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Pesticide monitoring of drinking water with the help of solid-phase extraction and high-performance liquid chromatography

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Abstract

This paper describes the results of a comparison of extraction efficiency between a new polymeric sorbent for solid-phase extraction (SPE) and classical RP-18. Pesticides and their hydrophilic metabolites in the low ppb range have been chosen as target compounds to be extracted from large volume samples. The pesticides were separated using HPLC and detected by diode array detection.

Furthermore, a method is presented regarding the determination of carbendazim in water, using SPE on a mixed RP/polymer phase and high-performance liquid chromatography. Recovery, working range, precision and the detection limit are presented.

Keywords: Water analysis; Environmental analysis; Pesticides

1. Introduction

In recent years, people have become more conscious of the risks and dangers arising from the intense use of pesticides on large areas of agricultural land. One consequence of this has been the implementation of drinking water guidelines in many European countries, with legal tolerance levels of 0.1 ppb for each individual substance and 0.5 ppb for the sum of pesticides (including their main metabolites) [1].

HPLC and GC, subsequent to appropriate enrichment processes, have become the procedures of choice for monitoring drinking water. With respect to enrichment, solid-phase extraction (SPE) is now established in the analytical chemistry laboratory and has largely replaced classical liquid–liquid extraction due to many advantages which are cited below [2,3]:

1. sampling in the field,
2. speed and simplicity in the off-line mode,
3. no formation of emulsion,
4. safety with respect to hazardous samples,
5. low costs
6. and flexibility.

Conventional SPE using RP solid-phases sometimes produces less than satisfactory results — based on the extraction of highly polar contaminants from large water volumes — as breakthrough of these analytes occur. Two criteria are responsible for breakthrough during the enrichment step; retention of the analyte and the sorbent capacity [4].

An alternative sorbent material for SPE in environmental analysis has been developed. This adsorbent is based on a highly cross-linked, porous ethylvinylbenzene–divinylbenzene copolymer and

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exhibits a unique adsorption efficiency due to its high specific surface area. This sorbent is characterized by an approximately ten-fold higher capacity for a variety of analytes than RP-18-modified silica gels have. This makes it especially suitable for environmental sample preparation [5–7]. Therefore, the sorbent capacities have been determined comparatively — for hydrophilic molecules by the caffeine capacity and for hydrophobic molecules by the capacity for diisodecyl phthalate [8].

This article has two further objectives. The first one is that of comparing the abilities of the new LiChrolut® EN sorbent and a conventional RP-18 solid-phase. The second is that of demonstrating a solution to the problem of extracting and analysing a selected carbamate fungicide, namely carbendazim, in drinking water.

2. Experimental

2.1. SPE

A LiChrolut extraction manifold with a drying attachment from Merck (Darmstadt, Germany) was used. LiChrolut extraction cartridges (LiChrolut EN, 200 mg, 3 ml and LiChrolut RP-18, 1 g, 8 ml) were conditioned with one column volume of methanol and one column volume of water, respectively. The moist adsorbent was used for the enrichment step. The column should not be allowed to run dry during the conditioning phase. For calculating the recovery rates, a sample of tap water (pH 5.5–6.0), containing a 33-multicomponent pesticide standard (200 ng/l per pesticide), was used. The water sample was run through the conditioned solid-phase material at a flow-rate of approximately 5 ml/min. Subsequent to this enrichment procedure, 1 ml of water was used to flush the column and any residual water was removed by purging with nitrogen (approx. 2 bar for 15 min in the case of EN and 40 min in the case of RP-18.). Batches of 2 × 3 ml methanol–ethyl acetate mixture (1:1, v/v) were used to perform the elution. During the initial elution phase, no vacuum was used; this allowed the process to proceed optimally. Excess solvent was evaporated off, the residue dissolved in 1 ml of a mixture of acetonitrile and ammonium acetate (20:80, v/v) and filtered through an Anotop® membrane filter (Merck) into an autosampler vial. A 100-μl aliquot of this sample was injected into the HPLC column.

