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Packed-column supercritical fluid chromatography coupled with solid-phase extraction for the determination of organic microcontaminants in water

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Abstract

A multiresidue method for the analysis of 35 common contaminants (including pesticides, polycyclic aromatic hydrocarbons and phenols) in lough and river waters from Castilla y León Spanish region, by using supercritical fluid chromatography (SFC) with five silica packed columns, is described. In order to decrease the detection limits, a preconcentration step by coupling solid-phase extraction to the SFC system was used. The different variables affecting the extraction procedure were studied and optimized, selecting the Isolute Env+ as the best sorbent. The detection limits achieved ranged from 0.4 to 2.6 $\mu g\,l^{-1}$.

Keywords: Water analysis; Environmental analysis; Solid-phase extraction; Pesticides; Polynuclear aromatic hydrocarbons; Phenols

1. Introduction

Multiresidue methods for water pollution control are gaining acceptance in the last few years because they obtain the most possible information in the shortest analysis time. Usually the compounds are present at trace levels, so the determination of their residues requires different preconcentration or clean-up procedures in order to reduce the detection limits of methods and/or to avoid interference of the matrix.

Traditionally, analyses of contaminants in waters have been performed by using capillary gas chromatography (cGC) with sensitive and selective detection methods [1–7] such as electron-capture (ECD), nitrogen-phosphorus (NPD) or mass spectrometric (MS) detection. High-performance liquid chromatography (HPLC) with UV, fluorescence or electrochemical detectors has also been used mainly to analyse polar compounds [8–14].

Packed-column supercritical fluid chromatography (pSFC) is nowadays competitive with HPLC and GC since it combines the speed and efficiency of GC and the wider selectivity adjustment of HPLC, making possible the analysis of polar and thermolabile compounds. Several papers concerning the use of solid-phase extraction (SPE) coupled to SFC for the analysis of contaminants in waters have been published in the last few years, when they focused on a single family they analysed pesticides [15–17], phenols [18,19], polycyclic aromatic hydrocarbons (PAHs) [20] or when mixtures of families were analysed only pesticides were included [21,22].

The aim of this work was to study the capabilities

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that SPE on-line coupled to pSFC has in the multiresidue analysis of a mixture of organic microcontaminants at trace level in water, including some of the most common pesticides found in Castilla y León region, phenols and PAHs.

2. Experimental

2.1. Reagents and standard

Standard of the compounds studied were supplied by Promochem (Wesel, Germany) and Sigma Aldrich (Madrid, Spain). Methanol was HPLC grade and provided by Lab-Scan (Dublin, Ireland). Stock solutions (100 mg l^{-1}) and the working solutions for direct injection were prepared in methanol in order to avoid problems of miscibility with the mobile phase. Working solutions for extraction experiments were prepared daily or weekly by diluting the stock solutions with deionized Milli-Q quality water (Millipore, Bedford, MA, USA). Carbon dioxide (SFC grade) and helium (99.999% purity) were obtained from Carburos Metálicos (Barcelona, Spain).

2.2. Apparatus and chromatographic conditions

SFC experiments were performed by using a G1205A model supercritical fluid chromatograph from Hewlett-Packard (Palo Alto, CA, USA), equipped with a diode array detection (DAD) system (HP1050) and a 7410 Rheodyne (Cotati, CA, USA) valve (5-μl loop volume).

Five 200×4.6 mm Hypersil silica columns, from Hewlett-Packard, were coupled in series for the contaminants separation. The modifier (methanol) percentage was varied from 2% (5 min) to 10% (29 min) at 0.5%/min. The initial pressure, 100 bar, was held for 15 min and then programmed to increase at 5 bar/min to 150 bar being held for 25 min. The columns were equilibrated for 20 min with the initial conditions. A constant flow-rate of 1.5 ml/min and a temperature of 40°C were used. For single wavelength monitoring, which was used to calculate all data, the detection was set at the optimum wavelength for each compound studied (see Table 1). The spectra were recorded from 190 to 350 nm.

