA), *o,p'*-DDE and *o,p'*-DDT (Aldrich, Milwaukee, WI, USA), tetradifon (Phillips Duphar, Amsterdam, Netherlands), dimethoate, diazinon and methidathion (Ciba-Geigy, Münchwilen, Switzerland) and fenitrothion and malathion (Sumitomo, Osaka, Japan). Captan was a gift from the Institute of Organic Industrial Chemistry (Warsaw, Poland), lindane from the Center for Disease Control (Atlanta, GA, USA) and deltamethrin from the Laboratoire de Répression de Fraudes (Massy, France). Bromophos (Dr Ehrenstorfer) was used as an internal standard.

All solvents were of residue analysis grade (Merck, Darmstadt, Germany). Water was purified with a Milli-Q water-purification system (Millipore, Bedford, MA, USA).

Stock standard solutions of the pesticides and the internal standard were prepared in acetone at 1 g/l, except *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT, which were prepared in hexane, and azinphos-methyl, which was prepared in toluene. Oxyfluorfen was provided at a concentration of 10 mg/l in cyclohexane. Aliquots of each stock solution were mixed in a volumetric flask and diluted to the mark with hexane, thus obtaining an intermediate solution. Different volumes of this intermediate solution were diluted with methanol to prepare spiking solutions at the five concentrations defined in Section 2.3. The amount of hexane in the five methanolic spiking solutions was negligible.

Anhydrous sodium sulfate (Merck) was extracted in a Soxhlet apparatus for 24 h with acetone and heated at 90°C for 1 h to remove the solvent.

Octadecyl (C_{18}) Bakerbond SPE cartridges of 3 ml with 500 mg of packing material were used (J.T. Baker, Phillipsburg, NJ, USA).

2.2. Chromatographic conditions

GC–ECD

A Model 5880 HP gas chromatograph (Hewlett-Packard Española, Seville, Spain), equipped with a split–splitless injector, an electron-capture detector and an HP 5880A integrator were employed. An Ultra 2 capillary column (5% phenylmethylsilicone, 25 m × 0.32 mm I.D., 0.33 μm film thickness) was used. Helium was selected as the carrier gas at a flow-rate of 2 ml/min. The injector and detector temperatures were 250 and 300°C, respectively. A 1-μl volume of the sample was injected in the split mode (splitting ratio 1:20) with the following temperature programme: 160°C (held for 1 min), increased at 4°C/min to 230°C (held for 2 min) and at 20°C/min to 280°C (held for 6 min).

GC–FPD

A Model 5890 HP gas chromatograph (Hewlett-Packard Española), equipped with a split–splitless injector, a flame photometric detector and an HP 3396 Series II integrator were employed. An HP 1 capillary column (methylsilicone, 12.5 m × 0.22 mm I.D., 0.33 μm film thickness) was used. Helium was selected as the carrier gas at a flow-rate of 1.5 ml/min. The injector and detector temperatures were 250 and 275°C, respectively. A 1-μl volume of the sample was injected in the splitless mode with the following temperature programme: 45°C (held for 1 min), increased at 30°C/min to 170°C (held for 2 min), at 4°C/min to 200°C (held for 2 min) and at 20°C/min to 270°C (held for 2 min).

2.3. Calibration

The linearity $[1 - s_r(b)]$, sensitivity (s), precision $[s_r(c)]$ and the limit of detection (LOD) for each compound were calculated by the applica-

tion of the linear regression method to the calibration graph [17,18]. To do this, water samples spiked with pesticide mixtures at five concentrations between 5 and 400 ng/l (Table 1) were extracted. The concentrations used to spike the water samples were in the proportions of 1, 5, 10, 16 and 20. Each concentration level was repeated five times and injected twice.

Equations used to define the previous parameters are as follows:

$$s_r(b) = s_b/b$$

$$s = s_{R,c}/b$$

$$s_r(c)\,(\%) = (s_c/c) \times 100$$

$$\mathrm{LOD} = 3(s_{R,c}/b)[(n-2)/(n-1)]^{1/2}$$

where s_b is the slope standard deviation, s_c is the concentration standard deviation, b is the slope, c is the concentration, R is the chromatographic response, $s_{R,c}$ is the regression standard deviation of R to c and n is the total number of pairs of points [17,18].