For SPE of carbendazim, LiChrolut extraction cartridges (EN, 200 mg, 3ml; RP-18, 500 mg, 3 ml; a mixture of 100 mg of EN and 100 mg of RP-18) were applied. Each of them was activated with 3 ml of methanol and subsequently was conditioned with 3 ml of water and with 3 ml of water which was adjusted to pH 4.0 with hydrochloric acid (32%, w/v). One liter of spiked tap water was forced to pass through the cartridge within 3 h. After washing with 1 ml of water and drying with nitrogen for 15 min, preconcentrated carbendazim was eluted twice with 3 ml of methanol and once with 1 ml of methanol.

2.2. HPLC analysis

Complete separation of the 33 pesticide components was achieved using a LiChroCART® 250-4 Superspher® 100 RP-18 column (Merck) and an acetonitrile–ammonium acetate gradient, at a temperature of 28°C (Fig. 1 and Fig. 2). For gradient analysis, a LiChrograph® HPLC system (Merck-Hitachi, Darmstadt, Germany) was used, including pumps and an autosampler. A diode array detector (DAD) was used and provided optimal detection of substances absorbing at different wavelengths (λ = 220 and 245 nm). The recovery rates of the individual pesticides were calculated by using the respective peak area and calibration line obtained for each compound of the multicomponent standard.

HPLC analysis of carbendazim was isocratically performed on a Purospher® RP-18 column (125 × 4 mm I.D., 5 μm, Merck) with a mobile phase consisting of acetonitrile–water (20:80, v/v) at ambient temperature (Fig. 3 and Fig. 4 and Fig. 5). The injection volume was 100 μl and the flow-rate was 0.7 ml/min. Carbendazim was detected by using UV–DAD detection at a fixed wavelength of 220 nm.

2.3. Reagents and chemicals

The pesticides added to the tap water were atrazine, bifenox, bromacil, carbendazim, carbetamide, chloridazon, chloroxuron, chlorpropham, chlor-

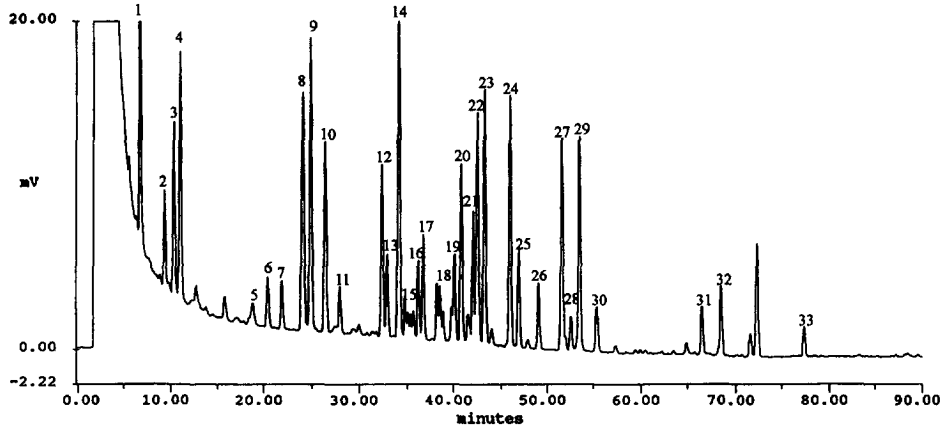

Fig. 1. HPLC chromatogram (UV 220 nm) obtained by analyzing a 1-l sample of tap water spiked with selected pesticides (200 ng/l) after SPE on LiChrolut EN. HPLC parameters: Column, LiChroCART 250-4 Superspher 100 RP-18, 4 μm I.D.; Mobile-phase A, acetonitrile; Mobile-phase B, ammonium acetate (1 mmol/l); Gradient, 25% A (at the start, then 10 min isocratic at 25% A, 60 min linear to 70% A, then 20 min linear to 90% A and 10 min isocratic at 90% A; Temperature, 28°C; Injection volume, 100 μl; Flow-rate, 0.7 ml/min. UV–DAD detection, 200–320 nm; spectral bandwidth, 4 nm. Mixing chamber volume, 1.1 ml.

