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Monitoring of priority pesticides and other organic pollutants in river water from Portugal by gas chromatography–mass spectrometry and liquid chromatography–atmospheric pressure chemical ionization mass spectrometry

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Abstract

Gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (LC–APCI-MS) were optimized and applied for the trace-level determination of 42 priority pesticides and 33 priority organic pollutants from European Union Directive EC 76/464. First, off-line solid-phase extraction of 200 ml of river water using an OASIS solid-phase extraction cartridge, followed by GC–MS was used. Next, selected samples that were positive to GC–MS were analyzed by LC–APCI-MS in order to detect further polar byproducts or to improve the determination of previously detected polar analytes. The transformation products of triazine pesticides like deethylatrazine (DEA) and deisopropylatrazine (DIA) and compounds such as diuron and several chlorophenols were positively identified by LC–APCI-MS. The present methodology has also been used for searching for new analytes not included in the EC 76/464 list, like Irgarol, DEA and DIA. In addition it was applied to target pollutants in 43 river water samples from Portugal during a pilot survey from April to July 1999. Atrazine followed by simazine and 2,4,6-trichlorophenol were the most ubiquitous compounds detected in this area. The levels detected of the different compounds were in the range of: 0.01–2.73 μg/l, 0.05–0.74 μg/l, 0.02–1.65 μg/l, 0.02–5.43 μg/l, 0.01–0.40 μg/l, 0.01–0.26 μg/l, 0.02–0.61 μg/l, 0.01–3.90 μg/l, 0.01–1.24 μg/l, 0.02–2.3 μg/l, 0.01–0.13 μg/l and 0.01–0.5 μg/l for atrazine, simazine, terbuthylazine, alachlor, metolachlor, Irgarol, propanil; tributhylphosphate, diuron, 2,4,6-trichlorophenol, deisopropylatrazine and deethylatrazine, respectively.

Keywords: Water analysis; Environmental analysis; Pesticides; Irgarol

1. Introduction

The organonitrogen herbicides atrazine, alachlor and metolachlor are among the most commonly used and detected pesticides in water streams around the world. They are among the top ten herbicides used in the USA and Europe. According to Portugese authorities, about $15 \cdot 10^6$ kg of atrazine, $22 \cdot 10^6$ kg of simazine, $17 \cdot 10^6$ kg of alachlor and 10^7 kg of propanil were applied in 1996 in Portugal, mainly in

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corn, rice and grape plantations. Although most of the non-point source pollution of waters by pesticides has an agricultural origin, in the last years particular attention has been devoted to the non-agricultural uses of pesticides (e.g. highways, railroads and golf courses). Diuron and Irgarol are pesticides used for non-agricultural purposes.

The need for monitoring some important groups of dangerous organic pollutants, such as organo-chlorinated compounds, polycyclic aromatic hydrocarbons (PAHs), pesticides, phenolic compounds, amines, phthalates, alkyl and aromatic sulfonates in surface waters by state-of-the-art methods is now recognized, being essential for achieving good water-quality objectives. The list of the priority organic compounds to be monitored from discharges (European Union Directive EC 76/464) was selected [1].

Mass spectrometry (MS) is a highly sensitive and specific technique suitable for use in environmental organic analysis. GC–MS is widely used and a well known technique and allows the identification and determination of pesticides in several matrices and is still the most popular technique for this purpose [4–10]. However, owing to their thermal instability and polarity, many pesticides are not directly amenable to GC analysis. They can be determined by liquid chromatography (LC), which can be applied equally well to most typical GC amenable compounds. LC coupled with atmospheric pressure ionization techniques, mainly atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) offers new opportunities for the determination of a wide range of pollutants [2,3,6,11–14].

Taking into account the different papers published in the literature about trace determination of organic pollutants in river water and the need to implement 76/464/EC Directive, we have optimized and applied a new analytical protocol that is able to determine up to 75 selected organic pollutants in river water samples. This new analytical protocol combines, after a common solid-phase extraction (SPE) procedure, two analytical methods involving GC–MS and LC–APCI-MS.

The objectives of the present paper were:

(i) to establish a single extraction procedure using an SPE cartridge OASIS (*N*-vinylpyrrolidane–divinylbenzene based sorbent) that will allow to enrich a broad range of organic pollutants from river water samples following previous work [1];

(ii) to analyze the SPE extracts by GC–MS under full scan – compound identification – and GC–MS with single ion monitoring (SIM) – quantitative analysis;

(iii) to use LC–APCI-MS to identify and quantify polar analytes and transformation products suspected to be present in the river samples following GC–MS determination;

(iv) to apply the developed methodology to a pilot survey of 43 different sampling points in river water of Portugal during a period of 4 months.

To our knowledge the application of this protocol for a wide range of organic pollutants to Portugal river water has been undertaken for the first time.

2. Experimental

2.1. Chemicals

The standards used were 98–99% pure. Seventy-five compounds were studied (Tables 1 and 3). Pesticide standards were obtained from Promochem (Wesel, Germany) and Riedel-de Haën (Seelze, Germany). Irgarol was from Ciba-Geigy (Barcelona, Spain) and terbuthylazine was from Dr. Ehrenstorfer (Augsburg, Germany). Anilines, nitrobenzenes, chlorotoluidines and phenols were from Aldrich (Germany). α- and β-endosulfan, α- and γ-chlordane were from Supelco. Stock solutions of 1000 ppm were prepared by weighing 10 mg of each of the solutes and dissolving them in chromatographic-grade dichloromethane (Merck, Darmstadt, Germany). They were then stored at −20°C. HPLC-grade solvents methanol and water were from Merck, acetic acid was purchased from Fluka.

2.2. Sampling

In order to determine the levels of some organic compounds in the river water samples from Portugal, 43 different sampling sites were established in the area. In the pilot study, samples obtained from each of the sites were collected monthly from April to July.

Table 1
Retention time, molecular mass (M_r) and qualitative (30–70%) m/z ions of organic pollutants studied after analysis by GC–MS in the SIM mode[a]

