Comparison of Extraction Methods to Monitor Pesticide Residues in Surface Water

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Abstract

A regular monitoring program to study the pesticide concentration in surface waters has been carried out since 1976 in Hungary by the National Plant Protection Organization of the Ministry of Agriculture and Regional Development jointly with the Regional Water Authorities. At the beginning of this program a liquid–liquid partition method is used to extract the pesticides from water samples. After checking the pH value, one sample aliquot is extracted to analyze the basic and neutral compounds. Another aliquot is acidified to pH 2 and extracted to analyze acidic compounds. Disadvantages of this method are high solvent consumption and the need to apply solvents (methylene chloride and diethyl ether) that are harmful to human health. Therefore, the solid-phase extraction method has been introduced. This method has another advantage in that by using the vacuum manifold a number of samples can be extracted simultaneously depending on the capacity (number of ports) of the manifold. Three types of cartridges (LiChrolut EN, ISOLUTE ENV+, and Carbograph) are tested. The suitability and reproducibility of the extraction on various cartridges is studied and compared through recovery experiments. Recoveries are done for 22 active ingredients at spiking levels of 1–5 times the limit of determination (in the range of 0.05–2.5 µg/L) with each extraction method. Individual recovery values as well as average recoveries for all methods are between 70% and 100%, with the relative standard deviation generally below 20%. Carbograph is the only cartridge among those studied that can be used to extract both neutral and acidic compounds in one sample loading step using two different consecutive elution steps.

Introduction

In 1976 a monitoring program was started in Hungary to evaluate the pesticide concentration in surface waters resulting from usual agricultural practice.

Samples of runoff water, drain water, and water from the streams and rivers on the catchment area of Lake Balaton (the most important resort place in Hungary) were analyzed. Later, the program was extended to the most important rivers, streams, canals, and lakes on each main agricultural cultivation and pesticide industrial area in Hungary. As a result of the monitoring data and changes in the agricultural practice (state farms and cooperatives transformed to private farms), today there are 35 sampling points. Samples are collected in every month from April to September (the spraying season) and analyzed for pesticides selected from those that were used in the relevant catching areas. Information on pesticide applications from the sale and spray records that must be maintained by the dealers and farmers are collected by field inspectors from the plant health stations.

Table I. Analyzed Pesticides, Detection Methods Used, and LOD

Name	Detection method	LOD (µg/L)
Lindane	GC–ECD	0.02
Endosulfan	GC–ECD	0.02–0.05
Diazinon	GC–NPD	0.05
Malathion	GC–NPD	0.05
Carbofuran	GC–NPD	0.5
Propachlor	GC–ECD	0.1
Acetochlor	GC–ECD	0.1
Propisochlor	GC–ECD	0.1
Metolachlor	GC–ECD	0.2
Pendimethalin	GC–ECD	0.05
Trifluralin	GC–ECD	0.02–0.05
Chlorbromuron	HPLC,GC–ECD	0.2,0.05
Isoproturon	HPLC	0.2
Bentazone*	GC–ECD	0.2
Atrazine	GC–NPD	0.1
Simazine	GC–NPD	0.1
Prometryne	GC–NPD	0.1
Metribuzin	GC–NPD	0.1
Terbutryne	GC–NPD	0.2
MCPA*	GC–ECD	0.2
2,4-DP*	GC–ECD	0.2
2,4-D*	GC–ECD	0.2

* After derivatization.

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Pesticides that are to be analyzed are selected on the basis of the experimental data obtained in the monitoring program and the conclusions of laboratory model experiments (studies on the pesticide concentration decrease in aquarium model systems and on the mobility and stability of the pesticides in soil). The analyzed pesticides are listed in Table I.

Because multiresidue methods are used, pesticides not specified in Table I can also be detected. Quantitative determinations are performed by gas chromatography (GC) using a capillary column, nitrogen–phosphorous selective detector (NPD), electron capture detector (ECD), and liquid chromatography with a UV detector. Confirmatory tests are completed on a GC–mass spectrometry system. The analyses are performed at fourteen regional laboratories. All laboratories used the same methods described in the standard operation procedures and were validated in accordance with guidelines for good laboratory practice (1).