toluron, crimidine, cyanazine, deethylatrazine (DEA), deethylterbutylazine, deisopropylatrazine (DIPA), dimefuron, diuron, isoproturon, karbutilate, linuron, methabenzthiazuron, metamitron, metazachlor, methoprotryne, metobromuron, metolachlor, monolinuron, pencycuron, pendimethalin, prometryne, propazine, sebutylazine, simazine, terbutryne and terbutylazine. These compounds were supplied by Riedel-de-Haen (Hannover, Germany). The 33-multicomponent standard solution was prepared by dissolving 20 mg of each pesticide (without carbendazim) in 100 ml of acetonitrile. A stock solution of carbendazim was prepared by dissolving 18.18 mg of carbendazim in 200 ml of acetonitrile (90.9 μg/ml). A second dilution yields a stock solution with a concentration level of 1.818 μg/ml. Further dilutions were prepared as calibration standards.

For HPLC analysis, gradient grade acetonitrile LiChrosolv® and water LiChrosolv were obtained from Merck. Ammonium acetate (analysis grade) and hydrochloric acid [32% (w/v), extra pure] were also supplied by Merck.

All LiChrolut extraction cartridges and LiChrolut

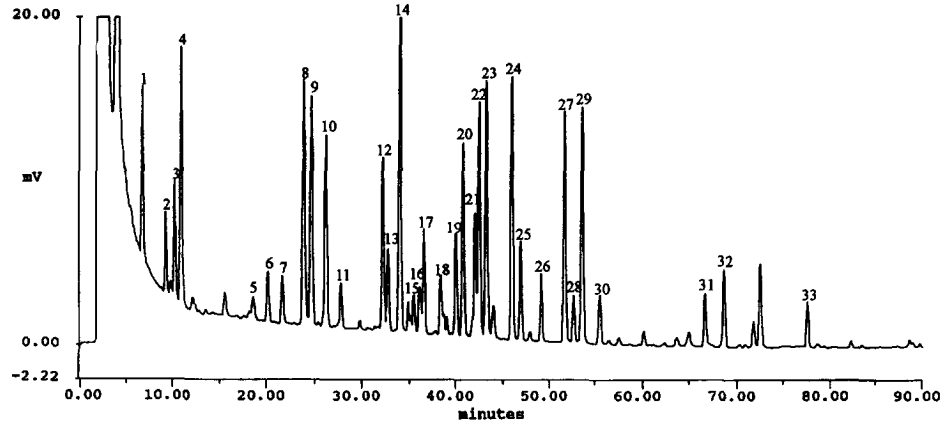

Fig. 2. HPLC chromatogram (UV 220 nm) obtained by analyzing a 1-l sample of tap water spiked with selected pesticides (200 ng/l) after SPE on LiChrolut RP-18 (for HPLC parameters see Fig. 1; for peak numbers see Table 1).

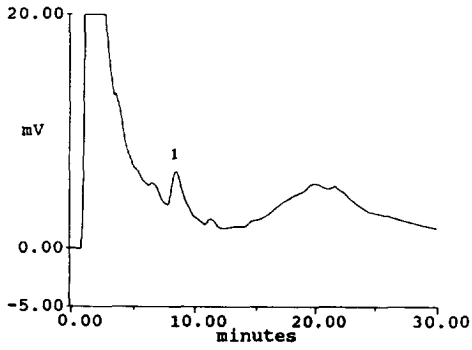

Fig. 3. HPLC chromatogram (UV 220 nm) obtained by analyzing a 1-l sample of tap water spiked with carbendazim (290 ng/l) after SPE on LiChrolut EN. HPLC parameters: Column, LiChroCART 125-4 HPLC cartridge, Purospher RP-18, 5 μm I.D.; Mobile phase A, acetonitrile; Mobile phase B, water (A/B, 20:80, v/v); Temperature, ambient; Injection volume, 100 μl; Flow rate, 0.7 ml/min; UV–DAD detection, 200–320 nm; spectral bandwidth, 4 nm; Fixed wavelength, 220 nm; Mixing chamber volume, 1.1 ml.

