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Analysis of Pesticide Residues in Water Samples by Gas Capillary Chromatography

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Several hundred pesticides of diverse chemical nature are widely used for agricultural purposes nowadays. It is therefore highly important to monitor surface, ground and drinking water in order to verify whether inadmissible levels of pesticides are present. Monitoring programs for pesticide residues are routinely conducted on a large scale and this poses the demand for methods simple, fast and sensitive. Sample preparation of water samples is performed by liquid-liquid extraction or by solid phase extraction (SPE), which has become popular as it is a simple and rapid technique. Octadecyl (C-18) bonded silica is applicable on non-polar and weakly polar analytes, as most pesticides are (Hennion MC and Scribe P, 1993). Capillary gas chromatography is suited for the separation of pesticides, as it provides high resolving power and quantitation of µg/L or ng/L levels of pesticides in environmental samples is attained by the use of the selective detectors, as the nitrogen-phosphorus (NPD) or the electron capture (ECD).

In the present study a multiresidue analytical method is presented, suitable for the simultaneous extraction and determination of approximately 60 pesticides in water samples. The extraction is performed by a solid phase extraction method previously described (Miliadis and Malatou, 1997), while the chromatographic separation and determination steps were developed by the use of two chromatographic systems running simultaneously. The aim of the study was to develop a rapid screening method, applicable for a great number of pesticides for monitoring natural waters of Greece.

MATERIALS AND METHODS

Stock solutions of 47 ECD-sensitive pesticides (1000 µg/mL) were prepared by proper dilution of analytical standards with “pesticide residue” grade ethyl acetate. Working solutions (10, 1, 0.1, 0.01 and 0.001 µg/mL) of the pesticides, as well as solutions of mixtures of them were prepared in ethyl acetate from the stock solutions according to the standard practices.

Sample preparation was performed by solid phase extraction with pre-packed reversed-phase octadecyl (C-18) bonded silica contained in cartridges. Isolute cartridges (International Sorbent Technology), containing 500 mg of packing material were solvated prior to loading the sample, by passing successively 1 mL/100mg sorbent (5 mL) ethyl acetate, 5 mL methanol and 10 mL of organic free water, with a glass syringe, 500 mL of water sample were cleaned by removal of any floating or insoluble material, and were passed through the cartridge. For extraction of samples a SPE large volume sampler was connected to a vacuum source equipped with a gauge and bleed valve to adjust the vacuum, in order to obtain a sampling rate of 15 mL water/min. Teflon tubes were inserted into the sample containers and the other ends were connected by adapters to the cartridges. In this way, up to eight water samples could pass through cartridges simultaneously. After passing the sample, suction continued for 30 min to dry out the packing material. The cartridges were disconnected and the adsorbed pesticides were eluted with ethyl acetate into 1-mL volumetric flasks. The extract was then stored in Teflon-sealed vials until chromatographic analysis.

A Fisons HRGC Mega 2 Series gas chromatograph was used with the following chromatographic systems: a) a cold-on-column injector, with a 30mX0.53mm i.d. DB-608 column, 0.83 μm film thickness and a nitrogen phosphorus detector for the determination of the NPD-sensitive pesticides. For the protection of the column 0.8m of empty precolumn was used. Operating conditions were: helium carrier gas, set at 1 mL/min and ambient temperature for the injection port, preserved with secondary cooling, and 280°C for the detector. b) a splitless injector, with a 30mX0.32 mm i.d. SE 54 capillary column, 0.25 μm film thickness and an electron capture detector for the determination of the ECD-sensitive pesticides. Operating temperatures were: 210°C for the injector and 300°C for the detector. The temperature program for succeeding separation of the compounds was the same for both chromatographic systems, allowing this way simultaneous injection of the sample in the two injectors and subsequent separation with the following temperature program: from 75°C to 180°C at a rate of 30°C/min, increased to 210°C at 1.8°C/min and then to 260°C at 30°C/min, and remain there for 20 min. The injection volume was 1 μL.

Quantification was carried out by use of a computer integrator. Identification of the unknown peaks in the samples' chromatograms was managed by comparing the relative retention time (RRT) of the unknown peaks to the RRTs of the reference standards. In order to confirm the identity of the pesticides found, the relative retention times obtained from another capillary column containing DB-1701 30m X 0.25 mm i.d. with 0.25 μm film thickness were used for both NPD-sensitive and ECD-sensitive compounds while further confirmation, whenever necessary was

achieved by the use of GC-MS Duplicate analysis was performed for the samples with pesticide residues detected.

RESULTS AND DISCUSSION

The extraction procedure using the C-18 cartridges is a well established technique applicable to different classes of pesticides (Miliadis et al, 1996). Cold-on-column injection, that was applied for the NPD-sensitive compounds, avoids losses caused by thermal degradation of components, as well as component discrimination and inaccuracies during the transfer of the sample to the column. This technique succeeds therefore lower detection limits and better reproducibility than the more traditional vaporisation injection techniques. On the other hand, the major advantage of splitless injection, that was applied for the ECD-sensitive compounds, is the ease in handling samples, especially the “dirty” ones, such as pesticide residues and environmental samples. Splitless injection offers better precision and sensitivity compared to split injection for quantitative analysis (Sandra, 1989). The application of the same temperature program for separating the NPD-sensitive and the ECD-sensitive compounds with the two detection systems on the same gas chromatograph allows simultaneous analysis of organochlorine and organophosphorous pesticides.