2.3. Solid-phase extraction process

The on-line trace enrichment experiments were performed by using two six-port rotatory valves (Rheodyne) connected in series [16] in order to make possible the different steps of the preconcentration process: conditioning and activation of the sorbent, retention of the analytes, drying of the sorbent and elution of the compounds. The sorbents tested were 40–63 μm LiChrolut RP-18, 40–63 μm LiChrolut EN both from Merck (Barcelona, Spain), 70–100 μm Isolute Env+ from IST (International Solvent Technology, Mid Glamorgan, UK), 20 μm PLRP-S from Polymer Labs. (Shorpshire, UK) and Envchrom P from Supelco (Bellefonte, PA, USA). These sorbents were packed into a 10×3 mm I.D. precolumn purchased from the Free University (Amsterdam, Netherlands). An Eldex pump from Waters (Millford, MA, USA) was used to deliver the sample and the conditioning solutions (10 ml methanol were used to clean the system and the sorbent and 10 ml of deionized water to activate the sorbent).

3. Results and discussion

The chromatographic conditions, including the number of columns, were chosen according to our previous experience [16] trying to obtain a good chromatographic resolution between the most of the compounds in the shortest analysis time (Fig. 1). The retention times and detection limits obtained are shown in Table 1. The compounds were eluted in

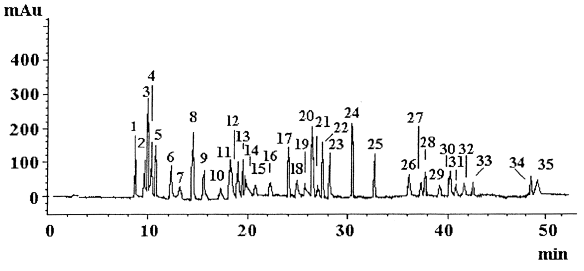

Fig. 1. Chromatogram of an 80 mg l^{-1} mixture of the compounds studied without using the SPE step. See Table 1 for peak identification and text for conditions.

Table 1
Retention times and detection limits of the compounds studied without using SPE[a]

Peak	Compound	t_R (min)	Family	Detection (nm)	Detection limits (mg/l)
1	Naphthalene	8.8	PAH	210	3.6
2	Biphenyl	9.6	PAH	250	2.5
3	Acenaphthene	9.8	PAH	220	0.3
4	Fluorene	10.4	PAH	210	3.2
5	Acenaphthylene	10.6	PAH	220	2.4
6	Phenanthrene	12.6	PAH	250	2.3
7	2-Chlorophenol	13.6	Phenol	280	2.4
8	Fluoranthrene	14.5	PAH	210	2.6
9	2,4-Dinitrophenol	16.0	Phenol	250	2.0
10	Propham	17.6	Carbamate	220	14.13
11	Benz[*a*]anthracene	18.2	PAH	280	0.3
12	2,4-Dichlorophenol	19.1	Phenol	280	3.4
13	Chlorpropham	19.6	Carbamate	210	3.11
14	Chrysene	19.9	PAH	250	2.7
15	2,4-Dimehylphenol	20.2	Phenol	280	3.6
16	Phenol	22.1	Phenol	280	3.9
17	Terbuthylazine	24.1	Triazine	220	1.52
18	Dinitre-*o*-cresol	25.0	Phenol	250	4.84
19	4-Chloro-3-methylphenol	26.0	Phenol	280	3.4
20	Napropamide	26.6	Amide	210	1.18
21	Carbofuran	27.4	Carbamate	210	5.55
22	Linuron	27.9	Carbamate	210	1.84
23	Chlorbromuron	28.1	Phenyl urea azine	210	1.85
24	4-Nitrophenol	31.7	Phenol	210	0.5
25	Desethylatrazine	33.7	Triazine	302	1.77
26	Desisopropylatrazine	36.2	Triazine	210	2.89
27	Aldicarb sulphone	37.8	Carbamate	210	7.95
28	Fenuron	38.1	Phenyl urea	210	4.60
29	Desmedipham	39.7	Carbamate	210	5.77
30	Phenmedipham	40.3	Carbamate	210	2.37
31	Warfarin	40.8	Coumarin	210	5.81
32	Chlortoluron	41.8	Phenyl urea	210	3.22
33	Monuron	42.4	Phenyl urea	250	3.31
34	Chloroxuron	48.5	Phenyl urea	250	3.12
35	Metoxuron	49.1	Phenyl urea	210	3.28