At the beginning of this program a liquid–liquid partition method was used to determine the pesticide concentration in surface water. Recently, the solid-phase extraction (SPE) method has been introduced.

The purpose of this study is to present a comparison between liquid–liquid and SPE methods as well as among the various cartridges tested by the analysis of water samples spiked with pesticides. All data are based on analyses carried out in four laboratories.

Experimental

The extraction methods were studied through recovery experiments.

Recoveries were done for 22 active ingredients at spiking levels of 1–5 times the limit of determination (LOD) (in the range of 0.05 to 2.5 µg/L) with each extraction method. The LODs are summarized in Table I.

One-liter water sample portions were spiked with 1-mL standard solutions containing the pesticides dissolved in methanol. After fortification the samples were extracted and analyzed by the relevant methods, and recovery values were determined.

Extraction

Liquid–liquid partition

The flow diagram of this method is given in Figure 1.

One sample aliquot was extracted without changing the pH of the natural water to analyze nonpolar compounds. Another aliquot was acidified to pH 2 and extracted to analyze polar compounds—2-methyl-4-chlorophenoxyacetic acid (MCPA), 2,4-dichlorophenoxyaceitc acid (2,4-D), and bentazone.

Before the GC analysis using an ECD, cleanup on neutral alumina (2) was performed.

SPE

First, the sample was homogenized and pretreated if necessary (pH adjustment). The cartridge was placed in a vacuum manifold and washed with conditioning organic solvent followed by water for equilibration. The next step was to apply the water sample for the enrichment of the compounds to be analyzed. Finally, the interfering contaminants were washed out and the compounds of interest eluted.

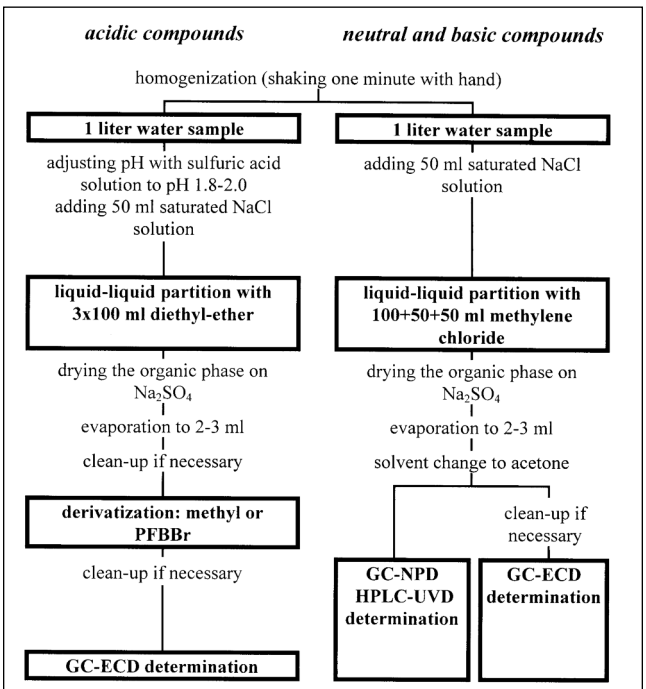

Figure 1. Extraction of water samples with liquid–liquid partition.

