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Journal of Chromatography A, 754 (1996) 145–157

JOURNAL OF CHROMATOGRAPHY A

On-line solid-phase extraction coupled to supercritical fluid chromatography with diode array detection for the determination of pesticides in water

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Abstract

Supercritical fluid chromatography (SFC) with ten silica packed columns in series has been assayed for the multiresidue analysis of 184 pesticides in water samples. As the detection limits achieved by the electron-capture and diode array detectors were somehow high (0.1 mg/l), a solid-phase preconcentration coupled on-line with SFC was finally used to reduce these limits till 0.01 μg/l. Before applying this solid-phase extraction–SFC system, a study of the variables that influenced the extraction step was made. After that, several small lough-water samples were analyzed identifying and evaluating 16 pesticides.

Keywords: Water analysis; Environmental analysis; Sample preparation; Pesticides

1. Introduction

Multiresidue pesticide analysis in water samples is usually carried out by using capillary gas chromatography (cGC) and sensitive and selective detection methods such as electron-capture (ECD) and nitrogen–phosphorus detection (NPD). Nevertheless, using mass spectrometric detection allows more reliable identification [1,2]. In some cases, there are compounds whose GC detector response or thermal lability make it difficult to apply the GC system, in those situations, HPLC with UV detection is a good alternative, but with this last technique the number of compounds analyzed is lower and the analysis runtimes are usually higher than those from GC [3,4].

As the supercritical fluid chromatography (SFC) system theoretically combines the performance and speed of cGC with the selectivity of HPLC [5] and it also makes possible the study of thermally labile compounds using both GC and HPLC detectors, it is possible that this technique could be used in pesticide multiresidue analysis.

In relation to the application of SFC for pesticide analysis, only small groups of pesticides have been analyzed until now. Using SFC with capillary columns, organochlorine and sulphur-containing pesticides have been analyzed [6–8]. Nevertheless, due to the speed, capacity and efficiency achieved with packed columns, the number of applications using them is higher [9,10]. Pesticides such as atrazine and their metabolites, organochlorines, phenylureas and carbamates have been evaluated in this way [11–14].

To analyze pesticides at trace level in water samples, an extraction–concentration step is commonly carried out before analysis [3]. With the aim of reducing analysis time, the solvent consumption or the detection limits, there are systems devoted to

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0021-9673/96/$15.00
PII S0021-9673(96)00547-X

on-line clean up-HPLC or clean up-GC. Moreover this aspect has scarcely been treated in SFC [8,15].

In this work, a multiresidue procedure based on the use of SFC equipment with diode array detection (DAD) and ECD, and ten silica packed columns in series has been assayed. Retention data of 184 pesticides, including some thermally labile ones, from different families, are given. The possibility of using an on-line coupling between solid-phase extraction (SPE) and the SFC equipment for multiresidue pesticide analysis in water samples has also been considered in this work, studying aspects such as the extraction on two types of sorbents and the breakthrough volume. Finally, the proposed procedure was applied to 40 water samples from loughs, in which 16 pesticides were found; then, a new system with seven columns in series was also optimized for this new situation.

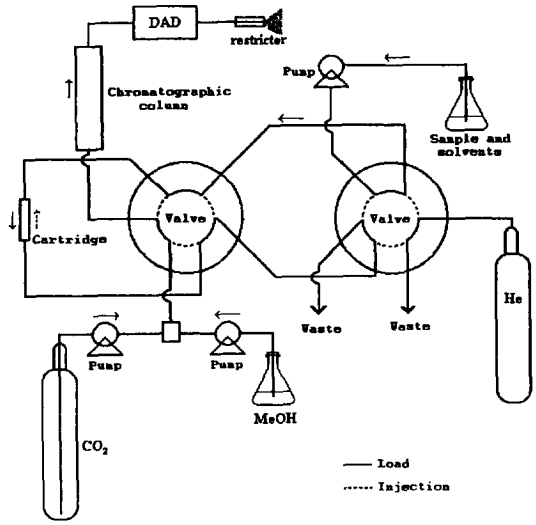

Fig. 1. Extraction–preconcentration system scheme.

2. Experimental

2.1. Chemicals

Chromatographic purity standards were supplied by Promochem (Wesel, Germany), Chemservice (West Chester, PA, USA) and Riedel de Haën (Hannover, Germany). HPLC-grade methanol was provided by Lab-Scan (Dublin, Ireland). Ultrapure water was obtained from a Milli-Q apparatus from Waters (Bedford, MA, USA). Carbon dioxide (99.999% purity) was purchased from Air Products (Sombreffe, Belgium), and helium (99.999% purity) from Carburos Metálicos (Barcelona, Spain)

For water sample preparation, disposable PTFE syringe filter units, 0.45 μm pore size, were obtained from Microfiltration Systems (Dublin, CA, USA).