The method’s efficiency, concerning 24 NPD-sensitive compounds, has been validated and found satisfactory (Miliadis and Malatou, 1997). Chromatography of 47 ECD-sensitive pesticides was performed with the described method and their relative retention times, as to parathion methyl, and their ECD sensitivities are listed in Table 1. As seen from the RRT values in this table separation is satisfactory in most cases; however compounds with insufficient separation must be further identified with the DB-1701 column. Validation of the method for the ECD-sensitive pesticides was performed by spiking, in the laboratory, HPLC-grade water with 37 of the compounds at various concentration levels and the results of the recovery study are also given in Table 1. As seen from these data, recoveries for 24 of the 37 pesticides are between 80 and 104%, values acceptable for residue analysis (Greve 1984). Recoveries of 7 pesticides are between 46 and 77%, and since they have satisfactory repeatability, as seen from their relative standard deviation values that are lower than 21%, quantitation can be performed for these compounds after multiplying the result with a factor that takes into account their recovery. For pp-DDE, pp-DDT, captafol, λ-cyhalothrin, premethrin and cyfluthrin recoveries were found lower than 20%, making their determination not feasible. These very low recoveries are attributed to sorption on C-18, as it is known that for the elution of more hydrophobic compounds, as the pyrethroids, ethyl acetate is not sufficient (Akerblom, 1995).

Table 1. Relative retention times (RRTs), as to parathion methyl, sensitivities and recoveries of the ECD-sensitive studied pesticides.

Pesticide	RRT	Sensitivity (ng)	Fortification level (μg/L)	Recovery %
propachlor*	0.60	0.002	0.01-0.4	83±10
diphenylamine	0.61	0.05		
trifluralin	0.66	0.0005	0.005-0.1	77±15
α-BHC	0.71	0.001	0.002-0.04	82±17
dicloran*	0.74	0.001	0.01-0.2	81±8
atrazine	0.75	0.05	0.1-2	99±20
β-BHC	0.79	0.001	0.01-0.1	98±4
lindane	0.79	0.0005	0.002-0.04	91±7
quintozene	0.81	0.0005		
propyzamide*	0.81	0.01	0.05-1	101±5
chlorothalonil	0.89	0.005	0.005-0.1	86±25
metobromuron	0.90	0.01		
propanil	0.96	0.005	0.02-0.4	120±13
vinclozolin*	1.00	0.001	0.01-0.4	101±7
parathion methyl*	1.00	0.005	0.01-0.2	102±13
alachlor	1.02	0.001	0.005-0.1	90±15
heptachlor	1.03	0.005	0.1-2	92±10
linuron	1.09	0.05		
op-dicofol	1.11	0.01		
dichlofluanide	1.14	0.001	0.01-0.4	48±19
aldrin	1.17	0.0005	0.005-0.1	62±17
parathion ethyl*	1.18	0.005	0.02-0.4	99±11
pp-dicofol	1.20, 2.26, 2.59	0.01		
pendimethalin*	1.33	0.005		
heptachlor epoxide	1.34	0.001	0.005-0.02	94±5
folpet*	1.42	0.001	0.02-0.4	104±12
procymidone*	1.45	0.01	0.05-0.2	88±7
γ-chlordane	1.47	0.0002	0.002-0.04	61±12
α-endosulfan	1.54	0.002	0.005-0.02	96±5
α-chlordane	1.55	0.0004		
dieldrin	1.67	0.0004	0.002-0.04	84±6
pp-DDE	1.69	0.001	0.02-0.4	15±22
op-DDD	1.74	0.0005	0.01-0.2	46±15
pp-DDD	1.78	0.002	0.03-0.6	48±21
endrin	1.80	0.001	0.005-0.02	96±7
β-endosulfan	1.87	0.001	0.01-0.8	87±9
op-DDT	1.99	0.001	0.02-0.8	80±12
endosulfan sulfate	2.15	0.004	0.02-0.4	99±6
pp-DDT	2.20	0.001	0.2-0.8	18±9
captafol	2.33	0.05	0.1-2	<10
propargite	2.36	1.0		
iprodione*	2.54	0.001	0.1-2	81±22
λ-cyhalothrin	2.89, 2.93	0.005	0.1-2	<10
fenarimol*	2.91	0.001	0.01-0.8	82±4
permethrin	3.09, 3.13	0.005	0.1-2	<10
cyfluthrin	3.27, 3.30, 3.32, 3.34	0.01		
cypermethrin	3.37, 3.41, 3.43, 3.45	0.005	0.1-2	<10

* compounds also sensitive to NPD

The described method was applied for analysing samples of natural waters from various regions of Greece. 69 samples were analysed, 44 of which were intended for human consumption. Residues of 11 pesticides were detected in 13 samples, all of them non-potable. The pesticides that were detected and the frequency of their appearance at different concentration levels are shown in Table 2. The most frequently appearing pesticides were lindane (γ-BHC) and its isomer α-BHC. Lindane is an insecticide that degrades slowly in the environment and its residues have also been found in the past in natural waters of Greece (Kilikidis et al., 1992; Miliadis, 1994). Heptachlor and y-chlordane residues were detected in the water of the Evros river, in Northern Greece. Since the use of these chlorinated pesticidesis banned in Greece for the last 20 years their appearance may be attributed to uses in the neighbouring countries that the rivers runs across. In all cases the concentration levels found in the samples were much lower than the European Union maximum acceptable concentration for surface waters.

Table 2. Pesticides detected in natural waters of Greece and frequency of appearance at different concentration levels.

Pesticide	Samples with residues at the reported area*, µg/L				
	<0.005	0.01	0.05	0.1	0.2
alachlor				1	
α-BHC	5	4	3		
β-BHC	1	1	1		
γ-BHC (lindane)	8	3	1		
γ-chlordane	1				
chlorothalonil		1			
dimethoate				1	
heptachlor			1	1	
propachlor					1
propyzamide		1	1		
trifluralin	1	2			

*** Each column covers concentrations which start from higher concentration values of the previous column, e.g. the column 0.05 covers concentrations from 0.01 to 0.05 µg/L.**

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