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Determination of organophosphorous and nitrogen-containing pesticides in water samples by solid phase extraction with gas chromatography and nitrogen–phosphorus detection

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ABSTRACT

Organophosphorus and nitrogen-containing pesticides were extracted from water using solid-phase extraction (SPE) with Sep-Pak C_{18} cartridges and eluted with acetone and hexane. Different methods were evaluated to concentrate the eluates and, finally, pesticides were determined in the concentrated eluates by gas–liquid chromatography with nitrogen–phosphorus detection. Recoveries varied with the physico-chemical properties of the pesticides, being from 0% to 91%.

INTRODUCTION

The presence of pesticides in groundwaters in an agricultural area demands a suitable way of detecting large numbers of those chemicals at concentrations below the EEC regulations [1].

Extraction and concentration of pesticides from water has evolved in recent years. Liquid–liquid partition [2–4] produces good results but is time-consuming, polluting, unhealthy and expensive. Solid phase extraction (SPE) [5–9] is becoming increasingly popular as it does not have these disadvantages. Different supports have been used in the determination of pesticides from aqueous solutions [6–9], although octadecyl-bonded porous silica (C_{18}) is one of the most common ones [5,7,10–12].

The "Vega de Granada" (Grenada, South of Spain) is an area with a high agricultural production. Groundwater is in some places present less than 2 m below the surface. Therefore, contamination of groundwater with pesticides is likely to occur, depending on, among other factors, soil type, adsorption of the pesticides to the soil and the water solubility and chemical nature of the compounds applied.

A multiresidue method of analysis for pesticides present in the soils and groundwater of the "Vega de Granada" is being developed to assess their contamination levels. An SPE method, with a quantitative study of its different steps, for the determination of organophosphorous and nitrogen-containing pesticides in water is presented here. Later, this method might be used to monitor the groundwater contamination in this area.

EXPERIMENTAL

Reagents

Pesticides were selected according to their use in the studied area and all of them were reagent grade: fonofos, formothion, fenthion, chlorpyrifos, phosmet, azinphos-methyl, phosalone and amitraz were acquired from Labor Dr.

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Ehrenstorfer (Augsburg, Germany). The following pesticides were gifts from the producers: dimethoate, diazinon, methidathion and simazine (Ciba-Geigy, Münchwilen, Switzerland), fenitrothion and malathion (Sumitomo, Osaka, Japan) and pirimicarb (ICI Agrochemicals, Yalding, UK). Propiconazole (Ciba-Geigy) was used as internal standard.

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

Stock solutions of the pesticides and the internal standard were prepared in acetone (Probus, Badalona, Spain) at 1 g/l, except fenthion, which was prepared in isopropanol (Carlo Erba, Milan, Italy), and azinphos-methyl, which was prepared in toluene (Merck, Darmstadt, Germany). Simazine was prepared in acetone at 0.5 g/l. Dilutions were conveniently prepared in hexane (Probus, Badalona, Spain).

Standard mixtures. A concentrated standard mixture at 10 mg/l was prepared from the individual pesticide stock solutions, by a 1:100 dilution. The standard mixture at a concentration of 0.5 mg/l was a 1:20 dilution of the concentrated solution.

Glass wool and anhydrous sodium sulphate (Merck, Darmstadt, Germany) were extracted in a Soxhlet apparatus for 24 h with acetone. Anhydrous sodium sulphate was heated at 90°C for 1 h to remove the solvent and the glass wool left at room temperature until the solvent had evaporated [13].

Sep-Pak Classic short-body C_{18} cartridges with 360 mg of packing material/cartridge were used (Waters, Milford, MA, USA).

Apparatus

A Hewlett-Packard (Seville, Spain) 5880 gas chromatograph equipped with a split/splitless injector, a nitrogen–phosphorus detection (NPD) system and a Hewlett-Packard 5880A terminal to integrate peak areas, was employed. An HP-1 capillary column (cross-linked methyl silicone gum), 12 m × 0.2 mm I.D. (0.33 μm), with helium as the carrier gas at 1 ml/min was used. Injector and detector temperatures were 250 and 280°C, respectively. A 1-μl aliquot of the sample was injected in the splitless mode with the following temperature programme: 45°C (2 min), increase at 30°C/min to 160°C (2 min), increase at 4°C/min to 190°C (2 min) and increase at 20°C/min to 250°C (3 min).

The microcolumn was from Afora (Barcelona, Spain) and the rotary evaporator from Heidolph (Germany).

Procedure

Linearity. Linearity of the responses in the gas chromatograph was studied with mixtures of the pesticides at concentrations between 0.12 and 1.00 mg/l. Every sample was injected three times.