sorbents were supplied by Merck. All solvents for solid-phase extraction (methanol and ethyl acetate) were of LiChrosolv HPLC quality and also were purchased from Merck.

2.4. Aqueous sample preparation

2 ml of the multicomponent standard solution were diluted to 200 ml with acetonitrile and 5 ml of this solution were further diluted to 50 ml with acetonitrile (200 ng/ml). Spiked tap water samples were prepared by adding 10 ml of the last multi-component standard concentration level to 10 l of drinking water to give a concentration of 200 ng/l.

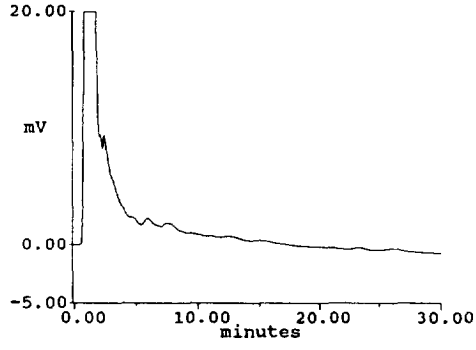

Fig. 4. HPLC chromatogram (UV 220 nm) obtained by analyzing a 1-l sample of tap water spiked with carbendazim (290 ng/l) after SPE with LiChrolut RP-18 (for HPLC parameters see Fig. 3).

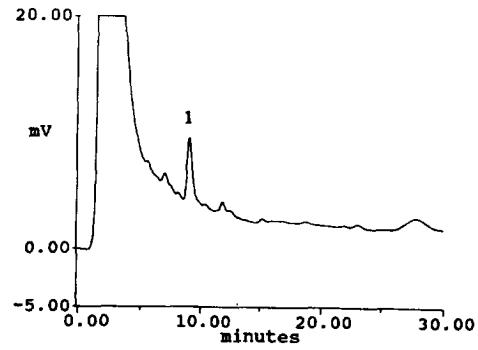

Fig. 5. HPLC chromatogram (UV 220 nm) obtained by analyzing a 1-l sample of tap water spiked with carbendazim (290 ng/l) after SPE with a mixture of LiChrolut® EN and LiChrolut® RP-18 (for HPLC parameters see Fig. 3).

For carbendazim trace analysis in tap water, 5 ml of standard solution with a concentration level of 290.9 ng/ml were added to 5 l of tap water, which was adjusted to pH 4.0 with hydrochloric acid.

3. Results and discussion

Off-line SPE of pesticides belonging to the triazine and phenylurea groups from 1 l water samples, using C_{-18} reversed phase supports has been reported [9]. Their retention on hydrophobic supports is mainly due to Van der Waals interactions. However, this adsorption mechanism does not apply to their hydrophilic metabolites (e.g. DIPA and DEA) which are very water-soluble and less retained on hydrophobic supports (log $k_{o/w} \approx 0$ or negative). To prevent the loss of *s*-triazine compounds in particular, a combination of stationary phases (C_{-18}–cation exchanger) should be selected [10] or the sample volume should be small. Consequently, low enrichment factors are obtained that are not sufficient for trace analysis. These drawbacks can be largely overcome by changing the sample volume/sorbent ratio, e.g. by increasing the quantity of adsorbent used (Table 2) and by adding a certain amount of an indifferent electrolyte (e.g. NaCl) to the aqueous sample ("salting-out-effect") [2]. Particularly in routine laboratories with a high sample throughput,

these two alternatives create additional waste and significantly increase the costs because bigger quantities of sorbent and organic eluents are needed.

