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Determination of pesticide residues in waters from small loughs by solid-phase extraction and combined use of gas chromatography with electron-capture and nitrogen–phosphorus detection and high-performance liquid chromatography with diode array detection

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Abstract

A procedure for the determination of pesticide residues in waters from small loughs surrounded by different crops has been developed. For this purpose, a solid-phase extraction procedure with octadecylsilane cartridges was used, optimizing the elution parameters as well as the breakthrough volume and the influence of the pesticide amount. The recoveries of the pesticides can be improved by about 10–20%. Extracts were analyzed either by gas chromatography with electron-capture and nitrogen–phosphorus detection or high-performance liquid chromatography with diode array detection to achieve a more reliable identification and determination of twenty-three pesticides from different chemical families, including triazines, phenylureas and organophosphorus. © 1997 Elsevier Science B.V.

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1. Introduction

Water from small loughs often used to irrigate the surrounding crops undergoes seasonal variations in its pesticide concentration. As a result, it is necessary to emphasize the frequent presence of variable amounts of pesticides used for protecting the different surrounding crops. Pesticide nature differs considerably according to the type of crops and they belong to very different chemical families. So, it is necessary to have procedures that encompass a wide range of concentrations and can provide information on as many different pesticides as possible to evaluate their residues. In a previous work, we addressed this problem by using supercritical fluid chromatography (SFC) which required a concentration step that we performed with SPE on-line due to the high values of the detection limits [1]. This was found to be an acceptable choice although the availability of this equipment is less widespread than that of conventional gas chromatography (GC) and high-performance liquid chromatography (HPLC). As consequence, this paper presents an alternative method based on more commonly used analytical techniques, combining them with a previous clean-up step.

For the isolation of pesticides from water samples, the use of solid-phase extraction (SPE) has been preferred in this work for its countless advantages in terms of simplicity, robustness and easy automation [2–17] in relation to liquid–liquid extraction [18–

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20], using octadecylsilane (ODS) as stationary phase [21–27], and studying aspects such as the elution mode, the breakthrough volume and the influence of the pesticide concentration in order to maximize recoveries.

As the presence of compounds of very different chemical properties (organochlorines, organophosphorus, triazines, benzimidazoles, acetamides and others) is foreseen on the basis of the prevailing crops in the area studied (cereal and sugar beet), and in order to find the best procedure, the combined use of HPLC–diode array detection (DAD) and GC with selective and conventional detection methods such as electron-capture detection (ECD) and nitrogen–phosphorus detection (NPD) has been tested. The ensuing procedure has been applied to waters from forty small loughs located next to one another.

2. Experimental

2.1. Reagents

Pesticide standards were obtained from Riedel-de Haën (Hannover, Germany) and Promochem (Wessel, Germany). Residue analysis grade acetonitrile, ethyl acetate, ethyl ether, methanol, dichloromethane, acetone and *n*-hexane were supplied by Lab-Scan (Dublin, Ireland). Ultrapure water was obtained by using a Milli-Q apparatus from Millipore (Bedford, MA, USA). Florisil of 60–100 mesh was purchased from Baker (Deventer, Netherlands). Octadecylsilane 500 mg cartridges (RP-18) from Merck (Darmstadt, Germany) were used for solid-phase extraction.

2.2. Study of the extraction on ODS cartridges

A study of SPE of pesticides on ODS cartridges has been carried out with a solid–liquid extraction system supplied by Varian (Harbor City, CA, USA). ODS cartridges were conditioned by successive elution of 15 ml of methanol and 10 ml of water, by means of a gentle vacuum, to avoid drying-out during the procedure. Then, the sample volume was percolated at 5 ml/min and the cartridge was dried with nitrogen and eluted by gravity. Finally, the extract was injected in one of the chromatographic systems.

Ethyl acetate, acetone, acetonitrile and methanol were used as eluents to collect the extract which was assayed on 100 ml of ultrapure water spiked with 1 μg of each pesticide by the addition of an acetone solution (0.5 ml) containing them, just prior to extraction. Those solvents were used in the study as eluents because they had theoretically an adequate polarity to achieve the complete elution of the pesticides retained on the cartridges.

The influence of an equilibrium (or soaking) time of 2 min between the solvent and stationary phase, before eluting the cartridge, and of the solvent volume eluted (2, 3 or 4 ml) on the recovery was also studied, carrying out the elution with the solvent previously selected.

0.25, 0.5, 1, 1.5, 2 and 2.5 μg of each pesticide were added to 100 ml of water in order to evaluate the capacity of the cartridges to retain different pesticide amounts. Volumes of 100, 200, 300, 400 and 500 ml spiked with 1 μg of each pesticide were also used to obtain the breakthrough volume.

2.3. Study of the elution through a Florisil packed-column

A study to verify the elution of the pesticides through a packed-column of Florisil as clean-up was also undertaken. Florisil was activated by heating at 120°C for 4 h. The column, 10 cm×1 cm I.D., was prepared from a Florisil (about 5 g) slurry in *n*-hexane and compacted with a rod. Once ready, the column was loaded with 1 ml of solution containing 0.5 mg/l of each pesticide, and eluted by gravity with 15, 20, 25, 30 and 35 ml of a *n*-hexane–dichloromethane (1:1, v/v) mixture, whilst avoiding drying-out of the column. Subsequently, the eluate was evaporated in a rotary evaporator from Büchi (Plawil, Switzerland) at 35°C and the residue dissolved in 2 ml of acetone.

2.4. Procedure proposed for lough-water analysis

Extraction was performed on ODS cartridges conditioned as described in Section 2.2. A water sample volume of 300 ml, previously filtered through glass plate, was percolated at a 5 ml/min flow-rate and the cartridge was dried with nitrogen for about 40 min. Then, 2 ml of methanol were added to the

cartridge, which was soaked for 2 min. The 2 ml volume was percolated, eluting further 2 ml of methanol, all by gravity. Finally, both eluates were combined and concentrated to 1 ml in a rotary evaporator at 35°C.