2.4. Extraction and concentration of samples

A C_{18} cartridge was conditioned by passing consecutively 5 ml of isooctane, 5 ml of ethyl acetate, 5 ml of methanol and 10 ml of Milli-Q water. Subsequently, 1 l of Milli-Q water, with 1 ml of the corresponding spiking solution added at the concentration levels indicated in Table 1, at pH 6.5, was passed through the column at a flow-rate of 10–15 ml/min under vacuum. Then,

Table 1
Extraction method calibration data set ($R = a + bc$)

Pesticide	Concentration range (ng/l)		a	s_a	b	s_b	$s_{R,c}$
	Upper	Lower					
ECD							
Trifluralin	100	5	9.6230	2.7655	0.6209	0.0418	8.9527
Lindane	100	5	25.9951	6.8258	2.7107	0.1068	26.8187
Triallate	200	10	6.8543	2.1232	0.4667	0.0176	8.1533
Alachlor	400	20	9.3137	1.8090	0.2127	0.0076	6.9443
Captan	200	10	5.5867	3.3615	0.5186	0.0236	10.9424
Folpet	200	10	−6.0288	1.1865	0.2235	0.0097	4.4887
o,p'-DDE	100	5	3.0138	1.6220	0.9786	0.0288	6.2734
p,p'-DDE	100	5	8.7465	1.6715	0.8902	0.0263	6.5123
Oxyfluorfen	100	5	18.2142	2.3292	1.2214	0.0380	9.0748
o,p'-DDT	100	5	10.3319	1.7886	0.9501	0.0270	6.8601
p,p'-DDT	100	5	10.0581	2.3875	1.0579	0.0385	9.3701
Bromopropylate	100	5	5.0582	1.1075	0.6638	0.0183	4.3177
Dicofol	200	10	17.2590	3.2658	0.6857	0.0256	12.8143
Tetradifon	100	5	10.7749	2.0197	0.9789	0.0329	7.8995
Deltamethrin	200	10	6.9198	1.9940	0.2327	0.0152	7.8285
FPD							
Dimethoate	200	10	−3.5458	2.0121	0.4747	0.0165	7.6648
Fonofos	200	10	−17.4454	11.2948	2.9367	0.0901	44.1780
Diazinon	200	10	15.1954	7.4303	2.2801	0.0613	29.0581
Fenitrothion	200	10	6.4958	5.5700	2.1522	0.0442	21.7885
Malathion	200	10	3.5513	3.1973	1.8142	0.0283	12.3143
Fenthion	200	10	−39.5727	12.3606	1.2262	0.0904	48.5416
Chlorpyriphos	200	10	12.1381	4.5725	1.6541	0.0393	17.7070
Methidathion	200	10	−2.2817	3.4742	1.4074	0.0295	13.4013
Phosmet	200	10	−23.6910	5.4184	1.3133	0.0479	20.7504
Azinphos-methyl	200	10	−7.1984	6.2450	1.1094	0.0510	24.5196
Phosalone	200	10	3.2536	7.0452	2.2393	0.0580	27.3255

the cartridge was washed with 10 ml of Milli-Q water and dried by aspirating air for 30 min.

Compounds retained in the cartridge were eluted with 3×0.5 ml of ethyl acetate and 3×0.5 ml of isooctane. The eluate was dried over anhydrous sodium sulfate and washed with an additional 0.5 ml of each eluting solvent. The combined fractions were concentrated to dryness under a gentle stream of nitrogen. The final residue was dissolved in 1 ml of hexane and 5 μl of 10 mg/l bromophos solution, carefully measured with a 10 μl Hamilton syringe, were added. Bromophos was added as a quantification internal standard to correct for possible differences in chromatographic analysis due to variations in the manual injection.