[a] See text for chromatographic conditions.

less than 50 min, the PAHs being the least retained followed by phenols, triazines, carbamates and phenyl ureas. The carbamates did not have a well defined time window and some of them appeared mixed with the other families. Although a small overlapping between some compounds could not be avoided, a general screening of the occurrence of these compounds can be done if the profile of the chromatogram and the time windows of the compounds are considered. For instance, a chromatogram with peaks in the region from 9 to 12 min suggests the presence of PAHs and so the individual method to analyse these compounds [20] can be used in order to make a correct quantification of the compounds avoiding some overlappings (biphenyl–acenaphthene). In the case of overlapping between compounds of different chemical families, DAD can be used for measurements at other wavelengths and checking peak purity.

The detection limits, corresponding to a signal-to-

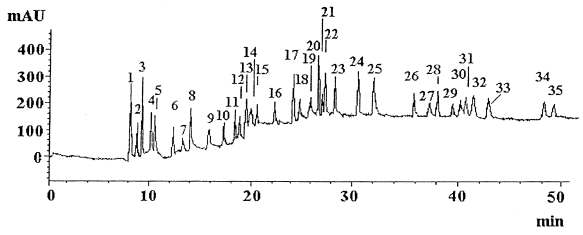

Fig. 2. Chromatograms obtained with 30 min drying time, using the Isolute Env+ as sorbent.

noise ratio of 3, ranged from 2.5 to 26.5 mg l^{-1}, which are high for analysing real water samples.

In order to decrease the detection limits, preconcentration of the compounds by on-line coupling SPE–SFC was assayed and five different types of sorbents were checked.

The drying time is one of the most important steps in the preconcentration procedure. As the polymeric sorbents need a drying time higher than those based on silica, due to the fact of the higher surface area, a

Table 2
Recoveries (%) obtained with the different sorbents[a]

Peak	Compound	RP-18	LiChroelut EN	Isolut Env+	Envichrom P	PLRP-S
1	Naphthalene	55.3	55.4	57.2	50.0	36.5
2	Biphenyl	64.5	60.3	70.3	61.2	33.0
3	Acenaphthene	107.0	109.5	105.0	89.5	72.5
4	Fluorene	59.4	51.0	96.2	65.0	101.2
5	Acenaphthylene	100.6	109.5	102.1	89.5	72.5
6	Phenanthrene	50.5	60.2	55.6	46.2	39.5
7	2-Chlorophenol	95.7	107.2	103.3	106.2	105.1
8	Fluoranthrene	32.6	33.7	34.6	34.3	32.5
9	2,4-Dinitrophenol	39.8	66.9	101.3	106.2	108.1
10	Propham	98.8	97.3	106.3	104.5	110.1
11	Benz[*a*]anthracene	51.0	37.1	44.8	44.3	32.7
12	2,4-Dichlorophenol	99.8	83.1	109.6	92.4	96.8
13	Chlorpropham	102.6	83.1	108.2	103.5	102.8
14	Chrysene	56.5	39.6	56.6	34.4	41.5
15	2,4-Dimethylphenol	60.5	101.2	102.3	105.1	103.1
16	Phenol	85.6	106.9	108.3	108.5	101.5
17	Terbuthylazine	107.2	84.7	97.8	106.1	106.3
18	Dinitro-*o*-cresol	96.5	85.6	97.8	87.1	83.4
19	4-Chloro-3-methylphenol	74.2	78.6	79.2	68.1	69.4
20	Napropamide	104.3	110.2	103.5	108.1	106.5
21	Carbofuran	104.2	106.3	99.3	101.2	101.5
22	Linuron	108.6	99.8	99.7	101.2	102.6
23	Chlorbromuron	81.6	98.6	89.8	98.5	88.4
24	4-Nitrophenol	108.2	106.3	106.5	105.2	109.1
25	Desethylatrazine	102.5	83.8	109.5	109.2	114.1
26	Desisopropylatrazine	102.8	56.9	99.8	67.8	54.6
27	Aldicarb sulphone	85.6	92.5	101.8	102.1	57.8
28	Fenuron	113.5	91.8	101.2	97.4	102.5
29	Desmedipham	111.2	82.6	81.8	79.5	97.1
30	Phenmedipham	80.1	54.2	79.4	79.4	79.3
31	Warfarin	97.2	71.4	99.5	90.1	105.2
32	Chlortoluron	102.5	106.5	103.2	104.1	101.5
33	Monuron	108.1	101.7	109.5	96.4	95.5
34	Chloroxuron	99.4	68.8	83.9	83.5	86.4
35	Metoxuron	107.4	96.8	92.5	92.8	86.3