Repeatability. The standard mixture, at 0.5 mg/l, was injected eight times.

Concentration. Two different methods for concentration of the Sep-Pak eluates were investigated. A 10-μl volume of the concentrated standard mixture was added to 8 ml of acetone–hexane (1:1) and concentrated to about 0.3 ml using a microcolumn in a water bath at 75°C, or to about 2 ml in a rotary evaporator at 40°C under a 400 mbar vacuum. Further concentration of both solutions to a final volume of 0.2 μl was carried out under a gentle stream of helium. To an aliquot of 100 μl, carefully measured with a 100-μl Hamilton syringe, 5 μl of the internal standard at a concentration of 20 mg/l were added.

Sample extraction. A Sep-Pak cartridge was conditioned by consecutive passing 2 ml of hexane, 2 ml of acetone–hexane (1:1), 2 ml of acetone and 16 ml of Milli-Q water. Later, a volume of 1 l of water, spiked with 10 μl of the concentrated standard mixture, at pH 6.5, was passed through the cartridge at 30–40 ml/min under vacuum. Then, it was dried by sucking air for 30 min.

Compounds retained in the cartridge were eluted with 2 ml of acetone, 2 ml of acetone–hexane (1:1) and 2 ml of hexane. The eluate was dried on anhydrous sodium sulphate, which was washed with an additional 1-ml volume of each eluting solvent. The combined fractions were concentrated in a rotary evaporator and added with the internal standard, as indicated above.

RESULTS AND DISCUSSION

Fig. 1 shows the separation of the pesticides at 0.5 mg/l. All the pesticides are basically resolved at the baseline.

In a multiresidue method, the components of the sample under investigation and the standard sample may not always be at the same concentration. Therefore, to confirm that results can be extrapolated from one concentration to the other, linearity of the NPD responses for all the pesticides, including the internal standard, was studied in the range 0.12–1.00 mg/l (Table I). The results show that the response to the different chemicals is linear in the range studied, with correlation coefficients between 0.996 and 1.000.

A sample at a concentration of 0.5 mg/l, *i.e.* in the linear range, was injected eight times to determine the repeatability of the response. The results presented in Table II are expressed as areas relative to that of propiconazole. As can be seen, relative standard deviation (R.S.D.) is in all cases, except for fonofos, below 10%, and in general around 6–7%.

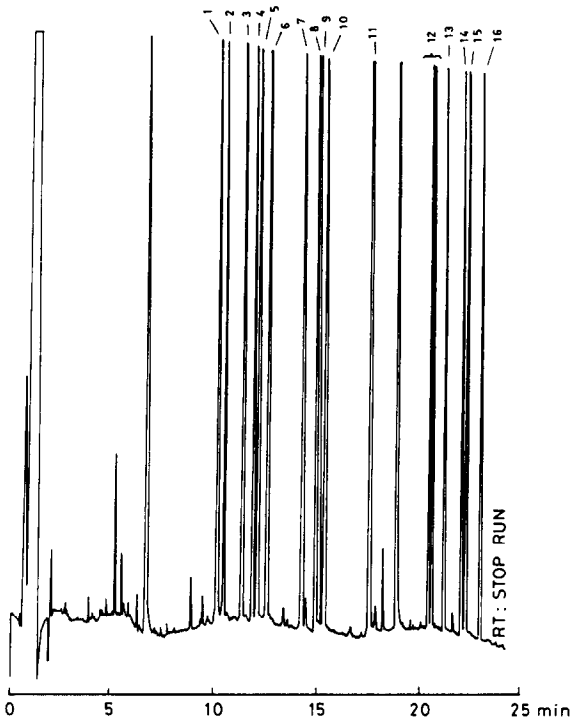

Fig. 1. Gas chromatogram showing the separation of organophosphorous and nitrogen-containing pesticides at about 0.5 mg/l. Volume injected: 1 μl. Chromatographic conditions as explained in the text. Peaks: 1 = dimethoate; 2 = simazine; 3 = fonofos; 4 = diazinon; 5 = formothion; 6 = pirimicarb; 7 = fenitrothion; 8 = malathion; 9 = fenthion; 10 = chlorpyrifos; 11 = methidathion; 12 = propiconazole (internal standard); 13 = phosmet; 14 = azinphos-methyl; 15 = phosalone; and 16 = amitraz.

Although high recovery levels have been reported for different pesticides using C_{18} silica cartridges [14,15], unexpectedly high deviations of the results were obtained in the first assays, so possible losses of the eluates during the concentration step were investigated.

