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# Comparison of different sorbents for on-line liquid–solid extraction followed by high-performance liquid chromatographic determination of nitrogen-containing pesticides

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## Abstract

LiChrolut EN [poly(styrene–divinylbenzene), PSDVB], Carbograph (graphitized carbon black, GCB), Isolute CN (cyanopropylsilica), Isolute C<sub>2</sub> (ethylsilica), and LiChrospher RP18 (octadecylsilica) were studied for on-line solid-phase extraction of twelve nitrogen containing pesticides from water. Determination (UV 210 nm) was performed with a Spherisorb C<sub>8</sub> analytical column and an acetonitrile–water gradient. The mean recoveries from 50 ml ranged from 83% for RP18 to 44% for GCB, and decreased in the following order: RP18, CN, LiChrolut EN, C<sub>2</sub>, and GCB. GCB showed poor recoveries due to incomplete desorption with the acetonitrile–water gradient used, and memory effects were detected. Backflush was needed to elute selected pesticides from GCB and from LiChrolut EN. Breakthrough volumes were 50–100 ml for most selected pesticides with silica-based phases, whereas LiChrolut EN allowed larger sample volumes without observable losses. © 1998 Elsevier Science B.V. All rights reserved.

**Keywords:** Extraction methods; Sorbents; Pesticides; Carbamates; Iprodione

## 1. Introduction

Since pesticides are present in water at low concentrations they need to be enriched before analysis. In recent years, liquid–liquid extraction (LLE) techniques have been displaced for this purpose by solid-phase extraction (SPE) procedures.

To determine low levels of pesticides, gas chromatographic techniques are largely preferred. However, compounds with low volatility and thermal instability such as polar pesticides are usually determined by liquid chromatography (LC). Among the detectors used for on-line LC procedures, the diode

array detector (DAD) [1–3] is the one most frequently cited because it can be economically used in setting up methods or analysing samples in routine methods. Because of its limited selectivity its positive samples must be confirmed.

In the present study, twelve pesticides (oxamyl, methomyl, dioxacarb, carbendazim, methylthiophanate, carbofuran, carbaryl, dietofencarb, molinate, fenothiocarb and thiobencarb), and a carboximide (iprodione) were included. They all have polar or medium polar properties. To extract them from waters by SPE with off-line procedures, several phases such as octadecylsilica C<sub>18</sub> [4–12], low carbon octadecylsilica C<sub>18</sub>/OH [4], octylsilica C<sub>8</sub> [4,8,10,13,14], cyanopropylsilica CN [10], ethyl

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silica C<sub>2</sub> [10], phases based on polystyrene divinylbenzene polymers (PSDVB) [6,15,16], and carbon phases (graphitized carbon black, porous graphitic carbon) [5–7,9,11,12,15] were used.

On-line procedures were developed to improve reproducibility, reduce manual work and avoid losses during the evaporation step. Earlier on-line procedures directly concentrated the target analytes at the head of the analytical column with a precolumn [17]. Recently, automated systems have made it possible to perform SPE with a true extraction cartridge followed by desorption and further analysis on the analytical column in an on-line process [1–3].

Reported on-line procedures for the determination of medium to polar pesticides include the use of PSDVB extractive phases coupled to C<sub>18</sub> [2,3,18], C<sub>8</sub> [1,19,20], PLRP-S [2] or PGC [3] analytical columns, C<sub>18</sub> extractive phases coupled to C<sub>8</sub> analytical column [1,20], low carbon C<sub>18</sub>/OH extractive phases coupled to C<sub>8</sub> analytical column [19], and C<sub>8</sub> extractive phases coupled to C<sub>8</sub> analytical column [21].

In the present study, the ability of several solid sorbents, including a poly(styrene–divinylbenzene) support (LiChrolut EN), a graphitized carbon black GCB (Carbograph), and the silica based phases LiChrospher RP18, Isolute C<sub>2</sub>, and Isolute CN, were examined for the sorption and desorption of eleven nitrogen containing pesticides and iprodione from water using the OSP-2A on-line extraction system.

## 2. Experimental

### 2.1. Reagents

Pesticide standards, carbaryl, carbendazim, carbofuran, dietofencarb, dioxacarb, fenothiocarb, iprodione, methomyl, methylthiophanate, molinate, oxamyl and thiobencarb were obtained from Promocem (Wesel, Germany). All standards were at least 95% pure.