2.2. Supercritical fluid chromatograph

SFC was performed with a Hewlett-Packard (Wilmington, DE, USA) G1205A supercritical fluid chromatograph equipped with an HP 7673A autosampler and a 7410 Rheodyne (Cotati, CA, USA) valve (5 μl loop). Two detection systems were used in parallel, an electron-capture detector and an HP1050 diode array detector, from these data it was found that 220 nm was a wavelength where most of the pesticides showed a good absorption, so this value was selected with the aim of making further comparisons between quantitative data. The split ratio of the column effluent was 200:1 to the ECD system. Chromatographic data were collected by means of an HP-SFC 3365 ChemStation.

For the multiresidue analysis, ten standard 200× 4.6 mm I.D. silica Hypersil columns (5 μm particle size) from Hewlett-Packard were coupled in series by means of low-dead-volume connectors from the same manufacturer. The initial pressure, 100 bar, was held for 30 min and then programmed to increase at 5 bar/min to 150 bar, being held for 30 min. The organic modifier (methanol) gradient profile was initially 2%, held for 5 min, and then programmed to increase at 0.5%/min to 10%. The chromatographic columns were equilibrated for 15 min with the initial pressure and mobile phase composition. The mobile phase flow-rate was 1.5 ml/min and the oven temperature was set at 40 °C. The ECD temperature was maintained at 300 °C; nitrogen was used as auxiliary gas.

When real water samples were analyzed only 16 pesticides were found by the multiresidue method, so the conditions of the system were changed trying to optimize the procedure. Seven packed columns, also coupled in series, and a new methanol gradient were used: initially 3%, held for 2 min, then a 0.3%/min

Table 1
Retention times of 184 pesticides and related compounds obtained in the SFC system

Pesticide	Family	Retention time (min)
Tepp	Organophosphorus	14.10
trans-Heptachlor	Organochlorine	14.85
1,2-Dichloropropene	Organochlorine	14.90
Difenzoquat methyl	Pyrazole	14.90
Imazamethabenz methyl	Imidazoline	14.91
Daminozide	Hydrazide	14.94
Thiram	Dithiocarbamate	15.14
Propamocarb	Carbamate	15.15
Flamprop methyl	Aminopropionate	15.30
HCB	Organochlorine	15.43
Biphenyl	Biphenyl	15.64
Amitrole	Triazole	15.79
Trifluralin	Dinitroaniline	16.34
Pentachlornitrobenz.	Organochlorine	16.41
trans-Nonachlor	Organochlorine	16.43
Butylate	Thiocarbamate	16.76
4,4′-DDE	Organochlorine	16.86
Vernolate	Thiocarbamate	17.43
Terbufos	Organophosphorus	17.49
cis-Heptachlor	organochlorine	17.58
Phorate	Organophosphorus	17.59
Aldrin	Organochlorine	17.60
Dinobuton	Nitrocompound	17.63
EPTC	Carbamate	17.64
Diallate	Carbamate	17.75
Pebulate	Thiocarbamate	17.84
Dinoseb	Nitrocompound	17.91
Endosulfan A	Organochlorine	18.02
Triallate	Carbamete	18.07
Vinclozolin	Oxazolidine	18.18
Chloridazon	Pyridazine	18.30
Chlorpyrifos	Organophosphorus	18.31
Thiometon	Organophosphorus	18.53
Fenclorfos	Organophosphorus	18.55
Chlorbensid	Organochlorine	18.59
2,4-D	Fenoxyacid	18.60
Chlorpirifos methyl	Organophosphorus	18.60
Bromoxynil octanoate	Nitrile	18.61
β-HCH	Organochlorine	18.70
Heptachlor epoxide	Organochlorine	18.70
Endrin aldehyde	Organochlorine	18.79
cis-Nonachlor	Organochlorine	18.89
4,4′-DDT	Organochlorine	19.15
Chlortal dimethyl	Phtalate	19.16
α-HCH	Organochlorine	19.20
2,4′-DDE	Organochlorine	19.24
γ-Chlordane	Organochlorine	19.26
Pentachlorophenol	Organochlorine	19.40
Cycloate	Thiocarbamate	19.47
2,4′-DDT	Organochlorine	19.70
α-Chlordane	Organochlorine	19.72
Sulfallate	Carbamate	19.73
Parathion ethyl	Organophosphorus	19.82

(*Continued on p. 148*)

Table 1 (*continued*)