The best recoveries, even for large water volumes and especially for polar pesticides, were obtained by using a thoroughly purified, highly cross-linked ethylvinylbenzene–divinylbenzene copolymer (LiChrolut EN) as the support for SPE. This outstanding sorbent has a specific surface area of approximately 1200 m^2/g. Due to its microporous structure, it exhibits a high adsorptive capacity, especially for components with low molecular mass. Therefore, in comparison to C_{-18}-modified silicas, a much higher number of active sites is available for the target analytes. For efficient extraction of organics from aqueous solutions it is necessary to obtain a maximum surface contact with the components being extracted. Conventional porous polystyrene resins also have a hydrophobic surface, as has C_{-18} sorbent itself. Chemically bonded polar acetyl or hydroxymethyl groups provide a more hydrophilic surface so that extraction of polar components becomes more effective [11]. In contrast, the new LiChrolut EN copolymer exhibits hydrophilic

Table 1
Recovery (%) of selected pesticides and pesticide metabolites extracted from 1 l of spiked tap water after SPE on 1000 mg of LiChrolut RP-18 and 200 mg of LiChrolut EN

	1000 mg LiChrolut RP-18			200 mg LiChrolut EN	
	Recovery rate (%)	R.S.D. (%) $n = 6$	Peak No.	Recovery rate (%)	R.S.D. (%) $n = 3$
DIPA (Deisopropylatrazine)	59	3.0	01	102	1.8
Metamitron	74	3.0	02	87	3.9
Chloridazon	67	3.5	03	95	2.2
DEA (Deethylatrazine)	108	0.8	04	108	2.5
Crimidine	90	7.8	05	93	6.9
Carbetamide	97	2.8	06	97	1.7
Bromacil	99	1.8	07	99	4.6
Simazine	97	2.2	08	95	2.6
Cyanazine ($n = 3$)	107	3.5	09	108	0.1
Deethylterbutylazine	97	3.0	10	99	4.0
Karbutilate	96	1.1	11	96	1.0
Methabenzthiazuron	97	1.4	12	99	1.7
Chlortoluron	97	0.7	13	97	2.6
Atrazine	102	2.3	14	100	3.2
Monolinuron	95	2.4	15	96	1.7
Isoproturon	97	0.8	16	97	1.7
Diuron	100	0.7	17	99	2.1
Metobromuron	96	1.5	18	96	3.1
Metazachlor	112	3.6	19	100	1.9
Methoprotryne	95	1.7	20	88	3.0
Dimefuron ($n = 3$)	121	3.6	21	108	1.8
Sebutylazine	101	2.4	22	101	2.3
Propazine	99	3.6	23	101	3.1
Terbutylazine	97	2.2	24	96	1.2
Linuron	98	1.1	25	97	2.5
Chloroxuron	103	1.9	26	103	3.8
Prometryne	94	2.8	27	89	6.0
Chlorpropham ($n = 4$)	93	6.7	28	81	4.3
Terbutryne	99	2.0	29	93	3.1
Metolachlor	95	3.0	30	95	3.2
Pencycuron	113	3.4	31	112	3.5
Bifenox	116	2.3	32	108	4.9
Pendimethalin	96	10.2	33	98	13.2

properties without any surface modification. Consequently, laborious conditioning prior to sample injection is not necessary. It could be shown that this adsorbent has the highest efficiency for enrichment of polar molecules. Various groups of compounds were tested, including nitroaromatics [12], phenolic compounds [13] and non-ionic detergents [14]. Dissociated organic compounds are only weakly adsorbed, but this can be circumvented by suppression of the ionization. In our investigations, parameters like flow-rate during sample injection onto the cartridge and elution strength and volume of the eluent have been optimized for the maximum recovery of the most polar pesticide, namely DIPA.