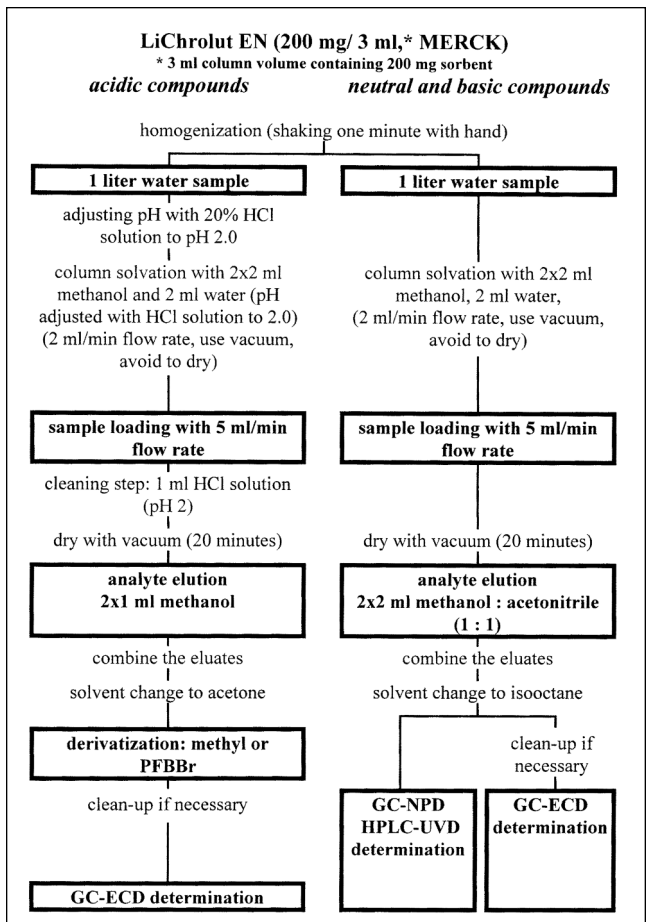

Figure 2. SPE of water samples on the LiChrolut EN cartridge.

One of the sorbents used was a LiChrolut EN (Merck, Darmstadt, Germany). This sorbent type was a nonionogenic, highly porous polystyrene–divinylbenzene polymer (3). It had an irregular particle shape, and the particle size distribution was 40–120 µm. Its specific surface was 1200 m^2/g with a 3-mL column volume and a 200-mg sorbent. The flow diagram of this method is given in Figure 2. Special sample preparations were not required, but the pH of one sample aliquot had to be adjusted for the extraction of the acidic compounds. The conditioning of the column was performed with 2 mL methanol and 2 mL water. The pH was adjusted with an HCl solution to 2.0 for acidic compounds. A flow rate of 5 mL/min was found to be the optimum for the sample loading. A cleaning step was performed only for the acidic compounds. One milliliter of distilled water was used, and the pH was adjusted with an HCl solution to 2.0. The drying step was conducted with vacuum for 20 min. In regards to an elution step, a 2- × 2-mL mixture of methanol–acetonitrile (1:1) was used, but for acidic compounds, 2 × 1 mL methanol was used. Before GC analysis with ECD, cleanup on neutral alumina (2) was performed.

Another sorbent that was used was the ISOLUTE ENV+ (International Sorbent Technology, Mid Glamorgan, U.K.) The sorbent type was a hyper-crosslinked hydroxylated styrene–divinylbenzene copolymer (4). It had an irregular particle shape, and the particle size distribution was 30–160 µm. The specific surface was 1000 m^2/g, and it had a 6-mL column volume and a 200-mg sorbent. The flow diagram of this method is given in Figure 3. Special sample preparation was not required, but the pH of one sample aliquot had to be adjusted for the extraction of the acidic compounds. The conditioning of the column was conducted with 5 mL methanol and 10 mL water. The pH was adjusted with an HCl solution to 2.0 for acidic compounds. A flow rate of 10 mL/min was found to be the optimum for the sample loading (the manufacturer's recommendation was 60 mL/min). The cleaning step involved 5 mL distilled water. The pH was adjusted with an HCl solution to 2.0 for acidic compounds. The drying step was conducted with vacuum for 20 min. A 2- × 2-mL mixture of acetone–ethyl acetate (1:1) (acidifying with 2% acetic acid for acidic compounds) was used for the elution step. Before GC analysis with ECD, cleanup on neutral alumina (2) was performed.

The final sorbent used was the Carbograph (Lida Manufacturing Corp., Kenosha, WI). The sorbent type was graphitized carbon black (5). The particle size distribution was 160–470 µm, and it had a specific surface of 100 m^2/g with a 6-mL column volume and a 300-mg sorbent. The surface of the carbon originally contained a positively charged, oxygen-containing complex (active centers) that could interact with the sufficiently acidic compounds; therefore, these acidic compounds were strongly bound to the Carbograph surface. Their separation from the base–neutral compounds was possible by stepwise elution (5). The flow diagram of this method is given in Figure 4. Special sample preparation was not required. The conditioning of the column involved a 5-mL mixture of methylene chloride–