	Time (min)	M_r	Diagnostic m/z ion	Qualitative m/z ion	Compounds		Time (min)	M_r	Diagnostic m/z ion	Qualitative m/z ion	Compounds
1	5.90	128	128	130	2-Chlorophenol	37	21.51	229	87	93	Dimethoate
2	5.97	128	128	130	3-Chlorophenol	38	21.79	202	201	186	Simazine
3	8.95	127	127	129	2-Chloroaniline	39	22.01	214	61	153	Monolinuron
4	9.81	162	162	164	2,4-Dichlorophenol	40	22.08	215	200	173	Atrazine
5	10.44	128	128	130	4-Chlorophenol	41	22.09	288	181	219	β-HCH
6	10.53	127	127	129	3-Chloroaniline	42	22.13	264	266	165	Pentachlorophenol
7	10.60	127	127	129	4-Chloroaniline	43	22.31	229	214	172	Propazine
8	11.22	157	157	111	1-Chloro-3-nitrobenzene	44	22.34	288	181	219	Lindane
9	11.35	141	141	106	2-Chloro-5-methylaniline	45	22.80	229	214	173	Terbuthylazine
10	11.42	161	161	163	2,6-Dichloroanline	46	23.63	288	181	219	δ-HCH
11	11.44	157	157	75	1-Chloro-4-nitrobenzene	47	23.65	274	88	153	Disulfoton
12	11.61	141	141	106	Chlorotoluidine	48	25.56	217	161	163	Propanil
13	11.62	157	157	75	1-Chloro-2-nitrobenzene	49	26.13	263	263	109	Parathion-methyl
14	11.76	257	109	79	Trichlorfon	50	26.66	269	160	188	Alachlor
15	12.49	142	142	144	4-Chloro-3-methylphenol	51	27.30	262	169	109	Oxydemethon methyl
16	12.98	171	154	173	4-Chloro-2-nitrotoluene	52	27.90	277	277	125	Fenitrothion
17	13.44	191	145	109	3,5-Dichloronitrobenzene	53	28.00	248	61	160	Linuron
18	13.45	161	161	163	2,4-Dichloroaniline	54	28.77	330	173	125	Malathion
19	13.90	161	161	163	2,5-Dichloroaniline	55	28.86	283	162	238	Metolachlor
20	13.91	196	196	160	2,4,6-Dichlorophenol	56	29.10	278	278	169	Fenthion
21	14.04	196	196	160	2,4,5-Dichlorophenol	57	29.27	291	291	109	Parathion
22	14.22	191	145	109	2,5-Dichloronitrobeozene	58	31.78	253	182	238	Irgarol
23	14.24	196	196	160	2,3,4-Trichlorophenol	59	32.28	406	373	375	γ-Chlordane
24	14.51	154	154	76	Biphenyl	60	32.76	184	184	92	Benzidine
25	14.53	162	162	127	1-Chloronaphtalene	61	32.85	316	246	176	*o,p′*-DDE
26	14.71	161	161	163	2,3-Dichloroaniline	62	32.98	404	195	339	α-Endosulfan
27	14.71	191	145	109	3,4-Dichloronitrobenzene	63	33.23	406	373	375	α-Chlordane
28	14.96	191	145	109	2,3-Dichloronitrobenzene	64	35.28	318	235	165	*o,p′*-DDD
29	14.96	161	161	163	3,5-Dichloroaniline	65	36.42	404	195	339	β-Endosulfan
30	15.56	161	161	163	3,4-Dichloroaniline	66	37.45	352	235	165	*o,p′*-DDT
31	15.63	224	127	192	Mevinphos	67	38.67	313	161	172	Triazophos
32	18.83	213	156	110	Omethoate	68	39.49	221	221	77	Pyrazon
33	18.99	172	172	126	4-Chloro-2-nitroaniline	69	42.54	252	252	254	3,3′-Dichlorobenzidine
34	19.90	266	99	155	Tributhylphosphate	70	44.58	317	160	132	Azinphos-methyl
35	20.59	335	264	306	Trifluralin	71	46.78	345	160	132	Azinphos-ethyl
36	20.88	288	181	219	α-HCH	72	50.69	362	362	226	Coumaphos

[a] HCH=Hexachlorocyclohexane.

Samples were collected in 1 l precleaned amber glass bottles, acidified with acetic acid to pH~4.0, filtered through 1.2 and 0.45 μm glass fiber filters in order to remove suspended particles and transported immediately to Barcelona by plane at 4°C to avoid degradation. Samples were kept at 4°C in the dark until analysis. SPE extractions were carried out at least 1 week after arrival.

2.3. SPE extractions

Automated SPE was performed with the ASPEC XL (Gilson) apparatus. The cartridges (OASIS 60 mg, Waters, USA) were washed sequentially with 6 ml of dichloromethane, 6 ml of acetonitrile and 6 ml of water at a flow-rate of 30 ml/min. A 200-ml aliquot of sample was passed though the cartridge at

a flow-rate of 6 ml/min and then washed with 1 ml of water. Water residues from cartridges were eliminated by 30-min vacuum. Elution was carried out with 2.5 ml of acetonitrile–dichloromethane (1:1) followed by 3.2 ml of dichloromethane at a flow-rate of 1 ml/min. Evaporation of the solvent was performed under a stream of nitrogen. The final sample volumes (0.2–0.5 ml) were weighed and corrected by solvent density.

2.4. GC–MS conditions

GC–MS analyses were performed in a Trace GC 2000 Series and Trace MS ThermoQuest Finnigan Instruments utilizing helium as carrier gas and the following conditions: fused-silica column HP-5MS (30 m×0.2.5 mm, d_f – 0.25 μm), 60°C for 1 min, 60–175°C (4 min) at 6°C/min, 175–240°C (5 min) at 3°C/min, 240–300°C (1 min) at 7°C/min, splitless, temperature of interface was 270°C, source temperature was 200°C, temperature of injector was set at 250°C. Electron impact ionization at 70 eV was used. All samples were analyzed in the SIM mode for quantification purposes of the compounds (major ions corresponding to the typical fragments of the compounds were selected, Table 1) and the scan mode in the range 70–450 u for confirmation of the spectral data against a real standard and library search.

2.5. LC–MS conditions

Selected positive samples (by GC–MS) were also analyzed by HPLC–APCI-MS in SIM and scan modes, utilizing a VG Platform system (Micromass, Manchester, UK) equipped with an APCI interface. The eluent was delivered by a gradient system from a Waters 616 pump, coupled to a Model Waters 600S controller (Waters, Milfrod, MA, USA). A LiChrocart cartridge column (250×4 mm I.D.) packed with Lichrospher 60RP-selected B of C_{18} (5 μm, Merck) was used. The gradient elution was carried out with a binary gradiente composed of methanol–water (1% acetic acid), from 20 to 100% of methanol in 30 min. After the analytical run, the column was rinsed with 100% methanol for 5 min and the mobile phase was returned to the initial conditions in 5 min. The flow-rate was 1 ml/min. A 20 μl volume of sample was injected each time.

The VG Platform APCI interface consists of a heated nebulizer probe and the standard atmospheric pressure source configured with a corona discharge needle. The different operating parameters included a drying gas (N_2) flow-rate of 150–300 l/h and a nebulizing gas flow-rate of 10 l/h. The cone voltage was set at 30 V, and the corona voltage was set at 3.5 kV. The ion source and the probe temperature were set at 150 and 350°C, respectively. The instrument control and data processing utilities included the use of the MassLynx application software installed in a Digital DEC personal computer 466. The chromatograms were recorded under scan and SIM conditions in positive or negative ion mode of operation. For scan conditions, the m/z ranged from 100 to 400, and for SIM conditions, the ions corresponding to the typical fragments of the compounds were selected (see Table 3).

2.6. Recoveries

Ground water samples were used. The compounds were spiked in 200 ml of water to give a final concentration of 1.0 μg/l and subsequently the water was acidified at lower pH (pH~4.0). Immediately after this operation, the water samples were extracted with the ASPEC XL.

The limits of detection (LODs) were calculated by using a signal-to-noise ratio of 3 (the ratio between the peak intensity under SIM conditions and the intensity of the noise was used). Recoveries and LODs are presented in Table 2.

2.7. Quantification

External standard calibration was used for quantification of the extracts. Calibration graphs for SIM mode were plotted using four to six points in the concentration 0.05, 0.10, 0.25, 0.5, 1.0 and 2 ppm. Calibration equations obtained in SIM mode and R^2 values are presented in Table 3 for LC–APCI-MS and Table 2 for GC–MS.