Methanolic extracts were cleaned-up by passage through a Florisil-packed glass column, made as described in Section 2.3. The column was loaded with 1 ml of extract and 30 ml of *n*-hexane–dichloromethane (1:1, v/v) mixture were percolated. Subsequently, the solvents were evaporated and the residue was dissolved in 2 ml of acetone, and subjected to chromatographic analysis.

2.5. HPLC system

The HPLC system was composed of a membrane degasser, a ConstaMetric 4100 quaternary pump, an AutoMetric 4100 autosampler and a 5000 diode array detector, all supplied by LDC Analytical (Riviera Beach, FL, USA). A 150×4.6 mm Spherisorb ODS-2 column, 5 μm pore size, from Phenomenex (Torrance, CA, USA) was used with the acetonitrile–water mobile phase gradient that follows: time 0 min, 10:90, time 10 min, 40:60, time 20 min, 45:55, time 30 min, 90:10. The mobile phase flow-rate was 1 ml/min and the volume injected was 25 μl.

2.6. GC system

A Hewlett-Packard (Avondale, PA, USA) 5890 gas chromatograph equipped with an HP7673 autosampler, two detectors, electron capture and nitrogen–phosphorus, and a 60 m×0.25 mm capillary column coated with a 0.25 μm film of 50% phenylmethylpolysiloxane (Quadrex Scientific, Surrey, UK) was used. The oven temperature programme was as follows: initial temperature 50°C, held for 1 min, a 15°C/min ramp to 200°C, and

Table 1
Detection mode and retention times (n=5) for the pesticides

Pesticide	Detection	Retention time (min)	R.S.D. (%)	Family	Use
Alachlor	ECD	55.67	0.05	Acetamide	Herbicide
Atrazine	NPD	50.57	0.04	Triazine	Herbicide
Azinphos methyl	NPD	100.23	0.06	Organophosphorus	Insecticide
Captan	ECD	68.55	0.09	Phtalimide	Fungicide
Carbaryl	220 nm	15.11	0.08	Carbamate	Insecticide
Carbendazim	220 nm	12.70	0.08	Benzimidazole	Fungicide
Cypermethrin[a]	ECD	107.10	0.08	Pyrethroid	Insecticide
Chloridazon	ECD	89.71	0.10	Pyridazinone	Herbicide
Chlortoluron	245 nm	17.10	0.07	Phenylurea	Herbicide
Chlorsulfuron[b]	NPD	9.70	0.05	Sulfophenylurea	Herbicide
Dicofol	ECD	61.12	0.06	Organochlorine	Acaricide
Dimethoate	NPD	53.85	0.05	Organophosphorus	Insecticide
Dinobuton	ECD	61.91	0.02	Nitrocompound	Fungicide
Diuron	245 nm	15.63	0.08	Phenylurea	Herbicide
Isoproturon	245 nm	18.10	0.06	Phenylurea	Herbicide
Malathion	NPD	59.82	0.04	Organophosphorus	Insecticide
Metalaxyl	NPD	58.85	0.04	Acylalanine	Fungicide
Metamitron	NPD	79.04	0.07	Triazinone	Herbicide
Oxadixyl	NPD	81.48	0.08	Oxazolidine	Fungicide
Permethrin[a]	ECD	95.15	0.11	Pyrethroid	Insecticide
Simazine	NPD	51.28	0.04	Triazine	Herbicide
Terbutryn	NPD	58.38	0.05	Triazine	Herbicide
Tetradifon	ECD	88.53	0.06	Organochlorine	Acaricide

R.S.D.: Relative standard deviation.
[a] Retention time of the last eluting isomers.
[b] Determined as 2-amino-4-methoxy-6-methyl 1,3,5-triazine.

finally an 1°C/min ramp to 275°C, held for 34 min. The carrier gas (He) flow-rate was 0.7 ml/min, measured at 50°C. Splitless injection (2 μl) was carried out at 200°C, the purge valve was on at 1 min. Hydrogen, air and helium were used as auxiliary gases for NPD, and argon–methane (90:10) for ECD. In both cases, the detector temperature was 300°C.

3. Results and discussion

3.1. Extraction on ODS cartridges

Table 1 shows the pesticides included in the study, the chromatographic technique used for their quantitative analysis and their retention times. Pesticides were preferentially determined by GC–ECD or GC–NPD due to their highest sensitivity in comparison with that obtained by HPLC–DAD. Chlorsulfuron was determined as 2-amino-4-methoxy-6-methyl 1,3,5-triazine, a thermal degradation product, because the parent compound had a very broad peak in the HPLC system. For permethrin and cypermethrin, which have isomeric compounds and present more than one chromatographic peak in GC, the most retained peak was considered in the study. For the HPLC detection, the most suitable wavelengths for each compound in terms of sensitivity and selectivity were selected from the DAD data.

Table 2 shows the recoveries obtained after eluting 2 ml of different solvents. The average recoveries for ethyl acetate, acetonitrile, acetone and methanol were 58.8, 64.1, 68.0 and 77.3%, respectively. Methanol was selected for the following experiments because it provided higher or acceptable recoveries for most of the pesticides, excepting carbaryl and cypermethrin. Ethyl acetate was the worst choice for the multiresidue analysis. The relative standard deviation (R.S.D.) of the results ranged from 3 to 5% ($n=5$).