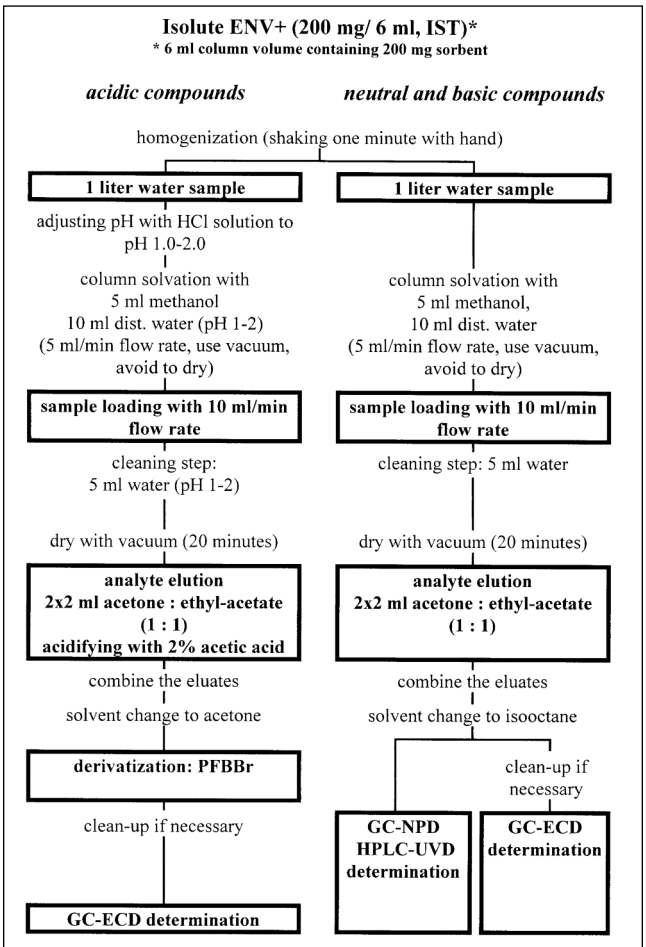

Figure 3. SPE of water samples on the ISOLUTE ENV+ cartridge.

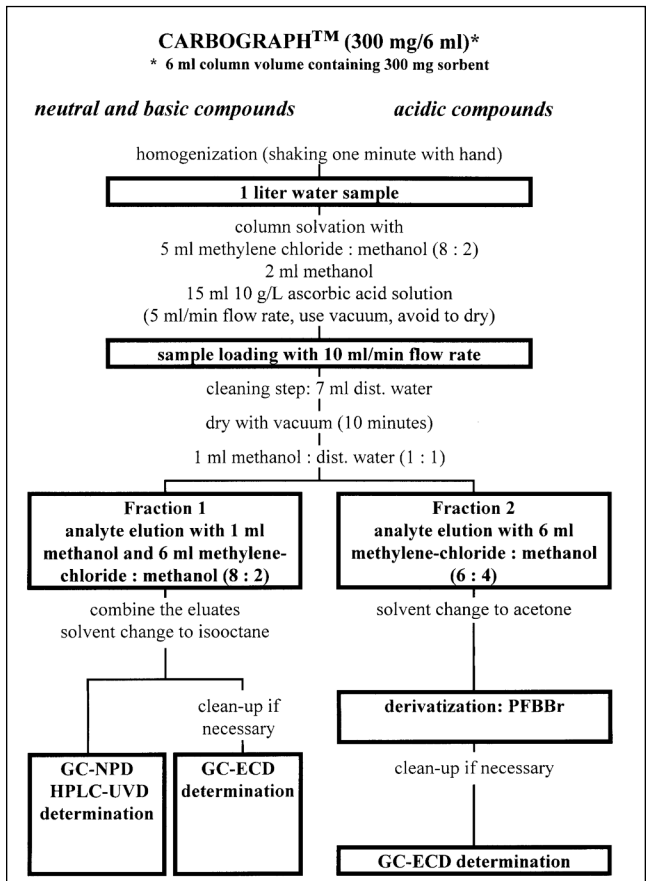

Figure 4. SPE of water samples on the Carbograph cartridge.

methanol (8:2), 2 mL methanol, and 15 mL of a 10-g/L ascorbic acid solution. A flow rate of 10 mL/min was found to be the optimum for the sample loading (the manufacturer's recommendation was 150 mL/min) (5). The cleaning step involved 7 mL distilled water, and the drying step was conducted with vacuum for 10 min. Rinsing was performed with a 1-mL mixture of methanol–distilled water (1:1). Elution 1 was 1 mL methanol followed by a 6-mL mixture of methylene chloride–methanol (8:2), and elution 2 was a 6-mL mixture of methylene chloride–methanol (6:4). Sample loading was performed in one step (only one sample portion had to be loaded), and the analytes were eluted in two consecutive steps. First, the neutral–basic compounds were eluted followed by the acidic compounds (6–8). Before GC analysis using an ECD, cleanup on neutral alumina (2) was performed.