Stock solutions were prepared by diluting standards with methanol to obtain a concentration of 500 µg/ml. A working standard solution containing 12 µg/ml of each pesticide was prepared in methanol. Water samples were spiked with the analytes at a concentration of 1.2 µg/l and were freshly used.

A supragradient HPLC grade acetonitrile was acquired from Scharlau (Barcelona, Spain). HPLC-grade water was obtained by filtering deionized water through a 0.45-µm filter with a Waters-Millipore (Milford, MA, USA) system. Acetonitrile and water were degassed for 15 min using a Branson 5200 (Branson Ultrasonic Corporation, Danbury, CT, USA) ultrasonic bath.

### 2.2. Apparatus

Two LaChrom L-7100 intelligent pumps, an OSP-2A automatic sample preparation appliance, a LaChrom L-7400 programmable wavelength detector and a D-2500 integrator all from Merck-Hitachi (Darmstadt, Germany) were used.

Extractive cartridges (10.0×4.6 mm I.D.) for the OSP-2A unit were packed, with LiChrolut-EN (Merck) (40–120 µm particle size, 1200 m<sup>2</sup>/g specific surface), Carbograph (Alltech Associates, Carnforth, UK) (37–150 µm particle size, 100 m<sup>2</sup>/g surface area), LiChrospher RP18 (Merck) (10 µm monofunctional not end capped C<sub>18</sub>), Isolute C<sub>2</sub> (International Sorbent Technology-IST, Hengoed, UK) (40 µm trifunctional end capped C<sub>2</sub>) and Isolute CN (IST) (40 µm difunctional end capped CN).

The analytical column was a Spherisorb C<sub>8</sub> (150×4.6 mm I.D., 3 µm) from Phase Separations, Wadinxveen, Netherlands.

### 2.3. Analytical procedure

Sorption, desorption and determination was performed on-line with the OSP-2A unit. The on-line system with forward and backward desorption is shown in Fig. 1. Extraction of water samples was carried out at a flow-rate of 5 ml/min. After extraction, the cartridge elution and analytical separation was achieved at 0.7 ml/min with a water–acetonitrile gradient. Solvent program was started with 23% acetonitrile, held for 0.1 min, followed by a linear gradient to 45% acetonitrile in 14.9 min, and another linear gradient to 75% acetonitrile in 25 min. The whole trace enrichment procedure was executed whilst the analytical column was re-equilibrated. Pesticides eluting from the analytical column were detected at 210 nm.

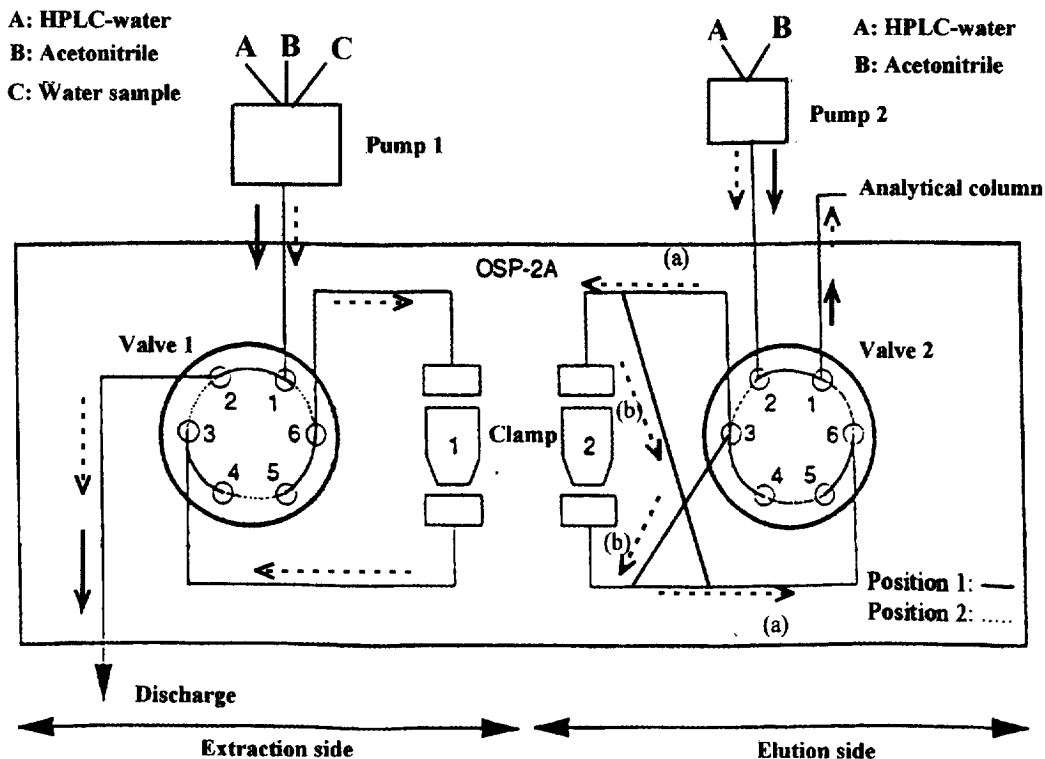


Fig. 1. Set-up of the on-line trace enrichment of aqueous samples with HPLC separation and UV detection in forward mode (a) and backflush mode (b).