Table 3 shows the influence of the equilibrium time (cartridge soaked for 2 min prior to elution), and

Table 2
Recovery (%) of pesticides from 100 ml of ultrapure water spiked with 1 μg of each pesticide by ODS cartridges eluted with 2 ml of different solvents ($n=5$)

Pesticide	Ethyl acetate		Acetone		Acetonitrile		Methanol	
	Recovery	R.S.D.	Recovery	R.S.D.	Recovery	R.S.D.	Recovery	R.S.D.
Alachlor	80.7	4.8	90.4	5.0	60.6	5.4	92.9	5.2
Atrazine	80.5	4.4	85.2	4.6	80.0	4.1	89.9	5.1
Azinphos methyl	81.3	4.2	98.5	4.0	101.8	3.5	96.8	4.5
Captan	45.2	5.1	76.0	3.9	69.1	4.2	66.1	4.8
Carbaryl	94.0	3.4	90.0	3.2	100.8	3.5	72.3	4.2
Carbendazim	51.3	5.8	74.6	4.5	80.2	5.1	86.3	5.0
Cypermethrin	34.2	4.8	31.7	4.5	30.6	4.6	21.1	4.7
Chloridazon	38.2	4.5	33.5	4.0	39.2	3.8	67.3	3.3
Chlortoluron	30.7	5.2	57.5	4.0	35.9	4.0	68.1	3.9
Chlorsulfuron	70.2	4.2	85.5	4.5	81.6	4.3	89.3	4.5
Dicofol	36.3	5.0	47.0	4.2	51.2	4.7	52.2	5.2
Dimethoate	86.3	3.8	62.1	4.5	99.9	3.5	91.9	3.4
Dinobuton	74.0	4.2	90.3	3.8	55.0	5.1	99.3	3.9
Diuron	65.5	4.7	43.7	4.8	64.4	5.0	57.5	5.5
Isoproturon	20.6	5.0	60.4	3.9	29.8	4.7	76.6	4.6
Malathion	56.9	4.4	92.5	3.7	64.9	4.1	101.3	3.8
Metalaxyl	71.5	4.1	92.7	5.0	87.4	4.6	95.6	5.6
Metamitron	55.3	5.1	89.7	4.2	70.3	4.0	93.6	4.9
Oxadixyl	41.3	4.1	83.2	3.5	45.8	3.7	100.1	3.2
Permethrin	11.9	4.4	17.0	4.6	10.2	4.3	20.9	4.1
Simazine	92.9	3.5	82.1	3.6	85.6	3.5	88.5	3.5
Terbutryn	92.5	3.6	71.4	3.9	81.3	3.5	89.1	3.5
Tetradifon	43.2	4.5	54.1	5.0	50.4	5.1	60.8	4.0

R.S.D.: Relative standard deviation (%).

Table 3
Recovery (%) of pesticides from 100 ml of ultrapure water spiked with 1 μg of each pesticide by ODS cartridges eluted with different volumes of methanol and equilibrium times ($n=5$)

Pesticide	Equilibrium time: 0 min		Equilibrium time: 2 min					
	2 ml		2 ml		3 ml		4 ml	
	Recovery	R.S.D.	Recovery	R.S.D.	Recovery	R.S.D.	Recovery	R.S.D.
Alachlor	92.3	5.2	97.3	4.8	100.7	4.5	100.3	4.2
Atrazine	89.9	5.1	89.0	4.6	95.8	4.2	97.0	3.5
Azinphos methyl	96.8	4.5	96.8	4.5	97.5	4.5	97.8	4.7
Captan	66.1	4.8	66.1	4.7	69.5	4.4	71.2	4.4
Carbaryl	72.3	4.2	80.4	4.0	88.3	4.1	93.6	4.2
Carbendazim	86.3	5.0	87.6	4.9	92.7	4.0	94.1	3.9
Cypermethrin	21.1	4.7	23.4	3.9	25.7	3.8	27.5	3.5
Chloridazon	67.3	3.3	66.8	3.5	72.3	3.6	84.9	3.4
Chlortoluron	68.1	3.9	77.7	4.1	88.3	4.0	96.0	3.8
Chlorsulfuron	89.3	4.5	90.3	3.8	92.4	3.4	95.7	3.3
Dicofol	52.2	5.2	58.8	3.7	64.1	3.8	69.3	3.5
Dimethoate	91.9	4.5	95.4	3.5	101.4	3.1	101.7	3.3
Dinobuton	99.3	3.9	97.4	3.4	98.9	3.0	99.0	3.4
Diuron	57.5	5.5	66.6	4.9	77.5	4.2	87.5	4.4
Isoproturon	76.6	4.6	83.4	5.1	88.5	4.2	94.8	4.5
Malathion	101.3	3.8	100.8	4.0	99.9	3.9	99.8	3.6
Metalaxyl	95.6	5.6	93.8	4.8	96.6	4.6	99.7	4.5
Metamitron	93.6	4.9	92.9	4.7	94.7	4.1	95.0	4.0
Oxadixyl	100.1	3.2	99.7	3.6	100.6	3.3	100.6	3.1
Permethrin	20.9	4.1	24.7	3.9	26.4	3.9	29.8	3.9
Simazine	82.1	3.5	98.0	3.8	99.3	3.5	99.8	3.4
Terbutryn	89.1	3.5	90.3	3.9	91.5	3.8	92.8	3.5
Tetradifon	60.8	4.0	63.8	4.1	65.4	4.2	66.7	3.9

R.S.D.: Relative standard deviation (%).

the eluent volume on the recovery. The equilibrium between the stationary phase and solvent improved the recovery of various pesticides when the cartridge was eluted with 2 ml of methanol; for instance, the recovery increased to 8% for carbaryl and chlortoluron and a 16% for simazine. Recovery data were submitted to analysis of variance (1-way ANOVA). Significant differences ($p<0.05$) were found for the recoveries of carbaryl, chlortoluron, diuron, isoproturon and simazine. On the other hand, the solvent volume also affected the recovery of the pesticides retained in the cartridge. Higher methanol volumes (from 2 to 4 ml) increased the recovery-rates up to ca. 12% for carbaryl, chloridazon, dicofol and isoproturon, and ca. 20% for chlortoluron and diuron. A 1-way ANOVA was applied to the recoveries obtained by elution with the 2, 3 and 4 ml, after the equilibrium time. Significant differences ($p<0.05$) were obtained for the recovery of atrazine, carbaryl, carbendazim, chloridazon, dicofol and isoproturon. When the ANOVA was performed on all the data obtained, combining the influence of the solvent volume and equilibrium time, alachlor, atrazine, carbaryl, carbendazim, chloridazon, chortoluron, chlorsulfuron, dicofol, dimethoate, diuron, isoproturon, permethrin and simazine also presented significant differences ($p<0.05$).