Pesticide	Family	Retention time (min)
Carbofenotion	Organophosphorus	19.86
Paraquat dichloride	Bipyridylium	19.95
4,4′-TDE	Organochlorine	20.07
Chlorfenson	Organochlorine	20.26
Fenoprop	Phenoxyacid	20.34
Fenthion	Organophosphorus	20.39
Dieldrin	Organochlorine	20.67
Chlordimeform	Formamidine	20.71
Fenitrothion	Organophosphorus	21.01
2,4′-TDE	Organochlorine	21.19
Ethion	Organophosphorus	21.24
Lindane	Organochlorine	21.30
Picloram	Pyridine	21.51
Parathion methyl	Organophosphorus	21.71
Molinate	Thiocarbamate	21.80
Iprodione	Dicarboximide	21.83
Tetradifon	Organochlorine	21.94
Endrin	Organochlorine	22.19
Phenthoate	Organophosphorus	22.22
Dinitro-*o*-cresol	Nitrocompound	22.26
Cypermethrin	Pyrethroid	22.70
Malathion	Organophosphorus	22.71
Propham	Carbamate	22.80
Fenpropathrin	Pyrethroid	22.86
Fenvalerate	Pyrethroid	23.09
Procymidone	Dicarboximide	24.00
Dichlobenil	Nitrile	24.86
Methoxychlor	Organochlorine	25.52
Bromopropylate	Bridged diphenyl	25.84
Chlorpropham	Carbamate	26.38
Quinalphos	Organophosphorus	26.85
Anilazine	Triazine	26.96
Alachlor	Acetamide	27.50
Pirimicarb	Carbamate	27.91
Imazalil	Imidazole	28.05
Piperonyl butoxide	Benzodioxole	28.27
δ-HCH	Organochlorine	28.30
Methidathion	Organophosphorus	28.43
Pirimiphos methyl	Organophosphorus	28.46
Phosalone	Organophosphorus	28.55
Dichlofluanid	Sulphamide	28.63
Dichlorvos	Organophosphorus	28.89
Endosulfan B	Organochlorine	29.00
Diazinon	Organophosphorus	29.07
Ethoprophos	Organophosphorus	29.41
Pyrazophos	Organophosphorus	29.44
2,4-Dichlorophenol	Organochlorine	29.58
Propachlor	Acetamide	29.76
Dibrom	Organophosphorus	30.00
Coumarin	Coumarin	30.81
Folpet	Phtalimide	30.86
Dialifos	Organophosphorus	31.14
Flamprop isopropyl	Aminopropionate	31.15

Table 1 (*continued*)

Pesticide	Family	Retention time (min)
Trimethacarb	Carbamate	31.16
Propazine	Triazine	31.28
Dicofol	Organochlorine	31.48
Benalaxyl	Acylaniline	31.62
Triadimefon	Triazole	31.94
Terbuthylazine	Triazine	32.04
Chlorfenvinfos	Organophosphorus	32.23
Fentin hydroxide	Organotin	32.41
Tetrachlorvinphos	Organophosphorus	32.58
2,4,5-Trichlorophenol	Organochlorine	32.71
Dicloran	Nitroaniline	32.91
Demeton-*S*-methyl	Organophosphorus	33.05
Captan	Phthalimide	33.18
Atrazine	Triazine	33.18
Phosmet	Organophosphorus	33.24
Cymoxanil	Urea	34.19
Coumaphos	Organophosphorus	34.57
Azinphos ethyl	Triazine	34.96
Aldicarb	Carbamate	35.05
Carbonalate	Carbamate	35.14
Propoxur	Carbamate	35.49
Metobromuron	Urea	35.54
Simazine	Triazine	35.72
Captafol	Phtalimide	36.00
Azinphos methyl	Triazine	36.11
Napropamide	Amide	36.72
Pirimiphos ethyl	Organophosphorus	36.75
Metalaxyl	Acylalanine	36.84
Methiocarb	Carbamate	37.09
Linuron	Urea	37.47
Triazophos	Organophosphorus	37.56
Carbofuran	Carbamate	37.77
Cyanizine	Triazine	37.86
Chlorbromuron	Urea	38.17
Terbutryn	Triazine	38.83
Tribenuron-methyl	Sulfophenylurea	39.18
Terbacil	Uracil	39.19
Mevinphos	Organophosphorus	39.29
Amitraz	Triazapentadiene	39.60
Fenamiphos	Organophosphorus	39.73
Bentazone	Benzothiadiazole	40.72
Bromacil	Uracil	40.87
Phosphamidon	Organophosphorus	41.54
Carbaryl	Carbamate	41.93
Trichlorfon	Organochlorine	44.00
Triadimenol	Triazole	44.16
Nuarimol	Pyrimidine	44.65
Propanil	Acetamida	45.72
Fensulfothion	Organophosphorus	45.91
Neburon	Urea	46.30
Oxadixyl	Oxazolidine	46.62
Siduron	Urea	46.71
Permethrin	Pyrethroid	47.20

(*Continued on p. 150*)

Table 1 (*continued*)

Pesticide	Family	Retention time (min)
Dimethoate	Organophosphorus	47.22
Isoproturon	Urea	48.77
Desmediphan	Carbamate	49.72
Fluometuron	Urea	50.47
Prometryn	Triazine	50.59
Carbendazim	Benzimidazole	50.90
Warfarin	Coumarin	50.97
Thiabendazole	Benzimidazole	51.31
Cyhexatin	Organotin	51.34
Thiophanate	Carbamate	51.05
Monocrotophos	Organophosphorus	51.45
Prochloraz	Imidazoline	51.47
Diuron	Urea	51.50
Phenmedipham	Carbamate	52.45
Coumachlor	Coumarin	52.74
Chlorsulfuron	Sulfophenylurea	52.90
Fenuron	Urea	53.06
Acephate	Organophosphorus	54.05
Triamifos	Organophosphorus	54.11
Omethoate	Organophosphorus	55.46
Chlorotoluron	Urea	56.22
Strychnine	Alkaloid	57.95
Monuron	Urea	59.65
Metamitron	Triazine	62.13
Ethylenethiourea	Carbamate	62.62
Chloroxuron	Urea	65.45

ramp to 7%, held for 15 min, and finally, a 10%/min ramp to 15%.