[a] See text for conditions.

drying time of 30 min was finally selected (see Fig. 2). Table 2 shows the recoveries obtained for the different sorbents tested, the experiments were performed by using 2 ml of deionized water spiked with 0.025 μg of each compound. A calibration obtained by direct injection of the standard was used to calculate the recoveries. Isolute Env+ provided the highest recoveries for most of the compounds studied but PAHs presented low recoveries which could be caused by the high retention of PAHs on this type of sorbent. Several attempts to increase the recoveries of PAHs by decreasing the polarity of the conditioning solution (using mixtures of isopropanol or acetonitrile with water) were made, but then the recoveries for the other compounds greatly decreased. So Isolute Env+ and deionized water as conditioning solvent were finally selected to continue the study.

The breakthrough volumes of the compounds studied, determined by preconcentrating different

Table 3
Breakthrough volumes obtained by using Isolute Env+ as sorbent[a]

Compound	Recovery (%)				
	2 ml	10 ml	15 ml	20 ml	40 ml
Naphthalene	57.1	53.3	27.4	28.1	24.3
Biphenyl	70.2	70.3	41.6	18.2	13.4
Acenaphthene	105.0	102.5	92.1	89.5	72.5
Fluorene	96.2	104.2	106.3	104.3	36.5
Acenaphthylene	102.2	89.9	55.4	53.6	55.2
Phenanthrene	55.6	56.1	42.5	31.4	34.6
2-Chlorophenol	102.2	103.3	100.2	106.2	97.2
Fluoranthrene	34.6	35.6	33.2	29.2	2.5
2,4-Dinitrophenol	101.3	96.9	76.3	70.2	43.2
Propham	106.3	97.8	98.13	91.25	91.4
Benz[*a*]anthracene	44.8	46.3	28.5	25.3	16.4
2,4-Dichlorophenol	109.6	98.2	99.1	76.4	42.3
Chlorpropham	108.2	99.8	99.1	98.25	89.2
Chrysene	56.6	58.2	27.6	15.4	16.2
2,4-Dimethylphenol	102.3	98.6	98.7	98.2	96.5
Phenol	108.3	104.2	104.3	94.2	92.3
Terbutylazine	97.8	101.2	104.2	102.1	101.3
Dinitro-*o*-cresol	97.8	98.7	101.2	99.6	99.7
4-Chloro-3-methylphenol	79.2	77.9	78.9	72.1	58.2
Napropamide	103.5	101.2	99.1	91.21	84.3
Carbofuran	99.3	97.83	96.1	86.22	88.2
Linuron	99.7	101.1	101.1	101.2	102.6
Chlorbromuron	89.8	88.5	89.8	71.2	57.4
4-Nitrophenol	106.5	102.7	105.1	101.0	96.2
Desethylatrazine	109.5	100.1	102.1	95.2	91.1
Desisopropylatrazine	99.8	99.5	100.1	102.4	101.2
Aldicarbsulphone	101.8	98.5	101.8	102.1	57.8
Fenuron	101.2	105.2	105.3	96.8	97.1
Desmedipham	81.8	80.6	65.3	56.3	38.4
Phenmedipham	79.4	77.6	72.3	77.1	76.5
Warfarin	99.5	100.2	91.4	89.5	55.2
Chlortoluron	103.2	100.5	104.2	108.1	109.6
Monuron	109.5	107.2	103.2	104.1	102.6
Chloroxuron	83.9	88.9	78.2	71.2	62.3
Metoxuron	92.5	91.2	92.3	85.4	89.2

[a] See text for conditions.

volumes of deionized water spiked with 0.025 μg of each compound, are shown in Table 3. The results obtained were different depending on the compound, and in the case of some PAHs (naphthalene, biphenyl, acenaphthylene and chrysene) there was a significant decrease in the recoveries when the volume of sample was higher than 10 ml. The detection limits obtained are shown in Table 4, they ranged from 0.4 to 2.6 $\mu g\,l^{-1}$, but although they are high to analyse tap water, according to the European Union (EU) directive, the technique can be useful for a general screening of polluted lough and river waters (Fig. 3) Table 5.