Table 4 lists the recoveries and precisions obtained in the extraction of different pesticide amounts contained in 100 ml of water. As can be seen, increasing the analyte amount led to similar or slightly lower (4–5%) recoveries in most instances. The sharpest decreases (10–16%) were obtained for azinphos methyl, permethrin, oxadixyl and dinobuton. Significant differences ($p<0.05$) in the recoveries of azinphos methyl, permethrin, oxadixyl, dinobuton and malathion were found when a 1-way ANOVA was applied to the data obtained for the

Table 4
Recovery (%) of pesticides from 100 ml of ultrapure water spiked with different amounts of each pesticide ($n=5$)

Pesticide	0.25 μg		0.50 μg		1.0 μg		1.5 μg		2.0 μg		2.5 μg	
	Recovery	R.S.D.	Recovery	R.S.D.	Recovery	R.S.D.	Recovery	R.S.D.	Recovery	R.S.D.	Recovery	R.S.D.
Alachlor	100.1	3.8	100.5	3.5	100.3	4.2	99.9	3.7	99.2	3.6	97.8	4.4
Atrazine	97.4	3.5	97.2	3.6	97.0	3.5	97.4	4.0	96.3	4.6	94.6	3.7
Azinphos methyl	97.7	4.1	97.7	3.5	97.8	4.7	98.2	4.2	96.3	3.8	86.8	4.2
Captan	65.3	4.0	69.9	3.8	71.2	4.4	70.1	4.3	60.5	4.0	61.2	4.0
Carbaryl	95.7	3.5	94.3	4.0	93.6	4.2	92.6	3.9	91.2	3.5	91.1	3.9
Carbendazim	93.5	3.2	94.1	3.5	91.1	3.9	94.8	4.2	93.3	4.0	92.4	4.2
Cypermethrin	29.0	4.3	27.9	3.8	27.5	3.5	26.3	4.0	24.1	3.8	23.0	3.9
Chloridazon	88.2	3.3	85.4	3.1	84.9	3.4	85.4	3.7	83.7	4.1	83.0	3.0
Chlortoluron	98.0	3.4	96.4	3.4	96.0	3.8	96.3	3.6	94.0	3.5	95.0	3.8
Chlorsulfuron	95.6	3.5	95.8	3.3	95.9	3.3	96.8	3.5	95.6	4.1	94.8	3.7
Dicofol	69.4	4.0	69.6	3.9	69.3	3.5	69.3	3.8	68.3	3.0	69.2	3.2
Dimethoate	101.5	4.1	101.4	3.5	101.7	3.3	101.7	3.4	101.0	3.1	93.7	3.7
Dinobuton	99.1	3.5	99.2	3.6	99.0	3.4	95.2	3.7	91.3	4.0	83.8	3.8
Diuron	93.0	3.5	88.0	4.2	87.5	4.4	89.0	3.4	87.0	3.9	86.6	3.6
Isoproturon	94.9	3.5	95.2	3.8	94.8	4.5	97.1	3.9	96.0	4.5	96.8	4.1
Malathion	100.4	3.9	99.9	3.8	99.8	3.6	97.4	4.0	96.5	3.8	94.4	4.0
Metalaxyl	99.7	4.2	99.8	4.0	99.7	4.5	97.8	4.1	96.2	4.3	96.1	4.2
Metamitron	95.4	4.0	94.8	3.9	95.0	4.0	94.2	4.2	93.0	4.9	92.3	4.0
Oxadixyl	100.5	2.9	100.0	3.2	100.6	3.1	94.4	3.3	89.8	3.4	87.6	3.6
Permethrin	31.0	3.6	30.2	3.2	29.8	3.9	22.7	4.0	21.8	4.5	21.4	4.7
Simazine	99.7	3.5	99.8	3.5	99.8	3.4	100.2	3.7	100.3	3.2	97.1	3.0
Terbutryn	93.0	3.8	92.6	3.7	92.8	3.5	93.2	3.5	90.0	3.7	90.3	3.2
Tetradifon	66.5	4.2	66.8	3.7	66.7	3.9	65.4	3.8	67.0	3.8	65.9	3.7

Elution with 4 ml of methanol after soaking for 2 min.
R.S.D.: Relative standard deviation (%).

different pesticide amounts. For diuron and dimethoate, their recoveries were statistically different ($p<0.05$) when the ANOVA was only applied to the 0.25 and 2.5 μg data.

Table 5 presents the results of the study performed for the breakthrough volume. Metamitron and dimethoate were the most affected compounds, their recoveries decreasing from 95 and 101% to 35 and 34%, respectively, when the volume increased from 100 to 500 ml, while the recoveries of azinphos methyl, chlorsulfuron, oxadixyl, malathion and dinobuton decreased by 10–20%. Lower variations were exhibited by the other compounds. Results were also submitted to ANOVA to test for statistical differences. So, the recoveries of azinphos methyl, chlorsulfuron, dimethoate, dinobuton, malathion, metamitron and oxadixyl were significantly different ($p<0.05$).

A volume of 300 ml was chosen as a compromise solution for the multiresidue analysis. 1-way ANOVA was also used to determine if the recoveries achieved with 100 or 300 ml were significantly different. The analysis revealed that the recoveries of chlorsulfuron, dimethoate, dinobuton and metamitron were significantly lower ($p<0.05$) when 300 ml were used in the experiments.

An anomalous behaviour, perhaps explained by adsorption phenomena, was found for dicofol and tetradifon which increased their recoveries from 69 and 67% to 85 and 97%, respectively, when higher water volumes (from 100 to 500 ml) were analyzed.

The recoveries of these compounds were different ($p<0.05$) after a 1-way ANOVA. As regards cypermethrin and permethrin (pyrethroids), their recoveries were always very low, below 35%, according to previous data [24].