Table 2
Mean percentage recovery, standard deviation (SD), limits of detection (LOD), calibration equation and coefficients of correlation (R^2) in the SIM mode of organic compounds using SPE followed by GC–MS, linearity was observed for all the compounds studied over a concentration level varying from 0.05 to 2 ng/μl, spiked sample at 1 ng/ml, river water volume: 200 ml

Compounds	Recovery (%)	SD	LOD (μg/l)	Calibration equation	R^2
Atrazine	95 (n=10)	12.9	0.009	$y=1\cdot10^6x-16\,463$	0.999
Simazine	92 (n=11)	9.4	0.02	$y=675\,739x-18\,404$	0.999
Alachlor	91 (n=11)	11.7	0.03	$y=1\cdot10^6x+8405.9$	0.997
Metolachlor	81 (n=7)	10.2	0.01	$y=4\cdot10^6x-8254.9$	0.998
Tributhylphosphate	102 (n=4)	9	0.005	$y=1\cdot10^7x-50\,202$	0.999
Terbuthylazine	100 (n=4)	6	0.01	$y=2\cdot10^6x-35\,281$	0.994
Propazine	117 (n=7)	11	0.006	$y=841\,761x-62\,126$	0.9989
Linuron	89 (n=3)	9.8	0.01	$y=3\cdot10^6x-302\,092$	0.995
Monolinuron	111 (n=6)	11	0.004	$y=2\cdot10^6x-532\,385$	0.9914
Irgarol	84 (n=6)	4.3	0.005	$y=2\cdot10^6x-9190$	0.999
Pentachlorophenol	81 (n=4)	17	0.03	$y=491\,451x-150\,105$	0.997
Disulfoton	91 (n=3)	14	0.03	$y=2\cdot10^6x-23\,998$	0.9979
Propanil	106 (n=7)	9.6	0.02	$y=2\cdot10^6x-516\,106$	0.991
2,4,6-Trichlorophenol	76 (n=7)	4.8	0.009	$y=2\cdot10^6x-35\,281$	0.994
2,4,5-Trichlorophenol	98 (n=4)	9.5	0.01	$y=1\cdot10^6x-470\,158$	0.9991
2,3,4-Trichlorophenol	94 (n=3)	3.6	0.01	$y=1\cdot10^6x-239\,825$	0.9989
2-Chlorophenol	74 (n=4)	28	0.01	$y=1\cdot10^6x-21\,725$	0.999
3-Chlorophenol	74 (n=6)	7.4	0.03	$y=3\cdot10^6x+93\,889$	0.996
4-Chlorophenol	73 (n=4)	8.6	0.002	$y=6\cdot10^6x-2\cdot10^6$	0.9977
4-Chloro-2-nitrotoluene	81 (n=3)	4	0.02	$y=1\cdot10^6x+16\,679$	0.992
2,6-Dichloroaniline	97 (n=4)	9	0.01	$y=3\cdot10^6x+42\,902$	0.998
2,5-Dichloroaniline	80 (n=4)	17	0.03	$y=4\cdot10^6x=740$	0.998
Biphenyl	65 (n=2)	25	0.3	$y=8\cdot10^6x+171\,356$	0.996
α-HCH	81 (n=4)	16	0.02	$y=26\,909x-464.4$	0.999
β-HCH	99 (n=4)	13	0.02	$y=335\,901x-5892$	0.9985
δ-HCH	107 (n=4)	12	0.01	$y=735\,538x-27\,297$	0.9994
Lindane	90 (n=4)	31	0.02	$y=894\,672x+18\,141$	0.999
2-Chloro-4-methylaniline	59 (n=2)	1.9	0.002	$y=2\cdot10^6x-75\,371$	0.996
2-Chloro-5-Methylaniline	53 (n=2)	2	0.003	$y=23+06x-32\,452$	0.9946
3,4-Dichloroaniline	110 (n=3)	8.7	0.05	$y=3\cdot10^6x-154\,777$	0.999
2,4-Dichloroaniline	117 (n=3)	11	0.02	$y=6\cdot10^6x-223\,909$	0.9986
2,3-Dichloroaniline	101 (n=3)	2	0.04	$y=219\,668x-55\,058$	0.9977
3,5-Dichloroaniline	110 (n=3)	8.7	0.007	$y=6\cdot10^6x-464\,675$	0.9973
4-Chloro-2-nitroaniline	105 (n=2)	1.7	0.27	$y=2\cdot10^6x-89\,918$	0.997
2-Chloroaniline	99 (n=3)	7	0.08	$y=88\,774x+4422$	0.999
3-Chloroaniline	97 (n=2)	2	0.02	$y=3\cdot10^6x-1\cdot10^6$	0.9984
4-Chloroaniline	65 (n=3)	2	0.02	$y=6\cdot10^6x-17\,481$	0.9987
Dimethoate	96 (n=4)	17	0.02	$y=2\cdot10^6x-215\,466$	0.997
2,4-Dichlorophenol	60 (n=7)	4.7	0.02	$y=2\cdot10^6x-303\,220$	0.999
4-Chloro-3-methylphenol	66 (n=4)	8.2	0.01	$y=2\cdot10^6x-303\,220$	0.9981
α-Endosulfan	74 (n=4)	5	0.01	$y=131\,053x+2887$	0.9999
β-Endosulfan	74 (n=4)	2	0.01	$y=131\,238x+1727$	0.9999
γ-Chlordane	131 (n=3)	5.4	0.03	$y=146\,586x-5805$	0.9999
α-Chlordane	147 (n=3)	2.7	0.04	$y=138\,618x-5301$	0.9999
o,p'-DDD	95 (n=3)	13	0.003	$y=1\cdot10^6x+20\,718$	0.9999
o,p'-DDT	105 (n=4)	14	0.004	$y=1\cdot10^6x-7899$	0.9999
o,p'-DDE	63 (n=3)	5.6	0.02	$y=2\cdot10^6x-47\,322$	0.9988
Azinphos-methyl	90 (n=4)	13	0.02	$y=283\,911x-81\,705$	0.9992
Azinphos-ethyl	125 (n=4)	13	0.02	$y=313\,343x-80\,943$	0.9954

Table 2. Continued

Compounds	Recovery (%)	SD	LOD (μg/l)	Calibration equation	R^2
Parathion	124 (n=4)	30	0.01	y=250 972x−83 995	0.9957
Parathion-methyl	118 (n=4)	28	0.01	y=407 270x−122 324	0.9943
Triazophos	111 (n=3)	19	0.06	y=353 202x−110 545	0.9979
Fenitrothion	99 (n=3)	18	0.03	y=411 818x−249 637	0.9935
Fenthion	70 (n=3)	30	0.009	y=736 202x−25 565	0.9997
Oxydemethon-methyl	130 (n=3)	30	0.04	y=563 243x−509 328	0.9913
Trifluralin	56 (n=5)	6.5	0.005	y=649 339x−46 329	0.9984
Coumaphos	117 (n=4)	10	0.03	$y=1\cdot10^6x$−42 815	0.9977
3,3′-Dichlorobenzidine	71 (n=5)	8	0.04	y=583 630x−158 180	0.9973
Mevinphos	96 (n=7)	9.8	0.005	$y=2\cdot10^6x$−695 051	0.9984
1-Chloronaphtalene	36 (n=3)	3	0.008	$y=2\cdot10^6x$−15 743	0.9958
3,5-Dichloronitrobenzene	91 (n=2)	4	0.007	y=966 824x−592 81	0.9993
2,5-Dichloronitrobenzene	64 (n=2)	1.6	0.01	y=687 878x−43 012	0.9994
3,4-Dichloronitrobenzene	61 (n=2)	2.0	0.01	y=618 473x−48 630	0.9997
2,3-Dichloronitrobenzene	65 (n=2)	2.8	0.01	y=689 031x−40 436	0.9987
1-Chloro-4-nitrobenzene	63 (n=2)	1.4	0.009	y=765 113x−98 532	0.9954
1-Chloro-2-nitrobenzene	68 (n=2)	2	0.07	y=788 899x−91 384	0.9931
1-Chloro-3-nitrobenzene	63 (n=2)	5	0.01	y=718 101x−209 401	0.999
Benzidine	132 (n=3)	30	0.08	y=48 276x−10 916	0.9961
Pyrazon	83 (n=4)	14	0.002	$y=2\cdot10^6x$−135 704	0.9918
Omethoate	14 (n=3)	2	0.01	$y=1\cdot10^6x$−102 383	0.993
Malathion	114 (n=3)	19	0.004	$y=2\cdot10^6x$−225 158	0.9965
Trichlorfon	75 (n=5)	17	0.08	y=66 502x−7793	0.9976