2.3. On-line extraction–preconcentration system

Water samples were spiked with the pesticides and analyzed by a solid-phase extraction–preconcentration system on-line with the SFC equipment. The pesticide enrichment was performed by two six-port rotary valves (Rheodyne) connected in series to enable the different steps of the preconcentration process: conditioning and activation of the packed sorbent, retention of the pesticides, dryness of the sorbent and elution of compounds. The 10×3 mm I.D. holder was obtained from the Free University (Amsterdam, Netherlands) and the sorbents were 10 μm Spherisorb ODS-2 from Tecnokroma (Barcelona, Spain) and 20 μm PLPR-S from Polymer Laboratories (Shropshire, UK). The sorbent weight contained in the holder was 20 mg. An Eldex/Waters (Milford, MA, USA) pump was used to deliver the sample and the conditioning solutions through the cartridge.

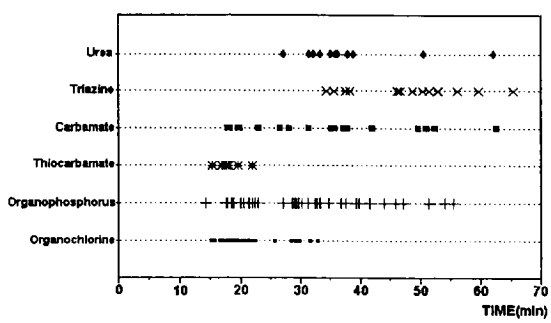

Fig. 2. Distribution of pesticide retention times according to their chemical families.

The scheme of the equipment used is shown in Fig. 1, the operation sequence is as follows. Firstly, the preconcentration system was rinsed with 5 ml of methanol to remove all solvents in the tubing. Then, the cartridge was cleaned and conditioned with 10 ml of methanol. Next, the tubing was rinsed with 5 ml

Table 2
Linearity, detection and quantitation limits obtained at 220 nm for 16 pesticides in the SFC–DAD system

Peak[a]	Pesticide	Retention time (min)	L.D.R. (mg/l)	r	Detection limit (mg/l)	Quantitation limit (mg/l)
1	Tetradifon	16.56	1.0–60.9	0.9964	0.25	0.80
2	Procymidone	18.11	1.5–50.9	0.9954	0.30	1.10
3	Dichlofluanid	20.06	1.5–49.8	0.9985	0.40	1.20
4	Dicofol	22.21	2.0–56.6	0.9936	0.40	1.30
5	Benalaxyl	22.59	1.5–46.0	0.9950	0.30	1.00
6	Atrazine	23.76	1.0–46.4	0.9983	0.09	0.30
7	Methiocarb	27.05	1.5–47.8	0.9997	0.25	0.80
8	Simazine	27.58	1.0–30.0	0.9968	0.15	0.50
9	Carbaryl	33.25	1.0–49.9	0.9948	0.08	0.20
10	Nuarimol	34.94	2.0–66.4	0.9977	0.50	1.50
11	Triadimenol	40.14	2.0–39.9	0.9984	0.50	1.60
12	Isoproturon	43.77	1.0–36.8	0.9991	0.20	0.60
13	Chlorsulfuron	44.41	2.0–45.0	0.9959	0.40	1.50
14	Chlorotoluron	45.08	1.0–38.0	0.9985	0.20	0.75
15	Diuron	47.34	1.2–52.8	0.9985	0.30	1.10
16	Metamitron	50.18	2.0–62.6	0.9960	0.30	0.90

Loop volume = 5 μl. See Section 2 for experimental conditions.
[a] Identification of the pesticides in the Figs. 2, 4 and 5.
L.D.R. = linear dynamic range. r = coefficient of regression.

of ultrapure water and the sorbent was conditioned with 10 ml of the same water. Later, the tubing was rinsed with 5 ml of the water sample, and the target water volume to analyze was preconcentrated. Then the cartridge was dried with 5 bar helium for at least 5 min, after drying the tubing. The pesticides trapped on the cartridge were desorbed in the backflush mode and transferred to the analytical column on-line.