### 3. Results and discussion

#### 3.1. Poly(styrene–divinylbenzene)

LiChrolut EN is a highly cross-linked styrene–divinylbenzene copolymer. Its structure allows it to establish strong interactions between the own  $\pi$  electrons and those from the pesticide. This characteristic makes LiChrolut EN a unique extractive phase with great possibilities for extracting a wide variety of pesticides.

Elution was carried out in backflush mode. Forward elution generated a very intense band broadening which overlapped the peaks, thus making the chromatograms useless. Most PSDVB phases were eluted by backflushing the cartridge [1,18,19,22]. In the present study, a band-broadening effect was observed on the chromatograms and, as a consequence, the dietofencarb–molinate and iprodione–thiobencarb pairs were not resolved. Therefore, to

quantify them, new runs had to be performed with a wavelength program monitoring dietofencarb at 240 nm and iprodione at 250 nm. In such conditions molinate and thiobencarb are not detected.

Band broadening occurs when a PSDVB based phase is coupled to a different kind of analytical column on which retention is quite different [3].

Recoveries obtained for a deionized water samples spiked at a level of 1.2  $\mu\text{g/l}$  are shown in Table 1. R.S.D. ( $n=4$ ) values are between 1 and 15%. Breakthrough volumes are reached for oxamyl and carbaryl with 200 ml, and for methomyl, dioxacarb, methylthiophanate, iprodione and carbofuran with 400 ml. For the other selected pesticides recoveries did not decrease even when 400 ml of sample was extracted. Junker-Buchheit et al. explain that LiChrolut EN has ten times the loading capacity of any bonded silica phase and the better loading capacity implies high breakthrough volumes [16].

Recoveries are good except in the case of moli-

Table 1

Limits of detection (LODs), recoveries and relative standard deviation (R.S.D.%,  $n=4$ ) of selected pesticides for the extraction with LiChrolut EN cartridges of 50–200 ml of spiked deionized water samples (1.2  $\mu\text{g/l}$ )

Pesticide	LODs (ng/l)	Recovery (%)		
		Extracted water volumes (ml)		
		50	100	200
Oxamyl	30	102	100	85
Methomyl	40	81	76	74
Dioxacarb	90	90	92	87
Carbendazim	260	81	80	79
Thiophanate-methyl	200	95	99	86
Carbofuran	60	83	80	81
Carbaryl	90	90	92	77
Dietofencarb	60	82	75	74
Molinate	110	65	59	57
Fenothiocarb	100	50	49	47
Iprodione	70	32	27	26
Thiobencarb	110	51	45	47

nate, fenothiocarb, iprodione and thiobencarb. The retention mechanism is unclear and the literature does not suggest any general behaviour.

### 3.2. Graphitized carbon black

Carbograph can be considered a nonpolar phase, but its retention mechanisms, which are not well understood, include strong interactions with polar, nonpolar and ionic compounds.

Elution was performed in the backflush mode. When 50 ml of spiked deionized water samples (1.2  $\mu\text{g/l}$ ) were extracted with GCB cartridge, only oxamyl, methomyl and methylthiophanate showed recoveries over 70%. The behaviour of oxamyl and methomyl was similar to that reported by other authors using Carbopack B [23].

In addition, the use of the carbograph cartridge increased peak widths. To minimise this band broadening effect some authors proposed the use of a PGC analytical column [24], but others reported persistent adsorptions on it [22].

### 3.3. Silica based phases

LiChrospher RP18, with a total carbon coverage about 18%, possesses the strongest nonpolar property. Thus, main interactions with pesticides are governed by Van der Waals forces. Nevertheless, since it

is not end capped, a few residual silanol groups could act with typical polar properties. Isolute C<sub>2</sub> (with a 2.7% total carbon coverage) has nonpolar properties weaker than those of C<sub>18</sub>. Isolute CN (with a 6.5% total carbon coverage) is classified as a moderately nonpolar neutral phase. Polar interactions include hydrogen bonds, and  $\pi$ - $\pi$  and dipole-dipole interactions.