Table 3
Molecular mass (M_r), major m/z ions, standard deviation (SD), limits of detection (LOD), calibration equation and coefficient of correlation (R^2) in the SIM mode of selected organic compounds after analysis by SPE followed by LC–APCI-MS in the PI or NI operation mode, linearity was observed for all the compounds studied over a concentration level varying from 0.05 to 2 ng/μl, river water volume: 200 ml

Compound	M_r	m/z of main ions	Calibration equation	R^2	Recovery (%)	SD (n=2)	LOD (mg/l)
PI mode							
Atrazine	215	216 $[M+H]^+$	$y=1\cdot10^6x$−1952	0.999	80	0.4	0.02
Simazine	201	202 $[M+H]^+$	$y=1\cdot10^6x$−19 610	0.999	76	4.3	0.02
Deisopropylatrazine	173	174 $[M+H]^+$	$y=2\cdot10^6x$−21 670	0.999	50	3.5	0.07
Deethylatrazine	187	188 $[M+H]^+$	$y=2\cdot10^6x$−11 304	0.999	86	1.1	0.01
Irgarol	253	254 $[M+H]^+$	$y=1\cdot10^6x$−20 103	0.999	82	0.8	0.03
Terbuthylazine	229	230 $[M+H]^+$	y=962 912x−18 246	0.999	96	4.7	0.008
Diuron	232	233 $[M+H]^+$	y=577 709x+12 007	0.998	84	1.4	0.02
Alachlor	269	238 $[M+H\text{-}MeOH]^+$	y=956 867x−20 499	0.999	92	10	0.008
Metolachlor	283	252 $[M+H\text{-}MeOH]^+$	y=811 590x−3854	0.999	84	3.9	0.003
NI mode							
2,4,6-Trichlorophenol	196	195 $[M-H]^+$	y=198 950x+66 918	0.996	61	30	0.05
Pentachlorophenol	264	263 $[M-H]^+$	y=139 590x−160 421	0.998	80	4.5	0.1
2,4-Dichlorophenol	162	161 $[M-H]^+$	y=56 762x+14 302	0.992	77	30	0.2
4-Chlorophenol	128	127 $[M-H]^+$	y=13 006x−37 827	0.963	91	31	2
2-Chlorophenol	128	127 $[M-H]^+$	y=13 558x−11 599	0.998	63	12	1
3-Chlorophenol	128	127 $[M-H]^+$	y=11 650x−12 923	0.998	60	12	1

3. Results and discussion

3.1. Optimization of two analytical methods – GC–MS and LC–MS

Recoveries for the majority of compounds studied were above 70% with standard deviations below 17% (see Table 2 for GC–MS and Table 3 for LC–MS results). Exceptions were omethoate and 1-chloronaphthalene.

Standard deviations around 10%, but always below 30%, according to the US Environmental Protection Agency (EPA) methods general characteristics that stipulate acceptable recoveries values in the range from 70 up to 130% with a maximum relative standard deviation of 30% each [17].

The calibration curves constructed were linear over the range of interest. The correlation coefficients were in all cases higher than 0.99, indicating good performance of the chromatographic method (Tables 2 and 3). The mass spectra of the compounds permitted their unequivocal identification in environmental samples (see Figs. 3, 5 and 6). As it can be seen in Table 2, detection limits for the majority of the compounds were in the range of 0.002–0.08 μg/l by GC–MS (far below 0.1 μg/l) being good enough for trace levels determination, taking into account that 200 ml of water were percolated through the cartridge. Exceptions are biphenyl and 4-chloro-2-nitroaniline, but this is acceptable, as the method involves different classes of compounds. Good results were also observed by LC–MS analysis (Table 3), mainly in positive operation mode. Recoveries were around 76–92%. Detection limits were in the range of 0.003–0.07 μg/l. A low recovery value was observed for deisopropylatrazine (DIA), 50% of recovery. In the negative operation mode, recoveries were lower, 60–

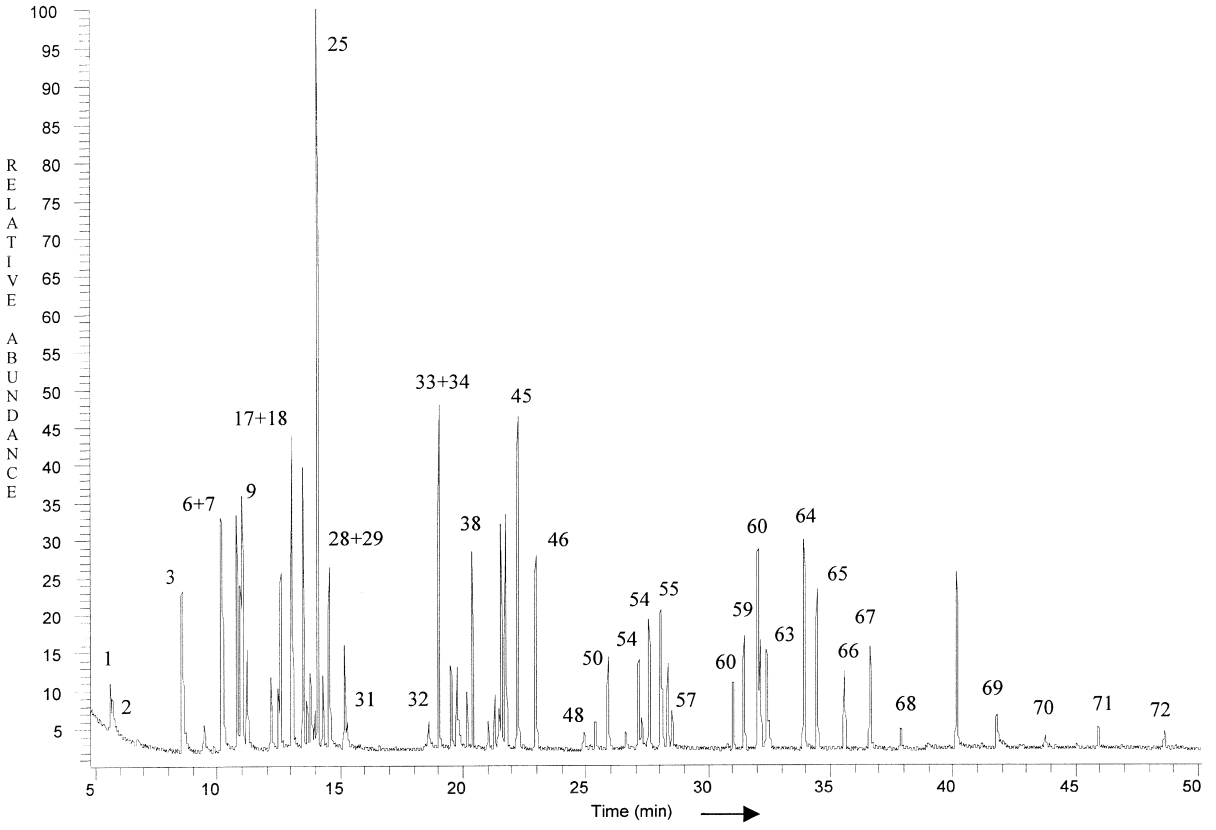

Fig. 1. GC–MS chromatogram from the standard solution sample at 2 ppm in the scan mode. For numbers, see Table 1.