Analysis

The pesticides were identified and quantitated by GC and high-performance liquid chromatography (HPLC).

Atrazine, carbofuran, diazinon, malathion, metribuzin, prome-

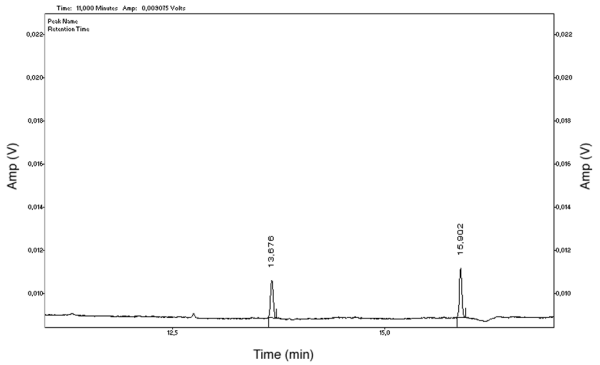

Figure 5. Chromatogram (NPD) of a water sample after enrichment with the Carbograph.

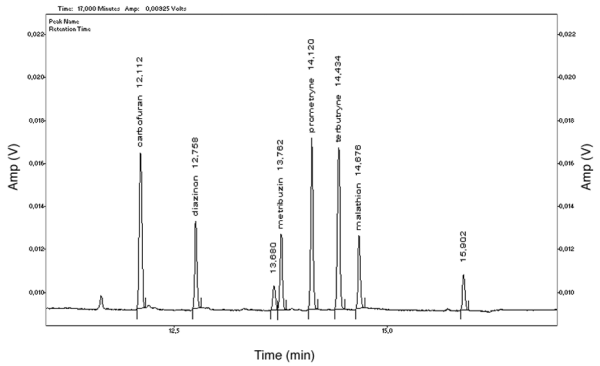

Figure 6. Chromatogram of a water sample spiked at 5 times the LOD with pesticides detectable with an NP detector after enrichment with the Carbograph.

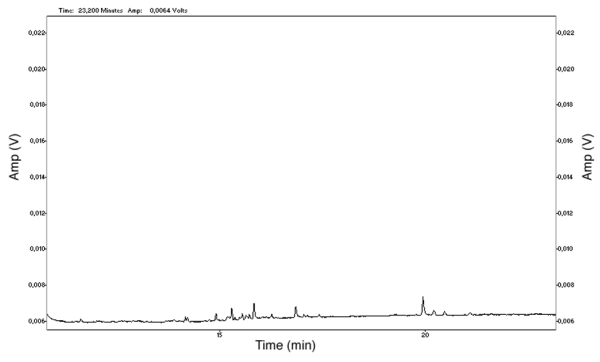

Figure 7. Chromatogram (ECD) of a water sample after enrichment with the Carbograph.

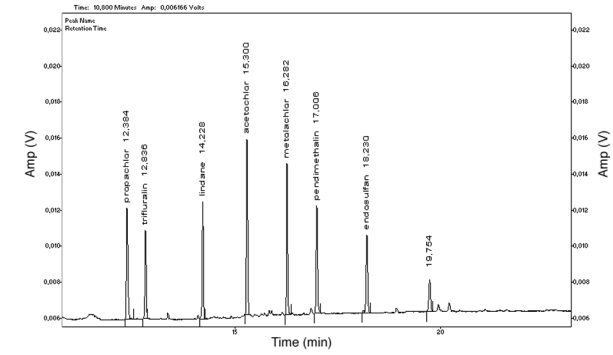

Figure 8. Chromatogram of a water sample spiked at 5 times the LOD with pesticides detectable with an ECD after enrichment with the Carbograph.