Table 4
Detection limits obtained with the SPE–SFC procedure (S/N=10)

Compound	Detection limit ($\mu g\,l^{-1}$)
Naphthalene	2.1
Biphenyl	2.6
Acenaphthene	0.4
Fluorene	1.4
Acenaphthylene	1.8
Phenanthrene	2.4
2-Chlorophenol	1.2
Fluoranthrene	2.6
2,4-Dinitrophenol	1.1
Propham	2.3
Benz[*a*]anthracene	0.9
2,4-Dichlorophenol	1.5
Chlorpropham	1.3
Chrysene	2.2
2,4-Dimethylphenol	1.5
Phenol	1.3
Terbutylazine	1.1
Dinitro-*o*-cresol	2.1
4-Chloro-3-methylphenol	2.6
Napropamide	1.0
Carbofuran	1.8
Linuron	0.4
Chlorbromuron	0.9
4-Nitrophenol	0.6
Desethylatrazine	1.1
Desisopropylatrazine	1.5
Aldicarbsulphone	2.5
Fenuron	2.2
Desmedipham	2.2
Phenmedipham	2.3
Warfarin	1.9
Chlortoluron	1.0
Monuron	1.0
Chloroxuron	1.6
Metoxuron	1.8

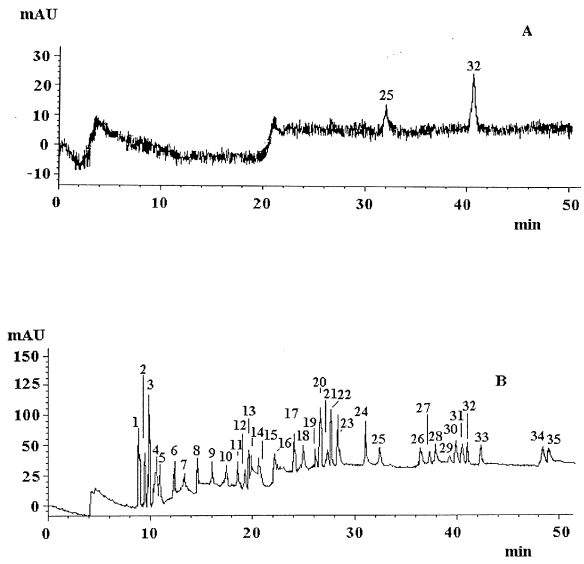

Fig. 3. Chromatogram obtained by using the SPE–SFC procedure; (A) 10 ml of a lough water sample, (B) 10 ml of river water spiked at 5 $\mu g\,l^{-1}$ level.

4. Conclusions

Information about the presence of a mixture of 35 organic contaminants (including phenols, PAHs and pesticides) in a time not longer than 50 min can be obtained by using SFC with five silica packed columns.

Sample preconcentration is necessary in order to decrease the detection limits. SPE is a useful tool for the extraction–preconcentration of these compounds and coupling it on-line to the SFC system, the analysis of waters containing these contaminants at a low microgram per litre level can be performed. In this case, Isolute Env+ was the sorbent which provided the highest recoveries for most of the compounds studied. The recoveries of some PAHs were low, probably due to a high retention on this kind of sorbent, which resulted in an increase of their detection limits, but in spite of this fact the method

Table 5
Repeatability and reproducibility obtained by applying the method on river water