3. Results and discussion

Figs. 1 and 2 show the chromatograms obtained after the extraction of a spiked water sample. All the pesticides are in general baseline resolved. In Fig. 1, the additional peaks observed correspond to organophosphorus pesticides detected by ECD.

The different parameters associated with the calibration graphs were studied using areas relative to that of the internal standard.

The calibration graph equations for the extraction method were calculated by linear regression. The values which define the calibration line (intercept and slope), together with the error estimators [s_a (intercept standard deviation), s_b and $s_{R,c}$] are given in Table 1. In all the cases studied the fitting was statistically significant ($P > 0.05$), but each has different standard errors of the estimate ($s_{R,c}$).

It has been shown previously that the chromatographic response for each pesticide is linear over the concentration ranges employed [17]. The alachlor concentration range is higher because it is not very sensitively detected by ECD owing to the presence of only one chlorine atom in its molecule.

Linearity for the extraction data was calculated from $1 - s_r(b)$. According to Cuadros et al. [18], a value of $s_r(b) = 0.1/t$ (Student's t corre-

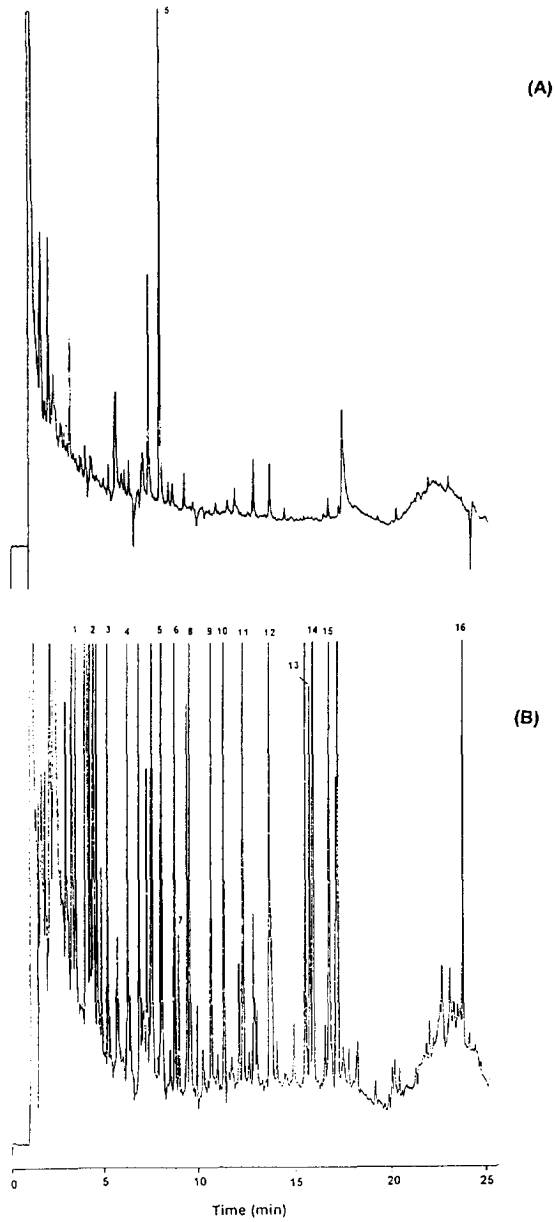

Fig. 1. (A) Water blank gas chromatogram and (B) gas chromatogram showing the separation of organochlorine and ECD-sensitive pesticides by GC–ECD, after the extraction of 1 l of Milli-Q water spiked at the concentration levels given below in parentheses (ng/l). Volume injected: 1 μl. Chromatographic conditions as explained in the text. Peaks: 1 = trifluralin (50); 2 = lindane (50); 3 = triallate (100); 4 = alachlor (200); 5 = bromophos (internal standard); 6 = captan (100); 7 = folpet (100); 8 = o,p'-DDE (50); 9 = p,p'-DDE (50); 10 = oxyfluorfen (50); 11 = o,p'-DDT (50); 12 = p,p'-DDT (50); 13 = bromopropylate (50); 14 = dicofol (100); 15 = tetradifon (50); 16 = deltamethrin (100).