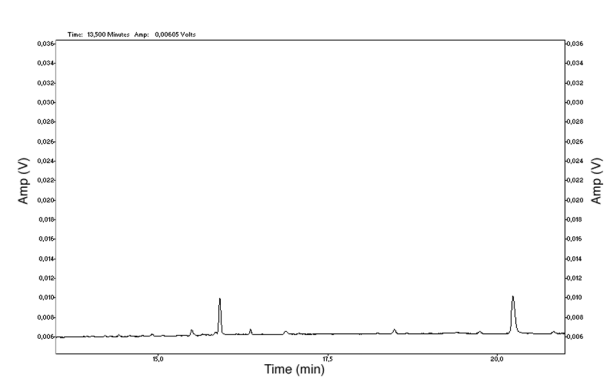

Figure 9. Chromatogram (ECD) of a water sample after enrichment with the Carbograph (derivatized with PFBBr).

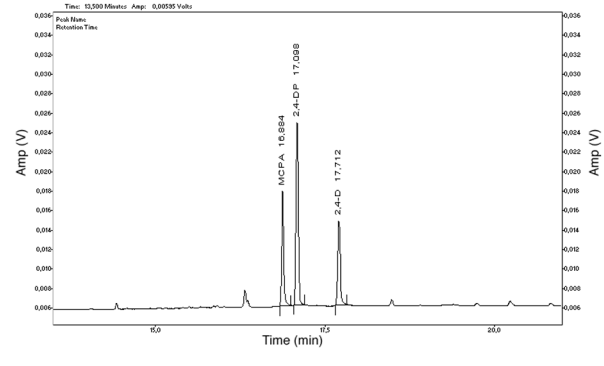

Figure 10. Chromatogram of a water sample spiked at 5 times the LOD with MCPA, 2,4-DP, and 2,4-DP (derivatized with PFBBr) after enrichment with the Carbograph.

tryne, simazine, and terbutryne were analyzed on a Chrompack 9001 GC (Delft, Holland) equipped with a nitrogen–phosphorus selective thermoionic detector. The column was 0.25 mm × 30 m containing a CP-SIL-8CB stationary phase with a coated film thickness of 0.25 µm (Chrompack). The column temperature was 120°C for 1 min and then increased at 10°C/min to 270°C. The temperature of the injector was kept at 80°C for 6 s and then programmed at 10°C/s to 270°C. The detector temperature was 280°C.

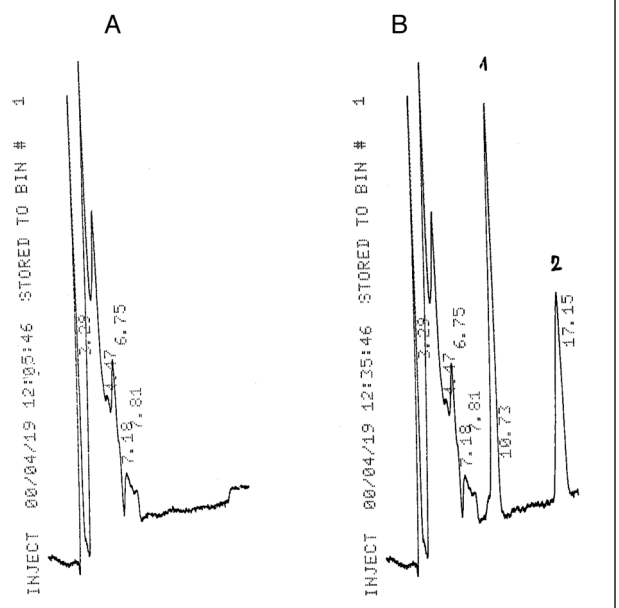

Figure 11. Chromatogram of (A) water and (B) spiked water with (1) isoproturon and (2) chlorbromuron at a level of 5 times the LOD after enrichment with the Carbograph.

The flow rates of nitrogen (carrier gas), hydrogen, and air were 0.75, 4.4, and 140 mL/min, respectively (chromatograms are shown in Figures 5 and 6).