91%, with standard deviations up to 30 and limits of detection too high for mono-chlorophenols, although for the polychlorinated phenols the limits of detection were better, 0.05–0.2 μg/l.

3.2. Monitoring study of target analytes

First, the analyses were carried out by GC–MS, which provided a primary screening of the samples collected. LC–APCI-MS was used to determine the polar compounds and identify the presence of positive transformation products formed [e.g., diuron, deethylatrazine (DEA), DIA].

The data obtained in this study have been useful for determining the occurrence and temporal distribution of these target compounds in the studied area. Although we have analyzed 75 organic compounds in 43 water samples in 4 months, we show here only the most significant results. Fig. 1 illustrates a gas chromatogram from the standard solution at 2 ppm in the scan mode.

The triazine herbicides, atrazine and simazine were found in the greatest number of samples and their values range from 0.01 to 2.74 μg/l and from 0.05 to 0.7 μg/l, respectively. Other pesticides such as alachlor, metolachlor and dealkylated metabolites of atrazine also appeared in some samples. Alachlor and metolachlor were found in the range of 0.02–5.4 and 0.01–0.40 μg/l, respectively.

The compounds were not distributed uniformly throughout the different sampling sites. The maximum concentration levels for the atrazine were observed at ''Alvalade do Sado'' (April), but we observed a decreasing tendency through the other months. The site ''Monte da Vinha'' presents the greater values to the majority of the compounds analyzed. A tendency of slight decreasing values was observed for atrazine and simazine, as illustrated in Fig. 2.

Among rice pesticides, propanil is one of the most applied, and Portugal is not an exception. Propanil and its major degradation product were monitored in surface water and soil samples from two rice fields of the Ebra Delta (Spain) area following agricultural application [15]. Concentration values of propanil were in the range of 0.02–0.61 μg/l. ''Ponte Aranha'' and ''Fervenças'' are plantations with rice cultivation so it is not surprising that propanil was detected in those areas.

Non-pesticide organophosphorus compounds are mainly industrial chemicals used as flame retardants, plasticizers and industrial hydraulic fluids and sol-

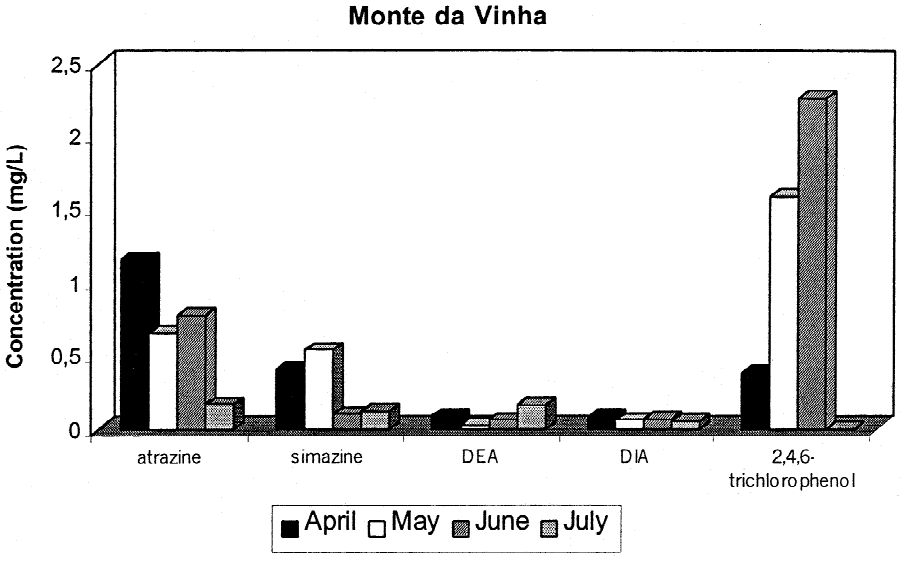

Fig. 2. Concentration values (μg/l) of atrazine, simazine and 2,4,6-trichlorophenol after analysis by GC–MS and deethylatrazine (DEA) and deisopropylatrazine (DIA) after analysis by LC–MS from a river water sample collected from April to July 1999 in Monte da Vinha (Portugal). Standard deviations were 13, 9, 1.1, 4.3 and 4.8 for atrazine, simazine, DEA, DIA and 2,4,6-trichlorophenol, respectively.

vents. In Italy, during 1985 and 1987, tri-isobutylphosphate (TiBP), tributylphosphate (TBP) and *tris*-2-chloroethylphosphate (TCEP) have been monitored. Levels varied from non-detectable (below 0.01 μg/l) up to 0.5 μg/l. In Spain, in the rivers Llobregat and Besos, concentrations of TiBP and TBP during 1985–1986 were below μg/l. In 1988, water samples of the Llobregat and Ebro rivers showed values of TBP up to 0.3 μg/l [16]. In the portuguese rivers studied, values of TBP varied in the range 0.01 to 0.36 μg/l, the higher concentration value observed only at the site Porto.

Other target compounds have also been detected in some samples: e.g. lindane (0.03–0.17 μg/l), linuron (0.13–0.68 μg/l), dimethoate (0.14–0.35 μg/l), 2-chloroaniline (0.03–1.9 μg/l) and 2-chlorophenol (0.03–0.08 μg/l). Mass fragmentograms in the SIM mode and mass spectra of selected compounds are shown in Fig. 3.

3.3. Monitoring study of non-target analytes

Major ions of the compounds studied by LC–APCI-MS in the positive (PI) and negative (NI) operation mode, calibration equations and R^2 values are shown in Table 3. Linearity was observed for all the compounds studied over a concentration level varying from 0.05 to 2 ng/μl with correlation coefficients greater than 0.99.

Fig. 4 illustrates the mass fragmentogram obtained under SIM conditions by HPLC–APCI-MS of atrazine, simazine and two transformation products (DEA and DIA) detected in April corresponding to the site ''Monte Real'', whereas Fig. 2 illustrates concentration values of DEA and DIA.