3. Results and discussion

3.1. Multiresidue system

Table 1 shows the retention times of the 184 compounds studied in the SFC system. The reproducibility of these times was generally in the ±0.03 min range. The chromatographic conditions used

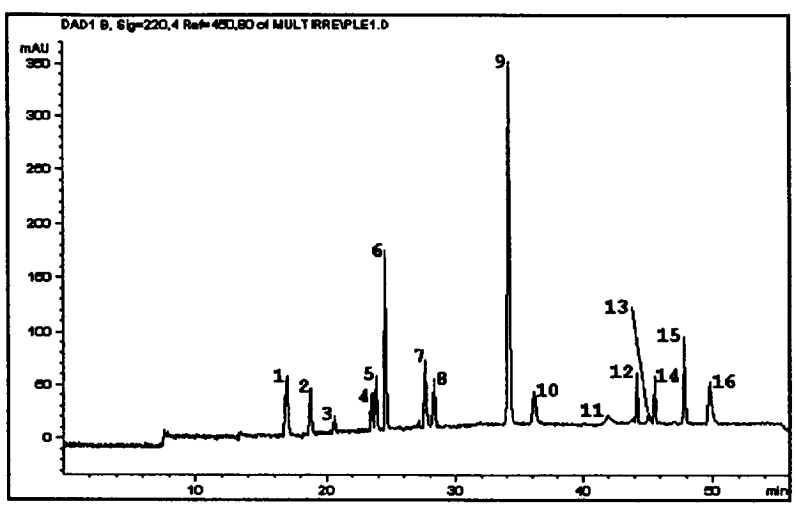

Fig. 3. Chromatogram obtained at 220 nm for a standard solution of pesticides. Concentration 50 mg/l. See Table 2 for peak identification.

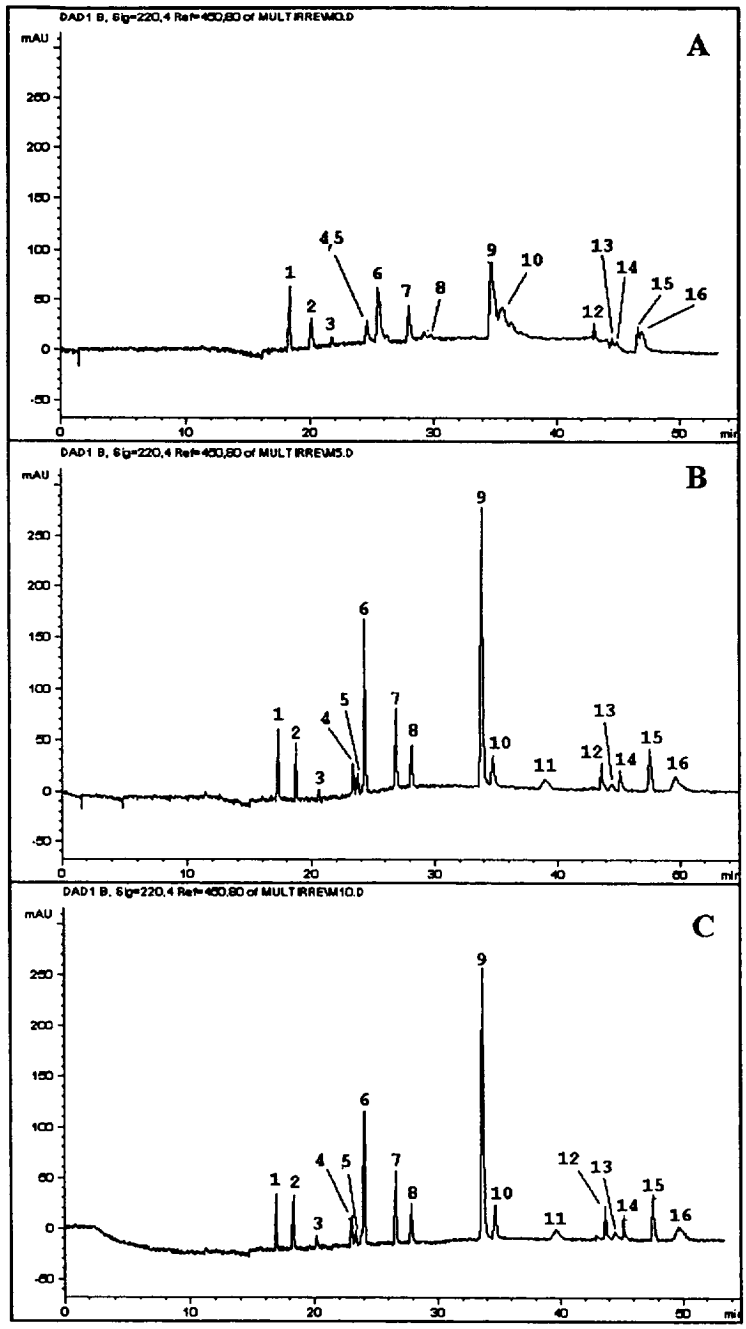

Fig. 4. Chromatograms obtained at 220 nm for a 2 ml water sample containing 0.25 μg of each pesticide, using the on-line preconcentration–SFC system after different drying times: (A) No drying time; (B) Drying time, 5 min; (C) Drying time, 10 min.

were chosen after carrying out previous assays; these assays were conducted to achieve a good chromatographic isolation for the most of the pesticides in the lowest run-time possible. The oven temperature and mobile phase flow-rate were found to be the least important factors. A 40 °C temperature, suitable for

thermally labile compounds, and 1.5 ml/min flow-rate were selected as the best ones. Increasing the temperature, a slight decrease in the retention time of the pesticides was produced, whereas a reduction in the flow-rate slightly enhanced the resolution between some compounds, but lower flow-rates increase considerably the chromatographic run-time.