Because acidic compounds (bentazone, 2,4-D, and MCPA) could not be detected directly in the GC system, they were derivatized and either the methyl (9) or pentafluoro benzyl bromide (PFBBr) (10) derivatives were analyzed. Before GC analysis, the derivatives were cleaned on a 2-g Woelm Silica of Super I activity (Silica Woelm, 100–200-µm particle size, Woelm Pharma GmbH, Eschwege, Germany). The silica was mixed with 10 mL *n*-hexane and filled into a 10-mm-i.d. column. After loading the sample into the column, the vial was rinsed with a 2-mL mixture of petrol ether–benzene (3:1). The rinsing mixture was put into the column. Interfering materials were washed with 14 mL of a mixture of petrol ether–benzene (3:1) followed by 4 mL of a 1:1 mixture. Derivatives were eluted with 40 mL of a 1:1 mixture of petrol ether–benzene. After evaporation the residue was analyzed on GC–ECD.

Acetochlor, endosulfan, lindane, metolachlor, propachlor, propisochlor, pendimethalin, trifluralin, and derivatives of bentazone, dichlorprop (2,4-DP), 2,4-D, and MCPA were analyzed on

Table II. Mean Recovery, Standard Deviation, and Relative Standard Deviation Values by the Various Methods Used*

	Liquid–liquid partition				SPE–LiChrolut EN				SPE–ISOLUTE ENV+				SPE–Carbograph			
	R† (%)	SD‡	RSD§ (%)	*n***	R (%)	SD	RSD (%)	*n*	R (%)	SD	RSD (%)	*n*	R (%)	SD	RSD (%)	*n*
Lindane	82	16.4	19.9	14	76	18.6	24.5	20	91	17.6	19.4	5	88	18.4	21.0	13
Endosulfan	90	14.9	16.4	13	75	11.5	15.2	20	88	13.1	14.8	3	89	10.6	12.0	11
Diazinon	82	15.2	18.6	13	79	15.8	19.9	22	83	16.4	19.8	6	79	13.4	17.0	14
Malathion	85	14.1	16.5	18	85	15.1	17.7	21	90	14.4	16.0	5	85	15.2	17.8	27
Carbofuran	87	15.7	17.9	13	82	17.9	21.8	13	94	11.6	12.3	5	76	12.6	16.6	22
Propachlor	82	13.9	16.9	14	80	14.5	18.3	21	87	11.2	12.9	5	76	17.3	22.7	12
Acetochlor	86	14.5	16.9	14	88	13.3	15.1	22	87	15.2	17.5	8	90	16.0	17.7	14
Propisochlor					86	14.3	16.7	3	94	17.1	18.3	3	95	16.4	17.2	4
Metolachlor	88	17.8	20.4	14	91	10.9	12.0	23	91	17.3	19.0	6	98	9.4	9.6	12
Pendimethalin	88	14.9	17.0	14	78	11.3	14.4	18	76	12.0	15.7	6	85	9.0	10.6	11
Trifluralin	79	12.3	15.7	12	64	12.3	19.3	12	70	20.2	29.0	4	79	13.5	17.0	14
Chlorbromuron	86	10.8	12.6	16	81	10.5	13.0	21	81	6.9	8.5	4	93	17.9	19.2	12
Isoproturon	82	8.8	10.8	16	79	14.6	18.5	20	84	10.8	12.9	8	89	14.5	16.2	26
Bentazone	75	14.0	18.6	11	72	14.9	20.6	11	81	16.3	20.2	3	79	15.2	19.2	10
Atrazine	82	8.6	10.6	21	81	11.9	14.7	21	84	14.1	16.9	6	89	9.6	10.7	21
Simazine	87	10.3	11.8	13	79	10.9	13.8	20	85	21.2	25.0	2	90	9.0	10.0	22
Prometryne	86	7.0	8.1	13	80	11.3	14.0	20	85	13.6	16.0	5	92	12.3	13.3	23
Metribuzin	86	7.0	8.1	13	80	11.5	14.3	19	86	14.3	16.7	4	70	10.2	14.6	19
Terbutryne	87	16.6	19.1	17	82	15.0	18.3	21	84	13.7	16.3	6	91	12.8	14.0	22
MCPA	89	10.8	12.2	7	74	15.2	20.5	17	85	10.4	12.3	7	75	13.4	17.8	29
2,4-DP	82	12.7	15.5	11	70	12.3	17.7	19	89	18.2	20.4	8	77	13.0	16.9	30
2,4-D	86	17.0	19.7	12	71	14.0	19.6	23	82	13.6	16.5	7	88	16.2	18.4	28