Tables 2–4 show the sorption and desorption behaviour of the studied silica based supports with the selected pesticides. When a sample volume of 50 ml (1.2  $\mu\text{g/l}$  level) was passed through the SPE cartridges, the recoveries obtained with RP18, C<sub>2</sub> and CN bonded silica were in the range 62–103% (R.S.D. 2–8%), 37–101% (R.S.D. 4–11%) and 33–104% (R.S.D. 2–14%), respectively. Comparing these data, the best overall results were obtained with the RP18 phase (83% as a mean). RP18 was the only cartridge that was not manually packed. This fact affects the R.S.D.%, which were slightly lower with RP18 than with the other silica based phases, but not the recoveries.

Of the selected silica-based phases, CN and C<sub>2</sub> are the most polar. These phases can be used in reversed-phase as well as in normal-phase mode due to their short alkyl-chain length [25]. Because of their polarity, both CN and C<sub>2</sub> extract hydrophilic pesticides such as oxamyl ( $\log K_{ow}$  -0.47) or methomyl ( $\log K_{ow}$  0.13) better than others with

Table 2

Limits of detection, recoveries and relative standard deviation ( $n=4$ ) of selected pesticides for the extraction with RP-18 cartridges of 50–200 ml of spiked deionized water samples (1.2  $\mu\text{g/l}$ )

Pesticide	LODs (ng/l)	Recovery (%)		
		Extracted water volumes (ml)		
		50	100	200
Oxamyl	10	99	71	46
Methomyl	30	71	45	28
Dioxacarb	30	79	80	64
Carbendazim	70	101	79	55
Thiophanate-methyl	70	103	100	77
Carbofuran	20	73	68	52
Carbaryl	40	83	67	44
Dietofencarb	20	96	64	36
Molinate	40	94	66	41
Fenothiocarb	40	69	72	49
Iprodione	20	68	51	31
Thiobencarb	50	62	57	35

greater octanol–water partition coefficients such as thiobencarb ( $\log K_{ow}$  3.4).

For the selected pesticides the breakthrough volumes with silica-based phases was reached, on the whole, before 100 ml. However, the RP18 allows sorption and desorption of 100 ml of dioxacarb, methylthiophanate, carbofuran, fenothiocarb and thiobencarb while maintaining maximum recoveries. The same observation is valid for carbofuran, moli-

nate, and fenothiocarb extracted with CN, and for methylthiophanate and carbofuran extracted with  $C_2$ .

Since the extractive cartridge is coupled on-line with the analytical column, the phase contained in the cartridge hardly influences the analytical chromatographic behaviour. In on-line processes, this observation has been extensively discussed when carbon or PSDVB extractive cartridges are coupled with  $C_{18}$  analytical columns [2,3,18]. However, the

Table 3

Limits of detection, recoveries and relative standard deviation ( $n=4$ ) of selected pesticides for the extraction with  $C_2$  cartridges of 50–200 ml of spiked deionized water samples (1.2  $\mu\text{g/l}$ )

Pesticide	LODs (ng/l)	Recovery (%)		
		Extracted water volumes (ml)		
		50	100	200
Oxamyl	10	99	88	66
Methomyl	20	99	84	54
Dioxacarb	20	71	59	41
Carbendazim	70	96	79	57
Thiophanate-methyl	90	77	79	57
Carbofuran	10	101	96	68
Carbaryl	30	71	45	31
Dietofencarb	20	56	48	<sup>a</sup>
Molinate	50	37	21	<sup>a</sup>
Fenothiocarb	30	60	47	38
Iprodione	20	47	33	<sup>a</sup>
Thiobencarb	50	49	30	<sup>a</sup>

<sup>a</sup> Recoveries <20%.

Table 4

Limits of detection, recoveries and relative standard deviation ( $n=4$ ) of selected pesticides for the extraction with CN cartridges of 50–200 ml of spiked deionized water samples ( $1.2 \mu\text{g/l}$ )

Pesticide	LODs (ng/l)	Recovery (%)		
		Extracted water volumes (ml)		
		50	100	200
Oxamyl	10	104	785	5
Methomyl	40	63	49	32
Dioxacarb	20	85	57	41
Carbendazim	80	90	70	53
Thiophanate-methyl	70	101	89	67
Carbofuran	20	89	87	76
Carbaryl	40	92	75	63
Dietofencarb	20	76	59	33
Molinate	20	95	93	72
Fenothiocarb	40	54	54	44
Iprodione	20	50	31	<sup>a</sup>
Thiobencarb	70	33	<sup>a</sup>	<sup>a</sup>

<sup>a</sup> Recoveries <20%.