3.2. Elution through Florisil

The recovery of pesticides for different volumes of *n*-hexane–dichloromethane eluted through a Florisil packed-column is shown in Table 6. As can be seen,

Table 5
Recovery (%) of pesticides from different volumes of ultrapure water spiked with 1 μg of each pesticide ($n=5$)

Pesticide	100 ml		200 ml		300 ml		400 ml		500 ml	
	Recovery	R.S.D.	Recovery	R.S.D.	Recovery	R.S.D.	Recovery	R.S.D.	Recovery	R.S.D.
Alachlor	100.3	4.2	100.5	4.0	100.1	4.1	100.2	3.8	98.8	4.0
Atrazine	97.0	3.5	97.1	3.7	97.3	3.6	97.0	3.6	96.4	4.7
Azinphos methyl	97.8	4.7	97.7	4.2	92.9	4.2	86.9	4.0	82.6	3.5
Captan	71.2	4.4	71.1	4.3	71.0	4.5	70.8	4.2	70.3	4.2
Carbaryl	93.6	4.2	94.0	3.5	93.8	3.9	93.5	3.8	92.4	3.4
Carbendazim	94.1	3.9	94.0	3.7	93.1	3.9	91.6	4.2	89.4	4.6
Cypermethrin	27.5	3.5	27.9	3.9	27.4	3.6	27.5	3.5	27.6	4.0
Chloridazon	84.9	3.4	84.3	3.6	83.3	3.7	83.0	3.9	82.5	3.8
Chlortoluron	96.0	3.8	96.2	3.8	96.0	3.5	95.9	3.4	93.1	4.1
Chlorsulfuron	95.7	3.3	90.9	3.4	89.5	3.7	88.4	3.8	76.1	4.9
Dicofol	69.3	3.5	72.6	3.6	76.7	3.5	81.5	3.3	84.6	3.3
Dimethoate	101.7	3.3	86.5	3.3	73.8	3.5	43.8	4.9	34.1	4.8
Dinobuton	99.0	3.4	93.1	3.7	81.8	3.9	78.5	4.0	81.4	3.9
Diuron	87.5	4.4	87.1	4.0	87.6	4.0	87.4	3.8	86.9	3.9
Isoproturon	94.8	4.5	95.2	4.2	94.6	3.7	95.2	3.9	94.7	4.7
Malathion	99.8	3.6	94.0	3.5	93.4	4.0	92.1	3.7	89.5	3.9
Metalaxyl	99.7	4.5	97.4	4.0	95.2	4.2	94.6	4.0	93.7	4.2
Metamitron	95.0	4.0	89.3	4.0	79.9	4.3	54.8	4.8	34.8	5.3
Oxadixyl	100.6	3.1	99.8	3.3	99.3	3.2	89.2	3.5	88.8	4.3
Permethrin	29.8	3.9	33.4	4.2	27.9	4.0	35.2	4.9	30.6	4.2
Simazine	99.8	3.4	100.2	3.1	100.0	3.5	99.4	3.5	99.8	3.8
Terbutryn	92.8	3.5	93.5	3.1	93.0	3.3	92.7	3.5	93.0	3.5
Tetradifon	66.7	3.9	83.2	3.5	88.1	4.0	90.0	3.5	96.5	3.6

Elution with 4 ml of methanol after soaking for 2 min.
R.S.D.: Relative standard deviation (%).

compounds such as tetradifon, permethrin, cypermethrin, metalaxyl, malathion, chlorsulfuron, carbendazim and carbaryl were eluted to an extent of about 80% or higher with only 15 ml of solvent. In all cases, the recovery increased gradually for higher eluent volumes. A volume of 30 ml was adopted as optimum to ensure high recoveries and minimize the coeluted interferences in dealing with real samples. Recovery data obtained for 30 ml were submitted to a 1-way ANOVA to test if they were different from those achieved for 15 and 35 ml. In the elution with 15 or 30 ml, all the recoveries were significantly different ($p<0.05$), except for permethrin and cypermethrin. For the elution with 30 or 35 ml, differences ($p<0.05$) were found in the recovery of dicofol, metamitron, oxadixyl and terbutryn. The precision (R.S.D.) for the elution with 30 ml was about 3% while it was worse for lower eluent volumes, about 4% ($n=5$).

3.3. Procedure evaluation

Table 7 shows the recoveries and precisions achieved in the application of the proposed procedure on waters spiked with 1 μg of each pesticide. On ultrapure water, the recoveries were above 70%, except for the pyrethroids, with R.S.D.s ranging from 3 to 8%, while on lough water the recoveries were comparable or lower than the previous ones, due likely, to the organic matter present in the real samples, which could affect the retention on the cartridges. However, notably higher recoveries were obtained for permethrin and cypermethrin in comparison with those obtained on ultrapure water, 45.8 and 39.6% against 28.3 and 27.4%, respectively. This fact, which has already been observed in the analysis of complex waters [24] could be also associated to the effect of the organic matrix which, in this case, would favour the adsorption on ODS.

Table 6
Recovery (%) of pesticides from a Florisil column for different volumes of *n*-hexane–dichloromethane (1:1) as eluent ($n=5$)