The presence of deethylatrazine and deisopropylatrazine in some of the samples can be attributed to the microbial degradation of the triazine herbicides in the soil samples and afterwards being transported

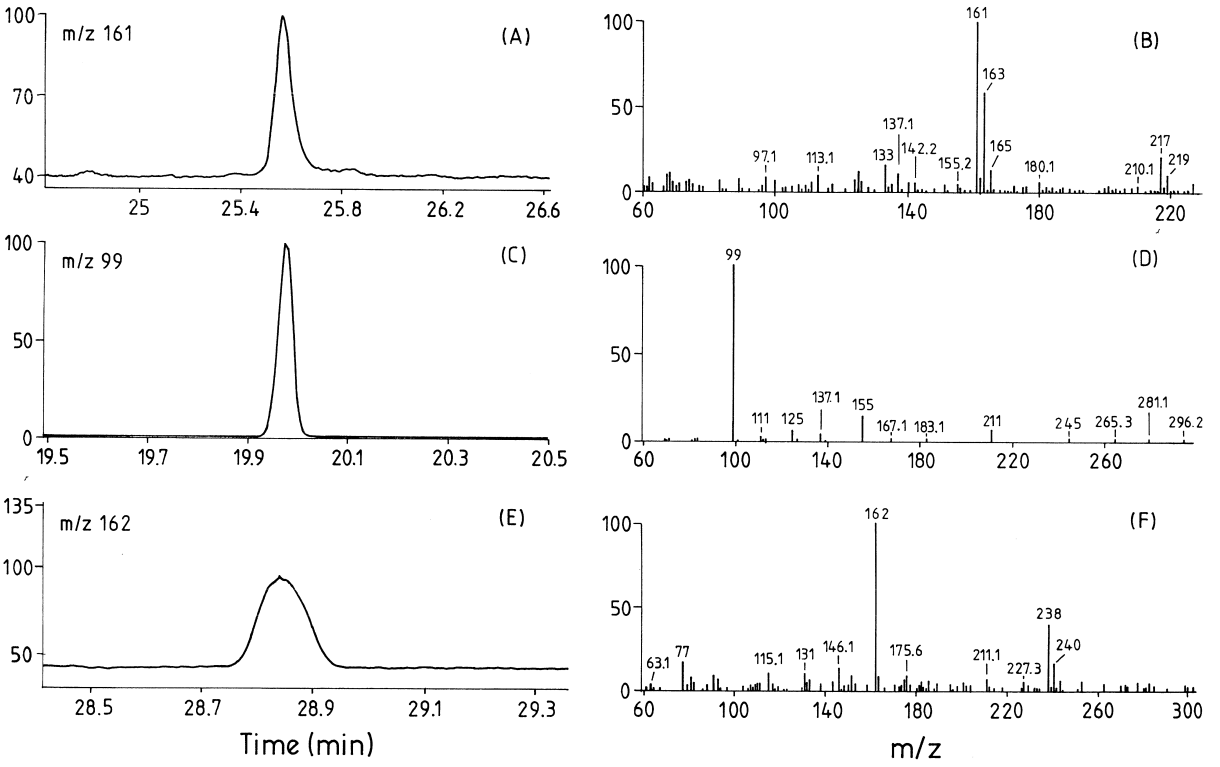

Fig. 3. GC–MS: (A) *m*/*z* 161 mass fragmentogram of propanil in the SIM mode; (B) mass spectrum of propanil; (C) *m*/*z* 99 mass fragmentogram of tributhylphosphate in the SIM mode; (D) mass spectrum of tributhylphosphate; (E) *m*/*z* 162 mass fragmentogram of metolachlor in the SIM mode; (F) mass spectrum of metolachlor.

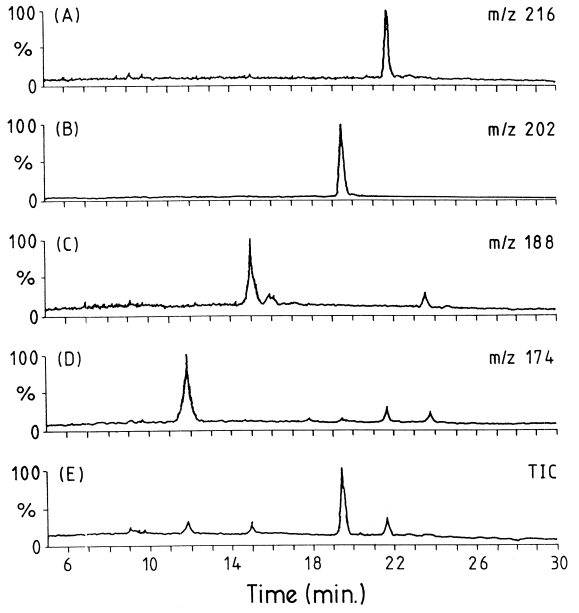

Fig. 4. LC–APCI-MS in the SIM mode in the sample Monte Real (April): (A) *m/z* 216 mass fragmentogram of atrazine; (B) *m/z* 202 mass fragmentogram of simazine; (C) *m/z* 188 mass fragmentogram of DEA; (D) *m/z* 174 mass fragmentogram of DIA; (F) total ion chromatogram.

through the river in the dissolved phase. Similar behavior was observed in the Ebro delta area [7].

Terbuthylazine was detected in two sites; ''Alb. Povoas e Meades'' and ''Porto Carvoeira''. In ''Alb. Povoas e Meades'' the values encountered are the highest ones. This may be diagnostic data that, in these sites, terbuthylazine is being applied preferentially to atrazine and simazine [16]. These values also showed a decreasing tendency from June to July at both sites, which correlates with the application period of these herbicides that occurs in April–May. Fig. 5 shows the mass fragmentogram obtained under SIM conditions by GC–MS and LC–APCI-MS and also the mass spectrum of terbuthylazine at 50 ng/l by GC–MS.

Irgarol, the herbicide 2-(methylthio)-4-(*tert*-butylamino)-6-(cyclopropylamino)-*s*-triazine (trade name Irgarol 1051) was also detected. This compound is used in antifouling paints as a biocide agent. The herbicide is added in paints in order to inhibit the primary growth of fouling organisms such as algal shines and seaweeds [18,19]. Few data are available concerning Irgarol contamination of aquatic environment. Studies had been undertaken in Côte d'Azur [20,21], the southern coast of the UK [22], Lake Geneva [23] and seawater from the Mediterranean Spanish coast [18,19]. Irgarol is added to ship/boat paints to prevent fouling and diffuse into the surrounding waters and contaminate environments. To our knowledge, the presence of Irgarol has never been reported in river water although a freshwater study has been performed at Lake Genova [23] and Southern England [22]. Some Portugese rivers are areas of recreational and commercial boating activities and thus is the cause of river contamination.

We have detected Irgarol in some river samples as has been pointed out in Fig. 6. The values found range from 0.01 to 0.26 μg/l. The greatest value was observed at the Fervenças site in May. The LODs and recoveries of Irgarol are also shown in Table 2 and were 5 ng/l and 84%, respectively by GC–MS and 80 ng/l and 82% by LC–MS. In Fig. 5 it is shown the *m/z* 182 and *m/z* 254 mass fragmentograms from Irgarol in the SIM mode by GC–MS and also the mass spectrum obtained at 50 ng/l by GC–MS. The detection of Irgarol in these samples is not surprising, some of these rivers are used for boating and Irgarol is released from boats to the water.

Diuron was encountered in some of the samples analyzed. It is also used as an antifouling agent in boat paints as well as for other applications. The levels of diuron range from 0.01 to 1.24 μg/l. This last value is higher than the ones reported by Ferrer and coworkers [18,19] and by Thomas [24]. Fig. 7 shows the mass fragmentogram *m/z* 233 of diuron and its mass spectrum obtained by LC–APCI-MS. In the sample ''Ponte Aranha'' Irgarol and Diuron in May–June, as illustrated in Fig. 7 had good correlation. Recovery and LOD for diuron, being determined by LC–APCI-MS, were 84% ($n=2$) and 0.02 μg/l, respectively.