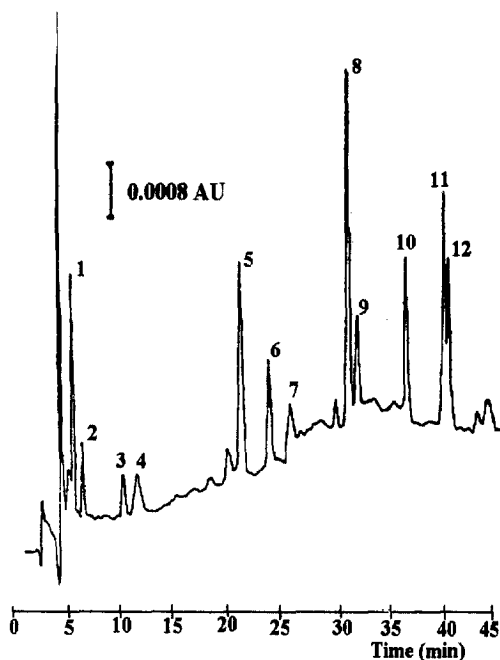


Fig. 2. HPLC–UV (210 nm) chromatograms obtained after on-line preconcentration of 50 ml of spiked deionized water ( $1.2 \mu\text{g/l}$  level). Forward elution of LiChrospher RP18 cartridges using the mobile phase program. Peaks: 1=oxamyl, 2=methomyl, 3=dioxacarb, 4=carbendazim, 5=methylthiophanate, 6=carbofuran, 7=carbaryl, 8=dietofencarb, 9=molinate, 10=fenothiocarb, 11=iprodione, 12=thiobencarb.

effect is evident even when coupling phases with similar polarity, and the resolution for some critical peaks is affected.

The narrower peak widths correspond to the use of RP18 and CN (see Figs. 2 and 3). The critical pairs dietofencarb–molinate and iprodione–thiobencarb can be correctly quantified with RP18, but new runs as described for the PSDVB are required with the CN cartridge.

#### 4. Conclusions

GCB cartridges extracted the selected pesticides efficiently from water, but desorption with an acetonitrile–water gradient was incomplete.

Strong retention of the selected pesticides on the carbon and on PSDVB made elution by backflushing necessary, and produced a band broadening which overlapped some peaks. This effect was more evident with the PSDVB phase than with the GCB.

Extracting 50 ml of spiked deionized water samples, the best recoveries were obtained with the RP18 phase, followed in decreasing order by CN, PSDVB, C<sub>2</sub> and GCB. Breakthrough volumes are reached earlier with silica-based phases than with PSDVB.

The RP18 phase appears to be a good choice for

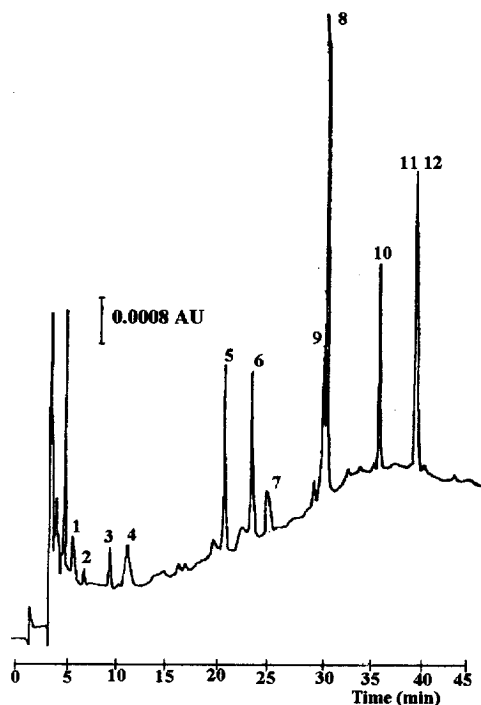


Fig. 3. HPLC–UV (210 nm) chromatograms obtained after on-line preconcentration of 50 ml of spiked deionized water (1.2  $\mu\text{g/l}$  level). Forward elution of Isolute CN cartridges using the mobile phase program. Identification of peaks as in Fig. 2.

preconcentrating nitrogen-containing pesticides from water. This technique is at present being tested by our laboratory on environmental water samples.

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