The CO_2 pressure and the organic modifier percentage were the factors that affected the separation in an important way. The time-programme for these variables was selected taking into account the following considerations: in the beginning their values ought to be weak enough to allow a better separation of compounds with low polarity, then the solvent strength of the mobile phase was increased, trying to reduce the retention times of the most polar compounds.

A distribution of the retention times on the basis of the pesticide families is shown in Fig. 2. As can be seen, the pesticides of the same family elute within a time range in some cases. Organochlorine compounds eluted between 12 and 30 min, triazines between 26 and 40 min, except for metamitron (triazinone), ureas between 34 and 68 min, and thiocarbamates between 15 and 22 min. On the other hand, carbamate and organophosphorus pesticides were eluted throughout the chromatogram. That could be due to the presence of heterogeneous substituents, such as halogens, sulphur, aromatic rings, alkyl groups on those pesticides. From the overall observation of the retention times, it can be pointed out that there is a certain parallelism between the analyte retention in SFC with silica columns and the normal-phase in HPLC.

DAD is more suitable than ECD for the multiresidue analysis as all pesticides absorb in the UV spectral region, whereas ECD was more selective for a screening of organohalogenated compounds. To distinguish between compounds with very similar retention times, it is advisable to use the comparison of their spectra with the other ones from the standards. It is also useful to check the peak purity and to know their ECD response as well.

3.2. Linearity: detection and quantitation limits

For the determination of the 16 pesticides found in water samples after an off-line SPE–SFC analysis, the silica column number was reduced from ten to seven. The methanol percentage gradient was also modified to achieve a better pair-peak resolution and performance. At the same time, a 15% methanol percentage at the end-programme was found to be

Table 3
Recovery and precision obtained in the analysis of pesticides (0.25 μg of each one) from 2 ml of water by the on-line preconcentration–SFC system with cartridges of ODS-2 and PLPR-S ($n=5$)

Pesticide	ODS-2		PLPR-S	
	Recovery(%)	Standard deviation	Recovery(%)	Standard deviation
Tetradifon	73.8	4.5	48.9	6.0
Procymidone	97.5	4.2	71.3	6.0
Dichlofluanid	99.0	2.1	57.7	5.3
Dicofol	83.4	4.3	81.0	8.7
Benalaxyl	87.6	6.1	77.5	7.1
Atrazine	91.5	4.2	79.2	6.3
Methiocarb	95.7	7.9	77.2	8.7
Simazine	103.6	6.8	70.7	4.4
Carbaryl	98.8	3.3	59.9	4.6
Nuarimol	100.8	8.5	70.3	9.4
Triadimenol	96.0	7.6	43.1	8.0
Isoproturon	96.8	6.5	70.5	7.0
Chlorsulfuron	95.6	6.1	67.1	6.7
Chlortoluron	89.5	4.9	81.6	5.3
Diuron	100.6	3.7	75.9	6.9
Metamitron	88.2	3.6	76.7	4.9

Table 4
Recovery (%) of pesticides (0.25 μg each one) obtained by the on-line preconcentration–SFC system with ODS-2 cartridges for different water volumes ($n=3$)

Pesticide	Volume (ml)				
	20	25	30	50	100
Tetradifon	76.3	78.6	77.7	75.8	71.9
Procymidone	98.2	99.0	96.0	92.6	79.0
Dichlofluanid	99.4	102.7	100.4	95.7	75.8
Dicofol	69.8	69.5	70.4	74.7	73.2
Benalaxyl	85.8	87.5	84.5	85.5	85.8
Atrazine	93.3	90.1	84.3	82.6	86.7
Methiocarb	89.3	90.2	87.0	82.5	76.0
Simazine	89.5	90.4	91.1	90.5	91.7
Carbaryl	87.7	86.3	81.8	77.4	72.8
Nuarimol	99.2	97.0	103.9	101.2	99.2
Triadimenol	95.1	102.7	99.3	71.3	20.0
Isoproturon	86.1	85.9	84.0	82.6	82.8
Chlorsulfuron	102.0	95.1	93.8	70.1	42.9
Chlortoluron	82.3	83.1	82.8	81.9	83.4
Diuron	91.7	90.8	87.5	81.5	70.9
Metamitron	69.1	71.3	69.4	62.7	10.9

adequate to remove interferences due to co-extracted substances. Table 2 gives the retention times of the 16 pesticides in the new chromatographic conditions and Fig. 3 shows a chromatogram of a standard mixture in methanol.

The coefficient of regression for the linear fitting, the considered linear dynamic range (L.D.R.) and the detection and quantitation limits of the 16 pesticides are also given in Table 2. For this purpose, a 5 μl sample volume was injected and the chromatographic peak area was quantified. The coefficient of regression (r) was higher than 0.99 for all the pesticides as deduced from the three calibrations made in the L.D.R. considered. The detection and quantitation limits were calculated as a signal-to-noise ratio of 3 and 10, respectively, and they are the average of seven determinations. The detection limits varied from about 0.1 to 0.5 mg/l, and the quantitation ones varied from about 0.2 to 1.5 mg/l. These values are high as consequence of the background inherent to the SFC system; the injection of a higher sample volume is not advisable owing to the peak-shape deformation observed with large injection volumes [15].