Some preliminary assays were undertaken with three different concentration processes, with or without a keeper (toluene). These were: microcolumn (MC), rotary evaporator (RE) and a gentle steam of helium. The addition of toluene led in general to lower recoveries [16] and to an increase in concentration times. In the RE, the increase in time was slight, from 1 to 1.5 min, but in the MC time was increased from 5 to 20 min. Concentration of the whole eluate in a stream of helium yielded good results but was time-consuming, and finally rejected. Therefore, MC and RE without toluene were studied more in depth.

The different conditions studied showed that the residence time of the solution in the water bath for both the MC and the RE had an influence on the recovery of the different pesticides. Temperatures had to be chosen so that the evaporation time was as short as possible, without being so high as to decompose the compounds. The final temperature for the water bath in MC was therefore 75°C with a total residence time of above 5 min, and for the RE 40°C with a time of about 1 min. In the latter case, the vacuum produced by the pump also had an influence on the concentration process. The higher the vacuum, the shorter the concentration time, but a certain loss of the chemicals was observed. A vacuum of 400 mbar was a good compromise in our conditions.

Table III shows the recoveries in both cases, between 87 and 137% for RE (R.S.D. 1–6%) and between 82 and 131% for MC (R.S.D. 3–14%). Both systems gave similar results. The RE concentration process was finally chosen for

TABLE I

LINEARITY OF THE RESPONSE FOR STANDARD SOLUTIONS AT DIFFERENT CONCENTRATIONS

A = Average of peak areas for three injections; R^2 = correlation coefficient.

	1.00 mg/l		0.50 mg/l		0.25 mg/l		0.12 mg/l		R^2
	A	R.S.D. (%)	A	R.S.D. (%)	A	R.S.D. (%)	A	R.S.D. (%)	
Dimethoate	280.74	19.81	122.50	11.96	62.93	2.80	24.37	17.74	0.997
Simazine	188.30	13.99	85.01	7.68	43.81	8.38	22.40	20.83	0.997
Fonofos	347.30	11.25	156.80	8.89	82.46	8.83	44.67	16.15	0.996
Diazinon	178.44	10.76	80.35	11.23	40.63	5.88	22.80	14.69	0.996
Formothion	225.82	42.72	117.33	10.21	60.37	2.59	31.53	10.89	1.000
Pirimicarb	215.22	12.90	98.07	8.94	52.23	5.07	27.76	15.15	0.997
Fenitrothion	206.18	13.60	97.72	8.27	51.44	8.72	28.54	15.61	0.998
Malathion	175.79	12.06	84.33	8.48	43.76	6.34	24.13	15.12	0.999
Fenthion	165.56	12.55	78.21	8.16	40.02	7.93	22.45	14.11	0.998
Chlorpyriphos	194.07	12.46	90.87	8.44	48.30	7.14	27.24	14.01	0.998
Methidathion	255.00	13.93	122.63	8.46	63.64	5.72	34.86	13.39	0.999
Propiconazole	185.54	21.11	93.32	13.45	54.30	3.35	24.66	3.12	0.998
Phosmet	258.20	17.77	124.36	8.87	62.65	4.06	31.94	7.76	1.000
Azinfos-methyl	223.75	19.63	109.35	7.67	57.05	3.04	28.63	8.11	0.996
Phosalone	239.92	16.91	115.08	8.51	58.11	5.94	31.40	10.58	0.999
Amitraz	150.45	19.91	71.72	6.98	37.45	3.83	20.24	14.64	0.999

this method because of its rapidity, lower R.S.D. and slightly higher recovery levels.

A 1-l volume of water spiked with 0.1 μg of the different pesticides was extracted and determined according to the proposed method. The results (Table IV) show different levels of recovery for the different pesticides: low (0–6%) for dimethoate, formothion, fenthion and amitraz; intermediate (19–56%) for simazine, fonofos and chlorpyriphos; and high (66–91%) for the others, diazinon, pirimicarb, fenitrothion, malathion, methidathion, phosmet, azinphos-methyl and phosalone.

The low recovery levels for dimethoate and formothion may be explained by their high solubility in water (25 and 2.6 g/l, respectively [17]). This indicates that both pesticides were not retained in the cartridge [3,4] and were eluted with the passage of water. Besides, as is known, formothion in aqueous solutions quickly degrades to dimethoate [17]. Better results for both organophosphorous pesticides may be obtained either with more polar beds (*i.e.* active carbon [6]) or with different eluents [14]. Fenthion, although not very water soluble (2 ppm), is extremely soluble in dichloromethane or propan-2-ol (>1 kg/kg), so that other elution processes might yield better results for this pesticide.