Phenolic compounds of environmental interest come from a wide variety of industrial sources. Phenols, and especially chlorophenols are toxic at concentrations of a few μg/l and are also persistent. For these reasons a number of phenolic compounds are listed in the EPA list of priority pollutants and in the European Union (EU) Directive 76/464/EEC [14]. 2,4,6-Trichlorophenol was ubiquitously encountered in the samples analyzed by GC–MS and

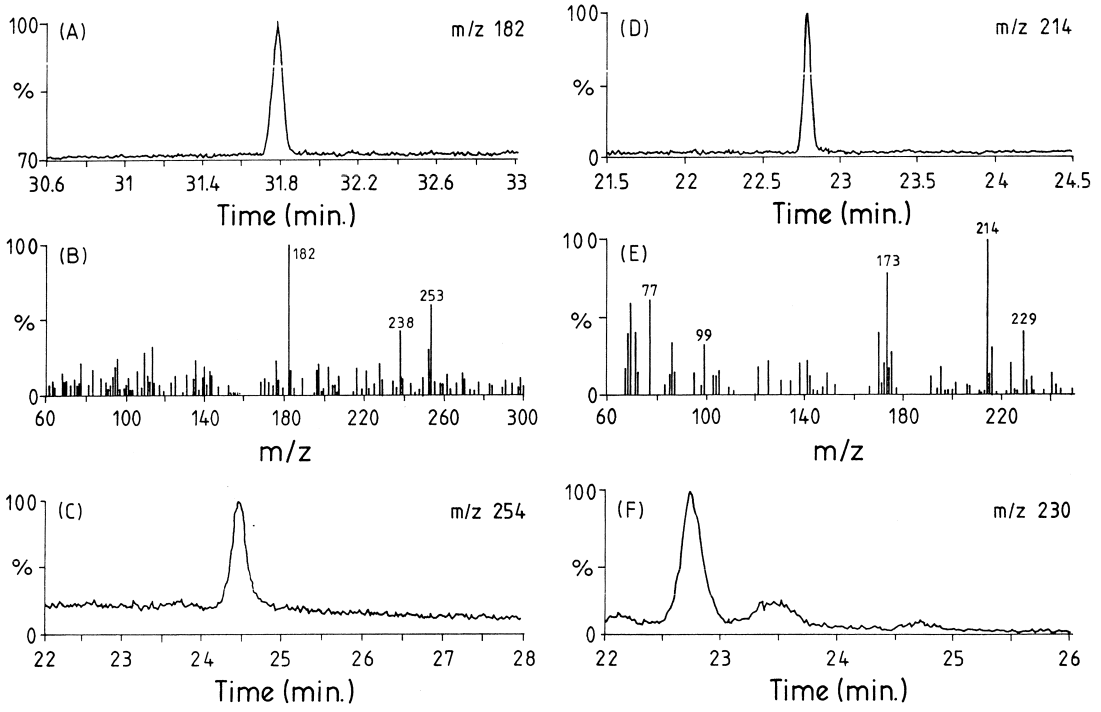

Fig. 5. (A) *m/z* 182 mass fragmentogram of Irgarol by GC–MS in the SIM mode in the sample Alb. Povoas e Meades; (B) mass spectrum of Irgarol by GC–MS in the sample Alb. Povoas e Meades (0.5 ppb); (C) *m/z* 254 mass fragmentogram of Irgarol by HPLC–APCI-MS in the SIM mode; (D) *m/z* 214 mass fragmentogram of terbuthylazine by GC–MS in the SIM mode in the sample Ponte Carvoeira; (E) mass spectrum of terbuthylazine by GC–MS in the sample Ponte Carvoeira (0.05 ppb); (F) *m/z* 230 mass fragmentogram of terbuthylazine by HPLC–APCI-MS in the SIM mode.

LC–APCI-MS. Other phenols such as 2-chlorophenol and pentachlorophenol were also detected in some samples. Although they can be detected and analyzed by GC–MS, LC–APCI-MS analysis is preferable, since the more polar phenols, such as pentachlorophenol, can be better determined. Fig. 7 shows the mass fragmentogram at *m/z* 195 and the mass spectrum of 2,4,6-trichlorophenol. The analysis of phenols had been well described in previous work [13,14,25].

3.4. GC–MS versus LC–MS determination

LC–APCI-MS conditions were adapted from an earlier paper of our group using positive (pesticides) [11] and negative ion mode (phenols) [4,13,14,25]. The eluent used was useful for both modes of operation. The cone voltage was set at 30 eV and provided less dissociation and a more abundant pseudo-molecular ion for the majority of the compounds studied.

Linear correlation was achieved ($R^2=0.8104$; $n=75$; $y=0.6271x+0.0557$) between both techniques, thus indicating a good agreement, as would be expected. The main reasons for the differences are that GC–MS is usually an automated technique, more sensitive, of easy manipulation and presents better calibration graphs than LC–MS. Furthermore, values are comparable and selected data from both methods are illustrated in Fig. 8. GC–MS permits a single analysis for the majority of the compounds. LC–MS was useful in the analysis of diuron, DEA, DIA. Combining both techniques allows analyzing a broader range of compounds. LC generally offers lower values than GC and this can be explained partly by the standard deviation of the samples. LC samples have been obtained from the dichloromethane extract of GC samples, so we changed solvent

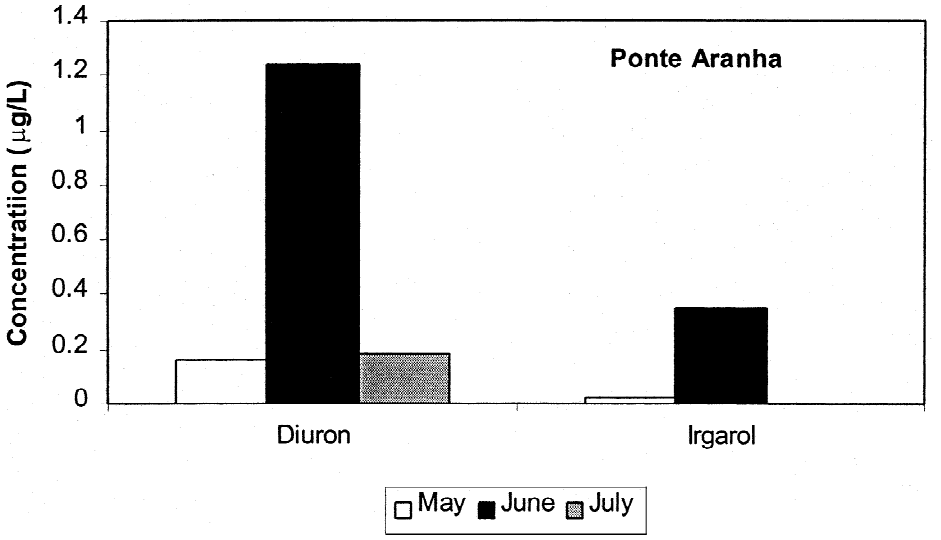

Fig. 6. Concentration values (μg/l) of diuron and Irgarol after analysis by SPE followed by LC–APCI-MS from river water samples collected from from May to July 1999 in Ponte Aranha (Portugal). Standard deviations were 1.4 and 0.8 for diuron and Irgarol, respectively.