The HPLC chromatograms of the pesticide standards, after addition to one liter of tap water and SPE on LiChrolut EN and RP-18, are shown in Fig. 1 and Fig. 2, respectively. The compounds are separated with good resolution and peak shape by gradient HPLC using a simple mobile-phase containing acetonitrile and ammonium acetate. This gradient is also useful for the baseline separation of some phenylureas e.g. of diuron and metobromuron which are well-known as problem substances. Furthermore, terbutylazine is well separated from linuron. To facilitate compound identification in unknown samples, diode array spectra taken from each peak were compared with the reference spectra of pure standards.

Table 1 shows the percentage recoveries together with relative standard deviations obtained for 33 selected pesticides from spiked tap water at the concentration level of 200 ng/l after SPE on RP-18 as well as on polymer EN sorbents. The comparison clearly reveals that 200 mg of polymer sorbent are sufficient to obtain quantitive recoveries, especially of the most polar pesticides, DIPA (102 ± 2%) and metamitron (87 ± 4%), which is in accordance with the significantly higher sorbent capacity for analytes. The recoveries of other pesticides were also satisfactory, varying between 108 ± 3% for DEA and 81 ± 4% for chlorpropham. Repeatability of this determination is good with the exception of crimidine (due to some impurities in the chromatogram and lack of detection sensitivity at the selected wavelength) and pendimethalin (due to some loss of analyte during evaporation and impurities in the HPLC chromatogram).

In Table 2 and Table 3, recoveries of pesticides are listed, which were obtained by applying higher eluent volumes and larger quantities of RP-18 sorbent mass. Generally speaking, for 200 mg of LiChrolut EN (Table 2) 6 ml of eluent volume are sufficient to obtain consistent recovery rates at a pesticide concentration level of 200 ng/l. For chlorpropham, the recovery was improved whereas for crimidine, carbetamide, bromacil and karbutilate, it was decreased. For metamitron, the recovery seems

Table 2
Percentage recoveries of pesticides after SPE on LiChrolut EN and HPLC analysis when applying a higher eluent volume

Stationary phase	LiChrolut EN 200 mg	LiChrolut EN 200 mg
Eluent volume	6 ml	10 ml
	Recovery (%) $n = 3$	Recovery (%) $n = 3$
Deisopropylatrazine	102	103
Metamitron	87	82
Chloridazon	95	94
Deethylatrazine	108	110
Crimidine	93	78
Carbetamide	97	73
Bromacil	99	92
Simazine	95	96
Cyanazine	108	125
Deethylterbutylazine	99	98
Karbutilate	96	84
Methabenzthiazuron	99	98
Chlortoluron	97	97
Atrazine	100	102
Monolinuron	96	93
Isoproturon	97	97
Diuron	99	100
Metobromuron	96	95
Metazachlor	100	112
Methoprotryne	88	93
Dimefuron	108	104
Sebutylazine	101	101
Propazine	101	102
Terbutylazine	96	97
Linuron	97	98
Chloroxuron	103	101
Prometryne	89	96
Chlorpropham	81	94
Terbutryne	93	101
Metolachlor	95	95
Pencycuron	112	103
Bifenox	108	128
Pendimethalin	98	90

Table 3
Percentage recoveries of pesticides after SPE on LiChrolut RP-18 and HPLC analysis when applying larger quantities of sorbent and a higher eluent volume