* Spiking levels were 1–5 times the LOD (0.05–2.5 µg/L).
† R, mean recovery.
‡ SD, standard deviation.
§ RSD, relative standard deviation.
** *n* = number of samples.

the Chrompack 9001 instrument equipped with a ^{63}Ni ECD. The column was 0.25 mm × 50 m containing a CP-SIL-8CB stationary phase with a 0.25-µm film thickness (Chrompack). The column temperature was 120°C for 1 min and then increased at 10°C/min to 270°C. The temperature of the injector was kept at 80°C for 6 s and then programmed at 10°C/s to 270°C. The detector temperature was 280°C.

The flow rates of the carrier gas and the make-up gas (nitrogen) were 0.77 and 34 mL/min, respectively (chromatograms are shown in Figures 7–10).

Chlorbromuron and isoproturon were determined by HPLC on a Waters 490E programmable multiwavelength UV detector set at 245 nm with a sensitivity setting at 0.05 absorbance units full scale. A 244- × 4-mm Lichrospher RP-18 column (Merck) was used with a 70:30 acetonitrile–water mixture as the mobile phase at a flow rate of 0.4 mL/min (chromatograms are shown in Figure 11).

Results and Discussion

Recovery studies were completed at spiking levels of 1–5 times the LOD. Average recovery values, standard deviations, and relative standard deviations for all methods were calculated (11–13), which are summarized in Table II.

Recovery values were well-reproducible and acceptable; the acceptance criteria for recovery prescribed by the Pesticide Residue Analytical Network of National Plant Protection Organization in Hungary (14) is between 70% and 110% with a relative standard deviation below 20%. The most uniform recovery data for the studied pesticides were obtained by the liquid–liquid partition method. Lower recovery values were obtained with the LiChrolut EN cartridge (especially for acidic compounds), but those still were acceptable. ISOLUTE ENV+ and Carbograph cartridges provided somewhat higher recovery values than the LiChrolut EN cartridge, very similar to the results obtained by the liquid–liquid partition method. However, the differences were not significant. The SPE technique seems to be as good as the liquid–liquid partition to extract the investigated pesticides from water samples, but requires less solvent and therefore is less harmful to human health. The application of cartridges is especially useful if a great number of samples have to be analyzed, because depending on the capacity of the vacuum manifold a few samples can be extracted simultaneously.

There are a few factors to be considered when using the SPE technique. A proper flow rate should be selected. If it is too fast, it can result in low recoveries because of breakthrough during the retention step, dirty extracts when interfering components are eluted, and inadequate elution during the elution step.

According to our findings water samples can be loaded at a flow rate of 5–10 mL/min, which means a 2–3-h loading time for a 1-L sample.

Another important point is to remove the residual water remaining in the column after extraction and before the elution. Drying by vacuum for approximately 20 min is required for each cartridge tested. If the cartridge is not dried properly it can result in low recovery (less than 40%) for many compounds.

In the case of the Carbograph SPE, this step is especially critical. Only a fraction of the residual water can be removed by vacuum. The remaining water then can be removed by passing 1 mL of methanol. However, methanol washing can cause some loss of the compounds showing relatively low retention on the cartridge. Therefore, we first washed the cartridge with 1 mL of a 1:1 mixture of methanol–water and then we passed 1 mL of methanol collected together with the mixture of methylene dichloride and methanol.

We have found that the Carbograph SPE cartridge was the most cost- and time-effective among the tested cartridges for our purposes.

The main advantages of using the Carbograph SPE technique are: (*a*) very polar compounds can be quantitatively extracted from large volumes of the water sample (5), (*b*) acidic compounds can be isolated from the basic–neutral compounds by two different consecutive elution steps using a single cartridge and sample loading can be performed in one step; and (*c*) before the extraction of the acidic compounds no pH adjustment is required.

Because more than one liter of the water sample can be passed through the Carbograph SPE cartridge (5), its applicability should be particularly aimed at the extraction of compounds having a very low concentration (i.e., sulfonil urea compounds) in surface water.

Acknowledgments

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