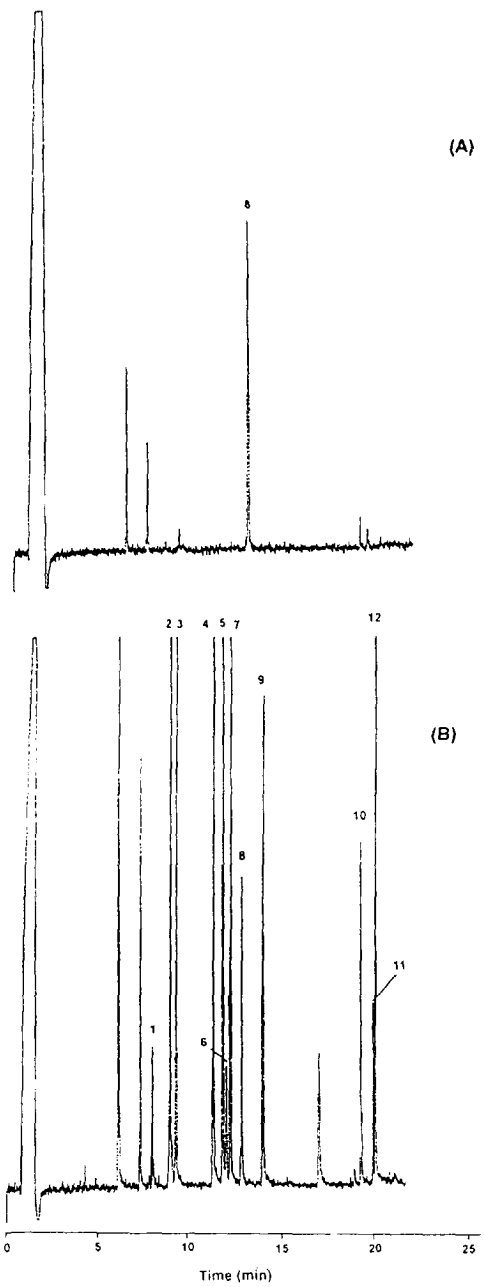

Fig. 2. (A) Water blank gas chromatogram and (B) gas chromatogram showing the separation of organophosphorus pesticides by GC–FPD, after the extraction of 1 l of Milli-Q water spiked with 100 ng/l of each pesticide. Volume injected: 1 μl. Chromatographic conditions as explained in the text. Peaks: 1 = dimethoate; 2 = fonofos; 3 = diazinon; 4 = fenitrothion; 5 = malathion; 6 = fenthion; 7 = chlorpyriphos; 8 = bromophos (internal standard); 9 = methidathion; 10 = phosmet; 11 = azinphos-methyl; 12 = phosalone.

sponding to $\alpha = 0.05$ with $n - 2$ degrees of freedom) can be used as an acceptable limit of calibration of an instrumental method. Only trifluralin, deltamethrin and fenthion showed a linearity value below 0.9503 (Table 2).

Fenthion and deltamethrin were not linearly recovered in the concentration range considered. Both compounds show poor recoveries by the method applied, as reported previously [17], fenthion because of its instability (unpublished data from our laboratory show a significant loss of this compound in the concentration process) and deltamethrin owing to its very low solubility in water (<0.2 μg/l at 25°C) [19]. In addition, both compounds show the lowest precisions, so the method is not appropriate for their determination. Variable recoveries depending on the concentration level have been reported previously for other pesticides [10].

Dimethoate is also poorly recovered [5,7,17] owing to the low octanol–water partition coefficient (log P_{ow} ranging from 0.50 to 0.78 [20]). Nevertheless, its recovery was improved with respect to previous results [5] by increasing the mass of sorbent and it presents a linear performance and a high precision; accordingly, the method could be appropriate for this compound.

Trifluralin was the first-eluting pesticide in the chromatographic run (Fig. 1) in a zone in which many interferences were observed, especially with such a sensitive detection method as ECD. Its linearity behaviour could be improved with a change in the chromatographic conditions. In this case, the lack of linearity is not associated with a low precision (Table 2).