Pesticide	15 ml		20 ml		25 ml		30 ml		35 ml	
	Recovery	R.S.D.	Recovery	RR.S.D.	Recovery	R.S.D.	Recovery	R.S.D.	Recovery	R.S.D.
Alachlor	71.2	4.2	93.0	3.2	99.3	3.0	99.5	3.1	99.6	3.0
Atrazine	61.0	4.3	78.3	4.0	91.4	3.4	94.9	2.9	95.6	2.8
Azinphos methyl	79.3	4.0	84.4	3.5	86.2	3.4	99.0	2.7	99.0	2.5
Captan	71.6	3.9	85.9	3.8	92.0	3.3	96.2	3.1	98.4	3.2
Carbaryl	85.4	3.5	91.6	3.8	95.5	3.1	95.8	3.4	94.8	3.3
Carbendazim	89.4	3.7	92.1	3.4	95.6	3.1	96.3	2.9	96.9	3.0
Cypermethrin	97.3	3.2	98.8	3.3	98.7	3.4	98.6	3.2	98.8	2.9
Chloridazon	41.9	4.7	53.2	4.5	72.3	4.0	93.3	3.2	95.7	3.2
Chlortoluron	74.9	3.7	77.9	3.8	95.4	3.1	95.6	2.9	95.7	2.7
Chlorsulfuron	85.3	3.8	95.6	3.5	97.6	3.3	99.9	3.3	102.1	3.1
Dicofol	78.0	3.8	88.3	3.7	91.6	3.7	85.0	3.4	97.8	3.0
Dimethoate	33.4	4.5	53.4	4.2	76.6	3.8	92.8	3.2	96.9	3.3
Dinobuton	76.3	4.0	93.8	3.4	100.0	3.0	100.1	2.9	100.3	2.8
Diuron	20.9	4.2	52.5	4.1	77.6	3.8	89.1	3.2	90.3	3.1
Isoproturon	28.9	4.6	48.1	4.0	61.2	3.5	89.8	3.0	94.4	2.6
Malathion	80.4	3.5	91.5	3.4	99.9	3.6	101.0	3.3	100.1	3.3
Metalaxyl	85.3	3.7	89.5	3.7	92.7	3.4	95.4	2.8	96.0	3.0
Metamitron	68.7	3.8	73.0	3.7	78.6	3.5	91.5	3.2	97.2	2.9
Oxadixyl	68.2	4.5	75.7	3.8	83.8	3.5	91.2	3.0	97.3	3.3
Permethrin	96.5	4.0	101.0	4.3	99.1	3.4	100.6	3.2	98.7	3.2
Simazine	59.1	4.2	70.5	3.9	95.6	3.1	94.3	2.8	93.9	3.3
Terbutryn	40.4	5.0	67.7	4.5	78.9	3.9	89.5	3.2	95.5	3.4
Tetradifon	89.5	3.5	98.2	3.7	100.3	3.3	100.4	3.0	100.1	3.2

R.S.D.: Relative standard deviation (%).

Table 7
Recovery (%) and precision obtained on ultrapure and lough waters by the proposed procedure ($n=7$)

Pesticide	Ultrapure water		Lough water	
	Recovery	R.S.D.	Recovery	R.S.D.
Alachlor	101.1	7.2	104.2	7.8
Atrazine	97.4	4.6	96.7	6.5
Azinphos methyl	92.0	4.4	94.8	6.0
Captan	70.6	6.6	69.5	7.1
Carbaryl	94.2	5.4	84.2	8.6
Carbendazim	93.1	4.6	87.5	8.2
Cypermethrin	27.4	3.4	39.6	3.4
Chloridazon	82.1	8.3	75.7	15.8
Chlortoluron	92.0	5.3	91.5	5.3
Chlorsulfuron	82.5	7.8	80.9	5.0
Dicofol	70.4	4.9	75.5	6.7
Dimethoate	75.0	3.9	67.3	7.8
Dinobuton	83.5	9.2	84.6	9.5
Diuron	73.4	8.5	72.3	13.8
Isoproturon	95.0	5.7	93.4	8.0
Malathion	98.5	3.5	98.5	7.9
Metalaxyl	93.0	4.3	89.5	4.1
Metamitron	79.5	8.4	68.4	11.8
Oxadixyl	98.2	7.5	94.3	3.9
Permethrin	28.3	3.4	45.8	6.6
Simazine	103.3	4.6	98.3	4.4
Terbutryn	94.5	5.3	95.5	5.2
Tetradifon	87.2	5.0	85.0	6.2

R.S.D.: Relative standard deviation (%).

The 1-way ANOVA showed differences ($p<0.05$) between the recovery values obtained in both instances for permethrin and cypermethrin, in addition to metamitron. As regards the precision, the R.S.D.s were higher on real samples, reaching values of 11–15% for compounds such as metamitron, diuron and chloridazon, as can be seen in Table 7. The recoveries on ultrapure water were similar to those achieved by the on-line coupled SPE–SFC system [1] for those compounds analyzed by both procedures, while the R.S.D.s were slightly lower in the on-line system, varying between 3 and 6%.

Fig. 1 shows the chromatogram for a lough water extract analyzed by GC–ECD. The chromatogram is fairly simple and has an acceptable baseline. The clean-up effect, which decreases the baseline noise, can be also observed. The efficiency of the clean-up was also reflected in the HPLC chromatograms while it was not as efficient in GC–NPD. Figs. 2 and 3 show chromatograms for a sample extract analyzed by GC–NPD and HPLC–DAD, respectively.

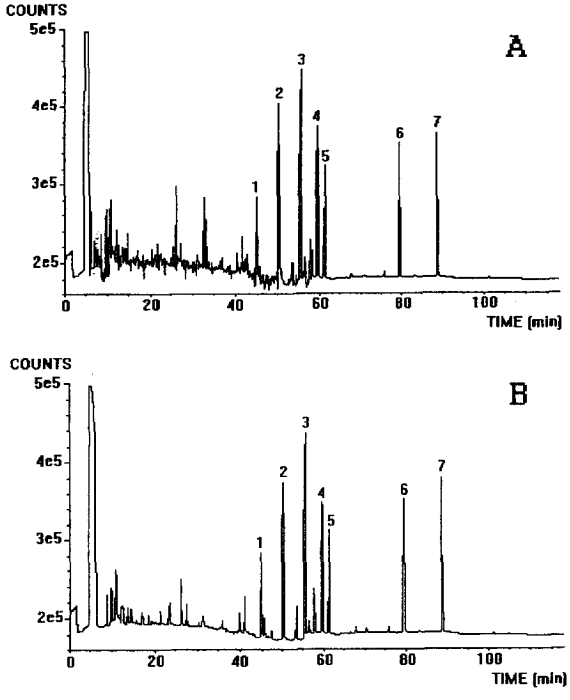

Fig. 1. Chromatograms of a spiked lough water sample obtained by the proposed procedure and GC–ECD. (A) Without Florisil clean-up; (B) with Florisil clean-up. (1) Chlortoluron, (2) atrazine, (3) alachlor, (4) malathion, (5) dicofol, (6) metamitron, (7) tetradifon.