The detection limit of the organohalogenated compounds, mainly tetradifon and dicofol, can be lowered by the use of ECD. However, the ECD quantitative response reproducibility is small, so the reliable designation of detection and quantitation limits and the dynamic range are unfeasible. The lack of reproducibility is attributed to the pressure and the mobile phase composition changes which

Table 5
Linearity, detection and quantitation limits obtained at 220 nm for 16 pesticides in the on-line preconcentration–SFC system

Pesticide	L.D.R. (μg/l)	r	Detection limit (μg/l)	Quantitation limit (μg/l)
Tetradifon	0.5–10.0	0.9984	0.03	0.08
Procymidone	0.5– 8.4	0.9990	0.02	0.08
Dichlofluanid	1.0–10.0	0.9985	0.1	0.30
Dicofol	1.0–11.1	0.9917	0.06	0.20
Benalaxyl	1.0– 9.3	0.9952	0.05	0.20
Atrazine	0.5– 7.7	0.9982	0.01	0.05
Methiocarb	0.5– 7.9	0.9955	0.03	0.10
Simazine	0.5–10.5	0.9974	0.03	0.09
Carbaryl	0.5– 8.2	0.9984	0.009	0.03
Nuarimol	1.0–11.0	0.9977	0.07	0.20
Triadimenol	1.0– 8.3	0.9977	0.08	0.30
Isoproturon	1.0–10.0	0.9951	0.07	0.30
Chlorsulfuron	1.0– 7.5	0.9969	0.13	0.30
Chlortoluron	1.5– 9.0	0.9980	0.07	0.20
Diuron	1.0– 8.7	0.9973	0.04	0.10
Metamitron	1.0–12.9	0.9991	0.17	0.40

See Section 2 for experimental conditions. Cartridge: ODS-2. Water volume: 30 ml.
L.D.R. = linear dynamic range. r = coefficient of regression.

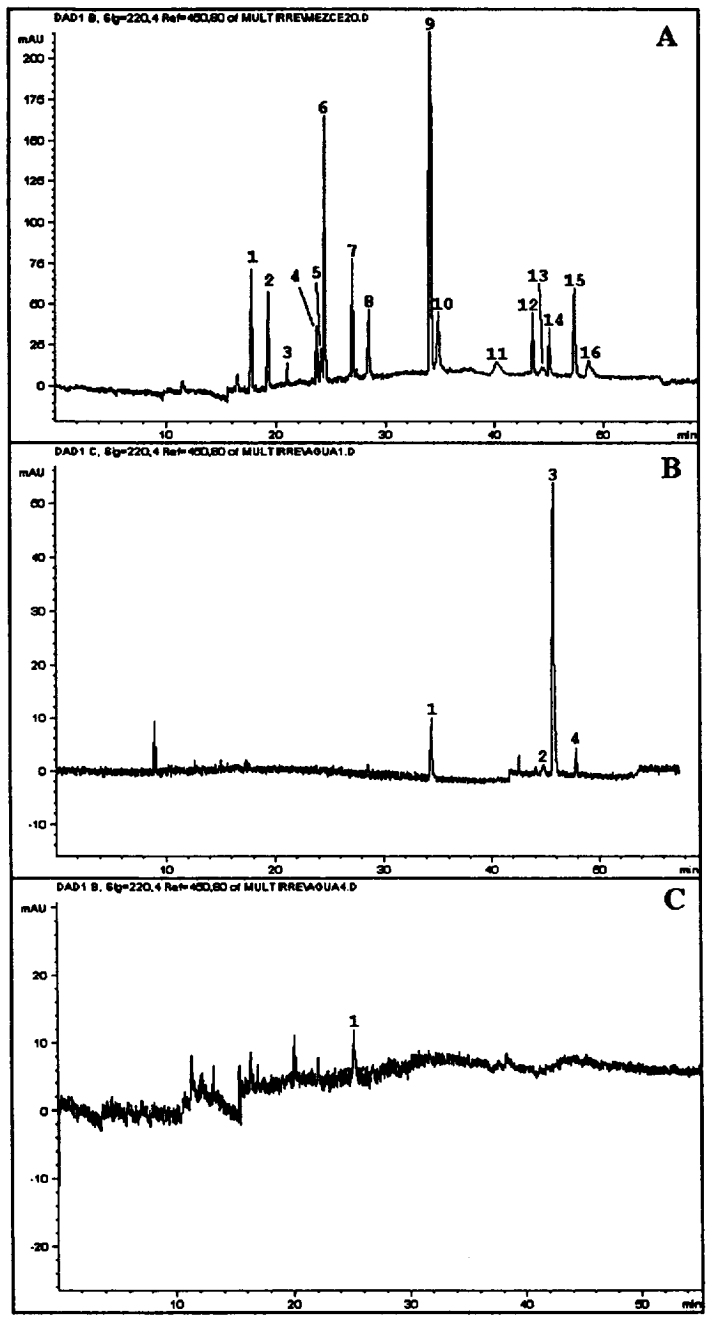

Fig. 5. Chromatograms obtained using the on-line preconcentration–SFC system. (A) Pesticide mixture in methanol. See Table 2 for peak identification; (B) Lough-water sample, 1. Carbaryl, 2. Chlorsulfuron, 3. Chlorotoluron, 4. Diuron; (C) Lough-water sample, 1. Methiocarb.

affect the split-ratio. Moreover, the obstruction of the ECD system is frequent. The use of ECD must be limited to confirm the identity of some compounds when the UV spectrum does not provide an unequivocal identification. So, the reliable identification of the pesticides at low concentration was difficult

owing to the background noise that affected the UV spectra.