Alachlor shows a high $s_r(c)$% value at a concentration of 100 ng/l and therefore a low precision (Table 2). Hence its response at this concentration is very low and its relative variability very high, as it was shown with pesticide standards [17] for the chromatographic response.

The LOD was calculated, after the whole processing of the water sample, both by means of an objective arithmetic calculation, derived from the calibration graph [17,18], and by a conventional method (signal-to-noise ratio = 3:1). The latter was obtained by measuring peak heights at the lowest concentration in the range

Table 2
Linearity, repeatability and limit of detection of the method

Pesticide	Linearity $[1 - s_r(b)]$	$s_r(c)$ (%) (100 ng/l)	LOD (ng/l)	
			Statistical	Conventional
ECD				
Trifluralin	0.9327	5.5	43	3
Lindane	0.9606	3.8	29	1
Triallate	0.9623	6.1	52	4
Alachlor	0.9643	11.7	97	10
Captan	0.9545	7.3	62	8
Folpet	0.9566	7.0	60	20
o,p'-DDE	0.9706	2.4	19	2
p,p'-DDE	0.9705	2.8	22	2
Oxyfluoren	0.9689	2.9	22	1
o,p'-DDT	0.9716	2.8	21	2
p,p'-DDT	0.9636	3.4	26	2
Bromopropylate	0.9724	2.5	19	3
Dicofol	0.9627	6.5	55	2
Tetradifon	0.9664	3.1	24	2
Deltamethrin	0.9347	11.7	100	5
FPD				
Dimethoate	0.9653	5.6	48	37
Fonofos	0.9693	5.2	45	7
Diazinon	0.9731	4.4	38	6
Fenitrohion	0.9795	3.5	30	6
Malathion	0.9844	2.4	20	8
Fenthion	0.9263	14.3	118	27
Chlorpyriphos	0.9762	3.7	32	7
Methidathion	0.9790	3.3	28	12
Phosmet	0.9635	5.5	47	33
Azinphos-methyl	0.9675	5.0	43	21
Phosalone	0.9741	4.2	36	8

used, except for those chemicals not detected at this concentration level.

Higher LODs are obtained with the statistically calculated method than when using $S/N = 3{:}1$ (Table 2). Nevertheless, for comparison among techniques, the statistical method should be used since it is based on mathematical data instead of personal appraisal, as has been reported previously [6,9].

Both systems have advantages and disadvantages. The statistical method is the result of the application of a calibration graph and the LOD reflects the errors associated with the fitting of this calibration. As a result, the LODs obtained may be too high when chromatograms are observed (Figs. 1 and 2). For example, alachlor in Fig. 1, at a concentration of 200 ng/l, is at twice its detection limit according to this statistical calculation. When measuring the peak height, for $S/N = 3{:}1$, an LOD of 10 ng/l is achieved.

In contrast, there are cases in which the conventional calculation of the LODs from a determined concentration may lead to erroneous values. This occurs, for example, with folpet. The peak height measurement for folpet in the chromatograms at 100 ng/l (Fig. 1) gives an LOD of 20 ng/l. Nevertheless, this fungicide is already lost in the extraction process at 50 ng/l. The statistical calculation gives an LOD of 60 ng/l, more in accordance with the predicted

value. Similar results were obtained with deltamethrin and fenthion, for which the conventional LODs are too low.

The use of calibration graphs for the recovery of pesticides from water samples has been applied in some cases [3,8,16,21]. It is advisable, from the previous results, to carry out a calibration experiment on the whole analytical process, with a 10–20-fold concentration. This would allow the calculation of the concentration of the pesticides present in the water sample more accurately. Likewise, this study has shown that the usual practice of calculating an LOD from a single concentration may lead to errors for some compounds, as is the case for fenthion, folpet and deltamethrin.

The performance parameters obtained by the calibration experiments using the statistical analysis of linear regression allows the detection of proportional and constant systematic errors, confirms the applicability of the method for each pesticide and permits one to compare easily the analytical parameters with those obtained by other methods.

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