Sorbent mass	LiChrolut RP-18 1 g	LiChrolut RP-18 2 g	LiChrolut RP-18 2 g
Eluent volume	6 ml	6 ml	10 ml
	Recovery (%) $n = 3$	Recovery (%) $n = 3$	Recovery (%) $n = 3$
Deisopropylatrazine	59	101	104
Metamitron	74	76	96
Chloridazon	67	91	91
Deethylatrazine	108	107	111
Crimidine	90	86	82
Carbetamide	97	98	92
Bromacil	99	97	100
Simazine	97	95	96
Cyanazine	111	113	103
Deethylterbutylazine	97	95	98
Karbutilate	96	94	91
Methabenzthiazuron	97	96	101
Chlortoluron	97	98	99
Atrazine	102	99	102
Monolinuron	95	90	92
Isoproturon	97	95	97
Diuron	100	100	100
Metobromuron	96	93	95
Metazachlor	112	108	106
Methoprotryne	95	95	96
Dimefuron	116	110	105
Sebutylazine	101	98	100
Propazine	99	96	99
Terbutylazine	97	94	96
Linuron	98	101	98
Chloroxuron	103	103	104
Prometryne	94	97	96
Chlorpropham	90	86	89
Terbutryne	99	103	98
Metolachlor	95	90	93
Pencycuron	113	116	118
Bifenox	116	124	126
Pendimethalin	96	93	100

to be unaffected by higher volumes, due to some kind of irreversible adsorption. Systematic tests demonstrated that metamitron was neither found in the water after soaking the sample through the cartridge nor in the eluent. It is possible that this component will be irreversibly retained in the micropores of the sorbent, due to steric effects during the drying procedure.

In the case of the RP-18 stationary phase (Table 3), the recovery rate for DIPA from 1 l of tap water increases from 59 to 101%, when the mass of sorbent is increased up to 2000 mg. It can be stated that the use of higher eluent volumes and larger quantities of RP-18 sorbent lead to almost quantitative recoveries in the case of the polar pesticides, e.g. metamitron and chloridazon.

The HPLC determination of benomyl (a systemic fungicide) and carbendazim (its degradation product) in water was recently published [15]. Carbendazim in particular, plays also an important role as a

fungicide controlling a wide range of pathogens of cereals, fruits and vegetables [16]. In our investigations carbendazim could be efficiently extracted from drinking water samples by SPE using LiChrolut EN. Optimum recovery (97 ± 2%, $n=4$) was achieved in weak acid medium at pH 4, which is found for tap water samples. RP-modified solid-phases are also suitable, although a lower recovery was found. Serious problems can occur arising from aqueous samples having a highly dissolved organic carbon (DOC) content. In this case, low recoveries are obtained as a result of the limited capacity of RP-18. This difficulty could be overcome by increasing the mass of the sorbent. Nevertheless, this does not represent economical SPE because the time needed for extracting the water sample and the time for drying the sorbent bed is significantly increased. On the contrary, LiChrolut EN has ten times the loading capacity of any bonded silica phase. However, at a pH of 4, humic acids are co-extracted and therefore will interfere with the future HPLC analysis. The most economical solution to this problem is to prepare a mixture of RP-18 and EN sorbent material — this means highest recoveries and lowest interferences from co-eluted components.

For calculation of the recovery of carbendazim, a linear calibration graph ($y = -1392 + 607.8\,x$; $r = 0.9999$) was used in the working range of 0.04 to 0.5 mg/l and the detection limit was 0.007 mg/l (determined for a signal/noise ratio of 4:1).

4. Conclusions

Pesticides in the low ppb range can be efficiently concentrated from large water samples by SPE with LiChrolut EN. Even for components that are difficult to extract, e.g. DIPA and DEA, quantitative recoveries were attained. Recoveries for metamitron, metoprotryne, prometryne and chlorpropham from water were in the range of 81 to 89%. Only in the case of chlorpropham was the recovery rate improved by using a larger eluent volume. Furthermore, for selected compounds, comparative extraction efficiencies between C_{18} and polymer SPE cartridges were obtained. One gram of LiChrolut RP-18 is not sufficient to achieve quantitative recoveries of polar components, e.g. of DIPA. This problem was circumvented by increasing the mass of the sorbent (up to 2000 mg).

The recovery of 0.29 ppb of carbendazim from a spiked tap water sample was 97% and the R.S.D. was 2.0%. The method was applied to the analysis of drinking water and surface water. Problems arising from high contents of humic acid compounds were circumvented by using a mixed RP–EN stationary phase.

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