TABLE II

REPEATABILITY FOR EIGHT INJECTIONS OF THE STANDARD MIXTURE AT 0.5 mg/l

	$CL^a = \bar{x} \pm \sigma t/n^{1/2}$	R.S.D. (%)
Dimethoate	0.48 ± 0.03	7.65
Simazine	0.69 ± 0.03	4.55
Fonofos	0.74 ± 0.06	10.11
Diazinon	0.46 ± 0.03	6.69
Formothion	0.43 ± 0.03	6.99
Pirimicarb	0.89 ± 0.03	4.55
Fenitrothion	0.49 ± 0.03	7.53
Malathion	0.41 ± 0.03	8.35
Fenthion	0.37 ± 0.02	7.56
Chlorpyriphos	0.49 ± 0.03	6.77
Methidathion	0.80 ± 0.04	6.38
Phosmet	0.43 ± 0.03	6.95
Azinphos-methyl	0.37 ± 0.02	7.02
Phosalone	0.39 ± 0.02	6.28
Amitraz	0.41 ± 0.01	3.41

[a] Confidence interval of the average. Peak areas relative to that of propiconazole. σ = Standard deviation; t = Student's t test for $\alpha = 0.05$.

TABLE III

RECOVERY LEVELS FROM THE CONCENTRATED ELUATE MIXTURE USING MC AND RE

	MC		RE	
	CL[a]	R.S.D. (%)	CL	R.S.D. (%)
Dimethoate	130.58 ± 59.13	14.48	136.95 ± 4.48	1.78
Simazine	94.18 ± 18.73	6.36	102.24 ± 2.37	1.26
Fonofos	86.21 ± 7.64	2.83	93.08 ± 2.32	1.36
Diazinon	86.95 ± 7.57	2.78	98.05 ± 1.87	1.04
Formothion	112.79 ± 34.70	9.83	124.85 ± 6.33	2.76
Pirimicarb	86.17 ± 11.08	4.11	94.55 ± 3.09	1.78
Fenitrothion	87.44 ± 8.13	2.97	97.94 ± 2.14	1.19
Malathion	86.42 ± 10.67	3.95	94.30 ± 2.55	1.47
Fenthion	81.56 ± 7.86	3.08	91.48 ± 6.23	3.71
Chlorpyriphos	85.45 ± 7.30	2.73	94.42 ± 1.35	0.78
Methidathion	87.84 ± 11.54	4.20	96.05 ± 1.74	0.99
Phosmet	91.45 ± 18.44	6.45	99.71 ± 3.15	1.72
Azinphos-methyl	92.54 ± 20.71	7.51	102.02 ± 3.68	1.97
Phosalone	90.20 ± 18.05	6.40	96.45 ± 2.42	1.36
Amitraz	85.15 ± 19.16	7.19	86.71 ± 9.30	5.84

[a] Confidence interval of the average, as in Table II; $n = 5$ for MC and $n = 4$ for RE. Each replicate was injected three times.
[b] Relative standard deviation ($\sigma/\bar{x} \cdot 100$).

Amitraz has been reported to be unstable at pH < 7 [17].

Other authors [18,19] have compared different methods of extraction and, although there is a general agreement about the slightly higher recovery of the liquid–liquid method, it is also generally accepted that it presents many disadvantages that may be avoided by using the SPE process, whose different steps are studied in this paper.

TABLE IV

RECOVERY LEVELS FROM 1 l OF WATER SPIKED WITH 0.1 μg OF EACH PESTICIDE

	CL[a]	R.S.D. (%)
Dimethoate	3.48 ± 7.67	182.77
Simazine	39.14 ± 15.14	32.90
Fonofos	18.75 ± 13.06	64.86
Diazinon	90.64 ± 15.81	27.50
Formothion	0.25 ± 0.65	387.30
Pirimicarb	85.67 ± 16.88	26.58
Fenitrothion	74.06 ± 11.69	22.76
Malathion	66.12 ± 14.76	25.22
Fenthion	0.80 ± 1.14	211.03
Chlorpyriphos	55.73 ± 9.02	26.49
Methidathion	69.77 ± 15.35	23.34
Phosmet	66.20 ± 7.41	17.67
Azinphos-methyl	82.00 ± 8.45	15.98
Phosalone	68.82 ± 16.65	36.29
Amitraz	6.16 ± 7.31	172.11

[a] Confidence interval of the average, as in Table II; $n = 5$ and each replicate was injected three times.

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