The preconcentration factor achieved by the extraction procedures commonly used for water sample analysis is 1000. Thus, the final detection limit was about 0.1–0.5 μg/l for each pesticide. With the aim of decreasing the detection limits and increasing the water analysis speed, the 16 pesticides were also determined by a SPE system coupled on-line with the SFC equipment, and the quantitative features of the on-line system were studied.

3.3. Solid-phase preconcentration–extraction coupled on-line with SFC

The above-mentioned operational procedure of the on-line SPE–SFC system was established after studying some influencing parameters. From the steps involved, such as tubing purge and conditioning of the cartridge, the drying time was likely to be the most important. Fig. 4 shows the chromatograms obtained for 2 ml of ultrapure water containing 0.25 μg of each pesticide, after applying different drying times with helium at 5 bar. A 5 min drying time is enough to achieve no distorted chromatographic peaks.

Two sorbents, ODS-2 and PLPR-S, were assayed to extract the 16 pesticides from water samples. Table 3 shows the recovery rate of the 16 pesticides and the precision of the analysis procedure (expressed as standard deviation) for both sorbents. The experiments were performed on 2 ml of water spiked with 0.25 μg of each compound. A calibration in mass obtained by direct injection of standards was used for the determination of the recoveries. The recoveries of pesticides on ODS-2 were higher than those obtained on PLPR-S in all cases. Only the recoveries of atrazine and dicofol on the two sorbents were comparable. With regards to the precision and in general terms, the recoveries provided by the ODS-2 cartridges had a somewhat lower standard deviation in comparison with the data obtained by the PLPR-S ones.

The results of a study about the breakthrough volume on ODS-2 cartridges are shown in Table 4. For this purpose, 0.25 μg of each pesticide were added to different water volumes. The results are different depending on the compound; however, a fair decrease in the recovery of some pesticides can be seen when the sample volume is higher than 30 ml. If the results are compared with the recoveries given in Table 3 it is stated that the recoveries from only 2 ml of water are notably better for most of them.

Data arising from our calibration experiences for the monitoring of pesticides in lough waters were used to make the Table 5, which shows the linearity, detection and quantitation limits reached in the on-line SPE–SFC system, by using ODS-2 cartridges, 30 ml of water and detection at 220 nm. The shown data are the average of 14 calibrations. The regression coefficients of the linear fittings were always higher than 0.99, as in the direct injection, for a dynamic range from about 0.5 to 13 μg/l. The detection limits varied between 0.01 and 0.17 μg/l for each pesticide whereas the quantitation limit was about 0.08–0.37 μg/l, with no significant differences in the inter or intra-day variation when the quality of the consumables was maintained. To assure good reproducibility it is very important to keep the cartridges in methanol after they have already been used. With the same cartridge and under those conditions, 20 consecutive extractions of lough-water samples can be made. The limits of some pesticides could be slightly lowered by monitoring on another wavelength. For example, this is the case for the phenylureas chlorotoluron, isoproturon and diuron, which can be analyzed at 245 nm. So, the linearity data were similar at both wavelengths. The preconcentration step of the on-line SPE–SFC system enables better detection limits on water samples than the off-line SPE–SFC procedure. However, it should be stated that the retention time reproducibility in this on-line preconcentration is worse (±0.5 min) in relation to the reproducibility obtained in direct injection, which could make the identification difficult, particularly at trace level.

Fig. 5 shows some chromatograms obtained with the preconcentration on-line system. As can be seen, the application of this system in the proposed conditions to environmental water samples provides simple chromatograms; no interferences arising from the matrix were observed.

4. Conclusions

The use of SFC equipment with ten silica packed columns coupled in series allows information to be obtained about the occurrence of 184 pesticides in a time no longer than 70 min.

ECD presents serious difficulties in its application to SFC; its use must be limited to confirm the identity of those not identifiable by DAD. The detection limits are higher than 0.1 mg/l.

A sample preconcentration is necessary to achieve more appropriate detection limits for the pesticide levels often found in water. The on-line coupling between SPE and SFC makes possible the simple and quick analysis of waters containing pesticide concentrations below 1 μg/l.

The extraction cartridges can be used for at least 20 consecutive samples, keeping them in methanol between runs. The drying step after loading the sample is very important as regards the reproducibility.

Acknowledgments

This work was sponsored by the Domingo Martinez Foundation (Spain).

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