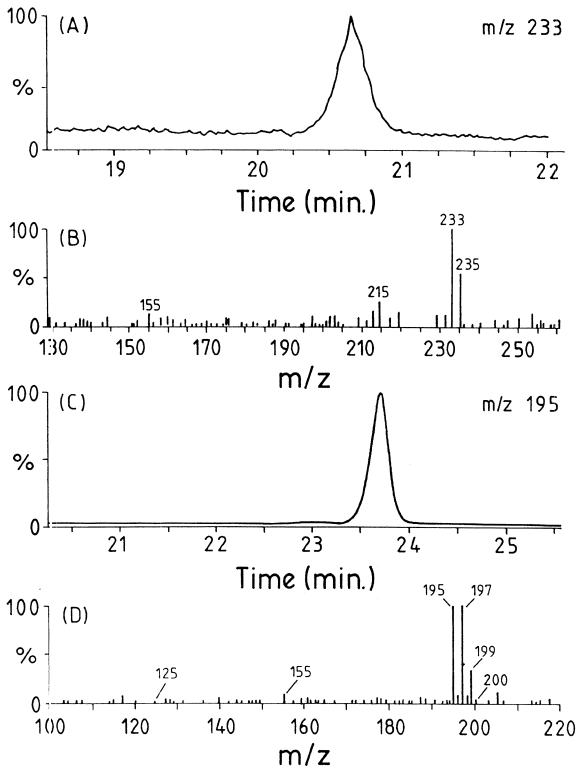

Fig. 7. LC–APCI-MS (A) *m/z* 233 mass fragmentogram of diuron in the sample Ponte Aranha (June); (B) mass spectrum of diuron; (C) *m/z* 195 mass fragmentogram of 2,4,6-trichlorophenol; (D) mass spectrum of 2,4,6-trichlorophenol.

to methanol, the sample being cleaner and with less interfering peaks.

3.5. General comments

The combined methodology involving SPE followed by GC–MS and LC–MS permitted the determination of 75 organic pollutants in the river water samples.

Among the organic pollutants studied and detected, alachlor, metolachlor, atrazine, terbuthylazine are caused by agricultural contamination mainly from corn; propanil is applied to rice plantation; Irgarol and diuron are used in boating paints and trichlorophenol and tributylphosphate have an industrial origin. Although we have detected only some agricultural, industrial and boating pollutants, this method was applied to a broad range of different organic compounds, which are commonly analyzed by different methods. The method presented here (combined SPE–GC–MS and LC–MS) permitted the simultaneous determination of 75 organic pollutants in water samples and offers relevant advantages: SPE automation, compound identification by GC–MS, quantitative determination by GC–MS-SIM and, when needed, further identification and quantification by LC–APCI-MS. Recoveries for the majority of compounds were between 70 and 130% with stan-

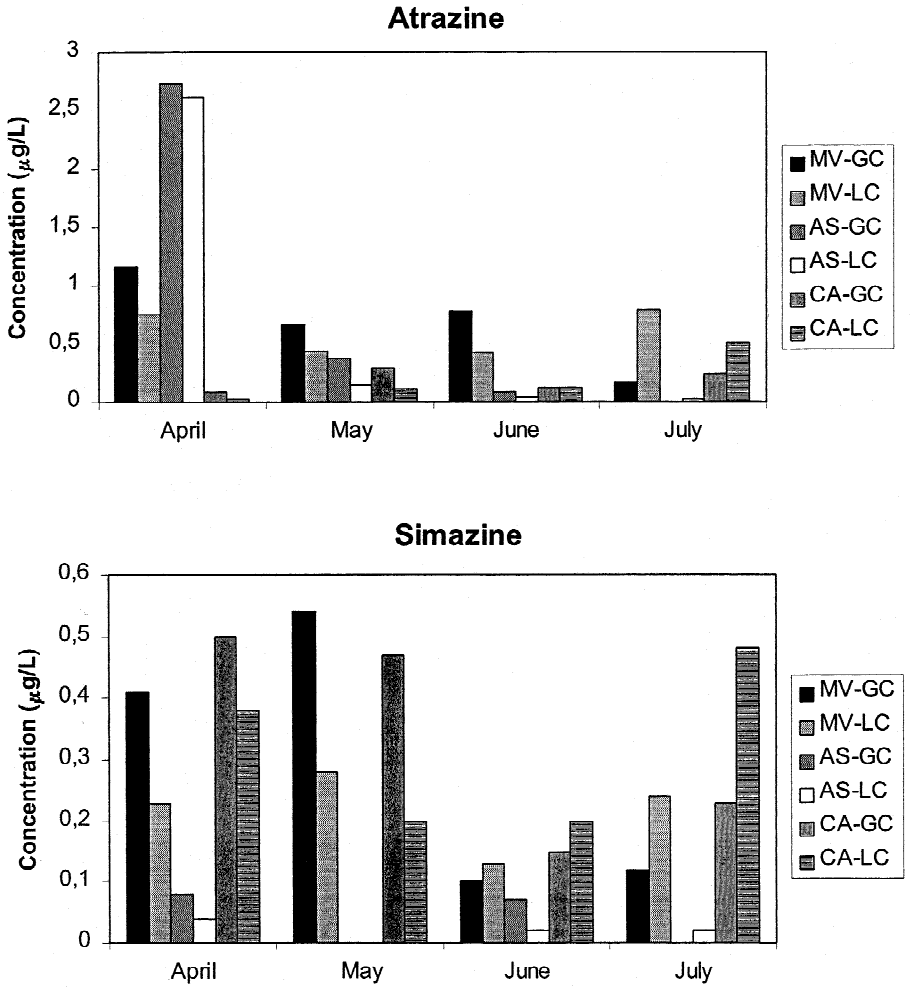

Fig. 8. Concentration values (μg/l) of atrazine and simazine after analysis by GC–MS and LC–MS for selected samples, from April to July. Standard deviations were 12.9 and 0.4 for atrazine; 9.4 and 4.3 for simazine by GC–MS and LC–MS, respectively. MV=Monte da Vinha; AS=Alvalade do Sado; CA=Cais do Alcoutim.

dard deviations below 30 for both chromatographic techniques, as stipulated by the EPA methods.

New pesticides are being developed to replace the more toxic ones or those that cause widespread contamination. This is the case for e.g., atrazine, which is being slowly replaced in some EU countries by terbuthylazine. In this sense, analytical developments need to be continuously carried out and should be improved to determine the new pesticides and the transformation products that are being released into the different types of environmental waters.

In this particular, the 76/464/EC list should be updated and incorporate new pollutants detected in samples such as Irgarol, terbuthylazine and some transformation products such as DEA and DIA.

4. Conclusions

We have optimized and applied an analytical method for analyzing 75 organic compounds in 43 river water samples from Portugal during 4 months by GC–MS whereas for the target polar compounds and their transformation products LC–APCI-MS was

used. The herbicides atrazine and simazine were found in the greatest number of samples. Other pesticides such as alachor, metolachor and dealkylated metabolites of atrazine also appeared in some samples. We also detected other triazines as Irgarol and terbuthylazine in relevant concentration levels. These results are not surprising as these sites have agricultural and boating activities. This is the first pilot study undertaken in Portugal that monitors 75 priority pollutants in different surface waters. By performing the present combined methodology used here we provide the analytical tools to carry out advanced monitoring strategies in environmental analysis. The present method permits to analyze the target priority pollutants and to incorporate new pollutants that are being used in the last few years.

Acknowledgements

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