Peak	Compound	Repeatability (R.S.D., %)	Reproducibility (R.S.D., %)
1	Naphthalene	15.60	12.44
2	Biphenyl	2.48	10.69
3	Acenaphthene	2.10	4.18
4	Fluorene	9.59	10.25
5	Acenaphthylene	6.40	7.48
6	Phenanthrene	2.98	8.32
7	2-Chlorophenol	2.52	6.70
8	Fluoranthrene	9.44	11.44
9	2,4-Dinitrophenol	7.32	8.65
10	Propham	6.28	14.02
11	Benz[*a*]anthracene	7.46	9.92
12	2,4-Dichlorophenol	5.08	6.05
13	Chlorpropham	13.09	9.82
14	Chrysene	8.84	5.46
15	2,4-Dimethylphenol	5.80	7.97
16	Phenol	4.89	3.46
17	Terbutylazine	7.57	11.38
18	Dinitro-*o*-cresol	4.77	4.39
19	4-Chloro-3-methyl phenol	4.68	6.59
20	Napropamide	6.71	7.85
21	Carbofuran	9.05	10.33
22	Linuron	7.58	10.67
23	Chlorbromuron	6.40	7.22
24	4-Nitrophenol	3.03	4.83
25	Desethylatrazine	6.65	8.29
26	Desisopropylatrazine	8.29	10.36
27	Aldicarb sulphone	6.16	5.56
28	Fenuron	5.28	6.16
29	Desmedipham	4.23	10.98
30	Phenmedipham	8.15	6.23
31	Warfarin	3.54	4.34
32	Chlortoluron	4.77	6.33
33	Monuron	4.32	6.59
34	Chloroxuron	2.50	9.28
35	Metoxuron	9.69	10.19

can be useful to detect the compounds in a general screening.

References

[1] C.L. Gabelish, P. Crisp, R.P. Schneider, J. Chromatogr. A 749 (1996) 165.
[2] J. Rodriguez, M.H. Bollain, R. Cela, J. Chromatogr. A 750 (1996) 165.
[3] M.L. Bao, F. Pantani, K. Barbieri, D. Bunini, O. Griffini, Chromatographia 42 (1996) 227.
[4] F.J. Santos, M.T. Galcerán, D. Fraisse, J. Chromatogr. A 746 (1996) 181.
[5] N.L. Olson, R. Carrell, R.K. Cumnings, R. Rirok, LC·GC 2 (1994) 142.
[6] P. Popp, K. Kalbitz, G. Oppermann, J. Chromatogr. A 687 (1994) 133.
[7] A. Zapf, R. Heyer, H.J. Stan, J. Chromatogr. A 694 (1995) 453.
[8] T. Monde, T. Kamiusuki, T. Kiroda, K. Mikumo, T. Ohkawa, H. Fukube, J. Chromatogr. A 722 (1996) 273.
[9] M. Kadota, M. Imanaka, K. Ikegawa, K. Kumashiro, T. Mori, S. Suzuki, H. Nakazawa, J. Food Hyg. Soc. 37 (1996) 48.
[10] S. Sennert, D. Volner, K. Levsen, G. Wunsch, Fresenius J. Anal. Chem. 351 (1995) 642.
[11] E.R. Brouwer, J. Liska, R.B. Geerdink, P.C.M. Fintrop, W.H. Mulder, H. Lingeman, U.A.Th. Brinkman, Chromatographia 32 (1991) 445.

[12] G. Marko-Varga, D. Barceló, Chromatographia 34 (1992) 566.
[13] C. Molina, M. Honing, D. Barceló, Anal. Chem. 66 (1994) 4444.
[14] K.M. Moore, S.R. Jones, C. James, Water Res. 29 (1995) 1225.
[15] T.A. Berger, W.H. Wilson, J.F. Deye, J. Chromatogr. Sci. 32 (1994) 179.
[16] T.A. Berger, Chromatographia 41 (1995) 133.
[17] P. Sandra, A. Kot, A. Medvedovici, F. David, J. Chromatogr. A 703 (1995) 467.
[18] J.L. Bernal, M.J. Nozal, L. Toribio, M.L. Serna, F. Borrull, R.M. Marcé, E. Pocurull, Chromatographia 47 (1997) 295.
[19] E.D. Ramsey, B. Minty, M.A. McCullagh, Anal. Commun. 34 (1997) 3.
[20] J.L. Bernal, M.J. Nozal, L. Toribio, M.L. Serna, F. Borrull, R.M. Marcé, E. Pocurull, J. Chromatogr. A 778 (1997) 321.
[21] T.A. Berger, Chromatographia 41 (1995) 471.
[22] J.L. Bernal, J.J. Jimenez, J.M. Rivera, L. Toribio, M.J. del Nozal, J. Chromatogr. A 754 (1996) 145.