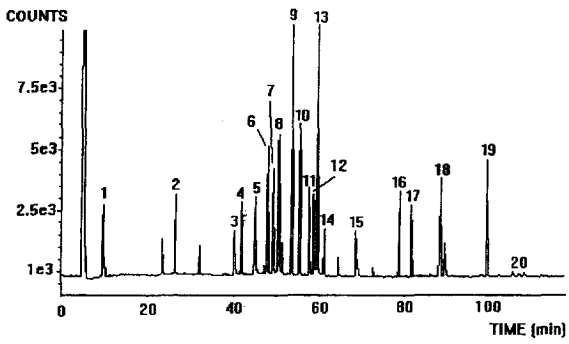

Fig. 2. Chromatogram of a spiked lough water sample obtained by the proposed procedure and GC–NPD. (1) Chlorsulfuron, (2) diuron, (3) carbaryl, (4) isoproturon, (5) chlortoluron, (6) omethoate, (7) atrazine, (8) simazine, (9) dimethoate, (10) alachlor, (11) terbutryn, (12) metalaxyl, (13) malathion, (14) dinobuton, (15) captan, (16) metamitron, (17) oxadixyl, (18) chloridazon, (19) azinphos methyl, (20) cypermethrin.

Table 8 shows the theoretical and experimental detection and quantitation limits, and the regression coefficients of the linear fittings in the mentioned linear dynamic range. The detection and quantitation limits of the procedure were calculated considering a signal-to-noise ratio of 3 or 10, respectively, a sample volume of 300 ml and a recovery of 100%. The theoretical limits were calculated by successive dilutions of a standard solution while the experimental limits were obtained by spiking extracts with the pesticides. As can be seen, the theoretical limits were lower than those obtained by spiking extracts. Detection limits varied between 1 and 370 ng/l. On the

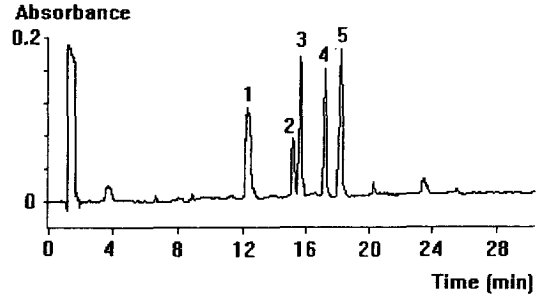

Fig. 3. Chromatogram of a spiked lough water sample obtained by the proposed procedure and HPLC–DAD at 220 nm. (1) Carbendazim, (2) carbaryl, (3) diuron, (4) chlortoluron, (5) isoproturon.

Table 8
Linear dynamic range, coefficient of regression and limits of detection and quantitation of the proposed procedure ($n=5$)

Pesticide	Linear dynamic range (mg/l)	Regression coefficient	Theoretical		Experimental	
			LOD (ng/l)	LOQ	LOD	LOQ
Alachlor	0.001–1.10	0.9997	1	3	2	3
Atrazine	0.001–1.25	0.9995	1	4	1	4
Azinphos methyl	0.075–1.50	0.9999	66	200	70	220
Captan	0.100–1.60	0.9983	71	310	90	390
Carbaryl	0.010–12.0	0.9999	10	22	16	30
Carbendazim	0.020–15.0	0.9998	16	41	28	62
Cypermethrin	0.150–2.00	0.9995	60	200	150	500
Chloridazon	0.050–1.20	0.9991	33	110	30	110
Chlortoluron	0.060–10.0	0.9998	13	39	50	160
Chlorsulfuron	0.030–1.60	0.9991	23	76	35	100
Dicofol	0.015–1.40	0.9995	2	10	3	11
Dimethoate	0.015–1.50	0.9999	1	3	2	5
Dinobuton	0.045–0.90	0.9998	43	145	58	165
Diuron	0.010–12.0	0.9996	2	9	3	9
Isoproturon	0.060–10.0	0.9998	13	40	61	200
Malathion	0.002–0.55	0.9997	1	4	1	5
Metalaxyl	0.200–2.20	0.9998	200	650	210	700
Metamitron	0.050–1.20	0.9996	25	95	30	110
Oxadixyl	0.300–2.80	0.9974	250	940	310	1020
Permethrin	0.300–2.10	0.9997	166	555	370	1240
Simazine	0.040–1.00	0.9999	30	100	30	100
Terbutryn	0.040–1.10	0.9998	20	95	30	110
Tetradifon	0.006–1.15	0.9999	5	10	5	14

LOD: Limit of detection.
LOQ: Limit of quantitation.

Table 9
Pesticides, concentration range and number of samples where they were found after analysis of forty lough water samples

Pesticide	Number of samples	Concentration range (μg/l)
Alachlor	3	<LOQ–0.10
Atrazine	15	0.01–1.20
Azinphos methyl	2	0.25–0.40
Carbaryl	4	<LOQ–0.20
Captan	4	<LOQ–0.65
Chloridazon	6	<LOQ–0.23
Chlortoluron	30	<LOQ–7.82
Chlorsulfuron	4	0.13–0.30
Cypermethrin	1	1.10
Dicofol	11	<LOQ–0.83
Dimethoate	3	<LOQ–0.14
Isoproturon	7	0.25–1.00
Malathion	5	<LOQ–0.05
Metamitron	6	<LOQ–0.34
Permethrin	10	<LOQ–2.17
Simazine	7	0.14–0.92
Terbutryn	10	0.06–0.75
Tetradifon	37	0.13–0.30

<LOQ: Concentration below quantitation limit.

other hand, the detection limits reached by the combined use of GC and HPLC were, at least, three times lower than those obtained by SFC [1], for those compounds analyzed by both methods. This was mainly motivated by the biggest concentration capacity of the ODS cartridges in relation to the minicartridge used in the SPE–SFC system.

3.4. Application to lough water samples

The analysis procedure has been applied to water samples from forty loughs in the province of Leon (Spain), collected in Autumn. Eighteen pesticides were found at very high concentrations, exceeding, in some cases, the value of 1 μg/l. Chlortoluron, and mainly tetradifon, were the most widely distributed compounds in the monitored loughs (Table 9).

The presence of many pesticides in the extracts was confirmed taking into account that those compounds were often monitored in more than one detector. So, for example, triazines quantified by NPD also supplied ECD and HPLC signals, organophosphorus quantified by NPD provided ECD signal, and phenylureas measured by HPLC exhibited signals in ECD and NPD. Fig. 4 shows the chromatograms obtained for the same extract in GC–ECD and HPLC–DAD; as can be seen, the presence of atrazine and chlortoluron is revealed in both chromatograms.

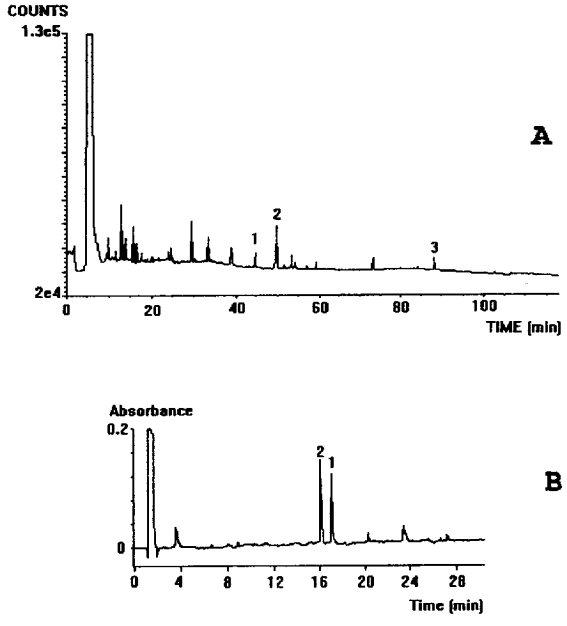

Fig. 4. Chromatogram of a lough water sample obtained by different detectors. (A) GC–ECD; (B) HPLC–DAD at 220 nm. (1) Chlortoluron, (2) atrazine, (3) tetradifon.

4. Conclusions

The combined use of GC and HPLC with conventional detectors makes possible the direct, reliable, efficient and economical determination of pesticides on water samples from small loughs, having some advantages in comparison with an on-line SPE–SFC system.

The optimization of the experimental variables that affect to the extraction–elution process is advisable in order to ensure high recoveries as consequence of the wide variability of properties of the potentially-present pesticides.

Eighteen pesticides have been detected in variable concentration among the twenty-three selected for this study on the basis of their wide use on the crops surrounding the loughs. Tetradifon and chlortoluron were found to be the most abundant pesticides in these samples.

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References

[1] J.L. Bernal, J.J. Jiménez, J.M[a]. Rivera, L. Toribio, M[a].J. del Nozal, J. Chromatogr. A 754 (1996) 145.
[2] G. Font, J. Mañes, J.C. Moltó, Y. Picó, J. Chromatogr. 642 (1993) 135.
[3] D. Barceló, J. Chromatogr. 643 (1993) 117.
[4] P. Parrilla, J.L. Martínez Vidal, M.M. Galera, A.G. French, Fresenius J. Anal. Chem. 350 (1994) 633.
[5] P.J.M. Kwakman, J.J. Vreuls, U.A.Th. Brinkman, R.T. Ghijsen, Chromatographia 34 (1992) 41.
[6] J.L. Bernal, M[a].J. del Nozal, J. Atienza, J.J. Jiménez, Chromatographia 33 (1992) 67.
[7] C. de la Colina, F. Sánchez Rasero, G.D. Cancela, E. Romero, A. Pena, Analyst 120 (1995) 1723.

[8] D. Barceló, J. Chromatogr. 643 (1993) 117.
[9] J. Schulein, A. Martens, P. Spitzauer, A. Kettup, Fresenius J. Anal. Chem. 352 (1995) 565.
[10] J.S. Salou, R. Alonso, G. Batllo, D. Barceló, Anal. Chim. Acta 293 (1994) 109.
[11] S. Chiron, S. Dupas, P. Scribe, D. Barceló, J. Chromatogr. A 665 (1994) 295.
[12] E.R. Brouwer, D.J. van Iperen, I. liska, H. Lingeman, U.A.Th. Brinkamn, Int. J. Environ. Anal. Chem. 42 (1992) 267.
[13] M.R. Driss, M.C. Hennion, M.C. Bouguerra, J. Chromatogr. 639 (1993) 352.
[14] C. Crespo, R.M. Marcé, F.J. Borrull, J. Chromatogr. A 670 (1994) 135.
[15] G. Font, J. Mañes, J.C. Moltó, Y. Picó, J. Chromatogr. 642 (1993) 135.
[16] Y. Picó, A.J.H. Louter, J.J. Vreuls, U.A.Th. Brinkman, Analyst 119 (1994) 2025.
[17] E.R. Brouwer, S. Kofman, U.A.Th. Brinkman, J. Chromatogr. A 703 (1995) 167.
[18] R. Hu, J.M. Berthion, I. Bordereau, J. Fournier, Chromatographia 43 (1996) 181.
[19] H.B. Lee, T.E. Peart, J.M. Carron, H.J. Tse, J. Assoc. Off. Anal. Chem. 74 (1991) 835.
[20] G. Durand, V. Bouvot, D. Barceló, J. Chromatogr. 607 (1992) 319.
[21] J. Velasco, M. Monteoliva, J. Bermudez, J. Romero, E. Hita, Toxicol. Environ. Chem. 43 (1994) 19.
[22] C. de la Colina, A. Peña, G.D. Cancela, F. Sánchez Rasero, J. Chromatogr. A 655 (1993) 127.
[23] Th. Heberer, S. Butz, H.J. Stan, Int. J. Environ. Anal. Chem. 58 (1995) 43.
[24] G.R. van der Hoff, F. Pelusio, U.A.Th. Brinkman, R.A. Baumann, P. van Zoonen, J. Chromatogr. A 719 (1996) 59.
[25] B. Nouri, B. Fouillet, G. Toussaint, P. Chambou, R. Chambou, Analyst 120 (1995) 1133.
[26] I. Liska, E.R. Brouwer, A.G.L. Ostheimer, H. Lingeman, U.A.Th. Brinkman, R.B. Geerdink, W.H. Mulder, Int. J. Environ. Anal. Chem. 42 (1992) 267.
[27] T.A. Albanis, D.G. Hela, J. Chromatogr. A 707 (1995) 283.